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Lipid extraction in the primary sludge generated from urban wastewater treatment: characteristics and seasonal composition analysis

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ABSTRACT

A seasonal study of the lipid composition of a primary sludge (dry and dewatered base) obtained from an urban wastewater treatment plant located in Aguascalientes (Mexico) is reported. This study assessed the variability in sludge composition to establish its potential as a raw material for biodiesel production. Lipid recovery was achieved by extraction using two solvents. Hexane was employed for lipid extraction from dry sludge, whereas hexane and ethyl butyrate were used for comparison with dewatered sludge. The formation (%) of fatty acid methyl esters (biodiesel) was determined using extracted lipids. The extraction results from the dry sludge showed 14 and 6% of recovered lipids and their conversion to biodiesel, respectively. For the dewatered sludge, the lipid recovery and biodiesel formation were 17.4 and 60% using hexane, and 23 and 77% for ethyl butyrate, respectively, on a dry basis. Statistical data indicated that lipid recovery depended on the physicochemical characteristics of sewage sludge, which were related to seasonal changes, population activities, and changes in plant configuration, among other factors. These variables must be considered in the design of large-scale extraction equipment for the application and commercial exploitation of biomass waste in biofuel production.

Key words: biodiesel, biomass, calcium soaps, ethyl butyrate, extracted lipids, sewage sludge

HIGHLIGHTS

- The primary sewage sludge was valorized as biodiesel feedstock.
- An annual sampling showed that lipid content depended on seasonal activities and weather.
- For dewatered sludge, lipid extraction with hexane was 17.4%.
- Lipid extraction with ethyl butyrate achieved 23%.
- Primary sewage sludge is an alternative green feedstock for biofuel production.

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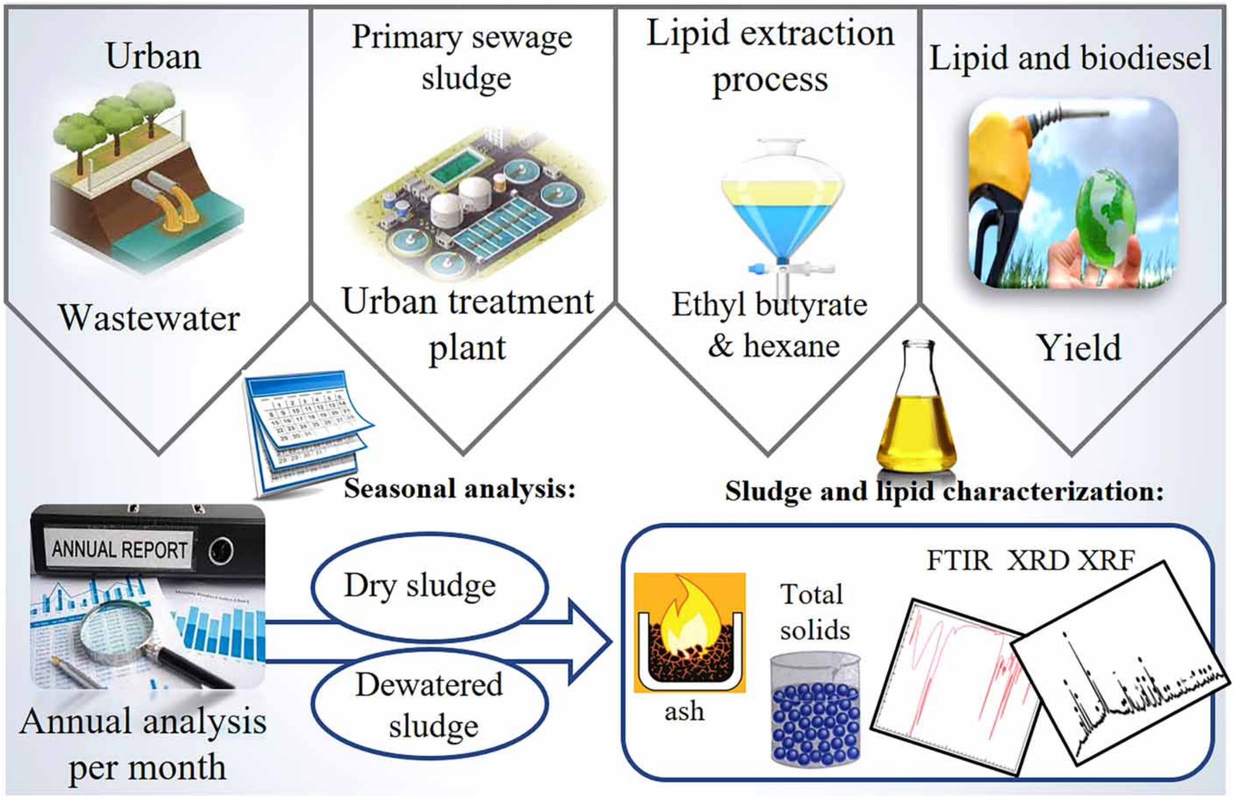
GRAPHICAL ABSTRACT



1. INTRODUCTION

The start-up and operation of urban wastewater treatment plants (WWTPs) have increased considerably in recent decades owing to population growth and urbanization. Sewage sludge (SS) is a secondary product of wastewater treatment. This residual biomass is defined by the Environmental Protection Agency (EPA) as a pollutant that generates relevant environmental problems because of the high amounts produced, its high water content (80–95%), and its physicochemical characteristics (Hu & Gaob 2020; Liu *et al.* 2021; Xiao *et al.* 2022). The sludge can be treated by thermal, biological, or incineration processes for its final disposal (Liu *et al.* 2021; Cecconet & Capodaglio 2022; Goldan *et al.* 2022; Xiao *et al.* 2022). However, these processes require additional steps, units, and energy, which increase its management cost. For example, it has been estimated that the management of this type of waste accounts for 50% of the total operating cost of WWTPs (Ogwuelek *et al.* 2021; Cecconet & Capodaglio 2022). This has led to the proposal of other attractive alternatives for the management, disposal, and utilization of SS (di Bitonto *et al.* 2020a, 2020b; Ogwuelek *et al.* 2021).

