MEASURING FAECAL SLUDGE STABILIZATION AND ITS RELATION TO DEWATERING PERFORMANCE

By

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ABSTRACT

Stabilization and dewatering are indispensable treatment mechanisms applied in the management of Faecal Sludge (FS) that accumulates in onsite containment facilities such as pit latrines and septic tanks. This is because FS is mainly comprised of 80 – 95 percent water and readily degradable organic matter. Based on field observations, Faecal Sludge is normally stabilized (offering limited scope for further stabilization at treatment) due to the longer storage times it spends in the onsite containment systems where it undergoes digestion in mostly anaerobic and facultative conditions. In addition, a potential linkage has been reported between the observed level of Faecal Sludge stabilization and its dewatering performance. It has been noted that more stabilized FS is easier to dewater than fresh sludge which is not stabilized. However, it is not clear how FS stabilization and its relation to dewatering can be measured with practitioners relying on qualitative information such as colour and odour to distinguish between the so-called stabilized and non-stabilized or fresh Faecal Sludge. The study evaluated rapid and low cost methods that can be used to measure FS stabilization, including criteria or index for characterizing a sample as stabilized or not stabilized. The study also assessed the relationship between FS stabilization and its dewatering performance. Methods that can be used for measuring FS stabilization were selected through a two-stage process i.e. screening using a decision matrix and laboratory evaluation to determine method performance and suitability. The relationship between FS stabilization and dewatering performance was determined through laboratory anaerobic digestion and dewatering experiments. A total of 27 faecal sludge samples including a fresh sample were collected and nine parameters related to stabilization and dewatering performance were analysed. The study found that FS stabilization can be measured using low cost methods such as the Volatile Solids to Total Solids ratio and the Specific Oxygen Uptake Rate and is associated with dewatering performance measured as capillary sanction time. Stabilization was correlated to the age/ type of faecal sludge, though the differences based on sludge age were not significant among samples from pit latrines and septic tanks. The observed differences in dewatering performance were associated with differences in level of stabilization. The majority of the FS samples become stabilized after 60 days of anaerobic digestion and a corresponding improvement in dewatering performance was also observed. Based on these results, FS from onsite containment facilities is not fully stabilized, despite the longer retention times at containment. Further, the associations between anaerobic digestion and improvement in dewatering performance as well as stabilization suggests that application of a biological stabilization step at treatment before FS dewatering can be beneficial.

Key Words: Faecal Sludge, Stabilization, Dewatering Performance, Anaerobic Digestion, Stability Index.

DEDICATION

This thesis is dedicated to my parents Sishumba Kapanda and Peggy Kalolu Kapanda. For their endless love, support and encouragement which made it possible for me to get an education.

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ABBREVIATIONS AND ACRONYMS

APHA	American Public Health Association
ATP	Adenosine Triphosphate
BMP	Biomethane Potential
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
CSO	Central Statistics Office
CST	Capillary Suction Time
DHA	Dehydrogenase Activity
DRI	Dynamic Respiratory Index
EA	Esterase Activity
EAWAG	Swiss Federal Institute of Aquatic Science and Technology
EC	Electrical Conductivity
EPA	U.S. Environmental Protection Agency
FS	Faecal Sludge
FSTP	Faecal Sludge Treatment Plant
FSM	Faecal Sludge Management
GRZ	Government Republic of Zambia
JMP	Joint Monitoring Program
LCC	Lusaka City Council
LSP	Lusaka Sanitation Program
LWSC	Lusaka Water Supply and Sanitation Company
OSS	Onsite Sanitation
pH	Potential Hydrogen
SD	Standard Deviation
SFD	Shit Flow Diagram
SMP	Sanitation Master Plan
SOUR	Specific Oxygen Uptake Rate
TAN	Total Ammonia Nitrogen
TOC	Total Organic Carbon
TS	Total Solids

TTC	Tetrazolium Chloride
UNZA	University of Zambia
UNICEF	United Nations International Children's Fund
VS	Volatile Solids
VVL	Vertical Vault Latrine
WHO	World Health Organization

OPERATIONAL DEFINITIONS

The following are the operational definitions applicable to this study:

Anaerobic Digestion: Anaerobic digestion is a process through which bacteria break down organic matter such as wastewater sludge in the absence of oxygen (EPA, 2022).

Onsite Containment: A sanitation system that collects, stores, treats and disposes of excreta/wastewater on or near the site of generation (Tilley et al., 2014).

Faecal Sludge: Is raw or partially digested, a slurry or semisolid that accumulates in OSS facilities and has not been transported through a sewer (Strande et al., 2014).

Sludge Dewatering: The removal of free water and water that is loosely bound in pores and interstitial spaces of sludge particles and flocs (Ward, *et al.*, 2021)

Sludge Age: The total time that sludge is retained in an onsite sanitation containment, usually determined as time scale between emptying events.

Stabilization: Stabilization is the breakdown of readily biodegradable organic matter in a substrate, leaving behind a more stable product with less degradable organics (Strande, *et al.*, 2014).

Stability Index: value above or below which a sample can be categorized as either stabilized or not stabilized

Practitioner: A person actively engaged in an art, discipline, or profession.

Wastewater: Used water which includes water from sinks, showers, bathtubs, toilets, industrial or agricultural activities.

CHAPTER ONE: INTRODUTION

1.1 Background

The sanitation needs of the majority living in urban settlements in most developing countries worldwide are served by onsite sanitation (OSS) technologies such as septic tanks and pit latrines (UNICEF, 2021). Typically in most of the growing cities, the greatest challenge is the lack of effective management systems for the resulting accumulation of Faecal sludge (FS), generating significant negative public health and environmental risks (Strande, *et al.,* 2014; Blackett, *et al.,* 2014). Stabilization and dewatering are indispensable treatment mechanism applied in the management of the accumulated FS (Strande *et al.,* 2014; *Gold et al.,* 2018). This is because FS is mainly comprised of 80 – 95 percent water and readily degradable organic matter (Strande, *et al.,* 2014; Gold *et al.,* 2016; Semiyaga *et al.,* 2017). The current understanding among most practitioners is that FS is normally stabilized (offering limited scope for further stabilization at treatment) due to the longer storage times it spends in the onsite containment systems (Tayler, 2018) where it undergoes digestion in mostly anaerobic and facultative conditions (Shaw and Dorea 2021; van Eekert *et al.,* 2019). However, the actual processes occurring inside the containment facilities are at the moment not well understood due to limited studies (Ward *et al.,* submitted). In addition, a potential linkage has been reported in the field between the observed level of FS stabilization and its dewatering performance. It has been noted that more stabilized FS is easier to dewater than fresh sludge which is not stabilized (Semiyaga *et al.,* 2016; Cofie *et al.,* 2006; Ward *et al.,* 2021;Ward *et al.,* 2019). In most cases descriptive and qualitative parameters such as colour, odour, place of origin (public or domestic) type of containment (septic or pit latrine) and how often the facility is emptied are used to predict the level of FS stabilization and its corresponding dewatering performance (Ward *et al.,* 2021; Ward *et al.,* 2019). In both studies, Ward *et. al.,* (2019 and 2021) noted that qualitative parameters such as light brown colour and fresh excreta odour which are associated with less stabilized FS corresponded with samples that were difficult to dewater.

Despite the above observations, practitioners are still faced with a challenge of inconsistency dewatering performance of FS at treatment which is attributed to the high variability in the physical-chemical characteristics of FS and its level of

stabilization (Gold *et al.,* 2018; Semiyaga *et al.,* 2017). This variation is as a result of the different types of containments (e.g. lined and unlined pits), differences in methods of emptying, usage/ user behaviour (e.g. wet and dry containments), duration of storage in onsite containment and locational environmental conditions (e.g. high water table, flooding) which all effect level of stabilization as well as physical-chemical characteristics (Ward *et al.,* 2019; Semiyaga *et al.,* 2017). Some researchers have also attributed the variations in level of stabilization to the differences in the moisture content or total solids (TS) concentration between different onsite containments (e.g. wet and dry latrines) (Van Eekert *et al.,* 2019; Couderc et al. 2008). Further, anaerobic digestion of organic substrates with high TS concentration (e.g. above 10 percent which is referred to as dry digestion is said to be problematic due to mixing, mass transfer and diffusion limitation issues (Bollon *et al.,* 2013; Liotta *et al.,* 2014). This is expected especially in dry latrines which have FS with higher TS concentration which can be as high as 20 percent and the contents are not mixed. Other parameters such as pH and total ammonia nitrogen also affect anaerobic stabilization of FS (Jiunn *et al.,* 1997; Zuo, 2021).

Generally, the variations in physical-chemical characteristics and dewatering performance (e.g. based on containment type and source) have been reported in literature (Ward *et al.,* 2019; Semiyaga *et al.*, 2017; Ward *et al.,* 2021; Strande *et al.*, 2018a) while that for level of stabilization has lagged behind due to the lack of methods for measuring FS stabilization. The development of low cost and rapid methods to measure FS stabilization can improve accurate prediction of its dewatering performance if the relationship between the two is established to exist as practitioners have been observing in the field. A number of studies have been conducted that have shown relationships between simple/ rapid field based measurements (e.g. pH, electrical conductivity and colour) and laboratory based measurements (e.g. total solids, ammonia, dewatering time) which have the potential to improve the prediction of variability of FS characteristics and dewatering performance (Ward *et al.,* 2021; Gold *et al.,* 2018 ;Bousek *et al.,* 2018; Kimwaga and Mayo, 2021). Ward *et al.* (2021) reported supernatant color to be the best predictor of dewatering performance while texture of sludge photographs was the best predictor of total solids (TS) through the use of predictive models developed based on a dataset of 421 samples collected from OSS in Lusaka. Such relationships and predictive models if further developed and

validated can aid adjustments and process controls to optimize and enhance FS treatment. When it comes to relationships between FS stabilization and dewatering performance, very limited research has been conducted. Ward *et al.* (submitted) and Sam *et al.* (2022) observed an improvement in dewatering performance of FS with anaerobic stabilization though the trend was not consistent. In addition, Ward *et al.* (submitted) also reported relationships between indicators of FS stabilization and dewatering performance, but did not report on how to measure stabilization. Further, inconsistent results have been reported when it comes to the effect of anaerobic stabilization on the dewatering performance of different types of wastewater Sludge(activate, primary and secondary sludges) (Pontoni *et al.,* 2018) and most recently FS (Ward *et al.,* Submitted). Generally, anaerobic digestion can either improve or worsen sludge dewatering performance (Ward *et al.,* submitted; Christensen *et al.,* 2015; Pontoni *et al.,* 2018). This is because of the different origins of the Sludge(e.g. conventional activated sludge system vs membrane biorector systems for wastewater Sludgeand predominantly anaerobic systems for FS) which affects the physical chemical characteristics (pH, electrical conductivity, surface charge), microbial community, particle size distribution and morphology of the sludge flocs differently (Jin *et al.,* 2004; Ward *et al.,* submitted). It is well accepted within wastewater sludge literature and most recently FS (based on one study by Ward *et al.,* (submitted)) that poor dewatering performance is caused by the presence of small particles (<100 µm) which clog filter beds and the interstitial spaces in the sludge cakes (Ward *et al.,* submitted; Christensen *et al.,* 2015). In addition, extracellular polymeric substances (EPS) (long charged polymer chains which keep sludge flocs together through bioflocculation) have also been reported to influence the dewatering performance of both wastewater sludge and FS (Ward , *et al.,* 2019; Dai, *et al.,* 2013; Christensen *et al.,* 2015; Sam *et* 2022). Most studies seem to agree that physical-chemical characteristics, EPS and particle size distribution greatly influence the dewatering performance of both FS and wastewater sludges. All these parameters are altered and affected as the sludge undergoes stabilization.

Overall, it is clear in literature that extensive knowledge has been developed over the years on the dewatering performance of wastewater sludge and the mechanisms as well as factors that govern it as compared to FS (Gold , *et al.,* 2018) where research is limited. Currently, research is being actively conducted to determine how FS

dewatering fits into this body of knowledge of wastewater sludge. The recent FS dewatering research has focused mostly on elucidating underlying factors and mechanisms that govern dewatering such as particle size distribution, the role of EPS, physical-chemical properties (pH, EC, surface charge), microbial community and how these change with anaerobic stabilization in line with literature on wastewater Sludge(Ward *et al.,* 2019; Ward *at al.,* 2021; Sam *et al.,* 2022; Ward *et al.,* Submitted; Gold , *et al.,* 2018). As mentioned ealier, one of the factors that has been observed to be related to the dewatering behavior of FS is its level of stabilization. It is not clear at the moment how FS stabilization can be measured with practitioners relying on qualitative information such as colour and odour to distiguish between the so-called stabilized and non-stabilized or fresh FS. On the contrary, methods and criteria for measuring stabilization of other organic substances such as wastewater sludge and composts have been well researched and published (Bożym and Siemiątkowski, 2020; Benito et al., 2005; Samson & Ekama, 2000; Cokgor *et al.,* 2012; Borglin et al., 2012; Bernal et al. 1998; Ferrer, 2006; Mangkoedihardjo, 2006).

Based on the current understanding on FS dewatering and how it has been observed in the field to be related to level of stabilization, this study was carried out to develop methods and criteria for measuring FS stabilization and its relation to dewatering performance. Ultimately the study will contribute to the body of knowledge on FS dewatering which is new and possibly enhance the prediction of the dewatering behavior for FS.

1.3 Statement of the Problem

The inconsistent dewatering performance of FS which has been observed in the field by most practitioners is a major bottleneck which hinders optimal design of FS dewatering technologies (e.g. sludge drying beds and settling thickening tanks) and efficient operations of existing Faecal Sludge Treatment Plants (FSTP). It has been suggested in literature that this inconsistency in dewatering behaviour is as a result of the high variability in the physical chemical characteristics and level of stabilization of FS. Further it has been observed in the field that FS dewatering behaviour is related to the level of its stabilization. However, it is not known yet how FS stabilization can be measured and practitioners in the field have always relied on the use of qualitative and descriptive information such as colour and odour to make technical judgement on weather a certain FS sample can be categorized as stabilized or not. Further, the

relationship between FS stabilization and its dewatering performance has not been extensively reported or published in literature. Thus development of knowledge on how FS stabilization can be measured/ quantified and how it is related to dewatering will contribute to the accurate prediction of the variations in the dewatering behaviour of FS to optimize design and operations of treatment units such as sludge drying beds.

Thus, the problem statement of this study can be summarized as follows:

"The determination of stabilization of FS and how it affects dewatering has not been researched and reported on in literature. In addition low-cost and relatively easy to implement methods that can be used by practitioners to predict the dewatering performance of FS to aid effective treatment have not been developed yet.

1.4 Research Questions

i How can we measure FS stabilization?

ii How is FS stabilization related to FS dewatering behaviour?

iii Do intrinsic physical chemical characteristics of FS influence its ability to undergo further stabilization under anaerobic conditions?

1.5 Hypotheses

This study had three hypotheses as follows:

i. Methods and criteria for measuring stabilization of wastewater sludge and compost can be applied to measure FS stabilization;

ii. Faecal Sludgefrom different types of onsite containments (e.g. dry pit latrines, wet/ pour flush latrine, and septic tanks) and fresh human excreta with different sludge age have varying levels of stabilization;

iii. The dewatering performance of FS improves with increasing level of stabilization.

1.6 Research Aim

The aim of the study is was to determine how to measure FS stabilization and its relation to dewatering performance. In order to address this aim, three objectives were developed and are presented in section 1.6.1.

1.6.1 Objectives

i. To establish how FS stabilization can be measured by using rapid and low cost methods;
ii. To ascertain the relationship between FS stabilization and dewatering performance; and
iii. To establish if intrinsic physical-chemical characteristics of FS influence its ability to undergo stabilization.

1.7 Significance of the Study

Dewatering and stabilization of FS are indispensable processes in the treatment of FS to ensure adequate public health and environmental protection (Gold *et al.*, 2018; Strande, 2014). Practitioners have observed in the field that the level of FS stabilization might be related to its dewatering performance. This stems from the observations that less stabilized FS (which is identified through its yellowish to brown colour and strong offensive smell of fresh feaces) dewaters more poorly than more stabilized FS (which is identified through its greyish to black colour and soil or compost smell) (Semiyaga *et al.*, 2016; Cofie *et al.*, 2006; Ward *et al.*, 2021;Ward *et al.*, 2019). This study is motivated by the need to develop low cost and rapid methods that can be used to measure FS stabilization to determine its relation to dewatering performance. In this regard, the study has identified and developed methods and criteria that can be applied to measure FS stabilization. Further, the study has also explored the relationship between FS stabilization and dewatering to confirm what has been observed in the field. Based on the information and data generated, this study contributes to the knowledge gap on measuring FS stabilization and its relation to dewatering performance which has the potential to optimize the design as well as operations of dewatering technologies such as drying beds in the field. The data also adds to the global FS quality database especially on the variability in the dewatering characteristics of sludge from different onsite containment systems as well as location.

1.8 Scope of the Study

The study was restricted to FS generated in OSS facilities located in Lusaka City. The OSS facilities that were adopted for the study included pit latrines and septic tanks. In addition fresh human faeces were also included to test the hypotheses on the degree or level of stabilization of FS samples. Since the aim of the study was to measure FS

stabilization and how it is related to dewatering, low cost and easy to measure methods were employed to ensure easy replication/application in middle and low income countries where treatment solutions for FS are greatly needed. In addition, the parameters of FS that were analysed were those that indicate the stability of organic matter and the dewatering performance of the sludge. All the samples were collected in the dry season i.e. from May 2021 to August 2021.

1.9 Study Assumptions

Two critical assumptions were made as follows:

- User-behaviour and characteristics of each household onsite containment (e.g. pit latrines and septic tanks) are similar within each respective type, hence the location of the facility where FS was collected did not matter.
- There are no major differences in the dietary intake of the households in the study area, hence there was no need to analyse this dimension in the determination of FS stabilization.

1.10 Study Limitations

The study involved field and laboratory work which was all conducted at the time when the COVID-19 pandemic was at peak. As a result, a number of challenges were encountered as follows:

- Study schedule delays due to two indefinite closures of the university in 2020 and 2021 due to the COVID-19 pandemic. During these closures, the researcher could not access the school laboratory to carry out the laboratory work.
- Disruptions in the supply chain for the lab consumables and chemicals that were required in the study as a result of the COVID-19 pandemic. It was challenging to procure the supplies during this time as most of the local suppliers had run out of stock. Most of the laboratory consumables had to be sourced from outside the country and the delivery time took long due to disruption in the supply chains. . In addition some of the required chemicals such as those required to perform the Dehydrogenase Activity (DHA) test could not be sourced as they were out of stock. Thus, some parameters that were intended to be tested could not be analysed.

- Lack of availability of adequately working equipment in the UNZA laboratory resulted in challenges when it came to conducting some tests such as Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). Thus arrangements were made for these tests to be done at another laboratory for Lusaka Water Supply and Sanitation Company (LWSC) limiting the number of samples that could be analysed at a time.

1.11 Ethical Considerations

This research was conducted as part of the ongoing bigger research in FS dewatering at the Swiss Federal Institute of Aquatic Science and Technology (Eawag). Thus, the ethical consideration was done within scope of this bigger research based on the MoU signed between UNZA and Eawag (see attached ethical clearance in Appendix 1). The MoU included a bigger FS qualities and quantities study that was completed in 2020.

Nonetheless, the study involved the collection of FS samples from private pit latrines and septic tanks. Thus In order to protect both the workers and residents from contamination of areas due to sampling activities, a protocol of ensuring adequate sanitization of the area after sampling was developed which included: ensuring the latrine was adequately cleaned with water and then disinfected; and ensuring all the equipment used and the areas around the latrine were disinfected after the sampling was completed. Finally, all laboratory analysis on the faecal sludge was done following standard protocols to ensure that all people working on the samples were not exposed to risks associated with faecal sludge.

1.12 Organisation of the Thesis

Chapter 1: Introduces the research background, problem statement, rationale, research questions, hypotheses, aim and objectives, and scope.

Chapter 2: Presents the literature review that was undertaken to develop the theoretical background around the research topic.

Chapter 3: Describes the methodology adopted for the study.

Chapter 4: Presents the study results.

Chapter 5: Discusses the study results.

Chapter 6: Presents the conclusions and recommendations arising from the key findings of the study

1.12 Chapter Summary

This chapter set out to put the study in context. It therefore provided a background to the areas of research and provided a clear research problem that required to be addressed. Research questions, hypothesis and objectives were outlined providing a contextual setting of the study. The following chapter provides a review of literature critical to the study.

CHAPTER TWO: LITERATUR REVIEW

2.1 Introduction

This Chapter presents the literature reviewed relevant to the study. It first highlights the sanitation challenge at both global and local levels and its impacts on environment and public health. It then proceeds to describe aspects of FS treatment and characterisation. Since a major component of the study was on FS stabilization and dewatering performance, a section looking at stabilization and how it can be measured therein follows. Towards the end, the chapter presents literature on sludge dewatering (covering both FS and wastewater sludge) and its potential linkages to stabilization/ anaerobic digestion.

