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THE FATE OF C_{10}-C_{40} HYDROCARBONS DURING ANAEROBIC DIGESTION OF SEWAGE SLUDGE DESTINED FOR LANDSPREADING

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to my family

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ABSTRACT

Among the countless consequences of world population growth, the increase in sewage sludge (SS) production, a waste product of urban and industrial wastewater treatment cycles, is acquiring increasingly essential proportions.

In addition to the high-cost items that the management of wastewater treatment plants (WWTPs) has to budget for the disposal of sludge, it should also be added that this latter contains such high quantities of nutrients and micronutrients that it is necessary to consider them as a resource to reintroduce into the production system, instead of waste material. From the perspective of the new paradigm of the Circular Economy, the use of SS as a source of nutrients based on nitrogen and phosphorus, essential for the agricultural sector, constitutes an important tool to address the other major problem caused by overpopulation, that is, the scarcity of raw material.

Since 1986 in Europe and 1992 in Italy, there has been the possibility of reusing SS for spreading on agricultural soil to use the precious nutrients and micronutrients contained therein for food production. To operate in this way, the sector operators must carry out treatments on the sludge to respect certain limits on the concentrations of some characteristic parameters imposed by law to protect the environment and public health.

Since 2018, with the so-called “Genoa decree”, the Italian legislator has introduced, for SS landspreading, the compliance with a parameter previously regulated at a regional level, namely the C_{10}-C_{40} hydrocarbon content, also known as petroleum hydrocarbons. The C_{10}-C_{40} hydrocarbons, as well as heavy metals and carcinogenic substances, represent an additional source of pollution that can accumulate in the soil and penetrate the aquifers.

Before landspreading, SS must be made stable and dense enough to be moved with a shovel and brought to a concentration of C_{10}-C_{40} hydrocarbons below the legal limit of 1,000 mg per kg of raw sludge. Therefore, it becomes necessary to verify whether the current treatment systems for stabilizing

sludge can adequately reduce the hydrocarbon concentration values in SS. The presence of hydrocarbons in SS is undeniable, whether they are of natural origin or petroleum derivation. Given the low affinity of hydrocarbons with water, when the urban drainage system is of a mixed type, almost the entire hydrocarbon component in the sewage treated in WWTP ends up in the sludge line. In this circumstance, thanks to the stormwater, urban wastewater is enriched with debris and slag deriving from the washing away road surfaces, vehicles, and building facades. Moreover, there is the water coming from contact with vegetation, which releases waxes, called cuticular, and rich in hydrocarbons of natural origin, used by the leaves as a protective layer. In addition to what is released by the water treated in the WWTP's sewage line, the sludge can receive a further contribution of hydrocarbons in the conditioning phase, used for settling and dewatering operations, a stage in which chemicals derived from petroleum synthesis products are very often used. Regarding industrial waters, the presence of hydrocarbons depends on the production process from which the wastewater derives.

Another fundamental point to consider is the non-availability, at the time of the start of the PhD, of a tailored, reliable, and validated measurement methodology that can uniquely quantify the hydrocarbon concentrations in SS. It was not uncommon for two different laboratories to give different results for the same sample, even of some order of magnitude. Therefore, many issues to overcome were mainly due to the high affinity between a lipophilic analyte with a complex matrix such as SS.

OBJECTIVES

The PhD research project aimed to overcome the limits of a not yet standardized method of measurement, set up an easy-to-use and reliable method, and investigate the ability of the anaerobic digestion (AD) treatments of SS to degrade C_{10}-C_{40} hydrocarbons and keep their concentration below

the legal limits. The latter is a problem of national impact, which could soon take on an international dimension, given the ever-increasing reliance on the landspreading of SS.

Two experimental lines have been set up:

- An analytical one, which lasted over two years and made it possible to develop a flexible and efficient analytical methodology with different operational solutions in the various analytical phases, depending on the complexity of the specific sample to be analyzed, capable of returning two distinct parameters, i.e., total (TH) and C_{10}-C_{40} hydrocarbon concentrations (C_{10}-C_{40}), which better identify the nature of the hydrocarbons under study, thanks to the use of a mixed gravimetric/gas-chromatographic determination.
- Reactor experiments to study the hydrocarbon degradation capacity of the AD processes used for sludge stabilization operations, especially those destined for landspreading. With the refinement of the analytical method developed, it was possible at this stage also to exploit the great potential of mass spectrometry associated with gas chromatography for carrying out speciation and characterization of each subgroup identified in the hydrocarbon mixture and better understand the nature and the degradability of the hydrocarbon mixture analyzed.

A case study also investigated whether using polyelectrolytes in chemical conditioning could affect SS's anaerobic degradation and hydrocarbon content.

The strong interaction of lipophilic substances such as hydrocarbons with natural organic matter (NOM), which constitutes the solid matrix of SS, is critical in understanding the processes underlying the phenomena object of our research. The compaction of NOM induced by chemical conditioning was indeed targeted as a potential factor affecting the fate of hydrocarbons during the biological treatments to which SS is subjected.

1. INTRODUCTION

1.1. Landspreading of sewage sludge as a solution to a multifaceted problem

The main problem with sewage sludge (SS) management is due to the considerable disposal costs of this waste from the water cycle. The production rate of SS from wastewater treatment plants (WWTPs) on a global scale is currently about 45 million tons per year (Zhang et al., 2017). Among the disposal choices at present most adopted, there is the destination of sludge for agricultural use as fertilizer. The landspreading of treated sludge is becoming common in several European regions, given the high nutrient and energy content retained in their organic matter (Collivignarelli et al., 2020). Due to a future increase in the demand for agricultural products to feed the growing world population, the exploitation of nutrients, such as phosphorus, present in limited quantities on our planet, will be increasingly necessary (Sattari et al., 2016). SS can be an essential resource for agricultural applications (Cincinelli et al., 2012), allowing the recycling of these nutrients and establishing a sustainable approach to waste management (Singh and Agrawal, 2008). The occurrence of valuable plant nutrients can help SS work as an effective soil amendment (Azizi et al., 2015), thereby minimizing the reliance on chemical fertilizers and significantly contributing to carbon sequestration (Torri et al., 2014). Up to a maximum dose of 40 $Mg \cdot ha^{-1} \cdot year^{-1}$, the contents of organic matter and nitrogen, as well as microbial activity, are increased in the soil by the organic amendments, with the improvement of carbon and nitrogen mineralization processes and enzymatic functions (Brisolara and Qi, 2013). The addition of SS could lead to an improvement in soil structure, with a reduction in bulk density and a promotion of soil aggregation, since SS, like other organic materials, is less dense than the mineral fraction of soil (NAS, 1996). However, to induce significant changes in the physical properties of the soil, much higher SS amounts would be required than are commonly needed to provide nutrients for crop growth (Keeney, 1984). Nevertheless, SS could also contain recalcitrant compounds, pathogens, heavy metals, and inorganic constituents, sometimes in potentially harmful concentrations (Jing et al., 2019; Khan et al., 2019; Rosińska and Karwowska,

2017; Sharma et al., 2017). The formation of phytotoxic metabolites by incomplete organic matter decomposition could also be detected (Ahadi et al., 2020).

Given the environmental and human health threats from pollutants and pathogens, SS cannot be applied directly to soil without treatment (Bondarczuk et al., 2016). Therefore, proper treatment of SS is necessary before land application to stabilize the organic matter, prevent nutrient immobilization and phytotoxicity, and inactivate pathogens for humans and plants for safe disposal.

1.2. Necessary treatments for the landspreading of sewage sludge

In recent years, many aerobic biotechnologies, such as bioremediation through composting, were developed for treating SS and soils (Ahadi et al., 2020; Alves et al., 2019; Namkoong et al., 2002; Zorpas et al., 2014). The high decomposition efficiency of processes involving oxygen as a final electron acceptor is a strong incentive to use such solutions; however, it should be noted that, in terms of the general economy of water cycle management, anaerobic processes could be more suitable. Anaerobic systems, having virtually no ventilation costs, lower plant volumes, and being able to produce biogas, undoubtedly bring general economic benefits. Processes developed for biological stabilization to degrade the organic content of sludges can involve anaerobic digesters that operate on SS from domestic wastewater treatment plants and generate methane gas as a valuable by-product (NAS, 1996). In the Part 503 Sludge Rule, U.S. E.P.A. (US EPA, 1993) identifies, under the aspect of the pathogenic quality of sludge, anaerobic digestion (AD) as a “Class B” (crop and site restrictions required) process, or “Process to Significantly Reduce Pathogens”. The anaerobic metabolic pathways for cyanide degradation were also investigated, with necessary confirmations (Fallon, 1992). Although less than in aerobic processes (Keeney, 1984), the inorganic nitrogen content (nitrate and ammonium) is present in AD-treated SS along with organic nitrogen. This latter, not directly accessible to the crop, is gradually mineralized in the years following the first cultivation year (NAS, 1996). Low-contaminated environments have an anaerobic potential furthermore to remove trace organic contaminants. Technological solutions can be applied to enhance this potential by enhancing

the temperature or combining biological and physicochemical processes. Each sludge stabilization process induces a particular dissipation of contaminants, including their transformation and their association with various matter compartments of the considered matrix, interacting with the fate of contaminants after SS landspreading (Barret et al., 2012).

1.3. C_{10}-C_{40} hydrocarbons in sewage sludge: an urgent problem for landspreading

The presence of organic contaminants such as petroleum hydrocarbons in SS from municipal WWTPs could be due to combined rainwater sewerage systems to sewerage connections from industrial areas. Petroleum hydrocarbons, for their negative impact on the environment, are typically used as an indicator of stormwater quality, although, due to the analytical difficulties in their determination, other indicators are often preferred. The range of determining petroleum hydrocarbons is usually between C_{10} and C_{40} in terms of the length of carbon atoms chains, which results from the composition of products of crude oil processing (Badowska and Bąk, 2017). Petroleum hydrocarbons are mainly aliphatic hydrocarbons, defined as open-chain methane derivatives, which are both non-aromatic and non-cyclic organic compounds containing carbon and hydrogen, and can be subdivided into three structurally different groups: alkanes, alkenes, and alkynes (Stroud et al., 2007). The alkane fraction of petroleum hydrocarbons, ranging from C_1 (methane) to C_{40} (tetracontane) or more, occurs as straight-chain or branched-chain compounds (Scullion, 2006). Alkanes are mainly saturated hydrocarbons, and diesel fuel, kerosene, gasoline, and heating oil are petrochemical products containing them (M. A. I. Khan et al., 2018). Alkenes are unsaturated hydrocarbons containing double C=C bonding, while alkynes have at least one triple C≡C bond (Stroud et al., 2007). Several aliphatic and polyaromatic hydrocarbons have origins in coal, petroleum, and their derived products. They have also been formed as combustion by-products. Aliphatic and polyaromatic hydrocarbons are sometimes highly toxic, highly environmentally persistent, low biodegradable, and high lipophile (Moreda et al., 1998) and fall between the persistent organic pollutants (Varjani and Upasani, 2017). The presence of hydrocarbon substances of natural origin with a high molecular weight and not at all

toxic, consisting of linear alkanes with an odd number (greater than 25) of carbon atoms, fundamental components of plant cuticular waxes must also be taken into account (Eglinton et al., 1962). The Carbon Preference Index (CPI) is the ratio of the sum of linear alkanes with an odd number of C atoms to the sum of linear alkanes with an even number of atoms of C (Kotianová et al., 2008). Hydrocarbons of vegetable origin are dominated mainly by odd congeners (CPI>1), while petrogenic hydrocarbons occur equally with even and odd numbers of carbon atoms (CPI≈1). These non-volatile hydrocarbons are associated with atmospheric particulate and move in the troposphere during this phase (Bacci, 2015).

1.4. Degradation of C_{10}-C_{40} hydrocarbons during anaerobic digestion of sewage sludge

Hydrocarbons are a quantitatively significant portion of organic matter on Earth, deriving almost entirely from biomass, either directly as natural products or indirectly as products of heat-driven transformation reactions. They have high C–H bond dissociation energies responsible for their low chemical reactivities. However, hydrocarbon degradation can occur under both aerobic and anaerobic conditions (Boll et al., 2020). The latter type has lower removal performance and speed than aerobic processes, where molecular oxygen is the primary electron acceptor, but, in turn, has undoubted economic advantages. Very often, the stabilization treatments of SS are focused on anaerobic processes. In aerobic organisms, the low chemical reactivity of hydrocarbons is always overcome by using highly reactive metal-bound oxygen species for the initial reactions. Instead, under anaerobic conditions, hydrocarbon degradation processes occur through anaerobic hydrocarbon-degrading bacteria, so-called “intra-aerobic” organisms. These last appear to produce O_2, or equivalent chemically reactive molecules, from anaerobic electron acceptors, such as chlorate or even nitrate or nitrite, in enough quantity to fuel standard mono- or dioxygenases for hydrocarbon degradation, even if it does not suffice for aerobic respiration (Boll et al., 2020). Anaerobic microorganisms degrading n-alkanes have substrate specificity for the chain length range of usable n-alkanes. Many of them have been successfully enriched and isolated from various habitats. Both bacteria and archaea may

be responsible for the initial activation of the hydrocarbon substrate as well as for consuming the reducing equivalents produced by their respective syntrophic partners. Catabolic pathways differ depending on the chemical nature of the initial activation product (Boll et al., 2020).

In SS from municipal wastewater, consisting of a highly complex solid substrate in aqueous suspension, the hydrolysis of macromolecules has been identified as the limiting phase of the degradation process (Angelidaki and Sanders, 2004; Morgenroth et al., 2002; Ramirez et al., 2009; Vavilin et al., 2008; Yasui et al., 2008). From experimental data, it has been observed that sludge biodegradation comprises two stages. In the first stage, the most easily hydrolyzable components are biodegraded, while the worst hydrolyzable fraction is only subsequently biodegraded (Jimenez et al., 2014). It is also evident that during AD, there is different behavior between indigenous hydrocarbons already present in the sludge and those eventually added. The hydrocarbons already present in the sludge bulk, in conditions of restricted accessibility, are biodegraded with greater difficulty than the added, more easily accessible hydrocarbons (Aemig et al., 2016). Microorganisms that anaerobically degrade hydrocarbons could be divided into at least two groups. The first group concerns the facultative anaerobic microorganisms, such as nitrate, iron, and manganese reducers, while the second consists of obligate anaerobic microorganisms, such as sulfate-reducers. Studies on the anaerobic oxidation of hydrocarbons by pure bacterial cultures have highlighted the bacterial ability to conduct this process in particular predetermined conditions, such as reducing conditions for sulfate, nitrate, iron, or manganese. Although the two groups have been identified, their specific roles in the biodegradation of hydrocarbons to methane are still being characterized (Boll et al., 2020). It has also been estimated that downstream of residence times of the order of months, a reduction in hydrocarbon concentrations by AD of about 15÷20% can be observed (Grishchenkov et al., 2000; Scherr et al., 2012). Several studies explored methods for optimizing the degradation of hydrocarbons in SS during AD with very diversified approaches. Some have focused on modifying the weight ratios of carbon and nitrogen in biodegradable organic matter. It is known that to have correct sustenance of metabolic processes, the C:N:P ratio in the municipal wastewater entering WWTPs must be approximately

100:5:1 for aerobic treatments and 250:5:1 for anaerobic treatments. The yield of biogas from any substrate strongly depends on the C:N ratio in the substrate, as well as on solid concentration, pH, and temperature, and can be increased by adjusting the C:N ratio (up to approximately 25:1) via the addition in co-digestion of organic matter with high biodegradability (Janajreh et al., 2020). Others have focused on the pre-treatment of sludge in AD with alkaline substances or ultrasounds to promote biogas production through the increase in chemical demand for soluble oxygen linked to the increase in the availability of the organic substance caused by cellular disintegration triggered by these pre-treatments (Joo et al., 2015). Concerning the degradation of hydrocarbons in the soil, it should be emphasized that adding sludge to the soil would increase the biological activity of the soil and the degradation of the hydrocarbons contained therein (Liu, 1980; Varanka et al., 1976).

It is also essential to underline the bioavailability of these contaminants in living systems. The key process of removing hydrocarbons in soils is microbial biodegradation, controlled by the physical and chemical properties of hydrocarbons, environmental conditions, bioavailability, and the presence of catabolically active microbes. The biodegradation of aliphatic hydrocarbons occurs through specialized absorption degradation mechanisms typical of the indigenous microbial community. However, significant concentrations of aliphatic compounds persist in soils due to their interaction with soil components, with consequent limitation of bioavailability. Natural organic matter (NOM), a heterogeneous mixture of organic compounds and reactive functional groups with acid-base properties, has high complexation capacities and is among the principal constituents of the soil and SS solid matrix (Yao et al., 2022). The high lipophilicity of aliphatic hydrocarbons causes a high interaction between them and the NOM in soil and SS. Remediation techniques try to improve the removal of aliphatic hydrocarbons from the soil through changes to the microbial community, enhanced biostimulation, and increased bioavailability through amendments (Stroud et al., 2007). The transfer rate of petroleum contaminants from a medium into microorganisms, the partitioning behavior of contaminants from water into the soil NOM, and the influence of the dissolved organic matter (DOM) on the contaminant level in the water and soil-water system are all relevant factors. It

has been proven that there is a correlation between the bioavailability of petroleum hydrocarbons, the content of soil NOM, and the level of DOM in soil-water systems. The content of soil NOM affects the degrading rate of hydrocarbons significantly. The hydrocarbon degradation rates in the water phase were found to be enhanced by the added level of DOM. A positive correlation exists between the contaminant bioavailability and the contaminant level in water as impacted by the content of NOM in soil and the level of DOM in the water (Chen et al., 2019).

An aging phenomenon has been observed, for which the bioavailability and extractability of pollutants decrease as the contact time between soil and pollutants increases. Aging has been described in studies of bacteria, earthworms, and other organisms. It has been shown that the bioavailable pollutant fraction can vary between organisms, hence the problem of choosing which organisms to mimic using an extraction procedure to assess bioavailability by chemical means (Ramadass et al., 2018; Reid et al., 2000). Three processes may occur for chemicals to be detained in soil: weathering, biodegradation, and sequestration. An action where the physical, chemical, and biological processes affect the fraction of hydrocarbons remaining in soil was defined as weathering (Loehr et al., 2001). Weathering leads to decreased bioavailability of pollutants to microorganisms through enhancing sorption of the contaminants into soil solid matrix and their diffusion into soil pores with increased contact time (Gao, 2009). Long-term oil-contaminated soil consists mainly of recalcitrant compounds of high molecular weight hydrocarbons formed in the soil after biotic and abiotic weathering of lighter hydrocarbons, which are more difficult to degrade by indigenous microorganisms. Weathered petroleum hydrocarbons are less or non-bioavailable (Ramadass et al., 2018). Furthermore, weathered hydrocarbons exhibit significant qualitative and quantitative differences compared to initial petroleum products (Jiang et al., 2016). Therefore, in oil-contaminated soils, it is necessary to evaluate the efficiency of hydrocarbon-degrading microorganisms in the context of weathered hydrocarbons (Ramadass et al., 2018).

Current approaches in studying hydrocarbon biodegradation in oil-contaminated soils suffer from several limitations, including polluting characteristics, poor capabilities of microbial communities in the field, lower bioavailability of pollutants, and growing conditions. The conditions of each oil-polluted site are unique, and a full site assessment is required to identify the most appropriate remediation method for a specific site (Varjani and Upasani, 2017). The non-bioavailable fractions of the compounds can be recovered by exhaustive extraction with organic solvents (Alexander, 2000; Gao, 2009; Kelsey et al., 1997). Attempts have been made to describe the sequestration process in various ways (Chung and Alexander, 1999; Gao, 2009), such as slow diffusion and entrapment with tiny pores in soil aggregates or the formation of strong chemical bonds with soil constituents (Chung and Alexander, 2002; Gao, 2009; Gevao et al., 2001; Megharaj et al., 2011; Palomo and Bhandari, 2006; Pignatello and Xing, 1996). It has been proven that interactions between compounds and soil are influenced by many factors, such as clay constituents of soils and organic matter (Gao, 2009; Reid et al., 2000). A complete understanding of the influence of soil properties on aging has not yet been achieved to assess the bioavailability of organic compounds in soil (Gao, 2009). To investigate the impact of aliphatic hydrocarbons carried by SS in the soil matrix, it is, therefore, necessary to deepen all the components playing a role in the interactions between petroleum hydrocarbons and soil, with particular attention to any contributions to these interactions by complex matrices as SS.

1.5. Chemical conditioning of sewage sludge: does it impact hydrocarbon quantification?

Water is the primary constituent of SS. The humidity of sludge can range from values of 99% for very diluted sludge, as in a biological process with activated sludge, up to 88% for some chemical precipitation processes, all values such to still allow easy hydraulic transport of the sludge. With the same dry matter weight, a variation in the humidity of 1% determines a weight variation of 50% SS. For disposal in agriculture, humidity values of 80-85% are generally sufficient, but for disposal in landfills, the humidity must drop to at least 75-80% through a good dewatering process. Dewatering operations reducing the quantity of SS sent for final disposal are therefore essential. Since SS is a

concentrated suspension of colloidal particles, more or less organized in flaky structures with a surface charge to establish a series of mutual repulsive forces, determining the stability of the suspension and such to bind large quantities of water through electrostatic forces or the incorporation in the flocked matrices, chemical conditioning of SS is very useful for dewatering purposes. SS chemical conditioning aims to reduce the repulsive forces between the particles (destabilization of the colloidal suspension) and, by increasing the probability of collision between the previously destabilized solids, to form larger and more resistant flocs. Organic polyelectrolytes are the primary type of organic additives used for sludge conditioning. They are polymers that dissociate in water into a large number of negative (cationic polyelectrolytes) or positive (anionic polyelectrolytes) ions, which increase the conductivity of the solution. They are monomers joined by polymerization in long linear or branched chains with functional groups placed periodically along the chain (the chains can count up to 2 or 3 million monomers). The functional group can have a negative (anionic polyelectrolytes), positive (cationic polyelectrolytes), or globally neutral charge (non-ionic polyelectrolytes) (Marinetti, 2007). Chemical conditioning in WWTPs, moreover, is not always carried out downstream of the AD processes of SS, given the need to increase the sedimentation of solids to optimize the costs of SS handling and storage within the sludge line, as well as for settling optimization reasons within the sewage line.

The strong interaction of lipophilic substances, such as hydrocarbons, with NOM in the solid SS matrix, could be further enhanced by the compacting effect due to conditioning chemicals, modifying the fate of hydrocarbons in SS by probably decreasing their mobility and bioavailability.

1.6. Analytical issues with the determination of C_{10}-C_{40} hydrocarbon concentration in sewage sludge

The expression of a given concentration value occurs downstream of a specific analytical chemistry procedure performed in a laboratory. The determination of C_{10}-C_{40} hydrocarbon concentration in SS, both to quantitatively characterize this data, and to verify the degradation of the substance within the matrix by a specific treatment process, gives rise to a series of problems to be addressed, which cannot always be uniquely overcome. A limited number of publications have dealt with the subject exclusively and exhaustively.

In 2007, a working group made up of ISPRA, ARPA/APPA, ISS, CNR-IRSA, and ex-ICRAM, defined as the "Hydrocarbons Working Group", identified the following operational definition of the analytical parameter Total Hydrocarbons: "The set of compounds which, after the extraction and purification processes reported in the method, can be detected by gas chromatography with flame ionization detector (GC/FID), on a non-polar capillary column with retention times between those of n-decane ($C_{10}H_{22}$) and n-tetracontane ($C_{40}H_{82}$)", a definition that also extends to the heavy hydrocarbon parameter C>12 present in Table 1, Annex 5, Title V, 4th Part of Legislative Decree 152/2006, starting however from the n-dodecane compound ($C_{12}H_{26}$) (Ispra, 2011).

For "method", the definition refers, depending on the matrix, to one of the two methods: ISO16703:2004 [Soil quality – Determination of content of hydrocarbon in the range C_{10} to C_{40} by gas-chromatography (> 100 mg/kg)] and UNI-EN14039:2005 [Waste characterization – Determination of the hydrocarbon content in the range between C_{10} and C_{40} by gas chromatography (> 100 mg/kg)] (BS EN ISO, 2004; ISO, 2011), however suitable for two different and much less complex matrices (i.e., soils and solid waste) than SS. The methods provide stable and repeatable results for soils, waste, sediments, and waters, which is not valid for SS. The inclusion, due to the "Genoa decree" in 2018, of the C_{10}-C_{40} hydrocarbon parameter attributable to the ISPRA definition of 2007, among the parameters to be monitored to spread stabilized SS on agricultural soil; however,

it introduces the need also to quantify this parameter in SS, a very complex matrix as it is solid, suspended and dissolved in aqueous solution. The two standard methods mentioned above remain an analytical reference, but they have not been validated yet for SS. In any case, it is possible to identify in the literature an entire line of research, in continuous evolution, dedicated to the analytical question of the hydrocarbon content in SS and the validation of a definitive analytical method.

The analysis of saturated hydrocarbons, as well as polycyclic aromatic hydrocarbons (PAHs), present in traces within the SS, constitutes a sort of "analytical challenge", becoming necessary to select, adapt and develop the appropriate methods for sampling, storage, extraction, clean-up, and isolation, as well as identifying a good technique for identification and quantification. Many authors have described methods for analyzing trace substances and discussed the problems associated with their execution. Unlike soil and waste, SS acts as a "trap" for petroleum-derived compounds from different sources and is often regarded as one of the worst environmental matrices to be extracted. It can contain a large variety of pollutants in addition to the analytes of interest and a very high moisture content, which gives rise to interference and interactions, both with the extraction and clean-up methods (Pavlova and Ivanova, 2003).

The main problems to be faced in the quantitative determination of the concentration of C_{10}-C_{40} hydrocarbons in SS can therefore be summarized in the search for the most suitable methodologies and strategies for (1) sampling of the sludge; (2) conservation of the sludge sample; (3) extraction from the sludge sample of a fraction as close as possible to the nature of C_{10}-C_{40} hydrocarbons, mostly saturated and a-polar; (4) purification (clean-up) of the extract from all the substances taken away by affinity with the extraction method, but alien to the aliphatic nature of C_{10}-C_{40}, such as polar, hydrolyzed, and carboxylated substances; (5) isolation of the substance to be measured.

The sample obtained from all the previous steps must then be subjected to elution to obtain a gas-chromatographic spectrum for quantifying the components of the C_{10}-C_{40} mixture in the original

sample. Further problems arise in defining the operating parameters adopted in the chromatographic analysis (Badowska and Bąk, 2017).

Regardless of the detection technology, whether by flame ionization (GC-FID) or by mass spectrometry (GC-MS), there is also an underlying problem that characterizes the gas chromatographic determination of hydrocarbon mixtures. The chromatograms of samples containing hydrocarbons tend to assume a hump shape caused by the presence of complex mixtures of unresolved hydrocarbons, called Unresolved Complex Mixtures (UCM). UCMs are composed of several types of recalcitrant aliphatic and aromatic petroleum hydrocarbons, which tend to be co-eluted and give overlapping peaks. The large and pronounced baseline elevation characterizing a UCM consists mainly of linear hydrocarbon chains having one or more branches; it has been observed that the humped shape increases as the degradation state of the UCM mixture increases (Gough and Rowland, 1990; Moreda et al., 1998; Peters et al., 2005).

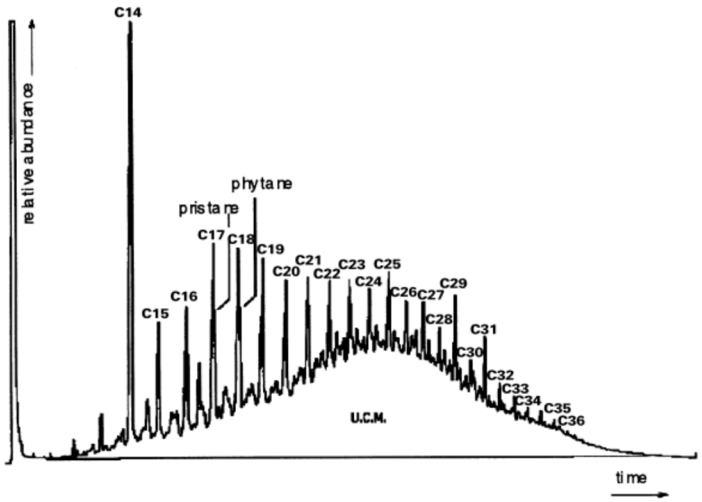

Figure 1.1. Gas chromatographic determination of an unresolved complex mixture of hydrocarbons (Moreda et al., 1998).

Some studies have considered various approaches to face the co-elution problem, comparing different types of detection, such as GC-MS and GC-FID with UV fluorescence systems or with Fourier Transform Infrared absorption spectrometers (FT-IR), obtaining benefits in terms of speed of determination, but not in terms of resolving capacity (Pavlova and Ivanova, 2003).

The system's peak capacity should be much higher than a given mixture's number of components. Since the number of hydrocarbon isomers increases exponentially with the number of carbon atoms, gas chromatography shows all its limitations when dealing with substances containing more than nine carbon atoms, making it impossible for detailed analyses of samples with a range of atoms of carbon from C_8 to C_{15} and impossible for samples from C_{15}-C_{30} and up. At the turn of the 80s and 90s, multidimensional chromatography (MDGC) was proposed to overcome these limitations, in which separations, called bi- or multidimensional, of all or part of the components of the sample are carried out through repeated elutions in two or multiple chromatographic columns characterized by different separation mechanisms (Vendeuvre et al., 2007). A primary column that separates compounds based on volatility is typically coupled to a second, shorter column that separates by polarity. The two columns are connected by a modulator, a device trapping, focusing, and re-injecting the peaks eluted from the first column into the second. Each peak that emerges from the first column, which may consist of a certain number of co-elution peaks, is further separated in the second column (Dallüge et al., 2003).

Given the complex problem underlying the quantitative determination of the concentration of C_{10}-C_{40} hydrocarbons in SS using gas-chromatographic methods, it is also interesting to investigate such a determination through a gravimetric method.

With a gravimetric method, the problem of the co-elution of the components of the hydrocarbon mixture is eliminated. The method verifies the weight of a given quantity of substance extracted and purified from the starting sample compared to the weight of the starting sample. The opportunity of determining a qualitative spectrum of the extracted mixture is lost. However, on the other hand, it is generally possible to have fair repeatability of the overall quantitative measure and, e.g., to quantify a possible degradation of hydrocarbons by a digestion process of the sludge, comparing measurements on the sample before and after the degradation process.

The IRSA CNR Q 64 method, notebook no. 21, was expressly written for the gravimetric determination of oils and fats in SS (CNR, 1983). However, the method lacks drafting dating back to 1988, which is why it must be necessarily updated, as, e.g., it recommends using Freon gas as an organic solvent operating the biphasic extraction, which has been banned for environmental impact reasons. An acceptable alternative, in terms of eco-sustainability, consists of the US method EPA 3540C, a method intended to extract semi-volatile or non-volatile organic compounds from solids such as soils, sludge, and waste (US EPA, 1996).

It can be concluded that the problems concerning the determination of the concentration of C_{10}-C_{40} hydrocarbons contained in a complex matrix such as a SS made the research wide open to investigations and insights, requiring the development of a definitive and validated measurement method that allows a unique quantification.

Among the main objectives of this PhD work is developing a reliable, cheap, and reproducible method, which we have addressed, developing a methodology involving a gravimetric determination and a GC-MS measurement simultaneously. The results gave rise to the quantification of two different parameters, able to better define the mixture of aliphatic hydrocarbon content in each SS sample, one evaluated gravimetrically, i.e., total hydrocarbons (TH), the other by GC-MS analysis, i.e., C_{10}-C_{40} hydrocarbons (C_{10}-C_{40}).

The developed methods were also applied to address the other critical issue examined in the PhD research, i.e., the degradation of aliphatic hydrocarbons during the AD of SS.

2. DETERMINATION OF C_{10}-C_{40} HYDROCARBON CONCENTRATIONS IN SEWAGE SLUDGE

The development of a methodology for measuring C_{10}-C_{40} hydrocarbon concentrations in SS was the first topic addressed during the PhD research project, given the absence of a standardized methodology for a complex matrix such as SS. The research progressed in successive analytical steps, described below.

1) The first round of experiments involved a series of initial considerations, including the choice of individual stages to divide the analytic process and to be addressed individually. We started with the deepening of three extraction methods, using a simple clean-up procedure and a single solvent solution. The first results from this experimental step have allowed us to direct the work of the subsequent steps profitably. The gravimetric determination kept the same approach developed in this stage until the final version about two years later.

2) The second round of the experimental sections of the PhD research project deepened the methodology for determining the C_{10}-C_{40} hydrocarbon concentrations in SS samples. New organic solvent mixtures were tested with all the previously investigated measurement procedures, and the clean-up stage was definitively structured. The most critical apport due to this analytical research step was verifying the results through GC-MS detection.

3) This section was dedicated to the definitive development of the measurement method, moving from a gravimetric method to a mixed gravimetric/gas chromatographic method. The definition of two quantitative parameters, TH and C_{10}-C_{40}, was made. They represent respectively the whole hydrocarbon mixture extracted and purified with the previously optimized methods and determined with gravimetry (TH), as well as the fraction of this mixture falling only in the range of aliphatic substances between n-decane ($C_{10}H_{22}$) and n-tetracontane ($C_{40}H_{82}$), determined thanks to the GC-MS (C_{10}-C_{40}).

2.1. Materials and Methods

2.1.1. Delineation of methodologic procedures

2.1.1.1. Sewage sludge and samples preparation

For the experiments of the two research mentioned above lines, various SS samples were used from two WWTPs in southern Italy and taken from different points of the sludge lines. From the first WWTP, SS was taken downstream of chemical conditioning and dewatering, with which the measurement of the concentration of C_{10}-C_{40} hydrocarbons through a gravimetric method was optimized. In contrast, from a second WWTP, pre-thickened SS was used to evaluate the effects of AD on hydrocarbon degradation.

For the tests to optimize the measurement of the concentration of C_{10}-C_{40} hydrocarbons, chemically post-conditioned and dewatered SS samples were used in the dry state. The raw SS sample was hermetically stored in a fridge at about 4 °C. Before being used, the sample was subjected to quartering to make it as homogeneous and significant as possible and was then dried in a thermostatic refrigerator at about 40 °C for six days. At the end of this period, the sample was weighed through a precision scale and returned to the device. In the following days, the sample was weighed again until it was verified that the weight had reached a constant value, effectively losing the free water content. The sample thus obtained was subjected to a manual particle size reduction before being analyzed.

To evaluate the effects of the degradation of hydrocarbons due to AD, samples of pre-thickened sludge, raw and then digested, were used. The raw SS sample was stored in a hermetically sealed container and placed inside a fridge at a temperature of about 4 °C. Before being used, it was stirred to facilitate the homogenization of the content, mainly liquid. AD was performed in small reactors made with laboratory bottles (Simax, Czech Republic) in borosilicate glass with a total capacity of 500 mL. In each reactor, a quantity of 250 mL of pre-thickened sludge was inserted, and a cap equipped with two sampling ports was used to facilitate the withdrawal of the gas produced during

AD and of liquid sludge, respectively. A STAR 3400 gas chromatograph from Varian (California, USA) was used to measure the biogas composition. The AD lasted 30 days and was carried out in mesophilic conditions (T ≈ 35 °C), guaranteed by a thermostatic bath. After AD, the sample was centrifuged and naturally dried under a hood before being subjected to subsequent analytical operations.

2.1.1.2. Solids content determination

The Total Solids (TS) and Volatile Solids (VS) contents of the SS samples were determined by following the APHA-AWWA-WEF Standard Method 2450 (APHA et al., 1999) through the use of a laboratory oven (Argolab TCN 115, Italy), a muffle furnace (Asal ZB/1, Italy), and a precision scale (Sartorius 1801, Germany).

2.1.1.3. Hydrocarbons extraction

It is customary to classify hydrocarbons into light hydrocarbons, with less than 12 carbon atoms, and heavy hydrocarbons, with more than 12 carbon atoms. The extraction techniques proposed by the Environmental Protection Agency (EPA) are different for the two groups of hydrocarbons: for hydrocarbons up to C_{10}, the EPA method 8015b suggests the use of headspace techniques (5021), distillation under vacuum (5032) or "purge and trap" (5035 and 5030b), while for those with more carbon atoms, less volatile, extraction procedures with solvent using a Soxhlet extractor (EPA 3540c and 3541 methods), under pressure (EPA 3545 method), with ultrasound (EPA 3550b method), or in the supercritical phase (EPA 3560 method) (Ispra, 2011), are suggested. Since light hydrocarbons are characterized by high volatility, their permanence in the soil matrix is considered unlikely (Ispra, 2011), and therefore, during the experiments, attention was paid to the analysis of the techniques for the extraction of heavy hydrocarbons (C_{10}-C_{40}). The techniques were performed based on the procedures suggested by the EPA and on the data available in the literature (Grimalt et al., 1984; Kuster et al., 2006; Pavlova and Ivanova, 2003; Suciu et al., 2015), suitably varying parameters such as solvents, reagents, extraction times and temperatures, to optimize and identify the best conditions

for achieving the set purpose. For the final determination, a gravimetric method was initially set based on the IRSA CNR Q 64 method, notebook no. 21, which allows the extraction of hydrocarbons using Freon-113 (CNR, 1983). This solvent is no longer used due to its dangerousness, classified as a toxic substance to the environment.

Three extraction methods were compared:

- Soxhlet extraction;
- Cold solid-liquid extraction;
- Microwave extraction.

The solvent used for all the extraction types for these experimental sessions was a mixture of toluene/methanol (in a 10:1 volumetric ratio), given the high availability in the laboratory of its components.

2.1.1.3.a. Soxhlet extraction

The Soxhlet extraction procedure, recognized as the EPA 3540c method, is suitable for extracting non-volatile and semi-volatile organic substances from solid matrices such as soils, sludge, and waste (US EPA, 1996). The Soxhlet extractor is a system capable of continuously extracting the components of interest from a solid matrix using a volatile solvent or a mixture of volatile solvents. This method ensures close contact between the extraction solvent and the matrix to be analyzed (Ispra, 2011). The Soxhlet equipment comprises three main parts: a flask with an emery neck, the extractor, and the condenser. The method is applicable for extracting non-volatile and semi-volatile organic substances from solid matrices such as soils, sludge, and solid waste, using different organic solvents, pure or mixed. The solid sample is positioned inside a thimble filter in cellulose fiber permeable to solvents and placed in the extractor's central section. The extractor consists of an upper section, the actual extraction chamber, and a lower chamber that allows continuity with the underlying balloon. The upper chamber communicates with the lower one through a duct, allowing the passage of the

vaporized solvent and a siphon for discharging the extract. The balloon is inserted inside an electric heating mantle, which allows for maintaining a controlled heat flow.

Inside the condenser, connected to the upper chamber, cooling water flows in counterflow, which allows the solvent that evaporates from the flask to condense directly into the extraction chamber, giving rise to a continuous recirculation of "clean" solvent. The solvent, positioned inside the balloon flask, is brought to a boil thanks to the heating mantle, reaches the extractor through the duct mentioned above, and then the condenser, precipitating inside the thimble containing the sample; it then passes through the walls of the filter, taking the solute with it. The extraction chamber fills with the solvent/analyte solution until it reaches a volume such as to trigger the siphon, which allows the solution to be transported and stored in the flask. At this point, the solvent is heated again, repeating the abovementioned cycle, and concentrating inside the flask the solute (analyte), not participating in the solvent evaporation and condensation cycles.

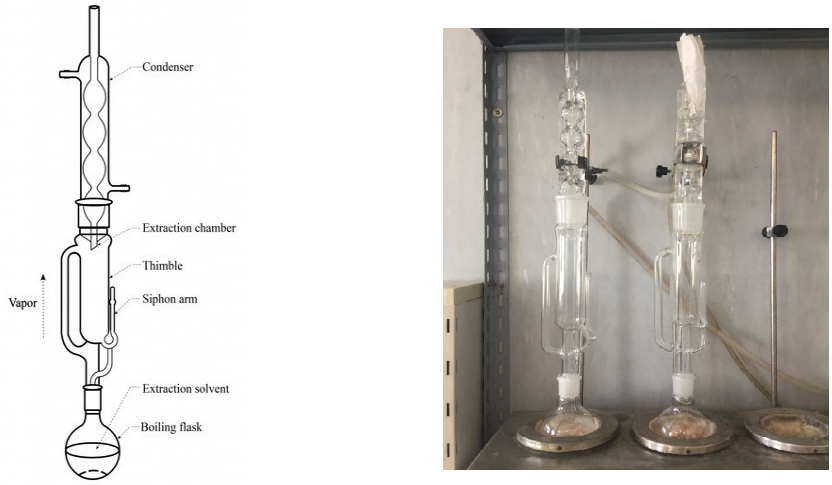

Figure 2.1. Soxhlet extractor scheme (left); Soxhlet extractors used (right).

The operating conditions used in the experiments were:

- Solid sample: SS sample after chemical conditioning and dewatering (about 15 g if raw, or about 5 g if dried);
- Flask: 250 mL ground-neck borosilicate glass flask;
- Solvent: 200 mL of a mixture of Toluene/Methanol – $C_6H_5CH_3/CH_3OH$ (10:1 v:v);

- Duration of extraction: 16 hours;
- Thimbles: in cellulose fiber (Whatman) with an external diameter of 32 mm, an internal diameter of 30 mm, and a length of 80 mm;
- Extraction temperature: about 65 °C.

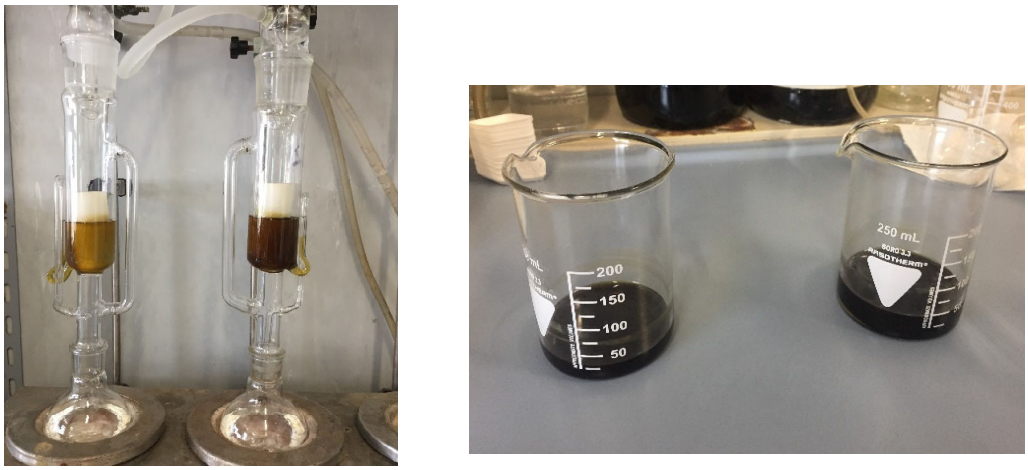

Figure 2.2. Soxhlet extractor in operation (left); Soxhlet post-extraction samples (right).

The advantages deriving from the use of this method consist of:

- low activation costs;
- most representative sample to be analyzed (15-30 g);
- no need to manipulate the sample loaded into the thimble;
- efficient extractions.

On the other hand, the disadvantages consist of:

- long extraction times (up to 16-24 hours);
- the need for a high volume of solvent (200-300 ml per sample);
- mandatory evaporation of the solvent from the extract;
- possible loss of volatile substances;
- possible degradation of thermolabile substances.

2.1.1.3.b. Cold solid-liquid extraction

Cold solid-liquid extraction, certified as ISO/TR11046 method, is an extraction process through which it is possible to separate the analytes of interest from the solid matrix in which it is contained without increasing the temperature of the system. In this way, it is possible to operate quickly without the risk of altering the thermolabile compounds present. In our case, the goal was to extract the hydrocarbons contained in a SS sample (solid matrix) placed in a Falcon tube with a certain amount of liquid organic solvent. The extraction process was divided into the following stages (Naviglio and Ferrara, 2008):

1) the solvent, added to the SS sample, penetrates through the pores of the solid matrix, occupying all accessible spaces;
2) the analytes contained in the matrix solubilize in the solvent by chemical affinity, giving rise in the solid to a solution with a specific concentration of hydrocarbons;
3) the concentrated solution inside the solid and the diluted one outside generate a solute diffusion towards the outside. This stage lasts until the solute concentration gradient between the inside and outside of the solid matrix is canceled;
4) once the equilibrium is reached, the solution enriched in extracted analytes is ready to be subjected to a phase separation from the now-exhausted solid matrix (by centrifugation).

It should be noted that the recovery of the analytes from the solvent is not total, as a certain part of it remains in the matrix (depending on the imbibition ratio between matrix and solvent), so it is necessary to repeat the extraction cycle several times by adding new solvent. In our case, it was considered to obtain good exhaustion of the solid matrix after repeating the cycle three times. The characteristic parameters of solid-liquid extraction are many: degree of crushing of the solid matrix, size and physical state of the particles of the solid matrix, quantitative solvent/matrix ratio, contact times, penetration mechanism of the solvent into the particles of the material being extracted, process pressure and temperature. In this type of extraction, it is considered essential to use a Vortex stirring device (Reax 2000, Heidolph) which, by increasing the turbulence in the matrix-solvent system,

allows the reduction of the thickness of the diffusion boundary layer, makes a more uniform the concentration of analytes in the solution and optimizes the exploitation of the analyte-solvent contact surfaces, also preventing sedimentation of the solid.

In our experiments, about 1-2 g of the SS sample (post chemical conditioning and dewatering), raw or dried, was placed inside a 50 mL Falcon tube and mixed with 20 mL of the toluene/methanol solvent mixture (10:1 v:v). The system was stirred by Vortex for 15 min.

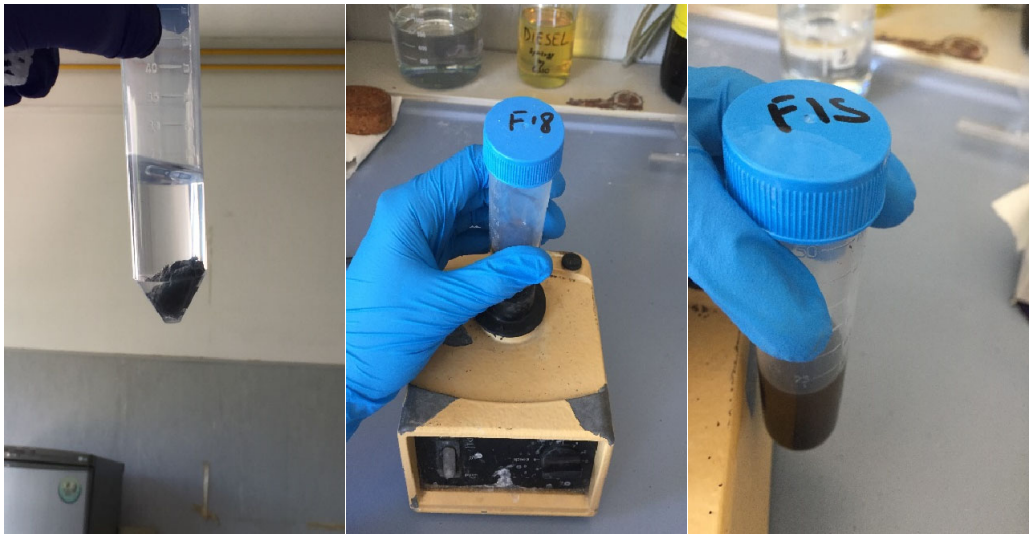

Figure 2.3. Sample before (left), during (center), and after cold extraction (right).