With this in mind, the interest in the reuse of this waste has increased in recent years (Xiao *et al.* 2022). This interest is based on the fact that SS mainly contains organic matter, which can be transformed into value-added products via the application of several technologies, following the philosophy of the circular economy and the concept of biorefinery (Dufreche *et al.* 2007; D'Ambrosio *et al.* 2021; Liu *et al.* 2021; Goldan *et al.* 2022; Capodaglio 2023). Therefore, SS can be used as the feedstock for biorefineries where the organic compounds contained in this residual biomass can be transformed into fertilizers, biopolymers, biofuels, and organic acids, among other interesting value-added chemicals (Cecconet & Capodaglio 2022; Goldan *et al.* 2022; Mohamed & Li 2023). On the other hand, the inorganic fraction of this biomass can also be employed for the preparation of ceramic materials (Mao *et al.* 2023).

Primary sludge typically contains high amounts of biodegradable compounds, including carbohydrates, proteins, and lipids (di Bitonto *et al.* 2020a). Additionally, this sludge has a higher lipid content than secondary sludge (Mondala *et al.* 2009), in which free fatty acids and salts of fatty acids (soaps) are the most abundant (di Bitonto *et al.* 2020a, 2020b). The amount of these compounds in the SS matrix mainly depends on the unit operations used in the water treatment plants

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(Fytili & Zabaniotou 2008; Pastore *et al.* 2015; di Bitonto *et al.* 2020a). Fats are mainly recovered in flotation tanks or grease traps, where less dense immiscible compounds are separated from water (Pastore *et al.* 2015). However, soaps tend to precipitate and are present in higher amounts in settled residues (Pastore *et al.* 2013). Both residues contain non-polar compounds such as waxes and sterols, which cannot be converted into fatty acid methyl esters (FAMEs) via transesterification (di Bitonto *et al.* 2020b).

The use of the lipid fraction of these residues is very attractive because they can be used to produce biodiesel (i.e., FAME) (Kech *et al.* 2018; di Bitonto *et al.* 2020a). Biodiesel can be produced by catalyzed esterification/transesterification of lipids in the presence of alcohol (Dufreche *et al.* 2007; Mondala *et al.* 2009; Kech *et al.* 2018). However, the main limitation to its commercial production is the lipidic raw material that represents 70–85% of the production cost (Kech *et al.* 2018). Therefore, the use of residues such as SS from WWTP as raw materials is an economically and environmentally competitive alternative for producing biodiesel (di Bitonto *et al.* 2020a).

The optimization of lipid recovery using primary sludge has been analyzed in several studies (Siddiquee & Rohani 2011; Pastore *et al.* 2013; Olkiewicz *et al.* 2014; di Bitonto *et al.* 2016; Kech *et al.* 2018; D'Ambrosio *et al.* 2021; Villalobos-Delgado *et al.* 2021). Extraction studies of these lipids with dried primary sludge have been carried out using different extraction methods (Mondala *et al.* 2009; Willson *et al.* 2010; Kech *et al.* 2018), types of solvents (Willson *et al.* 2010), operating conditions (Siddiquee & Rohani 2011), and drying methods (Olkiewicz *et al.* 2014), among other variables. In certain cases, similarities have been reported in terms of the lipid recovery percentage (approximately 30% of lipid recovery in dry sludge (DS)) and conversion to FAME (i.e., biodiesel) of 15%. Additionally, lipid recovery from dewatered sludge using hexane as the solvent has been reported to reduce the energy required for total water removal (Pastore *et al.* 2013). Other studies have explored liquid–liquid extraction of raw sludge (>95% moisture) with organic solvents and ionic liquids (Olkiewicz *et al.* 2014, 2015a; Kech *et al.* 2018). In addition, biorefinery schemes using primary sludge as the raw material (Olkiewicz *et al.* 2016) have been proposed, and thermodynamic data on lipid extraction from emulated sludges have been calculated (Villalobos-Delgado *et al.* 2021), which are useful for the intensified design of extraction equipment of lipids contained in dewatered primary sludge.

The main challenge associated with biodiesel production from SS is the efficient lipid extraction (Kech *et al.* 2018). However, a crucial design parameter is the variability of wastewater entering the WWTP. Seasonal and daily variations generate changes in the composition of SS during their course in the WWTP. Such a composition will depend on seasonal changes, as well as urban discharges from homes, markets, and other services that are related to the activities of the population during a specific period. The degree of variability in SS composition has not been studied in detail, despite its importance and impact on the design of processes and operation of equipment used for raw material processing. Therefore, it is important to study the effects of seasonal variability on the physicochemical composition of SS to determine the feasibility of its implementation as a large-scale raw material for biodiesel production.

In this study, monthly sampling of the primary sludge from a WWTP in the city of Aguascalientes, Mexico, was carried out to determine the variation in the amount of lipids recovered and its relation to the characteristics of the sludge, collection period, and other parameters. Hexane was used as an extraction agent for the lipid extraction from dried sludge. Extraction from dewatered sludge using both a conventional solvent (hexane) and an alternative solvent with less environmental impact (ethyl butyrate) is important because the cost of treating dewatered sludge is lower than that of the drying process (28% of the drying cost) of a raw sludge (Olkiewicz *et al.* 2014). In summary, this study focused on generating statistical data on the characteristics of primary sludge and the recovery of lipids for their conversion to biodiesel.

2. METHODOLOGY

2.1. Characteristics of the WWTP and sludge sampling

According to information from the inventory of the National Commission of Water (CONAGUA 2021), there are 2,872 WWTPs in Mexico, the majority of which are stabilization ponds and activated sludge. The total processing capacity of these plants is 198,603.55 L/s; however, they only process 145,341.0 L/s, which is equivalent to 67.2% of the residual water collected in the sewage systems in this country. A municipal WWTP located in the city of Aguascalientes (Mexico) was selected for this study. This plant has a capacity of 2,000 L/s and a primary SS generation of 400 m^3/day. Figure 1 shows a flowchart of the WWTP selected for this study.

