Development of a Methodology for the Physicochemical Characterisation of a Cutting Fluid based Sludge

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September 2014

A Thesis Submitted for the Degree of Master of Philosophy

School of Engineering

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Abstract

This thesis presents a physicochemical analysis of a cutting fluid based floating sludge that arose in the wastewater storage tanks at a metal machining factory in South Wales, UK. The sludge is causing a range of issues from damaging aspects of the wastewater treatment system to potentially stopping the wastewater treatment plant from operating, which in turn would cause the temporary closure of the metal machining factory.

As this type of cutting fluid based sludge has never before been experienced or studied, a structured order of analyses and calculations were developed by reviewing existing analytical methods and formulating new analyses where the existing methods were inapplicable or non-existent. The chemical components that make up 70% of the sludge are water, oleic acid and palmitic acid. The ratio between these three chemicals is believed to directly influence the physical properties of the sludge.

An assessment was undertaken to determine if the sludge could be described as 'grease' (formed by the interaction of calcium and sodium with palmitic acid and oleic acid). It was found that only up to 3.6% of the sludge could be grease. This has implications for preventative measures employed to stop the sludge from forming.

The routes of formation of the sludge from virgin products is thought to be via biological degradation of the cutting fluids into free fatty acids. Alongside the formation the design of the wastewater treatment facility is reviewed to understand why the sludge has accumulated in such large quantities.

DECLARATION

This work has not been previously accepted in substance for any degree and it is not concurrently submitted in candidature for any degree.

Signed……..

Date……..

STATEMENT

This thesis is being submitted in partial fulfilment of the requirements for the degree of Master of Philosophy.

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This thesis is the result of my own independent work, except where otherwise stated. Other sources are acknowledged by explicit references.

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Acknowledgements

I would like to thank everyone who contributed their time, knowledge and experience to help in the composing of this thesis.

- Dr Devin Sapsford
- Dr Mike Harbottle
- Mr Mike Jones
- Mr Steve Marney
- Castrol Ltd
- Ford Motor Company Ltd
- ECHA Microbiology Ltd
- Ravi Mitha
- Jeff Rowlands

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Nomenclature

AES	Atomic emission spectrometry
AOAC	Association of Analytical Communities
AOCS	American Oil Chemists Society
ASE	Accelerated solvent extraction
ASTM	American Society for Testing and Materials
AWWA	American Waste Water Association
BS	British Standard
DCM	Dichloromethane
DI	De-ionised (water)
ECN	Energy Research Centre of the Netherlands
EPA	Environmental Protection Agency
FAME	Fatty acid methyl ester
FID	Flame ionisation detector
FTIR	Fourier transform infra-red spectroscopy
FOG	Fats, oils and grease
GC	Gas chromatography
GC-FID	Gas chromatography-flame ionisation detection
GC-MS	Gas chromatography-mass spectrometry
HPLC	High pressure liquid chromatography
HSE	Health and Safety Executive
ICP-AES	Inductively coupled plasma-atomic emission spectroscopy

ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
IR	Infrared
LC	Liquid chromatography
MS	Mass spectrometer
NLS	National Laboratory Service
NMR	Nuclear magnetic resonance
OES	Optical emission spectrometry
PAH	Polycyclic Aromatic Hydrocarbon
PPB	Parts per billion
PPM	Parts per million
PPT	Parts per trillion
PSI	Pounds per square inch
TC	Total carbon
TIC	Total inorganic carbon
TOC	Total organic carbon
TS	Thermospray
UF	Ultra-filtration
UV	Ultraviolet
VOC	Volatile organic compound

1.0 Introduction

Sludge is a common waste product of wastewater/effluent treatment plants and in general does not cause a problem when it is intentionally generated. The problems occur when the sludge is not intentionally generated. Sludge build-up causes many issues, such as blocked pipework, reduction in wastewater storage capacity, increased maintenance costs and damage to wastewater treatment apparatus.

To overcome these problems, and even stop the formation of unintended sludge, it is essential to know the composition of both the sludge and the inputs into the system. The composition was determined using a range of chemical and physical analyses and analytical methods. These processes exist in a structured format for other types of sludge, solids and oils, but not oil-based sludge. From these compositions the route of formation of the sludge was determined. This shows which processes to target to stop the unwanted sludge from forming.

The aim of this thesis was to develop a methodology that will allow an analyst to be able to characterise the physicochemical properties of a cutting fluid based floating sludge. Existing methods to analyse similar environmental pollutants have been reviewed and new methods that are more suited to oil-based floating sludge have been developed. The areas of soils, fats, oils and greases (FOG) and wastewater sludge have analytical techniques and methods that prove to be applicable to the analysis of cutting fluid based floating sludge and therefore research of methodologies has been focused upon these areas.

This thesis generates a structured approach to the analysis of oil-based floating sludge, using the sludge found at a metal machining factory in South Wales, UK as the test subject. The sludge in question was appearing in the wastewater storage tanks prior to ultrafiltration treatment and has been apparent since 2006 when the factory changed from petroleum derived cutting fluids to vegetable oil derived cutting fluids.

1.1 Aim & Objectives

Aim

Develop a methodology for physicochemical characterisation of an oil-based sludge that was formed during the treatment of used cutting fluid, which is relevant for application in other floating oil-based sludge.

Objectives

Systematically determine the best methods and equipment to use for the physicochemical characterisation of cutting fluid based floating sludge.

Undertake physicochemical characterisation of oil-based floating sludge that was formed during the treatment of used cutting fluid.

Determine if grease or soap constitutes a significant fraction of the sludge.

Theorise on the route of formation of the oil-based floating sludge.

2.0 Cutting Fluid Review

Cutting fluids are used in the machining and forming of metals to lubricate and cool the processes. Several kinds of cutting fluid exist including neat oils, oil in water emulsions, pastes, aerosols and gases. Cutting fluids are made from petroleum distillate, animal fats, vegetable oils and various gases; however the focus in this thesis is upon vegetable based oil in water emulsions. This is the primary cutting fluid that the metal machining factory uses, with other minor processes using petroleum distillate derived cutting fluid. Cutting fluid is continuously circulated around the systems, with each system being topped up with water, cutting oil and surfactant. Occasionally the systems need to be completely drained and replenished; this was usually conducted during the summer shutdown period.

2.1 Petroleum Distillate Based Cutting Fluids

Cutting fluids based upon animal fats have been used for centuries, but are not commonly used in modern metalworking. Animal fat cutting fluids have been superseded by petroleum distillate based cutting fluids since the late 19th century and now make up the majority of cutting fluid used around the world.

2.1.1 Petroleum Distillate Cutting Fluid Chemical Composition

These cutting fluids are mineral oil, or can be manufactured to make a synthetic cutting fluid; alternatively they can be a mixture of the two, known as semi-synthetic oil. It is typically a mixture of hydrocarbons obtained from petroleum by distillation (Dictionary, 2014), with the addition of chemicals to achieve the desired physical properties.

Within the metal machining system it is used as an oil in water emulsion; however the oil itself is hydrophobic, so a surfactant is used to make the emulsion. The metal machining factory in South Wales uses triethanolamine as the surfactant. Depending upon the metal machining systems requirements the amount of water, oil and surfactant can vary, however it is commonly around 90% water, 5% oil and 5% surfactant, with minor other additives.

2.1.2 Petroleum Distillate Cutting Fluid Formation

Petroleum distillate based cutting fluid is formed much as the name suggests, by the distillation of crude oil.

A surfactant is usually added to the oil in the factory for ease of use, where it is poured directly into the system to meet its needs. Additional surfactants are added to the system when free floating oil, known as tramp oil is seen within the systems.

2.1.3 Petroleum Distillate Cutting Fluid Treatment and Disposal

Once the oil has been used for a number of weeks, months or years (depending upon the level of maintenance of the system) it will require disposal. Depending upon the size of the system and of the factory the oil may be disposed of into the sewers for treatment by a wastewater treatment company, sent offsite by lorry for treatment, or treated onsite. The site under study generates around 74,000m^3 of wastewater each year; therefore it treats all of its wastewater onsite.

The aim of treating the used cutting fluid emulsion is to separate the oil and other unwanted chemicals from the water. This can be achieved using a number of different processes, such as the addition of chemicals to break the emulsion, then the use of a settling tank to allow the swarf (metal solids) to sink and the oil to float, where it is removed using skimmers. Alternatively biological degradation can be used, although this is usually reserved for reducing the chemical oxygen demand in the latter stages of the treatment. The metal machining factory uses ultrafiltration to separate the emulsion, with the water being sent to a wastewater treatment company for further de-polluting, whilst the oil, which is now ~95% oil, 5% water is sent for refining.

2.2 Vegetable Based Cutting Fluids

Vegetable based cutting fluids are beginning to be used as an alternative to petroleum derived cutting fluid, due to their lower environmental impacts and lower toxicity for humans (Lawal, 2012; Clarens, 2008; McManus, 2004). Few companies have implemented their use on a large scale, except for the corporation, to which the metal machining factory in South Wales belongs. This means there is little or no documented evidence of the impacts it has to the wastewater treatment system, when used on a large scale.

2.2.1 Vegetable Based Cutting Fluid Chemical Composition

Many different types of vegetable oil-based cutting fluid exist, such as soybean, sunflower, rapeseed and palm oil amongst many others (Shashidhara and Jayaram, 2010). The majority are triglycerides, which are three fatty acid chains linked by a glucose molecule (Sefanescu et al, 2002), such as the example of trimethylolpropane trioleate shown in Figure 1. All vegetable oils will contain some other components such as monoglycerides, diglycerides and free fatty acids in varying concentrations; however their main constituent is triglycerides. The physical properties of the oils are influenced by which free fatty acids are bonded to the glucose molecule, which may not all be the same fatty acid (Shashidhara and Jayaram, 2010). For example the triglycerides in rapeseed oil are composed of oleic acid (60%), palmitic acid (4%), linoleic acid (20%) and alpha-linoleic acid (10%), with the brackets indicating the average percentage composition of the ester by named free fatty acid (Gunstone, 2009). All of the vegetable based cutting fluids used in the metal machining factory are produced from rapeseed oil.

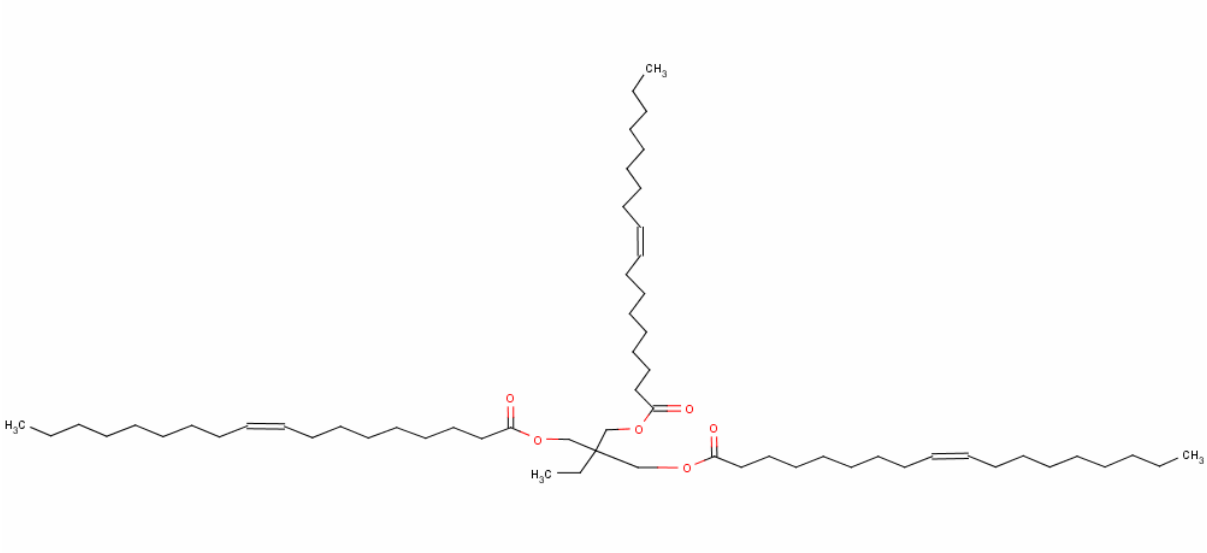


Figure 1 Trimethylolpropane Trioleate Chemical Structure (Chemnet, 2013)

2.2.2 Vegetable Based Cutting Fluid Formation

The natural vegetable based oils are usually extracted by crushing the seeds of the plant. The oil can either be used as it is, or can be refined depending upon the quality of oil required.

2.2.3 Vegetable Based Cutting Fluid Treatment and Disposal

As the vegetable based oils have not been previously implemented on a large scale no methods of disposal have been developed, except those at the metal machining factory. Here they separate the water and oil using an ultrafiltration process, which produces water with a chemical oxygen demand of around 12,000mg/L, which is sent to a wastewater treatment company for further treatment. The waste oil produced is around 50% purity and is transported by vacuum tanker offsite for acid splitting or incineration.

The only previous publication on pollution caused by rapeseed oil is from the International Tankers Owner Pollution Federation, which showed images of days old floating sludge formed when a tanker spilled its load in a dock. The images looked similar to the sludge seen at the metal machining factory; however this is no longer available online (ITOPF, 2010).

2.3 Other Chemical Additives Used at the Metal Machining Factory in South Wales

2.3.1 Hydraulic Oils

Also in use at the metal machining factory are synthetic vegetable based hydraulic and slideway oils, which are triglycerides, but have been synthetically produced by reacting free fatty acids with alcohol. These are designed to be more resistant to bacterial degradation (Castrol, 2010), however are more expensive than rapeseed based cutting fluids.

Small quantities of mineral hydraulic oil are also used at the factory.

2.3.2 Other Chemicals

To increase the longevity of the oil additional antibacterial agents are poured into the systems to control the levels of bacteria. These are in the form of biocides and acticides.

Once the component has been machined it is passed through a further set of machines to rinse off the oil. These machines use a surfactant-water mixture to clean the components and are drained and replenished more regularly than cutting fluid systems.

Along with these chemicals the systems use trace amounts of antifoam, pH adjusters and corrosion inhibitors.

2.4 Site Description

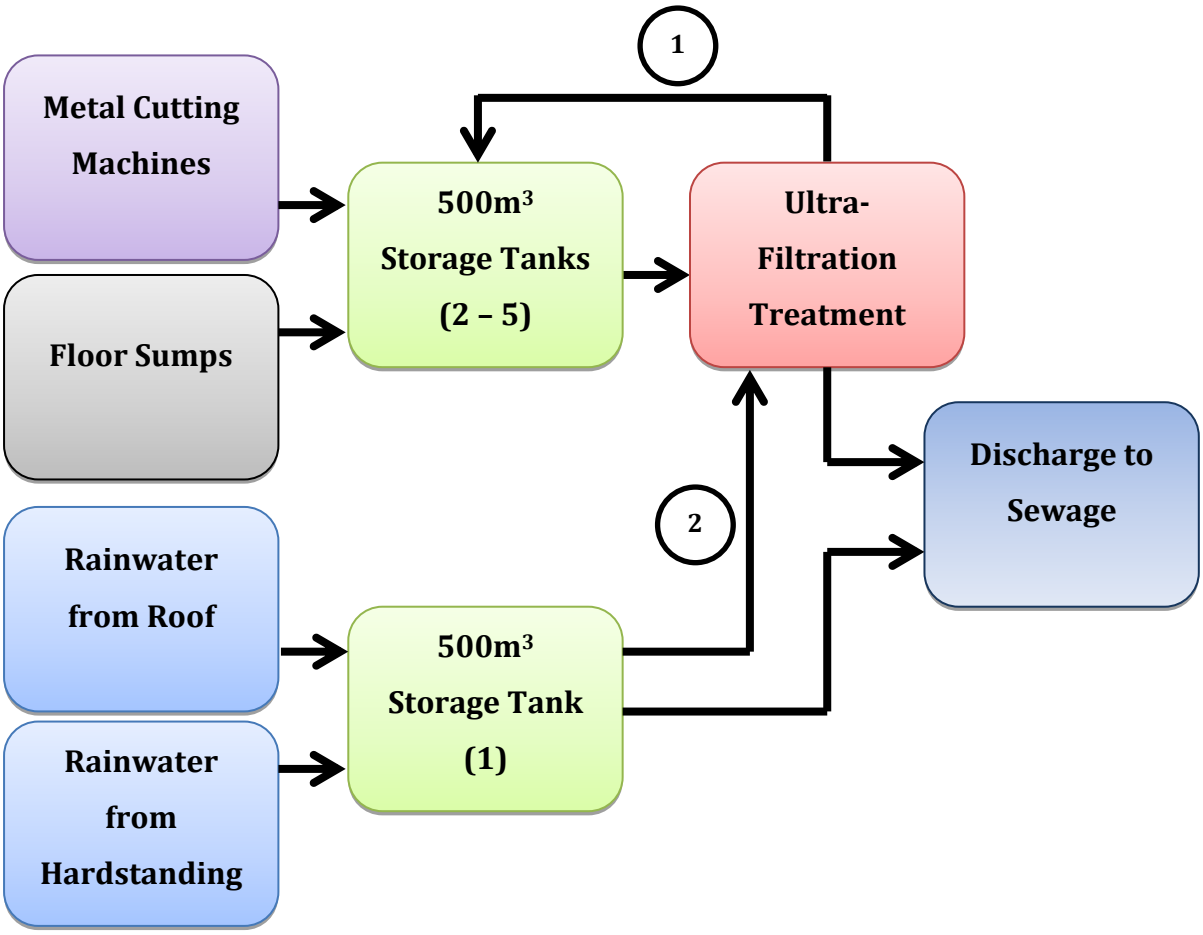
The floating cutting fluid based sludge is forming in the storage tanks of an onsite wastewater treatment facility at a metal machining factory in South Wales, UK. An example of this sludge is shown in Figure 2.

The onsite treatment plant treats consists of five 500m^3 storage tanks, into which all of the liquid wastewater (excluding raw sewage) flows, followed by a series of ceramic ultrafiltration membranes, a storage tank for the waste oil and a discharge pit for the wastewater. It treats around 74,000m^3 of wastewater per annum, of which around 50,000m^3 is rainwater that may be stored in tank 1, but is usually discharged directly to the sewer. The rainwater can be used to dilute the wastewater entering the ultrafiltration plant if necessary. The wastewater emulsion from the factory is stored in the 500m^3 storage tanks for up to one month, before it is processed through the ceramic ultra-filtration membranes and passed to the sewer. The liquid wastewater that passes to the onsite treatment facility originates from system drains, leaks and additional, but minimal floor cleaning waste and rest area sinks. The wastewater flowchart is shown in Figure 3, but excludes the floor cleaning and waste area sinks as these are insignificant inputs.

Cleaning of the ultrafiltration system (UF) occurs once a month using a sodium hydroxide solution to clean the ceramic membranes. This spent cleaning solution is passed into storage tank 4, as is any overflow from the treatment system, from which sludge 1 is extracted. Sludge 2 is taken from tank 5, shown in the lower image of Figure 2, which represents the sludge in the remaining tanks as it is seemingly identical.

Within the 500m^3 storage tanks the sludge is manifesting. The wastewater being sent to the wastewater treatment facility and entering the storage tanks comprises mostly of water, emulsifiers, vegetable based cutting fluid and hydraulic oil. It also comprises of trace quantities of swarf, food, mineral oil, additives and general dirt from the factory.

Figure 2 Examples of Floating Oil-Based Sludge



Notes

1. Overflow and NaOH cleaning solution is returned to tank 4 from the UF treatment system
2. Occasionally dilution of the emulsion into the UF treatment system is required, this is conducted using rainwater from tank 1

Figure 3 Flow Diagram of Wastewater at the Metal Machining Factory, South Wales, UK

2.5 Grease

Some minor analysis of the sludge has been previously undertaken by industrial engineers, so as they may gain a better understanding of what the sludge may be. Their results have brought them to the conclusion that the sludge is grease, as defined below. This theory that the sludge is grease is tested within this thesis. Correct identification of the sludge is necessary as measures to stop the formation of the sludge may not work if the sludge is wrongly identified.

Grease is defined as "any thick, fatty oil, especially one used as lubrication for machinery" (TheFreeDictionary, 2014). Its chemical composition is a thickening agent in a liquid lubricant, such as an oil. The thickening agent is generally an organic fat that has been treated with a strong alkali, such as sodium to produce soap. In other words grease is an emulsion of soap and oil (Nailen, 2004).

The chemical composition of oil has been described previously in this Chapter. Soap is defined as "a salt of a fatty acid, saturated or unsaturated, containing at least eight carbon atoms or a mixture of such salts" (IUPAC, 2014). Examples of such soaps include calcium stearate, sodium stearate and calcium palmitate.

Calcium bonds to two fatty acids to form soap. Its molecular mass is 40g/mol, therefore per mole of calcium 513g of palmitic acid, or 565g of oleic acid, or 569g of stearic acid is required.

Sodium requires only one fatty acid to form soap. Its molecular mass is 23g/mol, therefore per mole of sodium 256g of palmitic acid, or 282g of oleic acid, or 284g of stearic acid is required.

No data can be found on the ratio of soap to oil required to form grease, however this is assumed to be 1:1 by mass for this thesis. To calculate the mass of grease the mass of soap that has been determined is doubled.

3.0 Review of Methodologies for Sampling and Analysis of Sludge

Due to a lack of appropriate methods for the analysis of oil-based sludge, this Chapter has been included to review similar methodologies and draw together the most appropriate to apply to the sludge. Methodologies within this Chapter are extracted from journal articles, books and guidance documents, both from academia and industry.

The aim of this Chapter is to present methodological approaches derived from the literature and then justify the choice of each method, as later presented in Chapter Four.

The format for the justification and review is as follows: The reviews of existing methods are listed by number and are referred to by their preceding number in the review of that particular set of methods after they have all been listed. An example is given below for microwave digestion of samples.

[1] "1g of homogenised FOG was mixed with 10 ml of 50% HNO_3 and refluxed at 95°C for 15 min. After cooling a further 5 ml HNO_3 was added followed by 30 min under reflux. If brown fumes were produced a further 5 ml of HNO_3 was added and refluxed for a further 30 min, this cycle was repeated until no oxidation was apparent. The sample was then heated to 95°C for 2 h to reduce the volume to 5 ml. The samples were then diluted using distilled water to give 2% HNO_3 content" (Williams et al, 2012).

[2] Microwave assisted acid digestion of siliceous and organically based matrices: Up to 0.5g of sample is placed into a microwaveable container, along with 9ml of HNO_3 and 3ml of HF. This is heated to 180°C in less than 5.5 minutes and maintained at that temperature until the total heating time reaches 10 minutes. Use of the HF acid is not essential and other acids may be added to stabilise certain analytes. H_2O_2 may also be used to aid sample oxidation (EPA, 1996a).

Review of [1] is scientifically robust, [2] is missing critical analysis steps. [1] has been chosen to be conducted on the sludge.

3.0.1 Sludge

Sludge is a broad term used for describing "Thick, soft, wet mud or a similar viscous mixture of liquid and solid components, especially the product of an industrial or refining process" (Oxford Dictionaries, 2014).

It can also refer to sewage sludge, known as bio solids, which is defined as the "solids separated during the treatment of municipal wastewater" (EPA, 2013).

The first definition encompass the sludge that is manifesting at the metal machining factory as they are solids that are being separated during the treatment of wastewater and they are a viscous mixture of liquid and solid components. Bio solids would not be used to describe the sludge as it is not derived from municipal wastewater.

3.0.2 Stages of Physicochemical Characterisation

There are many steps that need to be taken when conducting a physicochemical analysis. The general steps taken in physicochemical analysis are documented in Table 1 . Each step is as important as the last. If one is step conducted incorrectly then the results in the later stages will be erroneous or meaningless. The sludge analysis methods section will present in the same structured format as Table 1.

Table 1 General Steps in a Chemical Analysis (Harris, 2010)

Step	Description
Formulating the question	Translate general questions into specific questions to be answered through chemical measurements.
Selecting analytical procedures	Search the chemical literature to find appropriate procedures or, if necessary, devise new procedures to make the required measurements.
Sampling	Sampling is the process of selecting representative material to analyse…If you begin with a poorly chosen sample, or if the sample changes between the time it is collected and the time it is analysed, the results are meaningless.
Sample preparation	Sample preparation is the process of converting a representative sample into a form suitable for chemical analysis, which usually means dissolving the sample. Samples with a low concentration of analyte may need to be concentrated prior to analysis. It may be necessary to remove or mask species that interfere with the chemical analysis.
Analysis	Measure the concentration in several different aliquots. The purpose of replicate measurements is to assess the variability in the analysis and guard against a gross error in the analysis of a single aliquot. The uncertainty of a measurement is as important as the measurement itself, because it tells us how reliable the measurement is. If necessary, use different analytical methods on similar samples to make sure that all methods give the same result and that the choice of analytical method is not biasing the result.
Reporting and interpretation	Deliver a clearly written, complete report of your results, highlighting any limitations that you attach to them. Your report might be written to be read only by a specialist, or it might be written for a general audience. Be sure the report is appropriate for its intended audience.
Drawing conclusions	Once a report is written, the analyst might not be involved in what is done with the information, such as modifying the raw material supply for a factory or creating new laws to regulate food additives. The more clearly a report is written, the less likely it is to be misinterpreted by those who use it.

3.1 Sampling & Storage

The importance of taking a representative sample is often under-estimated. Doing this incorrectly can produce results that do not represent the bulk of the sample, which leads to an incorrect conclusion and remediation strategy that will more than likely not solve the problem under study. There is little literature on the sampling of floating cutting fluid based sludge; instead sampling and analysis of materials with similar physical characteristics are reviewed.

3.1.1 Sampling

The process of sampling should be kept as simple as possible, yet still be representative. The following methods aim to be both simple and representative.

[1] Sampling of fats and oils are conducted in different manners, due to their differing physical characteristics. As such the methods for oil sampling cannot be applied to sludge, due to the increased viscosity of the sludge in comparison to the oil. However, the physical characteristics of a fat can be similar to those of sludge; therefore the same sampling techniques that are used for fats may be able to be used for sludge. Solid fats are sampled using a trier, following BS809 Section 4, but only if they cannot be melted and stirred. Three samples are extracted from the fat using the trier, with the sampling locations being diagonally across the sample; the top third of one, middle third of the second and bottom third of the third being used to form the composite sample for analysis (Hamilton and Rossell, 1986).

[2] Sampling strategies must also be considered taking into account time, cost and the quantity of material required/available, this leads to three strategies for sampling; targeted, spot and random (NLS, 2013). The following are sampling guidance for contaminated land.

Targeted – "It allows the use of knowledge and/or expertise to select the sampling points. For example, it may be knowledge of the position of buildings/plant and equipment on a site cleared for redevelopment or in the case of a known discharge a herring bone pattern, working away from the point of discharge, could be selected" (NLS, 2013).

Spot – “A single sample may be all that is required. It is likely that contamination or pollution will be apparent and that laboratory analysis is required to establish the nature and extent of the contamination at a particular location” (NLS, 2013). If the contaminant seems similar for the entire amount then three samples should be taken from differing locations i.e. one from the top, one internally and one from the base. These can be combined to form a composite sample. Larger masses can have multiple sampling points that are analysed separately. For contaminants that seem to differ across their structure, then the process above should be followed, along with spot samples from the distinctive areas (NLS, 2013).

Random – “Random sampling can be simple or stratified. Simple random sampling is achieved by laying a grid over the area and randomly selecting points on the grid or taking samples in a "W" pattern across the selected area. Stratified random sampling involves taking samples at selected depths at each of the randomly selected points” (NLS, 2013). Composite samples can be made from these specimens; however “Combining separate strata from stratified random sampling should be avoided. The reason for taking different strata is to assess the extent of variability with depth” (NLS, 2013).

[3] The American Waste Water Association (AWWA) states that “it is beyond the scope of this publication to specify detailed procedures for the collection of all samples because of varied purposes and analytical methods” (AWWA, 2005). Meaning that how each sample is taken should be tailored to the question under study and the analytical methods used (AWWA, 2005).

[4] Sampling of fats, oils and greases (FOG) from sewers “were taken using telescopic rods lowered into manholes to avoid risks associated with sewer entry. FOG samples were scraped or cored from deposits in sewers or sewage works, or collected as “fat balls” from the water surface in pumping stations” (Williams et al, 2012). These samples were then “homogenised in a blender (30s at the highest setting and 1 min at low) and split into sub –samples” (Williams et al, 2012).

[5] Sampling of FOG from grease traps was conducted manually from at least two separate points on the surface within the trap. After storage in a freezer the FOG was blended to ensure homogeneity (Nitayapat and Chitprasert, 2014).

The floating cutting fluid based sludge will most likely be of a substantial quantity to be causing problems significant enough to require scientific analysis, instead of just disposal of the sludge. It is also likely that the sludge is occurring in more than one location and with challenging access for sampling.

Taking into account the multitude of locations sludge could materialise, targeted sampling, as described in [2], was used as the sludge in each location was considered to be slightly different. Each different sludge that is analysed can help in deciphering the cause of the sludge formation as it may show the sludge in its varying stages of transformation from products to sludge. At each location the volume of the sludge will differ, however it is likely there is sufficient enough quantities that spot sampling would generate unrepresentative results, therefore random sampling to form a composite sample, as in [2] was conducted, with samples being homogenised afterwards. The sampling method in [1] could also be used for a large area, with composite samples being taken and homogenised. Both the sampling techniques in [1] and random sampling in [2] for larger volumes would create a representative sample. The only drawback of the random sampling is that only areas with high concentrations of a chemical may be sampled, leading to incorrect conclusions on the composition of the sludge. This can be reduced by doing more samples of smaller volumes across the sampling area. The random sampling across the surface of the sludge should also be done for the depth of the sludge. A trier [1] can effectively sample at different depths and can be used with extension pieces for the deeper materials. The samples taken from the different depths should also be homogenised with the rest of the samples from the bulk. The sampling of FOG in method [5] is in fewer places than required by method [1] and was only taken from the surface of the sample, not throughout the depth, as in method [1] and [2]. This may lead to an unrepresentative sample.

Practical aspects of sampling may need the analyst to use their ingenuity as no one physical sampling method can govern all sites [3]. Triers and telescopic rods, such as in [1] and [4] are some of the devices that are necessary when sampling, however, as long as the sample acquired is representative of the bulk and does not interact with the

sample chemically, then any device may be used to make the sampling easier for the analyst.

Spot sampling is used where the sludge is materialising in more than one location. At each of these locations random sampling using the grid method was used and included samples from different depths. These samples from the same location formed a composite and have been homogenised prior to analysis. Practical sampling may be a challenge; therefore an alternative sampling device was used, which did not chemically interact with the sample.

3.1.2 Storage

Storing the sample correctly is another essential step in successful analysis. "If the sample changes between the time it is collected and the time it is analysed, the results are meaningless" (Harris, 2010). This includes both the type of container used, how and where it is stored. The following methods are reviewed:

[1] The type of container best used for sampling sludge and other solids is a wide mouth glass or plastic beaker that is airtight. It should allow little or no light to penetrate and should have minimal headspace in the jar to minimise oxidation or gas build-up (NLS, 2013).

[2] "Where there is a possibility of photochemical decomposition of chemicals it is general good practice to keep them out of direct sunlight and to store them in brown bottles" (Furniss et al, 1989).

[3] For water analysis AWWA states that "sampling equipment must be clean and quality assured before use. Use sample containers that are clean and free of contaminants. Bake at 450°C all bottles to be used for organic analysis sampling…Fill samples containers without pre-rinsing…Except when sampling for analysis of volatile organic compounds, leave an air space equivalent to approximately 1% of the container volume to allow for thermal expansion during shipment" (AWWA, 2005).

[4] Samples of FOG were "stored in a refrigerated cool box and then refrigerated at 4°C for further analyses within 5 days" (Williams et al, 2012).

[5] Samples of FOG from other analyses were stored at 7°C for an unspecified period of time (Keener et al, 2008).

[6] The Environmental Protection Agency (EPA) guidelines for the determination of metals states: "Solid samples require no preservation prior to analysis other than storage at 4°C. There is no established holding time limitation for solid samples" (EPA, 1994).

[7] Samples of FOG were chilled on ice immediately prior to extraction and frozen as soon as was possible afterwards. Defrosting of the FOG was conducted in a water bath at 39°C (Nitayapat and Chitprasert, 2014).

[8] Sludge samples were stored at 5°C to reduce natural degradation and loss of volatile organic compounds (Zheng et al, 2013).

[9] Activated sludge "samples that cannot be analysed directly must be kept cool (4-7˚C), preferably in open bottles. The samples must not be frozen as this can affect the structure of the floc" (Eikelboom, 2000).

[10] Samples that are being used for carbon analysis are "stored at 4˚C and have a holding time of up to 28 days" (EPA, 2002).

[11] Samples that have been collected and processed by digestion for inductively coupled plasma (ICP) analysis, may be stored for up to 6 months, so long as they have been acidified to a pH less than 2 (EPA, 1994).

The duration that the sludge has been in its current state and location must be taken into account when storing the sample. Some samples may have only existed for a day or two before they are sampled, others may have been in their current state and location for several years, where they have most likely not greatly changed in chemical composition from when they were formed. Sludge that has been in the same location for many years may not need to be stored to stop degradation as the degradation may have reached its climax, however this is not known and to ensure that no further degradation is taking place, a storage methodology should be devised and followed.

Where possible, analysis of the sludge should be conducted immediately so that the sample has little time to degrade. If this is not practical, then storage that minimises sample degradation should be used. It is well known that the degradation of organics is most commonly caused by micro-organisms and that by reducing the temperature the activity of the micro-organisms is reduced (Pietikainen et al, 2005; Qiu et al, 2005). Longer term reduction of temperature is achieved through storage in a cold environment, such as a fridge. Temperatures listed in [4] – [10] are between freezing (-18°C) and 7°C, but most common is 4°C. Freezing is most effective at reducing the bacterial activity, however it may also affect the structure of the sample, with the structure of the activated sludge floc [9] being affected by freezing. Freezing crystallises any water within the sample and therefore may destroy any emulsion that may be

present. For this reason freezing is not suitable if microscopic or emulsion analysis is to be undertaken. Freezing also has the potential to degrade certain chemicals within the sample, such as the degradation of an appreciable amount of fatty acids in food that is frozen at -22˚C (Gonzalez-Castro et al, 1996). The degradation is of a moderate rate, occurring within one month of freezing, but can be reduced by blanching the food sample first. Samples that had been freeze-dried could be stored at room temperature for up to two months before significant degradation occurred (Gonzalez-Castro et al, 1996). Instead, storing at as low a temperature as possible, without the risk of freezing, will reduce, but not eliminate the degradation occurring to the sludge. This temperature, as listed in [4], [6], [9] and [10] is 4°C.

The duration of storage is also important as storing at low temperatures only reduces the activity of the micro-organisms, but does not stop them altogether (Pietikainen et al, 2005). Storage of samples also differs for the different analyses. FOG samples for analysis by gas chromatography-mass spectrometry (GC-MS), melting point, hardness and ICP were stored for 5 days to ensure that the degradation occurring did not affect the results [4]. Samples for carbon analysis were stored at 4°C for 28 days [10]. At low temperatures there should be little loss of volatile organic compounds and the production of carbon dioxide from the micro-organisms should also be low, therefore only insignificant amounts of carbon should be lost over a 28 day period, thus not greatly affecting the carbon analysis. Overall the shorter the duration between sampling and analysis the less likely the sample is chemically altered. As the EPA guidelines in [6] state “there is no established holding time limitation for solid samples” and there is no consistency in storage times across the methods. A maximum storage time of one week at 4°C should provide a balance between having enough time to prepare the samples for analysis and not allowing them to significantly change in chemical composition due to degradation. The degradation rates and routes can be monitored by preparing and analysing a sample straight after sampling and then comparing the results to the rest of the batch that has been stored at 4°C for a period of time.

Once processed for analysis, such as for ICP analysis in [11], samples can be stored for increased durations without significant chemical alteration. This is not completely correct as although the analytes that are being measured will not be lost, evaporation of the solvent may lead to elevated concentrations, creating false results. Reducing

evaporation losses can be achieved by storage at low, but not freezing temperatures, such as 4°C.

Storage equipment also affects the quality of sample and can chemically change it if it interacts, or does not sufficiently protect the sample. This should not be allowed to occur and can be minimised by following [3], ensuring that the vessels are glass, contaminant free and are 99% full when sampling to minimise the volume of air in the vessel, which should stop the majority of air based degradation of the sample. Also storage in an opaque container, such as brown glass, stops the effects of light degradation [1] and [2]. The effects of the degradation that may occur due to the air remaining in the jar can be overcome by using a large volume vessel and homogenisation of the sludge in the vessel prior to analysis. Any oxidative degradation may show up on the analysis, but should be negligible compared to the chemical concentrations within the sludge.

In summary the analysis was conducted in the days following the collection of the sludge. During which it was stored in its unprocessed state at 4˚C for a week maximum, this gave enough time for the sample to be prepared for all analyses. The samples were stored in clean, brown glass vessels that were filled to 99% capacity and stored in the dark. Storage of digested ICP samples was at 4°C as the ICP analysis was not conducted on the same day. They are stored for up to one month without significant chemical alteration.

3.2 Sample Preparation

The types of sample preparation will vary for each analysis, with some requiring no preparation, others requiring water separation, extraction of the organics, ashing of the sample or microwave digestion.

3.2.1 Water Separation

Separation of the water in the sample is required in most analyses. It not only allows the determination of the water content, but also stops damage to sensitive machines, such as GC columns, which can be affected by water.

[1] Water removal for fats "should be carried out in such a way that the fat properties are not affected" (Hamilton and Rossell, 1986). The properties of fats can be affected by excessive heat, chemical addition and oxidation. The fat is melted at temperatures between 60°C and 70°C and kept at that temperature until the water has settled out. The liquefied fat is then poured through a warmed filter, or warmed centrifuge to remove the final droplets of water (Hamilton and Rossell, 1986).

[2] The fat is melted at temperatures between 60°C and 70°C. The emulsion is then poured into a column packed with anhydrous sodium sulphate and washed with a solvent, which can be removed afterwards by vacuum dehydration. The difference in mass between the initial mass and the final mass after extraction is used to calculate the water content of the sample (Hamilton and Rossell, 1986).

[3] With particularly wet fat samples the free water should be allowed to settle and measured to the nearest 2-3% using a mass loss method. "Residual moisture may be removed by evaporation at 103°C provided that the heating has no influence on the subsequent analytical tests." If the sample does not clarify then it should be filtered at 10°C above its melting point (Hamilton and Rossell, 1986).

[4] Warm the solid fat in a container up to 35°C and shake to homogenise, being careful not to break the emulsion. Cool the sample to ambient temperature and stir for 10 seconds. A portion of the sample is placed into a dried dish and dehydrated at 102 ±2°C for 2 hours. The water content can be derived from the change in mass (BSI, 1984; Hamilton and Rossell, 1986).

[5] Warm the solid fat in a container up to 35°C and shake to homogenise, being careful not to break the emulsion. Cool the sample to ambient temperature and stir for 10 seconds. A portion of the sample is placed into an open beaker and dried until no water remains. The water content can be derived from the change in mass (BSI, 1984; Hamilton and Rossell, 1986).

[6] Determination of water content in fats and fatty oils utilises entrainment distillation using the Dean and Stark method (BSI, 1981; Hamilton and Rossell, 1986). This involves mixing 20-100g of sample with 100-300ml of solvent (xylene or toluene). The mixture is heated, with the solvent and water being distilled together in the condenser. The liquids condense and drip down into the graduated receiver, with water settling at the bottom as it has a higher density. The solvent flows back into the distillation flask, where distillation continues until the level of water in the graduated receiver does not increase. The heat is removed and apparatus allowed to cool. Once it has cooled a wire is passed down the condenser to free any water droplets (BSI, 1981; Hamilton and Rossell, 1986).

[7] Oven drying is another method used, where the sample is dried in the oven at 102 ±2°C until there is no mass change of the sample. This can cause oxidation of fatty acids, which can increase the mass of the sample (Hamilton and Rossell, 1986).

[8] FOG samples were dried by placing a 1g sample into an oven at 105°C for 18 hours and then left to cool in a desiccator (Williams et al, 2012).