The advantages of this extraction method are:

- reduction of extraction times;
- reduction of the amount of solvent used.

The disadvantages, on the other hand, consist of:

- the possibility of incomplete extractions;
- need to separate the solvent from the rest of the solid matrix;
- reduced quantity of analyzed sample;
- overheating of the vortex apparatus after four consecutive extractions.

2.1.1.3.c. Microwave-assisted extraction

Microwave-assisted extraction (MWAE), recognized as EPA 3546 method, is suitable for extracting insoluble or poorly soluble organic compounds in water from soils, sediments, sludge, or solid waste (US EPA, 2007). This methodology is based on the ability of some materials or solutions to heat up if immersed in a high-frequency electromagnetic field. Microwaves are non-ionizing electromagnetic waves with a wavelength between 1 mm (ν = 300 GHz) and 1 m (ν = 300 MHz), located in the area of the spectrum between the infrared and radio wave frequencies.

The sample absorbs the microwave energy through two mechanisms (Frecentese, 2014):

1) Ion conduction, a conductive migration of the ions present in the solution due to an electromagnetic field, increases its temperature and causes an increase in the kinetic energy of the ions;
2) Rotation of the dipoles, the alignment of the molecules that have non-zero dipole moments, caused by a magnetic field. Many molecular species, such as those of the solvents examined, have polarity; like electric dipoles, they have one end with a positive electric charge and the other with a negative electric charge. For this reason, they are sensitive to an alternating electric field, which, by continuously changing its direction, induces the molecules to repeatedly modify their orientation based on the frequency of the field. The result is a molecular excitation, transferring the motion to the rest of the substance through shocks, thus obtaining its heating.

When using this methodology, attention must be paid to the power of the microwaves, which must be such as to maximize extraction efficiency (concerning extraction times and yield) without, however, causing thermal degradation of the compounds present in the sample. The main parameters to consider are, therefore: power, temperature, pressure, extraction time, and type of solvent. Two types of microwave extractors are currently marketed: closed and open-vessel systems. Particular attention should be paid to explosion problems when using a closed system. Open systems are generally simple and safe. In our case, a closed system microwave oven (Start D, Milestone) was

used, and the SS samples, with or without the use of cellulose fiber thimbles, were loaded, together with the solvent, into special polytetrafluoroethylene (PTFE) cylinders, with a volume of about 65 mL, screwed into their respective reinforced ceramic supports to form sealed capsules. Thanks to a thermal probe inserted in one of the capsules containing the sample, the parameters were monitored on display. Thus, the only parameters to be optimized will be the type of solvent, the power to be applied, and the extraction time.

Unlike other extraction methods, all types of SS samples were used for MWAE. The SS samples in the capsule were in contact with 20 mL of solvent, a mixture of toluene, and methanol (10:1 v:v). Four test cycles were conducted to optimize the extraction at a constant temperature of 85 °C, which was monitored by the thermal probe. The first and second cycles were performed with an extraction time of 30 and 60 min, respectively, using SS samples after chemical conditioning and dewatering (as it is and dried). The third cycle was always carried out with an extraction time of 60 min, but by adding a cellulose fiber thimble inside the PTFE cylinder, containing the same type of SS sample as the first two cycles. As regards the last cycle, the first extraction of 60 min was carried out using only the solvent to wash the thimbles and was followed by the actual extraction of the same duration starting from SS samples, both pre-thickened (as it is and digested) and post-chemical conditioning and dewatering (as it is and dried).

The advantages deriving from the use of this technique are:

- a considerable reduction in processing time;
- reduction in the volumes of solvent used;
- local heating of solvent and sample more efficient and faster than external radiative heating;
- less chance of contamination;
- the possibility of carrying out up to twelve parallel extractions;
- reduction of heat losses;
- the precision of the thermal control action.

The disadvantages, on the other hand, consist of:

- the possibility of incomplete extractions;
- need to separate the solvent from the rest of the matrix;
- possible combustion of the sample;
- reduced quantity of sample analyzed (less significance).

Figure 2.4. Microwave extractor (left); PTFE cylinder and supports (center); closed capsule (right)

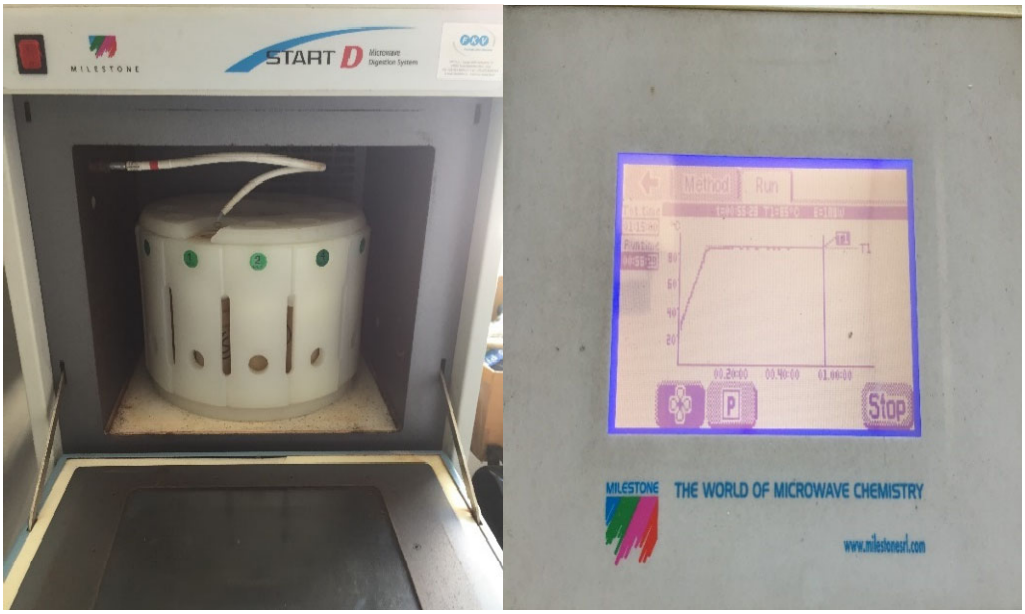

Figure 2.5. Capsules in the supports inserted in the MWAE (left); display with thermal trend (right)

2.1.1.4. Phase separation

At the end of the various extraction methods, liquid-solid phases were separated using a centrifuge treatment (IEC, Centra GP8R). The samples were centrifuged in 50 mL Falcon tubes at a speed of 4600 rpm and a temperature of 25 °C for 20 min. This way, the liquid part containing the extracted substances was separated from the solid part of the SS sample. Subsequently, the supernatant was transferred to a clean beaker through a pipette and subjected to the clean-up steps.

This operation was performed after all the cold extractions and the first two cycles of the MWAE. Instead of the extractions with Soxhlet and the last two cycles of the MWAE, the liquid-solid separation was guaranteed by the thimble in cellulose fiber which retains the solid component by passing only the liquid one then transferring it to a clean beaker.

2.1.1.5. Clean-up procedures

Procedures of fundamental importance allow the elimination of all those substances that can be extracted together with C_{10}-C_{40} hydrocarbons and can cause interference in the analysis process. A series of clean-up operations were performed to allow:

- water removal;
- removal of polar substances.

2.1.1.5.a. Water removal

The water was removed by adding anhydrous sodium sulfate powder (Na_2SO_4 – Carlo Erba, Italy) to the container with the extracted sample. It appears as a white, crystalline, and odorless powder and can absorb significant quantities of water when it comes into contact with it. The chemical reaction that develops involves the formation of sodium sulfate decahydrate, which, assuming a crystalline and no longer dusty appearance, is deposited on the bottom of the container.

$$\mathbf{Na_2SO_4\ (anhydrous) + H_2O \rightarrow Na_2SO_4 \cdot 10H_2O} \qquad (2.1)$$

Anhydrous sodium sulfate is added until, by shaking the container, the resuspension of decahydrate crystallized particles is observed.

2.1.1.5.b. Removal of polar substances

The clean-up of the polar substances was performed by elution in adsorption columns containing two different types of materials:

- Silica gel;
- Florisil®.

Silica gel

Silica gel is a polymer of silicon dioxide treated with sulfuric acid, which has weakly acidic properties and is generally used for separating compounds with different polarities. The silica gel used for the experimentation (granulometry 70÷200 μm) was subjected to a 105 °C for 24 h conditioning, which allowed the activation of the silica gel, to deactivate which 10% of water is enough during its storage. Subsequently, a slurry was prepared, using a magnetic stirring mixer (MR 2002, Heidolph), with 50 g of activated silica gel and 100 mL of toluene/methanol solvent mixture (10:1 v:v). The prepared slurry was then inserted into columns of different sizes depending on the extraction method used.

Soxhlet extracted samples were cleaned-up using a glass column 300 mm long, with a 15 mm internal diameter, packed with silica gel slurry in a toluene/methanol mixture (10:1 v:v). To further eliminate any traces of water present in the extract, a layer of anhydrous sodium sulfate was added at the top of the column. After the column has been packed, the sample extracted from above is introduced. At the sample extraction's end, a solvent volume equal to the packed column is added to allow the still present analytes' passage and wash the adsorbent surface. The eluate, consisting of analyte mixed with solvent, is collected in a 250 mL glass flask placed under the column. The clean-up procedure for this extraction method allowed two samples to be treated simultaneously, with a total duration of approximately 4 hours.

Figure 2.6. Clean-up with double silica gel column.

For the clean-up of samples extracted through cold extraction and MWAE, characterized by lower quantities of solvent, glass columns 80 mm long and with an internal diameter of 13 mm were used, packed with the slurry of solvent and silica gel. The extracted sample was inserted, the adsorbent column was subsequently washed, and the eluate was collected in 250 mL glass flasks. The clean-up procedure for these extraction methods made it possible to filter five samples simultaneously, with a total duration of about an hour.

Figure 2.7. Glass column (left); triple clean-up (center); clean-up detail – color gradient (right).

Florisil

Another material used to clean up polar substances is Florisil (2 g), a magnesium silicate treated with sulfuric acid with acidic properties, which is widely used on a laboratory scale. Florisil cartridges (2 g), used for experimentation, do not need to be packaged or conditioned. Clean-up treatment with Florisil was used only for the samples extracted by Soxhlet. Initially, we proceeded directly with the above introduction of the extracted sample and with the eluate collection in a 250 mL glass flask positioned below the column. This configuration was abandoned due to the continuous clogging of the cartridges. To solve the problem and improve the purity of the eluate, Florisil cartridges were coupled with silica gel columns.

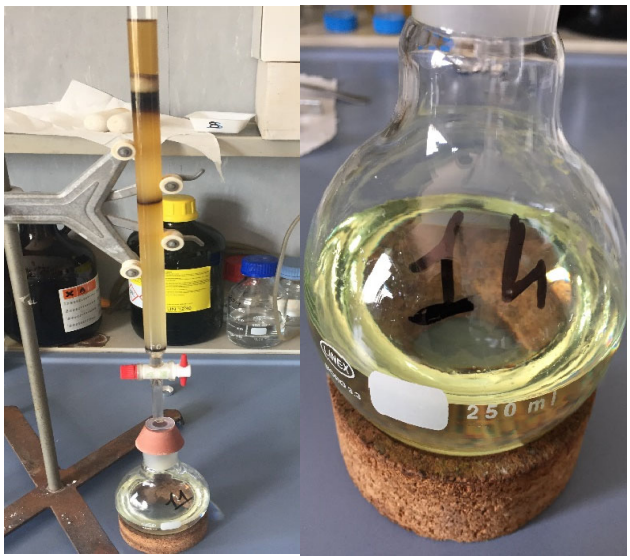

Figure 2.8. Clean-up with SiO_2 gel column + Florisil cartridge (left); post-clean-up sample (right).

2.1.1.6. Solvent removal

The sample, extracted and cleaned up, has a significant amount of solvent, which must be removed. For this purpose, the sample was concentrated up to a volume of about 5 mL in different ways:

- hood;
- distillation.

2.1.1.6.a. Hood

The sample was placed inside a hood with suction and carbon filters to absorb harmful and volatile substances. This operation allowed the removal of the excess solvent by natural evaporation at room temperature. However, this methodology was immediately abandoned for two main reasons:

1. quite long times, about 12-14 hours, for solvent removal;
2. impossibility of recovery of the removed solvent.

2.1.1.6.b. Distillation

The other method used to remove the excess solvent is to subject the sample to distillation. Distillation is a technique that allows the different components of a liquid mixture to be separated by exploiting their different boiling points. To use distillation, the substances to be distilled must be stable at the temperature required for vaporization. The procedure consists of solvent evaporation, followed by condensation and condensate (distillate) collection in a container different from the initial one. In our experiments, the sample, consisting of C_{10}-C_{40} hydrocarbons and the toluene/methanol mixture, was placed in a 250 mL glass flask and heated using an electrical heater to a temperature of about 65 °C. Given its high volatility, the solvent evaporates and precipitates through a condensation column before being collected in a 250 mL glass flask different from the initial one. In the latter, the concentrated sample remains to be subsequently analyzed. It was decided to use this method as it allows the solvent to be removed in a short time, about 45 min, and also allows the removed solvent to be recovered, which can thus be reused, for example, for clean-up operations.

2.1.1.7. Preparation of the extracted sample

The post-distillation sample, having a volume of about 5 mL, cannot be immediately analyzed, as it still contains a small amount of solvent. To be able to analyze it, it is necessary to perform the following operations:

- preparation and conditioning of the weighing boat;

- loading the sample in the weighing boat and drying it in an oven and bell dryer.

2.1.1.8. Preparation and conditioning of the weighing boat

The first operation involves using support on which to lay the sample then. In case of contact, a square-shaped plastic weighing boat was used as support and coated with aluminum foil to avoid degradation by the toluene solvent mixture. Subsequently, the weighing boat was conditioned by drying it in an oven at 45 °C for 30 minutes and in a bell dryer for another 30 minutes to remove all the moisture. Finally, the weighing boat is calibrated using a very high precision (1 μg) scale (Mettler MT5).

2.1.1.9. Loading and drying of sample

The sample was subjected to drying to remove the remaining part of the solvent. The post-distilled sample was taken, using a glass Pasteur pipette, from the 250 mL flask and placed inside the weighing boat on a stove. The sample dried at 45 °C for at least 24 hours; instead, for the first Soxhlet tests, it was dried for about 4 hours. The sample was finally moved to a bell dryer for about an hour to prevent the increase in humidity.

2.1.1.10. Weighing of extracted hydrocarbons

The dried sample was weighed with a very high precision scale (Mettler MT5). The sample was then placed again in the oven, in the dryer, and reweighed to verify a constant weight reaching. The difference between the weight of the dried sample and the tare weight of the weighing boat was determined to quantify the C_{10}-C_{40} hydrocarbons present in the analyzed sludge sample. The sample's initial weight percentage was calculated as the ratio between the weight of C_{10}-C_{40} hydrocarbons and the initial weight of the analyzed sample. By dividing this value by the percentage of TS at extraction, obtained from the characterization tests of the different types of SS used, the weight percentage relative to the TS was calculated. Finally, a comparison was made with the legal limit ("Genoa decree" of 28 September 2018, article 41), which provides for a concentration of C_{10}-C_{40}

hydrocarbons per matter of raw SS lower than 1,000 mg/kg, which, supposing a use of SS in landspreading with 80% of humidity, is equal to 5,000 mg/kg$_{TS}$.

2.1.1.11. Tests for the evaluation of the residual extractable fraction

Experiments were conducted to evaluate the residual extractable fraction to determine the possible presence of interferents and analytes of other origins and verify the yield of the individual extraction methods. These consisted of applying the standard procedure for determining C_{10}-C_{40} hydrocarbons using SS samples previously subjected to extraction. Before being used, SS samples already subjected to the extraction procedure were left under the hood to remove all the solvent used in the previous extractions.

2.1.1.12. Spiking tests

Analyses were conducted on samples subjected to spiking with diesel oil to verify the effectiveness and accuracy of the extraction methods used. These tests involve executing the various extraction techniques, using SS samples previously subjected to extraction and, therefore, without the analytes of interest and adding known quantities of hydrocarbons (Spikes). A solution was prepared with 500 mg of diesel oil diluted in 100 mL of acetone, obtaining a mixture with a concentration equal to 5 g/L. To add a quantity of diesel oil twice as much as the regulatory limit (10,000 mg/kg$_{TS}$) to the SS sample to be analyzed, 2 mL of the 5 g/L solution was taken from the solution for each gram of dry solid substance. The quantity withdrawn from the 5 g/L solution, using a micropipette (Transferpette), was mixed with the sample of SS extracted inside a small glass container. Subsequently, the sample was left under a hood for one night to evaporate the solvent and proceed with the extraction operations. The objective of this procedure is to return, at the end of the extraction method used, a quantity of diesel oil (C_{10}-C_{40} hydrocarbons); the closer to the added quantity, the more accurate the method is.

Figure 2.9. Samples previously extracted for spiking (left); spiking procedure (right).

2.1.2. Improvement of the methodology

2.1.2.1. Sewage sludge and samples preparation

The SS samples analyzed were all of the same typology, i.e., chemically post-conditioned and dewatered SS from a WWTP in the south of Italy. Before the subsequent analytical stages, all SS samples were stored at 4 °C, dried at 35 °C for 6÷7 days, and underwent a particle size reduction in a mortar.

Figure 2.10. Sample before (left) and after (center) drying; p. size reduction in a mortar (right).

2.1.2.2. Optimization of the sewage sludge drying process

The SS drying in a refrigerator thermostat at 35 °C is of fundamental importance for reducing the sample's humidity. Treating samples characterized by reduced quantities of water allows having less interference in the analysis. The drying carried out was performed for a minimum of 6 continuous days. A drying period of 15 days resulted in an average TS percentage of 91.68%. It was observed that, under the same operating conditions with Soxhlet extraction, to treat raw SS samples characterized by a TS percentage equal to 77.39%, unlike the dried SS samples characterized by a TS percentage equal to 91.68%, it was necessary to enhance the clean-up operations. In this case, two clean-up cycles were performed, each characterized by a column packed with silica gel slurry and a packed syringe with a dry bed of Florisil (2 g) placed in series. It is therefore considered appropriate

to reduce the sample's water content as much as possible, to avoid operations requiring long execution times.

2.1.2.3. Sample particle size reduction and homogenization

The particle size reduction of the dried sludge is of fundamental importance, as a high specific surface is a fundamental element for any biphasic extraction operation, ensuring the right degree of contact between the phases.

Below are some explanatory figures of the different grades of grain size achieved thanks to the use of a mortar.

Figure 2.11. Different grain sizes achieved.

Furthermore, after the particle size reduction, it was moved for a few seconds on a Vortex stirrer to best homogenize the sample. It was used to best prepare the samples for subsequent analysis stages.

2.1.2.4. Solids content determination

As the first analytical step, the TS and VS contents of the SS samples were determined by following the APHA-AWWA-WEF Standard Method 2450, using a laboratory oven (Argolab TCN 115, Italy), a muffle furnace (Asal ZB/1, Italy), and a precision scale (Sartorius 1801, Germany).

2.1.2.5. Hydrocarbons extraction

Also, in this round of experimentation, the extraction methods tested were:

- Soxhlet extraction;

- Cold solid-liquid extraction;
- MWAE;

using different configurations for extraction times, contact sample-solvent, and, mostly, testing different mixtures of organic solvents.

2.1.2.5.a. Soxhlet extraction

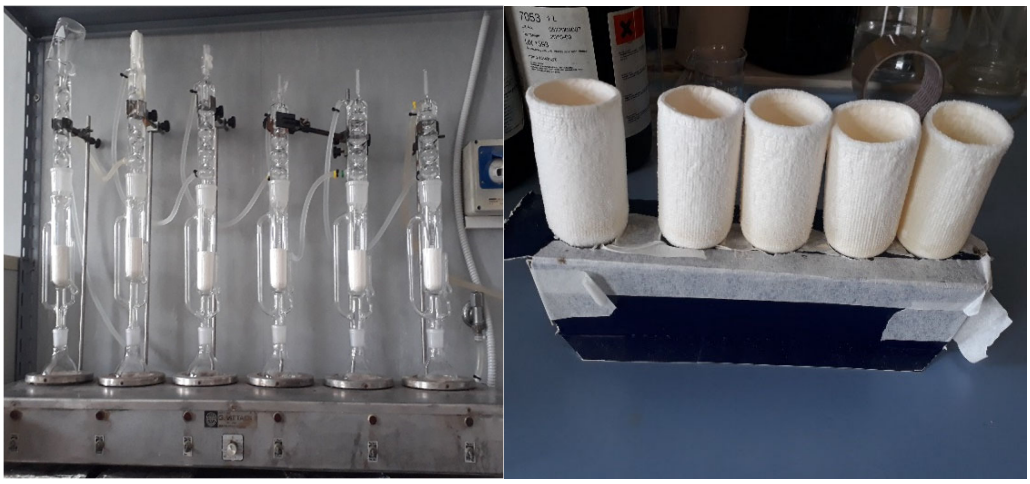

Figure 2.12. Soxhlet extractors (left) and thimbles used (right).

The Soxhlet extraction, in this experimental round, lasted 11 hours. It has been observed that each cycle (including boiling, evaporation, and condensation of the solvent, filling, and emptying of the extractor by siphoning motion) lasts about 30 minutes if dichloromethane is used as a solvent and 15 minutes if an acetone/hexane mixture is used as a solvent. Considering that, based on the instrumental operating mode, the substances to be analyzed will be increasingly concentrated in the flask at each cycle and will have a concentration in the sample contained in the thimble that will tend to zero as the extraction cycles increase, it is not considered necessary to iterate the process beyond 11 hours.

Materials and operating conditions used:

- solid sample: chemically post-conditioned and dewatered SS sample, 10 g;
- flask: 250 mL ground-neck borosilicate glass flask;

- solvents: (a) 200 mL of dichloromethane (CH_2Cl_2); (b) 200 mL of a 1:1 (v:v) acetone/hexane (CH_3COCH_3/C_6H_{14}) mixture; (c) 200 mL of a 10:1 (v:v) toluene/methanol ($C_6H_5CH_3/CH_3OH$) mixture;
- extraction duration: 11 hours;
- thimbles: cellulose fiber thimbles with 37 mm external diameter, 33 mm internal diameter, and 80 mm length, previously conditioned with dichloromethane;
- extraction temperature: depending on the azeotropic boiling temperature of the solvent mixture.

Figure 2.13. Post-extraction samples.

2.1.2.5.b. Cold solid-liquid extraction

For the cold extraction, it was necessary to repeat the extraction process since the recovery of the analytes through the solvent solution is not total, as a residual part remains in the matrix. It was considered that good exhaustion of the solid matrix was obtained after repeating the cycle three times. Therefore, the solvent loaded with the analyte was taken after each cycle, and a new solvent (equal quantity to the removed one) was added for the next cycle. This solvent/matrix separation process was carried out by taking the solvent with a glass pipette, and therefore, unlike a system that provides for the presence of a thimble to ensure a clear and precise separation of the phases, it is possible to

have small losses of solvent (which is not withdrawn and remains in the sample). Overall, the cold extraction method has been optimized by introducing the use of multiple tube stirring equipment (VX-2500 Multi-tube Vortexer), which allows the simultaneous treatment of 24 samples, and excluding the use of the Vortex stirrer (Reax 2000, Heidolph), which allows the treatment of a single sample at a time.

Operating conditions used:

- solid sample: chemically post-conditioned and dewatered dried SS sample, 2 g;
- container: 50 mL Falcon tube;
- solvent: 5 mL of dichloromethane (CH_2Cl_2) (to be added at each cycle);
- water to facilitate the separation of the phases: 5 mL of distilled water (to be added only to the first cycle);
- extraction duration: for each cycle, 15 min are carried out on vortex at speed such as to put the system in agitation and assure phase contact and 20 min in a centrifuge at 4600 RPM to separate the phases;
- temperature: room temperature.

Figure 2.14. Sample stirring devices.

This extractive configuration has the following advantages:

- low costs;

- use of reduced quantities of solvent;
- possibility of carrying out numerous extractions in parallel;
- fast extraction times.

Among the disadvantages are listed instead:

- reduced sample quantities that can be analyzed;
- need to iterate several extraction cycles;
- need of phase separation after the extraction.

2.1.2.5.c. Microwave-assisted extraction

For MWAE, various tests were performed with different temperatures and durations: 45 °C for 1 hour, 85 °C for 1 hour, 85 °C for 2 hours, and 95 °C for 2 hours. Compared with those proposed in the literature, these extraction durations are already considerable; therefore, subsequent extraction cycles are not considered necessary.

Operating conditions used:

- solid sample (all charged in thimbles): chemically post-conditioned and dewatered dried SS sample, 2 g;
- solvents: 20 mL of dichloromethane CH_2Cl_2; 20 mL of a 1:1 (v:v) acetone/hexane (CH_3COCH_3/C_6H_{14}) mixture; 20 mL of a mixture of toluene/methanol ($C_6H_5CH_3$/CH_3OH) 10:1 (v:v);
- extraction durations: 1 hour; 2 hours;
- temperatures: 45 °C; 85 °C; 95 °C.

This operational configuration of the extraction methodology has the following advantages:

- low costs;
- use of reduced quantities of solvent;
- modest extraction times;

- minimization of heat losses;
- no need for phase separation;
- reduced possibility of sample contamination;
- possibility of carrying out numerous extractions in parallel;
- precision in monitoring process parameters.

Among the disadvantages are listed instead:

- reduced sample quantities that can be analyzed;
- possibility of incomplete extractions.

2.1.2.6. Phase separation

This process was used in this experimental round only after cold solid-liquid extraction, as it was necessary to separate the solvent loaded with analytes from the exhausted solid matrix. Conversely, separation was not necessary downstream of the Soxhlet extractions and MWAE, as it is already guaranteed by using the cellulose fiber thimble, which can retain the solid part of the matrix. The separation of the phases took place by inserting the Falcon tubes, previously subjected to stirring, in a centrifuge (IEC, Centra GP8R) for 20 minutes at a speed of 4600 RPM and at room temperature. At the end of this process, the phases were separated; the solvent was extracted with a glass pipette from the Falcon tubes, transferred to clean ones, and subjected to the clean-up phase.

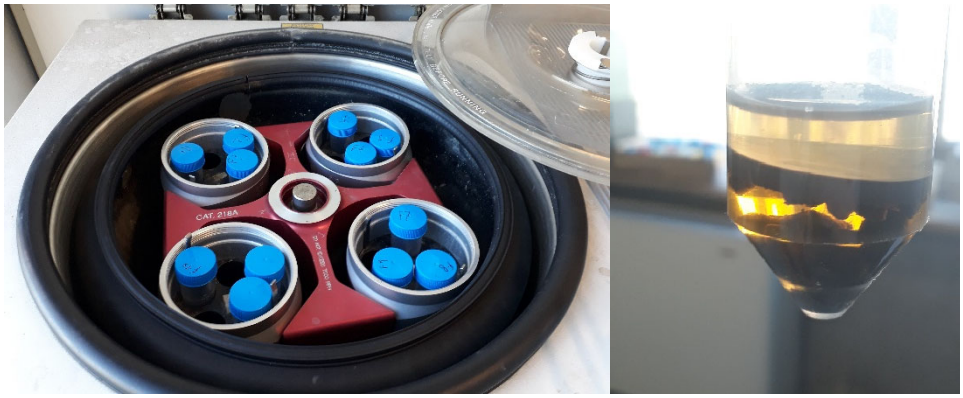

Figure 2.15. Samples in the centrifuge (left) and separate sample phases (right).

While for the Soxhlet extraction, a cellulose fiber thimble is necessary, no significant differences were found in the extracted quantities for the microwave-assisted one when comparing the data deriving from tests carried out in the presence and absence of a thimble inside the PTFE cylinder. However, considering that the presence of the thimble guarantees an excellent and precise phase separation, its use is recommended.

2.1.2.7. Clean-up procedures

The clean-up procedures are aimed at improving the purity of the extracted sample. They make it possible to remove all those substances that may have been extracted together with the C_{10}-C_{40} hydrocarbons, which can cause interference in the analyses, thus altering the results. Specifically, operations were carried out aimed at removing water and polar substances. In this experimental round, the removal of polar substances was notably deepened.

2.1.2.7.a. Water removal

Anhydrous sodium sulfate was added to each extracted sample so that, by shaking the container, resuspension of the crystallized particles was observed.

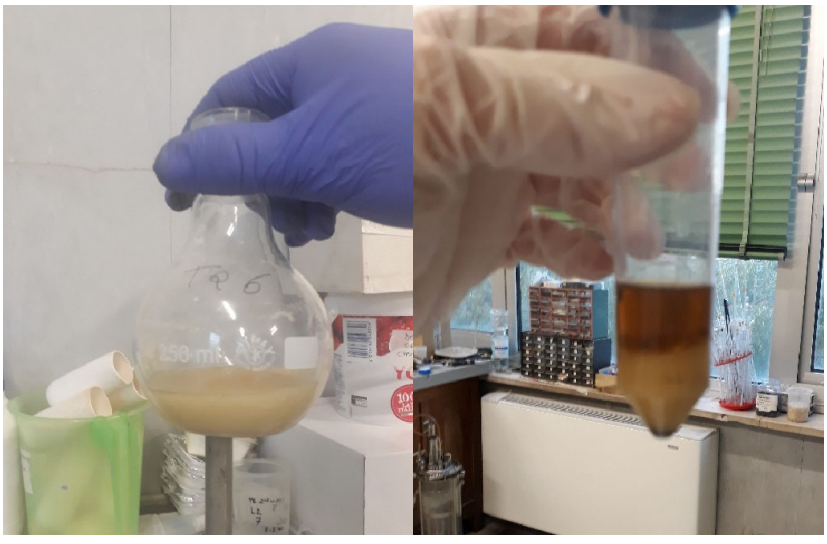

Figure 2.16. Crystallized Na_2SO_4 after treating a Soxhlet extracted sample (left); Na_2SO_4 treatment for a cold extracted sample (right).

2.1.2.7.b. Removal of polar substances

This process occurs according to the column chromatography technique, precisely adsorption chromatography. This technique separates the substances based on their different affinity with more or less polar substances. The glass column was packed with a solid phase (stationary phase) and a liquid phase (mobile phase). A slurry of silica gel (stationary phase) and solvent (mobile phase) was prepared to start from a 1:2 ratio of these substances and correct the doses until a homogeneous mixture was reached. The operating principle is based on the ability of some solid materials to adsorb (bond through weak interactions, such as Van der Waals forces and hydrogen bonds) the molecules on their surface. The highly porous granules with a very low particle size (70 ÷ 200 μm) have a high specific surface with a high number of active sites that can establish weak bonds with the molecules of the mixture to be separated. In this case, the siliceous adsorbent, previously activated, can interact with the polar groups transported by the eluent and retain them on its surface. The solvent loaded with analytes but free of polar substances will then be obtained at the outlet from the chromatographic column.

Operating conditions used for the preparation of the slurry:

- preventive conditioning (activation) of the silica gel in ceramic cups placed in the oven at 105 °C for at least 24 hours;
- cooling of the activated silica gel in a bell dryer for 1 hour to prevent it from acquiring moisture when it cools;
- solid phase: 50g activated silica gel (particle size 70 ÷ 200 μm);
- liquid phase: 100 mL of dichloromethane (CH_2Cl_2);
- stirring the slurry on a magnetic stirring mixer (MR 2002, Heidolph) until a homogeneous mixture is obtained.

Once ready, the slurry is poured into columns of different sizes according to the extraction method used.

Removal of polar substances for Soxhlet extraction

The samples extracted by Soxhlet, characterized by a considerable amount of solvent, were treated in a glass column 300 mm long with a 15 mm internal diameter. A layer of anhydrous sodium sulfate was placed at the top of the column to eliminate any traces of water still present in the extract. The columns used are equipped with a valve that allows the regulation of the sample flow through the stationary phase, and above all, it allows stopping the flow to avoid, during the preparation stage, the drying of the column and crystallization of the silica gel particles. Solvent and analytes were eluted through the packed column and collected in a 250 mL glass flask. A volume of solvent equal to the packed column's volume was added after the extracted sample's passage to allow the passage of the analytes still present and the washing of the adsorbent surface.

The clean-up procedure for this extractive methodology allowed the simultaneous treatment of 2 samples with a duration of about 40 min, corresponding to all examined solvents.

Figure 2.17. Packed column (left); running column (center); post-treatment column (right).

Figure 2.18. Simultaneous treatment of two samples (left); sample before and after clean-up (right).

It should be noted that it was necessary to enhance the clean-up operations to treat raw SS samples with a TS percentage equal to 77%, unlike the dried samples with TS>90%. In this case, two clean-up cycles were performed, each characterized by a column packed with silica gel slurry and a syringe packed with a dry bed of Florisil (2 g) placed in series. Florisil sorbent is particularly suitable for the adsorption of polar substances.

Figure 2.19. Serial clean-up (left); Florisil syringe before and after treatment (right).

Removal of polar substances for cold and microwave extractions

The cold and microwave-extracted samples, characterized by a reduced quantity of solvent, were treated in glass columns (syringes) 80 mm long and with a 13 mm internal diameter. A layer of anhydrous sodium sulfate was placed at the top of the column to eliminate any traces of water still present in the extract. Solvent and analytes were eluted through the packed column and collected in a 100 mL glass flask. A volume of solvent equal to the packed column's volume was added after the

extracted sample's passage to allow the passage of the analytes still present and the washing of the adsorbent surface.

The clean-up procedure for this extraction method allowed the treatment of only one sample at a time with a 10 ÷ 15 min duration.

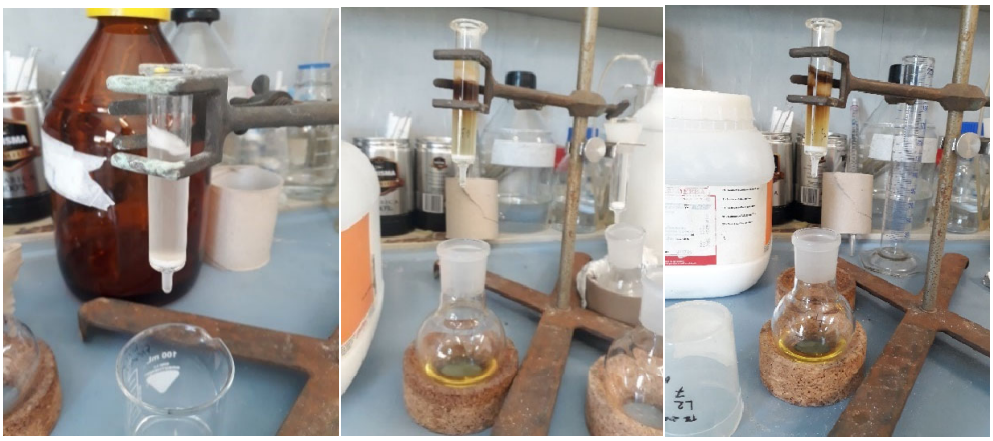

Figure 2.20. Packed syringe (left); syringe running (center); post-treatment syringe (right).

It was necessary to enhance the clean-up operations for the samples treated with cold extraction due to their high water content. A syringe packed with a dry bed of anhydrous sodium sulfate was then prepared for them and placed in series with the syringe packed with silica gel slurry.

Figure 2.21. Syringe packed with dry bed of anhydrous Na_2SO_4.

2.1.2.8. Solvent removal

The removal of the solvent from the extracted sample took place through the use of the distillation technique. This methodology allows the different components of a liquid mixture to be separated by exploiting their different volatility. The system used provides for the presence of 2 glass flasks (one containing the sample to be treated and one designed to collect the removed solvent), a condensation column with an annular water jacket, and a heating plate.

Figure 2.22. Distillation system.

The flask containing the extracted sample, consisting of analytes and solvent, was placed in the heating mantle, which provides a controlled heat output. This latter allows the solvent's evaporation, which, passing through a condensation column, condensed and was collected in a collection flask. The distillation was stopped when the sample reached a volume of about 5mL. The times required for this process vary according to the amount of solvent to be removed: for the samples extracted by Soxhlet (characterized by the presence of considerable amounts of solvent), at least 60 minutes of treatment were required; for the samples cold and microwave extracted (characterized by the presence of a reduced quantity of solvent) about 15 minutes of treatment were required. It should be noted that with this method, it is possible to recover considerable quantities of solvent, which can then be reused in subsequent experiments. In this case, the distilled solvent was used to perform the clean-up operations.

Figure 2.23. Post-distillation sample.

2.1.2.9. Validation of the clean-up and solvent removal stages

Clean-up with dichloromethane and distillation

All the methodologies used include downstream of the extraction, clean-up operations (aimed at removing all those substances that may have been extracted together with C_{10}-C_{40} hydrocarbons and which can cause interference in the analyses, thus altering the results), and distillation (aimed at removing a large part of the solvent from the extracted sample). Both phases were validated to verify that the solvents, adsorbents, set temperatures, and times were appropriate for the analytes of interest. Two tests were carried out to perform these checks, each one in triplicate, using sample solutions that simulate the post-extraction conditions:

- for the first test, 3 sample solutions were prepared, consisting of 20 mL of dichloromethane and 50 µL of evaporated diesel oil. They were then subjected to the clean-up and distillation phases, following the operating procedures described in the previous chapter;
- for the second test, 3 sample solutions were prepared, consisting of 20 mL of dichloromethane and 50 µL of evaporated diesel oil. Subsequently, they were subjected to the distillation phase only, following the operating procedures described in the previous chapter.

The results obtained are shown below:

Table 2.1. Results of the "clean-up + distillation" test.

	Tare [mg]	Duration [h]	Gross weight [mg]	Net weight [mg]
S1	4035.03	21	4064.98	29.95
S2	4050.68	20	4083.95	33.27
S3	3935.16	20	3967.69	32.52

In the first test, an average of 31.92 mg of diesel oil was recovered, corresponding to 84% of that added (considering the characteristic mass variation of the diesel oil described above).

Table 2.2. Results of the "distillation only" test.

	Tare [mg]	Duration [h]	Gross weight [mg]	Net weight [mg]
S1	3370.84	20	3406.36	35.52
S2	3818.08	20	3850.87	32.79
S3	3587.28	20	3624.37	37.09

In the second test, an average of 35.13 mg of diesel oil was recovered, corresponding to 92% of the added quantity (considering the characteristic mass variation of diesel oil described above).

From the results obtained, it is clear that neither the clean-up nor distillation phase significantly interferes with the analyte of interest.

Clean-up with acetone/hexane (1:1-v:v) mixture

In the treatment of the samples with a mixture of acetone/hexane 1:1 (v:v) using Soxhlet extraction and MWAE, difficulties were encountered in the clean-up phase. The main problem was related to the difficulty of the conditioned silica gel to remain in suspension in this mixture. Therefore, it was not possible to correctly prepare the slurry to pack the columns, and therefore, we opted to pack them with a dry bed of silica gel (for about 1/3 of the length of the column) and a thin layer of anhydrous sodium sulfate.

A second problem was the overheating of the column when the dry silica bed was wetted with the acetone/hexane mixture. The cause of this phenomenon is heat release due to a possible interaction between acetone and silica gel; therefore, it is preferable to perform the clean-up with hexane only.

It should be noted that, for the Soxhlet samples extracted with the acetone/hexane mixture, it was necessary to enhance the clean-up described above, repeating this operation three times.

Figure 2.24. Clean-up system after the first (left), second (center), and third clean-up cycle (right).

2.1.2.10. Preparation of the extracted sample

The post-distillation sample still contains a small amount of solvent that must be removed before it is subjected to hydrocarbon analysis. To this end, the sample is loaded into a small weighing boat and then dried.

2.1.2.11. Preparation and conditioning of the weighing boat

The weighing boat is a square-shaped tray made of plastic material that is coated with aluminum sheets to avoid its degradation in case of contact with the organic solvent. It is preferable to use cylindrical glass weighing boats to overcome this problem. A correct coating is of fundamental importance for plastic weighing boats. They should be carefully coated with a double aluminum sheet that covers them entirely and does not show wear. Otherwise, since the solvents used are very aggressive towards the plastic material, there is a risk that they will degrade the weighing boat. The glass weighing boats, made with a capacity of about 5 mL, represent a valid alternative. They do not require coatings and do not risk degrading. However, they need the following precautions:

- they must be carefully washed after each use to avoid that they have previously extracted analyte residues;
- they must be carefully conditioned and calibrated, for which it is recommended to carry out two conditioning cycles on the stove for 30 minutes and subsequent cooling in a bell dryer for a further 30 minutes to prevent them from reabsorbing moisture while reaching the room temperature;
- eventually, an additional conditioning cycle will be carried out to reach weight convergence.

Figure 2.25. Coated plastic weighing boat (left); glass weighing boats (right).

The weighing boat tare was carried out using a very high precision (1 μg) scale (Mettler MT5).

Figure 2.26. Weighing boat tare.

2.1.2.12. Loading and drying of sample

The post-distillation sample was taken from the flask with a glass Pasteur pipette and loaded into the weighing boat on the stove. The drying was performed at a temperature of 45 °C for about 24 hours. Thanks to the positioning in the stove of a weighing boat containing 5 mL of only solvent, it has been verified that this period was sufficient to allow the evaporation of all the solvent present. Before proceeding to the next weighing stage, the weighing boat was taken from the stove and placed in a bell-type dryer to cool without acquiring humidity.

2.1.2.13. Weighing of extracted hydrocarbons

The algebraic difference between the weight of the dried sample and the tare weight of the weighing boat was measured to quantify the hydrocarbons extracted from the analyzed SS sample. The hydrocarbons' weight percentage was also calculated by relating their weight to that of the initial sample and the weight percentage related to the total solids by relating this result to its TS percentage. It will therefore be:

$$\mathbf{E = (T+E) - T} \tag{2.2}$$

Where **E** is the weight (mg) of the extracted sample and **T** is the tare weight (mg) of the weighing boat. From which, the C_{10}-C_{40} hydrocarbon concentration ($mg_{C10\text{-}C40}/kg_{TS}$) will be calculated as:

$$\mathbf{[C_{10} - C_{40}] = \frac{E}{P * TS}} \tag{2.3}$$

P is the weight (kg) of the initial raw sample inserted into the extraction system, and **TS** is its total solids content (%).

2.1.2.14. Tests for the evaluation of the residual extractable fraction

Zero tests, i.e., tests for evaluating the residual extractable fraction on the samples treated with each method, were conducted to verify the yield of each methodology used. The SS samples already treated were then left in the hood for three days to allow the removal of the residual solvent used in the

previous tests. Subsequently, they were again subjected to the same extraction methodology to obtain information on the quantities of residual extractable hydrocarbons.

2.1.2.15. Spiking tests

Spiking tests were conducted using diesel oil as a reference analyte to evaluate the effectiveness and accuracy of the extraction methods. The SS samples already subjected to extraction, and therefore free of hydrocarbons, were left in the hood for three days to allow the removal of the residual solvent used in the previous tests. Subsequently, a known quantity of a solution containing 5 g/L of diesel oil in acetone was injected into the samples. Specifically, 1 mL of this solution was injected for each gram of dry substance of the sample to be treated to bring it to a known analyte concentration equal to 5,000 mg/kg$_{TS}$ (roughly equivalent to the regulatory limit of 1,000 mg/kg$_{as\text{-}it\text{-}is}$ provided by the Genoa Decree, assuming the humidity of a sludge destined to spread is equal to 80%, as typically happens). The solution with a concentration of 5 g/L (*spike solution*) was obtained by diluting a solution with a concentration of 50 g/L (*stock solution*).

However, not diluted, evaporated diesel oil was also used, injecting a 50 µL volume into the samples to be treated regardless of the weight of the sample. This way, we moved away from the instrumental zero during the verification of the accuracy of the measurement.

2.1.2.16. Variations in diesel oil characteristics

The behavior of diesel oil used as a reference analyte was studied. Tests were carried out to observe any variations in the diesel oil's main characteristics due to 24 hours spent on the stove at 45 °C. First, the density of the non-evaporated diesel oil was calculated: after measuring the weight of a volume of diesel oil equal to 500 µL (found to be equal to 420.163 mg), the density was obtained from the ratio between these two quantities (found to be equal to 840.326 mg/mL). Two glass lenses were tared, loaded with 10 mL of diesel oil each, weighed, and placed in an oven at 45 °C for 24 hours.

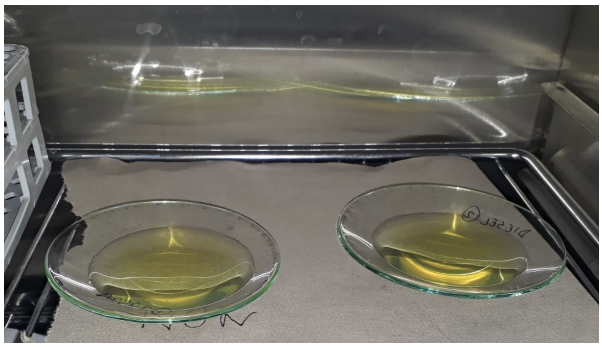

Figure 2.27. Lenses loaded with diesel oil in the stove.

After this time, 500 µL samples were taken from each lens to reassess density. No variations in density values from the starting conditions were found; therefore, it can be assumed that the diesel oil evaporates uniformly. Conversely, variations in the mass of the whole sample were observed:

Table 2.3. Diesel oil mass variations.

Diesel oil sample	D1	D2
Lens tare (g)	44.14	44.33
Lens+evaporated diesel oil weight (g)	50.66	50.61
Evaporated diesel oil weight (g)	6.52	6.29
Initial diesel oil weight (g)	8.40	8.40
Mass reduction (%)	22	25
Average mass reduction (%)	24	

Therefore, during the diesel oil vaporization test, variations in the mass of the whole sample equal to 24% were observed. This data will be considered in measuring the analytes in the spiking recovery tests.

Preparation of the stock solution

The stock solution was prepared in a 10 mL graduated flask; therefore, 500 mg of diesel oil (evaporated in a thermostatic refrigerator at 35 °C for 4 hours) and the remaining part (complement to 10 mL) of acetone were used. It should be noted that the diesel oil was inserted using a graduated automatic pipette; therefore, the corresponding necessary volume was previously calculated (found to be equal to 600 µL) downstream of the determination of the density of the evaporated diesel oil, as mentioned above, equal to that of the non-evaporated diesel, i.e., 840.326 mg/mL.

Preparation of the spike solution

The spike solution was prepared in a 10 mL graduated flask, taking 1 mL of stock solution and bringing this volume to 10 mL with the addition of acetone.

After carrying out the spiking tests, an extraction methodology will be deemed more effective the more the quantity of diesel oil extracted (representative of C_{10}-C_{40} hydrocarbons) approaches the known quantity added to the sample.

2.1.2.17. Validation of the gravimetric methods through gas chromatography/mass spectrometry

Gas chromatographic analysis with mass spectrometry detection (GC-MS) was used to validate the methods used so far and to qualitatively understand whether the analytes extracted from SS samples and gravimetrically measured fell into the category of C_{10}-C_{40} hydrocarbons or if the measured substances were not included in this classification. The samples collected immediately after the gravimetric determination inside the weighing boats were diluted with acetone, bringing the mixture to a volume of 10 mL in a graduated flask, and injected into a GC-MS device.

