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Lipid Extraction for Biodiesel Production from Al-Rumaitha Sewage Project Plant

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Keywords:
Liquid-liquid extraction; Biodiesel; Lipids; Free fatty acids; Water content; Dry matter content.

Highlights:

- *The organic solvent mixture, the Smedes mixture, is the best choice for extracting lipids from wet sludge.*
- *Sampling is unlimited in the WWTP's primary sedimentation tanks, as samples can be taken from the screening stage, and good quantities of lipids can be obtained.*
- *The amount and quality of extracted lipids are greatly affected by differences in air temperatures.*

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Abstract: The samples used in the study were taken from the WWTP (wastewater treatment plant) screening location. Lipids extraction from sewage sludge (a by-product of WWTP) grows in large quantities in cities. The wet sludge was adopted to extract the lipids by direct liquid-liquid extraction at room temperature using a mixture of organic solvents cyclohexane, isopropanol, and water for three extraction stages. The content of lipids was 36.3% wt DC and 34.78% wt DC for the first and second samples, respectively. Fourier Transform Infrared spectroscopy (FTIR) and gas Chromatography-Mass Spectroscopy (GC-MS) analysis determined the content of free fatty acids. FFAs (free fatty acids) content was 58% and 36.6% for the first and second samples, respectively. Oleic acid (C18:1) was the most abundant acid in the two samples, followed by palmitic acid (C16:0) and linoleic acid (C18:2).

استخلاص الدهون لأنتاج الديزل الحيوي من مشروع محطة معالجة الرميثة

فاطمة عدنان جمعة، علي عبد الحسين جازع الخالدي
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الخلاصة

العينات في هذه الدراسة تم أخذها من مرحلة السكرينك لمحطة معالجة مياه الصرف الصحي. استخلاص الدهون من حمأة الصرف الصحي (وهو ناتج ثانوي لمحطات معالجة مياه الصرف الصحي) المتزايد بكميات كبيرة في المدن. ثم اعتمد الحمأة الرطبة لاستخلاص الدهون. الاستخلاص المباشر السائل _ السائل. هيه الطريقة المستخدمة لاستخلاص الدهون من الحمأة الرطبة بدرجة حرارة الغرفة باستخدام مزيج من المذيبات العضوية (الهكسان الحلقي، وكحول الايزوبروبانول، الماء) لثلاث مراحل استخلاص. محتوى الدهون كان (٣٦,٣٪ في المادة الجافة و ٣٤,٧٨٪ في المادة الجافة) للعينتان الأولى والثانية على التوالي تم تحديد محتوى الاحماض الدهنية الحرة بواسطة فحص مطيافية الاشعة تحت الحمراء و كروماتوغرافيا الغاز. حيث كان محتوى الاحماض الدهنية الحرة (٥٨٪ ,٣٦,٦٪) للعينتان الأولى والثانية بالتتابع حيث وجد ان أكثر الحوامض وفره هو حمض الاوليك يليه حمض البالمتك ثم حمض اللينوليك.

الكلمات الدالة: استخلاص سائل-سائل، وقود الديزل الحيوي، الدهون، الأحماض الدهنية الحرة، محتوى الماء، محتوى المادة الجافة.