[9] Soil was oven dried at 105°C until no discernible decrease in mass was seen. This was determined gravimetrically (Gardner, 1986; Keener et al, 2008).

[10] For metals analysis the sample should be dried at 60°C to ensure no mercury is driven off. It should remain in the oven until it remains a constant mass (EPA, 1994).

[11] FOG samples were dried in an oven at 105°C overnight (He et al, 2011).

[12] “Molten FOG was obtained from fatty waste by heating at 100°C for 60 min. A portion of cooled, molten FOG (5 g, accurately weighed) then was heated (air oven) at 105°C for 120 min and the mass of the residue was determined after cooling. Heating of the residue for 60 min periods was repeated until constant mass (within less than 0.05%) was achieved” (Nitayapat and Chitprasert, 2014).

[13] Water determination in oil sludge, but not water separation is achieved using nuclear magnetic resonance (NMR) by the following method. "The T_2 distribution curves of the oil sludge samples were recorded, in triplicate and at 32 °C, on an NMI 20 NMR spectrometer, equipped with a permanent magnet and a 15 mm diameter probe, operating at 21.960 MHz" These were then analysed using chemometrics to determine the water and oil content (Zheng et al, 2013). This method takes around 5 minutes to determine the water and oil content of a sludge, which would otherwise take over a day.

[14] Karl-Fischer titration utilises re-agents to accurately measure the water content of a sample, without the losses of volatile organic compounds (VOC's) that can occur when heat drying. Following the coulometric method the sample and anode solution are transferred into the main compartment of the titration cell. The anode solution is made up of an alcohol, base, sulphur dioxide and iodine. The instrument is turned on and water content measured by means of I_2 generation and subsequent conductivity drop. This method is difficult to use with materials that slowly release their water i.e. chocolate (Fischer, 1935).

[15] Freeze drying is the process of removing water by freezing the sample and reducing the pressure to cause the water to sublimate. Freeze drying of multiple, or larger volume samples is best conducted using batch drying in a tray dryer. The samples must be stored in an oxygen and moisture free environment after drying, but can be stored outside of a fridge, however lower temperatures increase the lifespan of the sample (Labcono, 2004).

[16] Drying of soil in the microwave is carried out following the American Society of Testing Materials (ASTM) method D4643-93. The sample is placed in a suitable microwavable container and microwaved for three minutes. It is then cooled in a desiccator and weighed. This is repeated until no further decrease in mass is detected (ASTM, 2014).

[17] Vacuum dehydration using a vacuum desiccator with silica gel crystals. A sample is placed into the vacuum desiccator with freshly dried silica gel crystals in the base. A vacuum is then applied to the desiccator and the sample is left until there is no discernible difference in mass.

Quantification and separation of the water in the sludge can lead to loss of other components, such as VOCs (Fischer, 1935), which is to be avoided. Care is needed when choosing which technique/s to use when separating the water from the sludge as it varies based upon which analysis is being undertaken. Not only can components in the sludge be lost, but also components inherent in the water can be left behind, generating misleading results, however this is not mentioned in any of the analyses and has been developed further in this thesis.

Drying of the sample, whether it is by heat or vacuum [1] – [5], [7] – [12] and [16] – [17], leads to the loss of VOCs, if they are present (WHO, 1989) and thus over calculation of the water content. Separation or quantification by means that does not liberate the water content of the sample into the air is most beneficial as then both the water content and other chemicals content can be measured and compared to ensure that nothing has been lost in the separation. This should be managed by weighing the sample prior to separation and both the water fraction and the remaining fraction after the solvent has been removed by evaporation. The oven and vacuum drying can take a couple of days to complete, whilst [6], [13] and [14] take a few hours. Methods [6], [13] and [14] are non-destructive, however [13] and [14] only measure the water content, but do not separate it from the sludge, therefore does not prepare the sample for further analysis. Full separation is achieved by [6], although this may transfer the chemicals that usually reside in the water into the solvent solution at the base of the apparatus, where the organic and solids from the sludge are. Analysis of the water would show reduced concentrations of chemicals, whilst analysis of the solvent solution would show increased concentrations of chemicals. The remaining portion in the solvent solution from [6] could be used for carbon, GC-MS and liquid chromatography analyses as there should be insignificant amounts of organics within the water. A more sophisticated approach is needed in the separation for analysis by ICP, which is developed in Chapter Four.

As mentioned in the previous section, freeze drying [15] has the potential to degrade certain chemicals and alter the physical structure of the sludge (Gonzalez-Castro et al, 1996). This is not desired for organic or microscopic analysis, and once again may leave components in the sludge that were inherent in the water, generating unrepresentative results.

None of the above techniques are deemed applicable to the samples in this study. A new technique for separation of the water and its inherent components, such as metals, has been devised in Chapter Four. This is necessary as all of the current techniques only focus upon separating the water, but do not retain the components inherent within the water, instead leaving them within the remainder of the sludge. To ensure the accuracy of this technique it has been compared to entrainment distillation using the Dean and Stark method [6].

3.2.2 Organics Extraction

Extraction of the organic portion of the sample is usually necessary for analysis using precision instruments, such as GC-MS. For example, if pure sludge samples were analysed using GC-MS, they would most likely block the capillary column, damage the injection needle and cause other issues to the machine leading to inaccurate results. The following methods have been reviewed for their applicability in the current study. All of the solvent solutions generated by the methods detailed below can be analysed by GC-MS or high pressure liquid chromatography (HPLC).

[1] Water insoluble organics can be extracted from environmental solids, such as soils, clays, sediments and sludge using US EPA Method 3545. This method involves using high pressures and temperatures to achieve Soxhlet equivalent recoveries. Samples must be dried, or mixed with anhydrous sodium sulphate prior to being placed into the extraction cell. Higher extraction rates can be achieved using a sample that has been ground into a powder. The extraction cell is placed into the machine, where the solvent is passed through the cell under high pressure and temperature for 5-10 minutes. The solvent solution containing the dissolved organic compounds is collected and may be concentrated down. The extractor used is a Dionex Accelerated Solvent Extractor (ASE). A polar and non-polar solvent mix, usually of 1:1 ratio is advised as the extraction solvent due to being able to dissolve both the polar and non-polar organics. For extracting diesel range organics the following ASE operating conditions are used: 175°C oven temperature, 1500-2000 psi pressure, 5-10 minute static time, 60-75% of cell volume for flush, 60 second nitrogen purge at 150 psi and 1 cycle for extraction (EPA, 2007a).

[2] Extraction of polycyclic aromatic hydrocarbons from soil, sludge or sediment samples was conducted using a Dionex ASE 200. The samples were pre-dried, ground, or mixed with anhydrous sodium sulphate and 7g of the sample placed into the extraction cell. They were run through the ASE at a temperature of 100°C, pressure of 1500 psi, oven heat-up time of 5 minutes, static time of 5 minutes, flush of 60% of cell volume and nitrogen purge of 1MPa for 1 minute. The solvent used was a 1:1 mix of DCM and acetone. The solvent solution containing the organics can be placed straight into an HPLC or GC-MS after any necessary dilution (Dionex, 2011).

[3] Soxhlet extraction can be conducted on numerous environmental compounds to extract the organic proportion of the sample. A small sample is placed into a porous thimble, which is put into a Soxhlet extractor. The chosen solvent is poured into the apparatus and heated, which causes it to evaporate, condense in the condenser, drip down and mix with the sample in the thimble, extracting the organic proportion from the sample. The solvent solution now contains the organic compounds and is now ready for further analysis (Furniss et al, 1989).

As the sludge is to be quantitatively analysed the extraction needs to be highly efficient and not leave any residual soluble components. It should also be quick in its extraction as the analyst has many analyses to run.

ASE as in method [1] and [2] has reduced the extraction time significantly compared to the Soxhlet extraction used in method [3], where extractions can take over a day to finish (Furniss et al, 1989). ASE extraction still has as high an extraction efficiency as Soxhlet extraction (Bandh et al, 2000), but also reduces solvent consumption by up to 95% (Dionex, 2014). As ASE is simple, quicker, lower cost and still as efficient as Soxhlet extraction then ASE was used to extract the organic portion of the sludge.

The use of two solvents of with a large difference in polarity also aids in an efficient extraction as "no single solvent is universally applicable to all analyte groups" (EPA, 2007c). The most non-polar solvents include pentane, hexane and heptane at up to 0.1 polarity. The most polar solvent is water, but this is not desired as the solvent for use in GC-MS, therefore organic solvents, such acetonitrile, methanol and acetone all have a high polarity, of 5.1 or above. A combination of any of the above mentioned polar and non-polar solvents would work well to achieve high extraction efficiencies. DCM and acetone used in method [2] are not as polar opposite as solvents such as hexane and acetone, which are common lab solvents. The method in [1] allows the user to select the most applicable solvents for their needs, which was hexane and acetone. The sludge that was extracted with the ASE is already been separated from its water; however as a precautionary measure the ASE cell was packed with anhydrous sodium sulphate and cellulose acetate filter. The operating conditions follow that in method [1], except that

the extractions were repeated until the extracted solvent ran clear. This was a minimum of two extractions to ensure that the maximum extraction efficiency was achieved.

In summary the sludge that has been separated from its water was weighed and mixed with the anhydrous sodium sulphate in the ASE cell. The operating conditions were: 175°C oven temperature, 1500-2000 psi pressure, 5-10 minute static time, 60-75% of cell volume for flush and a 60 second nitrogen purge at 150 psi, using hexane and acetone in a 1:1 ratio by volume. The extraction was repeated twice, meaning the solution ran clear on the second run. The solution was then dehydrated on a heated block, with nitrogen being blown over the solution, until it was completely dehydrated. It was then re-weighed to assess the mass that was not soluble. It was then re-entrained in 10ml of hexane and acetone and diluted, ready for analysis by GC-MS or HPLC.

3.2.3 Digestion

Digestion of samples is undertaken so that they may be in a state which is able to be analysed using ICP, combined with a detector. Samples in a solid or semi-solid state would not successfully pass through the ICP, or would give erroneous spectra; therefore they must be extracted so the inorganic elements are dissolved into a solution.

[1] "1g of homogenised FOG was mixed with 10 ml of 50% HNO_3 and refluxed at 95°C for 15 min. After cooling a further 5 ml HNO_3 was added followed by 30 min under reflux. If brown fumes were produced a further 5 ml of HNO_3 was added and refluxed for a further 30 min, this cycle was repeated until no oxidation was apparent. The sample was then heated to 95°C for 2 h to reduce the volume to 5 ml. The samples were then diluted using distilled water to give 2% HNO_3 content" (Williams et al, 2012).

[2] Microwave assisted acid digestion of siliceous and organically based matrices: Up to 0.5g of sample is placed into a microwaveable container, along with 9ml of HNO_3 and 3ml of HF. This is heated to 180°C in less than 5.5 minutes and maintained at that temperature until the total heating time reaches 10 minutes. Use of the HF acid is not essential and other acids may be added to stabilise certain analytes. H_2O_2 may also be used to aid sample oxidation (EPA, 1996a).

[3] FOG digestion was achieved using "1.0-g dried, ground sample of FOG deposit was acidified using concentrated nitric acid. The sample was digested at 95°C for approximately 60 minutes. Hydrogen peroxide was then added, and the sample was heated for an additional 15 minutes" (Keener et al, 2008; EPA, 1994).

[4] The digestion of butter is undertaken as follows – 0.5g of sample is placed into a microwave suitable container along with 5ml of $HN0_3$ and 2ml of H_2O_2. The microwave is then powered up to 1400W over 10 minutes, held at this power for 15 minutes and then cooled for 20 minutes. This is conducted using an Anton Paar Multiwave 3000 (Anton Paar, 1997).

[5] The digestion of cottonseed oil is undertaken as follows – 0.2g of sample is placed into a microwave suitable container along with 5ml of HNO_3 and 2ml of H_2SO_4. The microwave is then powered up to 800W over 10 minutes, held at this power for 20 minutes and then cooled for 20 minutes. This is conducted using an Anton Paar Multiwave 3000 (Anton Paar, 1997).

[6] The digestion of edible oil is undertaken as follows – 0.6g of sample is placed into a microwave suitable container along with 5ml of HNO_3, 0.5ml of HCl and 3ml of H_2O_2. The microwave is then powered up to 1400W over 10 minutes, held at this power for 10 minutes and then cooled for 20 minutes. This is conducted using an Anton Paar Multiwave 3000 (Anton Paar, 1997).

[7] The digestion of domestic sludge is undertaken as follows – 0.5g of sample is placed in a microwave suitable container along with 1.5ml of HNO_3 and 4.5ml of HCl. The microwave is then powered up to 1400W over 5 minutes, held at this power for 20 minutes and then cooled for 20 minutes. This is conducted using an Anton Paar Multiwave 3000 (Anton Paar, 1997).

[8] Acid digestion of oils for metals analysis using ICP. This method is as follows. Place 0.5g of sample and 0.5g of $KMnO_4$ into a suitable microwaveable vessel. Heat at 20% power for 2 minutes then add 2ml of H_2SO_4 and reheat at 20% for 3 minutes. Add 10ml of HNO_3 and 2ml HCl and heat for 5 minutes at 15% power and then 3 minutes at 20% power, allowing to cool for 5 minutes afterwards. Add 5ml of HCl and heat at 15% power for 5 minutes. Alternatively using 0.5g of sample add 10ml H_2SO_4 and heat at 20% power for 5 minutes, then 25% power for 5 minutes, then 30% power for 5 minutes, then 35% power for 5 minutes, allowing to cool for 5 minutes afterwards. Add 8ml of H_2O_2 and heat at 50% power for 5 minutes and allowed to cool for 5 minutes afterwards. Finally add 8ml of H_2O_2 and heat at 60% for 5 minutes (EPA, 1996b).

[9] Acid digestion of sludge, sediments and soils. 1.0g of sample was put in a microwaveable container along with 10ml of 1:1 HNO_3 and de-ionised (DI) water. It was heated in a microwave for 2 minutes up to 95°C and kept at that temperature for 5 minutes and allowed to cool for 5 minutes afterwards. 5ml of HNO_3 was added and the previous heating profile repeated. Once again 5ml of HNO_3 was added and the temperature profile repeated again. 10ml of H_2O_2 was slowly added, with the sample being heated for 6 minutes up to 95°C and kept at that temperature for 5 minutes, allowing to cool afterwards for 5 minutes. Finally 5ml of HCl and 10ml of DI water was added and the sample heated for 2 minutes up to 95°C and remaining at that temperature for 5 minutes (EPA, 1996c).

[10] Microwave assisted acid digestion of sediments, sludge and soils. Between 0.25g and 0.5g of sample is placed into a microwaveable container along with 10ml of HNO_3. This is heated to 175°C in less than 5.5 minutes and maintained at that temperature until the total heating time reaches 10 minutes (EPA, 2007b).

[11] Microwave assisted acid extraction and dissolution of sediments, sludge and soils. Between 0.25g and 0.5g of sample is placed into a microwaveable container along with 3ml of HCl and 9ml of HNO_3. This is heated to 180°C in less than 5.5 minutes and maintained at that temperature until the total heating time reaches 10 minutes (EPA, 2007b).

ICP is used on aqueous samples, which are homogeneous and contain little or no organic matter. Sludge is far from this as it is rich in organic matter and heterogeneous. Therefore a number of steps are undertaken to convert the sludge into a form that is analysable by ICP. The first is homogenisation of the sample, followed by removal of the water, then degradation of the organic matter, suspension of the analytes in the solution and finally dilution if necessary prior to passing into the ICP.

Due to the organic content of the sludge, which is around 99% excluding water, digestion using an oxidising chemical, such as nitric acid or hydrogen peroxide is required. The most effective oxidising chemical is perchloric acid; however its salts can be explosive and can itself generate explosive gases when it degrades at 245°C. It is not suitable for the inexperienced chemist. Hydrogen peroxide is a less dangerous chemical to oxidise the organic matter (Kingston and Haswell, 1997). Using different oxidising chemicals can also help to reduce the time required to degrade the organic compounds and generate more accurate results. Using a variety of acids in the digestion process aids in stabilising different analytes, therefore generating more reliable results (EPA, 1996a; Kingston and Haswell, 1997). Methods [1], [3], [4] and [10] only utilise one type of acid, whilst methods [2], [5] – [9] and [11] use more than one type of acid. Methods [2], [6] and [9] use a variety of acids and oxidising chemicals, therefore should be able to fully oxidise the organics, whilst ensuring a broader range of analytes are dissolved into the solution.

Methods [9] – [11] are "not intended to accomplish total decomposition of the sample, [therefore] extracted analyte concentrations may not reflect the total content of the sample" (EPA, 2007b). The degradation in [1] is based upon visual assessment by the analyst and therefore has an elevated chance for error, compared to a standardised method. Addition of other analytes, such as potassium permanganate [8] and sulphuric acid [5], aids in generating extra complexity to the calculation of the analytes. Use of HF [2] will add the danger of life threatening injury to the analyst and if possible should be avoided, instead using lower toxicity acids.

Both methods [2] and [6] would offer acceptable digestion conditions as they both use H_2O_2 and HNO_3 to oxidise the large amount of organics that are expected to be present in the sludge. They also use two or more types of acid, however [2] is adaptable to the types of inorganic compound that are being determined, therefore there is no limit to the number of acids that can be used. Method [2] is an internationally recognised method as devised by the EPA and therefore was used, albeit slightly adapted to use 2ml±2 HCl instead of HF, as it is less dangerous and 2ml of H_20_2 to oxidise the organics, as these adaptations are permitted within the method. This method can be used with any analytical elemental analysis techniques for quantification of the following elements: aluminium, antimony, arsenic, boron, barium, beryllium, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, potassium, selenium, silver, sodium, strontium, thallium, vanadium and zinc.

To summarise, the sludge was homogenised following the method previously stated in this Chapter. The water extraction by drying of the sample will leave analytes that are inherent within the water, in the sludge, which will create misleading results. An effective and representative water separation technique is developed in Chapter Four. Once the water phase and organic phase are acceptably separated then the following methods are used:

For the water sample: 0.1ml of HNO_3 is added to 20ml of the water, left for 2 hours to fully dissolve the analytes. This produces a solution that is suitable for ICP analysis. HNO_3 is used as any it will oxidise any organic compounds remaining in the water, which could potentially affect the analysis.

For the remaining sludge: 9ml of HNO_3, 3ml of HCl and 2ml of H_2O_2 is added to 0.5g of sludge in a microwaveable container. The sample is heated to 180˚C in 5.5 minutes or under and maintained at that temperature until the total heating time reaches ten minutes. DI water can be used to dilute the solution if necessary.

3.2.4 Ashing

Ashing offers an alternative to oxidation during the digestion process; however the ash that results still requires digestion.

[1] A sample of solid FOG was placed in a clean crucible and put in a muffle oven, with the temperature being increased by 100°C every hour, up to 500°C. The sample remained in the oven at that temperature for 16 hours, whereupon it was removed and allowed to cool. After cooling, 2ml of DI water was used to rinse the ash towards the centre of the crucible. 4ml of 6N HCl was added to the solution and brought to 95°C on a hot plate for 45 minutes until completely dry. Once this was complete a further 4ml of 6N HCl was added and warmed on the hot plate for 15 minutes. The sample was allowed to cool and then filtered through a Whatmann filter paper. The solution was diluted to 25ml using DI water. The sample was further diluted to 10x dilution using a DI water solution of 1% HCl and 1% HNO_3, ready to be measured using ICP-OES (He et al, 2011).

The technique in method [1] is more time consuming for the analyst than digestion of the sludge and brings with it the risk of spontaneous combustion of the sludge in the muffle oven. It also offers the same problem with the water separation; analytes inherent within the water may be left in the ash, generating misleading results. The water would need to be separated prior to incineration; however this water separation technique is developed in Chapter Four. The water is also needed to be separated prior to incineration due to the likelihood of the water in the sludge boiling, causing sludge to be lost from the crucible by bubbling over the sides. Each step of the process must be accurately recorded so that the analytical results can be converted back to represent the sludge.

In summary 50g of sludge is separated from its inherent water, following the technique that is developed in Chapter Four. The dried sample is placed in a crucible and put in a muffle oven at 90˚C, with the temperature increasing at 100˚C per hour up to 500˚C, where it is held for 16 hours. Once it is removed and cooled 2ml of DI water is used to rinse the ash into the centre of the crucible. 4ml of 6N HCl is added to the solution and brought to 95°C on a hot plate for 45 minutes until completely dry. Once this iss complete a further 4ml of 6N HCl is added and warmed on the hot plate for 15 minutes. The sample is allowed to cool and then filtered through a Whatmann filter paper of

unspecified pore size. The solution is diluted to 25ml using DI water. The sample is then further diluted to 10x dilution using a DI water solution of 1% HCl and 1% HNO_3, ready to be measured by any analytical elemental analysis that is available (ICP-OES). This is repeated for a homogenised, but not solvent separated sample of sludge. The previous sludge digestion technique was compared to this for ease of application.

3.3 Analysis

To be able to identify the composition and structure of the sludge a number of physicochemical analyses are undertaken. These analyses include determination of the non-lipid solids content, melting point, microscopic structure, influence of bacteria/fungus, organic composition by gas chromatography and liquid chromatography, inorganics content by inductively coupled plasma-optical emission spectroscopy (ICP-OES), bonding by fourier transform infra-red spectrophotometry, nuclear magnetic resonance and carbon content.

3.3.1 Non-Lipid Solids Determination

Non-lipid solids are the solids that remain after all of the organic solids have been dissolved (Nitayapat and Chritprasert, 2014). These include items such as swarf, sticks and ear-plugs that have been rinsed or fallen into the wastewater storage tanks.

[1] After water separation a portion of FOG was added to 50ml of kerosene, which was then heated at 50°C until all of the FOG had dissolved. This solution was filtered through a Gouch crucible packed with pre-weighed glass fibre to capture the solids. The filter was washed five times with 10ml of hot kerosene, followed by petroleum ether to remove any remaining kerosene. The crucible, glass fibre and captured solids were dried together at 101±1°C until it remained a constant mass (Nitayapat and Chitprasert, 2014).

Method [1] should readily dissolve the majority of soluble organics within the sludge, however it does not utilise a mixture of polar and non-polar solvents to be most effective at dissolving the organics, therefore some may remain undissolved. It does heat the mixture, which will increase the effectiveness of the solvent and rinse it after to ensure that the solvent does not influence the results. A method to determine the non-lipid solids is developed in Chapter Four.

3.3.2 Melting Point

Knowing the melting point of the substance can aid in its identification. “When two different compounds are mixed together and the melting point of the mixture is determined, it is found that melting begins at a temperature several degrees below that of the lower-melting pure compound” (Criddle and Ellis, 1980). “Impurities will usually produce a marked increase in the melting point range and cause the commencement of melting to occur at a temperature lower than the melting point of the pure substance” (Furniss et al, 1989). These are known as eutectic systems. To be able to analyse samples composed of different chemicals a mixed melting point analysis should be used.

[1] Around 1g of sample is heated in a capillary tube using a suitable heating bath. The capillary tube is 8cm in length and sealed at one end. Any heating bath that can be altered to within 1°C will suffice as the temperature is measured using a calibrated thermometer. Figure 4 shows a typical setup for melting point apparatus; however this may be adapted to suit the equipment available. The powdered sample is placed into the capillary tube, where it must be lightly packed up to around the 5cm mark. The exterior is then wiped clean. When heating a non-convection bath, mix the water to ensure a uniform temperature across the bath. Slowly increase the temperature of the bath until the sample starts to liquefy and then solids fully disappear, this is known as the melting point range. The purer the compound the lower the melting point range, usually 0.5-1°C. Also record any sintering, softening or gas production prior to the melting point being reached. This will then be repeated with a new capillary tube. The solidification point after liquefaction should not be measured as the sample may have decomposed, or changed its crystalline structure (Furniss et al, 1989). This method was used to analyse the melting point of FOG (Williams et al, 2012).

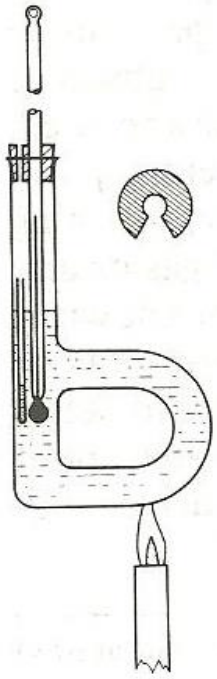


Figure 4 Example of Melting Point Equipment Setup (Furniss et al, 1989)

Mixed chemical compositions usually reduce the temperature at which the pure chemical would melt; however there exist mixtures that increase the melting temperature. If the chemicals within the sample are known, then they should be mixed proportionately and tested for their melting points. This can aid in quantitative analysis (Furniss et al, 1989).

[2] A derivative of method [1] is to pre-melt the FOG and pour into the capillary tube after filtration through filter paper. The part filled capillary tubes are chilled to 4-8°C and then heated (Nitayapat and Chitprasert, 2014).

[3] Automated melting point apparatus is also widely used. A small amount of sample is placed into the capillary columns, which are then inserted into the machine. These samples are heated at a rate that is decided by the analyst, up to 20˚C increase per minute. The machine records a video of the samples melting, along with the temperature of the machine and analyses the video using software to determine the melting point. This can be reviewed by the analyst afterwards (SRS, 2013).

Both [1] and [3] will generate equally valid results, as they are essentially the same test, except that [3] is using more complex machinery than [1] to acquire the results. If automated melting point apparatus is available it is best to use this as the results can be double checked afterwards, whereas with Thiele tubes the results cannot be altered. Methods [1] – [3] use capillary tubes to determine the melting point range. These are very difficult to fill with sludge as it is viscous and adhesive. This is most likely why the FOG in [2] was pre-melted and poured into the capillary column. Pre-melting the sample alters its melting point (Furniss et al, 1989); therefore an incorrect melting point is measured.

To summarise a homogenised, but otherwise unaltered 1mg of sample is placed into a capillary tube and pushed to the bottom of the tube using a rod. The capillary column is placed into a Thiele tube, which is heated at a rate of 5°C per minute from ambient. Once the sludge starts to melt the Thiele tube was allowed to cool by 10°C and the capillary tube was replaced by a new tube with fresh sludge in. The heating rate was altered to a 1°C per minute increase, where the initial and full melting points were measured to determine the melting range. This was repeated three times for each sample.

3.3.3 Microscopic & Biological

There are a number of factors to consider when viewing a sample under the microscope, such as whether it is needed to see what is within the sample and the size and type of particulates. Due to these factors there are a multitude of functions available when using a microscope. Functions include illumination, magnification, software packages for counting particle numbers, 3D imaging and preparation of the sample on the slide.

[1] Many activated sludge plants utilise bright-field illumination and either an eyepiece micrometer, or more commonly, software to calculate the number of bacteria in an activated sludge slurry. A magnification of at least 100x is commonly employed (Mesquita et al, 2013).

[2] Phase contrast illumination is used to enhance the image of some of the types of bacteria, which lack in contrast due to their transparency (Mesquita et al, 2013).

[3] Bright field microscopy staining is used for increasing the clarity of bacteria in the image, with the two most common staining techniques being Gram and Neisser (Mesquita et al, 2013).

[4] Dip slides are used to measure the aerobic bacterial activity. The solid or liquid is sampled by placing the dip slide into the liquid for thirty seconds, or by pushing both sides of the pad against the solid surface. Once this is done the dip slide is incubated at 30˚C for 48 hours and then compared to a bacterial development chart (HSE, 2013).

[5] The microscopic analysis of activated sludge slurry is conducted as follows; “Microscopic investigation should be carried out with sludge that is as fresh as possible” (Eikelboom, 2000). This is to give an accurate view of the current state of the sludge as the bacteria and structure of the sludge alters over time. The sludge should be well mixed prior to analysis. One drop of slurry is placed onto a clean microscopic slide and enclosed with another, avoiding entrapment of air bubbles. Choice of illumination is also important, with bright field and dark field illumination commonly used. The magnification power used was based upon the need for bacterial identification and accuracy in counting, however a 100x to 200x magnification is a good level to start viewing and 400x and higher magnification used for identifying the bacteria. Counting the bacteria is best achieved by comparing the image to bacterial counting charts.

Identification and clearer imaging of the bacteria is conducted using a staining technique. The slide must be viewed systematically, following the pattern on Figure 5, to avoid erroneous results. Experienced analysts can identify: form, structure, dimensions and composition of the floc in the slurry, also bacteria not bound to the floc, other organisms and species of bacteria (Eikelboom, 2000).

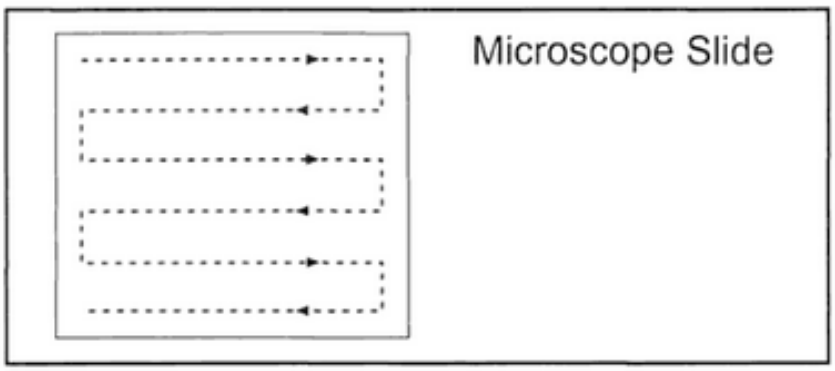


Figure 5 Systematic Viewing of a Slide (Eikelboom, 2000)

The structure of the sludge may contain clues as to why it shows its semi-solid, viscous and adherent characteristics. As such, there is the possibility that the sludge is formed of colonies of bacteria, that bind together forming a structure known as biofilm. This can be determined microscopically and with the aid of a dipslide.

The sludge is freshly sampled and homogenised [5]. It is analysed within a day and stored at 4˚C when not being used, as previously mentioned in this Chapter, to minimise any further degradation.

Methods [1] – [3] and [5] use three types of illumination, bright field, dark field and phase contrast. All three were used on the sludge, with varying degrees of magnification between 10x – 200x as individual bacteria need not be identified. Staining of the bacteria can be used as in [3]; however with the use of phase contrast illumination this is not necessary. Viewing of the slide in a methodical manner is essential so as to record the full structure of the sludge. Viewing is conducted by going back and forth over the slide as in [5].

Method [4] does not use microscopy to indicate the number of bacteria within the sludge; instead it uses a dipslide to calculate the numbers of living bacteria and fungus.

This technique is used alongside microscopic analysis to determine if the bacteria seen under the microscope are living and how many they number. In depth biological analysis is beyond the scope of this thesis, but would be beneficial in determining the route of the formation of the sludge.

In summary, a sludge sample is homogenised and analysed within a day and stored at 4˚C when not in use. A small sample is placed on a microscope slide, with another slide being placed carefully on top and compressed. The microscope uses dark field, light field and phase contrast microscopy at magnifications between 10x – 200x. A bacterial counting program is used if necessary. Alongside this, a dipslide is used to calculate the number of living bacteria within the sample. Both sides of the dipslide are wiped across the surface of the homogenised sludge and then incubated at 37˚C for 48 hours. Analysis is against the solid surfaces chart.

3.3.4 Gas Chromatography

Gas chromatography is a precise, yet delicate tool used for the analysis of organic chemicals. Careful sample preparation must be undertaken to ensure that the equipment is not damaged, or incorrect results derived from poor preparation. Due to the number of columns and operating conditions in existence, only ones used in analysis of similar materials, are reviewed in this Chapter.

[1] FOG samples were analysed for their fatty acid content only. This was conducted by hydrolysis of the triglycerides and methylation of the fatty acids. A portion of the sludge ~1.0g was placed into a beaker along with an internal standard solution of toluene and 10ml of 1% H_2SO_4 in methanol was added afterwards. This solution was refluxed for 2 hours to allow the triglycerides to hydrolyse and the fatty acids to methylate. The solution was cooled and a NaCl solution added. The upper layer of toluene was removed and mixed with a small amount of anhydrous sodium sulphate to remove and possible water contamination. This solution was analysed on a Varian CP-3800 gas chromatogram against certified standards, using these settings: injection volume: 0.6 ml, injection temperature: 200°C, column: 30m x 0.2 mm Solgel WAX, 0.25mm film, column temperature: 50°C for 1 minute, 10°C/min to 270°C, held at 270°C for 10 minutes, carrier gas: O_2-free N_2 at 2.0 ml/min, split ratio: 50:1, detector: flame Ionisation at 275°C (Williams et al, 2012).

[2] Samples of FOG were saponified to convert any fatty acids to methyl esters, following standard method AOCS CE 2-66. In brief 0.5-1.0g of sample was placed in a tube with 0.5mg of tridecanoin in ethanol to serve as an internal standard. 1ml of 0.5N sodium hydroxide in methanol was added and the tube heated at 85°C for 10 minutes and allowed to cool. 1ml of 14% boron trifluoride in methanol was added and the tube then vortexed and heated at 85°C for a further 10 minutes. 1ml of DI water and 1ml of hexane was added to the solution and the tube vortexed at full speed for 30 seconds. The tube was allowed to stand, where it formed layers in the solution. The top layer containing the FAME was removed and dried using sodium sulphate. This was analysed using a Perkin Elmer Autosystem XL gas chromatography-flame ionisation detector (GC-FID). An SGE BPX-070 column was used, with the sample being run against a fatty acid methyl ester (FAME) standard (He et al, 2011; AOCS, 2004).

[3] FOG was prepared for analysis on a GC-FID using the following method: "Dried FOG was dissolved in chloroform/diethyl ether (1:1 by volume) and treated with BF_3/methanol. A Supelco capillary column (100m x 0.25mm) fitted to an Agilent Gas Chromatograph (6890N) was used. The carrier gas was He with a split ratio of 100. The injector and the flame ionisation detector temperatures were 250°C. The temperature of the oven was varied according to the following programme. After application of the solution of methyl esters (1μL) the temperature of the oven was held at 140°C for 5 min. Then it was increased at the rate of 3°C/min until 250°C was reached and this temperature was maintained for 17 min. The system was calibrated by application of Supelco™ 37 Component Fatty Acid Methyl Ester Mix" (Nitayapat and Chitprasert, 2014).

[4] Analysis of fatty acids within FOG deposits was conducted following the Association of Analytical Communities (AOAC) method 996.06, which briefly is as follows: 0.2g of sample is blended with ethanol and a concentrated hydrochloric acid solution in a Mojonnier flask. The sample was heated to 75°C for 40 minutes, whilst being gently agitated. Chloroform and diethyl ether were added to the solution and the temperature increased to 100°C for 45 minutes and allowed to cool. Hexane was added causing the solution to layer. The top layer containing the FAME was extracted and analysed through GC-FID against certified standards. The column suited for use is the Sigma-Aldrich SP2560 100m x 25mm with a 20μm film thickness (Keener et al, 2008; AOAC, 2002).

[5] Analysis of vegetable based oil, butter and lard by GC-FID was conducted using a MET Biodiesel capillary column of 14m length, 0.53mm inner diameter with integrated guard 2m long, 0.53mm inner diameter. The chemicals were extracted using an unspecified method, assumed to be dissolving in an organic solvent and filtration prior to injection onto the column. The solution is injected onto the column using cool on-column injection, which is required for triglyceride analysis. The operating program varies for each chemical. Butter starts at 150°C, increasing at 30°C/min to 350°C, total time of 15 minutes, with the FID being 400°C. Lard starts at 150°C, increasing at 20°C/min to 350°C, total time of 15 minutes, with the FID being 400°C. Palm oil starts at 200°C, increasing at 20°C/min to 350°C, total time of 15 minutes, with the FID being 380°C. Olive oil wax starts at 200°C, increasing at 30°C/min to 350°C, total time of 15

minutes, with the FID being 380°C. This method does not require hydrolysis, or saponification of the esters prior to analysis (Buchanan, 2011).

[6] Semi-volatiles from solids were analysed after extraction using pressurised fluid extraction, EPA Method 3545. The extract solution is injected onto the GC column, which is a narrow-bore fused silica capillary column. The column should be 30m in length, 0.25/0.32mm inner diameter, 0.25/0.5/1µm film thickness, silicone coated fused silica capillary column. A mass spectrometer should be used for detection. An optional guard column of deactivated fused silica, 0.25mm inner diameter, 6m in length is permitted. This is measured quantitatively against certified standards (EPA, 2007d). An operating procedure suggested for this method uses a Supelco SLB-5ms column of 30m in length, 0.25mm inner diameter and 0.25µm film thickness uses the following settings: Oven start temperature of 40˚C, held for 1 minute, increasing by 22˚C/min until 240˚C then 10˚C/min to 330˚C, where it is held for 1 minute. Injection is at 250˚C, injection volume of 0.5µl, carrier gas helium at 1ml/min. (Sigma Aldrich, 2006a) An alternative operating procedure from Sigma Aldrich uses a SLB-5ms column of 30m in length, 0.25mm inner diameter and 0.50µm film thickness with the following settings: Oven start temperature of 40˚C, held for 1 minute, increasing by 12˚C/min until 250˚C then 25˚C/min to 340˚C, where it is held for 4.5 minutes. Injection is at 250˚C, injection volume of 1.0µl, carrier gas helium at 1.5ml/min (Sigma Aldrich, 2006b).

A GC-MS is highly adaptable so that it may analyse for a broad range of chemicals. This is achieved by altering the operating conditions and changing the columns. Each column and set of operating conditions determine only a fixed range of species of chemicals. This means that to identify a multitude of different chemicals, a number of different columns and/or operating conditions must be used. It must be noted that the columns and operation of the GC-MS are relatively expensive and time consuming and therefore care must be taken to not over analyse the samples, leading to increased analytical costs. Analysis of the sludge by GC-MS is expected to be complex, due to the fact that there are likely to be thousands of different chemicals present at varying concentrations. To aid in the decision of which column and set of operating conditions to use it is advisable to research the chemistry of the products that are used within the

metal machining systems that could contribute to the formation of the sludge. Alternatively a general column, such as the Perkin-Elmer Elite 5MS, using generalised operating conditions, should be used to give a basic overview of the compounds within the sludge. From the qualitative results found by this analysis and study of the virgin products, alternative, specialised columns and methods can be identified to be used. Once qualitatively identified, the compounds forming the largest peak areas must be quantitatively analysed against standards.

Not all organic compounds may be able to be determined by GC-MS, such as free fatty acids, which usually require being converted to fatty acid methyl esters to improve peak shape, resolution and absolute recoveries (Hamilton and Rossell, 1986). These compounds may have to be converted to be able to elute through the column. If these compounds are suspected to exist within the sludge then a conversion process must be used to convert them into compounds that can be eluted by the column. Alternatively the use of liquid chromatography may mean that these compounds can be quantitatively determined without the need to convert them (Mondello et al, 2001).

Methods [1] – [4] all use their own techniques to prepare the sludge for analysis, whilst it is not stated what technique is used in for the extraction of the organics in method [5]. The likely technique is described in the 'Organics Extraction' section of this Chapter. EPA Method 3545 is used to prepare the sludge in [6], which is also described in the 'Organics Extraction' section.

None of the analyses in [1] – [5] are conducted to determine the general chemicals found within the samples. They are all specific to individual chemicals, such as triglycerides and fatty acids. These methods may prove to be useful if the chemicals within the sludge are qualitatively analysed, or deduced to be fatty acids and triglycerides. Around 250 semi-volatile compounds can be determined by following [6]. This method should offer a basic qualitative overview of the compounds within the sludge. The stationary phase of the Sigma Aldrich SLB-5ms column is also used by other brands and is composed of 5% diphenyl and 95% dimethyl polysiloxane. The SLB-5ms column is not the only one containing that phase make up and any column that contains this phase make up and fulfil the column sizing criteria in EPA 8270D may be used for [6].