2.2 The Global Sanitation Challenge

The sanitation needs of the majority living in urban and rural settlements in most developing countries worldwide are served by OSS technologies such as septic tanks and pit latrines. According to UNICEF, (2021) about two out of five people (43 percent) of the global population rely on OSS technologies and in least developed countries only 4 percent are served by sewer systems. The use of the OSS systems results in accumulation of large quantities of FS which requires either to be emptied and transported to treatment (for continued use of the OSS system) or construction of a new one (Still and Foxon, 2012). Joint Monitoring Program (JMP) data shows that in 2020, 3.6 billion people worldwide lacked assess to safely managed sanitation services that ensure that human excreta is safely handled and treated before disposal or end use (UNICEF, 2021). The greatest challenge in most developing countries is the lack of effective management systems for the accumulated FS, generating significant negative public health and environmental risks (Strande *et al.,* 2014; Hawkins *et al.* 2014). A rapid assessment on the status of sanitation conducted by Peal *et al.* (2020) using the Shit Flow Diagram (SFD) approach and methodology implemented in 39 cities revealed that more than 50 percent of the excreta produced in these cities is not safely managed. The non-existence of emptying services and treatment of the FS were the major contributors to this existing situation (Peal *et al.,* 2020). This challenge is even more pronounced as these countries strive to meet the sanitation targets of the sustainable development goals (SDG) which aim at achieving

universal access to adequate and equitable sanitation for all by 2030 (Gambrill *et al.*, 2020).

Faecal sludge management (FSM) refers to the storage, collection, transport, treatment, and safe end use or disposal of faecal sludge (Strande *et al.,* 2014). FS is raw or partially digested, a slurry or semisolid that accumulates in OSS facilities and has not been transported through a sewer (Strande *et al.,* 2014). It is composed of excreta, but also anything else that goes into an onsite containment technology, such as flush water, cleansing materials and menstrual hygiene products, grey water (i.e. bathing or kitchen water, including fats, oils and grease), and solid waste (Velkushanova *et al.,* 2021). The importance of FSM has received numerous recognition over the recent past years, resulting in the need to develop sustainable solutions for the future to contribute to achieving SDG 6 (Strande *et al.,* 2014; Velkushanova *et al.,* 2021). One of the sustainable solutions that need to be developed are those related to FS treatment technologies and operations whose current understanding is limited as FSM is a relatively new field (Strande *et al.,* 2014; Strande *et al.,* 2018 (a)). Thus, it is imperative to generate knowledge in this area in order to optimize the design of FS treatment technologies and at the same time improve operations of existing treatment plants (Strande *et al.,* 2018; Velkushanova *et al.,*2021).

2.3 Sanitation Status in Zambia and in the Project Area

In Zambia, about 40 percent of the population live in urban areas; hence, it is marked as one of the fastest urbanizing cities in Sub-Saharan Africa (UN-habitat, 2015). The rapid urbanization has resulted in the formation of low-income settlements known as peri-urban areas (PUAs). These areas account for the highest number of Zambia's urban population and form a major feature of the country's city landscape (Tembo, 2014). The rapid increase of population has exerted pressure on infrastructure and this has resulted in many complex problems regarding settlement and waste management. Thus, as is the case in most sub-saharan African cities where access to safely managed sanitation services in 2020 was estimated at 21 percent (WHO/ UNICEF, 2021), the situation is not any different in Zambia. According to JMP data (WHO/ UNICEF 2021), only 32 percent of Zambia's population had access to at least basic sanitation services in 2020, meaning that the remainder (68 percent lacked assess to safely

managed sanitation services that ensure that human excreta is safely handled and treated before disposal or end use.

According to projections by the Zambia Statistical Agency (ZSA), the population of Lusaka District is estimated to be 3.5 million inhabitants in 2021. An estimated 70 percent of Lusaka's urban residents live in 33 "peri-urban areas", which are relatively high-density, unplanned neighbourhoods largely comprised of households who are in the low income bracket (LWSC, 2018). According to LWSC (2016), 90 percent of Lusaka's residents use on-site sanitation facilities, consisting of septic tanks (22 percent, pour flush latrines (10 percent), improved pit latrines (50 percent), and traditional latrines (8 percent). Only 9 percent of households are connected to a sewer and open defaecation is estimated at 1 percent. According to the Lusaka SFD only 17 percent of the total human waste generated in the city is safely managed (Kappauf *et al.,* 2018). Of this less than 1 percent of the FS generated in pit latrines (mostly found in low income communities (LICs) was safely collected and transported to treatment. This situation can be attributed to the non-existence of safely managed FSM services in the city as well as the lack of adequate containment and treatment infrastructure. With the majority of Lusaka's population relying on onsite facilities such as pit latrines, it has become imperative to put in place sustainable OSS and FSM systems/ solutions to safely handle the sludge produced in the city along the sanitation service chain. To achieve this, there are many interventions and programs that have been put in place in Lusaka with the major one being the Lusaka Sanitation Program (LSP). LSP is multi-donor funded program being implemented by LWSC which aims at improving access to safely sanitation in Lusaka City. It represents one of the first major investments in Lusaka's Sanitation Master Plan (SMP), the objective of which is to achieve 100 percent safe sanitation coverage by 2035 (LWSC, 2011).

2.3 State of the Art: Faecal Sludge Treatment

Protection of public health and the environment requires that the FS is emptied and transported to treatment before it is subsequently reused or disposed of (Akumuntu *et al.*, 2017; Tayler, 2018). Many of the treatment process and technologies used for FS are based on those developed for wastewater and wastewater sludge treatment, but the two differ in terms of characteristics and volumes or quantities (Tayler, 2018; Bassan, *et al.,* 2014). For example, the concentrations of total solids and organic matter such as COD in FS are up to two orders of magnitude higher compared to wastewater (

(Niwagaba, *et al.,* 2014; Gold *et al.,* 2018; Tayler, 2018). This is due to wastewater being relatively diluted and homogenously mixed by the large quantities of flush water during transportation in the sewer pipes to the treatment plant (Velkushanova *et al.,* 2021) while FS is not. Due this difference, the existing knowledge of wastewater treatment solutions which has been extensively researched on over the years cannot be directly applied to FS treatment (Niwagaba, *et al.,* 2014; Gold *et al.,* 2018; Tayler, 2018).

FS treatment commonly employ biological and physical treatment mechanisms such as drying, dewatering, nutrient removal, pathogen inactivation and stabilization (Bassan, *et al.,* 2014; Tayler, 2018). No single technology can achieve all the treatment objectives, thus typical treatment stages include preliminary treatment (course screening, stabilization of fresh FS), settling or solid-liquid separation (settling-thickening tanks), dewatering of the separated sludge (sludge drying beds), treatment of the separated liquid (anaerobic/ aerobic stabilization) and resource recovery or disposal of the dewatered sludge and treated liquid (Tayler, 2018; Klinger *et al.,* 2019). Both dewatering and stabilization of FS are indispensable mechanisms in FSM which are required to achieve adequate public health and environmental protection (Gold *et al.,* 2018; Strande *et al.,* 2014). This is because FS is mainly comprised of 80 – 95 percent water and readily degradable organic matter (Strande, *et al.,* 2014; Gold , *et al.,* 2016; Semiyaga *et al.,* 2017). Dewatering is a treatment objective that is aimed at separating the water from the solid part of FS through either evaporation, sedimentation or filtration reducing the cost for handling and transportation of the resulting sludge (Semiyaga *et al.,* 2017; Getahun *et al.,* 2020). On the other hand stabilization involves the breakdown of readily biodegradable organic matter, leaving behind a more stable product with less degradable organics (Strande, *et al.,* 2014). It aims at a product that is easy to handle through reduced unpleasantness of fresh FS, without odour nuisance, control vectors and improved settleability as well as dewatering characteristics to reduce volume before disposal (Tayler, 2018; Parravicini, *et al.,* 2006). As compared to Sludgefrom wastewater treatment, very limited studies have been conducted on treatment of FS (especially on stabilization and the dewatering characteristics of FS) to optimize the design and operations of FSTPs to ensure effective treatment (Ward *et al.,* 2019; Gold *et al.,* 2018; Klinger *et al.,* 2019). Currently, research is being actively conducted to determine how FS

treatment processes such as dewatering fits into the body of knowledge of wastewater sludge treatment (e.g. dewatering of activated sludge) that has been studied for many years (Ward *et al.*, 2019; Ward *et al.*, Submitted).

With the current state of knowledge, FS treatment covers technologies that are either established (where adequate knowledge exists e.g. drying beds and settling thickening tanks), transferring (not widely established e.g. mechanical dewatering and anaerobic digestion which have been widely applied in wastewater) and innovative (still under development e.g. black soldier fly larvae) (Ward , et al., 2021). Thus, the most common technologies employed for FS treatment include settling thickening tanks and sludge drying beds, which are established (Tayler, 2018; Ronteltap, *et al.*, 2014). However, these technologies have large land requirements which makes their application difficult in cities in developing countries where land is scarce due to rapid population growth and urbanization (Dodane & Bassan, 2014; Tayler, 2018; Dodane & Ronteltap, 2014; Gold *et al.*, 2016). Thus, experimentation is required to develop knowledge on how transferring technologies such as geotextile, the use of conditioners and mechanical presses can be optimally applied in FS dewatering to increase throughput and treatment performance and reduce the footprint (Ward, et al., 2021).

2.4 Faecal Sludge Characterization

The design of treatment solutions requires characterizing and understanding the properties of FS to be treated (Niwagaba, *et al.*, 2014; Bassan *et al.*, 2013). FS characterization involves measuring and evaluating the physical, chemical and biological properties of FS to e.g. understand stabilization processes, monitor treatment efficiency, or determine loadings for design and operation of a treatment plant (Velkushanova & Strande, 2021). There are various properties/ parameters of FS and the specific ones to be measured are determined based on the objectives of carrying out the characterization (Velkushanova & Strande, 2021). However, common properties include total solids (TS), Volatile Solids (VS), COD, BOD, nutrients, pathogens and metals (Niwagaba, et al., 2014). Other properties related to resource recovery of FS as a fuel (e.g. calorific value) or selection/ or design of a technology for emptying containment systems (e.g. particle size, viscosity) can also be determined (Tembo, 2019; Muspratt *et al.*, 2014).

From literature, there is high variability in the characteristics of FS due to a number of factors such as variations in the types of containment technologies (e.g. pit latrines, septic tanks), differences in household usage/ practices, containment retention time, frequency of collection, quality of construction and environmental factors such as type of soil and water table (Niwagaba, *et al.,* 2014 ; Velkushanova & Strande, 2021; Wanda *et al.,* 2021; Strande *et al.*, 2018b; Bassan *et al.,* 2013). In addition the accurate determination of characteristics of FS is complex due to FS heterogeneity, lack of standardized methods for sampling and analysis and differences in laboratory capacities (Velkushanova & Strande, 2021). To improve this, efforts are currently being made to develop standardized methods that can adequately determine the characteristics and quantities of FS. One such example is the recent publication: Methods for Faecal Sludge Analysis, which includes standard methods for sampling and characterization of FS. The publication also presents a methodology which is based on the use of Spatially Presented Demographic, Environmental and Technical Data (SPA-DET) as predictors of characteristics and quantities of FS (Velkushanova & Strande, 2021; Strande *et al.*, 2018b; Velkushanova *et al.,*2021). This method was recently applied in a study to estimate the characteristics and quantities of FS produced in Lusaka (i.e. the study area) to aid the design and operations of treatment plants that are earmarked for construction in Lusaka city. The results of the study showed high variability in the concentrations of TS and COD, however, they were comparable to what is observed in other cities in Sub-saharan Africa (Andriessen, et al., 2020). This variability in the characteristics of FS was also reported in another study by Tembo, (2019) conducted in Lusaka though it did not apply the SPA-DET methodology.

Other FS characterization studies have also included dewatering, settling and biodegradability or stabilization as parameters and how they are related physico-chemical parameters such as TS and COD. One study carried out in eThikwine Municipality (South Africa) included aerobic biodegradability as a parameter in the characterization and the results showed variations in the degree of stabilization of FS with less than 50 percent biodegradability (Bakare *et al.*, 2012). In another study conducted by Gold *et al.*, (2018) , it was found that the dewatering rates differed between sludge collected from different containment systems and was positively correlated to electrical conductivity (EC) and ammonia (NH_4-N). This is in agreement with the variability observed in other characterization studies. In both studies, Ward

et. al., (2019 and 2021) also reported similar results showing variability in FS dewatering rates based on source (e.g. public toilet, household toilet, pit latrine and septic tanks) and strong correlations with physico-chemical parameters such as EPS. Another study conducted in Kenya reported the ranges and upper limits of commonly measured parameters such as COD, TS and EC where the best settling performance of FS is expected to occur e.g. the limits for COD and TS were reported to be 42.8g/L and 32.9g/L, respectively (Junglen *et al.*, 2020).

Thus, a number of studies have been conducted that have shown relationships between simple/ rapid field-based measurements (e.g. pH, electrical conductivity and colour) and laboratory-based measurements (e.g. TS, ammonia, dewatering time) which have the potential to improve the prediction of variability of FS characteristics and dewatering performance (Ward *et al.,* 2021; Gold *et al.,* 2018; Bousek *et al.,* 2018; Doglas *et al.,*, 2021). Ward et al. (2021) reported supernatant colour to be the best predictor of dewatering performance while texture of sludge photographs was the best predictor of total solids (TS) through the use of predictive models developed based on a dataset of 421 samples collected from OSS in Lusaka. The characterization of FS settling and dewatering performance and its correlation with easy to measure physico-chemical parameters such pH, EC, TS, COD and stabilization can aid the planning of treatment steps and operations to increase treatment efficacy (Junglen *et al.*, 2020).

2.5 Faecal Sludge Stabilization

FS must be stabilized before it is disposed or reused. Stabilization involves the breakdown of readily biodegradable organic matter, leaving behind a more stable product with less degradable organics (Strande, *et al.,* 2014). It also results in reduced volume (for easier transportation of FS) and pathogen inactivation to prevent contamination of the environment and the public (Kazimierczak, 2013; Tayler, 2018).

Commonly applied stabilization processes in FS and wastewater treatment include biological (anaerobic and aerobic digestion), chemical (e.g. lime stabilization) and thermal (e.g. pyrolysis and incineration). Biological stabilization utilizes micro-organisms in controlled steady state natural conditions to reduce the biodegradable organic matter content of the sludge, a process termed as digestion. Biological stabilization options mostly applied in FS treatment include aerobic digestion, anaerobic digestion, compositing and emerging innovative technologies such as

vermicomposting and black soldier flies (Ronteltap, *et al.,* 2014;Yadav *et al.,* 2010). From these treatment processes, anaerobic digestion has a wider application in FS treatment processes as it leads to resource recovery through biogas production and biosolids for soil conditioning as well as biofuels (Madikizela, *et al.,* 2017).

FS which originates from OSS technologies such as septic tanks and pit latrines will normally offer limited scope for further digestion at treatment (Tayler, 2018) due to the longer storage times in the containment systems where it undergoes digestion in anaerobic and facultative conditions. On the other hand, FS collected from frequently emptied facilities such as those found at public places such as markets, bus stations and restaurants is likely to be poorly stabilized (Ward *et al.*, 2019), offering a wide scope for further digestion at treatment . However, the processes occurring during onsite treatment of FS in containment systems such as pit latrines are currently not well understood (Velkushanova & Strande, 2021; Nwaneri *et al.,* 2008) due to limited evindence. The processes have been theoretically conceptualized by Nwaneri *et al.*(2008) as physical (filling and accumulation of FS) and biological (degradation of organic matter content of FS). A few studies on processes in onsite containments suggest that both aerobic and anaerobic digestion contribute to removal of organics in containment systems (Still & Foxon, 2012; Nwaneri *et al.,* 2008), however, the bulk of the digestion can be said to be attained through anaerobic digestion (Van Eekert *et al.*, 2019; Shaw and Dorea, 2021).

Unlike biological processes, chemical stabilization involves the addition of chemical compounds such as lime and conditioners to FS to improve the performance of physical characteristics such as settling, dewatering or to inactivate pathogens and stabilize the sludge (Strande *et al.,* 2014; Tayler, 2018). Lime stabilization has mostly been shown to sanitize FS and septage at treatment through pathogen reduction (Greya *et al.,* 2016; Anderson *et al.,* 2015) with no direct organic matter reduction. However, Greya *et al.* (2016) observed a reduction in the readily biodegradable matter of FS (reported as reduction in volatile solids) with increasing alkaline conditions due to lime addition. Further chemical stabilization through the use of conditioners at treatment increase settling and dewatering of FS resulting in an increase in treatment capacity as well as reduced foot print of technologies such as drying beds (Gold *et al.,* 2016;Moto, *et al.,* 2018)

Lastly thermal processes for the treatment of FS are emerging (they include pyrolysis, thermal drying, hydrothermal oxidation) and offer complete destruction of pathogens while allowing for resource recovery (Krueger, *et al.*, 2021).

2.5.1 Factors Affecting Faecal Sludge Stabilization

FS accumulating in onsite containment systems undergoes biological processes that stabilize it during storage. It is subjected to further treatment processes (which can be chemical, biological or thermal stabilization) when transported to central treatment facilities (e.g. a-FSTP) before it is finally disposed in to the environment or reused. Factors affecting biological processes inside onsite containments include temperature, pH, Moisture content, presence of nutrients and presence of inhibitory or toxic substances (Van Eekert *et al.*, 2019; Shaw and Dorea, 2021; Doku, 2002). The same factors also apply when FS is subjected to further treatment at FSTPs using biological processes. However, the presence of readily biodegradable organics becomes important as well. When it comes to chemical and thermal stabilization, organic composition of FS (e.g. lignin, proteins and cellulose content), temperature, moisture content, solids concentration and pH have been reported to affect stabilization at treatment (Krueger *et al.*, 2021; Krueger *et al.*, 2021; Greya *et al.*, 2016; Gold *et al.*, 2016).

