Potential and Extraction of Wastewater Lipid for Biodiesel Production

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Abstract

Biodiesel holds significant promise as an alternative to conventional diesel fuel due to its renewability, environmentally friendly, and adaptability to existing engine systems. Despite its potential in various market applications, the competitiveness of biodiesel is limited by high production costs associated with expensive raw materials, primarily vegetable oil. Additionally, the reliance on vegetable oil feedstock raises conflicts with the demand for food consumption and sustainable land use for plantations. As efforts continue to harness the benefits of biodiesel, addressing these challenges becomes crucial for unlocking its full potential as a sustainable alternative fuel. These concerns have increased interest in developing wastewater lipids in sewage systems as an alternative feedstock for biodiesel production. These waste materials are continuously generated and contain substantial lipid concentrations, offering the potential for sustainable biodiesel production. This study investigates the potential of wastewater lipids as a viable raw material for biodiesel production.

The first study conducted in this thesis evaluates different types of sewage sludges obtained from two wastewater treatment plants (WWTPs) (A and B) in Japan. Several samples were investigated, including primary, waste-activated, mixed, and dewatered sludge, and primary and secondary scum. Lipids were extracted via the Soxhlet hexane method and converted into biodiesel via acid-catalyzed transesterification. Among the sludges tested, primary WWTP A scum had the highest lipid and biodiesel yields (28.5% and 11%, respectively). Analysis of fatty acid methyl esters revealed that palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) predominated, regardless of sludge type. Furthermore, the comparison of sludge fatty acid profiles with conventional biodiesel feedstocks confirmed their viability for biodiesel production.

From the previous research, it was found that primary scum contained high lipid and biodiesel potential. However, more research is needed to understand the characteristics of feedstocks, since the physical and chemical characteristics may vary with seasons and collection sites. In the present study, we compared the potential of fat balls which is a floating fats, oils, and grease (FOG) deposits collected from the sewage pumping station, with primary scum and primary sludge obtained from the WWTP primary clarifier. In addition, this study also investigated screen sludge from wastewater pre-

treatment processes. However, the results indicate that the complex biomass characteristics of screen sludge make it unsuitable for biodiesel feedstock. Samples were collected in four seasons (winter, summer, autumn, and spring) from two WWTPs (H and S) and one pumping station in Japan. The results show that average lipid recoveries from fat balls (48.6%) and primary scum (45.0–48.7%) were higher than the lipid recovery of primary sludge (12.4–12.9%). The average yield of biodiesel produced from the extracted lipids ranged from 4.5% to 19.9%. Radiocarbon analysis indicated the presence of fossil-derived carbon (26-42%) in the biodiesel obtained from wastewater lipids. There were considerable site and season-dependent variations in the characteristics of the lipid waste materials. Fat balls from the pumping station had higher solids and carbon contents, along with greater calorific values, than primary scum and primary sludge samples collected at the primary clarifier. Finally, we estimated the potential for biodiesel production from WWTP-derived lipids; about 447.0 metric tons of biodiesel per year could be produced from fat balls and primary scum in Japan. This presents a viable option to supplement used cooking oil, which has been the main traditional biodiesel source in Japan. The result indicates that primary scum has a higher moisture content, and initial concentration steps may be required prior to lipid extraction, potentially increasing energy requirements. In addition, it may be more practical to collect the fat balls as they can be skimmed out of the system without being mixed into the current sludge treatment process. Therefore, we concluded fat balls as the most favourable biodiesel feedstock from wastewater facilities.

Although conventional solvent extraction methods are commonly used for extracting wastewater lipids, alternative extraction method needs to be investigated to improve the lipid recovery process. This part of the thesis investigates the use of dimethyl ether (DME) as an alternative solvent to improve the recovery of lipids from fat balls. DME is a synthetic polar gas (at room temperature) that has gained prominence as an eco-friendly and non-toxic extraction agent. Response Surface Methodology (RSM) based on Box-Behnken design (BBD) was utilized to evaluate DME extraction and optimize the best potential combination of sample size, velocity, and DME/sample ratio for a higher yield of lipid. The highest percentage of lipid recovery was 65.2% under the optimal DME extraction conditions (sample size 1 mm, velocity 3.33 m/h, and DME/sample ratio 80 mL/g). Furthermore, a comparative study was performed using

mechanical shaking and the Soxhlet method. Lipid recovery by mechanical shaking extraction (49%) and Soxhlet extraction (62%) was lower than that of DME extraction.

Overall, this study reveals that the wastewater residues (FOG waste and sewage sludge) contain substantial amounts of lipids that can serve as alternative raw materials for biodiesel production. As a recommendation for the proposed biodiesel production from wastewater lipids, further experimental investigations are needed to optimize key parameters of the transesterification reaction. This is crucial for improving the conversion efficiency. The characteristics of lipids and biodiesel varied across seasons and sites as shown in this work. Although the lipids were successfully converted to biodiesel, further characterization is essential to confirm that the lipids meet the biodiesel standards. The assessment of available material and biodiesel potential indicated significant volume of wastewater lipids can be generated from wastewater facilities in Japan. In order to facilitate the practical implementation of biodiesel from wastewater lipids, future work should prioritize the study of the economic feasibility of material collection and biodiesel production.

CONTENTS

List of Abbreviations

WWTP	Wastewater treatment plant
GI	Grease interceptor
FOG	Fats, oils, and grease
SFE	Supercritical Fluid Extraction
FB	Fat balls
SC H	Primary scum of WWTP H
PS H	Primary sludge of WWTP H
SC S	Primary scum of WWTP S
PS S	Primary sludge of WWTP S
C	Carbon
H	Hydrogen
N	Nitrogen
TS	Total solids
VS	Volatile solids
HHV	Higher heating value
LHV	Lower heating values
TG	Thermogravimetric
FTIR	Fourier transform-infrared spectroscopy
GC-MS	Gas chromatography-mass spectroscopy system
FAME	Fatty acid methyl esters
MUFA	Monounsaturated fatty acids
SFA	Saturated fatty acids
PUFA	Polyunsaturated fatty acids
UCO	Used cooking oil
DME	Dimethyl ether

Chapter 1 Introduction and Literature Review

1.1 Background

As the world's population continues to grow, a significant amount of energy will be needed to meet the energy demands of the coming decades [1,2]. In addition, environmental deterioration contributed by fossil fuel and industrial processes is required to be addressed with the shift to renewable energy sources. Several types of biofuel have been developed, including bio-ethanol, biodiesel, biogas, biomethane, etc [3]. Among them, biodiesel seems to be an attractive renewable fuel because of its adaptability to the current engine infrastructures and can be mixed with petroleum diesel [4]. However, the main challenge of the biodiesel industry is that the manufacturing costs are expensive, the vegetable oil feedstock alone accounts for 70-85% of the total production cost [5]. To improve the economics of the manufacturing process, utilizing readily available wastewater-derived lipids, such as fats, oil, and grease (FOG) [6–10], and sewage sludge [11–14] can be considered to be less expensive feedstock to be used in the production of biodiesel.

FOG has been identified as a major problem for wastewater services. FOG refers to material that consists of a mixture of animal fats, waste oil, food residues, plastic substances, soaps, and other impurities that are primarily discharged from restaurants, households, and industries [8,15]. A significant amount of FOG precursors can enter the sewer system, either by direct disposal into the sewer or due to the inefficiency of the grease interceptor (GI), which is unable to capture these lipid-rich substances from the effluent, allowing them to pass to the sewer system [6,16]. The FOG precursors are mixed throughout the water phase with other disposed solids and reach wastewater treatment plants (WWTP) [17]. It is believed that the presence of FOG causes adverse effects on WWTP operation and infrastructure, because they damage both aerobic and anaerobic treatment [7,18], and clog sludge dewatering equipment [7]. Hence, the elimination of these fatty-rich substances from influent urban sewage is performed at the initial pretreatment stage of wastewater treatments.

WWTP mainly generates sewage sludge from sewage treatment processes. Depending on their generation source, sewage sludge is categorized as primary, secondary, mixed sludge, and sewage scum. Primary sludge consists of settled and

floatable scum while secondary sludge contains suspended solids and microbial cells. Sludge lipids are derived from absorption from sewerage FOG, phospholipids of microorganisms, and microbial cells [19].

The estimated operating cost for FOG treatment was about 25% of the wastewater treatment cost [20]. An additional cost would be required to transport the sewage sludge into the landfill or incineration plant. The disposal activity not only increases the cost of treatment facilities but also can potentially cause environmental impacts on the landfill [7,20,21]. Despite their negative effect, these waste materials could contain promising lipid fractions [7,8,22]. The common utilization of wastewater residuals is limited to anaerobic digestion and composting. Therefore, extensive effort has been made to investigate the possibility of treating these waste materials as lipid sources for biodiesel production.

Understanding different types of urban wastewater residuals could help define their properties. More research is needed for further investigation of characteristics, as their quantity and properties are influenced by several factors such as weather changes, different seasons, geographical distribution, and industrial activities [3,8,20].

1.2 Current energy situation and development of biofuels

The world is witnessing a substantial surge in energy consumption driven by both the expanding world's population and economic growth [23]. The energy, economy, and environment have emerged as a multidisciplinary concern [3]. Conventional fuels, mainly crude oil, natural gas, and coal continued to be the primary source of energy, with the majority of energy being utilized for power generation, transportation, and industries [3,24]. Figure 1.1 depicts the global energy consumption by fuel in 2019. Crude oil represents 30.9%, coal for 26.8%, and natural gas for 23.2% [25]. On the contrary, biofuel and nuclear energy make up relatively modest percentages, accounting for 9.4% and 5%, respectively. Moreover, the remaining renewable energy sources harnessed from natural occurrences accounted for less than five percent. Crude oil reserves are diminishing at a yearly pace of 4 billion tons. At the present consumption rates, the conventional crude oil stocks are projected to be depleted in roughly 50 years, as shown in Figure 1.2 [26].

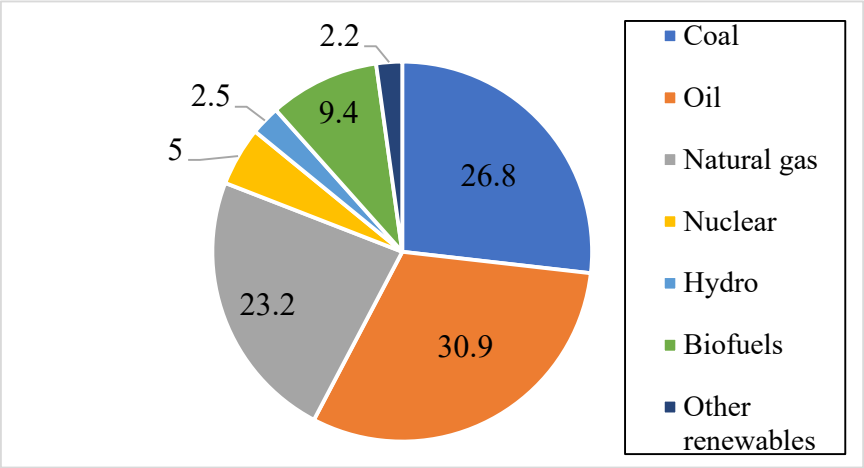

Figure 1.1. World's energy reserves for oil, coal, and gas [26].

The excessive consumption of fossil fuels and the rise of greenhouse gas emissions have intensified renewable energy development [27]. Researchers have been increasingly directing their focus toward identifying suitable alternatives to conventional fuels because renewable energy resources are expected to have a substantial impact on addressing the world's future energy needs. The energy present in the universe can be broadly classified into two main categories: renewable energy and non-renewable energy, as shown in Figure 1.3.

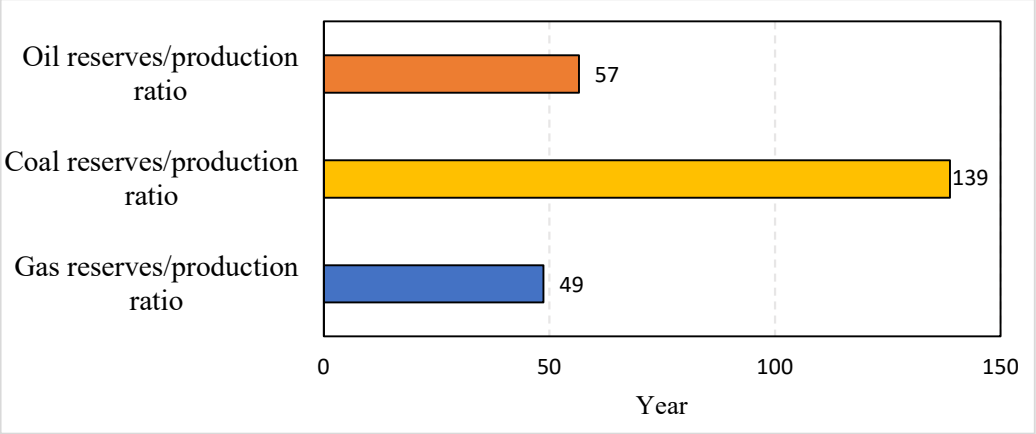

Figure 1.2 World primary energy consumption by fuel in 2019 [25].

Renewable energy encompasses both clean energy sources and bioenergy/biofuel (Figure 1.3). Biofuel holds significant importance due to its unique ability to provide liquid fuels for transportation, distinguishing it from other clean energy sources (wind energy, solar energy, hydro-energy) [28]. It ranks as the third most significant energy source globally, following oil, coal, and natural gas [26].

Biofuels can be categorized into two groups based on their utilization forms: primary and secondary biofuels [29]. Primary biofuels are derived directly from the combustion of unprocessed natural sources such as cellulosic plant matter, animal waste, and crop residue [30]. Primary fuels are mainly used for heating, cooking, or electricity production while secondary fuels are generated by processing primary fuels into the form of solid (charcoal), liquid (ethanol, biodiesel, dimethyl ether, bio-oil), or gases (biogas, syngas, and hydrogen). Secondary biofuels consist of three generations, depending on their feedstock and conversion technology (Figure 1.3). First-generation biofuels originate from edible oils or animal fats, with bioethanol and biodiesel being the main categories within this group.

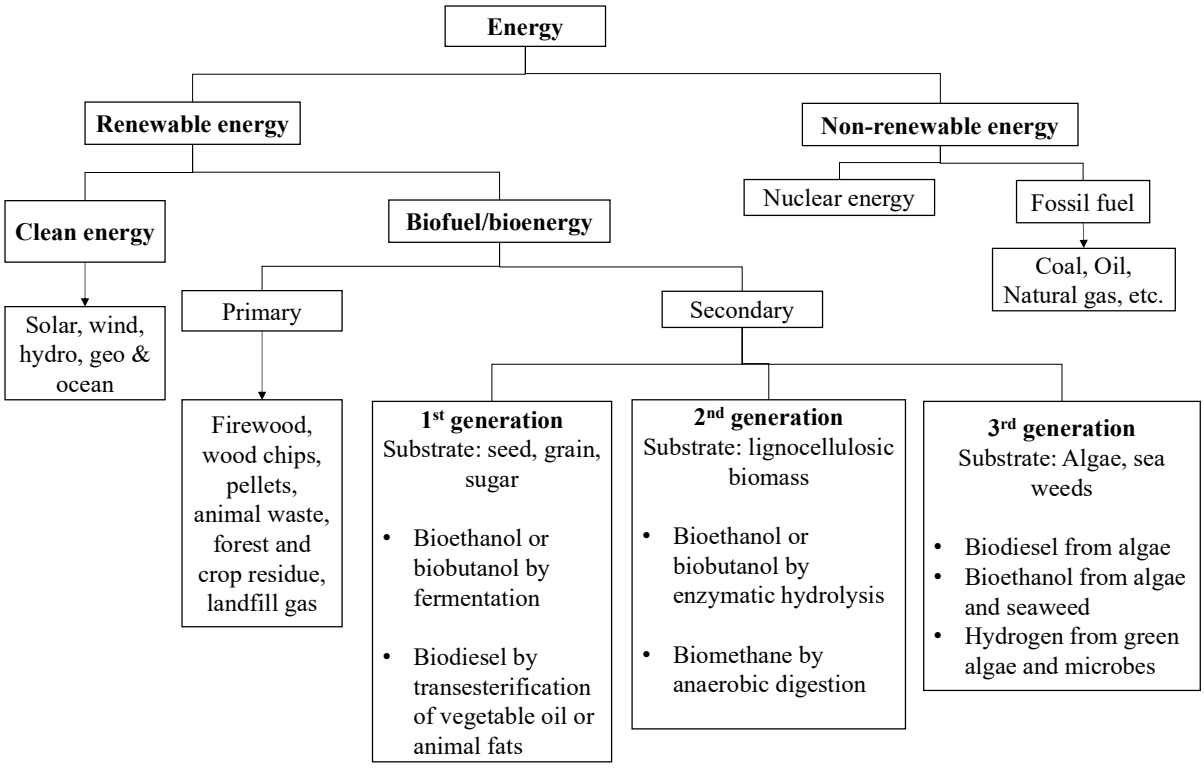

Figure 1.3. Classification of energy by source types [30,117].

The oil extraction of first-generation biofuel feedstock primarily depends on mechanical techniques (Figure 1.4). Nonetheless, due to the unsustainable nature of using edible oils, current research is shifting its focus towards the extraction of second and third-generation biofuel feedstocks. The second generation of biofuels is derived from non-edible oils. Different conventional organic solvents have been tested as extraction agents for second-generation biofuel production, including hexane, petroleum ether, chloroform, dichloromethane, and acetone [31]. Supercritical Fluid Extraction (SFE) utilizes a solvent in a supercritical fluid state, possessing physical and thermal characteristics that fall between those of a pure liquid and a gas [32]. The use of SFE was regarded as suitable for fractionating lipids in biomasses due to its excellent solvency characteristics. The most frequently employed supercritical fluids include carbon dioxide (CO_2) and water, both of which hold promise for potential use in biofuel production. Various novel extraction techniques are presently under development due to their potential efficiency in extracting biofuel feedstock. These methods include ozonation, ionic liquids, and liquefied dimethyl ether [33–35].

Extraction procedures commonly act as an intermediate stage following the cultivation process (considered upstream), and subsequently, the extracted materials typically proceed to undergo further refining steps (considered downstream) [31]. Extraction can be categorized into three basic methods:

- Mechanical techniques: traditional mechanical techniques are commonly employed in first-generation biofuels. The utilization of presses or expellers represents the oldest mechanical method for extracting oil from oleaginous materials.
- Physical techniques: this includes sonication, microwave, homogenizing, and heating.
- Chemical techniques: this approach involves the use of a variety of solvents to separate the desired extractable component from feedstocks. Physical techniques were often combined to aid the chemical extraction process.

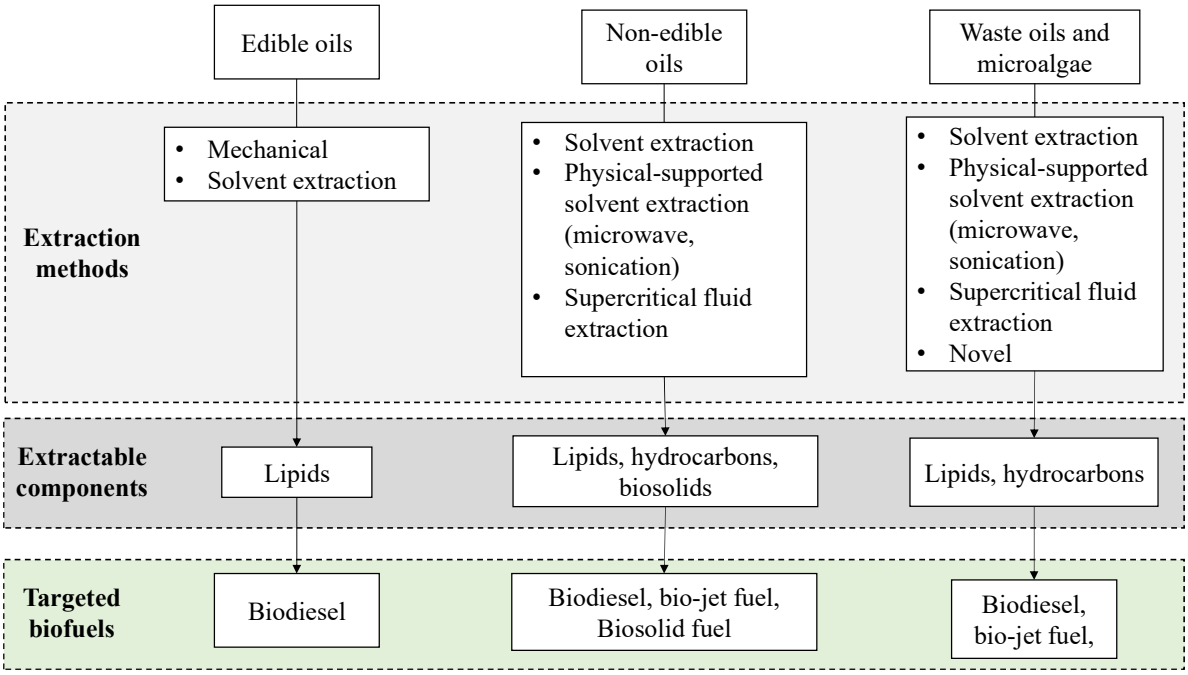

Figure 1.4 Relationships between different biofuel feedstocks with the classified extraction methods along with targeted biofuels [31].

The demand for biofuels is anticipated to rise, driven by advancements in transportation modes in some countries and by domestic policies favoring higher blend ratios [36]. Fuel consumption in countries like Brazil, Argentina, Colombia, Paraguay, and Indonesia is set to increase, leading to a corresponding rise in the use of ethanol and biodiesel. Among liquid biofuels, biodiesel is attracting global interest as a viable substitute for fossil diesel due to the advantages associated with lower emission contents, higher lubricity, and cetane values [37]. This liquid biofuel can be blended with petroleum diesel to decrease reliance on conventional fuels entirely and encourage the adoption of renewable energy sources. The designation B100 refers to a fuel composed entirely of FAME (Fatty Acid Methyl Ester), whereas lower percentages, like B20, are categorized as biodiesel blends [38]. In South and Southeast Asian nations, biodiesel is expected to grow significantly due to the demand from transportation and industrial sectors [36].

1.3 Overview of biodiesel

The majority of global biodiesel production (approximately 95%) is commercially reliant on the use of edible oils (soybean oil, sunflower oil, rapeseed oil, palm oil) [39]. These oils are predominantly utilized in various countries, including the United States of America, Argentina, Brazil, various European nations, Malaysia, and Indonesia (Table 1.1). However, the use of edible feedstocks is influenced by some concerns, including availability, sustainable land use, and ecological imbalances [40]. Furthermore, the expenses associated with using edible oils as fuel feedstock are prohibitively high. In present circumstances, the expense associated with the utilization of plant oils comprises approximately 60–80% of the overall production cost of biodiesel [39]. As a result, it is crucial to intensify a wide variety of biodiesel feedstock options, with particular attention focused on inedible oils, waste oil, and urban wastewater lipids.

Table 1.1 Biodiesel production ranking and major feedstock [36].

Country	Production ranking	Major feedstock
United States	2 (18.3%)	Used cooking oil, soybean oil
European Union	1 (32.2%)	Rapeseed oil/Palm oil/used cooking oils
Brazil	4 (12.3%)	Soybean oil
China	5 (3.6%)	Used cooking oil
India	15 (0.4%)	Used cooking oil
Canada	12 (0.7%)	Canola/used cooking oil
Indonesia	3 (17.6%)	Palm oil
Argentina	6 (3.3%)	Soybean oil
Thailand	7 (3.0%)	Palm oil
Colombia	10 (1.2%)	Palm oil
Paraguay	17 (0.02%)	Soybean oil

A broad spectrum of prospective sources is being assessed as a potential feedstock for biodiesel production. They can be categorized into various groups, including edible oils, inedible oils, waste oils and animal fats, and other lipid sources, as shown in Figure 1.5. Numerous lipid-rich raw materials have been identified as having significant potential for biodiesel production. However, several prerequisites must be met before selecting alternative lipid sources for biodiesel production. These include assessing the economic viability of the biodiesel production process, evaluating the technical feasibility of converting the raw material into biodiesel, and considering its performance in diesel engines during the combustion process [41].

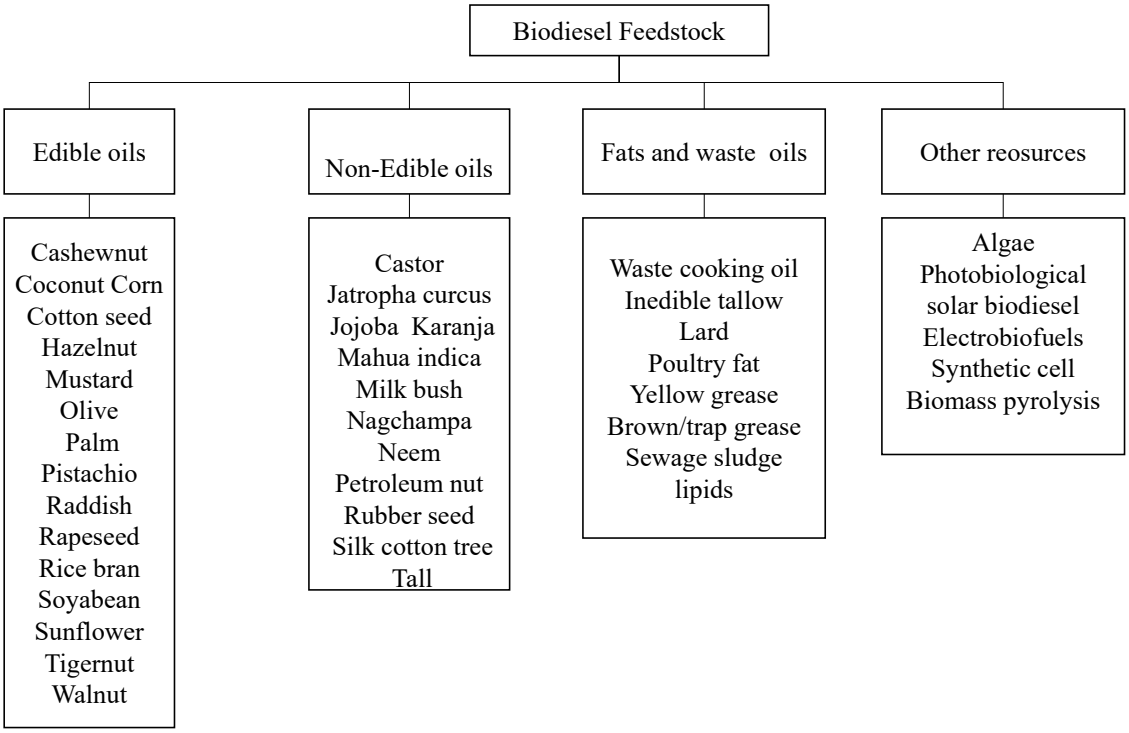

Figure 1.5 Biodiesel feedstocks based on different generations [38,42].

Non-edible plant oils have raised significant interest as a promising feedstock due to their abundant oil content, widespread availability, and ease of cultivation. Moreover, cultivating non-edible oil plants is less reliant on local weather conditions and demands fewer resources, which can significantly reduce cultivation costs [43].

Waste oils can be categorized into three groups: waste oils originating from the food industry, those from non-food industries, and those generated in households and restaurants. Animal fats are a lipid material derived from slaughtered animals during animal processing that converts waste animal tissue into value-added products. The animal fats consist of edible and inedible tallows, choice white grease from swine processing, and poultry fat from chicken rendering and processing [41,44].

Microalgae holds the potential to become the future source of biodiesel [45]. Currently, research is underway to improve both the biodiesel production rate from algal biomass and the extraction process. Regarding alternative biodiesel sources like photobiological solar, electro biofuels, and synthetic cells, they are currently in the initial phases of fundamental research and development. Table 1.2 shows the benefits and limitations of each group of biodiesel feedstocks.

Table 1.2 Benefits and limitations of alternative fuel options for different types of feedstocks [38].

	Edible Oils	**Non-edible oils**	**Waste oils and microalgae**	**Other resources**
Benefits	Easy biodiesel conversion process, less impurity	No effect on food supply, ease of cultivation on non-arable land, less production cost	Minimize disposal of oil waste, high crop yield for algae	High lipid content, rapid growth rate
Limitations	Affects the food chain, low crop, limited cultivation area, price volatility	High free fatty acids level, higher cost for conversion technology, low crop yield	Higher energy consumption for algae cultivation, less cost-effective in conversion technology	High investment

1.4 Urban wastewater lipids as an alternative biodiesel feedstock

Waste oil/lipids are often referred to as FOG and are generally classified into three major categories [9]:

- Yellow grease is derived from a mixture of waste oil (vegetable oil and animal fats) that has been heated and used during cooking operations [46]. Overheating of frying oils degraded the oil quality, resulting in oil oxidation and free fatty acids (FFA) formation, with the FFA content less than 15% [46–48].
- Yellow grease that is poured down a kitchen sink becomes brown grease, as it has commingled with wastewater or greywater in drainage systems. Grease traps/interceptors are designed to prevent the FOG from the effluent and separate it from the sewage before entering the sanitary sewer lines [46]. Grease traps must be maintained periodically, by collecting the grease and oil from the top of the container. After collection, the grease haulers send trap grease to a WWTP for dewatering and send it to a landfill.
- Brown grease is the oily material or lipid fraction captured in grease traps either from restaurants, food processing plants, or wastewater treatment plants [49,50]. Brown grease is typically composed of FOG as well as water, food particles, trash, and biosolids. About 80% of the lipids of brown grease are FFA, with

contents ranging from 15–100% [51]. Brown grease can be liquid or form solid particles, with a melting point between 35°C-45°C, and the share of usable brown grease from grease trap waste would be around 1-7 % [17,52].

Wastewater lipids consist of three major groups including FFA, phospholipids (cellular lipids), and waxes. Phospholipids are present in the waste oil which are derived from organisms [42,53]. Wax is considered an unsaponifiable fraction that originated from both vegetable and animal sources [1]. FFA are derived from triglycerides, as shown in Figure 1.6. When waste oil (FOG precursors) is released into the water, a hydrolysis reaction occurs in which a substantial fraction of triglyceride molecules are cleaved into glycerol and FFA.

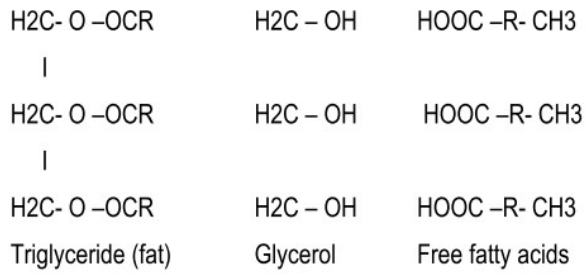

Figure 1.6 Molecular structures of triglyceride, fatty acid and glycerol [17].

FFA consists of several substances, such as carbon, hydrogen, and oxygen arranged in a sequential carbon series with a carboxyl group. The chain length, degree of saturation, and configuration play an important role in the properties of fatty acids. The chemical structure of the most common fatty acids is 16-18 as the number of carbon chain length. Saturated fatty acids of simple forms, having only single C-C bonds without any double bonds. These types of fatty acids are present in animal fat, coconut oil, palm oil, whole milk, and butter. Unsaturated fatty acids contain both single as well as one or more double bonds C=C. These are mostly found in vegetable oils, plants, avocados, and fish oil. Based on their double bonds (C=C), they are divided into two types: monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) [54].

There is a significant variation in the fatty acid profile of waste lipids compared to edible feedstocks. Table 1.3 presents the fatty acid profile of different FOGs in comparison with other edible feedstocks. The profile and concentration of total fatty acids largely depend upon the sources generating FOG [3]. In general, palmitic acid (saturated), oleic acid (monounsaturated), and linoleic acid (polyunsaturated) show dominant proportions in both edible and waste oil sources.

Table 1.3 Fatty acid profile of different types of FOG in comparison to the common edible feedstocks.