To summarise, the first analyses conducted on the GC-MS use the solution that has been previously extracted in the 'Organics Extraction' section of this Chapter. It is run on a capillary column composed of 5% diphenyl and 95% dimethyl polysiloxane. The column is 30m in length, has an inner diameter of either 0.25mm or 0.32mm, has a film thickness of either 0.25µm or 0.5µm or 1µm. An optional guard column of deactivated fused silica, 0.25mm inner diameter, 6m in length is used if it is available. The operating conditions follow those devised by Sigma Aldrich with an oven start temperature of 40˚C, held for 1 minute, increasing by 12˚C/min until 250˚C, at which point it alters to increase at 25˚C/min to 340˚C, where it is held for 4.5 minutes. Injection on the column is at 250˚C, with an injection volume of 1.0µl and carrier gas helium flow of 1.5ml/min. These operating conditions are designed to distribute the peaks more evenly across the spectra, allowing for easier analysis and identification. Once these results and the products used within the machining systems are analysed, other columns and operating conditions can be used that are more specific to the chemicals identified. The follow-up analyses are quantified against certified standards for the main chemical components in the sludge.

3.3.5 Liquid Chromatography

Liquid chromatography can be beneficial over gas chromatography for environmental pollutant analysis, especially when analysing for polar compounds, as these need not be derivatised before passing onto the column (Mondello et al, 2001). There are a vast range of columns available, therefore not all are covered in this thesis, however the ones which have been used to specifically analyse similar environmental pollutants are focused upon.

[1] Analysis of soil was conducted by extracting the soil overnight using acetonitrile, filtered and the filter rinsed with acetonitrile. The solution was concentrated and diluted with water. 100µL of sample was extracted and injected into the column. The columns used for determination of fenpropimorph in soil were: 5µm Hypersil SAS, 60m length, 4.6mm inner diameter, followed by a second column: 5µm Hypersil ODS, 150m, 4.6mm inner diameter, where it was passed into a UV detector. Using these columns in series aided in separation of the chemical constituents of the soil, creating a clearer and more definitive spectra (Mondello et al, 2001).

[2] One problem an analyst can encounter is lack of clarity of the spectra caused by insufficient separation of the compounds. This leads to problems with both qualitative and quantitative analysis of the sample. To reduce these effects and further separate the chemicals the use of two columns may be required. This can either be liquid chromatography of one column type, followed by liquid chromatography by another column type, or liquid chromatography followed by gas chromatography, depending upon the nature of the chemical that is being sought after. These can be used in conjunction with many different detectors, such as mass spectrometer, flame ionisation detector or a fluorescence detector (Mondello et al, 2001).

[3] HPLC was used to analyse for explosives in soil. The soil was dried at 60°C for 2 hours and sieved through a 60/250µm sieve. Acetonitrile was added to the sieved soil to a concentration of 0.2g/ml. This solution was sonicated and filtered through a 0.2µm filter and evaporated to dryness and resolved in HPLC grade acetonitrile to make a 1g/ml solution. This solution was again sonicated and transferred to 2ml HPLC vials containing 350µl sample tubes. The mobile phase was 65:35 acetonitrile: DI water. The systems calibration was checked daily against a standard. A Dionex HPLC was used with

a diode array detector. The column was an Alltech Widepore Econosphere C18 column, 5µm particle size, 250mm long and 4.6mm diameter. The guard column was Phenomenex Widepore C18 (Bommarito et al, 2007).

[4] Analysis of soils by HPLC for polycyclic aromatic hydrocarbons was conducted using an Agilent 1200 series HPLC connected to a fluorescence detector. Using an Agilent ZORBAX Eclipse polycyclic aromatic hydrocarbons (PAH) column, 4.6mm diameter, 50mm long and 1.8µm in size the analytes were separated. The soil samples were prepared by placing 5g of sample into a centrifuge tube along with 5ml of DI water and shaken vigorously for 1 minute. 10ml of acetonitrile, 6g of anhydrous magnesium sulphate and 1.5g of anhydrous sodium acetate were added to the solution and shaken vigorously again for 1 minute. The mixed samples were centrifuged at 4000rpm for 5 minutes. 6ml of the upper layer of solution was extracted into a solid phase extract vial, along with 400mg of primary secondary amine, 400mg of C18EC (high purity silica) and 1200mg of anhydrous magnesium sulphate. This solution was vigorously shaken for 1 minute and centrifuged at 4000rpm for 5 minutes. 4ml of this solution was filtered through a 0.45µm filter and put through the HPLC (Pule et al 2012).

[5] Solvent extractable non-volatile compounds were determined using reverse phase HPLC with a thermospray mass spectrometer (MS) as the detector and the following method: A solid sample is extracted using the chosen method in the 'Organics Extraction' section, except that the hexane and acetone solvent solution is substituted for methylene chloride and acetone. This solution was evaporated and replaced with either methanol or acetonitrile. An ODS–Hypersil, C_{18} reversed phase column of 100mm length, by 2mm inner diameter and 5µm particle size is to be used. Alternatively a MOS2-Hypersil C_8 reversed phase column, 100mm in length, by 2mm inner diameter and 3µm particle size or equivalent may also be used. The guard column is C_{18} reversed phase column of 10mm length, by 2mm inner diameter and 0.5µm particle size with the same stationary phase as the analytical column. The operating conditions are run as 0.8ml/min flow rate, 0.1M ammonium acetate for the post column mobile phase and 0.4ml/min for the post column flow rate. The MS measured in both positive and negative ionisation modes. Positive ionisation mode has the discharge electrode switched off, the filament can be either on or off, mass range of 150-450amu, scan time of 1.5secs/scan and an optional repellent wire at 170-250V. Negative ionisation mode

has the discharge electrode switched on, filament switched off, mass range of 135-450amu and scan time of 1.5secs/scan. The TS temperatures are 110-130°C for the vaporiser control, 200-215°C for the vaporiser tip, 210-220°C for the jet and 230-265°C for the source block. A sample injection volume of 20-100µl is used. Thermospray (TS) may cause interference with quantification of the analytes on MS; therefore a UV detector may be used to quantify analytes (EPA, 2007e).

Liquid chromatography (LC) can be used alongside, or combined with GC analysis to aid in qualitative and quantitative analysis of the sludge. Benefits of LC include the ability to detect certain chemicals that would otherwise require derivitisation for GC analysis and analysis of highly volatile substances that could not be analysed by GC (Mondello et al, 2001).

Methods [1], [3] – [5] all require that the sample is pre-extracted before passing into the column. Acetonitrile is the only solvent used to extract the sample in [1], [3] and [4], with DI water being used for dilution. This single solvent extraction may not extract all of the organics, or extract them fully, leading to incomplete and incorrect qualitative and quantitative analysis. Extraction using more than one solvent offers best results (EPA, 2007c). Samples should be extracted following the method in the 'Organics Extraction' section of this Chapter so the maximum extraction of organics is achieved.

As with GC, LC has an extensive selection of columns and operating conditions, which allows it to detect many thousands of organic chemicals. Initially a general column should be used, such as in [4] for PAH and [5] for non-volatile compounds, unless a specific chemical is being analysed for, such as those that would require derivitization using GC. Specific chemicals were analysed for using [1] and [3], which although may be a common chemical analysed for in soils, are unlikely to be chemical components of the sludge, therefore these methods will not be used for analysis of the sludge. HPLC is a modern method of LC that reduces the time the chemicals take to elute from the columns, thus greatly reducing the analytical time. If peaks on the spectra have merged and require greater separation more than one column may be used [2], or LC may even be coupled to GC to enhance the separation of the chemicals and produce a clean spectra.

In summary HPLC is used to detect the chemicals that cannot be detected by GC, and as with GC, a general column and operating conditions are initially used to give an overview of the chemicals within the sludge. The general column and operating conditions are those used in method [5]. The sludge is extracted following the method specified in the 'Organics Extraction' section of this Chapter, using methylene chloride and acetone as the solvents and methanol replacing the solvents above before passing the sample into the column. An ODS–Hypersil, C_{18} reversed phase column of 100mm length, by 2mm inner diameter and 5µm particle size is used with an ODS-Hypersil C_{18} reversed phase guard column of 10mm length, by 2mm inner diameter and 0.5µm particle size. The operating conditions are: 0.8ml/min flow rate, 0.1M ammonium acetate for the post column mobile phase and 0.4ml/min for the post column flow rate. The MS is measuring in both positive and negative ionisation modes. Positive ionisation mode has the discharge electrode switched off; the filament was off, mass range of 150-450amu and a scan time of 1.5secs/scan. Negative ionisation mode has the discharge electrode switched on, filament switched off, mass range of 135-450amu and scan time of 1.5secs/scan. The TS temperatures are 130°C for the vaporiser control, 215°C for the vaporiser tip, 220°C for the jet and 265°C for the source block. A sample injection volume of 100µl is used. Once this initial qualitative analysis is complete and combined with chemicals suspected to be in the sludge and GC results more specific columns and operating conditions are used.

3.3.6 Inductively Coupled Plasma

Inorganic elements are commonly analysed using inductively coupled plasma. Concentrations as low as ppb can be detected, depending upon the analyte and detector used.

[1] Analysis of wastewater treatment sludge was conducted by following this method: Using a pre-prepared (pre-digested) sample the concentrations of the inorganic elements occurring within the sludge are measured and compared against standard solutions of similar concentrations and against a blank sample. The manufacturers operating conditions for the ICP should be followed. Any appropriate measuring device may be used in conjunction with the ICP i.e. flame ionisation detector; however some detectors may not detect all inorganic elements (AWWA, 2005).

[2] After digestion of a solid sample the solution is put through an inductively coupled plasma-atomic emission spectrometer (ICP-AES). There are no specific instructions for analysis on the ICP-AES, only the instruction to correctly calibrate and test the machine using the certified standards prior to analysis of the samples (EPA, 1994; Keener et al, 2008).

[3] Calcium concentrations in FOG samples were determined by ICP-OES. Method [1] in the 'Ashing' section of this Chapter was used for the sample preparation, which is briefly: The samples were initially dried in an oven at 105˚C, and then incinerated in a muffle oven at 500˚C. The dry residue was then suspended in 6N HCl, filtered and diluted using DI water. The sample was then measured using a Perkin Elmer 2000 ICP-OES. No details are given on the operating conditions of the ICP-OES (He et al, 2011).

[4] Metal content of FOG was analysed by inductively coupled plasma-mass spectrometry (ICP-MS) after the sample had been digested following the method used by Williams et al, 2012. The analysis on the Agilent 7500ce ICP-MS was undertaken using the octopole reaction cell, following the semi-quantitative method in He mode against certified standards (Williams et al, 2012).

There are a number of detectors that can be used with ICP analysis. Each detector type has different detection levels and ability to speciate elements, therefore the detection

levels stated below are generalised for the detector (AWWA, 2005). The most common detectors used with ICP are: optical emission spectrometry (OES), mass spectrometry (MS), flame ionisation detection (FID) and atomic emission spectrometry (AES). MS can detect a wide range of elements at concentrations many orders of magnitude lower than OES can detect (EAG, 2007). This is the same for AES which can detect down to the parts per billion level, compared to MS, which is parts per trillion (Varian, 1994; Cetac, 2014). FID can detect ppb levels (Ametek, 2011). This means MS is preferable in ICP analysis as it can detect elements at lower concentrations that can be detected using FID, AES or OES. MS is used in [4], but can also be used with [1]. Detection levels of ppb should be sufficient in the analysis of the sludge as it is expected that concentrations of elements that significantly affect the chemical makeup of the sludge are in, or above the ppm concentration. Typical concentrations of analytes in FOG range from 15ppm – 49,000ppm for elements that significantly affect the chemical makeup of the sludge (Williams et al, 2012; He et al, 2011). Once the sludge is digested it can be diluted to reduce the concentrations to levels that match up to the standard calibration curves on the ICP. Any detector may be used with the ICP as the concentrations of analytes within the sludge are expected to be at least in the ppm range.

Only method [4] details some of the operating conditions of the ICP, though all methods are run against certified standards. The operating conditions will vary with the ICP and detector used and should follow the conditions detailed by the manufacturer, but must be run again certified standards. As there is no standard detector for ICP any detector that can measure in the ppm range can be used, however MS has greater sensitivity and should be used if available.

Prior to passing into the ICP the sludge must be processed into a liquid form. Methods [1] and [2] do not define the procedure that is required to process them, however the chosen methods in the ‘Ashing’ and ‘Digestion’ sections of this Chapter can be used. The use of any of the methods defined above, or in the ‘Ashing’ or ‘Digestion’ sections of this thesis may cause incorrect or misleading results, due to elements inherent in the water remaining within the sludge after the water has evaporated. An alternative method that prepares the sludge to generate more precise results is developed in Chapter Four. This does not affect the ICP method used, only the preparation of the sludge for the ICP analysis.

To summarise, the sludge is prepared according to the chosen methods in the 'Ashing' and 'Digestion' sections of this Chapter. Once in a liquid form it can be passed into the ICP. OES was used as the detector for the ICP due to it being the only detector available for use.

3.3.7 Fourier Transform Infra-Red Spectrophotometry

Fourier Transform Infra-Red spectroscopy (FTIR) is a non-destructive analytical technique. It works by emitting infra-red light, some of which is absorbed by the sample and some reflected. Each type of bond and the way it vibrates/rotates occurs at different wavelengths, meaning that chemicals can be identified when compared to the database. The drawback from this is that complex samples, those made up from more than one chemical can be difficult to positively identify as there are many different bonds present that may lead to incorrect chemical identification.

[1] Analysis of unaltered FOG samples was conducted using a Digilab FTS-6000 FTIR spectrophotometer using a mounted crystalline zinc selenide attenuated total internal reflection sampling attachment. The detector was a liquid nitrogen cooled, wideband mercury-cadmium-telluride detector with a linearized normal spectral response of 450-7000cm^{-1}. The spectra were converted into absorbance units by taking the negative of the log ratio of air (He et al, 2011).

[2] Sewage sludge was analysed by dewatering in a centrifuge and sterilising in an autoclave at 121˚C for 15 minutes. A tablet of the sludge was created with KBr using 1% (w/w) of the sludge and 99% of KBr. This tablet was analysed using a Shimadzu IR Prestige-21 spectrometer with a resolution of 4cm^{-1} and a spectral response of 250-3750cm^{-1} for 32 cycles (Silva et al, 2012).

[3] Activated sludge was analysed using a Perkin Elmer FTIR. The sludge was prepared by washing with DI water for five minutes and filtered to remove the bulk of the water. It was further dehydrated under a vacuum with pressure of 600mmHg overnight at ambient temperatures. Once dry the sludge was mixed with KBr in the ratio of 1g sludge to 50g KBr for thirty seconds. Pellets were then formed from the mix and analysed for 100 cycles at a resolution of 4cm^{-1} and wavelength range of 400-4000cm^{-1} (Liao, 2008).

Potassium bromide is used to pelletize the sludge as it does not absorb in the wavenumber 4000-400cm^{-1}. This allows easier handling of the sample for analysis on the FTIR. Alteration of the sludge should be avoided so that no chemicals can degrade or react prior to analysis. The sludge in [1] is analysed with no processing prior to analysis, whilst in [2] it is dewatered, causing a potential loss of organics in the centrifuged

water, although this is likely to be an insignificant amount compared to the degradation that may occur to the organics in the autoclave. Washing with DI water [3] may also cause a loss of organics; however it is not dried by exposure to heat, only vacuum dehydration at ambient temperatures. Vacuum dehydration may still cause the loss of VOCs, though these are thought to be minimal, especially in the sludge samples that have been in existence for extended periods of time. FTIR is commonly operated at 4000-400cm^{-1} wavenumber [3] and 4cm resolution [2] and [3] and has multiple cycles.

In summary, the sludge is not altered prior to analysis i.e. not dehydrated or mixed with KBr. It is placed directly on the cell ensuring that no air bubbles are between the cell and the sludge. Following part of the procedure in method [3], scans at 4cm resolution, 4000-400cm^{-1} wavenumber and 100 cycles are conducted. Due to the expected chemical complexity of the sludge it is anticipated that FTIR will not be able to fully characterise its chemical makeup, only assist in identifying possible chemicals and bonding structure.

3.3.8 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) is used to determine the structure of a compound. The two types of NMR that are mostly commonly used are; ^{13}C NMR, which measures the ^{13}C carbon isotope and H NMR, which measures the ^{1}H hydrogen isotope. Isotopes of other elements may also be studied. The chemical under study should be suspended in a deuterated solvent so it does not cause inference with the analysis for H NMR. Samples may also be in a powdered form.

[1] Fatty acids were analysed by H NMR using the following method: 6mg of homogenised pure sample was dissolved in 3ml of deuterated chloroform. The NMR acquisition time was 2 seconds, sweep width 4000Hz, pulse width 8.8μs and 20 transients (Hamilton and Rossell, 1986).

[2] Fatty acids were analysed by 13C NMR using the following method: 150mg of pure homogenised sample was dissolved in 3ml of deuterated chloroform. The NMR acquisition time was 1 second, sweep width 16000Hz, pulse width 9.9μs and 120 transients (Hamilton and Rossell, 1986).

H NMR and ^{13}C NMR can be used together to determine the chemical structure of the sample, however it is common to include other physical and chemical analyses alongside NMR analysis to determine the chemical structure of the sample (Hamilton and Rossell, 1986). Although ^{13}C NMR has better resolution of fatty acids than H NMR (Hamilton and Rossell, 1986), both techniques was used for the analysis of the sludge.

NMR is used to "identify functional groups, count hydrogen and carbon atoms, assign stereoisomers, work out bonding frameworks and locate the position of functional groups along the hydrocarbon chain" (Hamilton and Rossell, 1986). Identification of all of these characteristics greatly aids in the identification of a single chemical compound, however when multiple compounds are present within the sample complications may arise. These complications arise from multiple types of bonds, variations in carbon and hydrogen numbers and a multitude of functional groups. This is due to no pre-separation of the sample prior to NMR analysis. Due to the large variety of chemicals expected to be present within the sludge it is likely that NMR will not be able to distinguish between the individual chemical compounds, therefore producing incorrect

or inconclusive results, therefore NMR will not be used alone, instead was used alongside other analyses detailed within this Chapter.

To summarise, aspects of both methods [1] and [2] were used. For H NMR 6mg of homogenised sample is dissolved in 3ml of deuterated chloroform, using an acquisition time of 2 seconds, sweep width of 400Hz, pulse width of 8.8μs and 20 transients. For ^{13}C NMR 150mg of homogenised sample is dissolved in 3ml of deuterated chloroform, using an acquisition time of 1 second, sweep width of 16000Hz, pulse width of 9.9μs and 120 transients.

3.3.9 Carbon Content

Total carbon (TC) is the measure of the total mass of the carbon within the sample, expressed as a percentage. Total organic carbon (TOC) is the amount of this carbon bound in an organic form, such as glycerol. Total inorganic carbon (TIC) is the amount of carbon bound as carbonic acid salts and dissolved carbon dioxide. A number of different analyses can be used, which are usually conducted for only two out of the three carbon analyses, where the results are added or deducted from one another to give the result of the third. i.e. TOC = TC – TIC.

[1] TOC analysis of sediments involves removing and recording any large items within the sediment, i.e. sticks/stones and homogenisation of the sample. Wet determination of TOC is conducted by extracting the sample using the Walkley-Black procedure. $K_2Cr_2O_7$ and HCl are added to 0.5-1.0g of sample and swirled in a beaker to mix and heated at 150˚C for 30 minutes to allow complete oxidation of the organic carbon. Once the oxidation is complete then either of the following techniques can be used to determine the percentage of organic carbon within the sample. Excess Cr_2O_7 and Cr can be measured titrimetrically using Ferroin as the reagent to turn the solution reddish-brown, or it can be measured colorimetrically. CO_2 that has been released from the sample can be measured gravimetrically by its absorption onto Acarite, which is conducted by passing a CO_2 free gas over the reacting sample and over the Acarite, causing an increase in mass of the Acarite. It can be measured manometrically using Van Slyke-Neil apparatus that collects the CO_2, where the increased pressure is then measured using a manometer (EPA, 2002).

[2] TC analysis of sediments involves removing and recording any large items within the sediment, i.e. sticks/stones and homogenisation of the sample. Dry determination, if used without pre-removal of the inorganic carbon, measures the total carbon content. If there is no inorganic carbon present or if it has been removed, then organic carbon can be measured. All samples must be pre-dried before the carbon is measured by either drying in an oven at 105˚C, or allowing to air dry until no discernible difference is mass is seen. Initially a small sample ~0.5g is placed in a crucible, which is heated to up to 1500˚C to allow for complete oxidation of the organic and inorganic carbon. This is conducted in a pure oxygen atmosphere, with scrubbers built into the oven to remove particulates, water vapour, etc. A catalytic converter is used to convert any CO to CO_2.

The CO_2 can then be quantified by using the previous gravimetric, manometric or titration techniques. It can also be quantified using spectrophotometric or infrared (IR) detectors. If the dry determination has been conducted using a sample that contains water, this must be taken into account. The results are interpreted against a calibration curve created using the same technique against reference standards (EPA, 2002). TIC can be measured by adding 5ml of 40% H_3PO_4 to the sample in the crucible and placing in the combustion chamber at 200°C.

[3] Another TOC test on sediments by the EPA starts by drying 15g of the sample in a crucible, which is placed in an oven at 60˚C overnight, or until no mass change is seen. Allow the sample to cool and homogenise using a grinder or mortar and pestle. Sieve through a 100 or 140 mesh sieve. A test for inorganic carbon is now conducted. This involves placing a 1.0g sample onto a watch glass, adding 2ml of 4N HCl and observing for effervescence. If effervescence occurs, or inorganic carbon is believed to be present the sample must be treated with 5% H_3PO_4 before testing for TOC. To treat with 5% H_3PO_4 the sample must be placed in a crucible and 1ml of 5% H_3PO_4 added and monitored for effervescence. The crucible and sample are placed on a hot plate stirrer and heated/stirred at low power whilst wetting with DI water, add further 5% H_3PO_4 in 1ml portions until effervescence ceases. Add DI water at four times the amount of acid to the crucible and mix on hot plate for 1-2 minutes. Place in an oven at 60˚C overnight, or until no mass change is seen. Leave to cool and re-grind as above. Calibrate the LECO SC 444 using four different masses of a carbon standard between 0.05-0.15g. Analyse at least three samples of the sediment for TOC. An extended set of procedures is available from the EPA (EPA, 2005).

[4] Samples are homogenised by grinding to a particle size of <200µm. If they are moist or oily then they can be mixed with aluminium oxide powder until a granular material is obtained. This must be taken into account when the sample is analysed. To dry the sample it can be freeze dried if it contains volatile organic compounds, otherwise air drying, or oven drying at 105˚C may be used. Once dry and homogenised to measure the TC, the sample can be placed into a crucible to be oxidised in a furnace using oxygen as the carrier gas at up to 1500˚C. Samples must be repeated twice and if the carbon content is 10% different to the mean it must be repeated once again. To measure the TIC the sample has H_3PO_4 added in a sealed sample cell. The CO_2 released is

flushed and measured by a detector chosen by the user. Prior to analysis the machine must be calibrated using carbon standards, such as calcium carbonate for TIC and glycerol for TC (ECN, 2004).

[5] Samples are homogenised by grinding to a particle size of <200µm. If they are moist or oily then they can be mixed with aluminium oxide powder until a granular material is obtained. This must be taken into account when the sample is analysed. To dry the sample it can be freeze dried if it contains volatile organic compounds, otherwise air drying, or oven drying at 105˚C may be used. TOC determination firstly starts by removal of the inorganic carbon through the use of H_3PO_4 and DI water if required. This is left reacting for four hours to allow for the dissipation of the CO_2. Any moisture in the sample may be removed prior to combustion at temperatures not exceeding 40˚C. Full moisture removal is achieved in the analytical furnace by slowly increasing the temperature. CO_2 is measured using a detector chosen by the user (ECN, 2004).

Interferences in [1] include ferrous iron and chromate, which cause overestimation of the TOC, whilst if MnO_2 is present it causes an underestimation of the TOC (EPA, 2002). Ferrous iron is likely to be present in the sludge, due to the effluent coming from machining systems that wash the waste iron swarf from the items being machined. The levels of iron within the sample are expected to be high, but it is not known if they are in the ferrous or ferric form. Due to the high levels of iron expected, this technique will not be suitable for TOC determination.

If the sludge is dried at elevated temperatures, such as 105˚C, it may cause oxidation of fatty acids (Hamilton and Rossell, 1986) if they are present, increasing the mass of the sample and leading to an underestimation of carbon. The loss of VOCs by any form of drying (WHO, 1989) may cause an overestimation of carbon. Minimal change in mass of the sample needs to be ensured whilst drying so as not to significantly affect the results. As vacuum dehydration does not cause oxidation of the fatty acids, only potential loss of VOCs, which drying at 105˚C would also do, then vacuum dehydration shall be used to dry the sample prior to carbon analysis in methods [2], [4] and [5].

One advantage of [2] is that sample preparation is minimal (EPA, 2002), inhibiting potential for error that each stage of processing can add and a reduction in analysis time

for the analyst. Using a detector to measure the carbon dioxide, instead of gravimetrically or titrimetrically by the analyst also removes the potential for human error at that stage.

Addition of aluminium oxide in [4] and [5] adds another degree of complexity to the process, which is not necessary for analysis in [1] – [3]. Sieving and grinding with sludge is difficult, if not impossible even with the addition of aluminium oxide, with the adherence of the sludge being the main drawback.

Methods [2] – [4] all use phosphoric acid to remove the inorganic carbon from the sample, but using different techniques. The use of any of these techniques is valid, however only [4] measures TIC, whilst the other methods determine TIC as the difference between TC and TOC.

Carbon analysis using method [2] is the simplest as nothing is required to be added, sieved or ground, only dried, which is necessary for all but the wet determination in [1]. It is for this methods simplicity that [2] are used to determine the TC and TIC, with TOC being the subtraction of TIC from TC.

In summary, 10g of sludge was dried by spreading over a watch glass that was placed within a vacuum dehydrator until no discernible difference in mass was seen. To measure TC 0.5g of dried sludge was placed into a clean crucible. The sludge and crucible were placed in the furnace of the machine and sealed in, where a flow of pure oxygen was passed over the sample whilst it was being heated to 1500˚C. The gases that were driven off were passed over a catalyst to oxidise the carbon monoxide and through an IR detector to measure the carbon dioxide volume. TIC was measured by adding 0.5g of the dried sludge into a pre-incinerated crucible along with 5ml of 40% H_3PO_4. This was then placed into the combustion chamber at 200°C, with the carbon dioxide generated being measured by an IR detector. TOC was determined by deducting TIC from TC.

4.0 Methodology for Analysis of Sludge

The analytical methods detailed below include procedures that have been developed where existing methods are either inadequate for the task, or do not exist, see Chapter Three. Some of the existing procedures have been slightly adapted where, for instance, equipment was not available within the laboratory. These adaptations are clearly indicated when they occur.

4.1 Sampling & Storage

4.1.1 Sampling

Spot sampling was used where the sludge is materialising in more than one location. At each of these locations sampling using a bucket and rope, following the pattern shown in Figure 6 was undertaken instead of random sampling using the grid method at varying depths, following the method. The pattern was devised to try to replicate grid sampling so that each area of the sludge within the tank was sampled. This change in sampling method was due to poor access to the tanks, which are 9 metres tall by 9 metres in diameter, with a 1.5 metre drop into the tank from the walkway. This poor access also meant that sludge could not be taken from significantly different depths. Instead the bucket was thrown so that it penetrated the sludge to a depth of between 5cm and 20cm meaning that sludge from different depths was sampled. This throw and drag sampling technique was more effective than grid sampling as the surface area sampled is much larger, leading to a greater theoretical number of samples than would be taken by grid sampling and conducted in a shorter space of time. These samples from the same location form a composite and have been homogenised prior to analysis.

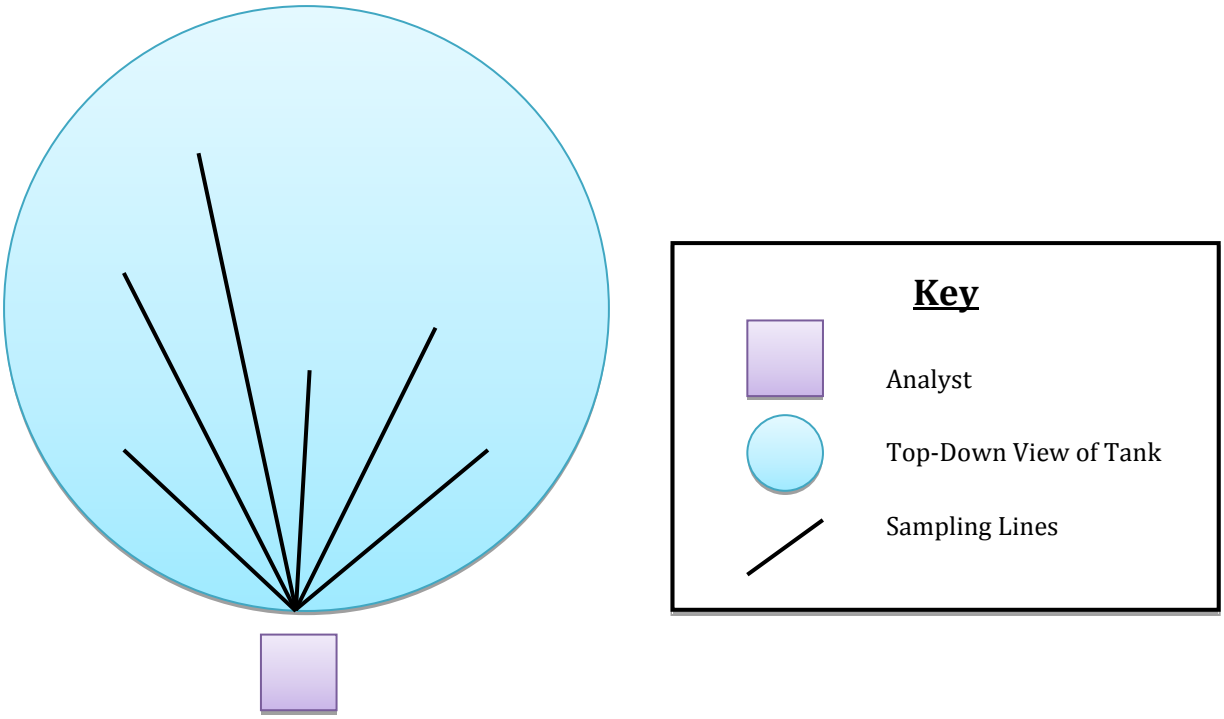


Figure 6 Sampling of the Tanks Using Bucket and Rope Method

4.12 Storage

The sludge was stored in its unprocessed state at 4˚C for a week maximum. This gave enough time for the sample to be prepared for all analyses. The samples were stored in clean 25 litre plastic containers instead of clean, brown glass vessels as the method dictated, due to no large glass vessels being available. Storing the sludge in the plastic containers was no different to storing in glass containers as the plastic is solvent resistant so no compounds adsorb to, or be adsorbed from the plastic. The containers were filled to 99% capacity and stored in the dark. Storage of digested ICP samples was at 4°C as the ICP analysis was not conducted on the same day. They are stored for up to one month without significant chemical alteration.

4.2 Sample Preparation

4.2.1 Water Separation

A new method for the separation of water and soluble organics is presented. It is needed as most techniques for separating the water from the bulk of the sludge may cause errors in the calculation of analytes within the relevant sections of the sludge. This is due to the analytes that are inherent within the water phase of the sludge remaining within the sludge once the water has been separated. For example, when the water is evaporated the ions that are within the water droplets contained in the sludge emulsion, are not transferred into the atmosphere with the water, instead remaining in the dried sludge. This does not alter the overall concentration of the analytes in the total mass of the sludge; however it does increase the concentration of the analytes within the dried sludge and does not proportionate the samples to where they were inherent before water separation.

Studies of FOG (He et al, 2011; He et al, 2013; Nitayapat and Chitprasert, 2014) separated the water by means of drying. No reference was made to the possibility of analytes, such as calcium, that were detected by ICP, being inherent within the water and not interacting with the free fatty acids in the sludge. Instead it was assumed by the authors that the analytes found within the sludge, were all bonded to the free fatty acids, as these are commonly found to do so. Free fatty acids, when bonded to metals such as calcium are known as soap, which when mixed with oil forms grease (Nailen, 2004). This assumption may lead to conclusions that over-estimate the mass of grease within the sludge, where in fact the free fatty acids may exist on their own without being bonded to the analytes to form soap and therefore grease.

It is for the reason above that a new method of water-sludge separation is devised that ensures the analytes inherent within the water stay within the water when it is separated, instead of being transferred to the dried sludge.

The new technique is as follows: Into a pre-cleaned, rinsed and dried separating funnel 25g of homogenised sludge is transferred. As the separating funnel has a narrow neck the sludge was put into a 20ml syringe, which was weighed with the sludge in and after transferring the sludge into the separating funnel. Along with this 50ml of hexane is poured into the separating funnel to dissolve the organic portion of the sludge. The

mixing of the solvent with the sludge was conducted by rigorous agitation for two minutes in a stoppered separating funnel that will then be allowed to settle for 15 minutes before removal of the solvent layer and its dissolved organics, leaving the water and sludge that was not dissolved in the bottom of the separating funnel. This step is repeated until the solvent that is being extracted runs clear. The solvent and dissolved organics are evaporated using a heating block set at 70°C (temperature dependent upon solvent), with nitrogen blowing onto the surface of the solution. Once the solvent has been evaporated and no further decrease in mass is seen from the separated sludge it is weighed and compared with the mass of the water that has been separated to the original mass of the sludge to see if any loss in mass has occurred during this process. Once the water has been separated both of the samples are prepared for analysis by ICP, the methods of which are detailed in the 'Ashing', 'Digestion' and 'ICP' sections of this Chapter.

The solvent used is non-polar so as to be the opposite polarity to water, which is polar, thus the analytes that are inherent within the water phase will remain within the water phase, likewise the analytes inherent within the organic portion of the sludge, are assumed to be non-polar, as it is expected to have significant oil content, which is non-polar. Analytes that are bonded to organics are not expected to be affected by this separation process. The process of evaporation of the solvent may also cause the loss of VOCs from the sludge, however this is expected to be minimal as due to the age and storage conditions of the sludge, the majority of VOCs would have already evaporated.

The afore mentioned water separation technique was found to need slight adjustments so that water could be separated. This was due to no distinguishable boundary layer being formed between the solvent and the water phases when the sludge and solvent was mixed in the separating funnel, although there was a deeper shade of brown for the upper portion of the solvent solution in the separating funnel. It is believed that this lack of distinguishable boundary layer was caused by the density of the hexane being less than that of the water, causing the organics layer to not fully separate. As an alternative DCM was used as it has a density greater than that of water, so therefore a more discernible boundary layer was formed. The drawback of using DCM is that it has a mid-range polarity so it may not dissolve all of the organics. The use of DCM meant that a boundary layer between the solvent solution and the water did occur, thus allowing

easy draining of the solvent solution through the bottom tap. This was repeated between 5 and 7 times until the solvent was clear, indicating that the majority of organics had been dissolved. Within the remaining water phase were suspended organics, including small roots, and what appeared to be minor quantities of sludge. During the separation process fine grains of dust settled at the bottom of the funnel. A magnet was placed against the side of the separating funnel and the fine grains were attracted to it, therefore were likely to be iron fillings from the machining process. These fines ended up in the organic phase as their density was higher than that of the solvent, however they did not seem to be bound to any organics. High levels of iron are expected to be found it the organic soluble portion of the sludge; however it is not likely to be a contributing factor to the structural formation of the sludge. It was decided not to analyse the water phase as the organics within it stuck to the sides of the separating funnel and could not be removed except through physically cleaning using detergent. Instead a sample of the sludge that has not had its water separated was analysed by ICP, with the ICP results from the separated sludge being deducted to give the concentrations of analytes in the water phase.

To ensure that this technique is accurate it was compared to entrainment distillation using the Dean and Stark method [6], which is run as follows: A 50g sample of sludge is used with 300ml of DCM/hexane solution in a 1:3 by volume ratio. Use of these two solvents means that a broader range of organics should be able to be extracted into the solution, leading to increased separation of the water from the sludge. The mass of the sample was weighed prior to separation and both the water and the remainder of the sludge was weighed after extraction, once the DCM/hexane has been evaporated using a block heated to 70˚C, with nitrogen blowing across the surface of the solution.

Water separation should not be conducted for melting point and microbial and microscopic analysis and is not necessary for analysis by FTIR. This new technique may be followed for GC, LC, NMR, carbon content and non-lipid solids determination, however separation of the water by means of vacuum drying can also be used for these analyses as the separation of analytes is not critical for these analyses. Removal of water in the sludge by vacuum drying may be a less time consuming process for the analyst, however it takes longer to remove all of the water from the sludge using this method – a number of weeks compared to a number of hours for solvent separation.

Repetition of the water extraction method should be conducted ten times for statistical validation and should be done to one standard deviation.

4.2.2 Organics Extraction

The sludge that has been previously separated from its water is weighed and mixed with the anhydrous sodium sulphate in the ASE cell. The operating conditions are: 175°C oven temperature, 1500-2000 psi pressure, 5-10 minute static time, 60-75% of cell volume for flush and a 60 second nitrogen purge at 150 psi, using hexane and acetone in a 1:1 ratio by volume. The extraction is repeated twice, so the solution runs clear. The solution is then dehydrated on a heated block at 70°C, with nitrogen being blown over the solution, until it is completely dehydrated. It is then re-weighed to assess the mass that was not soluble. It is then re-entrained in 10ml of hexane and acetone and diluted, ready for analysis by GC-MS or HPLC.

Once the ASE extracted organics had been re-suspended in an acetone/hexane solution and agitated it was found that a minor separate layer was forming on top of the solvent solution within the vial. No amount of shaking would re-dissolve this layer so the solution was vacuum filtered using a 0.2µm surfactant free cellulose acetate filter paper, so that the homogeneous solvent solution could be used for analysis. The layer of undissolved organics would cause quantification issues as the concentration and type of the chemicals within the layer of undissolved organics would differ from the solution of dissolved organics. The filter paper was dried at 105°C for one hour and allowed to cool in a desiccator. The filter paper was weighed and used to filter the solution directly into a clean vial. The filter was dried for another hour at 105°C and re-weighed. Analysis of the filter and its contents was conducted by ICP, detailed in Chapter Five. Analysis using the head space analyser of the filter and its contents was conducted using the GC-MS, which is also detailed in Chapter Five. It is thought the layer is forming due to the solubility of the organics in ambient conditions. The ASE utilises high pressure and temperature to reduce extraction time, thus dissolving more organics than would be dissolved under ambient conditions, such as using Soxhlet extraction.

Repetition of the ASE method should be conducted ten times for statistical validation and should be done to one standard deviation.

4.2.3 Digestion

Using the homogenised and solvent separated sludge that has been previously separated in this thesis the following method is used. 9ml of HNO_3, 3ml of HCl and 2ml of H_2O_2 is added to 0.5g of sludge in a microwaveable container. The sample is heated to 180˚C in 5.5 minutes or under and maintained at that temperature until the total heating time reaches ten minutes. DI water can be used to dilute the solution if necessary. This creates a solution suitable for analysis by ICP, with only minor residual organic matter that was stuck to the sides of the beaker, estimated to weigh <0.5g. This residual organic matter should not affect the ICP analytical results as it remains on the side of the beaker during analysis.

4.2.4 Ashing

Ashing is conducted using the sludge that has been homogenised and solvent separated from its water. Sludge that has not had its water separated prior to being placed into the muffle oven has the potential to boil and eject some of the sample out of the crucible.

A sample of 25g of homogenised but unseparated sludge is placed into a crucible, whilst into another crucible a 25g sample of homogenised and separated sludge is placed. These are put in a muffle oven at 90˚C, with the temperature increasing at 100˚C per hour up to 500˚C, where it is held for 16 hours. Once it is removed and cooled 2ml of DI water is used to rinse the ash into the centre of the crucible. 4ml of 6N HCl was added to the solution and brought to 95°C on a hot plate for 45 minutes until completely dry. Once this was complete a further 4ml of 6N HCl was added and warmed on the hot plate for 15 minutes. The sample was allowed to cool and then filtered through a Whatmann filter paper. The solution was diluted to 20ml using DI water ready for analysis by ICP. This technique is directly compared to that of digestion.

