

BIODIESEL PRODUCTION FROM PETROCHEMICAL INDUSTRY SLUDGE:
DIRECT LIQUID-LIQUID LIPID EXTRACTION METHOD

by

Elif Babayiğit

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ABSTRACT

BIODIESEL PRODUCTION FROM PETROCHEMICAL INDUSTRY SLUDGE: DIRECT LIQUID-LIQUID LIPID EXTRACTION METHOD

The oily sludges from petrochemical industry wastewater plant (WWTP) have high lipid content and can be used as lipid feedstocks. Standard drying method necessitates expensive sludge dewatering/drying steps, holding 50 % of overall biodiesel production cost, to remove high water content in sludge. This study explored lipid extraction from petrochemical industry WWTP sludges by using the novel direct liquid-liquid lipid extraction method, eliminating costly sludge dewatering and drying steps, and to compare it to standard drying method. The study also investigated the effect of pre-treatment methods on lipid and biodiesel yields of sludge samples. In both methods, hexane was used as an organic solvent. The direct liquid-liquid lipid extraction method, which does not require expensive sludge drying or dewatering steps, resulted with 53 % higher lipid and 56 % higher biodiesel yields for petrochemical industry WWTP sludges than the standard drying method. Acid pre-treatment increased lipid and biodiesel yields by using direct liquid-liquid and standard drying lipid extraction methods. The ultrasonication pre-treatment alone and microwave pre-treatment alone did not cause to an important change in lipid and biodiesel yields for the direct liquid-liquid lipid extraction method. The combined pre-treatment methods (acidification/ultrasonication and acidification/microwave) considerably increased the lipid and biodiesel yields for both extraction methods. The highest lipid (47.1 %) and biodiesel (32.6 %) yields were achieved by application of combined acidification/microwave pre-treatment to the thickener sludge samples and using the liquid-liquid lipid extraction method. Liquid-liquid lipid extraction seems to be an energy and cost efficient way of sludge management.

ÖZET

PETROKİMYA ENDÜSTRİSİ ÇAMURUNDAN BİYODİZEL ÜRETİMİ: DİREKT SIVI-SIVI LİPİT EKSTRAKSİYON YÖNTEMİ

Petrokimya endüstrisi atıksu arıtma tesisinin (AAT) yağlı çamurları yüksek lipit içeriğine sahiptir ve lipit hammaddesi olarak kullanılabilir. Standart kurutma yöntemi, çamurdaki yüksek su miktarını gidermek için, toplam biyodizel üretim maliyetinin % 50'sini tutan, pahalı çamur susuzlaştırma/kurutma aşamalarını gerektirmektedir. Bu çalışma, petrokimya endüstrisi AAT çamurlarından, yenilikçi direkt sıvı-sıvı lipit ekstraksiyon yöntemini kullanarak, pahalı çamur susuzlaştırma ve kurutma aşamalarını ortadan kaldırarak ve standart kurutma yöntemiyle karşılaştırmak suretiyle lipit ekstraksiyonunu araştırmıştır. Çalışma ayrıca, ön arıtma yöntemlerinin çamur örneklerinin lipit ve biyodizel verimleri üzerindeki etkisini araştırmıştır. Her iki yöntemde, hekzan bir organik çözücü olarak kullanılmıştır. Pahalı çamur kurutma veya susuzlaştırma aşamaları gerektirmeyen direkt sıvı-sıvı lipit ekstraksiyon yöntemi, petrokimya endüstrisi AAT çamurları için standart kurutma yöntemine göre % 53 daha yüksek lipit ve % 56 daha yüksek biyodizel verimi ile sonuçlanmıştır. Asit ön-arıtımı direkt sıvı-sıvı ve standart kurutma lipit ekstraksiyon yöntemleri kullanılarak lipit ve biyodizel verimini arttırdı. Tek başına ultrasonikasyon ön-arıtımı ve tek başına mikrodalga ön-arıtımı, direkt sıvı-sıvı lipit ekstraksiyon yöntemi için lipit ve biyodizel verimlerinde önemli bir değişikliğe neden olmamıştır. Birleşik ön-arıtma yöntemleri (asidifikasyon/ultrasonikasyon ve asidifikasyon/mikrodalga), her iki ekstraksiyon yöntemi için lipit ve biyodizel verimlerini önemli ölçüde artırmıştır. En yüksek lipit (% 47.1) ve biyodizel (% 32.6) verimleri, koyulaştırıcı çamur numunelerine birleşik asidifikasyon/mikrodalga ön-arıtımının uygulanması ve sıvı-sıvı lipit ekstraksiyon yönteminin kullanılmasıyla elde edilmiştir. Sıvı-sıvı lipit ekstraksiyonu, çamur yönetiminin enerji ve maliyet etkin bir yolu olduğu görülmektedir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
CO ₂	Carbon dioxide	
H ₂ O	Water	
H ₂ SO ₄	Sulfuric Acid	
HCl	Hydrochloric Acid	
NaOH	Sodium Hydroxide	
KOH	Potassium Hydroxide	
μm	Micrometer	

Abbreviation	Explanation	Unit
BTU	British Thermal Unit	
COD	Chemical Oxygen Demand	mg/L
CST	Capillary Suction Time	seconds
DM	Dry Matter	
FAME	Fatty Acid Methyl Ester	%
FFA	Free Fatty Acid	%
GC	Gas Chromatography	
IL	Ionic Liquid	
MLSS	Mixed Liquor Suspended Solids	mg/L
MLVSS	Mixed Liquor Volatile Suspended Solids	mg/L
MW	Microwave	
ORP	Oxidation Reduction Potential	mV
PAH	Polycyclic Aromatic Hydrocarbon	
PHC	Petroleum Hydrocarbon	
sCOD	Soluble Chemical Oxygen Demand	mg/L
VS	Volatile Solids	%
TS	Total Solids	%
US	Ultrasonication	
W/O	Water in Oil	
WAS	Waste Activated Sludge	
WWTP	Wastewater Treatment Plant	

1. INTRODUCTION

Energy has become a very important issue for the community. Today, with the rapid improvement of the society, the world population, industrialization and technology continue to grow, thereby leading to an energy crisis. According to reports of the International Energy Agency, the energy need of the world will increase by 39 % than the world's current situation (World Energy Outlook, 2017).

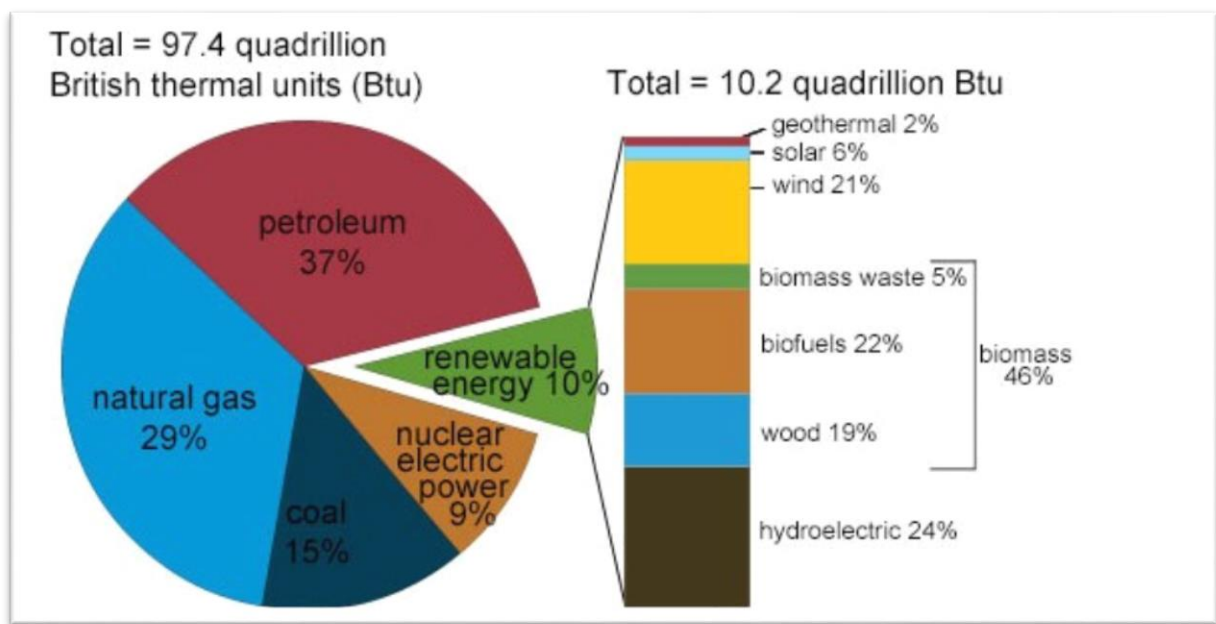


Figure 1.1. Distribution of the world energy consumption by fuel source in 2016 (EIA, 2016).

The source of 81 % of all energy used in the world is fossil fuels as seen in the Figure 1.1. It is known that in near future, these non-renewable fossil fuel energy sources will be depleted. Furthermore, the environmental problems related with over-consumption of fossil fuel sources such as greenhouse gas emissions (GHG), increased carbon footprint, natural resource depletion, global warming and air pollution, consequently, limit the access of these sources in the future. The alternatives to these fossil fuels, such as nuclear electric power (9 %) and renewable energy sources (10 %) currently constitute only 19 %.

As shown in the Figure 1.1., petroleum fuel, which is the major source of the world's energy, indicates 37 % of the world energy consumption among the fossil fuel sources. It is known that the transportation sector consumes the great majority of the world's oil need (IEO, 2017). The petroleum fuel demand for the transportation sector will increase very fast in the future. Thus, the demand for

petroleum fuels will increase day by day and with the effect of this, an expected increase in crude oil prices will occur. However, fossil fuels' depletion expectations resulting from excessive use and concerns arising from damages of the fossil fuels to the environment, will restrict their utilization in the near future. In this regard, it is necessary to identify and utilize renewable fuels having low impact on environment as alternative energy sources.

In Turkey, energy demand has been on a rising trend to satisfy the quickly growing energy and economic needs of the country over the last forty years. The use of fossil fuels was 113.6 Mtoe in 2015. Natural gas, coal and oil covered a third of total supply of energy in 2015: Natural gas (30.2 % or 39.2 Mtoe), oil (30.1 % or 39 Mtoe) and coal (27.3 % or 35.3 Mtoe). The supply of oil has increased from 28.7 Mtoe to 39 Mtoe, which is the main reason that the fuel requirement for transport increases by 68.7 %, during the last decade. Renewable resources comprising 48.9 % of all energy production are 10.1 % biomass (biofuels and waste), 17.9 % hydroelectricity, 14.8 % geothermal, 3 % solar and 3.1 % wind. According to the government, it is expected that total supply of energy will reach 222.4 Mtoe by 2020. In transport, 97.1 % of the total fuel demand is provided by petroleum oil and the rest is composed of natural gas (1.8 %), biofuels (0.7 %) and electricity (0.4 %). Biofuels were first offered to the Turkish transportation sector in 2006 (IEA, 2016).

Currently, there are 23 biodiesel facilities with operating licenses and 15 biodiesel facilities with distribution licenses. Even though Turkey has available feedstocks to produce biodiesel, existing biodiesel production capacity is not been fully utilized. Despite the biodiesel facilities in Turkey having 235 thousand tons of the biodiesel production capacity, the amount of biodiesel produced last year stayed at 70 thousand tons. Energy Market Regulatory Authority (EMRA) aims to produce approximately 115 thousand tons of biodiesel per year by making at least 0.5 % biodiesel blends in fuel oil compulsory from the beginning of 2018. It is anticipated that this production will increase by at least 50 percent by regulation (EMRA, 2016).

'Sustainable development' requires use of renewable fuels having low environmental impact (Hongyan et al., 2009). Biodiesel has some advantages like being biodegradable and environmentally friendly over the conventional diesel (Srivastava and Prasad, 2000). Biodiesel does not require any modification and can be used directly (Olkiewicz et al, 2012).

Currently, various types of lipid feedstocks such as animal fats, vegetable oils (canola, palm, coconut, soybean, and rapeseed), waste cooking oils and microalgae are used for biodiesel production. However, more than 70 % of the overall biodiesel production cost is spent on the raw materials for

lipid feedstocks (Kargbo, 2010; Pastore et al., 2013; Vicente et al., 2009). Sewage sludge has been considered as a promising lipid feedstock in the previous studies carried out for this purpose (Dufreche et al., 2007; Olkiewicz et al., 2012; Pastore et al., 2013; Shen and Zhang, 2003). On the other hand, petrochemical industry wastewater treatment plant (WWTP) sludge is another non-edible lipid feedstock that proposes significant lipid potential for biodiesel production.

High water content of the sludge is the major problem for the biodiesel production process. Water can constitute more than 95-98 %wt of the sludge and limits the lipid extraction efficiency. Sludge dewatering and drying steps are necessary in conventional reference drying lipid extraction method (Mondala et al., 2009). Almost half of overall biodiesel production cost is used for the dewatering and drying of the sludges making the process very expensive and hard to implement (Dufreche et al., 2007; Olkiewicz et al., 2014).

So far, few studies have used wet sewage sludge for lipid extraction by direct liquid-liquid lipid extraction and demonstrated applicability of this method (Kech et al., 2018; Olkiewicz et al., 2014). The direct liquid-liquid extraction method provides the simple lipid extraction from sludge samples (Olkiewicz et al., 2016). It is also reported that the yield of biodiesel can increase with this proposed method without the need for sludge drying and dewatering steps. Surprisingly, the direct liquid-liquid lipid extraction from petrochemical industry WWTP sludge has neither been reported. Thus, this study investigates the direct liquid-liquid extraction of lipid from petrochemical industry WWTP sludge to obtain high lipid yield without applying any sludge dewatering or drying steps. The direct liquid-liquid lipid extraction method was compared to reference drying method to demonstrate the liquid-liquid extraction method's feasibility. The liquid-liquid lipid extraction method was optimized varying the total solid concentrations of oily sludge samples and the sludge to solvent ratios (by volume). The study also investigated the effects of several sludge pre-treatment methods on the lipid extraction and so the biodiesel production by applying acidification, ultrasonic, microwave and combined pre-treatments to the sludge samples.

2. LITERATURE REVIEW

2.1. Biodiesel Characteristics

Biodiesel mainly comprises fatty acid methyl esters (FAMES), which can be produced from a variety of lipid sources through the esterification and/or transesterification process with an acid, base, enzyme or solid catalyst application (Bozaghian, 2014; Siddiquee and Rohani, 2011). The primary benefit of biodiesel is that it can use existing commercial diesel engine technology and does not require a new refueling station. It has major environmental advantages; it is renewable, sustainable, nontoxic, extremely biodegradable (fourfold faster than conventional diesel), safer for storage and transport, has lower harmful emissions (CO₂, CO, hydrocarbons, particulates) and almost no sulphur and aromatics. The use of biodiesel as an alternative energy source would reduce sulphur emissions by 30 % and carbon monoxide emissions by 10 %. Increased use of biodiesel has shown that air pollution and cancer types decrease by 90 % and 95 %, respectively (Sharp, 1996). Additionally, biodiesel has inherent lubricity, similar energy density and refueling technology to petroleum diesel. In commercial diesel engines, it can be used directly in a pure form (B100) or mixed with petroleum diesel at desired concentrations (Khan et al., 2014; Siddiquee et al., 2011). Considering all these characteristics, it can be observed that biodiesel is an excellent alternative to conventional petroleum diesel.

Table 2.1. Properties of biodiesel. (Boz et al., 2009; Demirbas, 2003; Monisha et al., 2013).

Properties	Values
Specific gravity	0.87 - 0.89
Kinematic viscosity @40°C (mm ² /s)	3.7 - 5.8
Cetane number	46 - 70
Higher heating value (Btu/lb)	16928 - 17996
Lower heating value (Btu/lb)	15700 – 16735
Sulphur wt %	0 – 0.0024
Cloud point °C	-11 - 16
Pour point °C	-15 – 13
Iodine number	60 – 135
Flash point °C	120 – 130

2.2. Biodiesel Feedstocks

The choice of feedstock is one of the most significant issues that determine the economy of the whole biodiesel production process. As a general rule, on each production line, the raw material cost should not exceed 50 % of the production price. The feedstock must meet two main requirements as much as possible: low production costs and large production scale. The availability of feedstocks for biodiesel production hinges on the geographical location of any country, its regional climate, agricultural practices and local soil conditions. According to the literature, feedstock by itself accounts for 75 % of total biodiesel production cost. For this reason, choosing the cheapest feedstock is very important to lower the cost of biodiesel production (Demirbas, 2008a; Lim and Teong, 2010; Masjuki, 2010; Pinto et al., 2005; Singh and Singh, 2010).

Biodiesel feedstocks can be divided into six main categories as below:

1. Edible plant oils : soybean, rapeseed, sunflower, palm oil, coconut oil and peanut.
2. Non-edible plant oils : jatropha, karanja, neem, castor and halophytes.
3. Waste cooking oil
4. Animal fats : chicken fat, tallow, yellow grease and by-products from fish oil.
5. Microalgae
6. Wastewater sludge

Some important factors need to be taken into account for the comparison of different feedstocks. Every feedstock should be figured out according to a full life-cycle analysis. This analysis contains: (1) land availability, (2) agricultural applications, (3) energy source and energy balance, (4) greenhouse gas emissions, (5) pesticide applications, (6) soil erosion and fertility, (7) effect on biodiversity, (8) transportation and storage cost, (9) direct effect of feedstocks on the economy, (10) creation or maintenance of employment, (11) water use, (12) effects of feedstocks on the air quality (Ahmad et al., 2011, Balat, 2011; Chisti, 2007).

Sources of edible plant oils such as soybean, rapeseed, palm oil, sunflower, aspir and coconut are supposed to be the first generation of biodiesel feedstocks because they are the first products to be used for biodiesel production. Currently, more than 95 % of world's biodiesel is produced by using edible plant oils. These include rapeseed (84 %), sunflower oil (13 %), palm and soybean oil (1 %) and others (2 %). Nevertheless, their use is accompanied by major environmental problems, such as the food crisis in response to the fuel crisis, significant devastation of land sources, desertification and the use of the majority of existing farmable soil. Moreover, over the past decade, the costs of

vegetable oil factories have increased significantly, affecting the economic viability of the biodiesel industry (Balat and Balat, 2010; Balat, 2011; Deng et al., 2011). It is not appropriate to use these edible plant oils for biodiesel production in the long-term because of the large difference between these countries' supply and demand of these oils in many countries. Furthermore, direct use of edible plant oils in diesel engines leads to many difficulties such as seizing and gumming of filters, lines and injectors; engine misfiring; coking of injectors on piston and head of engine; starting problem in cold weather; extreme engine wear; carbon accumulation in the piston and head of the engine (Suppes et al., 2001). One of the solutions to be decreased the use of edible plant oils for biodiesel production is the utilization of non-edible plant oils.