With gas chromatography, the various components of a mixture are separated, exploiting the different attitudes that each molecule or ion has in distributing itself between two different phases (stationary and mobile). The mobile phase is an inert carrier gas (such as helium or nitrogen) that has the sole function of transporting the sample through a column in which the stationary phase is located, which can be a porous granular solid or an adequately supported liquid. The column is positioned in the oven, a thermostatic chamber controlling the column's temperature and, therefore, the distribution of the species between the stationary and mobile phases. The mixture to be analyzed is inserted, using a special syringe, into the injector, ensuring the instantaneous vaporization of the sample. Each substance's time to elute through the column is called the retention time and depends on the chemical-physical characteristics of the substance, which determine its interactions with the two phases inside the column. Under the same experimental conditions, each substance's retention time is characteristic

and can be used to identify the species after comparing them with those of standard references injected under the same conditions. A detector is placed at the column outlet with the task of emitting an intensity signal proportional to the concentration of the detected individual components. The diagram showing the signal plotted against time is called a chromatogram: it appears as a sequence of peaks of different amplitude and height (intensity) distributed along the time axis. Under ideal conditions, the peak has a symmetrical trend and a Gaussian shape. This shape reflects every substance's inevitable dispersion process as it flows in the column. Thanks to the presence of the mass spectrometer, it is possible to measure the molecular masses and determine the structural equation of the compound, even if small quantities are available.

The mass spectrometer's operating principle is based on the ionization of the molecule due to the expulsion of an electron which occurs due to the passage of the molecule itself through an electron beam produced by a quadrupole consisting of four parallel metal rods on which is applied a combination of DC and RF voltages. The ionized molecules are projected into a mass analyzer whose task is to separate the ions according to their mass/charge ratio, which will finally be detected in sequence over time (detector). Mass spectrometers operate in high vacuum conditions to obtain a spectrum with good resolution, as the presence of any atmospheric gas molecules could interfere with the ions, varying their kinetic energy and altering their signal. The diagram showing the abundance of each ion as a function of the mass/charge ratio is called a mass spectrum, typical of each compound, as it is directly related to its chemical structure and the ionization conditions to which it was subjected. Specifically, an Agilent 6850 gas chromatograph coupled with an Agilent 5973 mass spectrometer was used for this research. The compounds, transported with helium as a carrier gas, were separated on a 30 m fused silica capillary column and NUKOL resin as a stationary phase, with an internal diameter of 0.25 mm and a thickness of 0.25 μm (Zebron™ ZB-Semivolatiles Guardian, Phenomenex, USA) with a flow of 1 mL/min and an injector temperature of 180 °C. The single quadrupole mass was maintained at 280°C and fed with an electron multiplier voltage of 2200 V.

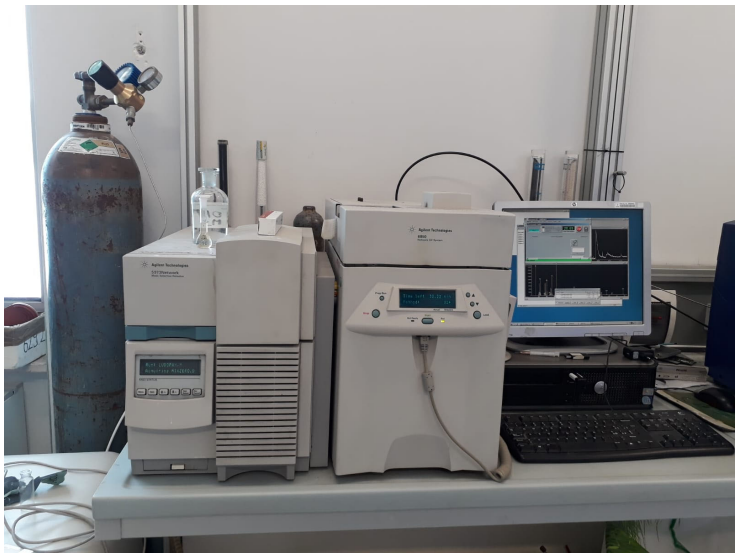

Figure 2.28. Agilent GC 6850 gas chromatograph and Agilent MS 5973 mass spectrometer.

A thermal curve for the automatic control of the oven was built by a constant 60 °C temperature for 10 min followed by an increase with 11 °C/min slope (dT/dt) up to 90 °C and 4 °C/min slope up to 320 °C. The last temperature was held for 20 minutes, resulting in a total elution time of 90.22 minutes.

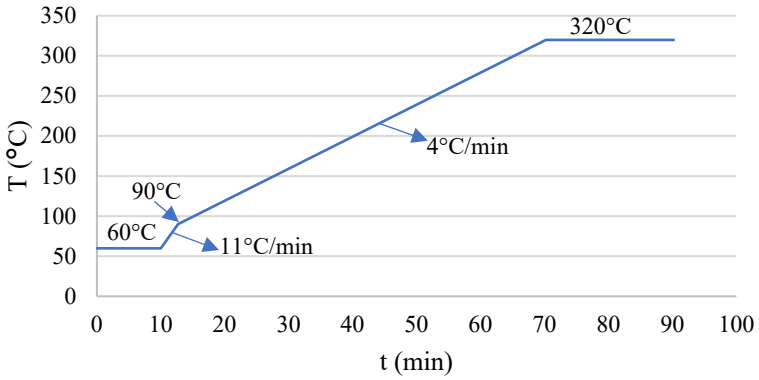

Figure 2.29. Thermal curve for the automatic control of the column oven.

2.1.2.18. Microwave extraction tests with bi-component solvent mixtures

In the present experimental step, the effects of two extraction methodologies for hydrocarbons, i.e., MWAE and Soxhlet extraction, have been investigated. In the case of MWAE, three solvents have been provided to guide the choice of the mixture that guarantees the best analyte yield. For this

purpose, six samples were prepared, divided into three zero samples and as many spiked samples. The zero samples were characterized only by the solid matrix, previously subjected to extraction cycles, and by the volume of 20 mL of the chosen solvent used for the extraction. On the other hand, the spiked samples were subjected to the spiking operation, which involves adding, using a high-precision calibrated micropipette (Transferpette), 50 µL of diesel oil into the solid matrix before pouring the 20 mL of solvent. A known quantity of hydrocarbons added to the sample must be returned as close as possible by the measuring system, measuring the method's accuracy.

The MWAE system was of closed type (Start D, Milestone), and the configured parameters were the temperature, extraction duration, and heating power. Based on the solvent used, treatment at a temperature of 150 °C was envisaged for 15 or 30 minutes, setting the maximum power of the oven at 800 W to ensure a constant temperature throughout the extraction phase.

The three solvents used are:

- Acetone/Dichloromethane 1:1 (v:v);
- Acetone/Hexane 1:1 (v:v);
- Acetone/Tetrachlorethylene 1:1 (v:v).

3 g of samples in contact with the 20 mL of solvent were placed in the PTFE capsules, previously calibrated. It would be possible to carry out extraction cycles using cellulose fiber thimbles, inside which 3 g of the solid matrix were poured and subsequently immersed in the solvent to facilitate the separation of the solvent and the recovery of the extracted sample. Although thimbles have the advantage of facilitating the recovery of the extracted solid sample, preventing it from being dragged by the solvent during the analysis phase, they mainly have two disadvantages in terms of method optimization:

- the thimble absorbs the solvent, retaining a large part of the analyte in it;

- several washes are required to recover the sample, using additional solvent, with a consequent increase in time and solvent consumption.

Therefore, the use of thimbles in MWAE was abandoned.

After a solvent-solid sample gravimetric separation, clean-up operations and water and polar substances removal were performed as described below.

- Water removal was performed by adding anhydrous sodium sulfate to the samples after extraction and removing the decahydrate ($Na_2SO_4 \cdot 10H_2O$) precipitate.
- Polar substance removal was used with syringes filled with dry beds of activated silica gel downstream of the anhydrous sodium sulfate layers.

Figure 2.30. Polar substances removal system (left); solvent-analyte mixture clean-up (right).

Due to a purely qualitative comparison, after clean-up, a distillation operation was performed to remove most of the solvent, after which the samples were dried on the stove at 45 °C for 24 h, diluted with pure acetone in a 10 mL graduated flask and directly injected in the GC-MS.

2.1.3. *Optimization of the gas chromatographic/mass spectrometric method*

A quantitative level of the gas chromatographic determination of C_{10}-C_{40} was refined by quantifying a calibration curve for the mass spectrometer by using mixtures of certified standards at different hydrocarbon concentration values. A first standard, consisting of the mixture of diesel oil and lubricating oil, purified and deprived of additives (Mineral Oil Standard mixture Type A and B – 69246, Sigma-Aldrich, Switzerland), was used to populate two different ranges of hydrocarbons covering the whole range of aliphatic hydrocarbons of interest. The two ranges typically are named Diesel Range Organic (DRO) for the C_{10}-C_{28} fraction and Lubrificant Range Organic (LRO) for the C_{18}-C_{38} fraction. A dilution solution made of n-heptane spiked with n-decane and n-tetracontane standards was used as a solvent (RTW Solution – 67583, Sigma-Aldrich, Switzerland) to identify the retention time window (RTW) for integrating chromatograms. Another standard, named Florida standard (Sigma-Aldrich, Switzerland), a mixture of 17 single hydrocarbon standards with an even number of carbon atoms, was also used. A table of the retention times using both standards is shown in Table 2.4.

Table 2.4. Retention Times Table for the GC-MS determination.

	Ret. Time (min)		Ret. Time (min)
$C_{10}H_{22}$	9.69	$C_{26}H_{54}$	52.23
$C_{11}H_{24}$	13.82	$C_{27}H_{56}$	
$C_{12}H_{26}$	17.04	$C_{28}H_{58}$	55.84
$C_{13}H_{28}$	20.23	$C_{29}H_{60}$	
$C_{14}H_{30}$	23.35	$C_{30}H_{62}$	59.19
$C_{15}H_{32}$	26.36	$C_{31}H_{64}$	
$C_{16}H_{34}$	29.26	$C_{32}H_{66}$	62.30
$C_{17}H_{36}$	32.03	$C_{33}H_{68}$	
$C_{18}H_{38}$	34.68	$C_{34}H_{70}$	65.22
$C_{19}H_{40}$	37.20	$C_{35}H_{72}$	
$C_{20}H_{42}$	39.62	$C_{36}H_{74}$	67.98
$C_{21}H_{44}$	41.93	$C_{37}H_{76}$	
$C_{22}H_{46}$	44.15	$C_{38}H_{78}$	70.63
$C_{23}H_{48}$	46.26	$C_{39}H_{80}$	
$C_{24}H_{50}$	48.36	$C_{40}H_{82}$	73.74
$C_{25}H_{52}$			

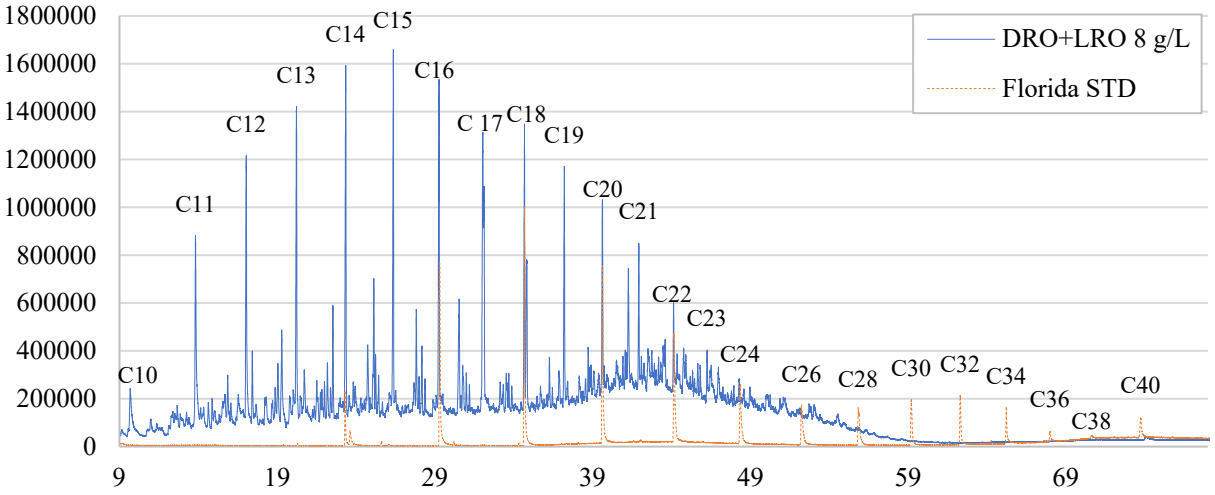

Figure 2.31. DRO+LRO Standard and Florida Standard in comparison to evaluate the RTW.

The DRO+LRO Standard used had a hydrocarbon concentration equal to 8.00(±0.15) g/L. Through a series of dilutions of DRO+LRO Standard, using the RTW Solution as a solvent, five standard solutions with decreasing hydrocarbon concentrations (8, 4, 2, 0.8, and 0.4 g/L) were obtained and measured with GC-MS.

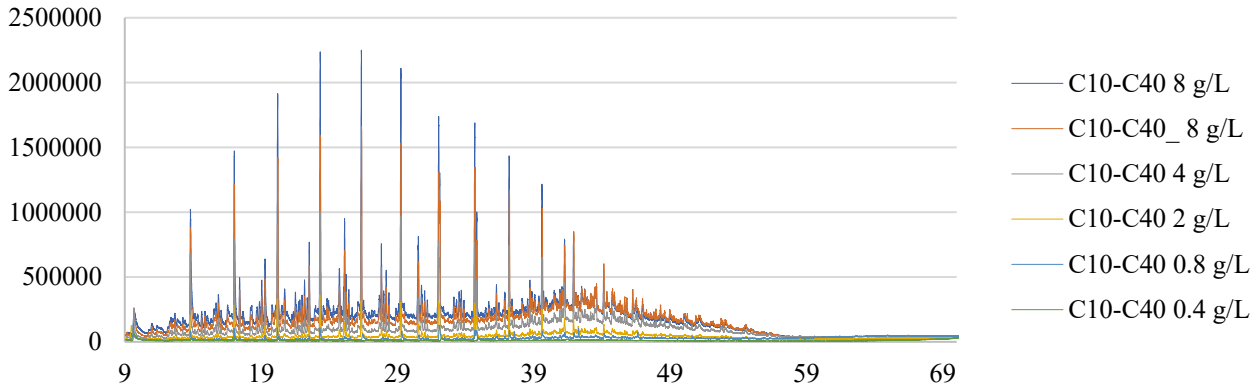

Figure 2.32. Subsequent dilutions of DRO+LRO Standard and instrumental response.

The gas chromatographic determination of aliphatic hydrocarbons does not provide net peaks for the single constituents of the mixture, but a baseline elevation, like a hump, named unresolved complex mixture (UCM) (Bruckberger et al., 2018; Jeon et al., 2017; Sutton et al., 2005). The integration of the area underlying the UCM constitutes the concentration of the cumulative parameter C_{10}-C_{40} hydrocarbons, a mixture of aliphatic hydrocarbons ranging from n-Decane ($C_{10}H_{22}$) to n-Tetracontane ($C_{40}H_{82}$) (Ispra, 2011). Integrating the areas below the chromatograms obtained by minimum square

regression, a calibration curve well approximated by a straight line was obtained, which made it possible to quantify the concentrations of C_{10}-C_{40} hydrocarbons for the eluted unknown samples.

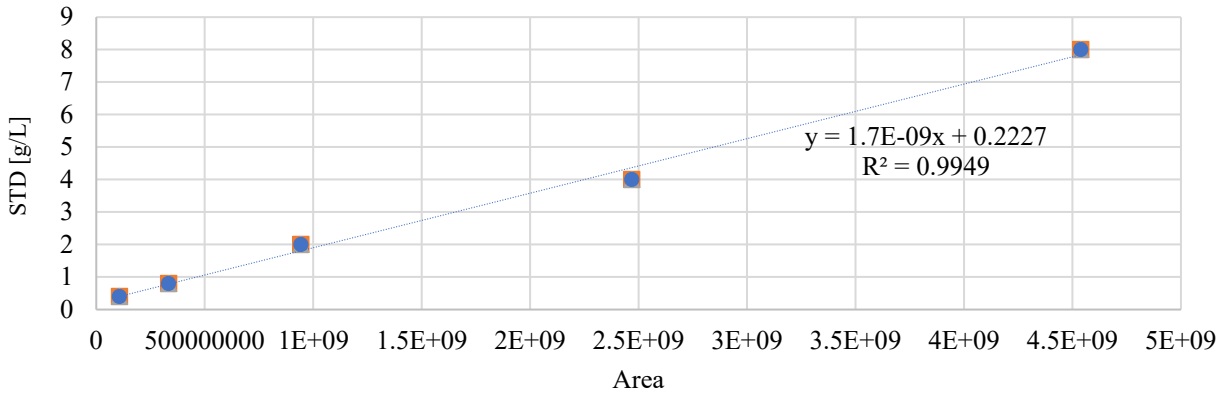

Figure 2.33. Calibration curve for C_{10}-C_{40} hydrocarbons determination with GC-MS.

Through the calibration curve equation, a regressive linear approximation on five points with R^2=0.9949, it is possible to calculate the value of the C_{10}-C_{40} hydrocarbon concentration in a sample, starting with the area underlying the chromatogram that the GC-MS analysis produces for that sample. It will be:

$$\mathbf{[C_{10}\text{-}C_{40}]_{(g/L)}=1.67882583950499*10\text{^}\text{-}9*AREA+0.222732827844396} \quad (2.4)$$

Where:

- $[C_{10}\text{-}C_{40}]_{(g/L)}$ is the C_{10}-C_{40} hydrocarbon concentration, expressed in g of C_{10}-C_{40} per L of solvent;
- AREA is the underlying area of the chromatogram, expressed in ionic unity (i.u.)*min.

According to a classification of the extractable petroleum hydrocarbons (EPH) made in the 1990s by the Massachusetts Department of Environmental Protection (MADEP speciation), we grouped the detected hydrocarbons into two classes, i.e., Lighter EPH (L_{EPH}) and Heavier EPH (H_{EPH}), targeting the C_{10}-C_{18} and C_{19}-C_{40} ranges (Bishop, 1997; Massachusetts D.E.P., 2004) respectively. These classes were evaluated by dividing the time integration interval of the UCM into two parts, with demarcation set at a retention time halfway between the retention times of C_{18} and C_{19}. These two times are given by the GC-MS response to the standard samples, with the specific optimized settings

of the analyzer (as column's status and thermal ramp, mass settings, transfer line's temperatures, and flow) at the moment of the measurement. LEPH and HEPH are quantified by integrating, in the two identified retention time intervals, the two halves into which the area underlying the UCM is divided. A comparison of LEPH and HEPH fractions between different SS samples allows the identification of profound differences in the global composition of the C_{10}-C_{40} hydrocarbon mixture due to different process treatment pathways SS underwent, such as different chemical conditioning paths, dewatering, and thickening.

2.2. Results and Discussions

The results of the various experimental phases carried out in this doctoral research are summarized in this section in chronological order, and discussions are provided to highlight their significance.

2.2.1. Delineation of methodologic procedures

2.2.1.1. Characterization of sewage sludge samples

Chemically post-conditioned and dewatered sewage sludge

The characterization of the chemically post-conditioning and dewatering sludge allowed the determination of TS and VS. Two solid characterizations were carried out in two different moments and are shown in Tables 2.5 and 2.6.

Table 2.5. Characterization of raw SS, chemically post-conditioned and dewatered.

Sample	A	B	C	Average	St. dev.
Sample initial weight (g)	26.8515	32.2917	21.4444		
Sample weight after drying in the oven at 105 °C for 24 h (g)	8.1041	9.7176	6.3388		
Total Solids Fraction (TS) (%)	30.18	30.09	29.56	29.94	0.34
Sample weight after incineration in the muffle furnace at 550 °C for 2 h (g)	4.5364	5.7807	3.4699		
Volatile Solids Fraction (VS) (%)	13.29	12.19	13.38	12.95	0.66

Table 2.6. Characterization of raw SS, chemically post-conditioned and dewatered.

Sample	D	E	F	Average	St. dev.
Sample initial weight (g)	22.7883	25.0252	29.5478		
Sample weight after drying in the oven at 105 °C for 24 h (g)	6.6654	7.1700	8.5025		
Total Solids Fraction (TS) (%)	29.25	28.65	28.78	28.89	0.32
Sample weight after incineration in the muffle furnace at 550 °C for 2 h (g)	3.6908	4.0683	4.8220		
Volatile Solids Fraction (VS) (%)	13.05	12.39	12.46	12.63	0.36

Subsequently, a characterization test was performed to determine the moisture loss in the analyzed SS samples after drying them in an oven at 35 °C for 6, 15, and 21 days. The results of this test are reported in Table 2.7.

Table 2.7. Moisture characterization of the sludge after chemical conditioning and dewatering.

Sample	A	B	C	Average	St. dev.
Sample initial weight (g)	27.3995	25.5124	26.2848		
Sample weight after drying at 35 °C for 6 days (g)	8.8266	7.7109	8.2147		
Total Solids Fraction (TS) (%)	32.21	30.22	31.25	31.23	1.00
Sample weight after drying at 35 °C for 15 days (g)	8.7762	7.6702	8.173		
Total Solids Fraction (TS) (%)	32.03	30.06	31.09	31.06	0.98
Sample weight after drying at 35 °C for 21 days (g)	8.7843	7.6774	8.1826		
Total Solids Fraction (TS) (%)	32.06	30.09	31.13	31.09	0.98
Final residual moisture (g)	0.55635	0.01648	0.28756		
Final residual moisture (% p/p)	2.06	0.09	1.13	1.09	0.98

Pre-thickened sewage sludge

The characterization of the pre-thickened sludge returned the values of the TS and VS parameters of the analyzed samples. The results of the tests, carried out in triplicate, are shown in Table 2.8.

Table 2.8. Characterization of pre-thickened raw SS.

	A	B	C	Average	St. dev.
Sample initial weight (g)	9.7132	9.7827	9.7063		
Sample weight after drying in the oven at 105 °C for 24 h (g)	0.2423	0.2449	0.2472		
Total Solids Fraction (TS) (%)	2.49	2.50	2.55	2.51	0.03
Sample weight after incineration in the muffle furnace at 550 °C for 2 h (g)	0.0893	0.0917	0.093		
Volatile Solids Fraction (VS) (%)	1.58	1.57	1.59	1.58	0.01

Subsequently, the pre-thickened sludge was characterized after being centrifuged and dried in a hood. The values of the duplicate test, in terms of TS, are shown in Table 2.9.

Table 2.9. Characterization of pre-thickened raw SS – centrifuged and dried.

Sample	T1	T2	Average	St. dev.
Sample initial weight (g)	1.4204	1.5061		
Sample weight after drying in the oven at 105 °C for 24 h (g)	1.2769	1.3492		
Total Solids Fraction (TS) (%)	89.90	89.58	89.74	0.22

Finally, the characterization of the pre-thickened sludge digested anaerobically for 30 days, centrifuged, and dried in a hood was performed. The results of the test carried out in duplicate are shown in Table 2.10.

Table 2.10. Characterization of pre-thickened digested SS – centrifuged and dried.

Sample	D1	D2	Average	St. dev.
Sample initial weight (g)	0.8379	0.7971		
Sample weight after drying in the oven at 105 °C for 24 h (g)	0.7560	0.7204		
Total Solids Fraction (TS) (%)	90.23	90.38	90.30	0.11

The results of the specific biomethane production are shown in Figure 2.34.

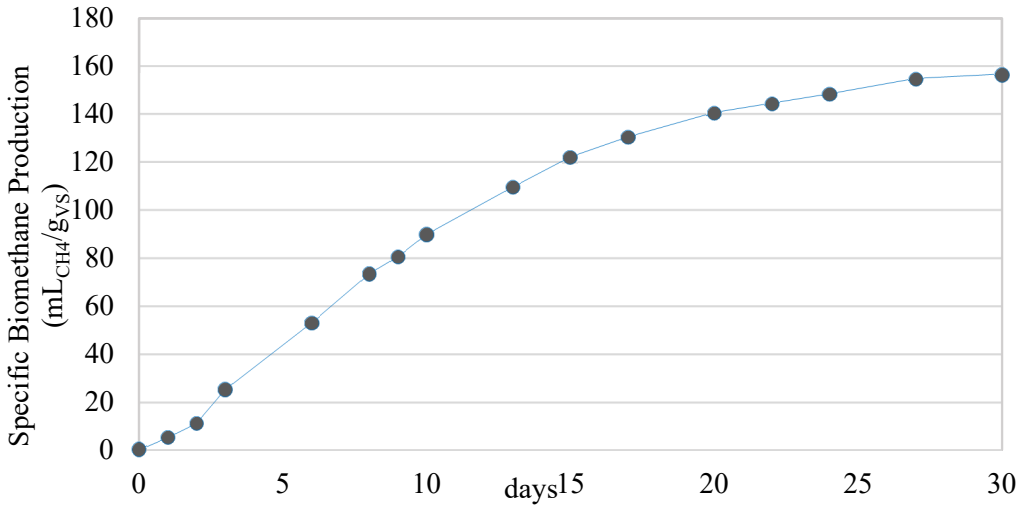

Figure 2.34. Biomethanation curve of the AD test of the pre-thickened SS.

The characterization of chemically conditioned and dewatered raw SS yielded TS and VS values higher than pre-thickened raw SS. Tables 2.5, 2.6, and 2.8 show these results, mainly due to the dewatering stage to which the first analyzed SS was subjected. For this reason, before the extraction

tests, the pre-thickened SS was subjected to centrifugation and drying actions to make it more concentrated and increase the percentage of TS (Tables 2.9 and 2.10). In addition, the chemical post-conditioned and dewatered SS was dried in a thermostatic refrigerator at a temperature of 35 °C for several days. This test was performed to study the initial humidity's interference with the analysis. Figure 2.35, obtained from the results in Table 2.7, shows how SS samples reached a constant weight in 6 days.

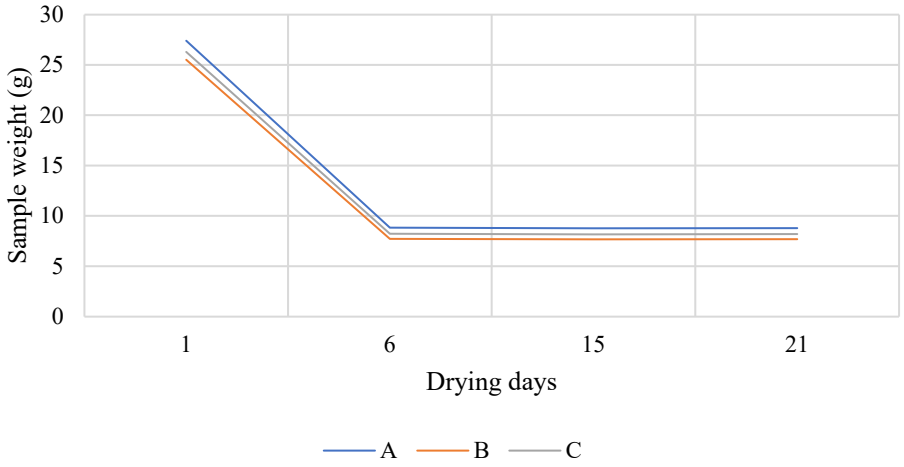

Figure 2.35. Chemically post-conditioned and dewatered SS drying test.

2.2.1.2. Soxhlet extraction

Chemically post-conditioned and dewatered raw sewage sludge

Eight tests were performed for the samples of raw SS chemically post-conditioned and dewatered extracted with Soxhlet, using two types of clean-up and two different drying periods. In particular, four samples were subjected to a clean-up with only Florisil, of which the first three were at a drying time of about 4 h and the last at a drying time of at least 24 h, while the other four samples were subjected to a clean-up with silica gel followed in series by one with Florisil, of which the first two with a drying time of 4 h and the last two with a drying time of at least 24 h. The results, obtained from the extraction tests and calculated according to equation (2.3), are shown in Tables 2.11 to 2.14.

Table 2.11. Soxhlet extraction tests from raw SS with Florisil clean-up, drying period of 4 h.

Sample	S01	S02	$S04_A$
Sample weight in thimble (g)	16.1658	16.0253	8.3405
C_{10}-C_{40} hydrocarbon weight (mg)	390.9870	636.4800	273.2150
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	24,186	39,717	32,758
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	80,620	132,391	109,192

Table 2.12. Soxhlet extraction tests from raw SS with Florisil clean-up, drying period of 24 h.

Sample	S12
Sample weight in thimble (g)	10.8609
C_{10}-C_{40} hydrocarbon weight (mg)	157.4810
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	14,500
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	48,333

Table 2.13. Soxhlet extraction tests from raw SS with silica gel and Florisil clean-up, drying period of 4 h.

Sample	S03	$S04_B$
Sample weight in thimble (g)	16.2310	8.3405
C_{10}-C_{40} hydrocarbon weight (mg)	569.5770	260.3790
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	35,092	31,219
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	116,973	104,062

Table 2.14. Soxhlet extraction tests from raw SS with silica gel and Florisil clean-up, drying period of at least 24 h.

Sample	S09	S10
Sample weight in thimble (g)	10.1692	10.4338
C_{10}-C_{40} hydrocarbon weight (mg)	79.6700	84.2300
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	7,834	8,073
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	26,115	26,909

Figure 2.36 shows the average values, and standard deviations of C_{10}-C_{40} hydrocarbon concentrations from the individual tests carried out with raw SS.

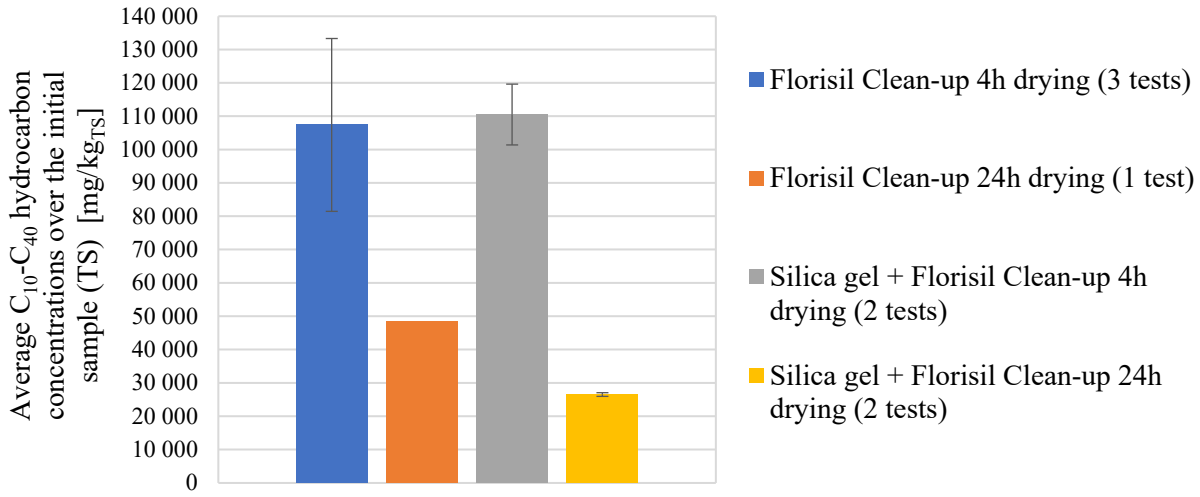

Figure 2.36. From tests, average values and standard deviations of C10-C40 hydrocarbon concentrations carried out with Soxhlet on raw SS.

The first Soxhlet extraction tests, using raw SS after chemical conditioning and dewatering, clean-up with only Florisil, and a drying period of about 4 h provided relatively high values of C_{10}-C_{40} hydrocarbon concentrations (Table 2.11). A further test was subsequently carried out, increasing the drying time to at least 24 h, obtaining a lower hydrocarbon concentration value than in the previous case (Table 2.12), which demonstrates the criticality of a complete abatement of the water content from the initial sample, otherwise the hydrocarbon measurement would be affected. To further improve the extraction methodology, new tests were carried out, applying a clean-up with silica gel followed in series by Florisil. Table 2.13 shows that the values of the C_{10}-C_{40} hydrocarbon concentrations, obtained with a drying period of 4 hours, are comparable with the values obtained for the samples treated with only Florisil and with a similar drying period. From Table 2.14, on the other hand, it can be observed that the values of the C_{10}-C_{40} hydrocarbon concentrations are almost halved compared to the values obtained for the samples treated with only Florisil and a drying period of 24 hours. It was decided to increase the drying period from about 4 h to at least 24 h to remove all the toluene/methanol mixture (10:1-v:v) and the initial humidity in the analyzed samples. The solvent, being of organic origin, should not take long to volatilize, considering that it was also previously subjected to distillation. It is hypothesized that, at this point, in the sludge, there is such a high quantity of C_{10}-C_{40} hydrocarbons to become solvent for the toluene/methanol mixture. The subsequent strong

interactions between the toluene/methanol mixture and the extracted organic substance could make solvent removal much more difficult.

Chemically post-conditioned and dewatered dried sewage sludge

The chemically post-conditioned and dewatered SS samples were dried in a thermostatic refrigerator at 35 °C for the durations of 7, 15, and 75 days and subsequently subjected to extraction with Soxhlet and treated with silica gel and Florisil clean-up. The results obtained according to equation (2.3) are reported in Table 2.15.

Table 2.15. Soxhlet extraction tests from dried SS for 7, 15, and 75 days, with silica gel and Florisil clean-up.

Sample	S13	S06	S07	S08
Drying time (days)	7	15	75	75
Raw SS sample weight in thimble (g)	12.0884	-	26.3103	24.9902
Dried sample weight in thimble (g)	3.6388	9.4950	8.4401	7.8220
C_{10}-C_{40} hydrocarbon weight (mg)	111.8050	453.1720	369.1000	381.0500
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	30,726	47,727	43,732	48,715
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	30,726	47,727	43,732	48,715

Figure 2.37 shows the average values, and standard deviations of the tests carried out on dried SS.

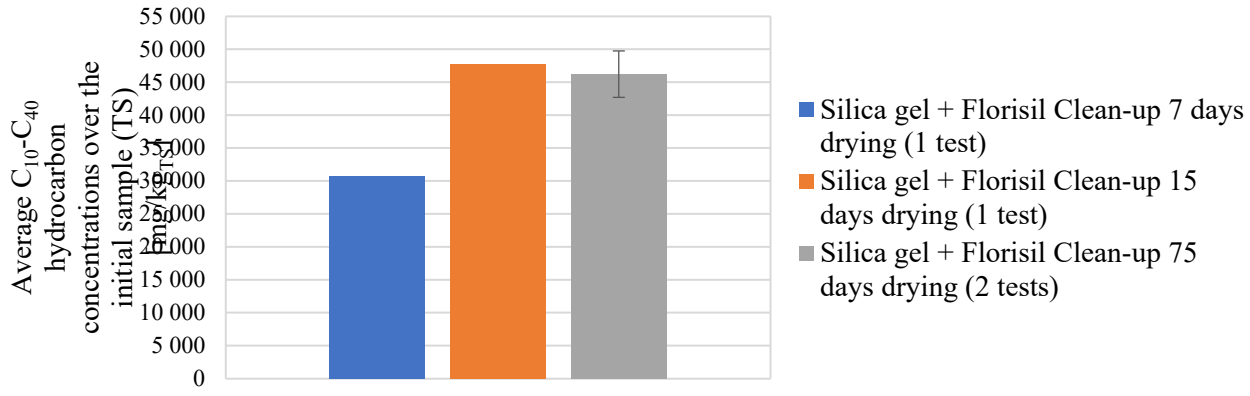

Figure 2.37. From tests, average values and standard deviations of C10-C40 hydrocarbon concentrations carried out with Soxhlet on SS dried at 35 °C.

A comparison was then made between the average values of the concentrations of the C_{10}-C_{40} hydrocarbons Soxhlet extracted from raw SS and dried; the results are shown in Figure 2.38.

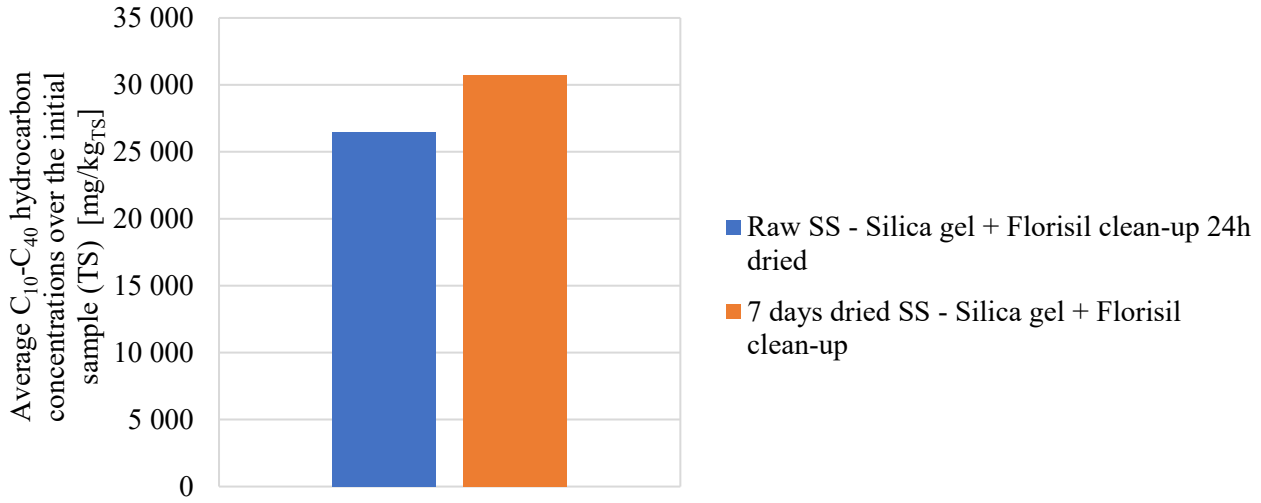

Figure 2.38. Comparison of average values of the concentrations of C_{10}-C_{40} hydrocarbons Soxhlet extracted from samples of SS raw and dried.

Concerning Table 2.15 and Figure 2.38, the Soxhlet extraction tests on SS dried at 35 °C and with clean-up with silica gel, and Florisil did not return significant changes in the values of the C_{10}-C_{40} hydrocarbon concentrations compared to extraction tests with raw SS samples and in the same operating conditions. Furthermore, it can be seen that as the drying days increase, the concentrations of extracted C_{10}-C_{40} hydrocarbons increase, and this could be due to the degradation of the organic substance that releases smaller molecules into the solvent, increasing interferences (Figure 2.37).

Evaluation of residual extractable fraction from chemically post-conditioned and dewatered sewage sludge after Soxhlet extraction

A test was performed on a chemically post-conditioned and dewatered SS sample, previously subjected to Soxhlet extraction, to verify the evaluation of the residual extractable fraction. The sample was subjected to a new Soxhlet extraction and treated with a clean-up of silica gel and Florisil. The results, obtained from the test and calculated according to equation (2.3), are reported in Table 2.16.

Table 2.16. Evaluation of the residual Soxhlet extractable fraction after extraction from chemically post-conditioned and dewatered SS and clean-up with silica gel and Florisil.

Sample	S15
Extracted sample weight in thimble (g)	5.1056
C_{10}-C_{40} hydrocarbon weight (mg)	15.4380

C_{10}-C_{40} hydrocarbon concentration over dried sample (mg/kg)	3,024
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	3,024

Subsequently, a comparison was made between the average value of the concentrations of C_{10}-C_{40} hydrocarbons and that obtained from the tests for the evaluation of the residual extractable fraction, extracted by Soxhlet, with the same clean-up operations, in the raw SS samples (Figure 2.39).

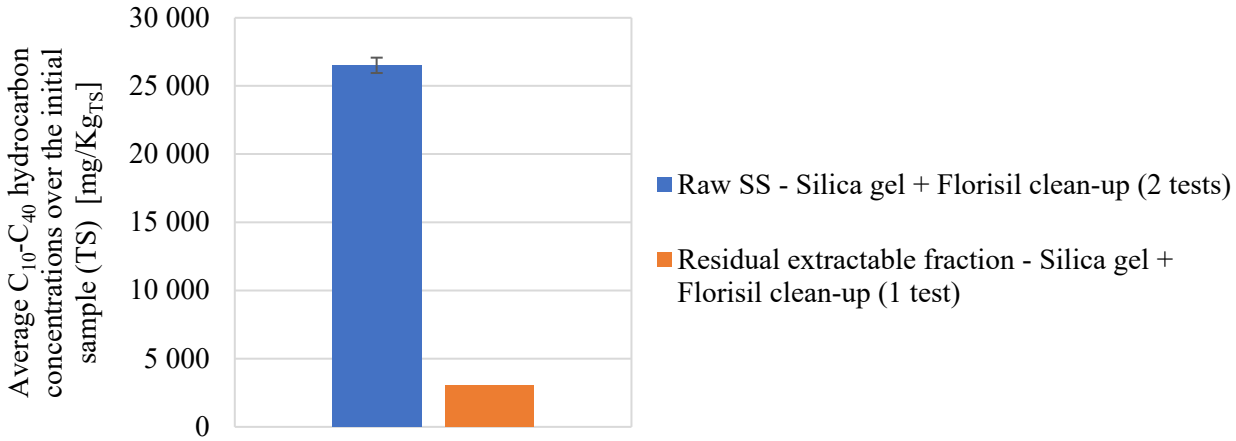

Figure 2.39. Comparison of the average value of the concentrations of C_{10}-C_{40} hydrocarbon, Soxhlet extracted from raw SS samples, with the residual extractable fraction.

From the results reported in Table 2.16 and Figure 2.39, it is possible to notice a very modest concentration of C_{10}-C_{40} hydrocarbons (3023.74 mg/kg_{TS}). Furthermore, comparing the first and second extraction, it can be observed that the concentrations of C_{10}-C_{40} hydrocarbons from the second extraction are negligible compared to those of the first extraction, testifying the efficiency of the Soxhlet extraction method.

2.2.1.3. Cold solid-liquid extraction

Chemically post-conditioned and dewatered raw sewage sludge

Fourteen samples of the chemically post-conditioned and dewatered raw SS were analyzed using cold extraction and performing clean-up only with silica gel. The results of the tests, calculated according to equation (2.3), are reported in Table 2.17.

Table 2.17. Cold extraction tests from raw SS with silica gel clean-up.

Sample	F01	F02	F03	F04	F05	F06	F07
Sample weight (g)	1.7883	1.7030	1.9646	2.0685	1.8167	2.0733	1.7744
C_{10}-C_{40} hydrocarbon weight (mg)	27.7850	27.2590	138.2750	33.4060	50.9820	17.3740	44.9120
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	15,537	16,006	70,383	16,150	28,063	8,380	25,312
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	51,790	53,355	234,611	53,833	93,543	27933	84,370

Sample	F08	F09	F10	F11	F12	F13	F14
Sample weight (g)	1.8764	1.8650	1.9118	1.7528	1.7030	1.8244	1.8672
C_{10}-C_{40} hydrocarbon weight (mg)	40.3720	45.1330	18.2990	18.9630	27.4870	29.5250	19.4970
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	21,516	24,200	9,572	10,819	16,140	16,183	10,442
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	71,719	80,667	31,905	36,062	53,801	53945	34,806

The first cold extraction tests were performed on raw SS and with clean-up with only silica gel. Table 2.17 and located below Figure 2.40 show pretty high values of C_{10}-C_{40} hydrocarbon concentrations, and a high standard deviation due to the heterogeneity of the extracted samples must be highlighted.

Chemically post-conditioned and dewatered dried sewage sludge

The chemically post-conditioned and dewatered SS samples were dried in a thermostatic refrigerator at 35 °C and subjected to cold extraction and clean-up with only silica gel. In particular, the test was performed in duplicate, and the results, calculated according to equation (2.3), are reported in Table 2.18.

Table 2.18. Cold extraction tests from dried SS with silica gel clean-up.

Sample	F15	F16
Dried sample weight (g)	1.9306	1.8600
C_{10}-C_{40} hydrocarbon weight (mg)	63.2510	67.8810
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	32,762	36,495
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	32,762	36,495

Referring to Tables 2.18 and located below Figure 2.40, cold extraction tests with SS dried at 35 °C and with silica gel clean-up returned values of the C_{10}-C_{40} hydrocarbon concentrations lower than the cold extraction tests with raw SS samples (Table 2.17), but with a statistically significant concordance ($p < 0.05$), also here proving an overestimation of the measure of the hydrocarbon content due to the water content. These results could be likely related to the higher amount of water in the extracts from the undried samples, requiring a longer removal time on the stove. The presence of water interferes with the clean-up operations, causing errors in the analysis of the gravimetric method. The homogeneity of the results obtained in the various tests must also be highlighted (Table 2.18).

Evaluation of residual extractable fraction from chemically post-conditioned and dewatered sewage sludge after cold extraction

Chemically post-conditioned and dewatered SS samples, previously cold extracted, were used to perform a second extraction to determine the residual extractable fraction. After being cold extracted again, the samples were subjected to a clean-up with only silica gel; test results, carried out in triplicate, with the calculations made according to equation (2.3), are reported in Table 2.19.

Table 2.19. Evaluation of the residual extractable fraction by cold extraction from previously extracted SS, with silica gel clean-up.

Sample	F17	F18	F19
Extracted sample weight (g)	1.7756	1.8581	1.8991
C_{10}-C_{40} hydrocarbon weight (mg)	50.1030	39.4190	278590
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	28,218	21,215	14,670
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	28,218	21,215	14,670

A comparison was then made between the average values of the concentrations of C_{10}-C_{40} hydrocarbons obtained from the cold extraction of dried SS samples and the residual extractable fraction (Figure 2.40).

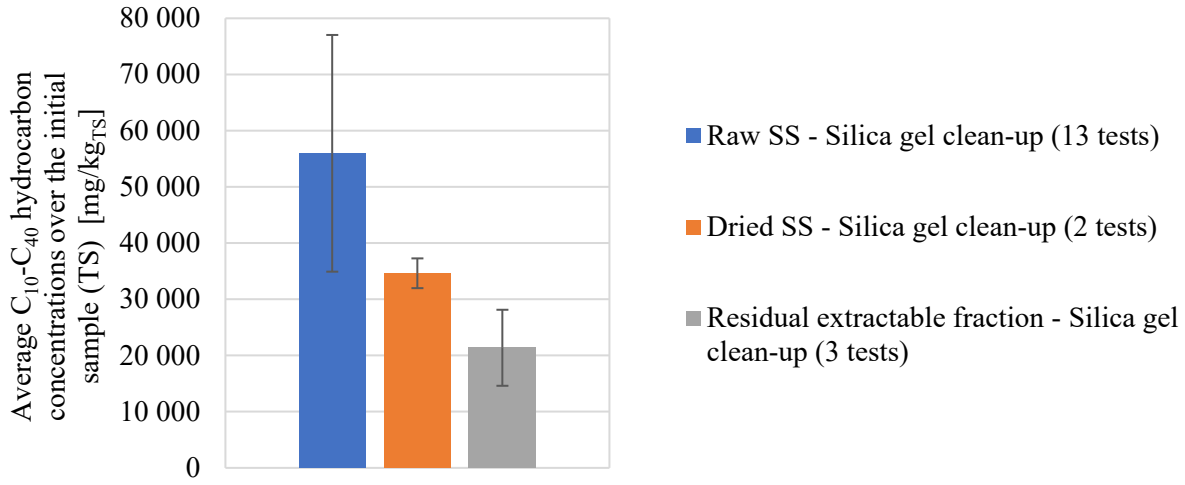

Figure 2.40. Comparison of average concentrations of C_{10}-C_{40} hydrocarbons obtained from the cold extraction of raw SS samples, dried and from the residual extractable fraction.