1.INTRODUCTION

Global warming, pollution problems due to the increase in greenhouse gases, especially carbon dioxide, and the high price of fossil fuels and their exhaustion in the short term [1]. Are the key reasons for finding alternative fuel sources. The most prominent of these alternatives and renewable is biodiesel [2]. Biodiesel is generally produced by trans\esterification of vegetable oils or animal fats with alcohols, yielding fatty esters (FEs), trace amounts of residual catalyst, Glycerin, and sops, from the lipid fraction. However, the competitive potential of biodiesel is currently limited by the high price of the common lipid feedstock, constituting between 70 and 85% of the overall biodiesel production cost, strongly influencing the final price of this biofuel [2-8]. Therefore, searching for alternatives is costly and invalid, so it does not cause shortage or scarcity in world food sources [4]. On the other hand, at the same time, finding a solution to the problem of sewage sludge growth where a huge amount of sludge accumulates, requires several treatment techniques such as electro-sedimentation, ion exchange, and reverse osmosis. These technologies have very large financial and operational requirements [5]. One of the main problems in many metropolitan populations worldwide is sewage treatment, which has recently gotten worse in some areas [6]. One of these alternatives, sewage sludge emerged strongly because it is low cost and a promising source of renewable lipids [7, 8]. Municipal sewage sludge, a waste product created during wastewater treatment, may be used as a substitute source of lipids for biodiesel manufacturing, reducing the operating costs of wastewater treatment plants (WWTPs) [8]. The most important reason for choosing sewage sludge as a feedstock for biodiesel production is that it contains a high free fatty acid (FFA) content, which can account for 70% of the lipid composition [3]. This high free fatty acid content is present in lipids feedstock, as biodiesel produces well using an acidic catalyst such as (HCL, H_2SO_4, DBSA) [9]. In two ways, on-site (one step) and traditional (two steps), alkaline catalysts, such as sodium hydroxide and potassium hydroxide, are unused to produce soap and water due to the high content of free fatty acids [9, 10]. Trans\esterification: The most common process of producing biodiesel from various sources [11]. Lipid extraction is the initial step in making biodiesel by traditional trans\esterification from the sludge of wastewater treatment plant sludge. Various techniques are currently available for extracting lipids from biological sources. Most of these techniques use organic solvents [12]. Direct liquid-liquid extraction is the method that will be used to extract lipids from the sludge because it is wet, and the water content in it reaches 98%. The drying or dewatering process; requires high energy and costs up to 50% of the total cost of biodiesel production [13]. Organic solvents are frequently used to extract lipids from sludge, generally in mixtures. The most widely used technique for lipid extraction from various materials is the Bligh and Dyer method, which uses chloroform, methanol, and water [14]. This technique constitutes the characteristics of ternary mixtures, creating a monophasic solution when the solvents are combined in the right proportions to improve contact between the lipids and the organic solvent in which they are soluble. Additional water or chloroform can produce a biphasic solution, straightforwardly separating the lipid-containing chloroform layer. One of the most characteristics of this way is the presence of water, but not exceeding 80% of the water is present, meanings that the sludge needs to be partially dried beforehand; chlorinated solvent has a toxic and environmentally harmful effect [15]. Kech et al. [14] attempted to develop this mixture of organic materials using multiple mixtures. They concluded that the Smedes mixture was the best non-chlorinated alternative to the Bligh and Dyer mixture. Pastore et al. [16] used dried primary sludge (dry matter content 14.4%) with hexane to extract lipids from it under the following conditions: ratio1:1, under acidic media (H_2SO_4), the temperature at 38°C, three consecutive extraction stages ware 1h for stage, and lipids content 20% (wt of lipids/wt of

dry sludge). Olkiewicz et al. [3] mentioned using the liquid-liquid extraction method with wet sludge (dry matter content 3.4%) also using hexane and the following conditions (ratio 2:1) sludge/hexane, acidic media by (HCL), at room temperature for 1h for each stage (three consecutive extraction stages), lipids content 26.7% (wt of lipids/wt of dry sludge). Since the goal is to produce biodiesel from wet sewage sludge, the liquid-liquid extraction method was developed based on what Olkiewicz provided at room temperature, however using a different solvent mixture, which is the Smedes mixture (isopropanol, cyclohexane, and water), with a longer contact time, the pretreatment of sludge in an acidic medium, the ratio of wet sludge to solvent. Also, the extraction from sludge is affected by many factors, including temperature, the nature of sludge composition, the microorganisms present, type and quantities of solvents, extraction time, mixing or stirring rate. Therefore, these factors will affect the nature of the extracted lipids. It must be mentioned that the lipids content cannot be measured accurately. The free fatty acid of lipids, saponification number, and the resulting esters are measured after biodiesel production [14]. The present study aims to extract lipids at room temperature from wet sewage sludge using a Smedes mixture: isopropanol, cyclohexane, and water.

2.MATERIALS AND METHODS

2.1.Reagents

The chemicals used in the present study were 0.1N of fuming hydrochloric acid M.Wt of 36.5gr/mol, from Carlo Erba, France; cyclohexane, M.Wt of 84.16gr/mol and purity 99.0%, from Thomas Beker, India; and Isopropanol, M.Wt of 60.10gr/mol and purity 99.5%, from Gainil and Chemical Company, UK.

2.2.Sludge Sample Collection

The wet sewage sludge sample (sewage residues resulting from heavy water treatment) was obtained from the Al-Rumaitha sewage project plant (25000 m^3\day, for 100K present) in Al-Rumaitha City, Al-Muthana, Iraq. The primary wet sludge sample was taken from screening. The screening stage was the first stage in which the sludge underwent a preliminary physical treatment, where large bodies were removed and floating fats in the form of foam. A large amount of water from the screening was taken and placed in plastic buckets and left to settle for 4h. The sediment was placed in a plastic jar (1750gr±50gr) and kept at a temperature of 5°C. to stop biological material from deteriorating and the characteristics of the sludge from changing. All of the bottles were frozen. The maximum storage period was 15 days. Figure 1 shows the sampling site in the sewage waste water treatment plant.