Non-edible plant oils are easily found in many parts of the world, especially in wastelands where the food crops are not suitable for planting. These oils can eliminate competition with the food market, reduce the rate of deforestation, produce useful by-products and they are more efficient, more environmentally friendly and much more economical compared to the edible plant oils. The major resources for biodiesel production from non-edible oils are karanja, jatropha, rubber seed tree, desert date, rice bran, castor, sea mango, neem, tobacco seed and jojoba. Non-edible plant oils are considered as the second generation of biodiesel feedstocks. However, it is known that the second-generation feedstocks will not be sufficient to meet global energy demand (Budiman et al., 2010). Furthermore, over cultivation of these feedstocks would result in deforestation and destruction of the ecosystem. Moreover, biodiesel obtained from non-edible plant oils has a comparatively inefficient performance in cold climates (Khan et al, 2014).

Biodiesel can also be produced by using waste cooking oils because of their low cost and elimination of the production step. Waste cooking oils gathered from large food processing units and service facilities enable the fatty acids ratio to increase as a result of the various chemical reactions during the food frying process (Canakci and Gerpen, 2001). However, as resources are often scattered, collection infrastructure and logistics are problematic to produce biodiesel in sufficient quantities (Balat, 2011; Demirbas, 2008a; Janaun and Ellis, 2010; Kumar and Sharma, 2011; Lin et al., 2011; Sharma and Singh, 2009; Singh and Singh, 2010).

Animal fats can be supplied more easily because the slaughter industry is generally well managed for product control and processing. There is no need to dispose of these feedstocks by the utilization of them. However, there are biosafety and biosecurity problems with the animal fats that may come from contaminated animals (Greene et al., 2005). Moreover, biodiesel produced from animal fats has a comparatively inefficient performance in cold weathers than vegetable oils (Canakci and Sanli,

2008). Since many types of animal fats contain high amounts of saturated fatty acids, the transesterification process required for biodiesel production is difficult to achieve (Atabani et al., 2012).

Microalgae have arisen as a third generation of biodiesel feedstocks in recent times. They convert sunlight, CO₂ and water into the algal biomass. They are more efficient in productivity and have higher lipid contents compared to the edible and non-edible plants. Moreover, microalgae can be grown more easily than many other plants, and even certain species can be obtained daily (Hu et al., 2008). Although there has been increased interest in the use of microalgae as feedstocks for biodiesel production, algae growth costs continue to arise due to the need for high-oil yielding algae strains for harvesting and the need for large-scale bioreactors (Ahmad et al., 2011; Hossain et al., 2008; Sharma et al., 2008).

Researchers focus on increasing the efficiency and reducing the cost of total biodiesel production (Ishak et al., 2017, Kamel et al., 2017, Srivastava et al., 2018). In this context, wastewater sludge is an effective feedstock for the production of biodiesel because it has a high content of organic matter (e.g. lipids), it is abundant, cost-free, readily and easily available (Boocock et al., 1992; Dufreche et al., 2007; Olkiewicz et al., 2012; Qi et al., 2016; Siddiquee and Rohani, 2011; Zhu et al., 2014). Moreover, wastewater sludge, as a waste source is economically available and environmentally friendly and not only brings about the development of biofuels, but also contributes to the resolution of the pollution problem. This sludge consists of various organisms consuming organic matter in wastewater. The amount of phospholipids present in the cells of these organisms have been evaluated to be 24 % to 25 % of the dry mass (Dufreche et al., 2007). Since phospholipids can be easily transesterified, they can be used as a ready-to-use source for biodiesel production. On the other hand, petrochemical industry wastewater treatment plant (WWTP) sludge (oily sludge) is another non-edible lipid feedstock that proposes significant potential for biodiesel production.

2.3. Petrochemical Industry WWTP Sludge as a Feedstock

A significant amount of oily sludge is generated from the petrochemical industry wastewater treatment plants (Xu et al., 2009). In general, a higher refining capacity is directly proportional to the amount of oily sludge production. Every year, more than 60 million tons of oily sludge can be generated worldwide and more than 1 billion tons of oily sludge accumulation has been occurred (BP, 2012; Silva et al., 2012; Tahhan et al., 2011). Moreover, the growing demand for refined petroleum

products worldwide will lead to the increase of total oily sludge production (BP, 2012; Bhattacharyya and Shekdar, 2003).

Petrochemical industry WWTP sludge can cause serious environmental and human health problems when it disposes without applying any treatment due to release of toxic and carcinogenic contaminants (Mishra et al., 2001). The treatment cost of oily waste sludge constitutes 60 % of the total cost of the wastewater treatment plant (Andrews et al., 2006; Liu, 2001; Wang and Wang, 2007). Because of its hazardous composition and global population growth, the effective treatment of oily sludge has gained importance in recent years (He and Zhou, 2004; Vaxelaire and Cezac, 2004). Given the relatively high lipid content in the petrochemical industry WWTP sludge, lipid recovery can be the most preferred management option since it cannot only make profit but also reduce waste volume and pollutant concentration (Elektorowicz and Habibi, 2005; Hu et al., 2013; Silva et al., 2012). Consequently, biodiesel production from this sludge as an effective option to common sludge treatment methods will be a solution to the energy and environmental problems.

2.3.1. Petrochemical Industry WWTP Sludge Production

Typical petrochemical industry wastewater treatment plants include an API separator for oil recovery, a dissolved air flotation (DAF) unit and an activated sludge process (ASP) for treating soluble and colloidal organic substances in the wastewater. Petrochemical industries use a complex process system that produce wastewater and oily sludges with varying physical/chemical properties. The complexity of the refining process determines the composition of the oily sludge produced. In these type of industries, primary treatment of the wastewater is a physical operation which removes floating and settleable solids in the wastewater obtained by either gravity separation or by flotation (Eckenfelder, 2000). The soluble and colloidal organic matters are made into biological sludge (waste activated sludge – WAS) in a typical activated sludge process in which wastewater is treated.

The oily sludge obtained from thickening unit of the petrochemical industry WWTP contains sludges from oil separators, primary clarifier, and the waste activated sludge from secondary clarifier and can be used as a feedstock for biodiesel production. It comprises of various organic and inorganic compounds consisting water-soluble metals, salts, phospholipids, suspended solids, petroleum hydrocarbons (PHCs), neutral lipids and free fatty acids (FFA) sourced from the oils that cannot be removed in WWTP and sourced from biological (waste activated) sludge in the plant (Mazlova and Meshcheryakov, 1999; Mulligan, 2009). Furthermore, the secondary clarifier tank of the activated sludge process produces biological sludge because of the biomass growth in the presence of organic

substances in the wastewater. The secondary clarifier sludge may include less organic compounds, higher water content, and biomass-derived organic solid materials and may be suitable for the biodiesel production process. (Bougrier et al., 2006; Kopp et al., 1997; Wang et al., 2014).

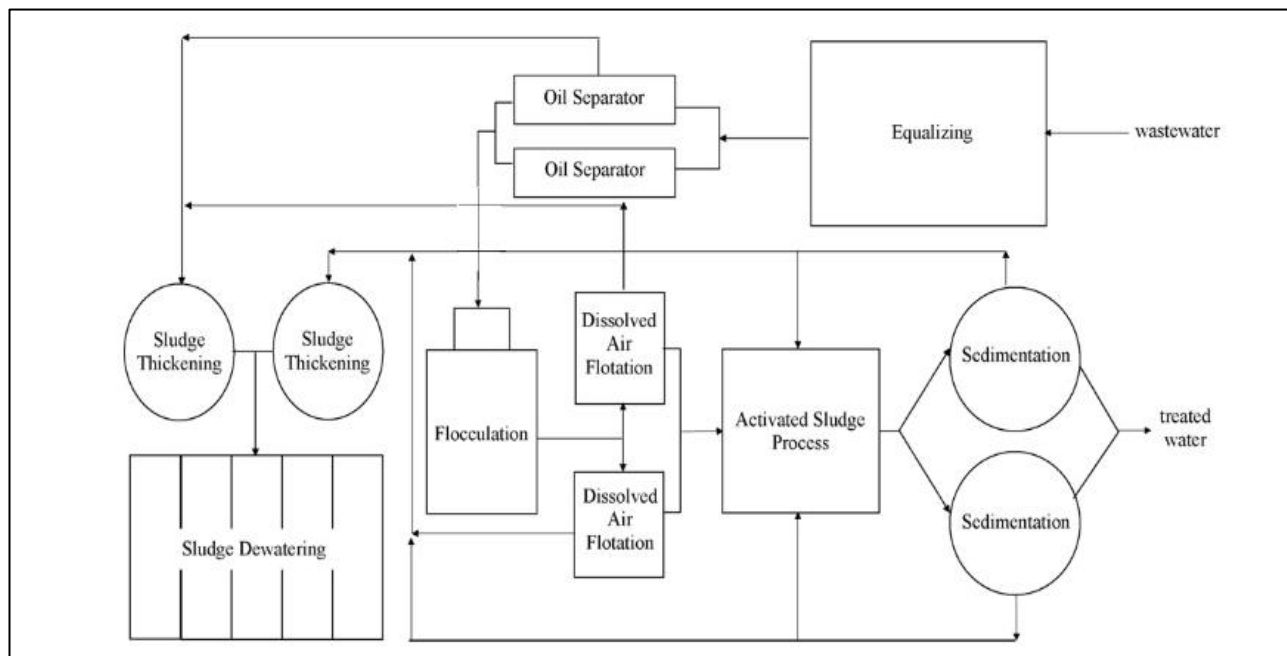


Figure 2.1. The flow scheme of a typical petrochemical industry wastewater treatment plant.

2.4. Lipid Extraction

Biodiesel production requires lipid extraction from the organic sources as a first step. Lipids cannot dissolve in water and they are extracted from fluids, cells or tissue by using organic solvents. The separation of different lipids into the organic phase and lipid composition of the sample play an important role in the efficiency of lipid extraction process. The most commonly used solvent systems for lipid extraction are Soxhlet extraction method, Bligh and Dyer method, Folch method and in situ lipid hydrolysis method.

When the wastewater sludges are used as a lipid feedstock, lipid extraction process might be a problematic due to the chemical complexity and diversity of the sludge produced in the WWTPs (Bharathiraja et al., 2014). The main challenges may arise from; (1) sludge pre-treatment selection for effective lipid extraction, (2) the lipid extraction methods, (3) the biodiesel production methods, (4) the biodiesel quality and (5) process economy and safety. On the other hand, lipid extraction from wastewater sludge for biodiesel production can provide significant environmental and economic benefits (Kwon et al., 2012). In literature, there are many studies on lipid extraction to produce

biodiesel from wastewater sludges utilizing different methods. Most of the lipid extraction methods require dewatering or drying of the sludge samples.

2.4.1. Conventional Extraction Methods Using Organic Solvents

Conventional organic solvent extraction method is the main method for extracting lipids from the wastewater sludges. Some studies have also explored the utilization of subcritical methods for lipid extraction, but it has been understood that extreme subcritical conditions are costly for extensive implementations (Dufreche et al., 2007; Huynh et al., 2010; Huynh et al., 2012; Siddiquee and Rohani, 2011). Comprehensive studies were carried out in organic solvent extraction to select the most efficient solvents. Various solvents such as hexane, toluene, ethanol, chloroform and methanol were investigated alone or in mixtures (Boocock et al., 1992; Dufreche et al., 2007; Pokoo-Aikins et al., 2010).

Various solvents have also been investigated for lipid extraction from oily sludge. Gazineu et al. (2005) used turpentine as a solvent for lipid extraction and found that the lipid yield for 13-53 % of the original sludge mass. Zubaidy and Abouelnasr (2010) used methyl ethyl ketone (MEK) and liquefied petroleum gas condensate (LPGC) as organic solvents and they compared these solvents to examine their effects on the lipid extraction. They obtained the highest lipid extraction yield of 39 % and 32 % by MEK and LPGC extraction, respectively. El Naggar et al. (2010) compared the effects of kerosene cut, naphtha cut, toluene, n-heptane, ethylene dichloride, methylene dichloride and diethyl ether as solvents to extract lipids from dry and semi-dry petroleum sludge and the toluene resulted with 75.94 % of the highest lipid extraction yield. Meyer et al. (2006) found that the virgin paraffinic diesel as a solvent was highly effective in extracting lipids from the petroleum sludge. Hexane and xylene as solvents have also been compared in terms of lipid extraction efficiencies for the oily sludge and both of them showed that about 67.5 % of lipid extraction yield could be achieved (Taiwo and Otolurin, 2009).

The selection of suitable solvent should depend on several features such as boiling point, polarity, miscibility with water, cost and environmental problem. Accordingly, toluene and chloroform are harmful to the environment and the utilization of a solvent mixture is not useful because it is very hard to recover the solvent alone from the mixture and is more energy efficient (Siddiquee and Rohani, 2011). With respect to polarity, non-polar solvents are well known to be the best solvent types for lipid extraction and biodiesel production experiments because they are capable of extracting non-polar/saponifiable lipids (free fatty acids and glycerides) that can be converted to

biodiesel. Moreover, polar solvents cause a high extractable portion because of the extraction of polar lipids and non-lipid portions, increasing biodiesel contamination by giving lipids which can be saponified at low levels. On the other hand, the ultimate cost of biodiesel production performing extraction with non-polar solvents (toluene, hexane etc.) is cheaper than extraction with polar solvents (ethanol, methanol etc.). When the extraction process is considered in its environmental aspect, it is seen that hexane has an environmentally friendly structure compared to the toluene (Pokoo-Aikins et al., 2010). Olkiewicz et al. (2012) proved that similar results were obtained from lipid extraction and biodiesel production studies with hexane and toluene.

The main obstruction in biodiesel production from wastewater sludges is an efficient lipid extraction from sludges having very high water content of about 95–98 %wt. This makes the conventional biodiesel production very expensive and difficult to scale up due to the necessary sludge dewatering and drying processes constituting about 50 % of the overall biodiesel production cost (Dufreche et al., 2007; Olkiewicz et al., 2014). Despite of this well-known fact, most of the studies have only been reported using solvent extraction method in lipid extraction or in situ transesterification of dried or dewatered sludge (Choi et al., 2014; Dufreche et al., 2007; Huynh et al., 2010; Mondala et al., 2009; Pastore et al., 2013; Perez et al., 2013; Revellame et al., 2011; Siddiquee and Rohani, 2011). Boocock et al. (1992) studied two different lipid extraction methods: Soxhlet extraction and boiling solvent extraction methods by using toluene and chloroform as solvent. They found the lipid yields to be about 12 %wt dry matter (DM) and 17-18 %wt DM for both of the solvents by using Soxhlet extraction and boiling solvent extraction methods, respectively. Although both solvents resulted with similar lipid extraction yields, they reported that toluene is a favorable solvent over the chloroform due to the economic and environmental reasons (Boocock et al., 1992). Dufreche et al. (2007) obtained a maximum lipid yield of 27.43 ± 0.98 %wt for lipid extraction from activated sludge by using pure and/or mixture of hexane (60 %), methanol (20 %) and acetone (20 %) as solvents. They also obtained lipid yields of 13.56 %wt and 3.55 %wt by using supercritical- CO_2 with methanol co-solvent technique and supercritical- CO_2 technique for lipid extraction experiments, respectively (Dufreche et al., 2007). Mustapha et al. (2017) optimized the lipid extraction from primary sewage sludge by using Soxhlet extraction method. 40.21 % of lipid yield was obtained by using methanol as a solvent in optimum conditions (Mustapha et al., 2017). Zhu et al. (2012) investigated the organic solvent extraction method by using different organic solvents to determine which solvent has the ability to extract the maximum lipid yield from sewage sludge. They concluded that mixed solvent has higher extraction efficiency than the single solvent (Zhu et al., 2012). Another study by Zhu et al. (2014) investigated the performance of three different extraction methods (acid hydrolysis extraction, soxhlet extraction, and the water bath shaking extraction) and

compared the lipid yields obtained from sewage sludge. They also studied the effects of different organic solvents on extraction efficiency. They concluded that Soxhlet extraction resulted with the best lipid yield compared to other methods (Zhu et al., 2014). Olkiewicz et al. (2012) investigated four different types of wastewater sludges (primary, secondary, blended and stabilized) for biodiesel production and obtained the highest lipid and biodiesel yields from the primary sludge using hexane as a solvent. 25.3 %wt DM of lipid yield by Soxhlet extraction was obtained from primary sludge (Olkiewicz et al., 2012). Moreover, conventional thermal drying or freeze-drying methods causes the loss of valuable organic compounds in sludge and reduces the biodiesel production yield (Cordero Esquivel et al., 1993).

2.4.2. Ionic Liquids as an Extracting Solvent

Ionic liquids (ILs) have attracted great interest in recent years for the utilization as a green substitute for harmful volatile organic solvents. They have outstanding properties such as perfect chemical and thermal stability, non-volatility except for low pressure and high temperature conditions and design possibilities (Plechova and Seddon, 2008). The ILs are described as salts present in a liquid form under 100° C. Successful results were obtained by using ILs in lipid extraction studies from dry biomass (Choi et al., 2014; Kim et al., 2012; Young et al., 2010). Also, the application of ILs for lipid extraction from wet biomass has also been recorded (Choi et al., 2014; Kim et al., 2012; Teixeira, 2012). The wet biomass was suggested that it could allow recovery of all organic components from the direct dissolution of ILs (Fujita et al., 2013; Teixeira, 2012). However, all investigations on the use of ILs in lipid extraction experiments have focused only on the use of microalgae biomass and imidazolium-based ILs. The availability and suitability of imidazolium-based ILs are also limited because of their high cost (Choi et al., 2014; Fraser and MacFarlane, 2009; Fujita et al., 2013).

2.4.3. Enzymatic Lipid Extraction

In the enzymatic lipid extraction method, appropriate enzymes are utilized to extract lipid from the crushed seeds. The use of enzymes makes this method feasible because this method is environmentally friendly and does not produce volatile organic compounds. In aqueous enzymatic oil extraction studies, it has been observed that the use of alkaline protease gave better results (Achten et al., 2008; Mahanta and Shrivastava, 2011). However, lower lipid yield, long process time, high energy required for water removal, high cost of enzymes and treatment of the resulting aqueous

effluent are the main disadvantages associated with this technique (Mahanta and Shrivastava, 2011; Rosenthal et al., 1996).