A second cold extraction was carried out on previously extracted SS samples to verify the yield of the extraction method, quantifying the residual extractable fraction. Regarding Table 2.19 and Figure 2.40, it can be seen that the values of the residual C_{10}-C_{40} hydrocarbon concentrations are pretty high, albeit lower, with a statistically significant difference than in the cold extraction tests with raw SS. Cold extraction should therefore be repeated several times, carrying out several extraction cycles, simulating the repeated siphoning happening in a single application of the Soxhlet extraction method.

2.2.1.4. Microwave-Assisted Extraction

Chemically post-conditioned and dewatered raw sewage sludge

Using chemical post-conditioned and dewatered raw SS samples, three MWAE cycles were performed. In particular, twelve samples were analyzed for cycle I, four for cycle II, and three for cycle III. All the extracted samples were subjected to a clean-up with silica gel only, and the test results, calculated according to equation (2.3), are shown in the following Tables.

Table 2.20. MWAE tests from raw SS with silica gel clean-up – cycle I.

Sample	M01	M02	M03	M04	M06	M07
Sample weight in PTFE capsule (g)	1.9205	1.8180	1.7947	1.7481	1.8244	1.9690
C_{10}-C_{40} hydrocarbon weight (mg)	14.4900	26.1480	8.6360	21.9100	19.4180	23.1080
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	7,545	14,383	4,812	12,534	10,644	11,736
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	2,5150	47943	16,040	41,779	35,478	39,120

Sample	M08	M09	M11	M12	M13	M14
Sample weight in PTFE capsule (g)	1.9750	1.8757	1.9057	1.8547	1.9295	1.9942
C_{10}-C_{40} hydrocarbon weight (mg)	7.5540	24.1250	20.7710	24.7930	37.4160	27.8040
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	3,825	12,862	10,900	13,368	19,392	13,942
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	12,750	42,873	36,331	44,559	64,639	46,475

Table 2.21. MWAE tests from raw SS with silica gel clean-up – cycle II.

Sample	M21	M22	M23	M24
Sample weight in PTFE capsule (g)	1.9713	2.0928	2.0355	2.0416
C_{10}-C_{40} hydrocarbon weight (mg)	18.2740	28.1690	27.7960	23.4560
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	9,270	13,460	13,656	11,490
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	30,900	44,867	45,519	38,297

Table 2.22. MWAE tests from raw SS with silica gel clean-up – cycle III.

Sample	M25	M27	M28
Sample weight in thimble (g)	1.9918	2.1761	2.0644
C_{10}-C_{40} hydrocarbon weight (mg)	14.7160	12.9810	12.1260
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	7,389	5,965	5,874
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	24,627	19,884	19,580

A comparison was then made between the average values of the C_{10}-C_{40} hydrocarbon concentrations obtained from the three MWAE cycles from raw SS (Figure 2.41).

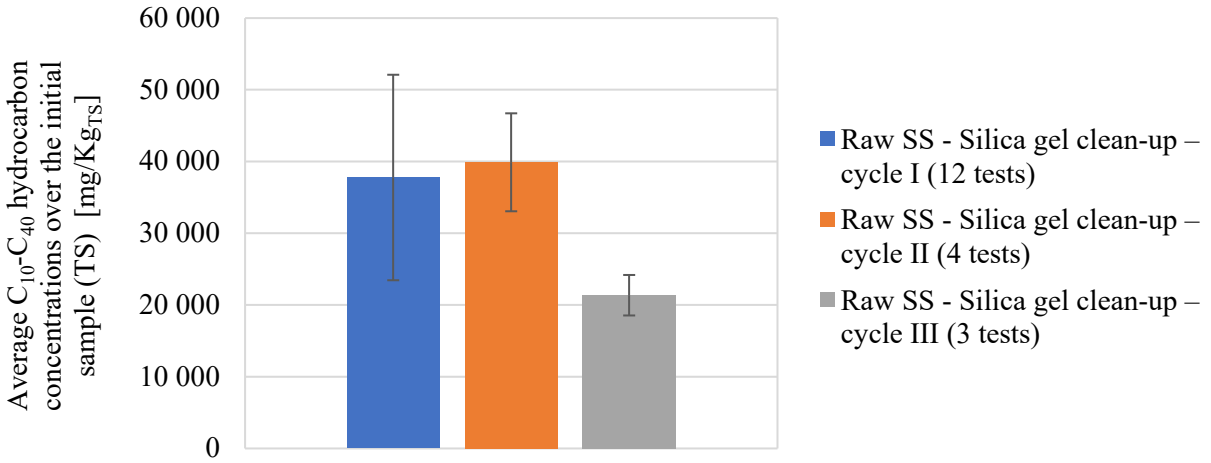

Figure 2.41. Comparison of average values of the C_{10}-C_{40} hydrocarbon concentrations obtained from the three MWAE cycles from raw SS.

For MWAE tests from raw SS, only the first three cycles were performed to optimize the extraction methodology. For the first cycle of extractions (Table 2.20 and Figure 2.41), values of C_{10}-C_{40} hydrocarbon concentrations with pretty high standard deviations were obtained due to the samples'

heterogeneity. The results obtained with the second extraction cycle (Table 2.21 and Figure 2.41) are comparable both with each other and, with excellent statistical accordance ($p < 0.05$), with the C_{10}-C_{40} hydrocarbon concentrations of the first cycle. Finally, the results of the third extraction cycle (Table 2.22 and Figure 2.41) yielded much lower values of the C_{10}-C_{40} hydrocarbon concentrations, almost halved compared to the first two cycles, with very low standard deviations and a statistically significant difference. This result could be due to the thimble inside the PTFE capsule, which could block any interfering particulate solid substances and allow for analysis of the only extractable component sought.

Chemically post-conditioned and dewatered dried sewage sludge

Chemically post-conditioned and dewatered dried SS samples were dried in a thermostatic refrigerator at 35 °C and subsequently subjected to MWAE and cleaned up with only silica gel. In particular, the test, performed in duplicate, gave the results calculated according to equation (2.3) and reported in Table 2.23.

Table 2.23. MWAE tests from dried SS with silica gel clean-up – cycle I.

Sample	M05	M10
Dried sample weight in PTFE capsule (g)	1.8229	1.8662
C_{10}-C_{40} hydrocarbon weight (mg)	24.8290	29.4050
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	13,621	15,757
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	13,621	15,757

A comparison was then made between the average values of the concentrations of the C_{10}-C_{40} hydrocarbons extracted with microwaves under the same operating conditions from raw SS and dried; the results are reported in Figure 2.42.

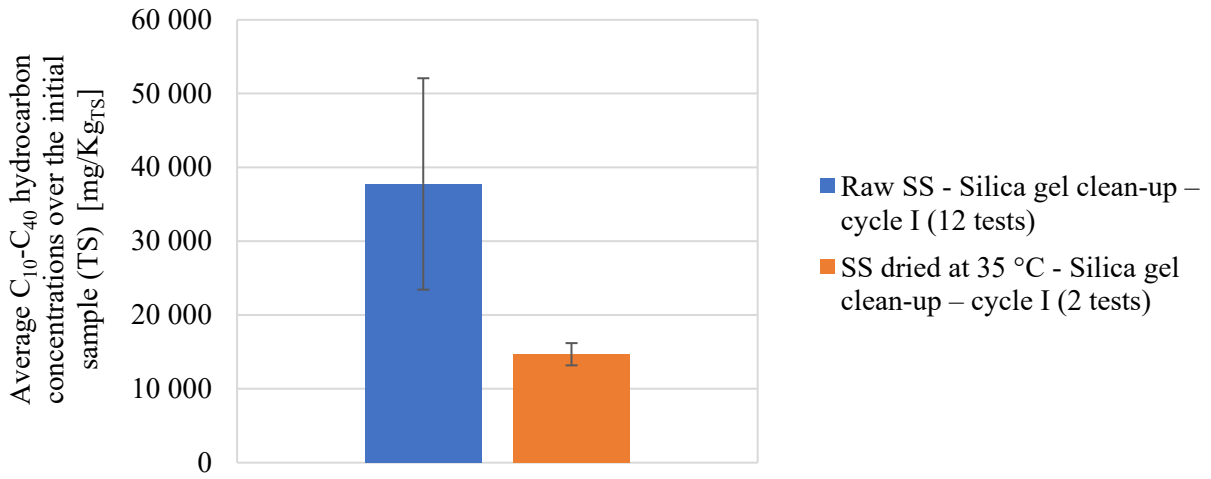

Figure 2.42. Comparison of average values of the concentrations of C_{10}-C_{40} hydrocarbons obtained by MWAE from raw and dried SS.

The results, reported in Table 2.23, show shallow values of the C_{10}-C_{40} hydrocarbon concentrations. Figure 2.42 shows a reduction in the average values of the concentrations of C_{10}-C_{40} hydrocarbons extracted from dried SS compared to the results obtained from extractions with dried SS samples. A possible explanation could be linked to the nature of the SS samples analyzed. Since this sample has been dried and deprived of water, lower temperatures and extraction yields may have been reached inside the PTFE capsule than those obtained on the raw sample. In fact, in the undried samples, there is water which, having a dipole moment, heats up more than the organic component, which could lead to more intense extractions. Furthermore, again regarding Figure 2.42, it can be noted that the standard deviation and the average values of the C_{10}-C_{40} hydrocarbon concentrations of the SS samples are very high and are probably due to the presence of residual water in the samples, which could interfere with clean-up operations and cause analysis errors. Finally, we cannot exclude that drying the SS sample causes a loss of the more volatile components; therefore, further investigations are necessary.

Evaluation of residual extractable fraction

Chemically post-conditioned and dewatered SS samples, previously extracted with microwaves, were used to perform a second extraction to evaluate the residual extractable fraction. For this test, four MWAE cycles were carried out, and, in particular, three samples were analyzed for cycles I, III, and

IV and four samples for cycle II. All extracted samples were subjected to a clean-up with silica gel only, and the test results, calculated according to equation (2.3), are reported in the following Tables.

Table 2.24. Evaluation tests of the residual extractable fraction with MWAE from previously extracted SS samples and with silica gel clean-up – cycle I.

Sample	M15	M16	M17
Extracted sample weight in PTFE capsule (g)	1.0163	1.0195	1.1693
C_{10}-C_{40} hydrocarbon weight (mg)	10.8910	15.4150	32.6180
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	10,716	15,120	27,895
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	10,716	15,120	27,895

Table 2.25. Evaluation tests of the residual extractable fraction with MWAE from previously extracted SS samples and with silica gel clean-up – cycle II.

Sample	M21$_{bis}$	M22$_{bis}$	M23$_{bis}$	M24$_{bis}$
Extracted sample weight in PTFE capsule (g)	1.9713	2.0928	2.0355	2.0416
C_{10}-C_{40} hydrocarbon weight (mg)	12.3660	15.9870	7.4760	16.1700
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	6,273	7,639	3,673	7,920
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	6,273	7,639	3,673	7,920

Table 2.26. Evaluation tests of the residual extractable fraction with MWAE from previously extracted SS samples and with silica gel clean-up – cycle III.

Sample	M25$_{bis}$	M27$_{bis}$	M28$_{bis}$
Extracted sample weight in PTFE capsule (g)	0.5374	0.5848	0.5559
C_{10}-C_{40} hydrocarbon weight (mg)	6.6300	7.6600	10.9000
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	1,2337	13,099	19,608
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	1,2337	13,099	19,608

Table 2.27. Evaluation tests of the residual extractable fraction with MWAE from previously extracted SS samples and with silica gel clean-up – cycle IV.

Sample	M32	M36	M37
Extracted sample weight in washed thimble (g)	0.5163	0.5460	0.5082
C_{10}-C_{40} hydrocarbon weight (mg)	3.5200	10.9170	4.1690
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	6,818	19,995	8,203
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	6,818	19,995	8,203

To verify the yield of the method and the effectiveness of the first extraction, a second MWAE was carried out on previously extracted SS samples. The results of the second extraction tests of the first cycle (Table 2.24) are very similar to those of the first extraction on raw SS samples under the same operating conditions. It is, therefore, possible to hypothesize the limited effectiveness of the first extraction. Table 2.25 shows that the values of the C_{10}-C_{40} hydrocarbon concentrations obtained from the evaluation tests of the residual extractable fraction of the second cycle are about six times lower than the first extraction on raw SS samples. The values are also repeatable and homogeneous. The results of the residual extractable fraction of the third cycle (Table 2.26) yielded still too high values of the C_{10}-C_{40} hydrocarbon concentrations. Finally, the evaluation tests of the residual extractable fraction of the fourth cycle (Table 2.27) show a decrease in concentrations compared to the first extraction, except for sample M36.

Spiking tests

Spiking tests were performed using chemically post-conditioned and dewatered SS samples previously extracted with microwaves. For these tests, two MWAE cycles were carried out, and, in particular, three samples were analyzed for each cycle examined. All the extracted samples were subjected to a clean-up with silica gel only; the test results, calculated according to equation (2.3), are reported in the following Tables.

Table 2.28. Spiking tests with MWAE from previously extracted SS and with silica gel clean-up – cycle III.

Sample	M29	M30	M31
Extracted sample weight in thimble (g)	0.5375	0.5848	0.5559
Added diesel oil (mg)	5.3740	5.8480	5.5590
C_{10}-C_{40} hydrocarbon weight (mg)	5.3980	9.3080	8.1160
C_{10}-C_{40} hydrocarbon concentration over dried sample (mg/kg)	9,945	15,759	14,455
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	9,945	15,759	14,455

Table 2.29. Spiking tests with MWAE from previously extracted SS and with silica gel clean-up – cycle IV.

Sample	M33	M34	M35
Extracted sample weight in thimble (g)	0.5622	0.5245	0.5009
Added diesel oil (mg)	5.6220	5.2450	5.0000
C_{10}-C_{40} hydrocarbon weight (mg)	6.8580	7.5900	6.2850
C_{10}-C_{40} hydrocarbon concentration over dried sample (mg/kg)	12,078	14,328	12,423
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	12,078	14,328	12,423

The results of the third cycle spiking tests (Table 2.28) are very similar to those obtained with the fourth cycle tests (Table 2.29). In both cases, C_{10}-C_{40} hydrocarbon concentration values are homogeneous and repeatable from one test to another, testifying to the goodness of the extraction methodology.

2.2.1.5. Choice of the extraction methodology

Regarding the previous Tables, the average concentration of the C_{10}-C_{40} hydrocarbons extracted with the various methodologies from samples of raw SS and samples of SS at the second extraction was calculated. With these values, it was possible to calculate the average recovery percentage for each extraction method (Table 2.30).

Table 2.30. Average recovery percentage for the various extraction methods on chemically post-conditioned and dewatered SS.

Extraction method	Operating conditions	Average C_{10}-C_{40} hydrocarbon conc. in raw SS (mg/kg_{TS})(*)	Average C_{10}-C_{40} hydrocarbon conc. in extracted SS (mg/kg_{TS})(*)	Average recovery percentage (%)
Soxhlet	Florisil – 4 h drying	107,401	-	-
	Florisil – 24 h drying	48,333	-	-
	Silica gel and Florisil – 4 h drying	110,518	-	-
	Silica gel and Florisil – 4 h drying	26,512	3024	88.54
Cold	Silica gel	55,979	21,367	61.83
MWAE	Silica gel – cycle I	37,761	17,911	52.56
	Silica gel – cycle II	39,896	6,376	84.01
	Silica gel – cycle III	21,364	12,718	40.47
	Silica gel – cycle IV	-	7,511	-

(*) F03 and M28bis were excluded from the calculation of the averages

From the results obtained, the best extraction method for analyzing the C_{10}-C_{40} hydrocarbon concentrations in the SS is Soxhlet, which has an average recovery percentage of 88.59%. The Soxhlet extraction technique, however, has a series of disadvantages, such as long extraction times and the use of high quantities of solvents, which make this method unsuitable due to the necessary reduction of the statistical sample. For these reasons, cold extraction and MWAE techniques have been considered, requiring an extraction time and a quantity of solvent lower than Soxhlet. The cold extraction methodology returned values of the average C_{10}-C_{40} hydrocarbon concentrations in raw SS samples pretty high compared with the other methods, with a recovery percentage of 61.83%. The MWAEs were carried out by varying the operating conditions and carrying out several cycles. From the results obtained, the values of the average C_{10}-C_{40} hydrocarbon concentrations in raw SS samples are very similar to the results obtained with the Soxhlet. Also, referring to cycles II and IV, very interesting recovery percentages were achieved and comparable with Soxhlet.

The MWAE method was chosen to simplify the application of the analytical method to an actual situation, as it achieves excellent results and extracts from eleven samples simultaneously, making the analysis process much faster.

2.2.1.6. Evaluation of the effects of anaerobic digestion on the degradation of C_{10}-C_{40} hydrocarbons

The pre-thickened, centrifuged, and dried SS samples were used only for cycle IV of MWAE. In particular, the extracted samples were subjected to a clean-up with silica gel only; test results carried out in duplicate, calculated according to equation (2.3), are reported in Table 2.31.

Table 2.31. MWAE tests from pre-thickened raw SS and with silica gel clean-up – cycle IV.

Sample	MT1	MT2
Extracted sample weight in washed thimble (g)	1.4582	1.4861
C_{10}-C_{40} hydrocarbon weight (mg)	13.4590	11.9090
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	9,230	8,014
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	10,285	8,930

The pre-thickened, centrifuged, and dried digested sludge samples were analyzed only by MWAE cycle IV. The extracted samples, also for this case, were subjected to a clean-up with only silica gel; the test results carried out in duplicate, calculated according to equation (2.3), are reported in Table 2.32.

Table 2.32. MWAE tests from digested pre-thickened sludge and with silica g. clean-up – cycle IV.

Sample	MD1	MD2
Extracted sample weight in washed thimble (g)	1.0371	1.0925
C_{10}-C_{40} hydrocarbon weight (mg)	9.2380	6.7880
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	8,908	6,213
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	9,864	6,881

The pre-thickened and digested SS samples were compared downstream of the MWAE to evaluate the effects of AD on the degradation of C_{10}-C_{40} hydrocarbons. By averaging the above results (Tables 2.31 and 2.32), the results reported in the following Table and Figure were obtained.

Table 2.33. Average percentage of degradation of C_{10}-C_{40} hydrocarbons in pre-thickened sludge after AD.

Sample	Raw SS	Digested SS	Degraded hydrocarbons	St. dev.
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	9,607	8,373	12.85%	1,892

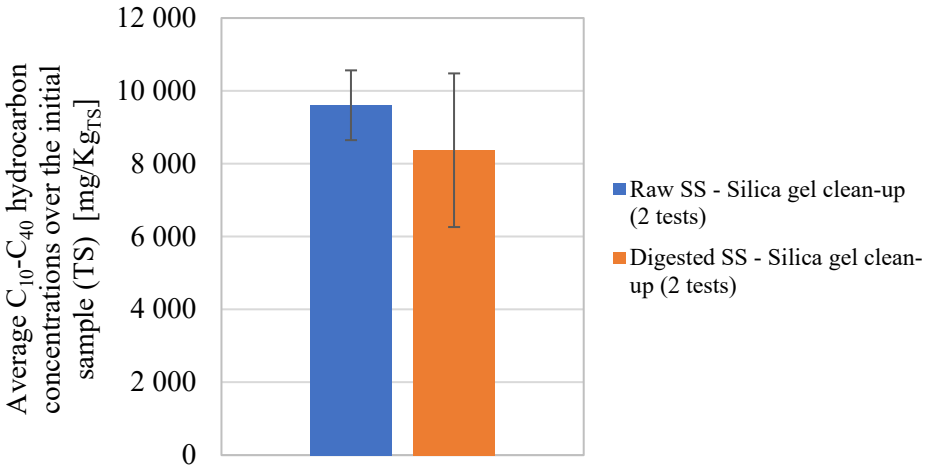

Figure 2.43. Comparison of average values of C_{10}-C_{40} hydrocarbon concentrations obtained with MWAE from pre-thickened and digested sludge.

After choosing the MWAE with cycle IV as the extraction methodology, the analytical method was applied to a real system to evaluate the effects of AD on the degradation of C_{10}-C_{40} hydrocarbons in a pre-thickened SS. Table 2.33 shows a degradation of the average C_{10}-C_{40} hydrocarbon concentration in SS of about 13 % after an AD period of 30 days. These results sufficiently agree with Grishchenkov et al., 2000, showing reductions in the concentrations of C_{10}-C_{40} hydrocarbons of about 15÷20 % through AD, although with a longer retention time, i.e., 180 days (Grishchenkov et al., 2000). In Figure 2.43, nonetheless, there would seem to be a not statistically significant reduction in the C_{10}-C_{40} hydrocarbon concentrations after AD ($p < 0.05$); therefore, further tests have been carried out in the following experimental steps (see paragraphs 3.1.1 and 3.2.1).

2.2.1.7. Evaluation of the effects of additives on C_{10}-C_{40} hydrocarbon concentrations in sewage sludge

MWAE tests performed on chemically post-conditioned and dewatered raw SS samples and on pre-thickened raw SS samples were compared to evaluate the effects of chemical conditioning; the results of the average values of C_{10}-C_{40} hydrocarbon concentrations are shown in Figure 2.44.

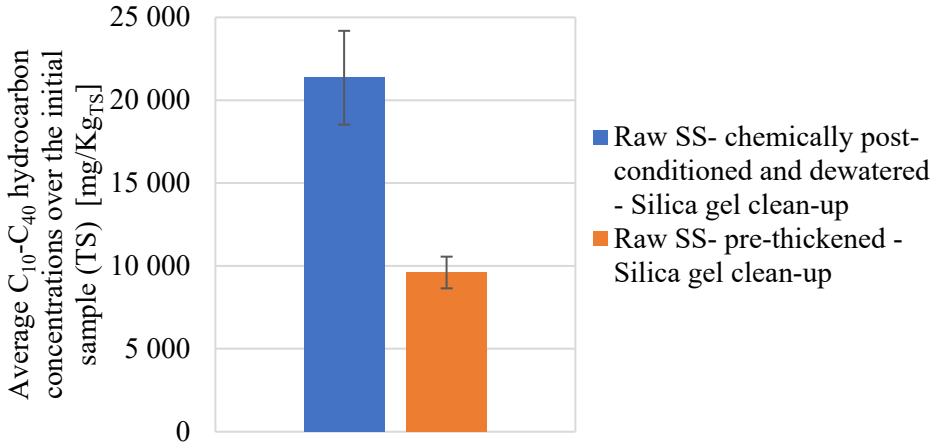

Figure 2.44. Comparison of average concentrations of C_{10}-C_{40} hydrocarbons between chemically post-conditioned and dewatered raw SS and pre-thickened raw SS.

A significant consideration that can be made is the verification of the difference in the C_{10}-C_{40} hydrocarbon concentrations between the chemically post-conditioned and dewatered SS and the pre-thickened SS. The values obtained from the extraction tests of the pre-thickened SS are unmistakably lower (almost half) compared to the extractions from chemically post-conditioned and dewatered SS (Figure 2.44), with a statistically significant difference. An explanation of the phenomenon could be linked to using large quantities of additives during the SS conditioning phase. These additives could cause interference problems with the measurement of C_{10}-C_{40} hydrocarbons or, being of a commercial nature, contain organic impurities which could lead to an increase in the C_{10}-C_{40} hydrocarbon concentrations in the chemically post-conditioned and dewatered SS.

2.2.1.8. Conclusions

- The drying phase is suggested to solve the problem of initial humidity.
- Soxhlet extraction is the ideal solution for measuring the C_{10}-C_{40} hydrocarbon concentrations in SS. This extraction method has been optimized through a clean-up with silica gel and Florisil and a drying period of at least 24 h, allowing an average recovery percentage of 88.59%.
- The cold extraction method returned higher C_{10}-C_{40} hydrocarbon concentration values than the others, with an average recovery percentage of 61.83%.
- MWAE, once optimized, represents a good alternative to using Soxhlet extraction. It allows multiple samples to be quickly extracted, to use small amounts of solvent, and to obtain reliable results, with an average recovery percentage, for the most optimized cycle, of 84.01%.
- AD in a pre-thickened SS, under mesophilic conditions and for a digestion period of 30 days, produced an average degradation of C_{10}-C_{40} hydrocarbon concentrations of 12.85%.
- For the examined samples, it was shown that the C_{10}-C_{40} hydrocarbon concentrations in the pre-thickened SS are considerably lower than in the chemically post-conditioned and dewatered SS.

2.2.2. Improvement of the methodology

2.2.2.1. Characterization and preparation of sewage sludge samples

The drying operations were carried out for a minimum of 6 continuous days. Data relating to the drying times of the samples and the relative percentages of TS obtained are presented below. All tests were carried out in triplicate. A drying period of 9 days resulted in an average TS percentage of 77.39%.

Table 2.34. TS characterization for 9 days dried SS.

Sample	1	2	3	Average	St. dev.
Crucible tare (g)	26.38	28.05	26.19		
Sample + crucible initial weight (g)	31.47	32.93	31.09		
Sample initial weight (g)	5.09	4.88	4.91		
Sample + crucible weight after drying at 105 °C (g)	30.46	31.98	29.70		
Sample weight after drying at 105 °C (g)	4.08	3.93	3.51		
Total solids fraction (TS) (%)	80.17	80.50	71.51	77.39	5.10

This SS was used for Soxhlet and cold extraction.

A drying period of 15 days resulted in an average TS percentage of 91.68%.

Table 2.35. TS characterization for 15 days dried SS.

Sample	1	2	3	Average	St. dev.
Crucible tare (g)	24.86	26.24	23.36		
Sample + crucible initial weight (g)	28.22	30.75	25.91		
Sample initial weight (g)	3.36	4.51	2.55		
Sample + crucible weight after drying at 105 °C (g)	27.95	30.37	25.69		
Sample weight after drying at 105 °C (g)	3.09	4.13	2.34		
Total solids fraction (TS) (%)	91.81	91.60	91.63	91.68	0.11

This SS was used for Soxhlet extraction.

A drying period of 17 days resulted in an average TS percentage of 93.35%.

Table 2.36. TS characterization for 17 days dried SS.

Sample	1	2	3	Average	St. dev.
Crucible tare (g)	28.18	25.27	26.24		
Sample + crucible initial weight (g)	32.98	29.79	30.67		
Sample initial weight (g)	4.80	4.52	4.43		
Sample + crucible weight after drying at 105 °C (g)	32.61	29.50	30.42		
Sample weight after drying at 105 °C (g)	4.43	4.22	4.17		
Total solids fraction (TS) (%)	92.38	93.48	94.19	93.35	0.91

This SS was used for MWAE.

A drying period of 33 days resulted in an average TS percentage of 94.94%.

Table 2.37. TS characterization for 33 days dried SS.

Sample	1	2	3	Average	St. dev.
Crucible tare (g)	24.83	28.05	26.19		
Sample + crucible initial weight (g)	28.93	32.34	31.58		
Sample initial weight (g)	4.10	4.29	5.39		
Sample + crucible weight after drying at 105 °C (g)	28.72	32.13	31.30		
Sample weight after drying at 105 °C (g)	3.89	4.08	5.12		
Total solids fraction (TS) (%)	94.96	94.96	94.89	94.94	0.94

This SS was used for Soxhlet extraction, using the acetone/hexane 1:1(v:v) mixture as solvent (CH_3COCH_3/C_6H_{14}).

2.2.2.2. Soxhlet extraction

Extraction from sewage sludge samples with TS=77.39% and dichloromethane solvent

For a second Soxhlet extraction cycle, 3 SS samples previously dried for nine days, which resulted in an average TS percentage of 77.39%, were used. The solvent used was dichloromethane. The extraction duration was 11 hours; then, the samples were subjected to a clean-up operation to remove water and polar substances. Anhydrous sodium sulfate was used for the removal of water, while for the removal of polar substances, the sample was treated with two clean-up cycles, each one characterized by the presence of a column packed with silica gel slurry followed in series by a syringe packed with a dry bed of Florisil (2 g). Finally, the sample was brought to a volume of about 5 mL

by removing the excess solvent through distillation. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.38. Soxhlet extraction from SS with TS=77.39%, CH_2Cl_2 solvent, silica gel, and Florisil clean-up.

Sample	S6	S7	S8
Drying time (days)	9	9	9
Sample weight in thimble (g)	9.23	10.38	9.41
TS fraction (%)	77.39	77.39	77.39
C_{10}-C_{40} hydrocarbon weight (mg)	31.21	33.92	40.99
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	3,382	3,270	4,358
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	4,369	4,224	5,631

Average (mg/kg_{TS})	Std. dev. (mg/kg_{TS})	Dev (%)
4,742	774.04	16

Where:

- "Average" is the average of the C_{10}-C_{40} hydrocarbon concentration values in the initial samples, considered in terms of total solids (mg/kg_{TS});
- "Std. dev." is the standard deviation of the C_{10}-C_{40} hydrocarbon concentration values in the initial samples, considered in terms of total solids (mg/kg_{TS});
- "Dev" is the standard deviation value compared to the average value, expressed in percentage terms (%).

Extraction from sewage sludge samples with TS=91.68% and dichloromethane solvent

For the first cycle of Soxhlet extractions, 5 SS samples previously 15 day-dried were used, which resulted in an average TS percentage of 91.68%. The solvent used was dichloromethane. The extraction duration was 11 hours, and the samples were subjected to a clean-up operation to remove water and polar substances. Anhydrous sodium sulfate was used to remove the water, while the sample was treated in columns packed with a silica gel slurry to remove polar substances. Finally, the sample was brought to a volume of about 5 mL, removing the excess solvent through distillation. The results obtained from the extraction tests, calculated according to equation (2.3), are reported below.

Table 2.39. Soxhlet extraction from SS with TS=91.68%, CH_2Cl_2 solvent, silica gel clean-up.

Sample	S1	S2	S3	S4	S5
Drying time (days)	15	15	15	15	15
Sample weight in thimble (g)	10.39	10.05	8.52	10.51	9.94
TS fraction (%)	91.68	91.68	91.68	91.68	91.68
C_{10}-C_{40} hydrocarbon weight (mg)	53.10	48.47	50.48	49.87	63.10
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	5,109	4,822	5,926	4,747	6,345
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	5,573	5,260	6,464	5,178	6,921

Average (mg/kg$_{TS}$)	Std. dev. (mg/kg$_{TS}$)	Dev (%)
5,879	774.02	13

Zero tests with dichloromethane solvent

A new test was performed using SS samples previously subjected to Soxhlet extraction to evaluate the residual extractable fraction. The test was carried out according to the same operating procedures as the previously described Soxhlet extractions. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.40. Soxhlet extraction from SS with TS=91.68%, CH_2Cl_2 solvent, silica gel clean-up, zero tests.

Sample	Z1	Z2	Z3
Drying time (days)	15	15	15
Sample weight in thimble (g)	7.50	7.94	8.44
TS fraction (%)	91.68	91.68	91.68
C_{10}-C_{40} hydrocarbon weight (mg)	16.96	27.51	16.57
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	2,262	3,465	1,964
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	2,467	3,780	2,143

Average (mg/kg$_{TS}$)	Residual percentage (%)
2,796	47.57

Where:

- "Average" is the average value of the C_{10}-C_{40} hydrocarbon concentration in the initial samples, considered in terms of total solids, obtained from zero tests (mg/kg$_{TS}$);

- "Residual percentage" is the index of the residual extractable fraction of C_{10}-C_{40} hydrocarbons, calculated as the ratio between the average concentration obtained from the Zero tests and that obtained from the extraction tests (%).

Spiking tests with dichloromethane solvent

A spiking test was conducted using diesel oil as a reference analyte, which was injected in the form of a spike solution into SS samples previously subjected to Soxhlet extraction to assess the accuracy of the measurement. The test was carried out according to the same operating procedures as the previously described Soxhlet extractions. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.41. Soxhlet extraction from SS with TS=91.68%, CH_2Cl_2 solvent, silica gel clean-up, spiking tests with spike solution.

Sample	S1	S2	S3
Drying time (days)	15	15	15
Sample weight in thimble (g)	7.85	7.78	7.85
TS fraction (%)	91.68	91.68	91.68
Added diesel oil (mg)	39.25	38.92	40.47
Expected diesel oil (mg)	29.92	29.66	30.85
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	29.33	33.70	25.83
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	3,718	4,309	3,176
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	4,055	4,700	3,464
Comparison with expected standard value (-)	0.98	1.14	0.84

Average (-)	Std. dev. (-)	Dev (%)
0.98	0.17	17

Where:

- "Average" is the average of the comparison values with the expected standard value (-);
- "Std. dev." is the standard deviation of the comparison values with the expected standard value (-);
- "Dev" is the standard deviation value compared to the average value, expressed in percentage terms (%).

Spiking tests were also carried out by injecting 50 µL of evaporated diesel oil into SS samples previously subjected to Soxhlet extraction. The test was carried out according to the same operating procedures as the previously described Soxhlet extractions. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.42. Soxhlet extraction from SS with TS=91.68%, CH_2Cl_2 solvent, silica gel clean-up, spiking tests with 50 µL of evaporated diesel oil.

Sample	S1	S2
Drying time (days)	15	15
Sample weight in thimble (g)	9.28	9.51
TS fraction (%)	91.68	91.68
Added diesel oil (mg)	42.02	42.02
Expected diesel oil (mg)	32.02	32.02
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	46.88	63.67
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	5,050	6,695.13
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	5,508	7,303
Comparison with expected standard value (-)	1.46	1.99

Average (-)	Std. dev. (-)	Dev (%)
1.73	0.37	21

From Table 2.42, it is possible to note that the value of diesel oil extracted is almost double the expected value. Most likely, the samples for the spiking operations were not properly prepared, with a residual fraction from previous extractions being unintentionally high.

Extraction from sewage sludge samples with TS=94.94% and acetone/hexane solvent

For this test, SS samples previously subjected to a drying period of 33 days were used, resulting in an average TS percentage equal to 94.94%. The solvent used was a mixture of acetone/hexane (1:1 – v:v). The extraction duration was 11 hours, and the samples were subjected to a clean-up operation to remove water and polar substances. Anhydrous sodium sulfate was used to remove water, while for the removal of polar substances, the sample, for the reasons set out above, was treated in columns packed with a dry bed of silica gel. Finally, the sample was brought to a volume of about 5 mL,

removing the excess solvent through distillation. The results obtained from the extraction tests, calculated according to equation (2.3), are reported below.

Table 2.43. Soxhlet extraction from SS with TS=94.94%, CH_3COCH_3/C_6H_{14}(1:1) solvent, silica gel dry bed clean-up.

Sample	E
Drying time (days)	33
Sample weight in thimble (g)	9.31
TS fraction (%)	94.94
C_{10}-C_{40} hydrocarbon weight (mg)	25.92
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	2,783
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	2,931

Zero test with acetone/hexane solvent

A new test was performed using SS samples previously subjected to extraction by Soxhlet to evaluate the residual extractable fraction. The test was carried out according to the same operating procedures as the previously described Soxhlet extractions, using a 1:1(v:v) acetone/hexane mixture as the solvent. The results obtained from the test, calculated according to equation (2.3), are reported below.

Table 2.44. Soxhlet extraction from SS with TS=94.94%, CH_3COCH_3/C_6H_{14}(1:1) solvent, silica gel dry bed clean-up, zero test

Sample	Z
Drying time (days)	15
Sample weight in thimble (g)	9.28
TS fraction (%)	91.68
C_{10}-C_{40} hydrocarbon weight (mg)	0.98
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	105.15
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	114.69

Residual percentage (%)
3.91

Spiking test with acetone/hexane solvent

A spiking test was performed by injecting 50 µL of evaporated diesel oil into SS samples previously subjected to Soxhlet extraction. The test was carried out according to the same operating procedures as the previously described Soxhlet extractions, using a 1:1(v:v) mixture of acetone/hexane as the solvent. The results obtained from the extraction test, calculated according to equation (2.3), are reported below.

Table 2.45. Soxhlet extraction from SS with TS=94.94%, CH_3COCH_3/C_6H_{14}(1:1) solvent, silica gel dry bed clean-up, spiking test with 50 µL of evaporated diesel oil.

Sample	S
Drying time (days)	15
Sample weight in thimble (g)	9.51
TS fraction (%)	91.68
Added diesel oil (mg)	42.02
Expected diesel oil (mg)	32.02
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	13.52
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	1,421
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	1,550
Comparison with expected standard value (-)	0.42

Extraction from sewage sludge samples with TS=30% and toluene/methanol solvent

For this test, a SS characterized by an average TS percentage equal to 30% was used. The solvent used was a mixture of toluene/methanol (10:1 – v:v). The extraction duration was 16 hours, and the samples were subjected to a clean-up operation to remove water and polar substances. Anhydrous sodium sulfate was used to remove the water, while the sample was treated in columns packed with a silica gel slurry to remove polar substances. Finally, the sample was brought to a volume of about 5 mL, removing the excess solvent through distillation. The results obtained from the extraction tests, calculated according to equation (2.3), are reported below.

Table 2.46. Soxhlet extraction from SS with TS=30%, $C_6H_5CH_3$/CH_3OH(10:1) solvent, silica gel clean-up.

Sample	E
Sample weight in thimble (g)	10.51
TS fraction (%)	30.00
C_{10}-C_{40} hydrocarbon weight (mg)	35.75
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	3,401
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	11,337

2.2.2.2.a. Investigation with gas chromatographic/mass spectrometry

All the chromatograms reported below were obtained by injecting into the GC-MS 1 µL of a solution composed of the extracted analytes diluted in 10 mL of acetone. Exceptions are the chromatograms relating to the analysis of evaporated and non-evaporated diesel oil, and those were obtained by injecting into the GC-MS 1 µL of a solution consisting of 50 µL of diesel oil brought to a volume of 10 mL with the addition of acetone.

GC-MS analysis of diesel oil

These chromatographic analyses were carried out to identify the main hydrocarbons in diesel oil, a substance we chose as the reference analyte in the spiking tests. Comparing these chromatograms and those obtained from the different extraction tests carried out with the various methods makes it possible to evaluate the latter's effectiveness.

Non-evaporated diesel oil

The chromatogram relating to the analysis of non-evaporated diesel oil assumes the trend shown in the Figure below.

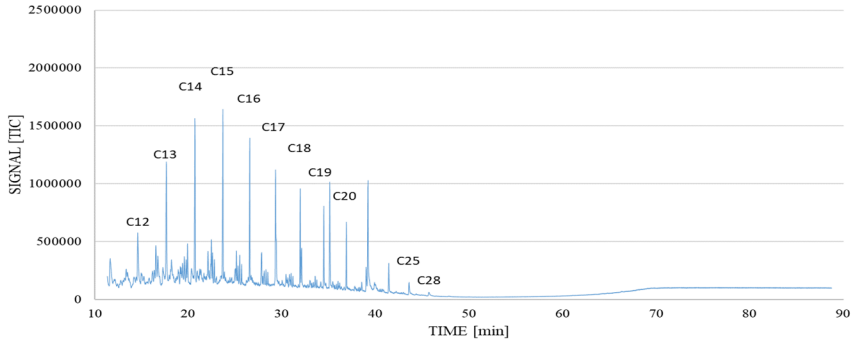

Figure 2.45. Non-evaporated diesel oil.

Evaporated diesel oil

The chromatogram relating to the analysis of evaporated diesel oil assumes the trend shown in the Figure below.

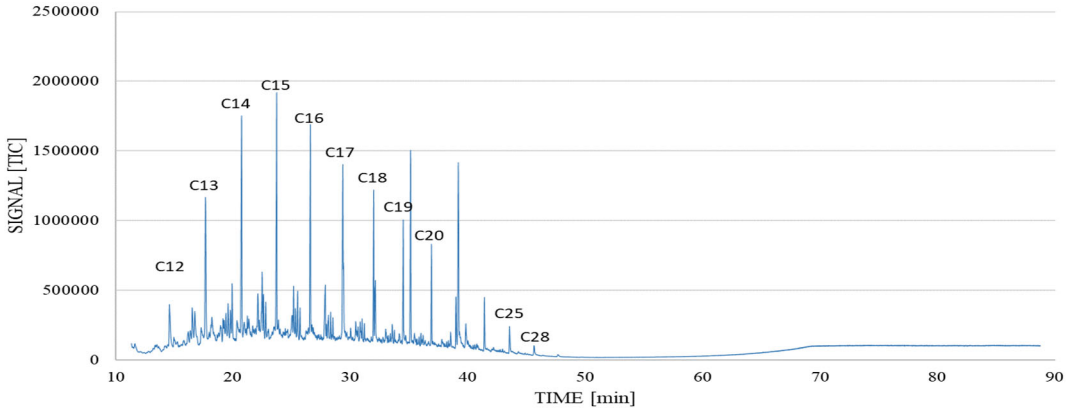

Figure 2.46. Evaporated diesel oil.

Comparison between evaporated and non-evaporated diesel oil

The comparison between the hydrocarbons constituting the evaporated and non-evaporated diesel oil is shown in the Figure below.

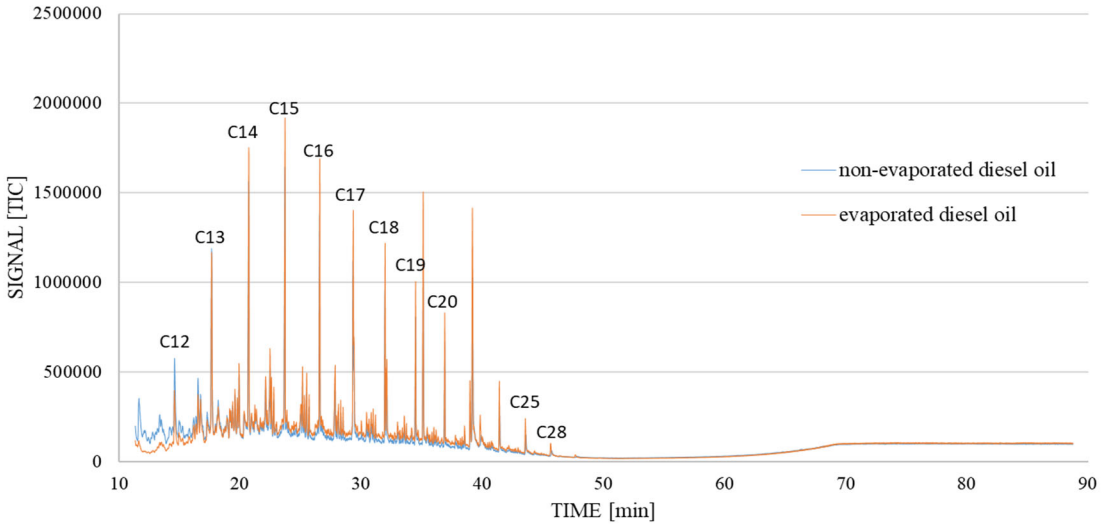

Figure 2.47. Comparison between evaporated and non-evaporated diesel oil.

2.2.2.2.b. GC-MS determination for Soxhlet extractions

Spiking test with 50 µL evaporated diesel oil and dichloromethane solvent

The chromatogram relating to the analysis of the substances extracted from dewatered SS using Soxhlet with dichloromethane during the spiking test with 50 µL of diesel oil injected into the sample assumes the trend shown in the Figure below.

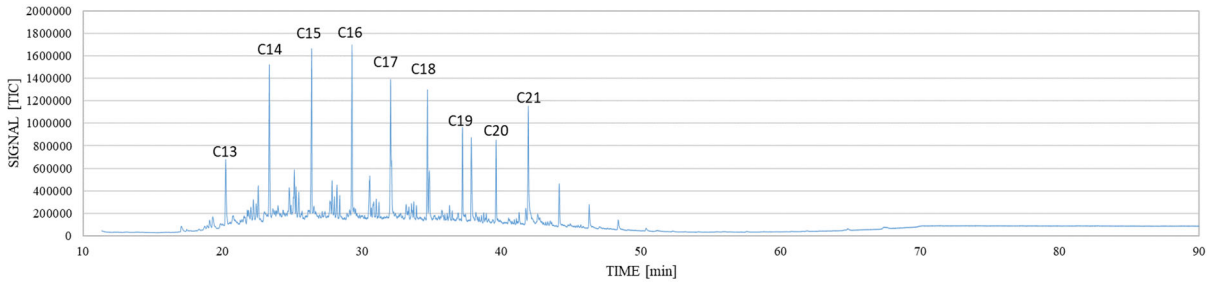

Figure 2.48. Spiking with 50 µL of diesel oil, dichloromethane solvent.

Extraction with dichloromethane solvent

During the extraction test, the chromatogram relating to the analysis of the substances extracted from dewatered SS using Soxhlet with dichloromethane assumes the trend shown in the Figure below.

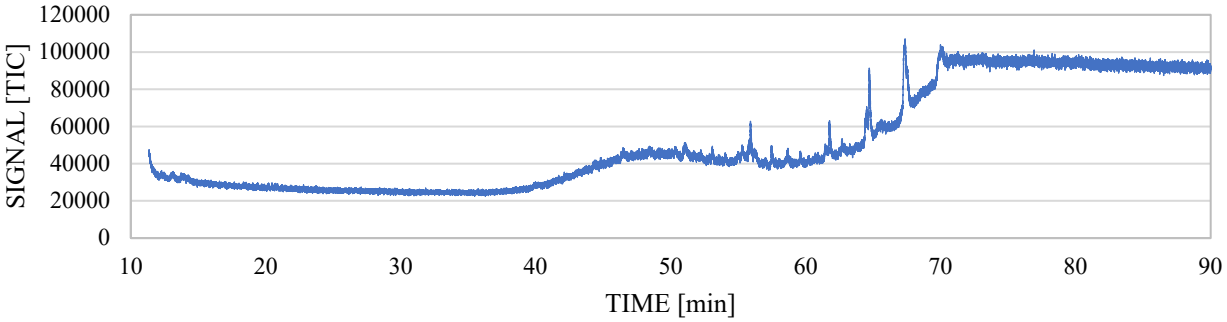

Figure 2.49. Soxhlet extraction, dichloromethane solvent.

Spiking test with 50 µL evaporated diesel oil and acetone/hexane solvent

The chromatogram relating to the analysis of the substances extracted from the dehydrated sludge using Soxhlet with a mixture of acetone/hexane 1:1(v:v) during the spiking test with 50 µL of evaporated diesel oil injected into the sample assumes the trend shown in the Figure below.

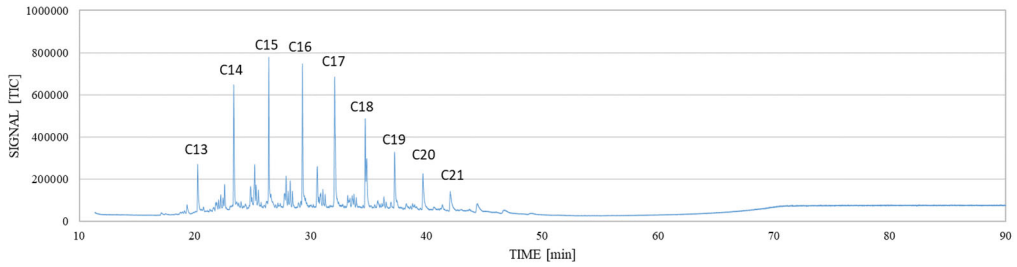

Figure 2.50. Spiking with 50 µL of diesel oil, CH_3COCH_3/C_6H_{14}(1:1) solvent.