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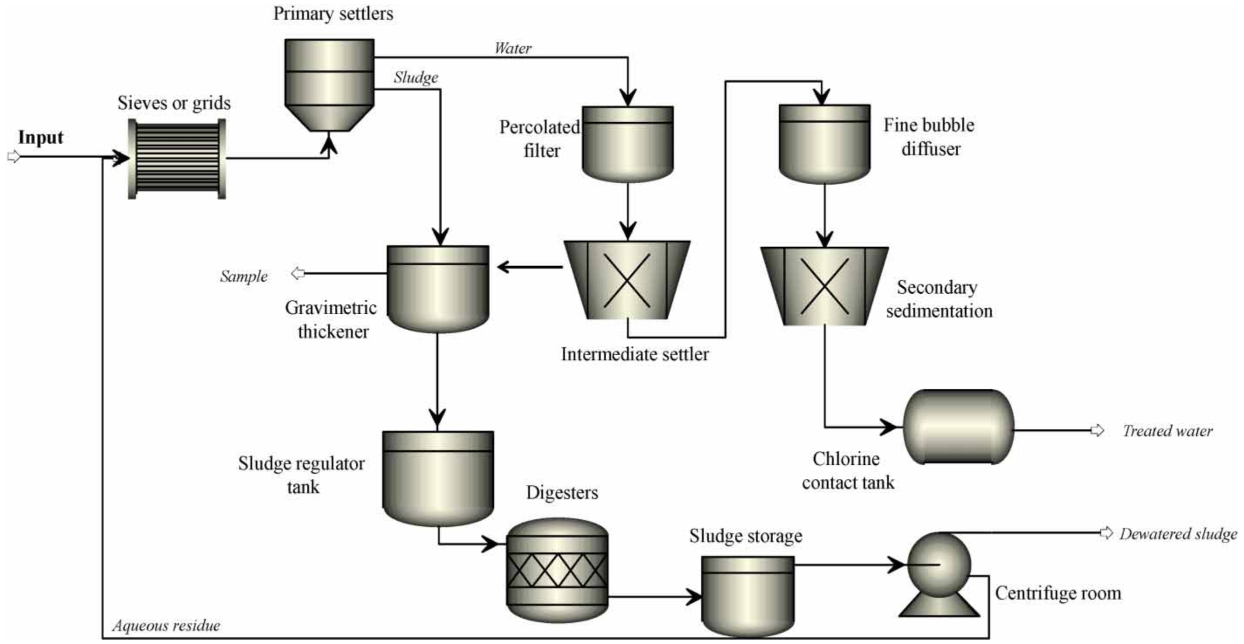

Figure 1 | Diagram of the urban WWTP in Aguascalientes, Mexico.

During the year, the sludge obtained from the feed lines (see Figure 1) of the primary sludge stabilization tanks was collected monthly. The collected samples were transported to refrigerated containers and stored in a refrigerator prior to characterization. A fraction of the sludge was dewatered by filtration until a water content of 88–93% was obtained. The wet sludge (WS) or raw sludge and partially dewatered sludge (PDS) were characterized, and each analysis was performed by triplicate.

2.2. Determination of ash and total solids in sludge samples

The moisture content of each sludge (WS and PDS) was determined by placing it in an oven at 100 °C for 48 h. Subsequently, the residual mass was weighed, and the water content and total solids (TS) were determined using a mass balance. On the other hand, the ash content was determined according to the methodology of di Bitonto *et al.* (2016). Briefly, a sample of DS (obtained from the previous process and weighed) was introduced into a muffle furnace at 550 °C for 3 h, and a mass balance was used to calculate the ash content.

2.3. Lipid extraction from SS samples

2.3.1. Solid–liquid extraction (DS)

Solid–liquid extractions were carried out using the DS. The sludge was ground and sieved until a particle size of 210 μm was obtained to achieve a higher contact area between the solid and the solvent. Then, 2 g of the DS was placed in a 15 mL vessel with 5 mL of hexane, and the mixture was vigorously shaken for 5 min. The system was allowed to settle to obtain a liquid phase (mixture of non-polar compounds) and a solid phase. The liquid phase was recovered by settling and deposition in a weighed container. Finally, the vessel containing the total liquid phase was placed in an oven at 50 °C to evaporate the solvent until a constant weight of the extract was obtained, and the extraction (percentage) of the organic fraction was determined. The lipid content was analyzed via an esterification/transesterification reaction to determine the fatty acid profile and conversion to biodiesel. Specifically, 50 mg of the lipid sample was reacted with 2 mL of a methanol solution with methyl heptadecanoate as an internal standard (2 g of standard/1 L of methanol) in the presence of a homogeneous acid catalyst (hydrochloric acid) at 100 °C for 4 h in a closed batch reactor. The non-reacted methanol was evaporated at the end of the reaction, and the upper phase of the mixture was analyzed in a gas chromatograph Thermo Scientific Trace 1300 GC equipped with a flame ionized detector (FID) and column TG-5SILMS with dimensions of 30 m × 0.25 mm × 0.25 μm. The instrument was programmed with an injection temperature of 250 °C in splitless mode, a detector temperature of

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300 °C, and a helium flow of 2.5 mL/min. The oven temperature was set at 40 °C for 2.5 min, with a heating ramp of 10 °C/min to achieve 280 °C, which was maintained for 10 min.

Methyl heptadecanoate was used as the basis for calculating the percentage of FAME formation according to the EN-14103 standard, using the following equation:

$$\%\mathrm{FAME} = \left(\frac{\sum A - A_s}{A_s}\right) * \left(\frac{C_s * V_s}{mb}\right) * 100 \tag{1}$$

where ΣA is the total area of FAME and internal standard, mb is the sample mass (g), V_s is the standard volume (mL), C_s is the concentration of the standard (mg/mL), and A_s is the area of the standard.

2.3.2. Liquid–liquid extraction (dewatered SS)

Ethyl butyrate and hexane were used as solvents for the extraction of the PDS. Specifically, 55 g of sludge was mixed with 50 mL of extractant (hexane or ethyl butyrate). The mixture was stirred at 400 rpm for 1 h to obtain a biphasic semi-solid suspension at the interface. The semi-solid phase was separated from the liquid phase. The light semi-solid phase mixture was recovered and centrifuged at 6,000 rpm for 2 min to separate the calcium soaps (semi-solid phase) from the liquid phase (non-polar compounds). The extraction procedure was repeated for the same sludge sample until 150 mL of solvent was added. The solvent was separated from the liquid phase by distillation. The solid was dried at 50 °C for 48 h to remove residual solvent. The weights of the non-polar compounds and solids are reported. The esterification/transesterification of the extracted lipids was performed according to the methodology described in Section 2.3.1.

2.4. Characterization of SS and lipids recovered by extraction

A set of representative samples of sludge ash and lipids recovered by extraction (calcium soaps and non-polar compounds) were analyzed by X-ray diffraction (XRD), infrared (FTIR) spectroscopy, and X-ray fluorescence (XRF) spectroscopy. An infrared spectrometer (Thermo Scientific Nicolet iS10) with an ATR configuration in the wavenumber range of 650–4,000 cm^{-1}, an X-ray diffractometer (Empyrean Panalytical using the Bragg-Brentano configuration with CuK-1 radiation), and an XRF spectrometer (Epsilon 4 with metallic ceramic X-ray tube, 50 μm Be window, and energy dispersive) were used.