2.4.4. Direct Liquid-Liquid Lipid Extraction Method Using Organic Solvents

Conventional methods for lipid extraction mainly include drying or dewatering steps prior to the extraction of lipids because high water content in the wet sludge prevents mass transfer of lipids from the cell and then leads to a reduction in the efficiency of lipid extraction. The energy consumed for sludge drying/dewatering constitutes the majority of the total process energy (Dufreche et al., 2007; Lardon et al., 2009; Mondala et al., 2009; Olkiewicz et al., 2014; Patil et al., 2012). These outcomes inhibit the application of conventional methods for large-scale lipid extraction applications despite of the high extraction efficiency. For this reason, it is important to develop a new approach to lipid extraction, which is an effective and environmentally friendly process. Compared with the conventional drying methods, extracting lipids from wet biomass is a more economical method that does not require energy to dry the biomass.

Olkiewicz et al. (2014) explored the novel direct liquid-liquid lipid extraction method in which there is no need for sludge drying and dewatering steps. In this method, hexane was used as an organic solvent after previous acidification in batch mixer settler reactor at room temperature. The results of this study demonstrated that higher lipid yield (27 %wt, dry sludge) was obtained from primary sewage sludge via direct liquid-liquid extraction technique, whereas 25 %wt (dry sludge) of lipid was produced by using the standard method (Olkiewicz et al., 2014).

Kech et al. (2018) improved the direct liquid-liquid extraction method proposed by Olkiewicz and co-workers. They obtained 32.8 % (on the basis of dry sludge) of lipid yield from wet primary sewage sludge by using hexane as solvent (Kech et al., 2018).

2.5. Sludge Pre-treatments

Wastewater sludges can be pre-treated for different purposes such as sludge minimization, removal of micropollutants, increase of sludge biodegradability, improvement of biogas production and lipid extraction efficiencies. Lipid extraction from wastewater sludges can be improved by the application of various pre-treatment techniques to the sludges leading to release of lipids from macromolecules that are not present in bound form in the solvent. These processes are chemical (acid, alkali, advanced oxidation), mechanical (ultrasound, high pressure and cell lysis), thermal

(microwave), biological (enzymatic) pre-treatments or a combination of these methods such as thermo-chemical pre-treatment. Although pre-treatment processes increase overall operating costs, they appear to have beneficial effects on extraction efficiency (Gude et al., 2013; Hu et al., 2013; Kech et al., 2018; Olkiewicz et al., 2015). In this section, an introduction to the generally used pre-treatment methods is made with the specific emphasis on detailed literature analysis conducted on acid, ultrasonic and microwave treatments.

Chemical pre-treatment allows the dissolution of the cell wall by applying chemicals to the sludge. Common chemical methods include acid or alkali pre-treatment, hydrogen peroxide addition and ozonation. HCl, H₂SO₄, NaOH, KOH, Ca(OH)₂ and Mg(OH)₂ are the chemical substances used to change the pH value for acid or alkali pre-treatment (De Franchi, 2005; Kim et al., 2003). Alkaline pre-treatment has full-scale applications worldwide because it requires simple devices and has a highly efficient operation (Cassini et al., 2006). Acid pre-treatment, however, has rather limited information in the literature and requires more attention.

Mechanical pre-treatment methods allow the sludge to disintegrate by physical methods such as translational, pressure or rotational energy. The principle of the mechanical pre-treatment of sludge is based mainly on the degradation of microbial cell walls by shear stress. The shear stresses of the solids stretch and deform the cell wall and thus cause cell lysis. To produce cell lysis, the stress applied must be higher than the cell wall strength (Neyens and Baeyens, 2003). Common mechanical pre-treatment methods include high-pressure homogenizers, ultrasonic homogenizers, stirred ball mills, high performance pulse technique, mechanical jet smash technique and lysis-centrifugation technique (Müller, 2001).

Thermal pre-treatment releases intracellular bound water and generally involves high-temperature thermal pre-treatment (>100°C) and low-temperature thermal pre-treatment (<100°C). Most researchers have studied thermal pre-treatment at high temperatures, because high temperature provides a more efficient treatment. However, when the temperature is higher than 180°C, the production of recalcitrant soluble organics or toxic/inhibitory intermediates occurs and these intermediates decrease the biodegradability (Wilson and Novak, 2009).

Biological pre-treatment disintegrates sludges with or without enzymes. In general, biological pre-treatment uses external enzymes, enzyme-catalyzed reactions and autolytic processes to crack components of the cell wall (Müller, 2001).

Combined sludge pre-treatment methods like thermal-acidic or mechanical-acidic pre-treatments can also be applied. For example, the combination of the ultrasonic pre-treatment and acid pre-treatment achieves better treatment efficiency, because they have different mechanisms of sludge dissolution; their combination has the advantages of both methods (Olkiewicz et al., 2015). Another thermochemical pre-treatment study has shown that 30-50 % hydrolysis yield was obtained by thermal acidic pre-treatment with HCl and H₂SO₄ (Smith and Göransson, 1992).

2.5.1. Acid Pre-treatment

The acid pre-treatment is carried out by addition of acid to reduce the pH of the sludge. This method enhances the solubility of the organic matter in the sludge, thereby reducing the amount of sludge and increasing its dewaterability (Neyens et al., 2003). Acid pre-treatment can facilitate the lipid extraction process by disintegrating the sludge when applied to the wastewater sludge, a processed sample where the lipid can bind to carbohydrates, proteins and minerals (Olkiewicz et al., 2015).

Müller (2001) studied the effect of acid pre-treatment on the sludge samples. He showed that sludge cells can be dissolved at low or ambient temperatures. Acid pre-treatment is considered to accelerate the hydrolysis step by breaking down cell walls, mineralizing microbial cells, improving dewaterability and improving the total performance of lipid extraction process. Moreover, according to Weemaes and Verstraete (1998) high temperatures increased the pre-treatment effects by creating aggressive reaction conditions.

Pastore et al. (2013) performed the lipid fraction extraction from the sludge with hexane as a solvent. They discovered that the main components consist of soaps (mainly calcium fatty acid salts) and therefore they have suggested that the extracted lipid fraction can be increased by using a strong acid to produce the FFAs which are more soluble in the organic phase (Pastore et al., 2013).

Olkiewicz and colleagues (2014) also evaluated the effect of acid pre-treatment on the direct liquid-liquid lipid extraction from wet sludge with hexane. They have reached a conclusion that the acid pre-treatment has caused a high increase in lipid yields for the sludge samples (26.6 %wt DM and 13.0 %wt DM for acidified and unacidified samples) (Olkiewicz et al., 2014).

The study of Kech et al. (2018) investigated the effect of acid pre-treatment by performing lipid extraction from the wet sludge using hexane and found the acidified and non-acidified lipid yields as

33.5 ± 0.6 %wt DM and 31.8 ± 0.6 %wt DM, respectively. These values indicate that acid pre-treatment provides only a slightly better extraction yield.

Acid pre-treatment was selected as one of the pre-treatment methods in this study because of these yield-enhancing effects in the literature.

2.5.2. Ultrasonic Pre-treatment

The ultrasonication process occurs at frequencies between 20 kHz and 10 MHz. It produces ultrasonic waves with the spread of this sound range, resulting in periodic compression and dilution of the medium. The compression pushes the molecules together by applying a positive pressure on the medium. Dilutions are extremely large negative pressure points where molecules are separated. Microbubbles which are also called as cavitation bubbles occur as a result of the large negative pressure (Pilli et al., 2011). The achievement of the ultrasonication process depends primarily on the cavitation mechanism. There are several factors affecting cavitation mechanism; viscosity and temperature of the liquid, ultrasonic density, duration of the ultrasound and the frequency of vibration (Bougrier et al., 2006; Pilli et al., 2011).

Ultrasonic pre-treatment comprises several factors such as sonication power, frequency and sonication time, converting ultrasound into a pre-treatment instrument. Ultrasonic energy can disintegrate the sludge flocs and large organic particles, break down the bacterial cell wall, and release the intracellular materials and extracellular polymeric materials in an aqueous phase (Appels et al., 2008; Metherel et al., 2009; Pernet and Tremblay, 2003; Pilli et al., 2011).

For extractions experiments, the ultrasonic effect can be described as follows: 1) a rapid movement of fluids caused by a change in the sonic pressure that results in rarefaction cycles and compression of solvents; 2) acoustic cavitation to provide energy for the liquid phase, when the large negative pressure gradient is applied to the liquid, the liquid will be break down and cavitation bubbles (microbubbles) will be formed. At high ultrasonic intensities, a small cavity can quickly grow with inertial effects. Thus, the bubbles grow violently and collapse. The formation and collapse of microbubbles are responsible for major chemical effects, and the mass transfer is increased by disrupting the interfacial boundary layers; 3) acoustic streaming mixing (Gogate, 2002; Ozcimen and Yucel, 2011).

According to Gogate (2002), the common sonication frequency is between 20 and 200 kHz, while cavitation occurs between 20 and 40 kHz. In another study, it has been shown that mechanical forces are the most efficient when the ultrasound is operated below 100 kHz (Tiehm et al., 2001).

Ultrasonic pre-treatment has been proven to be effective for removal of adsorbed materials from solid particles, solid/liquid separation in high concentration suspensions and reduction of the stability of water/oil (W/O) emulsion (Kim and Wang, 2003; Ye et al., 2008). Cavitation may increase the temperature of emulsion system and reduce its viscosity, may increase the mass transfer of the liquid phase, thereby causing the W/O emulsion to become unstable (Chung and Kamon, 2005). Other studies have proposed that under the effect of ultrasonic irradiation, larger droplets in the emulsion can move much slower than the smaller ones. This fact encouraged the separation of the water/oil phases by increasing the frequency of collisions to form clusters and coalescence of droplets (Gholam and Dariush, 2013; Yang et al., 2009). Ultrasonic irradiation can not only purify the surface of solid particles but also diffuse distinct areas of the inaccessible multi-phase system while using other separation methods (Swamy and Narayana, 2001). This phenomenon is called ultrasonic leaching, which allows solvents or leaching reagents to enter the interior of solid pores more easily and increases the mass transfer of contaminants through the matrix of solids (Feng and Aldrich, 2000).

Generally, the performance of pre-treatment of oily sludge by using an ultrasonic process can be influenced by various factors such as ultrasonic frequency, sonication power and intensity, temperature, ultrasonic treatment time, water content, solid particle size, and the presence of surfactant (Feng and Aldrich, 2000; Kim and Wang, 2003; Na et al., 2007).

Many studies have been conducted to investigate the efficiency of ultrasonic pre-treatment for lipid extraction from petrochemical industry wastewater sludges. Xu et al. (2009) performed ultrasonic cavitation at 28 kHz frequency in an ultrasonic cleaning tank to extract lipids from the solid particles in the oily sludge, and obtained 55.6 % of total lipid extraction yield. They also found that ultrasonic power, acoustic pressure and optimum temperature for lipid extraction from the sludge were 28 kHz, 0.10 MPa and 40°C, respectively. They have also noted that very high or very low temperature and ultrasonic energy input are not suitable for ultrasonic pre-treatment of oily sludge, because high ultrasonic energy input can hinder oil droplets from coalescing and low ultrasonic energy input makes it hard to separate lipids from solid particles (Xu et al., 2009). Zhang et al. (2012) stated up to 80 % of lipid extraction yield from the oily sludge-water matrix after 10 minutes of ultrasonic pre-treatment using a 20 kHz ultrasonic probe system with a power of 66 kW. Jin et al. (2012) obtained over 95 % of lipid extraction yield with the ultrasonic pre-treatment parameters of

28 kHz, 15 min, 400 W and 60°C, respectively, and when the power and treatment duration were increased above 400 W and 15 min in the ultrasonic pre-treatment, no more lipid extraction was observed.

The ultrasonic pre-treatment method has been chosen for this study because of the sludge disintegration capacity, the better homogenization of the sample and the better penetration of solvent to the sample. Moreover, ultrasonic irradiation is a "green" treatment method that allows the oily sludge to be processed in a relatively short period of time.

2.5.3. Microwave Pre-treatment

The microwave region of the electromagnetic spectrum corresponds to a 1 mm to 1 m wavelength with a frequency of 300 MHz to 300 GHz, but the industrial application is usually carried out at a frequency either near 900 MHz or near 2450 MHz (Appleton et al., 2005). Materials can be heated by the energy of microwaves by applying high-frequency electromagnetic waves (Jacob et al., 1995).

Microwaves can be used as a thermal pre-treatment or a process development technique for the extraction of lipids from biodiesel feedstock (Giese, 1992). Microwave pre-treatment is more efficient than other pre-treatment methods in many processes. It allows rapid and selective lipid extraction with low solvent and energy consumption (Letellier and Budzinski, 1999; Pare et al., 1994). Localized superheating rate is significant for the extraction yield under the microwave pre-treatment assisted extraction. Variable factors such as initial temperature, microwave power, frequency, and microwave applicator design can affect the heating rate and it can be selected for a specific processing application (Gude et al., 2013).

The higher dielectric constant of the water indicates a considerably lower spreading factor, which refers that the system absorbs more microwave energy than it can spread out. This phenomenon is called super heating. It occurs in the presence of water in the medium. This strong absorption increases the temperature inside the sample and causes the cells to break off by the water. In some cases, it may support degradation of the target compound or penetration of a solvent, and in other cases, it may improve the diffusivity of the target compound in the medium (Jain et al., 2009).

Microwave energy can be absorbed directly by the material through the electromagnetic field and molecular interaction. Compared to the conventional methods, microwave energy can also ensure a faster heating process with better heating performance. The temperature of the emulsions rises

quickly with this heating effect and the demulsification of the W/O emulsion occurs. This phenomenon leads to a decrease in viscosity which can speed up the placement of water droplets in the emulsion (Tan et al., 2007). For W/O emulsion, such as oily sludge, the microwave energy may penetrate better the internal phase than oil, which have a relatively higher dielectric loss. This type of energy absorption can cause water to expand and water-oil separation can make it easier to separate water/oil by making the W/O interfacial film thinner (Tan et al., 2007).

Various small-scale and large-scale studies have indicated the utilities of microwave pre-treatment for lipid extraction from oily sludge. Xia et al. (2003) obtained approximately 100 % demulsification efficiency over a very short period of time using microwave pre-treatment to process much higher W/O emulsions while using conventional heating methods. Fang and Lai (1995) applied the microwave irradiation to emulsify 188 barrel W/O emulsion in tanks, and the results demonstrated that the microwave irradiation could have a specific impact on the partial removal of polar compounds.

The efficiency of microwave pre-treatment on oily sludge demulsification can be influenced by many factors such as microwave time, microwave power, surfactant, salt and pH (Fortuny et al., 2007). Chan and Chen (2002) suggested that the optimal microwave irradiation power and duration of treatment were 420 W and 12 s in their experiments. Tan et al. (2007) explored that the efficiency of the microwave pre-treatment (700 W, 2450 MHz) on the W/O emulsions could be enhanced by the addition of chemical demulsifiers. In this direction, 50 ppm demulsifier was added to achieve a sufficient water-oil separation yield (95 % v/v).

The microwave power depends on the ambient temperature because high microwave power can raise the system temperature and the extraction efficiency can improve as these parameters decrease (Chemat et al., 2005; Hu et al., 2008; Xiao et al., 2008). The microwave power that manages the amount of energy provided to the matrix to be converted into heat energy in the dielectric material, controls the temperature.

The microwave pre-treatment method has been chosen for this study because this method not only decrease the reaction time and improve the biodiesel efficiency, but also significantly decrease period of the product separation (Refaat et al., 2008). Moreover, compared with other pre-treatment methods, the microwave irradiation can increase the energy of molecules in the medium more rapidly, which can lead to higher reaction rates (Robinson et al., 2008). The short heating period makes the

microwave pre-treatment a highly energy-efficient and simple to manage approach to disrupting emulsions.

2.6. Biodiesel Production

Biodiesel production from wastewater sludge consists of two steps: lipid extraction and conversion of extracted lipids into biodiesel. Due to the presence of various feedstocks to be used for biodiesel production, there is a great demand for the development of such processes which are suitable for the feedstock to obtain high yield of the product (Daud et al., 2015). For this reason, many studies have focused on finding various processes to increase efficiency of biodiesel and decrease the process cost. Among the several existing methods, nowadays two major technologies are performed for the production of biodiesel from wastewater sludge: pyrolysis and lipid transesterification (Atadashi et al., 2013; Siddiquee and Rohani, 2011; Vyas et al., 2010). Among them, the most commonly used method for biodiesel production from wastewater sudes is the transesterification (also known as alcoholysis) in the presence of a catalyst.

2.6.1. Pyrolysis

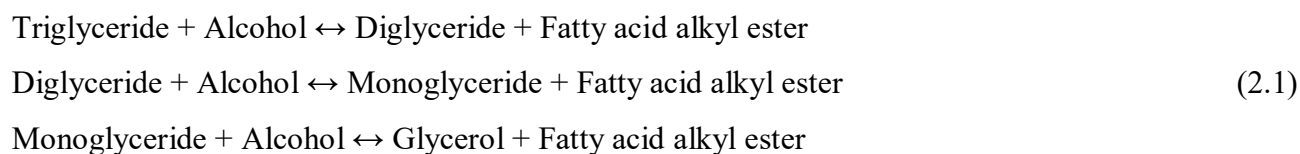
Pyrolysis is a promising method for the production of biodiesel from waste sludge (Leszczynski, 2006; Manara and Zabaniotou, 2012). It is a process in which a material is transformed into another form by thermal treatment (Mahmudul et al., 2017). This technique is also called thermal cracking. It can be performed in the presence or absence of a catalyst, but in the absence of oxygen. During the process, the sludge is heated in an inert atmosphere (300 to 900°C) and separated into oil (condensable volatile substances), solid carbonaceous residue and permanent gases. Biodiesel produced from this method is appropriate to diesel engines, but low-value materials are produced due to lack of oxygen in the environment (Abbaszaadeh et al., 2012). Pyrolysis is efficient, simple, wasteless and considered environmentally friendly and compared to common applications such as incineration and combustion, it is capable of concentrating heavy metals (except Hg and Cd) into the final residue when applied to sludge, in terms of clean gas emissions (Singh and Singh 2010). Generally, the pyrolysis process uses up a significant amount of energy, so care must be taken when adopting this process and it also requires expensive equipment.