Extraction with acetone/hexane solvent

The chromatogram relating to the analysis of the substances extracted from dewatered SS using Soxhlet with a mixture of acetone/hexane 1:1(v:v) as the solvent during the extraction test assumes the trend shown in the Figure below.

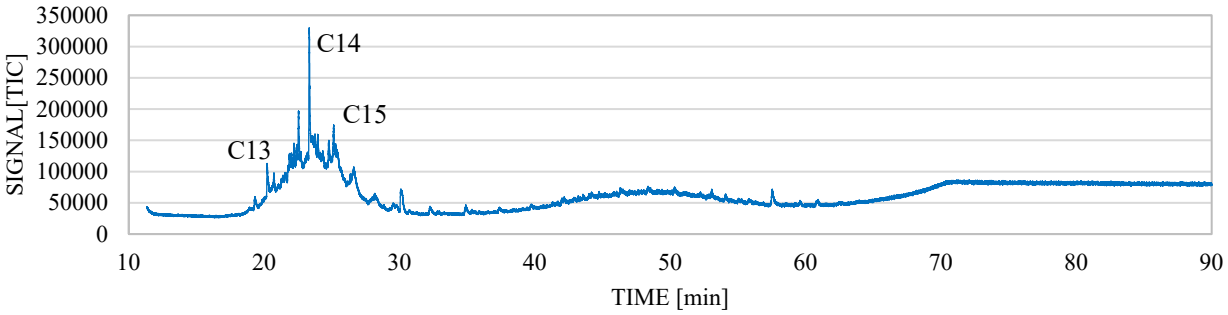

Figure 2.51. Soxhlet extraction, CH_3COCH_3/C_6H_{14}(1:1) solvent.

Zero test with acetone/hexane solvent

The chromatogram relating to the analysis of the substances extracted from dewatered SS using Soxhlet with a mixture of acetone/hexane 1:1(v:v) as the solvent during the zero test assumes the trend shown in the Figure below.

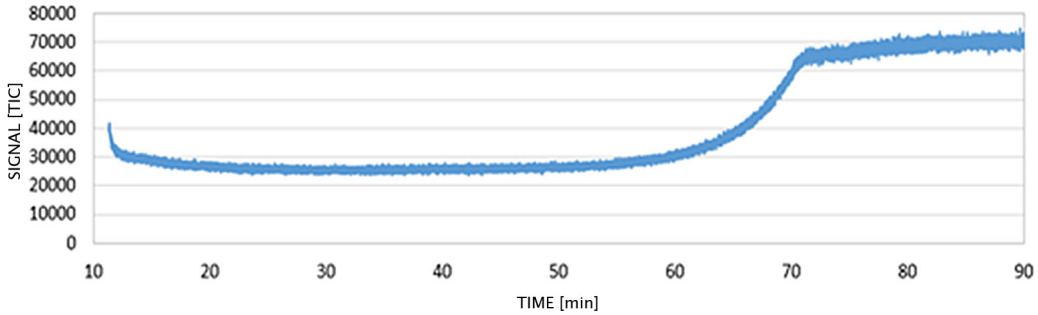

Figure 2.52. Zero test, CH_3COCH_3/C_6H_{14}(1:1) solvent.

Extraction with toluene/methanol solvent

The chromatogram relating to the analysis of the substances extracted from dewatered SS using Soxhlet with a mixture of toluene/methanol 10:1 (v:v) as the solvent during the extraction test assumes the trend shown in the Figure below.

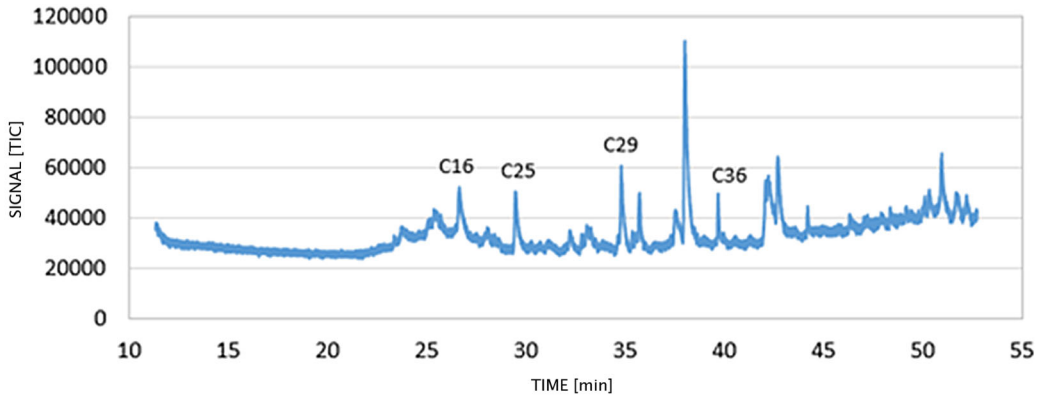

Figure 2.53. Soxhlet extraction, $C_6H_5CH_3/CH_3OH$ (10:1 v:v) solvent.

2.2.2.2.c. Discussion on Soxhlet extraction

Soxhlet extraction with dichloromethane

Zero tests conducted using dichloromethane as solvent (Table 2.40) gave a residual extractable fraction of the analytes of interest equal to 47.57%, as shown in Figure 2.54, which compares, with statistically significant difference ($p < 0.05$), the average value of the hydrocarbon concentration

obtained from the extraction tests with the average value of the residual hydrocarbon concentration obtained from the zero tests.

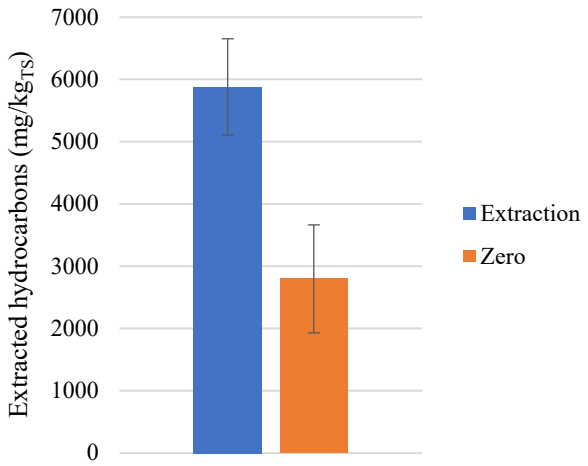

Fig. 2.54. Comparison between Soxhlet extraction and zero tests, CH_2Cl_2 solvent.

A residual percentage of 47.57%, considered quite high, would suggest that an extraction cycle lasting 11 hours is not sufficient to extract all the extractable analytes in the SS sample. However, from the spiking tests (Table 2.41), a recovery percentage of the reference analyte (diesel oil) injected in the sample equal to 98% is observed, as shown in Figure 2.55, in which the expected weight value of the diesel oil is compared, with statistically significant accordance ($p < 0.05$), with the extracted value.

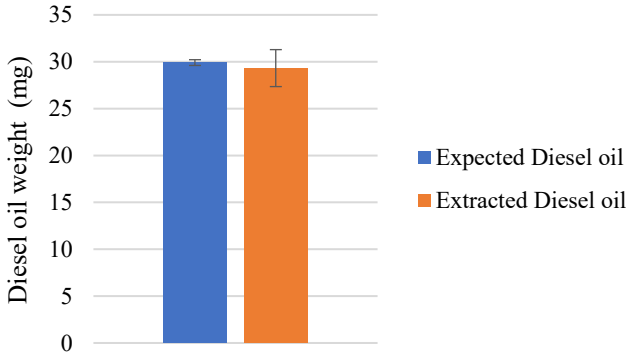

Fig. 2.55. Expected vs. extracted diesel oil in Soxhlet spiking tests, CH_2Cl_2 solvent.

Therefore, an extraction time lasting 11 hours is still sufficient for the Soxhlet extraction of C_{10}-C_{40} hydrocarbons. The confirmation of this statement can also be seen from the chromatogram relating to the spiking test (Figure 2.48), in which the part of the chromatogram relating to C_{10}-C_{40}

hydrocarbons rather faithfully follows that relating to evaporated diesel oil with a reasonable attenuation of the peak signals, as highlighted in the following figure.

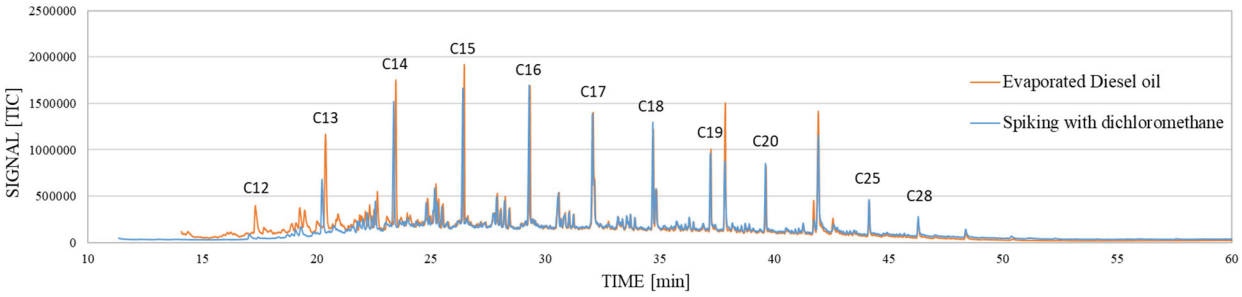

Fig. 2.56. Comparison between evaporated diesel oil and spiking in dichloromethane.

However, the chromatographic analysis of the substances extracted from the SS samples during the Soxhlet extraction tests (Figure 2.49) does not show the presence of C_{10}-C_{40} hydrocarbons. This probably suggests that dichloromethane can extract C_{10}-C_{40} hydrocarbons when injected in the form of diesel oil but cannot extract those possibly present in the SS, probably strongly retained by the NOM, which constitutes the SS matrix itself.

Soxhlet extraction with acetone/hexane

Zero tests conducted using the acetone/hexane 1:1 (v:v) mixture as solvent (Table 2.44) gave a residual percentage of the extractable analytes of interest equal to 3.91%, as shown in Figure 2.57, in which the average value of the hydrocarbon concentration obtained from the Soxhlet extraction tests is compared with the average value of the residual hydrocarbon concentration obtained from the zero tests.

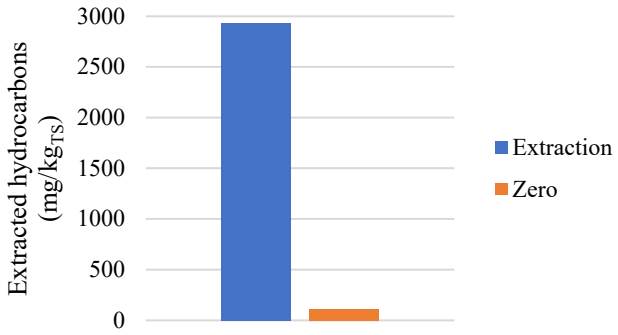

Fig. 2.57. Comparison between Soxhlet extraction and zero tests, CH_3COCH_3/C_6H_{14} solvent.

This result is considered entirely satisfactory, confirming that a Soxhlet extraction duration of 11 hours is sufficient to extract almost all the extractable analytes. It has been observed that each cycle (which includes boiling, evaporation, and condensation of the solvent, filling and emptying the extractor with siphoning) lasts about 30 minutes if dichloromethane is used as a solvent and 15 minutes if acetone/hexane mixture is used as a solvent. Therefore, under the same operating conditions and execution time, a double number of extraction cycles can be performed if a mixture of acetone/hexane is used. Therefore, a residual percentage of analytes with a mixture of acetone/hexane equal to 3.91%, compared to a residual percentage of analytes with dichloromethane equal to 47.57%, makes the mixture more favorable than the halogenated solvent.

From the chromatographic analysis of the substances extracted during the zero tests (Figure 2.52), there is no evidence of the presence of C_{10}-C_{40} hydrocarbons, testifying to the fact that they have already been extracted during the extraction tests.

However, compared to dichloromethane, this solvent gave lower yields (42% compared to 98%, as shown in Tables 2.45 and 2.41) in extracting the reference analyte (diesel oil) injected into the sample.

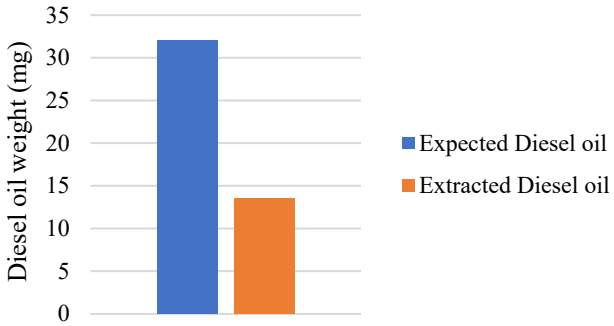

Fig. 2.58. Expected vs. extracted diesel oil in Soxhlet spiking tests, CH_3COCH_3/C_6H_{14} solvent.

The confirmation of this statement is also evident in the chromatogram relating to the spiking test (Figure 2.50), in which the intensity of the peak signals relating to C_{10}-C_{40} hydrocarbons is further attenuated compared to the case of dichloromethane, as shown in the following figure:

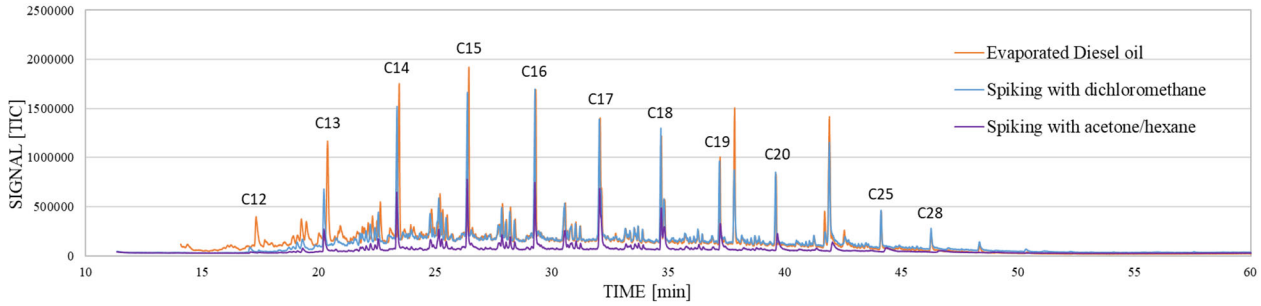

Fig. 2.59. Comparison between evaporated diesel oil and spiking in acetone/hexane.

However, from the chromatographic analysis concerning the substances extracted from the SS samples during the Soxhlet extraction tests (Figure 2.51), it is observed that the acetone/hexane mixture extracts only low molecular weight hydrocarbons (such as C_{13}, C_{14}, C_{15}). Heavier hydrocarbons (C_{16}, C_{25}, C_{29}, C_{36}) are, on the other hand, extractable using a 10:1 (v:v) toluene/methanol mixture as solvent.

Soxhlet extraction with toluene/methanol

From the chromatographic analysis of the substances extracted from the SS samples during the Soxhlet extraction tests (Figure 2.53), it is clear that the mixture of toluene/methanol 10:1 (v:v) extracts some hydrocarbons of the class C_{10}-C_{40} (C_{16}, C_{25}, C_{29}, C_{36}). From the same chromatogram, however, it is possible to understand that the presence of these substances in the extract is very modest, as shown by the weak peak signals detected.

2.2.2.3. Cold solid-liquid extraction

Extraction from sewage sludge samples with TS=77.39%

For the cold extraction, 10 SS samples were used previously subjected to a drying period of 9 days, which resulted in an average TS percentage of 77.39%. The solvent used was dichloromethane. The extraction process was iterated three times, removing the analyte-laden solvent and adding a new amount. Subsequently, the samples were subjected to the phase separation operation and then to the clean-up operation aimed at removing water and polar substances. Anhydrous sodium sulfate was

used to remove water, while, for the removal of polar substances, the sample was treated in columns packed with a silica gel slurry in series with a column packed with a dry bed of anhydrous sodium sulfate. Finally, the sample was brought to a volume of about 5 mL, removing the excess solvent through distillation. The results obtained from the extraction tests, calculated according to equation (2.3), are reported below

Table 2.47. Cold extraction from SS with TS=77.39%, CH_2Cl_2 solvent, silica gel + Na_2SO_4 dry bed clean-up.

Sample	F1	F2	F3	F4	F5
Drying time (days)	9	9	9	9	9
Sample weight in Falcon tube (g)	1.93	2.07	2.07	2.20	1.99
TS fraction (%)	77.39	77.39	77.39	77.39	77.39
C_{10}-C_{40} hydrocarbon weight (mg)	4.26	4.11	6.98	6.01	8.08
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	2,203	1,980	3,377	2,729	4,061
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	2,847	2,559	4,363	3,526	5,248

Sample	F6	F7	F8	F9	F10
Drying time (days)	9	9	9	9	9
Sample weight in Falcon tube (g)	2.02	2.22	2.00	2.07	1.85
TS fraction (%)	77.39	77.39	77.39	77.39	77.39
C_{10}-C_{40} hydrocarbon weight (mg)	3.83	3.82	5.55	3.10	2.91
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	1,895	1,726	2,770	1,501	1,576
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	2,448	2,230	3,579	1,940	2,036

Average (mg/kg_{TS})	Std. dev. (mg/kg_{TS})	Dev (%)
3,077	1,087	35

Zero tests

A new test was performed using 3 SS samples previously subjected to cold extraction to evaluate the residual extractable fraction. The test was carried out according to the same operating methods as the cold solid-liquid extractions described above. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.48. Cold extraction from SS with TS=77.39%, CH_2Cl_2 solvent, silica gel + Na_2SO_4 dry bed clean-up, zero tests.

Sample	ZF1	ZF2	ZF3
Drying time (days)	9	9	9
Sample weight in Falcon tube (g)	1.70	2.23	1.91
TS fraction (%)	77.39	77.39	77.39
C_{10}-C_{40} hydrocarbon weight (mg)	3.82	3.48	2.27
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	2,251	1,556	1,186
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	2,908	2,011	1,532

Average (mg/kg_{TS})	Residual percentage (%)
2,150	69.88

Spiking tests

A spiking test was conducted using diesel oil as a reference analyte, which was injected, in the form of spike solution, into SS samples previously subjected to cold extraction to evaluate the accuracy of the measurement. The test was carried out according to the same operating methods as the cold solid-liquid extractions described above. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.49. Cold extraction from SS with TS=77.39%, CH_2Cl_2 solvent, silica gel + Na_2SO_4 dry bed clean-up, spiking tests with spike solution.

Sample	SF1	SF2	SF3
Drying time (days)	9	9	9
Sample weight in Falcon tube (g)	1.86	2.04	1.77
TS fraction (%)	77.39	77.39	77.39
Added diesel oil (mg)	9.31	10.22	8.83
Expected diesel oil (mg)	7.10	7.79	6.73
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	5.02	4.75	3.57
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	2,698	2,323	2,022
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	3,486	3,002	2,613
Comparison with expected standard value (-)	0.71	0.61	0.53

Average (-)	Std. dev. (-)	Dev (%)
0.62	0.26	42

2.2.2.3.a. GC-MS determination for cold solid-liquid extractions

Spiking test with spike solution and dichloromethane solvent

The chromatogram obtained from the analysis of cold extracted substances from dewatered SS with dichloromethane during the test with 1 mL of spike solution injected into the sample for each gram of a dry substance assumes the trend shown in the Figure below.

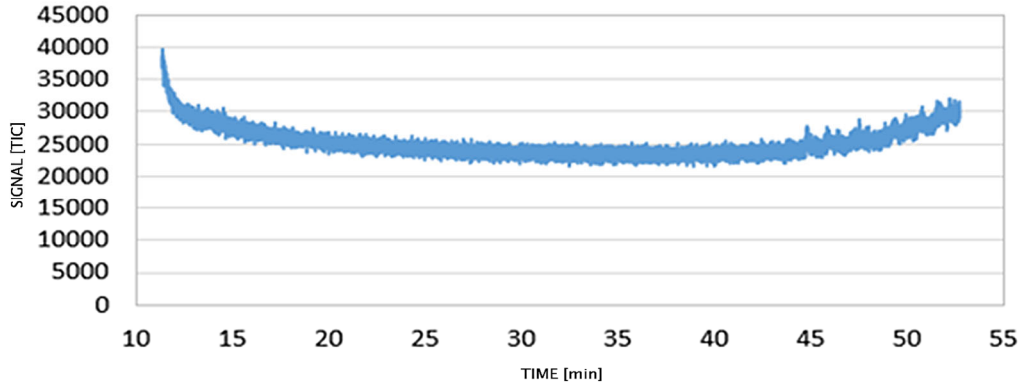

Figure 2.60. Spiking with spike solution, cold extraction, dichloromethane solvent.

Extraction with dichloromethane solvent

The chromatogram obtained from the analysis of cold-extracted substances from dewatered SS with dichloromethane during the extraction test assumes the trend shown in the Figure below.

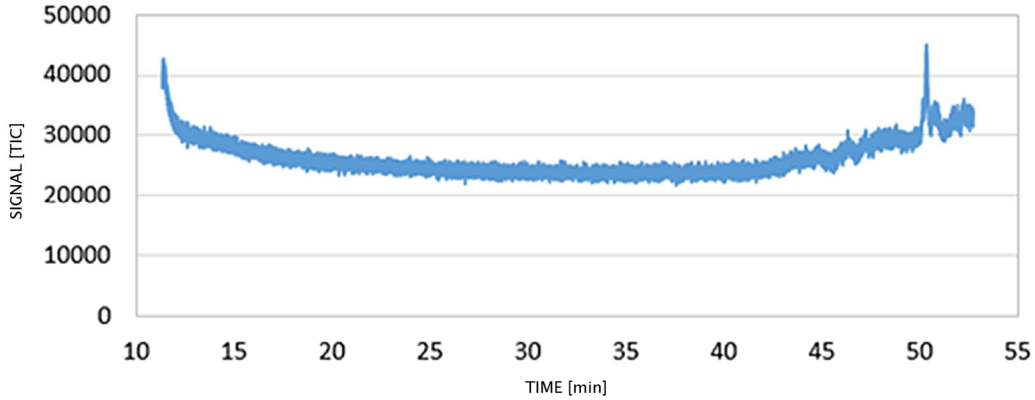

Figure 2.61. Cold extraction, dichloromethane solvent.

2.2.2.3.b. Discussion on cold solid-liquid extraction

Although optimized with 3 iteration cycles, the cold solid-liquid extraction does not produce satisfactory results. In fact, from the zero tests (Table 2.48), it can be deduced that the residual percentage of the extractable analytes of interest is equal to 69.88%, as shown in Figure 2.62, in which the average value of the hydrocarbon concentration obtained from the extraction tests is compared with the average value of the residual hydrocarbon concentration obtained from the zero tests.

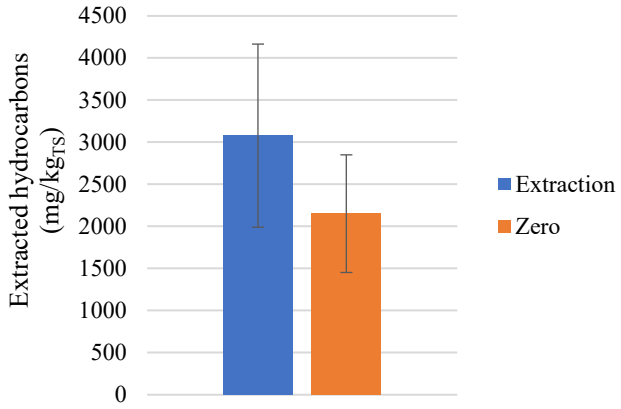

Figure 2.62. Comparison between cold extraction and zero tests, CH_2Cl_2 solvent.

From the spiking tests (Table 2.49), a percentage of recovery of the reference analyte (diesel oil) injected in the sample equal to 62% is observed, as shown in Figure 2.63, in which the expected weight value of the diesel oil is compared with the extracted value.

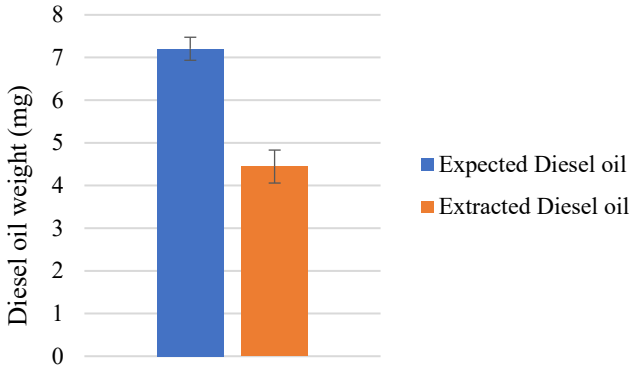

Figure 2.63. Expected vs. extracted diesel oil in cold spiking tests, CH_2Cl_2 solvent.

However, from the chromatographic analysis concerning the spiking tests (Figure 2.60), there is no evidence of the presence of C_{10}-C_{40} hydrocarbons. This could be because, for cold extractions, the spiking tests were carried out by injecting the sample with diesel oil in the form of spike solution, and, more precisely, 1 mL of spike solution was injected for each gram of dry sample. Therefore, since the expected quantity of diesel oil is very low (since it is related to a very small quantity of sample, about 2 g against about 10 g of the samples treated with Soxhlet extraction), and being the zero of the method very high, it is plausible that the diesel oil has not been extracted from the solvent, being below the reading threshold.

In addition, the chromatographic analysis of the substances extracted from the SS samples during the extraction tests (Figure 2.61) does not show the presence of C_{10}-C_{40} hydrocarbons. This suggests that dichloromethane cannot extract with the cold solid-liquid extraction method the C_{10}-C_{40} hydrocarbons present in the SS; those are probably strongly retained by the NOM, which constitutes the SS matrix itself.

2.2.2.4. Microwave-assisted extraction

Several cycles of MWAE tests were performed using SS samples previously subjected to drying for 17 days, by which an average TS percentage of 93.35% was reached. The solvent used was dichloromethane. Operating conditions, such as extraction temperature, extraction duration, and sample granulometry, distinguished the cycles. Subsequently, all samples were subjected to a clean-up operation to remove water and polar substances. Anhydrous sodium sulfate was used to remove the water, while the sample was treated in columns packed with a silica gel slurry to remove polar substances. The sample was finally brought to a volume of about 5 mL, removing the excess solvent through the distillation technique.

Extraction from sewage sludge samples, with TS=93.35%, duration=1h, T=45 °C and dichloromethane solvent

The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.50. MWAE from SS with TS=93.35%, T= 45 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up.

Sample	M1	M2	M4	M5	M6
Drying time (days)	17	17	17	17	17
Sample weight in thimble (g)	1.96	2.16	2.26	2.30	2.26
TS fraction (%)	93.35	93.35	93.35	93.35	93.35
C_{10}-C_{40} hydrocarbon weight (mg)	5.14	4.04	2.73	3.01	2.45
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	2,626.33	1,867.51	1,210.11	1,308.44	1,084.01
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	2,813.39	2,000.52	1,296.30	1,401.63	1,161.22

Sample	M7	M8	M9	M10
Drying time (days)	17	17	17	17
Sample weight in thimble (g)	2.18	2.00	1.93	2.17
TS fraction (%)	93.35	93.35	93.35	93.35
C_{10}-C_{40} hydrocarbon weight (mg)	0.51	3.57	4.19	2.57
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	234.81	1786.02	2,176.73	1,183.47
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	251.54	1913.22	2,331.76	1,267.76

Average (mg/kg$_{TS}$)	Std. dev. (mg/kg$_{TS}$)	Dev (%)
1,604.15	751.58	47

Extraction from sewage sludge samples, with TS=93.35%, duration=1h, T=85 °C and dichloromethane solvent

The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.51. MWAE from SS with TS=93.35%, T= 85 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up.

Sample	M1	M2	M3	M4	M5
Drying time (days)	17	17	17	17	17
Sample weight in thimble (g)	2.15	1.97	2.01	1.97	2.02
TS fraction (%)	93.35	93.35	93.35	93.35	93.35
C_{10}-C_{40} hydrocarbon weight (mg)	9.07	8.12	31.57	16.29	10.80
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	4,206.69	4,113.56	15,744.49	8,267.76	5,359.92
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	4,506.30	4,406.54	16,865.85	8,856.60	5,741.67

Average (mg/kg_{TS})	Std. dev. (mg/kg_{TS})	Dev (%)
5,877.78	2,076.65	35

Extraction from very fine-grained sewage sludge samples, with TS=93.35%, duration=1h, T=85 °C and dichloromethane solvent

The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.52. MWAE from very fine-grained SS with TS=93.35%, T= 85 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up.

Sample	M1	M2	M3	M4	M5	M6
Drying time (days)	17	17	17	17	17	17
Sample weight in thimble (g)	2.18	1.97	1.97	1.98	1.82	1.30
TS fraction (%)	93.35	93.35	93.35	93.35	93.35	93.35
C_{10}-C_{40} hydrocarbon weight (mg)	7.86	8.37	7.89	5.87	7.56	1.27
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	3,602	4,237	4,007	2,958	4,161	980
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	3,858	4,539	4,293	3,168	4,457	1,050

Average (mg/kg_{TS})	Std. dev. (mg/kg_{TS})	Dev (%)
3,561	1,330	37

Zero tests on sewage sludge samples, with TS=93.35%, duration=1h, T=45 °C and dichloromethane solvent

A new test was performed using SS samples previously subjected to MWAE to evaluate the residual extractable fraction. The test was carried out according to the same operating methods as the previously described MWAE. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.53. MWAE from SS with TS=93.35%, T= 45 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up, zero tests.

Sample	ZM1	ZM2	ZM3
Drying time (days)	17	17	17
Sample weight in thimble (g)	2.11	1.88	1.95
TS fraction (%)	93.35	93.35	93.35
C_{10}-C_{40} hydrocarbon weight (mg)	4.38	2.64	1.14
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	2,073	1,400	585
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	2,220	1,500	626

Average (mg/kg_{TS})	Residual percentage (%)
1,449	90.32

Zero tests on sewage sludge samples, with TS=93.35%, duration=1h, T=85 °C and dichloromethane solvent

A new test was performed using SS samples previously subjected to MWAE to evaluate the residual extractable fraction. The test was carried out according to the same operating methods as the previously described MWAE. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.54. MWAE from SS with TS=93.35%, T= 85 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up, zero tests.

Sample	ZM1	ZM2	ZM3
Drying time (days)	17	17	17
Sample weight in thimble (g)	1.51	1.59	1.86
TS fraction (%)	93.35	93.35	93.35
C_{10}-C_{40} hydrocarbon weight (mg)	1.77	1.08	1.51
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	1,176	681	812
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	1,260	730	870

Average (mg/kg$_{TS}$)	Residual percentage (%)
953	16.22

Zero tests on very fine-grained sewage sludge samples, with TS=93.35%, duration=1h, T=85 °C and dichloromethane solvent

A new test was performed using SS samples previously subjected to MWAE to evaluate the residual extractable fraction. The test was carried out according to the same operating methods as the previously described MWAE. The results obtained from the tests are reported below.

Table 2.55. MWAE from very fine-grained SS with TS=93.35%, T= 85 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up, zero tests.

Sample	Z1	Z2	Z3
Drying time (days)	17	17	17
Sample weight in thimble (g)	1.47	1.49	1.53
TS fraction (%)	93.35	93.35	93.35
C_{10}-C_{40} hydrocarbon weight (mg)	1.23	0.52	0.59
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	842	352	387
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	902	377	414

Average (mg/kg$_{TS}$)	Residual percentage (%)
564	13.89

Spiking tests on sewage sludge samples, with TS=93.35%, duration=1h, T=45 °C and dichloromethane solvent

A spiking test was carried out using diesel oil as a reference analyte, which was injected, in the form of spike solution, into SS samples previously subjected to MWAE to evaluate the accuracy of the measurement. The test was carried out according to the same operating methods as the previously described MWAE. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.56. MWAE from SS with TS=93.35%, T= 45 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up, spiking tests with spike solution.

Sample	SM1	SM2	SM3
Drying time (days)	17	17	17
Sample weight in thimble (g)	1.85	2.10	2.04
TS fraction (%)	93.35	93.35	93.35
Added diesel oil (mg)	9.24	10.50	10.19
Expected diesel oil (mg)	7.04	8.00	7.77
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	2.47	2.07	0.75
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	1,339	986	369
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	1,435	1,056	395
Comparison with expected standard value (-)	0.35	0.26	0.10

Average (-)	Std. dev. (-)	Dev (%)
0.24	0.13	55

Spiking tests on sewage sludge samples, with TS=93.35%, duration=1h, T=85 °C and dichloromethane solvent

A spiking test was carried out using diesel oil as a reference analyte, which was injected, in the form of spike solution, into SS samples previously subjected to MWAE to evaluate the accuracy of the measurement. The test was carried out according to the same operating methods as the previously described MWAE. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.57. MWAE from SS with TS=93.35%, T= 85 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up, spiking tests with spike solution.

Sample	SM1	SM2	SM3
Drying time (days)	17	17	17
Sample weight in thimble (g)	1.32	1.52	1.55
TS fraction (%)	93.35	93.35	93.35
Added diesel oil (mg)	6.60	7.61	7.75
Expected diesel oil (mg)	5.03	5.80	5.91
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	2.01	1.53	2.95
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	1,526	1,006	1,906
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	1,635	1,078	2,042
Comparison with expected standard value (-)	0.40	0.26	0.50

Average (-)	Std. dev. (-)	Dev (%)
0.39	0.12	31

Spiking tests on very fine-grained sewage sludge samples, with TS=93.35%, duration=1h, T=85 °C and dichloromethane solvent

A spiking test was carried out using diesel oil as a reference analyte, which was injected, in the form of spike solution, into SS samples previously subjected to MWAE to evaluate the accuracy of the measurement. The test was carried out according to the same operating methods as the previously described MWAE. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.58. MWAE from very fine-grained SS with TS=93.35%, T= 85 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up, spiking tests with spike solution.

Sample	SM1	SM2	SM3
Drying time (days)	17	17	17
Sample weight in thimble (g)	1.48	1.64	1.53
TS fraction (%)	93.35	93.35	93.35
Added diesel oil (mg)	7.39	8.21	7.66
Expected diesel oil (mg)	5.63	6.26	5.84
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	0.85	0.77	0.37
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	577	468	241
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg$_{TS}$)	618	502	258
Comparison with expected standard value (-)	0.15	0.12	0.06

Average (-)	Std. dev. (-)	Dev (%)
0.11	0.05	40

Spiking tests on very fine-grained sewage sludge samples, with TS=93.35%, duration=1h, T=85 °C, stock solution and dichloromethane solvent

A spiking test was carried out using diesel oil as a reference analyte, which was injected, in the form of stock solution, into SS samples previously subjected to MWAE to evaluate the accuracy of the measurement. The test was carried out according to the same operating methods as the previously described MWAE. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.59. MWAE from very fine-grained SS with TS=93.35%, T= 85 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up, spiking tests with stock solution.

Sample	SM1	SM2	SM3
Drying time (days)	17	17	17
Sample weight in thimble (g)	1.01	1.08	1.00
TS fraction (%)	93.35	93.35	93.35
Added diesel oil (mg)	50.66	53.93	50.04
Expected diesel oil (mg)	38.61	41.10	38.14
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	0.86	1.85	0.97
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	846	1713	967
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	906	1835	1036
Comparison with expected standard value (-)	0.02	0.04	0.03

Average (-)	Std. dev. (-)	Dev (%)
0.03	0.01	40

Spiking tests on very fine-grained sewage sludge samples, with TS=93.35%, duration=1h, T=85 °C, 50 µL evaporated diesel oil and dichloromethane solvent

Spiking tests were performed by injecting 50 µL of evaporated diesel oil into SS samples previously subjected to MWAE. The test was carried out according to the same operating methods as the previously described MWAE. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.60. MWAE from very fine-grained SS with TS=93.35%, T= 85 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up, spiking tests with 50 µL evaporated diesel oil.

Sample	SM1	SM2	SM3
Drying time (days)	17	17	17
Sample weight in thimble (g)	0.93	0.85	0.54
TS fraction (%)	93.35	93.35	93.35
Added diesel oil (mg)	42.02	42.02	42.02
Expected diesel oil (mg)	32.02	32.02	32.02
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	0.87	1.55	2.10
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	934	1,837	3,867
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	1,000	1,968	4,144
Comparison with expected standard value (-)	0.03	0.05	0.07

Average (-)	Std. dev. (-)	Dev (%)
0.05	0.02	41

Spiking tests on very fine-grained sewage sludge samples, with TS=93.35%, duration=1h, T=85 °C, 50 µL evaporated diesel oil, and dichloromethane solvent, without thimble

Spiking tests were performed by injecting 50 µL of evaporated diesel oil into SS samples previously subjected to MWAE. The test was carried out according to the same operating procedures described above, except for the cellulose fiber thimble. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.61. MWAE without thimble from very fine-grained SS with TS=93.35%, T= 85 °C, 1h, CH_2Cl_2 solvent, silica gel clean-up, spiking tests with 50 µL evaporated diesel oil.

Sample	SM1	SM2	SM3
Drying time (days)	17	17	17
Sample weight in PTFE capsule (g)	0.77	0.73	0.72
TS fraction (%)	93.35	93.35	93.35
Added diesel oil (mg)	42.02	42.02	42.02
Expected diesel oil (mg)	32.02	32.02	32.02
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	0.32	0.21	0.27
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	416	285	383
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	445	305	410
Comparison with expected standard value (-)	0.01	0.01	0.01

Average (-)	Std. dev. (-)	Dev (%)
0.01	0.00	21

Spiking tests on very fine-grained sewage sludge samples, with TS=91.68%, duration=2h, T=95 °C, 50 µL evaporated diesel oil and dichloromethane solvent

Spiking tests were performed by injecting 50 µL of evaporated diesel oil into SS samples previously subjected to MWAE. The test was carried out according to the same operating methods as the previously described MWAE. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.62. MWAE from very fine-grained SS with TS=91.68%, T= 95 °C, 2h, CH_2Cl_2 solvent, silica gel clean-up, spiking tests with 50 µL evaporated diesel oil.

Sample	SM1	SM2	SM3
Drying time (days)	15	15	15
Sample weight in thimble (g)	1.03	1.05	1.02
TS fraction (%)	91.68	91.68	91.68
Added diesel oil (mg)	42.02	42.02	42.02
Expected diesel oil (mg)	32.02	32.02	32.02
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	4.95	1.97	1.80
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	4,790	1,888	1,755
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	5,225	2,059	1,914
Comparison with expected standard value (-)	0.15	0.06	0.06

Average (-)	Std. dev. (-)	Dev (%)
0.09	0.06	61

Spiking tests on very fine-grained sewage sludge samples, with TS=91.68%, duration=1h, T=85 °C, 50 µL evaporated diesel oil and acetone/hexane solvent

Spiking tests were performed by injecting 50 µL of evaporated diesel oil into SS samples previously subjected to MWAE. The test was carried out according to the same operating methods as the previously described MWAE but using a 1:1(v:v) mixture of acetone and hexane as the solvent. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.63. MWAE from very fine-grained SS with TS=91.68%, T= 85 °C, 1h, acetone/hexane (1:1) solvent, silica gel clean-up, spiking tests with 50 µL evaporated diesel oil.

Sample	SM1	SM2	SM3
Drying time (days)	15	15	15
Sample weight in thimble (g)	1.18	1.14	1.15
TS fraction (%)	91.68	91.68	91.68
Added diesel oil (mg)	42.02	42.02	42.02
Expected diesel oil (mg)	32.02	32.02	32.02
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	8.80	8.52	12.38
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	7,453	7,445	10,795
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	8,130	8,120	11,774
Comparison with expected standard value (-)	0.27	0.27	0.39

Average (-)	Std. dev. (-)	Dev (%)
0.31	0.07	22

Spiking tests on very fine-grained sewage sludge samples, with TS=91.68%, duration=2h, T=85 °C, 50 µL evaporated diesel oil and acetone/hexane solvent

Spiking tests were performed by injecting 50 µL of evaporated diesel oil into SS samples previously subjected to MWAE. The test was carried out according to the same operating methods as the previously described MWAE, using a 1:1(v:v) mixture of acetone and hexane as the solvent. The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.64. MWAE from very fine-grained SS with TS=91.68%, T= 85 °C, 2h, acetone/hexane (1:1) solvent, silica gel clean-up, spiking tests with 50 µL evaporated diesel oil.

Sample	SM1	SM2	SM3
Drying time (days)	15	15	15
Sample weight in thimble (g)	1.23	1.30	1.07
TS fraction (%)	91.68	91.68	91.68
Added diesel oil (mg)	42.02	42.02	42.02
Expected diesel oil (mg)	32.02	32.02	32.02
C_{10}-C_{40} hydrocarbon weight (extracted diesel oil) (mg)	6.61	9.96	14.04
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	5,372	7,637	13,071
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	5,860	8,330	14,257
Comparison with expected standard value (-)	0.21	0.31	0.44

Average (-)	Std. dev. (-)	Dev (%)
0.32	0.12	36

Extraction from sewage sludge samples, with TS=30%, duration=1h, T=85 °C and with toluene/methanol solvent

The results obtained from the tests, calculated according to equation (2.3), are reported below.

Table 2.65. MWAE from SS with TS=30%, T= 85 °C, 1h, $C_6H_5CH_3$/CH_3OH(10:1) solvent, silica gel clean-up.

Sample	E
Sample weight in thimble (g)	1.98
TS fraction (%)	30.00
C_{10}-C_{40} hydrocarbon weight (mg)	7.55
C_{10}-C_{40} hydrocarbon concentration over the initial sample (mg/kg)	3,825
C_{10}-C_{40} hydrocarbon concentration over the initial sample (TS) (mg/kg_{TS})	12,749

2.2.2.4.a. GC-MS determination for microwave-assisted extraction

Spiking test with 50 µL evaporated diesel oil and dichloromethane solvent

The chromatogram relating to the analysis of the substances extracted from dewatered SS using MWAE at 85 °C for 1h, with dichloromethane, during the spiking test with 50 µL of diesel oil injected into the sample assumes the trend shown in the Figure below.

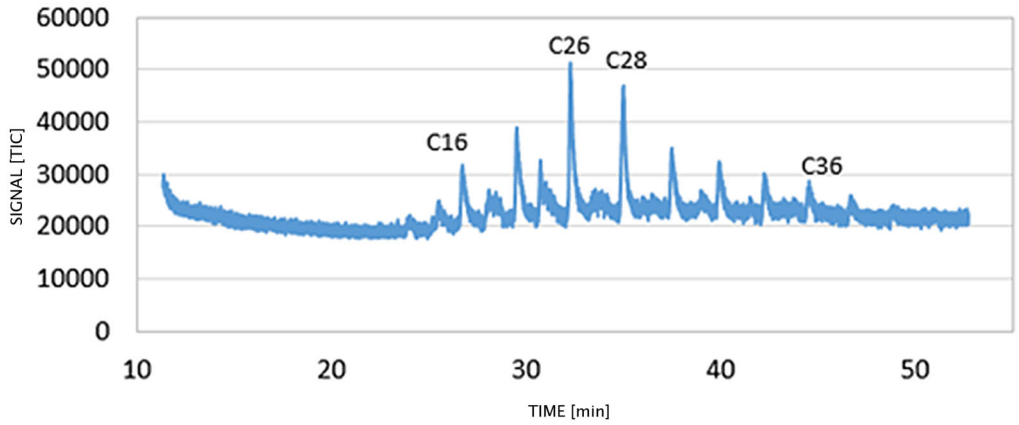

Figure 2.64. Spiking with 50 µL of diesel oil, 85°C 1h MWAE, dichloromethane solvent.

Extraction with dichloromethane solvent

The chromatogram obtained from the analysis of extracted substances from dewatered SS using MWAE at 85 °C for 1h, with dichloromethane, during the extraction test, assumes the trend shown in the Figure below.

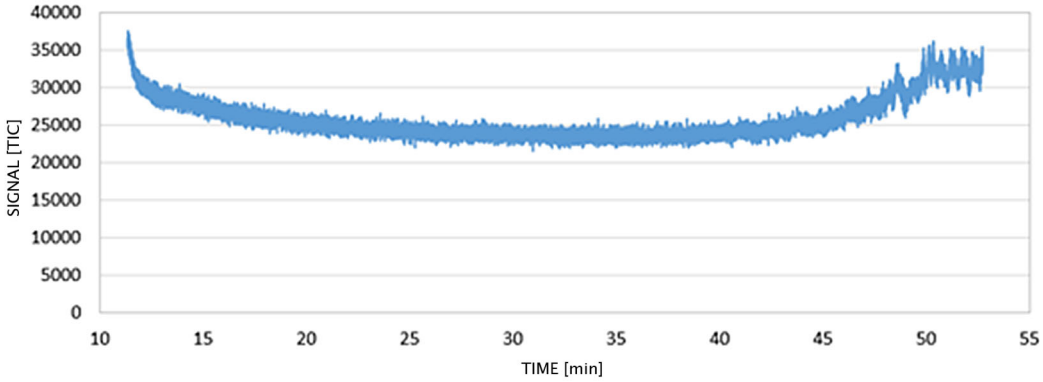

Figure 2.65. 85°C 1 h MWAE, dichloromethane solvent.

Spiking test with 50 µL evaporated diesel oil and with acetone/hexane solvent

The chromatogram obtained from the analysis of the substances extracted from the dehydrated sludge MWAE at 85 °C for 1 h with a mixture of acetone/hexane 1:1 (v:v) during the spiking test with 50 µL of evaporated diesel oil injected into the sample, assumes the trend shown in the Figure below.

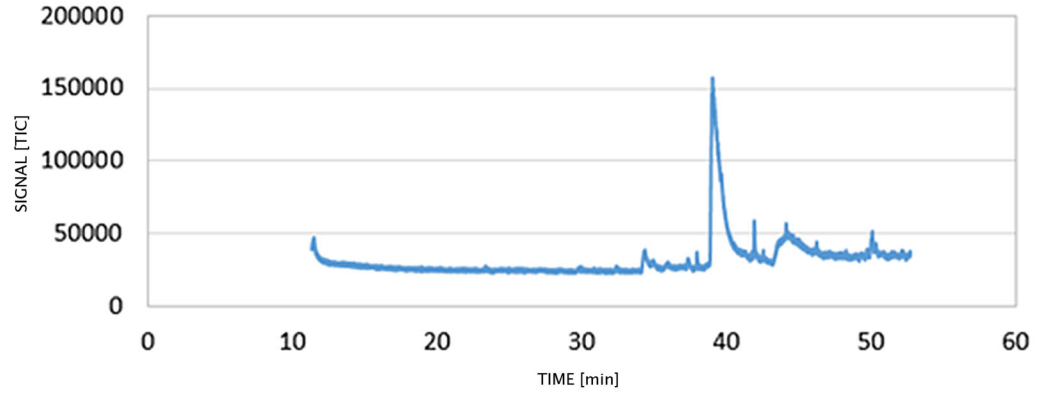

Figure 2.66. Spiking with 50 µL of diesel oil, 85°C 1 h MWAE, CH_3COCH_3/C_6H_{14} (1:1) solvent.

Zero test with acetone/hexane solvent

The chromatogram obtained from the analysis of the substances extracted from the dehydrated sludge MWAE at 85 °C for 1 h, with a mixture of acetone/hexane 1:1(v:v), during the zero test, assumes the trend shown in the Figure below.

