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# Potential of Lipids from Polymer-Based Dewatered Sewage Sludge as Feedstock for Biodiesel Production

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**Abstract:** Municipal wastewater treatment plants produce vast amounts of sewage sludge as waste, with more than 80% dewatered sludge (DS). DS is a polymer-based sludge containing flocculant and extracellular polymeric substances, including lipids. Lipids can be converted into biodiesel as an alternative energy that reduces dependency on fossil fuels while helping cities manage waste more sustainably. Past studies explored the potential of lipids from various sewage sludges in biodiesel production. However, the potential of DS remains largely unexplored. This study evaluates the lipid extracted from DS and the potential of its fatty acid methyl ester (FAME) to be used as biodiesel. Lipid extraction was conducted under varying parameters, including temperatures of 70, 80, and 90 °C, extraction time of 2, 4, 6, and 8 h, and sludge-to-solvent (S/L) ratios of 0.05, 0.075, 0.1, 0.125, 0.15, and 0.175 g/mL. The optimal extraction conditions of 70 °C for 4 h at S/L of 0.175 g/mL yielded 1.71 ± 0.10% lipid. FTIR and TGA revealed that the DS lipids contain triglycerides, fatty acids, glycerol, and proteins. Transesterification of DS lipids produced DS FAME with a fatty acid profile ranging from C4:0 to C22:0. The evaluation of DS FAME revealed a high ester content (94.7%) of fatty acids ranging from C14:0 to C24:1, surpassing the minimum standard of 90% for biodiesel. The elevated proportion of unsaturated fatty acids in DS FAME is expected to result in a low melting point, reducing the solidifying effect and enhancing its performance as biodiesel.

**Keywords:** polymer-based sludge; secondary raw materials; biowaste valorisation; lipid extraction; biodiesel feedstock; sustainable energy; circular economy

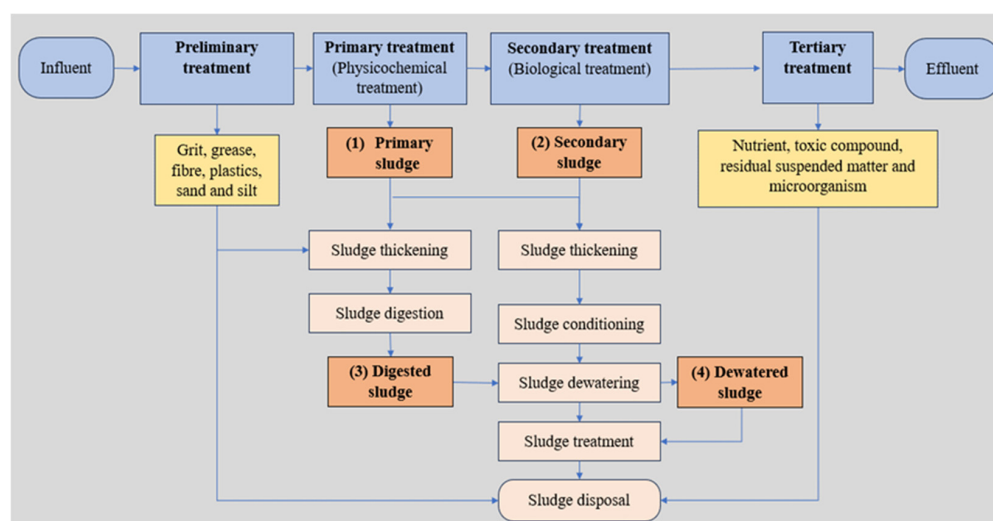
## 1. Introduction

The depletion of fossil fuel reserves has turned the world's escalating energy demands into a pressing challenge. The production and usage of fossil fuels are linked to high levels of environmental degradation, notably crude oil spills, CO<sub>2</sub> emissions, and inconstant prices of crude oil [1]. These issues related to fossil fuels highlighted the critical need for developing alternative sustainable fuels for energy consumption [1]. Biodiesel is a promising renewable fuel that offers a viable alternative to fossil fuels in diesel engines, which integrate seamlessly with existing engines and refuelling technology [2,3]. Various lipid sources from edible crops, non-food feedstocks, and microalgae were used in the production of fatty acid methyl ester as biodiesel by transesterification [4,5]. The concerns on the usage of these feedstocks related to food security and poverty [6], economy and environmental challenges [7], domestication difficulties [7] and compliance with biodiesel standards [7] urge the exploration of alternative feedstocks from non-edible sources that are both inexpensive and readily accessible [4,8,9]. Sewage sludge from wastewater treatment contains lipids comparable to some of the vegetal feedstock, which can be used as an alternative feedstock [10].

The increase in population and rapid industrialisation in recent decades resulted in the drastic generation of sewage sludge [9]. More than 3000 wastewater treatment plants in China produced around 60 million tonnes of sewage sludge in 2020, with a yearly increase of 13% [9,11,12]. The United States and European Union are generating around 40 and 50 million tonnes of sewage sludge annually [9]. This sewage sludge as biowaste is normally dumped into landfills or used in land applications, and it is hazardous to the environment if left untreated [13–15]. Figure 1 shows a typical wastewater scheme and the type of sludge produced [16,17]. Wastewater treatment plants produced four types of sludge: (1) primary, (2) secondary, (3) digested, and (4) dewatered sludges, in general. Primary sludge is produced from the primary treatment employing the physicochemical process [11]. Secondary sludge is formed in the secondary treatment of biological processes using microorganisms to stabilise the organic contents and remove non-colloidal solids [11]. Digested sludge is produced from both primary and secondary sludge in the wastewater treatment plant equipped with a digestion unit [18]. The primary and secondary sludge (with or without the addition of flocculant) passes through the digestion process prior to the dewatering process [19,20]. Dewatered sludge (DS) is polymer-based sludge generated from either primary sludge, secondary sludge, or both primary and secondary sludges, with the inclusion of polymer flocculant for conditioning before the dewatering process [19,20]. Over 80% of DS is produced as the final product of wastewater treatment, which requires further treatment or disposal [21]. Several nations, including Spain, Brazil, Jordan, and Turkey, used sewage sludge as a biosolid in agricultural applications [22]. The use of this material raised environmental concerns because of pollutants, which require further treatment. Sewage sludge is utilised in resource recovery by co-combustion with wood-producing ash that is rich in phosphorus and used as fertiliser [23]. These sewage sludges as a byproduct can be utilised in energy recovery as an alternative to low-cost energy sources in promoting affordable renewable energy and sustainable production practices as well as mitigating climate change, in line with SDGs 7, 12, and 13 of the United Nations Sustainable Development Goals (UNSDG) [19,24,25]. Sewage sludge treatment by anaerobic digestion is used to recover methane, while incineration is used for heat recovery, especially in Japan and Germany [26].