Menendez et al. (2002) have introduced a novel technique in which the sludge is pyrolyzed in a microwave oven. Their approach indicated that if the raw wet sludge was processed in a microwave oven by itself, only the sample was dried. However, if the sludge is mixed with a suitable microwave

absorber in a small amount (such as coal produced in pyrolysis itself), temperatures of up to 900°C can be obtained, thus pyrolysis takes place (Menendez et al., 2002). Xie et al. (2014) described a similar method in which the silica compounds as microwave absorbers were used. Microwaves provide important time and energy savings for an equivalent drying (or pyrolysis) degree according to the conventional heating methods. The Microwave-Assisted Pyrolysis (MAP) of the sludge, in addition to the gas and liquid fuel products, enables the base structure to achieve a volume reduction of more than 80 % by obtaining a porous carbonaceous residue (char). Capodaglio and Callegari (2017) have argued that MAP has the capacity to obtain higher biodiesel yields from waste sludge compared to other methods. However, the quality of the biodiesel produced with this method did not satisfy the diesel properties required for commercialization (Capodaglio and Callegari, 2017). Capodaglio et al. (2016) achieved the best conversion efficiency at about 280°C, while Menendez et al. (2002) studied at maximum 900°C and Xie et al. (2014) reached the best yields at 550°C.

2.6.2. Transesterification

Transesterification is considered to be the most appropriate process, and for this reason majority of industries are applied (Mahmudul et al., 2017). Due to its low cost and simplicity, it is very popular and by lowering the viscosity of the oil, makes it appropriate for engines and equipment (Abbaszaadeh et al., 2012, Koutsouki et al., 2016). Transesterification-derived biodiesel has the lowest viscosity compared to other biodiesel production methods (Capodaglio and Callegari 2017). Transesterification is the conversion of triglycerides and alcohol to fatty acid alkyl ester and glycerol, and occurs when an ester is converted to another ester (Koutsouki et al., 2016). The conversion is carried out by replacing an organic group R1 of an ester with an organic group R2 of an alcohol in the presence of an acid or a base as a catalyst (Demirbas, 2008b). The triglycerides are converted to diglycerides, followed by conversion of diglycerides to monoglycerides and finally monoglycerides to glycerol. At each step, a molecule of a fatty acid methyl ester is produced (Enweremadu and Mbarawa, 2009; Siddiquee and Rohani, 2011). Basic mechanism of the three consecutive steps is:



The general synthesization of triglycerides is shown in Figure 2.2.

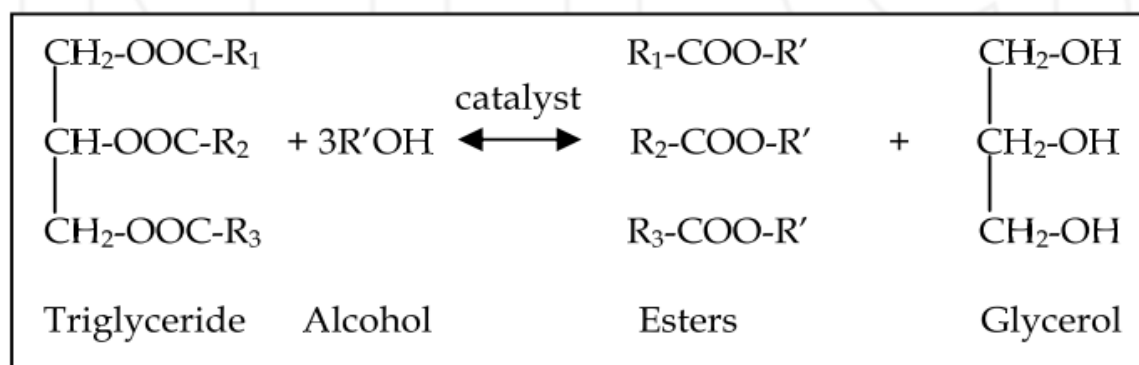


Figure 2.2. Transesterification of triglycerides with alcohols.

For a highly efficient transesterification, alcohol must be free of moisture and contain less than 0.5 % of free fatty acid. Transesterification is a reversible reaction, but in the biodiesel production, the backward reaction does not occur or can be neglected because the glycerol formed cannot mix with the product leading to a two-phase system (Formo, 1979; Ma and Hanna, 1999). After the reaction is performed, the glycerol is extracted from the alkyl esters. The low solubility of the glycerol in the esters quickly separates and can be achieved by settling or centrifugation processes (Ramadhas et al., 2004). To increase glycerol separation, after the transesterification process is terminated, water is added to the reaction mixture. After the glycerol has been removed, a neutralization step will begin by introduction of the alkyl esters and then excess alcohol will be removed and then washing with water will be carried out.

Various factors affect the efficiency of the transesterification reaction such as the amount and type of catalyst (acid, base, enzyme), temperature and reaction time, alcohol/lipid ratio, water and moisture content of fats or oils, free fatty acid content, purification of the final product and the mixing power throughout the chemical reaction (Agarwal, 2007; Balat and Balat, 2010; Freedman et al., 1984; Fukuda et al., 2001; Ma and Hanna, 1999; Meher et al., 2006; Shahid and Jamal, 2011; Sharma and Singh, 2008; Siddiquee and Rohani, 2011; Srivastava and Prasad, 2000; Verdugo et al., 2010).

Among the alcohols that can be used in the transesterification process, the most preferred alcohol for all commercial development is methanol due to its low cost and physical and chemical advantages such as polar and the shortest chain alcohol. When methanol is the alcohol used in transesterification, the process is called methanolysis (Siddiquee and Rohani, 2011). However, propanol, isopropanol, tert-butanol, octanol, butanol, and branched alcohols can also be used, but the cost is higher (Balat and Balat, 2010; Bisen et al., 2010; Demirbas and Demirbas, 2007; Fukuda et al., 2001; Shahid and Jamal, 2011; Sharma and Singh, 2008; Singh and Singh, 2010; Yusuf et al., 2011).

Transesterification process is classified as catalytic and non-catalytic process. As the name implies, the catalytic process requires the presence of a catalyst to improve the rate and time of the reaction. The non-catalytic process does not require the presence of any catalyst. The catalytic process can be separated by homogeneous catalysts and heterogeneous catalysts based on the type of catalyst used in the production process (Atadashi et al., 2013; Mardhiah et al., 2017).

2.6.2.1. Alkali/Base catalyzed transesterification. The bases can catalyze the reaction by removing a proton from the alcohol and thus become more reactive. Initially, the reaction of the catalyst with alcohol forms the alkoxy and then it reacts with the oil to form biodiesel and glycerol. The rate of this process, which is extremely efficient and very low corrosive, and is reasonably high even at a temperature of 60°C. When the free fatty acid (FFA) content is under the desired limit (between 0.5 % and 3 %), alkaline catalysts should be used (Atabani et al., 2012). It is known that the high FFA content of feedstock significantly reduces yield of the biodiesel production when a conventional basic catalyst (e.g., NaOH) is used due to the soap formation, and therefore, difficulties in biodiesel separation and purification. Alkali catalysts such as potassium hydroxide and sodium hydroxide are widely used catalysts (Atadashi et al., 2013; Bharathiraja et al., 2014; Churchill and Srinivasan, 2017). According to studies in the literature, this method is the most economical and fastest method for biodiesel production (Siddiquee et al., 2011). General equation of alkali catalyzed transesterification reaction can be found below:

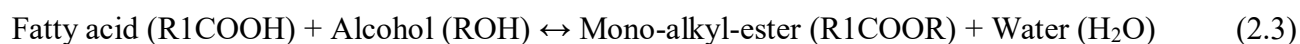


However, the alkali catalyzed transesterification process has various disadvantages: it is the most energy-consuming process, glycerol separation is hard, the reaction is a bit slow and very sensitive to both free fatty acids and water, soap formation is likely to take place, alkaline catalyst has to be removed after the reaction and the alkaline wastewater needs treatment (Agarwal, 2007; Al-Zuhair, 2007; Atadashi et al., 2011; Balat and Balat, 2010; Canakci and Sanli, 2008; Demirbas, 2008a; Janaun and Ellis, 2010; Ma and Hanna, 1999; Mahanta and Shrivastava, 2011; Marchetti et al., 2007; Shadid and Jamal, 2011; Sharma et al., 2008; Singh and Singh, 2010). In addition, the need for treatment of the produced alkaline effluents and the high water consumption during the washing in the purification stages indicate that the alkali-catalyzed transesterification is not so environmentally friendly (Robles-Medina et al., 2009).

2.6.2.2. Acid catalyzed transesterification. Acids can catalyze the reaction by linking a proton to the carbonyl group and thus become more reactive. Acid catalysts play an important role in the

transesterification of glycerides without any soap formation and simultaneous esterification of FFAs. They can both catalyze esterification and transesterification and contribute to more biodiesel production. The main acid catalysts are hydrochloric acid, sulfuric acid, phosphoric acid and ferric sulfate (Atadashi et al., 2013; Aransiola et al., 2014; Bharathiraja et al., 2014; Ishak et al., 2017). Acid catalysts are more suitable for feedstocks with high free fatty acids and water content because they help reduce the amount of free fatty acids (Mardhiah et al., 2017; Ishak et al., 2017). The acid catalyzed transesterification has been reported to give very high yields in esters. However, the main drawbacks of using acid catalysts are that the much slower rate of the reaction and much longer time of the reaction to reach a high conversion during the transesterification of glycerides and requires a large amount of water to wash biodiesel (Aransiola et al., 2014; Bharathiraja et al., 2014). When water accumulates, the reaction may stop. Therefore, a water management technique is a necessity for this approach.

General equation of acid catalyzed transesterification reaction can be given below:



However, there are several companies that commercialize this technology around the world because of their relatively low energy use, high conversion efficiency and low cost reactors and catalysts (Atadashi et al., 2010; Balat and Balat, 2010; Canakci and Sanli, 2010; Demirbas, 2008a; Janaun and Ellis, 2010; Ma and Hanna, 1999; Marchetti et al., 2007; Meher et al., 2006; Shadid and Jamal, 2011; Sharma and Singh, 2009; Singh and Singh, 2008).

Dufreche et al. (2007) obtained 4.41 % of biodiesel (based on the total dry weight of activated sludge) by acid-catalyzed transesterification reaction from solvent-extracted lipids. Olkiewicz et al. (2012) extracted lipids from sludges using the Soxhlet extractor with hexane as a solvent, and then converted the extracted lipids to biodiesel by acid catalyzed transesterification. The results showed that the primary sludge gave the largest biodiesel yield at 13.9 % (Olkiewicz et al., 2012). In the another study of Olkiewicz et al. (2014), lipids from sludge samples were extracted using two different extraction methods: the novel direct liquid-liquid lipid extraction method and the standard drying method. Lipids were converted to biodiesel via acid catalyzed transesterification. Higher biodiesel yield (19.2 %wt, dry sludge) was obtained from primary sewage sludge via direct liquid-liquid extraction technique, whereas 17.6 % of biodiesel were produced by using the standard drying method (Olkiewicz et al., 2014).

2.6.2.3. Transesterification with ionic liquids as a catalyst. Recent studies have shown that the biodiesel production with ionic liquids (ILs) can be a rising method (Andreani and Rocha, 2012; Olkiewicz et al., 2015). ILs play a major role in biodiesel production due to their properties such as easy separation of product from the reaction, the reuse of the catalyst and the low environmental effect (Andreani and Rocha, 2012; Muhammada et al., 2015; Zhaoa and Bakerb, 2013). Various studies have proven that both acidic and basic ILs can serve as a good catalyst for transesterification and/or esterification reactions to produce biodiesel (Earle et al., 2009; Han et al., 2009; Zhou et al., 2012). In the case of sludge lipids, acidic ILs are preferred because of their high FFA content. Successful studies of the utilization of Brønsted acidic ILs as a catalyst for the biodiesel production from lipid feedstocks with high FFA concentration are available in the literature (Elsheikh et al., 2014; Guo et al., 2011; Han et al., 2009; Li et al., 2014; Ullah et al., 2015). Therefore, it has been shown that ILs have a high capacity for biodiesel production as an environmentally friendly alternative to corrosive acid catalysts.

2.6.2.4. Enzymatic transesterification. An enzyme called lipase, is utilized as a catalyst for the production of esters (Freedman et al., 1984). Since the conversion of waste oils to biodiesel is highly complex and chemical catalysts are costly, lipases can be chosen (Guerreiro et al., 2006; Haas et al., 2002). Suitable conditions for the utilization of a particular enzyme are preferred according to the lipases origin and formulation (Rule, 1997). Enzymatic catalysts have many benefits over other catalysts because they are not affected by free fatty acids in higher levels. In the enzymatic transesterification reaction, mild reaction conditions such as low reaction temperature (between 30°C and 55°C for lipase), are essential to hinder loss of lipase activity (Gog et al., 2012). These conditions allow easy separation of the product. Nowadays, due to the high cost of lipases and slower reaction rate, the enzymatic method is not preferred much (Ghaly et al., 2010; Lam et al., 2010). They also cannot achieve the completion degree of the reaction required to satisfy ASTM fuel characterizations. Furthermore, alcohols, especially methanol, becomes an inhibitor of lipase when the alcohol/fatty acid molar ratio is greater than 0.5 (Marchetti et al., 2007).

2.6.2.5. In situ transesterification. The in situ transesterification process in which lipids are extracted and transesterified at the same time, has been improved to reduce the reaction time and the amount of solvent required compared to separate lipid extraction/transesterification processes. Higher ambient temperatures and enzymes must be added to reduce the transesterification reaction times (Kargbo, 2010). In situ transesterification requires a single step for extraction followed by conversion of lipids to biodiesel to simplify the process (Koutsouki et al., 2016; Qi et al., 2016). However, this

process is disadvantageous on an industrial scale as the operational conditions are difficult to maintain the process design and parameters (Bharathiraja et al., 2014; Mondala et al., 2009).

Revellame et al. (2011) carried out the in situ transesterification of activated sludge at four temperature levels (45, 55, 65 and 75°C), six methanol to sludge ratios (5, 10, 15, 20, 25 and 30, v/w) and five catalyst concentration levels (0.5, 1, 2, 4 and 6 %w). They found 4.88 %wt and 4.79 ± 0.02 %wt of biodiesel, respectively, by numerical and experimental optimization at 55°C, 25 methanols to sludge ratio and 4 %wt of sulphuric acid. The result of the study is that the unsaturated fatty acids or esters are significantly reduced at temperatures above 60°C due to acid-catalyzed polymerization (Revellame et al., 2011). Dufreche et al. (2007) produced 6.23 % of biodiesel from the dried secondary sludge by in situ acid-catalyzed transesterification. Mondala et al. (2009) used the primary and secondary sludge of a municipal wastewater treatment plant as a lipid feedstock to investigate the effect of the sludge type in the acid-catalyzed in situ transesterification process for biodiesel production. The maximum biodiesel yields for the primary and secondary sludge were 14.5 %wt and 2.5 %wt, respectively at 75°C, 12:1 methanol to sludge mass ratio and 5 % (w/w) H₂SO₄ (Mondala et al., 2009).

2.6.2.6. Supercritical methanol technique. Supercritical process is a non-catalytic transesterification technique. Kusdiana and Saka (2004) introduced a supercritical methanol technique in which biodiesel and glycerol were produced by the transesterification reaction of lipids without a catalyst. The supercritical methanol process consumes less energy than the catalytic transesterification processes and the reaction is completed within a few minutes (2-4 min) (Bharathiraja et al., 2014). Using this method, the disadvantages of the catalytic processes can be overcome by not using a catalyst, by the fact that the biodiesel purification stage and glycerol recovery are much easier (Aransiola et al., 2014; Atabani et al., 2012; Karmakar et al., 2010). Formation of a single-phase methanol and oil mixture emerges because the supercritical methanol technique consists of a very low dielectric constant. The purification of the products by this method is also easy, the reaction time is shorter and the process is environmentally friendly (Demirbas and Demirbas, 2007; Karmakar et al., 2010; Mittelbach, 1990; Shahid and Jamal, 2011). According to this method, the ratio of alcohol to oil must be high. Studies have shown that, at high temperatures and pressures, when the oil reacts with excess methanol, it gives alkyl esters in a very short time. This may occur within 3 to 5 minutes and the reaction should be quenched very quickly to avoid degradation of the products (Monisha et al., 2013; Zhang et al., 2003). However, the technologies used in this process are costly (due to high pressures and high temperatures) and there is an increase in the methanol consumption (Atabani et al., 2012; Deng et al., 2011; Shahid and Jamal, 2011).

2.6.2.7. BIOX process. BIOX process is one of the non-catalytic transesterification methods. It utilizes a co-solvent to solve the slow reaction time problem (Freedman et al., 1984). The tetrahydrofuran, which is known for its ability to dissolve the methanol easily and to provide a very rapid reaction, is utilized as a co-solvent in this BIOX process. The catalyst residues are not present in the glycerol as in the ester phase. The boiling point of methanol, close to the boiling point of tetrahydrofuran, caused to use of tetrahydrofuran as a co-solvent. After completion of the reaction, removal of excess methanol as well as tetrahydrofuran can be easily carried out in a single step. Operating temperature of approximately 30°C is sufficient for this process to take place. It has a clean separation of glycerol and ester and the products obtained are free of water and catalyst. At the same time, co-solvent recovery and recycling processes are also easily applied (Freedman et al., 1984). Several co-solvents such as methyl tert-butyl ether (MTBE) have also been investigated to perform for this process (Monisha et al., 2013).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Petrochemical Industry Wastewater Treatment Plant (WWTP) Sludge

In this study, petrochemical industry WWTP sludges obtained from the industrial wastewater treatment plant of a petrochemical industry in Turkey was utilized as a feedstock for the biodiesel production. The oily sludge samples were obtained from thickener and waste activated sludge (WAS) units of the industrial wastewater treatment plant. The plant has an approximate 50.000 m³/day wastewater treatment capacity and has activated sludge unit for biological treatment. The sludge thickening unit contains waste activated sludge from secondary clarifier and the sludges from primary clarifier and oil separators. After collection, the sludge samples were transported in a refrigerated container to the laboratory, stored at 4°C prior to use and brought to room temperature before using for analysis. Sludge samples were characterized by measuring their total solid (TS), volatile solid (VS), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), chemical oxygen demand (COD) and soluble chemical oxygen demand (sCOD) concentrations, viscosity, and pH. All of the analyses were conducted according to the Standard Methods of the Examination of Water and Wastewaters (APHA, AWWA, WEF, 2012). Depending on the experimental setup, the collected sludges were either used as received (in liquid form) or in dried form.