During the ashing process a number of significant issues arose. The first of these being that a significant amount of smoke was produced, which issued out of the top of the muffle oven. This would prove to be a serious issue in furnace rooms that have smoke detectors as there is enough smoke produced to set them off. It is also essential to not open the oven during the heating process as the oxygen entering the oven can cause a flash back, endangering the analyst and surroundings. Boiling of acid is another element of danger for the analyst. Through these elements of significant danger it is not advised that this procedure be used for the analysis of floating cutting fluid based sludge as an inexperienced analyst may not appreciate the dangers they will encounter.

Repetition of the ashing method should be conducted ten times for statistical validation and should be done to one standard deviation.

4.3 Analysis

4.3.1 Non-Lipid Solids Determination

Non-lipid solids can be measured indirectly by using ASE instead of by heating the sludge in kerosene until the sludge dissolves. Both ASE and heated kerosene work to dissolve the organic portion of the sludge and filter the solution, therefore both methods can be used to determine the non-lipid solids content. Use of the ASE to calculate non-lipid solids not only reduces the risk of heating a flammable substance, but also saves time for the analyst. The following method is used to analyse the non-lipid solids within the sludge.

A sample of 2g of vacuum dehydrated sludge was mixed with anhydrous sodium sulphate and placed into an ASE cell. This was then extracted in an ASE 100 using the following settings: 175°C oven temperature, 1500-2000 psi pressure, 5-10 minute static time, 60-75% of cell volume for flush and a 60 second nitrogen purge at 150 psi, using hexane and acetone in a 1:1 ratio by volume. This was repeated once to ensure full extraction of the sludge. The sludge/solvent solution was evaporated using a heated block at 70°C, with nitrogen blowing onto the surface of the solution to aid in evaporation. For increased clarity the non-lipid solids are called non-lipid solids, including solids over 0.2µm'.

Repetition of the non-lipid solids determination method should be conducted ten times for statistical validation and should be done to one standard deviation.

4.3.2 Melting Point

Trying to insert the sludge into the capillary column proved to be extremely difficult as its adherence meant it stuck to the sides along the entire length of the capillary column so more than 1mg of sample was required. Once into the Thiele tube and heated it was not possible to distinguish the melting range as the capillary column was coated in the sludge, therefore obscuring the view of the melting process.

To overcome this problem an adaptation of the Thiele tube method was developed. The sludge was placed at the bottom of a test tube via a syringe and plastic tube, which meant the sludge was only at the bottom of the tube and not coating the sides. Around 10g of sludge was used to fill the bottom of the test tube. As this tube was now too large for the Thiele tube a water bath was setup, as shown in Figure 7. This setup was a substitute for the Thiele apparatus, with the rest of the method being followed as before.

Repetition of the melting point method should be conducted ten times for statistical validation and should be done to one standard deviation.

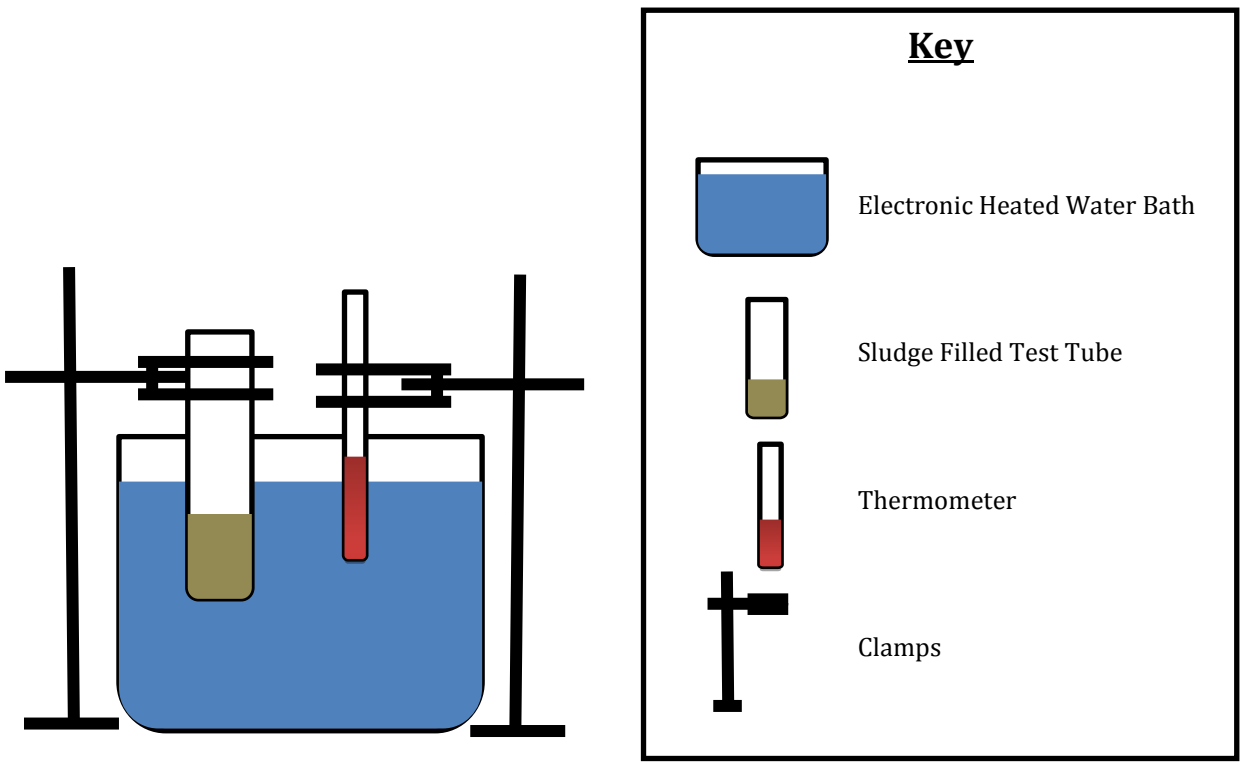


Figure 7 Water Bath Setup for Determination of Sludge Melting Range

4.3.3 Microscopic & Biological

A fresh (same day sampled) homogenised sample of sludge was used for analysis for all of these tests. A dipslide was wiped across the surface of the sludge and incubated at 37°C for 48 hours. Results are compared to the scale that accompanies the dipslide in Figure 8. A further sample was placed on a microscope slide and carefully compressed using another slide. Magnifications between 10x – 200x were used to observe the sample, using dark field, light field and phase contrast microscopy. Alongside this a dipslide was used to calculate the number of living bacteria within the sample. Both sides of the dipslide are wiped across the surface of the homogenised sludge and then incubated at 37˚C for 48 hours.

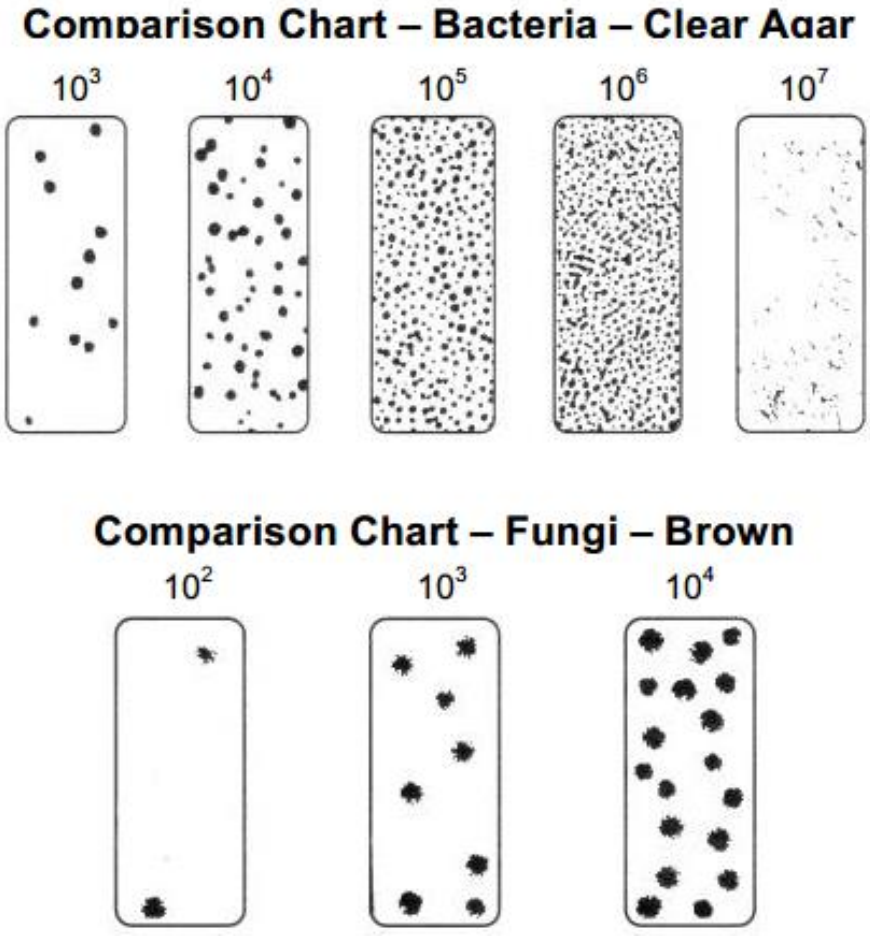


Figure 8 Dipslide Bacterial/Fungal Count (WET, 2005)

4.3.4 Gas Chromatography

The organics that were extracted from the sludge in the 'Organics Extraction' section of this Chapter were used for GC analysis. A Perkin and Elmer Clarus 500 GC with MS was used for the analysis. The column was a Perkin and Elmer Elite 5MS, length 30m, inner diameter 0.25mm, film thickness 0.25, column with the following settings: ~10.5 psi system pressure, 1ml/min helium, 250°C injection temperature and a temperature program of: 100°C initial temperature, hold for 2 minutes, increasing at 4°C/min until 310°C, hold for 4 minutes. A blank sample of DCM is used between samples to ensure little or no contamination remained on the column.

A number of operating conditions were altered from the conditions devised previously. The difference in the flow of helium should have no impact upon the results and was used at 1ml/min instead of 1.5ml/min as this was the standard flow rate used for analysis within this lab. The temperature limit for the GC and its components, such as the column connection valves, was 310°C, therefore the maximum temperature of the run was 310°C. The start temperature of the oven was increased to 100°C instead of 40°C due to the need to remove the solvent rapidly, which was the only compound eluted at that temperature due to the organics being exposed to a temperature of 70°C when the solvent was evaporated after ASE extraction, meaning that any VOCs of that temperature range were also driven off. The first 180 seconds of the elution was not measured using the MS (solvent delay) as during this period the elution of the solvent occurred, overwhelming the MS. A slower temperature increase profile was used, to improve the definition of the spectra, where the compounds have better separation. Qualitative analysis was conducted against NIST data.

Column flushing was an essential step between samples, as it was found free fatty acids were remaining on the column, causing contamination issues with the next sample to be run. Flushing with DCM removed any remaining sample from the column, leaving it uncontaminated for the next sample. This source of potential contamination was discovered prior to the qualitative and quantitative analysis used in this thesis, therefore these results are not affected by potential contamination from this source.

After qualitative analysis of the sludge on the Elite 5MS column a further column was used to qualitatively analyse the sludge. The chemicals that are aiming to be quantified

are esters and free fatty acids. Reviewing the methods in the 'Gas Chromatography' section in Chapter Three, there are a number which also analyse for fatty acids, however these methods need to convert the fatty acids to fatty acid methyl esters, which also convert the fatty acids that are bound within the esters to fatty acid methyl esters also, which would cause an overestimation of fatty acid concentration within the sludge, therefore these methods were not used.

A method to analyse cod oil fatty acids, which were underivatised was advised by SGE Analytical Science. This method focused upon the analysis of C14:0 to C24:0 free fatty acids. The method used a SGE BP21 FFAP column, which is a highly polar column with a phase comprised of Nitroterephthalic acid modified polyethylene glycol. This was designed to cause the polar fatty acids to better separate, producing a clearer spectra. The column was 30m in length, inner diameter of 0.25mm and film thickness of 0.25µm. The operating conditions include a pressure of 10.5psi and an initial temperature of 70°C, held for 0.5 minutes, increasing at 6°C/min up to 260°C, where it was held for 10 minutes. An injection temperature of 280°C and flow rate of 1ml/min was used (SGE, 2008).

A third column was available at the time of analysis, therefore to further reinforce the results found using the first two columns and methods an amines column was used with a general amines and phenols method. The column was a Restek RTX-5 Amine column, 30m in length, inner diameter of 0.25mm and film thickness of 1µm. The temperature profile started at 120°C , held for 2 minutes, increasing at 10°C/min until 310°C where it was held for 4 minutes. The flow rate was 1ml/min and injection temperature of 305°C (Restek, 2011). Flushing of the column with DCM between samples was used to limit contamination.

Analysis of the solids that have been filtered from the vial after extraction through the ASE could not be conducted by GC as they could not be suspended in a liquid solvent. If LC was available the organics would have been scraped from the filter paper, suspended in acetonitrile or methanol and analysed using HPLC following the operating conditions in the 'Liquid Chromatography' section of this Chapter. As LC was not available then headspace analysis on the GC was conducted using the following method: The filter paper and contents were placed into a headspace analysis vial and sealed. The sample

was then placed into the Perkin and Elmer Turbomatrix 40 Trap headspace analyser and run using these operating conditions: Injection volume of 1μL, initial temperature of 80°C, held for 1 minute, increasing at 10°C/min to 250°C, where it was held for 1 minute. The MS is run in the mass range of 300 El+ with a solvent delay of 115 seconds.

Quantitative analysis of the sludge was undertaken using the Elite 5-MS column, as this column produced the clearest spectra that included the greatest variation in chemicals found. The operating conditions of the GC and MS remained the same. The standards used in quantitative analysis were created using the method below.

Standards of the two main chemical compounds: palmitic acid and oleic acid were not available for purchase, therefore standards of these compounds were made in the lab using >99% purity oleic acid and palmitic acid. The palmitic acid standard was created by adding 0.1008g of palmitic acid to 10ml of DCM and allowing it to dissolve to create a concentration of 10.08g/L. The oleic acid standard was created by adding 0.1192g of oleic acid to 10ml of DCM and allowing it to dissolve. Diluted standards were created from these of 0.596, 1.192 and 2.980g/L for oleic acid and 0.4032, 1.008, 2.016g/L for palmitic acid, which were run through the GC-MS with peak integration to produce the calibration curves seen in Figure 9 and Figure 10. On the same day the sludge samples were also run so that any potential operational differences on the GC-MS were minimised. Solvent cleans were run in between each sample to minimise contamination.

Repetition of the GC-MS method should be conducted ten times for statistical validation and should be done to one standard deviation. The standards should be repeated three times to ensure they are correct and should use at least five different concentrations to generate the calibration curve.

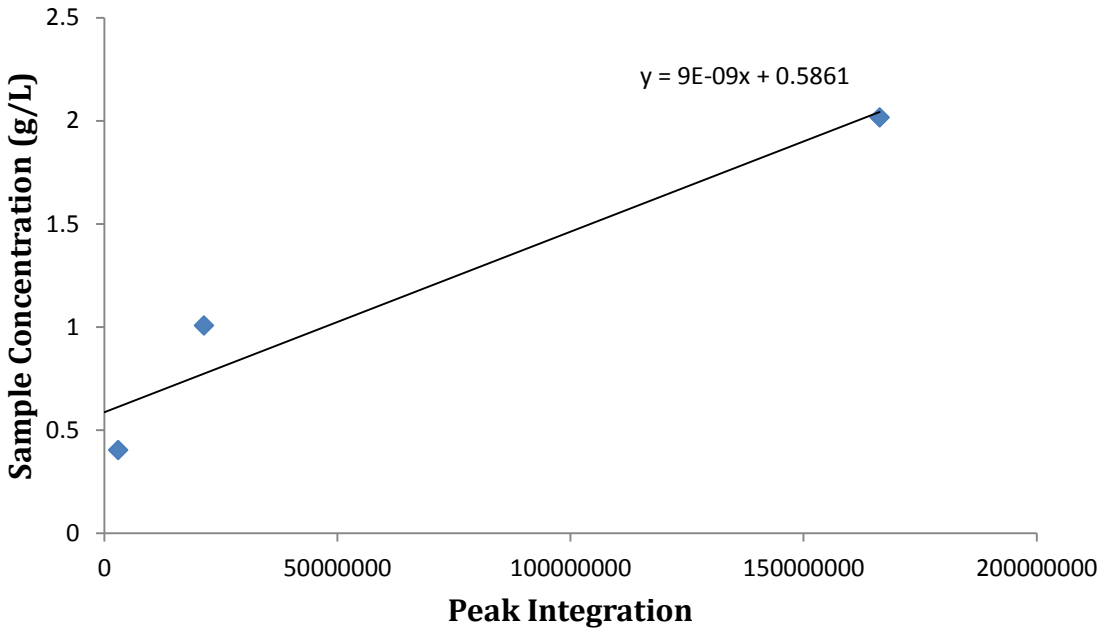


Figure 9 Calibration Curve for Palmitic Acid on the GC-MS

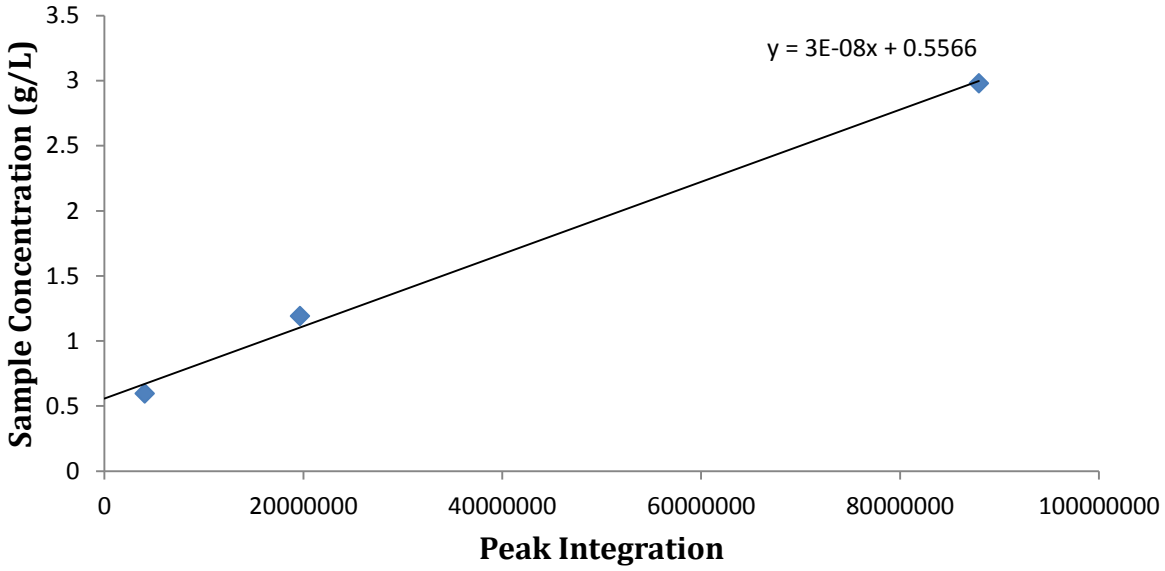


Figure 10 Calibration Curve for Oleic Acid on the GC-MS

The oleic acid calibration curve in Figure 10 has little deviation from the linear trendline, however its reliability as a calibration curve could be improved by using more samples with concentrations between 0.25-3.25g/L. The same statement stands for

palmitic acid in Figure 9, however there is greater deviation from the linear trendline. The accuracy of the trendline equation and reliability of the results could be questioned due to these findings. If the 2.016g/L peak integration was incorrect for palmitic acid then looking at the trend it would only be a higher peak integration, which would lead to higher concentrations of palmitic acid being calculated within the sludge. This means that the calibration curve for palmitic acid may underestimate the concentration of palmitic acid within the sludge.

Triglycerides are suspected to be in the sludge, however due to their high molecular mass and boiling point (851°C for trimethylolpropane trioleate) they do not elute from these columns using the methods above. Triglycerides are commonly analysed using GC, however they are only analysed up to T58 (number of carbon atoms) and this is at temperatures of around 360°C. Trimethylolpropane trioleate has 60 carbon atoms, therefore requires a higher temperature to be able to pass through the column, which the Perkin and Elmer Clarus 500 GC-MS cannot withstand. Analysis of triglycerides on the GC-MS is therefore not undertaken.

4.3.5 Liquid Chromatography

Liquid chromatography of the sludge was not undertaken due to the necessary equipment not being available. If it was available, the procedure described in Chapter Three would be undertaken.

4.3.6 Inductively Coupled Plasma

ICP-OES analysis was conducted on the sludge solutions that were prepared in the 'Ashing' and 'Digestion' sections of this Chapter. The ICP-OES that was used for analysis is a Perkin and Elmer Optima 2100DV with OES detector. All of the samples were run against certified 28 element standards, which were re-run every 10 samples to ensure accuracy of the machine.

Repetition of the microwave digestion and ICP-OES method should be conducted ten times for statistical validation and should be done to one standard deviation.

4.3.7 Fourier Transform Infra-Red Spectrophotometry

A small sample of homogenised sludge was placed directly upon the FTIR cell, ensuring that no air bubbles were between the cell and the sludge. Scans at a resolution of 4cm, wavenumber of 4000-400cm^{-1} and 100 cycles were conducted.

A Varian 3100 FTIR was used along with a Varian 600 UMA FTIR Microscope to analyse the 0.2ml samples of the sludge. The wavenumber was slightly wider at 6000-650cm^{-1}, scan resolution of 4cm and 100 cycles. The background spectrum was taken prior to every sample to remove its interference and between each sample the crystal was cleaned using ethanol, which was then evaporated.

4.3.8 Nuclear Magnetic Resonance

Nuclear magnetic resonance of the sludge was not undertaken due to the necessary equipment not being available. If it was available, the procedure described in Chapter Three would have been undertaken.

4.3.9 Carbon Content

Vacuum dehydrated sludge was used for carbon content analysis. TC was measured using 0.5g of dehydrated sludge. The crucible was pre-cleaned by heating to 900˚C in a muffle oven for 5 minutes. Once this was allowed to cool the sludge was added and the crucible placed into the furnace where it was heated to 1500˚C, with the gases being passed over a catalytic converter and past an IR detector to determine total CO_2 produced. TIC was measured by placing 0.5g of the dehydrated sludge into a clean crucible along with 5ml of 40% phosphoric acid. This was then heated to 200˚C and any CO_2 generated was measured using the IR detector.

Carbon analysis was carried out using a Shimadzu SSM-5000A solid sample module and Shimadzu TNM-1 detector. The Shimadzu used a pre-calibrated concentration curve that was checked against a glycerol standard for TC and against calcium carbonate standard for TIC prior to analysis of the sludge.

Repetition of the carbon content methods should be conducted ten times for statistical validation and should be done to one standard deviation.

4.4 Sludge Observations at the Factory

Sludge in various forms was apparent around the factory. Investigating exactly where the sludge was occurring provided an insight into its route of formation. The locations of its formation were; within dead spots of washing systems, in sumps and trenches that drain spills and leaks from the machines, filtered sludge from washing systems, filtered sludge from cutting systems, solid balls filtered from the chilled water system, within the waste oil storage tank overflow bund and finally within the wastewater storage tanks at the onsite wastewater treatment facility. Only visual analysis was carried out on these findings around the plant.

Biofilm appears to be growing in the dead spots of washing systems. It has a rubbery like consistency, with a green/grey colouring, shown in Figure 11 and a rancid odour. This type of sludge was also appearing in the swarf removal trenches beneath the factory floor, show in Figure 12.

Figure 11 Suspected Biofilm in a Washing System

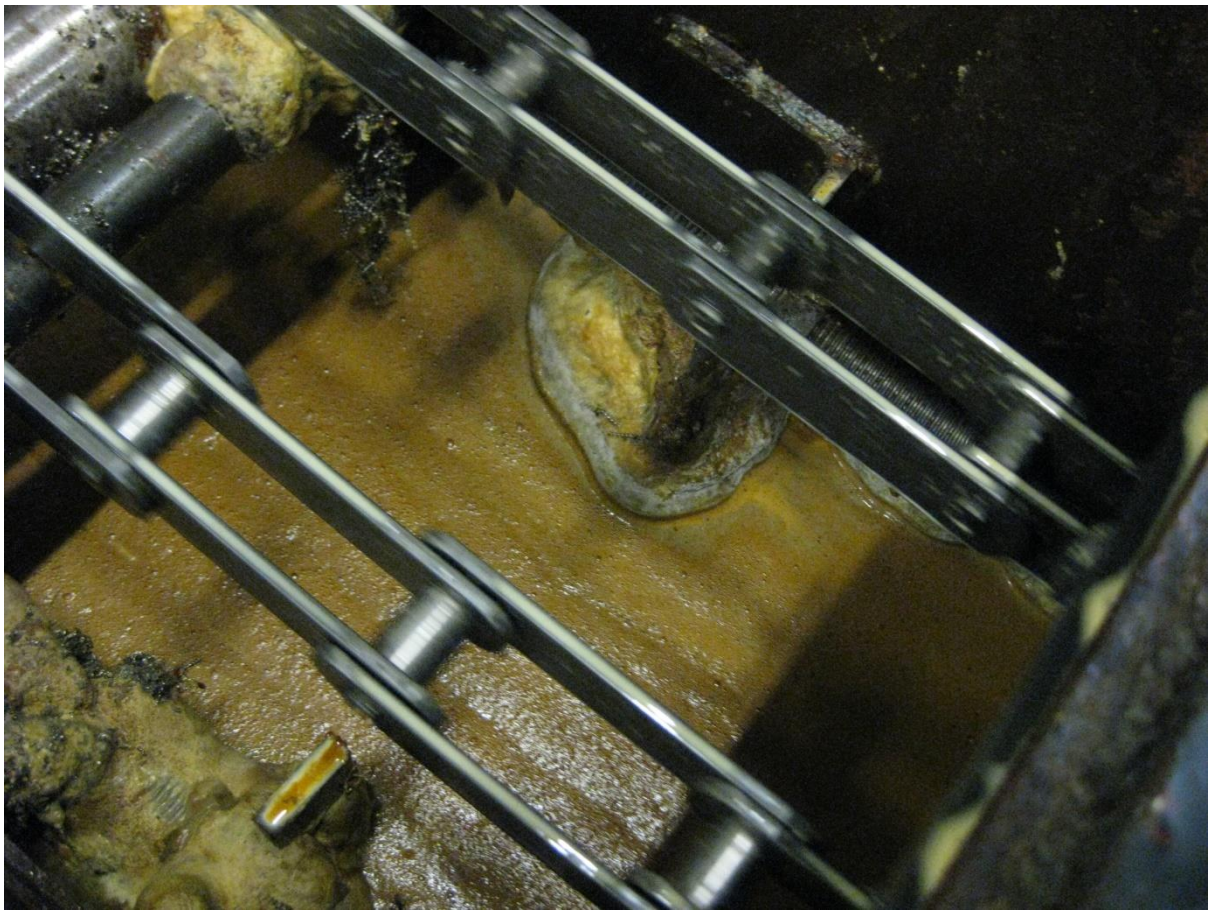


Figure 12 Suspected Biofilm in the Swarf Removal Trenches

Sludge was also present in sumps and trenches that drain leaks and spills from the machining and washing systems. The sumps were only fully emptied once per year, as they are pumped from near the bottom of the sump, therefore some fluid remains in the sump for up to one year. The sludge in the sumps, shown in Figure 13, was very similar to that found in the wastewater storage tanks.

Figure 13 Sludge within a Sump

An essential process within metal machining is the washing off of the cutting fluid from the products once they have been machined. This system requires constant filtration due to the contaminants that are washed into the fluid from the machined products. The filtration efficiency is enhanced by using cellulose powder, which is then filtered using a cloth filter and the sludge separated into a wagon, such as the one shown in Figure 14. This sludge is not transferred into the wastewater system, however it is similar to the sludge found at the wastewater storage tanks.

Figure 14 Sludge Filtered from a Washing System

Another filtration process was the filtration of swarf from the metal machining systems. This was achieved using similar cloth filters to those in the washing systems, but not using cellulose powder to aid in filtration as it is not needed. The sludge generated here was iron and aluminium rich, low in water and organic content and also did not get mixed into the wastewater, instead being disposed of into landfill.

Sludge was also in the waste oil storage tank bund. This had occurred due to the waste oil tank overflowing into the bund, which had then remained there for a number of years. The waste oil tank takes the oil that had been separated by ultrafiltration, which was then sent for further treatment or incineration. This sludge was virtually identical in physical characteristics to the sludge occurring in the wastewater storage tanks.

An interesting phenomenon found within the chilled water that cools the hydraulic oil in the systems, was the formation of large, solid, organically based balls, shown in Figure 15. They varied in diameter from 1cm up to 20cm and were accompanied by a

foul odour from the chilled water, which blocked the heat exchangers. It was found that several of the heat exchangers had been leaking vegetable oil-based hydraulic oil into the chilled water system, which was thought to lead to the formation of these balls.

Figure 15 A Sludge Ball Filtered from a Chilled Water System

The most prominent appearance of the sludge was within the wastewater storage tanks at the onsite wastewater treatment facility. It appeared as two types of sludge, with sludge 1 appearing in the rear tank and sludge 2 appearing in the fore tank of Figure 16. It was estimated that >99% of the sludge found at the metal machining factory was occurring within the confines of the wastewater treatment system.

Figure 16 Two Types of Sludge in the Wastewater Storage Tanks

The first appearance of sludge within the systems was where biofilm was suspected in the machining and washing systems, however the sludge in the wastewater storage tanks is not a biofilm. This gives the indication that the sludge may be biologically formed, but not formed of a biologically active membrane.

The next place of formation was the sumps and trenches around the machines, where the sludge was similar in appearance to the sludge in the tanks, rather than the biofilm. This was most likely due to the sumps being of a similar design to that of the tanks where the liquid was extracted from the base, thus allowing the sludge to remain floating on the surface.

The sludge filtered out using the cloth filters is thought to have little to do with the sludge in the wastewater treatment tanks as it was either mainly iron/aluminium, or formed due to addition of cellulose and subsequently filtered from the system.

Chilled water balls show that the sludge was derived from the vegetable based hydraulic oil as the only chemicals that enter those systems are water, hydraulic oil via plate heat exchanger leaks and trace quantities of anti-corrosion chemicals.

These observations point towards biological action that degrades the oils into chemicals that are solid at the temperatures experienced at the factory.

5.0 Results and Discussion

These experimental results were achieved by following the selected methods from Chapter Three and the newly created methods in Chapter Four. The sludge that was experimented on is from the wastewater settling tanks at the onsite wastewater treatment facility based within a metal machining factory in South Wales, UK.

5.1 Sampling & Storage

5.1.1 Sampling

The sludge, as can be seen in Figure 17, was occurring within settling tanks 2 – 4 of the effluent treatment system. It was also occurring in the rainwater tank, however this was due to the rainwater tank being used to store wastewater from the factory for a number of months, not from the rainwater itself. It was noted that the sludge within tank 4, Figure 18, which is also the tank that received the UF cleaning solution and overflow, had a higher viscosity and more clay like appearance than that of tanks 2, 3 and 5. The volume of the sludge was around 1000m^3, which was unevenly distributed between the five tanks. This volume may also contain settled solids that are not relevant to this thesis and is separate to the floating sludge seen in Figure 17 and Figure 18.

Figure 17 Access to 500m^3 Storage Tanks at the Metal Machining Factory, South Wales, UK

Figure 18 Higher Viscosity Sludge in Tank 4

Due to the significant differences seen between tank 4 and tanks 2, 3 and 5 it was decided that spot samples were to be taken from tank 4 and tank 5, with tank 5 representing the sludge found in tanks 2, 3 and 5. The sludge from tank 4 is known as 'Sludge 1' and from tank 5 is known as 'Sludge 2'.

Sampling of the sludge using the diagonal and grid methods would have offered the optimum conditions to ensure that the samples taken, when homogenised to provide a representative sample of the sludge within the tank. Due to access issues this was not possible and an alternative method was developed, which was more effective at getting an increased theoretical number of samples from within the tank by covering a larger surface area than would be covered using diagonal or grid methods. This technique cannot be directly compared to the grid or sampling methods due to the grid and sampling methods not being used, however based on the increased theoretical number of samples extracted when compared to the grid and diagonal methods it can be seen

that this is a far more efficient and representative method. The only drawback is that samples cannot be extracted from deeper than 20cm, which could be done using the grid and diagonal methods, however it would require the installation of scaffolding, therefore greatly increasing the cost and complexity of sampling.

The composition of the sludge may vary at different depths due to the sludge being up to four metres deep. As the sampling was not conducted at intervals across this depth then the results may be biased towards the type of sludge forming at the top of the layer, if it is any different to that lower within the layer. This cannot be confirmed or ruled out as no sludge was extracted from the lower depths. Also as the sludge was only sampled from two different tanks then the physicochemical composition may be only occurring within these two tanks.

Samples extracted on this date, time and weather conditions are not expected to have a physicochemical difference from samples extracted at any other date and time as the sludge has been present in its current state for six years and only changes its viscosity at different temperatures.

5.1.2 Storage

Storage of the sludge proved to be of little difficulty, with no foul odours escaping when the lids were sealed. It is deemed that due to the sludge being in the tanks undisturbed for six years that any major degradation that was going to occur to the sludge had already occurred within the tank and that no further degradation would occur whilst the samples were being stored at 4°C before being processed for analysis.

A visual inspection of sludge that had been stored in a pre-cleaned, air tight plastic bucket that was kept in a fridge at 4°C revealed that there had been no change to the samples over a two year period. The samples had not dried, nor lost their ability to flow, therefore storage in these conditions for two weeks should not cause any alteration in the chemical make-up of the sludge.

5.2 Sample Preparation

5.2.1 Water Separation

Following the amended solvent separation technique and in comparison to the Dean and Stark method the sludge was separated producing the results detailed in Table 2, Table 3 and Table 4. The mass of water from the solvent separation technique was determined by deducting the mass of organic soluble sludge from the initial sludge mass.

Calculation to one standard deviation of the average water content of the sludge is ±0.90% for sludge 1 and ±1.53% for sludge 2. For the solvent soluble sludge it is ±0.90% and ±1.53% for sludge 1 and sludge 2 respectively based upon three repetitions only. This means that there may be a greater deviation of the concentration of water within the sludge than measured in these three samples.

Table 2 Solvent Separation of Sludge Compared to the Dean and Stark Method

Repetition	Sample	Solvent Separation Sludge Initial Mass (g)	Dean and Stark Sludge Initial Mass (g)	Solvent Separation Water Mass (g)	Dean and Stark Water Mass (g)	Solvent Extraction Solvent Soluble Sludge Mass (g)	Dean and Stark Solvent Soluble Sludge Mass (g)
1	Sludge 1	24.87	51.20	7.62	15.39	17.25	35.46
	Sludge 2	24.57	49.46	8.54	18.22	16.03	31.04
2	Sludge 1	25.02	49.71	7.83	15.12	17.19	33.98
	Sludge 2	25.15	52.12	8.61	17.08	16.54	34.99
3	Sludge 1	25.85	50.86	7.53	14.22	18.32	36.12
	Sludge 2	25.80	49.30	9.73	19.00	16.07	29.87

Table 3 Solvent Separation of Sludge Compared to the Dean and Stark Method - Percentages

Repetition	Sample	Change in Mass After Solvent Separation (g)	Change in Mass After Dean and Stark (g)	Water Content Solvent Separation (%)	Water Content Dean and Stark (%)	Solvent Soluble Organics Solvent Separation (%)	Solvent Soluble Organics Dean and Stark (%)
1	Sludge 1	N/A	-0.68	30.64	30.27	69.36	69.73
	Sludge 2	N/A	-0.40	34.76	36.99	65.24	63.01
2	Sludge 1	N/A	-1.23	31.29	30.79	68.71	69.21
	Sludge 2	N/A	-0.10	34.23	32.80	65.77	67.20
3	Sludge 1	N/A	-1.02	29.13	28.25	70.87	71.75
	Sludge 2	N/A	-0.87	37.71	38.88	62.29	61.12

Table 4 Solvent Separation of Sludge Compared to the Dean and Stark Method - Averages

Sample	Change in Mass After Solvent Separation (g)	Change in Mass After Dean and Stark (g)	Water Content Solvent Separation (%)	Water Content Dean and Stark (%)	Solvent Soluble Organics Solvent Separation (%)	Solvent Soluble Organics Dean and Stark (%)
Sludge 1	N/A	-0.98	30.35 (±0.90%)	29.77	69.65 (±0.90%)	70.23
Sludge 2	N/A	-0.46	35.57 (±1.53%)	36.22	64.43 (±1.53%)	63.78

The results in Table 2, Table 3 and Table 4 show that there is a loss in mass of the sludge or water after it has been extracted by the Dean and Stark method. It cannot be distinguished whether it is water, or solvent extractable organics that are lost. It also cannot be shown at which point this mass was lost, whether it was during the physical extraction or during the evaporation of the solvent by heated block afterwards. It is possible that it may be a loss of VOCs, an inaccuracy of measurement, or human error. Due to the loss being 1% or less of the total mass of the sample and similar results occurring in the Dean and Stark method it can be said that this technique does not cause significant losses to the sludge.

The range for water content of the sludge by solvent separation is 3.48% at its greatest for sludge 2, whilst the Dean and Stark method has a range of 6.08% for water content, also in sludge 2. This shows that the solvent separation technique has greater precision than the Dean and Stark method. It can be said that the solvent separation technique for separating water from floating oil-based sludge is a success, accurate and can be easily replicated.

As for the water and soluble organic content of the sludge it is seen that sludge 1, the higher viscosity sludge has a lower water content of an average 30.35%, whilst the water content of the lower viscosity sludge 2 is on average 35.57%, both of which are within 0.65% of the Dean and Stark figure, showing that the water content from the solvent separation technique is more than likely correct.

Difficulties were encountered in the solvent separation of the sludge using hexane due to its density being less than that of water, therefore not allowing the development of a clear transition layer between the solvent solution and water. DCM replaced the hexane as the solvent, which allowed the solvent solution to settle beneath the water, creating a distinguishable boundary layer, allowing the water to be successfully separated from the sludge. Although DCM has a polarity nearer to water than that of hexane it still left analytes in the water phase, which are assumed to be inherent within the water phase. This was indirectly determined using ICP-OES analysis. Comparison to the Dean and Stark method showed little difference between the results, even though the Dean and Stark method used hexane in addition to DCM. This shows that the solvent separation technique developed in this thesis is as effective at separating water as the Dean and

Stark method, which is commonly used, reliable and accurate. The ICP-OES results derived from the separation of the water are a very important part of characterisation of the sludge. Other authors may have under-estimated the importance of this step, instead choosing to remove the water by dehydration, therefore leaving the analytes that are inherent within the water, now within the organic portion of the sludge. This may lead to false conclusions about the chemical composition of their sludge.

5.2.2 Organics Extraction

Processing of the organics through the ASE posed no problems that were not overcome. The vials used to dehydrate the samples were pre-weighed and re-weighed after the samples had been fully dehydrated on the heated block, which produced the results in Table 5. This was repeated three times for each sludge. One standard deviation for the ASE non-extractable solids is ±0.41% for sludge 1 and ± 0.28% for sludge 2. The mass of organics on the filter has an error of ±0.03% and ±0.01% for sludge 1 and 2 respectively based on three extractions only. This means that there may be a greater deviation of the concentration of solvent soluble organics and solids within the sludge than measured in these three samples.