3. RESULTS

3.1. Sludge sampling and characterization

The moisture content of all samples collected from the raw primary SS was higher than 95%. Figure 2(a) shows the percentage of TS for each sample. The percentage of TS ranged between 2.6 and 4.36%. During the hottest and rainiest months in the sampling area, a higher percentage of solids was found in the samples, which was the result of an increase in the dragging of pollutants into the sewer (Olkiewicz *et al.* 2014). The value of TS reported in the literature reveals differences in the types of waste generated in treatment plants (primary, secondary, mixed sludge, and slag or grease). Di Bitonto *et al.* (2020a) indicated that the TS content in the primary sludge was 4.2–4.4% in three different plants in southern Italy. Olkiewicz *et al.* (2012) reported similar amounts with mean values of 4.2 ± 1.2% in Spain, while Kech *et al.* (2018) reported 1.03 and 2.19–6.63% TS in the raw and dewatered sludge, respectively, obtained from a WWTP in Belgium.

Figure 2(b) shows the amount of ash in the solids, which varied between 22.68 and 51.5%. These values are consistent with those reported in the literature (Fytili & Zabaniotou 2008; Payá *et al.* 2019). The increase in the amount of ash and the consequent decrease in the organic matter identified in some samples were due to a change in the configuration of the treatment plant with the incorporation of an anaerobic reactor and the elimination of the aerobic digestor. Such new reactor received the primary and secondary sludges to generate biogas from the organic matter (Matheri *et al.* 2020). This new reactor offered a more efficient process that reduced the organic fraction contained in the aqueous residues recirculated to the plant input. This configuration change implied an increment of the inorganic matter content and, consequently, the ashes in the solids of the primary sludge also increased.

These data are interesting in terms of the design of biorefinery plants using SS as the feedstock. It is crucial to keep in mind that the composition of the sludge may vary due to various factors; the more information on municipal waste becomes available, the more the biorefineries will become a reality in the near future.

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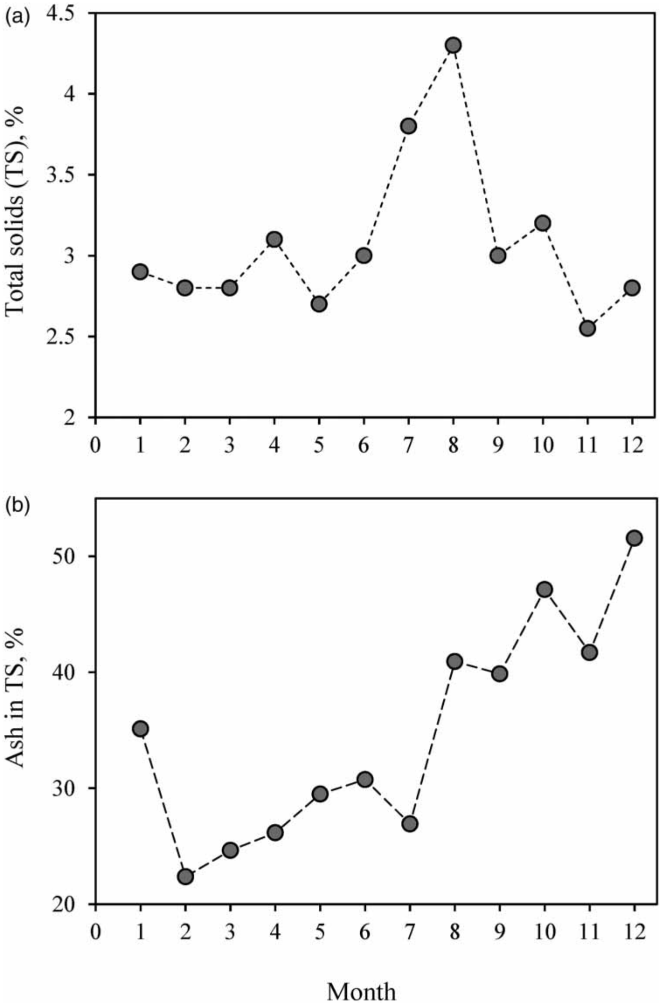

Figure 2 | (a) TS contained in the raw sludge from the WWTP and (b) ash content in TS.

It is convenient to indicate that the variation in the composition of the primary sludge could be due to both the modification of the primary process and the entry of wastewater, as well as the transportation and formation of new compounds through the municipal sewage system.

This could be mainly due to the presence of microorganisms that degrade long-chain organic compounds as well as the oxidation of sulfides (VandeWalle *et al.* 2012; Nielsen & Vollertsen 2021). These observations confirm that the composition of primary sludge is a function of multiple dynamic variables that affect its physicochemical characteristics (Yu *et al.* 2021).

Table 1 shows the characterization of the primary sludge ash by XRF, where the elements of the inorganic fraction of the sludge are indicated. Si is the most abundant element, followed by Ca (Coutand *et al.* 2007; Payá *et al.* 2019). Traces of metals, such as Cu, Ni, Cr, and Al, have also been observed (Payá *et al.* 2019). The X-ray diffractograms in Figure 3(a) show mineral compounds such as quartz (SiO_2, ICDD:01-086-2237), calcite ($CaCO_3$, ICDD:00-047-1743), and calcium aluminosilicate ($Al_{1.77}Ca_{0.88}O_8Si_{2.23}$, ICDD:00-052-1344) (Coutand *et al.* 2007; Baeza-Brotons *et al.* 2014; Payá *et al.* 2019). Diffraction

Table 1 | XRF results to determine the elemental composition of the ash contained in the residual sludge samples from the WWTP

Element	Si	Ca	Al	Fe	P	K	S	Ti	Mg	Zn	Cl
%	49.65	17.27	9.78	7.72	5.34	3.87	3.30	1.26	0.92	0.61	0.28