3.1.2. Chemicals

All chemicals used in this study were of analytical grade. For the lipid extraction experiments, hexane of laboratory reagent grade and magnesium sulfate monohydrate were purchased from Sigma-Aldrich. The esterification/transesterification experiments were conducted using sulfuric acid and anhydrous methanol from Merck. Hydrochloric acid was also purchased from Merck for sludge acidification experiments. Sodium bicarbonate, anhydrous sodium sulfate and sodium chloride were provided by Sigma-Aldrich for biodiesel production steps. For the free fatty acid (FFA) and free acid methyl esters (FAMES) analysis, 37 component FAMES mix standard solution was supplied by Supelco.

3.1.3. Instrumental Equipments

Instrumental equipments used in this study to conduct the analyses are given in Table 3.1.

Table 3.1. Instrumental equipments used in the experiments.

Instrumental Equipment	Experimental Analysis	Trademark/Model
Drying Oven	TS, VS, MLSS, MLVSS	NÜVE (FN 500)
Muffle Furnace	TS, VS, MLSS, MLVSS	Protherm
Analytical Balance	TS, VS, MLSS, MLVSS	Scaltec (SBA 31)
Water Bath	TS, VS	Julabo TW 12
Filtration Apparatus	MLSS, MLVSS	Schott Duran
COD Digester	COD, sCOD	HACH
Spectrophotometer	COD, sCOD	HACH (DR/2010)
Centrifuge	sCOD	Hettich Zentrifugen 16A
pH Meter	pH, ORP, Temperature	WTW 3110
Viscometer	Viscosity	Brookfield DV-I Prime
Capillary Suction Timer	CST	Triton 304M
Ultrasonic Homogenizer	Disintegration	Bandelin Sonopuls HD 3400
Microwave Digestion System	Disintegration	Berghof Speedwave MWS+3
Solvent Extractor	Lipid Extraction	Velp Scientifica SER 148
Jar Test Apparatus	Lipid Extraction	Jar Test F.6/S
Gas Chromatograph	FFA	Perkin Elmer Clarus 600
Gas Chromatograph-FID	FAME	Agilent HP 6850/6890

3.2. Methods

3.2.1. Pre-treatment of Sludge Samples

Sludge samples were disintegrated with acidification, ultrasonic and microwave pretreatments before the lipid extraction step. The pre-treatment techniques were performed to observe their effects on the lipid extraction and biodiesel production yields.

3.2.1.1. Acid pre-treatment (Pre-acidification). Lipid extraction experiments were performed with and without pre-acidification application to the sludge samples of 40 mL to estimate the effect of acid pre-treatment on the lipid extraction yields. The samples were acidified with 0.6 mL of concentrated hydrochloric acid (HCl) 0.1 N to reduce the pH to about 2, before the lipid extraction process.

3.2.1.2. Ultrasonic pre-treatment. The ultrasonic (US) pre-treatment was applied to the untreated sludge and acidified sludge samples, alone and in combination with the acidification process. Ultrasonic pre-treatment experiments were performed using an ultrasonic homogenizer (Bandelin-

Sonopuls HD 3400) as shown in Figure 3.1. The ultrasonic homogenizer is equipped with a generator (GM 3400), an ultrasonic converter (UW 3400), a booster horn (SH 3425) and a probe (VS 200 T).



Figure 3.1. The ultrasonic homogenizer used in the study (Bandelin-Sonopuls HD 3400).

Ultrasonic pre-treatment was conducted 20 kHz of working frequency, 70 % amplitude, 200 W of ultrasonic power for 13 min. In ultrasonic pre-treatment of the sludge samples, the specific energy input was adjusted to 35000 kJ/kg TS. The energy input per unit of sludge (as TS) to reach a certain degree of disintegration is described as the specific energy input (Khanal et al., 2007). The specific energy (E_s) is a function of the ultrasonic power, ultrasonic duration, volume of sonicated sludge and TS concentration and can be calculated using Equation 3.1 below (Bougrier et al., 2006):

$$E_s = \frac{P \times t}{V \times TS} \quad (3.1)$$

where,

E_s : the specific energy input in kW/kg TS (kJ/kg TS)

P: the ultrasonic power in kW

t: the ultrasonic duration in seconds

V: the volume of sludge in liters

TS: the total solids concentration in kg/L

The full characteristics of the ultrasonic equipment used are listed in Table 3.2.

Table 3.2. Characteristics of the ultrasonic homogenizer.

GM 3400 Generator	
Power supply	230V~50/60 Hz (alternatively 115V~50/60 Hz)
Ultrasonic frequency	20 kHz
Maximum power	400 W
Power setting range	60-300 W
Weight	3.1 kg
Dimensions (l × w × h)	324 × 230 × 131 mm
Time setting range	0:00:01-9:59:59 (h:mm:ss) or continuous operation
Amplitude setting range	10-100 % in 1 % increments
UW 3400 Ultrasonic Converter	
Frequency	20 kHz
Weight	2.2 kg
Dimensions	Ø 90 × 180 mm
Degree of protection	IP 20
VS 200 T Probe	
Diameter	Ø 25 mm
Connection to standard horn	SH 3425
Volume range	100-2500 mL
Maximum admissible amplitude setting	100 %
Immersion depth (recommended)	10-20 mm

3.2.1.3. Microwave pre-treatment. The microwave (MW) pre-treatment was performed to the untreated sludge and acidified sludge samples, alone and in combination with the acidification process. Microwave pre-treatment of sludge samples was performed by using microwave irradiation system, Berghoff Speedwave Microwave System (MWS+3) shown in Figure 3.2.



Figure 3.2. The microwave equipment used in the study (Berghoff Speedway MWS+3).

Microwave pre-treatment experiments were carried out at 175 °C for 30 minutes at 2000 kPa. The microwave unit has 12 teflon vessels, each with a capacity of 60 mL. Sludge samples with a maximum volume of 50 mL in each vessel were placed on the MW vessels and irradiated in the MW unit. The full characteristics of the microwave equipment used are sorted in Table 3.3.

Table 3.3. Characteristics of the microwave equipment.

Power supply	230 V/50 Hz/1350 W
Microwave output	1000 W
Frequency	2450 Hz
Weight/Dimensions (W × D × H)	Standart device: approx. 14 kg/520 × 460 × 330 mm Control unit: approx. 0.5 kg/188 × 35 × 114 mm
Oven chamber	Approx. 27 liter/350 × 340 × 215 mm (W × D × H)
Noise level	<60 dB
Ambient conditions	15-35°C / 85 % relative air humidity
Temperature measurements	Measurement range 50-260°C, accuracy 1°C at 200°C

3.2.2. Lipid Extraction

Lipid extraction is the first step of the biodiesel production process. In this study, two different lipid extraction methods; standard drying method and direct liquid-liquid lipid extraction method were used to compare lipid and biodiesel yields. The lipid extraction experiments were performed to the untreated, ultrasonically pre-treated and thermally pre-treated thickener and secondary clarifier sludge samples with and without acidification. Prior to extraction, the sludge samples were acidified with concentrated hydrochloric acid according to standard method 5520E (APHA, AWWA, WEF, 2012). The lipid yield for all extraction methods was calculated gravimetrically and defined by Equation 3.2:

$$\text{Lipid (\%)} = \frac{\text{Lipid (g)}}{\text{Sludge (g)} \times \frac{\text{TS}}{\%}} \times 100 \quad (3.2)$$

where,

Lipid: the amount of extracted lipids

Sludge: the amount of sludge used for extraction

TS: the total solids concentration of sludge used for extraction

3.2.2.1. Standard drying method (Soxhlet lipid extraction). For a comparison study, sludges were dried according to standard method 5520E (APHA, AWWA, WEF, 2012) to be the reference method. This method was studied since recent studies have demonstrated that other drying methods have an adverse effect on both extracted lipids and potential biodiesel yield when compared to MgSO₄·H₂O drying (Olkiewicz et al., 2014). In the standard drying method, sludge samples were acidified with hydrochloric acid before adding magnesium sulfate monohydrate for drying.

The lipid was extracted from dried sludge samples using a Soxhlet apparatus (Figure 3.3) with hexane as a solvent according to the standard drying method 5520E. A portion of the samples was used without acidification and the other portion was acidified with 0.3 mL of fuming hydrochloric acid to determine the acid pre-treatment effect on the yields. Before the extraction, to dry the sample, 25 g of magnesium sulfate monohydrate was added to 20 g of the sludge sample. The mixture was firstly crushed with a mortar and pestle to obtain a homogeneous mixture. For the Soxhlet extraction, after 7-10 g of the homogeneous sample was placed in a cellulose thimble, glass wool was added to the top of the thimble to prevent the scattering of the solid. 70 ml of hexane was added into an extraction cup previously weighed and then Soxhlet lipid extraction procedure consisting of immersion step for 20 min, washing step for 50 min and recovery step for 15 min was applied. As the boiling point of each solvent is different, the temperature of the heating plate in the Soxhlet was adjusted to 180°C for hexane. At the first stage of the extraction, a thimble involving the sample was immersed in the boiling solvent for the efficient extraction process. After the immersion step, the thimble was lifted up for 50 minutes and during the washing step, the condensed solvent was flowed over the sample to complete the extraction. After the first two steps, a large part of the hexane was recovered in a collecting container of Soxhlet apparatus for the purpose of using it the further stages in the last recovery step. After the lipid extraction, the lipids were stored in a desiccator overnight to be weighed the next day. After the quantification, the lipids were dissolved in hexane and kept them in the freezer at -20°C until further analysis.



(a)



(b)

Figure 3.3. Soxhlet apparatuses. (a) Traditional Soxhlet apparatus. (b) Automatic Soxhlet apparatus used in the study.

3.2.2.2. Direct liquid-liquid lipid extraction method. Direct liquid-liquid lipid extraction from petrochemical industry WWTP sludge samples was performed in a 6-paddle jar tester (Figure 3.4) to understand its applicability and evaluate the effect of process variables: sludge to solvent volume ratios, contact times, extraction stages and total solid contents. In the previous study, direct liquid-liquid lipid extraction method had been performed by using municipal WWTP sludge samples. Since hexane resulted in higher lipid yields for primary municipal WWTP sludge than other solvents like hexane, chloroform, petroleum ether and toluene, it was selected for the use as a solvent based on the results of solvent type-lipid yield experiments (Babayigit et al., 2018). Selection of suitable process variables provided the optimisation of liquid-liquid extraction and the lipid extraction experiments were continued by using optimum process conditions throughout the study. In this study, direct liquid-liquid lipid extraction method was performed according to the method described by Olkiewicz et al. (2014) with a few minor modifications. The sludge samples were mixed with hexane at 200 rpm for 20 min and let to settle at 60 rpm for 15 min in a 6-paddle mixer-settler batch reactor set at ambient temperature. After each consecutive extraction stage, samples were re-extracted with additional solvent and then the solvent phase was filtered using a 4 μm filter paper to remove residual solids and dried by using anhydrous sodium sulfate. Later, the solvent was recovered by using a Soxhlet apparatus and reused in each sequential extraction stages. After the extraction process, the solvent was removed and lipids were weighed to determine the extraction yield. There were three differences in this modified method: First, the sample size was 40 mL instead of 200 mL as used in their method, second; the mixing speed altered from 330 to 200 rpm and third, the hexane was recovered by using a Soxhlet apparatus instead of a rotary evaporator.

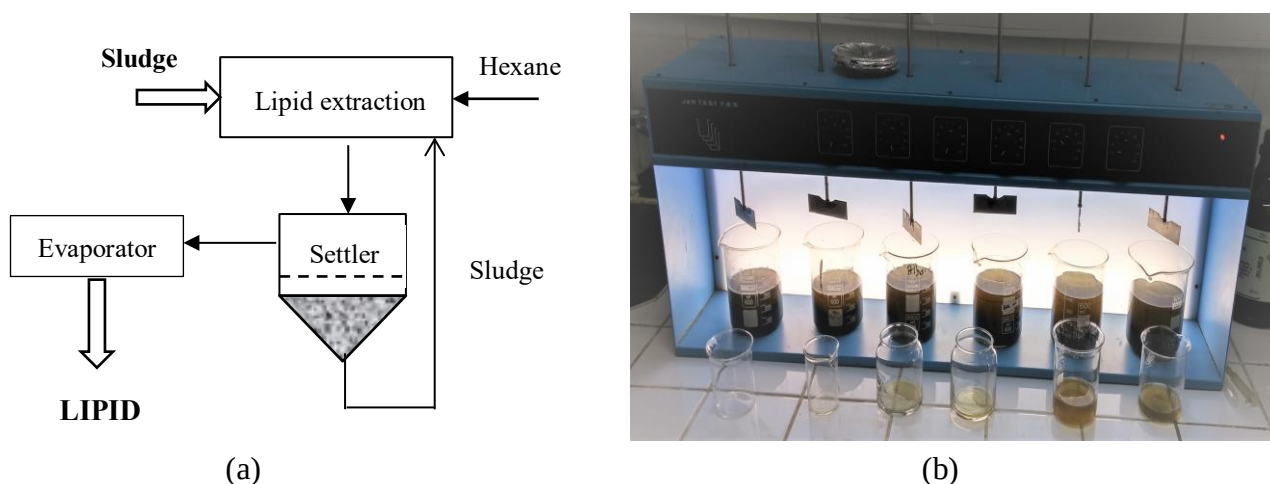


Figure 3.4. Direct liquid-liquid lipid extraction method. (a) Schematic diagram of the experimental liquid-liquid extraction setup. (b) 6-paddle jar tester used for the liquid-liquid extraction in this study.

3.2.3. Lipid Transesterification and Biodiesel (FAMES) Analysis

The lipids obtained from the petrochemical industry wastewater sludge were converted into biodiesel-FAMES through acid catalyzed transesterification by modifying Christie's method (Christie and Han, 2010; Dufreche et al., 2007). The reason for the application of this method is the high FFAs content of the sludge lipid part. Freedman et al. (1986) stated that an acid catalyst should be used in studies to be performed with lipid feedstocks containing more than 1 % FFA, so the transesterification method was chosen according to the FFA content of lipid.

Initially, 0.2 g of extracted lipids were dissolved in 10 mL of hexane in a vial and 20 mL of 2 % sulfuric acid in methanol was added. After that, the vial was capped, the mixture was put on a magnetic stirrer to mix and heat the mixture overnight at 50 °C. The mixture was cooled and then transferred to a separatory funnel. Then, 10 mL of 5 % sodium chloride (NaCl) in water was added and the FAMES were extracted three times with 20 mL of hexane by shaking for 3 minutes to recover the FAMES. The hexane phase as shown in Figure 3.5 was washed with 10 mL of 2 % sodium bicarbonate (NaHCO₃) in water followed by 10 mL of warm water and dried over anhydrous sodium sulfate. Finally, the samples filtered from Sartorius Stedim Minisart syringe filter (0.45µm pore size) and they were kept frozen with the addition of hexane until further analysis.



Figure 3.5. Transesterification reaction of biodiesel separation.

The biodiesel (FAMES) analysis was conducted by using an Agilent gas chromatograph with a flame-ionization detector (GC-FID). The separation was obtained in the HP-INNOWax column 30m

$\times 0.32 \text{ mm} \times 0.25 \text{ }\mu\text{m}$, with helium as a carrier gas and with a constant injector temperature 260°C . The injection volume of the sample was 1.5 mL with a split ratio 20/1. The FID was at 260°C for the process. The oven temperature programme was started from 150°C , retention for 1 min and then improved by $2.9^\circ\text{C}/\text{min}$ to 230°C , retention for 1 min. A 37 component FAMES standard mixture (Supelco 47885-U) was used for the calibration of the method. GC-FID gave results to figure out the quantity of saponifiable matter, Equation (3.3), because only the saponifiable portion of the total lipids can be converted into biodiesel and hence the yield of FAMES produced from sludge samples, Equation (3.4). The results that could not be determined by GC-FID are defined as ‘others’.

$$\frac{\text{Saponifiable}}{\%} = \frac{\left(\frac{\text{FAMES}}{\text{g}}\right)}{\left(\frac{\text{Lipid}}{\text{g}}\right)} \times 100 \quad (3.3)$$

where,

FAMES: the total FAMES produced after transesterification determined by the GC-FID run

Lipid: the amount of lipid used for transesterification

$$\frac{\text{FAMES}}{\%} = \frac{\text{Lipid}}{\%} \times \frac{\text{Saponifiable}}{\%} \quad (3.4)$$

where,

Lipid: the lipid content in sludge samples, Equation (3.2)

Saponifiable: the FAMES content in the lipid, Equation (3.3)

3.2.4. Analytical Methods

All analytical methods used in this study were performed according to the Standard Methods. All of the experiments were done in duplicates or triplicates and the arithmetic means of the experimental results are given in the study.

3.2.4.1. Total solids and volatile solids (TS & VS). TS and VS analyses were carried out according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2012) using method 2540B and 2540E, respectively. First, evaporating dishes were brought to a constant weight by heating in a muffle furnace (Protherm PLF 110/8) at 550°C for 1 hour to obtain accurate results. For TS measurement, 10 ml of well-mixed homogeneous sludge samples were placed in the evaporating

dishes, their weights were reported and put in the water bath (Julabo TW 12) to evaporate the moisture in the sludge and then dried at 103°C for 1 hour in the oven (Nüve FN 500). The evaporating dishes were then cooled in a desiccator for 30 minutes and weighed. For the VS measurement, the ignition of the sludge samples was carried out in a muffle furnace at 550°C for 30 minutes, then the samples were cooled in a desiccator and weighed. TS and VS concentrations of sludge samples were determined using calculations.

3.2.4.2. Mixed liquor suspended solids and mixed liquor volatile suspended solids (MLSS & MLVSS).

MLSS and MLVSS analyses were carried out according to the Standard Methods (APHA, 2012) using the method 2540D and 2540E, respectively. Firstly, filter papers and crucibles used in these analyses were brought to a constant weight. For MLSS analysis, sludge samples were filtered through a pre-weighed glass fiber filter paper (Whatman GF/C) and dried at 105°C for 1 hour in the oven. The filter papers were cooled in the desiccator for 30 minutes and weighed. The difference between the dry weight of sludge sample (residue + filter paper) and the weight of filter paper was divided into the volume of the sample to calculate MLSS in the sludge samples. For MLVSS analysis, the residue on the filter papers used in MLSS analysis in a crucible was ignited at 550°C for 30 minutes in the muffle furnace. The crucible with filter paper and inorganic residue on the filter paper were cooled in the desiccator and weighed. The difference between the weight of (sample residue + filter paper + crucible) before and after the ignition was divided by the volume of the sample to calculate MLVSS in the sludge samples.

