CHARACETERIZATION OF FAECAL SLUDGE AND ANALYSIS OF ITS LIPID CONTENT FOR BIODIESEL PRODUCTION

BY

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DECLARATION

I, Wilson Tamakloe, hereby declare that this thesis, "Characterization of Faecal Sludge and Analysis of its Lipid Content as a viable Precursor for Biodiesel Production", consist entirely of my work produced from research undertaken under supervision and that no part of it has been published or presented for another degree elsewhere, except for the permissible references from other sources, which have been duly acknowledged.

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DEDICATION

I wish to dedicate this work to three groups of person(s); the FS2BD (Faecal Sludge to Biodiesel) project out of which this work emerged, as well as team members of the project, my family who have stood by me offering moral support and God Almighty who has been my shield throughout this program.

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ABSTRACT

As urbanization continues to take place, the management of sanitation is becoming a major concern in urban areas of many African countries. For countries in Sub-Saharan Africa (SSA) to achieve the millennium development goal of halving the number of people without access to improved sanitation, a paradigm shift is needed. Aside sanitation, SSA countries lag behind the rest of the world in access to modern energy services, a situation that is militating against poverty reduction. This work looks at the characteristics that make faecal sludge (FS) from Kumasi a viable feedstock for the production of biodiesel, a renewable energy fuel. Faecal sludge from households were analysed for their lipid content, moisture content, total solids and pH.

On the average, the lipid content was found to be in the range of 8.84 – 9.71% of dry FS depending on the type of faecal sludge. The moisture content varied between 82.45 – 99%, while the pH was between 6.65– 8.49.

Lipids were extracted from dry faecal sludge using different methods; soxhlet extraction method (solvents: hexane, petroleum ether and methanol), 'Bligh and Dyer' method, Folch method and extraction with hexane-methanol-acetone combined-solvent system also known in this work as HMA extraction method. It was found that extraction with methanol yielded the highest amount of lipids, i.e., 12.67%w/w of the dry FS while petroleum ether extracted the least amount of lipids, i.e., 3.05%w/w of the dry FS. The lipids extracted with methanol contained 10.48% saponifiable matter while lipids extracted with petroleum ether contained 2.09% saponifiable matter.

HPLC analyses revealed that lipids extracted with hexane and petroleum ether using soxhlet method produced the highest amount of triglycerides, i.e., 16.6% and 16.0% respectively. Soxhlet extraction with these solvents showed that they contained lower levels of free fatty acids, 2.4% and 2.2% respectively, as compared the other methods.

GC analyses, indicated that all the lipids extracted from the different methods predominantly contained 16-carbon and 18-carbon fatty acids, which is a good sign since these are the kind of fatty acids in vegetable oil used as major precursors for biodiesel production.

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CHAPTER ONE

1.0 Introduction

As urbanization continues to take place, the management of waste is becoming a major public health and environmental concern in urban areas of many African countries. The concern is serious, particularly in the area of sanitation as many African countries are unlikely to meet the Millennium Development Goal (MDG) 7C of halving the number of people without access to basic sanitation [1]. In Ghana, only 13% of the population has access to improved toilet facilities according to the Water and Sanitation Monitoring Platform [2]. This means 87% of the over 24 million people lack basic sanitation in the form of improved toilet facilities. In places where there are no toilet facilities people practise open defecation. According to 'The Water and Sanitation Sector Monitoring Plan', about 20 % of Ghanaians practise open defecation. Provision of assess alone, however, is not sufficient to banish the faecal threat. The next challenge is in the mode of treatment and/or disposal. In Accra and most part of the country that lie close to the sea, faecal sludge is openly dumped in the sea untreated. This is indeed a worrying situation. Apart from Kumasi and Tamale which have an integrated faecal sludge treatment plant with its sanitary landfill, all other inland towns and villages dump faecal waste into rivers and water bodies.[4]

Faecal sludge (FS) needs adequate treatment and disposal to safeguard public health and the environment [5, 8]. One option that has not received much attenton in the treatment of faecal sludge is the recovery of resources which could eventually be channeled back into the life-chain stream for re-use. Development in the last hundred years have shown an exponential inversely proportional indication on the generation of waste as against energy production, which has led to the many proposal of waste-to-energy technologies [9]. Again, recent hikes in petroleum prices have raised questions about sustainability and

security of supplies of fossil based oil. A combination of these and other factors such as health effects and climate change have made biodiesel an attractive option as an alternative fuel source to fossil derived diesel. In the past, researchers focused their attention on conventional feedstock. In recent times, however, some researchers have looked at non-conventional feedstocks for the production of biofuels with most turning their attention to waste products like food waste, agricultural waste, and waste frying oils as reported by Math et al and Al-Zuhair et al [10, 11]. However, if an alternative source could be found in faecal sludge, then in addition to treating the faecal sludge, energy will be derived, making it a good way of dealing with sanitation.

Sewage sludge has been used to produce biodiesel via insitu trans-esterification, because it has been identified to contain some amount of lipids that could be used for this purpose.[9] Although there are some differences, sewage sludge is, to some extent, comparable with faecal sludge and night soil. This means that the technologies that are in use for treatment, resource recovery and reuse of sewage sludge may be appropriate for faecal sludge treatment as well. [6, 7]

It is important, therefore, to critically look at what faecal sludge contains, generally, and hence identify the opportunities it may present for resource recovery and/or reuse. In doing this, a 'qualitative and quantitative' analysis on the faecal sludge – characterization – will be a very important task to undertake, as it gives information that may further lead to technical recovery options to consider in dealing with the faecal threat. This is what this research work seeks to do; characterization of faecal sludge with the focus on faecal sludge collected in Kumasi and its environs and disposed of at Dompoase waste site for biodiesel production. The work examines the lipid content of the faecal sludge and its viability in the production of biodiesel.

1.1 Statement of Problem

Even though there has been some work done on characterization of sewage sludge, same cannot be said of faecal sludge as information on the characteristics of faecal sludge is hard to obtain, especially for those collected in Kumasi. This information if made available will provide the needed platform for further research and for decision making on the appropriate direction of faecal sludge treatment in Kumasi and in Ghana as a whole.

Also biodiesel, a biomass-derived fuel, has been realized as an interesting alternative fuel due to its environmental and technological advantages among others. Biomass, such as animal fats and grease, is one of the good sources for producing biodiesel. However if an alternative source could be found in faecal sludge, then in addition to treating the faecal sludge, energy will be derived, making it a better way of dealing with sanitation.

1.2 Objectives

Main Objective

The main objective of the work is to look at the characteristics of faecal sludge based on type and parameters considered, and also perform some qualitative and quantitative analysis on lipids to see the possibility of making biodiesel from them.

Specific Objectives

To characterize faecal sludge based on

- Type
- pH, moisture content, total solids, COD, Phosphorus content, Nitrogen content and lipid content

To compare various methods of extraction of lipids from faecal sludge

To perform speciation tests on the lipids obtained from the extractions

1.3 Justification

Sanitation is currently one major challenge for developing countries. A lot of efforts have been put into treatment of sanitary waste to prevent the danger they may cause to the society, however, much has not been done to recycle these wastes for reuse, especially, on faecal sludge. Faecal sludge characterization is expected to vary from place to place and with time, as many factors influence the real sludge quality. Hence, there is the need to look at faecal sludge collected from specific locations at specific periods in order to make some meaningful technological suggestions as to what it could be used for rather than dumping it in the water bodies, creating environmental and health inconveniences.

The rationale for this research is to fill the data gaps about the use of faecal sludge that limit scientific decision making and implementation. This will feed into Ghana's Environmental Sanitation Policy which seeks to develop a platform for adequate data collection to improve on the planning and management of environmental sanitation. [43]

Also, the Ghana Energy Commission's Strategic National Energy Plan targets 10% of total fuel consumed to come from renewable sources by 2020. Hence, it is eminent to find an alternative source to food grade oil in a cheaper and readily available source such as faecal sludge for production of biodiesel. [44]

More so, this work goes a long way to feed into a bigger project, 'faecal sludge fed biodiesel plant project' which seeks to build a pilot plant that convert faecal sludge into biodiesel. This project is funded by the Bill and Melinda Gates Foundation.

CHAPTER TWO

LITERATURE REVIEW

2.1 Key terms

2.1.1 Faecal Sludge

Faecal Sludge is the general term for the raw (or partially digested) slurry or solid that results from the storage of blackwater or excreta. The faecal sludge comprises varying concentrations of settleable or settled solids as well as of other, non-faecal matter [16].

2.1.2 Lipids

Lipids are a structurally diverse group of naturally occurring, water-insoluble compounds that can, for convenience, be divided into the following eight categories: fatty acyls (e.g., fatty acids), glycerolipids (e.g., monoacylglycerides, diacylglycerides, triacylglycerides), glycerophospholipids (e.g., phosphatidylcholine, phosphatidyl serine), sphingolipids, sterol lipids (e.g., cholesterol, bile acids, vitamin D), prenol lipids (e.g., vitamins E and K), saccharolipids, and polyketides (e.g., aflatoxin B1) [23].

2.1.3 Biodiesel

Biodiesel is a fuel for Diesel engines created by the chemical conversion of plant or animal oils with an alcohol to form the desired alkyl esters via transesterification. This transesterification is typically achieved using a base and/or acid as catalyst depending on the quality of the oil [24].

2.2 The Sanitation Situation

2.2.1 Sanitation Management Situation

Human settlements produce excreta that is either disposed of in nature or stocked in cesspits. This human waste is repugnant since it is known to be highly polluting and is the cause of many infections, which could lead to serious illness if poorly handled.

Management of faecal sludge is often neglected but essential for the functioning of sanitation systems and the well-being of urban dwellers [14].

Thousands of tons of sludge from on-site sanitation installations i.e. unsewered tanks, family and public toilets and septic tanks so called faecal sludges are disposed of untreated and indiscriminately into lanes, drainage, ditches, unto open urban spaces and into inland waters, estuaries and the sea in different parts of Ghana and many developing countries[15].

However, achieving a fully operational Faecal Sludge Management (FSM) chain requires well managed and sustainable services in all aspects of the collection (emptying), transport (haulage), disposal and treatment of faecal sludge [14]. Recent research into FSM has identified the broad range of criteria that need to be considered in this field. More current work has sought to tackle the wider context within which faecal sludge is managed, such as the institutional inter-relationships studied in work by Building Partnerships for Development and the role of private sector operators studied by SANDEC [16, 17].

In many towns and cities in Ghana, the two predominant forms of sanitation used are septic tanks and communal latrine facilities, although a significant number of families continue to use household pan latrines [18]. The Ghanaian government devolves powers to assemblies throughout the country and the responsibility for FSM falls to the Waste Management Department (WMD) within the assemblies. A gradual process of privatization of duties has, however, resulted in the responsibilities of the WMD, in the area of FSM, being reduced to:

- Managing the disposal/treatment facilities in Accra;
- Setting tariffs for emptying and disposal;

- Monitoring and regulating private operations; and
- Enforcing by-laws.

In this way, there is no conscious attempt on the part of government to invest into waste treatment and its possible reuse to regenerate resource to the benefit of the society. Pit latrines and septic tanks are typically emptied by large vacuum tankers and disposed of in several ways. Tankers arriving at one of the disposal points discharge faecal sludge directly into the sea or surrounding area without treatment, with resulting risks to health. Volumes disposed at Korle Gono and Lavender Hill, in Accra average 700m³/day, from an average of 100 tankers per day. [14, 18] This waste is discharged into the ocean or onto surrounding land completely untreated. In some urban areas, this faecal sludge is discharged into waste stabilisation ponds, but most of these ponds are said to be silted up and not working effectively.

2.2.2 Treatment of faecal sludge

Faecal sludge treatment can be a complicated process; several different designs are used, utilising mechanical, biological, and chemical methods, and in different combinations [29]

FS requires separation of solids and liquids, which will undergo further treatment in a second step, depending on FS characteristics, output criteria and available area. Separation of the FS solids from the liquids is the process-of-choice in FS treatment, unless FS is co-treated in an existing or planned wastewater treatment plant, and if the FS loads are small compared to the flow of wastewater. Solid-liquid separation may be achieved through sedimentation and thickening in ponds or tanks or through filtration and drying in sludge drying beds. The resulting solid and liquid fractions both require further treatment. Though the technologies used for solid-liquid separation, secondary treatment

and co-treatment with wastewater are often the same, their design and mode of operation vary. The schematic diagram below illustrates how after separation, the solid and liquid fractions of FS can be further processed or used.

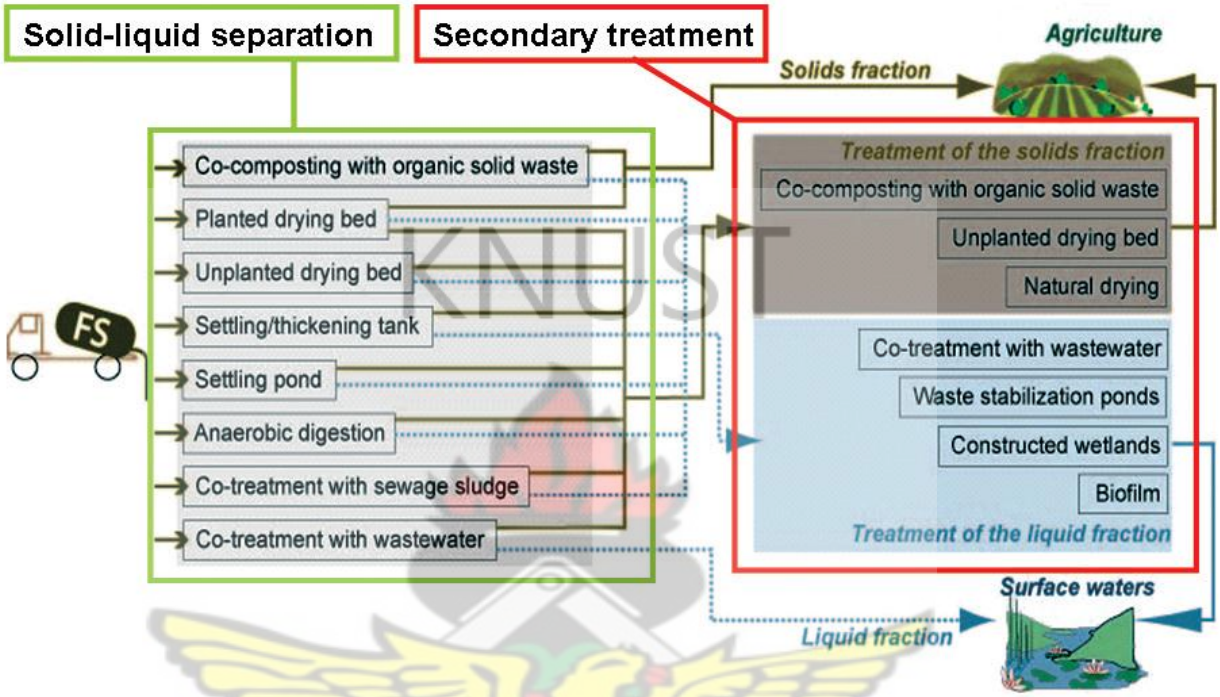


Figure. 2.1 Overview of Potential options for faecal sludge management

Source: Strauss et al., 2000 [15]

2.3 Resources in Sanitation

There is no doubt that with the aid of the UN, governmental agencies and other organisations worldwide, access to safe sanitation is gradually increasing globally. However, achieving access is not just the end of the problem itself; it is the beginning of new set of challenges which demand systematic and much higher levels of interventions. The goal of conventional waste management is to protect people and the environment from pollution at reasonable cost and dispose of waste safely. While successful in making waste seemingly disappear, this approach overlooks the valuable resources embodied in waste. Considering the fact that the availability of land for conventional disposal practices

such as landfills is likely to reach a point of scarcity, among other factors, it worthwhile then to look for new ways to reduce costs and recover revenues from waste.

2.3.1 Faecal sludge as a Resource

2.3.1.1 Co-composting of the sludge

Composting is the process with which biodegradable waste is biologically decomposed by microorganisms (mainly bacteria and fungi) under controlled aerobic and thermophilic conditions [17]. In co-composting, two or more raw materials are composted together – for instance, faecal sludge and organic solid waste. The process occurs most likely at ambient temperatures with concomitant inefficient inactivation of pathogens. In contrast, thermophilic composting, i.e. composting at 50–60 °C, is an effective process, which destroys pathogens, stabilises organic material and creates a valuable soil conditioner-cum-fertiliser [21]. Co-composting of FS and municipal solid waste is a most appropriate process, since the two materials complement each other. Human waste is relatively high in N content and moisture, whereas municipal solid waste has relatively high organic carbon (OC) content with good bulking qualities. [18]

2.3.2 Biogas from faecal sludge

Recycling and reuse of human excreta for biogas generation is an important way to get rid of health hazards from human excreta, besides uses of biogas for cooking, lighting and electricity generation. Biogas from faecal sludge has multiple benefits- improve sanitation, community health & hygiene, environment, make available quality liquid manure, in addition to uses of biogas for different purposes.