2.6 Methods for Measuring Stabilization

No studies have reported measuring and quantifying the level or extent to which FS is stabilized during the time it is stored in onsite containment facilities such as pit latrines and septic tanks. In most cases descriptive and qualitative parameters such as colour, odour, place of origin (public or domestic) type of containment (septic or pit latrine) and how often the facility is emptied are used to predict the level of FS stabilization (Ward *et al.*, 2021; Ward *et al.*, 2019). Thus, reference will be made as much as possible to the existing studies on the stabilization of wastewater Sludgeand compost. Methods and criteria for measuring stabilization of wastewater sludge and composts have been well researched and published (Bożym and Siemiątkowski, 2020; Benito *et al.*, 2005; Samson & Ekama, 2000; Cokgor *et al.*, 2012; Borglin *et al.*, 2012; Bernal et al. 1998; Ferrer, 2006; Mangkoedihardjo, 2006). From the literature, methods for measuring stabilization of organic substrates such FS, wastewater sludge and

composts after being subjected to biological treatment processes can be classified in the following categories:

i. Microbial Activity Methods

These methods are based on the premise that the biological stabilization process of organic matter in a sludge is strongly associated with the activity of living micro-organisms and enzymes (Pandey *et al.*, 2017; Nybroe *et al.*, 1992). This is because during the biological stabilization process, micro-organisms break down complex organic substrates resulting in more microbial biomass (Spanjers and Vanrolleghem, 2016). At the same time the biological activity of the micro-organisms during the stabilization process are controlled by enzymes, thus, the activity of the microorganisms and enzymes within the sludge floc can be used as an index for sludge stabilization (Renneberg, et al., 2017). The following methods exist in this category:

a) *Biomethane Potential (BMP) Test:* this method is used to determine the amount of methane that can be produced from organic materials providing information on how much and how fast the material can be degraded under optimal anaerobic conditions (Holliger *et al.*, 2016; Filer *et al.*, 2019; Strömberg *et al.*, 2014).
b) *Dehydrogenase Activity (DHA) Test:* this method is used to measure the biodegradability of organic substrates based on the measurement of the colour produced on reduction of the original substrate, INT 2-(p-nitrophenyl)- 5-phenyl tetrazolium chloride), to INT-formazan, by the oxidative effect of the dehydrogenase enzymes (Sánchez *et al.*, 2006; Xie *et al.*, 2008). Intercellular dehydrogenase enzyme is one of the main oxidoreductase enzymes which can be considered as an indicator of microbial activity. This enzyme plays an important role in the biological oxidation of organic compounds and causes the transfer of hydrogen from the organic substrate to the inorganic acceptor (Pourakbar *et al.*, 2020).
c) *Specific Oxygen Uptake Rate (SOUR):* SOUR is part of the respirometry methods for measuring microbial activity of organic substrates. SOUR aims at measuring the stability of a substrate by measuring oxygen consumption rate under normal conditions when the only limiting factor should be the amount of assimilable oxygen (Lasaridi and Stentiford, 1998).

ii. Elemental Composition Methods

These methods are based on the premise that the stabilization process of organic substrates results in a reduction of some physico-chemical and biochemical elements of the original sample (Godley *et al.*, 2004; Kazimierczak, 2013). The methods involve measuring the quantities of total organics contained in the substrate and estimating portions that are available for biological degradation. The following are some of the methods in this category:

a) *VS/ TS Ratio:* TS are the dry matter content of the substrate or sludge while VSis the proportion of TS that are organic and digestible. Thus, VS are a common indicator of the amount of organic matter content in the sludge. VS/TS ratio is defined as the ratio of the concentration of VS to dry solids and is an indicator of the level of stabilization for a sludge sample (Kazimierczak, 2013). In addition, reduction in VS during biological stabilization has been used to measure the degree of stabilization of sludge. Podedworna and Umiejewska (2008) reported that the reduction of VS concentration during stabilization process at the level of 38–40 percent can be assumed as the stabilization limit.
b) *COD/ BOD Ratio:* The organic matter content of substrates such as wastewater, FS and wastewater Sludgecan be determined or measured using BOD and COD. COD/ BOD ratio indicates the proportion of total organics in a substrate that are bioavailable for degradation (Mangkoedihardjo, 2006; Kewu and Wenqi, 2008; Changara *et al.*, 2018). Further, reduction in COD during biological stabilization has been used to measure the degree of stabilization of sludge. Sánchez *et al.* (2006) reported a reduction in the COD of 51 percent at the end of the experiment (after 135 days of sludge aeration in a 100L benchtop reactor). From day 70, the value of COD was constant.
c) *COD/ TOC ratio:* TOC is a measure of the total carbon in a substrate which is oxidizable and can also be used as energy source for microorganisms during stabilization. Thus, COD/ TOC ratio can be used as an indicator for stabilization because it represents the availability of organic carbon during biodegradation.

iii. Surrogate Methods

Since both the microbial activity and Elemental composition methods are based on biological tests that are time consuming, alternative methods that are rapid, simpler and cheaper can be useful as surrogates to biological tests to measure stabilization (Godley *et al.,* 2004). Several methods which include the lignin content determination (using the Klason method), Cellulase hydrolysis test and determination of humic substances (i.e. humification) have been used to stabilization (Hartenstein, 1981; Bernal *et al.,* 2009; Godley *et al.,* 2004). The widely applied is the humification method especially in the evaluation of maturity of composts (Humification index and rate of humification) which are determined through the fractionation of organic carbon of a sludge sample into humified substances (humic acid and fulvic acid) and non-humified substances (NH) (Bernal *et al.,* 2009; Ciavatta *et al.,* 1990). Most of these stabilization test methods have strengths and weaknesses and each is developed to determine stabilization for specific purposes and different situations. Since most of the widely applied stabilization test methods are time consuming and costly (Matsinhe, 2011), it is imperative to develop simple, rapid and low cost methods that can be used to determine the stabilization of FS for dewaterability.

2.7 Sludge Dewatering

FS is mainly comprised of about 80 - 95 percent water, making dewatering a requirement to ensure effective treatment (Strande, *et al.,* 2014; Gold, *et al.,* 2016). According to Ward, et al., (2021), dewatering is the removal of free water and water that is loosely bound in pores and interstitial spaces of sludge particles and flocs. The most common technologies employed in the dewatering of FS include settling thickening tanks and sludge drying beds, (Dodane & Bassan, 2014; Tayler, 2018; Gold , *et al.,* 2016) and can dewater FS (depending on the properties) to between 70 to 80 percent water content by weight (Ward, et al., 2021). Very limited research has been conducted on the dewatering performance of FS as compared to wastewater sludges. A recent study conducted by Ward *et al.* (2019) which was aimed at evaluating how dewatering of FS fits into the existing knowledge of wastewater sludge found out that FS had different dewatering behaviour than wastewater sludge and may be governed by different mechanisms. However, the mechanisms and factors that drive/ or influence dewatering performance in both wastewater sludge and FS have been reported to be the same and these are physico-chemical characteristics (TS, pH, EC and surface charge), EPS, particle size distribution and level of stabilization (Gold *et*

al., 2018; Semiyaga *et al.,* 2016; Ward *et al.,* 2019; Ward *et al.,* 2021; Ward *et at.,* Submitted; Sam *et al.,* 2022; Dai *et al.,* 2013; Christensen *et al.,* 2015). Among these, the concentration of smaller particles (i.e. particle size distribution) and EPS are reported to be major fundamental mechanisms. For example it is well accepted within wastewater sludge literature and most recently FS (based on one study) that poor dewatering performance is caused by the presence of smaller particles (<100 µm) which clog filter beds and the interstitial spaces in the sludge cakes (Ward *et al.,* submitted; Christensen *et al.,* 2015). Similarly, high concentrations of easily extractable or soluble polymers (also known as soluble EPS, slime EPS or soluble microbial products) that are released and dissolved into solution in a sludge have been reported to cause poor dewatering performance (Ward *et al.,* 2019; Ward *et al.,* 2021; Dai *et al.,* 2013; Sam *et al.,* 2022). The flocculated matrix of EPS also helps in keeping the bacteria responsible for digestion during biological stabilization and affects the physical-chemical characteristics of the sludge floc (Dai *et al.,* 2013). FS that appears to be less stabilized has been reported to have high concentrations of EPS and smaller particles which have been associated with poor dewatering performance (Ward *et al.,* 2019; Ward *et al.,* 2021; Sam *et al.,* 2022). In both studies, Ward *et. al.,* (2019 and 2021) noted that qualitative parameters such as light brown colour and fresh excreta odor which are associated with less stabilized FS corresponded with samples that were difficult to dewater. In addition, Ward *et al.* (submitted) reported correlations between dewatering performance and indicators of stabilization. These results are witth field observations, that more stabilized FS is easier to dewater than fresh sludge which is not stabilized (Semiyaga *et al.,* 2016; Cofie *et al.,* 2006).

Further, inconsistent results have been reported when it comes to the effect of anaerobic stabilization on the dewatering performance of different types of wastewater Sludge(activated, primary and secondary sludges) (Pontoni *et al.,* 2018) and most recently FS (Ward *et al.,* Submitted). Generally, anaerobic digestion can either improve or worsen dewatering performance depending on the origin of the sludge (Ward *et al.,* submitted; Christensen *et al.,* 2015; Pontoni *et al.,* 2018). Two studies by Ward *et al.* (submitted) and Sam *et al.* (2022) have observed some improvement of dewatering performance with anaerobic digestion which was associated with the degradation of EPS and smaller particles. However, in both studies the reduction was inconsistent and not statistically significant as was expected. On the contrary, one

study by Shahid*et al.*, (2022) reported an increase in all EPS fractions (i.e. Soluble EPS and tightly bound EPS) during FS thickening in anaerobic membrane-based thickening tank (MBTT), but it didnot result in a decrease in the dewatering rate of the sludge. This could be due to the higher fractions of tightly bound EPS (comprising high protein concentrations) which promote formation of larger sludge flocs countering the blinding effect of the small fraction of soluble EPS (Guo, et al., 2016). Other studies on anaerobic digestion of wastewater sludge have report an increase in the concentration of soluble EPS during the stabilization process (with higher fractions of humic-like substances) resulting in poor dewatering performance (Dai *et al.,* 2013; Tonanzi *et al.,* 2021; Guo *et al.,* 2020; Sakaveli *et al.,* 2021; Gebreeyessus, 2020). The increase in EPS fractions can be temporal as a result of their fast release/ accumulation during the start-up phase of the anaerobic digestion process and can decrease after longer periods of stabilization (Gebreeyessus, 2020; Shahid, et al., 2022; Novak *et al.,* 2003). This could explain why Ward *et al.* (2019) reported that the concentration of EPS in FS was an order of magnitude lower than that reported for wastewater sludges. This could explain the difference in the dewatering behaviour of FS compared to wastewater sludges.

2.8 Gap Analysis

Empirical and field observations suggest that the level of FS stabilization appears to be a good predictor of dewatering performance (Ward *et al.,* 2019; Shahid, et al., 2022; Cofie *et al.,* 2006; Gold *et al.,* 2018). These observations have been made through the use of descriptive and qualitative parameters such as colour, odour, place of origin (public or domestic) type of containment (septic or pit latrine) and how often the facility is emptied to distinguish between the so called less stabilized and stabilized FS. Further, various studies on FS and wastewater Sludgehave reported that characteristics/ parameters such as EPS, EC, pH and monovalent cations influence the underlying mechanisms (floc formation, particle size distribution) that govern the dewatering performance of various sludge types (Gold *et al.,* 2018; Ward *et al.,* 2019; Dai *et al.,* 2013; Tonanzi *et al.,* 2021; Guo *et al.,* 2020; Sakaveli *et al.,* 2021; Gebreeyessus, 2020; Novak *et al.,* 2003 ; Ward *et al.,* 2021; Sam *et al.,* 2022; Ward *et al.,* Submitted). In addition, these parameters are said to be altered during biological stabilization resulting in changes in the dewatering performance as well.

However, no specific studies have reported measuring and quantifying the level of FS stabilization and how it relates to dewatering (Ward *et al.,* 2021). The closest are studies by Shahid *et al.* (2022), Ward *et al.* (submitted) and Sam *et al.* (submitted) that looked at the evolution of EPS, particle size distribution and indicators of stabilization during anaerobic storage of simulant FS (made from urine, feces and water) and their respective correlations to dewatering performance. However, the studies did not measure the extent to which the simulant FS was stabilized during the anaerobic storage. It is therefore clear that there is a gap in the determination of FS stabilization (i.e. measuring the level of stabilization) and its relation to dewatering characteristics. This study therefore explored an area that had not been researched on before and was designed to address this identified knowledge gap.

2.9 Chapter Summary

This chapter reviewed literature relating to the area of study. It revealed that limited research has been conducted on the dewatering performance and measurement of stabilization of FS. The chapter indicated that a wealth of knowledge and information exists on measuring stabilization and dewatering performance from related fields such as the wastewater sludge and composts. The review revealed that despite this gap, practitioners in the field of FS treatment have observed correlations between the level of stabilization and the dewatering performance of FS. The review further revealed that descriptive information such as colour and odour of FS is used to characterize level of stabilization. This chapter therefore underlined the need to develop low cost and rapid methods to measure FS stabilization to improve accurate prediction of its dewatering performance if the relationship between the two is established to exist as practitioners have been observing in the field. The ensuing chapter discusses the methodological approach that was used to address the set objectives of the study.

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Introduction

This study aimed at answering several questions relating to stabilization and dewatering with the aim of developing rapid and low cost methods for measuring FS stabilization to enhance prediction of its dewatering performance. To this effect, the research sought to answer the research questions and prove the hypotheses presented in sections 1.4 and 1.5, respectively. A critical analysis of the available literature on the research topic which is comprehensively presented in Chapter 2 resulted in the identification of knowledge gaps thereby providing support for the significance of this research. The study adopted quantitative research methods since the purpose of the study is the scientific explanation of how FS stabilization can be measured and it is related to dewatering performance. Thus the study employed the use of fixed experimental design, laboratory analytical methods and representative samples to produce results that can be replicated and generalized. This section summarises the methodology and research design that was employed in this research.

3.2 Study Area

The study area Lusaka, is the capital city of Zambia. It is the largest city in the country and covers an area of approximately 360 km^2 (LCC, 2022). It is the smallest yet most densely populated of the 116 Zambian districts (CSO, 2016). The latest census (2010) estimated the city's population at 1,715,032 (CSO, 2012) with an annual average population growth rate of approximately 3.8 percent. According to projections by the Central Statistical Office (CSO), the population of Lusaka District is estimated to be 3.5 million inhabitants in 2021. Of this, an estimated 70 percent of residents live in 33 "peri-urban areas", which are relatively high-density, unplanned neighbourhoods largely comprised of low income earning residents (UN-HABITAT, 2007). A survey carried out by Lusaka Water Supply and Sanitation Company (LWSC) showed that 90 percent of Lusaka residents use on-site sanitation facilities, consisting of septic tanks (22 percent, pour flush latrines (10 percent, improved pit latrines (50 percent), and traditional latrines (8 percent) (LWSC, 2018). The survey estimated sewer connection coverage at 9 percent of city's total population. Overall, the shit flow diagram for Lusaka estimated access to safely managed sanitation at only 18 percent

(Kappauf *et al.*, 2018). The Figure below shows the map of Lusaka and the different types of sanitation systems.

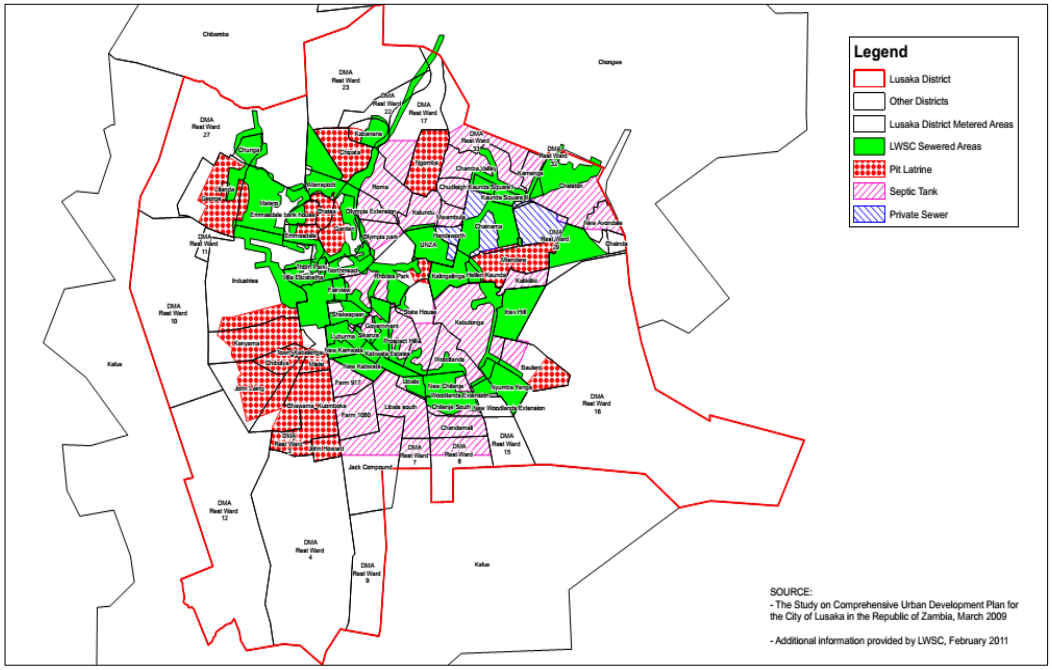

Figure 3-1: Map of Lusaka showing the coverage of different types of Sanitation Systems (Source: Lusaka Sanitation Master Plan, 2011)

From the foregoing, it is clear that proper management systems are required for FS that is accumulated in the OSS systems which serves the majority of the population.

3.3 Research Design

The experimental work under this research was carried out in two parts i.e. Part 1 which covered the selection of Methods for Measuring FS stabilization and Part 2 which covered the batch stabilization and dewatering experiments.

Part 1: Selection of Methods for Measuring FS Stabilization

Under part 1, methods for measuring FS stabilization were selected. The first step was conducting a detailed desk-based review of methods for measuring stabilization of organic substrates (e.g. FS, wastewater sludge and composts) that have been applied by various scholars mostly in wastewater sludge and composts. Based on the results of the desk review, a weighted criterion was developed upon which methods were selected for further evaluation in the lab to determine their suitability to be applied to measure the stabilization of FS. Generally, the selection of a particular test method was based on:

- local availability of the method requirements such as equipment, materials, and required laboratory techniques;
- easy and low-cost method; and
- ability of the method to allow a large number of replicates to be tested, thereby providing reproducible and accurate results.

Characteristics/parameters that are related to FS stabilization (those that reflect the concentration of organic matter) were also measured to determine correlations with the level of stabilization.

Part 2: Batch Faecal Sludge Stabilization and Dewatering Experiments

Under Part 2, batch laboratory FS anaerobic stabilization and dewatering experiments were conducted to determine how the two are related. FS samples were subjected to further stabilization under anaerobic conditions in lab scale reactors under controlled conditions. Stabilization measurements using the best evaluated methods in part 1 were conducted as well as dewatering tests using methods described in section 3.4 below.

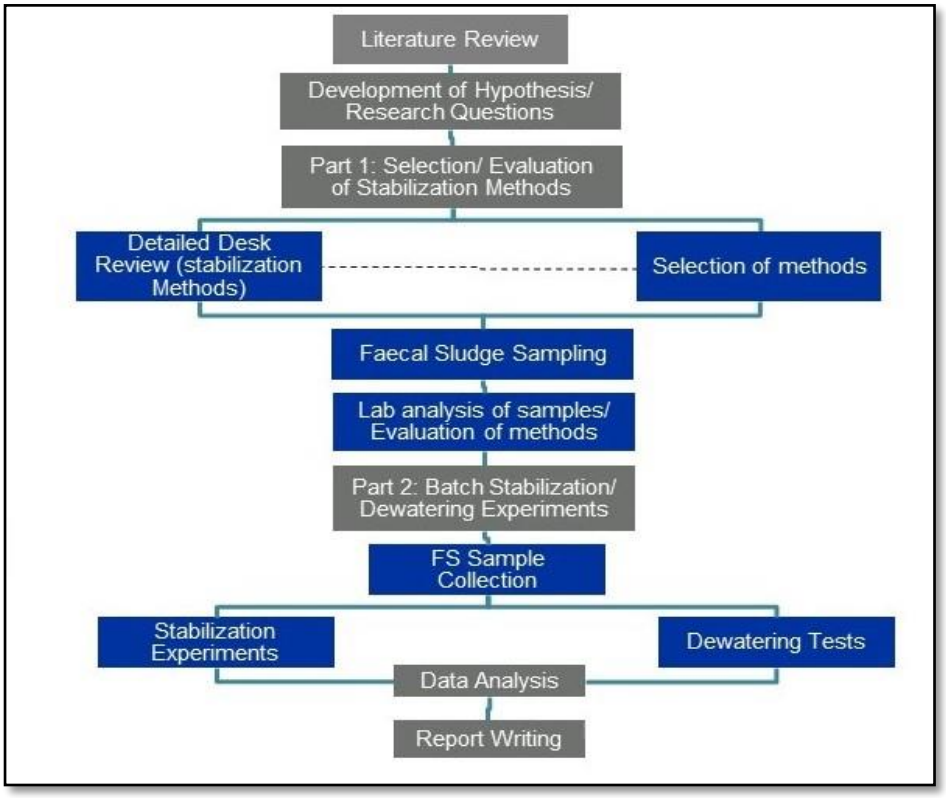

Figure 3-2: Graphical Representation of the Research Process Flow (Source: Author)

3.4 Materials and Methods

The research was carried out in parts i.e. Part 1: Selection of methods for measuring FS stabilization and Part 2: Batch stabilization and dewatering experiments.

3.4.1 Part 1: Selection of Methods for Measuring Faecal Sludge Stabilization

The selection of methods for measuring FS was done through a desk based literature search and laboratory evaluation of selected methods. The methods are detailed in the issuing sections.

3.4.1.1 Desk Study: Methods for Measuring Stabilization

A systematic literature search and review was conducted to identify available methods for measuring stabilization of organic substrates which have been applied/ used in published studies. In order to be eligible for inclusion, the publications were required to be in English, published after 1970 and before September 2020 and should be an article, a white or proceedings paper, a review, a dissertation or a book chapter. Searches were conducted in google scholar and web search to identify relevant literature to be included in the review. Additional studies were identified by searching the bibliography of identified studies. The search terms that were used included: Sludge stability index, sludge stabilization indicator and measuring sludge stabilization. Screening was conducted to ensure only literature containing laboratory methods or metrics for measuring stabilization as well as processes related to sludge biodegradability were included. The screening strategy included preliminary title screening and subsequent abstract and main text body screening to determine eligibility. The screening strategy followed the preferred reporting items for systematic reviews and meta- analyses (PRISMA) guidelines as shown in Figure 3-3.

