Research article

A new simpler and reliable method for determining mineral oil in sewage sludge: Influence of biogenic compounds for the quantitative analysis of C10–C40 hydrocarbons

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ABSTRACT

The characterization of organic contaminants in sewage sludge is a fundamental step to address the relevant most appropriate management practice. In this perspective, C10–C40 hydrocarbon content was considered in Italy a crucial parameter to be considered, in spite of its irrelevance in the literature. The very complex mixture of organic substances of both biogenic and anthropogenic origin the sludge is made up of makes sewage sludge a matrix of uniqueness nature, and the analytic determination of hydrocarbon content through conventional procedures may be subjected to overestimation. In this work, optimization of two conventional protocols for the determination of mineral oil (EN14039 and *IRSA CNR gravimetric method*) were run with attention to anthropogenic compounds potentially affecting the C10–C40 mineral hydrocarbons determination. Effects from the first manipulations of sewage sludge samples to extraction procedure and clean-up operations were investigated.

A new simple procedure was set up and tested on 30 samples from different wastewater treatment plants (WWTPs). Through a simple extraction with hexane (12 mL per 2 g of dried sludge, acidified with HCl conc.) at room temperature for 2 h, followed by a clean-up on Florisil column (10 mL–2 g) a confident determination of C10–C40 were obtained with respect to conventional optimized procedures. Variability within the range 0.06–9.49% was calculated with respect to the average value determined using three different methods, with an average value of 2.48 ± 2.37%, demonstrating the robustness of the determination. Up to 3% of the total hydrocarbons were identified as naturally occurring, namely terpenes, squalenes and deoxygenized sterols, passed through the clean-up Florisil column. A significant incidence (up to 75%) of the final overall C10–C40 content was found to be related to the C10–C20 component, originally present in the commercial polyelectrolytes in emulsion, widely used for conditioning before mechanical dewatering.

1. Introduction

Sewage sludge is the undesirable product of wastewater treatment and consists of a heterogeneous material composed of naturally occurring organic substances and organic and inorganic anthropogenic pollutants (Fijalkowski et al., 2017; Steele et al., 2022). The organic fraction of the sewage sludge comes predominantly from household wastes and faeces (Dubey et al., 2021; Rogers, 1996). From a chemical point of view, besides inorganic components, there is a complex mixture of fats, proteins, sugars, cellulose, hemicellulose, lignin, humic substances and microorganisms as well as their biotransformation products generated in wastewater treatment plant (WWTP) (di Bitonto et al.,

2020a). Currently, several sewage sludge management methods are employed. Reusing and recycling represent the most ambitious options considering the progressive reduction of landfill disposal due to the restriction introduced for biodegradable wastes (Mininni et al., 2019). Besides energetic applications (di Bitonto et al., 2020b), new alternatives have been recently investigated for using sewage sludge as a source of new composite materials (Palmieri et al., 2019) or brick making (from sewage sludge as it is (Areias et al., 2020), or after incineration (Weng et al., 2003). However, the use of sludge in agriculture still remains in many countries, the most used option in the perspective of circular economy (Gianico et al., 2021) in spite of its declining in citizens' acceptance. The European Union (EU) is currently regulating only the

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limits of heavy metals (Cd, Cu, Hg, Ni, Pb and Zn) listed in Council Directive 86/278/EEC (Kelessidis and Stasinakis, 2012). Several European countries have introduced more stringent requirements with respect to this directive and have adopted new limits for concentrations of other heavy metals, organic micropollutants and microbial contamination (Hudcová et al., 2019). The final fate of sewage sludge differs largely from country to country depending on national rules, opportunities, market possibilities and facilities equipment, even changing year by year. In 2015, in Italy, 9.9% was used in agriculture without further treatments than those used in the WWTPs, 26.4% was sent to external plants for compost production, 5.6% was sent to the external plant for the production of a soil conditioner called 'chalk of defecation' (end-of-waste admitted in Italy), 17.2% was disposed to landfill, and 5.9% was sent to incineration or co-incineration plants. The rest (35%) was sent to external sludge centres for further treatments (mainly chemical and physical processes) before recovery/disposal (Mininni et al., 2019). The huge amount of sewage sludge produced in Europe (the latest data show a yearly production of more than 10 million tons (on a dry basis), (Eurostat - Data Explorer)) pushes developing innovative reuse/recovery options in compliance with the European and national legislation and with the forthcoming transition towards the principles of a circular economy is absolute. Among the anthropogenic chemicals that are relevant as environmental contaminants, hydrocarbons C10–C40 content might be an emerging parameter to be considered, since hydrocarbons C10–C40 are well known to be neurotoxic to humans and animals. Several specific studies demonstrated that disposal costs for off-site solutions biological degradation often required a considerable effort for their degradation (Frutos et al., 2012; Oleszczuk, 2007; Tahhan et al., 2011; Tomczyk et al., 2020; Wang et al., 2011).

Petroleum oils in sewage sludge have many sources related to the common use of petroleum derivates in various commercial sectors (Tahhan et al., 2011) and the possible release of fuels and lubricants on roads and paved areas of petrol oil stations. In Italy, hydrocarbon contamination of sewage sludge by industrial effluents seems unlikely, considering that these are typically treated separately from urban wastewater. Even in the case of discharges of industrial effluents into the sewer, these must be pre-treated to reduce the concentration of hydrocarbons up to 10 mg/L. They are diluted with organic compounds brought with wastewater, only partially degraded in the WWTP and eventually accumulated in sewage sludge (Mansuy-Huault et al., 2009).