3.2.4.3. Chemical oxygen demand and soluble chemical oxygen demand (COD & sCOD).

COD determination of the sludge samples was carried out by using dichromate closed reflux colorimetric method in accordance with the Standard Methods for the Examination of Water and Wastewater, method 5220D (APHA, 2012). First, the sludge samples were diluted to some extent and then were refluxed with potassium dichromate (K_2CrO_7) and sulfuric acid (H_2SO_4) in a HACH COD digester at 150°C for two hours. Mercury sulphate ($HgSO_4$) was added to the samples to block the chloride inhibition and silver sulfate (Ag_2SO_4) was added as a catalyst. For sCOD determination, supernatant portions were obtained by centrifuging 40 mL of sludge samples at 2850 rpm for 15 minutes as described by Bougrier et al. (2008). The supernatant portions were removed using a syringe, diluted if necessary, and performed using the dichromate closed reflux method. After the samples were cooled, their absorbance values were measured at 600 nm using the HACH DR/2010 Portable Data Logging Spectrophotometer. COD and sCOD concentrations were determined using the calibration curve of potassium hydrogen phthalate (KHP), previously prepared with deionized water through the same method. All analyzes were performed in triplicate.

3.2.4.4. pH, oxidation reduction potential (ORP) and temperature. All pH, ORP and temperature determinations were carried out using a WTW 3110 Inolab pH meter. The pH meter was calibrated regularly with pH standard buffer solutions.

3.2.4.5. Viscosity. Viscosity experiments were conducted by using Brookfield RVDV-I Prime digital viscometer. For accurate measurement, the viscometer was turned on and adjusted using the apparatus under the three feet of the device controlling the bubble above the head. After leveling, viscometer was auto-zeroed. The setting was checked before each measurement. The viscosity of the 500 mL of sludge samples was measured by using RV Guardleg and #2 spindle (S02) of the RV spindle set. All viscosity experiments were performed at 25°C ambient temperature and the operating speed was set at 100 rpm. The results were reported as cP values.

3.2.4.6. Capillary suction time (CST). As described by Vesilind (1988), Capillary Suction Time (CST) analysis is a measure of sludge filterability. High CST values indicate the slow releasing of the liquid part of the sludge and low CST values indicate the easy separation of sludge water. CST analyses were performed using a Triton Electronics Type 304M capillary suction timer. The capillary suction apparatus comprises a sludge column contained in the sample cylinder centered in the middle of two concentric electrodes of D1 and D2 (diameters), which are based on a Whatman-17 filter paper. The diameters of the first circle having two sensors and second circle on which one sensor was present, were 3.2 cm and 4.6 cm, respectively. Different CST papers were used for each sample. A 2 mL sample was poured into the CST sample cylinder with Pasteur pipettes. When the water coming from the sludge begins to wet the paper and reaches the inner circle of the CST paper, the electrical timer begins counting with a signal, and then when the sample reaches the second circle, it bleeps again and the timer stops. The elapsed time between two circles is recorded as the capillary suction time (CST) in seconds.

The CST test is dependent on the sludge solids concentration and the instrument used. The temperature also influences the test due to its effect on the viscosity (Vesilind, 1988). During the experiments, the temperature of the samples was set at 25°C.

4. RESULTS AND DISCUSSION

This study investigated biodiesel production from petrochemical industry wastewater sludge (oily sludge) by using the direct liquid-liquid lipid extraction method, which does not require expensive sludge dewatering or drying steps. The study compared the lipid extraction through the liquid-liquid lipid extraction with the standard drying method. The study also investigated the effects of acid, ultrasonic and microwave sludge pre-treatments on the lipid extraction from the petrochemical industry WWTP sludge samples.

Experiments were performed by using sludge samples taken from both thickening unit and the secondary sedimentation tank of the industrial wastewater treatment plant in a petrochemical industry. The direct liquid-liquid lipid extraction method was optimized varying the total solid concentrations of oily sludge samples, the sludge to solvent ratios (by volume), contact times and extraction stages.

4.1. Sludge Characterization

The characteristics of the petrochemical industry WWTP sludge samples supplied from thickener sludge and waste activated sludge (WAS) units in a big petroleum refinery wastewater treatment plant were given in Table 4.1. Sludge samples were investigated for total solid (TS), volatile solid (VS), chemical oxygen demand (COD), soluble chemical oxygen demand (sCOD), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), viscosity, capillary suction time (CST), oxidation reduction potential (ORP) and pH. The values represent the average of the results obtained from two different samples collected in different months. The samples were immediately delivered to the laboratory and stored at 4°C prior to use. All of the measurements were performed for duplicate samples. The sludge samples were characterized according to Standard Methods of the Examination of Water and Wastewaters. Secondary clarifier sludge samples contain lower organic matter like lipids compared to the other sludge samples. The sludge composition depends on sludge type and varies during the wastewater treatment. Among the sludge samples tested, the thickener sludge samples have the highest COD content due to lipid content coming from oil separators in the WWTP. Secondary clarifier sludge samples contain lower organic matter like lipids compared to the other sludge samples.

Table 4.1. Characteristics of sludge samples used for different experiments in this work.

Parameter	Unit	Thickener sludge	Secondary clarifier sludge
TS	%	3.40	1.71
VS	%	2.07	0.65
MLSS	mg/L	25660	2300
MLVSS	mg/L	21460	1450
COD	mg/L	63220	7545
sCOD	mg/L	10800	175
pH	-	6.80	7.03
ORP	mV	10.6	10.9
Viscosity	mPa.s	52.80	18.40
CST	s	29.20	38.50

4.2. Effect of Extraction Methods on the Lipid Extraction Yield

The water content of sludge is a limiting factor in the biodiesel production and it increases the overall cost of the process. In this study, the novel liquid-liquid lipid extraction method which does not require sludge dewatering or drying and the standard drying method were investigated comparatively. In the liquid-liquid lipid extraction method, sludge samples are used directly in the wet form, while in the standard drying method, they are dried. Soxhlet apparatus and hexane as a solvent were used to perform a comparison study, applying the standard extraction method to sludge samples. In this study, hexane was selected to be used as solvent in both liquid–liquid and dry lipid extraction methods based on the results of solvent type-lipid yield experiments (Babayigit et al., 2018). In the study conducted by Babayigit et al. (2018), the use of hexane as a solvent in the liquid-liquid lipid extraction method led to higher lipid yields for the primary municipal WWTP sludges compared to other solvents. This can generally be explained in terms of the polarity. Based on the “like dissolve like” principle, non-polar solvents are the most suitable for the lipids extracted from the primary sludge, which are composed mostly of non-polar lipids derived from glyceride in wastewater (Dong et al., 2016). Since the hexane has the lowest polarity compared to other solvents, the highest lipid content from the primary sludge was obtained from extraction studies with the hexane (Babayigit et al., 2018). Furthermore, considering the environmental problems, safety and the possibility of interference with water, hexane was selected as the appropriate solvent for this study.

The lipid yields extracted from the two types of petrochemical industry WWTP sludge using standard drying and direct liquid-liquid extraction methods are presented in Table 4.2. The results indicate the average of at least three different samples collected in WWTP during different months.

Table 4.2. Lipid yields obtained from sludge samples (3 extraction stages with hexane as a solvent, S/H: 1:2, 200 rpm of mixing speed, ambient temperature).

Sludge type	Extraction method	Lipid yield (%) ^(a)	
		Acidified	Non-acidified
Thickener sludge	Standard drying method ^(b)	22 ± 1.8	19.80 ± 1.6
	Direct liquid-liquid lipid extraction method	33 ± 1.8	28.90 ± 1.6
Secondary clarifier sludge	Standard drying method ^(b)	4.60 ± 0.2	2.05 ± 0.1
	Direct liquid-liquid lipid extraction method	2.25 ± 0.1	1.98 ± 0.1
^(a) Each value is the average of at least 3 samples collected on different days.			
^(b) Extraction according to the standard MgSO ₄ .H ₂ O method, lipid yield on the basis of dry sludge.			

Irrespective of sludge acidification, Table 4.2 indicates the lipid extraction yields obtained by the standard drying and direct liquid-liquid methods. The higher lipid yields were obtained by direct liquid-liquid extraction method compared to the standard drying method for thickener sludge samples. These results are consistent with the results of the study by Olkiewicz et al. (2014) and the direct liquid-liquid extraction method enables easy removal of lipids from sludge samples (Olkiewicz et al., 2014). It has been found that by applying direct liquid-liquid lipid extraction method, the thickener sludge was increased by 45-50 % in lipid yield. Therefore, it can be said that the liquid-liquid lipid extraction method can extract most of the lipid content of the sludge samples compared to the drying method. It can also be said that the effect of the lipid extraction method depends on the lipid content in the sludge. When the lipid content of the sludge sample was low, there was almost no difference in lipid yields obtained by standard drying and direct liquid-liquid extractions methods.

As seen in the table, since secondary clarifier sludge (waste activated sludge) has low lipid content, the lipid yields by both extraction methods were lower compared to yields obtained from the thickener sludge. The lipid yields obtained by the standard drying method were 50 % higher than those obtained by the liquid-liquid lipid extraction method indicating that the standard drying method was more effective on the secondary sludge samples.

Based on the results of the study, standard drying lipid extraction method appears more suitable for materials in biological structure having lower lipid contents. However, direct liquid-liquid lipid extraction method was more efficient for materials having high lipid, low biological content. Supportively, Speight (2014) also reported that the standard method is used to extract low levels of lipid by chemically drying a wet sludge sample and then extracting it using Soxhlet apparatus. Speight (2014) also noted that this method is more suitable for biological lipids and mineral hydrocarbons (Speight, 2014). Therefore, it can be deduced that higher lipid extraction yield obtained from the

petrochemical industry WWTP sludge by direct liquid–liquid extraction method. The petrochemical industry WWTP sludges, consisting of primary and waste activated sludges and petroleum based waste oil were found to be an efficient source of lipids that can be used for biodiesel production. Although it is obvious that some part of the lipids are sourced from the petroleum based oil contamination, the rest comes from the primary and secondary (waste activated sludge) sludges produced in the WWTP.

There are various studies in the literature reporting that although it depends on the type and lipid content of the sludge, in general standard drying method necessitating sludge drying or dewatering steps, is more expensive than the direct liquid–liquid lipid extraction method (Kech et al., 2018; Olkiewicz et al., 2014; Olkiewicz et al., 2016). Furthermore, the drying of the sludge samples prior to extraction is economically disadvantageous for large-scale use due to the large energy requirement (Lardon et al., 2009; Xu et al., 2011). It was obvious that this novel liquid-liquid lipid extraction method was a simple and energy saving method because the drying and dewatering steps had been eliminated. It requires lower operating costs compared to the standard drying method. Beside these points, the liquid-liquid lipid extraction method has become a preferred method as it is economically feasible and has the potential to take the place of this conventional method.

4.3. Effect of Sludge Type on the Lipid Extraction Yield

The lipid yields of the two types of petrochemical industry WWTP sludge analyzed is shown in Table 4.2. As seen in the table, irrespective of the extraction methods and the sludge acid pre-treatment (acidification), the greatest lipid yield was obtained from the thickener sludge to be 33 %. The lipid yield for the secondary clarifier sludge was about 4.6 %. This was an expected result because thickener sludge has a high organic content, sourced from its high oil content (>50 %) and the solids in it (Hahn, 1994). Petrochemical industry WWTP thickener sludge comprises of sludges from oil separators, primary clarifier sludge, and the waste activated sludge from the secondary clarifier. It contains a mixture of various organic and inorganic compounds like water soluble metals, salts, phospholipids, suspended solids, petroleum hydrocarbons (PHCs), neutral lipids, and FFAs sourced from the waste activated sludge oils that cannot be removed in the WWTP. Secondary clarifier sludge (waste activated sludge) sourced from the aerobic biological treatment unit resulted with the lowest lipid yield because it composed of mostly the microbial cells containing polar phospholipids in their cell walls (Kech et al., 2018; Olkiewicz et al., 2012). There are both polar and non-polar lipids in these sludge types. However, the thickener sludge contains mostly non-polar lipids, whereas the secondary sludge consists mainly of polar lipids (Zhu et al., 2012). Hexane does not have the ability

to extract these polar compounds due to its non-polar structure (Dufreche et al., 2007). This may explain the reason for extracting lower lipids from the secondary sludge samples and high lipids from the thickener WWTP sludge samples. Since the liquid-liquid lipid extraction method was found to be more effective on the thickener sludge, the optimization experiments of this method were conducted by using the petrochemical industry thickener sludge samples.

4.4. Effect of Extraction Methods on the Saponifiable (Transesterifiable) Lipids

Total lipid content of sludges cannot be completely converted to the biodiesel (FAMES). It is composed of free fatty acids, acylglycerols, hydrocarbons, some waxes (saponifiable lipids), ketones, sterols, linear alkyl benzenes, trepans, and pigments (non-saponifiable lipids) (Pastore et al., 2013). As only the saponifiable lipids are transesterifiable to FAMES, non-saponifiable lipids are both inconvertible and lipid contaminants (Halim et al., 2012). With respect the polarity, effective results are obtained when using non-polar solvents like hexane in the transesterification reaction because they are capable of extracting non-polar/saponifiable lipids (free fatty acids and glycerides) that can be converted to biodiesel. However, polar solvents provide a high rate of extraction because of the polar lipid and non-lipid extraction, increasing biodiesel contamination by giving lipids which can be saponified at low levels. In a study by Olkiewicz et al. (2015), the decrease in the reaction temperature indicated that the productivity of saponifiable lipids increases, while higher reaction temperature caused more lipid contamination with non-saponifiable material that cannot be converted into biodiesel.

The total lipid content extracted from the thickener sludge is composed of polar lipids (glycolipids and complex phospholipids), non-polar (neutral) lipids, and non-lipids (carbohydrates and proteins) that could be extracted with hexane. Therefore, the lipid extraction process should be chosen according to both the structure of lipids and the structure of the saponifiable lipid fraction. The amount of saponifiable lipids and total FAMES of thickener sludge were analyzed by using two different extraction methods and the results are illustrated in Table 4.3. All transesterification experiments were conducted in duplicate.

Table 4.3. Saponifiable and FAMES yields from thickener sludge for each extraction method.

Sludge type	Extraction method	Yield (%) ^a			
		Saponifiable		FAMES	
		Acidified	Non-acidified	Acidified	Non-acidified
Thickener sludge	Standard drying method ^(b)	68 ± 1.3	66.50 ± 0.6	14.96 ± 0.9	13.17 ± 0.7
	Direct liquid-liquid extraction method	71 ± 0.7	69 ± 1.8	23.43 ± 1.3	19.94 ± 1.1
^(a) All transesterification experiments were performed at least twice.					
^(b) Extraction according to MgSO ₄ .H ₂ O method, lipid yield on the basis of dry sludge.					

Irrespectively of sludge acidification, slightly higher saponifiable lipids were obtained by the direct liquid-liquid extraction method compared to the standard drying method. With this extraction method, thickener sludge resulted with greater biodiesel yield (23.43 %) than that of the drying method (14.96 %). Although the obtained saponifiable yields of thickener sludge by the two types of extraction methods were similar, the overall FAMES yield obtained by the direct liquid-liquid extraction method was higher than the standard method due to a capacity to extract more lipids by the liquid-liquid extraction method. Moreover, the direct liquid-liquid lipid extraction method, having high selectivity for neutral/saponifiable lipids, resulted with higher lipid yields. Thus, for the petrochemical industry WWTP sludge samples, the direct liquid-liquid lipid extraction method yielded higher saponifiable fraction by reducing lipid contamination that could adversely affect the biodiesel production and led to higher biodiesel yields, compared to the standard drying method.

4.5. Effect of Extraction Methods on the Fatty Acids Composition

Fatty acids composition is very important for the characteristics of biodiesel. Thus, it is necessary to determine the effect of the extraction methods used on the fatty acids composition. Lipids extracted from thickener sludge samples include a high amount of FFAs (>25 %), refusing conventional base-catalyzed transesterification method. The reason is that these FFAs are converted into soap in a basic environment that leads to an emulsion that makes it difficult to separate from water. For this reason, only the acid-catalyzed process must be conducted in the esterification/transesterification process. The influence of direct liquid-liquid lipid extraction method on the fatty acids composition of the thickener sludge in comparison to the standard drying method is shown in Table 4.4.

Table 4.4. Fatty acids composition of the thickener sludge by each extraction stage.

Fatty acids	% (w/w)			
	Standard drying method		Direct liquid-liquid lipid extraction method	
	Acidified	Non-acidified	Acidified	Non-acidified
Lauric (C12:0)	1.4	1.6	1.5	1.8
Myristic (C14:0)	3.1	3.1	3.2	3.1
Pentadecanoic (C15:0)	0.4	0.7	0.8	1.9
Palmitic (C16:0)	48.2	48.0	44.3	43.8
Palmitoleic (C16:1)	3.5	3.3	3.6	3.8
Heptadecanoic (C17:0)	0.6	0.6	0.7	0.6
Stearic (C18:0)	15.3	14.8	14.4	13.6
Oleic (C18:1)	18.1	17.8	21.5	20.9
Linoleic (C18:2)	2.7	2.6	3.3	3.3
Arachidic (C20:0)	0.3	0.5	0.8	1
Eicosenoic (C20:1)	0.5	0.5	0.5	0.5
Behenic (C22:0)	0.9	0.8	0.8	0.7
Others	5.0	5.7	4.5	4.9

The fatty acid profile of thickener sludge showed a very similar and considerable amount of palmitic (44.3 % to 48.2 %), oleic (18.1 % to 21.5 %) and stearic (14.4 % to 15.3 %) acids for acidified sludges and each extraction methods.

Moreover, some remarkable differences can be noticed between standard drying and direct liquid-liquid extraction methods for thickener sludge according to Table 4.4, suggesting the effect of sludge drying and/or water content of sludge. The direct liquid-liquid lipid extraction method indicated an increment in the amount of palmitoleic, oleic and linoleic acids, counterbalanced by a reduction of stearic and palmitic acids. This situation affects the total saturated, poly-unsaturated and mono-unsaturated fatty acid content. Small amounts of both saturated and poly-unsaturated FAMES and high amounts of mono-unsaturated FAMES such as palmitoleic and oleic acids are main components of biodiesel properties. These essential fatty acids are very successful in the high oxidative stability of biodiesel (Hoekman et al., 2012). The polyunsaturated fatty acids are very sensitive to auto-oxidation, decreasing the oxidation stability of biodiesel. Moreover, saturated fatty acids, which are more than 60 % in the sludge, can cause problems such as the crystals formation and the solidification of saturated compounds in the cold flow properties of biodiesel. This can be overcome by the use of hydroxy fatty acid monoalkyl esters and branched-chain alcohols (Dailey et al., 2008; Dailey et al., 2009). Actually, these compounds are found in small amounts and are shown in Table 4.4 as “others”. This part is specified by GC–MS and is mainly consisted of some components like 1-decene, 1-tetradecene and cyclotetradecane; or methyl 10-hydroxyhexadecanoate, methyl 12-methyl-tetradecanoate, methyl 13-methyltetradecanoate, methyl-14methylhexadecanoate,

methyl 15-methylhexadecanoate, benzenoacetic acid methyl ester and benzenopropanoic acid methyl ester.