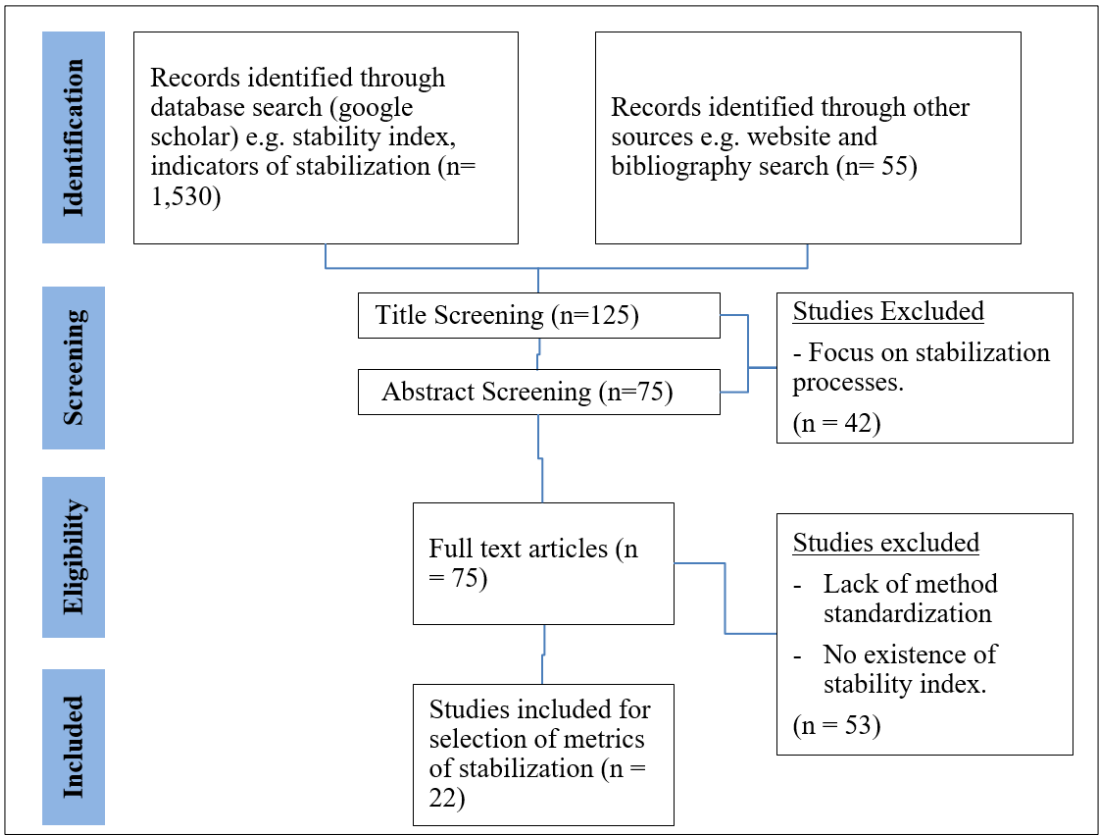

Figure 3-3: Process Flow Chart for selection of literature (Source: Author)

Fourteen (14) potential methods for measuring stabilization of organic substrates (such as wastewater sludge and FS) were identified. Then, a decision matrix (screening criteria based on method attributes and suitability for local application (see Appendix 3) which utilized a weighted score approach was used to screen each identified method. Table 3-1 details the screening criteria that was employed.

Each sub criterion was assigned a weighting (determined based on its viewed importance) and summed up to the total weighting of the main criteria. Each sub-criterion was scored using score values of 0, 2, 4, 6, 8 and 10. The score was multiplied by the assigned weighting for each sub-criteria and the product divided by the maximum possible score (i.e. 10) to get the weighted score. The sub-criterion scores were summed up to the score for each main criteria. The full criteria and scoring system are presented in Appendix 3.

Table 3-1: Screening Criteria

Criterion	Definition	Description
Appropriateness of the method based on findings from literature review		
Robustness	Control necessary at each stage of the method as an indication of reliability during normal usage	A method which is affected by small variations in method or experimental parameters scored less points.
Selectivity	Extent to which a method can determine stability through an analyte without interferences from other components.	A method which uses an analyte with least or no interferences from other components in the test sample as a measure of stabilization scored more points.
Defined units of stability index and limit	Stability index (limit of stabilization) is expressed in appropriate units of measurement and a limit is defined to indicate a well stabilized sample.	In literature the units of measuring the stability index are consistent among different researchers/ or studies and a limit is defined to indicate what is stabilization. Methods showing meeting this criterion based on literature scored high points.

Table 3-1 Continued

Criterion	Definition	Description
Demonstrated ability to measure Stabilization of wastewater / or faecal sludges	Application of the method to measure the stabilization of either wastewater or faecal sludge in at least two published scientific studies or papers	Based on literature review, methods meeting this criterion scored high points
Lab Attributes of the Method		
Simplicity of lab techniques and protocol	The lab techniques and protocol for the method are clear and easy to implement without the need for expert training and repeated exercise before one can confidently run the stabilization test. The method should also be suitable to be conducted in the Local Laboratory.	Existence of an SOP for the method.
Required effort	Estimated number of man hours per day required to successfully conduct the method.	Access effort based on SOP and indications from literature.
Working range	The range over which an analyte used to determine stability index for the method can be determined with reliability	Reported or accessed upper and lower detection limits for the method.

Table 3-1 Continued

Criterion	Definition	Description
Cost	Total cost of running the method based on local conditions i.e. Lusaka context. This includes the cost of laboratory consumables, equipment and allowances of helpers if required.	Expensive methods that required the use of high tech. equipment scored less points
Local application	Availability of all the required laboratory consumables, equipment and reagent locally i.e. in Lusaka.	Based on inquiries with local laboratory equipment and consumables suppliers in Lusaka
Application of the Method by Others		
Reproducibility and Repeatability	How close are the results of replicate measurements made on the same sample as reported in literature	A method that shows similarities in results reported by at least 3 studies scored more points.
Application in low-income settings	Suitability of the method for application in low-income countries with less advanced laboratories in terms of equipment and technology	Method complexity, required technical know-how and use of advanced equipment or technology

Based on this preliminary screening, five (05) methods which fulfilled the criteria best were selected for further evaluation using laboratory scale experiments (evaluation scores in Appendix 3). The methods were categorized as; (i) Elemental Composition Methods (BOD/ COD and VS/ TS ratio), and (ii) Microbial Activity Methods which

included the BMP, SOUR and the DHA. The number of methods to be selected was set at five (05) in order to balance the laboratory workload and time required to perform the tests on the samples to ensure that quality is not compromised.

3.4.1.2 Faecal Sludge Sampling

The raw materials used in the first part of the research included FS and fresh human excreta (made from urine, faeces and water). Lusaka city has three FSM zones with dedicated private operators providing emptying services in each respective zone. Thus, as an entry strategy to make sample collection easy, arrangements were made with the private operators to collect FS samples from selected facilities on their scheduled Jobs by accompanying the emptying teams to each respective site. A total of five FS samples were collected from onsite containment systems in the month of May 2021. The samples comprised four samples from pit latrines (located in Chazanga and Kanyama which are low income communities) and one septic tank (located in Handsworth court which is a high income residential areas) all located within Lusaka city.

The samples were collected from containment systems with varying sludge age i.e. a recently constructed fully lined pit latrine (vertical vault latrine) which had been in use for only six months (for collection of samples assumed to be less stabilized) and ordinary pit latrines and a septic tank which have been in use for more than three years (for collection of samples assumed to be stabilized). To test the hypothesis on variability of levels of stabilization (i.e. have a mix of samples assumed to be fresh, less stabilized and stabilized) one fresh human excreta sample was included which was made by mixing urine, feaces and water in a blender (250ml combination of urine and feaces to 500ml of tap water).

For pit latrine sample collection, the study team accompanied the private operators during the emptying of pit latrines. Approximately 10L of a composite sample was collected during the emptying of a specific pit latrine by using an elongated scooper (a prefabricated tool used to manually empty pit latrines with an approximate working volume of 4 Liters shown in Figure 3-4): 1 scoop at the start of the emptying job, 1 in the middle, and 1 at the end of the emptying Job. The composite sample was then homogenized in a 60L barrel and a 2L sample was taken for lab analysis. For septic tank samples, a 3m long core sampler based on the design presented in (Koottatep, et

al., 2021) was used (see Figure 3-4). A composite sample was produced by emptying the contents of the core sampler into a bucket, homogenizing the contents, and taking a 2 L sample for lab analysis. Lastly, the fresh sample (a mix of feaces and urine) was collected using a black plastic bag which was tied and transported to the lab immediately. The mixture of fresh human faeces, urine and tap water was homogenized in blender to form a paste like sample and taking a 1L for analysis. All samples collected were transport to the laboratory in a cooler box and stored in a fridge at 4°C until analysis.

Figure 3-4: Faecal Sludge Sampling (LHS: Sampling from a pit latrine using an elongated scooper; RHS: Sampling from a septic tank using a core Sampler). (Source: Author)

3.4.1.3 Laboratory Analysis

a) Sample Processing

Upon arrival at the lab, the samples were processed as depicted in the Figure 3-5 below. The samples were first homogenized thoroughly by shaking/stirring, and were divided into two portions – one to be blended for physical-chemical characterization, and the other to be unblended for stabilization test methods. Blending was avoided as it is likely to change sample characteristics and alter the rate of oxygen uptake significantly in the case of the specific oxygen uptake rate test.

b) Physical-chemical Characterization

A 300ml aliquot of the sample was homogenized in a blender for three minutes at medium setting. pH, EC, TS and VS were analyzed according to the standard methods (APHA 2017). Density was measured by determining the mass of 20 mL of sample. COD was measured using closed reflux photometric method - (APHA 2017). This was performed using medium range COD test cells obtained from Merck. Hydrotest Photometer HT1000 was used to read the results. BOD was measured using the 5-day BOD and membrane electrode method (APHA 2017). All samples were analysed within six days of sample collection and were stored at 4℃ in a refrigerator.

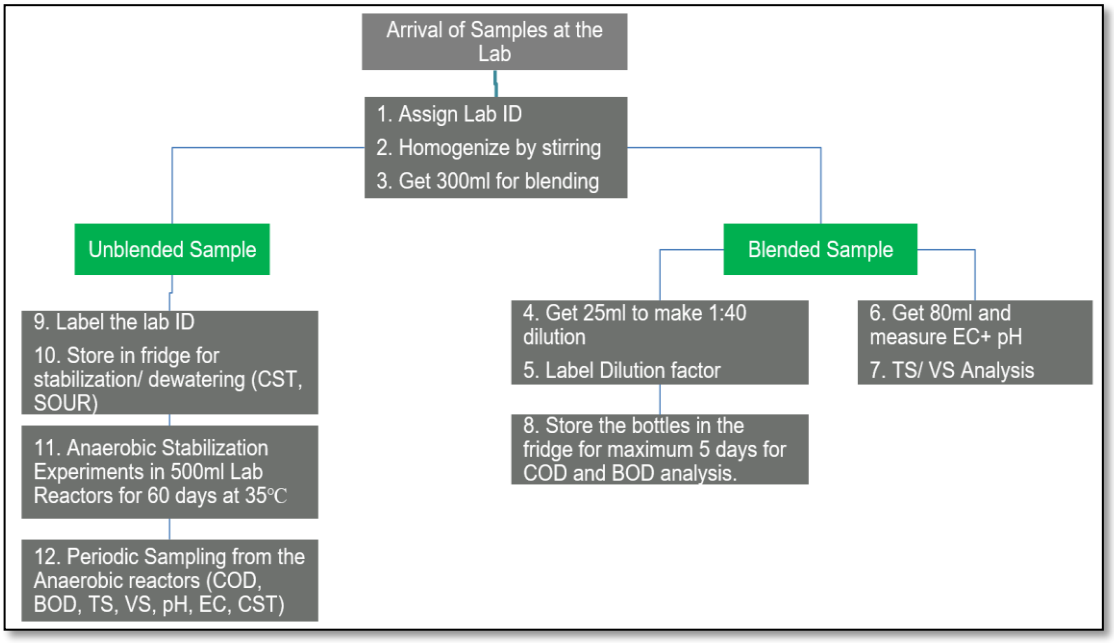

Figure 3-5: Graphical Representation of Sample Processing and Analysis Workflow: Part 1 (Source: Author)

c) Methods for Measuring FS Stabilization

As stated earlier, five methods were selected from literature as potential methods for measuring FS stabilization. They were categorized as follows:

- *Elemental composition methods:* There were two methods in this category i.e. VS/TS Ratio and BOD/COD Ratio
- *Microbial Activity Methods:* There were three methods in this category i.e. BMP, DHA and SOUR

The BOD/ COD and VS/ TS Ratio as measurements of stabilization were determined using the results from physical chemical characterization described above.

i. Biomethane Potential (BMP) Test

The BMP test was set-up following the guidelines by Holliger *et al.* (2016) and Filer *et al.* (2019). The inoculum was collected from a primary sludge bio-digester at Manchinchi Sewage Treatment Plant located in Lusaka City and operated by LWSC. The inoculum was collected in an 18.5L bottle which was tightly capped and stored at room temperature until use. A 175mL mixture of the inoculum and substrate (i.e. FS samples) at a ratio of 1:4 (inoculum to substrate ratio based on VS concentration) was placed in a serum bottle (with 200mL maximum capacity). The serum bottle was sealed with a butyl rubber stopper and capped with an aluminium crimp seal. Triplicate bottles were setup for all the samples including a set of positive controls. The positive controls were filled with microcrystalline cellulose and the inoculum and the blanks filled with the inoculum and water only to provide for the background methane generation for the inoculum. The bottles were incubated in a water bath at 35°C and were manually shaken once per day (Figure 3-6).

Figure 3-6: Water Bath used for the incubation of serum bottles at 35°C

Total gas production (methane + carbon dioxide) was measured intermittently using a Liquid Replacement System as described in Pham *et al.*, (2013) and shown in Figure 3-7. The concentration of methane in the biogas was measured by absorbing the carbon dioxide in an alkaline solution of 5M NaOH (Figure 3-7). A cylindrical flask was filled with the NaOH liquid and placed with the opening in the same liquid in a container, so that the flask remained full of liquid. A tube was inserted inside of the cylindrical flask with a provision for connecting a syringe at the other end. Biogas was then drawn from the serum bottles by using a 50ml syringe. The needle at the end of the syringe was injected through the butyl rubber by allowing the syringe plunger to move and equilibrate between the pressure in the serum bottle and atmospheric pressure at the

incubation temperature as described in Guwy, (2004). The volume of the biogas was recorded as (V1). The syringe was then connected to the end of the tube and the biogas was slowly injected in the cylindrical flask to displace the NaOH solution. The volume of the solution displaced corresponded to the volume of the Methane and was recorded as V2. The difference between V1 and V2 corresponded to the CO_2 content in the biogas and was used to calculate the methane content of the biogas**.**

Figure 3-7: Gas Measurement using Liquid Displacement (LH: Biogas measurement; RH: Methane Content Measurement)(Source: Author)

ii. Dehydrogenase Activity (DHA) Test

DHA is a simple and rapid test that has been applied to assess of the stability of wastewater sludge and composts as well as the studies in fields of microbiological control of water quality/ ecotoxicology (Sánchez *et al.,* 2006; Ghaly and Mahmoud, 2006; Nikaeen *et al.,* 2015; Kim *et al.,,* 1994; Dufour and Colon, 1992; Stier and Fischer 1998; Hongwei *et al.,* 2002; Zhao *et al.,* 2010). It is an indicator of the primary activity of microorganisms. However, it has not been applied before in the study of FS characteristics. Various studies have suggested different practical procedures to measure the DHA of different organic substrates (i.e. composts, activated sludge, anaerobically digested waste water sludge and fungal species). In all the procedures applied by different researchers, substrate pH, concentration of organic matter in the substrate, incubation temperature, concentration of tetrazolium salt and incubation time are critical parameters that have been reported to affect the accuracy of DHA test (Ghaly and Mahmoud, 2006; Dufour and Colon, 1992; Chung and Neethling, 1989; Lopez, *et al., 1*986). The method used is adapted to suit the substrate being tested. In this regard, preliminary 4^2 factorial experiments i.e. two factors (tetrazolium salt

concentration and VS concentration of the FS samples) with four levels each (0.05, 0.1, 0.2 and 0.3 mM for tetrazolium salt concentration and 1.3, 2.5, 5 and 15g/L for VS concentration) were conducted. The experiments determined the applicability of tetrazolium salt (TTC) test to quantify the DHA of FS samples and the optimum test conditions. Therefore, a modified method for DHA (see Appendix 2 for the laboratory SOP) of FS was proposed as follows based on the methods proposed by Chung and Neethling (1989) and Ghaly and Mahmoud (2006) as well as the preliminary 4^2 factorial experiments:

- The FS samples were diluted to have a comparable VS concentration in the range of 2.5 – 5g VS/l.
- The DO concentration and pH of the diluted FS samples were checked to ensure there were in the recommended range (DO in mg/l should be close to Zero and pH of 7 – 9) before performing the DHA test.
- Then a 5ml aliquot of the diluted sample was transferred into a 50ml centrifuge tube with a screw cap.
- 1.5ml of the 0.2 percent (v/w) TTC solution (prepared by dissolving 0.2g of TTC powder in 100ml of distilled water) was added to the centrifuge tube.
- The contents were then mixed by shaking and the tube was tightly capped and incubated in a water bath at 35°C for one hour.
- The centrifuge tubes were then removed from the water bath and the reaction was fixed by adding 0.2ml of 37 percent formalin.
- Extraction of the triphenyl formazan (TF) - a red coloured insoluble TTC reduction end product - was performed by centrifuging the tubes at 3500xg for 10 minutes to separate the TF pellet from the liquid portion. The supernatant was discarded and 5ml of ethanol was added to dissolve the TF. The tube was capped and the pellet was resuspended by manually shaking for 30 seconds. The TF was then extracted for 30mins in the dark at room temperature.
- The extract was then clarified by centrifugation at 1200xg for 5minutes. The absorbance of the red TF solution extract was read at 485nm using a spectrophotometer and the DHA results recorded as optical density.
- Samples were analysed in duplicates as a QA & QC measure.

iii. Specific Oxygen Uptake Rate (SOUR) Test

SOUR is part of the respirometry methods for measuring microbial activity of organic substrates. Few studies have been conducted on the application of respirometric methods to investigate sludge stabilization (Tas, 2010) and have mostly been applied to evaluate the stability of composts and activated sludge (Sánchez, *et al.*, 2006; Nikaeen *et al.*, 2015; Lasaridi and Stentiford, 1998; Samson and Ekama, 2000). Thus, SOUR test has not been applied before in the study of FS characteristics. In this regard, preliminary experiments to determine the optimal conditions such as solid concentration and aeration time were conducted. Therefore, a modified method (see Appendix 2 for the laboratory SOP) for SOUR of FS was proposed as follows based on EPA method 1683 (EPA, 2001):

- The FS samples were first diluted to have a comparable VS concentration of 2.5 – 5g VS/l. The dilution was necessary since FS has high concentration of suspended solids which can affect the oxygen transfer rate during the SOUR test (Lasaridi and Stentiford, 1998).
- Thereafter, 500ml of diluted sample was aerated for 1 hour in 500ml reactor glass bottles using a diaphragm lab vacuum pump (N820FTP Laboport vacuum pump). The prolonged aeration was done to acclimatize the substrate to aerobic conditions and activate the aerobic microbes since FS are mostly kept in nearly anaerobic conditions during the residence time in containment systems such as pit latrines and septic tanks.
- Then a 300ml aliquot of the aerated sample was placed in a 300ml BOD bottle and the SOUR test was performed following the procedure described in method 1683 (EPA, 2001).

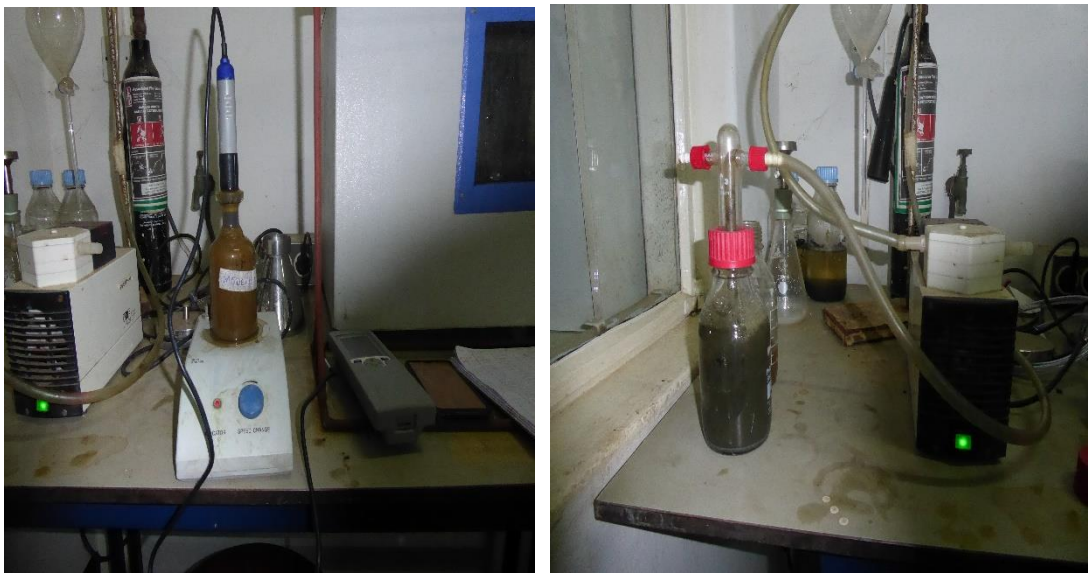

Figure 3-8: SOUR Setup (Left: SOUR Measurement using a DO meter probe; Right: Aeration of the sample before the performing the SOUR test) (Source: Author)

3.4.2 Part 2: Faecal Sludge Stabilization and its Relation to Dewatering

Under part 2 of the research, batch laboratory FS anaerobic digestion and dewatering experiments were conducted to determine how the two are related.