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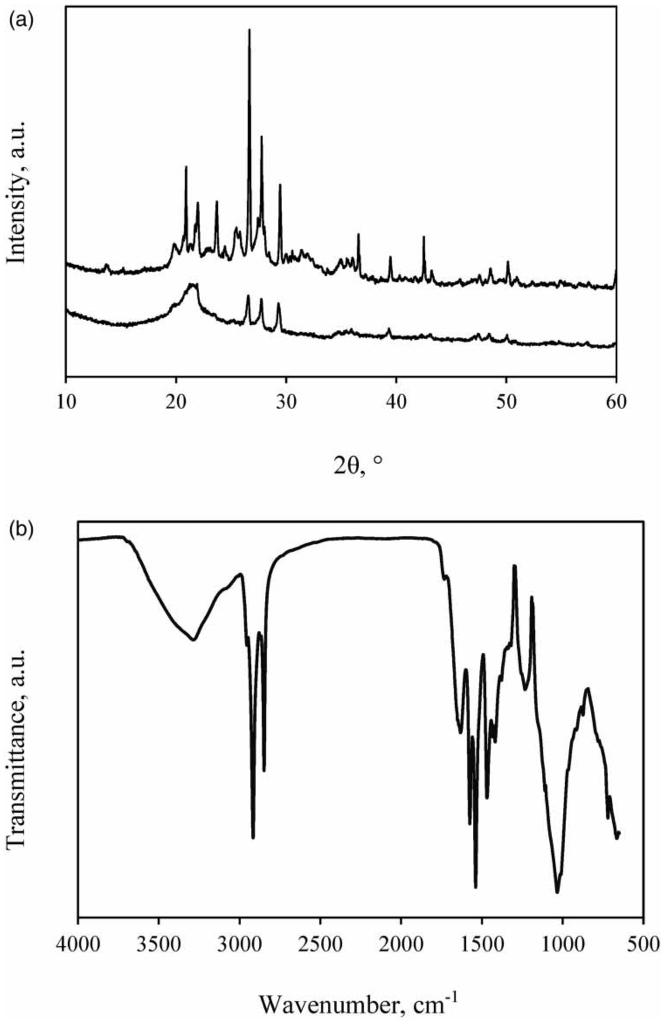

Figure 3 | (a) X-ray diffractograms of dry primary sludge samples and ashes contained in TS and (b) FTIR spectrum of the dry primary sludge.

peaks that identify the elements in quartz were found at 20.87°, 26.63°, 36.55°, 39.44°, and 50.129° 2θ; in the case of calcite, they corresponded to 23.66°, 29.96°, 36.01°, 39.44°, and 48.5° 2θ and for calcium aluminosilicate are 21.95°, 27.42°, 27.76°, and 28.03° 2θ, respectively. In general, these minerals are present in the soil and are easily transported through wastewater sewage systems to treatment plants. They are removed in primary processes where the residues obtained are disposed of as fertilizers or taken to sanitary landfills (Fytili & Zabaniotou 2008; Ohbuchi *et al.* 2008; Baeza-Brotons *et al.* 2014).

Figure 3(b) shows the infrared spectrum of the DS. An absorption band was observed at 3,300 cm^{-1}, corresponding to the OH group of the alcohols and humidity. Characteristic absorption bands of the CH aliphatic groups were found at the region 2,900–2,840 cm^{-1}, 1,410 cm^{-1}, and 800–700 cm^{-1} (Bahadi *et al.* 2016; Ahsaine *et al.* 2017). The absorption bands of the $\text{C}=\text{O}$ and C–O groups of glyceride-type esters or alkyl esters are located at 1,730 cm^{-1} and 1,300–1,000 cm^{-1} (Pastore *et al.* 2013; Bahadi *et al.* 2016; Han *et al.* 2020). The absorption bands at 1,468 and 1,631 cm^{-1} are associated with the $\text{C}=\text{C}$ and $\text{C}=\text{O}$ groups, respectively (Ahsaine *et al.* 2017; Han *et al.* 2020). Calcium soaps correspond to COO^{-} aliphatic groups (Pastore *et al.* 2013; Hao *et al.* 2017).

3.2 Solid–liquid extraction of lipids from DS using hexane

Figure 4 shows the percentage of lipid recovery and FAME formation obtained from the DS using hexane as the extraction agent. These percentages are in the range of 5.1–44.5% and 1.9–17.5% by weight of the DS, respectively. It is worth mentioning that a FAME conversion of 38.85–72.13% per gram of lipid can be obtained (see Table 2).

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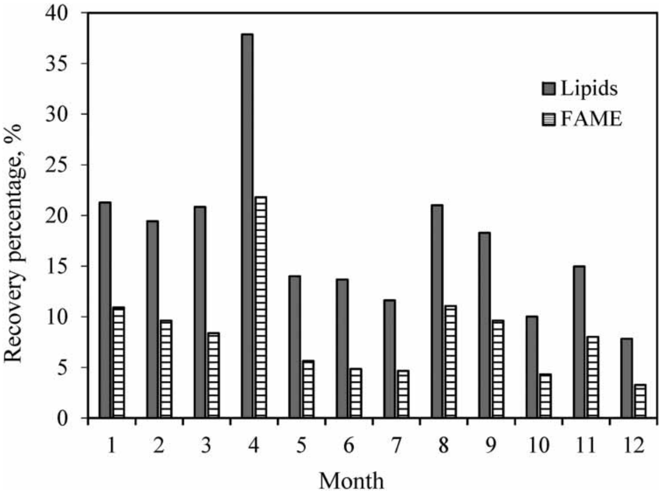

Figure 4 | Recovery of lipids and formation of FAME from the DS using hexane as the extraction agent.

Table 2 | Percentage conversion of FAME per unit lipid weight

Sampling month	FAME, % (per gram of lipids)
1	70.55
2	68.91
3	53.60
4	38.85
5	73.07
6	72.16
7	50.08
8	52.69
9	52.62
10	43.11
11	53.60
12	41.78

The FAME performance results are consistent with data reported in other studies, which show a biodiesel recovery between 4.78 and 18.9% by weight of the DS (Mondala *et al.* 2009; Willson *et al.* 2010; Siddiquee & Rohani 2011; Olkiewicz *et al.* 2014). However, in this study, it was observed that for some months, the variation between samples was considerable both in lipid recovery and FAME conversion.