Fatty acids	Yellow grease	Sewer grease/ FOG					Edible feedstock	
		restaurant grease	Trap grease	Trap grease	WWTP scum	WWTP Scum	Corn	Soybean
	Weight % fatty acid							
C14:0	1.4	1.7	0.2	1.3	5.63	4	0.2	ND
C16:0	37.5	22.8	10.92	38.3	32.45	37.3	13	11.6
C16:1	3.1	3.1	0.44	1.2	2.27	4	ND	0.3
C18:0	4.8	12.5	5.05	7.2	15.59	9.5	2.5	4.2
C18:1	36.3	42.4	33.47	36.9	41.35	30.1	30.5	21.6
C18:2	15.2	12.1	42.64	15.1		15.1	52.1	53.7
C18:3	ND	0.8	4.9	ND	ND	ND	1	7.5
C20:0	ND	ND	0.35	ND	ND	ND	0.5	0.8
C20:1	ND	ND	0.45	ND	ND	ND	0.2	0.3
C22:0	ND	ND	0.37	ND	ND	ND	ND	ND
Others	1.7	4.6	0.74	0	2.7	ND	0	0
SFAs	43.7	37	ND	46.8	53.67	ND	16.2	16.6
MUFAs	39.4	45.5	ND	38.1	ND	ND	30.7	22.2
PUFAs	15.2	12.9	ND	15.1	ND	ND	53.1	61.2
Ref.	[55]	[46]	[46]	[55]	[8]	[7]	[56]	[56]

ND: No Data.

1.5 Fats, oils, and greases (FOG) in sewer systems

Daily, a large volume of FOG is diverted into the sewer system from various sources. These FOG substances are found in the form of oily liquids and solid deposit that accumulates at different point such as pumping stations, sewer pipes, and WWTP [57]. FOG has been identified as one of the major contributors to sewer blockages in many wastewater collection systems that lead to sanitary sewer overflows (SSO). SSO can potentially release a high concentration of pathogens, nutrients, and solids that impose a risk to public health and the environment[58]. Several researchers have investigated FOG deposition based on the local conditions of sewers and lifestyles [59]. In general, FOGs in sewer systems are discharged from three main sources: domestic, commercial, and industrial [60].

Domestic sources are a significant contributor to FOG in the sewer system. The gray water composition that is discharged from domestic sources depends on the type of appliance and residential habits of the individual household. Problems with FOG blockages were particularly severe in sewers serving high-rise apartment buildings [59]. Commercial sources such as restaurants, hotels, fast food services, and convenience stores appear to be major contributors to FOG in the sewer. A large number of full-service and fast-food restaurants are being built both in large cities and rural communities. Restaurant wastewater discharges many oils and greases, suspended solids, and detergents. Uncontrolled disposal of this wastewater could release a large flow of FOG into the sewer system [61]. In the United States, Asian restaurants are identified as the major contributor of FOG, followed by seafood restaurants, and take-away establishments [62]. According to the Helsinki Region Environmental Services Authority, the total amount of sewer grease generated in 2013 was 40 tons that were collected from the restaurant in Helsinki [17]. Industrial sources, mainly food and beverage manufacturers and rendering plants are considered potential producers of wastewater with high FOG content [22,63,64]. Advanced removal techniques, such as primary sedimentation, dissolved air flotation units, and FOG screens, are applied at industrial food processing facilities [22].

1.5.1 Formation of FOG deposit in a sewer system

The formation of FOG deposits in sewers primarily originates from a saponification reaction involving lipids with the metal substances and an acid crystallization reaction [65–67]. Four major components contribute to FOG deposit formation on sewer pipe walls: calcium, free fatty acids (FFAs), FOG (or oil), and water [58]. The excess calcium forming aggregates with unreacted FFA, and other impurities in sewer pipe walls [58,65,68]. A study by Keener et al. (2008) characterized the chemical and physical properties of FOG deposits from 23 cities around the United States. The results found that high concentrations of calcium and saturated fatty acids existed in FOG deposits. Benecke et al. (2017) found that fatty acids were the predominant species in FOG deposits. Saturated fatty acids were generated from the cooking oil activities that were discharged into sewer pipelines [46]. Calcium is either naturally present in wastewater or released upstream from highly corrosive environments [58]. Figure 1.7 shows the mechanism for FOG deposit formation which occurred due to the combination of saponification and aggregation in sewer pipe walls.

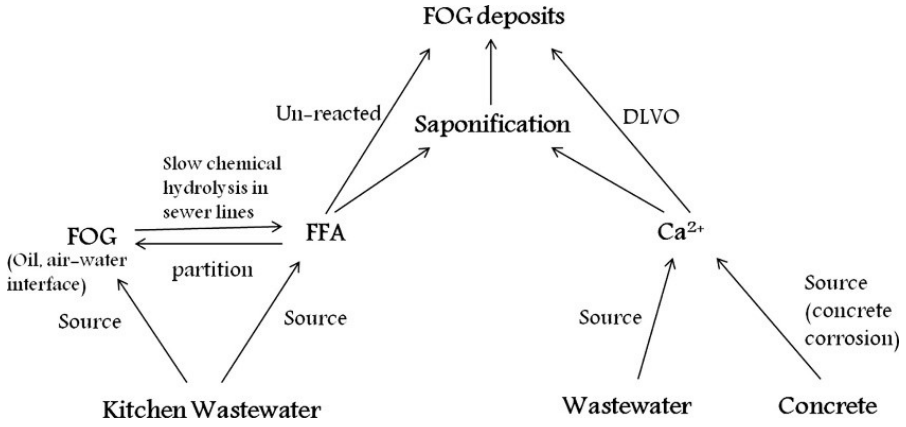

Figure 1.7 Mechanism of FOG deposit formation in sewer lines [68]. Note: Derjaguin, Landau, Verwey and Overbeek (DLVO) is theory of colloidal stability based on interactions between colloidal particles.

1.5.2 FOG generation in several countries

Table 1.4 illustrates the generation amounts from the literature for FOG from several countries.

Table 1.4 FOG generation amounts from various countries.

Sources	FOG generation percapita (kg/person/year)	Material potential								Ref.
		Brown grease		Yellow grease		Animal fats		Total		
		1000t/y	Tg/y	1000t/y	Tg/y	1000t/y	Tg/y	1000t/y	Tg/y	
United states (US)	4 (yellow grease) 6 (brown grease)	1500	1.5	1000	1	2800	2.8	5300	5.3	[44,49]
US	4 (yellow grease), 6 (brown grease)	1500	1.5	990	0.99	2880	2.88	5370	5.37	[70]
Finland	6.6 (brown grease)	36	0.04	N.D.	N.D.	N.D.	N.D.	36	0.04	[17]
Hongkong	N.D.	170	0.17	20	0.02	N.D.	N.D.	190	0.19	[61]
Norway	N.D.	8.2	0.01	N.D.	N.D.	124	0.12	132	0.13	[64]
United Kingdom (UK)	6.4 (brown grease)	189	0.19	N.D.	N.D.	N.D.	N.D.	189	0.19	[57]
South Carolina, USA	0.0545 (brown grease)	2.55	0.002	N.D.	N.D.	N.D.	N.D.	2.55	0.002	[71]

t/y: tonnes/year
Tg/y: Teragram/year
N.D.: No Data

1.5.2.1 Estimation of FOG in USA

It is estimated that more than 5.3 Tg of FOG (wet base) was produced in the US in 2012 [44,70]. The estimates of yellow and brown grease generation in the United States are based on population data for 2010 and per capita grease generation factors derived from Wiltsee (1998). Based on the report by Wiltsee (1998), FOG generation per capita of brown grease is 6 kg/person/year whereas the generation per capita of yellow grease is about 4 kg/person/year. The estimate for animal fats is based on 2012 state-level animal (cattle/calves, hogs, chickens, and turkeys) slaughter data obtained from the U.S. Department of Agriculture (USDA).

1.5.2.2 Estimation of FOG at catchment level in UK

Collin et al., (2020) observed several FOG samples collected from different locations including collected from households, food service establishments (FSEs), sewage pumping stations, sewers, and sewage treatment works (STWs). The results showed that around 94,730 tonnes/year (on a wet basis) of FOG materials could be recovered from the Thames Water Utilities' catchment, one of the most populated in the UK. Most of the FOG waste was produced by FSEs with an estimated average of 79,810 tonnes/year (on a wet basis) compared to 14,920 tonnes/year (on a wet basis) from private households. Furthermore, these materials could produce up to 221 GWh/year as biogas.

1.5.2.3 Estimation of grease trap waste (GTW) in Finland

The regional grease trap waste availability was evaluated by monitoring the amounts of received GTW during one year at two waste centers in Central and Northern Finland. GTW was mainly derived from restaurants and school kitchens (36%) and the food production industry (16%). Other sources were catering at industrial and retail stores and private properties.

In the Northern area, no GTW was received from the local municipal WWTP, as they mixed trapped grease waste onsite with dewatered sludge for composting. Researchers have found that in 2009, the annually collected amount of GTW from local grease traps and WWTPs was 6.6 kg per capita, with a total population of 367,500 inhabitants [17]. The quantity of annually collected GTW (6.6. kg per capita) correlates with the estimation from the Urban Waste Grease Resource Assessment (UWGRA) made in 1998 by Wiltsee (1998). Based on this estimation, approximately 36,000 brown grease is generated in Finland (with 5.4 million people).

1.5.2.4 Estimation of grease trap waste in Norway

The upscaling of the biodiesel potential based on the utilization of fat residue and grease trap waste in Norway was estimated by gathering information from three metropolitan districts (Bergen, Oslo, and Trondheim) and correlated with national population data. Researchers have estimated that around 8,200 tonnes of FOG could be collected from grease traps with a ratio of 1.62 tonnes per 1000 inhabitants on average. This FOG is not purified and contains water. By applying a yield of 95 % this could give 7,800 tonnes purified animal fat, which could result in 6400 tonnes biodiesel. The maximum available fat and oil from by-products of fish, slaughterhouses, and the poultry industry in Norway was approximately 124,000 tonnes.

1.6 Sewage sludge

Sewage sludge is a byproduct generated during the treatment of sewage in wastewater treatment plants which consists of complex organic and inorganic matters originating from wastewater [72]. This sludge must be disposed of in an environmentally safe manner. To improve the quality of effluent water, physical and biological transformations are employed during the treatment of wastewater. In the process of wastewater treatment (Figure 1.8), the pretreatment step involves the removal of debris and grit from the incoming wastewater. During the primary treatment phase, suspended solids settle out, generating what is known as raw primary sludge. Chemical flocculants are often utilized to improve the efficiency of the solid settling process. Primary sludge composed of undegraded organic matter with total solid (TS) concentration and volatile suspended solid (VSS) ranges from 2-7% and 60-85%, respectively [70,73].

Secondary treatment focuses on treating the additional dissolved organic matter using biological treatment, resulting in the production of secondary/ waste-activated sludge. The wastewater contains various organic substances, including detergents, pesticides, fats, oils, grease, dyes, solvents, phenols, etc. The TS and VSS of secondary sludge generally range from 0.5-1.5% and 70-80%, respectively [70].

The sludge from primary and secondary treatment can also be combined into a single waste stream, which can then be dewatered to reach an appropriate concentration. In addition, advanced wastewater treatment methods are implemented to eliminate nutrients or to disinfect the effluent. The subsequent treatment and stabilization of

sewage sludge involve reducing moisture content through thickening, drying, or dewatering, followed by the stabilization of organic matter through methods such as composting, digestion, or heat treatment [74].

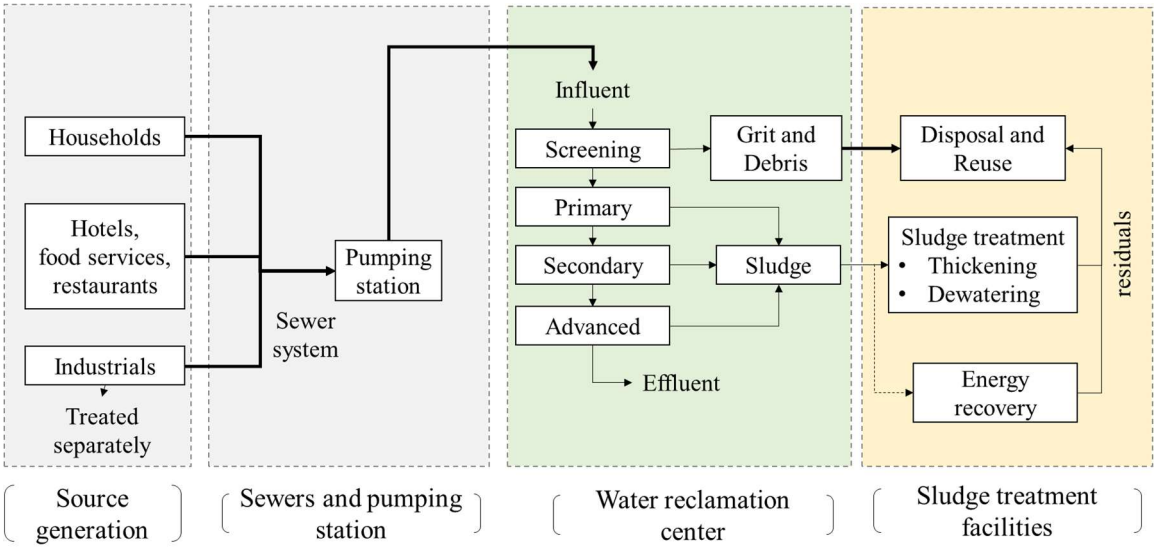

Figure 1.8 Sewerage system components.

1.6.1 Sewage Scum

Sewage scum is collected from the surface layer of clarifiers in wastewater treatment plants [75]. Around 60% of FOG enter the sewer systems are end up in WWTP in the form of scum [8]. Owing to their lower density than water, the scum floats on the surface of the primary and secondary clarifiers. In some wastewater treatments, the collected trap grease and sewage scum from urban wastewater are combined as sewer grease [47,76]. To avoid negative impacts on the WWTP operation, sewage scum is practically separated at the beginning of the wastewater treatment process and most of it is discarded in landfills. The scum disposal not only increases the expenses of treatment facilities but also causes a detrimental effect on the environment [1].

1.6.2 Sewage sludge production and management in Japan

Figure 1.9 presents the sewage sludge production and treatment in Japan. Currently, around 2,400 wastewater treatment plants are operated in Japan, which can generate >2,000 tons (dry basis) of sewage sludge and the recovery ratio is about 70% [77]. Several options for treating the sewage sludge include landfill, agricultural/land use, construction materials, and fuel production. As sewage sludge approximately consists of organic matter (80%), it has been practically used for energy production by incineration (73%), anaerobic digestion (16%), and composting (<10%).

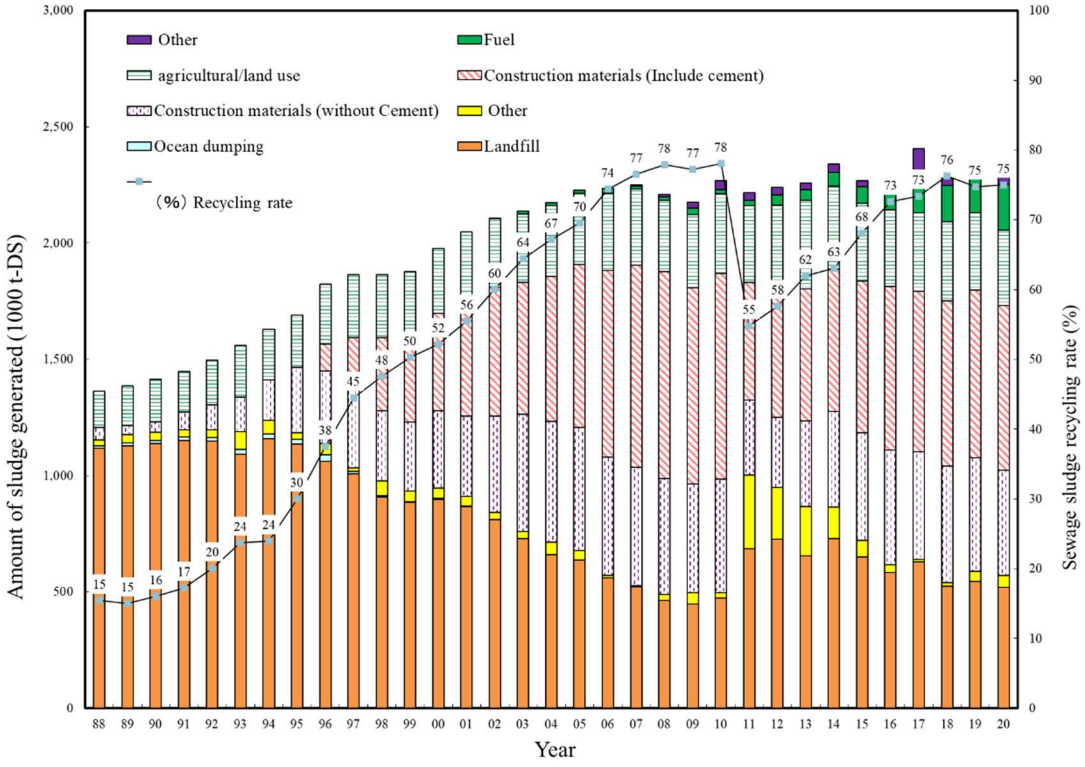

Figure 1.9 Sewage sludge generation and treatment in Japan [77].

1.7 Technology for biodiesel production

Figure 1.10 shows various existing methods for biodiesel conversion. Currently, several technologies have been developed for converting oil feedstock to biodiesel, these include cracking/pyrolysis, dilution, micro-emulsification, and transesterification [2,45,78].

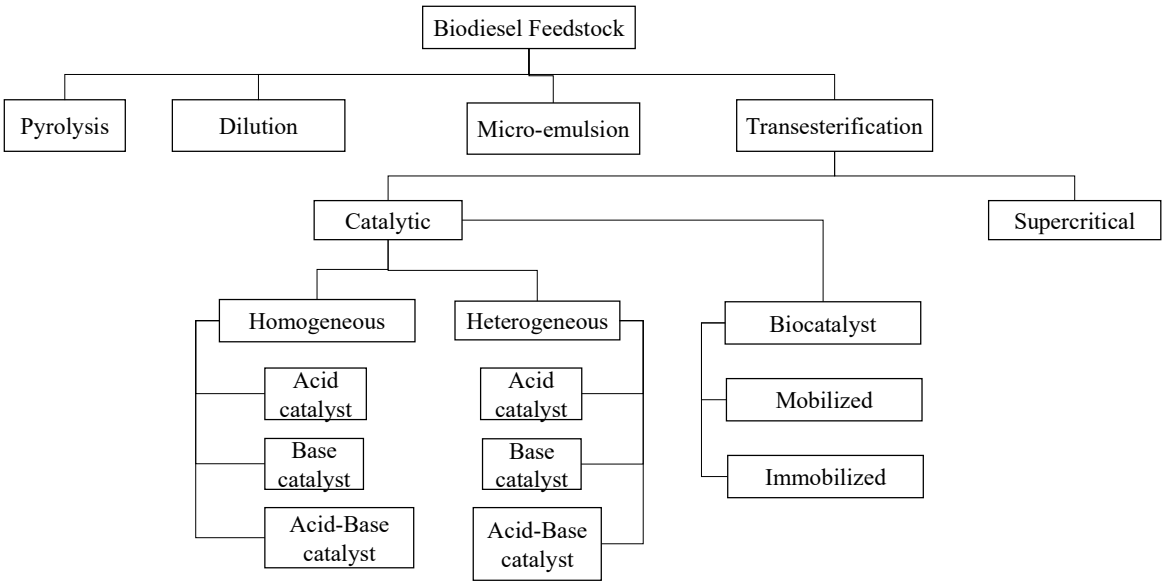

Figure 1.10 Methods for biodiesel production [39,78]

Table 1.5 presents a comparison of biodiesel technologies. Pyrolysis refers to a thermochemical process for the decomposition of organic substances at a high temperature (300−650 °C), in an oxygen-free environment. From the pyrolysis process, it will generate pyrolysis oil, gas products, and char [79]. Vegetable oil can be blended with petroleum diesel to reduce viscosity without adding any chemicals. It has been reported that blending 10-25% vegetable oil with diesel fuel is suitable for diesel engines [80]. Micro-emulsification of vegetable oils with aqueous phases (methanol, ethanol, butanol, ionic liquids, etc.) and surfactants were reported as one of the options to reduce the high viscosity of vegetable oils [37,81].

Although dilution and microemulsion offer simple processes, the processed oils still have high viscosity and low cetane numbers when used as fuel in diesel engines [19]. The pyrolysis method requires a high capital cost for equipment installation, and upgrading process of produced oil, which is not considered as efficient. Finally, transesterification/esterification appears as the most attractive method and widely available technique for the production of biodiesel. This technology has several advantages compared to other methods, such as cost-effectiveness, simplicity, lower energy consumption, and high conversion efficiency.

Table 1.5 Comparison of main biodiesel preparation technologies [1,19,82].

Technologies	Advantages	Disadvantages
Dilution or micro-emulsion	simple process	high viscosity, poor volatility, poor stability
Pyrolysis	simple process, non-polluting	require high temperature, expensive equipment, low purity
Transesterification	fuel properties are closer to diesel, high conversion efficiency, low cost, suitable for industrialized production	low free fatty acid and water content required for using a homogeneous base catalyst, inefficient during the purification step, the possibility of side reaction

The transesterification reaction is the current preferred method for biodiesel conversion [83]. This reaction involves the conversion of oil feedstock into biodiesel in the presence of alcohol and catalyst. Methanol is the predominant alcohol utilized in the production of biodiesel, where it facilitates the creation of methyl esters (main product) from triglycerides of oil feedstocks via transesterification [84]. The basic transesterification reaction is illustrated in Figure 1.11. During the transesterification process, glycerol (glycerin) is typically produced as a by-product.

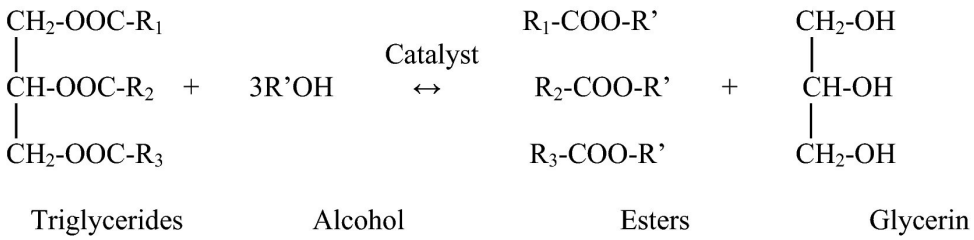

Figure 1.11 General transesterification reaction of vegetable oils [98,118]

The transesterification reaction can be optimized by adjusting several parameters, including reaction temperature, catalyst ratio, alcohol/oil molar ratio, stirring effect, etc. The catalyst increases the reaction rate of the transesterification and also enhances the solubility of alcohol. Transesterification can be catalyzed by homogeneous catalysts (acids and alkali) and heterogeneous catalysts [78]. Table 1.6 presents a comparison between homogeneous and heterogeneous catalysts.

Table 1.6 Comparison of homogenously and heterogeneously catalyzed transesterification [85].

Factors	Homogeneous catalyst	Heterogeneous catalyst
Reaction rate	fast, (high conversion for alkali catalyst)	moderate conversion
Post-treatment	require chemicals for a neutralization step	catalyst can be separated easily
Presence of water/free fatty acids	sensitive (for alkali catalyst)	not sensitive
Catalyst reusability	catalyst cannot be recovered	possible
Cost	Comparatively costly	potentially cheaper

The transesterification process employing homogeneous alkali catalysts including sodium hydroxide (NaOH) and potassium hydroxide (KOH) has gained widespread popularity due to their strong reactivity, cost-effectiveness, and ready availability. However, these catalysts may not be suitable for feedstocks with elevated levels of free fatty acids (FFA), such as lipids derived from urban wastewater [39]. This is because they can lead to soap formation between FFA and homogeneous base catalysts, resulting in reduced biodiesel production yields and a more intricate purification process. The base-catalyzed reaction is better in conversion performance than the acid-catalyzed reaction, but the yield of biodiesel is lowered due to the formation of soap. In addition to this, the separation of biodiesel from glycerol is quite difficult.

1.8 Extraction of lipids from urban wastewater residuals

Table 1.7 summarizes the lipid extraction process of urban wastewater lipids. The general information about the extraction methods has been described in Section 1.2. In general, solvent extraction is commonly used for extracting lipid from sewage sludge. Some pretreatment can be employed before the extraction process, such as gravity-settling, sonication, mechanical disintegration, freeze-dried, and acidification [86]. On the other side, the separation of lipids from FOG can be conducted by heating and filtration techniques [6,87].

Table 1.7 Extraction of lipids from municipal wastewater lipid sources.

Feedstock	Methods	Solvent	Lipid yield, wt%	Ref.
Primary sludge	solvent extraction (solid-liquid extraction)	hexane	26.3	[88]
Primary sludge	solvent extraction (liquid-liquid extraction)		25.2	
Secondary sludge			7.7	
Blended sludge (65% primary sludge and 35% secondary sludge)			21.1	
Primary sludge	solvent extraction (liquid-liquid extraction)	hexane and methanol	7.5-14	[89]
Primary sludge	solvent extraction (solid-liquid extraction)	hexane and methanol	58.9	[90]
Secondary sludge			51.22	
Secondary sludge	solvent extraction (solid-liquid extraction)	hexane	1.94	[53]
	supercritical carbon dioxide (SC-CO_2)		3.55	
	supercritical carbon dioxide (SC-CO_2)	methanol addition (1.96 wt.%)	4.19	
	supercritical carbon dioxide (SC-CO_2)	methanol addition (13.04 wt.%)	13.7	
Primary sludge	solvent extraction (liquid-liquid extraction)	hexane	0.48	[91]
Secondary sludge			0.48	
Mixed sludge			0.51	
Secondary sludge	solvent extraction (solid-liquid extraction)	chloroform-methanol (1:1 v/v)	95.3	[92]
		methanol	62	
		hexane	35.3	
		water	11.8	
Primary sludge	solvent extraction (solid-liquid extraction)	methanol:hexane (80:20)	27	[7]
Secondary sludge			16.9	
Primary scum			31.5	
Primary sludge	solvent extraction (liquid-liquid extraction)	hexane	21.2-24.4	[10]
Primary scum			37.9-46.9	

Table 1.7 Extraction of lipids from municipal wastewater lipid sources (cont.).

Feedstock	Methods	Solvent	Lipid yield, wt%	Ref.
Sewage sludge	solvent extraction (liquid-liquid extraction)	hexane	11.2	[57]
Sewage scum			13.7	
Secondary sludge	solvent extraction (solid-liquid extraction)	hexane	0.94-1.03	[93]
Waste activated scum		hexane	1.75	
Primary sludge	solvent extraction (liquid-liquid extraction)	isopropyl alcohol - cyclohexane (1.6:2)	34.5	[94]
Primary sludge	solvent extraction (liquid-liquid extraction)	ethyl butyrate	93.7	[95]
Sewage scum	filtering-acid washing	-	90	[8]
FOG from Food services establishment	solvent extraction (liquid-liquid extraction)	hexane	100	[57]
Fatberg			93.1	
FOG deposit from Pumping station			93.1	
FOG deposit in wastewater treatment plant			94.5	
Restaurant grease trap waste	heating	-	81-93	[6]
Restaurant grease trap waste	heating	-	0-98.3	[87]
Undigested sludge	liquefied DME extraction	DME	1.5-2.7	[96]

Table 1.7 Extraction of lipids from municipal wastewater lipid sources (cont.).

Feedstock	Methods	Solvent	Lipid yield, wt%	Ref.
Primary sludge	solvent extraction (solid-liquid extraction)	hexane	25.3	[11]
Secondary sludge			9.3	
Blended sludge (65% primary sludge and 35% secondary sludge)			21.9	
Stabilized sludge			10.1	
Sewage sludge	solvent extraction (solid-liquid extraction)	hexane, methanol, ethanol	62.5	[97]
Primary sludge	solvent extraction (solid-liquid extraction)	hexane	27	[86]
Secondary sludge			9	
Blended sludge (65% primary sludge and 35% secondary sludge)			21	
Stabilized sludge			9	
Primary sludge	solvent extraction with acidification (solid-liquid extraction)	hexane	25	[86]
Secondary sludge			7	
Blended sludge (65% primary sludge and 35% secondary sludge)			20	
Stabilized sludge			10	

1.8.1 Biodiesel production from waste lipids

According to the recent studies summarized in Table 1.8, the homogeneous acid catalyst is predominantly used for the conversion of wastewater lipids to biodiesel. Typically, non-edible oils or waste lipids contain higher FFA levels (>5%) when compared to refined oils. Consequently, it becomes necessary to initiate an esterification reaction for the free fatty acids (FFA). The FFA is subjected to a reaction with alcohol in the presence of a catalyst to yield fatty acid methyl esters. Sulfuric acid (H_2SO_4) exhibited high catalytic performance in the esterification reaction. The primary benefit of the esterification process catalyzed by acid is that it permits the utilization of

low-quality feedstocks with relatively high FFA and water content. However, the esterification process using an acid catalyst is considerably slower (approximately 4000 times) than an alkali catalyst [98]. A higher conversion could be achieved by increasing the reaction temperature and the reaction time.

Table 1.8 Summary of recent biodiesel production from urban wastewater lipids.

Raw materials	**Catalyst/ concentration**	**Time, h**	**Temp., °C**	**Methanol-to-sludge mass ratio**	**Yield, wt%**	**Ref.**
Sewage sludge	KOH-activated carbon/55.5 %	8	60	10	6.8	[99]
	KOH-CaO/6%	6	60	10	6	
	KOH/30%	6	60	10	1.2	
Anaerobic anoxic sludge	H_2SO_4/5% v/v	8	60	10	16.6	[100]
Membrane bioreactor sludge	H_2SO_4/5% v/v	8	50	8	4.2	
Secondary sludge	H_2SO_4/1%	12	50	2	6.23	[53]
Primary sludge	H_2SO_4/5%	24	75	12	14.5	[12]
Secondary sludge					2.5	
Wastewater sludge	SBA-15/15%	3	135	100 mL methanol:0.5 g lipid	30.14	[90]
Primary sludge	H_2SO_4/2%	24	60	10 mL methanol:0.2 g lipid	38-41	[101]
Secondary sludge					26-30	
Mixed sludge	H_2SO_4	8	105	300 mL/150g	8.12	[102]

Table 1.8 Summary of recent biodiesel production from urban wastewater lipids (cont.).

Raw materials	Catalyst/ concentration	Time, h	Temp., °C	Methanol-to-sludge mass ratio	Yield, wt%	Ref.
Primary sludge	H_2SO_4/1%	12	50	2 mL/0.02g lipid	13.9	[11]
Secondary sludge					1.0	
Blended sludge (65% primary sludge and 35% secondary sludge)					10.9	
Stabilized sludge					2.9	
Greasy sewage sludge	H_2SO_4 (7%), Novozym 435 (10%)	24	40	H_2SO_4:20, Novozym:4	57-61	[103]
Primary sludge	Zr-SBA-15/12.5%	3	209	10-20	15.5	[89]
Secondary sludge					10	
Blended sewage sludge	H_2SO_4/0.2%	8	70	20	3.1	[104]
Primary sludge	Bronsted ionic liquids/7%	5	100	1	90% (based on lipids)	[105]
Primary and secondary sludge	H_2SO_4	0.5	80	15 mg lipid and 4.5 mL methanol	34.5	[94]
Primary and secondary sludge	H_2SO_4/1%	12	50	20 mg lipid and 2 mL of 1% H_2SO_4	13.7	[106]
Scum sludge	H_2SO_4/5%	24	55	H_2SO_4 in methanol (5% v/v)	22.7	[7]
Primary sludge					9.0	
Secondary sludge					1.9	

Table 1.8 Summary of recent biodiesel production from urban wastewater lipids (cont.).