Upon the evidence of the adverse effects of some petroleum hydrocarbon on humans and animals (Boffetta et al., 1997), new and lower concentration limits for C10–C40 hydrocarbon in sewage sludge addressed to the land application have been significantly dictated, reducing the direct use on land of sewage sludge (in Italy, for instance, in 2020 was only the 2.9% against the 11.2% in 2015).

For this reason, the correct C10–C40 hydrocarbon quantification is considered of increasing importance in the near future, placing this parameter on the top list of organic micropollutants to be regulated.

Methods for assessing C10–C40 hydrocarbons in sewage sludge are generally based on the extraction of total oil and grease using organic solvents followed by a purification that allows the hydrocarbons to be isolated through an elution on a Florisil column and appropriately quantified (Moreda et al., 1998). Specifically, mineral oil determination on sewage sludge was reported by IRSA (1988) and consisted of a gravimetric method, whereas, more diffusely, the UNI EN 14039 (2005) was applied using a GC-FID determination. Both ways require long and tedious preparative operations and have some criticisms and limitations.

The gravimetric method is based on the extraction with 1,1,2-Trichlorotrifluoroethane Freon 113. It allows for determining not only mineral oil but also total oil and fats. Although gravimetry has no limits on oil quantity and requires no particular instrument, it has lower sensitivity and poor reproducibility (Wang et al., 2011). It does not offer any selectivity or information on the type of oil detected. Moreover, the use of this method in Europe is not recommended since the sale and use of Freon are no longer allowed being Freon toxic for humans and the environment.

On the other hand, EN14039 is not specifically designed for sewage sludge but has been optimized for general solid waste. It is based on the extraction of total grease and oil from a sample (with a mixture of heptane/acetone 1:2) followed by clean-up procedures which ended with an elution on a column containing sodium sulphate and activated Florisil with the scope of retaining polar components. The quantitative analysis of C10–C40 hydrocarbons is then carried out through capillary gas chromatographic measurements using a non-polar column and flame ionization detector (FID), cumulating the total peak area of compounds between n-decane ($C_{10}H_{22}$) and n-tetracontane ($C_{40}H_{82}$). Considering the unspecificity of this final determination, the preliminary manipulation of sewage sludge samples and the final clean-up has a pivotal role for a correct estimation. If the clean-up step is not effective in the removal of biogenic substances, an overestimation of petroleum hydrocarbon levels in sewage sludge could occur, leading in false exceedances that can trigger unnecessary and costly disposal measures. In detail, it is worth saying that among the warnings of EN14039, it is reported that a high concentration of total oil and grease (higher than 10,000 ppm) makes possible overestimation of C10–C40 content. This means that both the quantification of total polar biogenic compounds and the successive optimization of the clean-up step in terms of the correct amount of Florisil to be used is often required and could change sample by sample.

As a matter of fact, defining a protocol suited for the correct estimation of mineral oil in sewage sludge that meets the needs of all laboratories of analyses and the request for environmental safety is a critical analytical task. The present work aimed, first of all, at characterizing and identifying the major biogenic compounds that could affect mineral oil determination in sewage sludge. The gravimetric Bligh and Dyer method (BLIGH and DYER, 1959) was used to achieve the complete extraction of fats and oil from sewage sludge and the GC-MS technique was used to identify the most important constituents. Then, a study of optimization of both the protocols currently in use, namely the IRSA method and the EN 14039 method, was carried out to make them suitable for the analysis of hydrocarbon C10–C40 in sewage sludge. The analytical procedures were optimized with the aim of obtaining converging data. In particular, important factors which impact the trueness and precision of the methods were investigated, such as the sample pretreatment and the ratios sludge/solvent - Extract/Florisil, with the aim of preventing the biogenic compound contribution in the final determination of hydrocarbons.

Finally, a new reliable and simpler procedure based on only hexane/heptane extraction followed by purification on Florisil column and a GC quantitative determination was designed, optimized and tested. This analytical procedure, easier and faster than the previous ones, allowed converging results to be obtained. From a more specific analysis of the GC-FID profiles, the effect of the use of emulsions formulation of polyelectrolytes usually adopted as coagulants and flocculants in the final dewatering treatment of WWTPs' sludges was investigated in terms of contribution to the increase of the total hydrocarbon content in the final product.

2. Experimental section

All the used reagents were of a grade *per analysis* and suited for the specific purpose.

Florisil for the clean-up column was provided by Sigma Aldrich (particle size 150–250 μm, 60–100 mesh). It was activated at 140 °C for 16 h before the use, as required in EN 14039.

Hydrocarbons n-Decane and n-Tetracontane were purchased from Sigma and used to define the retention time window for gas-chromatographic determination. In particular, 15 mg of n-tetracontane and 15 μL of n-decane were dissolved in 0.5 L of organic solvent (n-heptane or n-hexane). The mixture was sonicated for 15 min. Such solution was used as an extractive solvent in the following procedure

(Standard Solution, STDS).

2.1. Instruments

Identification of the biogenic and anthropogenic products was carried out by using a PerkinElmer Clarus 500 GC-MS apparatus equipped with a Clarus MS spectrometer.

1H and 13C NMR spectra were recorded on a Bruker AV-400 (di Bitonto et al., 2020b).

FTIR spectra were collected on a Nicolet Summit Thermo Fisher Scientific FTIR spectrophotometer equipped with Everest Diamond ATR module and Czitek™ SurveyIR™ for the micro-ATR-FTIR analysis; the FTIR spectra were recorded in a frequency range from 4000 to 400 cm^{-1} with 16 scans at a 4 cm^{-1} resolution.