Table 1 presents the calorific value of several types of sludge. Sewage sludge, including primary, secondary, and dewatered sludges, is less favourable to be used as a heat source due to its low calorific value compared to other fuels such as bituminous coal (25.40–22.15 MJ/kg) and sawdust (18.13–20.40 MJ/kg) [11,27]. However, it holds potential for energy recovery and can be utilised as a cost-effective byproduct for energy genera-

tion. Sewage sludge presents significant potential for energy recovery through biodiesel production by leveraging its high organic matter, particularly its high lipid fraction of up to 25% [8,28–30]. Chan et al. [31] mentioned that the dewatered sludge and dried sludge exhibited positive residual energy ranging from 0.24 to 12.44 MJ/kg, indicating net recoverable energy. Wet sludge, including primary and secondary sludges, requires moisture content to be reduced below its critical threshold before energy recovery due to negative residual energy content (−0.89 to −1.91 MJ/kg) [31]. Energy balance analysis conducted by Zhang et al. [30] for two-step biodiesel production from primary sludge shows that 1 tonne of biodiesel can be produced from 5.26 tonnes of dry sludge, with a total energy input of 12.4 GJ [30]. Shifting transportation of the sludge to the biodiesel production plant instead of the landfill and the potential usage of the residual sludge as fertiliser results in energy credit, with a net energy input of 7.58 GJ [30]. The potential usage of sewage sludge in the production of biodiesel is highlighted by the energy gain per tonne of biodiesel of 595 MJ with landfill disposal and 601 MJ with fertiliser reuse [30]. The extraction process led to a reduction in the organic fraction in the residual sludge, while 55.51% of the sludge remained a thermally stable fraction [32]. There is an opportunity to utilise the residual sludge for material recovery via land application by mulching or ploughing due to the remaining organic content and thermally stable fraction, which can improve nutrient availability, exhibit weed germination, control soil temperature, and alter its physical properties [32]. Residual sludge with minimal water content is suitable for energy generation through thermal processes (gasification, combustion, incineration, and pyrolysis) compared to biological processes due to a higher energy efficiency [32].



**Figure 1.** Wastewater treatment plant scheme and the type of sludge produced [16,17].

**Table 1.** Calorific value of sewage sludges [31].

Type of Sludge	Higher Heating Value, HHV (MJ/kg)	Lower Heating Value, LHV (MJ/kg)	Residual Energy (MJ/kg)
Primary 1	17.53	16.34	−1.014
Primary 2	18.20	17.03	−1.074
Secondary 1	19.66	18.34	−0.880
Secondary 2	18.65	17.45	−1.109
Dewatered 1	17.41	16.15	0.244
Dewatered 2	13.19	12.36	1.629
Dewatered 3	15.66	14.44	0.445
Dewatered 4	17.48	16.29	0.688

Values are based on dry weight basis. Data from [31].

Previous studies have explored the potential of lipids for biodiesel from primary and secondary sludges of wastewater treatment plants, either untreated or pretreated. However, dewatered sludge (DS) generated as the end waste of the wastewater treatment process remains underexplored. Notably, 80% of sewage sludge is produced globally in the form of DS [21] and its appreciable lipid amount, underscoring its vast potential for lipid extraction. DS is characterised as polymer-based sludge due to its extracellular polymeric substances (EPS) polymer flocculant used during conditioning [19,20], representing a significant yet overlooked resource. DS has a higher total solids (TS) content of 17–20 wt.%, respectively, unlike primary and secondary sludges with 2.0–8.0 wt.% and 0.8–2.8 wt.% [32,33]. While DS exhibits lower organic content (volatile solid content of 14–60 wt.% of TS), this range is still comparable to raw primary and secondary sludges (30–80 wt.% and 2–88 wt.%) [32], making it a promising candidate for resource recovery. This study hypothesises that DS can serve as a viable substitute feedstock for sustainable biodiesel production due to its lipid content and suitability for conversion into fatty acid methyl esters (FAME). By investigating the influence of key extraction process parameters (temperature, time, and S/L) on lipid yield, the study addresses a significant research gap while exploring the feasibility of lipids for biodiesel production. The novelty of this research lies in its focus on repurposing an underutilised waste material of DS into a valuable resource of feedstock for biofuel production. This approach contributes to the circular economy by transforming the end waste of the wastewater treatment into valuable resources and further promotes sustainability through reduced reliance on conventional oil feedstocks and minimisation of waste. These findings align with global sustainability goals, offering a practical pathway for renewable energy development and improved waste management in the wastewater treatment sector.

## 2. Materials and Methods

### 2.1. Material and Reagent

Dewatered sludge (DS) was obtained from a regional sludge treatment plant (RSTP). Hexane [C<sub>6</sub>H<sub>14</sub>] of Exxsol<sup>®</sup> (ExxonMobil, Spring, TX, USA), boron trifluoride [BF<sub>3</sub>] (Sigma-Aldrich, Burlington, MA, USA), chloroform [CHCl<sub>3</sub>] (Sigma-Aldrich, Burlington, MA, USA), methanol [CH<sub>3</sub>OH] (Polyscientific, Malaysia), diethyl ether [(CH<sub>3</sub>CH<sub>2</sub>)<sub>2</sub>O] (Sigma-Aldrich, Burlington, MA, USA), toluene [C<sub>2</sub>H<sub>5</sub>CH<sub>3</sub>] (Sigma-Aldrich, Burlington, MA, USA), and sodium sulphate [Na<sub>2</sub>SO<sub>4</sub>] (Merck, Rahway, NJ, USA) were also used in this project. 37 compounds of FAME standard (Supelco, Bellefonte, PA, USA) and triundecanoin [C11:0] (Sigma-Aldrich, Burlington, MA, USA) as triglyceride standard were also utilised.

### 2.2. Preparation of Sludge Sample

Dewatered sludge was obtained from a sludge compartment within an RSTP. RSTP adopted a sequential batch reactor (SBR) for the secondary treatment process. The SBR process created waste-activated sludge, which was further mixed with polymer flocculant SediFloc (Metcon Water, West Bengal, India). DS was produced by dewatering the sludge mixture using a decanter and temporarily stored in a portable container within the sludge storage building before disposal. A shovel was used to collect the DS from the portable container. The DS was well mixed, sealed, and sent to the laboratory for further characterisation and treatment. The raw DS was mixed evenly and laid out on a tray before drying at 60 °C to a consistent moisture content using an oven (UM300, Memmert, Schwabach, Germany). A pestle and mortar were used to grind up the dry DS. A mechanical sieve (EFL2000, Endercotts, Hope Valley, UK) was used to separate the dry DS with a size range  $\leq 4.0$  mm as the sample for extraction experiment. Moisture

analyser (HR-250AZ, A&D, Tokyo, Japan) was used in determination of DS's moisture content before commencing the lipid extraction.

The properties of the raw DS are displayed in Table 2 according to the prior study conducted by Khalil et al. [32]. DS is comprised of various chemical components associated with carboxylic acids, carbonyl bonds, olefinic, esters, and amides, along with saccharides [32]. The elemental analysis observed the abundance of O, C, Fe, P, Al, Mg, and S, in addition to other elements such as Ca, Si, Na, Cl, and K [32].

**Table 2.** Properties of DS [32].

Properties	Value (wt.%)
Moisture content, MC	80.82 ± 0.94
Total solid content, TS	19.18 ± 0.94
Volatile solid content, VS	<sup>a</sup> 46.75 ± 0.74

Dry weight is the basis for the values. <sup>a</sup> % of TS.

### 2.3. Experiment of Lipid Extraction

Lipid extraction experiment was conducted using a 6-set of a parallel Soxhlet setup consisting of heating mantle (Biobase, Jinan, China), round-bottom flasks of 250 mL (Favorit, Milan, Italy), Soxhlet extractor, and condenser. A chiller (TC501D, Brookfield, Worcester, MA, USA) was connected to the condensers by hose to provide circulation of cooling water, maintained at 20 °C. DS of ranges ≤ 4.0 mm was dried to approximately 5% moisture content. A cellulose thimble (CT30100, Favorit, Milan, Italy) was filled with the dry DS sample according to the specified mass and placed into the extraction chamber (Favorit, Milan, Italy).