Theoretically, this option may be perfectly suited to treat higher-strength FS not yet subjected to substantial degradation. Such sludges may comprise the contents of unsewered public toilets whose vault contents are emptied at relatively high frequencies

of a few weeks. For biogas generation no manual handling of excreta at any stage is required. It involves an anaerobic digestion technology, where Hydraulic Retention Time (HRT) of feed material is maintained for 30 days. One cubic feet (cft) of biogas is produced from the human excreta of one person per day. Human excreta based biogas contains 65-66% methane, 32-34% carbon oxide about 1% hydrogen sulphide and trace amounts of nitrogen and ammonia. Biogas is stored inside the plant through liquid displacement chamber. Methane is the only combustible constituent which is utilised in different forms of energy. A thousand cubic feet (≈30m^3) of biogas is equivalent to 600 cft of natural gas, 6.4 gallons of butane, 5.2 gallons of gasoline or 4.6 gallons of diesel oil.

The municipal biogas systems globally known and operated exclusively with FS are those fed by public pour-flush toilets run by Sulabh, an Indian NGO. Below is a picture of a public toilet linked biogas plant made by Sulabh International Academy of Environmental Sanitation, Mahavir Enclave, New Delhi.

Figure 2.2: A public toilet linked biogas plant

2.3.3 Gasification of faecal sludge

Gasification can be broadly defined as the thermochemical conversion of a solid or liquid carbon-based material (feedstock) into a combustible gaseous product (combustible gas) by the supply of a gasification agent (another gaseous compound). The thermochemical conversion changes the chemical structure of the biomass by means of high temperature.

The gasification agent allows the feedstock to be quickly converted into gas by means of different heterogeneous reactions [25]. The combustible gas contains CO_2, CO, H_2, CH_4, H_2O, trace amounts of higher hydrocarbons, inert gases present in the gasification agent, various contaminants such as small char particles, ash and tars [26].

Biomass gasification is a clean, environmentally-friendly technology that has been in use for decades, but its use for human waste is still relatively new grounds in the light of scientific research.

2.4 Characterization of faecal sludge

Hypothetically one do not expect to be able to predict contents of FS as well as trends in the various parameters usually looked at during characterisation due to different kinds and source of sludge: private, public and community toilets. In contrast to sludge from wastewater treatment plant and to municipal wastewater, characteristics of faecal sludge differ widely by locality (from household to household; from city to city; from district to district). A basic distinction can usually be made between fresh, biochemically unstable and "thick" vs. "thin" and biochemically fairly stable sludges [14]. FS quality is influenced by:

- sources
- density and physical composition
- storage

- availability of oxygen during storage
- temperature and moisture

Generally certain parameters are considered in assessment of the main characteristics of FS. These are;

2.4.1 pH

The hydrogen-ion concentration is an important quality parameter for FS. The pH shows the level of acidity and alkalinity. The concentration range suitable for the existence of most biological life is quite narrow and critical (typically 6–9). Wastewater and FS with an extreme concentration of hydrogen ion are difficult to treat biologically.

2.4.2 Total solids (TS)

TS is the residue remaining after a wastewater sample has evaporated and dried at a specific temperature (103–105 °C). TS is used to assess the reuse potential of wastewater and to determine the most suitable type of treatment operation and process. Suspended solids can lead to the development of sludge deposits and anaerobic conditions if untreated wastewater is discharged into the aquatic environment.

2.4.3 Total volatile solids (TVS)

TVS are those solids that can volatise and be burned off when the TS are ignited (500 +/- 50 °C). Fixed solids (FS) comprise the residue remaining after a sample has been ignited. The ratio of the TVS to FS is often used to determine the amount of organic matter present.

2.4.4 Total Kjeldahl nitrogen (TKN)

TKN is the total amount of organic and ammonia nitrogen. Since nitrogen is an essential building block for synthesis of protein, nitrogen data is required to evaluate the biological treatability of wastewater. Insufficient nitrogen may require the addition of nitrogen to

render the waste treatable. Where algal growth in the receiving water or as part of the treatment has to be controlled (e.g. in facultative ponds), nitrogen in wastewater will have to be removed or reduced.

2.4.5 Ratio of biochemical oxygen demand and chemical oxygen demand (BOD/COD)

Typical BOD/COD ratios in untreated municipal wastewater lie within a 0.3 to 0.8 range. If the BOD/COD ratio for untreated wastewater is 0.5 or greater, the waste is considered to be easily treatable by biological processes. If the ratio is below about 0.3, either the waste may have some toxic components or acclimatised microorganisms may be required for its stabilisation.

2.4.6 Heavy metals

Heavy metals are usually found in commercial and industrial wastewater and may have to be source-controlled if the wastewater is to be reused. For example, cadmium, chromates, lead, and mercury are often present in industrial wastewater.

Table 2.1Typical characteristics of septage

Constituent	**Concentration, mg/l**	
	Range	Typical
Total solids (TS)	5,000 – 100,000	40,000
Suspended solids(SS)	4,000 – 100,000	15,000
Volatile suspended solids (VSS)	1,200 – 14,000	7,000
BOD_5	2,000 – 30,000	6,000
COD	5,000 – 80,000	30,000
Total Kjeldahl nitrogen (TKN)	100 – 1,600	700
Ammonia-nitrogen	100 – 800	400
Total phosphorus as P	50 – 800	250
Heavy metals[a]	100 – 1,000	300

[a] Primarily iron (Fe), zinc (Zn) and aluminium (Al)

Source: Metcalf & Eddy (2003)

2.5 Using Lipids to produce Biodiesel

Lipids could be used for several purposes, such as production of grease, waxes, etc. However, with the global growing demand on fossil fuel (a nonrenewable fuel source), which is the main source of fuels used worldwide, it will be prudent to consider its use as an alternative source of fuel to fossil fuel. More so, fuels from lipids source are renewable fuels, which when used will go a long way to assist in the global revolutionary against the carbon positive paths which developments in the past decades have been treading on, leading to excessive pollution of the atmosphere.

2.5.1 Lipids

Lipids are biological molecules that are insoluble in water but soluble in organic solvents. Lipids have a wider spectrum of compositions and structures because they are defined in terms of their physical properties (water solubility). Lipids are the waxy, greasy, or oily compounds found in plants and animals. However they can also be extracted from other sources, such as vegetable sources. [20]

Lipids can be grouped under two main categories (fig. 2.3):

2.5.1.1 Saponifiable lipids

These contain esters, which can undergo saponification (hydrolysis under basic conditions) (waxes, triglycerides, phospho-glycerides, sphingolipids)

2.5.1.2 Nonsaponifiable lipids

These do not contain ester groups, and cannot be saponified (steroids, prostaglandins)

Saponifiable lipids can also be divided into groups:

- Simple lipids —contain two types of components (a fatty acid and an alcohol)

- Complex lipids —contain more than two components (fatty acids, an alcohol, and other components)

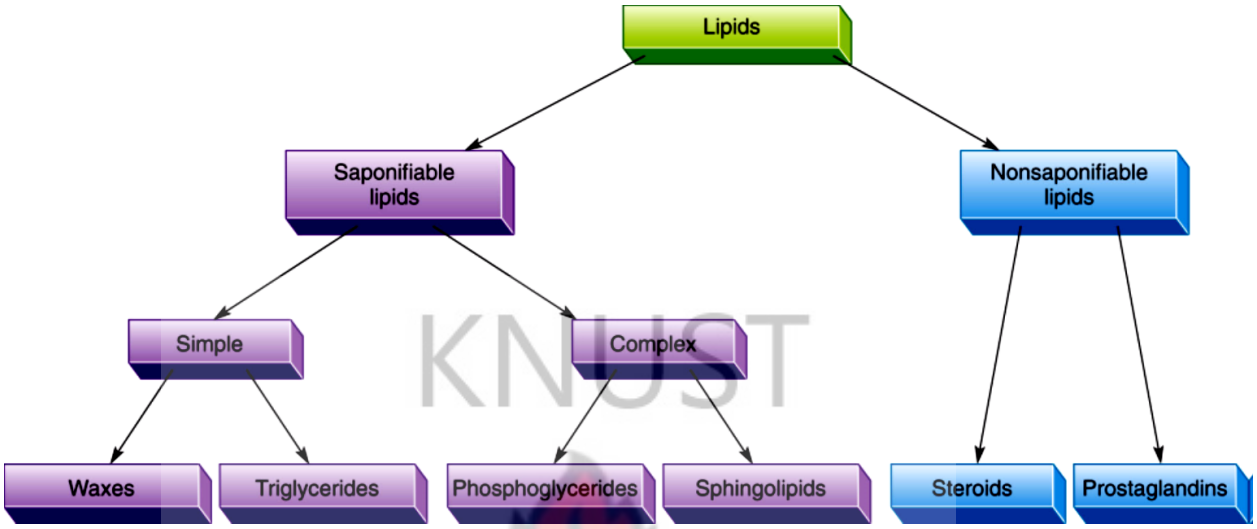


Figure 2.3: A flowchart showing the classification of lipids

A saponifiable lipid is one with an ester functional group that can be hydrolyzed under basic conditions. As a result, long chain fatty acids contained in these lipids are easily converted in transesterification process to produce biodiesel. These include triglycerides, phospholipids, glycolipids, sphingolipids, and the waxes. These lipids are known as simple lipids. [20]

2.5.1.3 Lipid classes: simple lipids and complex lipids

The lipids are generally classified into the following two groups: simple lipids and complex lipids. Simple lipids (including fatty acids, triacylglycerols, sterols, sterol and wax ester) are those that yield on hydrolysis at most two types of primary products per mole; complex lipids (including glycerophospholipids, glyceroglycolipids, ether lipids and sphingolipids) yield three or more primary hydrolysis products per mole[29].

2.5.1.3.1 Triacylglyerols

Nearly all the commercially important fats and oils of animal and plant origin consist almost exclusively of triacylglycerols (TAG) (Figure 2.4a). They consist of a glycerol

moiety with each hydroxyl group esterified to a fatty acid. Diacylglyerols (DAG) (Figure 2.4b) and monoacylglyerols (MAG) (Figure 2.4c) contain two moles and one mole of fatty acids per mole of glycerol, respectively, and are rarely present at greater than trace levels in fresh animal and plant tissues, but may be formed in stored products from hydrolysis of TAG.

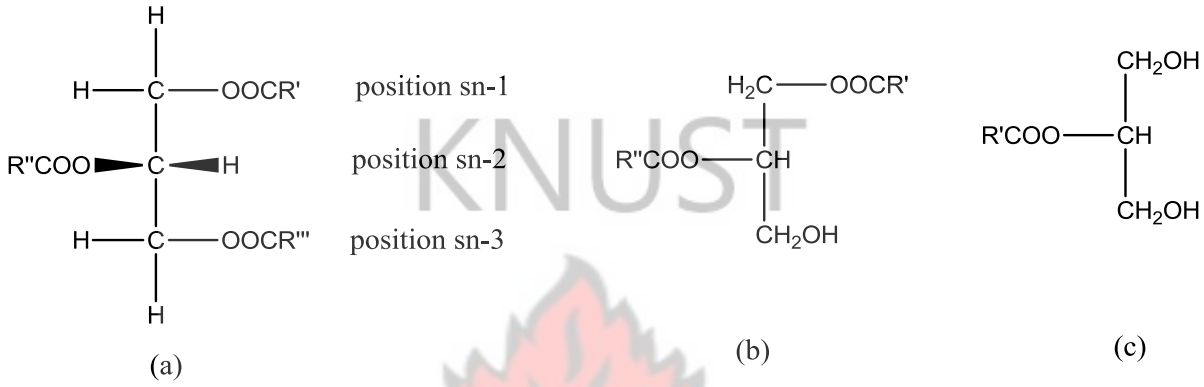


Figure 2.4 The structure of triacylglyerol, 1, 2-/2, 3-diacylglyerol and 2-monoacylglyerol

2.5.1.4 *Neutral and polar lipids*

Alternatively to the terms simple and complex lipids, ‘‘neutral’’ and ‘‘polar‘lipids, respectively, are used frequently to define lipid groups, although they are less precise and can be misleading. For example, phosphatidylethanolamin (PE) belongs to polar lipids, but it is less polar than some neutral lipids, FFAs is usually regarded as neutral lipids, but they typically have higher polarity than many polar lipids. This classification is convenient for chromatographic analysis. Neutral lipids mainly contain triacylglycerol (TAG) and cholesterol (C), free fatty acid (FFA), diacylglycerol (DAG) and monoacylglyerols (MAG) etc. The main polar lipids in most tissues are phosphatidylcoline (PC), phosphatidylethanolamin (PE), phoshatidylinositol (PI), phosphatidylserine (PS), lysopholipids. The polarity of lipids affect the solubility in solvents, thus affect the extraction efficiency of the solvents. Some lipids are acidic (free

fatty acids, Phosphatidic acid, PI and PS), and their phase distribution is therefore highly dependent of the pH in the polar phase.

2.5.1.5 *Fatty acids*

Fatty acids are compounds synthesized in nature via condensation of malonyl coenzyme A units by a fatty acid synthase complex. Fatty acids act as building blocks of lipids. In general, they contain even numbers of carbon atoms in straight chains (usually in the range C14 to C24), although the synthases can also produce odd- and branched chain fatty acids to some extent when supplied with the appropriate precursors; other substituent groups, including double bonds, are normally incorporated into the aliphatic chain later by different enzyme systems [18]. Fatty acids can either be saturated, monounsaturated or polyunsaturated depending on the number of double bonds.

2.5.2. *Biodiesel*

The components that support combustion in the oil are the basis for biodiesel. These components, called long chain fatty acids, have different properties that can be characterized by the number of hydrogen and carbon atoms and the way these atoms are bonded together. It is important to note that vegetables oils are usually composed of several fatty acids. This means that the properties of an oil will be a mixture of the properties of the fatty acids it contains. It follows that the biodiesel made from the oil will also exhibit a mixture of these properties [23, 24].

Table 2.2: the most common fatty acids with their melting and boiling points [2.12]

No. of Carbons and bouble bonds	Chemical Structure	Melting point (deg C)	Boiling point (deg C)
C8	CH3(CH2)6COOH	16.5	239
C10	CH3(CH2)8COOH	31.3	269
C12	CH3(CH2)10COOH	43.6	304
C14	CH3(CH2)12COOH	58	332
C16:0	CH3(CH2)14COOH	62.9	349
C16:1	CH3(CH2)5CH=CH(CH2)7COOH	33	-
C18:0	CH3(CH2)16COOH	69.9	371
C18:1	CH3(CH2)7CH=CH(CH2)7COOH	16.3	-
C18:2	CH3(CH2)4CH=CHCH2CH=CH(CH2)7COOH	-5	-
C18:3	CH3(CH2)2CH=CHCH2CH=CHCH2CH=CH(CH2)7COOH	-11	-
C20:0	CH3(CH2)18COOH	75.2	-
C20:1	CH3(CH2)7CH=CH(CH2)9COOH	23	-
C22:0	CH3(CH2)20COOH	80	-
C22:1	CH3(CH2)7CH=CH(CH2)11COOH	34	-

Figure 2.5 show the basic reaction involved in making biodiesel. The triglyceride is vegetable oil. R1, R2 and R3 represent any of the fatty acids listed in Table 2.2 above. Reacting one part vegetable oil with three parts methanol gives three parts methyl esters (biodiesel) and one part glycerol. In practical terms, the volume of Biodiesel will be equal to the input volume of vegetable oil.

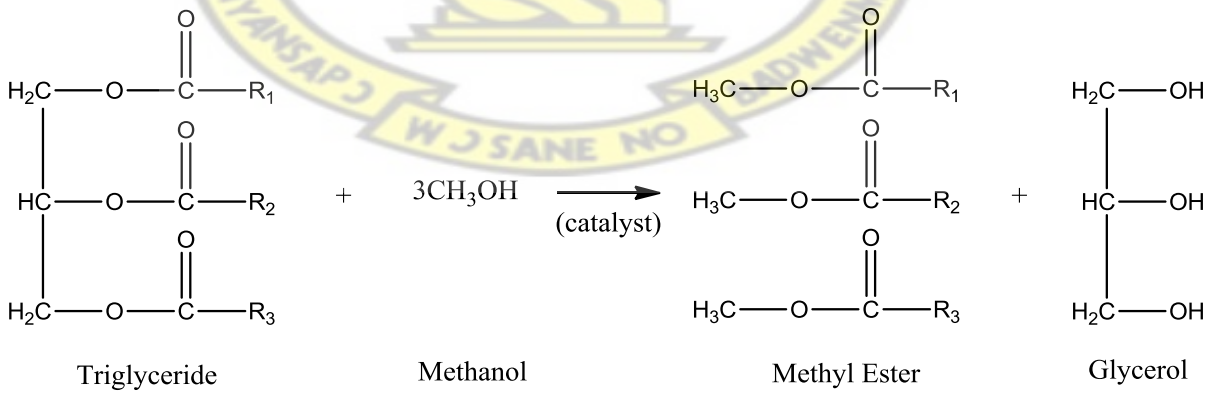


Figure 2.5: Basic Transesterification Reaction.

Free fatty acid (FFA) esterification and triglyceride (TG) transesterification with low molecular weight alcohols are the central reactions for the biodiesel production. Hence there is the need to know or determine how much of FFAs contained in a biodiesel precursor (lipids) to give an idea of what should go into the reaction.