It is important to highlight that the extraction method and type of extraction agent influence the amount of lipids recovered. For example, the addition of a mineral acid and methanol to sludge before the separation process favors the recovery of lipids or FAME, resulting in 14.5% (Mondala *et al.* 2009) and 10% of FAME (Pastore *et al.* 2013) from sludge dry basis. Willson *et al.* (2010) and Olkiewicz *et al.* (2014) used extraction via Soxhlet, obtaining a maximum lipid recovery of 23.1 and 26.3% with FAME formation of 51.04 and 71.8%, respectively. The use of ionic liquids as extraction agents has been reported where 18.5–23.4% of lipids have been recovered, with 74% saponifiable lipids (Olkiewicz *et al.* 2015a). Siddiquee & Rohani (2011) performed a detailed lipid recovery analysis by separating the solids from the organic phase via filtration using hexane as the solvent. The results showed a lipid recovery of 11.2% on a dry basis and a FAME conversion yield of 41.3% with respect to the extracted lipids.

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Suspended solids (calcium soaps) and low-density non-saponifiable solids were present in the samples with the highest percentage of lipid recovery. Therefore, the FAME yield was higher in this sampling and similar to the results reported by Olkiewicz *et al.* (2014) with 18.9% FAME with respect to the DS, as a higher amount of lipids (soluble lipids and calcium soaps) reacted with methanol in the presence of the acid catalyst. Previous studies have shown that 71–82% of the lipids contained in primary sludge are soaps, mainly calcium, although potassium, magnesium, and sodium are also present (Willson *et al.* 2010; Pastore *et al.* 2013). The presence of these organic salts is due to the use of domestic products and metabolic wastes transported by the sewage system (Pastore *et al.* 2013, 2015). These soaps are insoluble in water and are recovered during sedimentation in water treatment plants. In contact with solutions containing H^+ ion donors (HCl and H_2SO_4), soaps react to form salts and free fatty acids (Pastore *et al.* 2013, 2015). Free fatty acids are soluble in non-polar compounds, such as hexane, whereas fatty acid salts are scarcely soluble. The presence of suspended solids (calcium soaps) in the liquid phase and their subsequent conversion to FAME explain the differences observed in some samples. This is the case for the sample of month 4, which showed a higher lipid recovery than the rest of the samples during the year. This may be due to the presence of non-saponifiable lipids, especially calcium soaps, which play an important role in the beneficial liquid–solid equilibrium in lipid recovery. However, this behavior was not observed with other samples and this result can be considered as an outlier caused by process operation.

Therefore, for the design and intensification of the recovery processes of interesting compounds, it is essential to consider the variations in this type of raw material with respect to their composition (Siddiquee & Rohani 2011). The results of this study indicate that the composition of the residues circulating in the sewage system is not constant, and there may be variations in the amount of lipids extracted from SS. This result is very interesting because the primary sludge may have a different composition between regions and countries depending on waste disposal and government regulation of water discharge, among other factors. In this study, it was determined that each of the collected samples presented different characteristics in terms of color, percentage of solids, and content of fibrous compounds.

3.3. Liquid–liquid extraction in the dewatered sludge

According to the literature and data obtained in this study, the amount of moisture present in the total mass of raw primary sludge is higher than 95% (Olkiewicz *et al.* 2014; Bora *et al.* 2020). This parameter implies that the largest mass and volume of sludge is mainly water. The recovery of compounds of interest, such as lipids, from dried sludge is the most recommended process. However, the cost of drying alone, represents 50% of the total cost of biodiesel production from SS (Villalobos-Delgado *et al.* 2021). For this reason, some authors have conducted studies on the raw sludge without a moisture removal process and using hexane or other compounds (i.e., ionic liquids) in the extraction process (Olkiewicz *et al.* 2014, 2015a). Furthermore, dewatered sludge has been reported as an option for lipid recovery (Pastore *et al.* 2013). This dewatered process has a lower cost than the drying process.

Lipid extraction from the dewatered sludge was evaluated using hexane and ethyl butyrate as solvents. One of the main differences in the use of both solvents is the formation of an emulsion with sludge under the experimental conditions (Pastore *et al.* 2013; D'Ambrosio *et al.* 2021). Another difference observed in the samples was the color of the phases. Specifically, the color of the light phase was observed to be among yellow, green, and sometimes dark when hexane was used. In contact with ethyl butyrate, this phase presented a dark brown coloration with a reduction in solids in the semi-solid fraction. The semi-solid phase had a grayish color, as mentioned in the literature (Pastore *et al.* 2013, 2015).

Figure 5 shows the recovery of lipids and FAME in the dewatered sludge using hexane (Figure 5(a)) and ethyl butyrate (Figure 5(b)) as solvents for extraction. In the case of hexane, a higher recovery of lipids was observed compared to studies on lipid extraction from DS. The lipid recovery percentage of ethyl butyrate was higher than that of hexane with the dewatered sludge. The lipids extracted from such sludge were 7.9–27.8% for hexane and 10.1–36.1% for ethyl butyrate on a dry basis, while the percentage of FAME was 3.2–21.0% and 7.1–26.2% for hexane and ethyl butyrate, respectively, on a dry basis. On the other hand, the percentages of solid lipids (organic salts) and extracted non-polar lipids (dissolved in the solvent) are shown in Figure 6. Similar results for the behavior of both solvents were reported by D'Ambrosio *et al.* (2021). Notably, in some samples, the amount of lipids extracted was up to 50% lower than that obtained for most of the collected samples. This result was mainly attributed to changes in the configuration of the plant for the use of residual sludge in anaerobic processes.

In this context, calcium salts are poorly soluble in aqueous solutions and non-polar compounds such as hexane (Pastore *et al.* 2013). However, when a non-polar solvent (hexane) interacts with dewatered SS, the solvent wets calcium soaps

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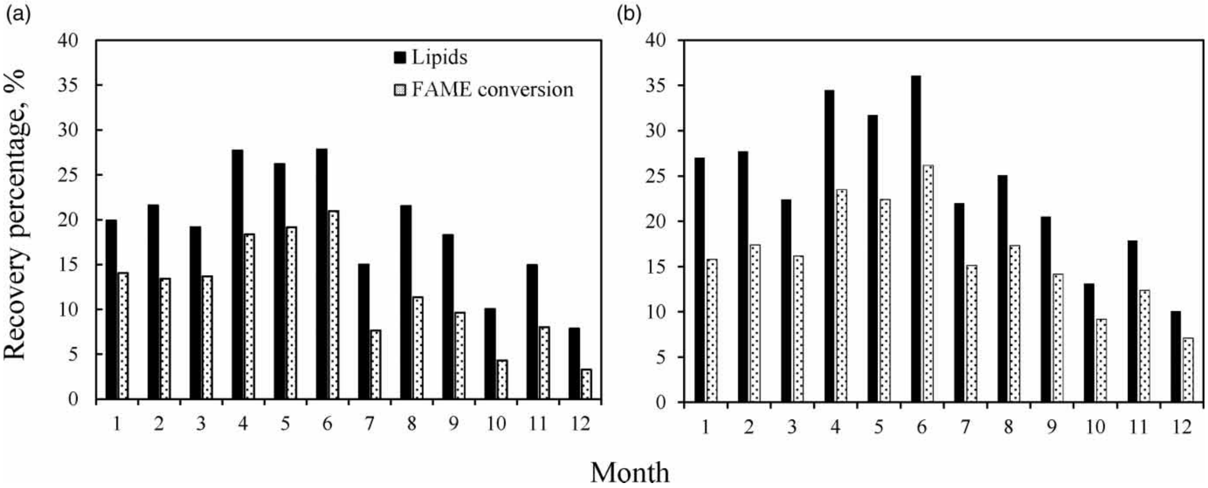