3.4.2.1 Faecal Sludge Sampling

The raw materials used in the second part of the research included FS collected from dry containment facilities (i.e. dry improved and unimproved pit latrines) and wet containment facilities (i.e. wet pit latrines with pour flush and septic tanks). The samples were collected from a mix of wet and dry containment facilities in order to test the hypothesis on the differences in levels of stabilization based on type of containment. The characterization of an onsite containment to be either a wet or dry was based on the physical consistency of the FS and not the usage or type of toilet. This is because in certain areas of Lusaka the groundwater table is low such that there is ingress of groundwater in pits (even those that are used as dry facilities) especially those that are partially lined. A total of 22 FS samples were collected from 20 containment systems (i.e. three septic tanks, nine wet pit latrines and nine dry pit latrines) located the city of Lusaka in the month of August and September 2021.

The samples were collected from Mtendere, Chazanga and Kanyama which are low income communities and Handsworth court and chudleigh which are high income residential areas all located within Lusaka city.

The sampling approach and tools were the same as described in part 1 of the study. All samples collected were transport to the laboratory in a cooler box and stored in a fridge at 4°C until analysis. One sampling triplicate was included from a pit larine as a Quality Assurance and Quality Control (QA&QC) measure to check the accuracy of the sampling methodology.

3.4.2.2 Laboratory Analysis

a) Sample Processing

Sample processing followed the same procedure described in part of the study. However, additional analysis which included dewatering tests and stabilization experiments were done as depicted in Figure 3-9 below.

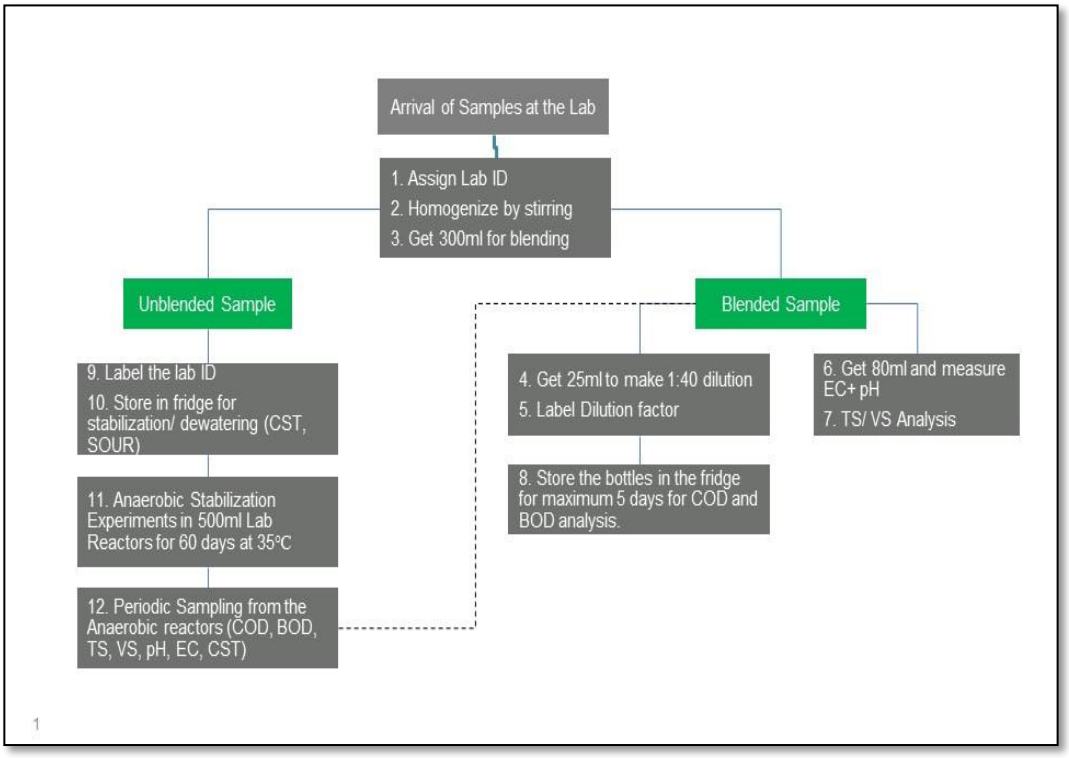

Figure 3-9: Graphical Representation of Sample Processing and Analysis Workflow: Part 2 (Source: Author)

b) Initial Physical-chemical Characterization, Dewatering and Stabilization Measurements.

The physico-chemical parameters analysed were the same as those in Part 1 of the study i.e. COD, BOD, pH, TS, VS and EC. A 300ml aliquot of the sample was homogenized in a blender for three minutes at medium setting for the initial physico-chemical characterization. The methods of analysis for all the physical chemical

parameter and stabilization measurements (i.e. VS/TS ratio, BOD/COD ratio and SOUR) were the same as described in part 1 of the study. Capillary suction time (CST) was quantified as a metric of dewatering performance. CST was measured using a Triton 319 Multi- CST apparatus with 18 mm funnel, according to Method 2710 G (APHA 2017), as adapted in Velkushanova *et al.* (2021). CST values were standardized by subtracting the CST of deionized water and normalized by TS concentration.

c) Batch Stabilization and Dewatering Experiments

10 samples (five from dry pit latrines and five from wet pit latrines) were selected for the controlled anaerobic digestion experiments to evaluate the changes in dewatering performance with stabilization. All the samples were selected from the 22 samples that were collected as described in Section 3.4.2.1. During these experiments, a total of 14 lab scale anaerobic digesters (10 samples with two setup in triplicates) were set-up using lab glass bottles. The digester had an overall volume of 500ml, with a working volume of 400ml. Thus, 400ml of FS (unblended and undiluted) was added to the pyrex glass bottles which were tightly sealed with rubber septa and plastic end cap with an opening to periodically vent gas from the reactor. A 50ml lubricated plastic syringe attached to needle was used to periodically vent the reactors as means of verification for anaerobic digestion through gas production.

The experimental period was 60 days, during which the digesters were operated under mesophilic conditions at 35°C in a water bath. Periodically, a 50ml FS sample was collected from each reactor and analysed for pH, EC, COD, BOD, VS, TS and CST. The sampling was done four times during the stabilization experiments i.e. at days 14, day 28, day 48 and on the last day which is day 60. SOUR was only measured on the last day i.e. day 60.

3.4.2.3 Quality Control and Quality Assurance

Measurement replicates for parameters were performed as a (QA/QC) measure. In addition, positive controls and blanks were also used for COD and BMP as standard check for the accuracy and performance of the test methods. The QA/QC measures were as follows:

Part 1 of the Study

In Part 1 of the study the parameters TS, VS, BOD were analysed in duplicates while COD, BMP and DHA were analysed in triplicates. For COD and BMP measurements one positive control (using a COD standard solution with concentration of 2g/L) and a blank (distilled water) was included in each batch and analysed in triplicates to check the accuracy of the test method. When it come to the SOUR test, one sample was analysed in duplicate per test batch as stipulated by EPA, (2001). Reported values are averages of measured replicates, and error bars in Figures represent the standard deviation of the replicates.

Part 2 of the Study

For the initial measurements on field FS samples, triplicate laboratory analysis were made for every 5^{th} measurement for VS, TS and COD while BOD and SOUR one sample was analysed in duplicate. For COD, a positive control and blank was included in every batch and analysed in triplicate. Every CST measurement was replicated three times.

During the anaerobic digestion experiment, three samples were analysed in triplicate on the third sampling (i.e. day 48) for TS and VS. For BOD five samples were analysed in triplicate on the third sampling. For COD one sample was analysed in triplicate on the second, third and fourth sampling day. Replicate analysis could not be done for every sampling due to the lack of sufficient quantity of FS in the reactors. For CST every sample was replicated three times on each sampling day. For the SOUR three samples were analysed in duplicate on the last day of the anaerobic digestion experiments.

3.4.2.4 Data Analysis

The main objective of Part 1 of the study was to select and evaluate methods that can be used to measure FS stabilization which is in with the first objective of this study (see section 1.6.1). In this regard, the data was plotted into bar graphs using excel and R studio version 1.4.1717. The plots were used to conduct visual comparative analysis to determine the consistency of the trends shown by the different stabilization methods.

The main objective of Part 2 of the study was to determine how stabilization is related to dewatering performance and if the intrinsic physical-chemical characteristics of FS affect its ability to undergo anaerobic digestion. In addition, the study was also aimed at determining any differences in the levels of stabilization among the different types of Sludgeas well as the changes in dewatering performance with anaerobic digestion in line with the study hypotheses (see Section 1.5). Descriptive statistics (means, median and standard deviations) were used to describe characteristics of FS from different types of containments (i.e. wet and dry containment facilities). Plots (scatter and box plots) were produced using R-studio (version 1.4.1717). For boxplots, the middle line represents the median, and the boundaries of the box represent the first and third quartiles (Q1 and Q3). The upper whisker extends to the last data point less than Q3 +1.5 * (Q3 – Q1) and the lower whisker extends to the first data point greater than Q1 – 1.5 * (Q3 – Q1). Outside of the whiskers, data are considered outliers and plotted individually as filled black dots. Statistical analysis was also performed using the R studio (version 1.4.1717). The Shapiro-Wilk test in R was used to test the normality of the data parameters. Statistically significant difference correlations among the types of FS was analysed using a non-parametric test (Wilcoxon test for medians at 95 percent confidence interval) for parameters that followed a non-parametric distribution. Further, to ascertain associations between stabilization and dewatering performance, spearman correlation analysis was conducted for non-parametric parameters. For datasets that followed a normal distribution parametric tests were used (t-test and person correlation analysis).

3.5 Summary of Methods and Materials

The methodology was designed to respond to the objectives of the study as detailed in the preceding sections. This can be summarised for each respective objective as shown in the Table 3-2 below.

Table 3-2: Summary of methods and tools applied for each objective

Objective	Methods Employed	Tools
To evaluate how to determine/quantify level of stabilization by using rapid,	Part 1 of the study Desk Study	Decision Matrix • FS Sampling Protocols

low cost methods, and its relevance to dewatering.	• Method Selection Criteria • FS Sampling • Lab FS Analysis	• Lab Analysis Protocols/ SOPs • Excel and R for results analysis

Table 3-2 Continued

Objective	**Methods Employed**	**Tools**
To evaluate how stabilization is related to FS dewatering performance and behavior	Part 2 of the study • FS Sampling • Lab FS Analysis • Stabilization Experiments	FS Sampling Protocols • Lab Analysis Protocols/ SOPs • Excel and R for results analysis
To determine if physical-chemical characteristics of FS influence stabilization	Part 2 of the study • FS Sampling • Lab FS Analysis • Stabilization Experiments	FS Sampling Protocols • Lab Analysis Protocols/ SOPs • Excel and R for results analysis

3.6 Chapter Summary

This Chapter highlighted the approach used to select and evaluate the methods used to measure the stabilization of FS. The methods for sample collection for the various parameters of interest namely physical chemical parameters and the metrics of stabilization and dewatering have been highlighted. The lab analytical methods for these parameters have been highlighted and the methods employed in the analysis of the collected data have been presented. The next chapter presents results obtained from the various data collection methods presented in this chapter.

CHAPTER FOUR: RESULTS

4.1 Introduction

This chapter presents the findings of the study. It is important to recall that the main focus of this study was to determine how to measure FS stabilization with the view that it can aid the prediction of dewatering performance. This can only be possible if the relationship between FS stabilization and dewatering performance is confirmed as practitioners have been observing in the field. In order to address this objective, the study sought to address the research questions and hypotheses which were set forth in this study. The ensuing sections therefore present results according to the research questions/hypotheses. The results are discussed in Chapter Five.

4.2 Part 1: Methods for Measuring Faecal Sludge Stabilization

The results for part 1 of the study are presented in the sections below.

4.2.1 Selection of Methods for Measuring Faecal Sludge Stabilization

Methods for measuring stabilization have mostly been applied in wastewater sludge and compost studies. Very few of these methods, if any have been applied to measure the stabilization of FS. The study therefore embarked on a desk-based literature study to identify potential methods that can be suitable to measure FS stabilization.

From literature, 14 potential methods for measuring stabilization of organic substrates were identified. From these 14 methods identified, only two methods have been applied in a few studies to indicate stabilization of FS. Table 4-1 below shows the methods that were identified from the literature search, which could be applicable for measuring stabilization in FS.

Table 4-1: Results for Literature Review on Methods for Measuring Stabilization

Method	Method Attributes	Comments	References
Biomethane Potential (BMP) Test	~ BMP tests are used to determine the amount of methane gas that can be produced from organic materials during anaerobic digestion. ~ The test is commonly performed in serum bottles (100ml – 2 l) closed with thick rubber caps. ~ The technical approaches and experimental set up of BMP test vary significantly. ~ Stabilization is measured through the normalized total volume of gas produced in 21 days (GS_{21}). ~ It has been applied mostly in the study of anaerobic digestion of wastewater sludge and municipal organic wastes. ~ GS_{21} < 20Nl/Kg TS indicates stabilized composited sewage sludge.	~ There is lack of standardization for the BMP test procedure, limiting the comparability of results. ~ Method has not been widely applied in the study on anaerobic digestion of FS.	*Filer, et al., (2019)* *Holliger, et al., (2016)* *Guwy, (2004)* *(Bożym and Siemiątkowski, 2020)*

Table 4-1 Continued

Method	Method Attributes	Comments	References
Specific Oxygen Uptake Rate (SOUR)	~ SOUR is part of the respirometry methods for measuring microbial activity of organic substrates. ~ SOUR measures the stability of a substrate by measuring dissolved oxygen (DO) consumption rate in a liquid medium. ~ SOUR test is standardized, inexpensive and easy to perform in most laboratories. ~ The SOUR has been widely applied to measure the stability of composts and sewage sludge. ~ SOUR of 2mg O_2/gVS/ h indicates a stable sewage sludge while 1mg O_2/gVS/ h is reported for composts.	SOUR test has been standardized i.e. Standard method 2710B and EPA method 1683. Method has not been applied in FS studies	*Ferrer, (2006)* *Nikaeen et al., (2015)* *Lasaridi & Stentiford, (1998)* *Samson & Ekama, (2000)*
Static Respiration Index (SRI) test	~ The SRI is also part of the respirometry methods. ~ SRI is a closed solid state method which measures oxygen consumption in the headspace of a vessel (using an O_2 electrode) on top of a solid material. ~ This method widely applied in the determination of stability for composts.	~ Method is most suitable for application using solid substrates such as composts ~ Method is reported to underestimate the O_2 consumption rate.	*Godley* et al., *(2004)* *Ferrer, (2006)* *Scaglia et al., (2000)* *Komilis and Kletsas, (2012)*

Table 4-1 Continued

Method	Method Attributes	Comments	References
Dynamic Respiration Index (DRI) test	~ The DRI is also part of the respirometry methods. ~ DRI is an open solid-state method involving passing air through the substrate and monitoring the difference in either O2 (consumed) or CO2 (produced) between the inflow and out- flowing air. ~ This method is widely applied in the stability of solid composts.	~ Method is most suitable for application using solid substrates such as composts ~ Equipment is expensive and expert training to operate.	*Godley* et al., *(2004)* *Ferrer, (2006)* *Scaglia* et al., *(2000)* *Komilis and Kletsas, (2012)* *Adani, Lozzi and Genevini, (2001)*

Table 4-1 Continued

Method	Method Attributes	Comments	References
Dehydrogenase Activity (DHA) test	~ DHA test assesses the stability of sludge based on the activity of the oxidoreductase enzymes (dehydrogenase) ~ It based on the measurement of optical density of the red compound (formazan) formed on reduction of the tetrazolium salt by the oxidative effect of the dehydrogenase enzymes ~ High Formazan crystal formation indicate high microbial activity and high organic matter content and vice versa. ~ Method mostly applied in the field of wastewater sludge, composts as well as the studies in fields of microbiological control of water quality/ ecotoxicology ~ 0.60 mg TF (Formazan)/ g DM/ d could indicate a stabilized compost.	~ Method has not been applied in FS studies ~ There is lack of standardization of the DHA test, however, it a fast a quick method. ~ DHA is reported in different units in different studies limiting the comparability of results.	*Sánchez et al., (2006)* *Xie et al., (2008)* *Pourakbar et al., (2020)* *Dufour & Colon, (1992)*

Table 4-1 Continued

Method	Method Attributes	Comments	References
Esterase Activity (EA) test	~ EA assess the stability of the sludge based on the activity of various enzymes i.e. esterases, lipases and proteases which represents the consumption of energy reserves/ secondary metabolic processes. ~ It based on the hydrolysis of fluorescein diacetate (FDA) into fluorescein which can be measured spectrophotometrically. ~ The technical approaches and experimental set up for the EA test are easy to perform, however, they vary significantly. ~ The EA in composits has been reported to peak and reduce as digestion progress. ~ Method mostly applied in the field of waste activated sludge, composts as well as the studies in fields of metabolic activity in soil.	~ Method has not been applied in FS studies ~ There is lack of standardization of the EA test procedure, limiting the comparability of results.	*Nikaeen, Nafez, et al., (2015)* *Fontvieille, Outaguerouine and Thevenot, (1992)* *Sánchez, Quiroga Alonso and Coello Oviedo, (2006)* *Nybroe, Jørgensen and Henze, (1992)*

Table 4-1 Continued

Method	Method Attributes	Comments	References
Adenosine Triphosphate (ATP) test	~ ATP is a compound produced during biological metabolic processes which carries energy that is required for cell growth, maintenance and reproduction and can be used to measure microbial activity. ~ The method involves extraction of ATP using Tris-EDTA buffer solution, addition of a firelight reagent, ATP light measurement in a photometer and determination of ATP concentration from a standard curve. ~ The concentration of ATP has been reported to reduce when sludge is digested. Digested waste water sludge can have ATP concentration as low as 0.1mg/l ~ Method mostly applied in the field of waste activated sludge and composts.	~ Method has not been applied in FS studies ~ There is lack of standardization of the ATP test procedure, limiting the comparability of results.	*Chung and Neethling, (1988)* *Atp, (2004)* *Tiquia, (2005)*

Table 4-1 Continued

Method	Method Attributes	Comments	References
VS/TS Ratio (including reduction in VS)	~ VS/TS ratio is defined as the ratio of the concentration of volatile solids to dry solids and is an indicator of the level of stabilization for a sludge sample. ~ Both TS and VS can be easily be measured according to standard methods. ~ In addition, TS and VS reduction rates are the most common assessment methods for the stabilization performance as they are easy to monitor. ~ a sludge can be considered to be well stabilized when it has a VS/ TS ratio of <= 0.5. ~ reduction of VS concentration during stabilization process at the level of 38–40 percent can be assumed as the stabilization limit	~ Method has been applied in FS studies ~ Standard methods exists.	*Kazimierczak, (2013).* *Podedworna and Umiejewska (2008)* *Cokgor et al., (2012)* *Maffo et al., (2019)* *Anderson et al., (2015)* Bassan *et al.*, (2013)

Table 4-1 Continued

Method	Method Attributes	Comments	References
BOD/ COD Ratio (including reduction in COD)	~ BOD and COD are parameters that are used to indicate the organic matter content of organic substrates e.g FS ~ Both BOD and COD can easily be measured according to standard methods. ~ BOD/ COD ratio has commonly been used as an indicator of the degree of biodegradation or stabilization in waste water research and it is suggested that stabilized waste water has a BOD/COD ratio of less than 0.1 ~ Reduction of COD concentration for waste water sludge during stabilization process at the level of 50 – 60 percent can be assumed as the stabilization limit.	~ Method has been applied in FS studies ~ Standard methods exists.	*Maffo et al., (2019)* *Bassan et al., (2013)* *Appiah-Effah et al., (2020)* *Tembo, (2019)* *Mangkoedihardjo, (2006)* *Sánchez et al. (2006)* *Bakare et al. (2012)*
Total Organic Carbon (TOC)	~ TOC is an empirical determination of the total carbon in a test substrate which is oxidizable ~ COD/ TOC ratio can be used as an indicator of the oxidation state of carbon (a representative of the organic compounds) present in a test substrate ~ Both TOC and COD can easily be measured according to standard methods.	~ Method has not been applied in FS studies ~ Standard methods exists.	*Kazimierczak, (2013)*