Raw materials	Catalyst/ concentration	Time, h	Temp., °C	Methanol-to-sludge mass ratio	Yield, wt%	Ref.
Mixed of primary and secondary sludge	SO_4^{2-}-Al_2O_3-SnO2/8%	4	130	128 mL methanol	73.3	[107]
Activated sludge	H_2SO_4/4%	24	55	30	4.79	[108]
Activated sludge	H_2SO_4/10%	24	75	30	3.93	[109]
Activated sludge	subcritical methanol-acetic acid/15%	1.5	250	5	30.11	[110]
Greasy sludge	H_2SO_4	8	60	15	63.9	[111]
Secondary sludge	Novozym 435	16		4	58.7	
Blended sewage sludge	H_2SO_4/0.7%	4	60	10	3.1	[112]
Secondary sludge	H_2SO_4/5%	1	55	H_2SO_4 in methanol (5 % v/v)	17	[93]
Sewage sludge	HZSM-5	3.23	285 (pyrolysis)	2.5	67.2	[113]

1.9 Summary

Global energy security and environmental degradation due to the use of fossil fuels have created an urgency to investigate renewable feedstocks for biofuels [3]. Among liquid biofuels, biodiesel is gaining interest as a viable substitute for petroleum diesel due to the benefits associated with lower emission, better lubricity, and combustibility [114]. Although biodiesel is an attractive renewable fuel, its dependence on vegetable oils is an obstacle to commercial use [37]. One of the current interests is to develop alternative, economical, and sustainable raw materials to provide more affordable biodiesel [42]. Utilization of wastewater lipids from municipal wastewater treatment facilities could be a potential feedstock for biodiesel production [19]. Those waste materials can be envisaged as a low-cost, sustainable, and non-edible feedstock, which can offer an alternative to waste disposal and alternative sustainable raw materials for

biodiesel production [42,115]. Exploration is underway to explore the feasibility of lipid extraction and biodiesel production from various wastewater residuals, including FOG and sludge. The characteristics of these wastewater residues also vary according to season, geography, and industrial activity [3,8,20]. Therefore, it is important to investigate their properties in order to assess their feasibility as a potential biodiesel raw material.

Japan's wastewater treatment system is well developed, and various treatments have been introduced to reuse excess sludge and wastes from wastewater treatment plants [116]. However, the approach of using wastewater residuals for biodiesel production has not yet been carried out here. Therefore, the study of wastewater residuals as biodiesel feedstock could provide a new option for waste treatment. In addition, evaluating the materials generated and their biodiesel potential in Japan needs to be investigated.

1.10 Research objectives and outline of dissertation

This thesis aims to investigate the potential of wastewater residues from wastewater treatment plants as a feasible raw material for biodiesel. We focus on the potential of extracting lipids from FOG and sewage sludge from pumping stations and WWTP, respectively. This involves evaluating different types of wastewater residues and investigating their lipid and biodiesel content. The properties of samples may vary depending on their site generation and seasonal differences. Further characterization should be carried out to assess their potential to produce biodiesel. There is also a need to investigate the optimization of lipid extraction processes to improve lipid recovery. The detailed objectives of this study are presented as follows:

1) To investigate the lipid and biodiesel content as well as the fatty acid profiles of various sewage sludges collected from WWTP.
2) To investigate the characteristics of lipid materials (fat balls, primary scum, and primary sludge) collected from WWTP in Japan, and to estimate the material production and biodiesel potential in Japan.
3) To investigate the lipid extraction of fat balls by dimethyl ether (DME) and to optimize the process parameters (size, velocity, and DME/sample ratio) using response surface methodology (RSM) with Box Behnken Design (BBD).

This doctoral thesis includes five chapters. The structure is shown in Figure 1.12.

Chapter 1 presents the literature on biodiesel development and the potential of urban wastewater lipids as an alternative biodiesel feedstock.

Chapter 2 presents the evaluation of different sewage sludges from WWTP as a potential biodiesel source in Japan. Lipid extraction was carried out by the Soxhlet method and converted to biodiesel via acid-catalyzed transesterification. The yield of lipids and biodiesel was determined along with the properties of methyl esters. Furthermore, economic feasibility studies for the conversion of wastewater lipid-derived oil to biodiesel were presented.

Chapter 3 investigates the valorization of several wastewater residues. After initially investigating the feasibility of wastewater lipids conversion to biodiesel, we focused on evaluating fat balls and primary scum, compared to primary sludge. Samples were collected in four seasons (winter, summer, autumn, and spring) from two WWTPs and one pumping station in Japan. Physical and chemical characterization were carried out. In addition, radiocarbon analysis was conducted to analyze the biogenic and fossil content of the biodiesel. Finally, the material and biodiesel potential of wastewater lipids in Japan was determined. In addition to investigating wastewater lipids as a biodiesel feedstock, we also examined screen sludge and its potential applications.

Chapter 4 reports the application of dimethyl ether (DME), an alternative to the conventional extraction techniques, to extract lipids from fat balls. The optimization was based on RSM-BBD. The performance of the liquefied DME method was compared to the mechanical shaking and Soxhlet extraction method. In addition, the characteristics of extracted lipids were also reported.

Chapter 5 summarizes the main conclusion of this thesis and outlines suggestions for future work in this field.

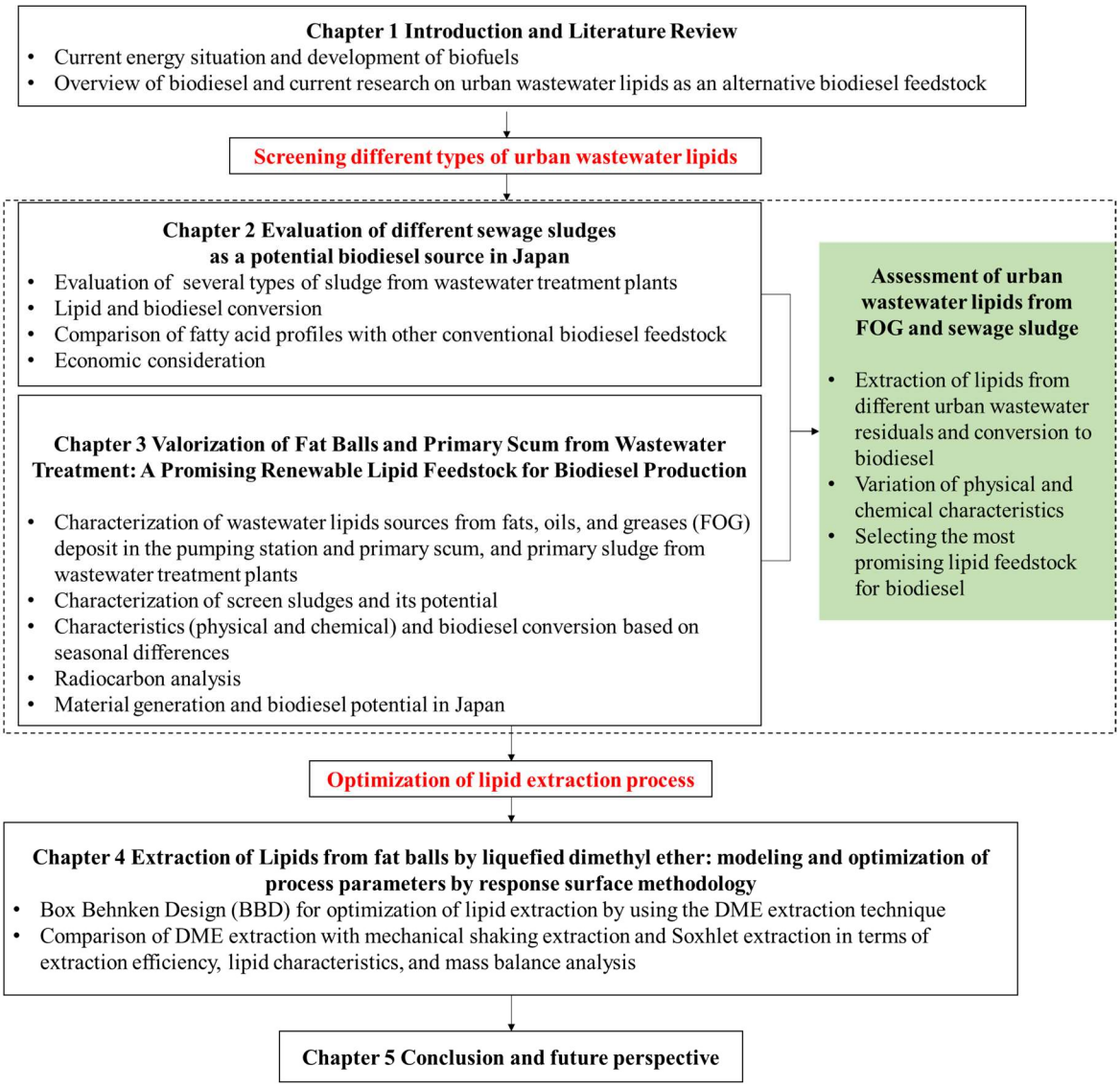

Figure 1.12 The structure of the doctoral thesis.

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Chapter 2 Evaluation of Different Sewage Sludges as a Potential Biodiesel Source in Japan

2.1 Introduction

Global energy demands are increasing because of rapid industrialization and metropolitan development [1,2]. Fossil-based fuels are non-renewable and becoming scarce [3]. Climate change caused by carbon emissions renders the discovery of environmentally benign energy resources imperative [4–6]. Biodiesel is more attractive than petroleum-based fuels because is renewable, biodegradable, associated with low greenhouse gas (GHG) emissions, and compatible with existing diesel engines [7–11]. Biodiesel is a combination of fatty acid methyl esters (FAMEs) obtained via transesterification of triglycerides from plant- or animal-based feedstocks. Despite the fact that biodiesel is a good alternative to conventional diesel, production thereof is constrained by the high costs of refined vegetable oil, which accounts for 70–85% of total production costs [12]. Furthermore, edible oil sustainability is not assured given the limited arable land available for cultivation; fuel production competes with human food production [13–15]. Thus, economically viable biodiesel production from inedible but abundant feedstocks is required [16].

Wastewater treatment plants (WWTPs) are generating ever-increasing volumes of sewage sludge that must be appropriately managed prior to disposal [17,18]. Sludge management accounts for 20–60% of the total operating costs of WWTPs [7,15]. In Japan, over 2.3 million tonnes (on a dry basis) of sludge were generated in 2018 [19]. Several sludge recycling techniques are employed; sludge is used to produce concrete, cement, solid fuel, and fertilizer, and is also incinerated [20]. However, sludge conversion from waste to energy remains limited. Sludge contains organic triglycerides, free fatty acids, phospholipids, sterols, and waxes, all of which are lipids [21–23]. Previous studies have demonstrated the potential of various types of sludge for biodiesel production. Transesterification is the most frequently used technique to produce biodiesel. Other methods include microemulsion and thermal cracking or pyrolysis [24–26]. Unlike edible feedstocks that are affected by market movements and prices, sewage sludge is competitively priced and is always available after wastewater treatment [22]. Thus, the use of sludge lipids for biodiesel production is attractive [26,27].

Biodiesel synthesis from sewage sludge may involve lipid extraction followed by acid-catalyzed transesterification, or direct in situ transesterification [28]. Mondala et al. [26] showed that in situ transesterification of primary and secondary sludge were associated with biodiesel yields of 14.5% and 2.5%, respectively (on a dry basis). In situ transesterification of municipal primary and secondary sludges in the presence of acidified methanol yields biodiesel. Willson et al. [29] found that the Soxhlet method extracted 23.1% (on a dry basis) of lipids and 11.88% (on a dry basis) of biodiesel from primary sludge. Olkiewicz et al. [30] investigated lipid extraction (by the Soxhlet method) from, and biodiesel production by, primary, secondary, blended, and stabilized sludges. Primary sludge yielded a maximum of 27% (on a dry basis) lipids and 19% (on a dry basis) biodiesel. Most research has focused on primary and secondary sludge; few authors have explored other sludges (such as the floating scum of primary and secondary sedimentation reactors). Wang et al. [31] found that lipids extracted from sewage scum had higher calorific value than those of primary and secondary sludge. As a first step toward biodiesel production from lipids of sewage sludge, we compared different sludges from two WWTPs in terms of the lipid and biodiesel yields, and the fatty acid profile. We assessed whether sewage sludge could serve as a second-generation biodiesel feedstock.

2.2 Materials and methods

2.2.1 Chemicals

Gas chromatography (GC)-grade hexane, methanol, hydrochloric acid (HCl), and sulfuric acid (H_2SO_4) were purchased from Fujifilm Wako Pure Chemical Corporation (Tokyo, Japan). Sodium chloride (NaCl), sodium bicarbonate (Na_2CO_3), and anhydrous sodium sulfate (Na_2SO_4) were from Nacalai Tesque, Inc. (Kyoto, Japan). A 37-component FAME mixture standard was supplied by Sigma-Aldrich (Tokyo, Japan) and used for identification and quantification during gas chromatography-mass spectrometry (GC-MS).

2.2.2 Sludge collection and preparation

Sewage sludges were collected from two WWTPs: A and B. Figure 2.1 shows a schematic diagram of the WWTP illustrating the sludge generation and sampling point in the WWTP facility. WWTP A processes 552,780 m^3 of wastewater per day delivered

by a combined sewer system and is located in Kyoto City, Japan. WWTP A has a sludge digestion system that produces biogas. WWTP B processes 52,500 m^3 of wastewater per day delivered via a simple sewer system, and is located in Shiga, Japan. We collected 11 samples, including primary, waste-activated, mixed, and dewatered sludge, and primary and secondary scum. All samples were immediately stored at 4°C and analyzed in terms of total solid (TS), water, and volatile solid (VS) contents using the standard 2540 G method [32]. C/H/N analysis was performed with an elemental analyzer (Micro Corder JM10; J-Science Lab Co., Ltd., Kyoto, Japan).

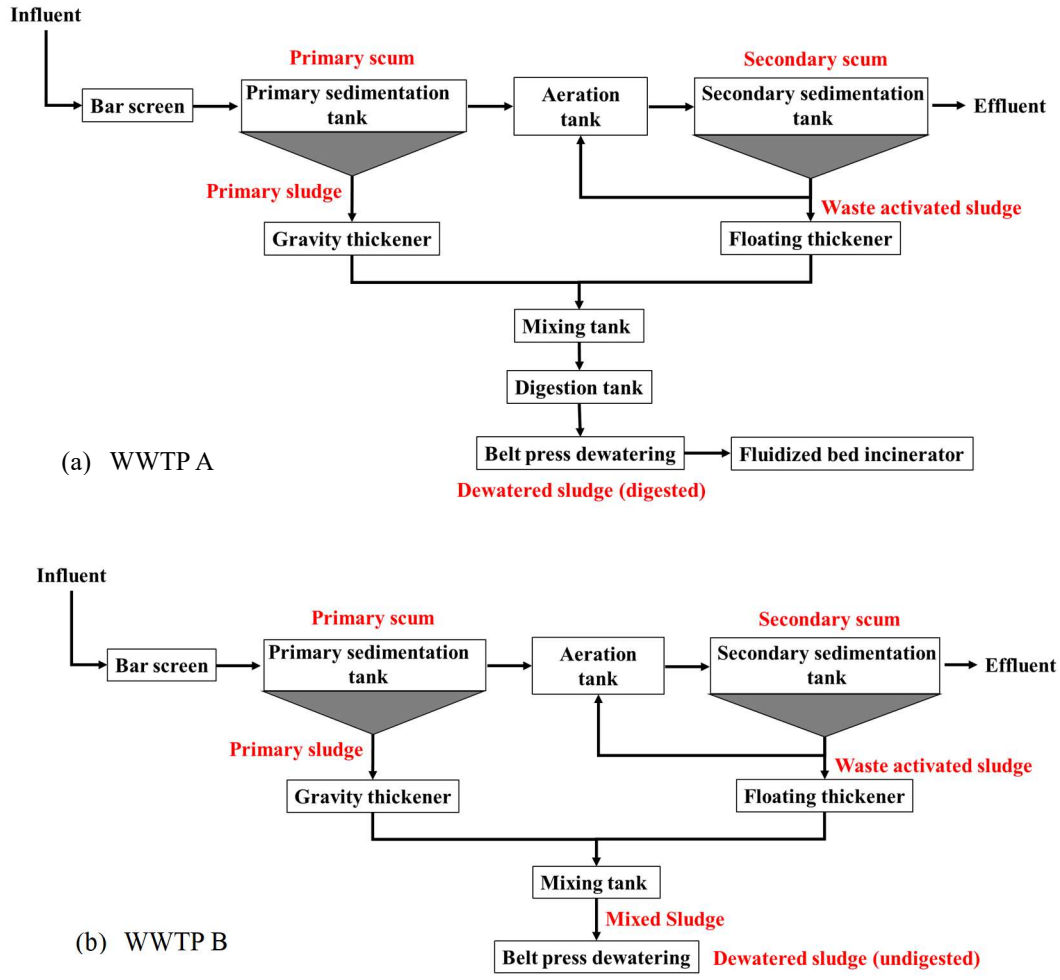

Figure 2.1 Schematic diagram of the current wastewater treatment plant (WWTP): (a) WWTP A, (b) WWTP B

Table 2.1 lists the data. Each sample was analyzed twice. The composition of sewage sludge varies greatly by treatment. The TS level ranged from 0.3 to 24.4%, with 69.7–96.3% VS. The C/H/N ratios also varied widely, as follows: 5.9% ≤ H ≤ 11.9%, 0.6% ≤ N ≤ 7%, and 33.9% ≤ C ≤ 69.2%.

Table 2.1 Characteristics of sludge used in the present study.

WWTP	Sludge type	TS, %	VS, %TS	Ultimate Analysis, %TS		
				C	H	N
A	Primary sludge	1.3	86.0	38.6	5.9	4.5
	Primary scum	1.4	77.1	37.1	6.0	4.9
	Waste activated sludge	0.4	69.7	38.1	6.0	6.0
	Secondary scum	0.3	80.3	48.6	7.7	4.6
	Dewatered sludge	24.4	80.8	33.9	6.2	3.2
B	Primary sludge	1.5	90.4	42.6	6.8	3.2
	Primary scum	8.9	95.5	69.2	11.9	0.6
	Waste activated sludge	0.7	77.8	37.7	6.6	7.0
	Secondary scum	2.5	96.3	53.7	8.5	3.5
	Mixed sludge	4.2	84.1	42.5	6.8	5.8
	Dewatered sludge	20.7	84.9	42.2	6.9	5.3

2.2.3 Soxhlet extraction

Prior to Soxhlet extraction, samples (20 g) were subjected to bath ultrasonication for 10 minutes and acidified to pH 2 by addition of concentrated HCl. Solid dewatered sludge was subjected to acidification only. Samples were dried in an oven at 105°C for 12 h and the desiccated sludges were then pulverized into fine powders using a pestle and mortar. Lipids were extracted into hexane in a Soxhlet apparatus [33]; the hexane removed via rotary evaporation at 40ºC; and each extract dried to constant mass in a vacuum desiccator. The lipid yields were calculated on a weight basis.

2.2.4 Lipid transesterification and biodiesel analysis

Extracted lipids were converted to FAMEs via acid-catalyzed transesterification [34]. Lipids (up to 50 mg) were dissolved in 1 mL hexane and 2 mL H_2SO_4 in methanol (1% v/v); the mixtures were heated overnight at 50˚C in an MG2200 bath (EYELA Co., Ltd., Tokyo, Japan); 5-mL amounts of 5% (w/v) NaCl in water were then added, followed by hexane extraction (2 × 5 mL). The hexane phases were washed with 4 mL of 2% (w/v) Na_2CO_3 and dried over Na_2SO_4. FAMEs were analyzed via GC/MS (QP2010 Plus; Shimadzu Corp., Kyoto, Japan; equipped with an SP-2560 capillary column [100 m × 0.20 μm × 0.25 μm]). Ultra-high-purity helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injection volume was 1 μL and the split ratio was 30/1. The temperatures of the injection and detector ports were 250ºC. The

GC oven temperature program was initially 100ºC (for 5 min); and then increased to 180ºC at a rate of 4ºC/min and 240ºC at a rate of 2ºC/min; this temperature was then held for 15 min. A calibration curve was created using the 37-component FAME standard. GC-MS was used to determine the amounts of saponifiable material among the lipids, and the biodiesel yield, of dry sludge.

2.3 Results and discussion

2.3.1 Lipid and biodiesel yields

The sludge lipid, saponifiable lipid, and biodiesel yields are listed in Table 2.2. Since the lipid from the sludge was extracted using a nonpolar solvent, the recovered raw lipids could also contain non-lipid substances (non-saponifiable matters) including pigments, hydrocarbons, sterols, benzenes, and waxes [35,36]. In this study, we mainly focused on the amount of lipid that could be converted into biodiesel (saponifiable matters), such as triglyceride and fatty acids which were usually used to produce biodiesel.

Primary scum had the highest lipid content, followed by primary sludge (26.7–28.5% and 14.8–22%, respectively). Primary sludge contains nonpolar lipids originating from fresh organic matter present prior to biological treatment [23,33]. Primary scum is a combination of floating grease, free fatty acids, human sewage, and unidentified solids with many undegraded organics [31,37]. Soxhlet extraction into hexane extracts nonpolar substances [28,38]. Thus, many lipids were extracted from both sludges. Activated sludge and secondary scum exhibited lower extraction efficiencies than primary sludge and scum; the average lipid contents were 2.1–7.0% and 2.6–11.2%, respectively. Primary sludge and waste-activated sludge contain different lipids [39]. The latter includes biological substances generated after wastewater processing, during which organic matter is partially decomposed by microorganisms. Secondary scum comprises a raft of floating microbes, the lipids of which are similar to those of waste-activated sludge. Dufreche et al. [21] attributed the lower lipid yields of waste-activated sludge to microbial encapsulation of polar phospholipids in cell walls, which makes membrane extraction difficult. However, hexane extracted neutral lipids such as triglycerides, diglycerides, monoglycerides, free fatty acids, hydrocarbons, and wax [40]. Cell membrane phospholipids are easier to extract into polar solvents. Kech et al. [39] suggested that lipid extraction using both

polar and nonpolar solvents would provide a higher lipid yield. Mixed sludge is a combination of primary and waste-activated sludge, with a lipid content of 4.8%. Our values are lower than those of Olkiewicz et al. [33], who reported a 21.1% lipid yield from mixed sludge. In this study, dewatered sludge of WWTP A contained more lipids than that of WWTP B; the average lipid contents were 12.2% and 1.5%, respectively. These values may reflect the differences in plant configurations (combined and separated systems, respectively).

Saponifiable lipids can be converted into FAMEs via acid-catalyzed transesterification. The differences in saponifiable levels reflect the types of lipids present, and in turn the quality of wastewater. The waste-activated sludge of WWTP B exhibited the highest saponifiable content, followed by the primary scums of WWTP A and B (about 40.5% and 38.9%, respectively). Thus, primary scum had the highest biodiesel yield (9.3–11%) Similarly, Wang et al. [31] reported that the biodiesel yield was highest (9.1%) from the lipids of scum sludge. Highly effective scum-to-biodiesel conversion was reported by Bi et al. [41]; the scum biodiesel yield was almost 60% after filtering, acid washing, acid-catalyzed esterification, base-catalyzed transesterification, glycerol washing, and oil refining. This yield was higher than that of saponifiable matters derived from primary scum in our experiment. That was a reasonable value because their study differed not only in terms of scum pretreatment but also in terms of biodiesel process conditions. For maximizing oil yield, the raw scum undergoes pretreatments before being subjected to base-catalyzed transesterification, however, this also increased processing time and energy. In the presented study, lipids were converted into biodiesel via an acid catalyst technique that is more tolerant for processing low-quality grease and fats but is much slower than base-catalyzed transesterification [42]. Further, it is known that the conversion ratio is limited due to some non-saponifiable lipids that cannot be transformed into biodiesel in the transesterification reaction.

Table 2.2 Lipid extraction and transesterification yields of sludge samples.

WWTP	Sludge type	Lipid yield [(a)], %	Saponifiable [(b)], %	Biodiesel yield [(a)], %
A	Primary sludge	22.0	18.7	4.0
	Primary scum	28.5	38.9	11.0
	Waste activated sludge	7.0	11.1	0.8
	Secondary scum	11.2	3.7	0.4
	Dewatered sludge	12.2	4.8	1.4
B	Primary sludge	14.8	30.6	4.5
	Primary scum	26.7	36.4	9.3
	Waste activated sludge	2.1	40.5	0.9
	Secondary scum	2.6	8.3	0.2
	Mixed sludge	4.8	7.4	0.4
	Dewatered sludge	1.5	5.7	0.2

[(a)] Lipid and biodiesel yields were based on sludge dry weight.

[(b)] The amount of transesterified (convertible into biodiesel) lipids on a mass basis (based on GC-MS analysis).

2.3.2 Fatty acid analysis

Sewage sludge lipid content varies by sludge origin [16]. Analysis of the acid profiles is important when determining the potential of sludge for biodiesel production. Fatty acids include both saturated and unsaturated types (mono- and poly-unsaturated). We found that the saturated fatty acid content of biodiesel produced from sludges of WWTP A and B ranged from 21.7 to 77.1%, and the unsaturated fatty acid content was 22.8–82.5%.

The FAME profiles of lipids in the sewage sludges are shown in Figure 2.2. The predominant components were palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), regardless of sludge type. The fatty acid profiles of the primary and waste-activated sludges of both WWTPs were similar: the C16:0, C18:1, C18:2, and C18:0 fractions accounted for more than 39%, 27%, 13%, and 6% of all biodiesels, respectively. Oleic acid was dominant in the FAMEs produced from the primary scum of WWTPs A and B (47% and 34%, respectively). The palmitic acid level was lower in the primary scum of WWTP A than that of WWTP B (about 10.8%

and 32.8%, respectively). C18:1 fatty acid predominated in biodiesels produced from primary scum (34-47.2%). Bitonto et al. [43] reported high levels of C16:0 and C18:1 FAMEs in primary scum (32.5% and 38.4% respectively). The fatty acid compositions of the secondary scums of WWTP A and B were relatively similar. However, the C16:0 content was slightly higher than that of C18:2 in biodiesel prepared from secondary sludge of WWTP B compared to WWTP A. Regarding dewatered sludge, the saturated fatty acid level of WWTP A sludge was much higher than that of WWTP B sludge, possibly because WWTP A dewatered sludge was digested and that of WWTP B not. These results are consistent with those of other studies reporting higher oleic and linoleic acid contents of undigested dewatered sludge compared to digested sludge [44].

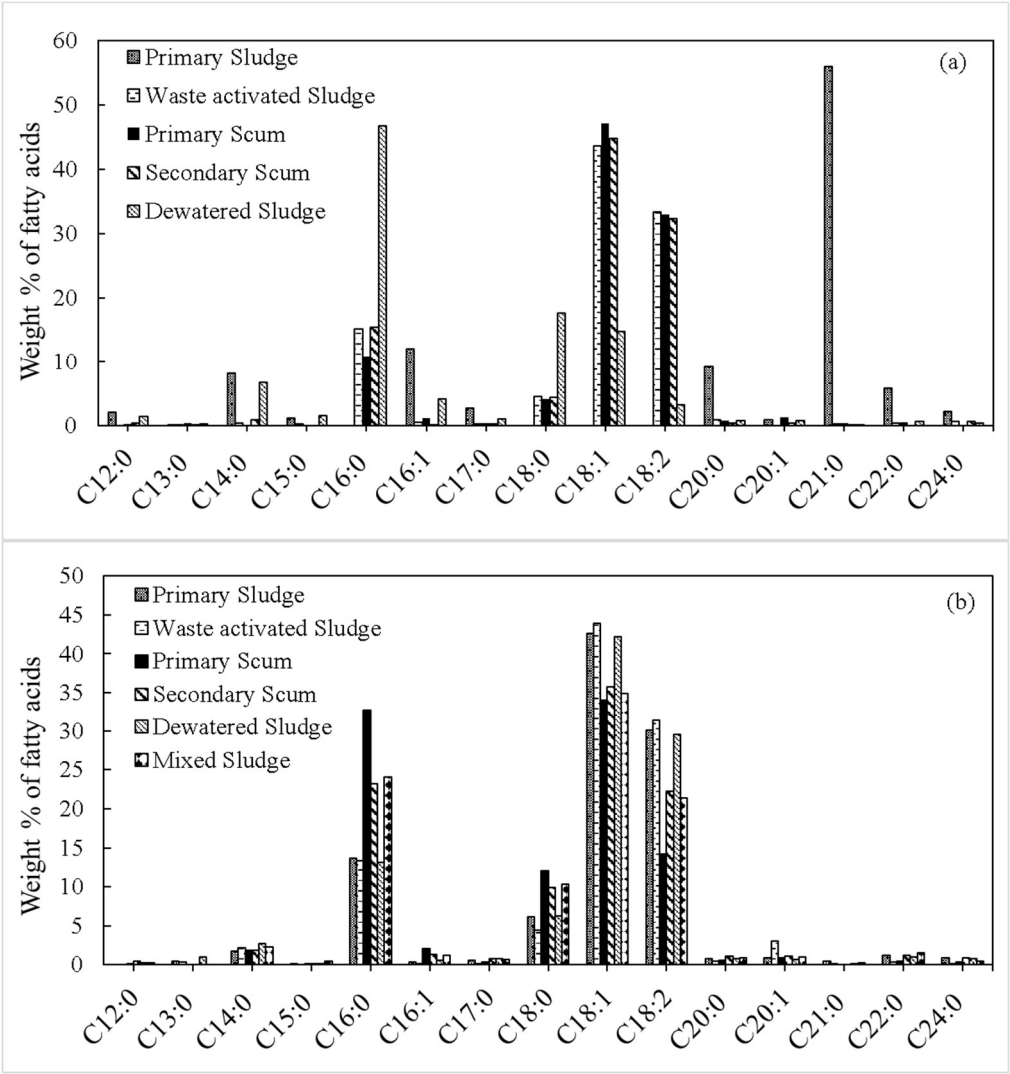

Figure 2.2 Fatty acid profiles of biodiesel from sewage sludges: (a) WWTP A, (b) WWTP B.

2.3.3 Fatty acid profiles of primary scum and primary sludges compared to soybeans, sunflowers, and animal fat.

The fatty acid profile of wastewater sludge (average values of primary sludge and scum) was compared to those of other biodiesel feedstocks in terms of saturation level (Figure 2.3). Soybean and sunflower oils contained more polyunsaturated fatty acids than other biodiesel sources (58.0% and 68.0%, respectively). Such fatty acids affect biodiesel liquidity and melting points [16]. Animal fat contained more (> 40%) saturated fatty acid than soybeans and sunflower seeds, and lower levels of polyunsaturated fatty acids, as did sludge. Biodiesel with high levels of saturated and mono-unsaturated fatty acids would be expected to be less prone to oxidation and to have more cetanes, than other biodiesels [41,45]. However, such biodiesels exhibit poor cold weather- resistance and tend to crystallize at low temperatures [13,46].

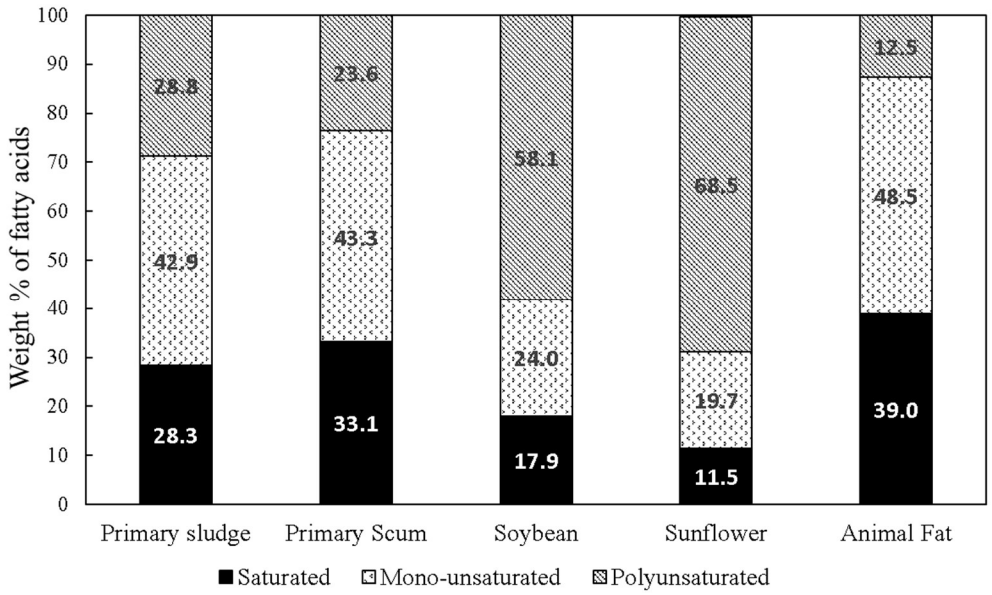

Figure 2.3 Fatty acid profile comparisons of primary sludge, primary scum, soybeans, sunflowers, and animal fat according to the saturation level.