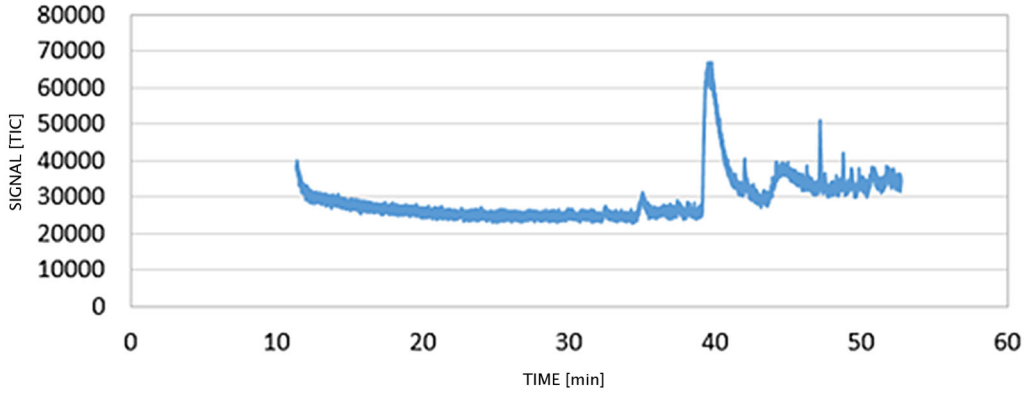

Figure 2.67. Zero test, 85°C 1h MWAE, CH_3COCH_3/C_6H_{14}(1:1) solvent.

Extraction with toluene/methanol solvent

The chromatogram obtained from the analysis of the extracted substances from dewatered SS using MWAE at 85 °C for 1 h, with toluene/methanol 10:1(v:v), during the extraction test, assumes the trend shown in the Figure below.

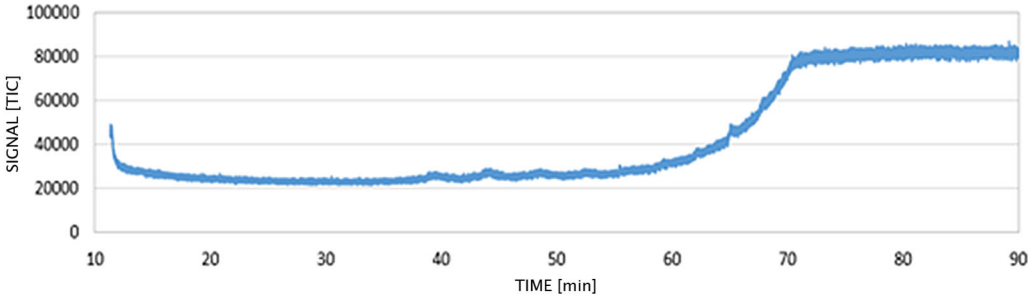

Figure 2.68. 85°C 1h MWAE, $C_6H_5CH_3/CH_3OH$ (10:1 v:v) solvent.

2.2.2.4.b. Discussion on microwave-assisted extraction

Extraction at 45 °C for 1 h with dichloromethane solvent

The results obtained from this methodology are not satisfactory, as the quantities of the extracted substance are meager (Table 2.50). The zero of the instrument is very high (Table 2.53), providing a residual percentage of the extractable analytes of interest equal to 90.32%, as shown in Figure 2.69, in which the average value of the hydrocarbon concentration obtained from the extraction tests is compared, with no statistically significant difference ($p > 0.05$), with the average value of the residual hydrocarbon concentration obtained from the zero tests.

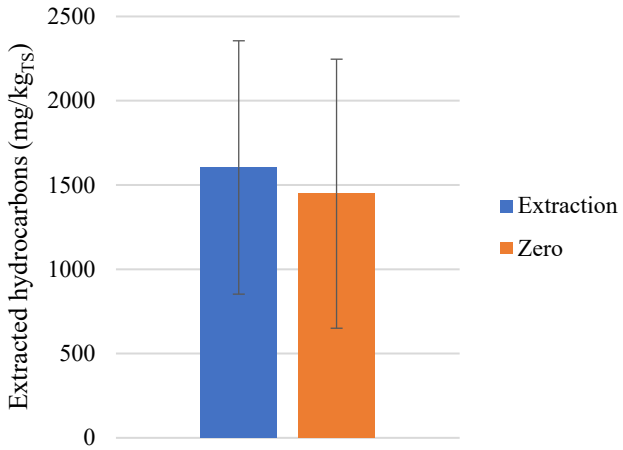

Figure 2.69. Comparison between MWAE and zero tests, 45°C, 1h, CH_2Cl_2 solvent.

Also, from the spiking tests (Table 2.56), a very low recovery percentage of the reference analyte (diesel oil) injected in the sample is observed, equal to 24%, as shown in Figure 2.70, in which the expected weight value of the diesel oil is compared with the extracted value.

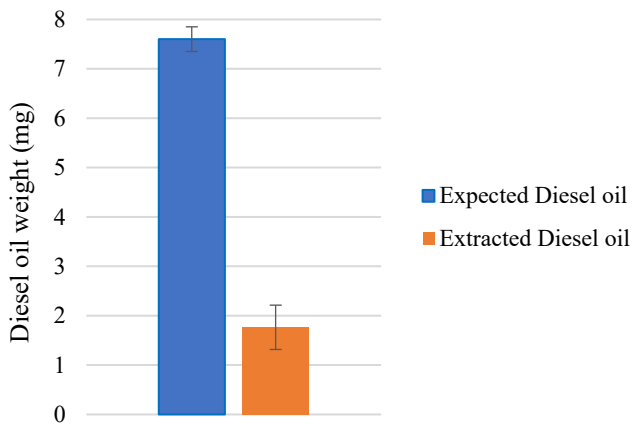

Figure 2.70. Expected vs. extracted diesel oil in 45°C 1h MWAE spiking tests, CH_2Cl_2 solvent.

Extraction at 85 °C for 1 h with dichloromethane

By increasing the extraction temperature, the quantities of substances extracted already from the first extraction increase (Table 2.52), and the zero of the instrument is low (Table 2.55), providing a residual percentage of the extractable analytes of interest equal to 13.89%, as shown in Figure 2.71, in which the average value of the concentration of hydrocarbons obtained from the extraction tests is compared, with statistically significant difference ($p < 0.05$), with the average value of the concentration of hydrocarbons obtained from the zero tests.

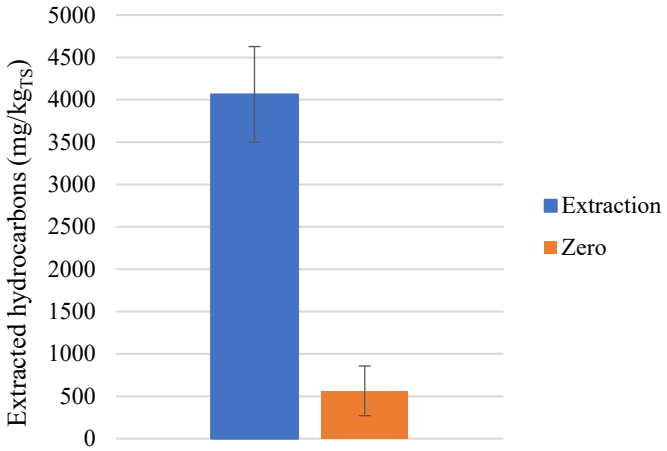

Figure 2.71. Comparison between MWAE and zero tests, 85°C, 1 h, CH_2Cl_2 solvent.

However, from the spiking tests (Table 2.58), a very low recovery percentage of the reference analyte (diesel oil) injected in the sample is observed, being equal to 11%, as shown in Figure 2.72, in which

the weight value of the diesel oil expected is compared, with no statistically significant accordance ($p > 0.05$), with the extracted value.

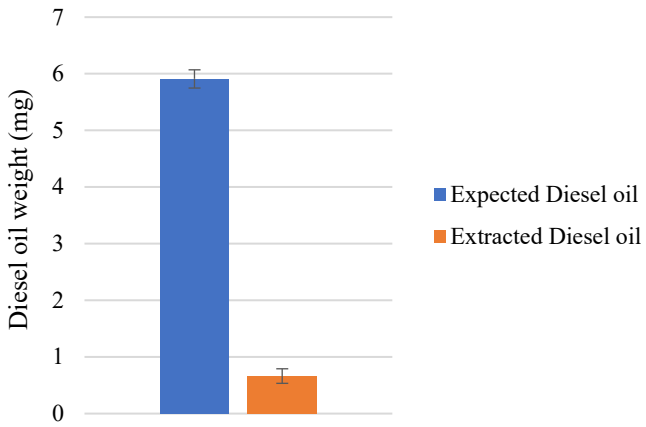

Figure 2.72. Expected vs. extracted diesel oil in 85°C, 1 h, MWAE spiking tests, CH_2Cl_2 solvent.

From the chromatographic analysis concerning the spiking tests (Figure 2.64), it is clear that the signals related to the presence of C_{10}-C_{40} hydrocarbons are weak, while they are null in the chromatographic analysis concerning the MWAE tests (Figure 2.65).

Further microwave extractions with dichloromethane solvent

Despite numerous attempts to optimize the method, carrying out spiking tests, in which various operating conditions from time to time were varied (injection of diesel oil in the form of stock solution, injection of 50 µL of diesel oil, absence of the thimble, increase in temperature to 95 °C and increase extraction time to 2h), Tables 2.59, 2.60, 2.61, and 2.62 show unsatisfactory results, with recovery percentages of the expected diesel oil even lower than 10%.

Extractions at 85 °C for 1 h and 2 h with acetone/hexane solvent

The spiking tests carried out using a mixture of acetone/hexane as solvent at 85 °C for 1 hour and 2 hours showed very similar extraction yields of the injected diesel oil (31% and 32%), as shown in Tables 2.63 and 2.64. In this case, optimizing the method to extend the extraction time over 1 hour is irrelevant. However, from the chromatographic analysis of the spiking tests (Figure 2.66) and the zero tests (Figure 2.67), there are no traces of C_{10}-C_{40} hydrocarbons in the extracted substances.

Extraction at 85 °C for 1 hour with a toluene/methanol solvent

From the chromatographic analysis of the extraction tests with a mixture of toluene/methanol (Figure 2.68), there are no traces of C_{10}-C_{40} hydrocarbons in the extracted substances. Therefore, it is believed that this solvent, as the others used for MWAE, cannot extract the hydrocarbons present in the SS, most likely strongly retained by the NOM, which constitutes the SS matrix itself, at the extraction temperature used.

2.2.2.5. Microwave extraction tests with bi-component solvent mixtures

With the MWAE method, a qualitative comparison was carried out between three different mixtures of organic solvents to evaluate which was the most suitable for extracting C_{10}-C_{40} hydrocarbons with this method. For this purpose, GC-MS analyses were conducted to make a qualitative comparison. In correspondence with the solvents used, the respective chromatograms obtained for the samples subjected to the spiking procedure were compared to compare the extractive efficiencies of the various mixtures.

The chromatograms present a series of peaks which, in the case in question, fall within the retention times between 20 and 40 minutes and whose intensities are read on the ordinate axis through an intensity parameter proportional to the concentration. The higher the peaks recorded, the better the extraction efficiency of the solvent.

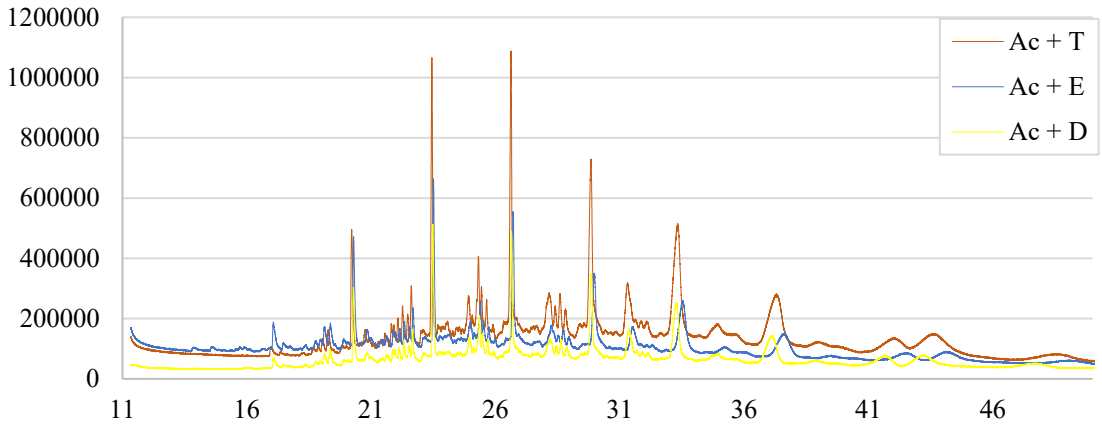

Figure 2.73. Comparison between solvents used for MWAE.

As can be seen from Figure 2.40, the highest intensity peaks were obtained with the solvent mixture Acetone/Tetrachlorethylene, which consequently is characterized by a better extraction capacity than the Acetone/Hexane and Acetone/Dichloromethane mixtures.

2.2.2.6. Conclusions

From the attempt to optimize the measuring methodology, at this point of the experimentation, the following observations emerged, being valid regardless of the extraction method used:

- drying sludge in a refrigerator thermostat at 35 °C is crucial for reducing the sample's humidity. Treating samples characterized by reduced quantities of water allows to have less interference in the analysis of the same and speeds up the clean-up operations, avoiding having to resort to further treatment cycles with columns packed with a dry bed of sulfate anhydrous sodium or Florisil;
- it is necessary to study the variations in the characteristics of the reference analytes used in the tests caused by the operating conditions adopted. In the cases studied, it is crucial to evaluate the diesel oil mass variation after its treatment on the stove at 45 °C for 24 h, following which an average reduction of its mass of 24% was recorded. This result was considered in the spiking tests when evaluating the quantities of diesel oil extracted versus those expected. If not, an underestimation of the quantities of diesel oil recovered and, therefore, of the tested methods would be made;
- in carrying out the spiking tests, it is advisable to inject 50 µL of reference analyte regardless of the weight of the sample to be analyzed. This choice derives from the need to move away from instrumental zero to have more reliable results. Acting in another way, i.e., injecting the reference analyte into the sample in the form of spike solution (having a concentration of 5 g/L) or stock solution (having a concentration of 50 g/L), would involve having comparable and therefore confusing quantities of recovered diesel oil, with the residual extractable fraction of the sample. It was also experimentally verified the uselessness of the injection of the spike or stock solution (a long and expensive process) compared to the injection of only diesel oil using a graduated automatic pipette (a more straightforward and faster process);

- it is necessary to validate all the phases to which the sample is subjected (as extraction, clean-up, and distillation) to verify that the solvents and reagents used and the relative methods of use, set temperatures, and established times are appropriate for the analytes of interest and that they do not produce alterations;
- it is essential to validate the gravimetric method with the gas chromatographic analysis method. This way, verifying that the extracted substances are the analytes of interest is possible.

Furthermore, the results obtained show that:

- the Soxhlet extraction method, regardless of the type of solvent used, is efficient, albeit with different yields, for the extraction of injected hydrocarbons (spiking tests). On the other hand, the extraction of hydrocarbons rooted in the SS matrix is more complex: some carbon compounds are extractable with dichloromethane, but the hydrocarbons belonging to the C_{10}-C_{40} class appear more challenging to extract, with the acetone/hexane mixture C_{10}-C_{40} hydrocarbons of modest molecular weight (such as C_{13}, C_{14}, C_{15}) are better extractable; with the toluene/methanol solvent, heavier hydrocarbons (such as C_{16}, C_{25}, C_{29}, C_{36}) are extractable;
- with the cold extraction method, even considering the multiple iterations of the process, it is not possible to extract the hydrocarbons of interest;
- the MWAE method is not suitable for this type of analysis, despite the numerous attempts made to optimize the method, modifying various operating conditions from time to time (extraction temperatures equal to 45 °C, 85 °C, 95 °C, extraction times equal to 1h, 2h, injection of diesel oil in the form of spike solution and stock solution, injection of 50 µL of diesel oil, absence of the thimble).

In conclusion, from the experimental step dedicated to improving the methodology, it emerged that the extraction of C_{10}-C_{40} hydrocarbons is a highly complex process since the analytes are strongly linked to the NOM, which constitutes the SS matrix. Although this condition makes the extraction operations challenging to perform, it could prove to be an advantageous condition for the agronomic use of SS: in fact, it could cause a difficult removal of the hydrocarbons from the matrix to which they are bound and, therefore, it would be difficult for them to mobilize into the soils and enter the biological and water cycles.

2.2.3. Optimization of the gas chromatographic/mass spectrometric method

Downstream the results obtained at this experimental stage, thanks to the calibration curve, which changes every time there is a single modification in the measurement system (as GC-MS settings and quality of the reactants), the GC determination returns a quantitative measure of the hydrocarbons which, once extracted, purified and measured by gravimetry, are diluted and injected into the GC-MS. However, the measured concentrations correspond only to the fraction of hydrocarbons falling within the RTW of the GC-MS, which from now on will be identified as C_{10}-C_{40}, as opposed to the TH, returned by the gravimetric measurement. As will be seen from the results of the following experimental stages, exposed in the next chapter, the method developed will always return, as expected, C_{10}-C_{40} values falling within a range of 30-50% of the TH values.

3. DEGRADATION OF HYDROCARBONS IN SEWAGE SLUDGE

3.1. Materials and Methods

3.1.1. Investigation of hydrocarbons degradation during mesophilic anaerobic digestion in sewage sludge treated with cationic polyelectrolytes

Two mesophilic AD cycles on two different SS were carried out during this experimental set. The aim was to verify the following:

- If the micro-aeration has an effect on the degradation of hydrocarbons during the mesophilic AD of SS;
- If the degradability and extractability of the hydrocarbons contained in SS are affected by cationic polyelectrolytes used for dewatering the SS.

3.1.1.1. Sewage sludge and samples preparation

Mesophilic AD was made using two different SS:

1) a mixture of a primary and secondary mixed sludge, taken from the pre-thickening phase within a WWTP in southern Italy, named Raw Sludge (RS), and
2) a thickened sludge, chemically conditioned, dewatered and centrifuged in the same WWTP as the first, named Conditioned Sludge (CS), subsequently subjected to dilution in the laboratory for experimental needs.

3.1.1.2. Anaerobic reactors setting

The lab scale reactors used for the AD tests were borosilicate glass bottles (Simax, Czech Republic) with a 1,000 mL volume filled with 630 mL of each sample. The reactors were sealed with caps, allowing liquid and gaseous sampling during the collection operations. The withdrawal of the biomethane produced took place through a 3 mm line with a valve to govern the flow. The liquid

withdrawal occurred through an 8 mm line sealed by a steel clip. Both lines were closed during operating conditions.

Figure 3.1. Sealed collecting cap (left) and Simax bottle as AD reactor.

3.1.1.3. Operating conditions

The experiments were characterized by mesophilic AD, lasting 80 days, in which the sample was divided into five sets of reactors made in triplicate to obtain statistically reliable measurements. The tests carried out were:

- AD of RS (RS tests);
- AD of CS (CS tests);
- AD of CS aerated with 5 mL of air (MA5 tests);
- AD of CS aerated with 10 mL of air (MA10 tests);
- AD of CS aerated with 20 mL of air (MA20 tests).

In addition to these 15 bottles, a sixteenth and a seventeenth bottle were prepared, containing respectively RS and CS samples, to carry out the initial conditions analyses.

A sample with an initial TS concentration of 27.25% was used to prepare the CS sludge reactors. The TS and VS contents for RS samples were equal to 3.87% and 2.37%, respectively; thus, to assure

comparable starting conditions, CS samples were diluted to lower the solids content to new TS and VS conditions. After several attempts, the average values reached were: TS=4.47% and VS=2.29%.

3.1.1.3.a. Hydraulic regime

The AD tests were carried out in batch conditions and triplicate, i.e., for each type of reactor, three homologous bottles were prepared to obtain the most reliable results possible. The gaseous withdrawal from the reactors and the consequent air insufflation for each mico-aerated set of reactors was periodically provided.

3.1.1.3.b. Temperature

Once the reactors were prepared, they were placed inside a thermostatic bath so that the sludge present in the reactors was completely immersed in water kept at a constant temperature of T=35 °C by an automated system of probes and electrical resistors.

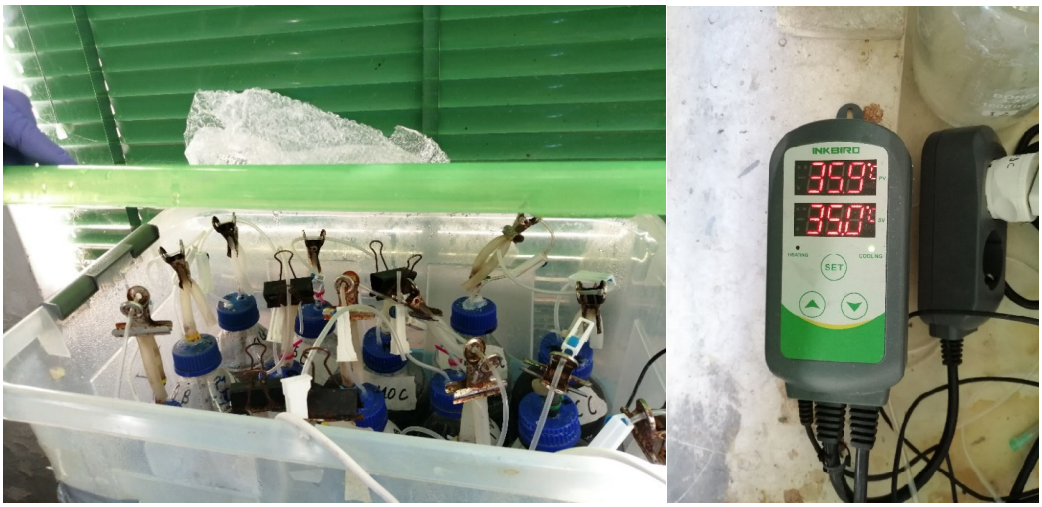

Figure 3.2. Reactors in the thermostatic bath (left) and thermostat (right).

3.1.1.3.c. Gaseous withdrawals

The biomethane withdrawn from the reactors during the digestion process was measured using a liquid displacement measuring instrument made with two glass columns inside which there was a 15% sodium hydroxide solution (NaOH, Carlo Erba Reagents, Italy) and water. The two columns were connected by a plastic tube.

Figure 3.3. Biomethane measuring system. Soda column on the left.

The soda column is equipped with a double outlet top cap. In one of these, the line from the reactor was connected, through which the biogas passes and reaches the diffuser, which can create microbubbles with the consequent advantage of guaranteeing a greater contact surface between biogas and the alkaline solution and facilitating the CO_2 capture. CH_4 passes through the soda column and, purified from CO_2, comes from the first column to the second; in this latter, the biomethane pushes out the water from the column. The displaced water volume, collected in a beaker and subsequently measured inside a graduated cylinder, represents the produced methane volume. The biomethane volume measurements must subsequently be reported at standard conditions, i.e., at the temperature of 0 °C (273 K) and 1 atm, and divided per gram of initial volatile solids contained in the reactor. Therefore, the measurement of the specific biomethane production was normalized through the equation:

$$\frac{\mathbf{NmL\ CH4}}{\mathbf{gVS}} = \frac{\mathbf{mL\ CH4}}{(\mathbf{VS} * \mathbf{Sample\ Volume})} * \frac{\mathbf{273}}{\mathbf{298}} \tag{3.1}$$

where:

VS initial volatile solids content (g/L).

3.1.1.3.d. Air insufflation

The operative conditions in the micro-aeration reactors were guaranteed by blowing different volumes of air with a 50 mL syringe through its connection to the bottle via the flexible plastic tube placed at the end of the cap. The operation was carried out only downstream of the periodic biomethane measurement to avoid sludge rising in the pipe due to the high pressure of the biogas produced in the reactor. Consequently, the air insufflation was carried out with a frequency of operations, higher at the beginning of the process due to the greater production of biogas, lower towards the end. After every air injection, the reactor was sealed and placed back into the thermostatic bath.

Figure 3.4. Reactor micro-aeration procedure.

3.1.1.4. Characterization of the samples

Both in the preliminary phase and at the end of the AD process, TS and VS values were determined on each sample by following the APHA-AWWA-WEF Standard Method 2450 through the use of a laboratory oven (Argolab TCN 115, Italy), a muffle furnace (Asal ZB/1, Italy), and a precision scale (Sartorius 1801, Germany).

3.1.1.5. Hydrocarbons measurement

Hydrocarbons in samples before and after the AD were analyzed to evaluate AD effects in micro-aeration conditions on the hydrocarbon content of SS, both chemically conditioned and not.

3.1.1.5.a. Sample preparation

The first step was preparing each sample in the initial and digested state for the subsequent analyte extraction operations. As happens in the sludge line of a WWTP, the digestate is subjected to a preliminary phase separation process to remove the liquid fraction, rich in colloidal suspended solids that would alter the subsequent operations from the solid fraction. For each bottle containing the digestate to be analyzed, the supernatant was removed following centrifugation at 4600 rpm at ambient temperature for 10 minutes.

Figure 3.5. Solid fraction after centrifugation.

Subsequently, only the solid part of the sample was transferred from the centrifuged 50 mL Falcon tube containing it into aluminum containers to be housed in a thermostatically controlled refrigerator set at a temperature of 35 °C, in which the samples were stored for no less than seven days, to remove the gravitational water present among the solid particles.

Figure 3.6. Fridge-thermostatic equipment (left); dried sample (right).

The dewatered sample has a remarkable consistency. Due to the drying in the fridge-thermostatic equipment, the only present fraction of water is “bound”, adhering to the solid particle by Van Der Waals forces, and therefore not removable by mechanical separation. The dried samples also have a very variable particle size and a vast inhomogeneity of the solid fraction. Since the extraction operations require samples with fine particle size, to ensure the maximum possible contact between the phases, it was necessary to subject the sample to a particle size reduction, using a steel mortar, to homogenize the sample.

Figure 3.7. Steel mortar and pestle (left); sample to the desired consistency.

3.1.1.5.b. Solvent preparation

The choice of the organic solvent fell on dichloromethane (CH_2Cl_2), given that the interaction with the aliphatic hydrocarbon fraction of this solvent during the analytical experiment set was the best.

3.1.1.5.c. Soxhlet extraction

Due to its greater extraction capacity, the Soxhlet extraction method was chosen to analyze in triplicate the hydrocarbon content in the SS samples relating to CS with and without micro-aeration and RS before and after AD.

- Solid sample: about 10 g of dried samples;
- Total Samples: 30. Six samples (3x IN and 3x OUT) for every sludge (RS, CS, MA5, MA10, MA20);
- Solvent: 200 mL of CH_2Cl_2;
- Duration of extraction: 11 hours;
- Thimbles: in cellulose fiber (Whatman) with an external diameter of 32 mm, an internal diameter of 30 mm, and a length of 80 mm;
- Extraction temperature: about 40 °C.

Figure 3.8. Soxhlet extraction operations (left); one of the thimbles used (right).

3.1.1.5.d. Clean-up procedures

Water removal

Water removal was performed by adding anhydrous sodium sulfate to the samples after extraction and removing the decahydrate precipitate, according to equation (2.1).

Removal of polar substances

Clean-up was carried out using 400mm long and 8mm internal diameter borosilicate glass columns filled with a silica gel and dichloromethane slurry downstream of an anhydrous sodium sulfate layer to remove the polar substance.

Figure 3.9. Clean-up operations (left); post and pre-clean-up samples (right).

3.1.1.5.e. Sample filtration

The sample was subjected to a filtration operation to remove further colloidal solids remaining in suspension in the solvent-analyte mixture obtained downstream of the removal of the polar substances. The mixture was then taken with 5 mL polypropylene syringes and filtered in a 0.45 µm polypropylene filter, specific for organic solvents (Whatman, U.S.A.).

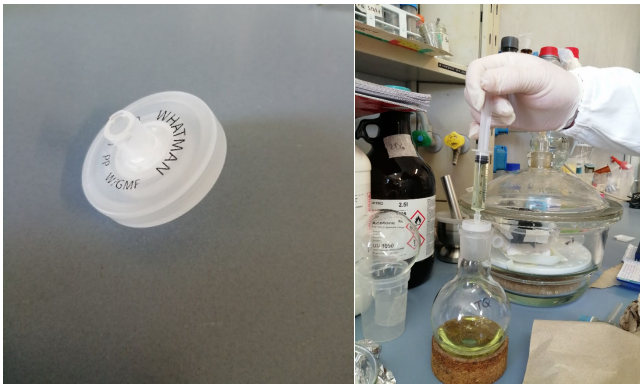

Figure 3.10. 45µm PP filter (left); sample filtration operation (right).

3.1.1.5.f. Solvent removal

Since the quantities of solvent used were very high, an analyte-solvent separation was opted for by distillation, which, being the solvent dichloromethane, takes place at 40 °C.

Figure 3.11. Distillation operation (left); post-distillation analyte (right).

The distillation operation was a relatively short phase, as the time required was 20-30 minutes per sample.

3.1.1.5.g. Total Hydrocarbons – Gravimetric determination

Following the distillation phase, the sample is transferred from the flask, with the aid of glass Pasteur pipettes, inside a borosilicate glass weighing boat, previously tared. Subsequently, the sample is dried at 45 °C on a stove for 24 h and in a bell dryer for 1 h. The weighing operations took place using a very high precision (1 µg) scale (Mettler MT5).

Figure 3.12. Weighing boats loaded with the samples (left); purified hydrocarbon analyte (right).

The weight of the analyte is derived from the following difference:

$$\mathbf{E = (T+E) - T} \tag{3.2}$$

Where **E** is the weight (mg) of the extracted purified analyte and **T** is the tare weight (mg) of the weighing boat.

Figure 3.13. Weighing the extracted sample with the very high precision (1 μg) scale.

The total hydrocarbon concentration (mg_{TH}/kg_{TS}) will be:

$$[\mathbf{TH}] = \frac{\mathbf{E}}{\mathbf{P} * \mathbf{TS}} \tag{3.3}$$

Where **P** is the weight of the initial raw sample, in kg, inserted into the extraction system, **TS** is its total solids content (%), and E is the weight of the sample, gravimetrically measured.

3.1.1.5.h. C_{10}-C_{40} Hydrocarbons – GC-MS determination

After setting the GC-MS to obtain quantitative determinations of the C_{10}-C_{40} fraction within the total hydrocarbon contained in the analyzed SS samples, this parameter was measured in correspondence with the samples, before (IN) and after AD (OUT), of CS and RS, therefore excluding micro-aerated samples.

Sample dilution was necessary inside a 10 mL graduated flask using a chromatographic grade pure acetone solvent, with which the weighing boat containing the purified hydrocarbon analyte was

washed to ensure that it could be analyzed on the chromatograph. Once this operation was carried out, the sample was ready to be introduced through a chromatography syringe, washed pre and post-injection with acetone, with which 1 μL of the diluted sample was taken.

Figure 3.14. Samples dilution operation (left); diluted sample ready for GC-MS analysis (right).

For the determination of the C_{10}-C_{40} hydrocarbon concentrations, the last update of the procedure, exposed in the 2.1.3 section, was used. Therefore, the calculations of C_{10}-C_{40} hydrocarbon concentrations are given by the linear curve expressing the GC-MS calibration:

$$\mathbf{[C_{10}\text{-}C_{40}]_{(g/L)}=1.67882583950499*10\text{^}\text{-}9*AREA+0.222732827844396} \quad (3.4)$$

to have:

- $[C_{10}\text{-}C_{40}]_{(g/L)}$: the C_{10}-C_{40} hydrocarbon concentration, expressed per volume unit of the solvent used to dilute the dried sample before injecting it in the GC-MS (g/L);

and by the equation:

$$\mathbf{[C_{10} - C_{40}] = \frac{E}{P*TS}} \quad (3.5)$$

where **P** is the weight of the initial raw sample, in kg, inserted into the extraction system and **TS** is its total solids content (%), and **E** is the weight of the C_{10}-C_{40} hydrocarbons, given by:

$$\mathbf{E = [C_{10} - C_{40}]_{(g/L)} \cdot V} \quad (3.6)$$

where **V** is the volume of the solvent used to dilute the dried sample before injecting it in the GC-MS to have:

- [C_{10}-C_{40}]: the searched C_{10}-C_{40} hydrocarbon concentration, expressed as ($mg_{C10-C40}/kg_{TS}$).

3.1.1.5.i. Determination of hydrocarbon mass removal yield

The biological process acting on the SS samples causes a variation of the hydrocarbon content in the SS matrix; however, a degradation of the solids content of each sample, in favor of biogas production, also occurs. Therefore it is useful to evaluate, in addition to the hydrocarbon concentration variation in the samples due to AD, also their mass removal yield, defined as follows:

$$\textbf{Hydrocarbons mass removal yield (\%)} = 1 - \frac{C_{OUT}}{C_{IN}} \cdot \left(\frac{TS_{OUT}}{TS_{IN}}\right) \qquad (3.7)$$

where:

- C_{IN} and C_{OUT} are the hydrocarbon concentrations (TH or C_{10}-C_{40}) respectively before and after AD expressed as mg per kg of TS;
- TS_{IN} and TS_{OUT} represent the TS concentrations expressed as g/L.

This parameter returns the mass variation of hydrocarbons due to AD, considering the variation of the total solid during AD.

3.1.2. Quantification of the hydrocarbons contained in sewage sludge using ultrasounds

This section shows the settings of an economical, fast, and reliable method of measuring the hydrocarbons contained in SS, which uses ultrasonic extraction.

Ultrasound technology is an effective and low-energy means of applying strong and intense stresses to liquids, sludges, liquid mixtures, and suspensions. The most common applications are mixing, dispersing, particle size reduction, chemical extraction, reactions, emulsions, etc. Sonication, a process that uses ultrasound in ultrasound-assisted extraction, is used to speed up and make the extraction process more efficient.

The ultrasounds efficiency is mainly due to the following reasons:

- Intensification of mass transfer: ultrasounds facilitate the formation of microparticles, the emulsion, and the exchange of the solvent around the walls of the considered SS sample;
- Breaking of particles: ultrasounds can break up the sludge, facilitating the extraction of the contents, thanks to the increase in the contact surface with the solvent;
- Increased solvent penetration: when the cavities collapse, the generated ultrasonic solvent jets force the solvent into the solid sludge, thus facilitating its passage.

When ultrasound is applied to a liquid, the waves propagate, generating a continuous succession of compressions and decompressions. The very rapid sequence of compression and decompression cycles, a phenomenon known as cavitation, generates millions of microbubbles, called cavities, which grow in volume with each cycle. When cavitation bubbles implode into irradiated liquids, their compression is so rapid that a small amount of heat can escape from the cavity as it collapses. The surrounding liquid, on the other hand, is still cold and will promptly extinguish the heating of the cavity. If the liquid jet hits a solid body, it yields energy, causing varying wave order and intensity effects. Ultrasounds affect treated systems in many ways. Among these is the direct effect of the ultrasonic waves that propagate in the liquid, the intensity of the induced cavitation phenomenon

(formation of a few/many bubbles), the implosion of the cavitation bubbles, the heat deriving from the implosion of the bubbles themselves. Hence, the type of probe used, the sonication vessel's size and shape, and the sonicated dispersed system's volume are all critical parameters.

3.1.2.1. Sonicated bath extraction

The sonicated bath works by immersion, in which the ultrasonic waves' power, the volume to be sonicated, and the time of application must also be considered. Ideal sample volumes to be the ultrasonic bath effective are typical of the order of a few hundred milliliters. The ultrasonic baths have one or more transducers connected to a steel tank that contains a liquid bath in which the samples to be analyzed are immersed. The mechanical waves are transmitted to the samples to be extracted from the tank through the liquid bat. Furthermore, the sonicated baths cannot reach the performance of ultrasonic probes, as the transducers do not operate directly in the solution but are connected to the liquid bath tank in which an additional container is immersed containing the solvent and the SS sample to be extracted.

3.1.2.2. Sample preparation

In this phase of the experimental activity, twelve SS samples were collected, of which six of unconditioned sludge RS and another six of chemically conditioned and dewatered sludge CS. Furthermore, half of the samples were digested (RS OUT E, RS OUT F, CS OUT A, CS OUT B, and CS OUT C), half in the initial condition (RS IN and CS IN). About 500 mg of each sample of the dried sludge, transferred into 2 mL Eppendorf-type tubes, were weighed using a very high precision (1 μg) scale (Mettler MT5).

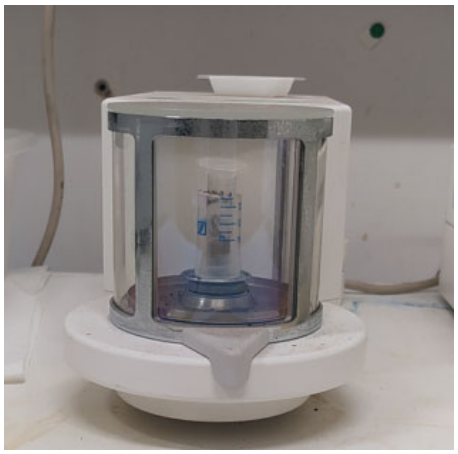

Figure 3.15. Sample Weighing with a very high precision (1 µg) scale (Mettler MT5).

3.1.2.3. Extraction operations

Subsequently, 1,000 µL of acetone was added to the sample by injection with a Transferpette micropipette. The large tank of the equipment used (Bandelin Sonorex™, Germany) allowed the simultaneous housing of multiple reaction chambers depending on the amount of sample to be extracted. In this case, 12 holes were made in a polystyrene plate and filled with the previously prepared Eppendorf tubes. In this way, in addition to the simultaneous processing of several samples, it is possible to obtain a comparable extraction efficiency between the samples processed in the same series, guaranteeing the possibility of comparison. The duration of the sonication process was 30 minutes.

Figure 3.16. Sonicated bath extraction applied to 12 samples.

The sonication was carried out to break any aggregate that may have formed and favor a greater dispersion in the solvent of the analytes present in the SS sample.

3.1.2.4. Phase separation

Once equilibrium was reached, the solution rich in extracted analytes was subjected to a phase separation from the now-exhausted solid matrix through centrifugation. The phase separation was carried out by centrifuging the Eppendorf tubes for 10 minutes at the speed of 7020 RPM and with variable temperatures.

Figure 3.17. Phase separation procedure by centrifugation.

At the end of this process, the phases were separated, and, using a Pasteur glass pipette, the solvent was extracted from the Eppendorf tube and transferred to a clean test tube to be subjected to the next phase. This procedure was repeated for a second time to ensure that everything was extracted from the SS sample by adding another 1,000 μL of acetone and a new separation by centrifugation.

Figure 3.18. Extracted sample (left); collected supernatant samples (right).

3.1.2.5. Organic phase transfer

The next step is the passage of the analyte to a new solvent, i.e., 2 mL of n-heptane and 2 mL of Milli-Q distilled water are added to the previously prepared samples to have the absorption of polar substances in water and non-polar substances in heptane. The contact between the three phases and the analyte was ensured using a vortex-stirring device (Reax 2000, Heidolph). This procedure was replicated for all the samples, recovering the supernatant each time and adding 2 mL of n-heptane to the previously prepared samples, followed by new vortexing.

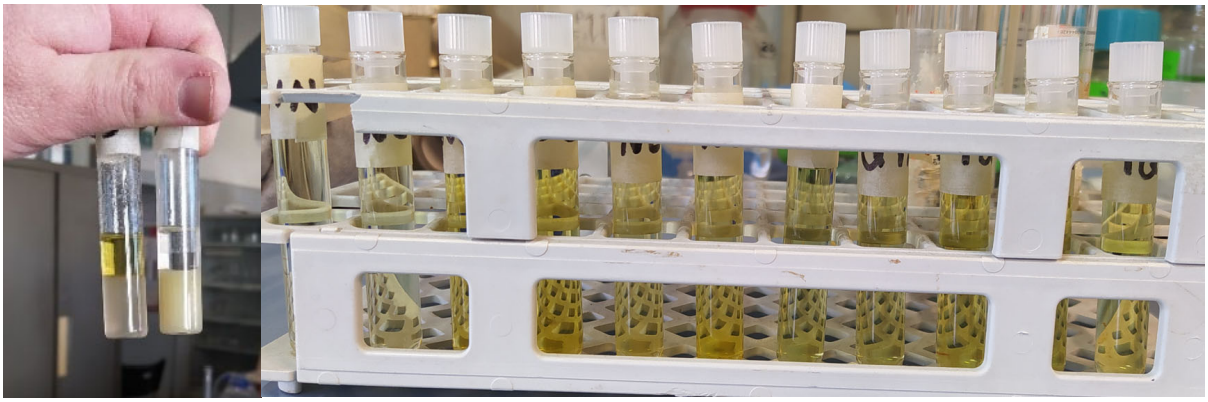

Figure 3.19. Tri-phasic solvent solution (left); collected analyte-full solvent samples (right).

3.1.2.6. Clean-up

A syringe packed with a dry bed (stationary phase) and a liquid phase (mobile phase) was used as the only clean-up stage. The stationary phase consists of two superimposed layers:

- Anhydrous sodium sulfate, about 600 mg, for absorbing large quantities of water when it comes into contact with it;
- Florisil, about 600 mg, previously activated in the oven at 140 °C for at least 16 hours, to interact with the polar impurities transported by the eluent and retain them on its surface.

Once the adsorption column has been conditioned, the solvent-analyte mixture is eluted from above. The sample was then recovered in a beaker while the column was cleaned and prepared for further passages of new samples.

Figure 3.20. Clean-up operation.

3.1.2.7. Measurement operations

The extracted samples followed the operational routine already seen for the optimized Soxhlet extraction method, to be analyzed with gravimetric and GC-MS methods and to quantify the TH and C_{10}-C_{40} concentrations. The only difference is the use of RTW solution (n-$CH_3C_5H_{10}CH_3$ spiked with $C_{10}H_{22}$ and $C_{40}H_{88}$) as the solvent for sample dilution before injection in the GC-MS, choosing different amounts (50, 100, or 500 μL of RTW solution), depending on the dilution necessity due to the sample characterization.

3.1.3. Matrix effect – deepening of the retention effect of the organic matter in a sewage sludge

In this phase of the experimental activity, the retention effect of the hydrocarbon particles by the natural organic matter present in the solid matrix of the SS was analyzed. For this purpose, a methanolysis reaction (Pontoni et al., 2021) of the organic substance was carried out, a critical step for getting new information on the composition of NOM. The methanolysis reaction allows the cleavage of glycoside bonds of polysaccharide shells, constituting the NOM present in the sludge, into o-methyl glycosidic monomers. At the same time, the methyl-esterification of the long-chain fatty acids present in the SS also takes place, allowing their volatilization into the GC system and hence their identification. The application of this reaction, new in the field, allows for considerably modifying the structure of the organic matrix of the SS, letting greater extractability of non-polar compounds retained in it. Below is the detailed analysis and preparation of three samples subsequently subjected to GC-MS analysis.

The samples examined were:

- Raw sludge (RS): unconditioned and undigested, not methanolized, raw sample;
- Spiked sludge (SP): unconditioned and undigested sample, not methanolized, with the addition of spike solution;
- Spiked and methanolized sludge (SK): unconditioned and undigested sample, methanolized, spiked solution added

3.1.3.1. Raw sludge sample preparation

Samples of 25 mg in triplicate of an unconditioned and undigested sludge were prepared using a very high precision (1 μg) scale (Mettler MT5).

The samples underwent three cold solid-liquid extraction cycles with a bi-phasic solvent according to the ISO/TR 11046 standard method. Our goal was to extract the hydrocarbons contained in the solid matrix of the SS sample, which was placed in a test tube with 2 mL of n-hexane and 2 mL of a

1M aqueous solution of HCl (biphasic liquid solvent). It is possible to identify different and separate operational phases in the process through which the extraction was carried out:

1) the solvent, added to the sample matrix, penetrates through the pores of the latter, occupying all accessible spaces;
2) the analytes contained in the matrix, by chemical affinity, solubilize in the solvent giving rise to a solution with a specific concentration of hydrocarbons;
3) the analytes solubilized in the solvent by osmotic effect or by simple gradient diffusion migrate towards the outside of the solid until the solute concentration gradient between the inside and outside of the solid matrix is zeroed;
4) once the equilibrium is reached, the extraction process stops as the solution, rich in extracted analytes, is ready to be subjected to a phase of separation from the solid matrix.

The recovery of the solute from the saturated solution is not total, as a certain part of it, defined by the imbibition ratio, remains in the matrix, so the extraction cycle with fresh solvent must be repeated until the solute in the matrix is exhausted.

In the case in question, it was decided to carry out extraction with two distinct phases and kept separate by a miscibility gap, with characteristics such that the organic fraction of the solvent brings into solution the related substances, such as hydrocarbons. In contrast, the aqueous fraction brings in solution, in its phase quite distinct from the organic phase, polar substances, and other substances of a different nature from that of hydrocarbons. In practice, an extraction with two solvents with a total miscibility gap is an operation that also includes a separation of the analyte from the interfering substances, an operation for which clean-up is usually used.

The characteristic parameters of solid-liquid extraction are many, such as the crushing degree of the solid matrix, size and physical state of the particles of the solid matrix, quantitative solvent/matrix ratio, contact times, mechanism of penetration of the solvent into the particles of the material being extracted, and finally, process pressure and temperature. In this type of extraction, it is considered

essential to use stirring equipment, which, by increasing the turbulence in the matrix-solvent system, allows the reduction of the thickness of the diffusion boundary layer, makes the analyte concentration in the solution more uniform, and optimizes the exploitation of the analyte-solvent contact surfaces, also preventing the sedimentation of the solid. A Heidolph Reax 2000 vortex stirring device was used.

Operating conditions used:

- solid sample: unconditioned and undigested SS sample, 25 mg;
- container: clear borosilicate glass test tube 100 mm long, with an internal diameter of 8 mm;
- solvent: 2 mL of n-hexane ($CH_3C_4H_8CH_3$) and 2 mL of 1 M aqueous solution of HCl (to be added to the first cycle);
- water to facilitate phase separation: 2 mL of Milli-Q® ultrapure distilled water (to be added only to the last two cycles);
- extraction duration: for each cycle, 120 seconds were carried out with the vortex stirrer at a speed such as to put the system in agitation to facilitate contact between the phases;
- temperature: room temperature.

After the first extraction cycle, the aqueous fraction of the 1M HCl solution was removed, after which a second step was carried out, which included:

- Addition of 2 mL of Milli-Q ultrapure distilled water;
- Extraction for 120 seconds with vortex stirrer at room temperature;
- Subtraction of water

The third and last step of extraction involved:

- Addition of 2 mL of Milli-Q ultrapure distilled water;
- Extraction for 120 seconds with vortex stirrer at room temperature;
- Subtraction of the organic solvent (n-hexane) enriched with the analyte.

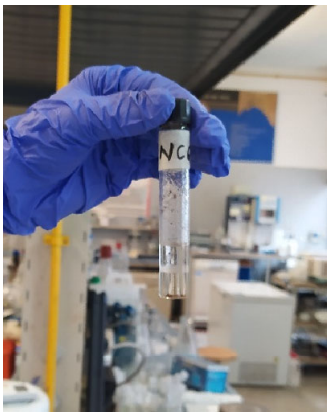

Figure 3.21. Result of a 120-second vortex extraction.

Once the three extraction cycles were completed, the sample was subjected to filtration with a 13 mm GD/X syringe filter in polypropylene with 45 µm of porosity (Whatman, U.S.A.) with a wash through new hexane addition to being subjected, in a subsequent phase, to gas chromatography.

Figure 3.22. GD/X syringe filter (Whatman, U.S.A.).