Table 5 ASE Extraction of Dehydrated Sludge

Repetition	Sample	Mass of Sludge Before ASE (g)	Mass of Organics After ASE (g)	ASE Non-Extractable Solids (g) (% dehydrated sludge)
1	Sludge 1	2.40	2.32	0.08 (3.3)
	Sludge 2	2.20	2.15	0.05 (2.3)
2	Sludge 1	2.22	2.16	0.06 (2.7)
	Sludge 2	2.43	2.39	0.04 (1.6)
3	Sludge 1	2.18	2.10	0.08 (3.7)
	Sludge 2	2.27	2.23	0.04 (1.9)

Table 6 Filtration of ASE Extracted Dehydrated Sludge

Repetition	Sample	Mass of Organics in Vial (g)	Mass of Organics Remaining in Vial (g)	Mass of Organics on Filter (g) (% of vial organics)
1	Sludge 1	0.6477	0.6430	0.0047 (0.73)
	Sludge 2	0.8768	0.8671	0.0097 (1.11)
2	Sludge 1	0.6832	0.6785	0.0047 (0.69)
	Sludge 2	0.8243	0.8153	0.0090 (1.09)
3	Sludge 1	0.6642	0.6591	0.0051 (0.77)
	Sludge 2	0.8458	0.8363	0.0095 (1.12)

The average ASE non-extractable solids are 3.2% (±0.4%) for sludge 1 and 1.9% (±0.3%) for sludge 2. It is assumed that there is minimal loss of VOCs from the ASE as it is a sealed unit and little or no VOCs should remain in the sludge after it has remained in the tanks for six years. It is therefore assumed that all of the ASE non-extractable solids are filtered solids that were in the sludge, such as analytes and larger solids.

The mass of organics filtered from the ASE extracted solution is lower than the ASE non-extractable solids. On average the solids on the filter are 0.73% (±0.03%) and 1.11% (±0.01%) of the mass of the organics within the vial for sludge 1 and 2 respectively. These were further analysed to determine their composition, which will hopefully explain why they passed through the ASE but would not re-dissolve in the solvent. It is possible that drying at 105°C for one hour not only caused the solvent to evaporate but may have altered the chemicals that were filtered, causing them to lose VOCs, or even oxidise and gain mass.

Extraction of the organics using ASE was simple to achieve, including the mass balance on the process so an inexperienced analyst should have no issues in undertaking this procedure. The main issue with this extraction and re-dissolving technique is the generation of an insoluble layer of organics on top of the solvent solution after extraction using the ASE. Although this insoluble layer accounts for less than 1.2% of the solids that are dissolved in the solvent it still requires careful filtration to be

undertaken. It is possible that the dehydration of the organic solvent and its subsequent re-suspension is the cause of the insoluble layer problem, but this is unlikely and is a necessary stage in quantifying the concentration of chemicals within the sludge, so therefore cannot be avoided. The ASE filtered up to 3.3% of the mass of the organics, which would be anything over 0.22μm in size that were not soluble at the high temperatures and pressures used by the ASE. This also aids in the deduction of the chemical composition of the insoluble layer of organics that passed through the ASE. Overall the ASE was successful in preparing the sludge into a form that was analysed using chromatography, with a loss of up to 4.4% of the total mas of the sludge that was extracted using the ASE, which is minimal and should not majorly affect the results.

5.2.3 Digestion

Digestion of the sludge was simple as this was undertaken by an experienced analyst. It is not suitable to be undertaken by an inexperienced analyst as the chemicals and method used has the potential to cause problems whilst being digested. The only issue with digestion is that the sludge is not fully digested and an estimated 10% or less remains as stuck to the sides of the vial, which is not a great problem as long as these remain stuck to the vials and do not enter the ICP.

The procedure was repeated ten times for statistical analysis, which is detailed in the ICP-OES section of this chapter.

5.2.4 Ashing

The ashing process reduced the mass of the sludge to that shown in Table 7, which acts as part of the conversion factor to turn the results to mg/kg of untreated sludge.

Table 7 Ashing of Solvent Extracted Sludge

Sample	Mass of Sludge in Crucible (inc Water) (g)	Mass of Ash in Crucible (g)	Ash: Sludge Ratio
Sludge 1	14.9334	0.1502	99.4
Sludge 2	25.4804	0.4039	63.1
Solvent Separated Sludge 1	11.1326	0.0575	193.6
Solvent Separated Sludge 2	6.8095	0.2081	32.7

Calculation of analytes within the sludge requires that the ICP results be converted to mg/kg of sludge from mg/L ICP results. This is done by producing the ratio of ash generated to sludge incinerated, as is seen in Table 7. Sludge 1 has a ratio of 99.4, whilst sludge 2 is far lower at 63.1. This higher mass after ashing for sludge 2 may be due to incomplete combustion, although this is unlikely after 16 hours at 500°C. It may be possible that there is a higher concentration of inorganics within sludge 2.

Ashing of the sludge was not as simple as digestion, with significant safety issues arising from heating of the sludge to 500°C. The first is the auto-ignition of the sludge, which could occur if the muffle oven is opened during the procedure. Another issue is the generation of black smoke, which has the potential to set any smoke detectors off in the room. The final safety issue is the boiling of acids, which is not a simple task to undertake for an inexperienced analyst as acids have the potential to pop and spit, launching acid outside of the boiling flask. The solution that was produced for analysis using ICP was clear and appeared to be homogeneous, with no issues of residual organics seen in the digestion technique. Due to these significant safety issues it would not be advisable for an inexperienced analyst to undertake ashing of the sludge and

digestion would be far more suitable if undertaken by an experienced analyst. This is also on the basis that good, reliable and accurate results are measured on the ICP for both preparation techniques.

Ashing of the sludge was not repeated for statistical analysis due to the danger of the procedure. This may mean that the ICP-OES results of the ashing could potentially have a great deviation from the actual concentrations found within the sludge.

5.3 Analysis

5.3.1 Non-Lipid Solids Determination

Processing of the non-lipid solids was straight forward and no problems were encountered with the method, which is far simpler than the method suggested for non-lipid solids determination in the previous Chapter. This method produced the results in Table 5, where the non-lipid solids are called ASE non-extractable solids.

The non-lipid solids account for a low portion of the sludge at only 3.2% (±19%) and 1.9% (±15%) of the dehydrated sludge by mass for sludge 1 and sludge 2 respectively. This cannot be extracted from the ASE cell for further analysis as the mixture with the anhydrous sodium sulphate and filter is too complex to separate. Error was calculated using only three repetitions, therefore the deviation in the non-lipid solids may be greater than calculated.

Determination of non-lipid solids is a by-product of the mass balance generated when using ASE, therefore is simple to undertake. Using the high temperatures and pressures that ASE requires no organics should remain undissolved, therefore any components that remains within the ASE cell should be non-lipid solids. The portion of the sludge remaining in the cell after it has been extracted may be too large to pass through the filter, but still may be an organic, such as a root, stick or feather. It may be better to call the non-lipid solids by another name that can include these items, such as 'non-lipid solids, including solids over 0.2μm' as this is the filter pore size. These solids that are too large to pass the filter will not contribute to the structure of the sludge as a semi-solid, therefore need not be included in the analysis as significant factors.

5.3.2 Melting Point

The adapted Thiele tube method was simple and successful to undertake, producing the results detailed in Table 8.

One standard deviation for the initial melting point is ±1.2°C for sludge 1 and ±0.5°C for sludge 2, whilst the error of the full melting point is ±0.0°C and ±0.8°C for sludge 1 and sludge 2 respectively. As the method was only repeated three times the deviation in melting point temperature may be greater than calculated.

Table 8 Melting Range of Sludge

Repetition	Sample	Initial Melting Point (°C)	Full Melting Point (°C)
1	Sludge 1	40	46
	Sludge 2	60	65
2	Sludge 1	42	46
	Sludge 2	59	64
3	Sludge 1	39	46
	Sludge 2	60	66

This adapted method proved to be successful and the melting ranges above were determined, however the results of this experiment cannot be compared to the standard Thiele tube method of melting point range determination for sludge. It is therefore not known if these results are fully accurate, however they should not be far from the melting points that would be determined using a Thiele tube.

The initial melting points for sludge 1 are in the range 39-42°C (±1.2°C), with the full melting point being stable at 46°C (±0.0°C). Sludge 2 has an initial melting point of 59-60°C (±0.5°C) and full melting point of 64-66°C (±0.8°C). None of these have a wide range of values, therefore this test is deemed to be accurate. The changes in melting temperatures seen between the two samples of sludge are most likely due to the difference in chemical composition of the samples.

Qualitative GC-MS analysis generated a list of chemicals that are found within the sludge. Table 9 lists these chemicals and their melting points, with the two chemicals in italics at the bottom being suspected of existing within the sludge.

Table 9 Melting Temperatures of the Compounds Discovered Using GC-MS

Chemical Compound	Melting Point (°C)
Cyclohexanamine, n-cyclohexyl- (Dicyclohexylamine)	-2
Pentadecane	8-10
Hexadecane	18
Tetradecanoic acid (Myristic acid)	52-54
Octadecane	28-30
Pentadecane, 7-methyl-	UNKNOWN
N-hexadecanoic acid (Palmitic acid)	63
Oleic Acid	14
Octadecanoic acid (Stearic acid)	69
Palmitic anhydride	61-64
Hexadecanoic acid, 2-methylpropyl ester	UNKNOWN
Decyl oleate	UNKNOWN
9, 12-octadecadienoic acid (Z,Z)-	-5
9-hexadecanoic acid	0
Heptadecane	21-22
Octadecane	28-30
Nonadecane	32-34
Calcium palmitate	*Decomposes above 155*
Calcium oleate	*84*

Around half of the compounds are solids at 12°C, including the compounds of palmitic and oleic acid, which have the largest peak areas of any of the identified compounds. These results start to indicate towards why the sludge is a semi-solid, in so much as some of the components within the sludge are solids, whilst others are liquids at 12°C.

As is seen the melting point of the sludge is far lower than that of calcium palmitate and still lower than that of calcium oleate. Palmitic acid is of a similar melting point, whilst

oleic acid is lower. Due to the sludge being composed of a number of chemical compounds a decrease in melting point temperature of several degrees was seen (Criddle and Ellis, 1980). This means that melting point as a sole analysis cannot be used to chemically identify the sludge, instead it must be combined in analysis with the results from all of the tests.

5.3.3 Microscopic & Biological

Sampling using the dipslide was quick and simple. Due to the adherence of the sludge a small amount stuck to the sides of the dipslide, though this was less than 1% of the total area of the dipslide so should not affect the results, shown in Table 10.

Analysis with the microscope using phase contrast microscopy and reflected light showed an amorphous grey mass with no distinguishing features, however the use of light transmitted though the sample showed up more detail as can be seen in Figure 19, Figure 20 and Figure 21.

Table 10 Microbiological Activity of the Sludge

Repetition	Sample	Bacteria/Yeast Count	Mould Count
1	Sludge 1	10^5 (moderate growth)	0.4 (slight)
	Sludge 2	10^5 (moderate growth)	0.4 (slight)
2	Sludge 1	10^5 (moderate growth)	0.4 (slight)
	Sludge 2	10^5 (moderate growth)	0.4 (slight)
3	Sludge 1	10^5 (moderate growth)	0.4 (slight)
	Sludge 2	10^5 (moderate growth)	0.4 (slight)

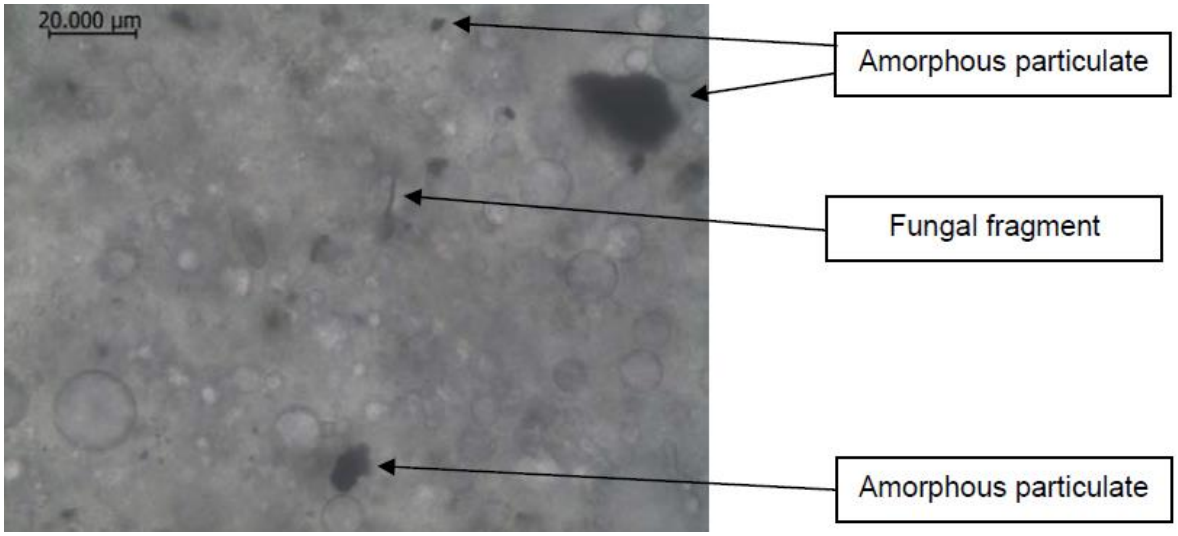


Figure 19 Microscopic Image of Emulsion within Sludge 2 (ECHA, 2012)

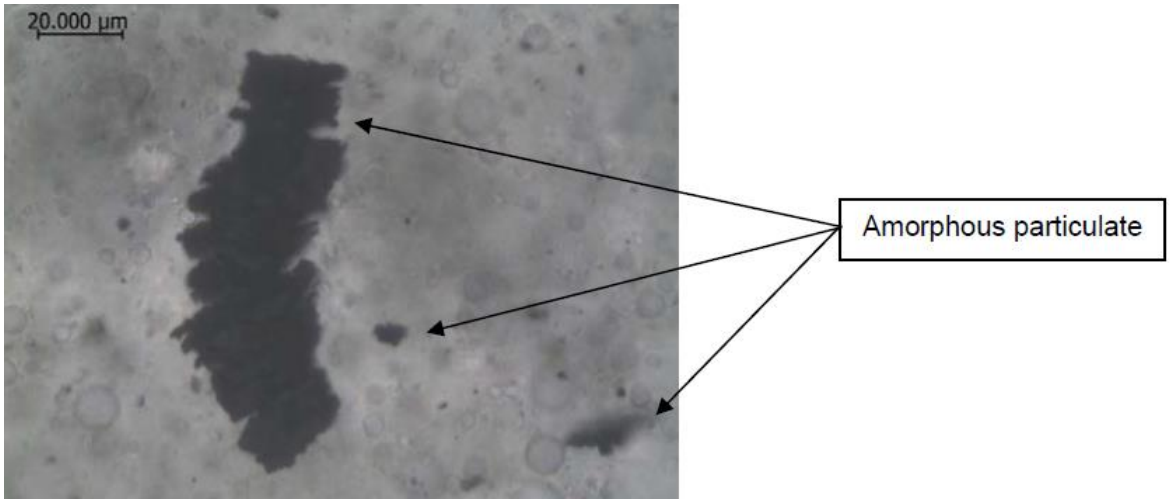


Figure 20 Microscopic Image of Amorphous Particulate in Sludge 2 (ECHA, 2012)

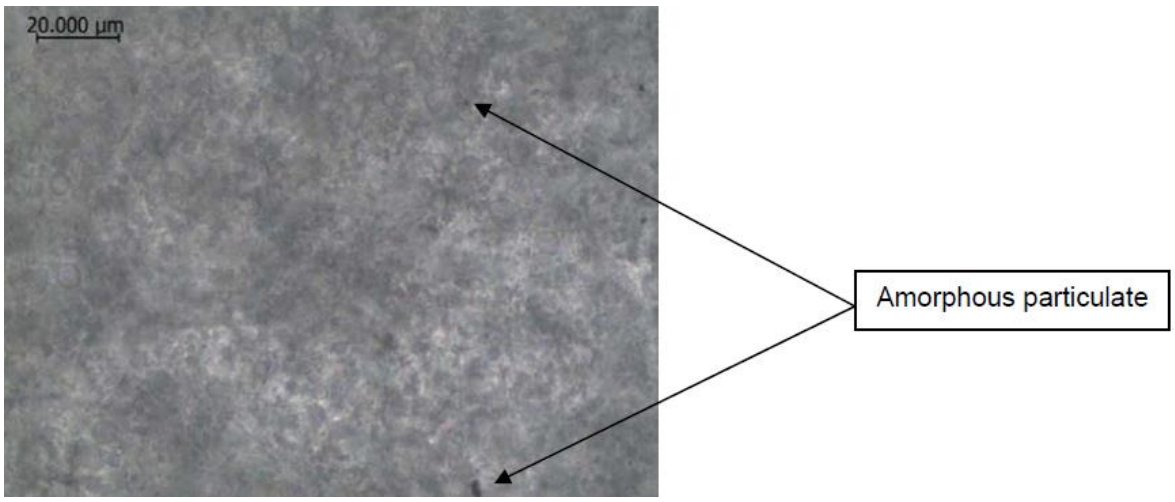


Figure 21 Microscopic Image of Sludge 1 (ECHA, 2012)

Dipslides and advice from an external company that specialises in microbiology proved simple to undertake and provided meaningful results to an analyst without a microbiological background. Although it may be costly, the analysis from a competent specialist may prove invaluable in identification of the sludge, or potential routes of formation.

The dipslide results differed slightly when reading, but not enough to fall into the next band of counting for the results detailed in Table 10. This shows that this test is

accurate. There is a moderate growth of bacteria, but not enough to conclude that the sludge is formed of, or a biofilm of bacteria/yeast. The mould count was slight.

Advice on the composition of the sludge was obtained from an external lab that specialises in microbiology, which was of benefit in identification of the sludge. The consultants examined the sample under the microscope and concluded that "Direct microscopy indicated that the sludge samples were not generated by the activity of microorganisms; they predominantly consisted of an emulsion of spherical liquid droplets, typical in appearance to an oil emulsion. We noted the presence of a very small amount of microbial biomass, namely some fungal fragments. We also noted presence of amorphous particulate" (ECHA, 2012). The full report is documented in Appendix B.

Microscopic visualisation of the sludge using top down lighting provided no great insight into the structure of the sludge, however using bottom up illumination of the sludge produced the images in Figure 19, Figure 20 and Figure 21, which show that the sludge is an emulsion and has varying sizes of amorphous particles embedded within it. It is not known if it is an emulsion of water in oil, or oil in water, however due to the water content being around 30% of the total mass of the sludge, with the majority of the remaining 70% being comprised of organics, it is most likely that it is an emulsion of water in oil. Staining of the water or oil within the emulsion would show if it was an emulsion of oil in water, or water in oil, which may help in understanding why it is a semi-solid. This was not undertaken, but may prove useful in future studies. The emulsion used within the metal machining systems, which is comprised of around 5% cutting oil, 5% surfactant and 90% water. It is seen that there has been a separation of the water from the cutting oil and surfactant, which can be caused by degradation of the surfactant. This would allow the oil with its lower density to float to the surface of the water, forming the floating sludge seen in the wastewater treatment tanks. Also within the structure of the sludge amorphous particulates are seen, which could be anything, but are most likely to be swarf that has been entrained into the sludge. Most swarf, owing to its density sinks to the bottom of the settling tanks, however due to the adherent properties of the sludge some swarf is trapped within it.

5.3.4 Gas Chromatography

Analysis using the Perkin and Elmer Elite 5MS column produced the spectra seen in Figure 22 and Figure 23, where the compounds are identified by comparison against the NIST database of compounds.

Initial analysis of the spectra revealed that the two main components of the sludge were oleic acid and n-hexadecanoic acid (commonly known as palmitic acid), with minor other components also present. Due to these chemicals being discovered it was decided to use a column suited to the identification of fatty acids, the SGE BP21 FFAP column.

Analysis of the sludge using the SGE BP21 FFAP column produced the spectra seen in Figure 24 and Figure 25, which show that once again the two main compounds are palmitic acid and oleic acid, although the oleic acid peak for sludge 1 is far less than that of sludge 2. Other fatty acids are also present, although their peaks are relatively minor. A Restek RTX-5 Amine column was available, therefore it was decided to analyse the samples on this column as well.

Analysis using the Restek RTX-5 Amine column produced the spectra shown in Figure 26 and Figure 27, which also showed the largest peaks as belonging to palmitic acid and oleic acid. The only problem that occurred whilst analysing the sludge on the RTX-5 column was an inadequate solvent delay on the MS (highlighted in blue on the spectra), which if run again would be extended to 300 seconds. Due to the use of an amine column the cyclohexanamine, n-cyclohexyl- peak is also prominent alongside palmitic acid and oleic acid peaks.

Headspace analysis of the filter paper with filtered organics produced the spectra seen in Figure 28 and Figure 29. During the analysis the filter blackened and smoke was produced in the vial. This is thought to be due to the oxidation of the filter and the filtered organics, which is not desirable as this would cause the chemical composition of the organic filtered solids to change. Future tests using the headspace analyser would involve using lower temperatures, or scraping the filtered organics from the filter paper into the vial for analysis.