Fig. 1 The Screening Stage from which the Sludge Sample was Taken.

3.SEWAGE SLUDGE CHARACTERISTICS

3.1.Water and Dry Content Determination

The sample of sludge was weighed before and after drying to determine the content concentration. A 5-gram wet sludge sample was dried in an oven (UNB400 Germany) for 30 min at 105°C to evaporate water from the sample. Sludge water and dry content were calculated using the Eqs. (1) and (2):

$$\text{Sludge water content} = \frac{\text{Weight of water}}{\text{weight of wet sample (before drying)}} \times 100\% \quad (1)$$

$$\text{Sludge dry content} = \frac{\text{weight of dried sample (after drying)}}{\text{weight of wet sample (before drying)} * 100\%} \times 100\% \quad (2)$$

The dry content (DC), the remaining sludge solid was calculated as (1 –water content).

3.2.Lipids Content

3.2.1.Lipids Extraction (Direct Liquid-Liquid Extraction Method)

1) A 5 gr of wet sewage sludge was placed in an ultrasonic bath (to improve extraction efficiency and facilitate extraction) for 30min.
2) 0.7 ml Fuming hydrochloric acid was added until the Ph value become 2 and shaken at 1000 rpm for 4 minutes.
3) 8 mL of isopropyl alcohol and 10 mL of cyclohexane were added, accounting for the amount of water in the sample.
4) 11 g of water was added. The following equation calculates the volume of water to be added.

$$V_{water} = 11 - m_{HCL} - \left(\frac{M * W}{100}\right) \quad (3)$$

where:
V_{water} = the added water (ml).
m_{HCL} = mass of fuming hydrochloric acid (gr).
M = mass of wet sample.
W = sludge water content (%).

5) The sample was shaken with a speed mixture of 1000 rpm for 2 hours at room temperature, then centrifuged at 3000 rpm for 10 min to separate the upper layer from the dark brown and lower layer. Finally, the upper layer was

transported and placed in a new flask. The remaining mixture was returned to the flask previously used in the first shaking process (first stage). Figure 2 shows the experimental work steps.

6) In the second stage of extraction, 10 mL of a cyclohexane combination containing 13% isopropanol (alcohol) is introduced into the residual mixture from the first stage, i.e., The wet sludge mixture and the solvent mixture (Smedes mixture) previously prepared and the oil was previously separated from it can still be extracted from this mixture. The resulting mixture was shaken for 2 hours. The subsequent processes were identical to those employed in the first stage.

7) The third extraction step involved repeating the second stage, encompassing its specific details.

8) After that the lipids extracted from the three stages were collected and placed in Rotary evaporator to separate the mixture of solvents from the lipids for 45 minutes at 90°C. The extracted lipids and solvents separation were kept in a sealed glass bottle.

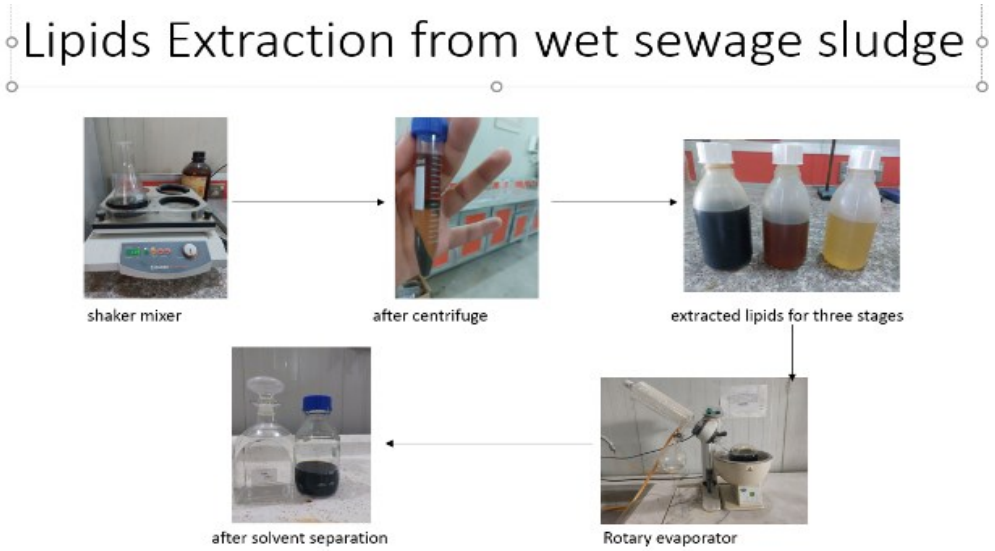

Fig. 2 The Steps of the Experimental Work.