2.3.4 Biodiesel economic consideration

Sewage sludge is a potential lipid feedstock for biodiesel generation. Prior to industrialization, the cost seemed to be the most concerned item. As sewage sludge is a byproduct of wastewater treatment, thus the cost of raw material is eliminated [51]. The cost of producing biodiesel is classified into two parts: the cost of oil feedstock and the

operational costs [52]. In this regard, Table 2.3 presents economic feasibility studies for the conversion of wastewater lipid-derived oil to biodiesel.

Mu et al., (2016) carried out an economic feasibility on a sewage scum-to-biodiesel plant with a capacity of approximately 1,657 tonnes/year of biodiesel. Before biodiesel conversion, the scum oil was pretreated to remove the impurities and converted into biodiesel in the presence of a base catalyst. The estimated capital cost for establishing the facility was around $1.2 million. The scum-to-biodiesel technology could generate annual revenue of approximately $467,539 ($3.1 per gallon of biodiesel produced). Olkiewicz et al. (2016), in their research on economic study of biodiesel showed that biodiesel production from liquid primary sludge could be more cost-effective, at an assumed yield of 70-85% biodiesel/dry weight, giving the break-even price (BEP) of 1,232 $/tonnes.

Table 2.3 Economic evaluations for biodiesel production plants.

References	[51]	[37]
Raw material	Sewage scum	Primary sludge
Flowrate	Not reported	60 m^3/h
Plant capacity	1,657 tonnes/year	4,000 tonnes/year
Process type	Acid-catalyzed esterification, base-catalyzed transesterification	Acid-catalyzed esterification/ transesterification
Biodiesel yield estimation	43%	70-85%
Biodiesel production	200 tonnes/year	4000 tonnes/year
Capital cost	$1,200,000	$4,385,208
Labor cost	$120,000	$661,500
Operation cost	$15,029	$7,455,447
Biodiesel break-even price	Not reported	$1,232/tonnes
Biodiesel price	$3.1/gallon ($467,539/year)	Not reported

2.4 Conclusion

We found that sewage sludges contained substantial amounts of lipids that could be converted into biodiesel through acid-catalyzed transesterification. Primary scum had the highest lipid and biodiesel yields. The gas chromatography analysis of fatty acid methyl esters (FAME) revealed that all sludge contained significant amount of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2). The comparison of sludge fatty acid profiles with conventional biodiesel feedstocks confirmed their viability for biodiesel production. It was found that the percentage of mono-unsaturated and saturated fatty acid was dominant in the FAME from primary sludge and primary scum. Sewage sludge could enhance Japanese biodiesel production. More research is needed to better understand the effects of sewage and seasonal differences on sludge characteristics. Such differences significantly impact lipid and biodiesel quality.

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Chapter 3 Valorization of Fat Balls and Primary Scum from Wastewater Treatment: A Promising Renewable Lipid Feedstock for Biodiesel Production

3.1 Introduction

The development of renewable energies has attracted worldwide attention due to increasing energy demands and decreasing fossil fuel reserves. Moreover, the general public has become increasingly conscious of climate change, which is driven by the consumption of conventional fuels [1]. Biodiesel, which is produced from lipids (including both edible or inedible sources) via transesterification reactions, is gaining attention for its renewability, biodegradability, and compatibility with current diesel engine systems [2–4]. However, progress in the biodiesel industry is hindered by the high cost of oil feedstock (primarily vegetable oils); additionally, biodiesel prices can exceed the price of petroleum-based diesel by threefold [5,6]. Consequently, research has focused on the identification of readily available, cost-effective raw materials to achieve sustainable biodiesel production.

There is increasing interest in the recovery of valuable organic components from the urban wastewater residuals that are continuously generated during wastewater treatment [7–9]. As a major organic component of wastewater, lipids are mainly derived from fats, oils, and grease (FOG) generated during cooking and food preparation in restaurants, households, and the food processing industry [10,11]. Large amounts of FOG can enter sewage systems due to uncontrolled disposal of waste oil or improper management of grease traps [12–14].

Primary sludge, activated sludge, and digested sludge have been investigated as potential lipid sources for biodiesel production [15–18]. Although sludge holds great potential as an alternative oil feedstock, a viable collection pathway must be established from wastewater treatment plants (WWTPs) because lipid extraction may impact conventional sludge treatment processes [19]. Within WWTPs, fat balls in pumping stations and primary scum in the primary clarifier contain substantial concentrations of lipids, which float on the water surface and cause operational issues such as foaming and system blockages [20–22]. These materials must be removed and treated separately to avoid problems in WWTPs.

Fat balls and scum are both composed of mixtures of FOG and solid materials; they demonstrate high moisture content. Fat balls are formed by the agglomeration of suspended solids (e.g., FOG, metals, and debris), which create a floating layer on the water surface in pumping stations [20,23]. In WWTPs, the primary clarifier separates settleable solids (primary sludge) and buoyant substances such as oil and grease (primary scum) from the influent after basic screening [24–27]. Collin et al. (2020) performed an energy assessment of various FOG waste materials obtained from a sewage catchment, which revealed that fat balls and floating scum had lipid contents ranging from 13 wt.% to 93 wt.%. Wang et al. (2016) explored the potential for producing biodiesel from sewage scum and primary sludge; they found that scum had a higher biodiesel yield (28.7 wt.%) compared with primary sludge (25.4 wt.%).

According to sewage statistics in Japan, approximately 75% of the total sewage sludge was allocated for recycling purposes in 2020, but only a small proportion (9%) was used for fuel generation [30]. In Japan, fat balls and primary scum are typically landfilled or incinerated [31]. However, to achieve a circular economy, there must be a shift from waste disposal toward resource recovery, while minimizing potential environmental risks [32–35]. Although several studies have reported that fat balls and sewage scum constitute potential biofuel feedstocks, there is limited available information regarding their characteristics and variability among seasons and collection sites, and few estimates have been published concerning their volume and potential as biodiesel feedstock [24,36]. Additionally, there has been no attempt to study biogenic C in biodiesel derived from wastewater lipids based on radiocarbon (^{14}C) content; according to Intergovernmental Panel on Climate Change guidelines, there is a need to distinguish between the fossil and biogenic C contents in biofuel feedstocks [37,38].

The aim of this study is to conduct a comprehensive comparison of waste materials obtained from wastewater treatment plants, including fat balls, primary scum, and primary sludge, with a focus on assessing their suitability as biodiesel feedstock. The samples were analyzed for their physicochemical properties, lipid composition, and biodiesel potential across different seasons. Additionally, this study aims to investigate the characteristics of screen sludge, a byproduct of sewage treatment processes collected during pretreatment stage, with a specific focus on characterization and exploring potential applications. The findings from this research are expected to make a significant contribution to the sustainable management of wastewater residuals.

3.2 Materials and methods

3.2.1 Sample collection and preparation

Samples were collected from two WWTPs (H and S) and one pumping station in Kobe, a major metropolitan area in the Kansai region of Japan. WWTPs H and S treat 171,500 m^3/day and 134,600 m^3/day of residential and industrial wastewater, respectively. The pumping station F (PS F) is located close to WWTP H and has a capacity of 37,000 m^3/day. Figure 3.1 shows the schematic diagram and sampling point in the WWTP facility.

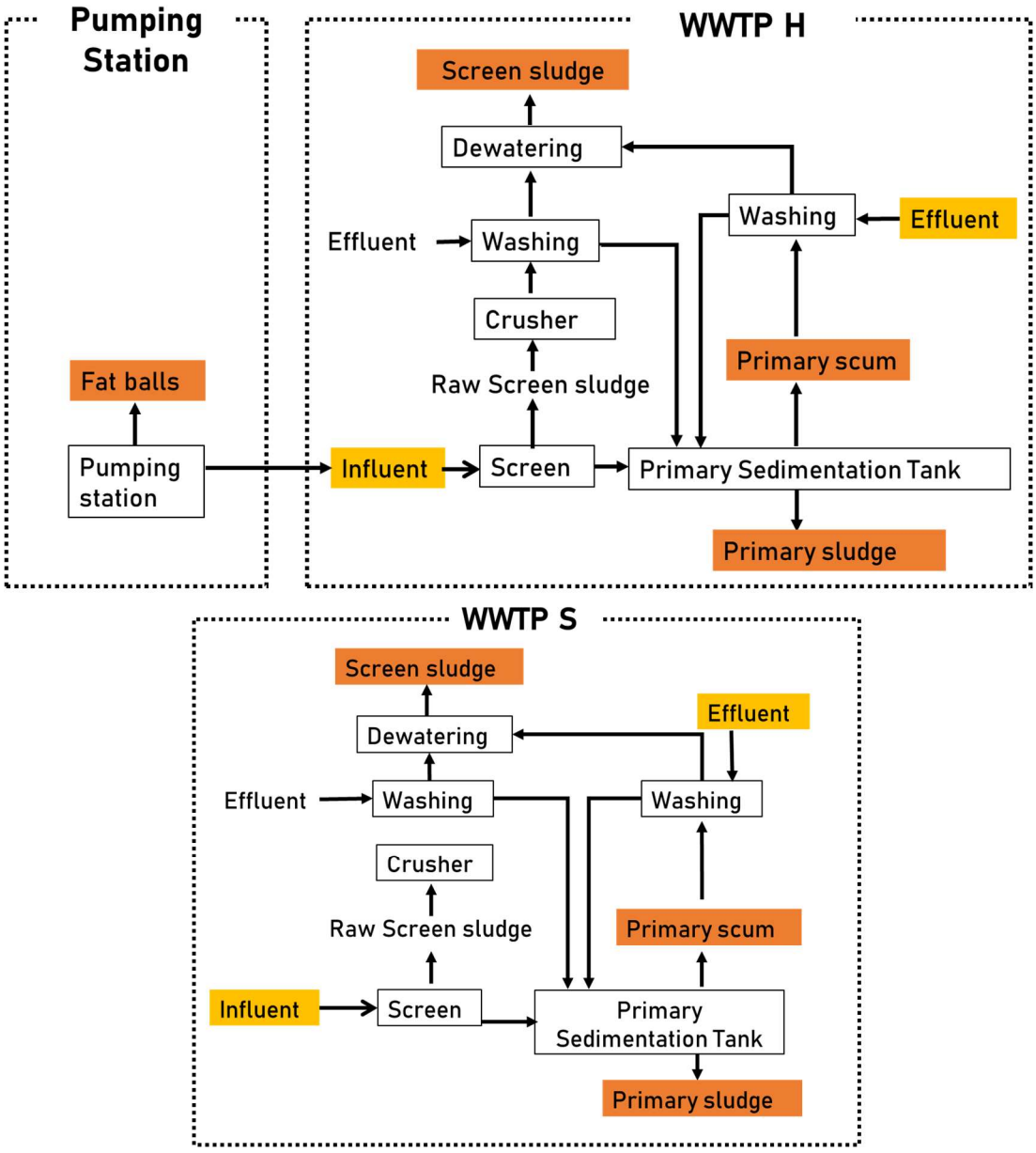


Figure 3.1 Schematic diagram of the current wastewater treatment plant (WWTP).

As summarized in Table 3.1, we collected fat balls from the pumping station (FB), primary scum (SC H), primary sludge (PS H), and screen sludge (SCR H) from WWTP H, and primary scum (SC S), primary sludge (PS S), and screen sludge (SCR S) from WWTP S. Samples were collected in four seasons (winter, summer, autumn, and spring).

Table 3.1 Summary of the sampling locations.

Location	Sampling source	Sample notation
PS F		
Fat balls	Water surface of pumping station	FB
WWTP H		
Primary scum	Water surface of primary clarifier	SC H
Primary sludge	Collected from primary clarifier	PS H
Screen sludge	Collected after pretreatment of influent by screens	SCR H
WWTP S		
Primary scum	Water surface of primary clarifier	SC S
Primary sludge	Collected from primary clarifier	PS S
Screen sludge	Collected after pretreatment of influent by screens	SCR S

3.2.2 Physical and chemical characterization

Samples were subjected to qualitative assessments of their physical appearance, considering characteristics such as texture and color. The contents of total solids (TS) and volatile solids (VS) were determined according to the Biosolids Analytical Methods and Sampling Procedures standard 2540 method G [39]. To ensure repeatability, each sample was measured in triplicate.

3.2.2.1 Element analysis

Trace elements in dried samples (Ca, Si, Na, P, Fe, Al, Mg, K, Zn, Cu, Pb, and Ni) were analyzed by inductively coupled plasma atomic emission spectrometry (ICAP-7000; Thermo Fisher Scientific, Waltham, MA, USA). Before the analysis, 0.2 g of dried sample was mixed with 6 mL of HNO_3, 1 mL of HCl, and 1 mL of HF, then digested using a microwave digestion system (ETHOS One; Milestone SCI, Inc., Shelton, CT, USA). The mixtures were digested for 20 min at 220°C and 1000 W, then for 20 min at 200°C and 1000 W.

Carbon (C), Hydrogen (H), and Nitrogen (N) analysis was performed using an elemental analyzer (Micro Corder JM10; J-Science Lab Co., Ltd., Kyoto, Japan). The Cl and combustible S contents were determined according to the Japanese Industrial Standard (JIS) [40]. The percentage of O was calculated using Eq. (1):

$$O = V - C - H - N - S \quad (1)$$

3.2.2.2 Thermogravimetry–differential thermal analysis

The thermogravimetric (TG) degradation of dried samples was measured using a differential thermal analysis balance (Thermo Plus EVO2, TG 8120; Rigaku, Tokyo, Japan). Each sample was heated from ambient temperature to 650°C at a rate of 10°C/min under an N_2 stream (50 mL/min).

3.2.2.3 Calorific value

The higher heating value (HHV) of dry solids was measured using an oxygen bomb calorimeter (CA-4J; Shimadzu Corp., Kyoto, Japan). The lower heating values of samples on a dry basis (LHV_d) and wet basis (LHV_w) were calculated from the measured HHV according to Eqs. (2) and (3):

$$LHV_d = HHV - 4.186 \times 600 \times 9 \times (H / 100) \quad (2)$$

$$LHV_w = (100 - w) / 100 \times LHV_d - 4.186 \times 600 \times (w / 100) \quad (3)$$

where LHV_d (kJ/kg) and LHV_w (kJ/kg) represent the LHVs of dried and dewatered samples, respectively; w is the moisture content of the dewatered samples; and H (% dry solids) is the H atom content of the sample.

3.2.2.4 Fourier transform-infrared spectroscopy analysis

The functional group compositions of the extracted lipids were determined via Fourier transform-infrared spectroscopy (FTIR; IRSpirit-T; Shimadzu) in attenuated total reflectance mode. The detection range was 500–4000 cm^{-1}.

3.2.2.5 Accelerator mass spectrometry ^{14}C analysis

The ^{14}C contents of dry solids and biodiesel were analyzed by accelerator mass spectrometry, in accordance with ASTM D6866-22, at Beta Analytic Testing Laboratory (Miami, FL, USA).

3.2.3 Lipid extraction

Lipids were extracted from samples using a mechanical shaker (SA300; Yamato Scientific Co., Ltd., Tokyo, Japan) with hexane (Guaranteed Reagent; Wako Co., Ltd., Tokyo, Japan). Wet samples were weighed in 50-mL tubes, and hexane was added at a 2:1 hexane:sample volumetric ratio [8,41]. All samples underwent three extractions. For each extraction, the sample was shaken at 200 rpm for 60 min at ambient temperature, then centrifuged at 3,000 rpm for 15 min. The supernatant phase (upper organic layer) was decanted and transferred to a pre-weighed round-bottom flask, then evaporated using a vacuum rotary evaporator at 40°C. The organic solvent remaining in the residue was further vaporized under an N_2 stream. Finally, the extracted lipid weight was recorded after the residue had cooled in a desiccator. The lipid yield was expressed as the lipid weight per unit sludge sample weight (dry basis).

3.2.4 Lipid transesterification and fatty acid methyl ester analysis

Lipids were converted by acid-catalyzed transesterification into fatty acid methyl esters (FAMEs; i.e., biodiesel) [8,42]. A 20-mg sample of extracted lipids was treated with 1 mL of hexane and 2 mL of 1% v/v H_2SO_4 in methanol (Guaranteed Reagent; Wako Co., Ltd.). The mixture was heated overnight at 50°C in a constant-temperature bath (MG-2200; EYELA Co., Ltd., Tokyo, Japan). After the reaction, 5 mL of 5% (w/v) NaCl (Nacalai Tesque, Inc., Kyoto, Japan) were added, followed by two extractions using 5 mL of hexane. Raw biodiesel in the hexane phase (upper layer) was then washed with 4 mL of 2% (w/v) Na_2CO_3 (Nacalai Tesque, Inc.) and dried at 60°C.

Fatty acids were analyzed using a gas chromatography–mass spectroscopy system (GC-MS; QP2010 Plus; Shimadzu Corp.) equipped with an SP-2560 capillary column (100 m × 0.20 μm × 0.25 μm; Supelco, Bellefonte, PA, USA). Ultra-high-purity He was used as the carrier gas with a flow rate of 1.0 mL/min and split ratio of 1:30. The sample injection volume was 1 μL. The injection and detector port temperatures were 250°C. The GC oven temperature program was initially 100°C, held for 5 min, then increased to 180°C at a rate of 4°C/min and 240°C at a rate of 2°C/min, and then held at 240°C for 15 min. The calibration curve was created using a standard comprising 37 FAMEs (Supelco). The GC-MS concentrations were used for FAME identification and quantification of transesterifiable lipids. The biodiesel yield (dry basis) was calculated based on the mass of lipids extracted and their content of transesterifiable material.

3.3 Results and discussion

3.3.1 Characterization of fat balls, primary scum, and sludge

3.3.1.1 Physical characterization of fat balls, primary scum, and sludge

Waste samples collected from the pumping station and primary clarifiers of the two WWTPs had distinct physical appearances. FBs from the pumping station were solid, spherical, and composed of a fatty outer layer and buoyant-material core (e.g., Styrofoam); they were light yellow and had a strong, unpleasant smell (Figure 3.2 a). The SC H and S samples were composed of a mixture of floating food scraps, solids, and oily waste (Figure 3.2 b, d). The PS H and S samples were brown-black in color and composed of a slurry of suspended solids (Figure 3.2 c, e).

We next characterized the SC, PS, and FB samples collected in winter, summer, and autumn (Table 3.2). Among the five samples collected across three seasons, FB samples from the pumping station generally had the highest TS content (49.9–56%). This was expected because fat balls are composed of solidified organic matter originating from discharged FOG. The SC and PS samples from the primary clarifiers had low TS contents of 0.7–6% and 1.5–2.5%, respectively. In comparison, reported TS for primary scum are higher, typically ranging between 8.9% to 62.5% [28,43,44] , whereas primary sludge was found between 1.03 and 9.09% [15,28,45–47]. The large difference between the pumping station and primary clarifier samples may reflect the different stages of treatment [48]. Samples collected from primary clarifiers might have undergone initial treatment steps, whereas pumping station samples may be more representative of raw wastewater, which could have higher FOG contents.

We also observed some seasonal variation in the TS contents. FB and SC samples had higher moisture contents in summer, possibly due to lower humidity under warmer temperatures and nature of these substances. The seasonal TS content of SC samples notably varied, whereas the TS content of PS samples remained relatively constant across seasons. This could be related to differences in the compositions of scum (i.e., floating matter) and primary sludge (i.e., settled organic and inorganic matter). Primary scum, comprised of grease, may exhibit fluctuations in TS due to its floating nature [49,50]. Furthermore, scum is often skimmed out from the surface of the primary clarifier. In contrast, primary sludge tends to maintain a more consistent TS level as it is generated after the primary treatment process [51].

The VS contents of all samples were ranged from 82.9% to 97.1% (dry basis), slightly higher than that reported in the literature for primary sludge, which typically falls between 60-80% [46]. These results indicate that the waste samples contain rich organics. Among all samples, the average elemental composition was in the range of 39.4–76.5 wt.% C, 5.9–13.2 wt.% H, and 0.16–5.6 wt.% N.

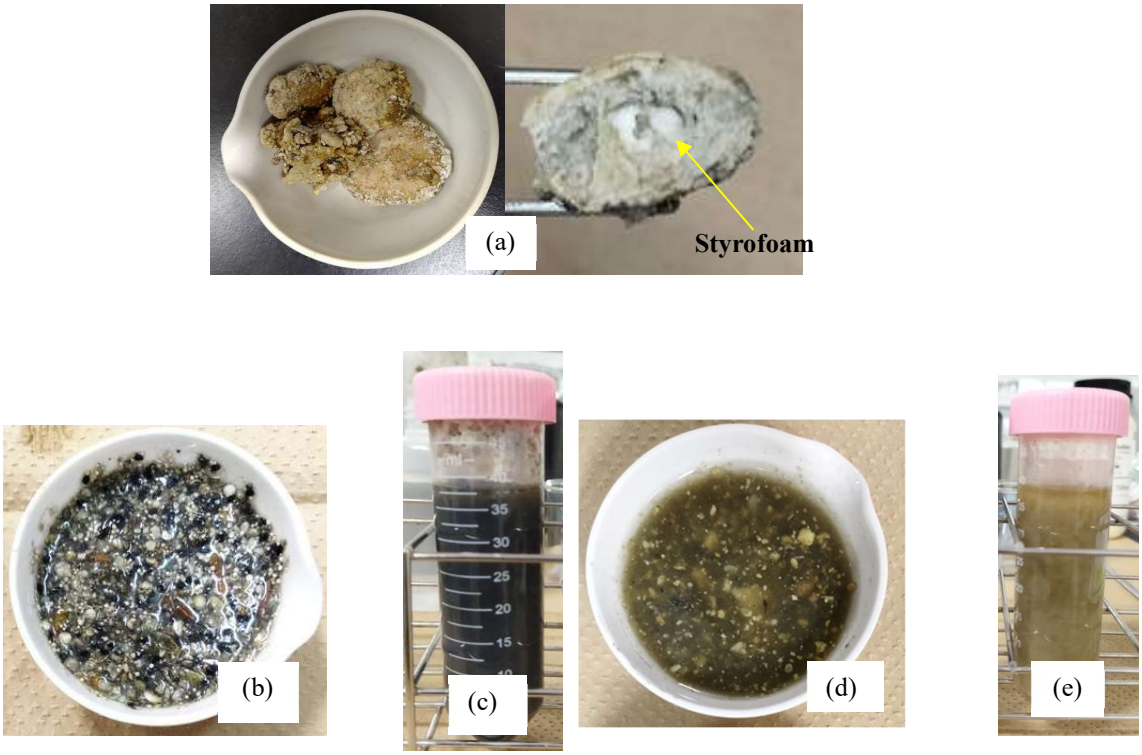


Figure 3.2 Samples collected from the pumping station and wastewater treatment plants (WWTPs): (a) fat balls from the pumping station; (b) primary scum from WWTP H; (c) primary sludge from WWTP H; (d) primary scum from WWTP S; (e) primary sludge from WWTP S.

Table 3.2 Characteristics of pumping station (fat ball) and wastewater treatment plant (scum and sludge) samples.

Source	Season	TS, %	VS, %TS	C, %	H, %	N, %	HHV, MJ/kg	LHV_w, MJ/kg
FB	Winter	56.0 ± 5.0	97.1 ± 0.5	71.1 ± 0.6	11.2 ± 0.2	0.23 ± 0.03	40.2 ± 9.6	20.0 ± 5.4
	Summer	49.9 ± 4.0	92.5 ± 1.3	76.5 ± 1.4	13.2 ± 0.3	0.57 ± 0.3	29.9 ± 15	12.2 ± 7.6
	Autumn	55.0 ± 5.0	92.4 ± 0.5	68.4 ± 0.6	11.4 ± 0.2	0.16 ± 0.03	39.2 ± 1.0	19.0 ± 0.5
	Spring	41.6 ± 3	90.6 ± 0.1	71.2 ± 0.5	12 ± 0.1	0.2 ± 0.1	42.3 ± 3	39.7 ± 3
Average		50.6	93.2	71.8	12.0	0.3	37.9	22.7
SC H	Winter	3.50 ± 2.0	86.5 ± 2.5	71.7 ± 0.6	11.3 ± 0.1	1.21 ± 0.04	37.4 ± 0.8	-1.20 ± 0.03
	Summer	1.30 ± 0.6	90.1 ± 3.0	47.5 ± 1.9	7.83 ± 0.4	2.38 ± 0.1	31.7 ± 2.1	-2.10 ± 0.14
	Autumn	0.70 ± 0.1	86.0 ± 3.0	56.9 ± 19	7.60 ± 2.7	2.66 ± 0.6	29.8 ± 3.4	-2.30 ± 0.02
	Spring	4.7 ± 2.8	85.7 ± 6.5	48.2 ± 2.7	7.8 ± 0.5	3.4 ± 0.5	33 ± 3	31.2 ± 3
Average		2.6	87.1	56.1	8.6	2.4	33.0	6.4
SC S	Winter	2.10 ± 0.8	91.0 ± 3.0	59.5 ± 1.0	9.30 ± 0.3	1.67 ± 0.1	31.9 ± 5.6	-1.90 ± 0.14
	Summer	1.40 ± 0.5	92.3 ± 5.0	54.1 ± 1.0	9.00 ± 0.2	3.41 ± 0.1	22.6 ± 6.4	-2.10 ± 0.01
	Autumn	6.00 ± 1.0	96.2 ± 0.6	65.0 ± 3.4	9.50 ± 0.5	1.85 ± 0.2	38.1 ± 4.3	-2.10 ± 0.1
	Spring	2.4 ± 0.1	89.9 ± 0.5	44.8 ± 0.5	7 ± 0.1	2.4 ± 0.3	25 ± 3	23.3 ± 3
Average		3.0	92.4	55.9	8.7	2.3	29.4	4.3
PS H	Winter	2.50 ± 0.1	82.9 ± 5.0	44.7 ± 4.0	6.90 ± 0.6	4.90 ± 0.5	24.4 ± 5.5	-1.80 ± 0.1
	Summer	2.20 ± 0.1	83 ± 0.3	42.1 ± 0.8	6.90 ± 0.1	4.40 ± 0.05	20.0 ± 0.6	-2.20 ± 0.1
	Autumn	1.70 ± 0.02	83.4 ± 1.0	45.2 ± 9.0	5.90 ± 1.1	5.60 ± 2.3	25.2 ± 4.3	-0.20 ± 0.3
	Spring	2.3 ± 0.01	86 ± 0.1	43.8 ± 0	7 ± 0.1	3.8 ± 0	23 ± 0.7	21 ± 0.7
Average		2.2	83.8	44.0	6.7	4.7	23.2	4.2
PS S	Winter	1.60 ± 0.1	91.6 ± 0.3	46.5 ± 0.3	7.30 ± 0.1	2.41 ± 0.1	20.0 ± 0.1	-2.20 ± 0.01
	Summer	1.70 ± 0.1	87.0 ± 3.0	44.9 ± 1.2	7.40 ± 0.2	3.18 ± 0.03	20.1 ± 0.9	-2.20 ± 0.02
	Autumn	1.50 ± 0.1	88.5 ± 2.0	51.2 ± 13	6.80 ± 1.8	3.00 ± 0.8	18.3 ± 0.8	-2.20 ± 0.01
	Spring	2.05 ± 0.02	87.4 ± 0.2	39.4 ± 1.2	6.5 ± 0.4	2.5 ± 0.2	21 ± 0.5	19.4 ± 0.8
Average		1.7	88.6	45.5	7.0	2.8	19.9	3.2

Samples are abbreviated as shown in Table 3.1

3.3.1.2 Calorific value of dry samples

The heating value represents the energy released when a specific volume of material is combusted. The composition of fuel impacts its energy content, and a larger HHV corresponds to a greater energy content [28]. The HHV was highest for FB (36.4 MJ/kg), followed by SC (29.4–33.0 MJ/kg) and PS (19.9–23.2 MJ/kg) samples (Table 3.2).

Among the three seasons, calorific values tended to be lower in warmer seasons because of the higher sample moisture contents. Accordingly, samples collected from the primary sedimentation tank had comparable LHV_w. The heating values tend to increase with higher levels of VS and ratios of C in samples (Folayan et al. 2019; Parikh et al. 2005). FB samples had higher VS (90.6-97.1) and C fractions (68.4–76.5%). Additionally, they exhibited lower moisture contents ranging from 44.0% to 58.4%. The comparison of calorific value of the waste samples evaluated in this study has been compared to literature, as summarized in Table 3.3. The variation in calorific value of waste samples is likely due to contact with sewage and other materials in the sewer.

Table 3.3 Comparison of the calorific value of fat balls, primary scum, and primary sludge in this study and previous literature.

Source	LHV_d, MJ/kg	LHV_w, MJ/kg	HHV, MJ/kg	References
Fat balls from pumping station	N.R.	14.0	N.R.	[28]
Fat balls from WWTP	N.R.	13.0	N.R.	[28]
Primary scum	N.R.	2.0	N.R.	[28]
Primary scum	26.5	N.R.	N.R.	[44]
Sewage scum	26.0	N.R.	28.0	[54]
Primary scum	N.R.	N.R.	17.6	[29]
Sewage scum	N.R.	N.R.	37.7	[43]
Primary sludge	N.R.	N.R.	12	[29]
Sewage sludge	N.R.	2.0	N.R.	[28]
Fat balls	N.R.	17.1	36.4	This study
Primary scum	N.A.	31.7	-2.0	This study
Primary sludge	N.A.	21.4	-1.8	This study

Abbreviations: N.R. = not reported, N.A. = not analyzed

3.3.1.3 TG–differential thermal analysis of dried samples

The TG and differential thermogram curves of the dried samples based on measurements in the three seasons are shown in Figure 3.3. The thermal decomposition of sewage sludge can be divided into three stages: dehydration (up to 180°C), organic matter degradation (180–580°C), and inorganic matter decomposition (580–1000°C) [55,56]. Smaller molecules are thermally degraded at lower temperatures, whereas larger molecules require higher temperatures (Chen et al., 2020).

We observed considerable thermal weight loss in the second stage, although there were differences in the specific temperature at which organic matter exhibited thermal decomposition among the samples. FB and SC have higher C content, indicating a greater proportion of organic matter. In contrast, PS decomposes earlier in the second stage of TGA, suggesting a distinct thermal decomposition profile likely due to its lower C content. The differential thermogram curves of the PS H and PS S samples showed similar trends, with maximum weight loss around 330–340°C. The SC H, SC S and FB samples required a higher temperature (442–460°C) to completely decompose the organic substances.