When the oil has less than 2.5% FFA, the single step (transesterification) as shown in Figure 2.5, works well. However, for oils with higher free fatty acid content, especially waste oils, this may pose a problem [23]

2.5.2.1. Oils with high FFAs

A free fatty acid is one that has already separated from the glycerol molecule. This is usually the result of the oil breaking down after many cycles of use.[20] The presence of these FFAs in transesterification results in soap formation in a base catalysed medium and for an acid catalysed medium more catalyst need to be used in order to buffer the system for the reaction to proceed. More so water becomes a byproduct which retards the main reaction and hence has to be removed [24].

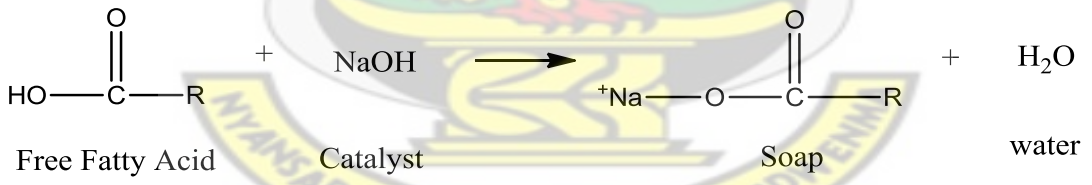


Figure 2.6: The reaction of FFA's and the catalyst NaOH.

There are several methods to treat high FFA waste vegetable oils in small-scale systems. The easiest is to mix the high FFA oil with low FFA oil. This will work for an occasional high FFA batch. Other options require esterification (two-stage process) or intentionally making soap and removing them before proceeding with the transesterification process [23]

2.6 The focus of the research

This work focuses on a feasibility study on the use of lipid in faecal sludge for the production of biodiesel. In doing this it seeks to provide information on parameters such as pH, moisture content, lipid content and the free fatty acid percent of the lipid. These parameters if determined will be crucial in predicting the path to consider in the production of the biodiesel.

The work also falls under the project, 'Faecal Sludge-Fed Biodiesel Plant: The Next-Generation Urban Facility'. This project seeks to develop a technology for the production of biodiesel from faecal sludge with an accompanying social enterprise model that channels the revenues generated to the benefit of those who cannot afford safe sanitation emptying and treatment services. The entire project is funded by Bill and Merlinda Gates Foundation [13]

CHAPTER THREE

METHODOLGY

3.1 Study Area/Location

This work was done in Kumasi the capital of Ashanti Region and the second largest city in Ghana. The Kumasi metropolis is second to the Accra metropolis, with a total geographical area of 245km^2 and population of 2,035,064 as at 2010, which is directly governed by the Kumasi Metropolitan Assembly, a government representation [30]

Kumasi has a centralized waste management system where wastes generated in its metropolis are collected and sent to the Dompoase Waste Treatment Site for treatment. These wastes include sanitary waste of all forms; thus, faecal sludge from latrines and septic tanks are all received at the site and treated. Faecal sludge was therefore collected at this site and used for the experimental work and analysis

3.2 Sampling

Faecal sludge (FS) was collected from trucks at the point of delivery at the site. Classification of Faecal sludge was based on EAWAG/SANDEC classification [31]. Three types of FS were considered in the analysis of the FS based on source; pit latrine, public septage and private septage. Sampling was done for 10 continuous weeks between September-October 2011. On each day of sampling three samples from different trucks from different locations for each type of Faecal Sludge was collected, making 9 different samples collected on each sampling day.

In taking the samples from the trucks, a three-point sampling was implored, thus taking portions of the sample at the beginning of discharge, at about mid-point and at about the end of discharge, homogenizing the portions and drawing the amount needed for storage and/or analysis.

3.3 Storage

Apart from the pH analysis which was done at point of sampling to avoid any changes that may occur between point of sampling and storage which could not be accounted for, the samples after collection were taken to the lab to continue experimental analysis. However, due to the volume of work that needed to be done on each sample, there was the need to store the samples in a freezer to preserve them and use them on subsequent working days in the lab. The samples, therefore, after every lab working day were stored in a freezer at $5^{O}C$.

3.4 Characterisation of Faecal Sludge (FS)

In characterizing the samples a number of parameters were considered: pH, moisture content/total solid, chemical oxygen demand, total posphorus content and total nitrogen content. These are parameters normally considered in assessing the use of any waste stream. In addition to these parameters, Soxhlet method of lipid extraction was used to determine the amount of lipids each sample taken contained. See APPENDIX A for the equipment, reagents and experimental procedures used for the various tests mentioned above.

3.4.1 Parameters Considered

3.4.1.1 pH

The testing was done on site just after sampling to avoid as much as possible degradation of the FS that may not be accounted for. A digital pH measuring kit with a probe was used. The electrode was first calibrated against pH buffer of 4, 7, and 10.

3.4.1.2 Moisture Content (MC)/Total Solids (TS)

The total solids (TS) was analysed based on the method described in the Standard Methods of water and wastewater analyses, APHA – AWWA – WE [35]. Moisture

content was also determined in the same process by calculation, subtracting the percent total solid from 100.

3.4.1.3 Chemical Oxygen Demand (COD)

COD was determined on the raw FS using the standard method described by APHA – AWWA – WE for water and waste water analysis. [35]

3.4.1.4 Nitrogen Content

Total nitrogen content also known as Kjeldahl nitrogen content was determined as described by the ASTM, EN 13342-Characterization of sludges, for the determination of Kjeldahl Nitrogen in soil, biowaste and sewage sludge. [34]

3.4.1.5 Phosphorus Content

Total phosphorus was determined using the method described in the EN 13342 Characterization of sludges for the determination of total phosphorus content in soil, biowaste and sewage sludge. [33]

3.4.1.6 Lipid content

This experiment was done on the dry solids of each FS sample collected. Soxhlet extraction with petroleum ether was the method used for this purpose.

3.5 Statistical Analysis of data on Characterisation of FS

A two-way ANOVA statistical tool was used to analyse the data on characterization of faecal sludge, to determine the variation in the means of the data obtained based on the sources and the weeks of sampling. A t-test was performed for the multiple comparison analysis to assess if there are differences between each of the pair means. Kruskal Wallis methodology was used for the datasets that do not satisfy the assumptions of the parametric methods. Statistical Package for Social Sciences (SPSS) software was used for the analysis. To establish relationship between the variables, the linear regression analysis was used.

3.6 Extraction and Analysis of Lipids from Faecal Sludge (FS)

In these experiments, FS from public septage sources were collected, dried, blended and used in the lipid extraction methods. Gas chromatography and high performance liquid chromatography were run on the lipids extracted from these dried FS.

Though pit latrine FS was found to contain lipids slightly more than that of public septage FS, public septage FS was chosen to work with at this stage because pit latrine contained a lot of garbage and was difficult to handle. Private septage was also not considered in this case because of its extremely high moisture content, which meant drying a lot to obtain the amount of dried faecal sludge needed for this stage.

In extracting lipids from the faecal sludge, six different solvent systems were considered, however these were used in four distinct methods; petroleum ether, hexane and methanol were used in the soxhlet extraction method, a solvent combination system of chloroform-methanol-water (2:2:1) was used in the Bligh and Dyer method, chloroform-methanol (2:1) was used in the Folch method, hexane-methanol-acetone (6:2:2) was used in the HMA method. These experiments were performed on dry faecal sludge samples.

3.6.1 Methods of Lipid Extraction

The three commonly used method for lipid extractions are Soxhlet method, Folch method and 'Bligh and Dyer'. A cold maceration method of lipid extraction involving a multi-solvent system of hexane-methanol-acetone has been found to extract lipids better than other ratios of the same constituents.

Petroleum ether, hexane and methanol were used in the Soxhlet method. Hexane is a non-polar organic solvent and hence expected to extract mostly non-polar lipids. Methanol on the other hand is a polar solvent and was also expected to give an idea of how much polar

lipids the FS contained. However, petroleum ether, which is a mixture of alkanes is a solvent commonly used on industrial scale to extract lipids from biomass.

Folch method [37] of extraction is a multi-solvent extraction system that uses chloroform-methanol (2:1). Bligh and Dyer [37] also uses chloroform-methanol-water (2:2:1) as the solvent system for its extraction. These are more balanced systems in terms of polarity, since the solubility of chloroform and methanol in water are **0.815% and 100%** respectively. They are commonly used on lab scale experiments for lipid extractions.

3.6.2 Lipid Analysis (speciation)

3.6.2.1 Analysis of Neutral lipids

The lipids extracted from the various methods were subjected to GC analysis as well as HPLC analysis. This was to determine the amount of neutral lipids- free fatty acid, monoglycerides, diglycerides and triglycerides -there are in the lipids. It was also to determine the kind of neutral lipids that were extracted with the various lipids.

3.6.2.2 Analysis of Polar lipids

Liquid chromatography was used in this experiment to identify and quantify polar lipids in the lipid extracts. There are several polar lipids that may exist in a particular lipid sample, however phospholipids have been found to be the major polar lipids and also the useful polar lipids in biodiesel production. Hence this experiment focused more on determining phospholipid content.

See APPENDIX A for the equipment, reagents and experimental procedures used for the various tests mentioned above.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Characteristics of FS

4.1.1 pH

Generally, the pH of the FS was found to be slightly basic, crossing the neutral mark by a small margin. This means that all the FS types considered (pit, private and public) have their hydrogen ion concentrations falling within the range suitable for the existence of most biological life (typically 6–9), see Chapter 2 Session 2.4.1.

Table 4.1: pH of FS type

FS type	PH
Pit	7.87±0.37
Public septage	7.48 ± 0.33
Private septage	7.41 ± 0.36

4.1.2 Moisture content / Total Solids

Generally, moisture content in all the three categories of FS was found to be very high, which also account for the low total solids content in them. Private septage was found to have the highest moisture content of 99.46%. This may be due to the type of cisterns used in Ghana. Most of the cisterns used in Ghana are about 4(l), this means a considerable amount of water is used to flush the faecal matter [40]. As shown in Table 4.2, pit latrines had the lowest moisture content. Even though traditionally pit latrines do not use water closet facilities, during empting of the latrine, water is pumped into the pit to allow for easy suction. This introduces large quantities of fresh water into the FS. Besides, in most cases, FS from sources other than pit latrines are added during collection, thus diluting the original content. For a research work like this one which may need more of the total

solid content (the dry faeces), a lot of energy will have to be invested in order to separate the solids from the liquid (e.g. using a drying process). It is necessary at this point to note that the composition of the FS sampled included other non-faecal matter e.g. papers, plastic bags and other garbage. These could affect the results significantly.

Table 4.2: Moisture content of FS type

FS type	Moisture Content (%)	Total Solids (%)
Pit	95.53 ± 2.26	4.47±2.26
Public septage	98.01 ± 1.63	1.99±1.63
Private septage	99.46 ± 0.46	0.54±0.46

4.1.3 Lipid content in FS

The amount of lipid in the various FS classification varied as presented in Table 4.3. All values are percentages of the dried matter. While public and private septage contained almost the same quantities of lipid, pit FS was different. On the average, public and private septage contained more lipids than pit FS. This may be attributed to the fact that most private and public septage are located in relatively more affluent neighbourhood than pit latrines. At 95% confidence interval, the amount of lipids in the different FS types did not differ significantly. Results do not differ significantly from widely reported values of lipid content in dry faecal matter. Human faeces are quoted to contain averagely 10±5 % of lipids by mass of the dried matter [39]. This translates to between 2.5 - 5% of the total mass of the faeces since total solids constitute about 25% of the total mass of faeces matter, the remaining 75% being water [38]. The lower lipid content reported in this work may be due to the age of the sludge and the possibility of degradation by some bacteria activities in the sludge over time. It was impossible to determine the age of the

FS sampled. All FS types had different ages ranging from two weeks to four years storage in the septic tanks.

Table 4.3: lipid content of FS type

FS type	Lipid content (%w/w of dried FS)
Pit	7.24±2.98
Public septage	9.84±3.43
Private septage	9.71 ± 0.87

4.1.4 COD of FS

The chemical oxygen demand in FS was found to be generally very high. This shows that the biological activities taking place in the FS is also high which explains why the pH ranges in Table 4.1 were recorded. High standard deviation figures were also recorded, which also indicates the high variability of COD in the various samples regardless of the type. Pit latrine FS was found to have the highest COD level, almost twice the level in Public septage FS whose COD level is also almost three times that of Private septage FS (see Table 4.4 below). Though the COD values obtained are very high, they fall within the expected levels, see Figure 2.1 in chapter two.

Table 4.4 Chemical Oxygen Demand of FS

Type of FS	COD (mg/l)
Pit	45611.67 ± 30819.27
Private	9495.36 ± 2309.09
Public	26765.85 ± 20870.52

4.1.5 Nitrogen and Phosphorus Contents of FS

The various FS were found to contain significant amounts of nutrients (N and P). Nitrogen content was generally higher compared to phosphorus content. Private septage FS recorded the lowest nitrogen content of 649.40mg/l, followed by public, 1396.63mg/l while pit latrine FS recorded the highest of 4181.58mg/l. However, the phosphorus content of public and private septage FS were almost the same even though pit latrine recorded the highest. See Table below.

Table 4.5 Nutrient content of FS

Type of FS	Nitrogen (mg/l)	Phosphorus (mg/l)
Pit	4181.58 ± 2830.22	514.47 ± 346.29
Private	649.40 ± 278.39	248.94 ± 69.79
Public	1396.63 ± 1083.73	261.74 ± 151.45

4.2 Statistical Analysis of data on Characterisation of FS

Looking at the mean and the standard deviation of the samples, which are shown in Tables 4.1, 4.2, 4.3, 4.4 and 4.5, one can see that the data is not stable, thus the variances are larger than their means. Considering the kurtosis and the skewness of the samples, it can be seen that the Total Solids (%w/w), Lipid Content (%w/w), Chemical Oxygen Demand (g/ml), Nitrogen Content (mg/l) and Phosphorus Content (mg/l) are not normally distributed. Hence it was necessary to subject the data to natural log transformation, which makes the data normally distributed for the analysis. Analysis of variance (ANOVA) method was applied on the data to see if there are differences in the mean of the various samples.

The mean, variance, kurtosis and the skewness of the original data are show in Table 4.6.

Table 4.6: Skewness of the data for public septage

	PH	*MC*	*TS*	*Lipid Content*	*COD*	*Nitrogen Content*	*Phosphorus Content*
Mean	7.477667	98.84133333	1.190667	9.842233	23839.73	2064.351	296.4103
Standard Deviation	0.325679	0.517232245	0.480237	3.434119	20870.52	1283.733	151.4473
Kurtosis	-0.92959	-0.88332638	-1.004	-1.16677	0.213102	1.318397	-0.00458
Skewness	-0.30728	-0.09964788	0.259123	-0.21995	1.031122	1.569652	0.893

The skewness explains how symmetric the data is, and a value of zero shows that the data is symmetric. However, the skewness for the datasets in Table 4.6 are different from zero, since some are negative and others positive. Also, kurtosis measures how peak or flatten the distribution of each dataset is. Moreover, a kurtosis of 3 implies the dataset is normally distributed. On the contrary, the kurtosis for each of the datasets in Table 4.6 are less than 3, which implies that the datasets have flattened normal distribution which again means that the variances are very large for these datasets.

4.2.1 Analysis of PH

Table 4.7: Levene's Test of Equality of Error Variances

Dependent Variable :PH

F	df1	df2	Sig.
1.060	8	81	.400

Table 4.7 shows the test of the equal variance assumption. From the test, since the p-value is greater than the level of significance of 0.05, we fail to reject the null hypothesis that the variances of the independent samples are the same, hence the assumption is proved. Therefore, the ANOVA test can be used to fit the faecal sludge data on log pH.

Table 4.8: ANOVA

Dependent Variable :PH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sources	.063	2	.032	14.149	.000
Weeks	.001	2	.001	.324	.724
Error	.189	85	.002		
Total	369.304	90			

a. R Squared = .254 (Adjusted R Squared = .219)

The ANOVA table 4.8 shows that pH for faecal sludge collected at the three sources is significantly different since the p-value<0.05. That is faecal sludge for Pit is different from that of Public and Private. But faecal sludge collected for weeks is not significantly different (p-value > 0.05).

Therefore it can be seen that sources of faecal sludge can actually determine the level of pH.

Table 4.9: Lack of Fit Tests

Dependent Variable:PH

Source	Sum of Squares	Df	Mean Square	F	Sig.
Lack of Fit	.001	4	.000	.059	.993
Pure Error	.189	81	.002		

The ANOVA model is good.

Table 4.10: Estimated Means of log pH based on Data Source

Dependent Variable :PH				
Data Source	Mean	Std. Error	98% Confidence Interval	
			Lower Bound	Upper Bound
Pit FS	2.062	.009	2.042	2.082
Public FS	2.011	.009	1.991	2.031
Private FS	2.002	.009	1.981	2.022

From table 4.10, we are 98% sure that the mean of the natural log pH for pit FS falls within 2.042 and 2.082. Also, we are 98% sure that the mean of the natural log pH for public FS falls within 1.991 and 2.022. Finally, we are 98% sure that the mean of the natural log of pH for private falls within 1.991 and 2.022.