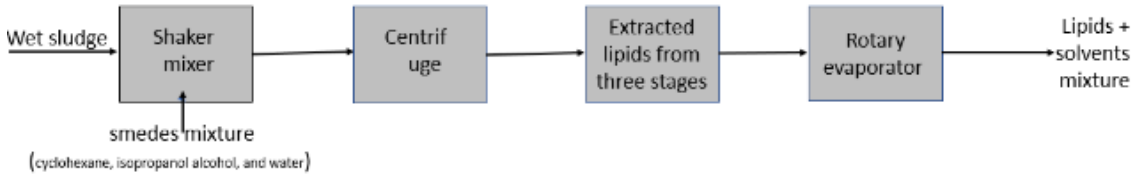

Fig. 3 The Diagram of the Experimental Work.

3.2.2.Determined Lipid Content

This characteristic is the most important characteristic that must be determined for the sludge, as it shows the amount of lipids obtained from sludge. Thus, the extraction method can be evaluated from the amount of lipids obtained. The outcome was given as the weight of lipids per unit weight of wet sludge and DC. using the following equations:

$$\textit{Lipids dry content} = \frac{\textit{weight of lipids}}{\textit{weight the of dried sample (after drying)}} \times 100\% \quad (4)$$

$$\textit{Lipids content in wet sample} = \frac{\textit{weight of lipids}}{\textit{weight of wet sample (before drying)}} \times 100\% \quad (5)$$

4.ANALYSIS METHODS (FTIR AND GAS CHROMATOGRAPHY ANALYSIS)

For Fourier Transform Infrared (FTIR) analysis, the sample was scanned using a BRUKER TENSOR27 spectrometer Germany. The resulting spectra were in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} cell. The fatty acid composition was determined using Gas Chromatography-Mass Spectrometry (GC-MS).

1) The QP2010 GC-MS system analyzed lipids from Shimadzu, Tokyo, Japan.

2) The DB-5 column with the 30 m, 0.32 mm i.d., and 0.25 m from J&W Scientific, Folsom, CA, USA .

3) The carrier gas was helium, flowing at 1.8 ml per minute.

4) The temperature program was as follows: Initial temperature of 90 °C for 4 minutes, increased at a rate of 25 ◦C min−1 to 160 ◦C, held for 2 min followed by an increase of 25°C per minute to 180 °C and a third rise of 20 °C per minute to 280 °C, all held for 5 minutes. All injections were conducted

in spitless mode at 280 °C for the injector.

To validate the retention periods of the analytes, the detector was scanned across the m/z range of 50–500. The temperature between the surfaces was fixed at 200 °C.

5.RESULTS AND DISCUSSION.

5.1.Water Content (W%), Dry Content (DC%), and Lipid Content of Sludge

Table 1 shows the properties of tow sewage sludge samples from the WWP Al-Rumitha sewage project plant in Al-Muthana-Iraq. Water and dry content have been determined by the drying method at 105℃. Lipid content has been determined by the weight of lipids per unit of dry sludge and unit of wet sludge. Note that the dry content (DC) in both samples was DC<5% [14]. The two samples' lipid contents for the same extraction method were 36.3 and 34.78, respectively. The sludge, taken from the same location (screening for WWTP) in different months, was pretreated and acidified. The result was expected since sludge mostly comprises organic material extracted from raw wastewater, a mixture of floating grease and solids (the largest lipid proportion). Lipids extracted from sewage sludge included free fatty acids (FFAs), triglycerides, acylglycerols, and phosphorous lipids. The extraction mixture contained nonpolar and polar solvents, which extracted other substances, such as pigments, hydrocarbons, trepans, polycyclic aromatic hydrocarbons, sterols, linear alkyl Benzene's, and other different waxes. For biodiesel, only triglycerides and free fatty acids that constitute the saponifiable portion of lipids (Trans/esterification to FAMEs) are appropriate and determine the content of FFAs for the two samples by FTIR and gas chromatography analysis.