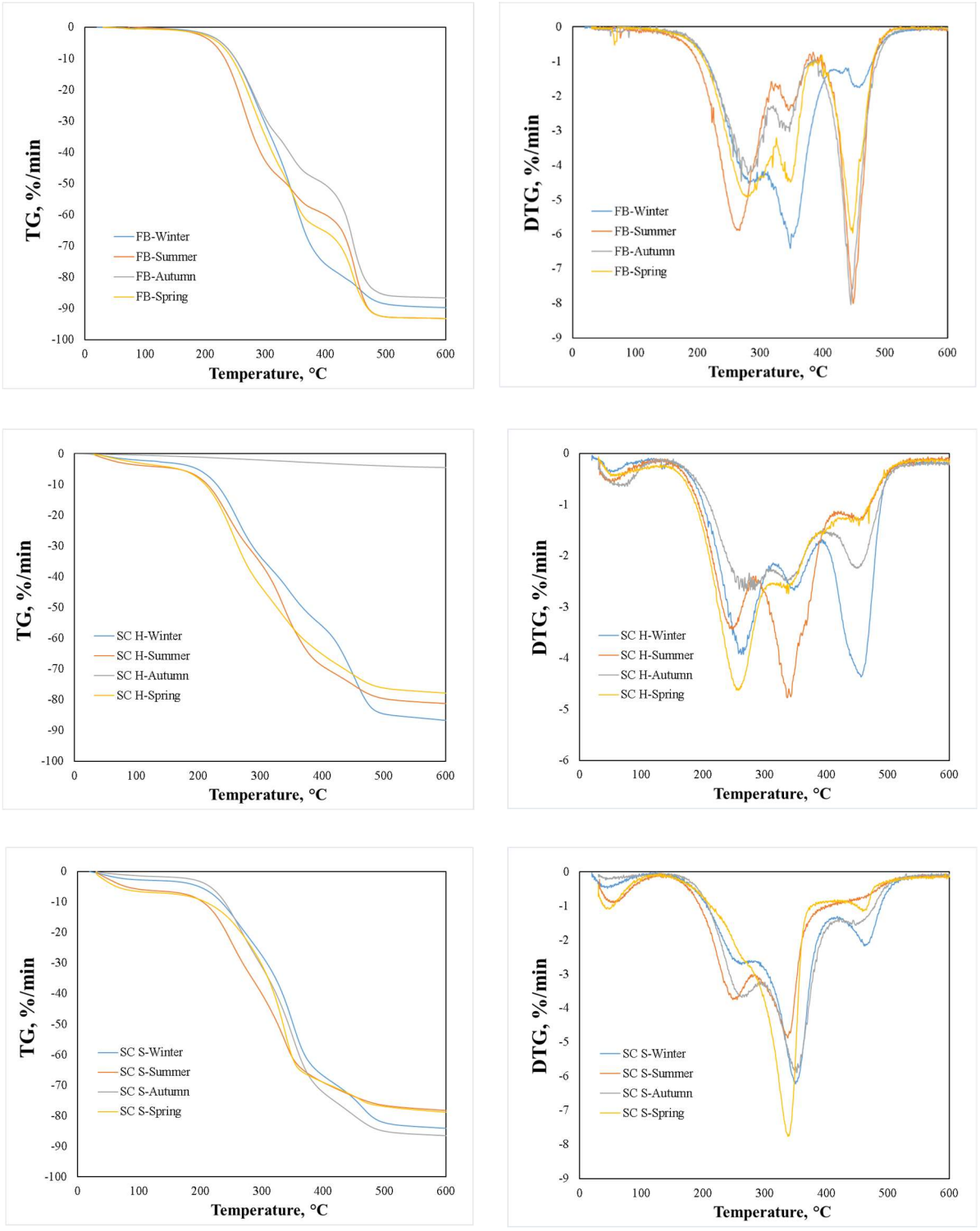


Figure 3.3 Thermogravimetric (TG) and differential thermogram (DTG) curves of fat balls, primary scum, and primary sludge. Samples are abbreviated as shown in Table 3.1.

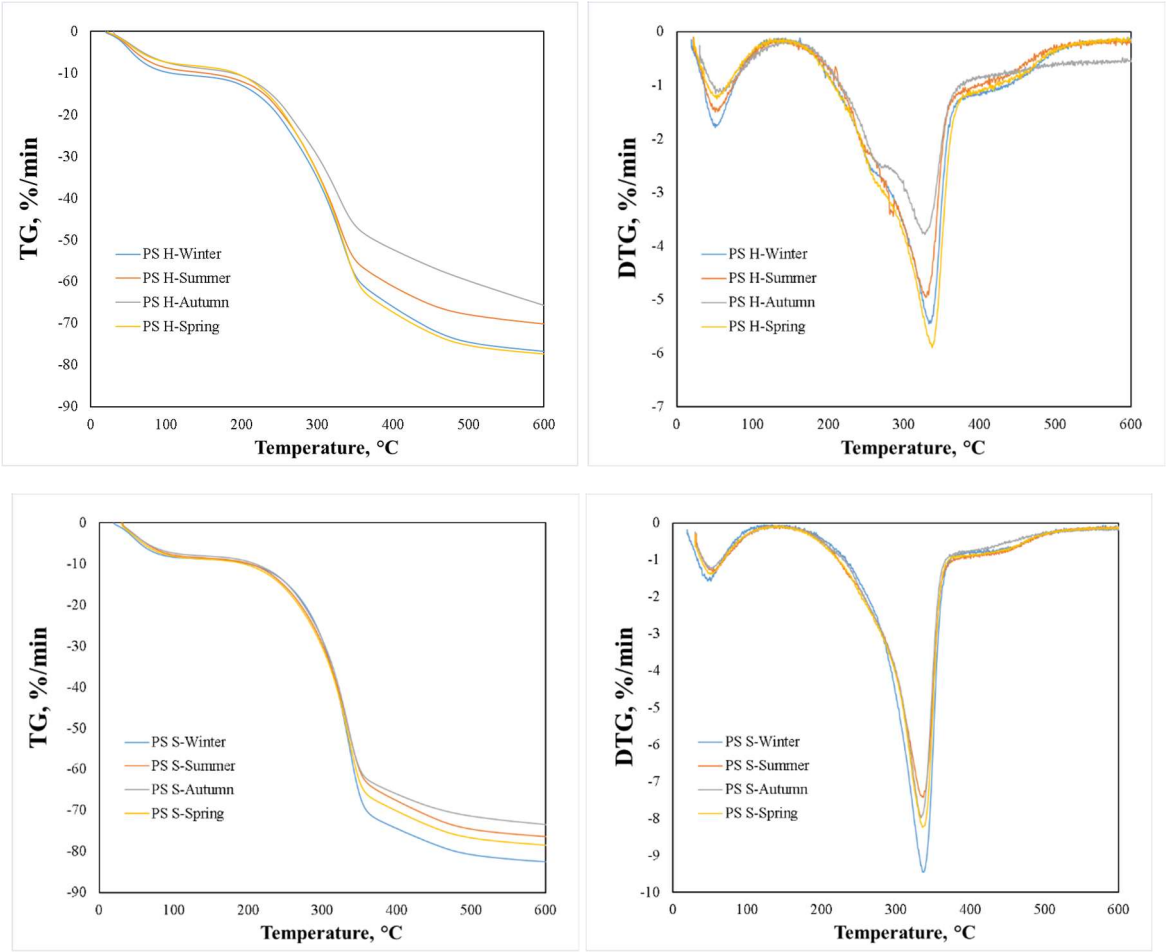


Figure 3.3 Thermogravimetric (TG) and differential thermogram (DTG) curves of fat balls, primary scum, and primary sludge (cont.). Samples are abbreviated as shown in Table 3.1.

3.3.1.4 Trace metal contents in dried samples

Table 3.4 displays the trace element concentrations measured in the solid samples. The samples contained relatively high contents of some major elements, including Ca, Si, Na, P, Fe, Al, and Mg, but lower contents of elements such as Zn, Cu, Ni, Pb, Cl, and S. Some elements presumably underwent natural release into the water from rock, soil, or concrete corrosion (e.g., Ca, Si, P, and S) [58,59]. Effluent from human activities also likely increased the concentrations of some elements, such as household products (Na, K, and Cl), agricultural runoff (P), and industrial effluent (Cu, Fe, Zn, Ni, Al, Mg, and Pb) [60–62].

The element concentrations were highly variable among samples and across seasons, although the SC and PS samples generally contained much higher trace element contents than the FB samples. Numerous studies have reported high levels of heavy metals and sulfur in lipids derived from grease trap waste [27,62–64]. Hence, further treatment is necessary before wastewater lipids can be utilized for biodiesel production.

Among the trace ions, Ca was the most prominent (9,200.0–24,700.0 mg/kg), consistent with the literature (Table 3.5) [23,24,65]. FB samples had the highest Ca concentrations, which may have contributed to the solidification process [23,65,66]. FOG agglomerates via saponification when a large concentration of Ca reacts with liquid fat (as a precursor of free fatty acids) [17,58,67]. We also observed that Styrofoam acted as a nucleus in the FB samples (Figure 3.2 a); FOG likely adheres to Styrofoam, leading to the formation of fat balls.

Table 3.4 Elemental contents of fat balls, primary scum, and primary sludge samples collected in winter, summer, autumn, and spring.

Season		Winter					Summer				
Source		FB	SC H	SC S	PS H	PS S	FB	SC H	SC S	PS H	PS S
Elements (mg/kg)	Na	1,950.0	4,500.0	7,950.0	12,200.0	5,920.0	2,350.0	8,740.0	8,400.0	10,900.0	10,100.0
	Fe	230.0	1,880.0	1,740.0	17,000.0	2,310.0	89.0	1,610.0	2,540.0	18,100.0	2,820.0
	Ca	11,300.0	16,990.0	21,930.0	9,770.0	7,560.0	28,400.0	10,700.0	10,640.0	9,830.0	8,600.0
	K	250.0	2,110.0	1,750.0	2,970.0	1,930.0	280.0	1,280.0	2,070.0	2,400.0	1,940.0
	Al	130.0	4,200.0	2,050.0	5,980.0	2,770.0	400.0	2,370.0	5,190.0	6,930.0	4,100.0
	Mg	200.0	830.0	1,140.0	2,620.0	1,570.0	280.0	1,530.0	1,970.0	3,150.0	2,560.0
	Zn	N.D.	180.0	160.0	300.0	160.0	20.0	140.0	360.0	400.0	250.0
	Cu	5.7	53.2	60.2	187.0	80.9	52.2	72.0	144.0	179.0	127.0
	Ni	N.D.	1.7	3.0	18.0	13.0	N.D.	N.D.	0.2	N.D.	6.0
	Pb	N.D.	18.3	N.D.	4.0	25.0	N.D.	4.4	13.3	61.0	430.0
	P	300.0	1,680.0	3,360.0	13,260.0	5,560.0	530.0	3,290.0	6,850.0	12,100.0	6,200.0
	Si	1,480.0	1,740.0	1,090.0	2,340.0	4,950.0	2,820.0	10,300.0	12,000.0	25,930.0	11,470.0
	Cl	1.1	3.0	9.0	5.9	5.9	2.1	37.0	11.0	10.2	11.0
	S	8.2	27.5	45.0	23.5	32.0	13.0	45.0	40.0	20.2	30.0

Abbreviations: N.D. = not detected; samples are abbreviated as shown in Table 3.1.

Table 3.4 Elemental contents of fat ball, primary scum, and primary sludge samples collected in winter, summer, autumn, and spring (cont.).

Season		Autumn					Spring				
Source		FB	SC H	SC S	PS H	PS S	FB	SC H	SC S	PS H	PS S
Elements (mg/kg)	Na	2,550.0	29,300.0	4,930.0	16,200.0	13,900.0	1,800.0	14,580.0	4,810.0	12,900.0	16,000.0
	Fe	208.0	5,960.0	900.0	17,690.0	3,760.0	92.0	14,040.0	4,140.0	12,660.0	4,290.0
	Ca	34,840.0	22,730.0	8,740.0	10,770.0	7,960.0	24,240.0	13,770.0	4,780.0	9,900.0	9,239.0
	K	290.0	4,350.0	910.0	3,160.0	1,970.0	100.0	2,050.0	1,090.1	2,550.0	2,190.0
	Al	520.0	4,160.0	1,630.0	6,540.0	4,070.0	680.0	6,920.0	6,030.0	7,030.0	4,870.0
	Mg	280.0	4,860.0	670.0	3,810.0	2,940.0	150.0	2,490.1	1,230.0	3,310.0	3,080.0
	Zn	34.0	500.0	180.0	450.0	260.0	21.6	660.0	299.3	390.0	270.0
	Cu	15.2	134.0	51.0	155.0	94.0	4.0	160.0	97.3	86.0	90.0
	Ni	33.2	8.0	16.0	5.0	1.2	N.D.	11.8	2.5	5.0	10.3
	Pb	N.D.	1.1	N.D.	7.4	N.D.	N.D.	0.4	N.D.	N.D.	N.D.
	P	630.0	6,900.0	2,100.0	13,900.0	6,820.0	320.0	11,180.0	5,690.0	11,949.9	7,370.0
	Si	5,390.0	25,590.0	4,720.0	16,090.0	10,420.0	10,960.0	45,400.0	24,350.0	34,460.1	21,170.0
	Cl	2.0	30.0	6.0	2.5	11.0	8.0	9.0	5.0	5.0	60.0
	S	16.0	40.0	17.0	26.2	18.0	19.0	193.0	57.0	72.0	120.0

Abbreviations: N.D. = not detected; samples are abbreviated as shown in Table 3.1.

Table 3.5 Comparison of the elemental characteristics of fat balls, primary scum, and primary sludge in this study and previous literature.

Source	This study			[23]	[65]	[24]
	Fat Balls	Primary scum	Primary sludge	FOG deposits in the United Kingdom	FOG deposits in the United States	Primary scum in Canada
Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/L	mg/kg
Na	2,170.0	10,400.0	12,260.0	1,900.0	139.0	930.0
Fe	155.0	4,100.0	9,830.0	1,850.0	387.0	4,130.0
Ca	24,700.0	13,790.0	9,200.0	10,900.0	4,260.0	16,500.0
K	230.0	1,950.0	2,390.0	N.R.	N.R.	980.0
Al	430.0	4,070.0	5,290.0	1,080.0	257.0	3,550.0
Mg	230.0	1,840.0	2,880.0	910.0	117.0	1,060.0
Zn	25.0	310.0	310.0	N.R.	N.R.	N.R.
Cu	20.0	95.0	126.0	N.R.	N.R.	N.R.
Ni	33.0	12.0	8.5	10.0	N.R.	N.R.
Pb	N.D.	9.6	126.0	60.0	1.4	N.R.
P	445.0	5,130.0	9,650.0	N.R.	447.0	4,620.0
Si	5,160.0	15,650.0	15,850.0	N.R.	101.0	N.R.
Cl	3.3	14.0	14.0	N.R.	N.R.	N.R.
S	14.0	60.0	44.0	N.R.	N.R.	N.R.

Abbreviations: FOG = fats, oils, and grease; N.D. = not detected; N.R. = not reported

3.3.2 Characterization of screen sludge

The pretreatment stage in wastewater treatment involves the removal of large debris (wood, fabric, plastic, and other solids) from the influent [68]. The solid materials are efficiently separated through screens and subsequently undergo a process of shredding and dewatering, known as screen sludge (Figure 3.4).

The comparison between screen sludge characteristics from two different treatment sites reveals distinct seasonal trends and compositional differences (Table 3.6). In the WWTP H, screen sludge exhibited higher TS and VS during summer, indicating increased organic matter in the wastewater. In contrast, the WWTP S displayed lower TS values overall, with winter exhibiting the highest TS. Screen sludge of WWTP S had notably higher carbon content in summer. Carbon and hydrogen levels are highest in summer.

Figure 3.4 Screen sludge collected from: (a) WWTP H; (b) WWTP S

Table 3.6 Characteristics of screen sludge samples.

Source	Season	TS, %	VS, %TS	C, %	H, %	N, %	HHV, MJ/kg	LHV_w, MJ/kg
SCR H	Winter	38.3 ± 2	97.5 ± 1.5	54.1 ± 1	7.8 ± 0.2	2.6 ± 0.4	N.A.	N.A.
	Summer	47.3 ± 2.8	96.5 ± 0.6	61 ± 4.1	9.6 ± 1.3	2 ± 2	N.A.	N.A.
	Autumn	45.3 ± 8	94.6 ± 1.4	56 ± 10	8.9 ± 3.4	0.9 ± 0.7	29.9 ± 15.3	12.2 ± 7.6
	Spring	26.2 ± 0.8	95.5 ± 0.7	54.1 ± 2	8.2 ± 0.5	1.5 ± 0.6	40.2 ± 9.6	20 ± 5.4
Average		39.3	96	56.3	8.6	1.8	35.1	16.1
SCR S	Winter	28.6 ± 0.7	97 ± 0.4	47.5 ± 0.5	7.3 ± 0.5	1.4 ± 0.1	N.A.	N.A.
	Summer	20.7 ± 1	96.8 ± 0.3	63 ± 3.6	10 ± 0.8	1.5 ± 1.1	N.A.	N.A.
	Autumn	19 ± 2	93.6 ± 2.5	47 ± 3.8	7.1 ± 0.4	0.8 ± 0.3	N.A.	N.A.
	Spring	19 ± 3	96.3 ± 0.7	43 ± 2.5	6.6 ± 0.2	0.8 ± 0.3	42.3 ± 3	20.7 ± 1
Average		21.8	95.9	50.1	7.8	1.1	42.3	20.7

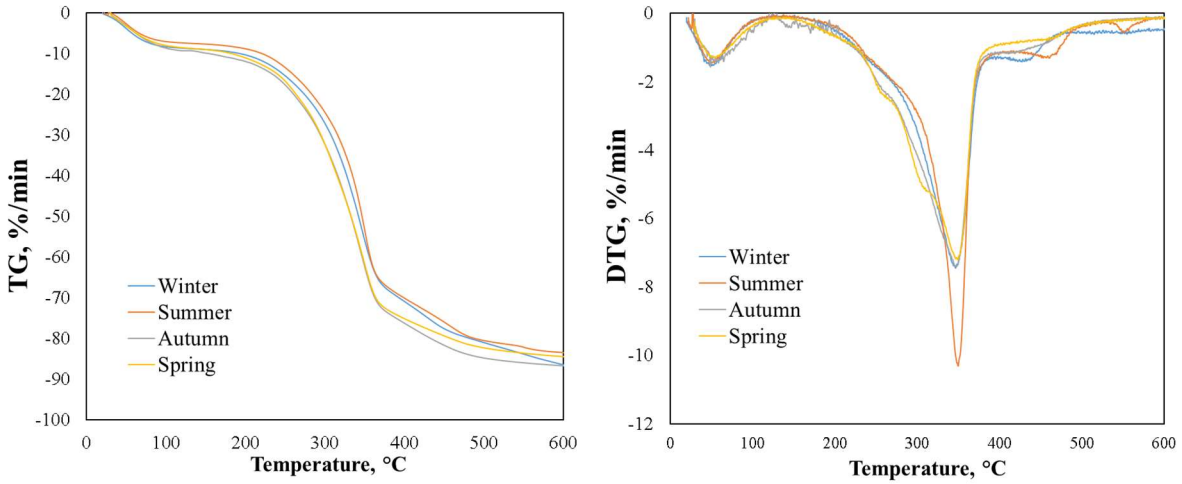
Abbreviations: N.A. = not analyzed; samples are abbreviated as shown in Table 3.1.

The elemental analysis of screen sludge revealed a diverse composition with varying concentrations, displaying notable seasonal and site-specific variations (Table 3.7). The thermal decomposition behaviour of the screen sludges remains relatively similar pattern throughout the season (Figure 3.5). This trend could be influenced by the woody biomass (cellulose and hemicellulose) which is the dominant component present in the screen sludge. In addition, the lignin may take higher temperatures (>400ºC) to degrade due to its complex and heterogeneous polymer [69].

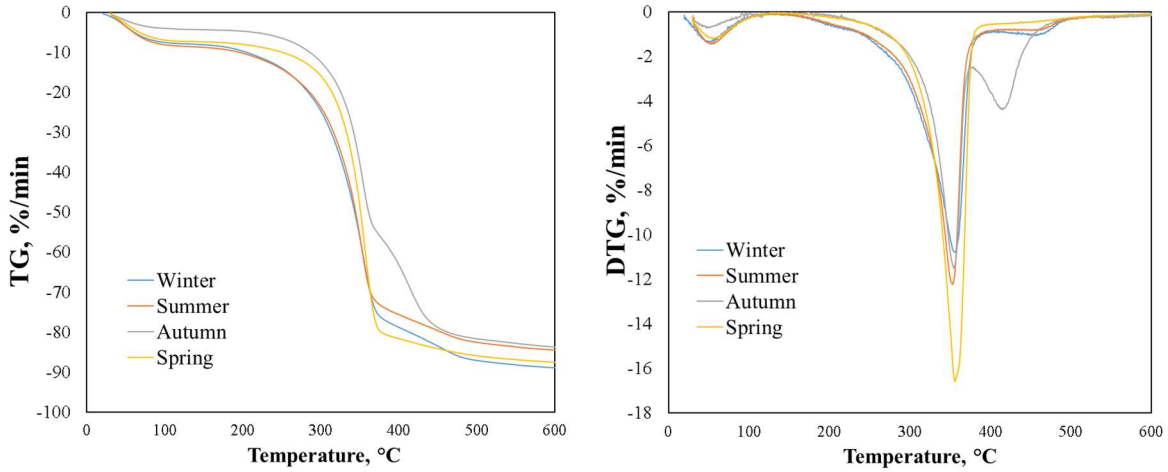
Table 3.7 Elemental contents of screen sludge

Season		Winter		Summer		Autumn		Spring	
Source		SCR H	SCR S	SCR H	SCR S	SCR H	SCR S	SCR H	SCR S
Elements (mg/kg)	Na	1,810.0	2,310.0	2,170.0	2,100.0	3,280.0	3,720.0	2,993.0	2,530.0
	Fe	1,110.0	380.0	1,570.0	870.0	1,380.0	2,570.0	1,240.0	1,990.0
	Ca	8,345.0	5,820.0	8,430.0	6,970.0	7,470.0	13,150.0	7,700.0	6,200.0
	K	306.0	330.0	250.0	180.0	655.0	550.0	373.0	335.0
	Al	450.0	690.0	870.0	640.0	2,390.0	1,770.0	910.0	1,290.0
	Mg	870.0	825.0	810.0	480.0	730.0	890.0	1,090.0	710.0
	Zn	130.0	43.5	140.0	140.0	66.0	160.0	86.0	205.0
	Cu	26.9	48.0	60.0	64.0	N.D.	N.D.	N.D.	N.D.
	Ni	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
	Pb	N.D.	N.D.	11.0	N.D.	N.D.	N.D.	N.D.	N.D.
	P	1,180.0	1,070.0	1,440.0	840.0	940.0	1,950.0	1,200.0	1,305.0
	Si	2,700.0	3,940.0	7,780.0	3,500.0	21,180.0	6,740.0	11,800.0	12,900.0

Abbreviations: N.D. = not detected; samples are abbreviated as shown in Table 3.1.



(a)



(b)

Figure 3.5 Thermogravimetric (TG) and differential thermogram (DTG) curves of screen sludges: (a) WWTP H; (b) WWTP S. Samples are abbreviated as shown in Table 3.1.

In this study, to better understand the composition of screen sludge, we conducted a fractionation process, categorizing the materials into two major groups: biomass and non-biomass substances (Table 3.8). The biomass substances identified in screen sludge predominantly comprise woody biomass, a complex material consisting of cellulose, hemicellulose, and lignin. The other substances in screen sludge present a complex mixture, including plastics, rubber, metals, and various solid materials (Figure 3.6).

Table 3.8 Fractionation of screen sludge components

Season	Sample	Biomass[a], %	Others[a], %
Winter	SCR H	96.0	4.0
Summer	SCR H	89.2	10.8
Average		**92.6**	**7.4**
Winter	SCR S	91.6	8.4
Summer	SCR S	86.5	13.5
Average		**89.1**	**10.9**

(a) Based on sample dry weight.Samples are abbreviated as shown in Table 3.1.

Figure 3.6 Separated non-organic matters found in screen sludge.

The fractionation analysis has revealed a significant composition in screen sludge with approximately >80% constituting woody biomass and the remaining composed of non-biomass components. The presence of woody biomass components may pose challenges for using as biodiesel feedstock. Typically, biodiesel production involves the extraction of lipids or fatty acids from triglycerides found in oils and fats. Due to the complex organic composition of woody biomass in screen sludge, biodiesel conversion may not be feasible. Taking into account the characteristics of screen sludge, alternative applications could be proposed such as for solid fuel [70].

The pretreatment process of screen sludge involves a cutting and washing process to breakdown the larger solids into smaller particles. This preparation is essential to facilitate the handling before screen sludges are directed toward incineration facilities. However, this process introduces the possibility of releasing smaller particles into the effluent, potentially contributing to an elevated concentration of microplastics within the wastewater treatment plant. Therefore, further analysis may be required to assess the microplastic's presence and investigate its potential implications.

3.3.3 Lipid content

We used a mechanical shaking method to extract lipids from waste samples, with hexane as the solvent. The average lipid contents extracted from SC and FB samples were generally comparable, ranging from 45.0% to 48.7% (Table 3.9). This finding may have occurred because chemically, FB and SC originate from discharged FOG that mixes with water and other contaminants in the sewage network [23,24]. Consequently, these samples are primarily composed of lipids. Such a relationship would explain the higher lipid concentrations in both SC and FB samples. In contrast, lipids in primary sludge mainly consist of settled organic compounds from wastewater influent [71]. Moreover, during the precipitation process in the primary clarifier, some organic matter may be microbially decomposed or transformed [28]. Therefore, the PS samples had relatively low lipid contents (6.7–19.9%).

The lipid yields of the different samples exhibited seasonal variation. FB and SC samples showed similar trends, with lower lipid yields in summer. Conversely, the lipid yield of PS was slightly higher in summer, possibly because that FOG accumulates more readily in cold temperatures. FOG tends to form a layer on the surface of water; in colder temperatures, it may more readily accumulate and combine with other sewage debris, whereas in warmer temperatures, it may oxidize and undergo more rapid decomposition [36,44].

Table 3.9 Lipid, transesterifiable, and biodiesel yields of pumping station and wastewater treatment plant samples.

Sample	Season	Lipid yield[a], %	Transesterifiable yield[b], %	Biodiesel yield[c], %
FB	Winter	56.6	36.9	20.9
	Summer	36.2	37.6	13.6
	Autumn	51.7	44.1	22.8
	Spring	50.0	44.7	22.4
average		**48.6**	**40.8**	**19.9**
SC H	Winter	50.6	44.0	22.3
	Summer	48.4	36.9	17.9
	Autumn	64.6	27.1	17.5
	Spring	16.2	46.3	7.5
average		**45.0**	**38.6**	**16.3**
SC S	Winter	74.1	29.2	21.6
	Summer	34.9	49.2	17.2
	Autumn	65.1	44.9	29.3
	Spring	20.5	55.4	11.4
average		**48.7**	**44.7**	**19.9**
PS H	Winter	12.9	46.5	6.0
	Summer	19.9	41.0	8.1
	Autumn	8.0	58.4	4.7
	Spring	10.7	46.4	5.0
average		**12.9**	**48.1**	**5.9**
PS S	Winter	14.9	40.3	6.0
	Summer	16.7	32.7	5.4
	Autumn	6.7	38.8	2.6
	Spring	11.4	35.6	4.1
average		**12.4**	**36.8**	**4.5**

Samples are abbreviated as shown in Table 3.1.

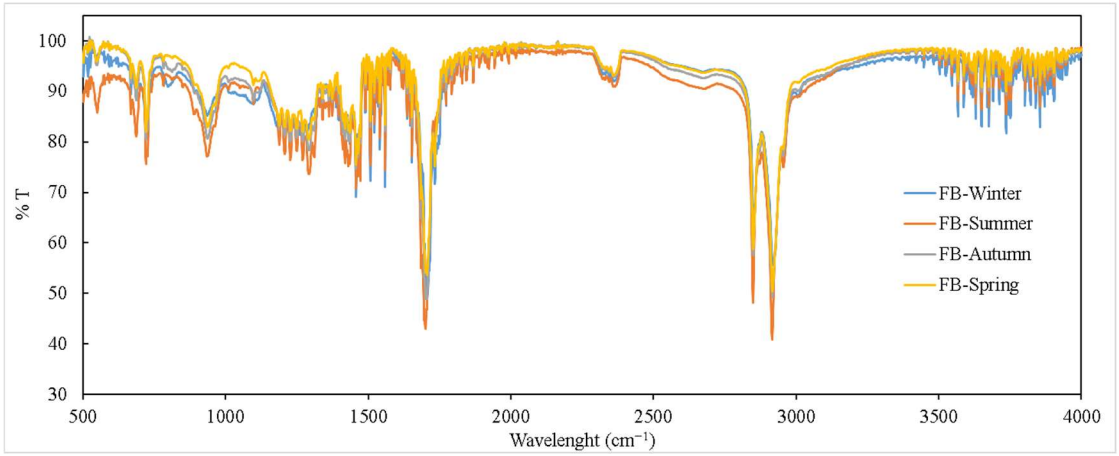
(a) Based on sample dry weight.

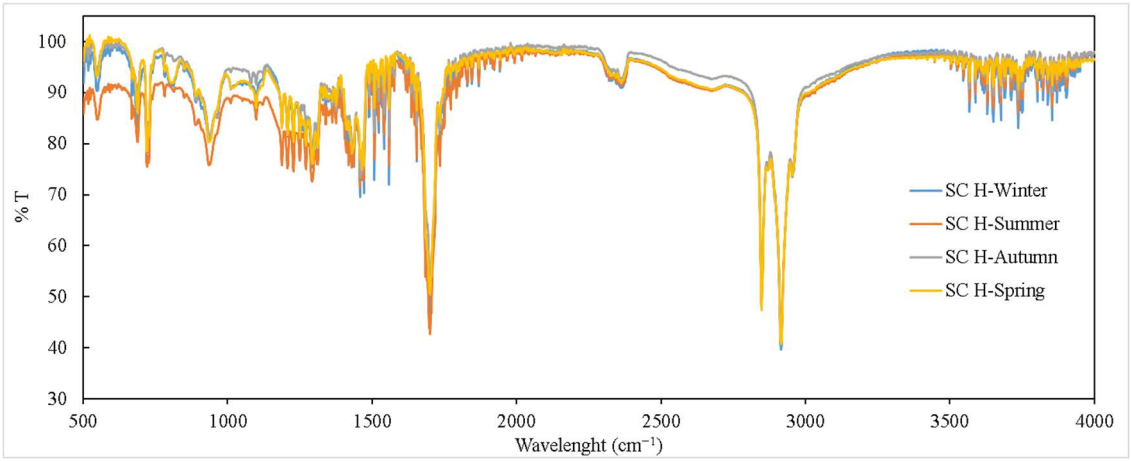
(b) Transesterifiable lipids (convertible into biodiesel) based on GC-MS analysis.

(c) Based on lipid and transesterifiable contents on a dry weight basis.

3.3.4 FTIR analysis of the lipid fraction

We used FTIR analysis to qualitatively characterize the functional groups of the lipid samples. Figure 3.7 presents the seasonally averaged FTIR spectra of the samples. All samples shared common peaks, indicating that the lipids had several similar functional groups across all samples. The absorption bands at 2,848 cm^{-1} and 2,915 cm^{-1} represented $–CH_2–$ stretching, indicating the presence of fatty acids. The band around 1,700 cm^{-1} represented axial deformation of the C=O group in esters [72]; the dominance of this peak indicated the presence of fats (e.g., free fatty acids and triglycerides) [23,73]. The band at 1,010–1,260 cm^{-1} represented the C–O stretching of aliphatic chains in crude soap mixtures (e.g., triglyceride, soap, and residue alkali) [66,74]. Finally, the absorption peaks between 900 cm^{-1} and 650 cm^{-1} indicated the presence of aromatic compounds (Chen et al., 2020).