Table 4.11: Multiple Comparison of FS types based on logPH

PH LSD				
(I) Data Source	(J) Data Source	Mean Difference (I-J)	Std. Error	Sig.
Pit FS (1)	Public FS	.05104*	.012183	.000
	Private FS	.06011*	.012183	.000
Public FS (2)	Pit FS	-.05104*	.012183	.000
	Private	.00907	.012183	.459
Private FS (3)	Pit FS	-.06011*	.012183	.000
	Public FS	-.00907	.012183	.459

From table 4.11 we can see that PH of FS from Pit is significantly different from Public and Private. But PH of FS from Public is not significantly different from Private.

4.2.2 Analysis of Total Solids

Table 4.12: Levene's Test of Equality of Error Variances

Dependent Variable: Total solids(%w/w)			
F	df1	df2	Sig.
2.442	8	81	.020

Table 4.12 shows the test for the equal variance assumption. From the test, since the P-value is greater than 0.05, which made the test significant.

Table 4.13: Tests of Between-Subjects Effects

Dependent Variable: Total solids(%w/w)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Sources	64.292	2	32.146	55.518	.000
Weeks	.231	2	.115	.199	.820
Error	49.217	85	.579		
Total	115.216	90			

a. R Squared = .567 (Adjusted R Squared = .547)

The ANOVA table, Table 4.13, shows that total solids for faecal sludge collected at the three sources is significantly different. That is faecal sludge for Pit is different from that of Public and Private. But faecal sludge collected for weeks is not significant.

Therefore we can see that sources of faecal sludge can actually determine the level of Total solids.

Table 4.14: Lack of Fit Tests

Dependent Variable: Total solids (%w/w)

Data Source	Mean	Std. Error	98% Confidence Interval	
			Lower Bound	Upper Bound
1	1.183	.139	.853	1.512
2	.088	.139	-.241	.418
3	-.887	.139	-1.216	-.557

Table 4.15: Estimated Means of logTS based on Data Source

Dependent Variable: Total solids (%w/w)

Source	Sum of Squares	df	Mean Square	F	Sig.
Lack of Fit	4.879	4	1.220	2.228	.073
Pure Error	44.338	81	.547		

Table 4.16: Estimated Means of logTS based on Weekly Sampling

Dependent Variable: Total solids(%w/w)

Week	Mean	Std. Error	98% Confidence Interval	
			Lower Bound	Upper Bound
1	.179	.139	-.150	.509
2	.146	.139	-.184	.475
3	.059	.139	-.270	.389

Table 4.17: Comparison of FS types based on logTS

Multiple Comparisons

Total solids(%w/w)

LSD

(I) Data Source	(J) Data Source	Mean Difference (I-J)	Std. Error	Sig.	98% Confidence Interval	
					Lower Bound	Upper Bound
1	2	1.0943*	.19647	.000	.6285	1.5602
	3	2.0691*	.19647	.000	1.6033	2.5350
2	1	-1.0943*	.19647	.000	-1.5602	-.6285
	3	.9748*	.19647	.000	.5090	1.4407
3	1	-2.0691*	.19647	.000	-2.5350	-1.6033
	2	-.9748*	.19647	.000	-1.4407	-.5090

From Table 4.17, FS is significantly different for Pit and Public, Pit and Private, and Private and Public.

4.2.3 Analysis of Lipid Content (LC)

Table 4.18: Levene's Test of Equality of Error Variances

Dependent Variable :Lipid content(%w/w)

	df1	df2	Sig.
.987	8	81	.452

Table 4.18 shows the test of the equal variance assumption. From the test, since the P-value is greater than 0.05, the test is not significant hence the assumption is satisfied. Thus, the ANOVA method can fit the faecal sludge data.

Table 4.19: Tests of Between-Subjects Effects

Dependent Variable :Lipid content(%w/w)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sources	2.561	2	1.281	8.445	.000
Weeks	.277	2	.139	.914	.405
Error	12.891	85	.152		
Total	369.070	90			

R Squared = .180 (Adjusted R Squared = .142)

The ANOVA table, Table 4.19, shows that Total solids for faecal sludge collected at the three sources is significantly different. That is faecal sludge for Pit is different from that of Public and Private. But faecal sludge collected on weekly bases is not significantly difference. Therefore we can see that sources of faecal sludge can actually determine the level of Lipid content.

Table 4.20: Lack of Fit Tests

Dependent Variable:Lipid content(%w/w)

Source	Sum of Squares	Df	Mean Square	F	Sig.
Lack of Fit	.375	4	.094	.607	.659
Pure Error	12.516	81	.155		

Table 4.21: Estimated Means of log LC based on Data Source

Dependent Variable :Lipid content(%w/w)

Data Source	Mean	Std. Error	98% Confidence Interval	
			Lower Bound	Upper Bound
1	1.899	.071	1.730	2.067
2	2.217	.071	2.048	2.385
3	1.829	.071	1.661	1.998

Table 4.22: Estimated Means of log LC based on Weekly Sampling

Dependent Variable :Lipid content(%w/w)

Week	Mean	Std. Error	98% Confidence Interval	
			Lower Bound	Upper Bound
1	1.992	.071	1.823	2.160
2	1.909	.071	1.740	2.078
3	2.044	.071	1.875	2.212

Table 4.23: Comparison of FS types based on logLC

Lipid content(%w/w)
LSD

(I) Data Source	(J) Data Source	Mean Difference (I-J)	Std. Error	Sig.
1	2	-.3181*	.10055	.002
	3	.0694	.10055	.492
2	1	.3181*	.10055	.002
	3	.3875*	.10055	.000
3	1	-.0694	.10055	.492
	2	-.3875*	.10055	.000

Table 4.23 shows that FS from Pit is significantly different from FS from Public, but is not significantly different from the FS from Private.

Also we can see that FS from Public is significantly different from FS from Pit, and is also significantly different from the FS from Private.

Finally FS from Private is significantly different from FS from Pit, but is not significantly different from the FS from Public.

4.2.4 Analysis of Chemical Oxygen Demand (COD)

Table 4.24: Levene's Test of Equality of Error Variances

Dependent Variable: Chemical Oxygen Demand(g/ml)

F	df1	df2	Sig.
1.339	8	81	.236

Table 4.24 shows the test of the equal variance assumption. From the test, since the P-value is greater than 0.05, the test is not significant; therefore, the assumption is satisfied. Hence the ANOVA method can fit the log COD data of the FS.

Table 4.25: Tests of Between-Subjects Effects

Dependent Variable: Chemical Oxygen Demand(g/ml)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sources	89.165	2	44.583	43.946	.000
Weeks	1.995	2	.998	.983	.378
Error	86.231	85	1.014		
Total	7804.487	90			

R Squared = .514 (Adjusted R Squared = .491)

The ANOVA table, Table 4.25, shows that Chemical Oxygen Demand for faecal sludge collected from the three sources are significantly different ($p<0.05$). That is faecal sludge for Pit is different from that of Public and Private. But faecal sludge collected for weeks is not significant. Therefore we can see that sources of faecal sludge can actually determine the level of Lipid content.

Table 4.26: Lack of Fit Tests

Dependent Variable: Chemical Oxygen Demand (g/ml)

Source	Sum of Squares	df	Mean Square	F	Sig.
Lack of Fit	1.525	4	.381	.365	.833
Pure Error	84.706	81	1.046		

Table 4.27: Estimated Means of logCOD based on Data Source

Dependent Variable: Chemical Oxygen Demand(g/ml)

Data Source	Mean	Std. Error	98% Confidence Interval	
			Lower Bound	Upper Bound
1	10.170	.184	9.734	10.606
2	9.612	.184	9.176	10.048
3	7.835	.184	7.399	8.271

Table 4.28: Estimated Means of logCOD based on Weekly Sampling

Dependent Variable: Chemical Oxygen Demand(g/ml)

Week	Mean	Std. Error	98% Confidence Interval	
			Lower Bound	Upper Bound
1	9.022	.184	8.586	9.458
2	9.208	.184	8.772	9.644
3	9.387	.184	8.951	9.823

Table 4.29: Comparison of FS types based on logTS

Chemical Oxygen Demand(g/ml)
LSD

(I) Data Source	(J) Data Source	Mean Difference (I-J)	Std. Error	Sig.
1	2	.5581	.26006	.035
	3	2.3344*	.26006	.000
2	1	-.5581	.26006	.035
	3	1.7764*	.26006	.000
3	1	-2.3344*	.26006	.000
	2	-1.7764*	.26006	.000

From above, FS is significantly different for Pit and Public, Pit and Private, and Private and Public.

4.2.5 Analysis of Phosphorus Content (P)

Table 4.30: Levene's Test of Equality of Error Variances

Dependent Variable: Phosphorus content (g/ml)

F	df1	df2	Sig.
1.380	8	81	.218

Table 4.30 shows the test of the equal variance assumption. From the test, since the P-value is greater than 0.05, the assumption is proved. Hence the ANOVA method can fit the faecal sludge data.

Table 4.31: Tests of Between-Subjects Effects

Dependent Variable: Phosphorus content(g/ml)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sources	30.212	2	15.106	32.837	.000
Weeks	.559	2	.279	.607	.547
Error	39.103	85	.460		
Total	2688.265	90			

a. R Squared = .440 (Adjusted R Squared = .414)

Table 4.31 shows that Phosphorus content for faecal sludge collected at the three sources is significantly different. That is faecal sludge for Pit is different from that of Public and Private. But faecal sludge collected for weeks is not significant. Therefore we can see that sources of faecal sludge can actually determine the level of Total solids.

Table 4.32: Lack of Fit Tests

Dependent Variable :Phosphorus content(g/ml)

Source	Sum of Squares	df	Mean Square	F	Sig.
Lack of Fit	.641	4	.160	.338	.852
Pure Error	38.461	81	.475		

Table 4.33: Estimated Means of logP based on Data Source

Dependent Variable: Phosphorus content(g/ml)

Data Source	Mean	Std. Error	98% Confidence Interval	
			Lower Bound	Upper Bound
1	6.000	.124	5.706	6.293
2	5.569	.124	5.275	5.862
3	4.613	.124	4.319	4.907

Table 4.34: Estimated Means of logP based on Weekly Sampling

Dependent Variable :Phosphorus content(g/ml)

Week	Mean	Std. Error	98% Confidence Interval	
			Lower Bound	Upper Bound
1	5.303	.124	5.010	5.597
2	5.495	.124	5.202	5.789
3	5.383	.124	5.089	5.676

Table 4.35: Comparison of FS types based on logP Multiple Comparisons

Phosphorus content(g/ml)
LSD

(I) Data Source	(J) Data Source	Mean Difference (I-J)	Std. Error	Sig.	98% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.4308*	.17512	.016	.0156	.8460
	3	1.3865*	.17512	.000	.9712	1.8017
2	1	-.4308*	.17512	.016	-.8460	-.0156
	3	.9557*	.17512	.000	.5404	1.3709
3	1	-1.3865*	.17512	.000	-1.8017	-.9712
	2	-.9557*	.17512	.000	-1.3709	-.5404

Table 4.35 shows that FS from Pit is significantly different from FS from Public, and is significantly different from the FS from Private.

Also we can see that FS from Public is significantly different from FS from Pit, and is also significantly different from the FS from Private.

Finally FS from Private is significantly different from FS from Pit, and is significantly different from the FS from Public.

4.2.6 Non Parametric ANOVA model for Nitrogen and Moisture Content data

The data for Moisture Content and Nitrogen Content did not satisfy the assumptions of the parametric ANOVA test. Hence a non-parametric approach was adapted.

Table 4.36 Descriptive Statistics for datasets based sample weeks

	N	Mean	Std. Deviation	Minimum	Maximum
Moisture content(%w/w)	90	4.58411	.024387	4.492	4.604
Nitrogen content(g/ml)	90	7.2612	1.01796	5.41	9.35
Week	90	2.00	.821	1	3

Table 4.37 Test Statistics for datasets based sample weeks

	Moisture content(%w/w)	Nitrogen content(g/ml)
Chi-Square	.220	.151
df	2	2
Asymp. Sig.	.896	.927

Kruskal Wallis Test

From Table 4.37, Moisture content in faecal sludge collected over week1, week2 and week3 are not significantly different.

The same applied to Nitrogen content.

Table 4.38 Descriptive Statistics for datasets based on Data Sources

	N	Mean	Std. Deviation	Minimum	Maximum
Moisture content(%w/w)	90	4.58411	.024387	4.492	4.604
Nitrogen content(g/ml)	90	7.2612	1.01796	5.41	9.35
Data Source	90	2.00	.821	1	3

Table 4.39 Test Statistics for datasets based on Data Sources

	Moisture content(%w/w)	Nitrogen content(g/ml)
Chi-Square	49.141	48.235
df	2	2
Asymp. Sig.	.000	.000

Table 4.39 shows that Moisture content for faecal sludge collected at the three sources is significantly different. That is faecal sludge for Pit is different from that of Public and Private. Therefore we can see that sources of faecal sludge can actually determine the level of Moisture content in the FS. The same applied to Nitrogen content.

4.2.7 Regression Analysis

Table 4.40 Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.895[a]	.801	.769	.491841	.801	25.164	4	25	.000	2.730

From Table 4.40, the Adjusted R Square of 77%, shows that the model is good and is able to predict precisely 77% of the variations in the Total Solids.

Table 4.41: ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	24.350	4	6.087	25.164	.000
	Residual	6.048	25	.242		
	Total	30.397	29			

From the ANOVA table, the P-value is less than 0.001 implying that all the coefficients of the independent variables are significant in the model.

Table 4.42: Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
1 (Constant)	106.499	22.244		4.788	.000		
PH.Pit	1.151	1.915	.054	.601	.553	.982	1.019
Moisture Content(%w/w).Pit	-23.758	4.727	-.650	-5.026	.000	.476	2.100
Lipid Content (%w/w).Pit	.833	.317	.336	2.628	.014	.488	2.049
Chemical Oxygen Demand (g/ml).Pit	-.094	.088	-.102	-1.074	.293	.886	1.129

From Table 4.42, the intercept, moisture content and Lipid content are significant in determining the Total solids of the faecal sludge of from the Pit. Therefore the regression model will be given as, $y = 106.499 - 23.758Moisture + 0.833Lipid$

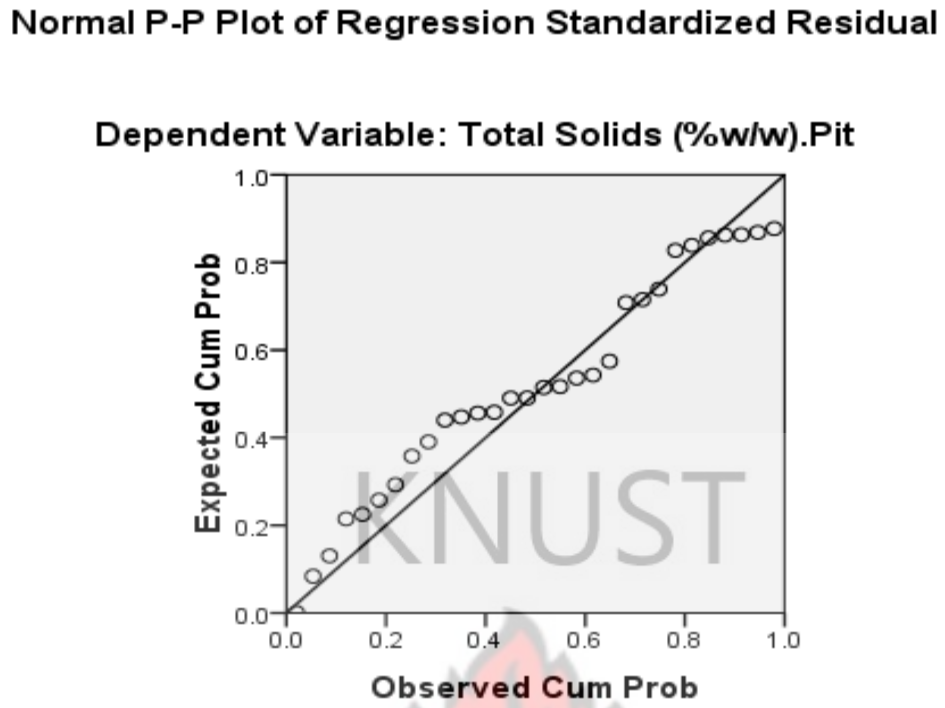


Figure 4.1: Normal probability plot of regression standard residue for TS content

From the normal probability plot above, it is clear that, the error terms are normally distributed.

Table 4.43: Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
1	.957[a]	.917	.903	.135351	.917	68.682	4	25	.000	1.986

From table 4.43, the Adjusted R Square of 90%, shows that the model is good and is able to predict precisely 90% of the variations in the Total Solids.

Table 4.44: ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	5.033	4	1.258	68.682	.000
	Residual	.458	25	.018		
	Total	5.491	29			

From the ANOVA table, Table 4.44 the P-value is less than 0.001 implying that all the coefficients of the independent variables are significant in the model.