The effect of extraction parameters on lipid yield was evaluated using the one-factor-at-a-time (OFAT) approach. Every parameter (temperature, time, S/L ratio) in the OFAT approach has a baseline level, which was then gradually changed across its range while keeping the other parameters constant [34]. Lipid extraction using hexane as a solvent was conducted based on parameters at various levels in duplicate. The experiment was started by varying the system temperatures of 70, 80, and 90 °C for 4 h and 0.1 g/mL of S/L. The following experiment focused on varying the extraction time from 2, 4, 6, and 8 h at 70 °C and 0.1 g/mL of S/L. The next experiment involved varying S/L ratios of 0.05, 0.075, 0.1, 0.125, 0.15, and 0.175 g/mL for 4 h at 70 °C.

### 2.4. Separation of Lipid

The resulting mixture in the flasks was allowed to cool after the extraction. A rotary evaporator (Rotavapor R-200, Büchi, Uster, Switzerland), coupled with a vacuum pump, was used to separate lipids from the mixture by evaporation. The flask containing lipids was cooled in a desiccator and weighed. The processes were repeated to obtain a consistent mass of lipids.

### 2.5. Lipid Yield

The lipid yield ( $Y_{ds}$ ) was calculated gravimetrically using the mass of lipid ( $M_{lipid}$ ) and dry sludge ( $M_{ds}$ ) [35]. Equation (1) was used to compute the lipid yield [36,37]. The analysis and visualisations were performed in Microsoft Excel (Microsoft 365).

$$\text{Lipid Yield, } Y_{ds}(\%) = \frac{M_{lipid}(g)}{M_{ds}(g)} \times 100\% \quad (1)$$



## 2.6. Characterisation of Lipid

Fourier transform infrared (FTIR) spectroscopy analysis of the lipid was conducted utilising an Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectrometer (Nicolet™, Thermo Fisher Scientific, Waltham, MA, USA), which is outfitted with an ATR diamond crystal. Omnic software version 9.8 (Thermo Fisher Scientific, Waltham, MA, USA) was employed for the operation and spectral collecting throughout the range of 4000–400  $\text{cm}^{-1}$  utilising 26 scans at a resolution of 8.0  $\text{cm}^{-1}$ .

A thermogravimetric analyser (Mettler Toledo, Columbus, OH, USA) was employed to conduct thermogravimetric analysis (TGA) of DS lipid over a temperature range of 30–900 °C, with a heating rate of 10 °C/min. Nitrogen ( $\text{N}_2$ ) gas served as the protective gas at 70 mL/min with this temperature range, whereas purge gas was introduced at a higher range of 900–1000 °C at 20 mL/min.

## 2.7. Conversion of Lipid to Fatty Acid Methyl Esters (FAME)

Lipid was converted to FAME by transesterification of lipid using  $\text{BF}_3$  as catalyst, in accordance with AOAC Official Method 996.05 [38,39]. The lipid was solubilised in a vial containing 2 mL of chloroform and 2 mL of diethyl ether. The vial was then placed in a water bath at 40 °C and evaporated to dryness under nitrogen gas. The vial was filled with 2 mL of  $\text{BF}_3$  (7% in methanol) and 1 mL of toluene and sealed. The mixture was heated in an oven to 100 °C for 45 min, and gently mixed every 10 min. Subsequently, the vial was permitted to cool to ambient temperature, after which it was filled with 5 mL of distilled water, 1 mL of hexane, and 1.0 g of  $\text{Na}_2\text{SO}_4$ . The vial was capped, shaken for one minute, and left to allow the separation of layers. The upper layer was then transferred to a fresh vial containing 1.0 g of  $\text{Na}_2\text{SO}_4$ . This upper layer was utilised in the subsequent characterisation of DS FAME.

## 2.8. Characterisation of FAME

ATR-FTIR spectrometer (Nicolet™, Thermo Fisher Scientific, Waltham, MA, USA), equipped with an ATR diamond crystal was used to analyse the FTIR spectra of FAME. Omnic software (Thermo Fisher Scientific, Waltham, MA, USA) was utilised for the operation and spectral collecting throughout the range of 4000–400  $\text{cm}^{-1}$ , employing 26 scans at a resolution of 8.0  $\text{cm}^{-1}$ .

A gas chromatography system incorporating a flame ionisation detector (GC-FID) (6890, Agilent, Santa Clara, CA, USA) was employed to analyse DS FAME and generate chromatographic spectra for fatty acid ester profiling in accordance with AOAC 996.06 [38,39]. A 1.0  $\mu\text{L}$  amount of sample containing DS FAME was injected into the GC system in split mode (50:1), with the inlet heater temperature of 225 °C and inlet flow of 105 mL/min. The analysis was performed using a polar column (RT 2460, Restek, Bellefonte, PA, USA) with carrier gas at 1.0 mL/min of flow rate and 18 cm/s of an average linear velocity. The temperature of the oven was set at 100 °C with a rate of 4.5 °C/min to 200 °C and 2.0 °C/min to 240 °C (hold time of 15 min). The detector was operated at 250 °C, with hydrogen at 40.0 mL/min of flowrate, air at 450 mL/min flowrate and nitrogen gas at 45.0 mL/min flowrate. FAME standards consist of 37 compounds, with triundecanoin (C11:0) being used as the triglyceride internal standard [38,39].

# 3. Results and Discussion

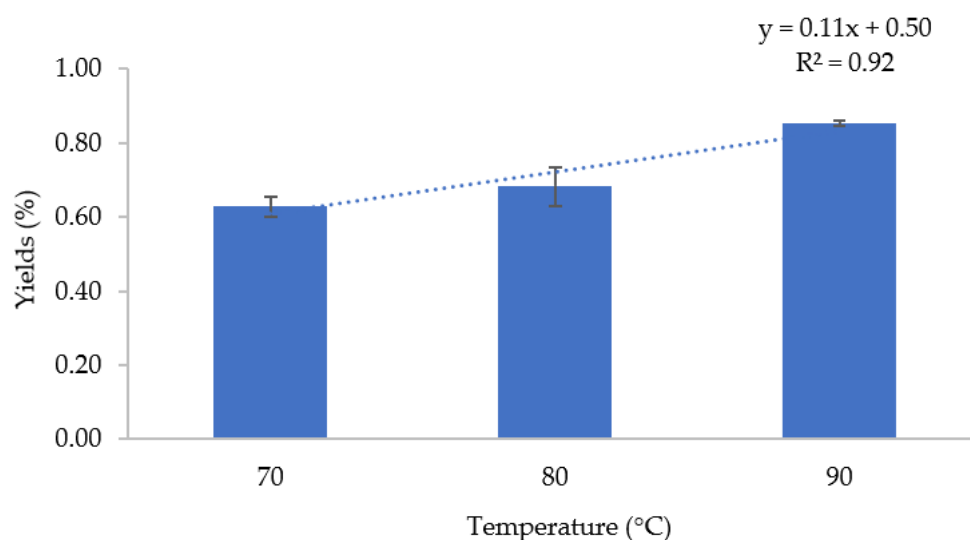
## 3.1. Influence of Extraction Parameters on Lipid Yield

The extraction parameters, including system temperature, time, and sludge-to-solvent (S/L) ratio, were evaluated to assess their influence on the yield of lipid extraction. While previous research has investigated most of these factors in primary and secondary sewage

sludges, a detailed analysis of the extraction process involving dewatered sludge (DS) remains valuable due to its unique characteristics compared to those sludges [32]. Hence, the influence of these extraction parameters on the yield of lipids is presented and discussed in the following sections.