Table 1 Sewage Sludge Characteristics Samples from the Al-Rumitha Sewage Project Plant in (Al-Muthana-Iraq).

Type of sludge	First Wet sludge	Second Wet sludge
Date	1November2022	15 December 2022
Water content (W)%	95.2	96
Dry matter content (DC)%	4.8	4
Lipids content in dry matter%	36.3±1.21	34.78±0.87
Lipid content in wet sample%	1.39±0.284	1.58±2.2

5.2.Characterization of the Lipids Extracted from the Sludge

5.2.1.The Extracted Lipids' Amount of Free Fatty Acids (FFAs)

For the first sample Fig. 4, note that there are slight changes in the peaks, and this did not affect the functional aggregates and the substance that creates the lipids extracted from the sludge. The absorption of groups bands between 2923.35 and 2852.89 for C-H stretch vibration Methylene >CH2 in lipids. Furthermore, at a weak peak, 1463.21C-H bend vibration methyl -CH3, these three most prominent and obvious peaks represent groups of alkene chains in lipids amount of biodiesel. As for 1709.01, a peak referred to the function group of C=O stretch vibration of carboxylic acid for free fatty acid (FFA). With the peak C=O stretch vibration, a slight bend was noticed almost at 1739, representing triglycerides esters. The essential difference here is in the type of beak, where the strength of the beck was noticed, representing free fatty acids and triglycerides, and its clarity, with the beck retreating at 1464.21, where it is clearer than the first sample, on the contrary, as it is in this sample. The concentration of fatty acids was higher in this sample than in the previous one. However, a gas chromatography test must be performed to ensure the level of free fatty acids to produce a fairly good amount of biodiesel.

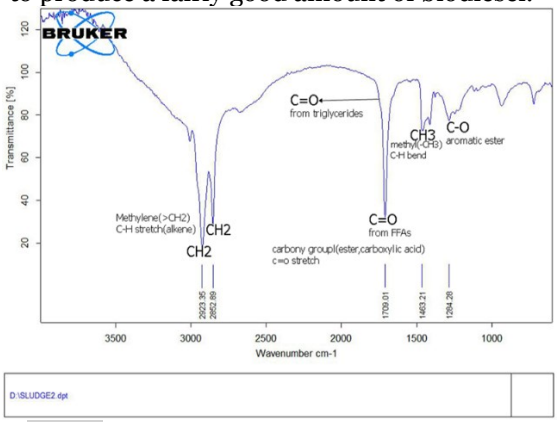

Fig. 4 FTIR Spectra of Sludge Lipids for the First Sample.

For the second sample, Fig. 5, the main peaks are three. The first and second peaks of the infrared spectra for the lipids extracted at 2929.77 and 2851.61cm-1, respectively. The functional group at peaks 2920.77 and 2851.61 was found to be C-H stretch vibration Methylene >CH2 in lipids; likewise, the peak was 1448.85. In the third peak, the functional group was referred to as C-H bend vibration methyl -CH3. These peaks represent groups of alkene chains in lipids. The weak peak at 1710.58, the function group of C=O stretch vibration of carboxylic acid-free fatty acid (FFA). At the same weak peak, a slight bend was noticed almost at 1738, i.e., the function group C=O stretch vibration, representing triglycerides esters.

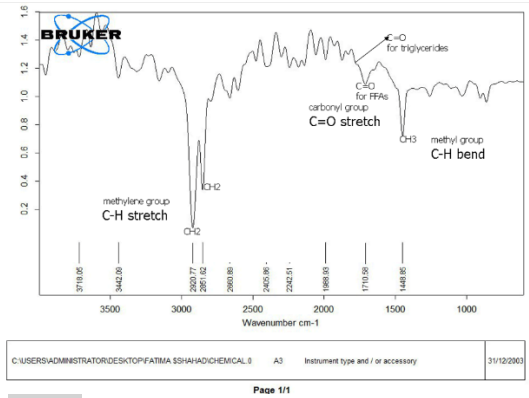

Fig. 5 FTIR Spectra of Sludge Lipids for the Second Sample.