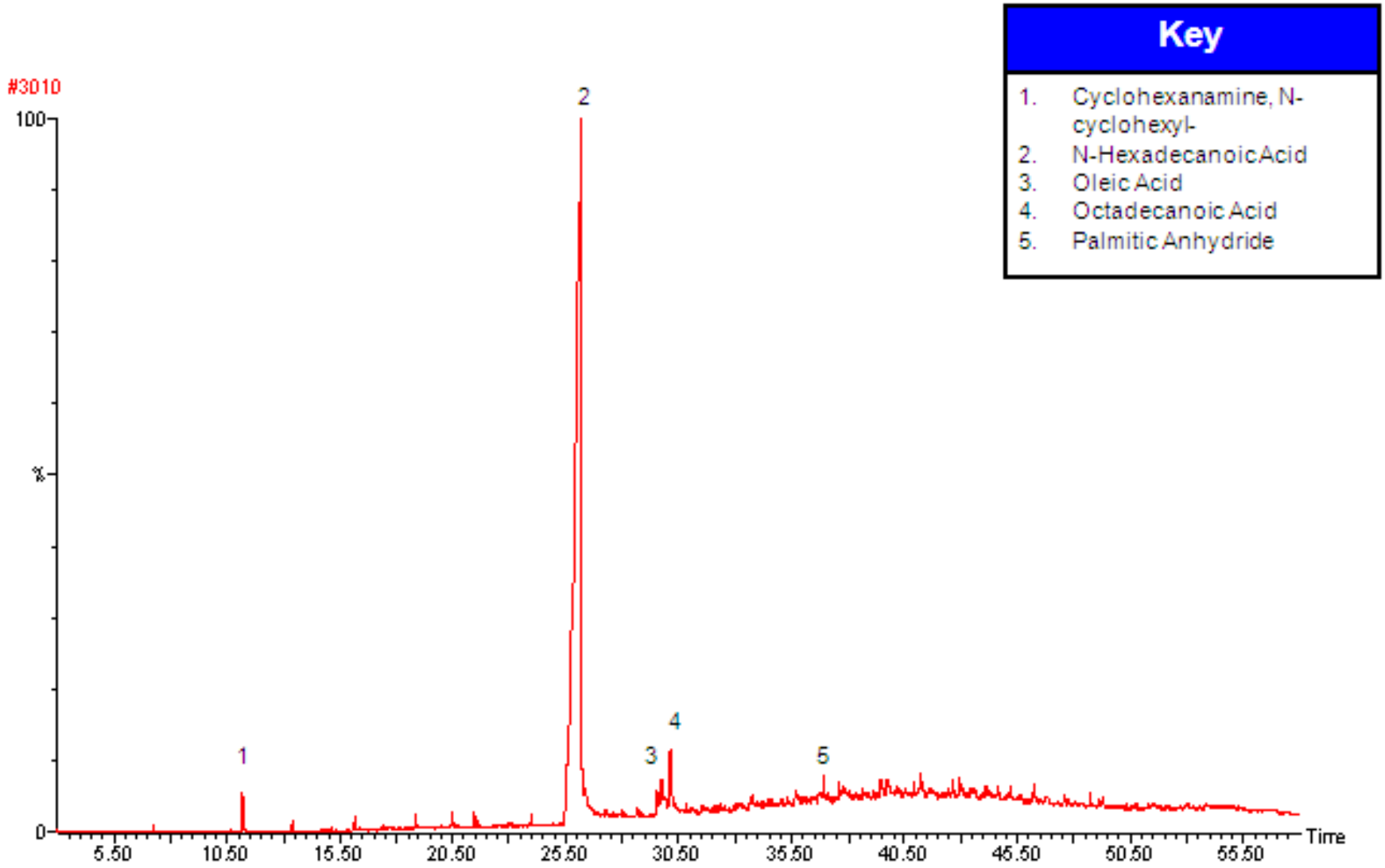


Figure 22 GC-MS Spectra of Sludge 1 Using Elite 5MS Column

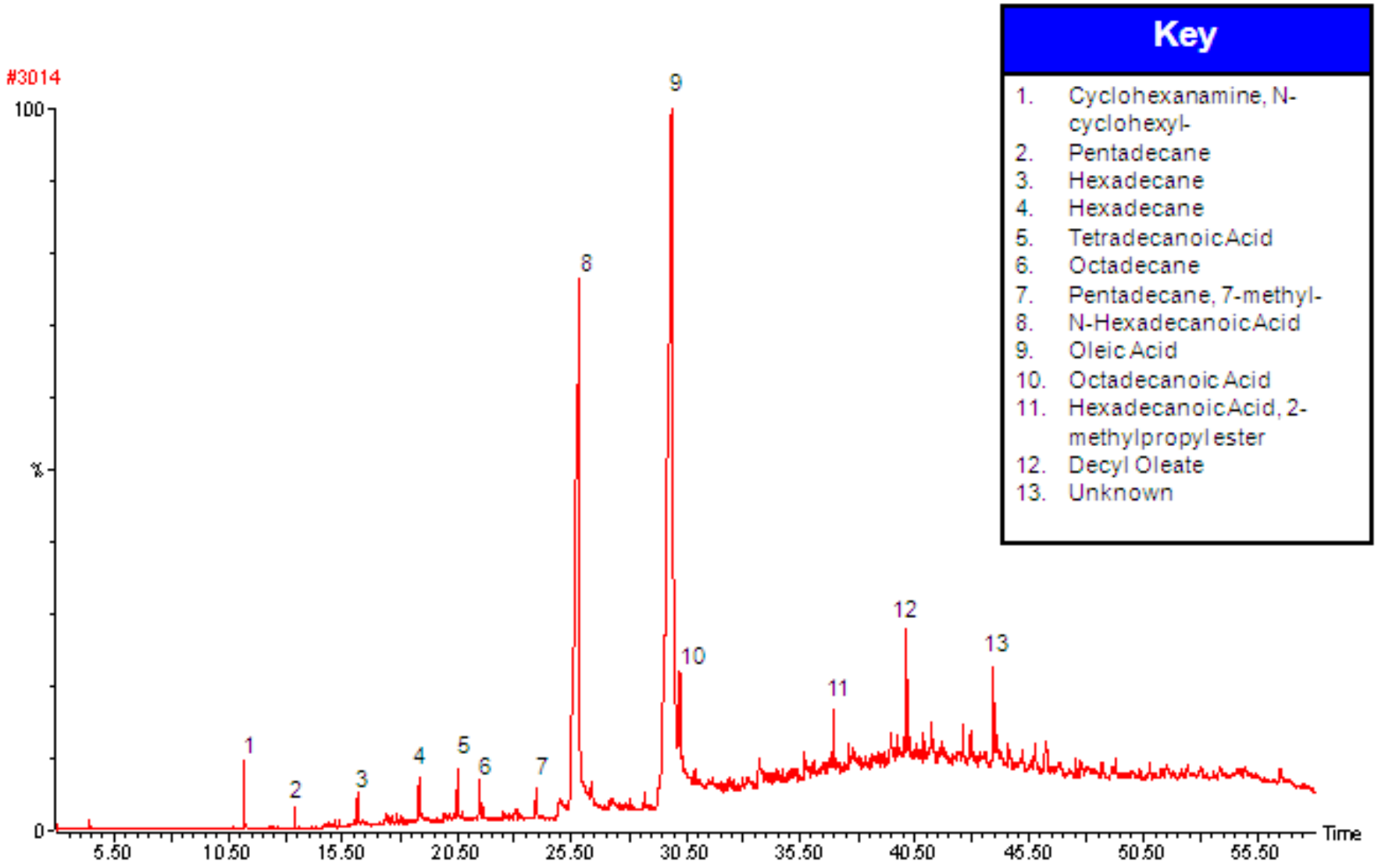


Figure 23 GC-MS Spectra of Sludge 2 Using Elite 5MS Column

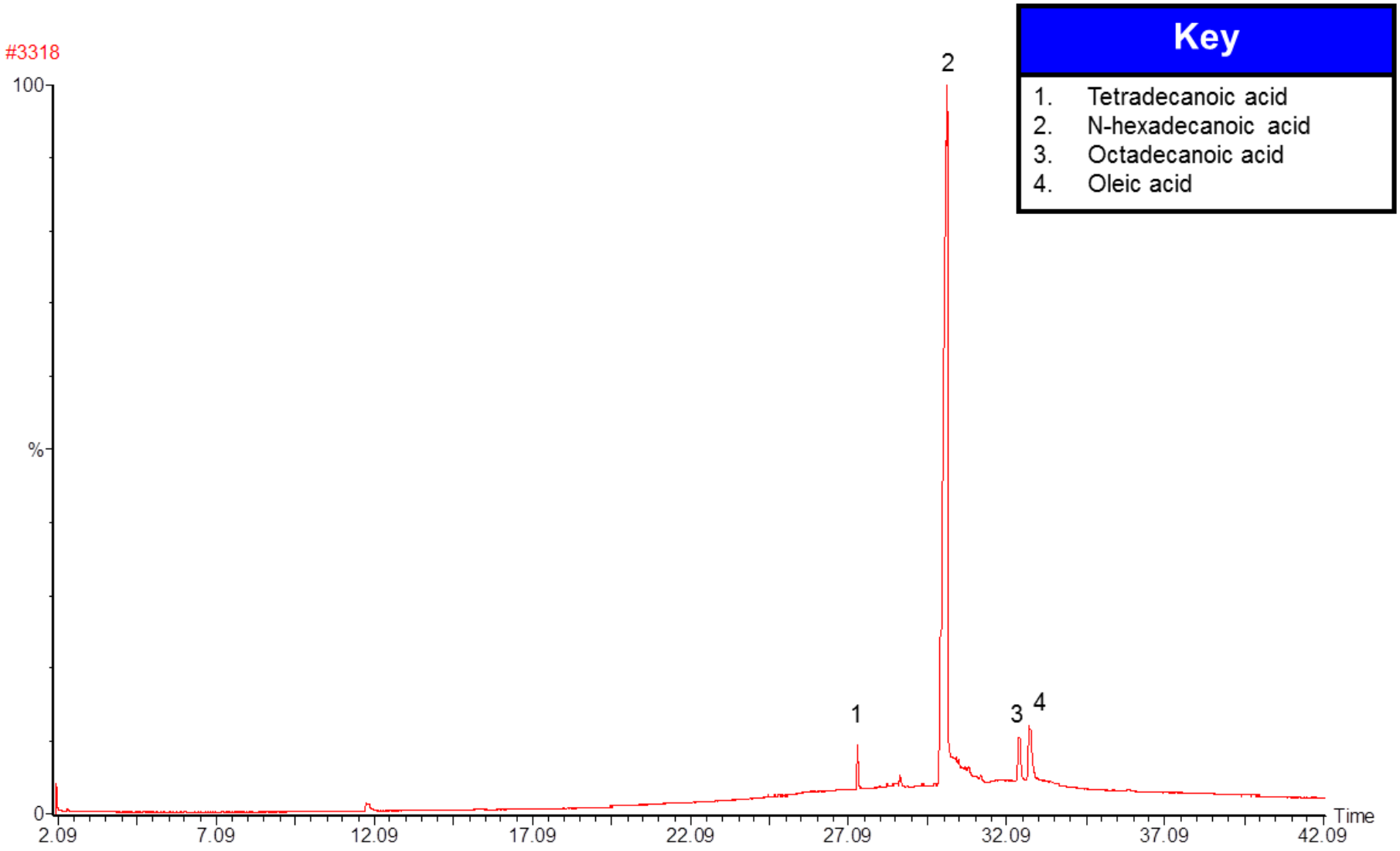


Figure 24 GC-MS Spectra of Sludge 1 Using BP21 Column

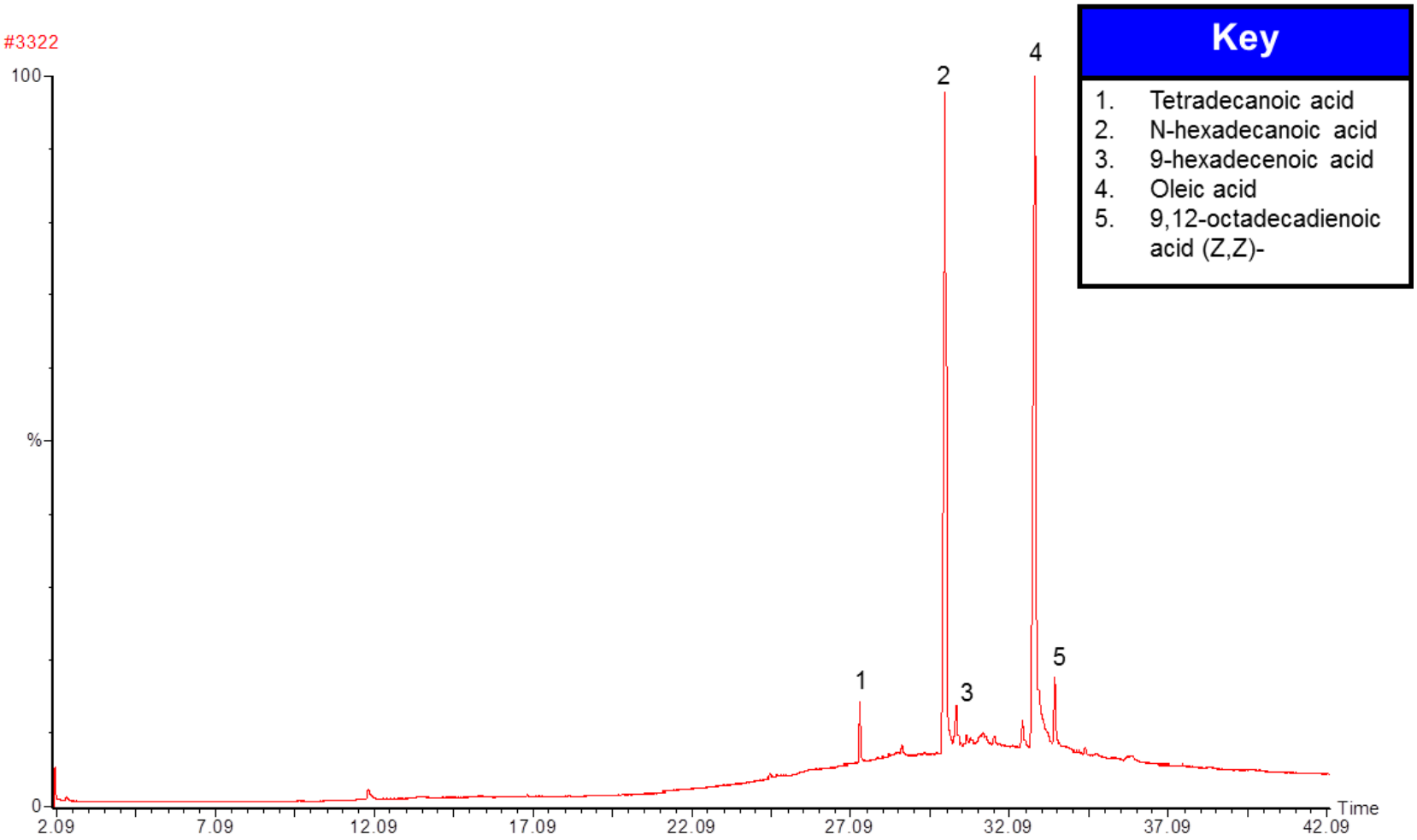


Figure 25 GC-MS Spectra of Sludge 2 Using BP21 Column

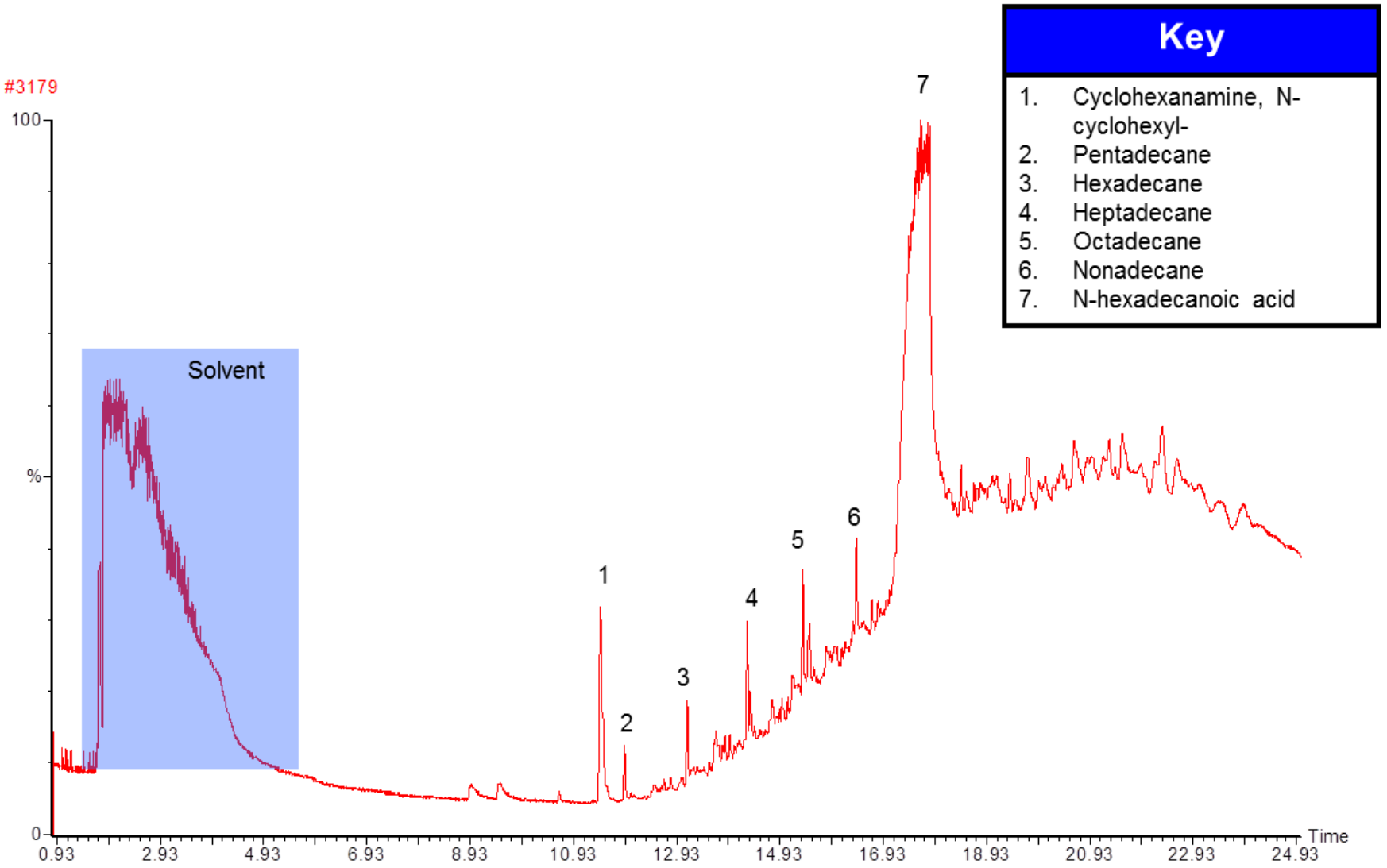


Figure 26 GC-MS Spectra of Sludge 1 Using RTX-5 Column

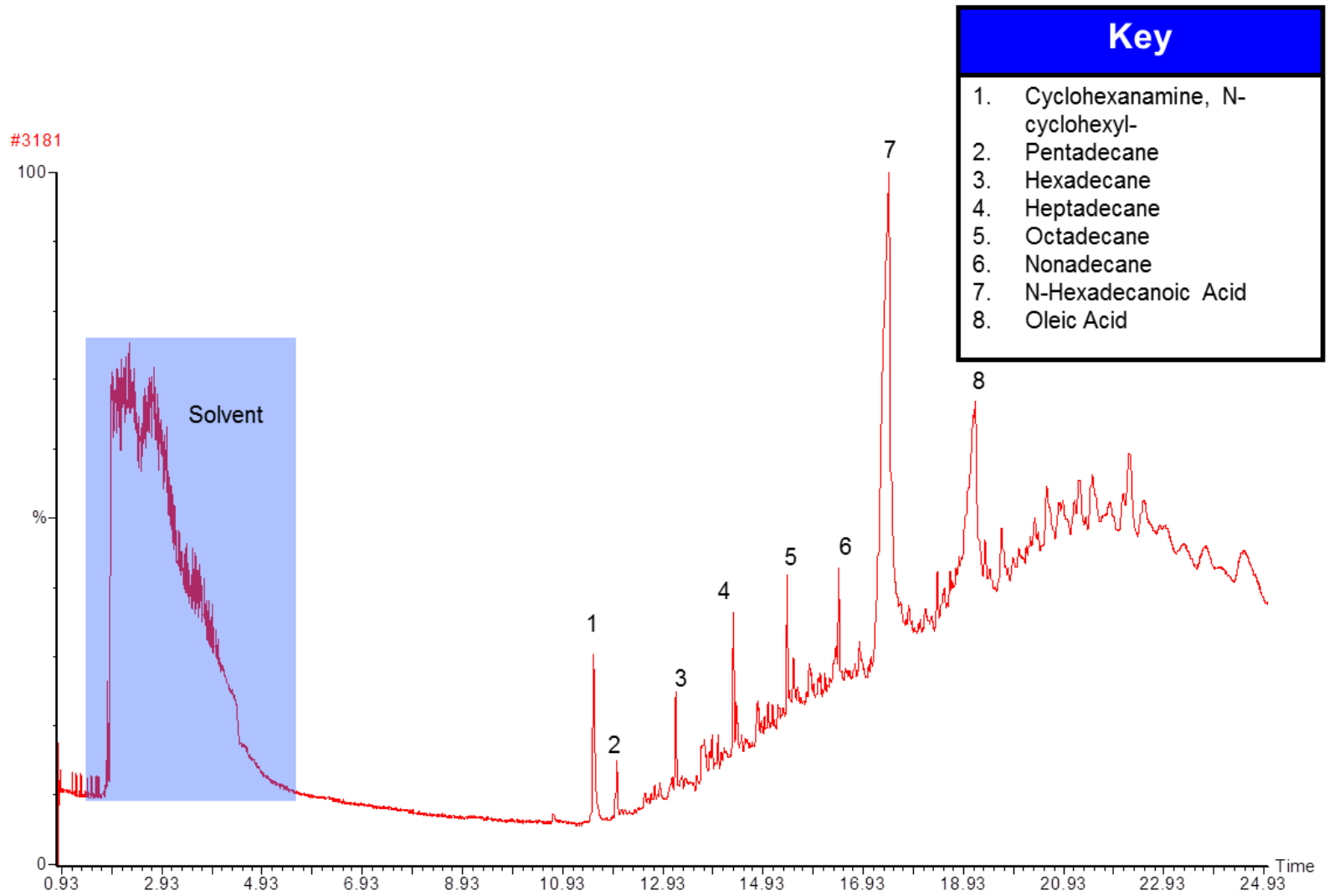


Figure 27 GC-MS Spectra of Sludge 2 Using RTX-5 Column

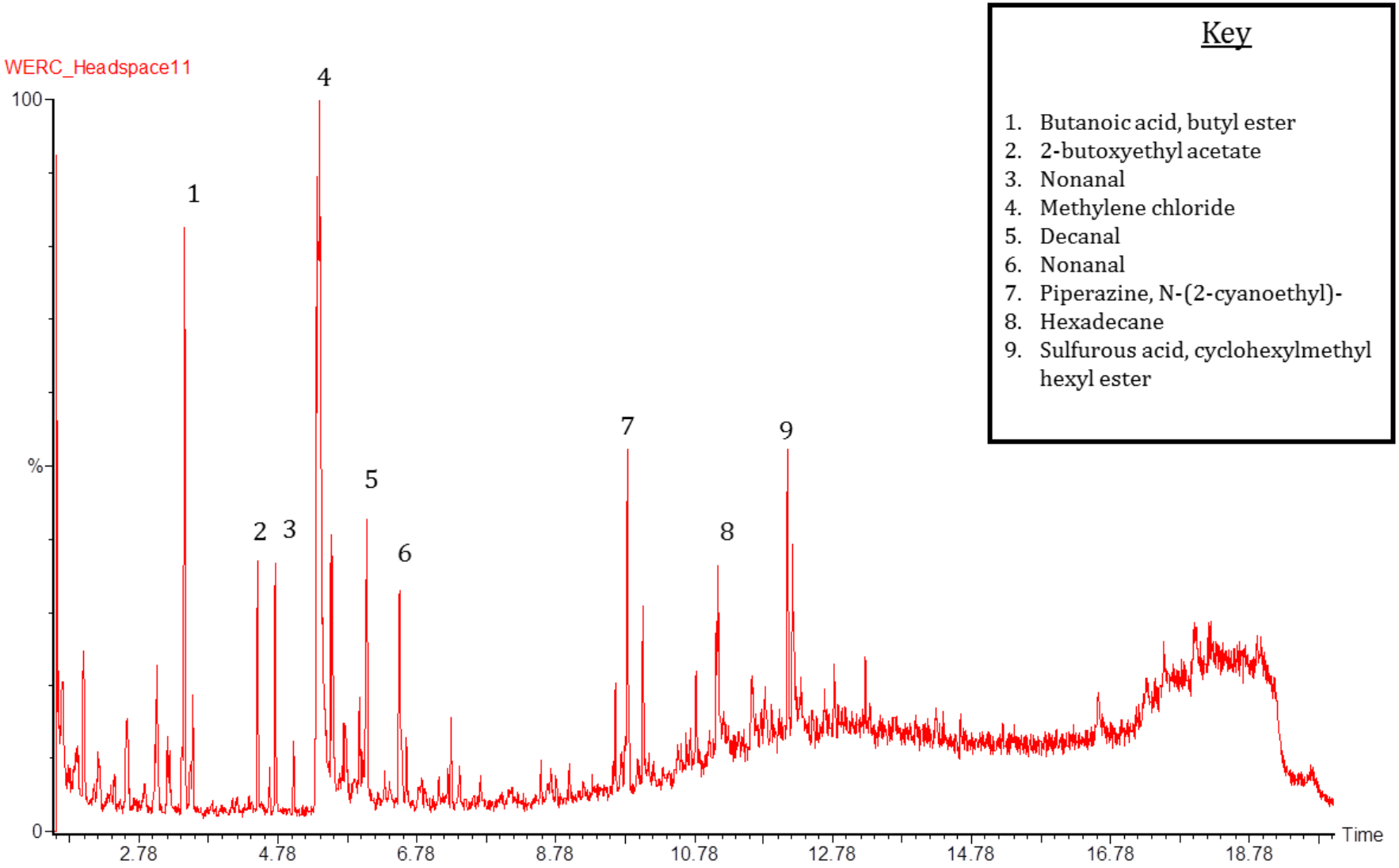


Figure 28 Headspace Analysis Spectra of Sludge 1 Filtered Organics

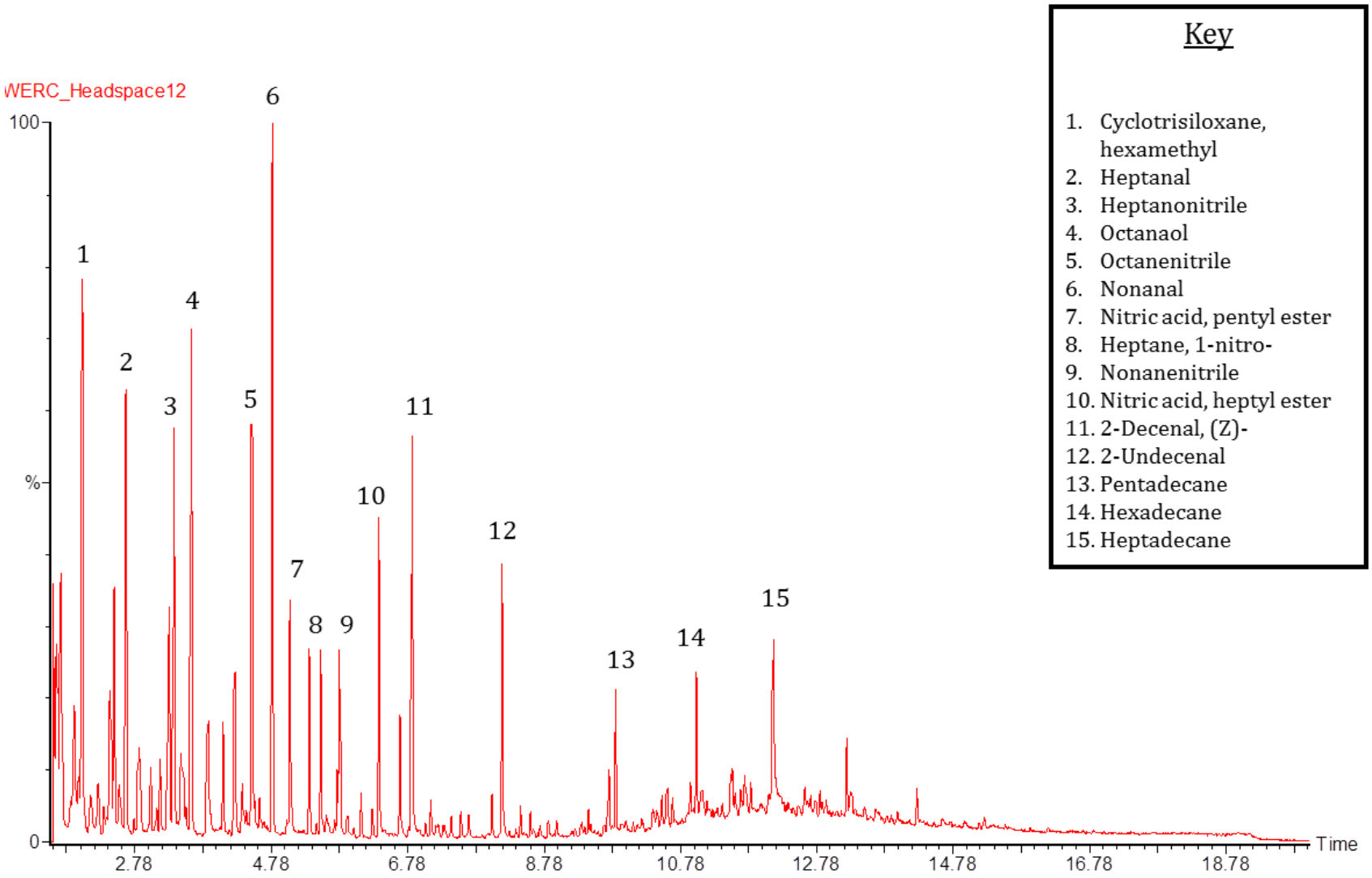


Figure 29 Headspace Analysis Spectra of Sludge 2 Filtered Organics

Qualitative analysis of the sludge spectra showed that the Elite 5MS column provided the clearest spectra, with well separated peaks and a stable baseline, when compared to the other two columns. Within the series of spectra seventeen different compounds were able to be identified between the two samples of sludge. These seventeen different compounds may not all be correct as GC-MS identification is complex and may not be exact. The chemicals that appeared several times were dicyclohexlyamine, palmitic acid, oleic acid and octadecanoic acid, however the largest peaks were from palmitic acid and oleic acid. All of these, except dicyclohexylamine have melting points above 12°C, which may be why the sludge has a semi-solid consistency. Oleic acid and palmitic acid were quantified using the Elite 5MS column against standards as they had the largest peak areas on the spectra.

Quantitative analysis of the sludge produced the results that are shown in Table 11, which uses Equation 1 to calculate the concentrations of palmitic acid and oleic acid within the sludge.

$$A = \frac{\big((B\ x\ C) + D\big)\ x\ 1000}{E}$$

Where:

A Concentration within extracted sludge/ g/kg

B Peak integration

C Multiplication number on trendline equation (differs for each compound)

D Addition number on trendline equation (differs for each compound)

E Sample concentration/ g/L

Equation 1 Calculation of Chemical Compound within Sludge

Table 11 Quantitative Analysis of Sludge on the GC-MS

Chemical Compound	Sample	Sample Concentration (g/L)	Peak Integration	Concentration within Extracted Sludge (g/kg)
Palmitic Acid	Sludge 1	4.305	107181808	360.22
	Sludge 2	3.900	16222613	187.72
Oleic Acid	Sludge 1	4.305	18538757	258.48
	Sludge 2	3.900	29376830	368.69

Concentrations of oleic and palmitic acids within the extracted sludge show that they are significant components, totalling 61.87% of the total mass for the ASE sludge 1 and 55.64% of the total mass for the ASE sludge 2. This does leave 38.13% and 44.36% of the extracted sludge unquantified. This unquantified mass is most likely to consist of triglycerides and compounds that have been identified by GC-MS, although this cannot be proven using these results. Out of the total mass of the sludge these two chemicals account for at least 41.7% of sludge 1 and 37.2% of sludge 2 by mass.

Qualitative analysis of the filter paper using a headspace analyser produced the spectra in Figure 28 and Figure 29, which show many different compounds that were not previously discovered in the analysis of the sludge samples. The majority of these are single chain hydrocarbons, up to C17 in length. These may be the breakdown products from heating of the organic solids, or may make up the organic solids, though the second theory is unlikely as they are liquids in ambient conditions. There is the possibility that the layer of organics that was filtered from the vial was grease, which have very low solubility in organic solvents (Harrison, 1924). A duplicate of this sample is analysed by ICP to determine the inorganics content. These filtered solids are discussed further in the 'Grease/Soap Determination within the Sludge' section of this Chapter.

Statistical analysis of the GC-MS results was not able to be undertaken due to a number of faults with the GC-MS. This means the concentrations measured may be greatly different to those actually present within the sludge, which has significant implications for the physicochemical makeup of the sludge. However it is likely that the palmitic and oleic acids are present in quantities similar to those measured by the GC-MS due to the

base oils being liquids at ambient temperature and containing significant amounts of oleic acid in the chemical makeup of the esters. Along with this the sludge when separated from the water is still a solid, therefore ruling out the solidity being caused by the sludge being a gel (water, surfactant and oil mix). No other reason for the solidity of the sludge has been discovered using all of the previous analyses, therefore it would be fair to say that the sludge does comprise of significant concentrations of palmitic and oleic acid, even though one standard deviation has not been calculated for the palmitic or oleic acids.

5.3.5 Liquid Chromatography

Liquid chromatography of the sludge was not undertaken due to the necessary equipment not being available.

5.3.6 Inductively Coupled Plasma

Digested sludge, ashed sludge and filtered solids from sludge were measured using ICP-OES and converted into the form of mg/kg of sludge using Equation 2, Equation 3, Equation 4 and Equation 5. The ash content of the unprocessed sludge is determined in the 'Ashing' section of this Chapter, whilst the fraction of total sludge mass is determined in the 'Water Separation' section of this Chapter, where the sludge used for this analysis is from repetition 1. The filtered solids content of solvent extracted sludge is found in the 'Organics Extraction' section of this Chapter.

To generate a breakdown of the components that make up a sludge there are a number of sub-divisions when analysing using ICP-OES. A flowchart of these sub-divisions is shown in Figure 39 in the following Chapter. The mass of components within the dotted line have not been directly measured. The results are expressed in mg per kg of sludge, therefore dividing by 10,000 will produce the total percentage that they account for of the sludge. As it is seen in the flow diagram the analytes in the solvent separated sludge also include the analytes from the filtered solids from sludge, which in the compositional make up was deducted from the solvent separated sludge analytes to give the filter passable solvent separated sludge analytes.

$$A = \frac{B\ x\ C\ x\ D\ x\ 1000}{E}$$

Where:

A Concentration of element within sludge/ mg/kg of sludge

B Measured concentration of element on ICP/ mg/L

C Litres of sample/ L (always 0.02L)

D Fraction of total sludge mass/ %

E Mass of sample digested/ g

1000 Conversion factor from mg/g to mg/kg

Equation 2 Calculation of Elemental Concentrations within Digested Sludge

$$F = \frac{B\ x\ C\ x\ D\ x\ 1000}{E\ x\ G}$$

Where:

B Measured concentration of element on ICP/ mg/L

C Litres of sample/ L (always 0.02L)

D Fraction of total sludge mass/ %

E Mass of sample digested/ g

F Concentration of element within sludge/ mg/kg of sludge

G Ash to sludge ratio

1000 Conversion factor from mg/g to mg/kg

Equation 3 Calculation of Elemental Concentrations within Ashed Sludge

$$J = K - L$$

Where:

J Concentration of element within water portion of sludge/ mg/kg of sludge

K Concentration of element within sludge/ mg/kg of sludge

L Concentration of element within solvent separated portion of sludge/ mg/kg of sludge

Equation 4 Calculation of Elemental Concentrations within Water Portion of Ashed and Digested Sludge

$$F = \frac{(B - M)\ x\ C\ x\ D\ x\ 1000}{E\ x\ N}$$

Where:

B Measured concentration of element on ICP/ mg/L

C Litres of sample/ L (always 0.02L)

D Fraction of total sludge mass/ %

E Mass of sample digested/ g

F Concentration of element within sludge/ mg/kg of sludge

M Blank filter measured concentration of element on ICP/ mg/L

N Filtered solids content of solvent extracted sludge/ %

Equation 5 Calculation of Elemental Concentrations within Filtered Solids from Sludge

One standard deviation was calculated for total sludge from sludge 2 using microwave digestion and ten repetitions of the method. This standard deviation will be applied to all of the microwave digested samples. Individual elements were; sodium 43.0mg/kg, calcium 25.5mg/kg, magnesium 0.9mg/kg, potassium 1.0mg/kg, iron 5.1mg/kg, manganese 0.1mg/kg, arsenic 0.1mg/kg, copper 0.1mg/kg, nickel 0.9mg/kg, lead 0.1mg/kg, antimony 1.4mg/kg, selenium 0.8mg/kg, zinc 0.9mg/kg, aluminium 10.7mg/kg, titanium 0.1mg/kg, vanadium 0.1mg/kg, strontium 0.2mg/kg and barium 0.8mg/kg. Elements not listed have a standard deviation of <0.1mg/kg. The standard deviation for the total mass is of inorganic elements is 51.3mg/kg, or 3.1% of the mass of the inorganic elements. No standard deviation was calculated for ashing as this method has proven to be too dangerous to conduct.

Table 12 Mass of Samples for Run on the ICP-OES

Sample	Mass (g)	Ash: Sludge Ratio	Filtered Solids Content of Solvent Extracted Sludge (%)	Fraction of Total Sludge Mass (%)
Digested total sludge 1	0.4750	N/A	N/A	100.00
Digested total sludge 2	0.5893	N/A	N/A	100.00
Digested solvent separated sludge 1	0.4622	N/A	N/A	69.36
Digested solvent separated sludge 2	0.4819	N/A	N/A	65.24
Filtered solids from sludge 1	0.0047	N/A	0.73	69.36
Filtered solids from sludge 2	0.0097	N/A	1.11	65.24
Ashed total sludge 1	0.0976	99.4	N/A	100.00
Ashed total sludge 2	0.1408	63.1	N/A	100.00
Ashed solvent separated sludge 1	0.0921	193.6	N/A	69.36
Ashed solvent separated sludge 2	0.1002	32.7	N/A	65.24

Table 13 ICP-OES of Digested Sludge

Analyte	Total Sludge 1 (mg/kg)	Sludge 1 Solvent Separated (mg/kg)	Sludge 1 Water (mg/kg)	Total Sludge 2 (mg/kg)	Sludge 2 Solvent Separated (mg/kg)	Sludge 2 Water (mg/kg)
Ca	932.33	135.46	796.87	1972.61	1302.47	670.14
Na	303.59	96.85	206.74	77.74	33.97	43.77
Mg	26.15	9.38	16.76	84.61	39.43	45.18
K	13.76	4.67	9.09	25.35	11.11	14.24
Al	131.63	76.91	54.71	775.53	439.57	335.96
Fe	124.09	47.59	76.50	1257.79	854.73	403.06
Mn	1.36	0.27	1.09	22.38	13.63	8.75
Ag	0.13	0.08	0.04	0.11	0.14	-0.03
As	0.47	0.47	0.00	0.12	0.36	-0.23
Be	4.77	1.42	3.34	6.33	1.83	4.50
Ba	16.35	9.09	7.26	32.01	17.53	14.48
Bi	-0.02	-0.01	-0.01	-0.02	-0.01	-0.01
Cd	3.99	2.08	1.91	3.88	2.07	1.81
Co	0.60	0.34	0.25	0.71	0.43	0.28
Cr	0.28	0.13	0.15	0.65	0.39	0.27
Cu	2.01	1.03	0.97	6.32	3.80	2.52
Li	24.46	13.40	11.06	66.98	36.92	30.06
Mo	0.69	0.32	0.37	0.73	0.40	0.33
Ni	4.69	2.80	1.89	7.34	4.84	2.49
Pb	3.24	1.56	1.68	8.44	4.54	3.90
Sb	11.58	5.82	5.76	29.09	19.23	9.85
Se	2.47	1.34	1.12	2.57	1.54	1.03
Si	6.07	3.51	2.56	5.92	3.85	2.07
Sr	4.75	1.53	3.22	4.27	2.47	1.80
Ti	1.34	0.71	0.63	4.48	2.29	2.19
Tl	-22.53	-12.09	-10.44	-19.17	-13.11	-6.06
V	0.22	0.10	0.12	0.64	0.38	0.26
Zn	18.56	11.35	7.21	108.04	69.75	38.29

Table 14 ICP-OES of Ashed Sludge

Analyte	Total Sludge 1 (mg/kg)	Sludge 1 Solvent Separated (mg/kg)	Sludge 1 Water (mg/kg)	Total Sludge 2 (mg/kg)	Sludge 2 Solvent Separated (mg/kg)	Sludge 2 Water (mg/kg)
Ca	578.60	92.98	485.62	616.94	725.85	-108.92
Na	88.52	45.43	43.08	10.04	26.04	-16.00
Mg	20.29	5.86	14.42	32.72	30.96	1.76
K	2.89	2.50	0.39	7.00	8.26	-1.25
Al	86.32	31.74	54.58	323.60	351.69	-28.09
Fe	699.42	359.01	340.40	892.99	2153.80	-1260.81
Mn	24.20	9.08	15.12	19.62	54.06	-34.44
Ag	0.44	0.07	0.37	1.40	0.33	1.08
As	0.22	0.07	0.15	0.02	0.28	-0.27
Be	-0.02	-0.01	-0.02	-0.01	-0.03	0.02
Ba	14.46	4.78	9.68	9.26	14.89	-5.63
Bi	0.29	0.09	0.20	0.19	0.45	-0.26
Cd	-0.01	0.00	-0.01	0.03	0.00	0.03
Co	0.54	0.61	-0.07	0.57	2.32	-1.75
Cr	202.17	45.77	156.40	50.35	237.93	-187.58
Cu	8.47	7.12	1.35	24.78	32.49	-7.71
Li	0.04	0.02	0.03	0.07	0.09	-0.02
Mo	2.22	1.54	0.69	2.60	5.14	-2.54
Ni	9.87	29.68	-19.81	7.97	65.75	-57.78
Pb	0.68	1.32	-0.63	5.41	8.79	-3.38
Sb	2.22	0.54	1.67	0.66	2.58	-1.91
Se	-0.07	-0.04	-0.03	-0.10	-0.38	0.28
Si	138.76	56.61	82.16	254.97	292.61	-37.64
Sr	3.11	0.82	2.29	1.59	1.93	-0.35
Ti	2.25	0.93	1.32	4.84	5.47	-0.63
Tl	0.16	0.05	0.11	0.04	0.23	-0.19
V	0.86	0.17	0.69	0.24	1.00	-0.75
Zn	5.49	4.36	1.13	32.98	39.31	-6.32

Table 15 ICP-OES of Filtered Solids from Sludge

Analyte	Filtered Solids from Sludge 1 (mg/kg)	Filtered Solids from Sludge 2 (mg/kg)
Ca	82.25	136.08
Na	-1.96	-7.33
Mg	0.57	1.52
K	0.16	-0.07
Al	2.37	1.00
Fe	1.92	40.96
Mn	0.05	1.15
Ag	0.00	-0.01
As	0.01	-0.05
B	0.34	0.26
Ba	0.43	0.66
Be	0.00	0.00
Bi	0.18	0.00
Cd	0.02	0.00
Co	0.01	0.01
Cr	0.06	0.05
Cu	0.32	0.41
Li	0.02	0.00
Mo	0.09	0.09
Ni	0.02	-0.01
Pb	0.28	1.83
Sb	0.13	0.03
Se	-0.01	0.04
Sr	0.20	0.19
Ti	0.01	0.07
Tl	-0.10	0.17
V	0.01	-0.01
Zn	1.12	3.58

Analysis of the solutions using ICP-OES was simple due to an experienced technician being available to run the ICP-OES. No problems were experienced during the analysis.

Two forms of processing the sludge prior to ICP-OES analysis exist. These are ashing of the sludge using a muffle oven and digestion of the sludge using a microwave and solvents. As a process digestion is simpler and safer than ashing as it does not involve the risk of auto-ignition and boiling of acids. In terms of ICP-OES results when comparing the digested sludge to the ashed sludge, it is seen that there is a significant difference in concentrations of the analytes. The digested sludge has up to 3x higher concentrations than the equivalent ashed sludge concentrations. This is most likely due to losses in the combustion process, or an error with the ash to total sludge ratio. The ashed total sludge 2 has concentrations of analytes lower than the ashed solvent separated sludge 2, which is not possible. This is most likely an problem in the combustion process, with the possibility that the sludge bubbled causing the ejection of analytes. With digestion this is not a problem as it is in a sealed container so no sludge was ejected. Overall digestion is a more user friendly and reliable method for the processing of sludge into a form ready for ICP analysis.

ICP analysis of the digested sludge is used to calculate the full chemical breakdown of the sludge in this Chapter, instead of ashing due to the issues encountered with the results, documented in the previous paragraph. Alongside this the digested sludge results have the benefit that they and the filtered solids from sludge both used the same microwave digestion technique to prepare them for ICP analysis. They can therefore be directly compared to one another to generate the chemical composition of the sludge on a percentage basis without the substantial differences in analyte concentrations that are seen between the digested sludge and the ashed sludge.

Analysis of the digested sludge shows that the analytes with the highest concentrations are calcium, sodium, magnesium, potassium, aluminium, iron, chromium and silicon. The remaining 20 analytes are most likely not present in significant enough concentrations to be the cause of the semi-solid characteristic of the sludge. As the iron was present in the form of iron fillings when the sludge was in the solvent solution, this is concluded to not be bonded to any organics within the sludge. Some of the other analytes, such as calcium, may be bonded to the organics to form chemicals such as

calcium palmitate, commonly known as soap. This bonding between organics and inorganics was explored further in the 'Grease/Soap Determination within the Sludge' section of this Chapter.

Separation of the water from the sludge by means of the newly developed solvent separation technique has proven that some of the inorganic analytes are inherent within the water phase, as shown in both Table 13 and Table 14. Not only this, but during the solvent separation process the iron appeared in the form of iron fillings that were not bound to any organic compounds. This is important as other authors (Nitayapat and Chitprasert, 2014; He et al, 2011; Williams et al, 2012; Keener et al, 2008) have assumed that the inorganic analytes, specifically calcium, are bound to the free fatty acids within their FOG samples, which has been proven here to not always be true. These authors do not separate the FOG using the solvent separation technique, instead it is vacuum or heat dried, leaving all of the analytes within the dried sludge. The concentration of calcium within the samples can vary greatly as Sludge 1 has only 15% of its total calcium inherent within the organic portion of the sludge, whilst sludge 2 has significantly more, but not all, at 66% of its calcium inherent within the organic portion of the sludge. This brings into question whether the work by other authors is correct, especially as the water content of their sludge ranges from 0.0-77.7% (Nitayapat and Chitprasert, 2014; Williams et al, 2012; Keener et al, 2008) meaning that there is a high potential for the calcium and other inorganic analytes to be inherent within the water phase, instead of bound to the fatty acids.

A minor constituent of the sludge appears to be the filtered solids from the sludge, which make up only 0.5-0.7% of the total mass of the sludge, yet account for 4-5% of the total inorganic analytes. They also account for 6-21% of the total inorganic analytes in the solvent separated sludge, which is made up only 0.7-1.1% of filtered solids from sludge. It shows that these filtered solids have a much higher than average concentration of analytes within them. This combination of high concentrations of inorganic analytes and the abundance of palmitic acid and oleic acid suggest that this part of the sludge is a soap or grease, however this is explored further in the 'Grease/Soap Determination within the Sludge' section of this Chapter.

5.3.7 Fourier Transform Infra-Red Spectrophotometry

No problems were encountered during the FTIR analysis of the sludge, which produced the spectra seen in Figure 30 and Figure 31. Above 4000cm^{-1} the spectra has what appeared to be significant interference, with no clear peaks present. It has therefore been decided to remove the 4001cm^{-1} and above wavelength results from the spectra below to aid in visual analysis of the remaining peaks.

As GC-MS and ICP-OES analysis had already been run and analysed prior to using the FTIR it was decided that a number of chemicals should be analysed and compared to the sludge. These chemicals were calcium palmitate, calcium stearate, oleic acid, palmitic acid, stearic acid and the mineral oil used in the factory.