The thickener sludge, which is made up of a small amount of microbial cells due to its biological content, contains palmitoleic acid (3.3 to 3.8 %), which is known to be bacterial, in an unnecessary amounts (Jardé et al., 2005). Linolenic acid (C18: 3), however, is absent in both extraction methods. These outcomes are supported with the literature data (Kech et al., 2018; Olkiewicz et al., 2015) and can be attributed to the fact that the sludge is a suitable feedstock for biodiesel production.

According to these results, it can be suggested that the direct liquid-liquid extraction method for the thickener sludge is more efficient and suitable than the standard drying method due to its high content of mono-unsaturated fatty acids, thereby improving the oxidative stability of biodiesel.

4.6. Effect of Sludge Pre-treatments

Acidification, ultrasonication and microwave techniques were applied as a pre-treatment to the petrochemical industry WWTP sludge prior to the lipid extraction step. These pre-treatment methods were chosen so as to increase the sludge disintegration capacity to allow mixing equal masses of the solvent by better penetration into the sample and to extract lipids bound to macromolecules that cannot be reached by the solvent (Olkiewicz et al., 2015; Pernet and Tremblay, 2003). Each method was first applied on its own, and then ultrasonic and microwave applications were combined with acid pre-treatment to determine their activity on their own and in combination. It is expected that the pre-treatment methods applied will give rise to release the lipid in extracellular polymeric substances of bacterial sludge flocs, disintegrate the sludge flocs and break down the bacterial cell wall (Pilli et al., 2011).

The lipid, saponifiable and biodiesel yields obtained from petrochemical industry thickener sludges by different pre-treatment methods are illustrated in Table 4.5.

Table 4.5. Effect of different pre-treatment methods on lipid, saponifiable and biodiesel yields obtained from thickener sludge by two different extraction methods.

Sludge type	Extraction method	Yield (%) ^a	Pre-treatment method					
			Unpre.	Acid.	US	MW	Acid. + US	Acid. + MW
Thickener sludge	Standard drying method ^(b)	Lipid	19.80	22	27	27.70	30.85	32.37
		Saponifiable	66.50	68	68	68	68	68.50
		Biodiesel	13.17	14.96	18.33	18.90	21	22.10
	Direct liquid-liquid extraction method	Lipid	28.90	33	29.32	30.33	42.87	47.07
		Saponifiable	69	71	69	69.5	69	69.5
		Biodiesel	19.94	23.43	20.29	21.02	29.58	32.62

^(a) All transesterification experiments were performed at least twice.

^(b) Extraction according to standard MeSO₄·H₂O method, lipid yield on the basis of dry sludge.

4.6.1. Effect of Acid Pre-treatment

Lipids obtained from carbohydrates and/or proteins could be released by hydrolysis, thereby obtaining more saponifiable lipids and biodiesel yields by acidifying sludge with the concentrated hydrochloric acid (Table 4.5). Additionally, non-saponifiable matters in the sludge such as triglycerides, phospholipids, sterols and wax esters could hydrolyze into free fatty acids (Zhu et al., 2014). It can be expected that the extraction of lipids from the sludge samples will be increased by the acid pre-treatment (pre-acidification), as well as the saponifiable fraction, thereby increasing the biodiesel yield.

The results of lipid yields from the two sludge types with and without previous sludge acidification can be seen in Table 4.2. The acid pre-treatment resulted with slightly lower lipid yield in standard drying method for all sludge types tested as compared to the direct liquid-liquid extraction method. Thickener sludge resulted with the highest lipid yield with the acid pre-treatment followed by the secondary clarifier sludge, irrespective of lipid extraction method. It seemed that the pre-acidification was more effective on the thickener sludge. This can be explained by the release of the lipids in bonded form, resulting in higher saponifiable lipid yields, hydrolysis of the phospholipids, triglycerides, sterol esters and wax esters present in the sludge into FFAs and letting the polar materials unextracted (McNichol et al., 2012; Olkiewicz et al., 2015; Zhu et al., 2014). Furthermore, insoluble soaps in thickener sludge samples are formed by the reaction between cations such as calcium and magnesium with well dispersed fatty acids or fatty acid salts during primary treatment. The insoluble soaps can be converted into free fatty acids which are soluble in the solvent, thus improving the lipid yield, saponifiable matter and biodiesel due to the acid pre-treatment application. Since the secondary clarifier sludge mostly comprises microbial cells, it does not contain insoluble

soaps as much as thickener sludge samples, so its lipid yield is lower. Only some additional lipids attached to cells may release by acid pre-treatment, thus slightly increasing the lipid yield (Pastore et al., 2013).

In the direct liquid-liquid lipid extraction method, petrochemical industry WWTP thickener sludge contains FFAs in an unbounded form that can be esterified directly, so the improvement was just 14 % by the effect of acid pre-treatment for the thickener sludge. In the standard drying method, the increase in lipid yields remained in a range of 11 % to 13.5 % for all of the sludge samples. Supportively, Olkiewicz et al. (2015) explained that an extraction by standard drying method produces heavily contaminated lipids with the non-saponifiable material, leading to a decrease in the biodiesel yield.

Moreover, the increase of FFA in the thickener sludge is connected with the conversion of insoluble soaps, found in the sludge, to FFAs presence of an acidic medium (Olkiewicz et al., 2014). Due to the increase of FFAs in the thickener sludge after acid pre-treatment, the amount of saponifiable lipids increased and led to an increase in the biodiesel yield (Table 4.4 and 4.5).

4.6.2. Effect of Ultrasonic (US) Pre-treatment

This study also investigated the ultrasonication (US) and combined acidification and ultrasonication pre-treatments on the lipid extraction and biodiesel production. The lipid, saponifiable matter and biodiesel yields obtained from thickener sludge samples by these pre-treatment methods are illustrated in Table 4.5. The reason for choosing ultrasonic pre-treatment method is a high sludge disintegration capacity and allowing for better penetration of the solvent (Pernet and Tremblay, 2003). In addition, the ultrasonication may facilitate deagglomeration of microorganisms and thus, increase the efficiency of extraction solvents (Hua and Thompson, 2000; Phull et al., 1997). Moreover, these pre-treatment methods are able to break down the sludge flocs and microbial cells, release the intracellular organic matters (lipid, protein etc.) to the liquid phase so that the lipid extraction from sludge is improved (Pilli et al., 2011).

In the study of Jin et al. (2012), when the power and duration of treatment in the ultrasonic pre-treatment were increased over 400 W and 15 min, a decrease in the lipid extraction efficiency or no more lipid extraction was observed. The reduction in lipid extraction yield was probably due to the damage of some substances in the cells when applied US pre-treatment for a long period of time

(Prommuak et al., 2012). According to these results, 200 W and 13 min of duration time for ultrasonic pre-treatment were selected as the pre-treatment duration and power for this study.

Acid pre-treatment method alone is not sufficient for lipid extraction, therefore by combining this method with ultrasonic pre-treatment method, a much better lipid extraction was targeted. As shown in Table 4.5, pre-treatment methods were so effective in thickener sludge for both lipid extraction methods. Both ultrasonication and combined acidification and ultrasonication pre-treatments increased the lipid and biodiesel yields obtained from petrochemical industry thickener sludge samples. The combined acidification and ultrasonication pre-treatment increased the lipid yields of the sludge samples by about 48 % and 55 % for the direct liquid-liquid lipid extraction method and standard drying method, respectively. As it can be seen, the effect of combined acidification and ultrasonication pre-treatment on the direct liquid-liquid lipid extraction method was slightly smaller compared the standard drying method. In the direct liquid-liquid lipid extraction method, while the application of ultrasonication pre-treatment itself slightly improved the lipid and biodiesel yields of thickener sludge samples, in the standard drying method the improvement was about 36 % and 40 % for lipid and biodiesel yields, respectively. These results are believed to be achieved because the ultrasonic pre-treatment alone could not effectively break the emulsifying film of surfactant molecules in the liquid sludge (Zhang et al., 2012). Especially, when compared to untreated sludge sample, the application of combined acidification and ultrasonication pre-treatment caused to an important increase in the lipid and biodiesel yields for the thickener sludge samples and using the liquid-liquid lipid extraction method. It increased the lipid and biodiesel yields by about 1.5 folds for thickener sludge samples. The results of direct liquid-liquid lipid extraction experiments showed that the effect of pre-treatment methods depends on the lipid concentrations in the samples and decreases with the decreasing lipid concentrations in the sludge samples. This explains the reason for obtaining higher yields from pre-treated thickener sludge samples having high lipid content.

4.6.3. Effect of Microwave (MW) Pre-treatment

This study also investigated the microwave (MW) and combined acidification and MW pre-treatments on the lipid extraction and biodiesel production. The lipid, saponifiable and biodiesel yields obtained from thickener sludge samples by different pre-treatment methods are illustrated in Table 4.5. Microwaves are utilized as a thermal pre-treatment method or process development method for the lipid extraction for biodiesel production (Giese, 1992). Recent laboratory scale microwave pre-treatment experiments in biodiesel production demonstrated that this pre-treatment method is more efficient than other pre-treatment methods in extraction experiments (Letellier and Budzinski,

1999; Pare et al., 1994). The rapid and selective extraction of organic compounds takes place with microwaves with a low solvent requirement and low energy consumption. The fact that the microwave pre-treatment method allows a higher sludge disintegration capacity and better penetration of the solvent is the reason for choosing this pre-treatment method (Pernet and Tremblay, 2003). Furthermore, this pre-treatment method can break down the sludge and microbial cells, release the intracellular organic substances (lipid, protein, etc.), and thus improve the lipid extraction from the sludge (Pilli et al., 2011).

The strong microwave energy absorption created by the MW irradiation increases the temperature inside the sample and causes the cells to be separated by the water. In some cases, it may induce degradation of the target compound or penetration of a solvent, and in other cases, it may improve the diffusivity of the target compound in the medium (Jain et al., 2009). For oily sludge, the internal phase may absorb more microwave energy than oil, which has a relatively higher dielectric loss. This type of energy absorption can cause water to expand and water-oil separation to be easier. (Tan et al., 2007).

Acid and ultrasonic pre-treatment methods alone are not sufficient for lipid extraction, therefore by applying microwave pre-treatment method alone and combining this method with acid pre-treatment method, a much better lipid extraction was targeted. As can be seen in Table 4.5., microwave pre-treatment alone was so effective in lipid and biodiesel yields for standard drying method, whereas it slightly improved the lipid and biodiesel yields of thickener sludge samples for direct liquid-liquid lipid extraction method. Combined acidification and MW pre-treatment method was also so effective in thickener sludge for both lipid extraction methods. The results of direct liquid-liquid lipid extraction experiments showed that the effect of pre-treatment method depends on the lipid concentrations in the samples and decreases with the decreasing lipid concentrations in the sludge samples. This explains the reason for obtaining higher yields from pre-treated thickener samples having high lipid content.

Microwaves used as pre-treatment techniques were efficient and yielded a higher amount of lipids compared to that obtained from the non-pretreated sludge biomass. The microwave and combined acid and microwave pre-treatments improved the lipid and biodiesel yields obtained from petrochemical industry thickener sludges. The application of combined acid and microwave pre-treatment to the petrochemical industry WWTP thickener sludge increased the lipid yields by about 63 % for both extraction methods when compared to unpretreated sludge sample. This combined pre-treatment method also increased the biodiesel yields by about 68 % and 63.5 % for standard drying

and direct liquid-liquid lipid extraction methods, respectively. The effect of this combined pre-treatment method can be explained by the fact that the emulsions such as polar lipids and re-flocculated large-sized particles formed in microwave processes can be hydrolyzed at the same time as acidification, resulting in more effective lipid extraction (Dong et al., 2016).

When the combined acid and MW pre-treatment compared to the acid pre-treatment, an increase of about 47 % was observed in both biodiesel and lipid yields for standard drying method, on the other hand, in the direct liquid-liquid lipid extraction method, increases of about 42.5 % and 40 % were observed in lipid and biodiesel yields, respectively. When this combined method compared to the combined acid and US pre-treatment, only slight increases of about 5 % and 10 % were obtained in both of the lipid and biodiesel yields for standard drying and direct liquid-liquid lipid extraction methods, respectively. These results indicated that microwave pre-treatment performance of sludge was slightly better than the performance of the ultrasonic pre-treatment in terms of lipid extraction and biodiesel production. Comparing among these three pre-treatment techniques, microwave pre-treatment method seems to be favorable for extraction of lipid from thickener sludge as it resulted in higher lipid yield. The highest lipid and biodiesel yields were achieved by the application of combined acid/microwave pre-treatment to the petrochemical industry thickener sludge samples and using direct liquid-liquid lipid extraction method.

4.7. Optimization of Direct Liquid-Liquid Lipid Extraction from Thickener Sludge

The optimization study of direct liquid-liquid lipid extraction for acidified thickener sludge samples was performed for nine stages at different contact times of 10, 20 and 40 min, different sludge to hexane volume ratios of 1:2 and 1:1 and the different total solids content of thickener sludge samples. The experiments were performed at ambient temperature by using 40 mL of sludge samples at 200 rpm mixing speed. These operative conditions were maintained constant during the experiments.

4.7.1. Effect of Contact Time and Sludge to Hexane (S/H) Ratio

Figure 4.1 shows the effect of different extraction time and sludges to hexane (S/H) ratio on the lipid yields of acidified thickener sludge.

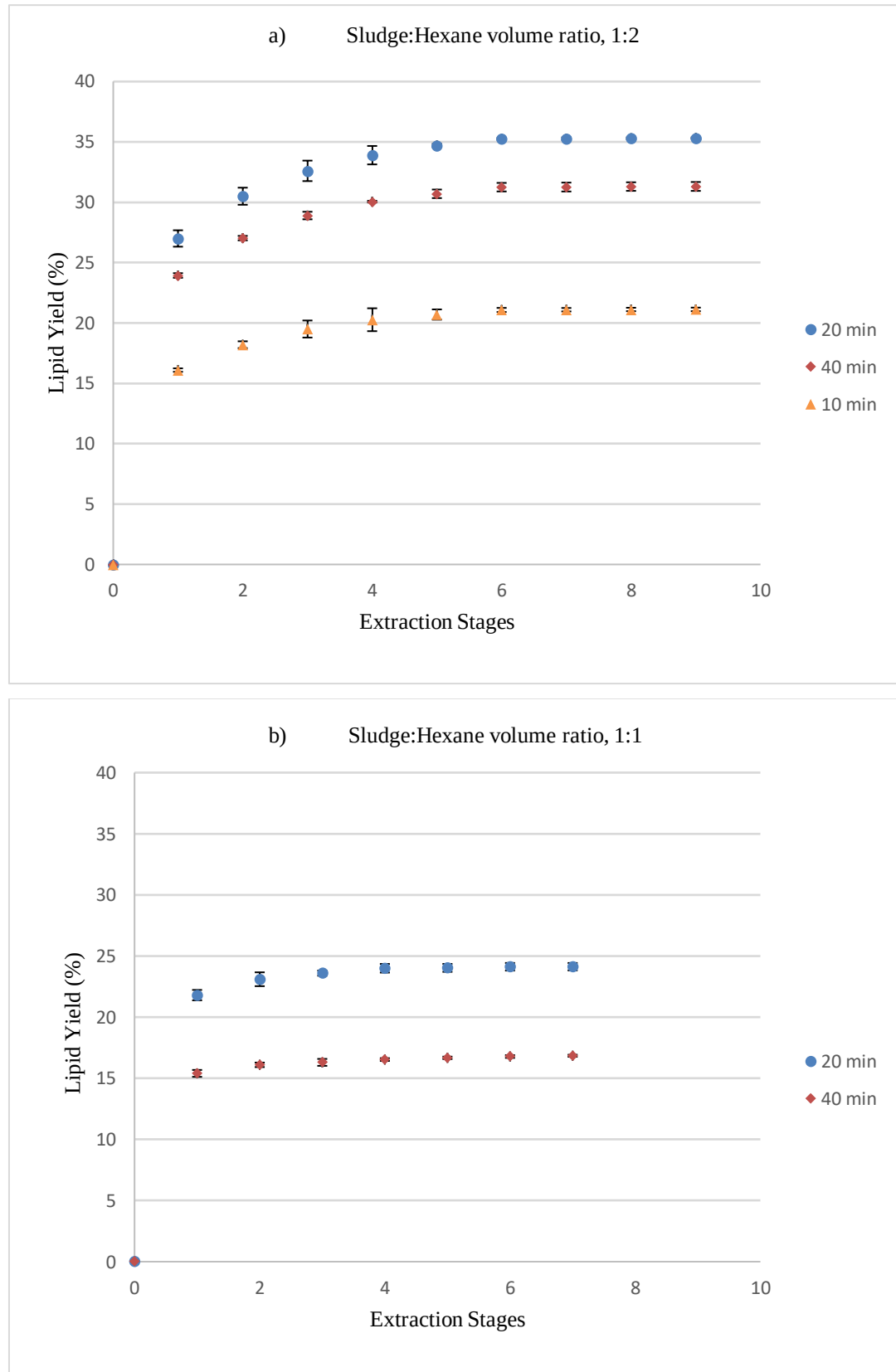


Figure 4.1. Effect of extraction time and sludge to hexane volume ratio on the lipid yields of acidified thickener sludge. a) S/H 1:2. b) S/H 1:1 (200 rpm of mixing speed, ambient temperature and acidic media).