Table 4.45: Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
1 (Constant)	178.402	41.839		4.264	.000		
PH.Pub	.195	.619	.020	.314	.756	.855	1.170
Moisture Content(%w/w).Pub	-39.245	8.939	-.472	-4.390	.000	.289	3.466
Lipid Content (%w/w).Pub	.466	.106	.427	4.396	.000	.353	2.833
Chemical Oxygen Demand (g/ml).Pub	.055	.036	.139	1.522	.141	.398	2.513

From Table 4.45, the intercept, moisture content and Lipid content are significant in determining the Total solids of the faecal sludge of from the Public.

Hence the regression model is given by $y = 178.402 - 39.245\text{moisture} + .466\text{lipid}$

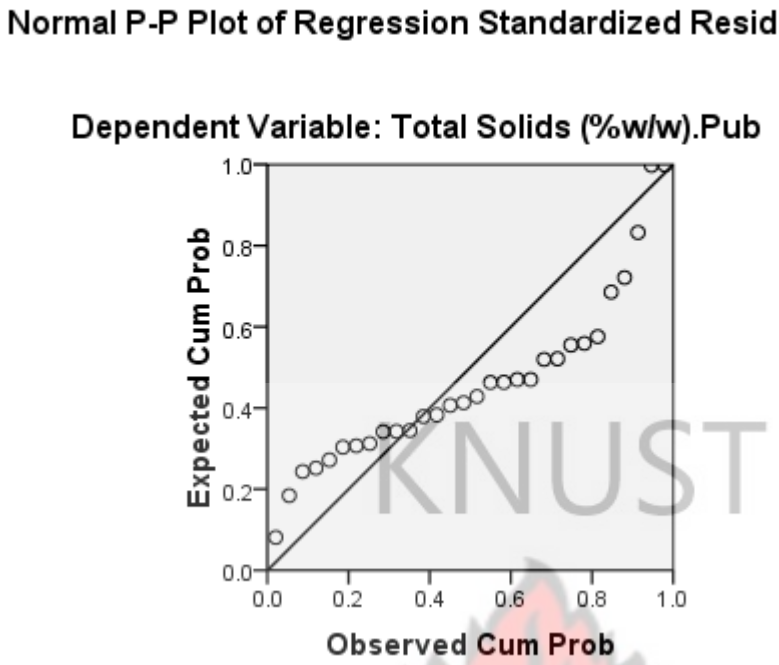


Figure 4.2: Normal probability plot of regression standard residue for TS content

From the normal probability plot above, it is clear that, the error terms are normally distributed.

Table 4.46: Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics			Durbin-Watson
					df1	df2	Sig. F Change	
1	.960[a]	.922	.909	.206202	4	25	.000	1.483

From Table 4.46, the Adjusted R Square of 91%, shows that the model is good and is able to predict precisely 91% of the variations in in the Total Solids.

Table 4.47: ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	12.497	4	3.124	73.476	.000
	Residual	1.063	25	.043		
	Total	13.560	29			

From the ANOVA table, Table 4:47 the P-value is less than 0.001 implying that all the coefficients of the independent variables are significant in the model.

Table 4.48: Coefficients

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
	B	Std. Error	Beta			Tolerance	VIF
1 (Constant)	633.380	38.753		16.344	.000		
PH.Pri	1.787	.797	.126	2.242	.034	.989	1.011
Moisture Content(%w/w).Pri	-138.721	8.395	-.943	-16.524	.000	.963	1.038
Lipid Content (%w/w).Pri	.081	.110	.042	.732	.471	.972	1.029
Chemical Oxygen Demand (g/ml).Pri	.012	.049	.013	.236	.815	.976	1.025

From Table 4.48, the intercept, and Moisture content are significant in determining the Total solids of the faecal sludge of from the Private. The regression model can be written as $y = 633.380 - 138.721\text{Moisture}$.

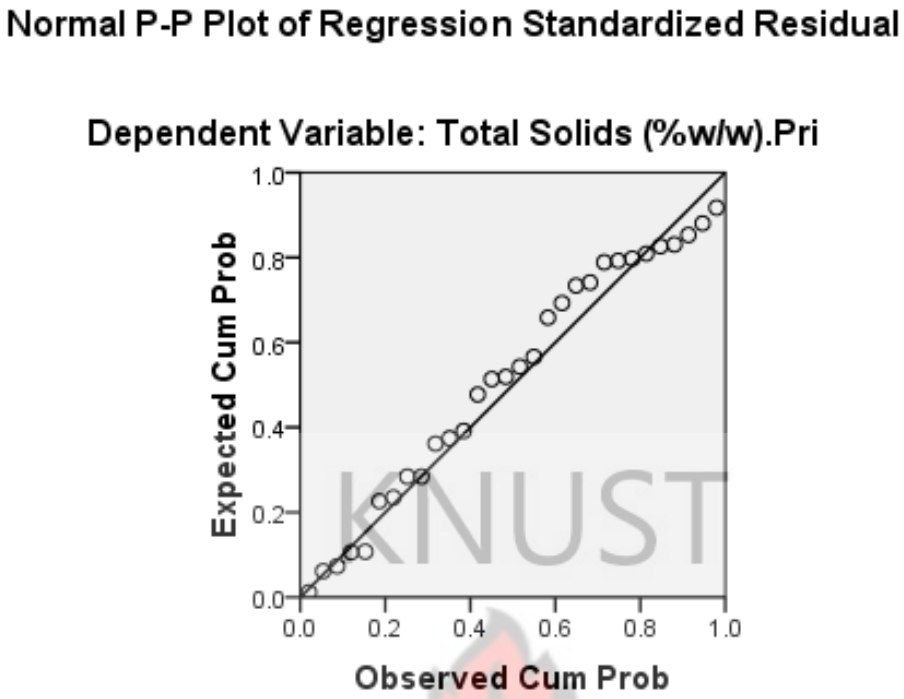


Figure 4.3: Normal probability plot of regression standard residue for TS content

From the normal probability plot above, it is clear that, the error terms are normally distributed.

4.3 Comparison of methods for lipid extraction

Among all the extraction methods and solvent systems used, soxhlet extraction with methanol gave the highest lipid content on the average, 12.67%w/w of dry FS. Lipid extractions using Folch, HMA and B&D methods also gave 6.86%w/w, 6.09%w/w and 5.63%w/w respectively. These values are almost half that of the amount of lipids obtained by methanol through soxhlet extraction. However hexane and pet-ether extracted the least amounts of lipids, 3.66%w/w and 3.05%w/w of dry FS respectively.

Though hexane and pet-ether extracted the least amounts of lipids, their lipids were found to contain very high portions of saponifiable matter, 2.69%w/w (out of 3.656%w/w total lipids) and 2.09%w/w (3.053%w/w total lipids) respectively. However, methanol lipid

extract contain the highest amount of saponifiable matter, 10.48%w/w of its total lipids. See table below.

Table 4.49: Lipid content of FS using the various extraction methods

Extraction Method	Solvent System	Average Lipid Content (%w/w)	Standard Deviation	Average Saponifiable Matter (%w/w) *
Soxhlet	Hexane	3.656	0.2297	2.69
Soxhlet	Pet-ether	3.053	0.4604	2.09
Soxhlet	Methanol	12.67	0.7104	10.48
HMA	Hexane-Methanol-Acetone (6:2:2)	6.089	0.7977	3.25
Bligh and Dyer	Chloroform-Methanol-Water(2:2:1)	5.633	1.167	3.48
Folch	Chloroform-Methanol(2:1)	6.857	0.6620	3.85

* normalized data for average saponifiable matter on average lipid content

Why hexane, methanol and petroleum ether are used in sox extraction?

Methanol is an organic chemical comprised of a single carbon surrounded by hydrogen with an alcohol group. Methanol is a polar molecule and therefore miscible in water. Hexane is another organic chemical composed of six carbons and hydrogen. Hexane is non-polar and therefore does not mix with water. Petroleum ether is a mixture of different alkanes, including hexane. Petroleum ether is also non-polar. The pre-cursors for biodiesel are called neutral lipids, which include triglycerides (TGs), diglycerides (DGs), monoglycerides (MGs) and free fatty acids (FFAs) [9]. TGs, the most desirable biodiesel pre-cursor are non-polar; MGs and DGs are considered polar molecules while FFAs are

both polar and non-polar [10]. With that being stated, it would seem that TGs would be most likely found in non-polar solvents, like hexane and petroleum ether. However, many of the fecal sludge loads received are not what one would consider fresh and could have been sitting in public septage for quite some time. This means that the TGs are most likely to have degraded to DGs, MGs, and FFAs. Therefore, it would make sense that, because these lipids are polar (at least in part), methanol extracted the highest yield of lipids.

4.3.1 Speciation of lipids: Qualitative and Quantitative analysis of fatty acids in lipids

4.3.1.1 Neutral Lipids (NL)

4.3.1.1.1 HPLC analysis of Neutral Lipids

Essentially lipids are made up of fatty acids. Fatty acids can be polar or non-polar depending on the chemical structure and solubility. Non-polar fatty acids, which are the major component of neutral lipids and sometimes referred to as such, comprises of triglyceride (TG), diglycerides (DG), monoglycerides (MG) and free fatty acids (FFA). From the table below, the weight percent amounts of the four components, as obtained from the HPLC analysis, are given for the lipids from the various methods of extraction. Hexane extracts were found to contain the highest amount of total fatty acid by mass percent followed closely by petroleum ether and HMA extracts. However, hexane and petroleum ether extracts were found to have most of its fatty acids as triglycerides, diglycerides and free fatty acids. On the other hand HMA extracts contained very high amounts of FFAs. This could be due to the presence of methanol in the HMA solvent system, which has created the enabling environment for more FFAs to be extracted. The large amount of FFAs could be as a result of hydrolysis that occur during storage or extraction process to release unesterified products.

Table 4.50: Percent (w/w) TG, DG, FFA and MG in Lipid samples

Sample	%TG	%DG	%FFA	%MG	Total % Neutral Lipids
H1	16.6%	17.9%	12.8%	2.4%	49.7%
PE1	16.0%	14.8%	13.5%	2.2%	46.5%
M1	3.3%	3.9%	11.7%	3.9%	22.8%
BD1	4.0%	5.7%	18.1%	6.8%	34.6%
F1	3.8%	5.2%	16.0%	7.1%	32.1%
HMA1	5.4%	7.9%	25.8%	6.8%	45.9%

From table 4.49 above, the results of the study showed that using Soxhlet extraction with methanol as the chemical solvent yielded 12.67% lipids based on dry weight, higher than any other method performed on the same fecal sludge sample. However, the total neutral percent lipids extracted using methanol was only at 22.8%. Using HMA yielded only 6.09% lipids based on dry weight, but 45.9% of those lipids were neutral lipids. This means that the methanol extracts contains 2.89% neutral lipids by dry mass, while the HMA solvent system extracts contains 2.80% neutral lipids by dry mass. These numbers show that even though at first glance it seems methanol extracted the most lipids, it only extracted a slightly larger percentage of usable lipids than HMA. Also, while methanol still extracted the highest percentage of neutral lipids, only 3.3% of those lipids are TGs. HMA, on the other hand, extracted 5.4% TGs. This shows the importance of looking at the types and percentage of biodiesel precursors extracted by each method. The other two methods, Bligh and Dyer and Folch, extracted 1.95 and 2.20% neutral lipids on a dry mass basis.

4.3.1.1.2 GC Analysis of neutral lipids

This experiment was to determine the kind of lipids that are in the various lipids extracted. The fatty acids content of total lipids recovered were variable and depended on lipid class composition and solvent system. It was realized that the fatty acids in the lipids ranged between $C_{13} – C_{24,}$ most of which were 16-carbon and 18-carbon fatty acids; $C_{16:0}$ (palmitic acid), $C_{16:1}$ (palmitoleic acid), $C_{18:0}$ (Stearic acid), $C_{18:1}$ (Oleic acid), $C_{18:2}$ (Linoleic acid). Predominantly palmitic acid and stearic acid comes in relatively high quantities in all the lipids. It must be noted that polar lipids are not accounted for in this analysis.

The NL of Methanol extract contained 82%w/w of total fatty acids. The total fatty acid is made of 38.74% $C_{16:0,}$ 8.66% $C_{16:1,}$ 11.33% $C_{18:0,}$ 6.68% $C_{18:1,}$ and 16.57%$C_{18:2.}$ It was identified that the NL of methanol extracts had more MGs and DGs than TGs and FFAs. This was expected since methanol is a less non-polar organic solvent and hence will follow this trend. The NL of Pet-ether extract was found to be made of fatty acids entirely, thus 100%w/w. The total fatty acid is made of 44.45% $C_{16:0,}$ 0.4% $C_{16:1,}$ 25.82% $C_{18:0,}$ 8.31% $C_{18:1,}$ and 3.08%$C_{18:2.}$ FFAs and MGs were the main lipid structures in the Folch and 'Bligh and Dyer' extracts.

In the production of Biodiesel, the two key benefits sought for are improved oxidative stability and improved cold flow properties. These properties are linked; in some cases biodiesel has to be heated to ensure flow. The warm temperatures increase the rate of fatty acid oxidation. The key fatty acids limiting cold flow quality of biodiesel are palmitic acid and stearic acid. Polyunsaturated fatty acids improve cold flow properties. Based on this analogy, the methanol extract which contains the highest amount of

Linoleic acid and relatively low amount of palmitic and stearic acid will make a more quality biodiesel.

Table 4.51: Major Fatty Acids in the FS Lipids

Lipid Sample ID	Palmitic Acid $C_{16:0}$ (%)	Palmitoleic Acid $C_{16:1}$ (%)	Stearic Acid $C_{18:0}$ (%)	Oleic Acid $C_{18:1}$ (%)	Linoleic Acid $C_{18:2}$ (%)
Hexane	50.98	2.47	28.99	5.11	1.69
Pet-ether	44.45	0.4	25.82	8.31	3.08
Methanol	38.74	8.66	11.33	6.68	16.57
Bligh and Dyer	31.89	14.4	10.66	16.9	6.42
Folch	28.01	13.44	10.08	15.25	7.49
HMA	34.23	6.76	21.82	6.79	7.88

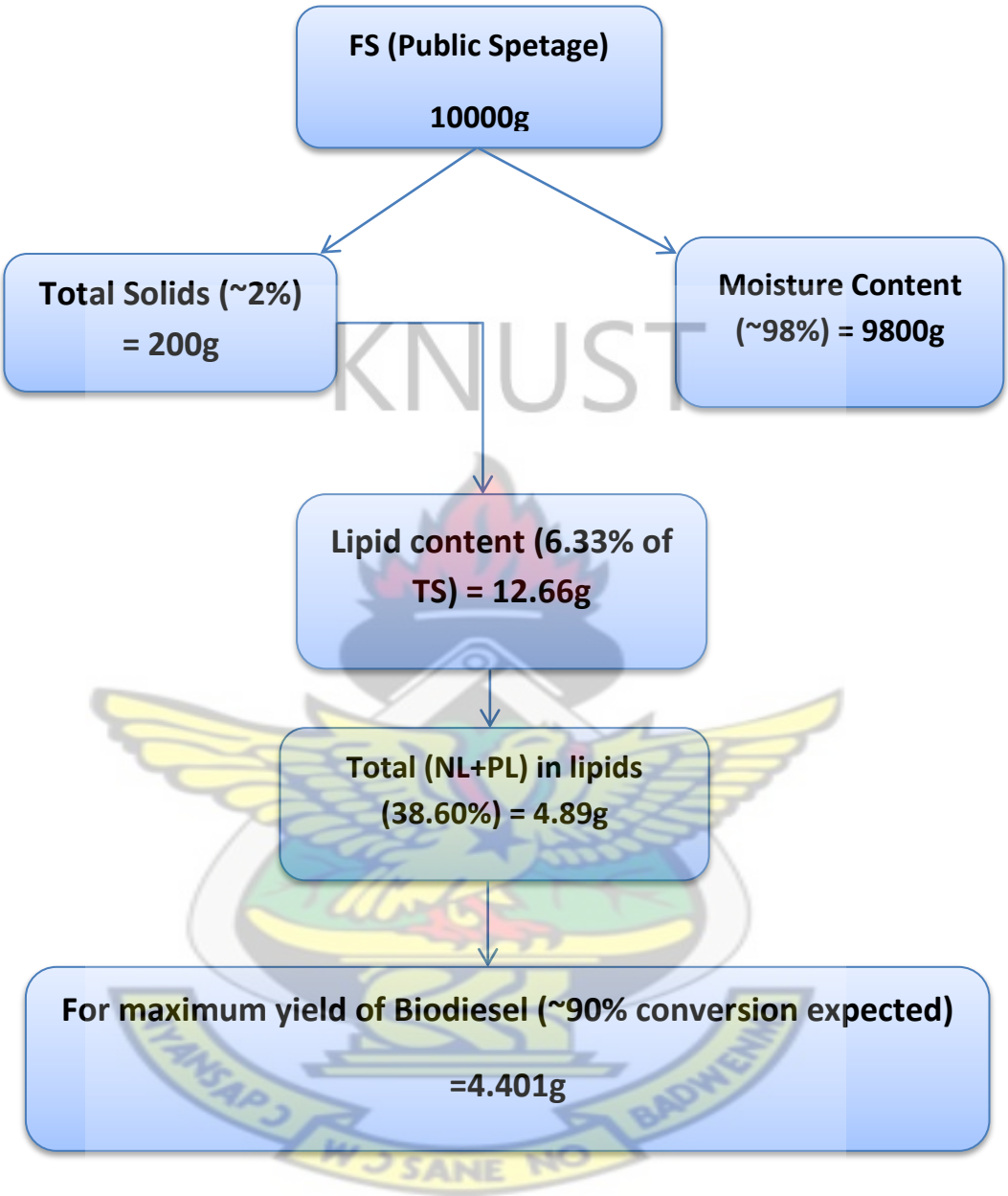
4.3.1.2 Polar lipids (PL)

The analysis of phospholipids in the various lipid extracts revealed that they contain generally, phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylsphingomyelin (PS) phosphatidylglycerol (PG), lysophosphatidylserine (LPS), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylinositol (LPI), lysophosphatidylsphingomyelin (LPS), lysylphosphatidylglycerol (LPG) and sphingomyelin (SM). The average total amount of phospholipids in the various lipids was determined to be 4292.25µg/g which represents 0.004%w/w of the total lipids. HMA extracts contained the highest amount of phospholipid, 5981.6µg/g, with petroleum ether extract extracting the least amount of 2757.9µg/g. These values are quite small and almost negligible compared to total amount lipids extracted and the average saponifiable matter content of these lipids. It was found that the content of phospholipids in crude soybean oil ranges around 1.5-2.5% [41]. These values are very small compared to the 95-97% of triglycerides in crude soybean oil. Phospholipids are considered to be

inhibitors to biodiesel production since they form emulsions between the biodiesel and glycerol layers and are difficult to handle [42]. The negligible amount and the bothersome characteristic of phospholipids justify the removal of phospholipids in conventional biofuels production. However recent research has proven that fatty acid methyl esters (FAME) can be produced from them. Hence its analysis was necessary in maximizing the possibility of making biodiesel from our lipid samples.