Table 4-1 Continued

Method	Method Attributes	Comments	References
Carbon/ Nitrogen (C/N) Ratio	~ C/N ratio is an important parameter in composting and a good balance is required to ensure stabilization. ~ An initial C/N ratio of 20 – 30 is recommended to achieve good rate of stabilization and the ratio can drop to below 12 at the end of the processes indicating a well stabilized compost. ~ Both Carbon (as TOC) and Nitrogen as total kjeldahl nitrogen (TKN) can be measured according to standard methods. ~ Applied mostly in the study of compost maturity and stability.	~ The method has been applied on FS by one study, however, it didn't not give meaningful results. ~ Standard methods exists.	*Ward et al., (2021)* *Nikaeen, Nafez, et al., (2015)* *Bernal, Alburquerque and Moral, (2009)*

Table 4-1 Continued

Method	Method Attributes	Comments	References
Humification	~ The humified fraction of organic matter in a substrate can be used as an indicator of stabilization as it is the most resistant to microbial degradation ~ During stabilization, humic substances are produced as humic acids (HA) while fulvic acid (FA) decreases due to microbial degradation, thus, the ratio of the non humic substances to humic substances can be used as a stability index. ~ The method involves the extraction of humic substances using an alkaline solution and determination of the TOC content of the humified and non humified fractions. ~ ratio of non-humic substances to humic substances (HI) of <1.0 indicates a good stability of OM in a substrate. ~ Applied mostly in the study of compost maturity and stability.	~ The method has not been applied in FS studies ~ There are no standard methods in existence. ~ It has been demonstrated to better indicate stability of composts and not non composted sludge	(Bernal, Alburquerque and Moral, 2009) (Hartenstein, 1981) (Tiquia, 2005)

Table 4-1 Continued

Method	Method Attributes	Comments	References
Cellulose and Lignin Content (Acid Detergent Test)	~ The "cellulose" (sum of cellulose and hemicellulose) and lignin content (LC) organic matter can be used to indicate stability of organic substrate. ~ During stabilization, cellulose reduces due to degradation while lignin increases as it is resistant to microbial degradation. ~ Method involves determined by several acid detergent fibre (ADF) methods. ~ The cellulose to lignin (C/L) ratio can be used to indicate stabilization of organic matter in a substrate. ~ Method is mostly applied in compositing of MSW and pulp industries.	~ No application in FS or waste water. ~ No defined limit of LC or C/L ratio of a stabilized substrate. ~ Suitable for substrates with plant origin which have high contents of lignin in the cell structure	*Edwards (1973)* *Lewin et al. (1996)* *Barlaz et al. (1997)* *Eleazer et al. (1997)* *Stinson and Ham (1995)*

Table 4-1 Continued

Method	Method Attributes	Comments	References
Cellulase Hydrolysis Test	~ Cellulose and hemicelluloses are a major component of plant derived organic wastes. ~ Thus the method involves enzymatic treatment of the substrate with commercial cellulose and hemicellulose. ~ Stabilization is determined in terms of the organic carbon released due to the hydrolysis by the commercial enzymes	~ Best suited for plant derived organic wastes such as pulp/ MSW ~ No reported limit to show a well stabilized sample.	*Rodriguez et al. (2001)* *Rodriguez et al., (2005)* *Godley et al., (2004)*

Based on the above, the current values evaluated in the literature for characterising the stabilization of composts and wastewater sludge can be summarised as in shown in Table 4-2 below:

Table 4-2: Criteria Evaluated in the Literature to Characterize Stabilization of wastewater sludge and composts

Method Category	Methods	Stability Index[1]	References
Microbial Activity	1. BMP 2. SOUR 3. DHA 4. ATP 5. EA 6. SRI 7. DRA	1. GS_{21} (<20 NL /Kg TS) 2. O_2 uptake (2g O_2/kg VS/h) 3. Formazan 0.60 mg TF/ g TS 4. Not specified 5. Not Specified 6. O_2 uptake (<3mgO_2/g VS/h) 7. O_2 uptake (<1mgO_2/g VS/h)	*1. (Bożym and Siemiątkowski, 2020)* *2.Samson & Ekama, (2000)* *3.Benito et al. (2005)* *6.Ferrer, (2006)* *7. Ferrer, (2006)*
Elemental Composition	8. BOD/COD Ratio 9. VS/TS Ratio 10. C/N Ratio 11. TOC	8. Ratio <= 0.1 9. Ratio <=0.5 10. Ratio <=12 11. Not Specified	*8.Mangkoedihardjo, (2006) 8.Borglin et al., (2012)* *9. Cokgor et al., (2012)* *10.Bernal et al. (1998a)*
Surrogate Methods	12. Humification 13. C/L Ratio 14. Cellulase Test	12. Humification Index (HI) <1 13. Not Specified 14. Not Specified	*12. Tiquia, (2005)*

[1] Stability index is the limit value above or below which a sample can be categorized as either stabilized or not stabilized.

4.1.1.1 Decision Matrix Results

Figure 4-1 below shows the results of the screening that was done in order to select methods for further evaluation through lab experiments. Five methods that scored the highest were selected and these were:

1. BOD/COD Ratio
2. VS/TS Ratio
3. DHA
4. SOUR
5. BMP

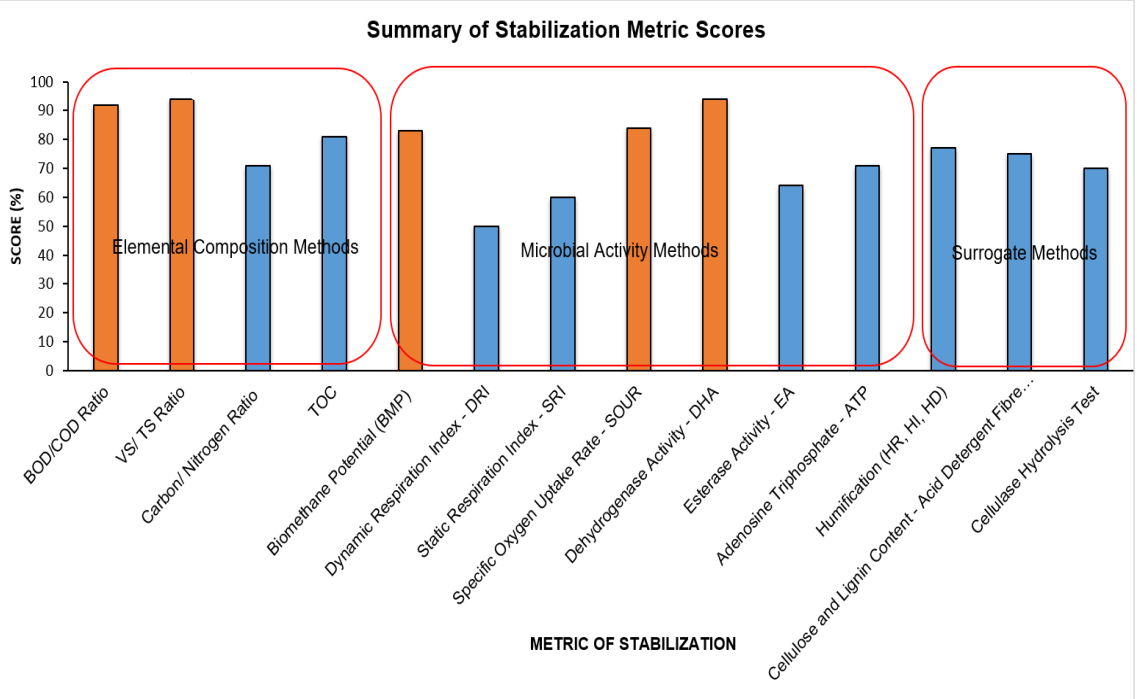

Figure 4-1: Summary of the scores for the identified metrics of stabilization. The metrics were grouped into "Elemental Composition", "Microbial Activity" and "Surrogate" Methods. Five Metrics (orange bars): (2) in Elemental Composition, and (3) in Microbial Activity which scored the highest were selected for evaluation in the Laboratory.

The screening was done based on the criteria and scoring system as presented in the methods section (see table 3-1). The screening tool is attached in Appendix 2. As shown in Figure 4-1 above, in the microbial activity category of methods, DHA and SOUR scored 94 and 84 respectively because both are easy to perform, do not require expensive equipment and have been widely applied to assess stabilization of waste water sludges. The same applied to the BMP method which scored 83, despite it being

a lengthy method which is not appropriate for rapid application and decision making. The other attribute for the BMP is that it is performed under anaerobic conditions which is the dominant pathway for FS stabilization during storage in containment systems (Shaw and Dorea 2021; van Eekert *et al.,* 2019). Thus, the BMP offered an opportunity in this study to understand the processes through which FS is stabilized in containment systems and how it might be related to dewatering performance. The EA and ATP though they are relatively easy and cheap methods, they have not been widely applied in stabilization studies in the wastewater sector and composting. Further, there were serious gaps in literature on how the two can be used to measure stabilization because; i) no stability indices for the two methods could be found in literature (see table 4-2) and ii) they are both related to the generation of energy (ATP) and utilization of energy reserves (EA) which means they are not direct indicators of microbial activity. However, the patterns produced by the two have been reported to be correlated to direct microbial activity methods such as DHA. SRI and DRI scored the least among all methods in the microbial activity category because they are both solid state methods (meaning they cannot be used for liquid samples like liquid FS). Hence they are customarily applied in compost maturity and stability studies. In addition the methods require expensive equipment and expert skills for operation which means they cannot be easily performed in laboratory found in low income countries. Due to this, these methods were not included in this study.

When it comes to the elemental composition method category, BOD/COD and VS/TS ratio scored the highest (i.e. 92 and 94 respectively) because all parameters can easily be measured using standard methods. The parameters also form part of the common physico-chemical characteristics of FS that are measured in most FS characterization studies. In addition the methods are easy and cheap to perform i.e. they don't require expensive equipment making it easy for them to be performed in local laboratories in developing countries. Stability indices for BOD/COD and VS/TS ratios (mostly for wastewater sludge) have also been reported in literature. The C/N ratio scored less than the target because the method is mostly applied in compositing.

All the methods under the surrogate methods category scored less than the target. There were serious gaps in literature regarding these methods which can be attributed to the fact that they have not been widely applied and at the same time they can be categorized as developing substitute methods. In this regard, no stability indices could

be established for Cellulose/ lignin content and the cellulase hydrolysis test (see table 4). In addition the methods were evaluated to be best suited for determining stability of wastes or substrates with plant origin which have high contents of lignin in the cell structure. The humification methods are more suited for composts and microbial activity for soils and from literature the methods may not produce tangible results when applied to FS and sewage sludge samples which are less humified (Ciavatta *et al.* 1990).

4.1.2 Laboratory Evaluation of Stabilization Methods

Table 4-3 below shows the characteristics of the FS samples that were used to evaluate the performance of the five methods that were selected (see Appendix 4 for laboratory results for all parameters analysed). The parameters BOD and VS traditionally indicates the fraction of the total organic matter content which are biodegradable. The results obtained from this study did not show consistent marginal decrease in the concentration of these parameters in containments with higher sludge age. Nonetheless, the fresh excreta registered the highest BOD and VS as compared to the older pit latrine and septic tank sludge samples as shown in Table 4-3. This was an indication that FS undergoes some form of stabilization during the time it is stored in containment systems resulting in a reduction in organic matter content as compared to fresh excreta.

Table 4-3: Characteristics of Sludge Samples used to evaluate the performance of Stabilization Methods

Sample	Sludge Age	pH	EC (mS/cm)	COD (g/L)	BOD (g/L)	VS (%TS)	TS (%ds)
Pit Latrine 1	3 years	7.9	5.6	104.8±10.7	16.9±0.5	37.5±0.2	20.4±0.2
Pit Latrine 2	3 years	8.0	10.3	113.1±11.7	12.7±1.6	60.6±0.3	13±1.9
Pit Latrine 3	3 years	8.1	16.1	90.8±1.6	12.2±0.3	38.2±0.9	18.6±0.1
Vertical Vault Latrine	0.5 years	8.1	12.0	90.6±5.1	16.4±0.5	74.6±0.01	10.8±0.01
Septic Tank 1	3 years	7.6	4.3	46.4±1.1	5.2±0.6	56.1±1.2	7.1±0.3
Fresh Excreta	-	8.3	8.3	87.2±4.2	39.8±0.5	81.6±0.3	4.8±0.1

In part 1 of the study, the sludge age was used as a proxy indicator of sludge stabilization (i.e. storage time in the onsite containment system). The samples were also grouped into Fresh Excreta, Dry FS – VVL, Dry FS (pit latrine sample 1 and 2 which were dry containment facilities) and Liquid FS (septic tank and pit latrine 2 which were wet containment facilities). The samples were grouped based on sludge consistency instead of type of technology (e.g. septic tank or pit latrine) as the majority of the containments do not conform to the standard technical descriptions/ features especially in the case of septic tanks. This was done in line with the hypotheses that stabilization increases with sludge age and different types of FS have varying levels of stabilization (see section 1.5). Going by this hypothesis, it was expected that the fresh excreta will be the less stabilized followed by the VVL sample and lastly the pit latrine and septic tank samples.

4.1.2.1 Evaluation and Performance of BOD/COD Ratio

The BOD/COD ratio as a measure of FS stabilization was determined using the BOD and COD results of the samples as shown in Table 4-3. Figure 4-2 below shows the performance of the BOD/COD ratio as a measure of FS stabilization assessed against the sludge age and the stability index identified from literature and presented in Table 4-2.

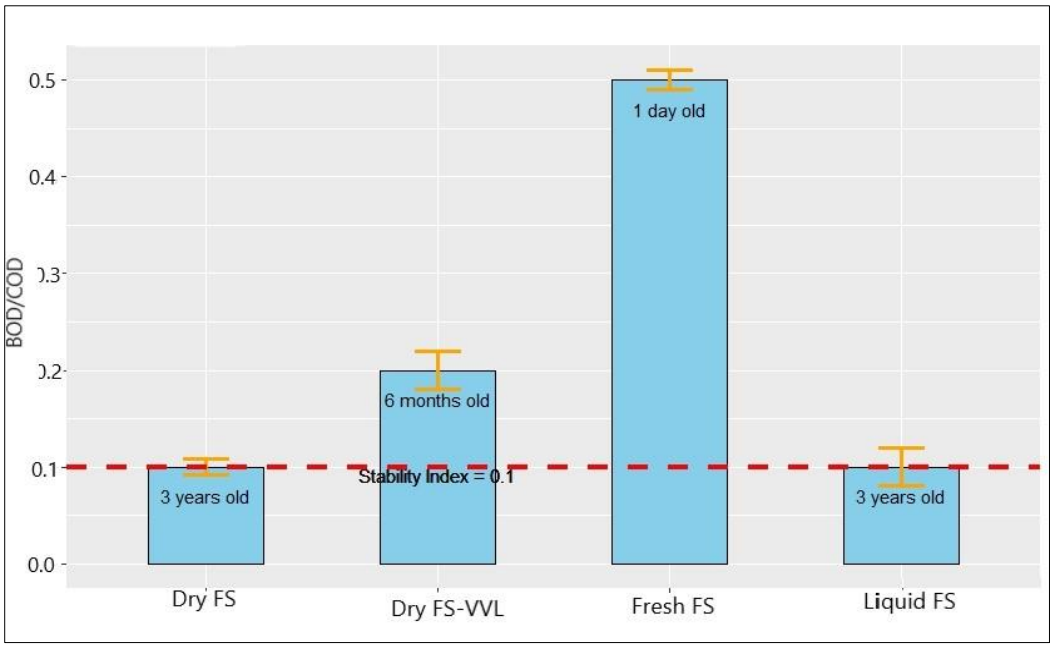

Figure 4-2: BOD/COD Ratio bar graph (redline represents stability index = 0.1)

From the graph, fresh excreta recorded the highest BOD/COD ratio followed by the VVL and lastly the dry/ liquid FS samples. The trend shown by the BOD/COD ratio was corroborated with the age of the FS sample. Further, assessed against the stability index (indicated by the red line on the graph), the results revealed that the fresh sample was the least stabilized followed by the Dry FS - VVL and lastly the dry/ liquid FS. Based on this index, the dry FS and Liquid FS (with a sludge age of three years) can be categorized to be stabilized as they had a BOD/COD ratio of 0.1. Further no difference was observed between the BOD/COD ratio of the of the liquid and dry FS samples with sludge age of three years.

4.1.2.2 Evaluation and Performance of VS/TS Ratio

Similarly, the VS/ TS ratio as measure of FS stabilization was determined using the VS and TS results of the samples as shown in Table 4. The bar graph in Figure 4-3 shows the performance of the VS/TS ratio as a measure of FS stabilization assessed against the sludge age and the stability index identified from literature and presented in Table 4-2.

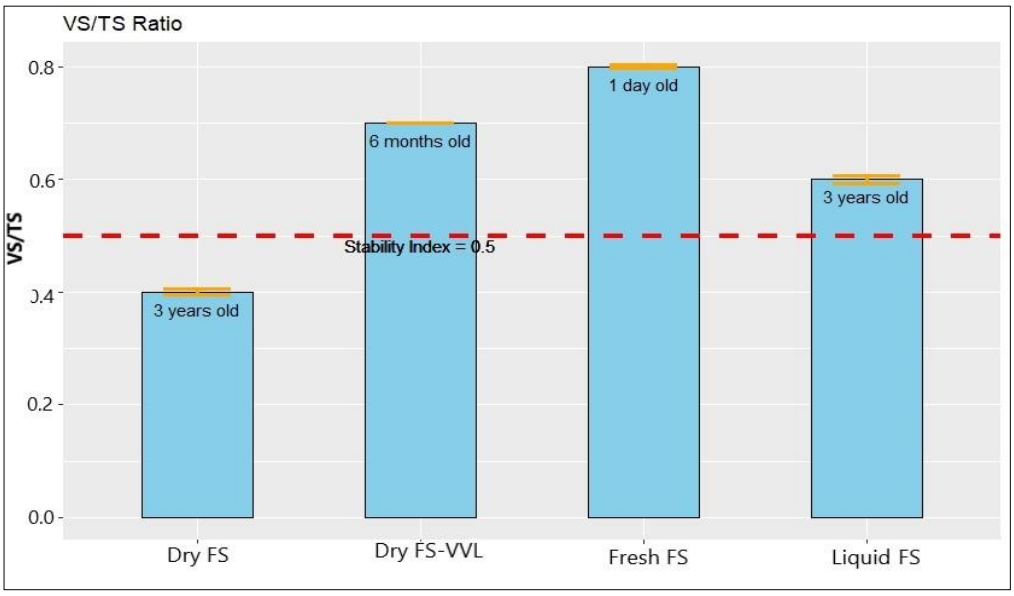

Figure 4-3: VS/TS Ratio Bar Graph (red line represents stability index = 0.5)

The trend shown by the VS/TS ratio was collaborated with the age of the FS sample. Further, assessed against the stability index (indicated by the red line on the graph) the results revealed that the fresh excreta sample was the least stabilized followed by the Dry FS - VVL, the liquid FS and lastly the dry FS. Based on the stability index for VS/TS ratio, the dry FS can be categorized as well stabilized as they all had a ratio

below 0.5. Further, the liquid FS samples recorded a higher VS/TS ratio as compared to the dry FS samples.

4.1.2.3 Evaluation and Performance of DHA Test

i Method Development and Optimization

In all the procedures applied by different researchers, substrate pH, concentration of organic matter in the substrate, incubation temperature, concentration of tetrazolium salt and incubation time are critical parameters that have been reported to affect the accuracy of DHA test (Sánchez *et al.*, 2006; Dufour & Colon, 1992). Thus, preliminary experiments to determine the optimal conditions such as the concentration of the TTC and concentration of the organic matter in the substrate as VS were conducted. For uniformity with the BMP, the incubation temperature was also set at 35°C. Figure 4-4 below shows the results of the preliminary 4^2 factorial experiments that were conducted to determine the optimum concentration of the TTC and VS.