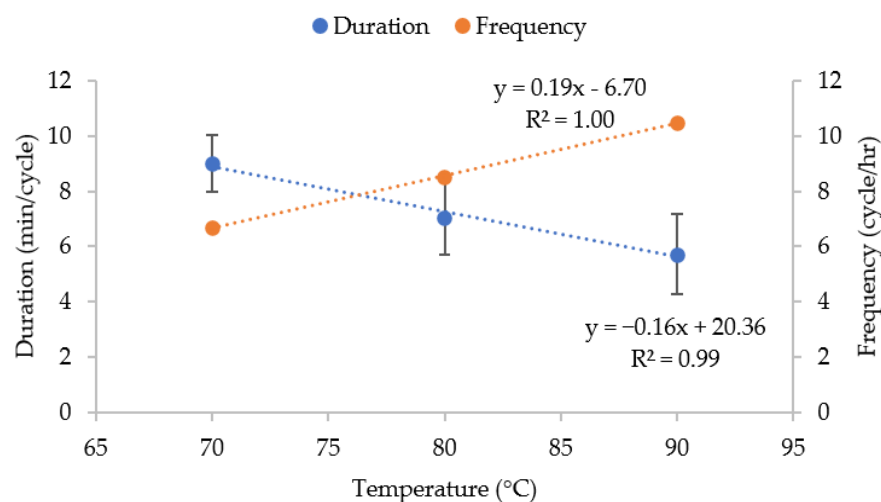
### 3.1.1. Extraction Temperature

Figure 2 illustrates the effect of the system temperatures (70, 80, and 90 °C) on the yield of lipid at 4 h of extraction time with an S/L ratio of 0.1 g/mL. The graph shows a positive linear correlation between temperature and lipid yield, with a strong correlation ( $R^2 = 0.92$ ). Extraction at 70 °C, slightly above the boiling temperature of hexane, yields a lipid content of  $0.63 \pm 0.03\%$ . Extraction at elevated temperatures of 80 °C and 90 °C increases the production of lipids to  $0.68 \pm 0.05\%$  and  $0.85 \pm 0.01\%$ .



**Figure 2.** Effect of temperature on the lipid yield at 4 h of extraction time and S/L of 0.1 g/mL.

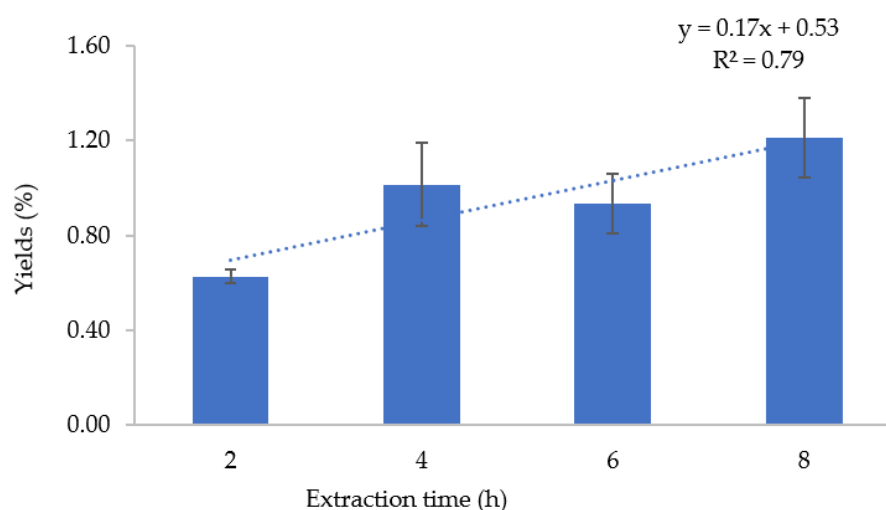
The efficiency of the extraction process can be explained by the relationship between system temperature and the frequency of siphoning cycles, as presented in Figure 3. The duration required for each siphoning cycle reduces as the temperature increases from 70 to 90 °C, resulting in an increase in the frequency of the siphoning cycle. The increase in the siphon cycle promotes rapid solvent cycling, resulting in an increase in contacts between fresh solvent and DS within a given time period [40]. Although the extraction system was set at elevated temperatures (70, 80 and 90 °C), the solvent temperature decreased upon reaching the extraction chamber, stabilising at  $43.8 \text{ °C} \pm 0.98$ ,  $48.7 \text{ °C} \pm 0.74$  and  $51.6 \text{ °C} \pm 0.15$ , respectively. This temperature drop occurs due to heat loss during solvent vaporisation, condensation, and transfer within the Soxhlet apparatus. Despite this reduction, an increase in the solvent temperature causes its viscosity to drop, which improves the ability of the solvent to wet and saturate the target component [41]. Additionally, the extra thermal energy supplied encourages analyte migration to the matrix surface and contributes to the disruption of analyte–matrix interactions [41]. Extraction at elevated temperatures of solvent is essential to enhance diffusion efficiency and mitigate the significant presence of organic lipophilic pollutants, particularly in primary sludge [42].



**Figure 3.** Effect of temperature on the siphon cycle at 4 h extraction time and S/L of 0.1 g/mL.

### 3.1.2. Extraction Time

Figure 4 illustrates the effect of different extraction times (2, 4, 6, and 8 h) on the yield of lipid when the extraction was conducted at 70 °C and 0.1 g/mL S/L ratio. The graph shows an increasing interaction between temperature and lipid yield, with a strong correlation ( $R^2 = 0.99$ ). The shortest extraction time of 2 h shows the lowest lipid yield of  $0.63 \pm 0.03\%$ . The lipid yield has an increasing trend with a longer extraction time of 4, 6, to 8 h, with the longest duration of 8 h producing the highest lipid yield of  $1.28 \pm 0.08\%$ . Lipid yield increased with extraction time from 2 to 8 h, and it remained constant beyond this point for the extraction at 70 °C and S/L ratio of 1:5 [43]. The trend may be evaluated by relating the duration of extraction with the frequency of the siphoning cycle of the Soxhlet extraction as presented previously in Figure 3. When the extraction was conducted at 70 °C, the frequency of siphon cycles rose to 14, 28, 42, and 56 cycles, with a longer period of time from 2, 4, 6, to 8 h. A higher frequency of siphon cycles improves the incorporation of fresh solvent into the system. The higher mass transfer by diffusion brought on by the increased contact between DS and the fresh solvent results in the extraction of more lipids [44].