3.1.3.2. Spiked sludge sample preparation

Same procedure as the RS sample, a triplicate of the 25 mg unconditioned and undigested sample was prepared. The only difference was that, before undergoing the three extraction cycles, 300 µL of spiking reference analyte was added to the samples using a high-precision calibrated micropipette (Transferpette) to add a known quantity of C_{10}-C_{40} hydrocarbons in the sample.

The spiking preparation consists of adding 20 µL of diesel oil to a 20 mL graduate flask, brought up to volume with acetone. Diesel oil was chosen as it is a mixture of hydrocarbons derived from petroleum distillation. Furthermore, this oil cut presents the same information regarding aliphatic hydrocarbons as that of Naphtha oil for agricultural use, although they are esthetically very different: diesel oil has a yellow color and the other petrol green. The treated SS samples were then left under

the hood for 3 hours and blown with air to allow the removal of the acetone introduced with the spiking operation. Then we proceeded with the three extraction cycles described above to prepare the sample for gas GC-MS analysis. Below are some descriptive images of the extraction phases that the SP samples have undergone.

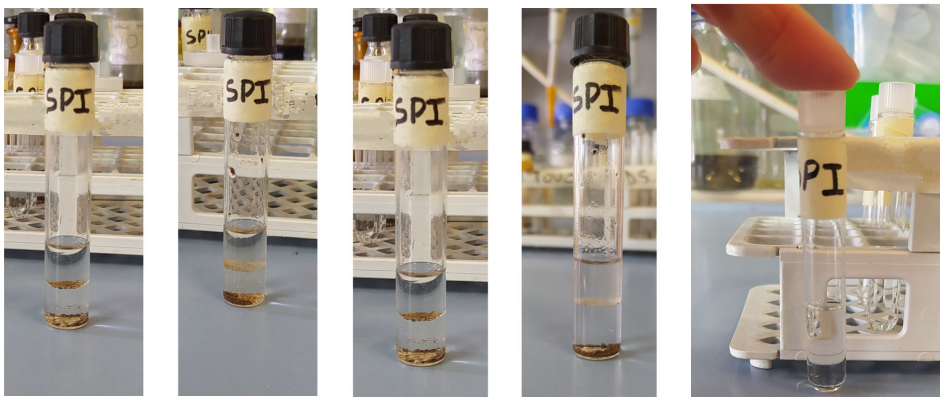

Figure 3.23. The extraction phases (from left to right) and filtered sample (extreme right).

3.1.3.3. Spiked and methanolized sludge sample preparation

The preparation of the SK samples has some differences compared to that of the RS and SP samples. The procedure adopted was carried out as follows:

- Weighing of 200 mg of unconditioned, undigested sample;
- Addition of 2 mL of 1M of HCl and CH_3OH solution;
- Mineralization at 100 °C overnight. This phase allows for speeding up the kinetic conditions and makes possible a better optimization of the lysis reaction promoted by methanol in acid and high-temperature conditions without losing the volatile elements;
- The methanolised sample is dried for 24 hours by blowing air under the hood;
- As described for RS and SP, three extraction cycles were performed, changing the solvent and extraction duration. Each extraction cycle is composed of:
    - container: 100 mm long borosilicate glass test tube with an internal diameter of 8 mm;
    - solvent: 2 mL of hexane, pure organic solvent;

- water for the extraction of polar substances and other interferers, for cycles II and III: 2 mL of ultrapure Milli-Q distilled water;
- extraction duration: for each cycle, 120 seconds are carried out on the vortex stirrer at speed such as to put the system in agitation, and 2.5 min in the centrifuge at 2500 RPM, to separate the phases and subtract the first two cycles' water. The third time of each cycle, on the other hand, the centrifuge is set at 4 minutes at 2500 RPM to subtract, then, also the organic solvent;
- temperature: room temperature.

The so far described procedure represented the conditioning of the SK sample, as the solid has received the treatment of:

- methanolysis;
- biphasic extraction of all the products of methanolysis.

Overnight solvent evaporation under the hood in a forced airflow from three samples brought each sample to about 25 mg. Then, before undergoing the three extraction cycles, diesel oil was added as a reference analyte by injecting a 300 µL volume with a 1,000 µL Transferpette micropipette as a spike solution. Then the sample was concentrated with forced airflow for 6 h under the hood to adsorb only the spike without solvent. This operation was followed by the extraction cycles described for RS samples. Once the three extraction cycles were completed, the sample was subjected to filtration with a GD/X syringe filter and hexane addition.

3.1.3.4. Clean-up operations

The clean-up procedures are critical, as fundamental is the removal of substances not of interest but also with an affinity with the organic solvent, which could have been extracted together with C_{10}-C_{40} hydrocarbons, and consequently, they would risk altering the final measure. The sample was then subjected to the removal of the humidity with anhydrous sodium sulfate, followed by the abatement

of the polar substances. The clean-up procedure was performed, according to the column adsorption chromatography technique, using a glass column packed with a dry bed of activated Florisil with a superimposed layer of anhydrous sodium sulfate.

3.1.3.5. GC-MS analyses

Subsequently, all the previously prepared samples were subjected to complete drying under a hood, diluted with 100 µL of acetone with a Transferpette micropipette, and then underwent the GC-MS analysis.

3.1.3.6. Investigation of toxic substances

Since one of the objectives of the thesis is to safeguard soil subjected to fertilization by spreading stabilized SS, the presence of potentially toxic substances in a SS sample and detectable thanks to the methanolysis process was therefore evaluated. Therefore, the analytical data relating to the extracts in the presence and absence of methanolysis treatment were compared to identify "hidden" and potentially toxic compounds not detectable with conventional analysis procedures. Thanks to a GC-MS, it was possible to identify the components within the sample, determine their molecular weight, and perform quantitative analyses. The identification of the substances in every chromatogram is followed by a comparison on the Material Safety Data Sheet (MSDS), a legal document listing all the dangers and safety to human health and the environment of a chemical product. These substances, moreover, are also reflected in various scientific references that underline their dangerousness. This way, it was possible to identify which of the two samples of unconditioned sludge, one raw and the other subjected to the methanolysis process, had more toxic substances. We can define the toxicity of a substance as its ability to damage soil organisms, landspreading treated SS.

Within the "Guidelines for the Safe Use of Wastewater, Excreta and Greywater in Agriculture" drawn up by the World Health Organization (World Health Organization, 2006), risk analysis plays a fundamental role in identifying the microbiological risk for workers and consumers due to the reuse

of purified wastewater in agriculture. Furthermore, risk analysis has recently become the tool for establishing adequate quality standards relating to emerging micropollutants within the proposed European regulation on the minimum quality requirements of wastewater intended for irrigation reuse or groundwater recharge.

3.1.4. Thermophilic anaerobic digestion of chemically conditioned and dewatered sewage sludge

An experiment was carried out which provided for the thermophilic AD of two SS from WWTP in northern Italy and destined for a centralized sludge treatment plant that produces biosolids for agricultural spreading. The two sludges, F1 and F2, were subjected in the laboratory to thermophilic digestion with a high solid rate (HSAD) for one month. Downstream the biological treatment, pre- and post-AD SS samples were treated for the analyses of TH and C_{10}-C_{40}. Together with the digested sludge, the experimental procedure was also carried out on a third sludge (F3) subjected to thermophilic AD by the centralized sludge plant.

3.1.4.1. Samples characterization and preparation

Analyses to determine TS and VS contents on SS samples, before and after AD, were performed following the APHA-AWWA-WEF Standard Method 2450 through the use of a laboratory oven (Argolab TCN 115, Italy), a muffle furnace (Asal ZB/1, Italy), and a precision scale (Sartorius 1801, Germany). The samples were left in the initial conditions for the AD procedure without altering the solids content with dilution operations. Therefore, the implemented AD procedure was necessarily of HSAD type, with the initial TS fractions equaling 13.39(±0.48)% and 22.15(±0.44)%, respectively, for F1 and F2.

3.1.4.2. Thermophilic anaerobic digestion

The AD was performed by placing the two SS samples in bioreactors at a temperature of 55 °C for 26 days. For the biogas produced, measurement was carried out for each reactor through a volumetric counter (MGC-1, Ritter, Germany). Manual stirring of the mixture was carried out every day.

Figure 3.24. Bioreactors during AD (left); thermostatic system (center); biogas measurement system (right).

3.1.4.3. Hydrocarbon determinations

Total and C_{10}-C_{40} hydrocarbon measurements in the SS sample, before and after AD, were performed following the below-exposed steps.

- Sample drying and particle size reduction through a mortar;
- New TS determination;
- Ultrasound extraction in CH_2Cl_2, for 40 minutes of each SS sample weighed (1g) and kept in contact with 5 mL of solvent;
- Solvent/analyte separation by two vortex stirring cycles, for 1 minute, with 2 mL of dichloromethane added in each cycle, to retrieve all the residual analyte fractions from the solid matrix;
- Clean-up in a syringe filled with a double-layer dry bed of activated Florisil (600 mg) superimposed by 600 mg of anhydrous sodium sulfate, with a final wash adding 1 mL of CH_2Cl_2;
- Filtration with a 0.45 μm polypropylene filter, specific for organic solvents (Whatman, U.S.A.);
- Reduction of the solvent quantity by evaporation with forced airflow under the hood;
- Gravimetric determination, as seen above, for TH content;

- GC-MS determination of C_{10}-C_{40} content, with a sample dilution, after gravimetric determination, with 500 µL of RTW solution (n-$CH_3C_5H_{10}CH_3$ spiked with $C_{10}H_{22}$ and $C_{40}H_{88}$ standards) as the solvent.

3.2. Results and Discussions

3.2.1. Investigation of hydrocarbons degradation with mesophilic anaerobic digestion in sewage sludge treated with cationic polyelectrolytes

3.2.1.1. Characterization of sewage sludge samples

From the analysis of the TS and VS contents conducted in triplicate, the starting conditions for all the SS samples are shown in Table 3.1.

Table 3.1. TS and VS content of initial sludges.

Sample	TS (g/L)	VS (g/L)	VS/TS
RS IN	38.65 ± 1.52	23.72 ± 0.76	0.61
CS IN	44.71 ± 0.89	22.89 ± 0.46	0.51

The conditioned sludge CS was delivered from the WWTP centrifuged and with TS=27.25%, a value too high to conduct a mesophilic AD and utterly different from the total solids load present in RS. It was, therefore, necessary to dilute the CS solids content, up to the value of TS=4.47%, to have practically the same VS content for all the SS samples, i.e., equal to about 23 g/L. The CS IN sample is representative of all the samples loaded into the CS, MA5, MA10, and MA20 reactors, the latter characterized by a micro-aeration treatment of 5, 10, and 20 mL, respectively. All sludges, therefore, start from an almost identical VS content. However, it should be considered that given the VS/TS ratios found, equal to 0.61 for RS and 0.51 for the others, it is reasonable to expect higher biomethane productions for the RS samples, with a greater quantity of volatile solids on the totality of the solids.

3.2.1.2. Specific biomethane production tests

The specific biomethane production (SBP), expressed in NmL of CH_4 per gram of initial VS according to equation (3.1), was evaluated for each SS sample during the 80 days of digestion. Specific cumulative average production curves were calculated and shown in Figure 3.25.

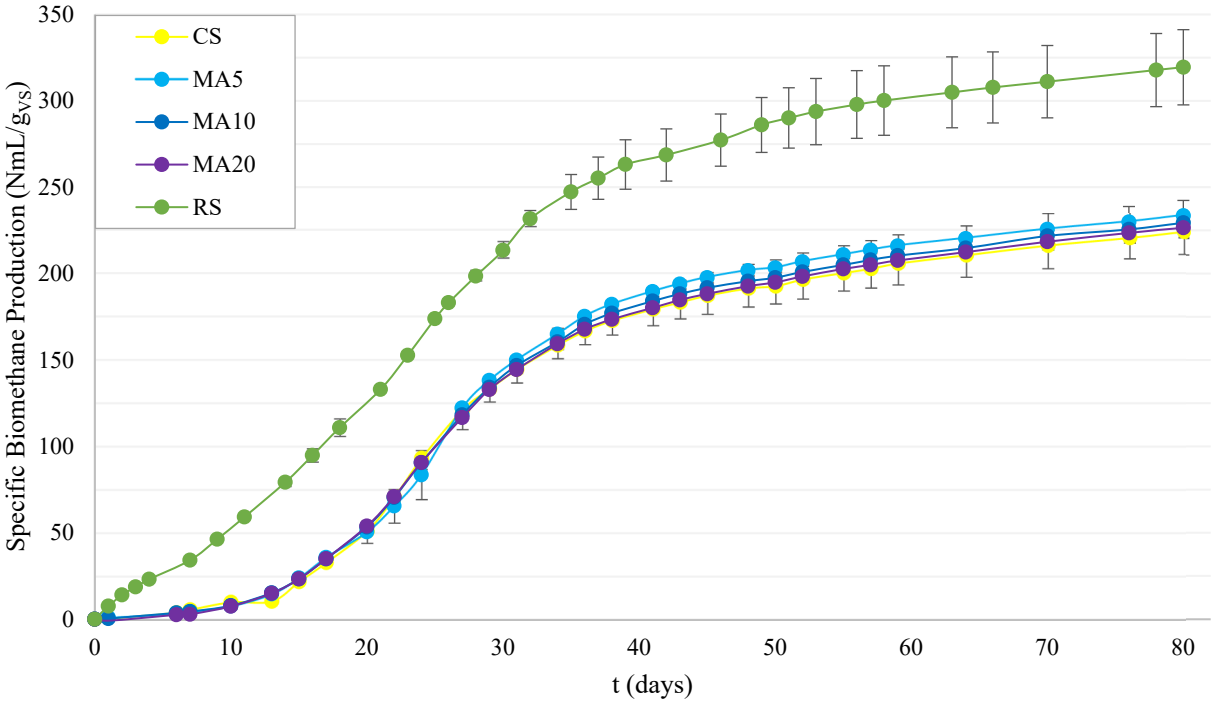

Figure 3.25. Specific biomethane productions for 80 days AD of SS.

From Figure 3.25, it can be observed that for the first 30 days of experimentation, the trends of the curves for CS and micro-aerated sludges are very similar, so the effect of micro-aeration is felt only after this time interval. In particular, from this point onwards, the curves tend to distance themselves, identifying a higher specific production for the reactors aerated with 5 mL of air, followed by the reactors blown with 10 and 20 mL, respectively. As clearly visible in Tables 3.2 and 3.3; however, the micro-aeration conditions do not involve a statistically appreciable variation compared to pure anaerobiosis, as no significant increases in final SBP were found.

Table 3.2. Specific biomethane productions at 80 days.

Sample	CS	MA5	MA10	MA20	RS
SBP (NmL_{CH4}/g_{VS})	223.89	233.51	229.47	226.60	319.47

Table 3.3. SBP increment on CS of micro-aerated sludges.

Sample	MA5	MA10	MA20
SBP increment (%)	4.30	2.49%	1.21%
St. dev.	0.07	1.9	0.71

Nevertheless, the most evident data is the difference in biomethane production between conditioned and non-conditioned sludge. In fact, from Figure 3.25 and Table 3.2, the production of biomethane relative to the RS sludge is approximately 50% higher than that obtained in correspondence with the conditioned sludge for all digestion conditions. One explanation could be due to the main effect of chemical conditioning, namely the formation of compact and dehydrated flakes, resulting in an obstacle to biomethane production during digestion processes (Di Capua et al., 2020; Pontoni et al., 2021).

3.2.1.3. Volatile solids degradation

Once the digestion process was completed, TS and VS analyzes were performed to determine the VS removal rates. The results obtained are reported in Table 3.4.

Table 3.4. VS removals.

Sample	VS post AD (g/L)	St. dev. (g/L)	VS removal (%)
CS	15.50	2.194	32%
MA5	13.53	0.110	41%
MA10	14.38	0.560	37%
MA20	15.14	0.921	34%
RS	11.23	0.228	53%

In Table 3.4, it is visible how reactors with higher biomethane production also have higher VS removal. The highest removal rates were found in reactors subjected to micro-aerated digestion (41% in MA 5 reactors), compared to pure AD (32% for CS). However, the maximum VS removal yield was found in the RS sludge, for which 53% of VS removal was found.

The differences in the various VS removals between the various conditioned sludges can be attributed to the effect of micro-aeration, which accelerated the kinetics of the microorganisms operating in the initial hydrolysis phase. The latter appears to be the limiting stage of the AD process of SS (Di Capua et al., 2020), whose efficiency was probably stimulated by micro-aeration, consequently motivating the higher biomethane productions and the higher VS removals.

3.2.1.4. Measures of hydrocarbon concentration variations in sewage sludges due to anaerobic digestion

To evaluate the effect of the AD process on the concentration of aliphatic hydrocarbons present in the SS, the choice of the extraction method used in the case in question fell on Soxhlet, opting for dichloromethane as an organic solvent.

The gravimetric method was used for the final determination of TH concentrations, while a measurement through the GC-MS, after calibration with standard solutions, allowed for quantification of the C_{10}-C_{40} hydrocarbon concentrations.

3.2.1.4.a. Total Hydrocarbon concentrations measurement – gravimetric determination

Table 3.5 shows the results of the gravimetric determinations concerning the TH concentrations before the biological process; Table 3.6 instead shows the values of the TH concentrations downstream of AD.

Table 3.5. TH concentrations in SS before AD.

Sample	P (g)	TS (%)	E (mg)	[TH] (mg/kg_{TS})	Avg. (mg/kg_{TS})	St. dev. (mg/kg_{TS})	Dev. (%)
CS IN 1	8.8941	93.73	100.727	12,082	11,533	549	4.76
CS IN 2	10.1322	93.73	104.318	10,984			
RS IN 1	10.2116	92.55	199.869	21,148	22,235	1,087	4.89
RS IN 2	6.9741	92.55	150.532	23,322			

Table 3.6. TH concentrations in SS after AD.

Sample	P (g)	TS (%)	E (mg)	[TH] (mg/kg_{TS})	Avg. (mg/kg_{TS})	St. dev. (mg/kg_{TS})	Dev. (%)
CS A	9.7133	94.12	120.266	13,155	13,029	138	1.06
CS B	9.9972	93.79	120.370	12,838			
CS C	9.9585	94.00	122.585	13,096			
MA5 B	9.8957	94.32	129.954	13,924	13,501	422	3.13
MA5 C	10.0446	94.00	123.488	13,079			
MA10 A	9.7171	94.17	106.847	11,676	12,102	301	2.49
MA10 B	9.9288	93.73	114.385	12,291			
MA10 C	9.9439	94.20	115.568	12,337			
MA20 A	9.9798	94.35	116.487	12,372	13,336	732	5.49

MA20 B	9.9865	93.59	132.197	14,144			
MA20 C	10.1901	93.98	129.200	13,491			
RS E	10.1383	94.04	145.748	15,287	15,579	292	1.87
RS F	9.9740	94.21	149.131	15,871			

Where:

- P: weight of the solid matrix in the thimble (g);
- TS: total solids percentage of the starting sample (%);
- E: weight of TH hydrocarbons extracted and purified with clean-up operations (mg);
- [TH]: mass concentration of total hydrocarbons per kg of total solids in the sample, calculated according to equation (3.3);
- Avg.: average value of the concentrations of total hydrocarbons in the samples (mg/kg$_{TS}$);
- St. dev.: standard deviation of the concentration values of total hydrocarbons in the samples (mg/kg$_{TS}$);
- Dev.: standard deviation value compared to the mean value, expressed in percentage terms.

Table 3.7. TH concentration variations in SS due to AD.

CS	MA5	MA10	MA20	RS
+12.97%	+17.07%	+4.93%	+15.63%	-29.94%

In all conditioned sludge samples, the AD process has led to an increase in the concentration of hydrocarbons compared to the initial condition. The initial concentration in CS of total hydrocarbons was equal to 11,533 mg/kg$_{TS}$ (Table 3.5), while downstream of AD, the concentration values obtained are 13,029 mg/kg$_{TS}$, 13,501 mg/kg$_{TS}$, 12,102 mg/kg$_{TS}$, and 13,336 mg/kg$_{TS}$ respectively for the CS, MA5, MA10 and MA20 samples (Table 3.6). In particular, in percentage terms, digestion led to an increase in the TH concentration of 12.97% in the reactors under conditions of pure anaerobiosis, while for the micro-aeration conditions, the increase was respectively 17.07%, 4.93%, and 15.63% in reactors with micro-aeration equal to 5 mL, 10 mL and 20 mL (Table 3.7).

To try to give an interpretation of the result obtained, we must focus on the substantial difference between the types of SS used in this study, relating to the presence or absence of chemical conditioning. The results show that all digestion processes involving conditioned sludge have increased TH concentrations, while the AD of unconditioned sludge (RS) showed a decrease of 29.94% of this fraction, following what happens to volatile solids (-53%). The chemical treatment to which the sludge was placed in the conditioning phase could explain the increased concentration of total hydrocarbons downstream of AD. The chemical additives used mainly separate the aqueous phase from the solid one, which hinders the transfer of matter, similar to what happens in correspondence with biomethane production. In such a matrix, aliphatic hydrocarbons would tend to be complexed inside a more compacted NOM due to the influence of the polyelectrolytes and, in general, all types of additives used during the conditioning phase (Al-Jasser, 2009; Pontoni et al., 2021), with the effect of making them more difficult to extract through the method developed.

Furthermore, downstream AD of the chemically conditioned sludge, the degradation action by the microbial consortia acting during digestion, both in anaerobic and micro-aerobic conditions, could produce an increase in the extractability of aliphatic hydrocarbons.

3.2.1.4.b. C_{10}-C_{40} Hydrocarbon concentrations measurement – GC-MS determination

The SS samples, conditioned and not, before and after AD (only CS and RS, IN and OUT, without micro-aeration cases) were measured through GC-MS analysis, which complies with quantifying the concentration of the C_{10}-C_{40} hydrocarbons fraction, i.e., the fraction of aliphatic hydrocarbons ranging from n-decane to n-tetracontane. The calibration procedure, examined in section 2.1.3, consents, knowing the values of the areas underlying the chromatograms obtained by the injection of each sample in the GC-MS, to know the value of the concentration of the C_{10}-C_{40} hydrocarbons content into that sample.

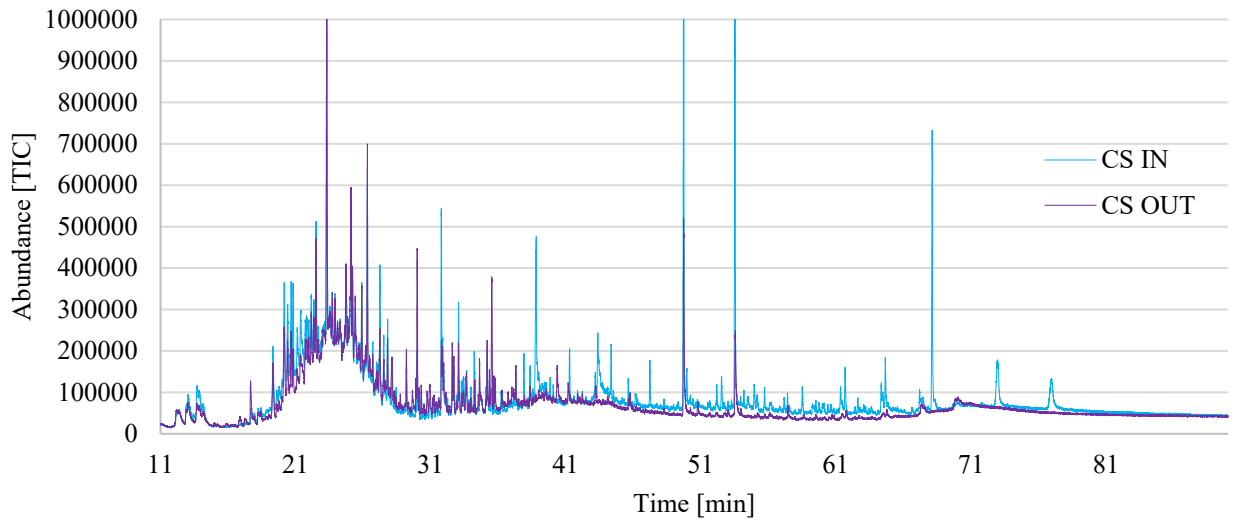

Figure 3.26. Chromatograms related to two CS samples, before and after AD.

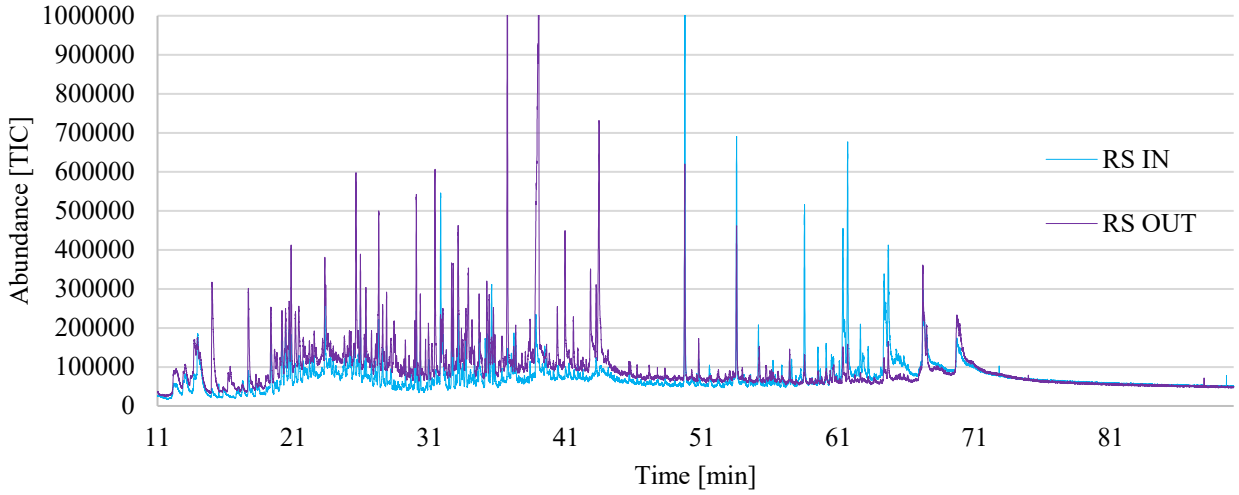

Figure 3.27. Chromatograms related to two RS samples, before and after AD.

By integrating the areas underlying the chromatograms obtained for each sample and applying the equation (3.4) exposed in sections 2.1.3 and 3.1.1, the results shown in the Tables below were obtained.

Table 3.8. C_{10}-C_{40} hydrocarbons weight, in 10 mL of solvent, before AD.

Sample	Chromatogr. Area	Conc. (g/L)	E (mg)
CS IN1	2592362093	4.57	45.75
CS IN2	3458367516	6.03	60.29
RS IN1	5650955140	9.71	97.10
RS IN2	4918665929	8.48	84.80

Table 3.9. C_{10}-C_{40} hydrocarbons weight, in 10 mL of solvent, after AD.

Sample	Chromatogr. Area	Conc. (g/L)	E (mg)
CS OUT A	3264787236	5.70	57.04
CS OUT B	3993284157	6.93	69.27
CS OUT C	3502456124	6.10	61.03
RS OUT E	4053705658	7.03	70.28
RS OUT F	2924127778	5.13	51.32

Where:

- Chromatographic Area: area underlying the GC-MS chromatogram (i.u.*min);
- Conc.: concentration values obtained through the linear calibration equation (3.4), expressed in mass (g) of C_{10}-C_{40} hydrocarbons per volume unit (L) of solvent used for dilution and injection in GC-MS;
- E: C_{10}-C_{40} hydrocarbons weight diluted in 10 mL of CH_3COCH_3 to be injected into the GC-MS, calculated according to equation (3.6) (mg).

The weights thus obtained (E) must be related to the solid mass fraction of the starting samples' weight to obtain the mass concentration of the C_{10}-C_{40} hydrocarbons on kg of the sample's total solids.

Table 3.10. C_{10}-C_{40} hydrocarbon concentrations in SS before AD.

Sample	P (kg)	TS (%)	P_{TS} (kg_{TS})	[C_{10}-C_{40}] (mg/kg_{TS})	Avg. (mg/kg_{TS})	St. dev. (mg/kg_{TS})	Dev. (%)
CS IN1	0.0089	93.73	0.0083	5,488	5,918	430	7.27
CS IN2	0.0101	93.73	0.0095	6,348			
RS IN1	0.0102	92.55	0.0095	10,274	11,706	1,432	12.24
RS IN2	0.0070	92.55	0.0065	13,139			

Table 3.11. C_{10}-C_{40} hydrocarbon concentrations in SS after AD.

Sample	P (kg)	TS (%)	P_{TS} (kg_{TS})	[C_{10}-C_{40}] (mg/kg_{TS})	Avg. (mg/kg_{TS})	St. dev. (mg/kg_{TS})	Dev. (%)
CS OUT A	0.0097	94.12	0.0091	6,239	6,715	489	7.28
CS OUT B	0.0100	93.79	0.0094	7,388			
CS OUT C	0.0100	94.00	0.0094	6,520			
RS OUT E	0.0101	94.04	0.0095	7,372	6,417	955	14.89
RS OUT F	0.0100	94.21	0.0094	5,461			

Where:

- P: weight of the sample (g);
- TS: percentage of total solids of the sample (%);
- P_{TS}: weight of the total solids of the sample (kg_{TS});
- [C_{10}-C_{40}]: mass concentration of C_{10}-C_{40} hydrocarbons per kg of total solids in the sample, calculated according to equation (3.5);
- Avg.: average value of the concentration of C_{10}-C_{40} hydrocarbons in the samples (mg/kg_{TS});
- St. dev.: standard deviation of the concentration values of C_{10}-C_{40} hydrocarbons in the samples (mg/kg_{TS});
- Dev.: standard deviation value compared to the mean value, expressed in percentage terms.

The following Table shows the percentage change in the concentration of C_{10}-C_{40} hydrocarbons due to digestion:

Table 3.12. Percentage variation in the concentration of C_{10}-C_{40} hydrocarbons due to AD.

CS	RS
13.5(±11.0)%	-45.2(±14.7)%

The AD process, starting from the same sample weight, increased the concentration of C_{10}-C_{40} hydrocarbons in the conditioned SS samples. This result is probably due to the degradative effect on the sludge matrix since digestion has increased the extractability of the hydrocarbons, which did not happen for the unconditioned samples since they do not present the compacting phenomenon of the organic matter that the chemical conditioning has induced in CS. The initial concentration of C_{10}-C_{40} hydrocarbons is equal to 5,918(±430) mg/kg_{TS} for CS IN, while for the samples downstream of AD, the concentration value obtained is equal to 6,715(±489) mg/kg_{TS}. Conversely, for the SS that have not undergone conditioning, there is a decrease in the concentration of C_{10}-C_{40} hydrocarbons, which

goes from 11,706(±1,432) mg/kgTS for RS into 6,417(±955) mg/kgTS for the samples downstream of the AD. In percentage terms, digestion increased C_{10}-C_{40} hydrocarbon concentration of 13.5(±11.0)% for CS (an increase which, due to the near coincidence between the average and the standard deviation, is practically an invariance) and decreased hydrocarbons for the unconditioned sludge of -45.2(±14.7)%. We also found a very similar result for the total hydrocarbons, measured with the gravimetric method, as shown in the graphs in Figure 3.28, while Figure 3.29 show the same results as Table 3.12, related to C_{10}-C_{40} hydrocarbons.

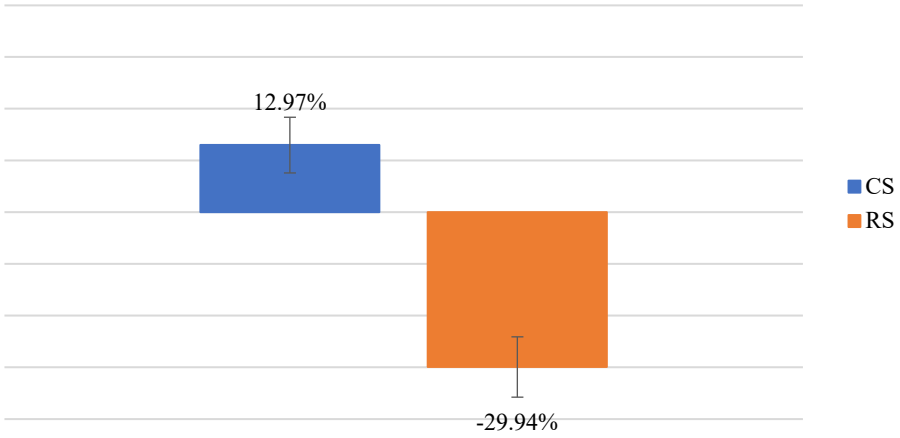

Figure 3.28. TH concentration variations before and after AD with gravimetry.

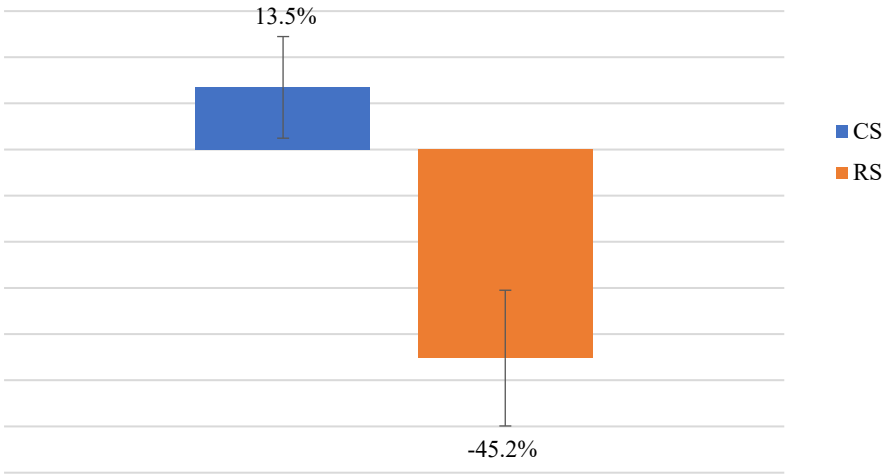

Figure 3.29. C_{10}-C_{40} concentration variations before and after AD with GC-MS.

Tables 3.5, 3.6, 3.10, and 3.11 show C_{10}-C_{40} hydrocarbon concentrations of about half TH for both SS, before (C_{10}-C_{40}/TH IN equal to 52.65±6.96% and 51.31±4.47 for RS and CS, respectively) and after AD (C_{10}-C_{40}/TH OUT equal to 41.19±6.18% and 51.55±3.79 for RS and CS, respectively).

The chemical treatment to which CS was subjected could cause the measured increase in the concentration of C_{10}-C_{40} hydrocarbons downstream of AD. The chemical additives used have the main effect of separating the aqueous phase from the solid one, with the consequence of hindering the mass transfer and reducing the mobility and bioavailability of aliphatic hydrocarbons trapped inside a more compacted NOM, due to the chemical conditioning, with the effect of making them more challenging to extract. NOM, indeed, has been widely described for its surfactant activity and capability of forming hydrophobic niches able to sequestrate hydrophobic compounds, which were proven to be exceedingly difficult to access even by the action of organic solvents (Pontoni et al., 2021; Yao et al., 2022).

3.2.1.4.c. Hydrocarbon mass removal yields

For evaluating hydrocarbon mass removal yield, it has to be taken into account that the total solids content in the samples decreased due to AD, as seen in the following Table.

Table 3.13. TS contents in the SS samples.

Sample	TS (%)
CS IN 1 CS IN 2	4.47
RS IN 1 RS IN 2	3.86
CS OUT A	3.55
CS OUT B	3.10
CS OUT C	3.24
RS OUT E	2.44
RS OUT F	2.39

The hydrocarbon mass removal yields in the analyzed SS samples, calculated according to equation (3.7), then take on the appearance shown in the following Figures.

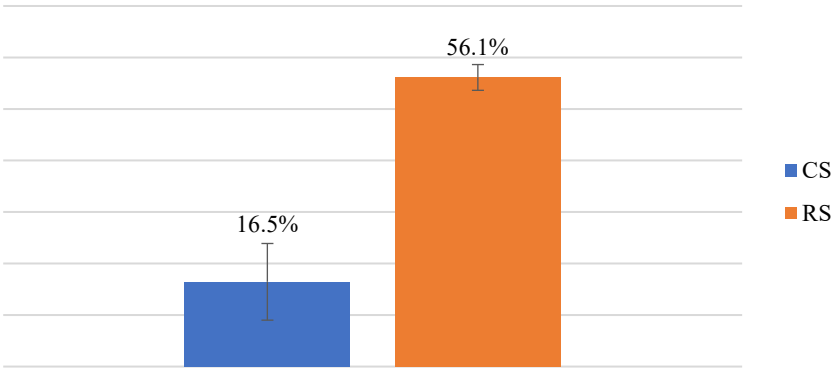

Figure 3.30. TH mass removal yields due to AD.

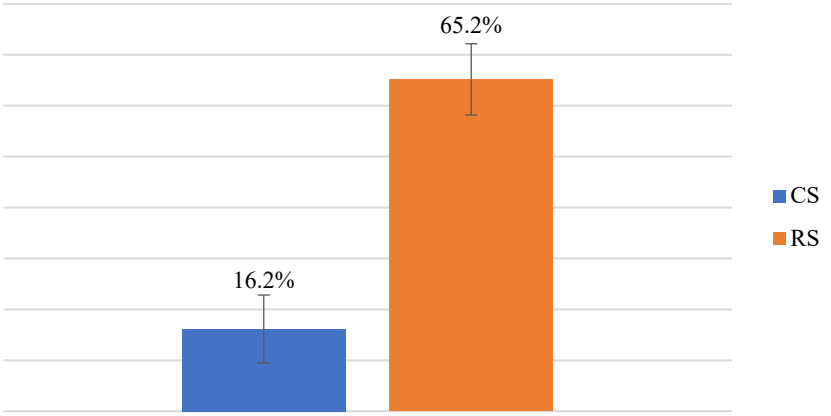

Figure 3.31. C_{10}-C_{40} mass removal yields due to AD.

Figures 3.30 and 3.31 show how AD affected all SS samples, reducing the hydrocarbon content for both CS and RS. Hydrocarbon removal is for RS about three times than for CS, both for TH and C_{10}-C_{40} (65.2±7.0% and 56.1±2.5% for RS against 16.2±6.7% and 16.5±7.4% for CS, respectively), further confirming what happens for the VS variations and the SBP. It should also be noted that, for CS, C_{10}-C_{40} reduction is almost the same as TH, while for RS, the difference between the two hydrocarbon fractions' reductions is within the tolerance of the standard deviations, with statistically significant accordance ($p < 0.05$). According to studies on hydrocarbon degradation during fermenting processes, degradative pathways with electron acceptors alternative to oxygen lead to the formation of aliphatic compounds of smaller size, as well as substances such as fatty acids (Boll et al., 2020; Ji et al., 2013; Sieber et al., 2012). Moreover, TH concentration expresses the whole aliphatic hydrocarbon content in the sludge, while the C_{10}-C_{40} fraction includes alkane compounds

from n-decane to n-tetracontane; from the obtained results, it must be admitted that most likely AD was more effective in the hydrocarbons reduction in RS than in CS. This different behavior could be explained by considering that the SS solid matrix consists mainly of NOM (Pontoni et al., 2021) and that, unlike RS, in CS, the main effect of the chemical conditioning was the compacting of the NOM in the form of flocs, where much of the water necessary for the biological degradation processes came out, and the sludge-water interface is reduced (Al-Jasser, 2009).

3.2.1.4.d. MADEP Speciation

Given the evident qualitative differences between the two types of sludge, as can be seen by comparing the chromatograms in Figures 3.26 and 3.27, using MADEP speciation (Bishop, 1997; Massachusetts D.E.P., 2004), we grouped the detected hydrocarbons into two classes, i.e., Lighter EPH (L_{EPH}), and Heavier EPH (H_{EPH}), targeting the ranges of C_{10}-C_{18} and C_{19}-C_{40} respectively. The chromatograms in Figures 3.26 and 3.27 show a higher L_{EPH} content for CS than for RS both before and after AD, which could be due to the presence of low molecular weight hydrocarbons in the chemicals added in CS during the conditioning treatment. AD process, moreover, causes a qualitative shift in hydrocarbons, with an increase in the L_{EPH} fraction at the expense of H_{EPH}. This effect agrees with several studies that report how the degradation pathways of hydrocarbons lead to the reduction of molecular complexity by increasing the amount of smaller molecular groups (Boll et al., 2020; Ji et al., 2013).

3.2.2. Quantification of the hydrocarbons contained in sewage sludge using ultrasounds

This procedure followed what was done previously, except that the extraction was done with a sonicated bath.

3.2.2.1. Characterization of the samples

The weighing operation on the SS samples showed the results in the Table below.

Table 3.14. Samples weighing.

Sample	Weigh (mg)
RS IN 1	501.16
RS IN 2	501.51
RS IN 4	500.07
RS OUT E 1	497.31
RS OUT E 2	503.12
RS OUT F 1	501.40
RS OUT F 2	505.10

Sample	Weigh (mg)
CS IN 1	458.81
CS IN 2	501.46
CS OUT A	500.11
CS OUT B	501.87
CS OUT C	500.65

3.2.2.2. Hydrocarbon measurements

Below are the results of the gravimetric and chromatographic determinations of the hydrocarbons contained in the 12 examined SS samples.

Table 3.15. TH concentrations in SS before AD.

Sample	P (g)	TS (%)	E (mg)	[TH] (mg/kg$_{TS}$)	Avg. (mg/kg$_{TS}$)	St. dev. (mg/kg$_{TS}$)	Dev. (%)
CS IN 1	458.81	93.73	4.16	9,673	9,038	898	9.93
CS IN 2	501.46	93.73	3.95	8,404			
RS IN 1	501.16	92.55	1.39	2,997	3,538	663	18.75
RS IN 2	501.51	92.55	1.55	3,339			
RS IN 4	500.07	92.55	1.98	4,278			

Table 3.16. TH concentrations in SS after AD.

Sample	P (g)	TS (%)	E (mg)	[TH] (mg/kg$_{TS}$)	Avg. (mg/kg$_{TS}$)	St. dev. (mg/kg$_{TS}$)	Dev. (%)
CS OUT A	500.11	92.63	6.15	13,276	10,570	2,346	22.19
CS OUT B	501.87	92.81	4.25	9,125			
CS OUT C	500.65	92.48	4.31	9,309			
RS OUT E 1	497.31	94.04	5.17	11,055	9,738	1,721	17.67
RS OUT E 2	503.12	94.04	5.17	10,927			
RS OUT F 1	501.40	94.21	3.47	7,346			
RS OUT F 2	505.10	94.21	4.58	9,625			

Where:

- P: weight of the solid matrix in the thimble (g);
- TS: total solids percentage of the starting sample (%);
- E: weight of TH hydrocarbons extracted and purified with clean-up operations (mg);
- [TH]: mass concentration of total hydrocarbons per kg of total solids in the sample, calculated according to equation (3.3);
- Avg.: average value of the concentrations of total hydrocarbons in the samples (mg/kg$_{TS}$);
- St. dev.: standard deviation of the concentration values of total hydrocarbons in the samples (mg/kg$_{TS}$);
- Dev.: standard deviation value compared to the mean value, expressed in percentage terms.

Table 3.17. C_{10}-C_{40} hydrocarbons weight, in 10 mL of solvent, before AD.

Sample	Chromatogr. Area	Conc. (g/L)	E (mg)
CS IN 1	3645566956	2.588	1.294
CS IN 2	4348572505	3.077	1.538
RS IN 1	1483795788	1.0847	0.5424
RS IN 2	926217097	0.6970	0.3485
RS IN 4	1596442122	1.1630	0.5815

Table 3.18. C_{10}-C_{40} hydrocarbons weight, in 10 mL of solvent, after AD.

Sample	Chromatogr. Area	Conc. (g/L)	E (mg)
CS OUT A	5408539706	3.813	1.907
CS OUT B	3645407617	2.588	1.294
CS OUT C	4069713617	2.883	1.441
RS OUT E 1	3627339875	2.575	1.288
RS OUT E 2	4398532777	3.111	1.556
RS OUT F 1	3023726585	2.155	1.078
RS OUT F 2	2429917153	1.743	1.307

Where:

- Chromatographic Area: area underlying the GC-MS chromatogram (ionic. u.*min);
- Conc.: concentration values obtained through the linear calibration equation (3.4), expressed in mass (g) of C_{10}-C_{40} hydrocarbons per volume unit (L) of solvent used for dilution and injection in GC-MS;
- E: C_{10}-C_{40} hydrocarbons weight diluted in 10 mL of CH_3COCH_3 to be injected into the GC-MS, calculated according to equation (3.6) (mg).

The weights thus obtained (E) must be related to the solid mass fraction of the starting samples' weight to obtain the mass concentration of the C_{10}-C_{40} hydrocarbons on kg of the sample's total solids.

Table 3.19. C_{10}-C_{40} hydrocarbon concentrations in SS before AD.

Sample	P (kg)	TS (%)	P_{TS} (kg_{TS})	[C_{10}-C_{40}] (mg/kg_{TS})	Avg. (mg/kg_{TS})	St. dev. (mg/kg_{TS})	Dev. (%)
CS IN 1	0.00046	93.73	0.00043	3,009	3,141	187	5.95
CS IN 2	0.00050	93.73	0.00047	3,273			
RS IN 1	0.00050	92.55	0.00046	1,169	1,059	270	25.53
RS IN 2	0.00050	92.55	0.00046	751			
RS IN 4	0.00050	92.55	0.00046	1,256			

Table 3.20. C_{10}-C_{40} hydrocarbon concentrations in SS, after AD.

Sample	P (kg)	TS (%)	P_{TS} (kg_{TS})	[C_{10}-C_{40}] (mg/kg_{TS})	Avg. (mg/kg_{TS})	St. dev. (mg/kg_{TS})	Dev. (%)
CS OUT A	0.00050	92.63	0.00046	4,116.205	3,336	696	20.88
CS OUT B	0.00050	92.81	0.00047	2,777.740			
CS OUT C	0.00050	92.48	0.00046	3,112.946			
RS OUT E 1	0.00050	94.04	0.00047	2,753.03	2,767	411	14.87
RS OUT E 2	0.00050	94.04	0.00047	3,287.86			
RS OUT F 1	0.00050	94.21	0.00047	2,281.43			
RS OUT F 2	0.00051	94.21	0.00048	2,746.37			

Where:

- P: weight of the sample (g);
- TS: percentage of total solids of the sample (%);
- P_{TS}: weight of the total solids of the sample (kg_{TS});
- [C_{10}-C_{40}]: mass concentration of C_{10}-C_{40} hydrocarbons per kg of total solids in the sample, calculated according to equation (3.5);
- Avg.: average value of the concentration of C_{10}-C_{40} hydrocarbons in the samples (mg/kg_{TS});
- St. dev.: standard deviation of the concentration values of C_{10}-C_{40} hydrocarbons in the samples (mg/kg_{TS});
- Dev.: standard deviation value compared to the mean value, expressed in percentage terms.

Other interesting data come from the ratio between C_{10}-C_{40} and total hydrocarbons, as shown in the next Table.

Table 3.21. C_{10}-C_{40} on total hydrocarbon ratios in SS samples.

C_{10}-C_{40}/TH (%)	RS	CS
IN	30.29±6.78	35.02±3.92
OUT	28.65±2.34	31.60±1.30

Both in RS and CS, indeed, AD causes a reduction in C_{10}-C_{40} fraction on TH, probably due to its greater bioavailability compared to the whole hydrocarbon mixture.