Figure 5 | Extraction of lipids and formation of FAME from the dewatered sludge using the following extraction agents: (a) hexane and (b) ethyl butyrate.

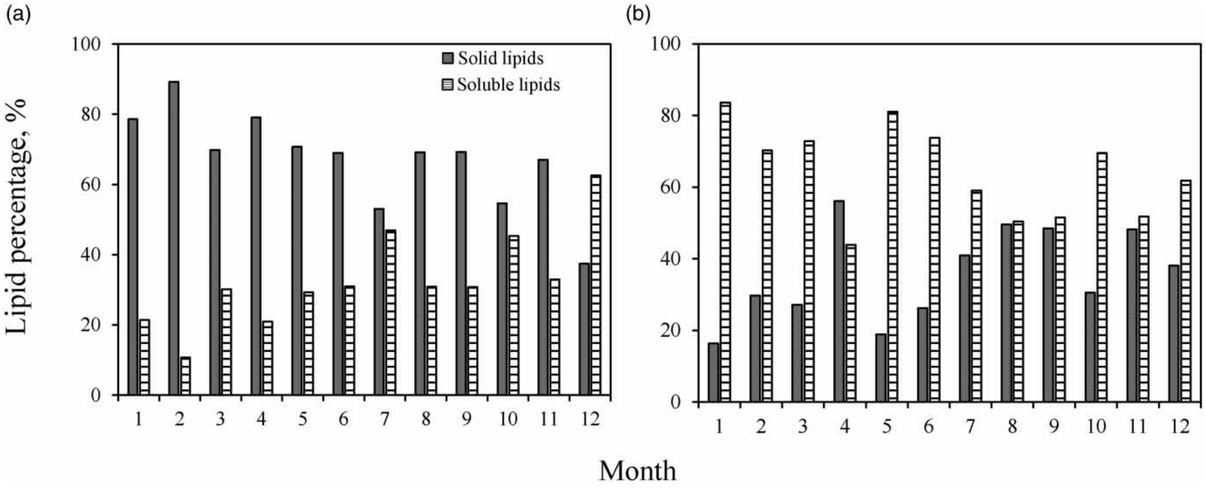

Figure 6 | Soluble lipids and solid lipids extracted from the dewatered sludge using (a) hexane and (b) ethyl butyrate.

and binds because calcium salts have one or two non-hydrophilic organic chains, causing the flotation of soap particles, which allows the wetted soaps to move to the interface (Pastore *et al.* 2013). Olkiewicz *et al.* (2015a) concluded that in minimal amounts of moisture, the viscosity is high, and agitation focused on highly viscous fluids is required to facilitate the extraction agent to wet all the small particles (Lotito *et al.* 1997; Pastore *et al.* 2013). Therefore, an adequate amount of water (88–94% humidity) allows all the solids to be distributed homogeneously in a certain volume and, in this way, facilitates solid–liquid–liquid separation. The opposite case was solid–liquid extraction, where hexane was in direct contact with the soaps. However, most organic salts remained in the solid phase. Thus, in the process, only the soaps that were soluble in the extraction agents and/or suspended in them were recovered.

The results reported in Figure 6 indicate that solid lipids were found in a higher proportion when hexane was used, while a higher amount of soluble lipids was observed when ethyl butyrate was used. It can be inferred that solid lipids react in the presence of ethyl butyrate to form free fatty acids (soluble lipids), favoring a higher FAME formation (D'Ambrosio *et al.* 2021). Lipid extraction using ethyl butyrate was higher when the dewatered sludge was used (Figures 5(b) and 6(b)). D'Ambrosio *et al.* (2021) demonstrated that lipid extraction using ethyl butyrate is higher than that achieved with other organic solvents such as hexane. The recovery of soluble lipids was higher than that obtained for insoluble lipids (calcium

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Table 3 | Fatty acid profile of lipids contained in the primary sludge recovered using hexane

	Sampling month											
Fatty acids	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
C16	42.70	45.32	49.30	51.93	47.44	50.52	50.88	49.64	50.12	58.11	48.45	52.45
C16:1	1.08	1.40	0.64	0.97	1.09	0.77	2.45	1.18	1.12	0.0	3.12	0.59
C14	5.40	5.85	5.56	6.61	5.37	5.64	6.54	4.72	4.39	5.96	5.82	6.56
C12	1.05	0.80	1.24	1.22	0.98	1.11	0.98	0.83	0.55	0.81	1.41	1.28
C18:1 + 2	20.46	22.83	17.18	12.32	15.62	13.64	15.65	12.56	15.41	9.46	16.56	14.05
C18:0	20.33	18.36	20.70	23.17	22.92	23.82	18.33	24.49	23.35	21.1	21.01	20.72
C20	1.06	0.90	1.44	1.33	1.31	1.32	1.34	1.92	1.48	1.64	1.36	1.20
C22	0.32	0.48	0.39	0.32	0.70	0.43	0.42	0.78	0.6	0.42	0.45	0.40