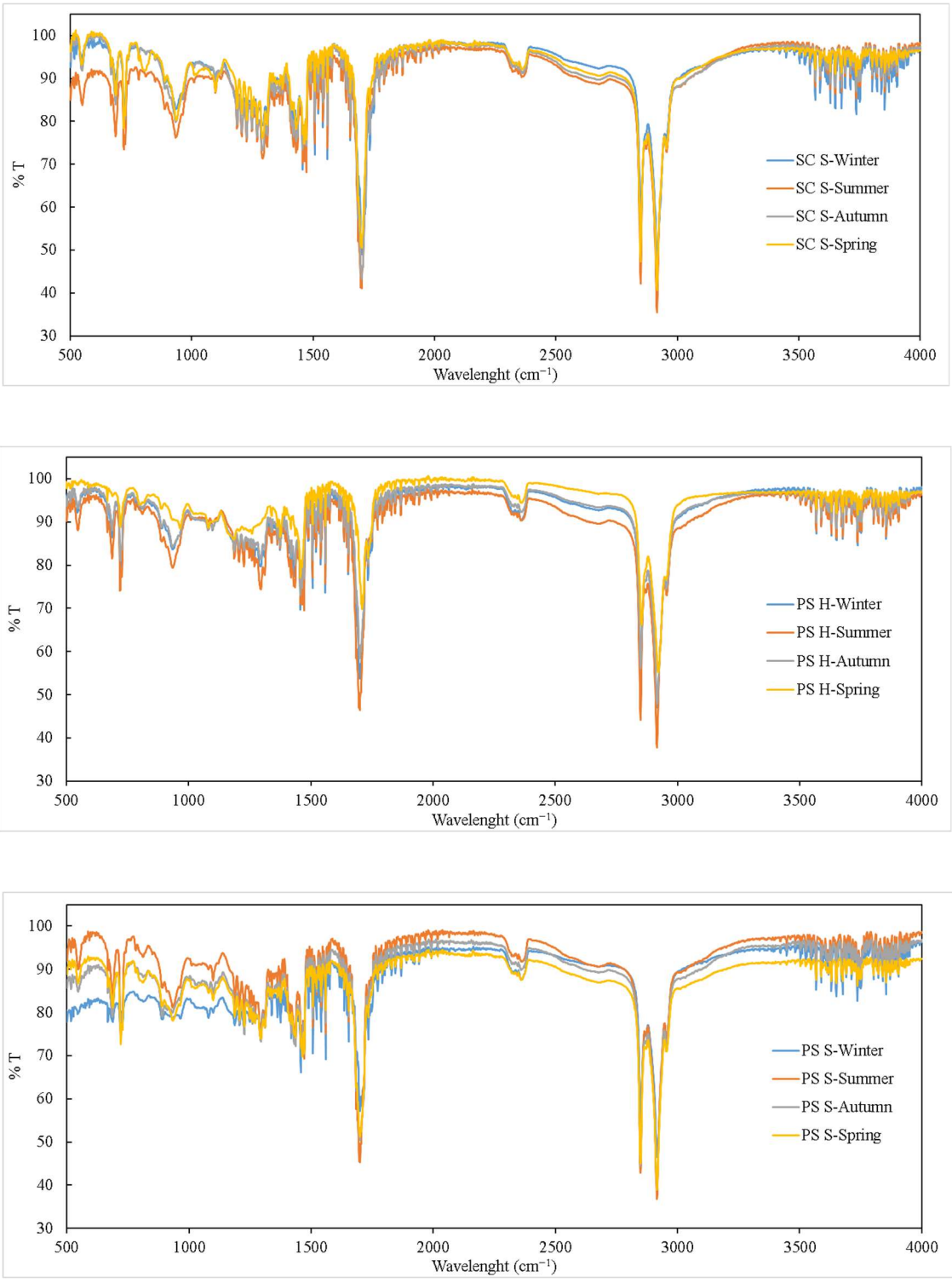


Figure 3.7 Fourier transform-infrared spectra of lipids extracted from fat balls, primary scum, and primary sludge. Samples are abbreviated as shown in Table 3.1.

3.3.5 Biodiesel potential and methyl ester analysis

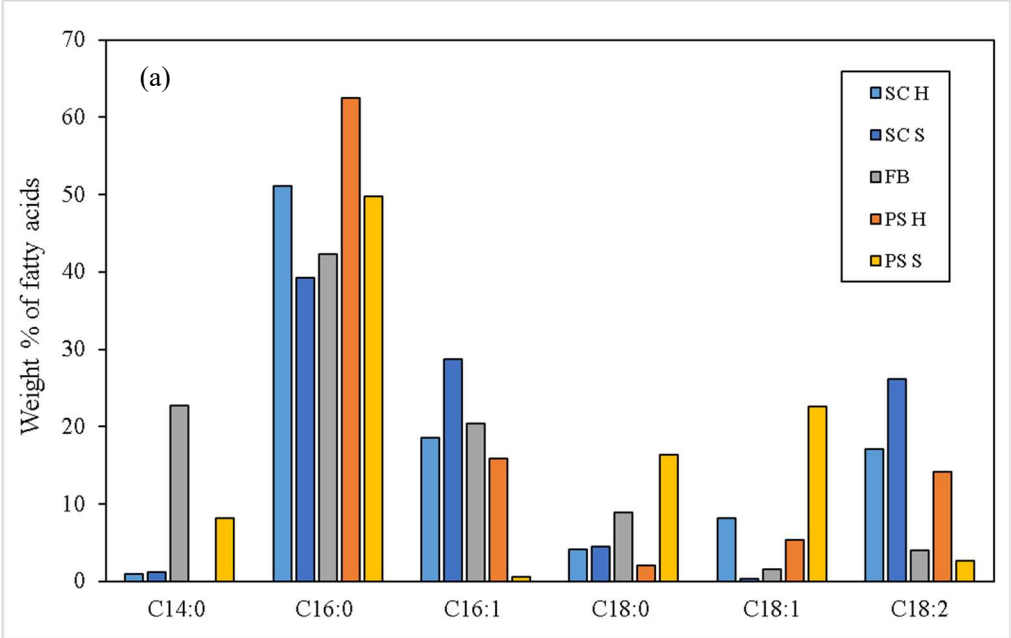
Extracted raw lipids are composed of acylglycerol and free fatty acids (i.e., transesterifiable fraction), which can be transformed into FAMEs/biodiesel, along with hydrocarbons, wax esters, steroids, terpenoids, and other non-polar compounds, which cannot be converted into FAMEs [15,17,41]. The average proportion of transesterifiable compounds considerably varied from 36.8% to 48.1% (Table 3.9); these yields were greater than the yields obtained by Dufreche et al. (2007), who converted FAMEs from activated sludge lipids. However, the present findings are generally consistent with other research concerning biodiesel conversion from different types of sludge, in which transesterifiable yields ranged from 36% to 76% [15,48,75].

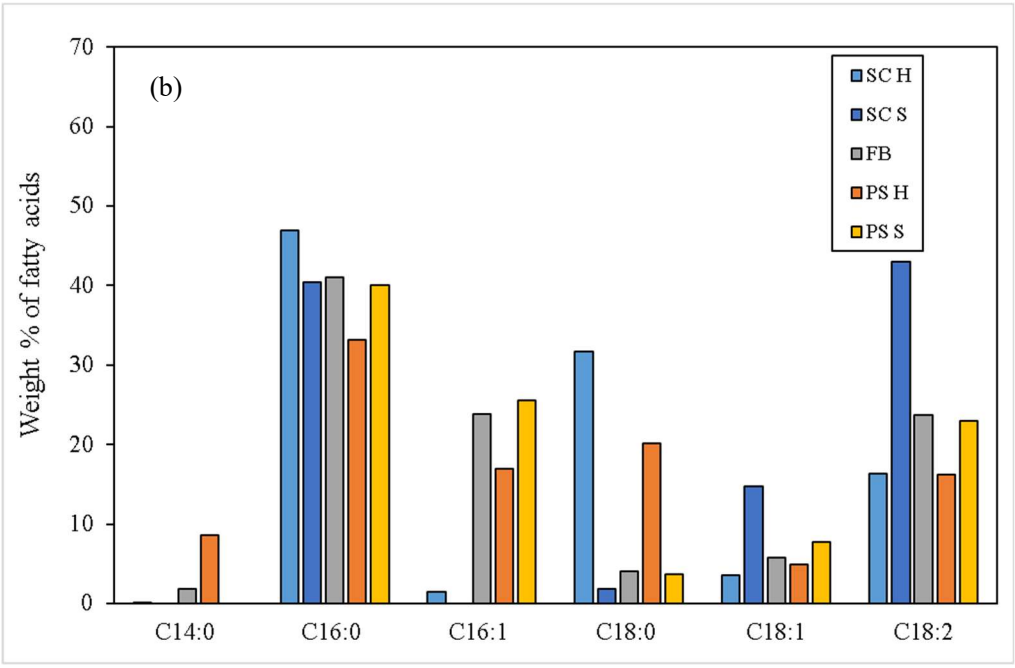
In the present study, the transesterifiable contents in the samples slightly fluctuated among seasons. Kobayashi et al. (2014) investigated the effects of seasonal variation in lipids extracted from restaurant grease trap waste. Their findings reported that lipid characteristics were influenced by oil sources, rather than seasons. This suggests that seasonal changes likely influence the utilization of different cooking oils in restaurants.

Total biodiesel yields were calculated based on the lipid and transesterifiable contents of the samples. The biodiesel yield varied from 4.5% to 19.9% (Table 3.9). The winter SC S sample had the maximum biodiesel yield (29.3%), whereas the autumn PS S sample had the lowest biodiesel yield (2.6%). The biodiesel yields of the FB and SC samples were higher than the biodiesel yields of PS, regardless of season. Our results are similar to a previous study, which showed that the total biodiesel yield declined as the wastewater treatment stage progresses from physical treatment to biological treatment stages [7]. This concluded that FOG-rich substances (fat balls and scum) are preferred for lipid feedstocks compared to primary sludge.

The methyl ester compositions according to sample and season are presented in Figure 3.8. The methyl ester contents considerably varied among samples and seasons. Methyl ester concentrations and compositions may be affected by the source oil [10]. [10]. Additionally, the composition of lipids in wastewater varies depending on wastewater treatment plant and the collection site where various lipid precursors accumulate [76]. Environmental factors can induce changes in the characteristics of fatty acids [77,78]. Despite variations in ester profiles, palmitic acid (C16:0) was

predominant in most samples, followed by stearic acid (C18:0) and linoleic acid (C18:2).





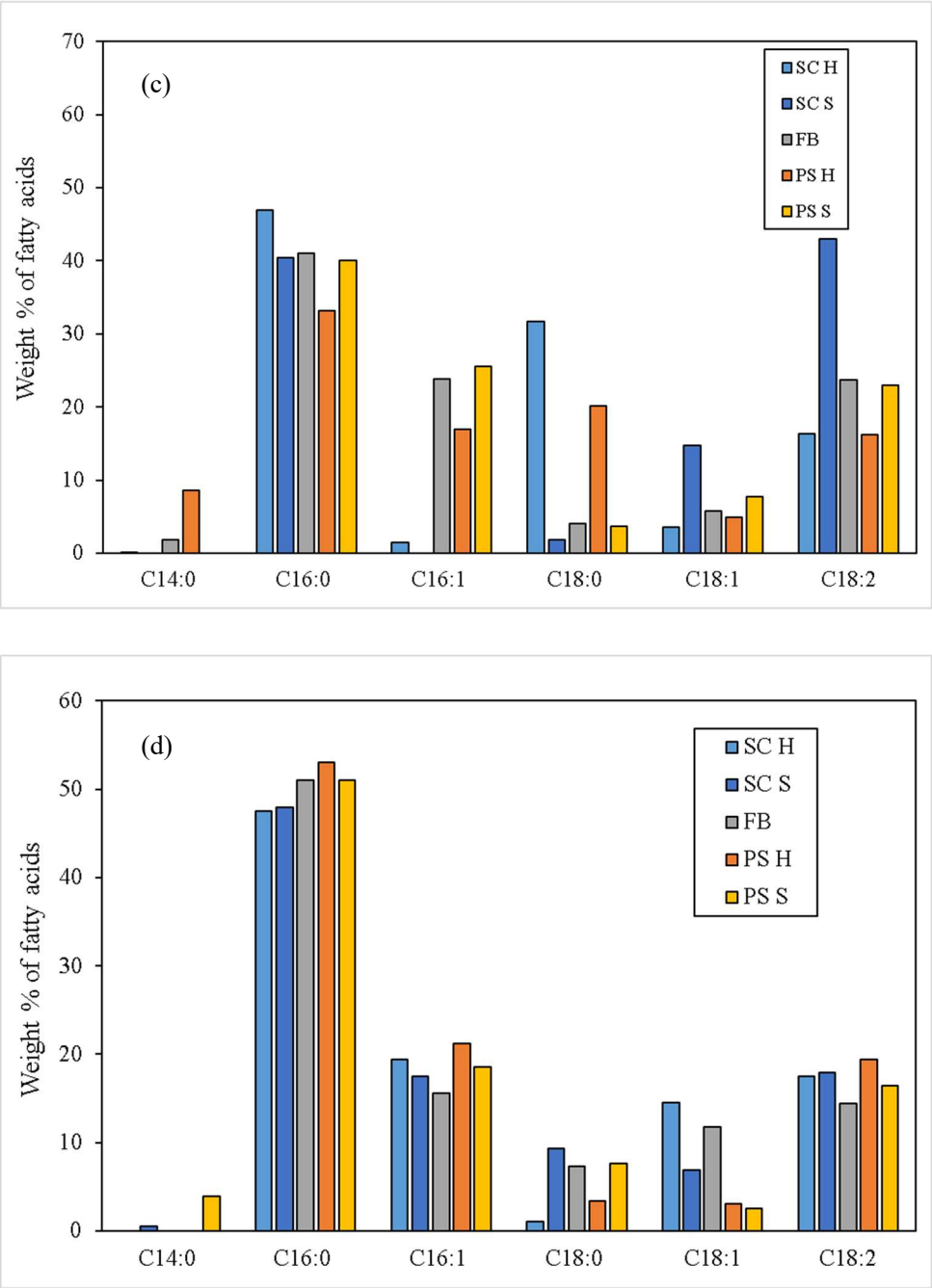
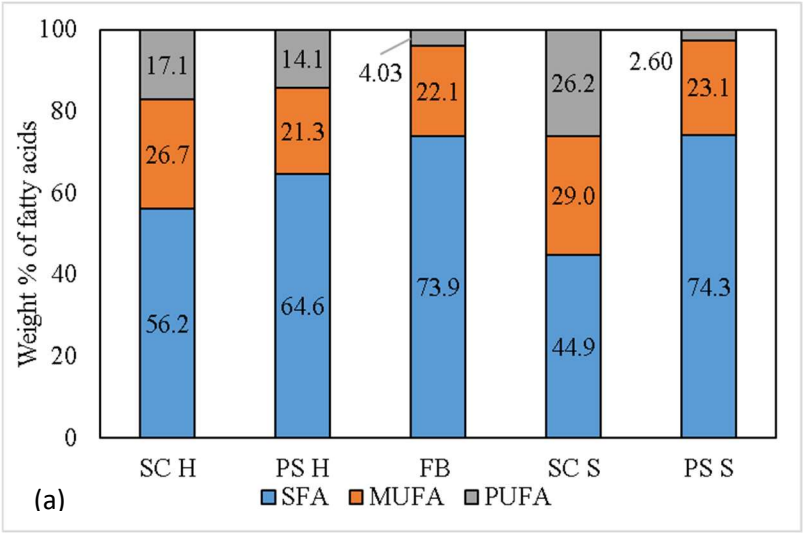
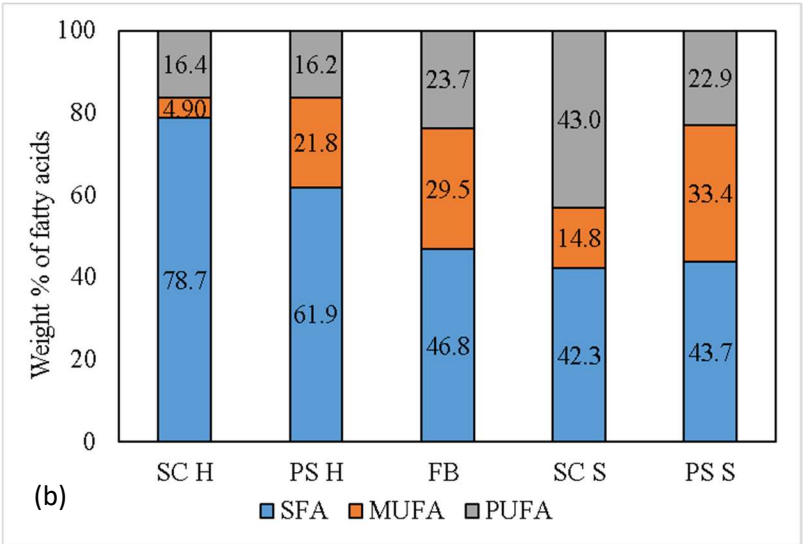


Figure 3.8 Profiles of the fatty acids myristic acid (C14:0), palmitic acid (CC16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) in biodiesel obtained from different samples during (a) winter, (b) summer, (c) autumn, and (d) spring. Samples are abbreviated as shown in Table 3.1.

The proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in biodiesel from wastewater lipids significantly varied among samples. However, all biodiesel samples mainly consisted of SFA (42.3–79.7%), followed by monounsaturated fatty acids MUFA (4.90–33.9%) and PUFAs (2.85–43.0%) (Figure 3.9). The saturation trends were consistent with previous reports [16,18,41].





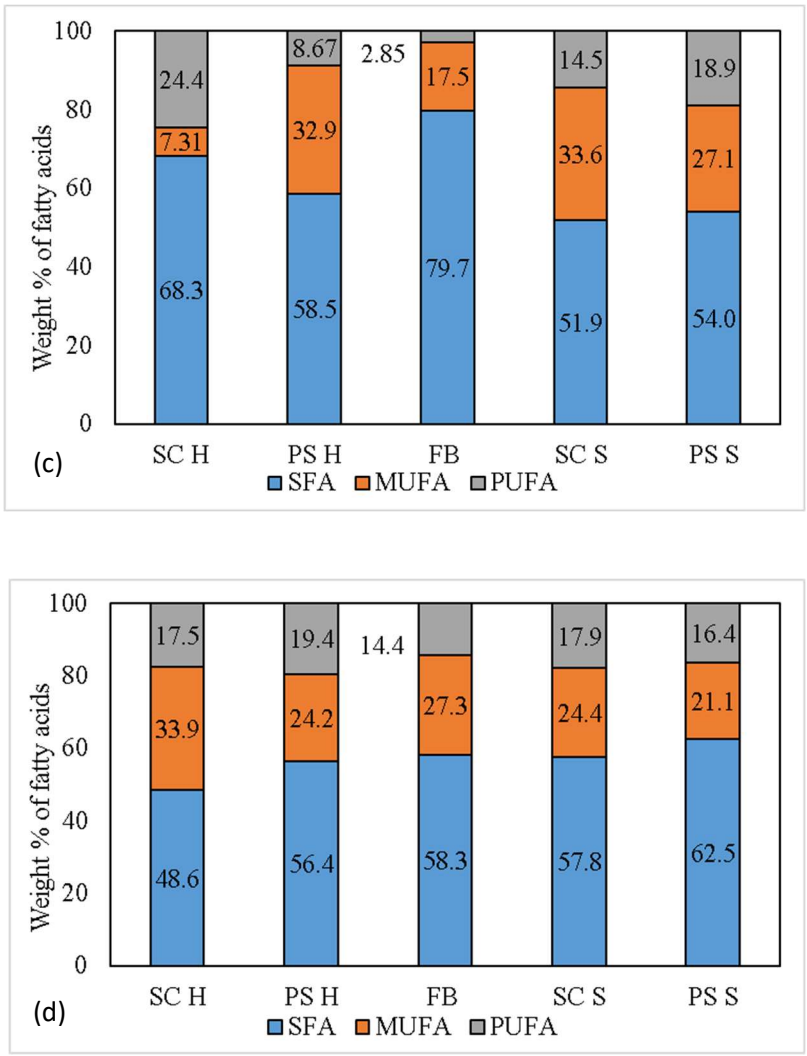


Figure 3.9 Profiles of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in biodiesel obtained from different samples during (a) winter, (b) summer, (c) autumn, and (c) spring. Samples are abbreviated as shown in Table 3.1.

The levels and composition of SFA vary, possibly due to the utilization of different types of fats and oils in warmer versus colder months [76]. Furthermore, the seasonal consumption of food might still be influenced by cultural or ritual connections, such as Thanksgiving, Christmas, and New Year celebrations [79]. Figure 3.10 shows some possible reasons for the variation in the fatty acids profiles.

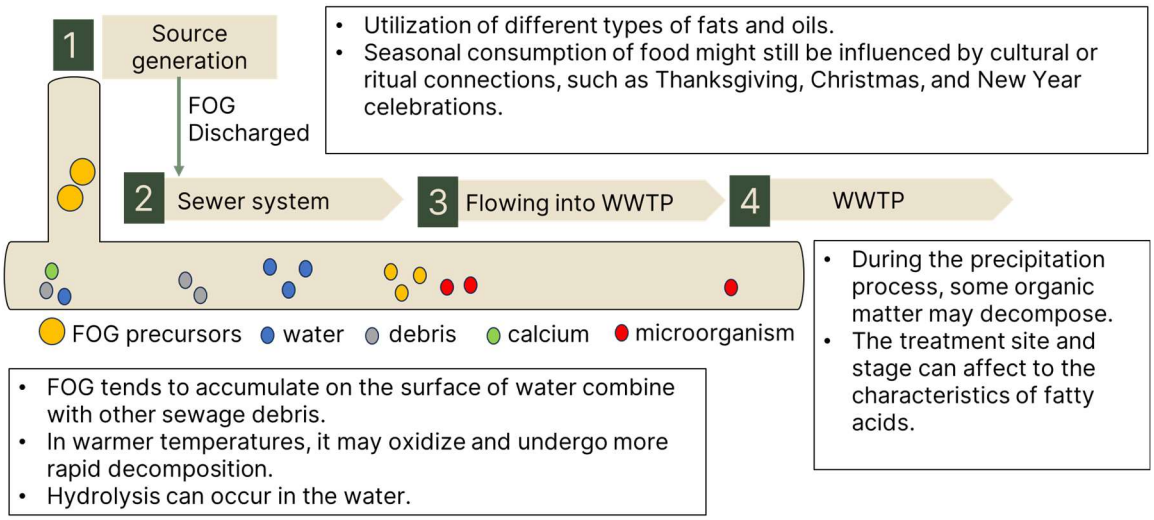


Figure 3.10 Possible factors involved in the variation of fatty acids profiles.

The methyl ester composition impacts biodiesel characteristics, including viscosity, stability, cold flow properties, and ignition quality. Biodiesel containing higher levels of SFAs and MUFAs is preferable due to its oxidative stability and improved combustion properties [8,80]. Our findings confirm that the FB and SC samples had desirable properties as potential lipid sources for biodiesel production. However, considering the high moisture content in the SC samples, initial concentration steps may be required before lipid extraction, which could increase production costs. Therefore, we recommend fat balls as the most promising feedstock for biodiesel production.

3.3.6 Contributions of biogenic and fossil C in wastewater lipid-derived biodiesel

Wastewater contains large amounts of organic C, which can originate from biogenic or fossil sources [81]. The fossil C fraction is typically derived from anthropogenic particulate matter (e.g., surfactants, pharmaceuticals, personal health care products, and industrial effluent) in wastewater [82,83]. The biogenic C fraction is derived from biomass (e.g., plants or animal byproducts). The use of lipids from wastewater as a potential biofuel feedstock requires assessing the proportion of biogenic C according to ^{14}C content. Therefore, we analyzed the biogenic and fossil C fractions of dry solids and biodiesel from summer samples by accelerator mass spectrometry.

Table 3.10 compares the contents of biogenic C between the present study and previous studies. The FB samples did not contain fossil C, indicating that they were primarily derived from natural sources. The SC samples had higher fossil C contents (4–8%), compared with the PS samples (1–5%). Our findings also reflected the impacts of different catchments; samples from WWTP H had higher fossil C contents (5–8%), compared with samples from WWTP S (1–4%). This difference was likely due to the distinct treatment capacities and operating conditions of the primary treatment systems [81].

Conversion of the extracted lipid samples into biodiesel enhanced the fossil C proportions, from 0–8% (dry solids) to 26–42% (biodiesel). This enhancement could be due to the inclusion of methanol (a fossil-derived reagent), which chemically bonded with methyl esters ($ROOCH_3$), contributing to 5–10% of fossil C [37,38]. Previous studies reported that commercial 100% biodiesel (B100) had a biogenic C content of 92.4–94.6% due to the use of methanol during biodiesel production [37,38]. Based on our initial feedstock (< 10% fossil C), the biogenic fractions of our biodiesel samples should have been comparable with B100, assuming that most of the fossil C was derived from methanol. However, our biodiesel samples contained lower biogenic C contents. The reason for this discrepancy is unclear, but it may have resulted from variations in C fractions driven by the fatty acid composition. Lee et al. (2022) noted that the ^{14}C content of biofuel can vary according to factors such as biomass age, type, and geographical source. Additionally, we cannot rule out the possibility that other fossil-derived chemicals remained in the biodiesel after processing, thus impacting the fossil C fraction. Nevertheless, our findings highlight the successful conversion of wastewater-extracted lipids into biodiesel containing biogenic C levels of 58–74%, representing substantial improvements relative to the biogenic C contents of B20 (22%) and petroleum diesel (2%).

Table 3.10 Biogenic and fossil C contributions in wastewater treatment plant samples and biodiesel from this study and diesel in previous studies.

Source		C fraction	
		Biogenic, %	**Fossil, %**
Dry solids	FB	100	0.0
	SC H	92	8.0
	PS H	95	5.0
	SC S	96	4.0
	PS S	99	1.0
Biodiesel	FB	74	26
	SC H	58	42
	PS H	67	33
	SC S	72	28
	PS S	64	36
Biodiesel 100% (B100)[1]		92.4	7.6
Biodiesel 100% (B100)[2]		94.6	5.4
Biodiesel 20% (B20)[1]		22	78
Petroleum diesel[1]		2.0	98

Samples are abbreviated as shown in Table 3.1.

[1] Lee et al., 2022

[2] Sebos, 2022

3.3.7 Estimation of biodiesel potential and CO_2 emissions

Based on information obtained from the wastewater facilities in Kobe, Japan, where we collected samples, the facilities generated 8.3 kg (pumping station FBs), 77.2 kg (SC H), and 47.7 kg (SC S) of materials per day (wet basis). Using these estimates and our experimental data, we calculated that a total of 97.5 kg of lipids per day could be extracted from the facilities (pumping station and WWTP H and S); these lipids could produce 35 kg of biodiesel per day (Table 3.11).

Table 3.11 Estimated daily biodiesel potential of materials from a pumping station and two wastewater treatment plants (WWTPs) in Kobe, Japan.

Source	Sample	Wastewater flow rate, m^3/day	Dry matter, kg/day	Lipids, kg/day	Biodiesel, kg/day
Pumping station	FB	37,000	4.40	2.10	1.00
WWTP H	SC H	172,000	1.40	0.710	0.300
	PS H		380	60.0	23.0
WWTP S	SC S	135,000	1.51	0.820	0.300
	PS S		230	34.0	10.3

Samples are abbreviated as shown in Table 3.1.

We found that primary sludge exhibited the largest lipid and biodiesel amount (about 34-60 and 10.3–23.0 kg/day, respectively). However, primary sludge would need to be collected from the sedimentation reactor prior to lipid extraction. In this regard, the study of Kargbo (2010) reported that biodiesel production from sludge faced huge challenges including collecting sludge, extracting lipids, and performing optimum transesterification. Additional equipment and process considerations are necessary if the sludge would be fed directly to the lipid extractor on-site of WWTP. In contrast, although fat balls and primary scum exhibited lower lipid potential than sludge, isolation of these waste materials is possible; they can be skimmed out of the system without being mixed into the current sludge treatment process and then treated outside of WWTPs [25]. Furthermore, the biodiesel process for these fat balls and primary scum will have little impact on conventional sludge treatment systems or sewage treatment systems. Therefore, if the economics of this biodiesel process can be assured, continued operation of the sewage treatment plant is feasible.

Japan has over 2,100 WWTPs and 3,700 pumping stations, which can process ~40,120,000 m^3 of wastewater per day [31]. Extrapolating from our above-described calculations, we estimated that 447.0 metric tons of biodiesel per year could be produced from wastewater fat balls and primary scum waste in Japan (Table 3.12). Importantly, this value may be an overestimation because fat balls and primary scum likely are not frequently skimmed at treatment facilities. Nevertheless, this value suggests that efficient management of wastewater-derived lipids could make them a

valuable resource for biodiesel production, which would match the goals of achieving a circular economy.

The biogenic and fossil CO_2 emissions associated with biodiesel can be calculated according to Eqs. (4) and (5) [37]:

Biogenic CO_2 in biodiesel =

$$\text{biodiesel quantity} \times \text{C content} \times \text{biogenic fraction} \times (44/12) \quad (4)$$

Fossil CO_2 in biodiesel =

$$\text{biodiesel quantity} \times \text{C content} \times \text{fossil fraction} \times (44/12) \quad (5)$$

By assuming a biodiesel C content of 0.83 g C/g [38], we estimated that the potential maximum amount of biodiesel produced annually in Japan from fat balls and primary scum (447 metric tons/year) would release 360 metric tons CO_2eq of fossil CO_2 emissions per year; petroleum-based diesel production would result in almost four times more CO_2 emissions. (Table 3.12). Unlike the emissions from petroleum-based fuels, biogenic C is presumed to contribute zero additional atmospheric CO_2 emissions [64]. Therefore, the use of wastewater lipids as an alternative feedstock for biodiesel production is expected to reduce greenhouse gas emissions.

Table 3.12 Biodiesel potential and CO_2 emissions based on pumping station (fat balls) and wastewater treatment plant (primary scum and sludge) samples.

Source	**Wet quantity, metric tons/year**	**Dry matter, metric tons/year**	**Lipids, metric tons/year**	**Biodiesel, metric tons/year**	**CO_2 emissions from biodiesel, metric tons CO_2eq/year**		**CO_2 emissions from petrodiesel[1], metric tons CO_2eq/year**
					Biogenic C fraction	**Fossil C fraction**	
Fat balls	3,300.0	1,660.0	810.0	410.0	920.0	320.0	1,240.0
Primary scum	6,600.0	184.0	86.0	37.0	72.0	40.0	110.0
Primary sludge	251,000,000.0	1,500,000.0	190,000.0	78,300.0	156,000.0	82,200.0	240,000.0

[1] CO_2 emissions of petrodiesel were estimated at 3.15 kg CO_2 per kg [85]

Between 2009 and 2020, the demand for diesel fuel in Japan was consistently within the range of 21,600,000.0 to 23,800,000.0 metric tons [86], as indicated in Table 3.13. However, as of 2022, the biodiesel production capacity in Japan was reported to be between 18,000.0 and 20,000.0 metric tons [86,87]. This highlights a substantial disparity between biodiesel production capacity and the demand for diesel fuel in Japan. Teixeira et al. (2018) noted that over 0.5 million metric tons of used cooking oil is generated in Japan and could potentially be used for biodiesel feedstock; however, the establishment of a cost-effective and efficient collection network remains a challenge in optimizing the valorization of used cooking oil (UCO) [89]. Japan has been importing used cooking oil to meet the demand for biodiesel production. However, this reliance on imports presents challenges including cost implications and transportation logistics [90]. Therefore, the extraction of lipids from wastewater could potentially offer a solution to supplement traditional biodiesel sources. This approach not only addresses energy security concerns but also aligns with waste management targets by converting wastewater residuals into a valuable resource.

Table 3.13 Japan's petroleum diesel demand and used cooking oil potential for biodiesel production.

	Amount, metric tons/year	**References**
Japan's petroleum diesel demand	21,600,000.0–23,800,000.0	[86]
Japan's Used cooking oil (UCO) potential	500,000.0	[88,89]
Japan's biodiesel production from UCO	18,000.0–20,000.0	[86,87]
Japan's Imported UCO for biodiesel production	800.0–880.0	[90]
Biodiesel potential from fat balls and primary scum in Japan	447.0	This study
Biodiesel potential from primary sludge in Japan	78,300.0	This study

3.4 Conclusion

We investigated the characteristics of and potential for biodiesel production using lipid sources from a pumping station (i.e., fat balls) and WWTP primary clarifier (i.e., primary scum and primary sludge) in Kobe, Japan. FB samples from the pumping station had higher TS and C contents, along with greater calorific values, than SC and PS samples collected at the primary clarifier. FB and SC samples had higher lipid contents (45.0–48.7%) and biodiesel yields (16.3–19.9%), compared with PS samples (lipid yield: 12.4–12.9%; biodiesel yield: 4.5–5.9%). Considering that primary scum has a higher moisture content, our findings suggest that fat balls are the most promising biodiesel feedstock from wastewater facilities. Analyses of methyl ester composition revealed that FAMEs (i.e., biodiesel) produced from the samples contained high SFA and MUFA contents. Moreover, ^{14}C analysis revealed that the produced biodiesel contained 58–74% biogenic C. Biodiesel conversion of screen sludge may not be feasible due to the complex organic composition. Further analysis may be required to assess the presence of microplastics following treatment of screen sludge. Taken together, our findings highlight the potential for alternative resource management strategies at wastewater facilities to recover valuable resources for the production of value-added products. Future research efforts should focus on optimizing lipid recovery to further enhance biodiesel yields.