The results of lipid extraction optimization studies indicated that the rate of increase in lipid yields decreased gradually in each extraction stage and stopped at the end of the ninth step. The optimum extraction stage number was selected to be three for petrochemical industry sludge. The optimization study also demonstrated that, after three extraction stages, 92 % of lipid from petrochemical industry thickener sludge was recovered. Accordingly, Olkiewicz et al. (2014) showed that three sequential extraction steps are enough to achieve 91 % of lipids existing in wastewater sludge samples. In this study, the highest lipid yields were obtained at sludge to hexane volume ratio of 1:2 for the 20 min mixing time for sludge samples. The mixing time plays an important role in terms of lipid extraction yield. As it can be seen in the Figure 4.1.a, the lowest lipid extraction yields were taken for 10 min of extraction time for sludge to hexane volume ratio 1:2. The contact time of 10 min was not sufficient for lipid extraction. In general, the lipid extraction yield is expected to increase with the increasing mixing time (Olkiewicz et al., 2014). However, the lipid extraction efficiency at the mixing period of 40 min was found to be less than that of obtained at 20 min. When the mixing time increased, the loss of the hexane by evaporation from the open extraction system also increased, limiting the extraction efficiency. An evaporation test was performed to see the extent of the hexane evaporation. Since hexane is a highly volatile organic solvent, at the end of the 40-min mixing period, the volume of hexane is severely reduced from 80 mL to 10 mL. Based on the results of the optimization studies given in Figure 4.1, the lower the amount of solvent, the lower the lipid extraction yield was attained. The lowest lipid yields were obtained at sludge to hexane volume ratio of 1:1 for thickener sludge samples, suggesting that the amount of hexane was not enough to extract high quantities of lipid in the sludge. Agreeing with Olkiewicz et al. (2014), in this study, the optimum extraction conditions for thickener sludges were found to be S/H of 1:2 and extraction time of 20 min in an open system. Olkiewicz et al. (2014) found the best extraction conditions for municipal WWTP sludges as; S/H 2:1 for 60 min extraction time, regarding the minimization of solvent use and as S/H 1:2 for 20 min extraction, regarding the minimization of extraction time. Kech et al. (2018) also carried out liquid-liquid lipid extraction method to extract lipids from primary and secondary sewage sludges at three sequential extraction steps in a closed system. Differently, they used isopropyl alcohol/cyclohexane mixture as an extraction solvent and mixed the sludge samples and solvent at 1900 rpm at a closed system and let them settle by using centrifugation at 3000 rpm. They found the best extraction conditions as 60 min and sludge to solvent ratio of 1:1.6:2 lipids (Kech et al., 2018). Based on the results of the optimization studies obtained, all experimental studies in this work were carried out under optimum conditions of sludge to hexane volume ratio of 1:2 and 20 min mixing period.

4.7.2. Effect of Total Solids Content

The power input, specific energy input, frequency, time of the pre-treatment method applied as well as sludge characteristics such as solids concentration and sludge type are also significant factors affecting the pre-treatment of sludge (Tyagi et al., 2014). Experiments have been performed for two different sludge total solids (TS) concentrations as shown in Figure 4.2.

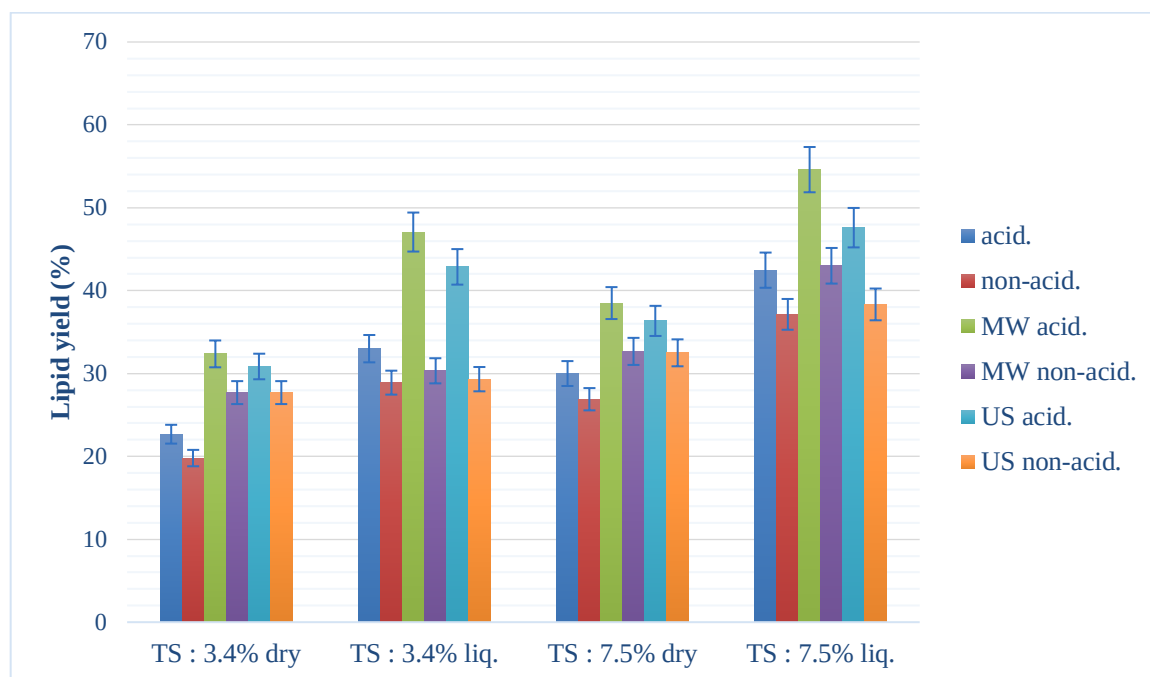


Figure 4.2. Lipid yields of thickener sludge samples for different TS concentrations in both of the extraction methods.

Lipid extraction yield depends on the solids concentrations of sludge samples. The results showed that the lipid yields increased at the higher TS concentrations. However, the increase in lipid yields was lower in pre-treated sludge samples. At the sludge TS concentration of 3.4 %, application of microwave and ultrasonic pre-treatments increased the lipid yields by about 45 % and 35 %, respectively, while at TS concentration of 7.5 %, the lipid yields were improved by about 25 % and 20 %, respectively. Increasing TS concentrations negatively affected the performance of the pre-treatment methods by reducing the applied specific energies. In ultrasonication pre-treatment, this can be explained with a decrease in power applied to the liquid portion as a result of increased TS concentration (Alagoz et al., 2015; Gholam and Dariush, 2013; Hu et al., 2013; Kidak et al., 2009; Swamy and Narayana, 2001; Yang et al., 2009). The sludge having a higher total solids (TS) concentration will absorb the acoustic energy and reduce the performance of the pre-treatment process when the total solids content increases more than a certain point (Kidak et al., 2009). The sludge

absorbance constant is directly proportional to the solid content of sludge, so that when the sludge TS is high, the sound intensity can be quickly lowered, resulting in a reduction in the sludge pre-treatment performance (Tyagi et al., 2014). In water-in-oil (W/O) emulsions such as oily sludge, water is present in the inner phase and oil is in the external phase. The inner phase-water, which has a comparatively higher dielectric loss than oil, can absorb more microwave energy in the microwave pre-treatment mechanism. This kind of energy absorption can cause water phase to expand and make the water-oil interfacial film thinner, making water/oil separation easier (Chan and Chen, 2002; Hu et al., 2013; Shang et al., 2006; Tan et al., 2007). In a solid-liquid environment such as a sludge, these pre-treatment methods require substantial amounts of liquid for evaporation. If the solids concentration is too high, liquid evaporation may be inhibited and this may reduce the effectiveness of these pre-treatment methods (Show et al., 2007).

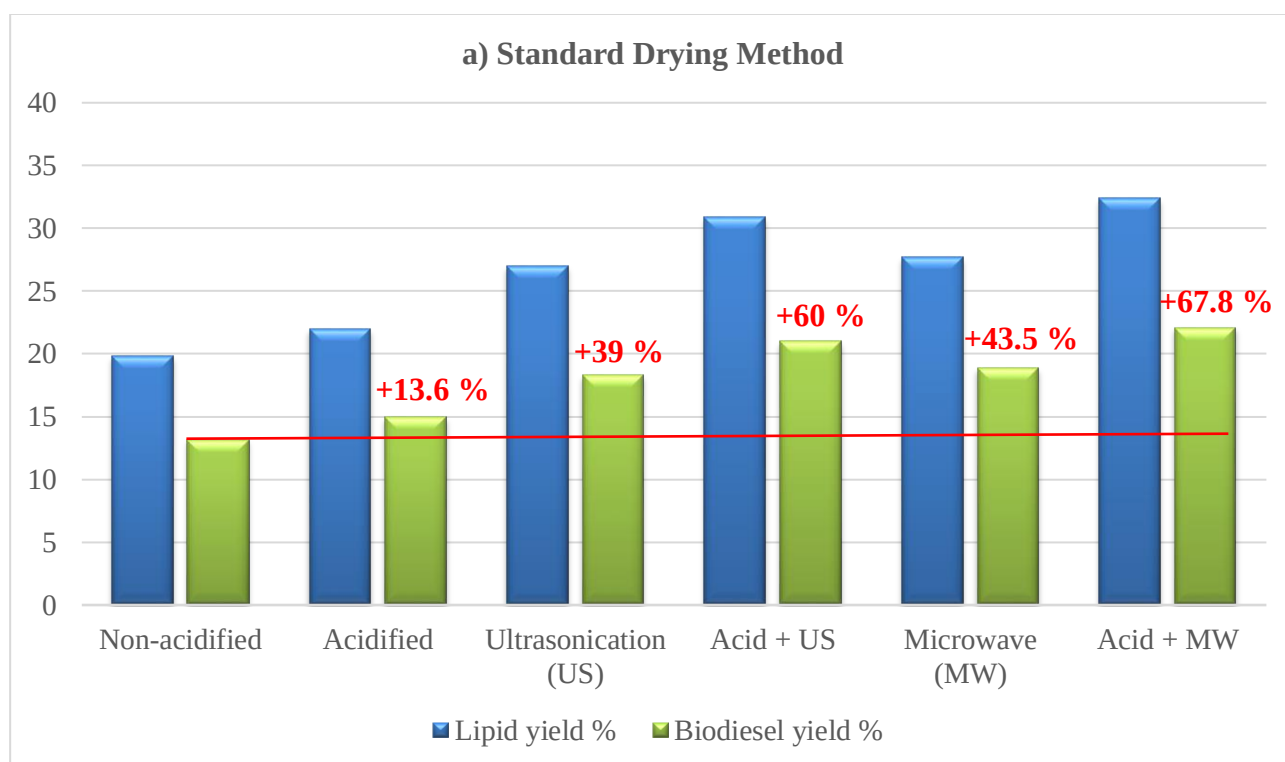
Furthermore, Zhang et al. (2008) showed that the higher VS/TS ratio provided higher energy efficiency for the inorganic materials to absorb energy. For this reason, pre-treatment of the sludge with a lower VS/TS ratio (more inorganic matters) becomes more energy-consuming than the sludge with higher VS/TS ratio (more organic matters) (Zhang et al., 2008). Consequently, it is important to select the optimum solids concentration range for an affordable and effective pre-treatment.

4.8. Biodiesel Production

The total lipid yield is not equal to the total biodiesel (FAMES) yield as only a part of the lipid extracted from the sludge can be converted into biodiesel. The lipidic compound extracted from the sludge contains not only acylglycerols and free fatty acids but also polycyclic aromatic hydrocarbons (PAHs), linear alkyl benzenes, hydrocarbons, sterols, pigments, trepans and some waxes. Only the acylglycerols and free fatty acids (FFAs), which form the saponifiable portion of the lipids, can be converted to biodiesel (can be transesterifiable/esterifiable to FAMES). Hydrocarbons, other non-polar materials (non-lipids) that can be extracted with hexane, and also some of the lipids, such as sterols and waxes are the non-saponifiable lipids known as lipid contaminants and not convertible to biodiesel (Dufreche et al., 2007; Jardé et al., 2005; Olkiewicz et al., 2012; Olkiewicz et al., 2014; Pastore et al., 2013; Siddiquee and Rohani, 2011).

The results of lipid transesterification and biodiesel production yields from thickener sludge samples were presented in Table 4.5. The biodiesel (FAME) yields of the lipids extracted by using two extraction methods (standard drying method and direct liquid-liquid lipid extraction method) were found to be 14.96 % and 23.43 % (on the basis of dry sludge) for the acidified petrochemical

industry WWTP thickener sludge samples, respectively. As shown in the Figure 4.3, in the standard drying method, the increase in biodiesel yields was in a range of about 39 % to 68 % for ultrasonicated, microwave-irradiated and combined pre-treated sludge samples; however, in the optimized direct liquid-liquid lipid extraction method, the improvement remained in a range of 2 % to 63.5 % for only the ultrasonic, microwave pre-treatment and combined pre-treatment applied sludge samples. The combined acidification and ultrasonication pre-treatment and the combined acidification and microwave pre-treatment caused an important increase in the biodiesel yields for both of the lipid extraction methods. The highest biodiesel yields were obtained from the combined acidification and MW pre-treatment methods. Compared to standard drying method, the direct liquid-liquid lipid extraction method resulted with 57 % increase in acidified samples, 41 % increase in combined acidification and US pre-treated samples and 48 % increase in combined acidification and MW pre-treated samples.



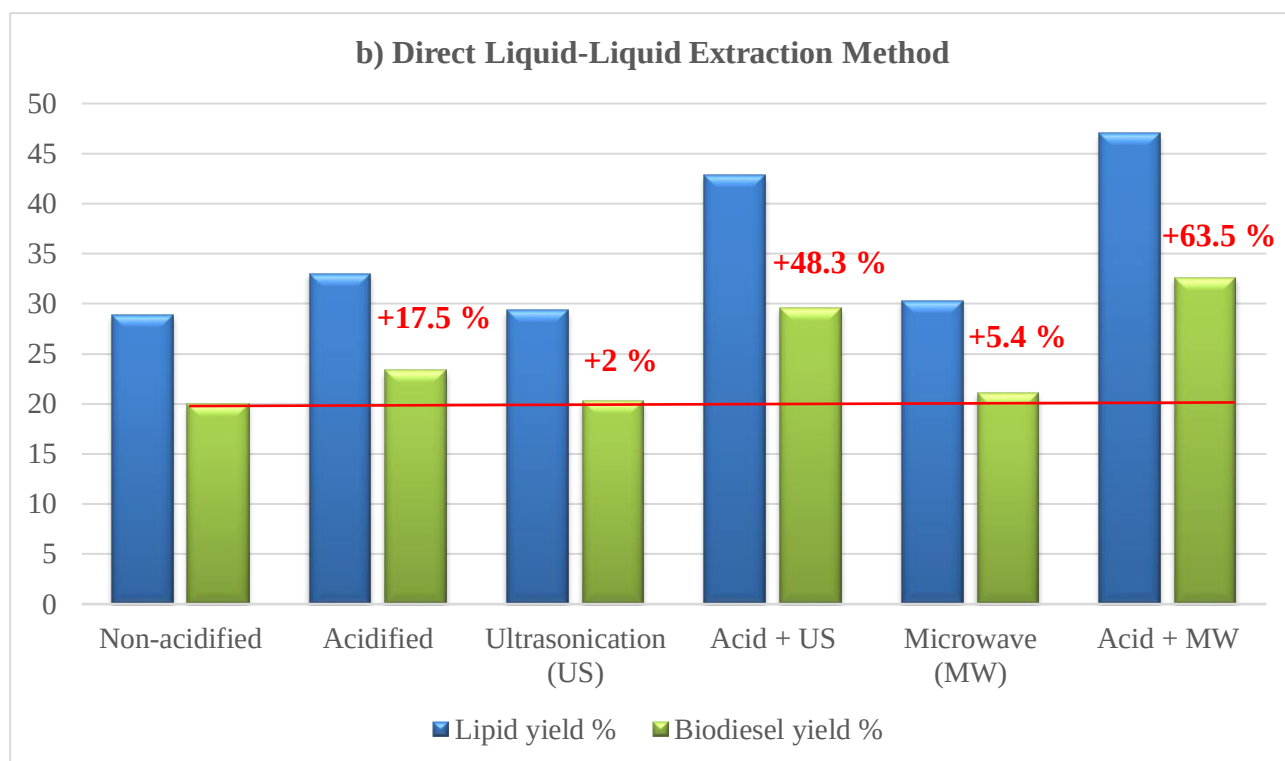


Figure 4.3. Comparison of lipid and biodiesel yields. a) Standard drying method. b) Optimized liquid-liquid lipid extraction method.

Parallel with the lipid yields, the highest biodiesel yields were also obtained from pre-treatment applied thickener sludge samples by using direct liquid-liquid lipid extraction method. Compared to the standard drying method, the direct liquid-liquid lipid extraction method was found to be much more efficient, in terms of biodiesel production yield for the sludge samples.

On the other hand, the biodiesel properties are mainly based on the fatty acid methyl ester (FAME) composition. FAME analysis of biodiesel samples obtained from standard drying and direct liquid-liquid lipid extraction methods showed that the major components of biodiesel obtained from the sludge samples are palmitic acid (C16:0), oleic acid (C18:1) and stearic acid (C18:0) (Table 4.4). The acid pre-treatment also did not have a noticeable effect on the FAME composition. This result also makes the direct liquid-liquid lipid extraction method efficient since it does not have a negative effect on the biodiesel properties obtained by this method. These outcomes showed compatibility with studies in the literature (Kech et al., 2018; Mondala et al., 2009; Olkiewicz et al., 2012; Olkiewicz et al., 2014; Olkiewicz et al., 2015).

5. CONCLUSIONS

The results indicated that petrochemical industry WWTP sludges having high lipid content can be used as efficient feedstocks for biodiesel production. However, petrochemical industry secondary clarifier sludges were found to be inefficient to be used as lipid feedstocks for biodiesel production due to their low lipid contents. Compared to standard drying method, direct liquid-liquid lipid extraction method was found to be more efficient for petrochemical industry WWTP sludge samples in terms of both lipid extraction and biodiesel production yields. The direct liquid-liquid lipid extraction method, which does not require expensive sludge drying or dewatering steps, resulted with 53 % higher lipid and 57 % higher biodiesel yields for petrochemical industry WWTP sludge samples than standard drying method.

Acid pre-treatment caused to an increasing effect on the lipid and biodiesel yields by using direct liquid-liquid and standard drying lipid extraction methods. The ultrasonication pre-treatment itself and microwave pre-treatment itself did not cause to an important change in the lipid and biodiesel yields obtained from petrochemical industry thickener sludge samples in the direct liquid-liquid lipid extraction method. On the other hand, the combined pre-treatment methods (acid/US and acid/MW) considerably increased the lipid and biodiesel yields for both extraction methods. The highest lipid and biodiesel yields were achieved by application of combined acidification/microwave pre-treatment to the thickener sludge samples and using the liquid-liquid lipid extraction method.

The results revealed that, standard drying lipid extraction method appears more suitable for materials in biological structure having lower lipid contents. However, direct liquid-liquid lipid extraction method was more efficient for materials having high lipid, low biological content.

It seems, direct liquid-liquid lipid extraction method makes the biodiesel production effective in terms of time, energy and cost compared to conventional standard drying method requiring expensive sludge drying or dewatering steps. The optimization of lipid extraction process improved both lipid and biodiesel yields and the economy of whole process. In both of the methods, hexane recovery contributes to the economy.

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