Quantitative analyses were carried out using two gas chromatographs (VARIAN 3800 and Agilent 8890) equipped with a flame ionization detector (FID), an autosampler and a capillary column DB-5HT 15 m in length, 0.32 mm in diameter and 0.10 film thickness. Helium was used as carrier gas.

GC-FID's performances were checked and optimized using a commercial standard solution provided by Sigma Aldrich containing all n-alkanes between C10–C40 range (C10–C40 included) at 50 mg/L each. The suitability of the gas chromatographic system for the resolution of n-alkanes as well as for the detector FID response, was verified.

Each parameter of the gas chromatographic method was set in order to obtain a chromatogram similar in shape to that presented in EN 14039. Upon optimization, the following parameters were set: on-column injection, constant pressure fixed at 20 psi, injected volume 2 μL, detector temperature 360 °C, oven ramp: 80 °C 1min, 20 °C/min up to 360 °C, 360 °C 15 min.

Commercial diesel oil and lubricating oil mixture (Sigma Aldrich 8000 ppm) were used to prepare calibration solutions in the range 100–4000 ppm. According to EN14039, hydrocarbons were determined as a sum area of resolved and unresolved components lying between the retention times of n-decane and n-tetracontane.

2.2. Sewage sludge collection and manipulation

Sewage sludge used for the set-up of the analytic method was collected in Martina Franca (S_MF) and Bari Est (S_BE) WWTPs. Other sludge samples, analyzed for the set-up of the new optimized methods, were provided by several companies associated with Utilitalia, that operate WWTPs on Italian territory. Each wet sample was screened (spreading thickness less than 2 cm), air-dried at 40 °C for 48–72 h in an air-ventilated oven (TS40) and ground to make it totally sieved (≤250 μm), in accordance with procedures reported in Federal Compost Quality Assurance Organisation (FCQAO) for the determination of organic pollutants in composts. 0.1–0.2 mL/g HCl 30% were added and kept under stirring for 30 min. The extraction chosen for the investigation was applied, and the final extract was analyzed for the determination of hydrocarbons or biogenic matter.

The preparation of the sample for the gas chromatographic determination of C10–C40 needed the use of a reasonably low temperature for preserving the evaporation and the loss of light hydrocarbons during drying operation. On the other hand, considering that TS40 determination brought to samples having a residual humidity (from 6.5 to 12.3%), the sludge was also dried at 105 °C in the oven for 24 h to determine the TS105. Final results were expressed as ppm of C10–C40 (determined on samples dried at 40 °C) referred to TS105.

2.3. Procedures for the analysis

Blank for each procedure was carried out using all the reagents in identical amounts and in the same way but without the sample. The blank value was subtracted from the value of interest.

2.3.1. Bligh and Dyer method adapted to sewage sludge

Lipid extraction from pre-treated sewage sludge was carried out according to the Bligh and Dyer protocol taking into account the difference in terms of water content between the tissue for which this method was developed and sludge that was going to be analyzed. Briefly, 5 g of dry sludge were homogenized with 50 mL of CHCl$_3$ and 50 mL of MeOH for 1 h. The mixture was filtered and then washed two times with 10 mL of CHCl$_3$/MeOH 1:1 mixture, and the collected liquid was extracted with 50 mL of water using a separatory funnel. The organic portion was then dewatered using sodium sulphate. The solution was filtered, and the volume was reduced in a bath of water at 50 °C. Lipid content was then determined gravimetrically after evaporating the chloroform phase to dryness. Finally, the obtained oil was dissolved in 2 mL of n-hexane and analyzed by GC-MS.

2.3.2. 21 IRSA CNR method optimization

5 g of homogenized and dry sample (40 °C) were treated with 0.5 mL of HCl (30%) and then added to 100 mL of a mixture of n-hexane and methyl-tert-butyl ether (80:20). The extraction was carried out in a separatory funnel for 3 min or in a glass extraction vessel under magnetic stirring as specified in the following test. The extraction was repeated twice. Then, the mixture was filtered and the filter washed with 10 mL of solvent. 3 g of Na$_2$SO$_4$ were added to the liquid solution that was then filtered again using 10 mL of n-hexane/ether mixture for washing. Finally, the volume was reduced quite completely in a bath at 50 °C. The oily residue was taken for 1 h in the oven at 50 °C and weighed after cooling in a glass desiccator. The amount of total oil was determined gravimetrically.

For the determination of mineral oil, the obtained residue was dissolved in 10 mL of n-hexane and eluted in a column 20 cm long containing about 8 g of Florisil for the removal of polar compounds. 10 mL of n-hexane were used to wash the beaker and other 80 mL for eluting the column. The collected solution was then put in a bath at 50 °C in order to leave the solvent to evaporate and then transferred in the oven at 50 °C for 1 h to reach dryness. The final residue was weighed, and the mineral oil portion was determined gravimetrically. These residues were also analyzed through FTIR and NMR. Final results were reported as mg of mineral oil per kg of dry sludge (ppm) according to the following Equation (1):

$$C_{\text{IRSA C10-C40}} = m_{\text{C10-C40}} / m_{\text{Dry Sludge}} \text{TS105} / \text{TS40} \qquad [1]$$

where $m_{\text{C10-C40}}$ is the C10–C40 fraction determined gravimetrically (in mg), $m_{\text{Dry Sludge}}$ is the mass of dry sewage sludge used for the determination (in kg), TS105 and TS40 are the total solids obtained from the sample at 105 °C 24 h and 40 °C 48–72 h, respectively, as mentioned above.