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Chapter 4 Extraction of Lipid from Fat Balls by Liquefied Dimethyl Ether: Modeling and Optimization of Process Parameters by Response Surface Methodology

4.1 Introduction

Global concerns over the depletion of fossil fuels and the environmental pollution problem have led to the development of carbon-neutral energy sources [1]. Biodiesel, as one of the most promising biofuels, has attracted attention due to lower emissions, renewability, and biodegradability [2,3]. Biodiesel consists of fatty acid methyl esters (FAMEs) that can be produced from the transesterification reaction of vegetable oil or animal fat [4]. The current biodiesel feedstock relies mainly on edible vegetable oil sources, which account for 70–80% of the total production cost, thus limiting the growth of biodiesel commercialization [5–7]. Therefore, there is an urgent need for cost-effective and sustainable biodiesel feedstocks. In this context, the utilization of fat balls from wastewater treatment plants has emerged as a viable alternative for biodiesel production, promoting the valorization of waste sources.

The term fat balls refers to fats, oils, and grease (FOG) deposits that accumulate on the water surface of the sewage treatment process. This waste is considered a nuisance and disposed of by incinerating or landfilling [4]. The raw fat balls consist primarily of fats, oils, and greases (FOG), along with water, plastics, and other impurities. William et al. (2012). examined FOG waste from different locations such as pumping stations, sewers, and sewage works. The study confirmed the fat balls from the pumping station had a low moisture content (44%) and a significant proportion of extractable oils (181 mg/g). Collin et al. (2020) analyzed fat balls obtained from a pumping station and inlet of a sewage treatment plant in London, UK, and recorded about 93-94% (dry base) of lipids. Due to their high lipid content and availability, fat balls have the potential to serve as an alternative lipid feedstock for biodiesel.

The various conventional methods used for lipid extraction from wastewater residual include Soxhlet extraction, the Bligh–Dyer method, and liquid–liquid extraction [9]. These methods employ various organic solvents such as hexane, chloroform, isopropyl alcohol, dichloromethane, and methanol [10–12]. However, these processes are affected by solvent characteristics and are less effective for raw materials with high moisture content; in addition, the post-extraction process consumes

high amounts of energy, which is not sustainable [13,14]. Although a pretreatment step can be employed to enhance extraction performance, it tends to be energy-intensive. Consequently, finding an alternative extraction method would be preferable.

Dimethyl ether (DME) is a synthetic polar gas (at room temperature) that has gained prominence as an eco-friendly and non-toxic extraction agent [10]. It possesses the ability to be liquefied under pressure and vaporize at standard temperatures [11]. In its liquefied state (0.51 - 0.59 MPa at room temperature), DME has a strong affinity for oil-based substances, with a solubility in water of about 7.8%. Consequently, it has proven to be effective in the extraction of neutral and complex lipids from wet feedstock [12,13]. Several studies have reported lipid recovery by DME from various feedstocks including microalgae, sewage sludge, and biomass [14–19]. It is reported that extraction using liquefied DME can achieve comparable lipids yield and properties close to the conventional extraction methods [13,17,19,20]. Furthermore, organic matter can be easily recovered after extraction and DME can be recycled, reducing the energy required. While numerous researchers have performed lipid extraction procedures using DME, the impact of the processing parameters, the possible interactions among parameters, and extraction optimization have not been adequately explored. Processing parameters can affect the properties and enhance extraction efficiency [13].

In the present study, we investigated the performance of lipid extraction from fat balls using the DME technique and optimized the process parameters (sample size, velocity of liquefied DME, and DME/sample ratio) using response surface methodology (RSM) through a Box–Behnken design (BBD). The performance of DME was compared to mechanical shaking and Soxhlet extraction in terms of the lipid and biodiesel yield and FAME profiles. The results of this study have significance for the development of lipid extraction technology and the better use of wastewater residuals for sustainable biodiesel feedstock.

4.2 Materials and methods

4.2.1 Sample preparation

Fat balls were collected from the water surface layer of a pumping station in Kobe, Japan. The fat balls appeared as floating, yellowish, spherical substances. The samples

were stored at 4°C immediately after collection. Table 4.1 presents the characteristics of fat balls.

Table 4.1 Characteristics of fat balls.

Total solids (TS), %	Moisture (TS), %	Higher heating value (HHV), MJ/kg	Lower heating value wet base (LHVw), MJ/kg	C, %	H, %	N, %	O, %
72	28	39.2	20.1	68.4	11.4	0.2	12.5

DS: dry sludge.

4.2.2 Dimethyl ether (DME) Extraction methods

Figure 4.1 shows a schematic diagram of the DME apparatus. The experimental device was a fixed bed extraction system consisting of three main parts: the DME supply tank/vessel 1 (TVS-1-100; volume: 100 cm3, Taiatsu Techno Corp., Japan), the extraction column/vessel 2 (HPG-10-5; volume:10 cm3, Φ 11.6 mm × 190 mm, Taiatsu Techno Corp.), and the recovery tank/vessel 3 (HPG-96-3, volume: 96 cm3, Taiatsu Techno Corp.). A needle valve was installed to control the liquefied DME flow rate.

The experiments were carried out according to the conditions specified in Table 4.2. Fat balls and glass beads were packed into vessel 2. A filter (polytetrafluoroethylene; pore size: 0.8 μm; Advantec Toyo Kaisha Corp., Japan) was placed at the outlet of vessel 2. Liquefied DME was produced by cooling pure gaseous DME (Tamiya Ltd., Japan) to −12°C with ethanol (Guaranteed Reagent; Wako Pure Chemicals Ltd., Japan) and ice and then stored in vessel 1. Vessel 1 was then placed in a water bath, and the temperature was maintained at 37°C. Liquefied DME was transferred from vessel 1 to vessel 2 under pressurization at 0.7 MPa at room temperature. The flow rate was adjusted by a needle valve, and the liquefied DME passed through vessel 2 at different velocities. A color change in vessel 2 indicated the presence of lipid extract. The total consumed liquefied DME was set at around 150 mL per batch.

After the extraction process, the lipids were recovered by reducing the pressure of vessel 3, allowing for complete gasification of the DME. The adhered raw lipid in vessel 3 was flushed with hexane and dried to determine the lipid yield. The lipid extraction yield is given by Eq. (1):

$$\text{Lipid yield (\%)} = \frac{W}{W_0}\, x\ 100\% \ , \qquad (1)$$

where W_0 is the weight of the initial fat balls (g), and W is the weight of the lipid extracted (g).

The mass balance for DME extraction was calculated by measuring the weight variation of vessels 1–3 before and after the experiments.

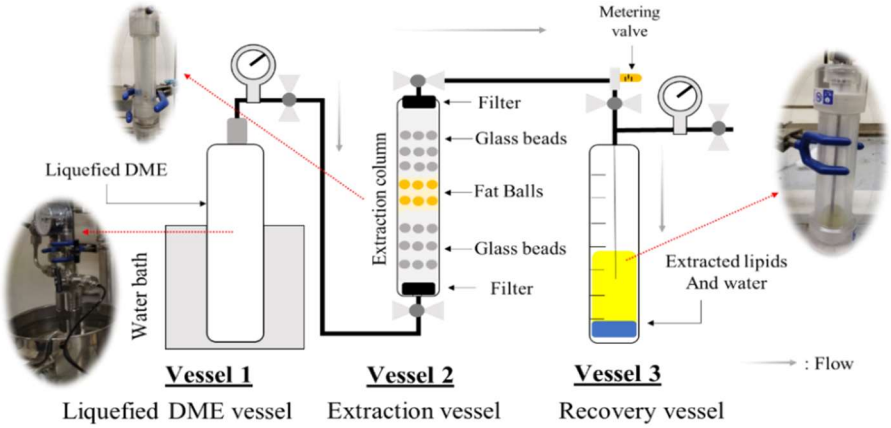


Figure 4.1 Schematic diagram of the lipid extraction process using liquefied dimethyl ether.

4.2.2.1 Optimization of DME extraction using RSM

RSM through the BBD was used for the optimization of lipid extraction of fat balls by DME. Three operational parameters, sample size, velocity, and DME/sample ratio, were chosen to study the independent and interactive effects of the variables on the lipid extraction yield. Table 4.2 illustrates the factors and levels for lipid extraction by DME.

Table 4.2 Independent variables: units and range of actual values.

Parameter		Unit	Level		
			−1	0	1
X_1	Sample size	Mm	1	3.3	5.6
X_2	Velocity	m/h	2.8	5.7	8.5
X_3	DME/sample ratio	mL/g	10	45	80

Minitab software (Version 21; US) was utilized to conduct the statistical analysis. The experimental outcomes were developed with a second-order polynomial equation using response surface regression analysis, as given by Eq. (2):

$$Y = b_0 + \sum_{t=1}^{3} biXi + \sum_{t=1}^{3} biiXi^2 + \sum_{t=1}^{3} \sum_{t=1}^{3} bijXiXj \quad , (2)$$

where Y is the response factor (lipid yield); b_0 is the constant coefficient; X_i is the independent variable; and b_i, b_{ii}, and b_{ij} are the coefficients of linear, quadratic, and interaction terms, respectively.

4.2.3 Conventional extraction of lipids with hexane

Mechanical shaking extraction and Soxhlet extraction were utilized to analyze the extraction performance of the DME technique. Hexane was selected due to its advantage in extracting organic substances [11].

4.2.3.1 Mechanical shaking with hexane method

Liquid-liquid extraction of lipid was carried out using a mechanical shaker (SA300; Yamato Scientific Co., Ltd., Tokyo, Japan) with hexane (Guaranteed Reagent; Wako Co., Ltd., Tokyo, Japan) [19,21]. Hexane was selected due to its advantage in extracting lipid substances [22]. The extraction process was divided into three consecutive experiments with a ratio of 5:1 (hexane/fat balls) for 60 minutes at ambient temperature (200 rpm). After shaking, the tube was centrifuged at 3,000 rpm for 15 minutes. The supernatant phase was collected and dried a stream of N_2. The remaining solid content represents the lipid content (Eq.1).

4.2.3.2 Soxhlet extraction

The extraction of lipids was carried out using a Soxhlet apparatus [23]. Fat ball samples (1 g) were placed into a thimble filter (26 × 30 × 100 mm, No. 84, Advantec Toyo Kaisha, Ltd., Japan) and extracted using 50 mL of hexane. The extraction was performed at 80°C for eight hours. After extraction, hexane was removed by rotary evaporation at 40°C. The lipid extract was then dried to constant mass in a vacuum desiccator. The lipid yield was calculated according to Eq.1.

4.2.4 Characterization techniques

The content of total solids (TS) and volatile solids (VS) were determined according to the standard method 2540 G [24]. The C/H/N analysis was measured using an elemental analyzer (JM10; J-Science Lab Co., Ltd., Kyoto, Japan). The calorific value

was determined using a bomb calorimeter (CA-4J; Shimadzu Co. Ltd.). The lower heating value (LHV) of dry solids was calculated from the measured higher heating values (HHV) [25]. The functional group compositions of the fat balls, residue, and extracted lipids were measured via Fourier transform-infrared spectroscopy (FTIR; IRSpirit-T; Shimadzu) in attenuated total reflectance (ATR) mode. The detection range was 500–4000 cm^{-1}.

For fatty acids analysis, the extracted lipids (up to 50 mg) were converted into fatty acid methyl esters using a fatty acid methylation kit (06482-04; Nacalai Tesque, Kyoto, Japan); the mixtures were heated in a constant temperature bath (EYELA Co., Ltd., Tokyo, Japan) at 37ºC. The analysis of methyl ester composition was done by gas chromatography-mass spectrometry (GC-MS; QP2010 Plus; Shimadzu Corp.) equipped with an SP-2560 capillary column (100 m × 0.20 µm × 0.25 µm; Supelco, Bellefonte, PA, USA). The GC oven temperature program was initially 100°C, held for 5 min, then increased to 180°C at a rate of 4°C/min and 240°C at a rate of 2°C/min, and then held at 240°C for 15 min. The calibration curve was created using the fatty acids standard (Supelco 37 Components FAME Mix, Sigma-Aldrich, Japan). Quantitative analysis was conducted to measure the quantity of transesterifiable substances. The biodiesel yield (dry basis) was calculated based on the mass of lipids extracted and its transesterifiable material.

4.3 Result and discussion

4.3.1 Development of the regression model equation for lipid extraction by DME

4.3.1.1 Box-Behnken design experiments

The lipid extraction using the dimethyl ether (DME) was performed using a Box Behnken experimental design (BBD) with three levels of three factors consisting of 15 experiment runs, as shown in Table 4.3. The lipid yield (response variable) is expressed in terms of the percent of dry base. The results show lipid yield varied from 46.6 to 65%.

Table 4.3 Box Behnken design containing coded and actual levels of main variables and response variables for the model of lipid extraction from fat balls by DME method.

Run order	Coded			Uncoded			Lipid yield, %	
	X_1	X_2	X_3	X_1	X_2	X_3	Experimental	Predicted
1	-1	-1	0	1	2.8	45	61	59.9
10	1	-1	0	5.6	2.8	45	59	58.3
7	-1	1	0	1	8.5	45	60	60.8
11	1	1	0	5.6	8.5	45	46.6	47.7
2	-1	0	-1	1	5.7	10	55	55.2
8	1	0	-1	5.6	5.7	10	53	52.8
4	-1	0	1	1	5.7	80	65	65.2
13	1	0	1	5.6	5.7	80	53	52.8
9	0	-1	-1	3.3	2.8	10	55	55.9
6	0	1	-1	3.3	8.5	10	55	54.1
5	0	-1	1	3.3	2.8	80	63	63.9
3	0	1	1	3.3	8.5	80	57	56.1
12	0	0	0	3.3	5.7	45	59	59.3
14	0	0	0	3.3	5.7	45	59	59.3
15	0	0	0	3.3	5.7	45	60	59.3

Note: X_1= sample size (mm), X_2=velocity (m/h), X_3= DME/sample ratio (mL/g)

The coefficient of the full model was evaluated with the second-order polynomial regression analysis. The regression equation for lipid yield (%) obtained by regression analysis in coded terms was given as:

$$Y= 59.333 - 3.680\ X_1 - 2.430\ X_2 + 2.500\ X_3 - 1.847\ X_1*X_1 - 0.847\ X_2*X_2 - 0.987\ X_3*X_3 - 2.860\ X_1*X_2 - 2.500\ X_1*X_3 - 1.500\ X_2*X_3$$

Where X_1= sample size (mm), X_2=velocity (m/h), X_3= DME/sample ratio (mL/g).

According to Table 4.4, the linear effect of three factors for lipid extraction, size (X_1), velocity (X_2), and DME/sample ratio (X_3) are considered important terms affecting lipid extraction by the DME method. The coefficients of X_1 (-3.68) and X_2 (-2.43) have negative signs (antagonistic effect) while X_3 (2.5) has positive signs

(synergistic effect). This means, using the lower level of X_1 and X_2 and higher level of X_3 is necessary to obtain high lipid yield. The interaction of X_1*X_3 (-2.5) and X_1*X_2 (-2.86) was found to be a statistically significant factor for the model. Moreover, quadratic parameters of the X_1 (-1.84) are considered significant.

Table 4.4 Estimated regression coefficients for the BBD model for lipid extraction by DME.

Term	**Coef**	**SE Coef**	**T-Value**	**p-value**
Constant	59.333	0.723	82.04	0
X_1	-3.68	0.443	-8.31	0
X_2	-2.43	0.443	-5.49	0.003
X_3	2.5	0.443	5.65	0.002
X_1*X_1	-1.847	0.652	-2.83	0.037
X_2*X_2	-0.847	0.652	-1.3	0.251
X_3*X_3	-0.987	0.652	-1.51	0.191
X_1*X_2	-2.86	0.626	-4.57	0.006
X_1*X_3	-2.5	0.626	-3.99	0.01
X_2*X_3	-1.5	0.626	-2.4	0.062

Note: X_1= sample size (mm), X_2=velocity (m/h), X_3= DME/sample ratio (mL/g)

Coeff.: coefficient, SE: Standard error

Figure 4.2 presents the perturbation plot of the operational variables to lipid yield. The perturbation plot compares the effects of all the factors at a specific point within the range of design. It can be seen that X_2 and X_3 show equal effects on lipid yield while X_1 shows more curvature, indicating the most sensitive factor on the lipid yield.

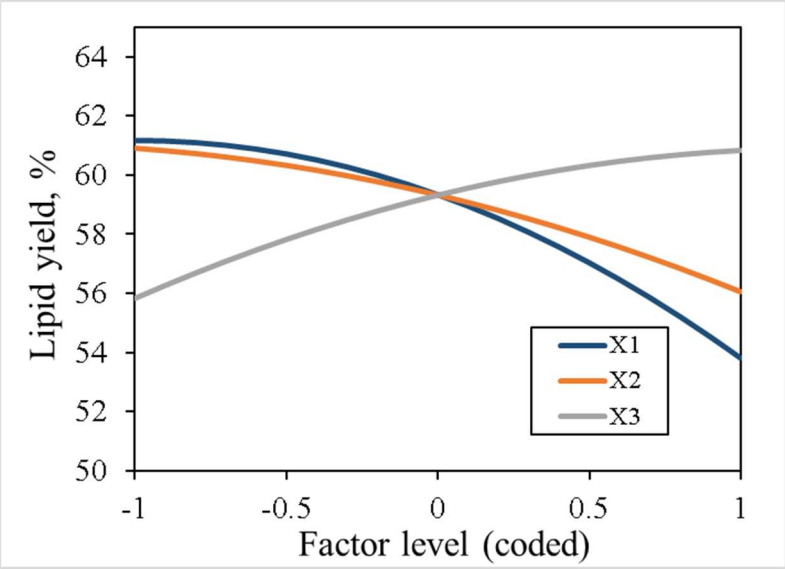


Figure 4.2. Perturbation plot for lipid extraction yield of fat balls by DME method.

4.3.1.2 ANOVA for lipid extraction yield

The experimental results were evaluated using an analysis of variance (ANOVA) to determine the fitness and significance of the model, which is shown in Table 4.5. The regression coefficient is significant when the probability of error value (p-value) is less than 0.05 ($p<0.05$) [31]. The ANOVA result showed an F-value of 20.47 with a p-value < 0.005 implying that the model was significant. The fit of the model was assessed using the coefficient of determination (R^2), resulting in 0.973 and 0.926 for the R-value and R^2 value (adjusted), respectively. This indicates that more than 97.3% of the variability in the response could be predicted by the model. R^2 (adjusted) value indicates that the model accounts for 92.6% due to the addition of ineffectual predictor variables. The regression model shows a good fit if the regression coefficient (R^2) is more than 80% [32]. The lack of fit test yields an insignificant p-value, this suggests that the model adequately fits the data [33].

The predicted and experimental plots of lipid yield are presented in Figure 4.3. It is observed that the actual and predicted data show no significant discrepancies, indicating that the model fits the observed data well, generating a good estimate of response within the range studied.

Table 4.5 Analysis of variance (ANOVA) results for lipid yield by DME method.

Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**p-value**
Model	9	289.088	32.121	20.47	0.002
Linear	3	205.578	68.526	43.67	0.001
X_1	1	108.339	108.339	69.05	0
X_2	1	47.239	47.239	30.11	0.003
X_3	1	50	50	31.87	0.002
Square	3	16.791	5.597	3.57	0.102
X_1*X_1	1	12.591	12.591	8.03	0.037
X_2*X_2	1	2.647	2.647	1.69	0.251
X_3*X_3	1	3.595	3.595	2.29	0.191
2-Way Interaction	3	66.718	22.239	14.17	0.007
X_1*X_2	1	32.718	32.718	20.85	0.006
X_1*X_3	1	25	25	15.93	0.01
X_2*X_3	1	9	9	5.74	0.062
Error	5	7.845	1.569		
Lack-of-Fit	3	7.178	2.393	7.18	0.125
Pure Error	2	0.667	0.333		
Total	14	296.933			
S	1.2526	R^2	0.9736	R^2(adj)	0.9260

Note: X_1= sample size (mm), X_2=velocity (m/h), X_3= DME/sample ratio (mL/g)

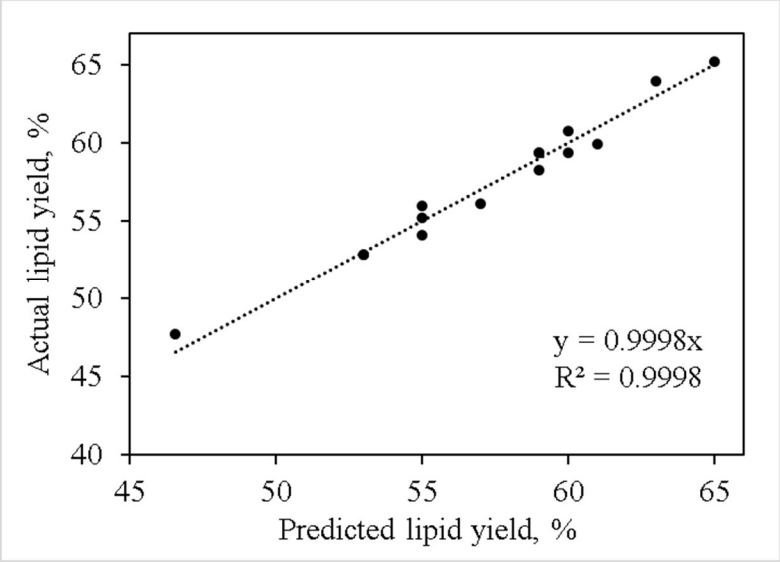


Figure 4.3 Actual vs predicted plots for lipid extraction from fat balls using DME method.

4.3.2 Interactions between process variables

To illustrate the main and interactive effects of the process variables on the lipid yield, the two-dimensional contour plot as well as three-dimensional response surface plots were generated based on the developed model.

4.3.2.1 Interaction of size and velocity

The sample size was varied at 1, 3.3, and 5.6 mm to study the influence of fat ball size on lipid extraction. Figure 4.4 describes contour plots and surface plots of the combined effect of sample size and velocity on lipid yield while the DME/sample ratio was fixed at center level (45 mL/g). A reduction in fat balls size and velocity of DME had a positive influence on lipid yield. However, a bigger fat ball size and higher DME velocity contribute to the lower yield of lipids due to the limitation of mass transfer which prevents the contact between DME and lipids [34,35]. It has been reported that the lipid yield increased almost linearly with a decrease in sample diameter (<3.7 mm) due to an increase in contact area between the DME and sludge ball sample [21].

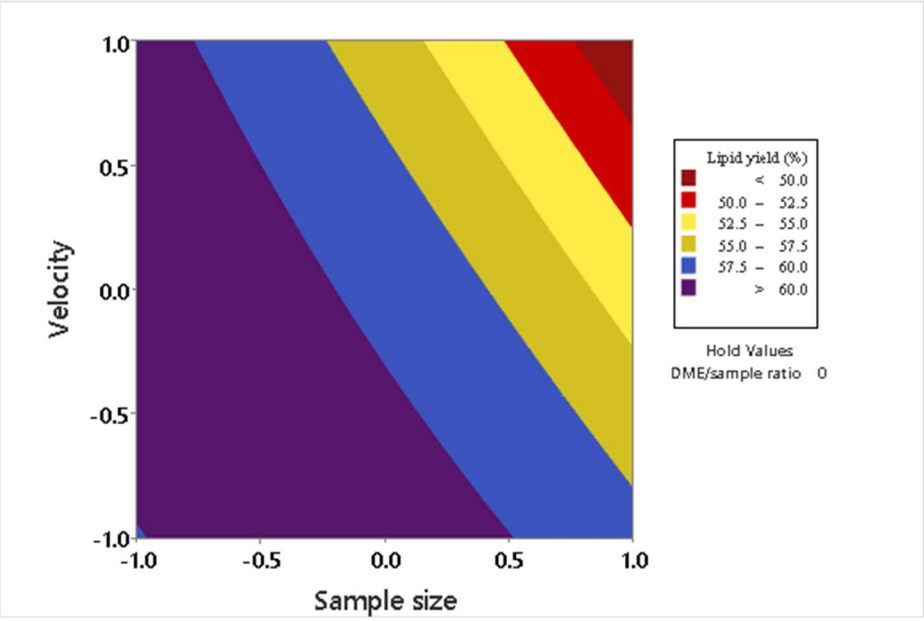
4.3.2.2 Interaction of DME/sample ratio and size

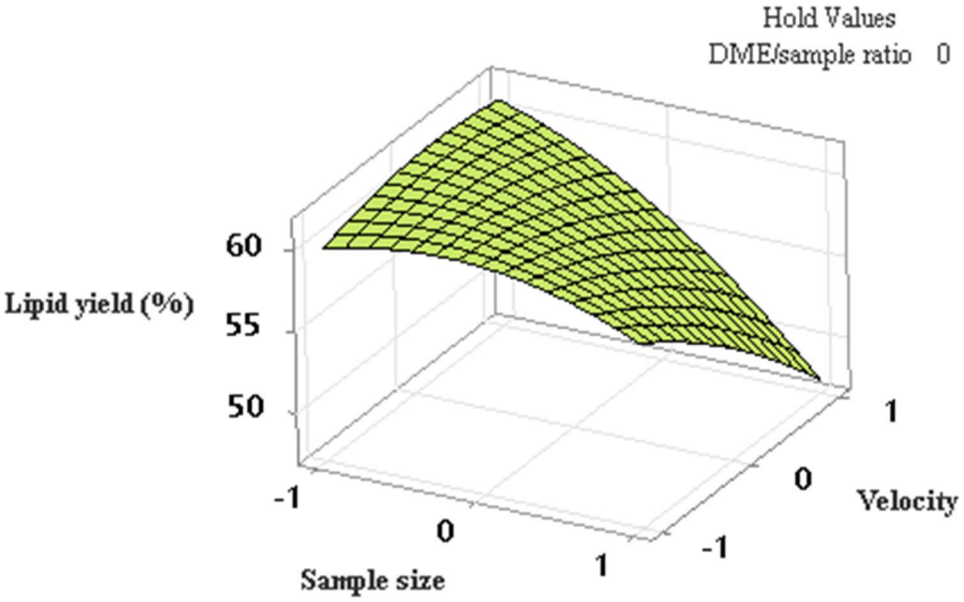
Figure 4.4 shows contour plots and surface plots of the interactive effect of DME/sample ratio and sample size on lipid yield when velocity was fixed at 4.3 m/h.

DME/sample ratio was varied from 10 mL/g, 45 mL/g, and 80 mL/g to observe the effect of DME/sample ratio on the lipid yield. The lipids recovery increased gradually when the DME/sample ratio was greater than 40 mL/g. It is clear that an increase in DME/sample ratio enhances the solubility of lipids which eventually raises the amount of extracted lipid [26,33]. However, increasing the DME ratio would require higher expense and energy, therefore, the DME reusability could be implemented to compensate for this.

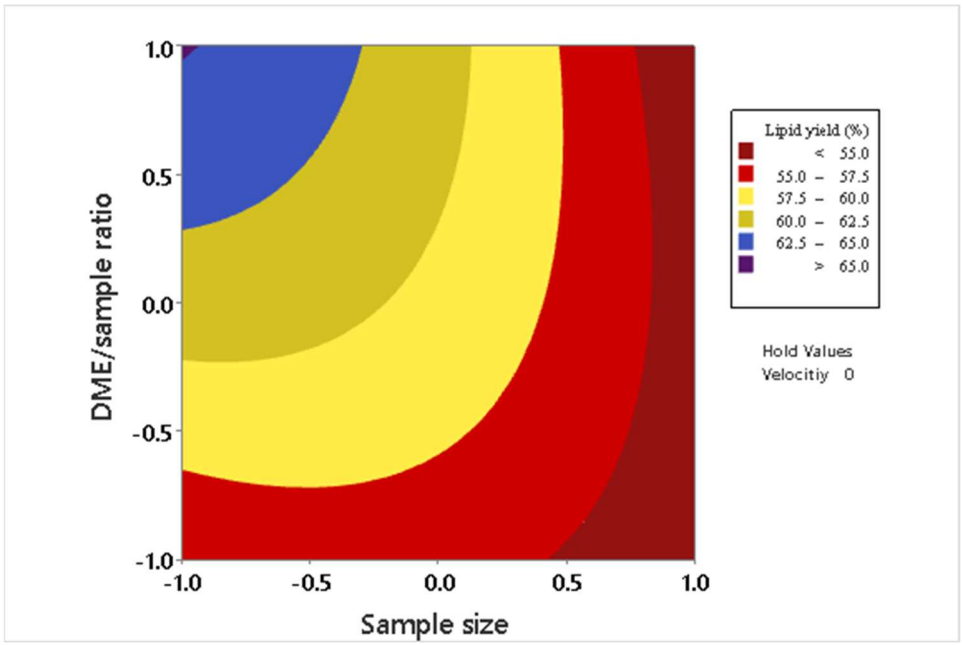
4.3.2.3 Interaction of velocity and DME/sample ratio

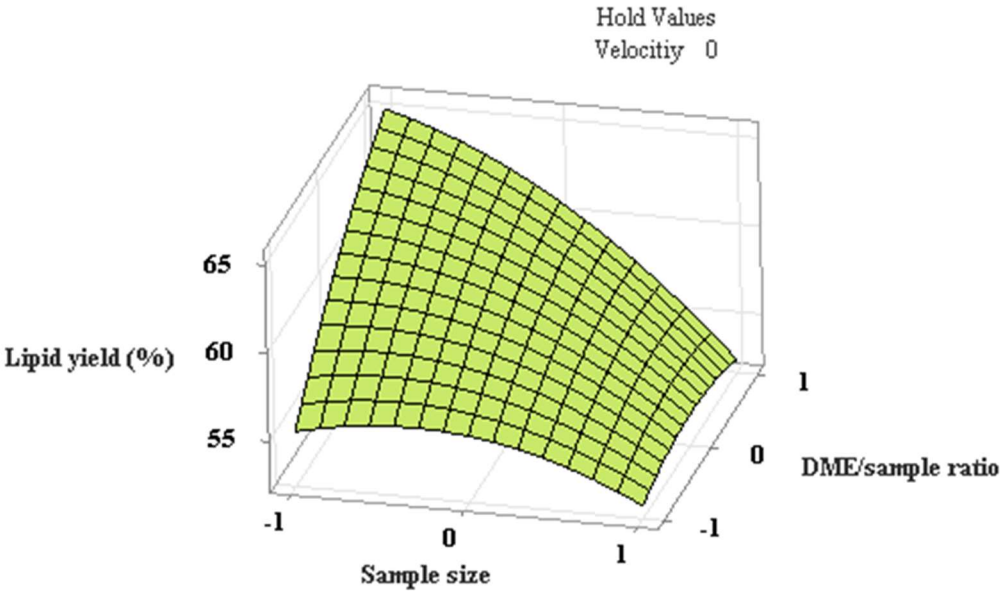
Figure 4.4 presents contour plots and surface plots for the yield of lipids as an interactive function of velocity and DME/sample ratio, while the sample size was maintained at 3.3 mm. The velocity varied from 2.8 m/h, 4.3 m/h, and 5.7 m/h. The effect of the DME/sample ratio had a greater influence on the lipid content as compared with velocity. It can be seen that the influence of low velocity is not substantial at low DME/sample ratio because an insufficient amount of DME results in an incomplete extraction of lipids. However, it resulted in better extraction performance with the increment of DME amount. Similar results are observed in literature for process optimization for lipid extraction using the DME technique in which the lipid content decreased at the high level of velocity due to less penetration time of DME, leading to insufficient to achieve the extraction equilibrium [35–37].