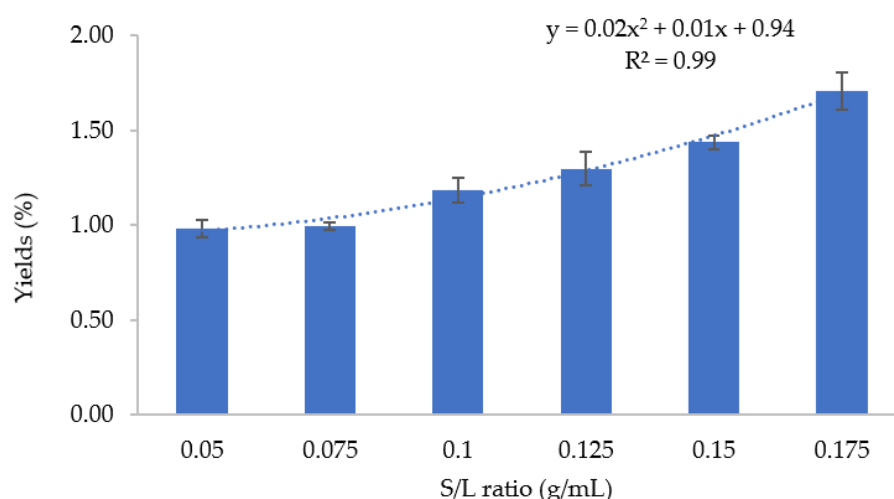
**Figure 4.** Effect of extraction time on the yield of lipid at 70 °C and S/L of 0.1 g/mL.

### 3.1.3. Sludge-to-Solvent (S/L) Ratio

Figure 5 shows the lipid yield of the extraction from DS based on different sludge-to-solvent (S/L) ratios. The graph shows an increasing parabolic relationship between the



S/L ratio and lipid yield, with a strong correlation ( $R^2 = 0.99$ ). The S/L ratios of 0.05 and 0.075 g/mL show a constant lipid yield ranging from  $0.98 \pm 0.04\%$  to  $0.99 \pm 0.02\%$  due to the excess solvent volume, which limits the lipid concentration gradient and mass transfer efficiency. As the S/L ratio increases from 0.1 g/mL onwards, the system reaches a more favourable balance where the solvent achieves higher lipid solubility and enhanced extraction efficiency, resulting in an increase in lipid yield. The highest lipid yield ( $1.71 \pm 0.10\%$ ) was achieved at an S/L ratio of 0.175 g/mL. Further, the increase in the S/L ratio was limited by the Soxhlet extraction set-up, particularly the size of the extraction thimble and the extraction chamber's capacity. A 30 mm  $\times$  100 mm thimble used in this study can only hold up to 35 g of DS, which represents a 0.175 g/mL S/L ratio. The increase in the S/L ratio beyond 0.175 g/mL requires a reduction in the volume of solvent, which must be cautiously considered. The gradual lipid accumulation in the boiling flask increases the viscosity of the solvent, potentially reducing evaporation efficiency due to slower solvent cycling. The solvent volume should also be considered to account for the losses during evaporation and condensation due to the high system temperature and long duration of the process. A minimum solvent volume of 80 mL should be considered to have a siphoning effect. Meanwhile, the solvent used also plays an important role in ensuring the efficiency of the extraction process [45]. Hexane used in the extraction in this project is a non-polar solvent that appears to be a good extraction agent for non-polar glycerides such as triglycerides [46]. The low-polar matrix of the sludge can be efficiently penetrated by this non-polar solvent since it has little to no charge or none at all [47].



**Figure 5.** Effect of sludge-to-solvent (S/L) ratio on the yield of lipid ratio at 70 °C and 4 h extraction time.

### 3.2. Optimum Lipid Yield

Extraction temperature, duration, and solid-to-liquid ratio are crucial factors in achieving optimum yield and ensuring efficient process operation [48]. The previous analysis in Section 3.1 found that extraction at a temperature of 70 °C for 4 h at an S/L ratio of 0.1 g/mL resulted in a 0.63% yield. Temperature increases to 90 °C resulted in the highest lipid production of 0.85%. Nonetheless, extraction at 70 °C for 4 h produced 0.87% yield, and a prolonged extraction period of 8 h resulted in the highest lipid yield of 1.21%. Despite the high lipid yield at 90 °C, the possibility of solvent loss increases at high temperatures due to vaporisation and raises the cost of the extraction process [48,49]. Furthermore, longer extraction times have a detrimental effect on economising energy and the cost of the process [32]. Conducting extraction at 70 °C for 4 h at S/L ratio beyond 0.1 g/mL increases lipid yield due to an enhancement of the availability of the volatile organic fraction in the

sample, including lipid. Thus, the highest yield of  $1.71 \pm 0.10\%$  of lipid was obtained with the optimal system temperature of  $70\text{ }^{\circ}\text{C}$ , 4 h of extraction time and S/L ratio of  $0.175\text{ g/mL}$ .

### 3.3. Lipid Yield from Various Types of Sludge

Further evaluation was conducted by comparing the optimal lipid yield obtained from DS to the other types of sludge (primary sludge, secondary, and sludge cake). Table 3 shows that the extraction from DS yielded the lowest yield of  $1.71\%$  relative to the dry sludge compared to the other types of sludge. The low lipid yield from DS is attributed to the lower composition of the volatile matter content, with  $46.75\% \pm 0.74$  compared to primary sludge (60–80%), secondary sludge (59–88%), and sludge cake (35.98%) [32,33,36]. The properties of sludges, including their volatile matter content, vary depending on the source and production conditions [11]. Despite the lower yield, the extraction of lipids from DS required a minimal extraction temperature ( $70\text{ }^{\circ}\text{C}$ ) compared to sludge cake ( $80\text{ }^{\circ}\text{C}$ ) and a shorter time (4 h) in comparison to primary sludge, secondary sludges, and sludge cake (5.5 h and 6 h), which minimises the impact of process costing. Achieving a balance between yield and cost efficiency in the optimisation of lipid extraction is challenging. The parameters (temperature, time, and S/L) and the conditions that maximise yield often conflict with those that reduce costs [48]. Hence, the strategy in optimisation can be further refined in future work by focusing on varying several parameters together to produce the highest lipid yield in a cost-effective manner [34]. The evaluation of the lipid yield from various sludge is further discussed in the subsequent sections.

**Table 3.** Comparison of lipid yield extracted by Soxhlet using hexane as solvent from various types of sludge.

Type of Sludge	Point of Collection	TS (wt.%)	Extraction Parameters	Lipid Yield, Y (%)		Ref.
				$Y_{ds}$	$Y_{ww}$	
Primary	Primary treatment, after partial gravity thickening	$4.2 \pm 1.2$	Temperature = NA Time = 5.5 h S/L = $0.1\text{ g/mL}$ Particle size = NA	26	1.09	[50,51]
Secondary	Activated sludge process, after partial thickening by flotation	$3.1 \pm 0.7$		9	0.28	
Sludge cake	Sludge storage yard of CSTF	33..32	Temperature = $80\text{ }^{\circ}\text{C}$ Time = 6 h S/L = $0.1\text{ g/mL}$ Particle size $\leq 100\text{ mesh}$	5.15	1.72	[36]
DS	Sludge compartment of RSTP	$19.18 \pm 0.94$	Temperature = $70\text{ }^{\circ}\text{C}$ Time = 4 h S/L = $0.175\text{ g/mL}$ Particle size $\leq 4\text{ mm}$	$1.71 \pm 0.10$	0.33	This study

CSTF = centralised sludge treatment facility, RSTP = regional sludge treatment plant, NA = not available, ds = relative to dry sludge, ww = relative to wastewater,  $Y_{ww}$  was estimated with an assumption that dry sludge content is similar to TS.