2.3.3. EN14039 method optimized for sewage sludge

4 g of the homogenized dry sample (40 °C) were put into a glass extraction vessel together with 0.4 mL of HCl. After 1 h under stirring, 40 mL acetone was added. After short shaking by hand, 20 mL of the n-heptane STDS (containing n-decane and n-tetracontane standard solution 30 ppm each) were added. The extraction was carried out for 1 h or as specified by shaking with magnetic stirrer or sonication. After settling of the solid material or centrifugation, the supernatant was transferred into a separatory funnel and 100 mL of water were added twice in order to remove the acetone. The organic phase was then collected and dehydrated with Na$_2$SO$_4$. 10 mL of the extract was then sent to the clean-up column filled with 2 g of Na$_2$SO$_4$ and 2 g of activated freshly prepared Florisil. The column was not pre-washed with an organic solvent. At the end, the purified eluate was analyzed by GC and GC-MS.

It was stated that the above volumes can be scaled down without affecting the final results as long as the critical ratios of n-heptane, sludge and Florisil are kept identical. Final results were reported as C10–C40 concentration (ppm) deriving from the ratio of mineral oil in

mg per kg of dry sludge (Eq. (2)):

$$C_{EN14039\,C10-C40} = m_{EN14039\,C10-C40} / m_{Dry\,Sludge}\,TS105/TS40 \qquad [2]$$

where $m_{EN14039\,C10-C40}$ is the C10–C40 fraction determined gas-chromatographically in mg, $m_{Dry\,Sludge}$ is the mass of dry sewage sludge used for the determination (in kg), TS105 and TS40 are the total solids obtained from the sample at 105 °C 24 h and 40 °C 48–72 h, respectively, as mentioned above.

2.3.4. Simplified extraction protocol

The simplified extractive procedure was carried out as follow: 2 g of dry homogenized sewage sludge were transferred into a glass vessel together with 0.25 mL HCl conc. After being kept under gentle agitation for 1 h, 10 mL of n-hexane (or n-heptane) and 2 mL of n-hexane/n-heptane STDS were added. The extraction was carried out under magnetic stirrer for 2 h. The solid was left to settle and 10 mL of the liquid phase was completely transferred in the clean-up column containing 2 g of Na$_2$SO$_4$ and 2 g of activated and freshly activated Forisil.

The purified solution was collected out of the column and analyzed by GC-FID and GC-MS analysis. Final results were reported as mg of C10–C40 gas-chromatographically determined per kg of initial dry sludge (Eq. (3)).

$$C_{Hexane\,C10-C40} = m_{Hexane\,C10-C40} / m_{Dry\,Sludge}\,TS105/TS40 \qquad [3]$$

3. Results and discussion

The unavailability of a prompt analytical method to determine the C10–C40 mineral oil content in sewage sludge required a critical investigation of different methods (optimized and compared) on two representative samples. The two sewage sludge samples were collected from the large Bari Est (S_BE) wastewater treatment plant (432,492 p.e.) after anaerobic digestion, and from the smaller wastewater treatment plant of Martina Franca (S_MF) (75,294 p.e.) after aerobic digestion treatment. The choice was made taking into consideration the different amounts of liquid waste entering the plants and the different sludge stabilization treatments. This allowed to include such aspects in the anthropogenic hydrocarbon content and the kind of biogenic species possibly present in the relevant sample of sludge. A conventional extraction (Bligh&Dyer method was applied) on these two samples of sludge allowed the relevant lipid components to be recovered, chemically characterized and cleaned up to isolate the hydrocarbon fractions (Section 3.1). In a successive step, the gravimetric protocol was optimized by replacing the Freon with a more appropriate mixture of solvents (hexane:methyl-tert-butyl ether 80:20, Section 3.2). As the third step, the EN 14039 protocol was also optimized and investigated (Section 3.3). Finally, the optimized gravimetric protocol, the optimized EN14039 method and a simplified procedure of extraction operated using hexane or heptane directly from sludge (exsiccated at 40 °C) were processed and applied on 30 samples of sludge uptaken from different Italian WWTPs.

3.1. Total oil and grease determination in sewage sludge: the Bligh and Dyer method

Natural lipids in sewage sludge generally consist of a mixture of non-polar components such as fatty acids and sterols. Usually, extraction of lipids from a natural matrix is performed with the use of various organic solvents or solvent mixtures that, in principle, should be adequately polar but also not so polar to remove lipids of different strains. The classic and most reliable methods for the quantitative extraction of lipids reported in the literature are the so-called "Bligh and Dyer" (BD) and "Folch" methods (BLIGH and DYER, 1959) in which a CHCl$_3$/CH$_3$OH/H$_2$O phase system is used as an extractive mixture. Among both, the former is the most recommended method (Payne et al.,

1999) and has become the standard protocol for lipid determination from different biomasses in many studies. In the present study, such protocol was used to ensure the total recovery of lipids from sewage sludge in order to determine their concentration and nature, with the scope of identifying the components that could affect the C10–C40 hydrocarbon determination.

Applying the adapted BD method to the sewage sludge of S_MF and S_BE, it was found that the total oil fraction (25,172 ± 553 and 17,430 ± 496 ppm, respectively) exceeded the critical limit of 10,000 ppm reported in EN 14039.

Extracts were then dissolved in 100 mL n-hexane and purified on a Florisil column: the oily residue that, in principle, should correspond to the mineral oil fraction was quite one order of magnitude lower than the total oil (3825 ± 256 and 3243 ± 315 ppm). The results changed when a further purification using a second column was performed (2944 ± 233 and 2521 ± 283 ppm).