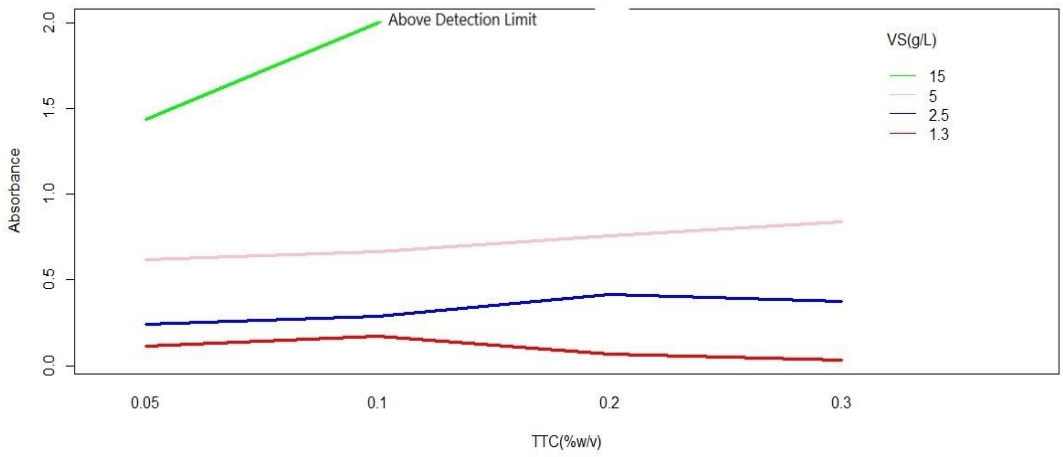

Figure 4-4: Effect of TTC Dosage on DHA at varying VS concentration of the fresh excreta

Results as shown in the interaction plot indicated that at VS concentration of 1.3g/L, TTC concentrations greater than 0.1 (percent w/v) resulted in a reduction in the microbial activity which was reflected by the reduction in DHA (the red line on the graph). Similarly, at VS concentration of 2.5g/L, TTC concentrations greater than 0.2 (percent w/v) resulted in a reduction in the microbial activity which was reflected by the reduction in DHA. From the graph, it was also observed that DHA was insensitive to the concentration of TCC at VS concentrations of 5g/L and above. However, at a

VS concentration of 15g/L, the microbial activity was very high at TCC concentrations above 0.1 (percent w/v) resulting in DHA (read as absorbance) above the detection limit of the spectrophotometer (i.e. the absorbance was greater than 2). It was also observed that at VS concentration of 1.3g/L, the concentration of the solids was too low which was problematic when it come extraction of the red formazan crystals through centrifugation. Based on these results the best VS concentration for FS samples to conduct the DHA test was chosen to be between 2.5 – 5g/L and TTC dosage of between 0.1 – 0.2 (percent w/v).

ii Performance of the DHA Test

The DHA did not perform well in terms of quantitative measurements of the response variable i.e. colour intensity of the red formazan solution which is read and reported as absorbance. The absorbance readings were not reproducible in repeated measurements i.e. repeated readings on the same formazan solution in the same cuvette gave different results each time. High variability was also observed in lab replicate samples. It was observed that the best approach for conducting the DHA test on FS samples is by reading the actual concentration of formazan (red compound dissolved in an organic solvent such as ethanol) and the results reported as mg TF/ g VS and not absorbance as it was the case in this study. This required a standard TF compound/ reagent in order to make the standard calibration curve. This could not be done within this study due non-availability of the reagent locally.

Nonetheless, Figure 4-5 below shows a visual illustration of the colour intensity of the formazan solution that were obtained at the end of the DHA test procedure.

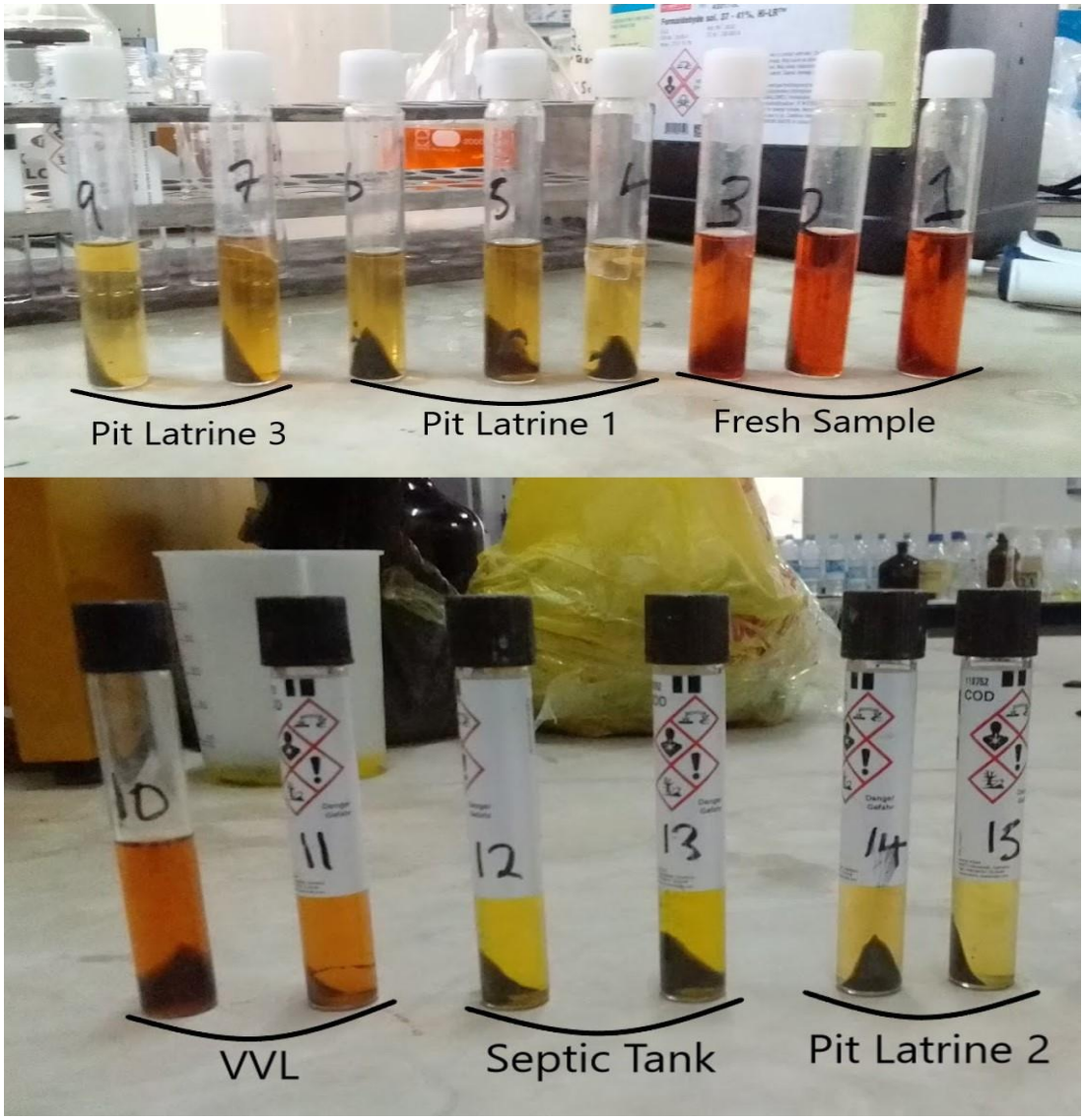

Figure 4-5: Colour Intensity of Formazan Solution after one hour sample incubation with TTC.

As shown in Figure 4-5, the variation in the colour intensity indicated the difference in the microbial activity of the samples. The fresh excreta produced the solution with a darker red colour (as compared to the other samples) which was an indication of high microbial activity. It was followed by the VVL. The pit latrine and septic tank samples produced lighter yellow to reddish solutions indicating a less production of formazan which was attributed to less microbial activity in the samples.

4.1.2.4 Evaluation and Performance of SOUR

i Method Development and Optimization

The SOUR test has mostly been used in the study of stability of activated sludge samples from wastewater treatment and composts. The Standard method 2710B and

EPA method 1683 provide guidance on how to perform the SOUR test on activate sludge and biosolids from aerobic waste water treatment processes.

Since FS samples have total solids concentration greater than 2 (which could limit oxygen transfer rate in sample during the mixing) (EPA, 2001) and are mostly kept under anaerobic conditions in the containment systems (sample origin conditions are anaerobic which is different from the SOUR test conditions i.e. aerobic, the observed measurement may not be identical with actual SOUR), preliminary experiments to determine the optimal conditions such as dilutions and aeration time to obtain the maximum SOUR were conducted. All samples were diluted to have comparable VS concentration between 2.5 - 5g/L before conducting the SOUR test. This was chosen based on the results from the DHA test on VS concentration for optimal microbial activity. This is because SOUR and DHA both indicate the metabolic pathway of microorganisms and are strongly correlated (Chung and Neethling, 1989; Sánchez, *et al.*, 2006).

Figure 4-6 below shows the results of the aeration time test that were conducted. As shown in Figure 4-6 (A), the maximum SOUR for the fresh excreta was obtained at 180 minutes aeration time. When it come to the pit latrine sample (Figure 4-6 B) which had a sludge age of greater than three years, the maximum SOUR was obtained after 60 minutes of aeration. Thus, all the samples were first subjected to a prolonged aeration for not less than 1 hour by bubbling air through the sample using a vacuum pump before conducting the SOUR test.

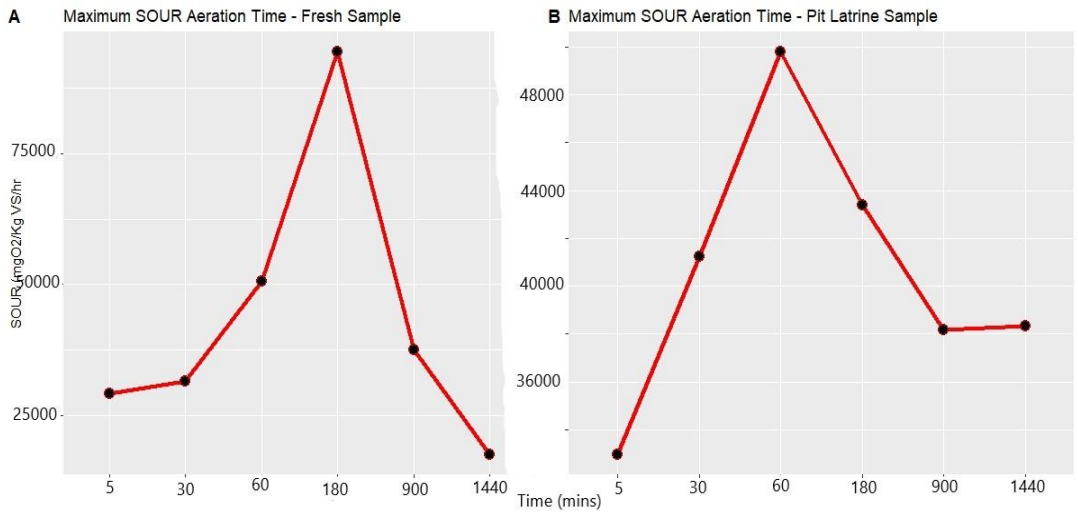

Figure 4-6: Maximum SOUR Aeration Time Experiment Results

In this study, the need for prolonged aeration was also reflected in the SOUR results obtained after 5 minutes aeration as required in the standard method. When samples were aerated for only five minutes, the results showed a very low microbial activity in the fresh excreta sample as compared to the pit latrine samples. At five minutes aeration, the fresh sample recorded SOUR of 1,109.14mgO_2/Kg VS/hr which was lower than that for the pit latrine sample which recorded a SOUR of 3,3703.20 mgO2/Kg VS/hr. The SOUR for the fresh excreta after 5 minutes of aeration would also mean that the sample is stabilized based in the SOUR stability index in Table 4-2 which was not expected to be the case.

ii Performance of the SOUR Test

The bar graph in Figure 4-7 below shows the performance of the SOUR as a measure of FS stabilization assessed against the sludge age and the stability index identified from literature and presented in Table 4-2.

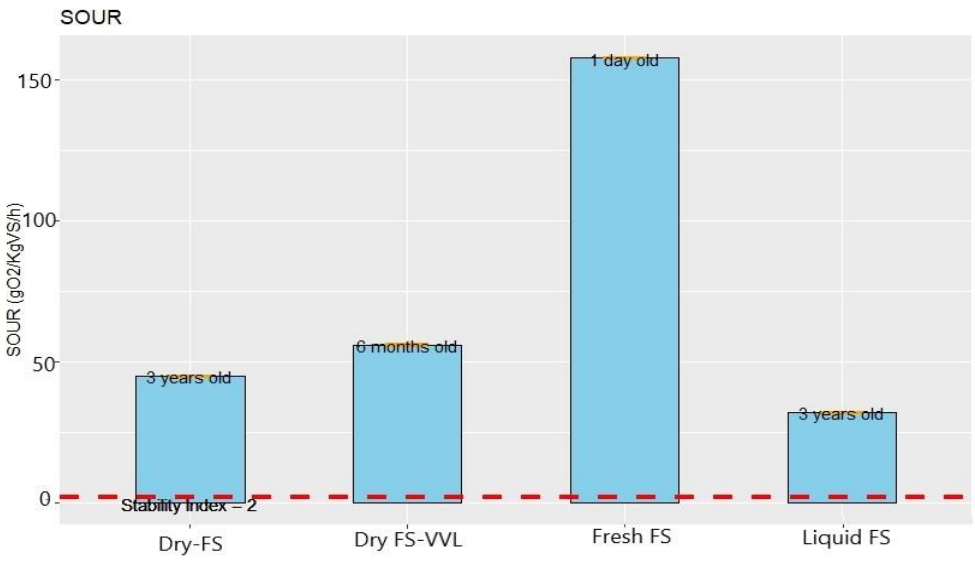

Figure 4-7: SOUR Bar Graph (redline represents stability index = 2)

From the graph, the fresh excreta recorded the highest SOUR followed by the Dry FS-VVL, Dry-FS and lastly the liquid FS samples. The trend shown by the SOUR results was collaborated with the age of the FS samples. Further, assessed against the stability index (indicated by the red line on the graph) the results revealed that the fresh excreta sample was the least stabilized followed by the Dry FS - VVL, Dry FS and lastly the Liquid FS sample. Based on the stability index for SOUR, none of samples can be

categorized as stabilized as they all had a SOUR above 2g O_2/kg VS/hr. Further, the dry FS recorded a slightly higher SOUR as compared to the liquid FS sample.

4.1.2.5 Evaluation and Performance of BMP

The cumulative methane production of the respective samples during the anaerobic digestion process is shown in Figure 4-8. The gas production for pit latrine, VVL and septic tank sludge samples increased gradually from the beginning of the BMP test and reached a stable state within the 21st day of the experiment (gas measurement were taken every after two – three days). On the other hand, the fresh excreta sample had a lag to start producing gas and was only able to reach a stable state within the 30th day of the experiment. The longest to stabilize was the positive control (sample MCC on the graph) as shown in the graph which went up to day 38 to stabilize. In addition, the positive control had a BMP of 358.23 NL CH_4/ Kg VS which was within 85 percent of the theoretical BMP for the microcrystalline cellulose. This was a confirmation that the BMP test performed well as recommended by Holliger *et al.*, (2016)

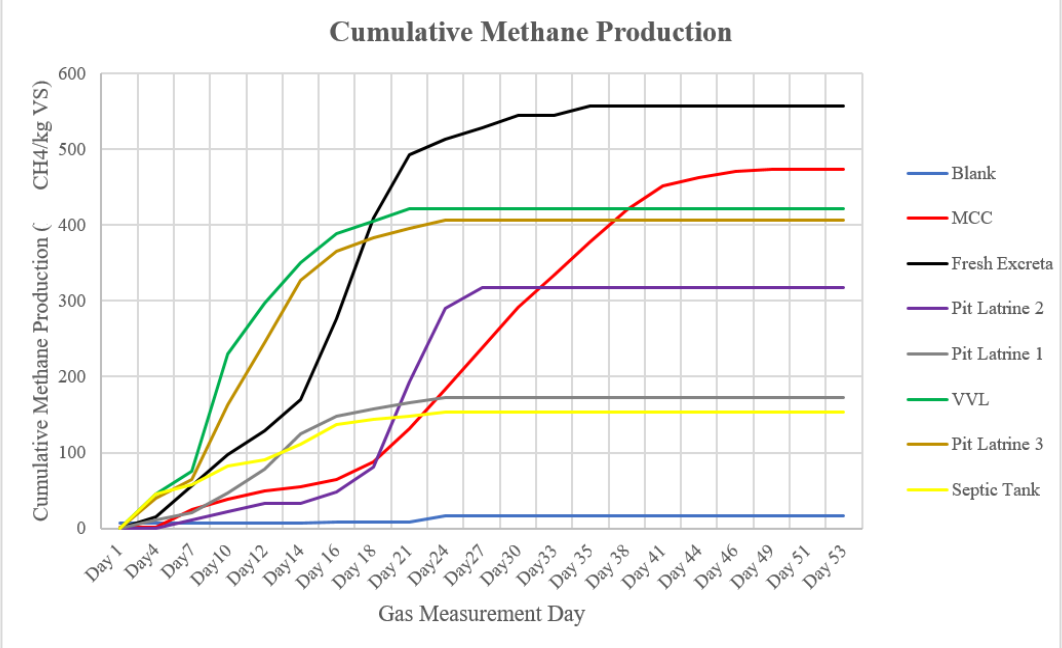

Figure 4-8: Cumulative Methane Production

Further, as shown in the graph, the fresh excreta produced the highest cumulative methane gas followed by the VVL, pit latrine and septic tank sludge samples. The trend shown by the BMP measurement results was collaborated with the sludge age of each sample.

The bar graph in Figure 4-9 below shows the performance of the BMP as a measure of FS stabilization assessed against the sludge age and the stability index identified from literature (table 4-2). The normalized gas production sum in 21 days of the BMP test was used as an index of stabilization. Based on the stability index identified from literature (GS_{21} < 20Nl/Kg TS), it can be stated that none of the samples can be categorized as stabilized. Further from the Figure, the fresh excreta was the most unstabilized sample followed by the Dry FS – VVL, the dry FS sample and lastly the liquid FS samples. Further, Dry FS samples recorded a higher GS 21 value as compared to the liquid FS samples.

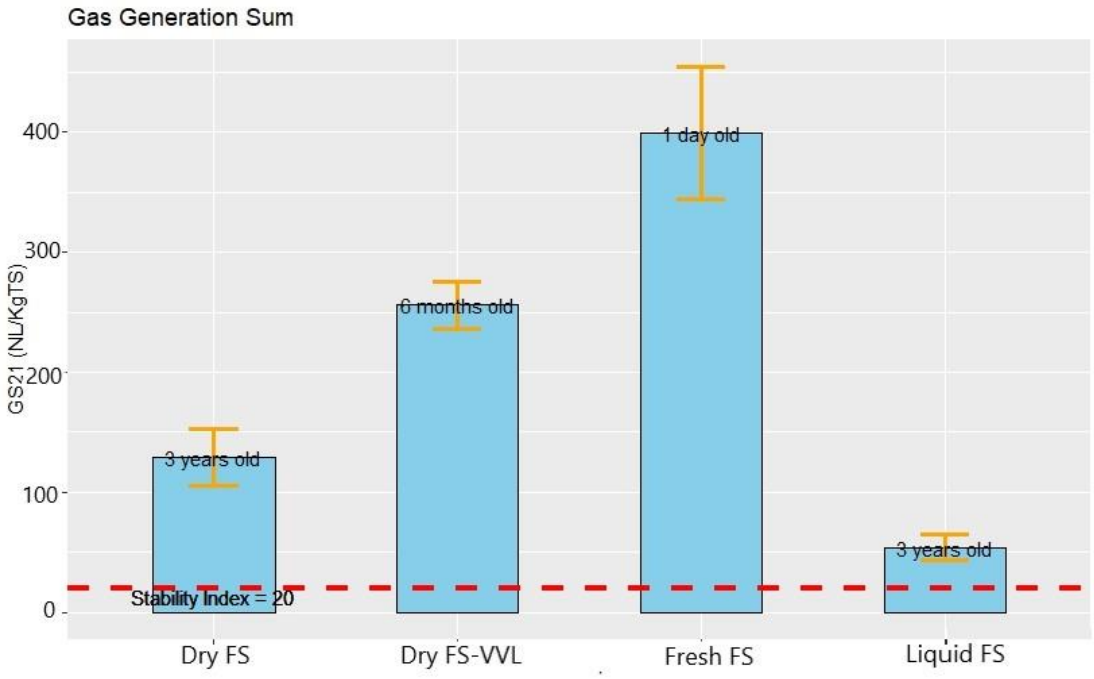

Figure 4-9: GS21 for the Samples (red dotted line indicates the stability index)

4.1.2.6 Comparative Analysis and Method Selection

Since the DHA did not generate quantitative results in this study, it was left out from this comparative analysis. Comparison of the results for the SOUR and BMP (as GS_{21}) (see Figure 4-7 and 4-9), indicated that none among all the sludge samples can be categorized as stabilized in relation to the stability indices identified from literature. When it come to the VS/TS and BOD/COD ratio, the results were different. The BOD/COD ratio showed that both the dry and liquid FS can be categorized as stabilized while the same was only true for the liquid FS when it come to the VS/TS ratio.

Based on these results, it was concluded that BOD/COD ratio, VS/TS ratio, SOUR and BMP can be applied to measure FS stabilization despite the slight variations that were observed when the method results were assessed against the stability indices identified from literature. However, the disadvantage of the BMP test is that takes longer to be completed (i.e. time consuming) and is labour intensive. Based on this, the other methods i.e. the SOUR, VS/TS and COD/BOD ratio were preferred for application in the second part of the study. The DHA was not selected due to the issues encountered with the method as stated earlier.