4.4 The possibility of producing biodiesel from FS

Biodiesel is conventionally produced from lipids (oils) from various sources. From the analysis above, the presence of fatty acids the various lipids (predominantly palmitic, oleic and stearic acids), points to the fact that scientifically biodiesel can possibly be prepared from lipids. Below is a flowchart the outlines the quantitative plausibility of obtaining biodiesel from a Public septage raw FS. The percentage figures used are based on the average values obtained for the various parameters considered in this work.



*oleic acid was used as the bases of the calculation.

*Source: (Kargbo, 2010)

Figure 4.4: Flowchart showing the quantitative plausibility of making biodiesel from a Public septage raw FS

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Currently, there is global interest and demand for biodiesel and other fuels derived from renewable biomass. However, the conventional way of making biodiesel with pure vegetable or seed oils is expensive, as the oil used contributes between 70% and 85% of the overall biodiesel production cost. Therefore oils (lipids) from waste sources need a lot of research attention.

In determining the characteristics of faecal sludge, it was found that faecal sludge from Kumasi contains averagely 98% of moisture, with the solids containing averagely 9%w/w of lipids in the dry FS. Generally the data obtain from characterization of the FS indicates a significantly high level of variation, hence could not be subjected directly to statistical analysis. However, when transformed to its log forms, it became possible to apply Analysis of variance (ANOVA) method on the data to see if there are differences in the mean of the various data obtained for the sample types given the parameters considered. Using the parametric approach of ANOVA, it was noticed that FS collected from the three sources (pit latrine, private and public septics) varied significantly in total solid, lipid, COD and phosphorus contents as well as pH. The non-parametric on the other hand showed that the moisture and nitrogen content of the FS varied also in these three sources.

In accessing the various methods of extraction, public septage FS was used as the feedstock for the extraction of lipids from various methods. It was found that the soxhlet extraction with methanol gave the best yield of lipids, extracting 12.67%w/w of the dry FS. However, this contains almost 20% of unsaponifiable matter which are not viable in the production of biodiesel. More so, the neutral lipids which contain the non-polar fatty

acids which are readily convertible to FAME contained about 82% of fatty acid. Again, only 0.004% of the lipids were made phospholipids from which FAMEs can be obtained.

In the speciation of lipids it was also found that lipids extracted with the HMA method contained the highest amount of free fatty acid (25%) with soxhlet extraction with methanol yielding the least amount of FFAs (11.7%). This may not be a major problem because, even though vegetable oil (lipids) containing a low FFA level (<1%) is currently used for commercial biodiesel production, grease traps containing 33% FFA are considered as the attractive feedstock for biodiesel synthesis because of its wide availability and low cost. In addition, acid catalysis followed by base catalysis in the reaction process has proven to work well for these high FFA lipid feedstock, and is recommended in industrial processes. Hence faecal sludge which has a maximum of 25% FFA and is currently dumped in the sea can be considered a better option than grease oil trap.

Generally the lipids obtained from the FS were found to contain palmitic, palmitoleic, stearic, oleic and linoeliec acids in relatively significant quantities. These fatty acids form key components of oils used for biodiesel production. The methanol extract which contains the highest amount of Linoleic acid and relatively low amount of palmitic and stearic acid will make a more quality biodiesel. This is due to Linoleic acids' ability to improve cold flow and oxidative stability properties of the biodiesel produced.

It is scientifically reasonable to produce biodiesel from FS, however due to the energy input in drying to obtain the solids and the minimal lipids produced upon extraction, it makes no economic sense.

5.2 Limitations

Faecal sludge contains pathogens and other disease causing agents [21, 22] and therefore too much exposure could result in serious health implications.

Information from domestic sources about the faecal sludge is hard to obtain, since most inhabitant are not literate and have no need to keep data on even when their pits and septic tanks were emptied.

5.3 Recommendation

In other to gain a better overall understanding of the composition of FS, there is the need to conduct further studies in different locations at different seasons.

Drying of the faecal sludge was one of the major challenges that was faced in this work. In the process a lot of energy is being consumed. It is recommended that a more energy efficient approach be used in drying the FS to reduced energy input which eventually affects the cost of the product. In view of this, it is recommended that investigations be conducted into wet extraction as it is less energy intensive.

The amount of lipids extracted from dry faecal sludge is less than a tenth of the mass of the faecal sludge. It is recommended that more research work be done to explore the possibility of maximizing the lipid content of the faecal sludge. The use of microbial activity could be one of approaches that may be considered for this purpose.

As a result of the high FFA levels obtained, its recommended that lipid from fresh faecal sludge be analysed in other to reduce the influence of biological and chemical degradation.

Due to the huge variability in the results obtained for the characteristics of faecal sludge, it is also recommended that a more specific sampling approach, targeting smaller communities or groups of people be used.

Finally, it is recommended that a cost analysis work be done to compare the chemical and material input to the biodiesel produced.

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APPENDICE

APPENDIX A: Experimental Procedures

A1.1: Determination of pH of the faecal sludge

Equipment:

1. pH meter
2. 250ml glass beaker
3. Magnetic stir plate/bar

Reagents:

1. Standard buffer solutions of known pH values - 4.0, 7.0 and 10.0
2. Distilled water

Procedure:

The pH meter was standardized with buffer solutions of pH 4, 7 and 10.

About 50ml of the sample, after homogenous mix, was measured and transferred into the glass beaker. A magnetic stir bar was placed into the beaker and the beaker placed on the stir plate. The sample was stirred a while to ensure homogenous mixture and then allowed to stand for few minutes to allow the pH of the samples to stabilize.

The pH electrode was then lowered into the sample in the beaker, making sure it doesn't touch the bottom of the beaker. The meter readings were allowed to stabilize and the readings were recorded appropriately.

The electrodes were then rinsed and the procedure repeated two more times to obtain triplicate results.

A1.2: Determination of Total Solid and Moisture Content of the Faecal Sludge

Equipment:

1. Ohaus Scout Pro Balance
2. Thermometer, $0^{O}C$ to $200^{O}C$ range, graduated to $1^{o}C$
3. Desiccator
4. Oven
5. Evaporating dishes, porcelain, 90 mm, 100 ml capacity

6. Water bathReagent:

1. Sample (faecal sludge)

Procedure:

The oven was allowed to equilibrate at 105^{o}C overnight. A clean evaporating dish was heated for an hour at 105^{o}C and cooled in a desiccator for 15 minutes. The weight of the dish was taken and recorded and the dish kept back in the desiccator until it was ready to be use.

The sample was homoginised by stirring and a 100ml of it was measured and transferred into the pre-weighed dish. The dish and its content were weighed and the weight recorded.

The dish and its content were then placed on the water bath and evaporated to near dryness. Drying was continued by placing the sample in the oven and heating for an hour at 105^{o}C. The sample was then cooled in the desiccator for 15 minutes and weighed.

The drying cycle of drying in the oven, cooling in the desiccator and weighing, was repeated until a constant weight was obtained.

Calculation:

$$\text{Total Solids, \%} = \frac{(B - D) \times 100}{(A - D)}$$

$$\text{Moisture Content}(\%) = 100\% - Total\ Solid(\%)$$

Where:

A = weight of moist sample + dish, g

B = weight of dried sample + dish, g

D = weight of evaporating dish, g

A1.3: Determination of Chemical Oxygen Demand on FS

Dichromate Reflux Technique Standard Method.

Equipment

1. 500-millilitre (ml) Erlenmeyer flask with standard (24/40) tapered glass joints
2. Friedrichs reflux condensers (12-inch) with standard (24/40) tapered glass joints
3. Electric hot plate or six-unit heating shelf
4. Volumetric pipettes (10, 25, and 50ml capacity)
5. Burette, 50 ml - 0.1 ml accuracy
6. Burette stand and clamp
7. Analytical balance, accuracy 0.001gram (g)
8. Volumetric flasks (1000ml capacity)
9. Boiling beads
10. Magnetic stirrer and stirring bars

Reagent

1. Potassium dichromate (K2Cr2O7) 0.25N
2. Sulphuric acid (H2SO4) / silver sulphate (Ag2SO4) solution
3. Mercuric sulphate (HgSO4) crystals
4. Ferrous ammonium sulphate (FAS) [Fe(NH4)2(SO4)2], approximately 0.01N
5. Ferroin indicator (1, 10-phenanthroline and ferrous ammonium sulphate)

Procedure

50ml of the sample (faecal sludge) was measured and placed in a 500ml refluxing flask. A blank was prepared using 50ml of distilled water. About 5-7 glass boiling chips were added to the flask. 1g of mercuric sulphate ($HgSO_4$) was then added followed by 5ml of concentrated sulphuric acid-silver sulphate solution. The content of the flask was mixed well until the $HgSO_4$ dissolved in solution.

25ml of 0.25N $K_2Cr_2O_7$, potassium dichromate was added and whole mixture stirred. While stirring, an additional 70ml of concentrated sulphuric acid-silver sulphate solution was added.

After thorough mixing, the flask was attached to a reflux condenser and heat was applied at 100^oC. It was then allowed to reflux for 2 hours.

Five sets of samples were run at the same time. A reagent blank prepared under the same conditions was made to reflux with each set of samples.

The whole set-up was made to cool to room temperature after the refluxing period. The content of flasks were transferred into volumetric flasks and made to 350ml with distilled water.

5 drops of Ferroin indicator was added to each flask and with a magnetic stir bar and plate, each flask was titrated against 0.1N Ferrous Ammonium Sulphate to the first red-brown endpoint.

Calculation:

$$\mathrm{COD}\left(\frac{\mathrm{mg}}{\mathrm{l}}\right) = \frac{(a-b)(N)\times 8000}{Sample\ size(ml)}$$

Where:

a = ml Fe $(NH_4)_2(SO_4)_2$ used for blank

b = ml Fe $(NH_4)_2(SO_4)_2$ used for sample

N = normality of FAS titrant (Fe $(NH_4)_2(SO_4)_2$)

ml sample = the actual volume of sample used before dilution

A1.4: Determination of Phosphorous

Equipment:

1. A digestion block
2. A spectrophotometer
3. Beakers (250ml)
4. Volumetric flasks (50ml)

Reagents:

1. 5N H_2SO_4 (Sulphuric acid):
   A litre clean beaker was placed in cold water and 500ml of distilled water was measured and transferred into it. 148ml of Conc HCl was measured and poured down the side of the beaker into the distilled water while stirring, to avoid explosion.Ammonium molybdate/ antimony potassium tartrate solution:

Made by dissolving 12 g ammonium molybdate (NH4)6Mo7O24. 4H2O) in 250 ml of warm (50^{O}C) distilled water. Then separately dissolving 0.291g of antimony potassium tartrate ($KSb.C_4H_4O_6$) in 100ml distilled water. Both solutions were added to 1000 ml of 5 N H2SO4, mixed thoroughly and diluted with distilled water to 2 litres. The final solution was the transfer to a reagent bottle for storage.

2. Ascorbic acid reducing agent:

   Prepared by dissolving 2.108-g ascorbic acid (C6H8O6) in 400 ml of ammonium molybdate/antimony potassium tartrate solution and mixing well.

3. Standard phosphate solution, 1000 ppm P:

   Prepared by weighing 1.0982 g of oven- dry KH_2PO_4; dissolving and making to 250 ml mark with distilled water (1 ml = mg P).

   10 ppm P working solution: 10 ml of the standard stock solution above (1000 ppm P) was diluted to 1 litre with distilled water.

Procedure:

5 ml of the supernatant clear wet-ashed digest solution was pipetted into a 50 –ml volumetric flask. About 20-ml distilled water was added to each flask. 10 ml of the ascorbic acid reducing agent was added to each flask, beginning with the standards (see below). Made to 50ml with water; it was given a stopper and shaken well. Allowed to stand for 1 hour to permit colour development. The standards and sample absorbance (blue colour) were measured at 420nm wavelength setting in a spectrophotometer.

Standards.

The standards were prepared by pipetting 0, 1, 2, 3, 4, 5, and 6 ml of the 10 ppm P working solution (above) into 50 ml volumetric flasks. 10 ml of the ascorbic acid reducing solution was added to each flask and made to the mark with distilled water. Allowed to stand for 1 hour and read the absorbance exactly like the sample solutions above. The standards contained 0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ppm P respectively.

Calculations.

P content (µg) in 1g of sample $= C \times df$

P content (g) in 100g of sample (%P) $= \frac{C \times df \times 100}{1000000} = \frac{C \times 1000 \times 100}{1000000} = \frac{C}{10}$

Where

C = concentration of P (µg/ml) as read from the standard curve;

df = dilution factor, which is 100 × 10 = 1000, as calculated below:

- 1g of sample made to 100ml(100 times)
- 5 ml of sample solution made to 50ml(10 times).

1000000 = factor for converting µg to g

A1.5: Determination of Total Nitrogen

Equipment:

1. A Kjeldahl digestion and distillation unit
2. Conical flasks
3. Burettes
4. Pipettes

Reagent:

1. Borate buffer, Indicating boric acid, 6N Sodium hydroxide solution, 0.02N Sulphuric acid,
2. Conc. Sulphuric acid (Conc. H_2SO_4)
3. Copper Sulphate ($CuSO_4.H_2O$) (AR – grade)
4. Potassium sulphate or anhydrous sodium sulphate (AR – grade)
5. 35% NaOH, sodium hydroxide solution: dissolve 350g of solid NaOH in water and dilute to 1Litre
6. 0.1M NaOH by dissolving 4g of NaOH in water and make the volume up to 1Litre. Standardize against 0.1N potassium hydrogen phthalate or standard H_2SO_4
7. 0.05M H_2SO_4: prepare approximately the standard acid solution and standardize against 0.1M sodium carbonate
8. Methyl red indicator

Procedure:

1g of the sample was weighed and placed in a Kjeldahl flask. 0.7g of copper sulphate was weighed and added to it, followed by 1.5g of K_2SO_4 and 30ml of Conc. H_2SO_4. The whole mixture was heated gently until frothing ceased. The mixture was then boiled briskly, until the solution was clear and digestion continued for at least 30minutes.

The flask was removed from the heater and allowed to cool. 50ml of distilled water was added to the flask and it was transferred to a distilling flask.

25ml of the standard acid 0.05M H_2SO_4, was placed in the receiving conical flask so that there was an excess of at least 5ml of the acid. 3 drops of methyl red indicator were added to the flask. 30ml of 35% NaOH was then added to the distilling flask in such a way that the content do not mix. The content was heated for 30-40minutes to distil the ammonia.

The receiving flask was removed and outlet tube rinsed into the receiving flask with small amount of distilled water. The excess acid in the distillate was then titrated against 0.1M NaOH. The blank on the reagents was determined using the same quantity of standard acid in the receiving conical flask.

Calculation:

$$N(\%) = \frac{1.401\left[\left(V_{1M_1 - V_2M2}\right) - \left(V3_{M1} - V4_{M2}\right)\right]}{W} \times df$$

Where:

V_1 – millilitres of standard acid put in receiving flask for samples

V_2 – millilitres of standard NaOH used in titratin blank

V_3 – millilitres of standard acid put in receiving flask for blank

V_4 – millilitres of standard NaOH used i titrating blank

M_1 – molarity of standard acid

M_2 – molarity of standard NaOH

W – weight of sample taken (g)

df- dilution factor of sample (if 1g was taken for estimation, the dilution factor will be 100)

Note : 1000ml of sample 0.1MHCl or 0.05M H_2SO_4 corresponding to 1.401g of N

A2: Lipid Extraction using different Methods

Sample Preparation

Public septage samples were collected from trucks at point of disposal at the Dompoase Sanitary landfill site. The samples were brought to the lab and dried in the oven at 105^{o}C, in aluminum containers lined with tin foils, till consistent weights were obtained. The dried samples were then grinded to coarse particles with an electric blender, which were then used in the extraction experiments.