GC-MS analyses (Fig. 1) allowed identifying species recovered in each extraction and purification step, explaining these differences. Upon the extraction with the CHCl$_3$/CH$_3$OH mixture, fatty acids and sterols derivatives, some of which in their oxidized form, were detected. During the first clean-up step, only fatty acids were efficiently retained, whereas oxidized sterols (that are not supposed to elute) were able to elute from the first Florisil column, because of an overloading (Fig. 1), but were efficiently removed by the second column. Washing the Florisil column with eluents of increasing polarity allowed oxidized sterol derivatives and sterols to be completely eluted: n-hexane/methyl tert-butyl ether (80:20), pure methyl tert-butyl ether without and with concentrated HCl were used in sequence to recover the different components up to fatty acids (the most polar species).

On the other hand, several forms of sterols derivative such as cholestan-2-ene and cholestan-4-ene were also present in all the collected oily residues. They are clearly of biogenic origin as the precursor coprostanol (5β-cholestan-3β-ol), epicoprostanol (5β-cholestan-3-αol), cholesterol (5,6-cholesten-3β-ol), cholestanol (5α-cholestan-3β-ol), stigmastanol (24β-ethyl-5α cholestan- 3β-ol) and coprostanone (5β-cholestan-3-one) come from the intestinal microorganism action on cholesterol (Froehner and Sánez, 2013; Juste and Gérard, 2021) and can be found in human and animal faeces (Derrien et al., 2012; Harrault et al., 2019; Jardé et al., 2005). Nevertheless, from a chemical point of view, they belong to the hydrocarbon family and there is no reason they should be removed during the clean-up treatment on Florisil. Thus these compounds affect the quantitative determination of C10–C40 hydrocarbons in all the analytical procedures that are based on the use of a chromatographic Florisil column for the separation of mineral oil from polar oil and fats. However, it is worth underlining that, such a biogenic class of compounds never overcome 3% of the total hydrocarbon
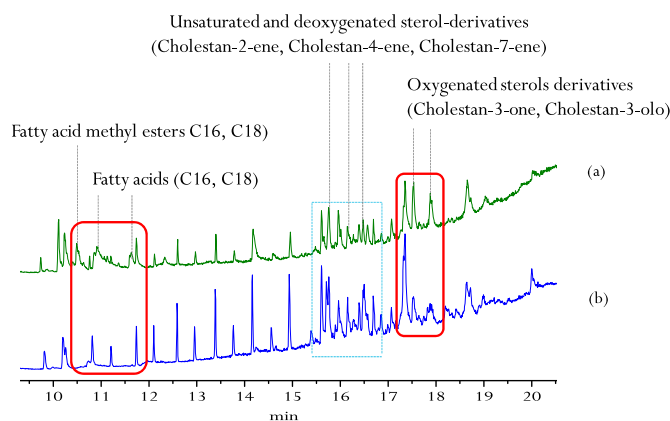

Fig. 1. GC-MS spectra of a) total oil obtained upon extraction with CHCl$_3$/CH$_3$OH; b) oily residue obtained after clean-up on Florisil using n-hexane as eluent.

content.

3.2. Optimization of gravimetric IRSA protocol (1988)

The gravimetric method for the determination of total oil and hydrocarbon-based on the use of FREON 113 is described in IRSA (1988). Since the use of Freon is not more allowed because of environmental and health reasons, it needs to be substituted with other solvents or mixtures without changing the extractive capability. The use of a mixture of n-hexane and methyl-tert-butyl ether (80:20) was then considered for the extraction of total oil since it was already introduced in the total grease determination in wastewater (APAT IRSA 5601 A1-A2). This extracting mixture combines the requirement of non-polarity with n-hexane and that of low polarity with methyl-tert-butyl ether matching well the range of polarity of the whole lipids present in sewage sludge.

A study on the impact of the extraction operative conditions (method and time) was carried out. Table 1 reports the concentration of total and mineral oils for S_BE and S_MF and the effect of preliminary acidification of sewage sludge with HCl. The use of HCl was based on the assumption that, in the presence of a mineral acid, lipids and soaps, in particular, could be converted into fatty acids, which usually show higher solubility in organic solvents with respect to the former (Pastore et al., 2013), and that the acid could promote floc and cellular membrane lysis making the lipid constituents of the membrane more available for the extraction.

The results summarized in Table 1 show clearly that the use of the separatory funnel and the manual stirring for 3 min suggested in the IRSA protocol were not efficient enough to extract all the lipid fraction from sludge, so that an underestimation of the mineral oil content was obtained.

Moving the set-up of extraction from the separatory funnel to a glass vessel with magnetic stirring, the total oil concentration greatly increased in both sludges reaching the highest yield after 2 h.

The mineral oil content follows the same trend as the total oil and fats, highlighting the importance of the first extraction step.

Nevertheless, by comparing the total oil concentration after 2 h with that obtained applying the BD procedure (see Section 3.1), it is quite evident that there is a great leak in the extraction with the mixture n-hexane/methyl-tert-butyl ether. On the contrary, the acidification of the sludge with hydrochloric acid before the extractive phase assured an increase in the yield extraction, giving a total oil concentration comparable to that obtained using the BD method. Hydrochloric acid acts mainly on the extraction of fatty acids as revealed from GC-MS spectra (Fig. 2). Moreover, the use of HCl in the pretreatment step affected also
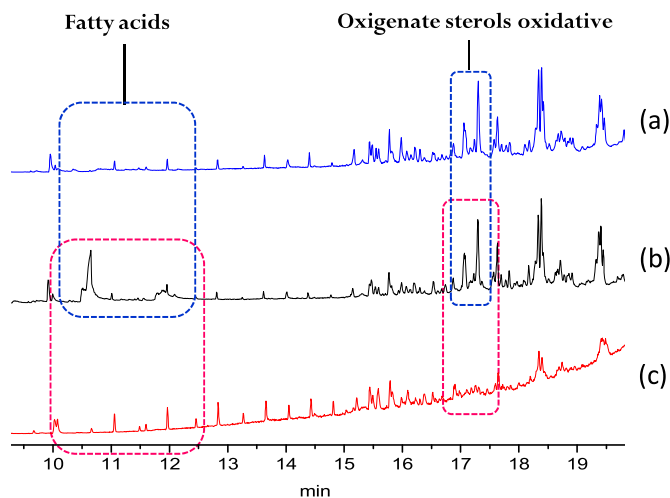