The variation of the lipid yield can be further discussed by comparing the type of sludge used in the extraction. The comparison was made by assuming that the lipid yield from dry sludge is similar to that from total solid (TS) in the wastewater. Table 3 shows that the DS in this study yielded a lower lipid of  $0.33\%$  relative to the wastewater compared to the primary sludge ( $1.09\%$ ) and sludge cake ( $1.72\%$ ). Despite the lower yield ( $0.32\%$ ) from DS when compared to the primary sludge and the sludge cake, the lipid yield is comparable to the secondary sludge ( $0.29\%$ ). Comparable lipid yield relative to wastewater for both DS and secondary sludge can be attributed to similarities in their organic content as observed by the similar thermal degradation profiles between  $202\text{ }^{\circ}\text{C}$  and  $491\text{ }^{\circ}\text{C}$  based on the TGA analysis [18,32]. The source and composition of the sludge can contribute to lipid yield. Primary sludge was collected from the primary treatment following the partial

gravity thickening of wastewater [50]. Sludge cake was produced as the end waste of a centralised sewage treatment facility (CSTF) that processes wastewater originating from individual and communal septic tanks (IST and CST), including sewage treatment plants (STP) [36]. The sludge cake was separated from the influent wastewater by centrifugation for drying and disposal [36]. Both primary sludge and sludge cake are expected to consist of a significant quantity of organic matter because the treatment was designed to collect the majority of the particulate organics from the influent wastewater [50,52].

### 3.4. Characteristics of DS Lipid and DS FAME

The characteristics of lipids extracted from DS play a crucial role in evaluating their potential as a feedstock for biodiesel production. The characteristics of lipids, primarily composed of triacylglycerol and fatty acids, vary depending on the extraction parameters. Understanding the properties of lipids and the composition of their fatty acid methyl esters (FAME) is essential to assess their suitability for sustainable energy production, particularly as biodiesel.

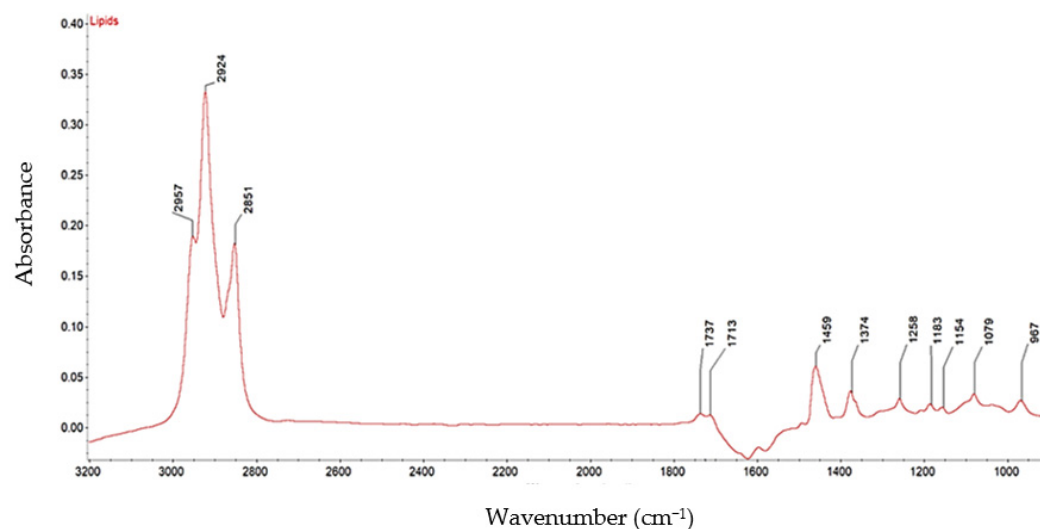
The variation of the system parameters is expected to influence the characteristics of DS lipids. Physical changes in lipid produced are essential to deduce the impact of specific process parameters [34]. Extraction at optimal conditions of 70 °C, 4 h, and 0.175 g/mL produced double-layer lipids, with the upper layer appearing in brownish liquid form and lighter-coloured solid seen at the bottom layer. The extraction at a higher temperature of 90 °C or a longer period of 8 h produced lipids with a darker colour that solidified with time. These physical changes might be due to the oxidation and degradation of biocompounds in lipids [49,53]. Extraction at high temperatures carries the risk of thermal degradation of temperature-sensitive components and might deteriorate the quality of the fatty acids extracted [54,55]. Longer extraction time at 8 h significantly influences the solubility and mass transfer of the compounds in lipids such as ceramide, diacylglycerol (diglyceride), and PUFA (eicosanoids) due to their structure and molecular weight [49,53,56]. Some lipid fractions, such as PUFAs, have a tendency for autooxidation and degradation at a higher temperature of 90 °C [57]. A prolonged extraction duration yielded a greater lipid quantity. However, the lipid fraction may contain a greater proportion of non-saponifiable matters that are unsuitable for biodiesel conversion [58]. Hence, DS lipids extracted at an optimal parameter of 70 °C, 4 h, and 0.175 g/mL of S/L were used in the evaluation of the potential of DS lipids as biodiesel feedstock. The characteristics of these DS lipids based on FTIR and TGA, as well as the composition of DS FAME, are further discussed in the next sections.

#### 3.4.1. FTIR Analysis of DS Lipid

FTIR spectra for DS lipids extracted from the dewatered sludge are shown in Figure 6. The peaks of the spectra were analysed for lipids, particularly the presence of triacylglycerol and impurities after the extraction and separation processes. There were more than five absorption bands seen, indicating the extract consists of complex molecules [59]. No broad absorption band was found between 3650 and 3250  $\text{cm}^{-1}$ , indicating there was no hydrogen bond (-OH) [59,60]. The absence of this peak indicated that there was no water fraction in the extract [59,60].

Peaks at 2957, 2924, and 2851  $\text{cm}^{-1}$  were observed, attributable to -CH<sub>3</sub> asymmetric, -CH<sub>2</sub> asymmetric, and -CH<sub>2</sub> symmetric stretching. These peaks correspond to the aliphatic bond presence in the compound [60]. A peak was detected within the 1750–1725  $\text{cm}^{-1}$  region, specifically at 1737  $\text{cm}^{-1}$ , indicating a simple carbonyl compound (C=O) of ester [61,62]. The peak, which occurred within 1750–1735  $\text{cm}^{-1}$ , is an indication of the presence of saturated esters [63]. A peak at 1737  $\text{cm}^{-1}$  corresponds to the C=O ester [64,65]. This peak is a bit lower compared to the FAME ester peak (1750–1745  $\text{cm}^{-1}$ ), suggesting

the carbonyl absorption is due to the triacylglycerol linkage of the saturated ester with a characteristic peak at  $1735\text{ cm}^{-1}$  [64,65]. The peaks at  $1183\text{ cm}^{-1}$  and  $1079\text{ cm}^{-1}$  are associated with C-C-O stretching in the saturated ester and C-O stretching, attributable to O-CH<sub>2</sub>-C asymmetric stretch with a characteristic peak at  $1100\text{ cm}^{-1}$  [60,64,66]. A peak near  $1183\text{ cm}^{-1}$  corresponds to the existence of saturated acyl groups [65]. The appearance of these peaks suggested the presence of triacylglycerol in the DS lipid.