A2.1: Soxhlet Method

Equipment:

1. Soxhlet apparatus;
    a. Quick-fit 250ml round-bottomed short necked flask with 24/29 socket.
    b. Quick-fit 100ml soxhlet extractor
    c. Quick-fit condenser
2. Paper thimble(22*80mm)
3. Cotton wool
4. Heating mantle
5. Ohaus Scout Pro Balance
6. Laboratory oven

Reagent:

1. Solvent;
    a. Petroleum ether
    b. Hexane
    c. Methanol

Procedure:

A known weight of the sample, FS (20±3g) was transferred into a 22*80mm paper thimble. A small ball of cotton wool was then placed into the thimble to prevent loss of the sample. 150ml of the solvent was added to the round bottom flask and the Soxhlet apparatus assembled. The set was then placed on the heating mantle and refluxed for 4 hours at the boiling point temperatures of the various solvent, depending on which one was used.

After extraction, the thimble was removed and the remaining solvent-lipid in the extractor column added to that in the round bottom flask. The solvent-lipid mixture was then

transferred to the rotary evaporator to recover the solvent, leaving a near dry lipid in the flask. This was then transferred into a pre-tarred crucible and finally dried in the oven at 105^{o}C till a constant weight was obtained.

Calculation:

$$Lipid\ Content = \frac{mass\ of\ lipid(g)}{mass\ of\ faecal\ a\ sludge(g)} \times 100$$

A2.2: HMA Method

Equipment:

1. 1 liter Erlenmeyer flask and condenser that fits into it.
2. Isotemp stir hot plate
3. Ohaus Scout Pro Balance
4. 1 liter separatory funnel
5. Buchner funnel
6. Retort Stand
7. Filter paper 90mm

Reagents:

1. 99.99% methanol
2. Hexane
3. Acetone

Procedure:

1 litre solvent mixture of hexane, methanol and acetone in the ratio 6:2:2 was prepared into a beaker.

50g of the sample (FS) was weighed into a 1 litre Erlenmeyer flask and 300ml of the solvent mixture added to it. The whole mixture was stirred at 400rpm with a stir plate and stir bar. The mixture was then filtered and the filtrate which contains the lipids collected.

The solvent was then recovered under a rotary evaporator, leaving the lipids to near dryness. Drying was completed by transferring the near dry lipids into a clean dry pre-weighed crucible and heating in the oven at 105^{o}C. The crucible with the lipids was weighed after every one hour till consistent weight was obtained.

Calculation:

$$Lipid\ Content = \frac{mass\ of\ lipid(g)}{mass\ of\ faecal\ a\ sludge(g)} \times 100$$

A2.3: Folch Method

Equipment:

1. 1 liter Erlenmeyer flask and condenser that fits into it.
2. Isotemp stir hot plate
3. Ohaus Scout Pro Balance
4. 1 liter separatory funnel
5. Buchner funnel
6. Retort Stand
7. Filter paper 90mm

Reagents:

1. Distilled water
2. Chloroform
3. 99.99% methanol

Procedure:

A mixture of 2 parts of chloroform and 1 part of methanol was prepared to a total volume 500ml.

20g of the sample (FS) was weighed into 1 litre Erlenmeyer flask. 400ml of the chloroform-methanol mixture was measured and added to the content of the flask. The whole mixture was agitated for 30 minutes at room temperature at 400rpm.

A Buchner funnel with a vacuum pump was then used to recover the liquid phase. The liquid phase was then washed with 20ml of distilled water, by gently swirling for 30 seconds. The two phases were allowed to settle and separate in a separatory funnel and the lower (water) layer was drained out. When necessary, the interface was rinsed once or twice with methanol/water (1:1), without mixing the whole preparation. The washing process was repeated to more times.

The resulting liquid phase was then placed back into the Erlenmeyer flask and vortexed for 5 minutes at 600rpm. The mixture was then returned into the separatory funnel and allowed to settle for 15 minutes. The lower(chloroform) phase was drawn off into a round bottom flask which was then attached to a rotary evaporator and the chloroform recovered as much as possible under vacuum at 70^{o}C.

The almost dried lipids in the round bottom flask was then transferred into a clean dry pre-weighed crucible and drying continued in an oven by heating at 105^{o}C and weighing after every one hour till consistent weight was obtained.

Calculation

$$Lipid\ Content = \frac{mass\ of\ lipid(g)}{mass\ of\ faecal\ a\ sludge(g)} \times 100$$

A2.4: Bligh and Dyer method

Equipment:

1. 1 liter Erlenmeyer flask and condenser that fits into it.
2. Isotemp stir hot plate
3. Ohaus Scout Pro Balance
4. 1 liter separatory funnel
5. Rotary evaporator
6. Filter paper (90mm)
7. Buchner funnel

Reagents:

1. Distilled water
2. Chloroform
3. 99.99% methanol

Procedure:

100g of sample containing 80g water and 20g dried faecal sludge was combined with 100ml of chloroform and 200ml methanol into the 1 litre Erlenmeyer flask. The quick-fit condenser was fixed onto it and the content of the flask mixed at 400rpm for 1 hour.

After mixing, 100ml of chloroform and 100ml of distilled water were added to the content of the flask and the whole mixture stirred again for 30 minutes at 400rpm.

A Buchner funnel and a vacuum pump were used to filter the solid phase from the liquid phase. The residue from the filtration was re-homogenised with 100ml chloroform by gently swirling for 30 seconds. The mixture was filtered again and the filtrate added to the separatory funnel holding the previous filtrate.

The final biphasic system in the separatory funnel was allowed to separate well, and lower chloroform phase collected, ensuring none of it remained in the upper phase. The chloroform in the lower phase containing the lipids was then evaporated under vacuum in a rotary evaporator at 70^{o}C to obtain a near dry lipid-chloroform mixture.

Drying was completed by transferring the near dried lipids into a clean dry pre-weighed crucible and heating with the oven at 105^{o}C, weighing after every one hour till consistent weight is accomplished.

Calculation:

$$Lipid\ Content = \frac{mass\ of\ lipid(g)}{mass\ of\ faecal\ a\ sludge(g)} \times 100$$

A2.5: Determination of Saponifiable Matter content in the Lipids

Based on the AOCS method Ca 6a-40

This experiment was performed on the various lipids extracted with the different solvent systems. It was intended to actually determine the amount of saponifiable matter in the lipid samples. This was obtained by first determining the weight percent of unsaponifiable matter using the Ca 6d-53 AOCS method and subtracting the results from 100%.

Determination of unsaponifiable matter content based on the AOCS method Ca 6d-53

Apparatus:

- 2-500ml Separatory funnels
- 1 liter separatory funnel
- 1-500ml Erlenmeyer flask
- condenser for top of Erlenmeyer flask
- 2-250 ml beakers
- hot water bath
- Hot plate
- Oven

Reagents:

- 96% ethanol
- Aqueous 50% by weight potassium hydroxide solution. Made by dissolving 60g of reagent grade KOH into 40ml distilled water.
- Aqueous potassium hydroxide, 0.5N
- Sodium hydroxide, 0.02 N solution
- Phenolphthalein, 1% in 95% ethanol
- Diethyl ether – reagent grade
- Acetone - reagent grade
- Distilled water

Procedure

About 2.00g of sample (lipids) was weighed into a 500ml Erlenmeyer flask. 25ml of 96% ethanol was measured and added to the flask. This was followed by an addition of 1.5ml of 50% KOH and whole content of the flask placed under reflux for an hour at 160^{o}C. A complete saponification was expected to occur at this stage.

The content of the flask was transferred into a 500ml separatory funnel. The flask was rinsed with a total of 50ml of distilled water. The rinsing process was then completed with 50ml of diethyl ether. This also began the extraction of unsaponifiable matter into the diethyl ether medium. The mixture was allowed to cool for an hour.

A stopper was inserted onto the separatory funnel and shaken vigorously for one minute and allowed to settle for 15min. At this stage, two layers were observed. The bottom layer (aqueous layer) was drained off into another separatory funnel making sure that none of it remained in the upper layer. The diethyl ether layer was left in the separatory funnel and the extraction process repeated until there were no more extracts in the aqueous phase. (About 7 extractions has been found to be enough to ensure complete extraction.

The diethyl ether layers were combined in a 1000ml separatory funnel and rotated gently with 20ml of distilled water, as violent shaking might result in the formation of an emulsion. The layers were allowed to separate as much as possible and the aqueous (bottom) layer discarded. This was repeated two more times, discarding the lower aqueous layer after each wash.

The combined extracts were then washed again with 20ml of 0.5N KOH, shaking vigorously. This was followed by washing with 20ml of distilled water. The layers were

allowed to settle and separate as much as possible and the aqueous(bottom) layer was discarded. This was also repeated two more times.

Finally washing was completed by washing with successive 20ml portions of distilled water until washes were no longer alkaline to 1% alcoholic phenolphthalein solution.

At this stage, a rotary evaporator was used to recover the diethyl ether, leaving the almost dry unsaponifiable matter. The near dry diethyl ether was transferred into a pre-tarred beaker. 5ml of acetone was added and drying continued to ensure there were no traces of solvent and a constant weight obtained. This result was recorded as **A** in the calculation.

5 drops of phenolphthalein indicator were added to a 10ml measure of warm ethanol. This was titrated to the phenolphthalein end point. The residue (A) was then dissolved in 2ml diethyl ether and added to the ethanol previously neutralized to the phenolphthalein end point. This also titrated with 0.02NaOH to the same final colour. Each milliliter of 0.02NaOH solution is equivalent to 0.0056 grms of oleic acid. The resulting figure corresponded to **B** in the formulae.

C was obtained by performing a blank procedure with no sample but following through the same procedure outlined.

$$Unsaponification\ matter(\%) = \frac{A - (B + C)}{Mass\ of\ Sample} \times 100$$

$$Saponifiable\ matter(\%) = 100\% - Unsaponifiable\ matter(\%)$$

Where:

A=Mass of residue (g)

B=Mass of fatty acids (g)

C=Mass of blank (g)

A2.6: Determination of free fatty acid in the Lipids

AOCS Official Method Ca 5a- 40

Equipment:

1. 50ml Burette
2. 50ml pipette
3. 250ml beaker

Reagents:

1. 1% phenolphthalein in 95% ethanol
2. 0.1N potassium hydroxide
3. Neutral Solvent: Mix 25mL ether, 25mL 95% alcohol and 1mL of 1% phenolphthalein solution and neutralize with N/10 alkali

Procedure:

Approximately 1.00g of the sample (lipids) was weighed and dissolved in 50 ml of the neutral solvent in a 250ml Erlenmeyer flask. About 5 drops of the phenolphthalein indicator was added to the content of the flask and titrated against 0.1N KOH. The content of the flask was consistently swirled while titrating until a pink colour which persisted for 15 seconds, was obtained.

Calculation:

$$\text{Acid Value } (\text{mg}\frac{\text{KOH}}{\text{g}}) = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1}{weight\ of\ sample}$$

$$\text{Free Fatty Acid(wt \%)} = \frac{\text{Acid value}}{2}$$

A2.7: Analysis of lipids (Speciation) : Qualitative and Quantitative analysis of fatty acids in lipids

Identity

H represents lipid extracted with hexane using the soxhlet method

PE represents lipids extracted with petroleum ether using the Soxhlet method

M represents lipids extracted with methanol using the Soxhlet method

HMA represents lipids extracted using HMA method

BD represents lipids extracted using the Bligh and Dyer method

F represents lipids extracted using the Folch method

Sample Preparation

Approximately 5mg-10mg of each sample were used for analysis. The samples were dissolved in 3.0mL of the original extraction solvents (initial diluents), centrifuged and the clear supernatants were collected. The solvents were evaporated under Nitrogen and the Lipids were diluted appropriately in 97:3 Toluene: Methanol to obtain a final concentration of about 5.0mg/mL. the samples were further diluted 1:1 and 1:4 and the original undiluted sample solution and the two diluted solutions were used for HPLC analysis. Table 1 summarizes the amounts of sample, solvents used and the final volumeTable 1

Sample	Amount (mg)	Initial diluent	97:3 TM Vol.	Conc (mg/mL)
PE	9.0	Heptane*	2.0	4.5
M	7.0	Methanol	1.5	5.0
BD	5.2	1:2 (C:M)	1.0	5.2
H	8.2	Hexane	1.6	5.06
F	9.0	2:1 (C:M)	2.0	4.5
HMA	5.3	H:M:A	1	5.3

*Pentane or petroleum ether was not available and hence Heptane was used dissolve the sample.

C, chloroform; M, methanol; H, hexane; A,acetone

HPLC analysis of Neutral Lipids

The method was calibrated with synthetic standards of Trianglycerides (TG), Diglycerides (DG), Monoglycerides (MG) and Free fatty acids (FFA).

The standards and the samples were injected on a normal phase HPLC column and analysed using an evaporative light scattering detector. Seven-level calibration curves were used to calculate the amounts of TG, DG and MG in the samples. HPLC standards of TG, DG, MG and FFA each at 1000ug/mL-15.625ug/mL.

Analysis of Neutral lipids by GC/FID:

The samples were removed from 20C-80C storage. Each of the 6 samples were weighted out into srew cap test tubes. The weights were recorded are as follows:

PE: 868.5mg

M: 715.2mg

BD: 407.1mg

H: 318.1mg

F: 427.0mg

HMA: 170.1mg

3mL of the appropriate solvents (listed in Table 1 on page 1) were added sample. The samples were then centrifuged at 2500rpm for 20 minutes before removing the solvent layer into a pre-tared screw cap test tube. This solvent layer was then dried down under a gentle stream of N2 before re-weighing. The sample weights recovered were as follows:

PE: 751.0mg

M: 237.5mg

BD: 398.2mg

H: 216.7mg

F: 305.4mg

HMA: 164.2mg

The samples were reconstituted in 1mL of HPLC Grade Chloroform before vortexing and centrifuging at 2500rpm for 20 minutes. The solvent layer was then transferred to a screw cap test tube to separate lipid fractions by TLC. Lipid fraction separation by TLC was performned by plating each sample with a neutral lipid TLC standard. 50uL of sample was streaked across half of the plate with a syringe. The plate was developed in 80:20:1 Hexane:Diethyl Ether: Acetic Acid. The 6 plates were each developed twice in the afore-mentioned mobile phase. At this point, the sample portion of the plate was completely covered with aluminum foil before placing the plate in an Iodine tank for 15 minutes. The spots from the neutral lipid standard were circled befoe removing the aluminum foil and scraping the corresponding sections of the sample portion of the plate. These silica fractions were collected into appropriately labled screw cap test tubes. 1mL of chloroform was added to each of the 24 tubes before vortexing and centrifuging at 2500rpm for 20 minutes. The chloroform layer was then transferred to pre-tarred screw cap text tubes and

re-weighted. Due to low recovery weights, each of the 24 fractions was reconstituted to increase the concentration by a factor of 5.

Fatty acid methyl ester analysis was performed on an Agilent 7890GC with flame ionization detector and 20M x 0.18um, DB-225 capillary column with hydrogen carrier. Total fatty acids were analyzed by the preparing extracted lipids using acid hydrolysis in methanol. Calibration of analyte retention times and identification was performed utilizing a fatty acid methyl ester standard from Nu-Chek Prep which contains C8 to C¬24:1 fatty acids. The relative amounts of detected fatty acids per sample fraction are report as % of total fatty acid methyl ester area.