Fig. 2. GC-MS spectra of a) Total Oil and Fats upon extraction of 2 h without HCl pretreatment on sewage sludge; b) Total Oil and Fats upon extraction of 2 h with HCl pretreatment on sewage sludge; c) Mineral Oil upon clean-up of total Oil and Fats in n-hexane mixture on Florisil column (8 g).

the mineral oil extraction that resulted completed already after 1 h. The time of extraction was optimized again to find the most advantageous conditions: when the time of contact was 2 h, most (>95%) of the lipids and fats were extracted from the sample. If the extraction was repeated on the sludge for the second time (total time of extraction 4 h) only a slight increase of the value was observed.

Final purified extracts were also analyzed through ^1H, ^{13}C NMR and FTIR (Fig. S1): only signals typical of C–H moiety were identified, without any appearance of heteroatoms (O, N and S). As results the extraction yield obtained using the optimized method was comparable to what obtained using the BD method. The complete extraction of all the lipid fraction was ensured and a mineral oil fraction free of oxygenated species was collected for the mineral oil quantitative determination.

3.3. Optimization of EN 14039 protocol

The suitability of the EN 14039 protocol for the quantitative determination of C10–C40 hydrocarbons in sewage sludge is a matter of discussion because of the particular nature of the matrix, which very often, due to its origin, contains a high amount of biogenic substances that could interfere with the quantitative determination of mineral oil. On the basis of what was found applying the previous gravimetric methods (Paragraph. 3.1, 3.2), the substances that mainly could affect the analysis were fatty acids and oxygenated sterol derivatives that were demonstrated to overcome the limit of 10,000 ppm, recommended for correct use of EN14039 protocol (Table 2). At the same time, the use of such protocol for the determination of C10–C40 hydrocarbon is greatly advantageous with respect to the other gravimetric methods as it allows the direct determination by gas chromatography of mineral oil saving time and solvent and limiting the error of the final determination thanks to a lower amount of steps of analysis with respect to the gravimetric method. The aim of the work was, then, the optimization of the EN 14039 protocol to make it suitable for the C10–C40 hydrocarbon determination in sewage sludge in spite of the high amount of biogenic components present in the matrix. The optimization consisted of the study of the influence of various operating parameters such as the solvent-to-solid mass ratio, the contact time during the extraction, the technique of extraction, and the amount of Florisil to be used.

Table 2 reports the results obtained by changing the sludge/solvent ratio in the determination of C10–C40 hydrocarbon in both S_BE and S_MF. Using the amount of sludge defined in the EN 14039 protocol

Table 1
Extraction set-up for gravimetric determination of Total Oil and Mineral Oil (Extraction of Total oil and Fats: Sewage Sludge = 5 g, n-Hexane/Methyl-tert-butyl ether 80:20 = 100 mL; Clean-up procedure: n-Hexane = 100 mL, Florisil = 8 g).

Extraction	Time	S_BE		S_MF	
		Total oil and fats	Mineral oil	Total oil and fats	Mineral Oil
Separatory funnel	3 min	2338	1355	2873	718
Magnetic stirring	1 h	7084	1510	5153	1486
Magnetic stirring	2 h	12,937	2332	8035	1579
Magnetic stirring[a]	1 h	11,043	2459	18,465	2189
Magnetic stirring[a]	2 h	16,015	2118	23,414	2155
Magnetic stirring[a]	4 h	17,363	2239	24,454	2160

[a] Extractions operated on acidified samples (0.5 mL HCl conc. per 5 g of dry sewage sludge).

Table 2
Study of the influence of Sludge/Solvent ratio on the C10–C40 hydrocarbon determination.

Protocol	Dry Sludge (g)	n-Heptane (mL)	sludge/solvent (g/mL)	S_BE	S_MF	GC-MS identification
				C10–C40 (ppm)	C10–C40 (ppm)	
EN 14039	20	20	1	6706	5443	FA (C16, C18) Oxygenized sterol derivatives Deoxygenized sterol derivatives
EN 14039 optimized	4	20	0.2	2191	2048	Deoxygenized sterol derivatives

(sludge:solvent:Florisil of 10:10:2), the final determination was affected by the presence of polar compounds of biogenic origin, namely fatty acids and sterol derivatives. This suggested that, upon the extraction with organic solvent, a too much concentrated solution containing both non-polar hydrocarbons and polar substances was obtained so that the Florisil column was going to be saturated and the clean-up step was not performed well in the purification of the extract. By decreasing the initial amount of sludge down to a final ratio sludge:solvent:Florisil of 2:10:2, any of such contaminants were detected in the GC-MS spectrum of the final extract. Deoxygenized sterol derivatives, squalenes and terpenoids were the only biogenic species observed but, as clarified before, these compounds are hydrocarbons and there are no possibilities to separate them from the mineral hydrocarbons fraction. Nevertheless, the amount of such species was estimated to be less than 3% of total hydrocarbon in both the analyzed samples suggesting that the anthropogenic hydrocarbon content could be overestimated by no more than 3%, analogously to some sediments of the river (Méjanelle et al., 2017) or lagoon (Tarozo et al., 2010).