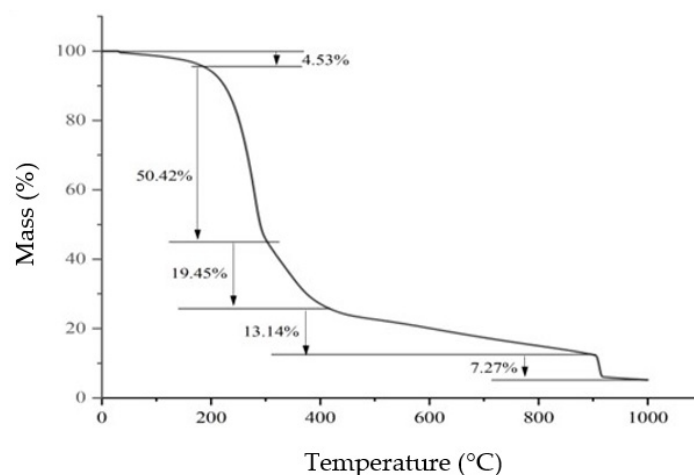


**Figure 6.** FTIR spectra of DS lipid.

Further analysis was conducted on the remaining bands to identify impurities in DS lipids. A weak free fatty acid peak was seen at  $1713\text{ cm}^{-1}$ , corresponding to the C=O bond of the carboxylic acid group that belongs to fatty acids [59,67]. The peaks at  $1374$  and  $1079\text{ cm}^{-1}$  attributed to the O-CH<sub>2</sub> and C-O stretching vibrations of the glycerol group in the lipid [68]. Peaks at  $1459$  and  $1258\text{ cm}^{-1}$  were observed due to the CH<sub>2</sub> bending and deforming vibration of NH<sup>+</sup>, indicating possible protein secretion from EPS in the lipid [62,68]. The peak at  $967\text{ cm}^{-1}$  attributable to the trans-C-H out-of-plane bending (C=C-H<sub>trans</sub>,  $970\text{--}960\text{ cm}^{-1}$ ) indicated the possible existence of the unsaturated ester in the lipid [69].

### 3.4.2. TGA Analysis of DS Lipid

Thermal degradation of lipids is expected to occur in the temperature ranges of  $200\text{--}635\text{ °C}$  [70]. Thermal degradation of DS lipid is presented in Figure 7. The thermal degradation pattern of DS lipid is further discussed based on four stages of mass loss. Stage 1 began at  $33\text{ °C}$  and continued up to  $188.23\text{ °C}$ , resulting in a 4.53% mass loss. This mass loss in the given temperature range corresponds to water evaporation [71]. The degradation of glycerol also took place in these temperature ranges, specifically at  $116.01\text{--}237.75\text{ °C}$  [72]. Stage 2, in the range of  $188.23\text{--}280.50\text{ °C}$ , observed a mass loss of 50.42% due to the degradation of fatty acids. Fatty acids such as oleic acid and palmitic acid thermally degrade at  $138\text{--}294\text{ °C}$  and  $117\text{--}265\text{ °C}$ , respectively [72]. Thermal degradation for proteins also occurs within this temperature range, specifically between  $209$  and  $309\text{ °C}$  [70]. Stage 3 corresponds to the degradation of triacylglycerol with 19.45% mass loss at a maximum temperature of  $417.79\text{ °C}$  [72]. Other components in oil and fat are expected to degrade at temperatures beyond  $417.79\text{ °C}$ , resulting in a mass loss of 13.14%. All components in lipids were expected to be converted to ash upon combustion beyond  $900\text{ °C}$  with a mass loss of 7.27% and a final residue of 5.19%.



**Figure 7.** TGA of DS lipid.

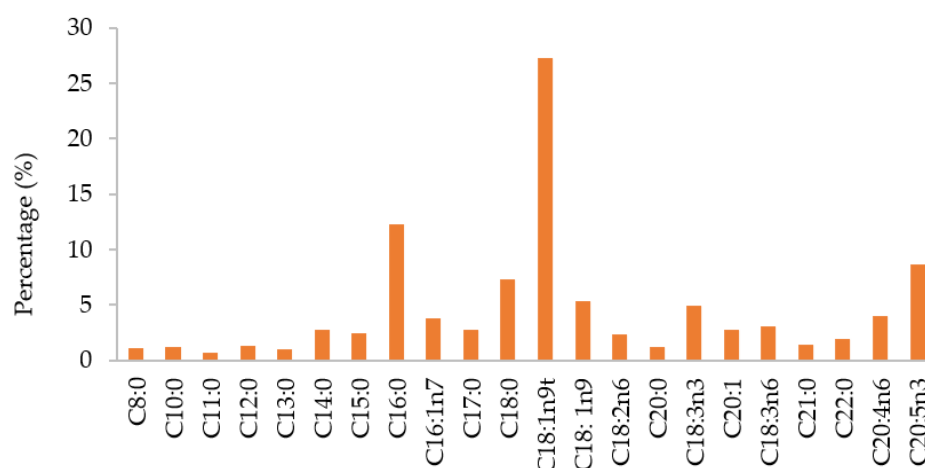
Lipid extracted from DS consists of high fatty acids (50.42%) and triacylglycerol (19.45%), with impurities such as moisture, glycerol, and proteins. Hence, the conversion of DS lipid to DS FAME by esterification and transesterification processes requires the use of acid catalysts due to high amounts of fatty acids and triacylglycerol [73]. Impurities in lipids not only interfere with the transesterification process but also serve as a source of contamination in the final FAME product [74]. Impurities such as water, undesired byproducts, and glycerol must be removed to meet the intended purity of FAME and satisfy the biodiesel standard [47,75,76]. Moisture may be reduced using heat or a vacuum in order to decrease the emulsion effect and produce a high biodiesel production [73,74].

### 3.5. Characteristics of DS FAME

The quality of biodiesel fuel is determined by the feedstock composition, the production process (including reaction and purification), and the storage and handling [74]. Practically, biodiesel properties can be anticipated by understanding the fatty acid ester composition [77]. Thus, this section aimed to determine the profile and composition of the fatty acids in DS FAME and further assess the suitability of lipids extracted from dewatered sludge as oil feedstock for biodiesel [78].

#### 3.5.1. Fatty Acid Profile of DS FAME

Figure 8 shows the fatty acid profile of DS FAME transesterified from DS lipid. The DS FAME consists of fatty acids of C8:0 to C22:0 with the highest fractions of elaidic (C18:1n9t), palmitic (C16:0), eicosapentaenoic (C20:5n3), stearic (C18:0), oleic (C18:1n9),  $\alpha$ -linolenic (C18:3n3) and arachidonic (C20:4n6) with 27.3%, 12.3%, 8.7%, 7.3%, 5.4%, and 4.9%, besides the other fractions such as myristic (C14:0), palmitoleic (C16:1n7), and margaric (C17:0). The fatty acid profile of the DS is expected to have a profile similar to the original lipid source, which is the secondary sludge [79]. FAME produced from lipids extracted from the secondary sludge consists of C16:0, C18:1, C18:0, C16:1, C18:2, C14:0, and C20:1 [80]. Lipids found in municipal wastewater are a result of human waste and kitchen waste, including animal and vegetable oils [81]. Human excreta constitutes lipids with three dominant fatty acids of C16:0, C18:0, and C18:1n9, whereas kitchen waste consists of animal fats and vegetable oil with high amounts of C16:0, C18:0, 18:1n9 and 18:2n6, as well as C14:0 originated from bacteria within the sludge [81].