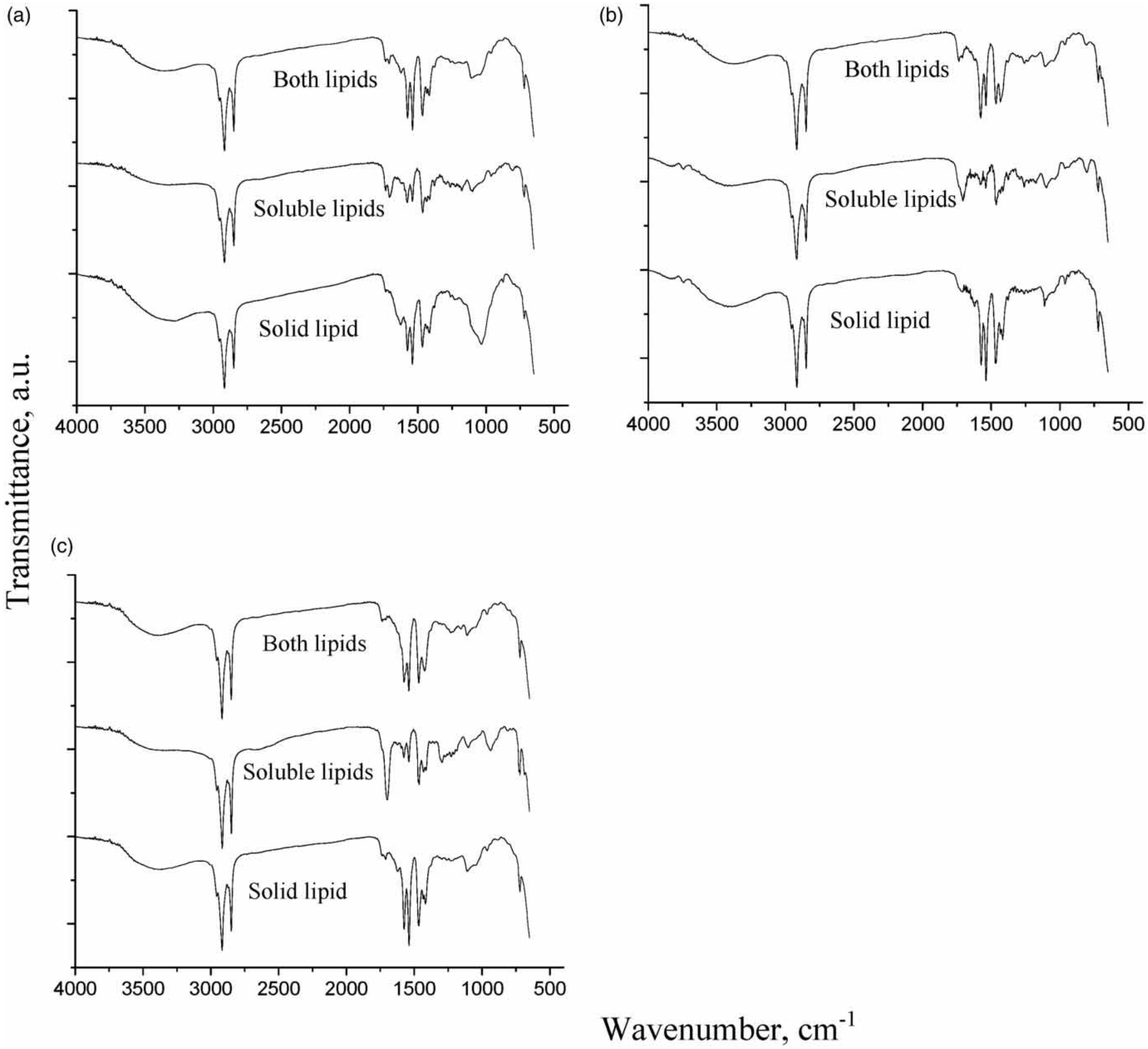

Figure 7 | FTIR spectra of lipids from the primary sludge. (a) Extraction from the DS using hexane, (b) extraction from the dewatered sludge using hexane, and (c) extraction from the dewatered sludge using ethyl butyrate.

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soaps) (Figure 6(a)), indicating that the reaction of such soaps to form free fatty acids (soluble) was due to the presence of ethyl butyrate.

3.4. Characterization of lipids

Table 3 shows the profiles of the fatty acids contained in the lipids extracted with hexane, with palmitic acid being the compound present in the highest amount (42–58%), followed by stearic acid (18.3–24.5%), and oleic and linoleic acids (9.5–22.3%). These fatty acids originate mainly from fecal waste and kitchen residues. This profile is similar to those reported in the literature (Mondala *et al.* 2009; Siddiquee & Rohani 2011; Olkiewicz *et al.* 2014, 2015b).

Figure 7 presents the FTIR spectra of the lipids extracted with hexane and ethyl butyrate from the dry and dewatered sludges.

It is interesting to observe variations in the intensities of the characteristic absorption bands of the extracted lipids. In the first instance, the absorption bands of aliphatic CH groups were identified at 2,900–2,840 cm^{-1}, 1,410 cm^{-1}, and 700 cm^{-1} (Bahadi *et al.* 2016). The absorption bands located at 1,300–1,000 cm^{-1} and 1,735 cm^{-1} are associated with the C–O and C = O groups of glyceride esters or alkyl esters (Pastore *et al.* 2013; Bahadi *et al.* 2016). In addition, an absorption band was observed at 1,710 cm^{-1} (more evident in soluble lipids), related to the C = O group of carboxylic acids in the free fatty acid fraction (Pastore *et al.* 2013; Bahadi *et al.* 2016). Calcium soaps are COO– carboxylic salts with absorption bands in the region of 1,580–1,530 cm^{-1}, and their presence is more noticeable in the spectra of solid lipids extracted with hexane (Pastore *et al.* 2013; Hao *et al.* 2017). In the spectra of the lipids recovered with ethyl butyrate, a decrease in the absorption bands associated with calcium soaps and an increase in the absorption band associated with free fatty acids were observed, thus confirming the reaction of the calcium soaps to form free fatty acids during the mixing of the solvent and primary sludge (D'Ambrosio *et al.* 2021).

4. CONCLUSIONS

The composition of the residual sludge generated in an urban WWTP in the City of Aguascalientes, Mexico was investigated to determine the degree of variation in its composition. The results of the study indicated that the lipid content that can be extracted from the residual sludge can vary significantly due to seasonal factors, changes in the configuration of the treatment plant itself, municipal waste management at the local level, and government regulation regarding water discharges, among other factors. These lipids are an inexpensive source of raw material for biodiesel production; however, as demonstrated, it is important to determine the variation of the composition of this raw material during the year to suitably design and intensify the processes and operations involved for the recovery and exploitation of such feedstock. The valorization of this type of waste must consider possible changes in its composition to scale the use of this raw material to an industrial level and take advantage of its potential to produce value-added compounds.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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