3.2.3. Matrix effect – deepening of the retention effect of the organic matter in a sewage sludge

3.2.3.1. Samples characterization

The sample preparation procedure made it possible to obtain the following sample types:

- RS, unconditioned and undigested SS, not methanolized, raw sample;
- SP, unconditioned and undigested SS, not methanolized, spiked;
- SK, unconditioned and undigested SS, methanolized, spiked.

Table 3.22. Samples weighing.

Sample	Weight (mg)	Sample	Weight (mg)	Sample	Weight (mg)
RS G	25.341	SP G	25.175	SK A	26.639
RS H	26.092	SP H	25.597	SK B	29.272
RS I	27.593	SP I	25.080	SK C	28.736

3.2.3.2. GC-MS determinations

In Figure 3.32, which shows the overlapping of the chromatograms, an entrapment effect implemented by the NOM is evident, which is reduced with the methanolysis reaction on the sample before making the spike (SK) since, in this case, the NOM is chemically degraded. It loses its three-dimensional structure, resulting in less complex and letting a more pronounced release of extractable non-polar substances.

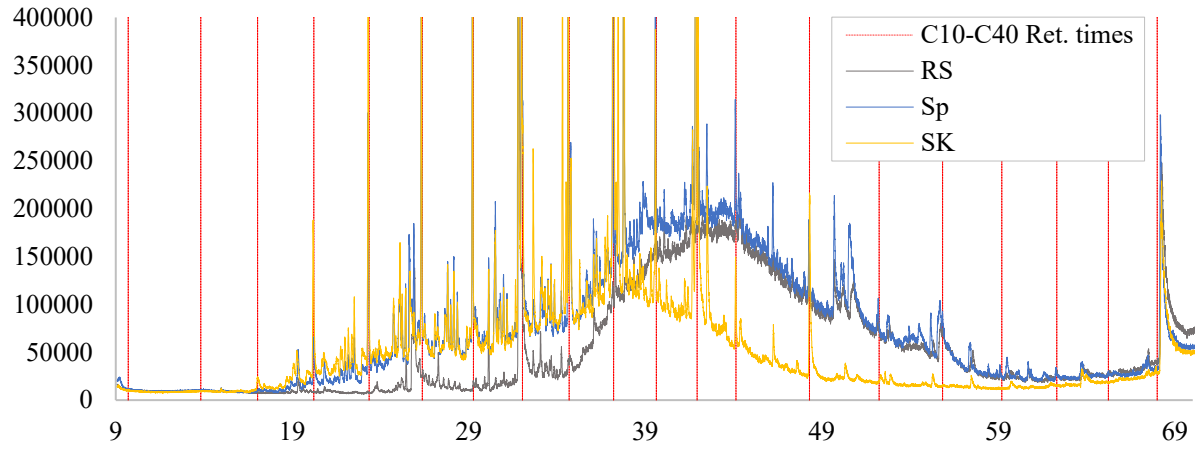

Figure 3.32. Unconditioned sample spiked (10,000 mg/kg$_{TS}$) and not, with and without methanolysis, without clean-up.

In Figure 3.32, where the retention times of the individual aliphatic hydrocarbons belonging to a standard mixture have also been represented, the UCMs of the three chromatograms are very different. While the RS sample has a particularly pronounced UCM between minutes 35 and 59, with very few resolution peaks related to single hydrocarbons possibly extractable from the matrix, both spiked samples, SP and SK, have a much more pronounced hump in the initial part, as early as minute 18, with evident resolution peaks mainly linked to the hydrocarbons added with the spike. While SP nevertheless presents a UCM that, between minutes 43 and 59, overlaps quite a lot with those obtained for RS, the UCM of the SK sample, which has undergone methanolysis reaction, in this section has a much less accentuated shape, with a greater resolution of the individual hydrocarbons added. This latter characteristic represents the interesting result: the effect of the methanolysis reaction on the solid matrix of SK samples is highlighted by the less accentuated UCM and by the more prominent resolution peaks of the added hydrocarbons to analytically manifest the reduction of the entrapment effect, due to the NOM of the SS solid matrix (Chen et al., 2022; Pontoni et al., 2021), obtained through the breaking of complex molecules carried out by the methanolysis process.

Qualitatively this phenomenon is even more evident in Figure 3.33, where the chromatograms of a sample that has never undergone spiking before and after the methanolysis process are compared. In this comparison, the number of substances released downstream of the cleavage of the polysaccharide shells that constitute the NOM is very high.

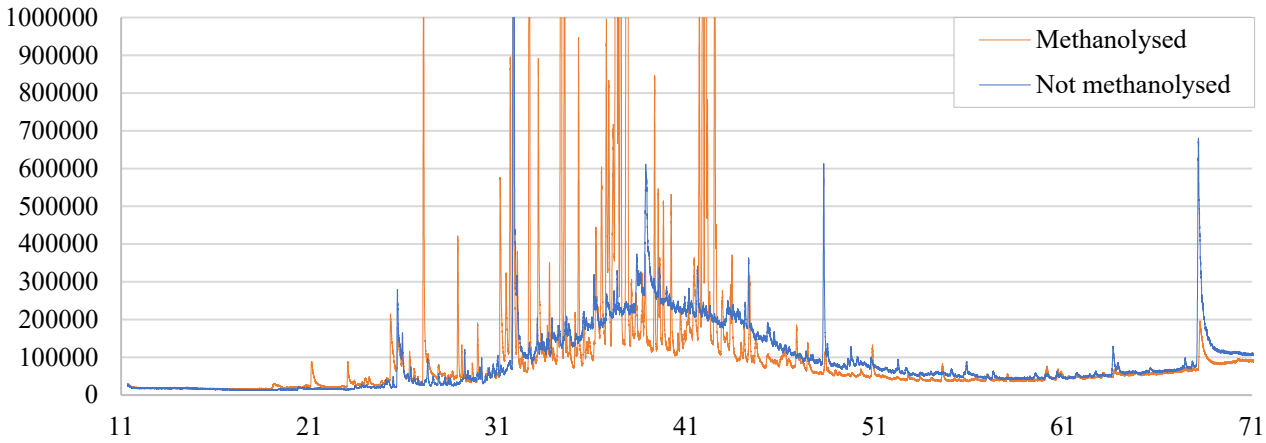

Figure 3.33. Unconditioned sample, with and without methanolysis, no clean-up.

3.2.3.3. Effect of Clean-up procedures on the findings

The clean-up procedures are critical, as, in this phase, the sample is subjected to a series of treatments aimed at removing substances that could have been extracted with C_{10}-C_{40} hydrocarbons, which consequently would risk altering the estimations. The samples were then cleaned-up, and their subsequent gas chromatographic analysis was carried out. In Figure below is the chromatogram of the samples that have undergone this treatment.

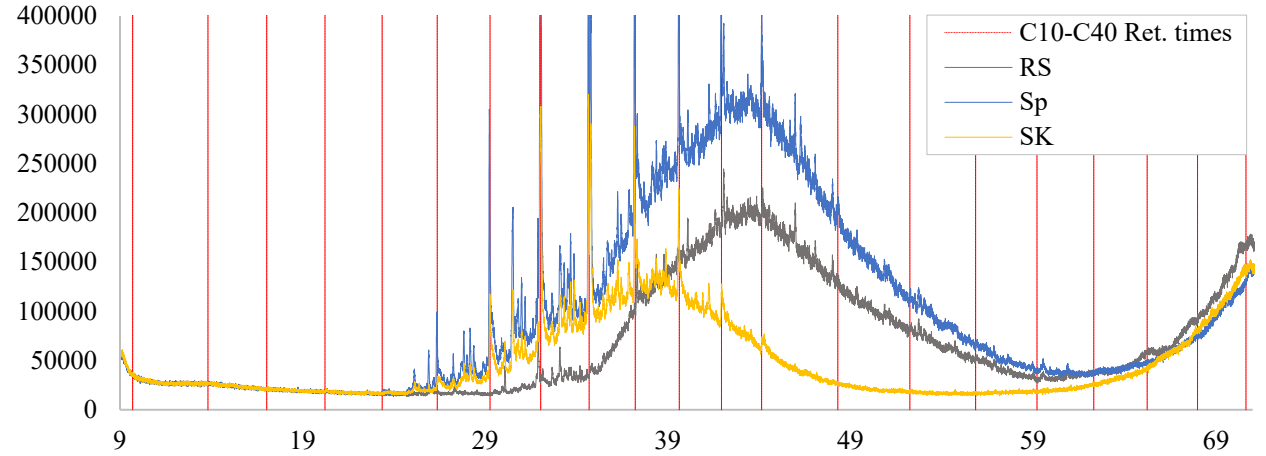

Figure 3.34. Unconditioned sample spiked (10,000 mg/kg_{TS}) and not, with and without methanolysis, with clean-up.

As seen in Figure 3.34, the trend is somewhat similar to that described previously in Figure 3.32, except that the clean-up acts very limitedly on the chromatogram of the sample that has not undergone spiking and therefore has fewer hydrocarbons. In contrast, for the samples with added hydrocarbons,

the effect of both spike addition and methanolysis is even more evident than in the case without clean-up.

3.2.3.4. Investigation of toxic substances

NOM, the principal component of the solid matrix of a SS, acts as a natural accumulator and transport mean of organic pollutants that can affect the soil and environmental matrices. As previously discussed, the modification of the organic matrix, carried out by the methanolysis reaction, makes any compounds trapped in it easily extractable in organic solvents and consequently detectable through GC-MS analysis. These substances would not have been identified using conventional extraction techniques.

These substances are the object of in-depth study in various scientific articles that show that these can have harmful effects on all animal and plant species; these substances also have safety data sheets that almost always testify to their tangible impact on environmental matrices.

Tables 3.23 and 3.24 show the substances identified using GC-MS, based on retention times and the NIST98 database, for samples not subjected to digestion, with and without methanolysis. Related chromatograms are shown in Figure 3.33.

Table 3.23. Substances found in the sample not subjected to methanolysis.

M.W.	R.T.	Denomination	Equation
518	25.44	Cycloheptasiloxane, tetradecamethyl	$C_{14}H_{42}O_7Si_7$
428	30.13	3,4-Dihydroxymandelic acid, ethyl ester, tri-TMS	$C_{19}H_{36}O_5Si_3$
282	31.80	2-Propenoic acid, n-pentadecyl ester	$C_{18}H_{34}O_2$
444	34.24	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	$C_{13}H_{39}O_5SiO_6$
149	36.09	Benzene, 1-isocyanate-4-methoxy-	$C_8H_7NO_2$
262	37.11	1,3,12-Nonadecatriene	$C_{19}H_{34}$
180	37.92	1-(2-Furoyl)piperazine	$C_9H_{12}N_20_2$
262	38.36	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	$C_{19}H_{34}$
274	39.01	D-Homoandrostane, (5.alpha.,13alpha)-	$C_{20}H_{34}$
262	41.59	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-,(E,E)-	$C_{18}H_{30}O$
340	48.26	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl)-4-methyl-	$C_{23}H_{32}O_2$
330	50.47	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$
370	65.91	Cholest-8(14)-ene, (5.alpha.)-	$C_{27}H_{46}$
514	68.22	Propanoic acid, 3,3'-thiobis-, didodecyl ester	$C_{30}H_{58}O_4S$
370	70.35	Cholest-3-ene, (5.beta.)-	$C_{27}H_{46}$

Table 3.24. Substances found in the sample subjected to methanolysis.

M.W.	R.T.	Denomination	Equation
88.2	23.86	Hydrazine, 1,2-diethyl-	$C_4H_{12}N_2$
206	26.54	Phenol, 2,4-bis(1,1-dimethyl ethyl)-	$C_{14}H_{22}O$
297	27.58	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$
212	29.61	Tetradecanal	$C_{14}H_{28}O$
232	30.03	Benzene, (1-butylheptyl)-	$C_{17}H_{28}$
278	30.13	Tris(trimethylsilyl)borate	$C_9H_{27}BO_3Si_3$
240	32.03	Methyl E-11-tetradecenoate	$C_{15}H_{28}O_2$
240	32.23	Oxacyclohexadecan-2-one	$C_{15}H_{28}O_2$
242	32.55	Oxirane, [(dodecyloxy)methyl]-	$C_{15}H_{30}O_2$
239	34.17	1-Heptadecene	$C_{17}H_{34}$
254	34.65	9-Hexadecenoic acid	$C_{16}H_{30}O_2$
269	35.08	Octadecanal	$C_{18}H_{36}O$
258	35.56	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	$C_{18}H_{26}O$
278	36.1	Dibutyl phthalate	$C_{16}H_{22}O_4$ or $C_6H_4(COOC_4H_9)_2$
267	38.31	1-Nonadecene	$C_{19}H_{38}$

140	42.59	2-Methyl-3-isopropylcyclopentanone	$C_9H_{16}O$
134	44.6	1,2,3,4,5,8-Hexahydronaphthalene	$C_{10}H_{14}$
177	48.31	Benzo(b)thiophene-2-carboxamide	C_9H_7NOS
254	48.85	Z-10-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$
267	50	5, 10-dihydro-11H-dibenzo[b, e][1, 4]diazepin-11-one	$C_{13}H_{10}N_2O$
234	60	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methyl ethyl)-. (4alpha,7beta,8beta)-	$C_{14}H_{24}O$
207	62.7	1H-Indole, 2-methyl-3-phenyl-	$C_{15}H_{13}N$
218	63.05	4-n-Hexylthiane, S, S-dioxide	$C_{11}H_{22}O_2S$
178	68.18	Thianaphthene-2-carboxylic acid	$C_9H_6O_2S$

Where:

- M.W.: molecular weight;
- R.T.: retention time, or the instant in which the highest point of the peak is recorded in the chromatographic plot (min);
- Denomination: Name of the substance identified by the mass spectrometer, based on the mass spectra databases stored in the NIST98 database;
- Equation: the brute equation of the identified substance.

From these Tables, it is evident that a more significant number of detectable substances is extractable from the methanolized sample than from the other. In the sample subjected to methanolysis, the cleavage products of the polysaccharide shells, constituting the structure of the NOM, are also extracted. Polar substances from NOM degradation were removed in the clean-up phase. So, it could be noted that some esters are naturally present in the extracts of the non-methanolized sample, as the substances identified at minutes 30.13, 31.80, 50.47, and 68.22. Indeed their presence is not ascribable to the methanolysis reaction.

Tables 3.25 and 3.26 show the results from scientific references on the toxicity or dangerousness of some substances identified in both samples.

Table 3.25. Substances identified in the sample not subjected to methanolysis.

R.T.	Denomination	Description
25.44	Cycloheptasiloxane, tetradecamethyl	Antifungal agent (Moustafa et al., 2013)
30.13	3,4-Dihydroxymandelic acid, ethyl ester, tri-TMS	One of the possible phytoconstituents present in the ethanolic extract of *E. variegata* bark (EBE) responsible for antidepressant activities (Martins and Brijesh, 2020)
31.8	2-Propenoic acid, n-pentadecyl ester	One of the compounds present in the grass of *Lactuca runcinata,* which in turn, has pharmacological potential (College et al., 2015)
34.24	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	Siloxanes, emerging contaminants
36.09	Benzene, 1-isocyanate-4-methoxy-	
37.11	1,3,12-Nonadecatriene	Known to have antifungal or antibacterial activities or both (I. H. Khan et al., 2018)
37.92	1-(2-Furoyl) piperazine	Deriving from benzamide, which has enzymatic inhibition and homolytic activity (Abbasi et al., 2020)
38.36	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	One of the compounds present in *Syzygium benthamianum* leaves which have antimicrobial, antioxidant, and antitumor activity (Kiruthiga et al., 2011)
39.01	D-Homoandrostane, (5.alpha.,13alpha)-	Steroid inhibitor of enzyme activity (Garrido et al., 2011)
41.59	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-,(E,E)-	Terpenoids present in the essential oils of various higher plants (Souza dos Santos, 2019)
48.26	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl)-4-methyl-	Studies of a synthetic antioxidant found acute, subchronic, and chronic toxicity in rats
50.47	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Present in a medicine that is used to treat nerve-related diseases such as paraplegia, hemiplegia, and muscle atrophy (Prabhu et al., 2020)
65.91	Cholest-8(14)-ene, (5.alpha.)-	A potent inhibitor of sterol biosynthesis in cultured animal cells (Tsuda et al., 1979)
68.22	Propanoic acid, 3,3'-thiobis-, didodecyl ester	A chemical component present in the dried seeds of *Parkia speciosa* (Salman et al., 2006)
70.35	Cholest-3-ene, (5.beta.)-	A potent inhibitor of sterol biosynthesis in cultured animal cells (Tsuda et al., 1979)

Table 3.26. Substances identified in the sample subjected to methanolysis.

R.T.	Denomination	Description
23.86	Hydrazine, 1,2-diethyl-	One of the compounds that, after repeated injections in adult rats, caused neuraesthesium-epithelioma products of the olfactory bulb, brain tumors, hepatic hemangioendotheliomas, mammary carcinomas, and leukemias, respectively (Druckrey et al., 1968)
26.54	Phenol, 2,4-bis(1,1-dimethyl ethyl)-	Present in *Spermacoce hispida L.*, one of the medicinal plants. This and other bioactive compounds have many applications in antioxidant, anticancer, anti-inflammatory, and anti-ulcer properties (Belgiorno et al., 2007)
27.58	Octadecane, 1-(ethenyloxy)-	
29.61	Tetradecanal	A repellent used by ant queens to prevent host worker aggression (Ruano et al., 2005)
30.03	Benzene, (1-butylheptyl)-	One of the bioactive compounds produced by marine *Pseudoalteromonas piscicida* (Hassan et al., 2017)
30.13	Tris(trimethylsilyl)borate	Electrolytic additive to improve the interfacial stability of the electrolyte based on cathode/lithium oxide carbonate stratified at high voltage (Mahalakshmi et al., 2013)
32.03	Methyl E-11-tetradecenoate	
32.23	Oxacyclohexadecan-2-one	Sexual and individual signals in the perianal gland secretum of crested porcupines (Massolo et al., 2009)
32.55	Oxirane, [(dodecyloxy)methyl]-	
34.17	1-Heptadecene	Copulation-inducing 1-heptadecene has been found in the extracts of confused birds of meal beetle (*Tribolium confusum*) (Keville and Kannowski, 1975)
34.65	9-Hexadecenoic acid	
35.08	Octadecanal	The levels of dimethyl acetal (DMA) in the muscle of *Octopus vulgaris Cuvier* are identified and quantified as they are derived from ethereal glycerophospholipids, including plasmalogens (PLM), emphasizing the importance and role of this class of phospholipids (Rosa et al., 2004)
35.56	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	It resulted in enzymatic, antioxidant, and lipid peroxidation activities in the earthworm *Eisenia fetida*
36.1	Dibutyl phthalate	It exhibits pollution and degradation characteristics in two types of soils harvested from uncultivated fields, cultivated fields, greenhouses, and vegetables in the Harbin and Handan districts of China (Xu et al., 2008)
38.31	1-Nonadecene	One of the possible phytocompounds present in the ethyl acetate extract of *Enhalus acoroides* (Amudha et al., 2018)
42.59	2-Methyl-3-isopropylcyclopentanone	
44.6	1,2,3,4,5,8-Hexahydronaphthalene	
48.31	Benzo(b)thiophene-2-carboxamide	Its derivatives are powerful antagonists of the urotensin-II receptor (Lim et al., 2016)
48.85	Z-10-Tetradecen-1-ol acetate	One of the compounds present in the leaves of *Apium graveolens*, in which foliar oil, has significant toxic effects on *A. aegypti larvae* (Nagella et al., 2012)
50	5, 10-dihydro-11H-dibenzo[b, e][1, 4]diazepin-11-one	A potent and selective antagonist of the muscarinic M2 receptor (Watanabe et al., 1998)
60	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methyl ethyl)-. (4aalpha,7beta,8abeta)-	
62.7	1H-Indole, 2-methyl-3-phenyl-	Identified among the bioactive compounds of medicinal plants against *C. Albicans* causing oral candidiasis in humans (Rani et al., 2016)
63.05	4-n-Hexylthiane, S, S-dioxide	
68.18	Thianaphthene-2-carboxylic acid	

From Tables 3.25 and 3.26, many organic compounds were found, and the type and extent of damage that they can cause depend on the chemical and physical characteristics of the molecule, the concentration, and bioavailability, as well as the characteristics of the exposed organism, such as age,

sex, mode of absorption and metabolic capacity. Each of these parameters, both of the molecule and the host, in this case, animal or plant, influences its absorption, the dynamics of distribution, the kinetics of metabolism, and consequently, the accumulation or excretion in the exposed organism, thus varying the extent of the damage.

In conclusion, the NOM constituting the SS solid matrix could be considered as having an entrapment effect on the lipophilic micropollutant substances, including hydrocarbons, that makes the extractability of these substances very complex. Data show that many of the hazardous compounds identified were strongly bound to the NOM matrix, to the point that their extractability with organic solvents was sensibly hindered. This may probably affect the bioavailability and hence the toxic activity of the biota. Indeed, the effect of the complexation of contaminants into SS NOM certainly needs further investigation to assess its impact on living organisms.

Nevertheless, with this research, it was possible to demonstrate how, by chemically modifying the organic matrix of sludge, it is possible to extract and identify a series of otherwise “hidden” substances with a potentially high level of toxicity for both autotrophic and heterotrophic organisms.

3.2.4. Thermophilic anaerobic digestion of chemically conditioned and dewatered sewage sludge

3.2.4.1. Characterization of samples undergone anaerobic digestion

TS and VS were measured and are shown in Table 3.27.

Table 3.27. Characterization of SS samples undergone AD.

Sample	F1 IN	F2 IN
TS (%)	13.39 ± 0.48	22.15 ± 0.44
VS (%)	11.09 ± 0.40	17.37 ± 0.28
VS/TS (-)	0.83 ± 0.01	0.78 ± 0.01

In comparable conditions with F1 and F2, the sludge treatment plant digested the F3 sludge. Therefore, after the biological treatments, TS and VS for the three sludge were shown in Table 3.28.

Table 3.28. Characterization of SS samples after AD.

Sample	F1 OUT	F2 OUT	F3 OUT
TS (%)	11.38 ± 0.60	20.98 ± 0.30	9.88 ± 0.06
VS(%)	9.17 ± 0.71	15.82 ± 024	5.69 ± 0.03
VS/TS (-)	0.78 ± 0.03	0.75 ± 0.01	0.58 ± 0.01

As can be seen in Tables 3.27 and 3.28, the AD affected both TS and VS for the two SS samples processed in the laboratory.

The samples were dried and subjected to a particle size reduction for the subsequent operations. TS values are now as in Table 3.29.

Table 3.29. Characterization of dried SS samples for the measurements of hydrocarbon content.

	F1 IN	F1 IN	F1 OUT	F2 OUT	F3 OUT
TS (%)	85.66 ± 0.12	90.55 ± 0.06	92.30 ± 0.32	93.31 ± 0.05	92.39 ± 0.06

The samples were brought to a very high solids rate to simplify the extraction and clean-up operations.

3.2.4.2. Total and C_{10}-C_{40} hydrocarbon content evaluations and discussions

Through the consolidated ultrasonic method, with dichloromethane as the solvent, the gravimetric determinations gave the concentration values shown in Table 3.30.

Table 3.30. TH concentrations in SS samples, before and after AD.

Sample	P (mg)	TS (%)	E (mg)	[TH] (mg/kg$_{TS}$)	Avg. (mg/kg$_{TS}$)	St. dev. (mg/kg$_{TS}$)	Dev. (%)
F1 IN A	988.70	85.66	11.428	13,494	13,954	869	6.23
F1 IN B	1,029.80	85.66	11.830	13,411			
F1 IN C	1,007.70	85.66	12.910	14,957			
F2 IN A	1,033.70	90.55	16.641	17,778	18,782	1,497	7.97
F2 IN B	1,009.70	90.55	16.517	18,065			
F2 IN C	1,013.30	90.55	18.812	20,502			
F1 OUT A	1,156.60	92.30	5.829	5,460	5,385	518	9.61
F1 OUT B	1,028.60	92.30	5.564	5,861			
F1 OUT C	1,096.70	92.30	4.893	4,834			
F2 OUT A	1,066.30	93.31	8.992	9,038	8,072	1,183	14.66
F2 OUT B	1,101.70	93.31	6.941	6,752			
F2 OUT C	1,044.00	93.31	8.208	8,426			
F3 OUT A	1,111.50	92.39	9.853	9,595	9,466	1,443	15.25
F3 OUT B	1,136.20	92.39	8.358	7,962			
F3 OUT C	1,085.70	92.39	10.873	10,840			

Where:

- P: weight of the solid matrix in the thimble (g);
- TS: total solids percentage of the starting sample (%);
- E: weight of TH hydrocarbons extracted and purified with clean-up operations (mg);
- [TH]: mass concentration of total hydrocarbons per kg of total solids in the sample, calculated according to equation (3.3);
- Avg.: average value of the concentrations of total hydrocarbons in the samples (mg/kg$_{TS}$);
- St. dev.: standard deviation of the concentration values of total hydrocarbons in the samples (mg/kg$_{TS}$);
- Dev.: standard deviation value compared to the mean value, expressed in percentage terms.

The big difference in the hydrocarbon content between one SS and another immediately strikes. The values obtained for the F1 and F2 sludges are perfectly in line with what was expected, given that they had been subjected to chemical conditioning in their respective WWTPs with very different cationic polyelectrolytes, from whose safety data sheets (SDS) and from WWTPs' laboratory information, the following Table can be deduced.

Table 3.31. Characterization of the Polyelectrolytes from the WWTPs of origin.

SS sample	Polyelectrolyte used	Polyelectrolyte composition (from section 3 of the SDS)	Concentration (%)	Polyelectrol. dosing (kg/ton SS)	C_{10}-C_{40} hydrocarbon concentration (mg/kg_{TS})
F1	DRYFLOC 652	adipic acid – sulphamidic acid	< 2.5	16.66	2,400
F2	SEDIFLOC 6080C	C_{11}-C_{14} hydrocarbons n-alkanes, iso-alkanes and cyclic (Aromatics content < 2%)	20-30	27.73	8,900

As evident by observing Table 3.31, for F1 and F2, the hydrocarbon contents also have very different sources, as there was a direct addition of aliphatic hydrocarbons in F2 during the conditioning phase. Furthermore, the data about the efficacy of the biological process implemented in the laboratory assumes considerable prominence, given the evident reductions in the total hydrocarbon concentrations of F1 and F2 due to AD. As can be observed in Table 3.30, TH undergoes about a halving, passing from 13,954(±869) mg/kg_{TS} to 5,385(±518) mg/kg_{TS} in the F1 sludge and from 18,782(±1,497) mg/kg_{TS} to 8,072(±1,183) mg/kg_{TS} in the F2 sludge.

For the C_{10}-C_{40} fraction, GC-MS analysis has returned the results shown in the Table below.

Table 3.32. C_{10}-C_{40} concentrations in SS samples before and after AD.

Sample	P (mg)	TS (%)	Chromatogr. Area	Conc. (g/L)	E (mg)
F1 IN A	988.70	85.66	1648880104	7.37351903	3.686759515
F1 IN B	1,029.80	85.66	2323864500	9.901653912	4.950826956
F1 IN C	1,007.70	85.66	2686036591	11.25815919	5.629079596
F2 IN A	1,033.70	90.55	4532901370	18.17553876	9.087769380
F2 IN B	1,009.70	90.55	3814154751	15.48349371	7.741746857
F2 IN C	1,013.30	90.55	4447089137	17.85413148	8.927065739
F1 OUT A	1,156.60	92.30	1042543366	5.102502002	2.551251001
F1 OUT B	1,028.60	92.30	923081686	4.655061673	2.327530836
F1 OUT C	1,096.70	92.30	888069541	4.523924678	2.261962339
F2 OUT A	1,066.30	93.31	1656403262	7.401696805	3.700848402
F2 OUT B	1,101.70	93.31	1359690485	6.290367527	3.145183764
F2 OUT C	1,044.00	93.31	1663041294	7.426559365	3.713279683
F3 OUT A	1,111.50	92.39	2771150851	11.57695223	5.788476117
F3 OUT B	1,136.20	92.39	2174280604	9.341391679	4.670695839
F3 OUT C	1,085.70	92.39	2686354400	11.25934954	5.629674768

Sample	[C_{10}-C_{40}] (mg/kg$_{TS}$)	Avg. (mg/kg$_{TS}$)	St. dev. (mg/kg$_{TS}$)	Dev. (%)	C_{10}-C_{40} /TH (%)	Avg. C_{10}-C_{40} /TH (%)	St. dev. C_{10}-C_{40} /TH (%)	Dev. (%)
F1 IN A	4,353	5,496	1,089	19.81	32.26	39.24	6.11	15.56
F1 IN B	5,613				41.85			
F1 IN C	6,521				43.60			
F2 IN A	9,709	9,302	723	7.77	54.61	49.65	4.31	8.68
F2 IN B	8,468				46.87			
F2 IN C	9,729				47.45			
F1 OUT A	2,390	2,359	112	4.74	43.77	43.94	2.20	5.01
F1 OUT B	2,452				41.83			
F1 OUT C	2,235				46.23			
F2 OUT A	3,720	3,530	410	11.62	41.16	43.90	2.38	5.42
F2 OUT B	3,060				45.31			
F2 OUT C	3,812				45.24			
F3 OUT A	5,637	5,233	679	12.97	58.75	55.47	3.50	6.32
F3 OUT B	4,449				55.88			
F3 OUT C	5,613				51.78			

Where:

- P: weight of the sample (g);
- TS: percentage of total solids of the sample (%);
- Chromatographic Area: area underlying the GC-MS chromatogram (ionic. u.*min);

- Conc.: concentration values obtained through the linear calibration equation (3.4), expressed in mass (g) of C_{10}-C_{40} hydrocarbons per volume unit (L) of solvent used for dilution and injection in GC-MS;
- E: weight of C_{10}-C_{40} hydrocarbons in 500 μL of RTW solution in which the sample was diluted to be injected into the GC-MS, calculated according to equation (3.6) (min);
- [C_{10}-C_{40}]: mass concentration of C_{10}-C_{40} hydrocarbons per kg of total solids in the sample, calculated according to equation (3.5);
- Avg.: average value of the concentration of C_{10}-C_{40} hydrocarbons in the samples (mg/kg_{TS});
- St. dev.: standard deviation of the concentration values of C_{10}-C_{40} hydrocarbons in the samples (mg/kg_{TS});
- Dev.: standard deviation value compared to the mean, expressed in percentage terms;
- C_{10}-C_{40}/TH: ratio between C_{10}-C_{40} and total hydrocarbons, expressed in percentage terms;
- Avg. C_{10}-C_{40}/TH: average value of the C_{10}-C_{40}/TH ratios (%);
- St. dev. C_{10}-C_{40}/TH: standard deviation of the C_{10}-C_{40}/TH ratios (%);
- Dev. C_{10}-C_{40}/TH: standard deviation value compared to the mean value of the C_{10}-C_{40}/TH ratios (%).

In Table 3.32, it can be seen that due to AD, C_{10}-C_{40} concentration also undergoes about a halving, passing from 5,496(±1,089) mg/kg_{TS} to 2,359(±112) mg/kg_{TS} in the F1 sludge and from 9,302(±723) mg/kg_{TS} to 3,530(±410) mg/kg_{TS} in the F2 sludge. From this Table, it is also possible to observe how the fractions of C_{10}-C_{40} in TH pass from 39.24(±6.11)% to 43.94(±2.20)% for F1 and from 49.65(±4.31)% to 43.90(±2.38)% for F2, as if the biological process had carried out a sort of equalization on the two SS, acting more on the "unnatural" initial content of C_{10}-C_{40} in F2 compared to F1 and bringing, at the end of AD, the C_{10}-C_{40}/TH ratios in the two sludges to the same value. The

same considerations can be deduced from the removal yield calculations, which, as seen above, considering the TS variations during AD, represent the mass variations of the hydrocarbons and are shown in the Figures below.

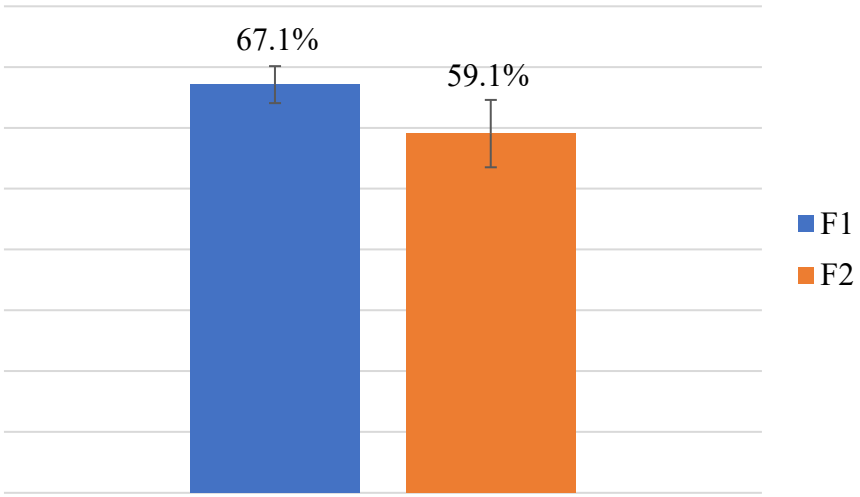

Figure 3.35. TH mass removal yields due to AD.

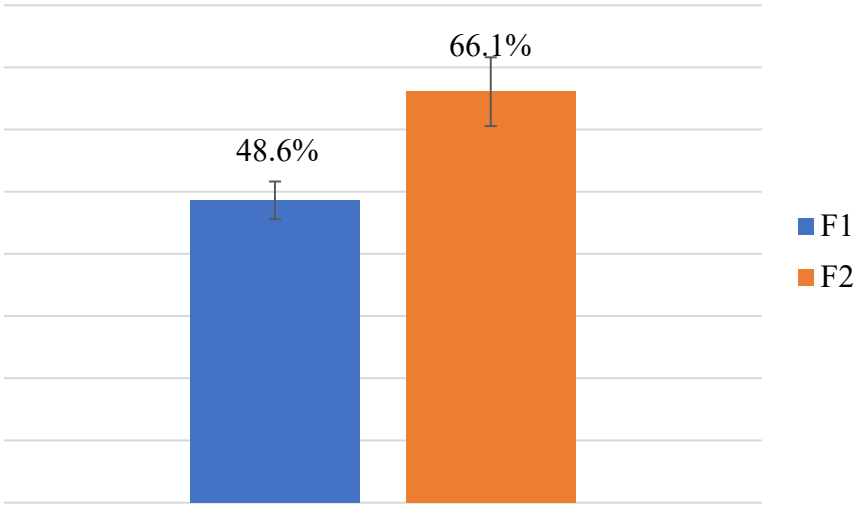

Figure 3.36. C_{10}-C_{40} mass removal yields due to AD.

Figures 3.35 and 3.36 show how the AD process affected the two sludges differently, with a more significant mass reduction of TH for the F1 sludge. In F1, probably, the effect of a less "aggressive" polyelectrolyte led to lower dewatering than in F2 sludge (13.39±0.48% and 22.15±0.44% for F1 and F2, respectively) and, therefore, to a lower compaction effect on the NOM of F1, with evident better

bioavailability of hydrocarbons and larger contact surfaces between water and sludge, compared to F2. On the other hand, the reduction of C_{10}-C_{40} in F2 was greater than in F1. From the initial information on the two different conditioning agents, it is clear that the one used in F2 was characterized by a greater quantity of C_{10}-C_{40} hydrocarbons (and by a higher C_{10}-C_{40}/TH ratio), as shown in Tables 3.31 and 3.32. Therefore, the C_{10}-C_{40} hydrocarbon content in F2 before AD was much higher than in F1, mainly of added type. In literature, it has long been known that AD is more effective on added hydrocarbons than on hydrocarbons already present and aged in NOM (Aemig et al., 2016). The obtained result thus follows what was expected from previous studies and, by comparison with section 3.2.1 results, demonstrates that a thermophilic AD has greater effectiveness than a mesophilic one, in which the degradation of C_{10}-C_{40}, even if added, it has traced that of the TH.

Examining the Figures below, showing some chromatograms related to the various sludges before and after AD, other interesting considerations can be made.

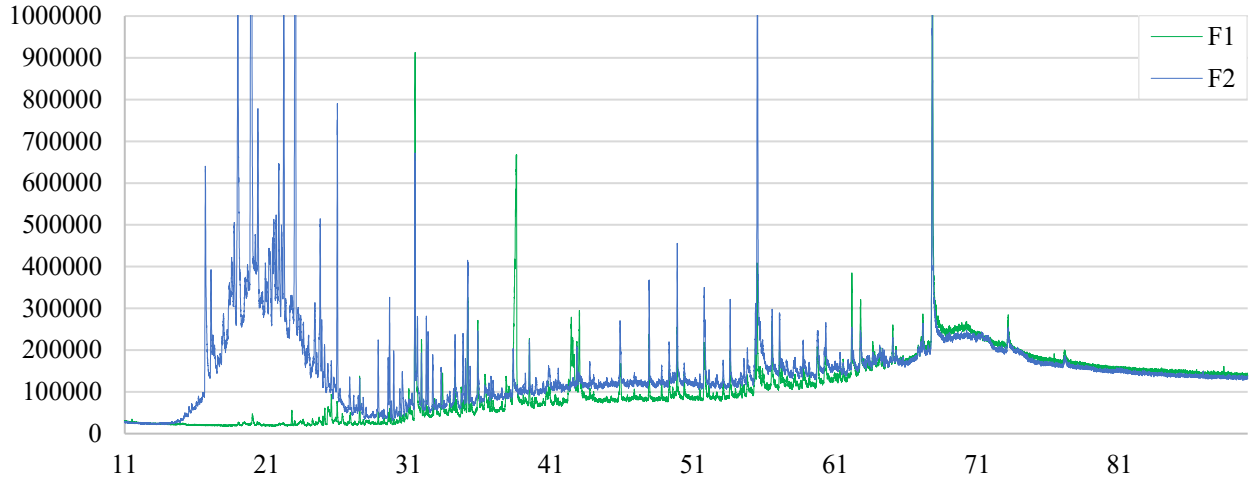

Figure 3.37. Comparison of F1 and F2 before AD.

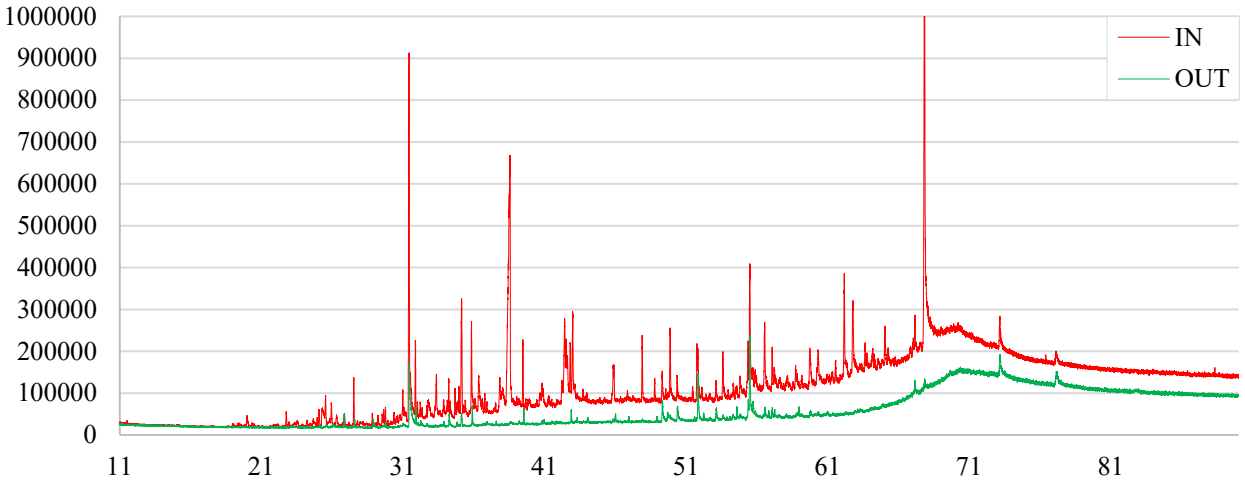

Figure 3.38. AD effect on F1 sludge.

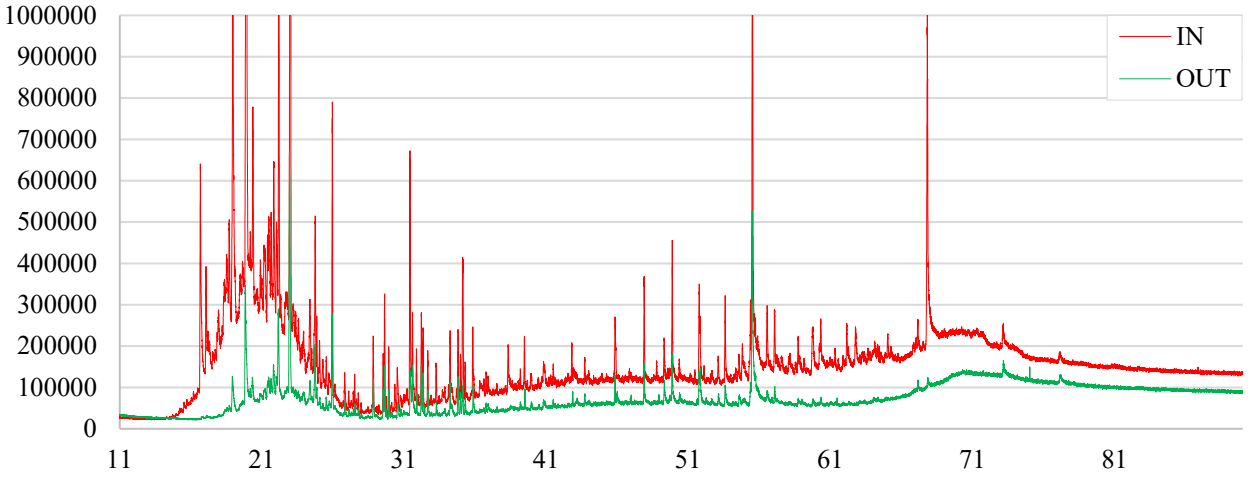

Figure 3.39. AD effect on F2 sludge.

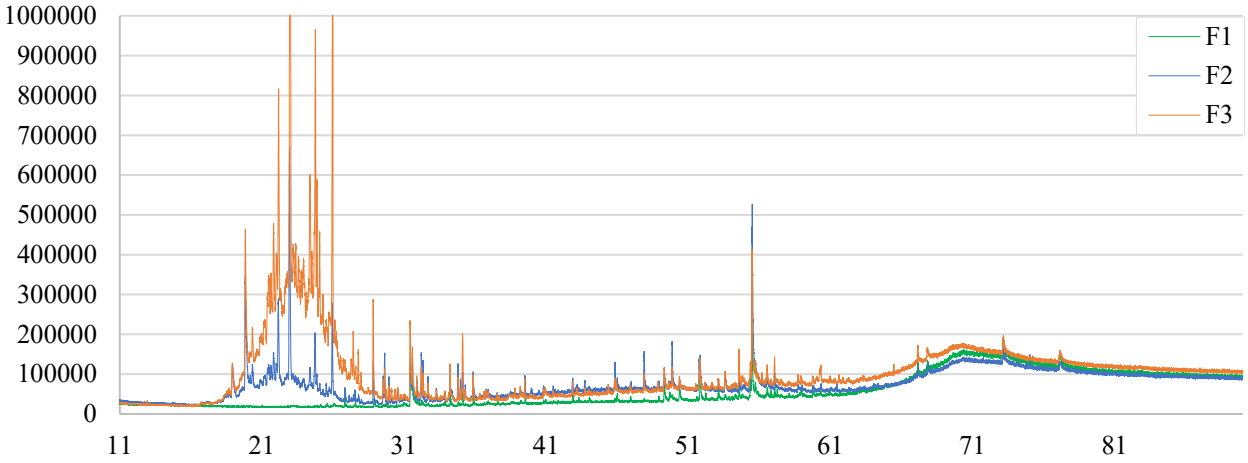

Figure 3.40. Comparison of all digested sludge.

Figure 3.37 further confirms what has been stated so far, pinpointing the high amount of low molecular weight C_{10}-C_{40} hydrocarbons present in the F2 sludge (eluted with retention times between minutes 14.47 and 28.23), which are almost absent in the F1 sludge. As shown in Figure 3.39, AD has a powerful effect on reducing this hydrocarbon fraction, evidently added during chemical conditioning. The degradation due to AD occurs on the whole hydrocarbon mixture, as also happens in F1 (Figure 3.38), but on the fraction of light hydrocarbons prominent in F2, it seems to be more pronounced.

At the end of AD, the two sludges have a chromatographic profile much closer than the initial conditions, as shown in Figure 3.40 (green and blue lines), according to the C_{10}-C_{40}/TH ratios obtained in Table 3.33 for the two sludges downstream of AD.

Figure 3.40 also shows a digested sludge in a centralized plant (F3), which, according to Table 3.32, has an even higher C_{10}-C_{40} hydrocarbon concentration than for F1 and F2, as is also evident from its C_{10}-C_{40}/TH ratio.

These three SS samples have subsequently been used for research on the toxicological implications deriving from their agricultural spreading, which will not be considered in the present PhD work.

4. CONCLUSIONS

The main idea behind this PhD research project was to investigate whether SS's hydrocarbon content could limit treated SS's landspreading given the new limit on C_{10}-C_{40} hydrocarbons imposed by Italian legislation. The study also aimed to fine-tune an analytical method coupling gravimetric and GC-MS determinations for hydrocarbon quantification in SS and provide helpful support for practitioners and analysts working with this complex organic matrix.

After the first two years of research, to overcome the criticality of the absence of a standardized measurement method for the aliphatic hydrocarbon content in SS, an innovative analytical methodology capable of measuring the hydrocarbon concentration with a good approximation and repeatability, testified by average recovery percentages of around 90% and standard deviations of about 10-15%, was developed. This new method can quantify two different parameters, i.e., TH and C_{10}-C_{40} hydrocarbons, which better describe the aliphatic content of SS. Moreover, the method provides a qualitative characterization of the hydrocarbon mixture according to MADEP speciation and identifies the light and heavy hydrocarbon fractions (L_{EPH} and H_{EPH}), which can provide interesting insights about their biodegradability.

During this research, AD of SS was performed at a laboratory scale under both mesophilic and thermophilic conditions to assess the effectiveness of the anaerobic degradation to reduce the content of aliphatic substances in SS and enable landspreading. A case study examined during the research considered whether chemical conditioning could affect SS's anaerobic degradation and hydrocarbon content. It has indeed been found that a mesophilic AD can degrade hydrocarbons by more than 60%, but on chemically conditioned sludge, this degradation drops to 16%, which could be partly attributed to the compaction effect on the NOM of the chemical conditioning. Thermophilic AD was instead capable of also degrading chemically conditioned sludge by about 60-65% and was more effective than a mesophilic one in degrading SS hydrocarbons. The necessary deepening of the interaction between hydrocarbons and NOM also allowed us to verify that by chemically cleaving the polysaccharide shells constituting this latter, many otherwise inaccessible lipophilic substances can

be extracted and analyzed, giving a further demonstration of the trapping capacity of NOM towards trace substances. Experiments designed to investigate the toxicity of the substances trapped in the NOM are currently underway.

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