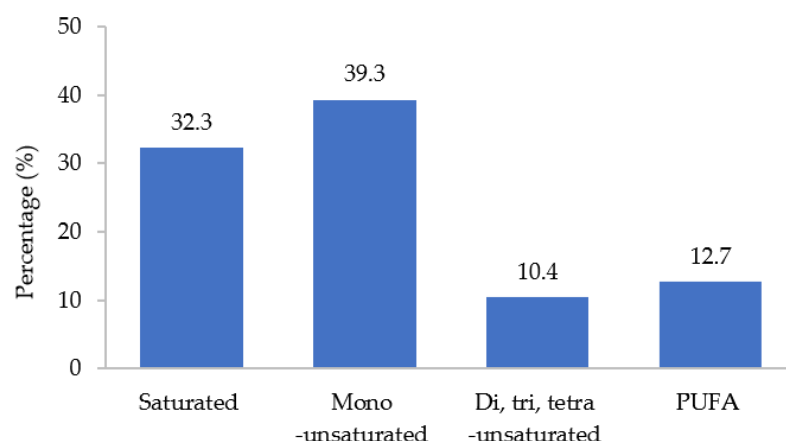
**Figure 8.** Fatty acid profile of DS FAME.

### 3.5.2. Potential of DS FAME as Biodiesel

The FAME content of the lipid as feedstock may be used to identify the properties of biodiesel as well as the fuel quality [74,77]. DS FAME produced from DS lipid shows a higher unsaturated fatty acid component of 62.4% compared to 37.6% saturated components of its C8:0–C22:0. Previous study by Hag. Ibrahim [37] focussed on the extraction of lipids from scum sludge utilising methanol–hexane resulting in lipids with high unsaturated fatty acids (59.3%) compared to its saturated fraction (40.7%). A high unsaturated fatty acid fraction indicates a higher content of free fatty acids in the lipid [37]. A lipid with high unsaturated fatty acid fractions is expected to produce FAME as biodiesel with a low melting point and contribute to enhancing its overall cold flow properties [4,82,83]. The unsaturated fatty acid fraction in FAME also minimises the solidifying effect of the saturated fraction, thus further easing the handling and distribution processes [4,82]. However, a higher unsaturated fatty acid fraction causes lower oxidation stability [84]. The existence of polyunsaturated fatty acids (PUFA) might lead to self-polymerisation, causing clogging of fuel lines or filters as well as engine fouling [84,85]. The fatty acid composition in FAME can also be used to predict the cetane number (CN), where a higher CN value indicates a high ignition quality of a diesel engine [77]. It is expected that the increase in saturation of the fatty acid compound from C4:0 to C18:0 of FAME increases the CN value up to 80 [4,77]. The increase in the degree of unsaturation or number of double bonds will lower the CN value [4,77].

Further evaluation of the DS FAME as biodiesel based on its C14:0 to C24:1 composition is presented in Figure 9. The extraction of lipid from DS and transesterification managed to produce DS FAME of carbon chain C14–C24:1 with an ester content of 94.7%. FAME, with an ester content of more than 90%, can be used as a pure biofuel or as a blending component for heating and diesel fuel based on EN14103 [86]. Ester content of DS FAME may be improved by eliminating impurities in the lipid feedstock or ensuring sufficient reaction conditions [74]. The DS FAME also consists of 8.0% linolenic acid (C18:3), within the acceptable range based on EN14103 and EN14214 biodiesel standards [86,87]. Biodiesel standard EN14214 allows FAME with properties meeting its requirements to be marketed and delivered as B100 diesel fuel for automobiles [87]. Restriction of the use of FAME with high linolenic acid content in automobiles based on EN14214 is due to its potential to oxidise [74,87,88]. Furthermore, the DS FAME exhibits a PUFA content of 12.7%, higher than the maximum limit (1.0%) specified by EN14214. High PUFA concentrations exhibit poor oxidation stability and a higher rate of self-polymerisation, resulting in fuel line or filter clogging as well as engine fouling [74].





**Figure 9.** Composition of DS FAME of C14:0 to C24:1.

### 3.6. Production of Biodiesel from Sewage Sludge and Its Economic and Environmental Implications

The usage of primary and secondary sludge in the production of biodiesel poses challenges, especially in pre-treatment to reduce the water content prior to extraction [32,80].

Liew et al. [89] conducted a lifecycle assessment of biodiesel production using secondary sludge. The thermal pre-treatment stage depends on electricity as input and significantly contributes to environmental burdens, as reflected in its high allocation across multiple midpoint impact categories. The substantial energy consumption required for drying sludge leads to elevated climate change and particulate matter formation impacts, primarily due to emissions from coal-based electricity production [89]. Additionally, sludge pre-treatment is linked to increased freshwater and marine eutrophication and ecotoxicity, as well as land occupation, highlighting its broader ecological effects [89]. These environmental burdens underscore the need for energy-efficient drying technologies or alternative feedstock substitutions to mitigate the impact. By utilising dewatered sludge (DS) instead of secondary sludge, the overall energy demand for pre-treatment could be lowered, thereby reducing its environmental footprint [31,89]. The usage of DS with low water content is expected to minimise the operation and maintenance (O&M) cost in biodiesel production by up to 13.8% by eliminating the need for a dewatering process prior to drying [32]. Dufreche et al. [80], Mondala et al. [90], and Pokoo-Aikins et al. [91] obtained very similar price estimates (USD 3.11, USD 3.23, and USD 2.79–USD 3.39) per gallon of biodiesel from sewage sludge, which are fairly comparable with the average prices of petroleum diesel and refined soy biodiesel [92]. Additionally, the abundant availability of DS and its negligible procurement cost make it an economically viable feedstock alternative for biodiesel production. This cost-effectiveness and sustainable sourcing highlight the environmental benefits of using sewage sludge as a biodiesel feedstock.

## 4. Conclusions

The potential of lipids extracted from dewatered sludge (DS) as a viable feedstock for biodiesel production presents a promising avenue for sustainable energy recovery. Due to its appreciable organic matter and lipid content, DS serves as a valuable feedstock for biodiesel synthesis, reducing reliance on conventional fossil fuels. The findings highlight the significant influence of extraction parameters of temperature, extraction time, and sludge-to-solvent ratio (S/L) on the lipid yield. The DS lipid produced consists of high fatty acids and triacylglycerol, besides impurities such as glycerol and proteins. DS FAME comprises a fatty acid profile of C4:0–C22:0 with a high fraction of elaidic (C18:1n9t), palmitic (C16:0), eicosapentaenoic (C20:5n3), stearic (C18:0), oleic (C18:1n9),  $\alpha$ -linolenic (C18:3n3) and arachidonic (C20:4n6), in which high ester content (94.7%) exhibit the potential to be

used as pure biodiesel or as a blending component for heating and diesel fuel. DS FAME is expected to exhibit stability with good cold flow qualities due to a higher unsaturated fatty acid fraction compared to a saturated acid fraction. A high amount of PUFA limits the usage of DS FAME in automotive engines due to its oxidative properties, hence the opportunity for process improvement. The positive outcome from this work served as a benchmark for future research on sustainable biofuels made from polymer-based sludge of DS generated from municipal wastewater treatment plants. Nonetheless, additional research is required to enhance pre-treatment and extraction technology, improve lipid and FAME quality, and evaluate the economic and environmental viability of large-scale implementation. Moreover, employing DS for biodiesel not only provides an alternate lipid source but also mitigates sludge disposal challenges in waste management and enhances environmental sustainability.

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