APPENDIX B: Tables and Figures

B1: DATA ON THE CHARACTERISATION OF FS

Table B1.1: Pit Latrine FS

Sample ID	Source	PH	Moisture Content (%w/w)	Total Solids (%w/w)	Lipid Content (%w/w)	Chemical Oxygen Demand (g/ml)	Nitrogen Content (mg/l)	Phosphorus Content (mg/l)
1	Nima Adukrom	8	96.5	3.5	5.73	84,800	770.97	728.36
2	Pankrono Estate	7.5	99.6	0.39	2.99	5120	252.6	1232.61
3	Breman	7.2	98.83	1.17	4.04	26800	376.9	1666.83
4	Aboabo	8	97.6	2.4	6.52	1400	4181.08	578.04
5	Moshi Zongo	8.3	96.9	3.1	5.05	60800	8922.42	555.2
6	Abuakwa	8.3	99.89	0.11	3.01	89600	11527.8	715.2
7	Aboabo	7.9	95.4	4.6	7.62	53200	7808.9	692.9
8	Mamponteng	8.5	94.2	5.8	7.02	11652	7066.53	718.2
9	Ayigya	8.3	93.6	6.4	7.99	13280	4755.38	646
10	Moshi Zongo	8.2	96.01	3.99	5.01	18160	1288.64	127.8
11	Gyakye	8.4	96.95	3.05	6.21	26400	3011.51	284.33
12	Bremanfie	8.1	98.68	1.32	5.9	28900	1512.76	83.83
13	Tafo	8.4	95.12	4.87	7.8	10640	3445.72	823.1
14	Ahwia	8.3	96.95	3.05	5.08	50800	6023.01	416.24
15	Trede	7.9	98.68	1.32	5.92	5520	448.22	54.52
16	Aboabo	7.7	95.226	4.774	11.62	53200	4972.84	145.39
17	Asokwa	7.1	99.54	0.46	3.11	8280	532.67	41.62
18	Ampayo	7.7	89.28	10.72	7.6	74400	4356.18	385.75
19	Buokrom	7.4	94.099	5.901	10.6	13280	1652.82	515.9
20	Mamponteng Zongo	7.7	96.47	3.53	5	21760	4146.07	269.68
21	Sobolo	7.9	91.1954	8.8046	15.2	88000	7227.61	690.02
22	Bantama	7.9	91.744	8.255	7.58	96,800	6835.41	684.15
23	Bantama	7.2	95.296	4.703	6.507	74400	5994.99	664.22
24	Ahenfo Boboano	8	92.768	7.232	13.12	76000	5574.78	702.33
25	Ahwia	7.5	92.448	7.552	6	7840	1708.85	91.46
26	Adeambra	8	95.68	4.32	10	43200	5210.6	593.87
27	Sabo Zongo	7.6	96.077	3.923	10.857	34400	4118.06	385.17
28	Tanoso	7.9	94.309	5.691	7	4720	5322.66	528.21
29	Ahodwo	7.7	92.168	7.832	11	72800	6891.44	595.04
30	Gyinyase	7.8	94.747	5.253	6	57600	3445.72	497.73

Table B1.2: Public Septage FS

Sample ID	Source	PH	Moisture Content (%w/w)	Total Solids (%w/w)	Lipid Content (%w/w)	Chemical Oxygen Demand (g/ml)	Nitrogen Content (mg/l)	Phosphorus Content (mg/l)
1	Maakro	8.06	98.24	1.76	12.89	42400	1731.68	397.3
2	Aboabo	7.48	98.42	1.58	12.22	12240	3277.63	330.09
3	Kronom	7.8	98.28	1.72	12.6	51200	1495.9	154.17
4	Aboabo	7.8	98.05	1.95	13.5	41200	4818.4	562.2
5	Pankrono	7.37	98.97	1.03	9.75	28400	1490.24	554.6
6	Abuakwa	6.93	98.98	1.02	9.52	24000	1078.53	217.5
7	Kwadaso	7.16	98.99	1.01	8.86	20800	2609.3	155.9
8	Oforikrom	7.77	97.94	2.06	15.82	63600	1477.73	177.6
9	Central Prisons	7.52	98.42	1.58	12.327	14400	1981.04	283.2
10	Emena	7.85	99.11	0.89	9.24	10400	1560.28	123.7
11	Krofrom	7.23	99.48	0.52	5.71	4040	1476.24	347.49
12	Ahodwo	7.71	99.36	0.64	5.83	4280	1486.24	283.25
13	Asuyeboa	7.83	99.11	0.89	9.18	1360	1576.24	240.45
14	Tafo new road	7.9	99.48	0.52	4.44	7280	1736.89	250.92
15	Moshi Zongo	7.72	99.36	0.64	6.7	1752	4314.16	199.33
16	Maakro	7.4	99.27	0.73	6.92	13600	798.4	263.32
17	Alaba	7.68	98.52	1.47	11.18	69200	5182.59	208.71
18	Asawase	7.6	98.08	1.92	13.475	74400	5252.63	426.79
19	Adum	7.68	98.7	1.29	7.576	47600	1644.32	172.94
20	Akyease	7.05	99.48	0.52	4.328	4040	1260.63	614.98
21	Asuoyeboa	7.66	98.72	1.27	12.381	31200	1308.15	123.11
22	Aboabo	7.56	99.41	0.59	4.86	4080	1448.22	265.66
23	Tanoso	7.23	99.3	0.69	6.25	4880	3529.76	646.63
24	Santasi	7.68	98.92	1.08	4.29	5760	840.04	411.62
25	Moshi Zongo	7.23	98.55	1.45	13.39	17440	1652.83	100.25
26	Ayigya	7.33	98.69	1.31	12.68	17040	1092.55	281.4
27	Maakro	7.22	98.83	1.17	10.98	24800	952.48	132.49
28	Amakom	6.93	97.99	2.01	15	29600	1840.42	188.19
29	Kejetia	7.1	99.86	1.14	11.11	11000	1308.15	340
30	Bantama	6.85	98.73	1.27	12.26	33200	1708.85	438.52

Table B1.3: Private Septage FS

Sample ID	Source	PH	Moisture Content (%w/w)	Total Solids (%w/w)	Lipid Content (%w/w)	Chemical Oxygen Demand (g/ml)	Nitrogen Content (mg/l)	Phosphorus Content (mg/l)
1	Aboabo	7.14	99.79	0.21	5.79	6905.45	1184	136.08
2	Ahinsan Estate	7.12	99.78	0.22	5.83	6720	720.83	54.52
3	Bokrom	7.47	99.82	0.18	4.78	1880	913.45	123.85
4	Santasi	7.28	99.68	0.31	6.6	8080	1162.58	153
5	Tafo	7.3	99.48	0.52	6.87	1304	918.95	74.5
6	Dikyemso	7.02	98.87	1.13	9.79	2560	322.16	139.9
7	Dikyemso	7.72	99.57	0.43	6.79	6400	484.74	246.8
8	Prempeh Assembly	7.89	99.14	0.86	7.14	1368	708.65	115.4
9	Adum Post Office	7.02	99.69	0.31	6.65	1350	800.17	170.6
10	Kronom	7.6	99.59	0.41	6.76	1576	364.18	132.24
11	Boadi	7.75	99.11	0.89	7.34	4320	574.29	45.73
12	Pankrono	8.16	99.66	0.34	8.75	3360	434.22	59.8
13	Esereso	8.14	99.59	0.41	6.7	1216	308.15	45.14
14	Pankrono	7.67	99.11	0.89	7.48	4400	448.22	84.42
15	Gyinyase	7.83	99.56	0.44	7.24	5520	308.15	170.59
16	Adum	7.23	99.86	0.14	6.67	528	336.17	121.11
17	Ahwia	7.32	99.73	0.27	9.63	1752	364.18	113.48
18	Ayigya	7.22	99.8	0.19	9	8760	714.36	95.56
19	Ahodwo	7.23	99.64	0.26	5.38	1488	280.1	43.97
20	Stadium, Asokwa	7.13	99.63	0.37	5.89	4920	224.11	61.56
21	Ahwia	7.11	99.66	0.34	6.67	736	504.25	110.73
22	Ahwia Nkwanta	7.7	99.67	0.33	7.39	448	400.07	52.76
23	Kwadaso	7.45	98.24	1.76	5.28	2480	1008.5	379.3
24	Kwadaso	7.8	98.34	1.66	4.98	3440	840.42	121.46
25	Kwadaso	7.14	99.64	0.36	4.29	1600	308.15	136.93
26	Ankaase	7.74	99.72	0.28	2.17	1432	532.27	132.24
27	Asuofia	7.21	99.75	0.25	8.18	3280	350.18	44.56
28	Patase	7.2	99.67	0.32	2.27	4256	974.99	58.81
29	Atonsu	6.65	98.29	1.71	9.62	3904	680.84	181.4
30	Bomso	7.11	99.74	0.26	4.72	3120	644.33	92.04

B2: DATA ON LIPID EXTRACTION

Extraction Method	Solvent System	No. of Replicates ran	Average Lipid Content (%w/w)	Standard Deviation
Soxhlet	Hexane	5	3.66	0.230
Soxhlet	Pet-ether	5	3.05	0.460
Soxhlet	Methanol	5	12.67	0.710
HMA	Hexane-Methanol-Acetone (6:2:2)	3	6.09	0.798
Bligh & Dyer	Chloroform-Methanol-Water(2:2:1)	5	5.63	1.17
Folch	Chloroform-Methanol(2:1)	5	6.86	0.662

Table B3: DATA FOR UNSAPONIFIABLE MATTER EXPERIMENTS ON THE LIPIDS, BASED ON OLEIC ACID

Extraction Method	Solvent System	No. of Replicates ran	Average Unsaponifiable Matter (%w/w)	Standard Deviation	Average Saponifiable Matter (%w/w)	Standard Deviation
Soxhlet	Hexane	3	26.41	0.827	73.59	0.827
Soxhlet	Pet-ether	3	31.60	0.746	68.40	0.746
Soxhlet	Methanol	3	17.24	0.557	82.76	0.557
HMA	Hexane-Methanol-Acetone (6:2:2)	3	46.61	0.867	53.39	0.867
Bligh and Dyer	Chloroform-Methanol-Water(2:2:1)	3	38.26	2.70	61.74	2.70
Folch	Chloroform-Methanol(2:1)	3	43.91	0.920	56.09	0.920

B4: The fatty acid contents (mg/g) of the neutral lipid fractions in different samples.

Table B4.1: Fatty acid content (mg/g) of NL fraction in Methanol sample

Sample	Fatty Acid	FFA fraction Mg/g	MG fraction Mg/g	DG fraction Mg/g	TG fraction Mg/g	
M	$C_{13:1}$	0.3	0	0	0	
	$C_{14:0}$	0.9	0	0	0	
	$C_{14:1}$	0.5	5.4	0	0	
	$C_{15:0}$	0.3	0	0	0	
	$C_{16:0}$	5.9	34.3	12.8	19.5	
	$C_{16:1}$	3.5	8.1	4.6	0	
	$C_{17:0}$	0.6	0	0	0	
	$C_{17:1}$	3.8	0	0	0	
	$C_{18:0}$	0.7	12.2	8.3	0	
	$C_{18:1\ Cis}$	1.1	9.8	0	0	
	$C_{18:1\ trans}$	1.6	0	0	0	
	$C_{18:2}$	2.4	9.8	5.3	13.5	
	$C_{18:3(\wedge^{9,12,15})}$	6.1	6.7	0	0	
	$C_{20:5}$	0.9	0	0	0	
	$C_{22:3}$	0	0	7.9	0	
		28.7	86.4	39.0	33.0	
					Total FFA FFA/NL	187.1 0.82

Table B4.2: Fatty acid content (mg/g) of NL of fraction in HMA sample

Sample	Fatty Acid	FFA fraction Mg/g	MG fraction Mg/g	DG fraction Mg/g	TG fraction Mg/g	
HMA1	$C_{13:1}$	0	0	0	0	
	$C_{14:0}$	1.5	9.9	0	0	
	$C_{14:1}$	1.3	0	0	0	
	$C_{15:0}$	0	34.0	0	0	
	$C_{16:0}$	5.5	54.9	35.4	20.6	
	$C_{16:1}$	6.9	11.0	0	5.1	
	$C_{17:0}$	1.3	0	0	0	
	$C_{17:1}$	10.2	0	0	0	
	$C_{18:0}$	2.0	20.4	43.7	8.1	
	$C_{18:1\ Cis}$	1.5	11.4	0	10.2	
	$C_{18:1\ trans}$	0	0	0	0	
	$C_{18:2}$	3.2	13.6	0	10.0	
	$C_{18:3(\wedge^{9,12,15})}$	14.9	0	0	0	
	$C_{20:4}$	0	0	0	0	
	$C_{20:5}$	2.1	0	0	0	
	$C_{22:0}$	1.6	0	0	0	
			0	0	0	
		51.9	155.2	79.0	54.0	
					Total FFA FFA/NL	340.1 0.74

Table B4.3: The fatty acid contents (mg/g) of NL fraction in Folch lipid sample

Sample	Fatty Acid	FFA fraction Mg/g	MG fraction Mg/g	DG fraction Mg/g	TG fraction Mg/g	
F1	$C_{13:1}$	0	2.9	0	0	
	$C_{14:0}$	1.3	1.7	0	0	
	$C_{14:1}$	1.0	1.5	0	0	
	$C_{15:0}$	0.5	17.7	4.0	0	
	$C_{16:0}$	16.4	24.0	13.8	0	
	$C_{16:1}$	6.7	4.2	15.1	0	
	$C_{17:0}$	0.6	0	0	0	
	$C_{17:1}$	1.6	0	0	0	
	$C_{18:0}$	3. 0	8. 0	8.5	0	
	$C_{18:1\ Cis}$	3.8	7.8	2.6	0	
	$C_{18:1\ trans}$	4.6	10.7	0	0	
	$C_{18:2}$	5. 0	6.1	3.4	0	
	$C_{18:3}(\wedge^{9,12,15})$	5.7	4.0	0	0	
	$C_{20:4}$	0.5	0	0	0	
	$C_{20:5}$	0.8	0	0	0	
	$C_{22:0}$	0.3	2.2	0	0	
	$C_{24:0}$	0	3.1	0	0	
		51.9	94.1	47.5	0	
					Total FFA	193.5
					FFA/NL	0.60

Table B4.4: Fatty Acid content (mg/g) of NL fraction in Bligh and Dyer lipid sample.

Sample	Fatty Acid	FFA fraction Mg/g	MG fraction Mg/g	DG fraction Mg/g	TG fraction Mg/g	
BD1	$C_{13:1}$	0	0	0	0	
	$C_{14:0}$	1.9	0	0	0	
	$C_{14:1}$	1.2	5.02	0	0	
	$C_{15:0}$	0.6	8.4	3.4	0	
	$C_{16:0}$	14.1	41.7	14.2	0	
	$C_{16:1}$	6.4	8.5	16.7	0	
	$C_{17:0}$	0.8	0	0	0	
	$C_{17:1}$	3.9	0	0	0	
	$C_{18:0}$	2.5	12.5	8.4	0	
	$C_{18:1}$ Cis	2.8	12.3	4.6	0	
	$C_{18:1}$ trans	3.1	10.7	3.6	0	
	$C_{18:2}$	4.2	9.9	0	0	
	$C_{18:3}(\wedge^{9,12,15})$	8.0	8.7	0	0	
	$C_{20:4}$	0	0	0	0	
	$C_{20:5}$	1.0	0	0	0	
	$C_{22:0}$	0	0	0	0	
	$C_{24:0}$	0	0	0	0	
		50.8	117.9	50.9	0	
					Total FFA	219.5
					FFA/NL	0.63

Table B4.5: Fatty acid content (mg/g) of NL fraction in H1 sample

Sample	Fatty Acid	FFA fraction Mg/g	MG fraction Mg/g	DG fraction Mg/g	TG fraction Mg/g	
H1	$C_{13:1}$	0	0	0	0	
	$C_{14:0}$	0.8	0	0	0	
	$C_{14:1}$	0.2	0	10.2	0	
	$C_{15:0}$	0.4	0	5.8	0	
	$C_{16:0}$	15.6	65.6	66.6	105.6	
	$C_{16:1}$	0	12.3	0	0	
	$C_{17:0}$	0.2	0	0	0	
	$C_{17:1}$	0	0	0	0	
	$C_{18:0}$	5.1	40.1	38.5	60.4	
	$C_{18:1}$ Cis	1.0	10.1	14.3	0	
	$C_{18:1}$ trans	0	0	0	0	
	$C_{18:2}$	0.5	0	7.9	0	
	$C_{18:3}(\wedge^{9,12,15})$	0	0	0	0	
	$C_{20:4}$	0	0	0	0	
	$C_{20:5}$	0	0	0	0	
	$C_{22:3}$	0	0	35.6	0	
	$C_{24:0}$	0	0	0	0	
		24.0	128.0	179.0	166.0	
					Total FFA FFA/NL	497 1

Table 4.6: Fatty acid content (mg/g) of NL fraction in Petroleum ether lipid sample

Sample	Fatty Acid	FFA fraction Mg/g	MG fraction Mg/g	DG fraction Mg/g	TG fraction Mg/g	
PE1	$C_{13:1}$	0	0	0	0	
	$C_{14:0}$	0.6	3.4	3.7	2.4	
	$C_{14:1}$	0.1	2.3	2.3	0.9	
	$C_{15:0}$	0.2	1.7	0	1.2	
	$C_{16:0}$	9.3	68.0	68.0	69.7	
	$C_{16:1}$	0.1	1.9	0	0	
	$C_{17:0}$	0.1	0	0	1.4	
	$C_{17:1}$	0	0	0	0.4	
	$C_{18:0}$	3.5	35.4	36.6	49.4	
	$C_{18:1}$ Cis	1.8	11.4	10.8	12.7	
	$C_{18:1}$ trans	0.1	1.8	0	1.6	
	$C_{18:2}$	0.4	4.5	6.2	3.8	
	$C_{18:3}(\wedge^{9,12,15})$	0	0	0	0	
	$C_{19:0}$	0	0	0	0.3	
	$C_{20:0}$	0.1	1.5	0	2.8	
	$C_{20:2}$	0.3	0	0	0	
	$C_{20:3}(\wedge^{5,8,12})$	2.0	0	29.4	0	
	$C_{20:4}$	0	0	0	0.3	
	$C_{20:5}$	0.3	0	0	0	
	$C_{22:0}$	0.1	0	0	3.1	
	$C_{23:0}$	0	0	0	0.9	
	$C_{22:3}$	0	0	20.5	0.8	
	$C_{24:0}$	0	0	0	3.4	
	Sum	19.2	131.9	177.4	155.2	
					Total FFA	483.7
					FFA/NL	1.0