1.0
0.5
0.0
-0.5
-1.0
Velocity
-1.0
-0.5
0.0
0.5
1.0
Sample size
Lipid yield (%)
< 50.0
50.0 – 52.5
52.5 – 55.0
55.0 – 57.5
57.5 – 60.0
> 60.0
Hold Values
DME/sample ratio 0

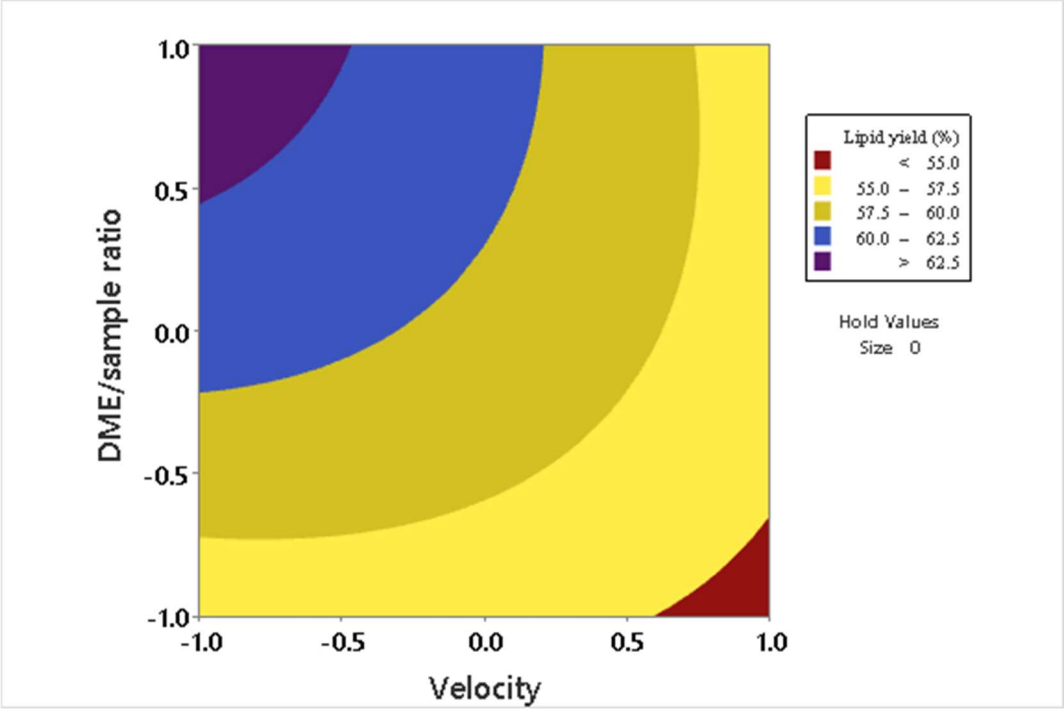

Hold Values
DME/sample ratio 0
Lipid yield (%)
60
55
50
-1
0
1
Sample size
1
0
-1
Velocity

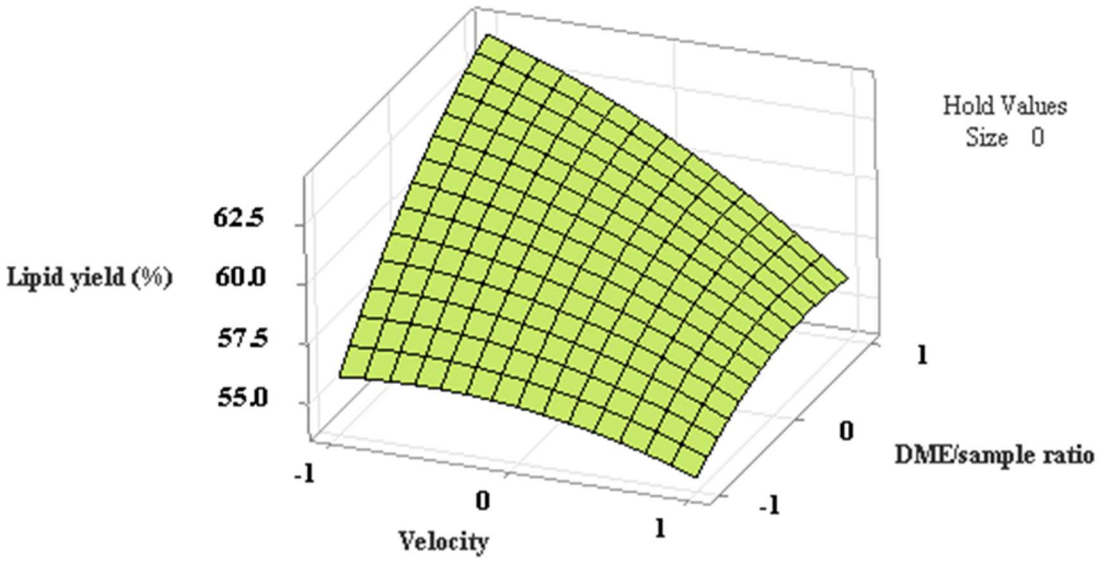
(a)


1.0
0.5
0.0
-0.5
-1.0
DME/sample ratio
-1.0
-0.5
0.0
0.5
1.0
Sample size
Lipid yield (%)
< 55.0
55.0 – 57.5
57.5 – 60.0
60.0 – 62.5
62.5 – 65.0
> 65.0
Hold Values
Velocitiy 0


Hold Values
Velocitiy 0
65
60
55
Lipid yield (%)
-1
0
1
Sample size
1
0
-1
DME/sample ratio

(b)





(c)

Figure 4.4 contour plot and surface plot: (a) sample size and velocity, (b) DME/sample ratio and sample size, and (c) Velocity and DME/sample ratio

4.3.3 Optimization and validation of reaction parameters

The optimization of lipid extraction was determined using a response optimizer in Minitab software to obtain the optimum combination within the specified range of variables (Figure 4.5). The optimal conditions for the lipid extraction by the DME method were as follows: sample size 1 (mm), velocity 3.3 (m/h), and DME/sample ratio 80 (mL/g). The predicted value at optimum conditions is validated with experimental results. The observed value was 65.2%, which is in good agreement with the value estimated by the model (65.5%). The contact time during optimum conditions is calculated at approximately 0.001 min/g.

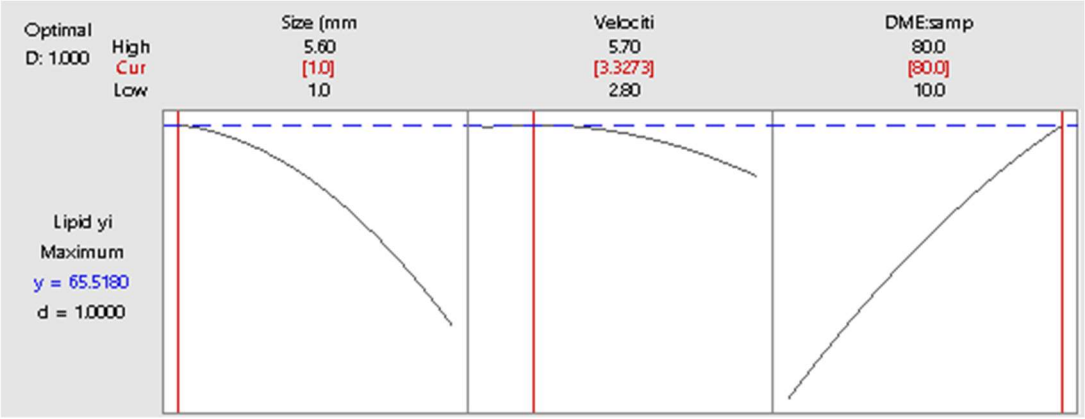


Figure 4.5 Optimization plot for lipid extraction using DME method.

4.3.4 Comparison of DME, mechanical shaking, and Soxhlet methods

4.3.4.1 Lipid and biodiesel yield

To compare the extraction performance and biodiesel yield of DME, the lipid extraction method was conducted by mechanical shaking and Soxhlet extraction with hexane (Figure 4.6). The maximum lipid yield for fat balls using DME was higher compared to the yields obtained by mechanical shaking (49%) and Soxhlet extraction (62%). The possible reason for the superiority of DME extraction was due to high solubility and low viscosity, allowing a better diffusion of solvent into the solid phase of fat balls [38]. DME has a medium polarity, which can be used to extract neutral and complex lipids [39]. In contrast, hexane can only extract non-polar lipids [11,40]. This

shows that differences in polarity contribute to their varied solubility properties and applications in different extraction processes.

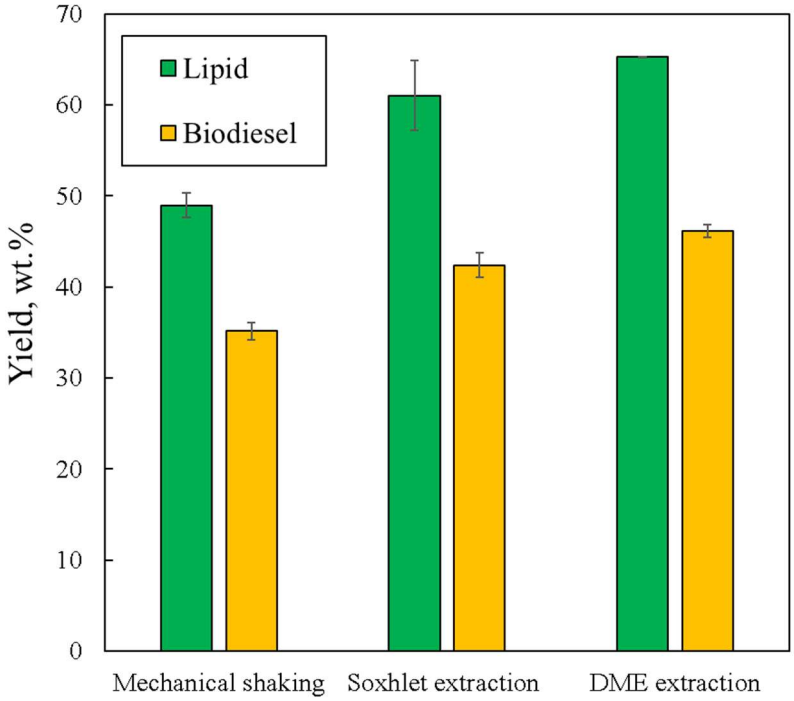


Figure 4.6 Comparison of the yield and purity of biodiesel produced by the three methods.

Although hexane was used in mechanical shaking and Soxhlet extraction, the latter was more efficient due to the use of a reflux condensation system [12,34]. Furthermore, the effectiveness of the solvent for extracting lipids through mechanical shaking reduces as the lipid concentration increases, consequently limiting its potential. The lipids were converted into biodiesel. The transesterifiable matters are about 69-72%. Overall biodiesel yield was 46.2%, 42.4%, and 35.2% for lipids derived by DME, mechanical shaking, and Soxhlet, respectively.

Figure 4.7 shows the mass composition (initial and after) treatment of three different extraction methods. The DME treatment concurrently extracted lipids and water during the extraction process, generating dried residue that can be proposed for waste-to-energy incineration [41]. Figure 4.8 shows the photos of raw fat balls and their residue after DME

extraction. Conversely, the mechanical shaking and Soxhlet methods still retain considerable water, which means further treatment is required to dry the residues due to their higher water content.

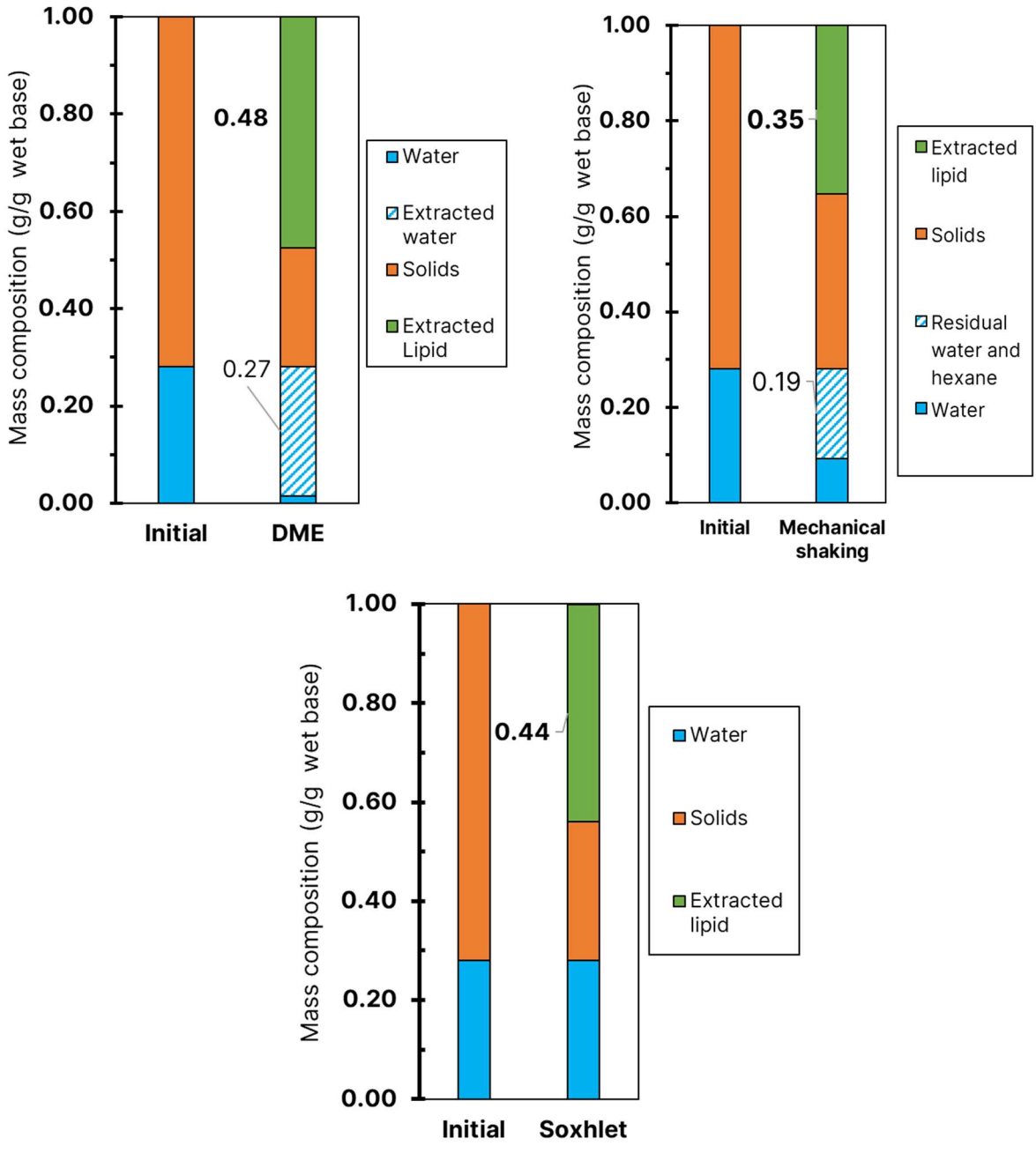


Figure 4.7. Mass balance (initial and after) treatment using different methods.

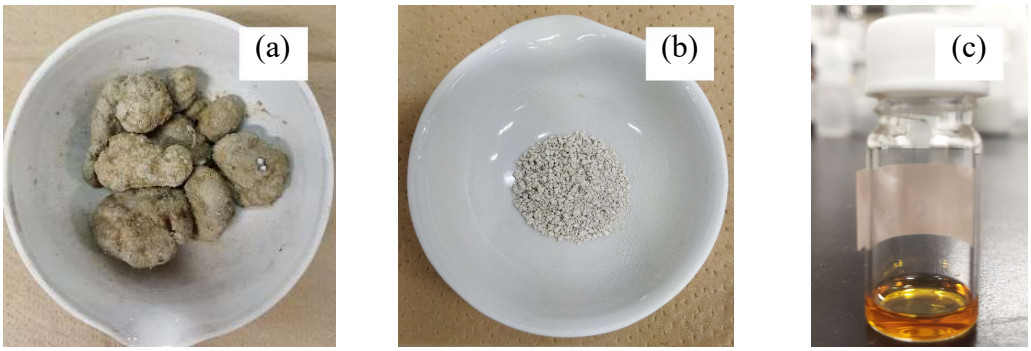


Figure 4.8 Photo images after DME extraction: (a) raw fat balls, (b) residue after DME extraction, (c) the extracted lipids.

4.3.4.2 Fatty acid methyl esters (FAME) profiles of recovered lipids

The fatty acid methyl esters (FAME) from the fat balls lipids extracted from the three methods were compared to those of other biodiesel feedstocks, as presented in Figure 4.9. Irrespectively of extraction techniques, the palmitic acid (C16:0) was most abundant (>50%) followed by oleic acid (C18:1), and linoleic acid (C18:2). The predominant value of palmitic acid in sewage lipids was also reported by other researchers [11,42]. The percentage of saturated fatty acids (SFA) in the lipid obtained by DME extraction was higher (71%) compared to the mechanical shaking and Soxhlet process (53.7-58.3 %). However, they are showing similar fractions, in which the lipids predominantly consist of SFA. Meanwhile, edible oil feedstock (corn and soybean) showed more dominant polyunsaturated fatty acid (PUFA), followed by monounsaturated fatty acid (MUFA), and SFA. The saturation levels of fatty acids affected the biodiesel properties, including oxidative stability, cetane numbers, and cold-flow properties [6,28]. Biodiesel with a high amount of SFA had higher cloud points, cetane number, and oxidative degradation [43]. On the other hand, more PUFA reduces the cetane number and oxidation stability [44,45].

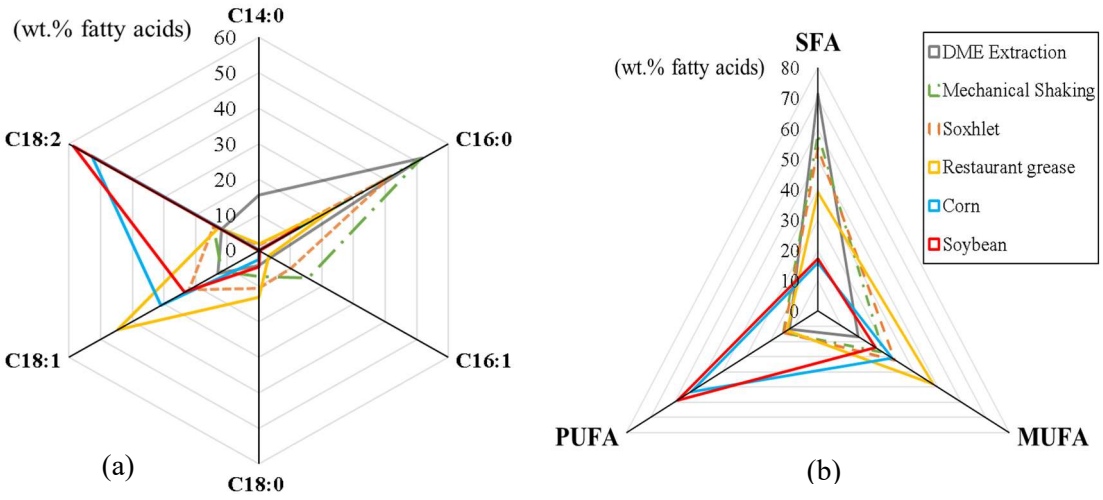
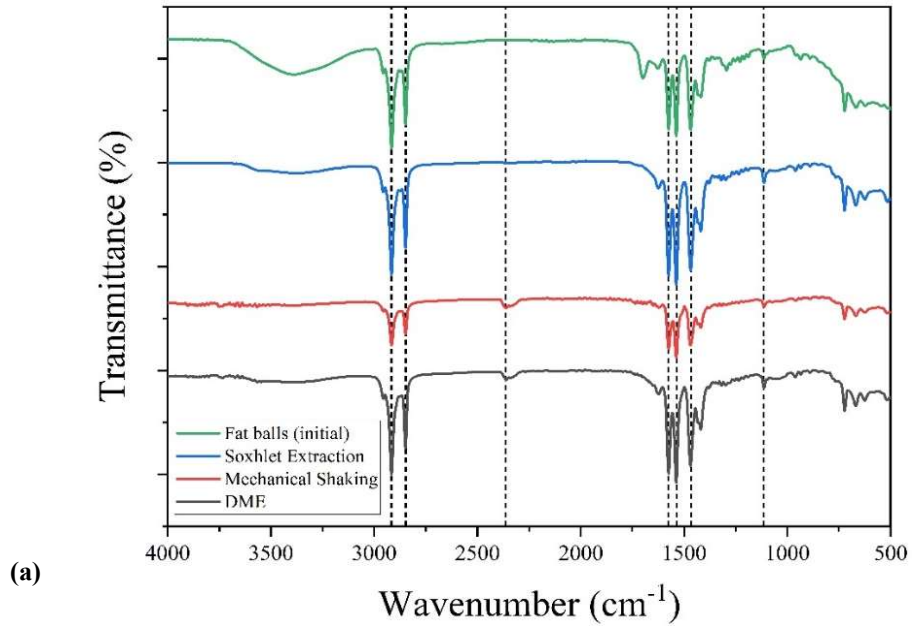


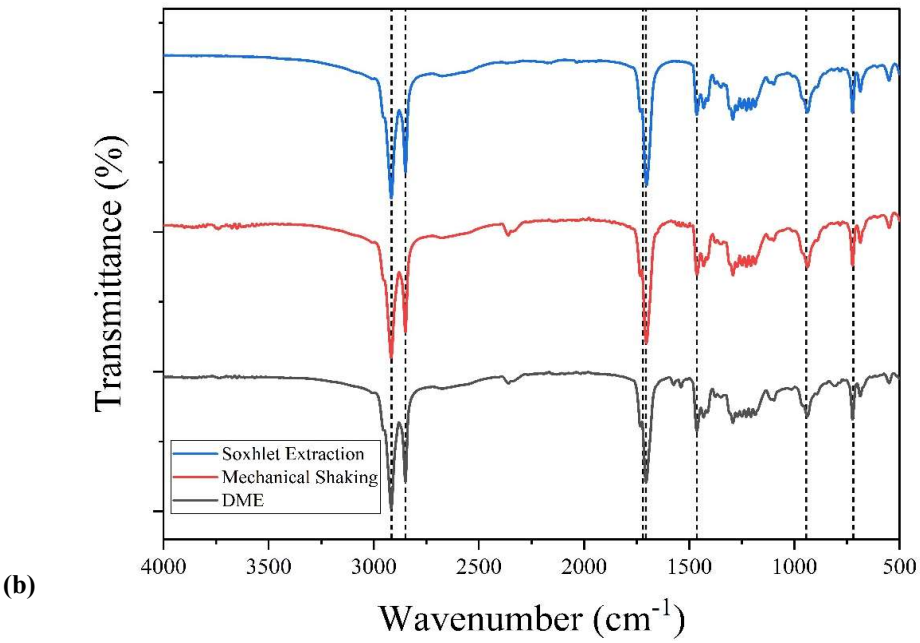
Figure 4.9 (a) FAMEs profiles (b) FAMEs based on saturated levels.

4.3.4.3 FTIR analysis

The FTIR spectrum of raw fat balls, recovered lipids, and the fat balls residue is shown in Figure 4.10. The peak observed between 3100-3600 cm^{-1} and the strong sharp band at 1720-1740 cm^{-1} were assigned to the stretching vibration of hydrogen bonds and the carboxyl group of esters, respectively [46,47]. Upon lipid extraction, these peaks were absent in the spectra of fat balls residue, confirming the removal of water and lipids. The peaks detected at 2950 and 2800 cm^{-1} are attributed to C-H stretching absorptions of the methylene and methyl groups in fatty acids [44,48]. The bands of carboxylate group between 1530-1630 cm^{-1} and 1300-1420 cm^{-1} were evident in FTIR spectrum [49]. The peak near 720 cm^{-1} could be attributed to vibration of methylene [46]. The FTIR spectra of lipids extracted via DME exhibit similarities to those obtained through mechanical shaking and Soxhlet extraction method, indicating the presence of similar components.



(a)



(b)

Figure 4.10 Fourier transform-infrared spectra: (a) raw fat balls and residue, (b) extracted lipids.

4.4 Conclusion

This work mainly aims to extract lipids from fat balls using DME and RSM-BBD was applied to investigate the influence and interaction of the process parameters such as size, velocity, and DME/sample ratio. The use of a smaller sample size, lower velocity of liquefied DME, and higher DME/sample ratio enhances the efficiency of lipid extraction of DME technique. The highest percentage of lipid recovery was 65.2% under the optimal DME extraction conditions (sample size 1.0 mm, velocity 3.3 m/h, and DME/sample ratio 80 mL/g). The lipid yield by DME extraction was better than that of mechanical shaking and Soxhlet extraction. The methyl ester characteristics of biodiesel were investigated and found to be similar among those three methods. This study provides a viable pathway for highly efficient techniques for lipid extraction of wastewater residuals with a view to producing biodiesel.

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Chapter 5 Conclusions and Future Perspectives

5.1 Conclusions

Biodiesel is a renewable liquid biofuel that has a promising prospect as a substitute for petroleum-based diesel. In order to increase the competitiveness of biodiesel, research is being carried out to find alternative lipid feedstocks. This study investigated the potential of wastewater residues, focusing on fats, oils, and grease (FOG), and sewage sludge as viable lipid sources for biodiesel production.

Since biodiesel quality is influenced by the properties of the feedstock, the variation between waste materials in terms of their properties, lipid, and biodiesel potential was investigated. The material generation and biodiesel potential in Japan were assessed. In addition, optimization of lipid extraction with dimethyl ether (DME) was carried out to improve lipid recovery efficiency. The main conclusions from this study are summarized as follows:

Chapter 2 evaluated sewage sludges from two wastewater treatment plants (WWTPs) in Japan. Several samples were investigated, including primary, waste-activated, mixed, and dewatered sludge, and primary and secondary scum. Among the sludges tested, primary WWTP A scum had the highest lipid and biodiesel yields (28.5% and 11%, respectively). The analysis of the fatty acid methyl esters showed that palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2) were predominant, regardless of the sludge type. Compared to vegetable oil feedstock, sludge fatty acid has a lower content of polyunsaturated fatty acids, similar to animal fat feedstock.

Chapter 3 investigated the biodiesel potential of fat balls, a FOG deposit collected from the sewage pumping station, with primary scum and primary sludge obtained from the WWTP primary clarifier. The samples were collected and characterized through four seasons. The results demonstrated that the characteristics of samples are considerably varied due to seasonal differences and site generation. The concentrations of trace elements were highly variable between samples and between seasons. However, the primary scum and primary sludge samples generally contained much higher levels of trace elements than the fat balls. Fat balls and primary scum had higher lipid and biodiesel yields compared to primary sludge. This is due to the fact that fat

balls and scum originate from similar types of lipids (discharged FOG on the surface of water) with comparable chemical characteristics. Our findings highlight the successful conversion of wastewater-extracted lipids into biodiesel containing a biogenic C content of 58–74%. The fat balls from the pumping station had a higher content of solids and carbon, as well as a higher calorific value than the primary scum and primary sludge collected at the primary clarifier. We suggested that fat balls are the most promising biodiesel feedstock from wastewater treatment facilities due to the higher moisture content of primary scum, which can lead to additional dewatering steps. Finally, we estimated about 447 metric tons of biodiesel per year can be generated from fat balls and primary scum in Japan. In addition, this study investigated screen sludge obtained from the pretreatment of sewage processes. The presence of woody biomass components poses challenges for its use as a biodiesel feedstock. Furthermore, the pretreatment process may release smaller particles into the effluent. Considering these characteristics, alternative applications, such as for solid fuel, could be considered.

Chapter 4 investigated the use of dimethyl ether (DME) for lipid extraction of fat balls. The optimization of the process parameters (size, speed, and DME/sample ratio) was carried out according to the response surface methodology (RSM) with the Box-Behnken design (BBD). The RSM-BBD model was evaluated with a coefficient of determination (R^2) and R^2 (adjusted) value of 0.973 and 0.926, respectively. This study clearly shows that the RSM was suitable for optimizing the process parameters. The optimum conditions for lipid extraction by DME were sample size 1 (mm), velocity 3.3 (m/h), and DME/sample ratio 80 (mL/g). When compared with conventional lipid extraction, DME gave a higher lipid yield (65.2 %) than both mechanical extraction (49 %) and Soxhlet extraction (62 %). The lipids recovered by DME had similar fatty acid fractions, with the lipids consisting predominantly of saturated fatty acid (SFA) compared to unsaturated fatty acid (UFA). Overall, using DME could be a viable way of recovering lipids from wastewater to produce biodiesel.

5.2 Future perspectives

In this study, we investigated the feasibility of wastewater lipids from FOG waste and sewage sludge as alternative raw materials for biodiesel production. For future research prospects, we propose the following:

(1) As demonstrated in this thesis, we are still focusing on evaluating different wastewater residuals and eventually concluding the recommended feedstock. Further experimental investigations are necessary to optimize key parameters of the transesterification reaction. This is crucial for improving biodiesel conversion efficiency.

(2) The characteristics of lipids and biodiesel varied across seasons and sites as shown in this work. Therefore, to confirm whether they meet the biodiesel standards, future work should focus on the characterization of biodiesel, including heating value, density, viscosity, and sulfur content.

(3) In the assessment of material generation and biodiesel potential, we estimated that Japan has a significant volume of wastewater lipids that can be harvested from their wastewater facilities. However, the study of the economic feasibility of material collection and biodiesel production should be assessed to facilitate the practical implementation of biodiesel from wastewater lipids.

(4) Our investigation into screen sludge revealed seasonal variations in characteristics, comprising biomass and non-biomass fractions. This prompts further analysis to understand the implications of screen sludge in potentially releasing microplastics into the effluent. These findings underscore the complexity of screen sludge composition, emphasizing the importance of detailed assessments for effective environmental management in wastewater treatment processes.

(5) In terms of utilizing DME for lipid extraction techniques, it would be interesting to assess feasibility by examining the energy balance (input and output) of the system.

Appendix

Supelco 37 component FAME mix components (Sigma-Aldrich):

Methyl butyrate 400 µg/mL

Methyl hexanoate 400 µg/mL

Methyl octanoate 400µg/mL

Methyl decanoate 400 µg/mL

Methyl undecanoate 200 µg/mL

Methyl laurate 400µg/mL

Methyl tridecanoate 200µg/mL

Methyl myristate 400 µg/mL

Methyl myristoleate 200 µg/mL

Methyl pentadecanoate 200 µg/mL

Methyl cis -10-pentadecenoate 200 µg/mL

Methyl palmitate 600µg/mL

Methyl palmitoleate 200 µg/mL

Methyl heptadecanoate 200 µg/mL

cis-10-Heptadecanoic acid methyl ester 200µg/mL

Methyl stearate 400µg/mL

trans-9-Elaidic acid methyl ester 200µg/mL

cis-9-Oleic acid methyl ester 400µg/mL

Methyl linolelaidate 200 µg/mL

Methyl linoleate 200 µg/mL

Methyl arachidate 400µg/mL

Methyl γ-linolenate 200 µg/mL

Methyl cis -11-eicosenoate ≤ 200 µg/mL

Methyl linolenate 200 µg/mL

Methyl heneicosanoate 200µg/mL

cis -11,14-Eicosadienoic acid methyl ester 200 µg/mL

Methyl behenate 400µg/mL

cis -8,11,14-Eicosatrienoic acid methyl ester 200 µg/mL

Methyl erucate 200 µg/mL

cis -11,14,17-Eicosatrienoic acid methyl ester 200 µg/mL

cis-5,8,11,14-Eicosatetraenoic acid methyl ester 200 µg/mL

Methyl tricosanoate 200µg/mL

cis -13,16-Docosadienoic acid methyl ester 200 µg/mL

Methyl lignocerate 400µg/mL

cis -5,8,11,14,17-Eicosapentaenoic acid methyl ester 200 µg/mL

Methyl nervonate 200µg/mL

cis -4,7,10,13,16,19-Docosahexaenoic acid methyl ester 200 µg/mL

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"**Whoever strives shall succeed**".