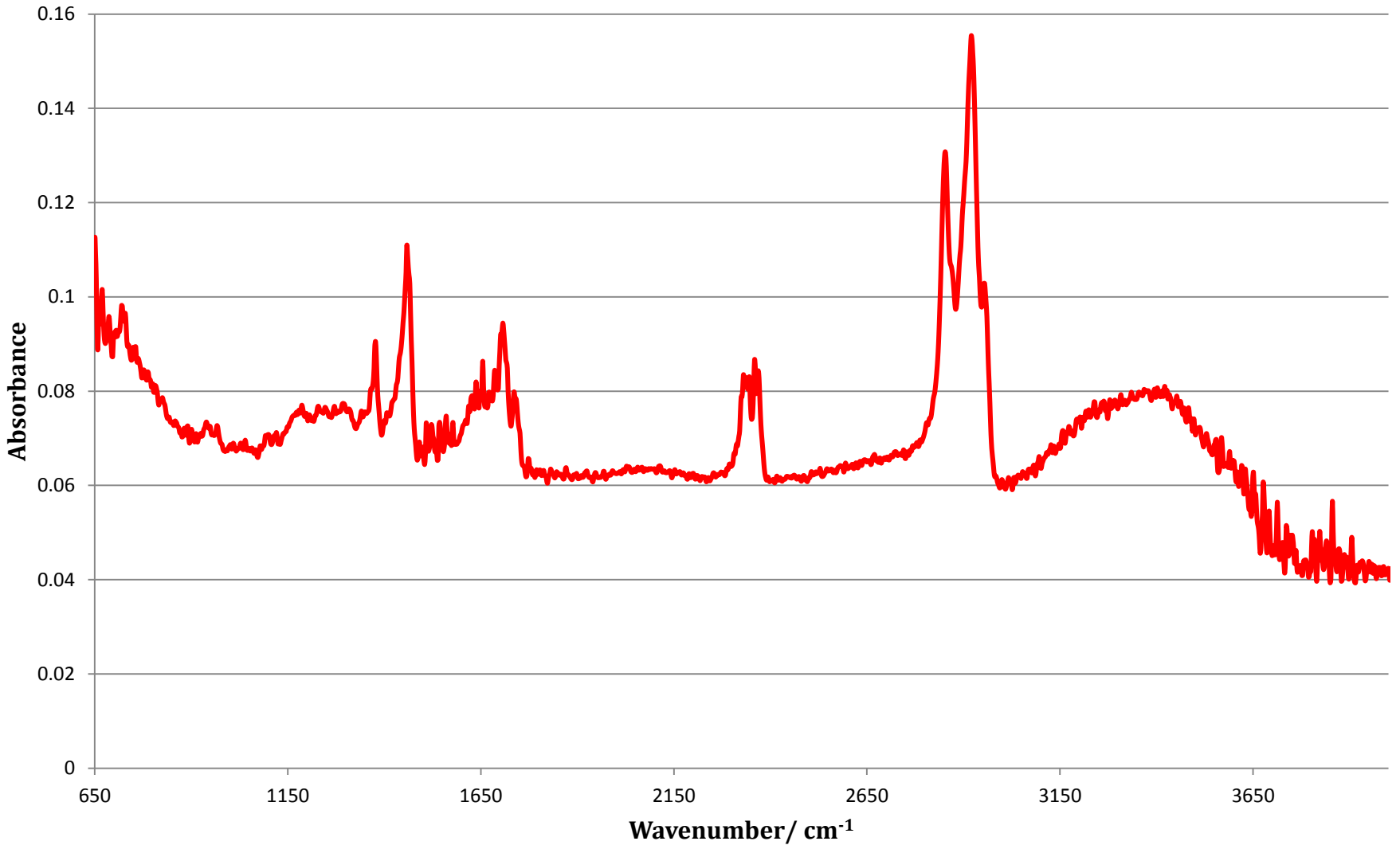


Figure 30 FTIR Spectra of Sludge 1

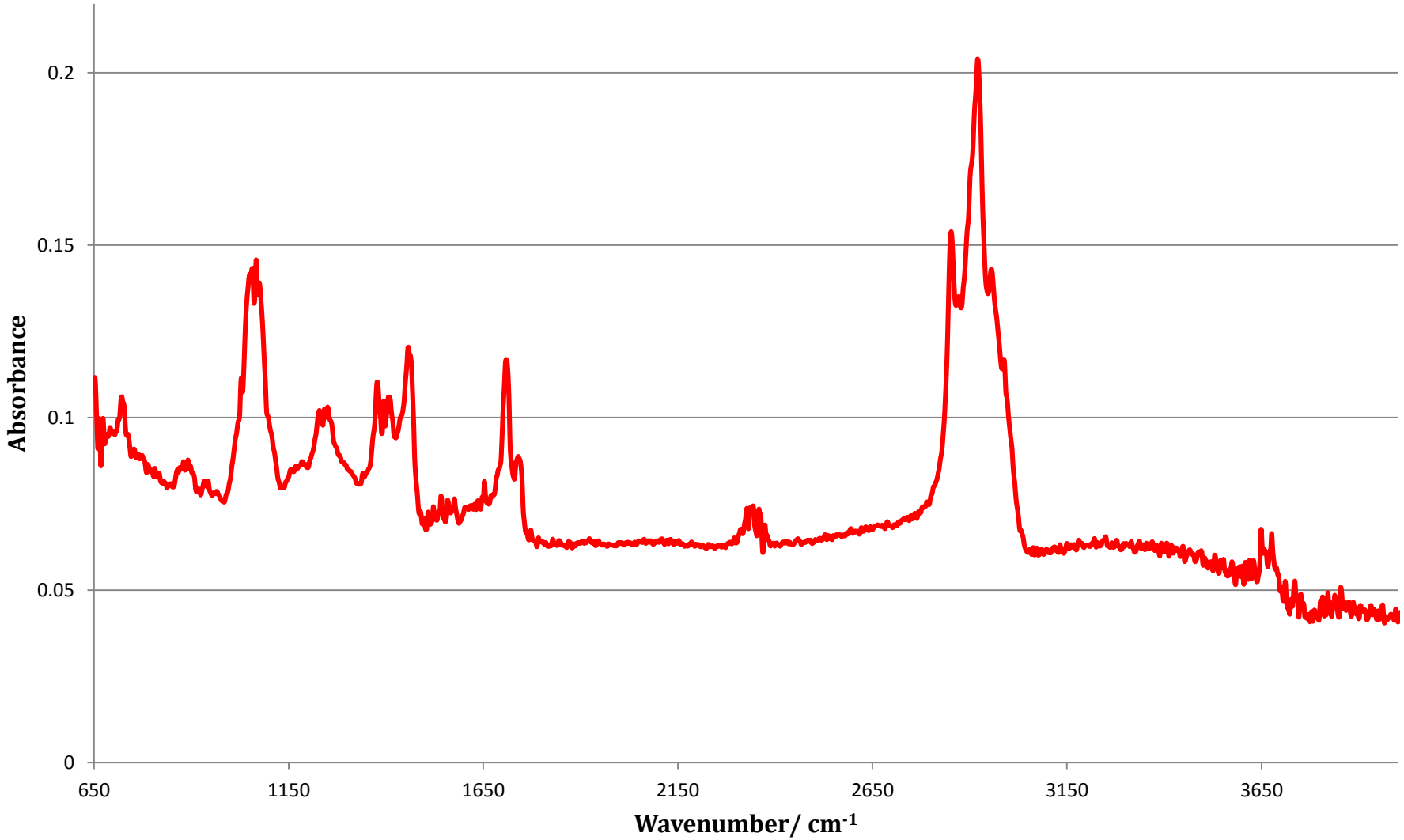


Figure 31 FTIR Spectra of Sludge 2

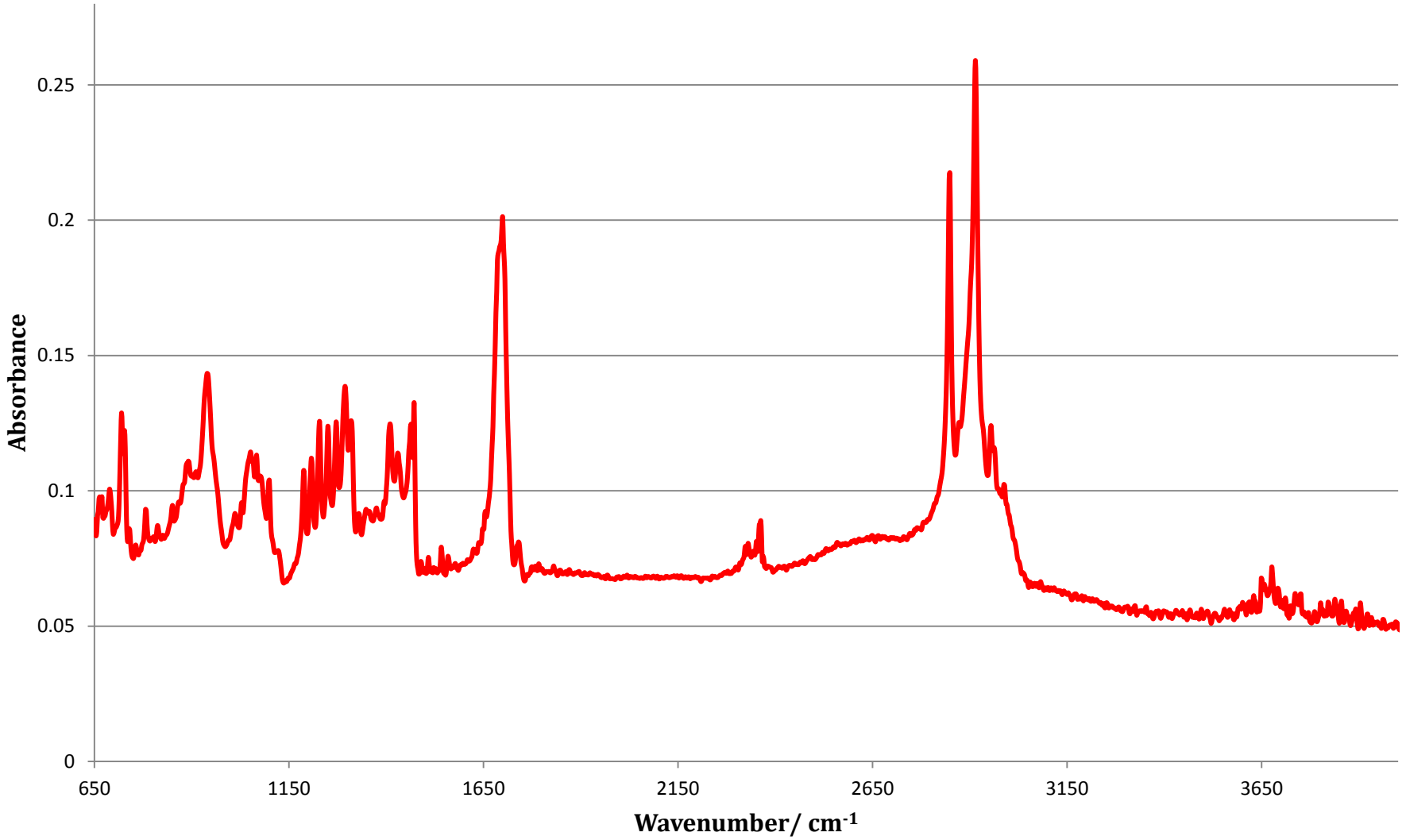


Figure 32 FTIR Spectra of Calcium Palmitate

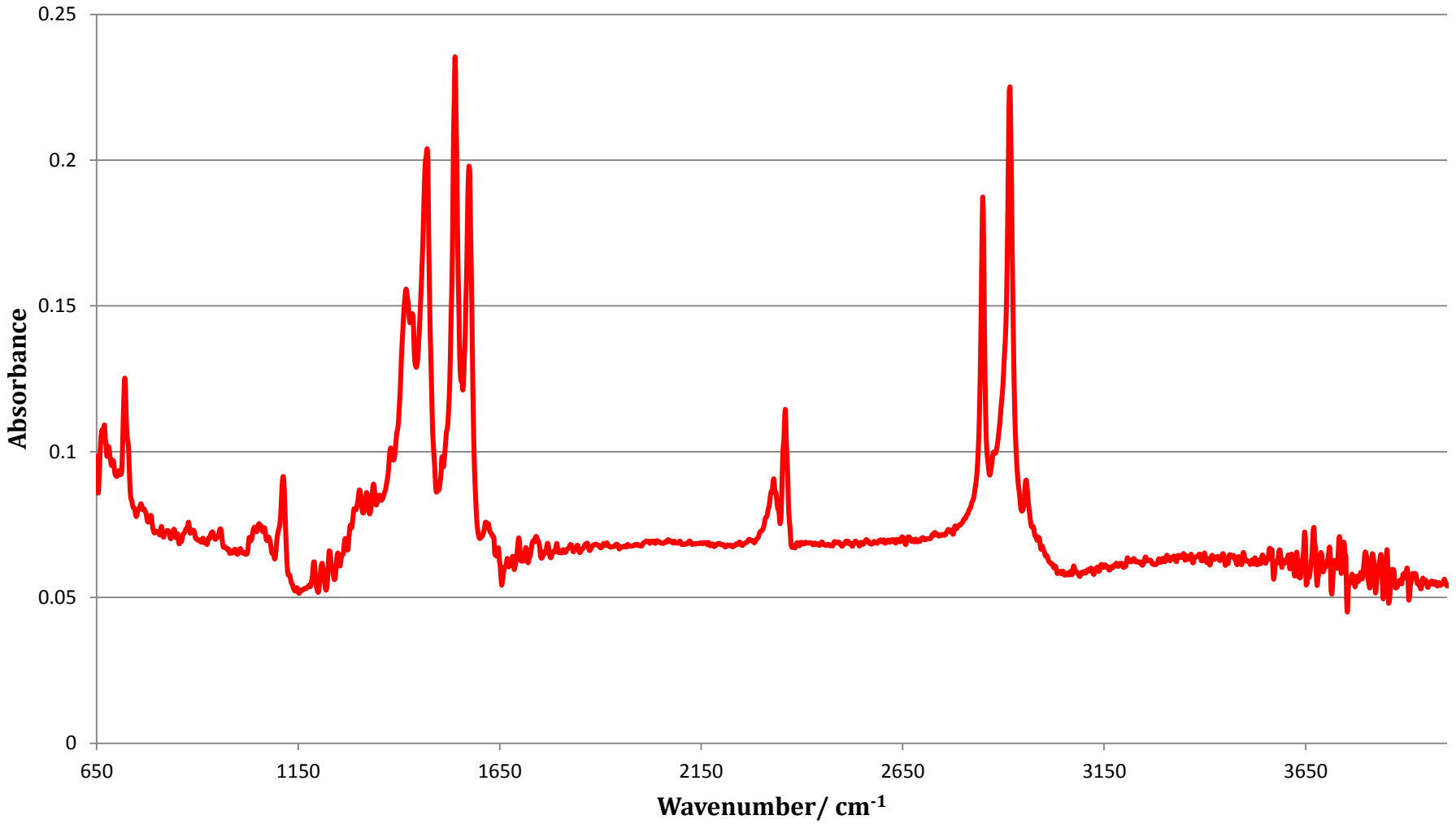


Figure 33 FTIR Spectra of Calcium Stearate

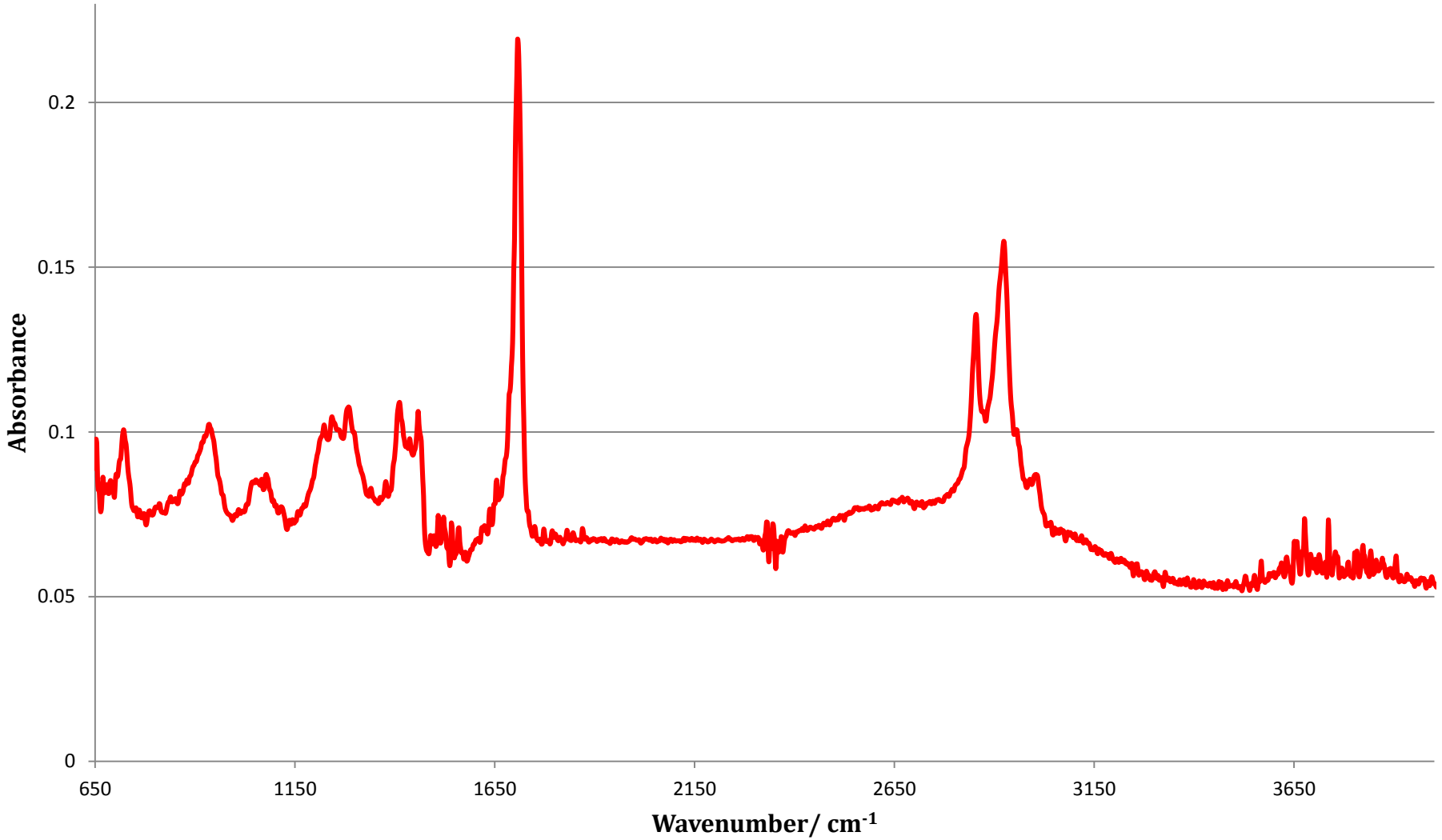


Figure 34 FTIR Spectra of Oleic Acid

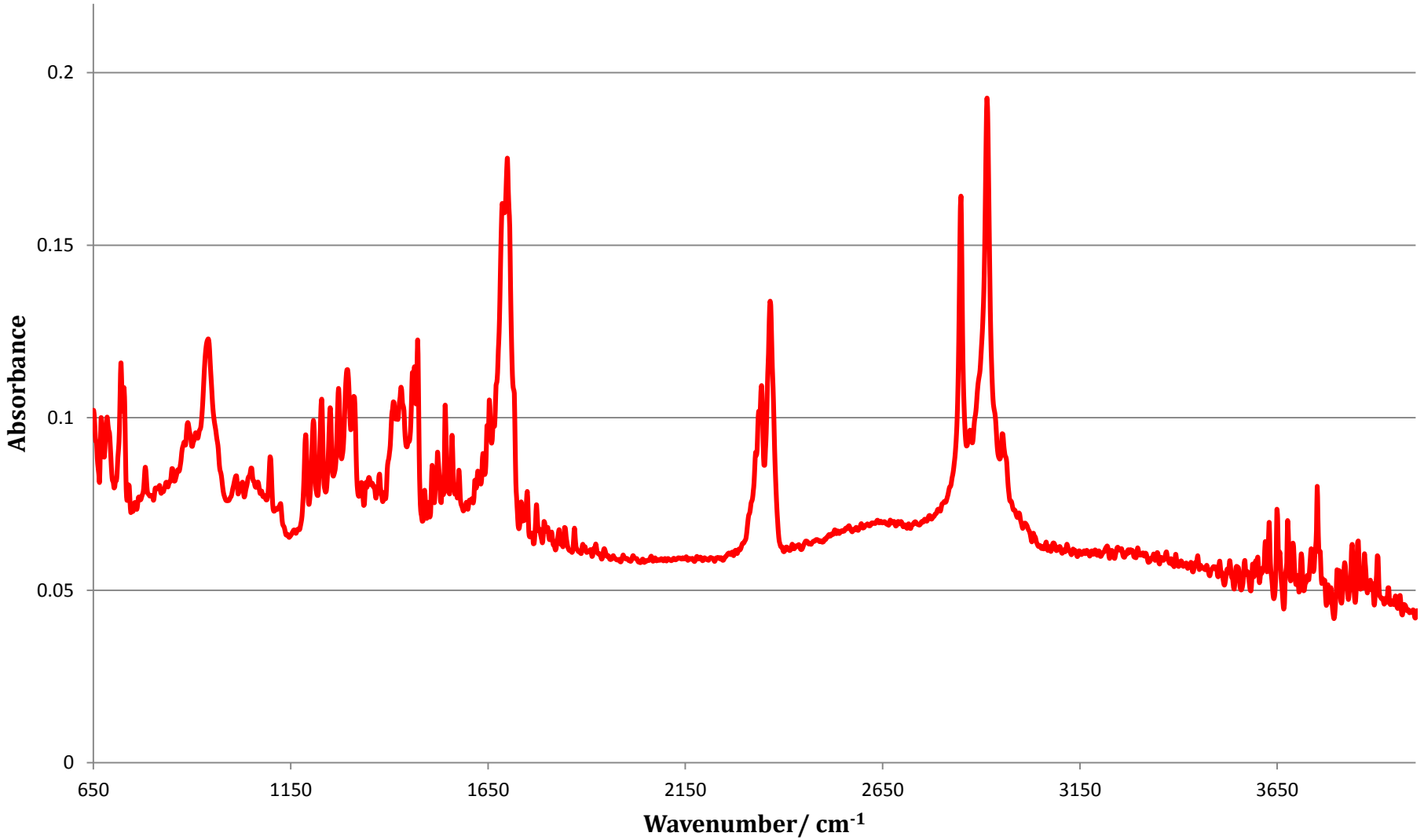


Figure 35 FTIR Spectra of Palmitic Acid

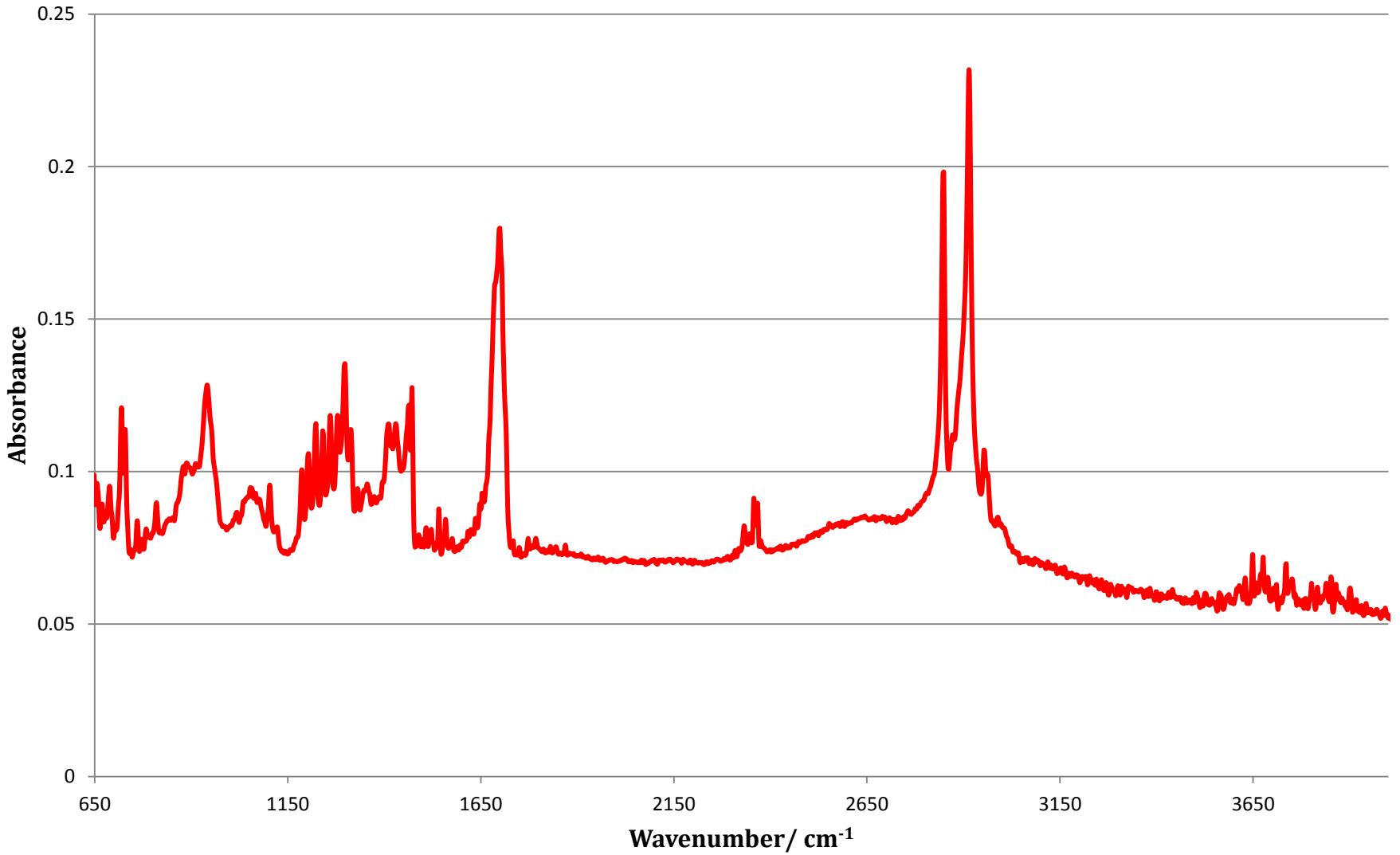


Figure 36 FTIR Spectra of Stearic Acid

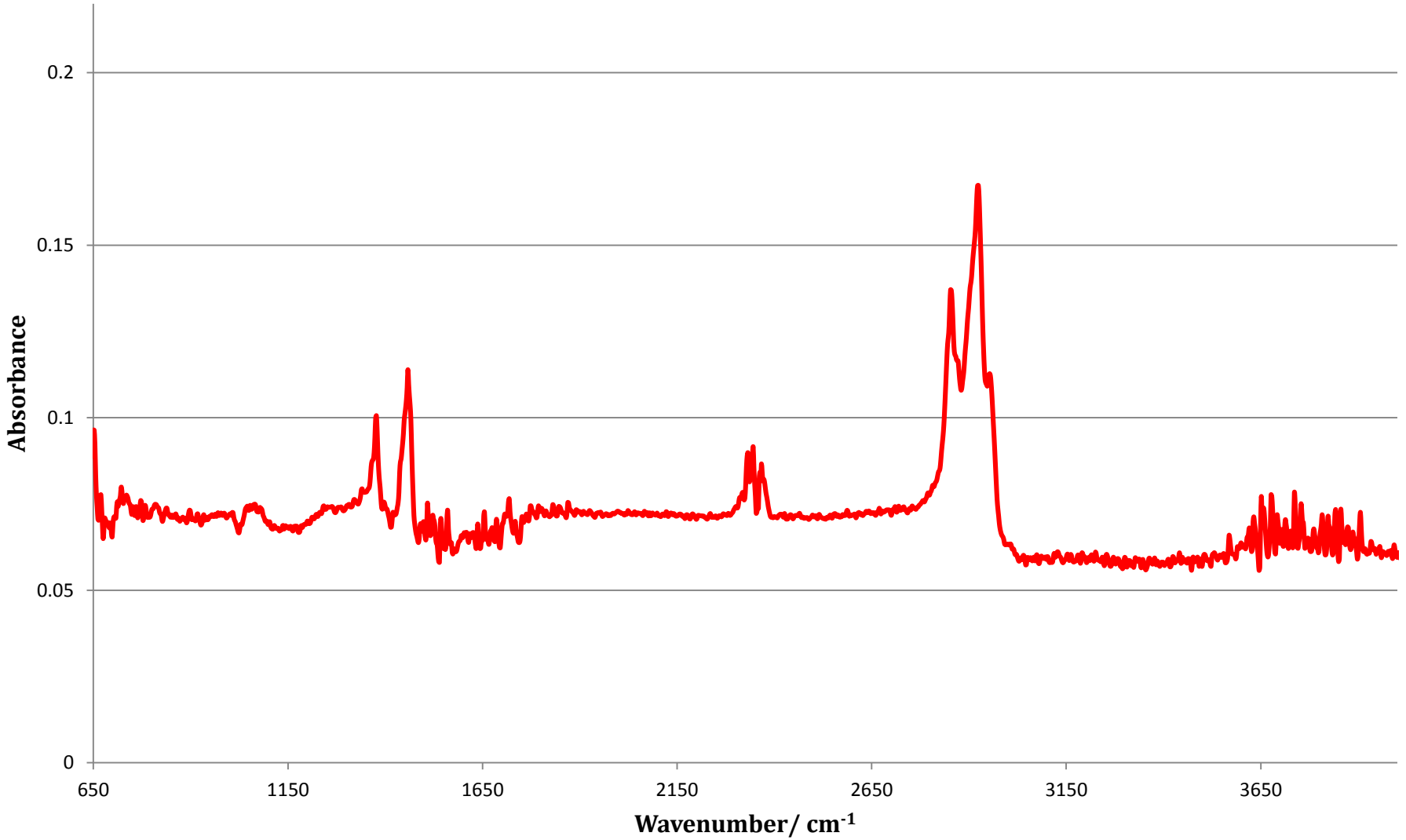


Figure 37 FTIR Spectra of Mineral Oil

Comparing the spectra of sludge 1, Figure 30 and sludge 2, Figure 31 shows that they are almost identical. All peaks are present in both of the sludge samples, however some are more pronounced than others, showing that one sludge may have higher concentrations of that particular bond.

Intense, sharp peaks are shown at 2800-3000cm^{-1}, which corresponds to a C-H bond. Smaller, but still sharp peaks are seen at 1600-1800cm^{-1}, which is a C=O bond, more precisely the peak is between 1650cm^{-1} and 1750cm^{-1}, which relates to acids and esters. Another C-O bond is detected at 1200-1300cm^{-1}. Overall there are C-O bonds, C-H bonds and C=O bonds, along with acids and ester bonds. All of these bonds are present within palmitic and oleic acid, however the most common bond in these chemicals is the C-H bond, which relates to the intense peak seen at 2800-3000cm^{-1}. The detection of esters confirms that not all of the oil has been degraded into free fatty acids.

Comparing the sludge samples directly to calcium palmitate in Figure 32 shows that they are also very similar. This is expected due to the high concentration of palmitic acid within the sludge. Additional peaks are visible at 800-1000cm^{-1} and at 1150-1300cm^{-1} there is an abundance of peaks that are not present within the sludge. These may be to do with the bonding on the calcium to the palmitic acid and shows that little, or no calcium palmitate is present within the sludge.

Calcium stearate is analysed in Figure 33, which when compared to the sludge samples has similar peaks. The main difference is the peak at 1470-1570cm^{-1}, which the sludge samples lack. This peak is usually attributed to a C=C bond, however this is not the case as stearic acid has no C=C bonds. The possible cause of these peaks is the bond between the calcium and the stearic acid, although little or no stearic acid has been found to be present within the sludge.

Comparisons between oleic acid in Figure 34 and the sludge shows that they are very similar, with few differences, except a larger peak at 1650-1750cm^{-1}, corresponding to acids and esters, of which it is an acid in its pure form, so a larger peak would be expected. These results match up to the results found by GC-MS showing high concentrations of oleic acid present within the sludge.

Palmitic acid in Figure 35 also closely resembles the sludge and like oleic acid has a large peak at 1650-1750cm^{-1}. The main difference is at 1130-1300cm^{-1}, where no clear

peaks are visible. This is similar to calcium palmitate, therefore these peaks are likely to be caused by bonds within the palmitic acid and not the bond joining it to calcium.

Comparing the sludge samples to Stearic acid in Figure 36 also shows the peaks visible in the palmitic acids at 1130-1300 cm^{-1}. It is not known what bond these peaks correspond with, but they are present in several of the acids. Also present is the large acid peak at 1650-1750cm^{-1}. One further additional peak is at 870-1000 cm^{-1}, which is also unknown. Other than these peaks the spectra in very similar to the spectra of the sludge.

Mineral oil in Figure 37 has the most different spectra to the sludge. It lacks the acid peak at 1650-1750cm^{-1}, which is expected, but it also lacks many of the peaks in 650-1300cm^{-1} range. It is unlikely that mineral oil makes a significant contribution to the chemical make-up of the sludge.

Overall most of the fatty acids are very similar in composition to the sludge, but not the mineral oil used at the factory. No one chemical matches the sludge spectra, but each has their similarities on different peaks, meaning that it is likely that the sludge is formed of a mix of these chemicals, along with others.

5.3.8 Nuclear Magnetic Resonance

Nuclear magnetic resonance of the sludge was not undertaken due to the necessary equipment not being available.

5.3.9 Carbon Content

Analysis of the carbon content was simple, with both of the standards aligning with the calibration curves on the first run. Solvent separated sludge was analysed, producing the results in Table 16, using Equation 6 to calculate TIC. To convert this back to wet mass Equation 7 is used, which produces the results in Table 17 and Table 18.

$$\mathrm{TOC} = \mathrm{TC} - \mathrm{TIC}$$

Where:

TOC Total organic carbon

TIC Total inorganic carbon

TC Total carbon

Equation 6 TOC Calculation Equation

Table 16 Carbon Content

Repetition	Sample	TC (%)	TIC (%)	TOC (%)
1	Sludge 1	80.00	0.00	80.00
	Sludge 2	76.36	0.00	76.36
2	Sludge 1	79.91	0.00	79.91
	Sludge 2	75.48	0.00	75.48
3	Sludge 1	79.60	0.00	79.60
	Sludge 2	74.57	0.00	74.57

$$A = \frac{B \times C}{100}$$

Where:

A Wet carbon content/ %

B Carbon content (TOC/TIC/TC)/ %

C Solids content of sludge after dehydration/ %

Equation 7 Wet Mass Carbon Content Conversion

Table 17 Wet Mass Carbon Content

Repetition	Sample	Solids Content (%)	TC (%)	TIC (%)	TOC (%)
1	Sludge 1	69.36	55.49	0.00	55.49
	Sludge 2	65.24	49.82	0.00	49.82
2	Sludge 1	68.71	54.91	0.00	54.91
	Sludge 2	65.77	49.64	0.00	49.64
3	Sludge 1	70.87	56.41	0.00	56.41
	Sludge 2	62.29	46.45	0.00	46.45

Table 18 Wet Mass Carbon Content Averages

Sample	Average TC (%)	Average TIC (%)	Average TOC (%)
Sludge 1	55.60 (±0.6%)	0.00 (±0.0%)	55.60 (±0.6%)
Sludge 2	48.64 (±1.5%)	0.00 (±0.0%)	48.64 (±1.5%)

Variation within the results was a maximum of 1.79% for sludge 2, which is low, demonstrating that the results are accurate. One standard deviation for average TC (dry) based upon three repetitions was 0.6% and 1.5% for sludge 1 and sludge 2 respectively, which related to 0.0% for average TIC and 0.6% and 1.5% for the average TOC standard deviation values. As only three repetitions were undertaken the deviation

in carbon content of the sludge may be greater than calculated, however it is fairly low, therefore would not make a great difference to the overall results.

No inorganic carbon was detected within sludge 1 or sludge 2, therefore all of the carbon is organically based, so TOC have the same values as TC. There is a high level of carbon present within the solvent extracted samples, averaging 79.84% and 75.47% for sludge 1 and sludge 2 respectively.

Comparing these values measured using the carbon analyser to the carbon content of the chemicals in Table 19, which are either known to be in the sludge, or suspected to be in the sludge, shows that they are closely related. As the sludge is a mix of chemicals the carbon content analysis on its own cannot define what the sludge is composed of, but as with the melting point, it aids in its identification. Carbon content analysis has ruled out any of the carbon being inorganically bonded, therefore no chemicals such as calcium carbonate are present within the sludge.

Table 19 Carbon Content of Sludge Chemical Components

Chemical	Chemical Composition	Carbon Content (%)
Trimethylolpropane Trioleate	$C_{60}H_{10}O_6$	87%
Dicyclohexylamine	$C_{12}H_{23}N$	80%
Palmitic Acid	$C_{16}H_{32}O_2$	74%
Oleic Acid	$C_{18}H_{34}O_2$	76%

5.4 Chemical Composition of Sludge

The chemical composition of the sludge has been calculated based upon the results from all of the analyses that have been conducted within this thesis. To aid in the readers understanding of how the sludge has been separated for each analysis flow diagrams have been produced, with Figure 39 showing the breakdown of the sludge for ICP-OES analysis and Figure 40 showing the breakdown for GC-MS and organics analysis. The dashed red circle indicates the components of the sludge that have been calculated from other results, but not directly measured. The colours within the flow chart correspond to the colours within the chemical breakdown in Table 20.

Calculation of the final values for the quantitative GC-MS analysis uses Equation 8, which is based upon the removal of filtered solids and ICP analytes from the sludge prior to calculation. Values are taken from Table 11 and Table 20.

$$A = \frac{B\ x\ (C - D - E - F - G)}{1000}$$

Where:

A Percentage of organic chemical within the total sludge/%

B Concentration of chemical within extracted sludge/ g/kg

C Percentage of ASE filtered solids within the total sludge/ %

D Percentage of filtered solids from sludge within the total sludge/ %

E Percentage of filtered solids from sludge analytes within the total sludge/ %

F Percentage of ASE filtered sludge analytes within the total sludge/ %

Equation 8 Calculation of Organics Percentage of Sludge

Table 20 Sludge Compositional Breakdown

Sludge Component	Sludge 1 (%)	Sludge 2 (%)
Water	30.58 (±0.90%)	34.60 (±1.53%)
Water Inorganic Analytes (Total)	0.12 (< ±0.01%)	0.16 (< ±0.01%)
Non-Lipid Solids, including Solids Over 0.2µm	2.29 (±0.41%)	1.50 (±0.28%)
Filtered Solids from Sludge	0.49 (±0.03%)	0.71 (±0.01%)
Filtered Solids from Sludge Inorganic Analytes (Total)	0.01 (< ±0.01%)	0.02 (< ±0.01%)
Unquantified Organics	25.35	27.83
Palmitic Acid	23.95	11.78
Oleic Acid	17.18	23.13
ASE Filtered Sludge Inorganic Analytes (Total)	0.03 (< ±0.01%)	0.27 (< ±0.01%)

With ~70% of the sludge quantified conclusions are drawn on the physicochemical composition of the sludge and why it is of a semi-solid consistency.

The main chemical components within the sludge, that make up 97.06% of sludge 1 and 97.34% of sludge 2 are water, unquantified organics, palmitic acid and oleic acid. Excluding the unquantified organics 71.71% of sludge 1 and 69.51% of sludge 2 are of known chemical composition. Some of the unquantified organics have been qualitatively analysed, but are not quantitatively known, such as the esters found using FTIR. Low concentrations of inorganic analytes are present within the sludge, accounting for only 0.16% of sludge 1 and 0.45% of sludge 2. The majority of these are within the water and filtered solids, with little remaining in the rest of the sludge. Calcium, sodium, magnesium, potassium, aluminium, iron, chromium and silicon are present in more significant concentrations than the other analytes. Of these, iron does not appear to interact with the organic portion of the sludge, however it is likely that calcium does and may be forming a grease or soap, which is the filtered solids from sludge, shown in orange in Table 20. This theory is explored further in the 'Grease/Soap Determination within the Sludge' section of this Chapter. Mineral oil used within the plant is seen not to be a significant contributor to the chemical make-up of the sludge, as shown using FTIR.

Consistency of the sludge is an important factor, as if the sludge was less viscous and more fluid it could be disposed of easily, thus not causing the problems for the wastewater treatment plant that it currently does. During the winter sludge 2 has a thicker, more viscous consistency than it does in the summertime, which contrasts with sludge 1, which does not change consistency at any time of the year. The reason why sludge 2 appears to change consistency is most likely due to its oleic acid content being higher and palmitic acid content being lower than that of sludge 1. Temperatures in the UK in the winter are generally below the melting temperature of oleic acid at 14°C, whilst in the summer they are warmer. This could cause the oleic acid to solidify and therefore increase the viscosity of the sludge during the winter. This change in consistency with change in seasons seems to directly contradict the melting point results, which showed that sludge 2 has a significantly higher melting point than sludge 1, which does not seasonally change in consistency. It is not known why there is this difference between the sludge within the tanks and a small sample extracted, except that it may be an unrepresentative sample.

The statistical validity of the results is high, even for the organic components that were unable to be repeated to have a statistical analysis undertaken upon them. As can be seen in Table 20 the error is low for all of the components, with many having an error lower than 0.01% of the total mass of the sludge. This means that the results are valid and the physicochemical composition is correct.

Overall it is likely that the ratio between water, palmitic acid, oleic acid and the unquantified organics within the sludge is the reason why the sludge is a semi-solid, due to having an emulsion of waxy solids and liquids. This ratio between the chemicals will also influence the viscosity of the sludge, with higher water and oleic acid content creating a less viscous sludge than with higher concentrations of palmitic acid, which is solid in ambient conditions.

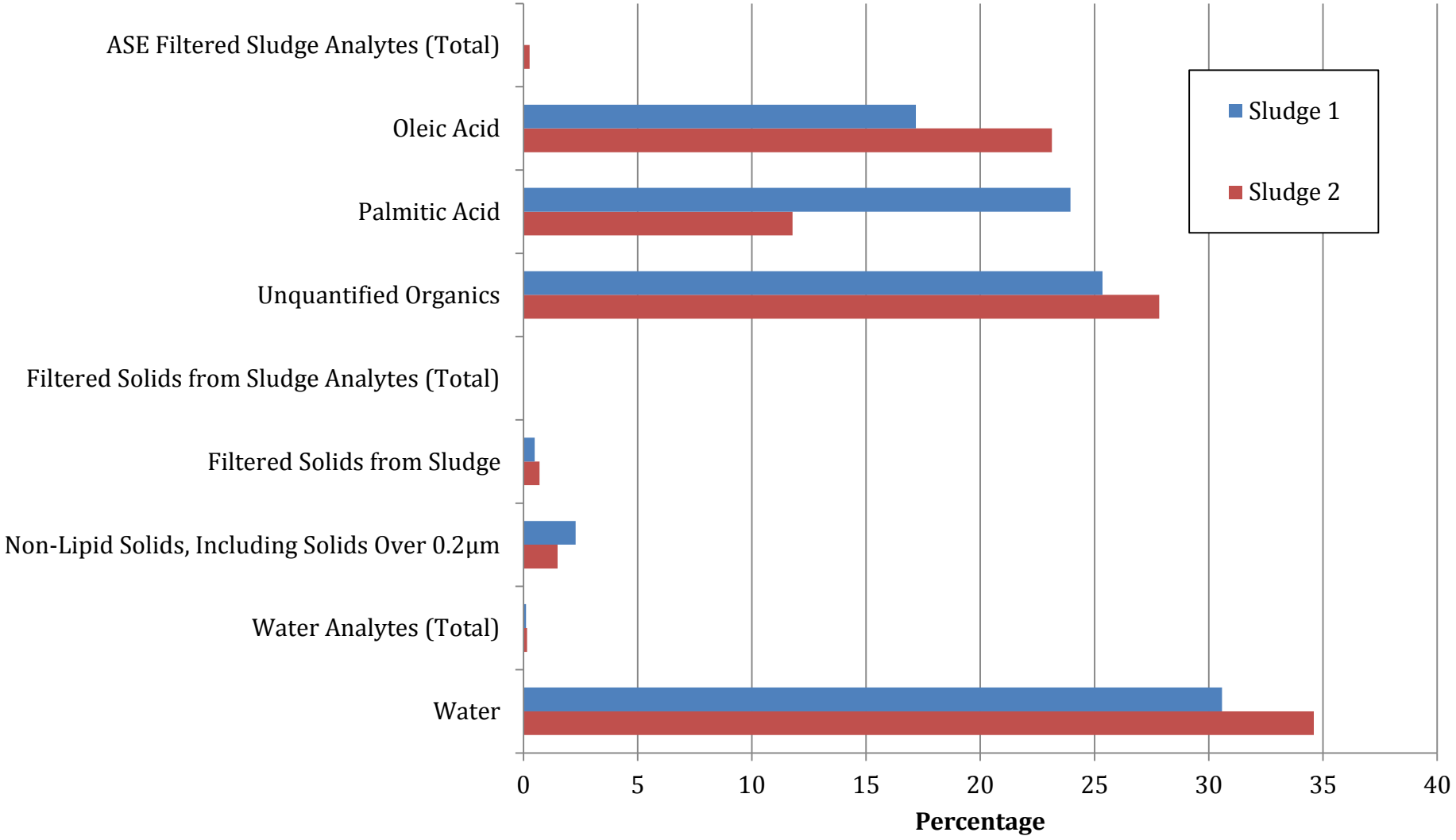


Figure 38 Sludge Compositional Breakdown

5.5 Grease/Soap Determination within the Sludge

It has been proposed by other analysts who are also researching the sludge that it is a grease/soap. The calculations below show that even if all of the calcium were to be bound to the palmitic/oleic acid to form grease, it would account for only 0.60% of the total mass of the sludge 1 and 3.59% of the total mass of sludge 2. A concentration of grease/soap this low will only contribute minimally to the structure of the sludge, not be the reason why it is of a semi-solid consistency.

Calculations in Table 21 are based upon sodium and calcium only as these are the inorganic analytes with the highest concentrations that are commonly used to make soap, which in turn form an emulsion with the oil, thus forming grease. A ratio of 1:1 palmitic acid to oleic acid is assumed for bonding of calcium/sodium to fatty acids, which gives a molecular mass of free fatty acid of 269.44g/mol. Included in the organic portion of the sludge is the ASE filtered sludge.

Table 21 Calculation of Soap Concentrations within Sludge

Inorganic Analyte	Sample	Concentration in Organic Portion of Sludge/ mg per kg of Sludge (mol)	Mass of Fatty Acid Required (mg)	Soap Percentage of Total Mass of Sludge (%)	Grease Percentage of Total Mass of Sludge (%)
Calcium	Sludge 1	135.46 (3.39)	1826.80	0.18	0.37
	Sludge 2	1302.47 (32.56)	17545.93	1.75	3.51
Sodium	Sludge 1	96.85 (4.21)	1134.34	0.11	0.23
	Sludge 2	33.97 (1.48)	398.77	0.04	0.08

It is likely that a substantial proportion of this grease is found within the filtered solids from sludge due to the high concentrations of calcium in the samples and the high concentrations of free fatty acids found within the rest of the sludge. This theory is

further backed up by the fact that calcium palmitate and calcium oleate are insoluble in solvents at standard temperatures and pressures (Harrison, 1924), which would explain why they manifest themselves after ASE. Unfortunately the full composition of the filtered solids from sludge is not known as they were not able to be characterised using any of the analytical techniques undertaken within this thesis.

Suggested methods to stop the formation of the sludge also included softening of the water used within the systems so the calcium and sodium ions would not be present to create the grease. The finding that only a small portion of the sludge may be grease has saved the factory time and money by showing that the implementation of water softening would not significantly impact upon the formation of the sludge.

6.0 Proposed Separations for the Physicochemical Analysis of a Cutting Fluid based Sludge

To undertake a high quality analysis of floating cutting fluid based sludge, which delivers an accurate and true set of results, a number of chemical separations of the sludge are required.

These divisions aim to be able to suitably separate the sludge in order to quantify the mass of all of the components in Table 20, including all of the associated inorganic analytes.

Not all of the subdivisions in the flowchart are measured directly, some are calculated using measured data from other aspects of the sludge. The aspects of the sludge that are calculated are circled in a dashed red line.