5.2.2.Comparison between the lipids Extracted from the First Sample and Second Sample

The gas chromatography analysis for the extracted lipids was to determine the content of free fatty acid (FFAC), triglyceride, and fatty acid components to be used as feedstock for biodiesel products. Lipids extracted from both sewage sludge samples contained a high fraction of FFA > 25%. Therefore, a certain acid-catalyzed mechanism can only catalyze the esterification/transesterification reaction. Because these FFAs are transformed into soaps in a basic media and produce an emulsion, the separation from water is challenging, where the free fatty acid (FFAs) content in the first sample was more than 58%. In comparison, the second sample amounted to more than 36%. Where it was found that oleic acid (C18:1) was the most abundant acid in the two samples, followed by a little less palmitic acid (C16:0) than linoleic acid (C18:2) with a decrease in stearic acid (C18:0) with an absence or semi-existence of palmitoleic acid(C16:1) in the two samples, like Fig. 6 shows.

Table 2 Fatty Acids Composition of the Two Sludge Samples from WWTP Located in Al-Rumitha (Al-Muthana-Iraq) (wt%).

Free fatty acids	First sample (15 October 2022)	Second sample (1 December 2022)
C12:0	5.76	2.88
C14:0	4.72	5.49
C15:0	0.62	0.54
C16:0	34.77	31.3
C16:1	-	0.03
C18:0	0.25	0.59
C18:1	39.31	34.2
C18:2	6.24	7.17
C20:0	0.49	5.08
Others	7.66	12.72

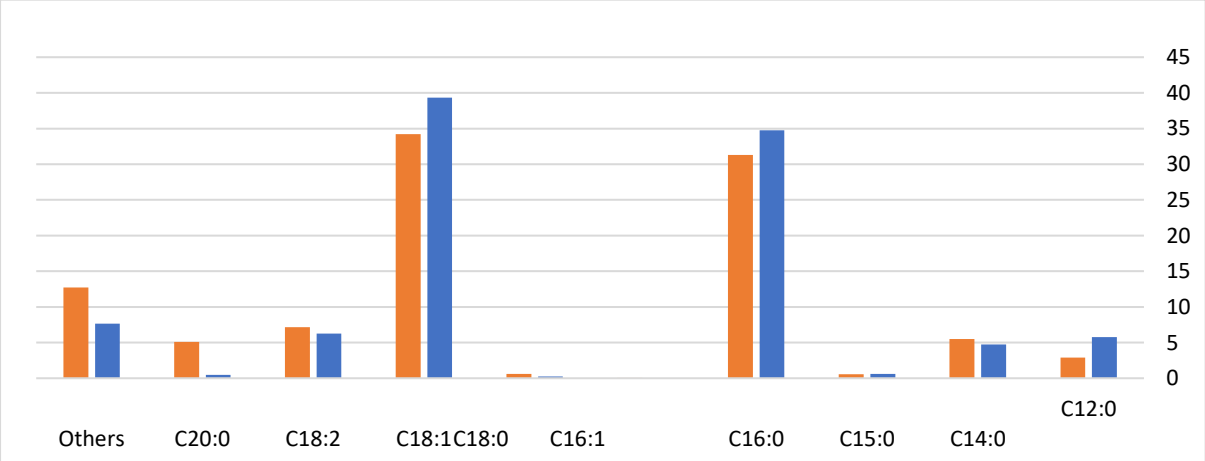

Fig. 6 Fatty Acids Composition of the Two Sludge Samples.

6.CONCLUSIONS

Although drying is the most suitable approach for producing biodiesel from sewage sludge, it consumes up to 50% of energy. Therefore, there has been a shift toward using organic solvents to extract fats from wet sludge, and these solvents have been developed to be environmentally friendly and non-harmful. The best is a mixture of organic solvents, i.e., Smedes mixture (isopropanol, cyclohexane, and water). It gave an extraction efficiency of up to 36.3% wt for the first sample at a high temperature compared to low temperatures. The extraction efficiency reached 34.78% wt for the second sample. Gas chromatography testing revealed that oleic acid (C18:1) was both samples' most abundant fatty acid. Thus, it was concluded that the air temperature plays an important role in the amount of lipids extracted. Also, taking samples from the screening of the sewage treatment plant proved that taking primary sludge samples is not limited to the primary sedimentation tanks only.

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NOMENCLATURE

C12:0	Lauric acid
C14:0	Myristic acid
C14:1	Myristoleic acid
C15:0	Pentadecanoic acid
C16:0	Palmitic acid
C16:1	Palmitoleic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C20:0	Arachidic acid
DC%	Dry content
FAMEs	Free Fatty Methyl Esters
FEs	Fatty Esters
FFA	Free Fatty Acid
FITR	Fourier Transform Infrared
GC-MS	Gas Chromatography-Mass Spectrometry
W%	Water content
WWTPs	Wastewater treatment plants

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