The influence of the technique of extraction was also investigated: taking the sludge under magnetic stirring allows to extract much more hydrocarbons with respect to using sonication. In particular, it was found that, under such conditions, 1 h of extraction is enough to completely solubilize hydrocarbon in the organic solvent. The increasing amount of Florisil used in the clean-up process did not influence the final quantitative determination of C10–C40 hydrocarbon, confirming that the analysis was not affected by the presence of polar species. All the data are reported in Table 3.

3.4. Set-up of a new simplified method for the determination of C10–C40 hydrocarbon in sewage sludge and comparison with optmized gravimetric and EN14039 methods

Conceptually, the most appropriate way to set-up the analytical protocol should consider i) a correct preliminary manipulation of sewage sludge that preserves from loss of analytes and their modifications, ii) a quantitative recovery and extraction of the total grease and oil (and analytes) from the solid samples, iii) an efficient and selective purification of C10–C40 hydrocarbons and iv) a robust quantification.

The use of desiccated sludge at 40 °C, instead of a wet sludge had put in discussion the necessity of using acetone together with n-heptane during the extraction of C10–C40 hydrocarbons, typical of the EN14039

Table 3
Study of the operative conditions for the optimization of the EN 14039 protocol (Sludge/Solvent ratio = 0.2).

Technique	Time of extraction (h)	Florisil (g)	S_BE C10–C40 (ppm)	S_MF C10–C40 (ppm)
Sonication	1 h	2	1546	2008
Magnetic stirring	1 h	2	2203	2117
Magnetic stirring	2 h	2	2191	2048
Magnetic stirring	1 h	4	2212	2133
Magnetic stirring	1 h	6	2210	2104

method. In fact, acetone, miscible with n-heptane, should facilitate the path of the organic solvent (not miscible with water) through the wet sludge. The absence of most of water due to the use of dry matter, should allow to avoid the use of acetone and make simpler all the analytical procedure. So, a new procedure in which the extraction was carried out using only n-hexane or n-heptane instead of a mixture n-heptane/acetone was evaluated. In detail, saving the set-up study run for EN14039 in terms of sludge:solvent:Florisil ratio (2:10:2), the direct extraction was tested on desiccated sewage sludge using the conditions reported in Fig. 3.

A comparison between the quantification of C10–C40 hydrocarbon through the optimized IRSA protocol, the optimized EN14039 and the simplified protocol using only n-hexane as extractive solvent was carried out. Tests on different sewage sludge were performed in order to check the reliability of the new method. Both GC and GC-MS analyses revealed that hexane and heptane extract the same kind and the same amount of compounds in all the analyzed samples. More in detail, the as obtained optimized procedures were used to analyze 30 samples of sewage sludge collected from different Italian WWTPs in terms of dimensions (from 35,000 to 2,000,000 p.e.), sludge treatment processes (anaerobic and aerobic digestions) and location (from the north to the south of Italy). The relevant data are reported in Table S1.

It is possible to observe that there is a very good agreement among results obtained with these three optimized protocols, even considering the large range of mineral oil content of the analyzed samples (893–10669 mg/kg ss) (Table S1).

In order to assess a measure of the confidence related to these determinations, the average value of the C10–C40 content of every single sample was calculated by considering the three different procedures, together with the relevant standard deviation expressed as a percentage calculated with respect to the medium value (σ_1). This was in the range of 0.73 and 9.63%, with an average value of 3.73 ± 1.98%, which is a very narrow interval, stating the good convergence of data, especially considering the nature of determinations (gravimetric and gas-chromatographic data using the external standard calibration) typically characterized by this range of indetermination.

Analogously, the deviation (Δ) between the C10–C40 content determined through the simplified method and the average values was calculated and expressed as percentage with respect to the medium value. This was in a very restricted range 0.06–9.49%, with an average value of 2.48 ± 2.37%, demonstrating the robustness of the determination.

The use of this new simplified protocol is advantageous from several points of view.