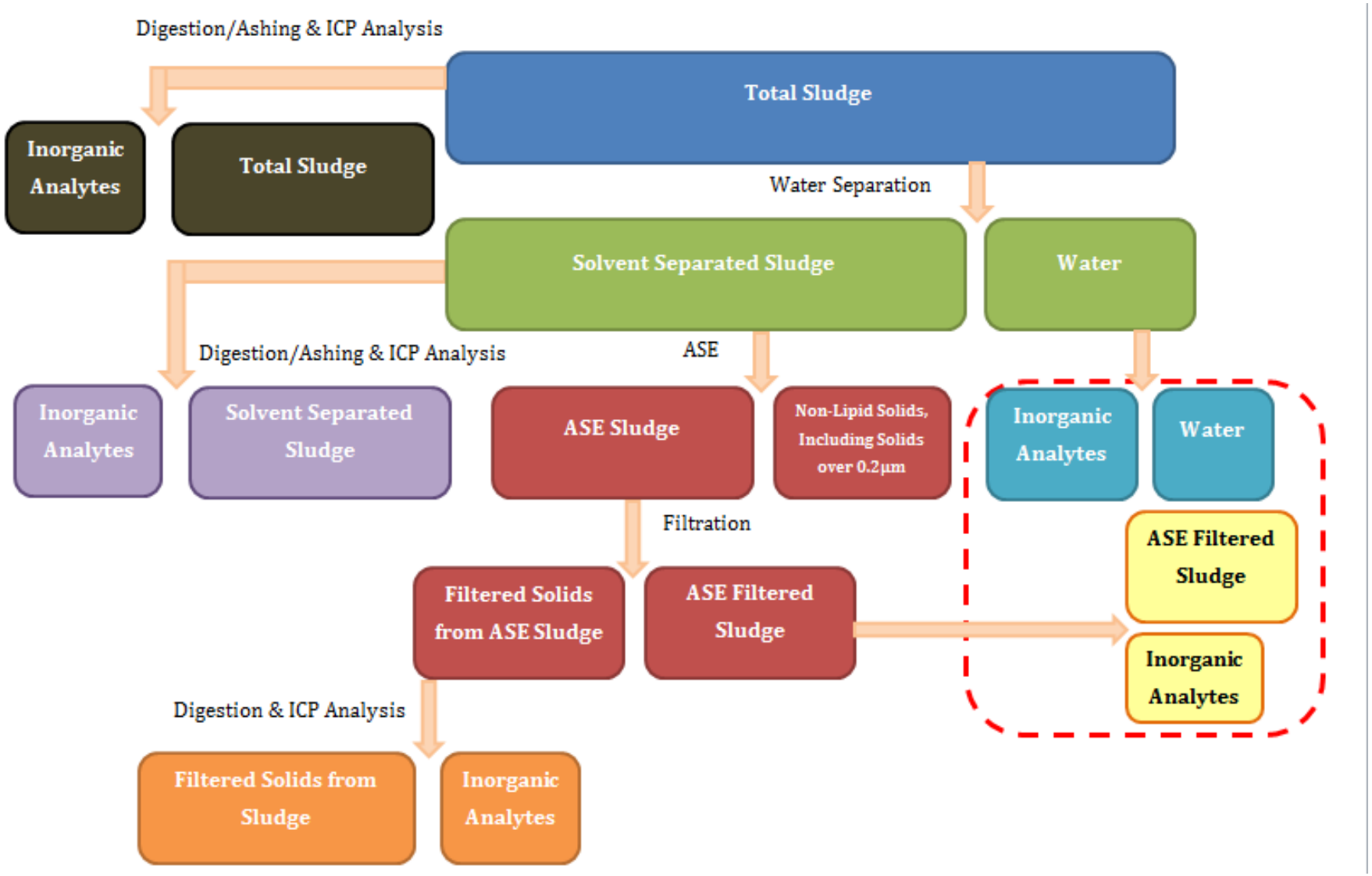


Figure 39 Flow Diagram Portraying the Divisional Analysis of the Sludge for ICP Analysis

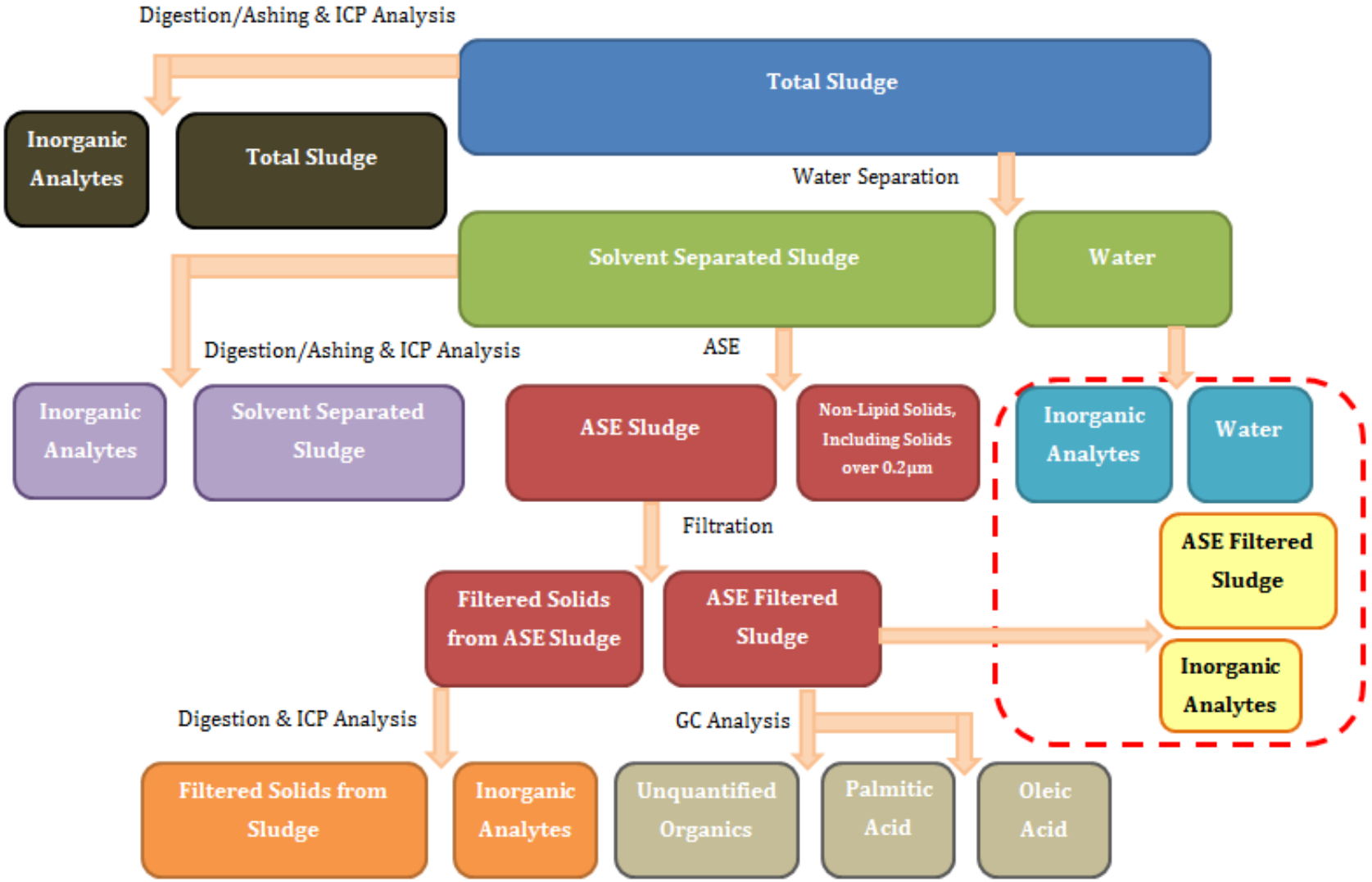


Figure 40 Flow Diagram Showing the Compositional Breakdown of the Sludge Based Upon Organic Analyses Conducted

7.0 Routes of Formation

Understanding the routes of formation is an important step in being able to halt the formation of the floating cutting fluid based sludge. If each stage of the degradation is known, then a strategic and targeted approach can deployed to halt that stage of degradation, therefore stopping the formation of the sludge.

To determine the routes of formation the chemical composition of the products needs to be known, along with the chemical composition of the sludge, both of which have been previously documented within this thesis.

The chemical composition of the two main products used at the metal machining factory are trimethylolpropane trioleate and rapeseed oil, which is also an ester, but uses glycerol as the base compound, instead of trimethylolpropane. As covered in the literature review trimethylolpropane trioleate is a triglyceride containing three oleic acids and trimethylolpropane as the base. The fatty acids in rapeseed oil vary, but on average are composed of oleic acid (60%), palmitic acid (4%), linoleic acid (20%) and alpha-linoleic acid (10%) (Gunstone, 2009).

The route of formation is theorised by the author to likely be trimethylolpropane trioleate and rapeseed oil degrading to free floating oleic acid and palmitic acid. This route is mostly biologically achieved, however low and high pH can also assist in the free fatty acid formation. Conditions within the factory machining systems are constantly monitored, with the pH being kept above 9, but most importantly the emulsion is dosed with biocide to stop the degradation of the cutting fluids. During prolonged storage in the wastewater storage tanks it is common for the biocide to be depleted, allowing the micro-organisms to become active and commence biodegradation of the oils.

The first stage in the degradation is bacterial hydrolysis of the triglyceride (VIA, 2010). This is also known as microbial rancidity. Figure 41 and Figure 42 show how hydrolysis with a neutral water solution is achieved. The equilibrium reaction in Figure 41 is relatively slow, this being due to only being in a neutral water solution. Bacterial degradation using lipases is faster (Wang et al, 2011), but still follows the same reaction. Figure 42 shows the different reactions that can take place to cleave the bond

and form the free fatty acids (VIA, 2010) and glycerol/trimethylolpropane. Evidence for this mechanism occurring is the detection of palmitic acid esters and decyl oleate within sludge 2, which are not present in the cutting fluids.

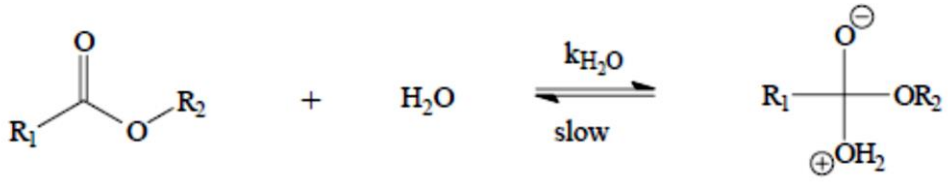


Figure 41 Primary Stage of Triglyceride Hydrolysis (VIA, 2010)

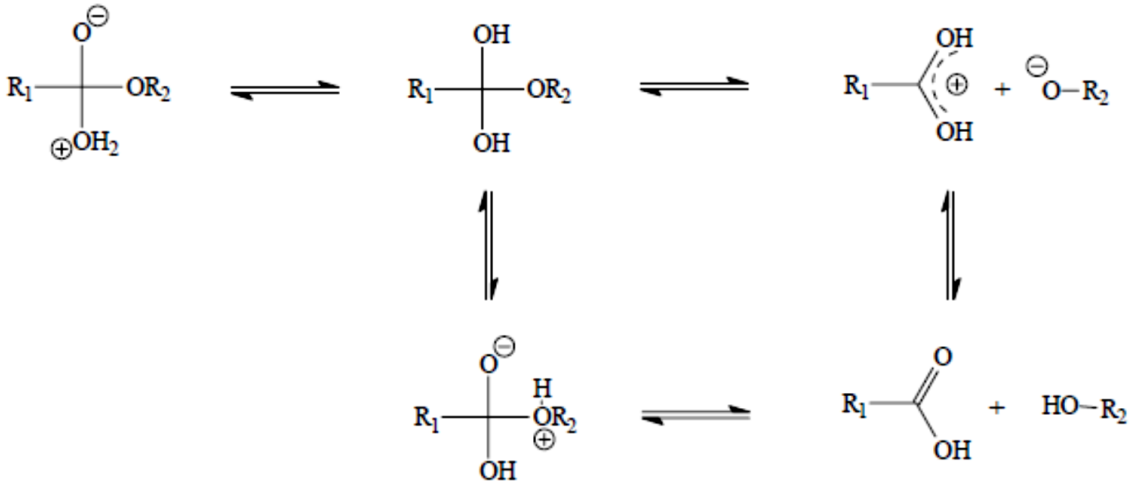


Figure 42 Further Stages of Triglyceride Hydrolysis (VIA, 2010)

The reactions seen in Figure 41 and Figure 42 will mostly occur whilst the oil is still in an emulsion, due to the requirement of water as within the reaction. During the triglyceride degradation mechanism other biodegradation processes can take place, including that of the degradation of the surfactant stabilising the emulsion, triethanolamine (West and Gonsior, 1996; Speranza et al, 2006). This degradation causes a decrease in the concentration of surfactant in the solution, which also causes a decrease in the pH of the solution caused by the generation of acid from the microbial activity. This in turn reduces the stability of micelles, thus allowing the oil and its components to separate from the solution, due to their hydrophobic nature. As the free

fatty acids densities are less than that of water they float to the surface where they agglomerate into the mass of sludge seen in the wastewater storage tanks. This phenomenon is known as creaming. The glycerol/trimethylolpropane is miscible in water and therefore stays in solution, where it is passed through ultrafiltration, thus not being detected within the sludge. This stage of the degradation is visualised in Figure 43.

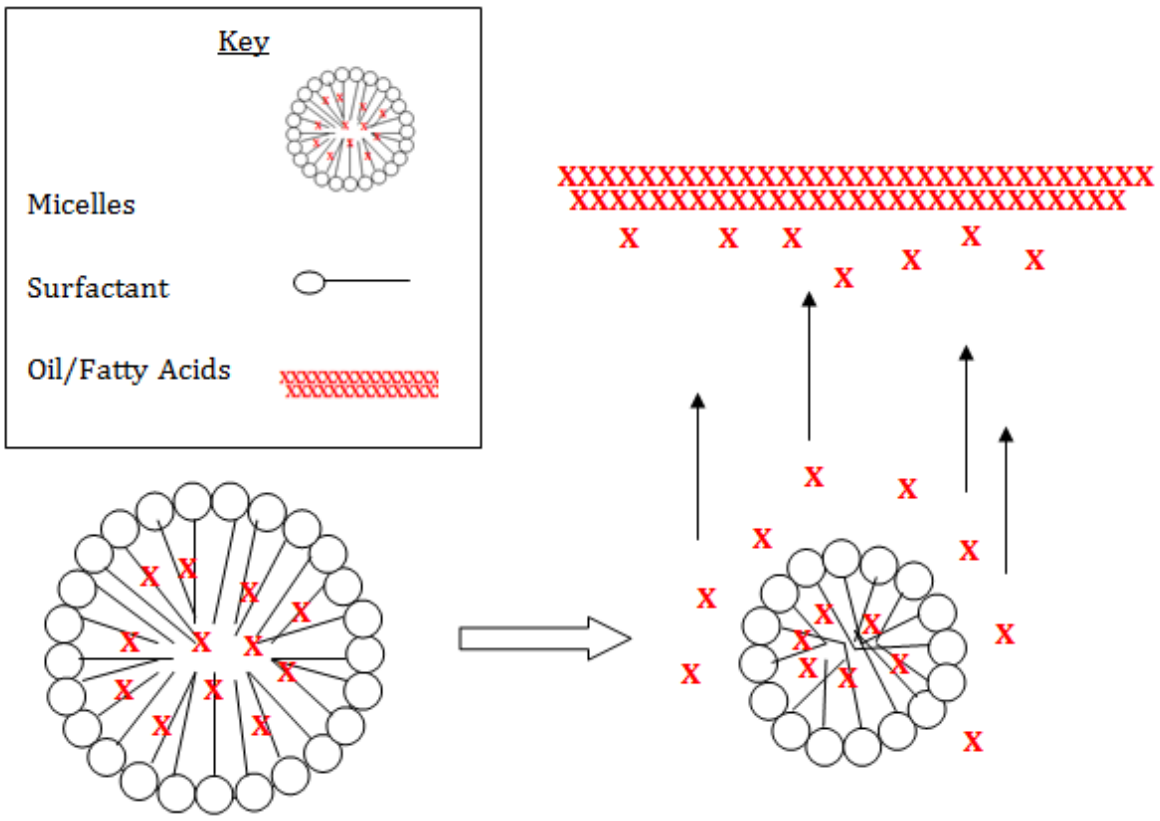


Figure 43 Emulsion Splitting

The final stage of the sludge formation is via the generation of palmitic acid through breakdown of the oleic, linoleic and alpha-linoleic acids. This step occurs as the concentration of palmitic acid found within the sludge could not solely come from the fatty acid separation of the rapeseed ester, which contains on average only 4% palmitic acid (Gunstone, 2009). The palmitic acid content of the sludge is 121m^3, based upon 1000m^3 of sludge being present, with a density of 0.85kg/m^3 an average palmitic acid concentration of 142g/kg of sludge, which is the average concentration between the five

wastewater storage tanks. The theoretical maximum palmitic acid content, in the form of esters entering the system, totals 33m^3 for the six years between 2006 and 2012.

The first stage in the process of fatty acid degradation starts by the breaking of the double bonds in the order seen in Figure 44. This may be why there is no linoleic or alpha-linoleic acid found within the sludge. Once all of the double bonds have been hydrogenated by means of biohydrogenation, then no further biohydrogenation can occur, which leaves stearic acid. Stearic acid, also known as octadecanoic acid, is then broken down to palmitic acid by means of the thioester Acteyl CoA, also known as the citric acid cycle (Gurr et al, 2002), demonstrated in Figure 45.

Alpha-linoleic, linoleic and oleic acid have almost identical retention times on the GC-MS columns. This may be why no alpha-linoleic or linoleic acid is seen in the sludge, as it may all be within the oleic acid peak on the GC-MS spectra.

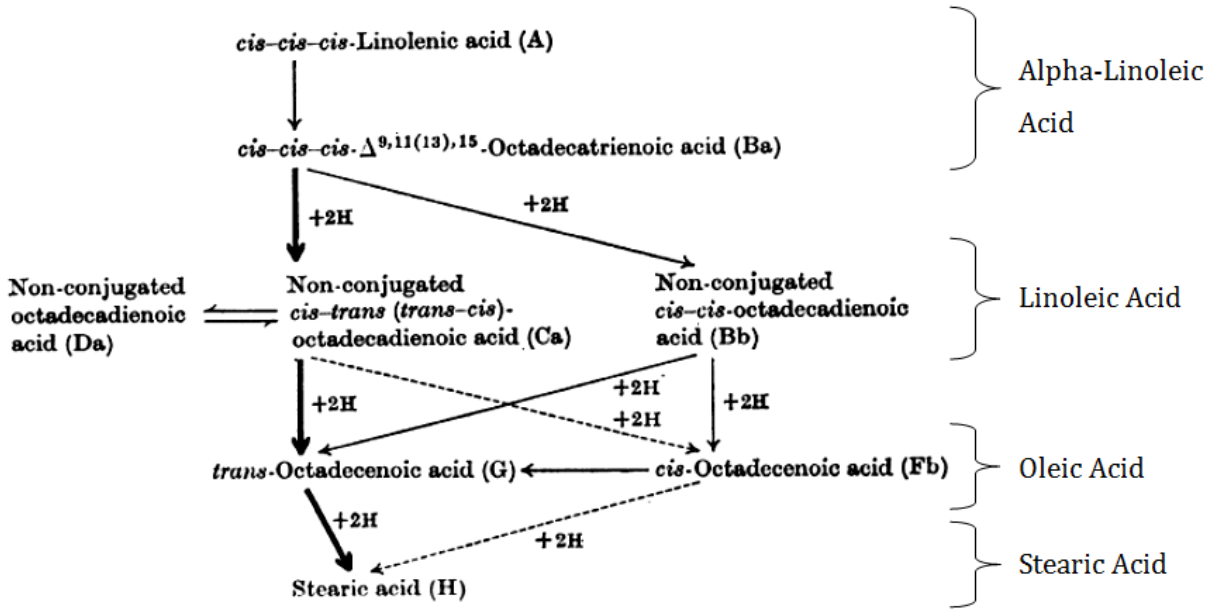


Figure 44 Biohydrogenation Order of C18 Fatty Acids (Wilde and Dawson, 1966)

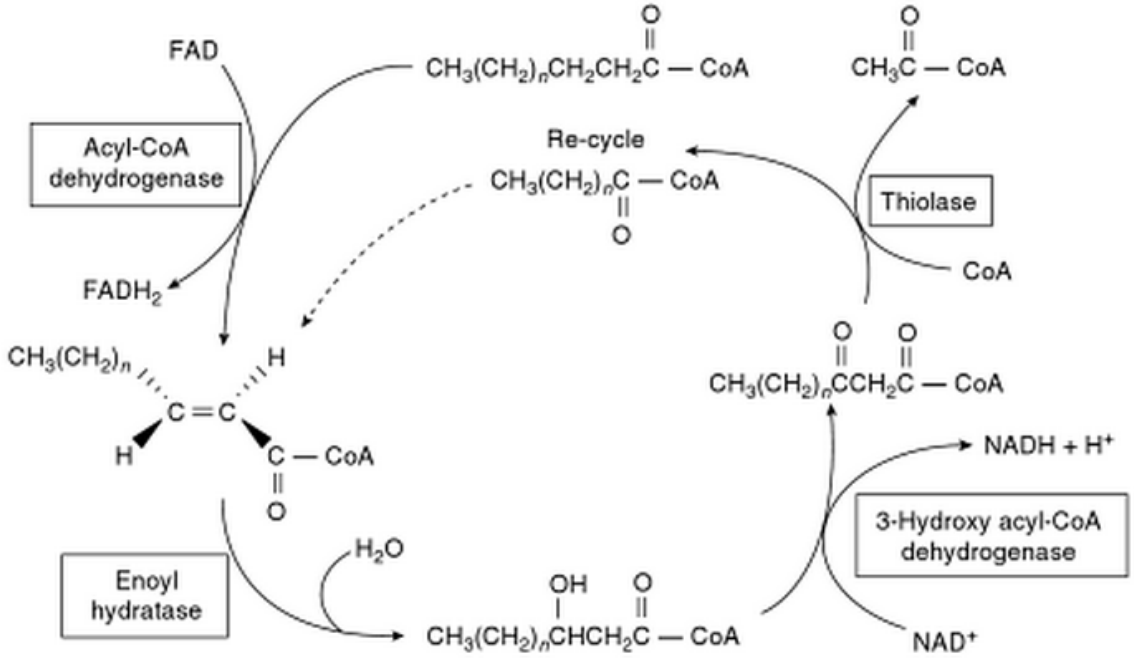


Figure 45 Beta Oxidation (Citric Acid Cycle) (Gurr et al, 2002)

The citric acid cycle is a constant cycle that breaks the fatty acids down to citric acid, which in turn breaks down to carbon dioxide and water if the conditions are favourable. The sludge has a minor component of myristic acid, which is the next breakdown product of palmitic acid via the citric acid cycle. The myristic acid peak has little area compared to the palmitic and oleic acids, which would be associated with a lower concentration. Not only is there a low concentration of myristic acid, but there is also a low concentration of stearic acid in the sludge, showing that beta oxidation is occurring.

The high concentration of palmitic acid found within the sludge is due to the inhibition of the citric acid cycle at the point of palmitic acid degradation. This is primarily caused by reduction in the surface area of sludge adjacent to the water, thus not allowing the lipases to interact with the fatty acids and degrade them further (Gurr et al, 2002). This water to fatty acid surface area is especially important as the majority of micro-organisms live within the water phase. The reduction in surface area relative to the water is caused by the build-up of the floating sludge on the surface of the water and the subsequent exclusion of water from its physical structure.

Other processes that can limit the full biodegradation of the sludge include; sludge flotation (Hwu et al, 1998), lack of nutrients and high oleic acid concentrations (Pereira et al, 2002).

Biological degradation gives a possible explanation for why the sludge is composed of the chemicals identified, but it does not explain how the sludge has accumulated to such severe levels that are seen within the tanks.

Due to the unreliability of the ultra-filtration wastewater treatment system and the large quantities of used cutting fluid that are transferred to the wastewater treatment plant during factory shutdown periods, the wastewater ends up being stored for long periods of time within the wastewater treatment tanks. To keep it from stagnating too much it is infrequently transferred from tank to tank. This creates the extended storage periods required for the microbial activity to degrade the oils and surfactants, thus releasing the oils and free fatty acids to float to the surface and form the sludge.

The accumulation of the floating sludge has originated from the design of the wastewater treatment tanks and the prolonged storage periods. The wastewater treatment tanks are designed, such that the wastewater enters the tank and is piped to a low level within the tank to avoid splash-back and spray in windy conditions, as shown in Figure 46. This allows the wastewater to be pumped in below the surface of the liquid in the tank and be extracted at the base of the tank, thus creating minimal turbulence on the surface. It is this lack of turbulence that causes the initial sludge layer to form. Once the initial sludge layer has formed it cannot be extracted as it causes blockages in the piping and valves. This layer of sludge is left to accumulate within the tank, where it builds up, forming the thick layers of sludge shown earlier in this thesis.

Accumulation of oil on the surface of wastewater treatment tanks from metal cutting processes is not uncommon and is managed by extraction using an oil skimmer. The tanks at the metal machining factory are all fitted with oil skimmers, but these do not work. They were trialled at removing the sludge when it first appeared, however due to the consistency of the sludge they were overwhelmed and so the trial was abandoned.

The micro-organisms that breakdown the oil and surfactants are abundant at the sludge-water interface and therefore are able to reproduce in greater numbers, causing a faster breakdown of the oil and surfactant. Without the sludge present the breakdown

would perhaps be slower as the bacteria would not remain in the tank in such vast quantities so as to easily seed the fresh wastewater, but instead would be disposed of into the sewer with the wastewater after treatment.

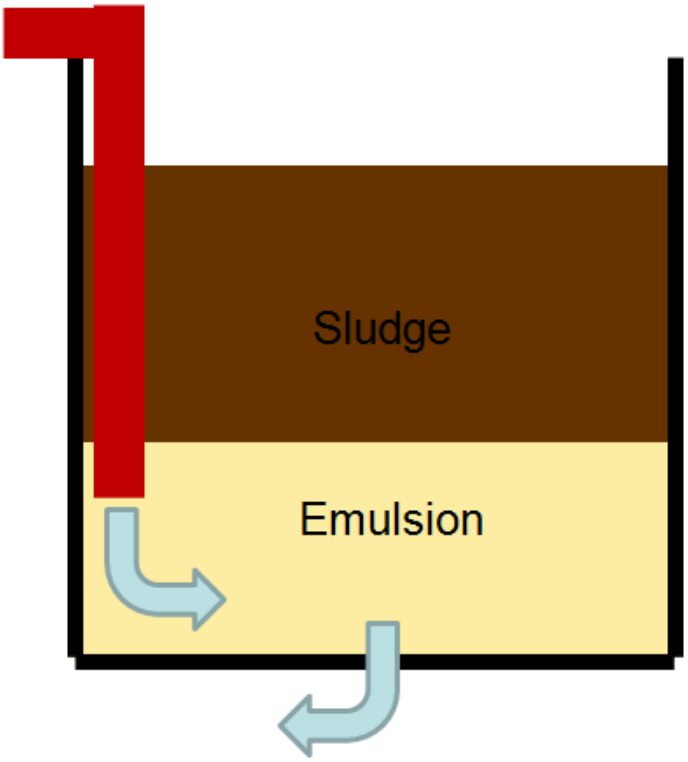


Figure 46 Cut-Out View of a Wastewater Storage Tank at the Factory

8.0 Conclusions

Based upon this study of the floating cutting fluid based sludge originating from a metal machining factory in South Wales, the following conclusions are drawn.

- A method has been developed that allows an analyst to analyse a cutting fluid based sludge to determine its chemical composition and to an extent, its physical structure.
- The example sludge that is used in this thesis is composed mostly of; water, oleic acid, palmitic acid and in low concentrations; calcium, aluminium, iron, sodium, along with traces of other elements and chemical compounds.
- Grease/soap does not form a substantial component of the sludge.
- The suspected route of formation is via the biological degradation of trimethylolpropane trioleate and rapeseed oil, which are the cutting oils predominantly used at the factory.

The findings presented in this thesis have implications on a global scale for machining with cutting fluids. It has delivered new knowledge on cutting fluid sludge that has never been experienced prior to its appearance at the metal machining factory in South Wales, UK. This is especially important as vegetable based cutting fluids, along with other techniques are seen to be the next step in machining fluids. Using the theorised routes of formation, processes can be put in place so the formation of sludge can be stopped, meaning that vegetable based cutting fluids can be continued to be used. This is beneficial to the environment as vegetable based cutting fluids have lower environmental impacts than petroleum derived cutting fluids.

Further Research

Methodologies

This study has developed a methodology for the simple analysis of floating oil-based sludge, however there are a few areas within the methodologies that require further research and development. These areas are listed below.

The water bath method for determination of the melting point range should be compared to the Thiele tube melting range method for a compound that is analysed in the capillary column. This should demonstrate the accuracy of the water bath method for the determination of the melting point range.

Staining of the emulsion will bring the benefit of knowing if the sludge is an oil in water emulsion, or water in oil emulsion, which may contribute to the understanding of why the sludge is a semi-solid.

Quantitative analysis of the sludge was conducted using a calibration curve that used only three different concentrations. To ensure accuracy in the calibration curve a minimum of five different concentrations at even spacing should be used. If available a certified standard should be used as the stock solution.

Sludge Composition

The results that are generated from the analysis of the sludge may be of great use to the analyst, however they do not include the routes of formation of the sludge from its original counterparts. This stage in the research of the sludge is particularly important as it greatly aids in developing an effective method to stop, or reduce the formation of the sludge. From this statement two stages of further research are deemed important. These being: research into the route of formation of the oil-based floating sludge and research into halting, or reducing the rate at which the sludge is formed.

Research into the route of formation would require laboratory trials that replicate the processes occurring where the sludge is forming, from the introduction of the pure chemicals into the system to the where the sludge is manifesting, in the wastewater

storage tanks. This would focus on each stage of the process, focusing on bacterial action, heat input and other forces acting upon the chemicals and water solution.

Research into developing a method to halt the formation of the sludge would be most beneficial if the conclusions from both this thesis and the previously suggested further research were combined. This would generate enough knowledge of the problem to be able to develop bespoke techniques to be trialled in a lab environment, then pilot scale and finally full scale. This would greatly benefit the industry as they would be able to continue using a more environmentally friendly product (Clarens et al, 2008; McManus et al, 2004; Willing, 2001) whilst not having the sludge formation issues they are currently experiencing.

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Appendix A – Unedited Data from Experiments

Table 22 Ashed Sludge ICP Concentrations

Element	Solvent Separated Sludge 1 (mg/L)	Sludge 1 (mg/L)	Solvent Separated Sludge 2 (mg/L)	Sludge 2 (mg/L)
Ca	119.5209539	280.7306332	182.3957939	273.9963999
Na	58.40305668	42.94677919	6.543819624	4.457583354
Mg	7.53336673	9.842241249	7.779186118	14.53109933
K	3.214426643	1.404510006	2.074437001	3.110268922
Al	40.79979319	41.88277825	88.37387226	143.7175295
Fe	461.4890371	339.3485231	541.2181588	396.5968381
Mn	11.67544348	11.74163853	13.58413218	8.713950862
Ag	0.091977225	0.215542649	0.082014612	0.62354089
As	0.085341342	0.106386639	0.070681978	0.00692303
Be	-0.006833404	-0.009941711	-0.006897522	-0.003139304
Ba	6.143523545	7.015230269	3.742160881	4.112418206
Bi	0.114260288	0.138852255	0.113303818	0.083350091
Cd	-0.000876652	-0.003210609	-0.000477079	0.01247617
Co	0.789897835	0.263511435	0.583359563	0.254084899
Cr	58.8292122	98.08857845	59.78802552	22.36049075
Cu	9.149712151	4.109335743	8.165491563	11.00662851
Li	0.025592405	0.021798805	0.02288714	0.030507956
Mo	1.979085266	1.079485515	1.291635605	1.153999698
Ni	38.14642906	4.788740976	16.52080745	3.538937984
Pb	1.694169771	0.331414581	2.208294259	2.402130762
Sb	0.696724751	1.075257506	0.647376575	0.295125174
Se	-0.055035556	-0.033436196	-0.095568957	-0.043666943
Si	72.76332801	67.32571085	73.52854325	113.2367579
Sr	1.059123407	1.509476688	0.485934376	0.704289176
Ti	1.196997779	1.092360717	1.37576949	2.149547929
Tl	0.061670813	0.076689008	0.057560342	0.016472086
V	0.217170571	0.416167004	0.250818587	0.108199636
Zn	5.60131993	2.663417427	9.876865737	14.64912215

Table 23 Digested Sludge ICP Concentrations

Element	Sludge 1 (mg/L)	Solvent Separated Sludge 1 (mg/L)	Sludge 2 (mg/L)	Solvent Separated Sludge 2 (mg/L)
Ca	22.14289286	5.75466492	45.58704038	48.10381804
Na	7.210265885	4.114230923	1.79661307	1.254663049
Mg	0.621044486	0.398663836	1.955378882	1.456224254
K	0.326710086	0.198436007	0.585873597	0.410358574
Al	3.126118971	3.267374542	17.92241282	16.23439693
Fe	2.947215656	2.021735324	29.06762054	31.56771193
Mn	0.032309072	0.01144885	0.517183634	0.503469984
Ag	0.002983296	0.003439442	0.002569146	0.00518104
As	0.011246236	0.019907092	0.002864242	0.013206474
B	0.11322117	0.060512688	0.146353929	0.067763999
Ba	0.388332874	0.386030662	0.739647718	0.6473368
Be	-0.000485313	-0.000393216	-0.000380726	-0.000393913
Bi	0.094818014	0.088514624	0.089742858	0.076542891
Cd	0.014179406	0.014633795	0.016487722	0.015893664
Co	0.006730266	0.00545753	0.015076637	0.014272314
Cr	0.047639636	0.043869982	0.146040718	0.140409318
Cu	0.580994881	0.569432248	1.547944965	1.363551503
Li	0.016456308	0.013799745	0.016875818	0.014866095
Mo	0.11138284	0.118832552	0.169553022	0.178876261
Ni	0.0770208	0.066433875	0.194973814	0.167545042
Pb	0.274927027	0.247204988	0.672197588	0.710395883
Sb	0.058565087	0.056991463	0.059304409	0.05675581
Se	0.144128274	0.149140492	0.136895711	0.142201138
Sr	0.112863098	0.06520012	0.098621351	0.091156891
Ti	0.031830531	0.030370486	0.103511342	0.08464291
Tl	-0.535093065	-0.513721622	-0.443061832	-0.48420554
V	0.005146879	0.004230977	0.014891132	0.014052405
Zn	0.440691979	0.482105958	2.49680974	2.576195076

Table 24 Filtered Solids from Sludge ICP Concentrations

Element	Filtered Solids Sludge 1 (mg/L)	Filtered Solids Sludge 2 (mg/L)	Blank Filter (mg/L)
Ca	4.175910482	9.480213998	0.335731226
Na	1.242581367	0.841613051	1.333992595
Mg	0.055689236	0.130789345	0.028940188
K	0.078704852	0.066548163	0.071264204
Al	0.267312236	0.22389103	0.156848213
Fe	0.172908237	2.835770742	0.083332432
Mn	0.014477558	0.08895834	0.011987855
Ag	0.005833445	0.004924062	0.005755607
As	0.012106637	0.008570722	0.011631536
B	0.04356515	0.044665579	0.027508241
Ba	0.014887165	0.039309441	-0.005232853
Be	0.017781905	0.017843869	0.017790721
Bi	0.015398749	0.007231661	0.00721864
Cd	-0.006054878	-0.00687453	-0.007033979
Co	0.010038626	0.01024223	0.00966994
Cr	0.008141926	0.008650748	0.005408256
Cu	0.063401038	0.076184671	0.048398835
Li	0.010973426	0.010156214	0.009982573
Mo	0.005299022	0.00688968	0.001077141
Ni	0.010959844	0.009365069	0.010143656
Pb	0.03883692	0.148908029	0.025764898
Sb	0.010775676	0.007116574	0.004869421
Se	0.023392525	0.02615804	0.023630287
Sr	0.013849343	0.017264015	0.004718097
Ti	0.020847173	0.025176961	0.02048013
Tl	-0.003226872	0.013144652	0.00165957
V	0.011931012	0.010648781	0.011282302
Zn	0.110389259	0.298795607	0.058240779

Appendix B – Reports from External Analysts

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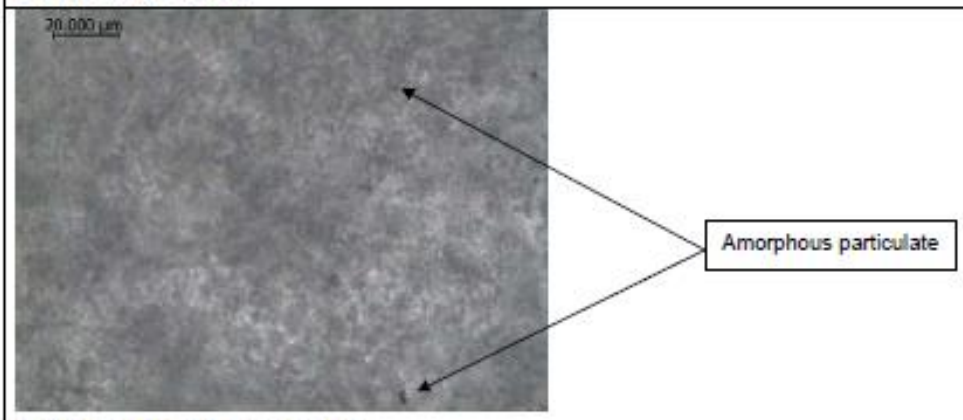
ECHA Ref.:	17212	**Issue / Rev. No.**	1.01
Client Ref:		**Date Reported:**	30th January 2012

MICROSCOPIC EXAMINATION OF SLUDGE FROM EFFLUENT STORAGE TANKS

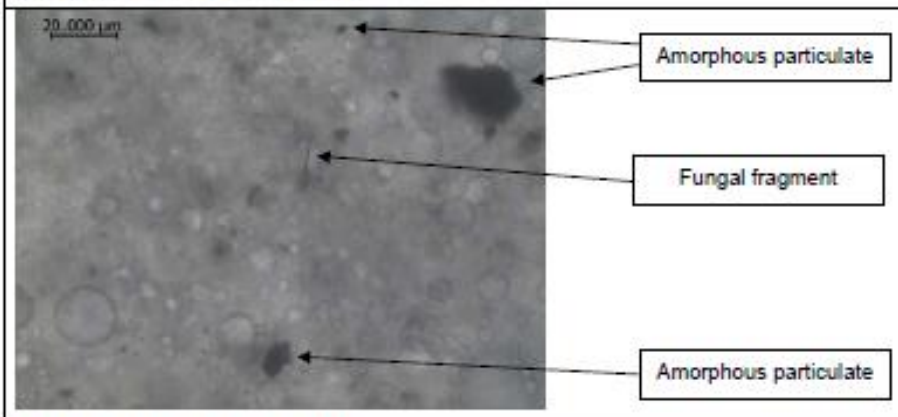
Reported by:	**Approved by:**
Dr Gareth Williams, Microbiologist	Edward Hill, Managing Director

MICROSCOPE EXAMINATION OF SLUDGE SAMPLES

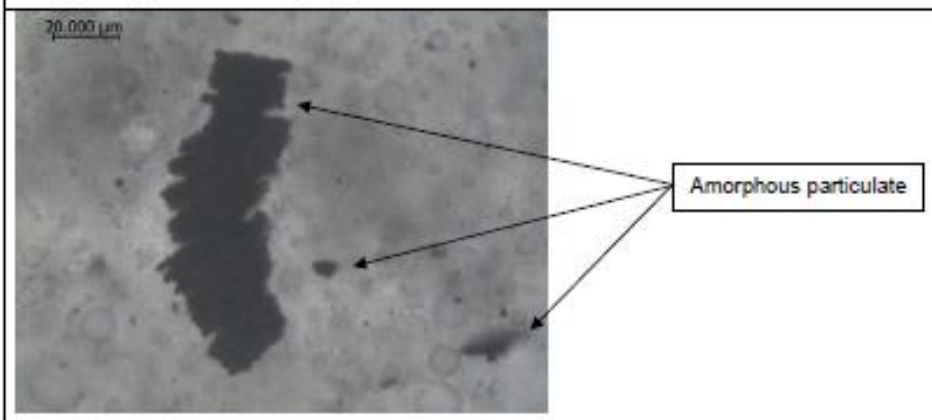
Sludge Sample Tank 4



Sludge Sample Tank 5: image (a)



Sludge Sample Tank 5: image (b)



COMMENT

We understand the sludge samples were taken from effluent storage tanks which receive spent cutting fluid emulsion. We noted a strong odour of sulphide when samples were opened (see notes on health & safety issues below). Direct microscopy indicated that the sludge samples were not generated by the activity of microorganisms; they predominantly consisted of an emulsion of spherical liquid droplets, typical in appearance to an oil emulsion. We noted the presence of a very small amount of microbial biomass, namely some fungal fragments. We also noted presence of amorphous particulate.

Health & Safety Issues

Before any clean up of sludge is undertaken, we believe that it would be necessary to consider the following specific hazards in the safety risk assessment for the remediation operation;

- Hydrogen sulphide gas
- Infection or exposure to microbial endotoxins

While we understand that the effluent tanks are open tanks, it is possible that any disturbance of the sludge may intensify the release of hazardous odours. This should be considered in a risk assessment.

A wide variety of microorganisms can grow in cutting oils and a few can cause health problems. A more specific assessment of microbiological hazards present could be established by a full microbiological analysis of sludge samples. It is quite possible Hazard Category 2 microbial pathogens e.g. *Pseudomonas aeruginosa* and *Aspergillus fumigatus*, may be present in the sludge and these present a potential infection hazard through inhalation, ingestion, eye contamination or contamination of open wounds. This should be considered in a risk assessment.

Other hazards, such as those presented by treatment chemicals and chemicals in the effluent will, of course, also need consideration in the safety risk assessment.

We would be happy to assist in any further work.