a) is reagents- and solvent-saving with respect to both the previously described analytical procedures so that it is advantageous both from an environmental and economic point of view; b) is easy to carry out because of the reduced number of operating steps being time-saving and reducing the operator's error. So this is a potential method to be applied in future for the C10–C40 hydrocarbon determination in sewage sludge as it is fast and instrumentally not demanding and, therefore, easily accessible by all laboratories of analysis.
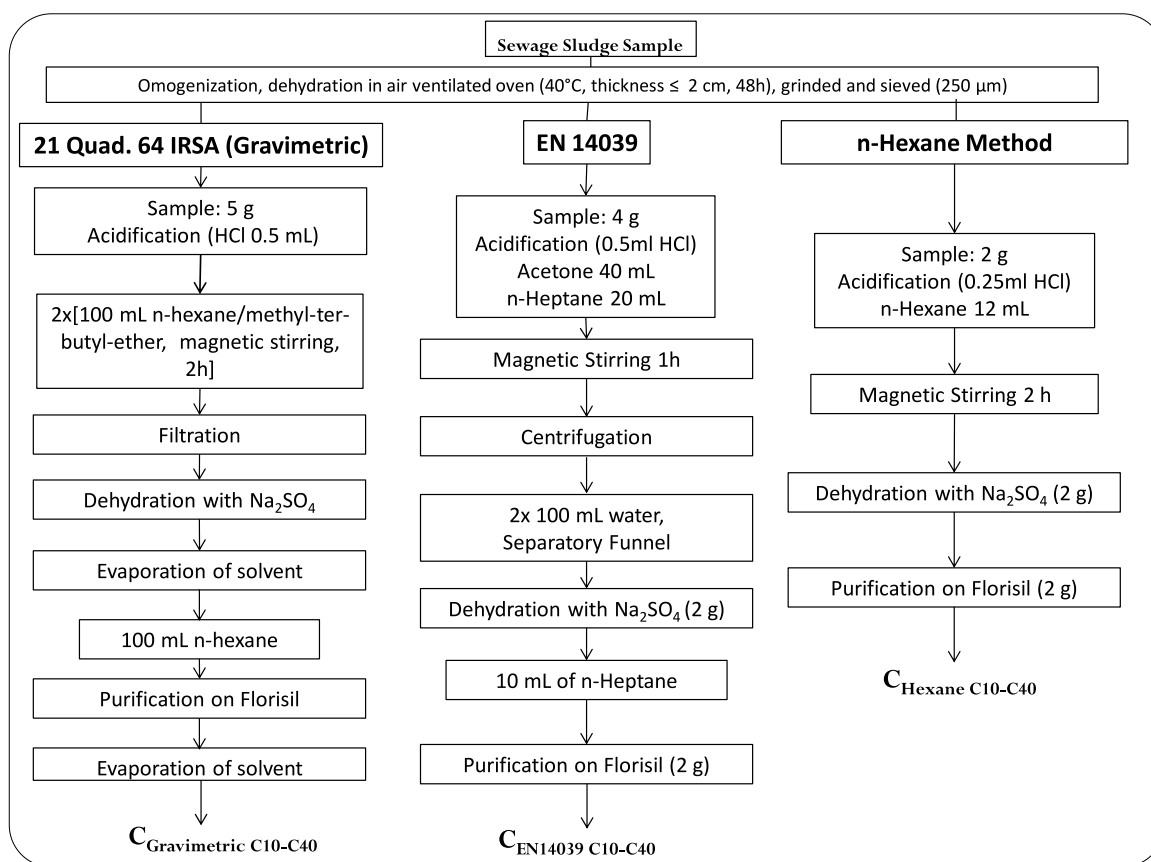
Fig. 3. Scheme of operations of the three quantifying methods for C10–C40 determination.

3.5. Determination of C10–C40 hydrocarbon in sewage sludge with new optimized methods: role of HCl in the pretreatment step

The effectiveness of the acid pretreatment in the extraction of hydrocarbons from different sewage sludge samples was evaluated in all the optimized methods studied in this work. As already described in paragraph 3.2., GC-MS analyses revealed that the use of hydrochloric acid influenced mainly the extraction of total oil, in particular of fatty acids, from sewage sludge, but it was observed some impact also on the mineral oil recovery. This was observed not only for the gravimetric procedure (par. 3.2) but also adopting the other gas-chromatographic protocols (Table S2): the extent of such influence ranged from 15 to over 100% depending most probably on the nature of the starting sludge (pH, amount of total fats and oil or presence of surfactants) that could influence the ability of desorption of hydrocarbon from the sludge.

For this reason, the pretreatment with hydrochloric acid is strongly recommended for total extraction of mineral oil from samples of sewage sludge in order to avoid the influence of other unpredictable parameters related to the nature and the composition of the sludge and make the mineral oil determination more reproducible. In addition, data reported in Table S2 show that the use of n-heptane instead of n-hexane allowed the effective extraction to be realized.

3.6. Effect of manipulation of sewage sludge on the final C10–C40 content

Sewage sludge samples used for this work were uptaken from different WWPTs in terms of dimensions (from 35,000 to 2,000,000 p. e.), the layout of sludge treatment processes (including anaerobic and aerobic digestions), location (from the north to the south of Italy) and seasonality. The most significant variable affecting the GC profile of

C10–C40 was related to the final dewatering step of digested sludge. In detail, the C10–C20 fraction represented up to 75% of the total C10–C40 content, and in general, never less than 50%, in sludge samples dewatered by dosing poly-electrolytes dispersed in hydrocarbon emulsion. On the other hand, when the dewatering of sewage sludge was operated through an emulsion-free polyelectrolytes, the incidence of the C10–C20 fraction on the C10–C40 value, dropped down to about 10–25% (see Fig. S2). However, all the samples whose C10–C40 content was higher than 3500 ppm, presented a big incidence of the C10–C20 fraction. In any case, the use of poly-electrolytes in emulsion could become critical for partially digested sludge. In that case, the undigested residual fraction of volatile solids could favour the adsorption of the hydrocarbons contained in the poly-electrolyte emulsion, resulting in dewatered sludge with a high content of C10–C40.

4. Conclusion

In this study, different protocols for the determination of C10–C40 hydrocarbons in sewage sludge have been considered. The aim of the work was to evaluate if any, the overestimation of the hydrocarbon content due to the presence of biogenic compounds that could be co-extracted and saturate the clean-up Florisil column before the analysis.

In particular, the gravimetric IRSA protocol and the EN 14039 methods were tested and optimized in order to obtain the most reliable result. GC-MS analyses coupled to all the quantitative methods, allowed to determine what kind of compounds could affect the hydrocarbon determination. Among all, the non-oxidized sterols derivatives of biogenic nature belong to the hydrocarbon family so it is impossible to separate them from the anthropogenic hydrocarbons. However, it was estimated that they could exceed the real hydrocarbon content of less than 3%.

Finally, a new simplified analytic method has been set up: it consists of a simple n-hexane extraction followed directly by purification on a Florisil column. Thanks to its simplicity, this method is advantageous from both economic and environmental points of view and gives results consistent with that obtained with all the other methods.

CRediT authorship contribution statement

Carlo Pastore: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Antonella Angelini:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Giuseppe Mininni:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft. **Camilla Maria Braguglia:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.118533.

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