4.2 Part 2: Faecal Sludge Stabilization and its Relation to Dewatering

This section presents the results for Part 2 of this research which covered batch anaerobic digestion and dewatering experiments.

4.2.1 QA/QC and Normality Results

The results for the normality test and replicate analysis of the samples as a quality control measure are presented in the Section 4.2.1.1 and 4.2.1.2.

4.2.1.1 QA/QC Results

Laboratory replicate (duplicates/ triplicates) measurements had average relative standard deviation of 4.11 percent for TS, 6.08 percent for VS, 9.38 percent for COD and 7.45 percent for BOD. For the SOUR, the standard method recommends duplicate analysis of one sample per batch and determination of the relative percent difference. In this study, the relative percent difference among the duplicate measurements for SOUR was 21.15 percent. The lab replicate measurements for samples during the anaerobic stabilization experiment had a relative standard deviation of 30.50 percent for TS, 37.13 percent for VS, 20.66 percent for COD and 15.69 percent for BOD. The results indicated the inherent heterogeneity of FS as the samples were not blended into a homogenous mixture to prevent changing the characteristics of the samples in relation to the SOUR and CST tests. In the study, one sampling replicate was included to measure the accuracy of the sampling method and the measurements had a relative standard deviation of 6.4 percent for TS, 24.63 percent for VS, 15.79 percent for COD and 2.53 percent for BOD. Further, the CST measurements were replicated four times and the standard relative deviation was 7.1 percent.

4.2.1.2 Normality Test Results

The results of the normality test (Shapiro-Wilk test) for FS stabilization and dewatering parameters are shown in the table 4-4 below. The results showed that the VS/TS ratio results were normally distributed.

Table 4-4: Results of Shapiro-Wilk Test for Stabilization and Dewatering Parameters.

Parameter	P-1[2]	P-2[3]	Conclusion
CST (normalized)	0.0004531	5.112e-07	Non-parametric
SOUR	0.0008971	4.633e-06	Non-parametric
BOD/COD Ratio	0.001174	0.0007381	Non-parametric
VS/TS Ratio	0.8219	0.05151	Parametric

4.2.2 Characteristics of Faecal Sludge Samples

Results of the physical chemical characteristics, stabilization and dewatering performance of the FS samples used in the second part of the study are shown in Tables 4-5 and 4-6 below (see Appendix 5 for the laboratory results for analysed parameters). The samples are grouped into liquid FS samples which were collected from wet containment facilities (i.e. wet pit latrines and septic tanks) and dry FS samples which were collected from dry containment facilities (i.e. dry improved or unimproved pit latrines).

[2] P-Value for untreated FS Samples

[3] P-Value for anaerobically digested FS Samples

Table 4-5: Results of Physical chemical, Stabilization and Dewatering Analysis of Dry FS Samples.

	Physico-chemical Parameters						Stabilization Metrics			Dewatering Performance
	pH	**EC (mS/cm)**	**COD (g/L)**	**BOD (g/L)**	**VS (%TS)**	**TS (%ds)**	**BOD/COD Ratio**	**VS/TS Ratio**	**SOUR (gO_2/kg VS/hr)**	**CST (s.L/gTS)**
Mean	7.3	13.8	152.5	12.3	55	14.8	0.08	0.6	31.9	65.2
Median	7.5	12.9	130.2	12.6	54.8	15.8	0.08	0.6	21.9	79.2
SD	0.5	5.3	61.9	1.1	16.5	4.9	0.03	0.2	26.2	46.7
N	10	10	11	10	11	11	10	11	10	11

Table 4-6: Results of Physical chemical, Stabilization and Dewatering Analysis of Liquid Samples.

	Physico-chemical Parameters						Stabilization Metrics			Dewatering Performance
	pH	**EC (mS/cm)**	**COD (g/L)**	**BOD (g/L)**	**VS (%TS)**	**TS (%ds)**	**BOD/COD Ratio**	**VS/TS Ratio**	**SOUR (gO_2/kg VS/hr)**	**CST (s.L/gTS)**
Mean	7.7	14.8	78.4	7.2	50.3	2.3	0.2	0.5	12.7	12.4
Median	7.7	15.8	36.6	6.9	52.1	2.4	0.3	0.5	7.2	9.4
SD	0.4	8.6	77.5	4.4	6.0	1.2	0.2	0.09	14.3	9.2
N	10	10	9	10	11	11	8	11	10	11

4.2.3 Stabilization and Dewatering Characteristics of Undigested Samples.

Higher variability was observed in the CST and VS/TS ratio values of dry FS samples as compared to the liquid FS samples. In contrast, a higher variability was observed in the BOD/COD ratio values for liquid samples as compared to the dry samples. However, when it come to the SOUR, both the liquid and dry FS samples showed equal variability in the values (Figure 4-10).

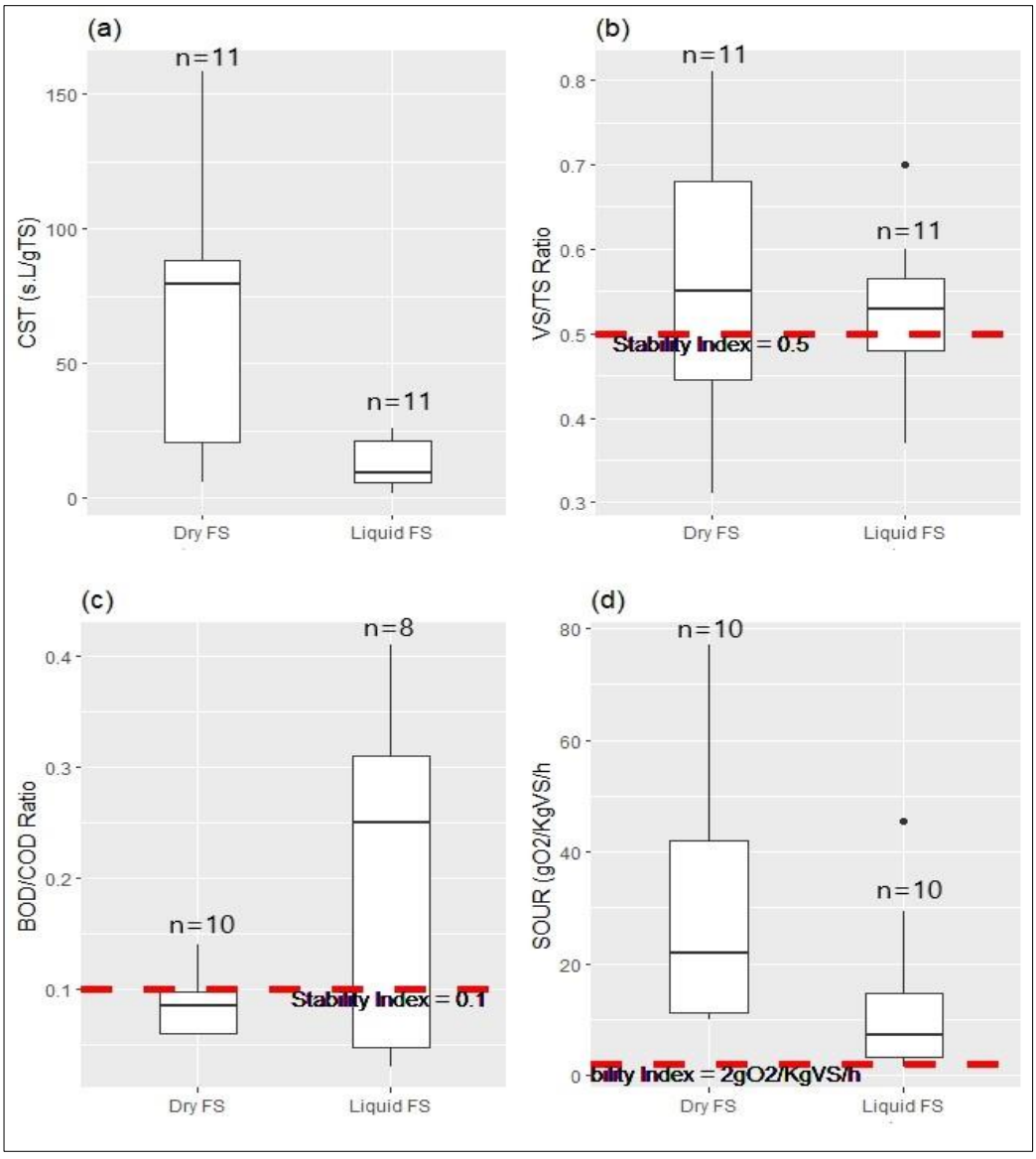

Figure 4-10: Box Plots showing the relationship between type of FS and (a) CST, (b) VS/TS Ratio, (c) BOD/COD Ratio and (d) SOUR (Red line represents the stability index)

In this study, the liquid FS samples had a lower normalized CST as compared to the Dry FS samples. Results of the Wilcoxon test showed statistically significant difference between the two groups (w=16, p=.0030). No significant difference was observed in the VS/TS ratio (p=.6151) and BOD/COD ratio (p= .3949) as metrics of stabilization between the two groups of FS samples. On the contrary, the liquid FS samples had a lower SOUR as compared to the dry FS samples. Results of the Wilcoxon test showed statistically significant difference between the two groups (w=16, p=.03). Assessment of the results for the VS/TS ratio, BOD/COD ratio and the SOUR, against the stability indices as determined in Part 1of the study showed that the majority of the dry and liquid FS samples can be categorized as not stabilized with an exception of the BOD/COD ratio of the dry FS samples. This trend was consistent with that observed in Part 1 of the study.

Comparisons between the trends shown in the dewatering performance (CST normalized) of the two types of FS samples and three stabilization metrics showed conflicting results. Overall the dry FS samples recorded higher SOUR values as compared to liquid samples which was correlated to the trend shown in the CST values as well. Results of spearman correlation analysis indicated a statistically significant medium positive correlation between SOUR and CST (R = .48; p=.033) as shown in Figure 4-11 below. A similar trend was also observed between VS/TS ratio and CST. However, the correlation was weak and not statistically significant (R=.13, p=.57).

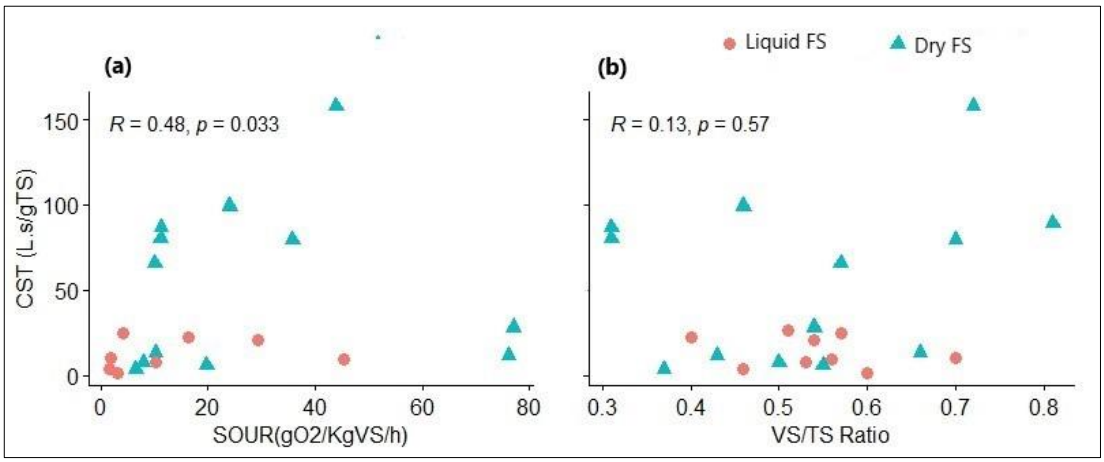

Figure 4-11: Scatter Plot showing Correlation between CST and Stability Metric (a) SOUR and (b) VS/TS Ratio

On the contrary the BOD/COD ratio values of the dry FS samples were lower than those for liquid FS samples which was the opposite of trend observed in the CST and

SOUR values. Further the BOD/COD ratio was negatively correlated to CST, however, the correlation was weak and not statistically significant as shown in Figure 4-12 (R=-.42, p=.079).

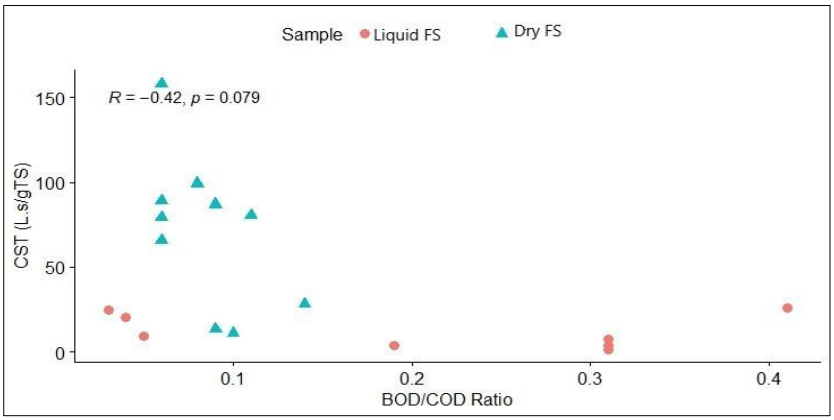

Figure 4-12: Scatter Plot Showing Correlation between CST and BOD/COD Ratio

4.2.4 Anaerobic Digestion of Faecal Sludge Samples

4.2.4.1 Effect of Anaerobic Digestion on Physical Chemical Characteristics

In this study the effect of anaerobic digestion on the physical chemical characteristics of the samples was monitored by observing the changes in the organic matter parameters (i.e. BOD, COD and VS) during the entire process. The graphs in Figure 4-13 show the effect of anaerobic digestion on BOD, COD and VS of the individual samples. The graphs show a reduction in concentration of COD, BOD and VS during the 60 days of anaerobic digestion. The organic matter removal efficiency ranged from 19 to 96 percent 30 to 70 percent and 10 to 78 percent for COD, BOD, and VS respectively. Based on this, there was a clear indication that the FS samples underwent biological stabilization under anaerobic conditions during this period. This was further confirmed through the production of gas from all the samples. Gas was actively produced up to day 24 and it eventually reduced drastically thereafter until it stopped around day 40.

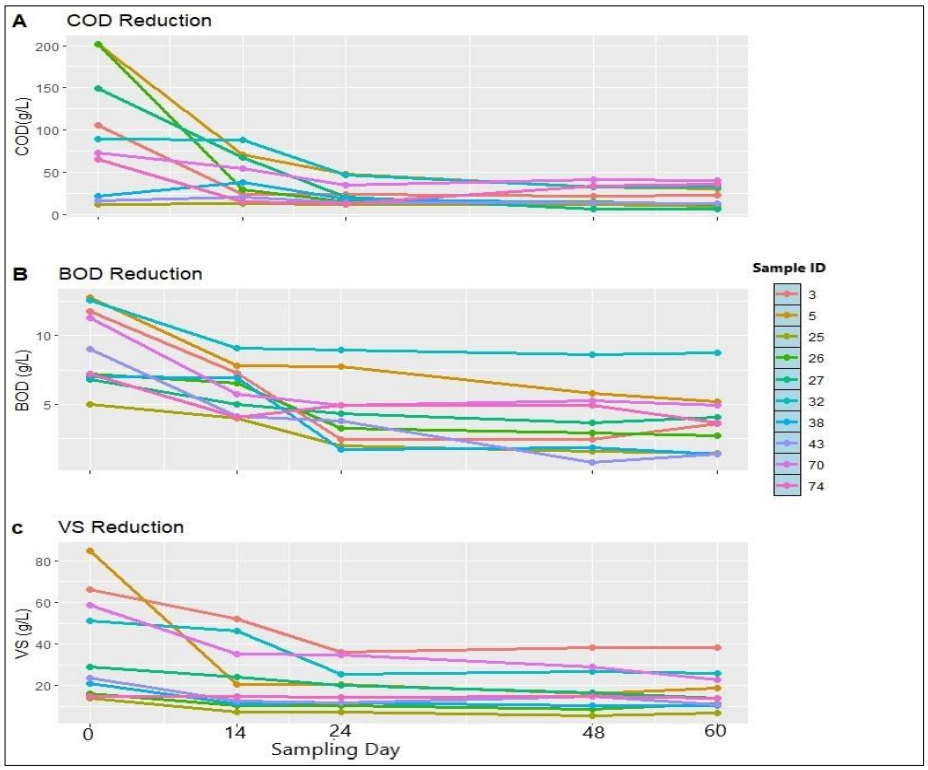

Figure 4-13: Graphs showing COD, BOD and VS Removal during Anaerobic Digestion

In addition, differences were observed between the grouped initial (Day 0) and final (after 60 days of anaerobic stabilization) median values of the COD, BOD and VS for the liquid and dry FS samples respectively (Figure 4-14). Results of the Wilcoxon test indicated statistically significant differences in the said parameters after and before the anaerobic digestion process (i.e. w=82; p=.017 for COD, w=94; p=.0009 for BOD and w=82; p=.017 for VS).

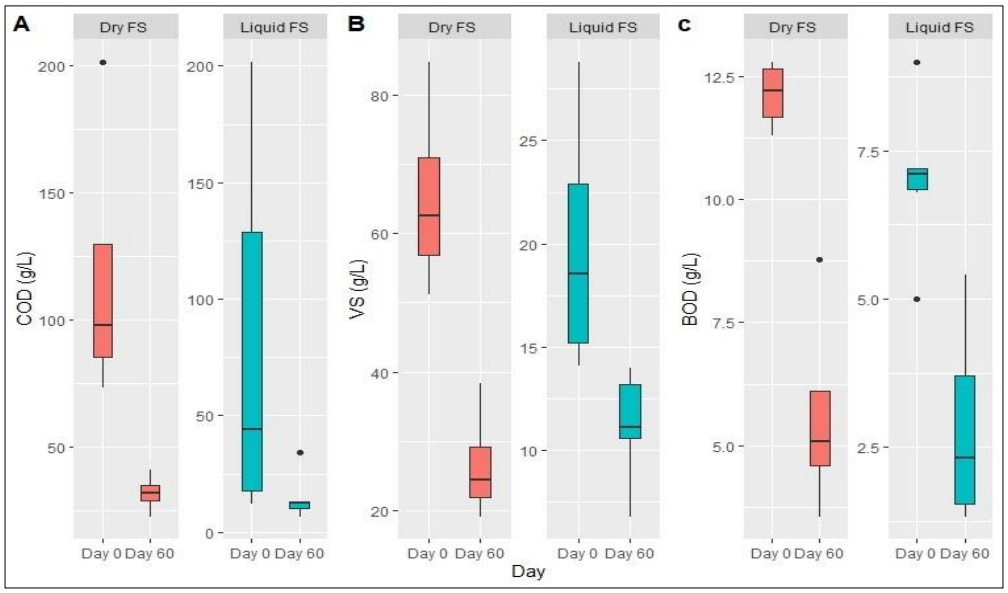

Figure 4-14: Box Plot Showing Effect of Anaerobic Digestion on (a) COD, (b) BOD (c) VS

4.2.4.2 Influence of Physical Chemical Characteristics on the Anaerobic Digestion of Faecal Sludge.

TS concentration (which is an indication of the moisture content), Total Ammonia Nitrogen (TAN) and pH have been reported to as the major physico-chemical parameters that can influence anaerobic stabilization of sludge (Liotta *et al.*, 2014; Couderc *et al.*, 2008; Jiunn *et al.*, 1997; Van Eekert *et al.*, 2019). In this regard the influence of these parameters on the anaerobic digestion of the FS samples was investigated. This was done in order to determine if they affected the ability of the FS to undergo intrinsic anaerobic digestion on its own without the addition of inocula or nutrients (i.e. without altering the characteristics of the field samples collected from onsite containments). TAN was calculated from the EC values using the linear model (EC (mS/cm) $*$ 0.2 = NH4+ -N (g/L)) as reported by Ward *et al.* (2021).

In this study, the pH values for all the FS samples (Figure 4-15(a)) remained within the range that has been reported to be suitable for anaerobic digestion (i.e. 6.1 – 8.3) by Forbis-Stokes *et al.*, (2016) and Wu *et al.*, (2021) before, during and after the anaerobic digestion process. When it comes to the TAN concentration, a difference was noticed between the dry and liquid FS samples. The liquid FS samples recorded a high TAN concentration which was above the limit reported to be favourable (i.e. above 3g/L) for anaerobic digestion except for one sample (See Figure 4-15(b)). However, no inhibition was observed probably because the FS samples were stored

for a long time under anaerobic conditions and the microbes were already acclimatized to the high TAN conditions (Colón *et al.*, 2015). All the dry FS samples recorded TAN concentrations which were favourable for anaerobic stabilization throughout the entire duration of the stabilization experiments. Based on these results, the pH and TAN concentration favoured further stabilization of the FS under mesophilic and anaerobic conditions.

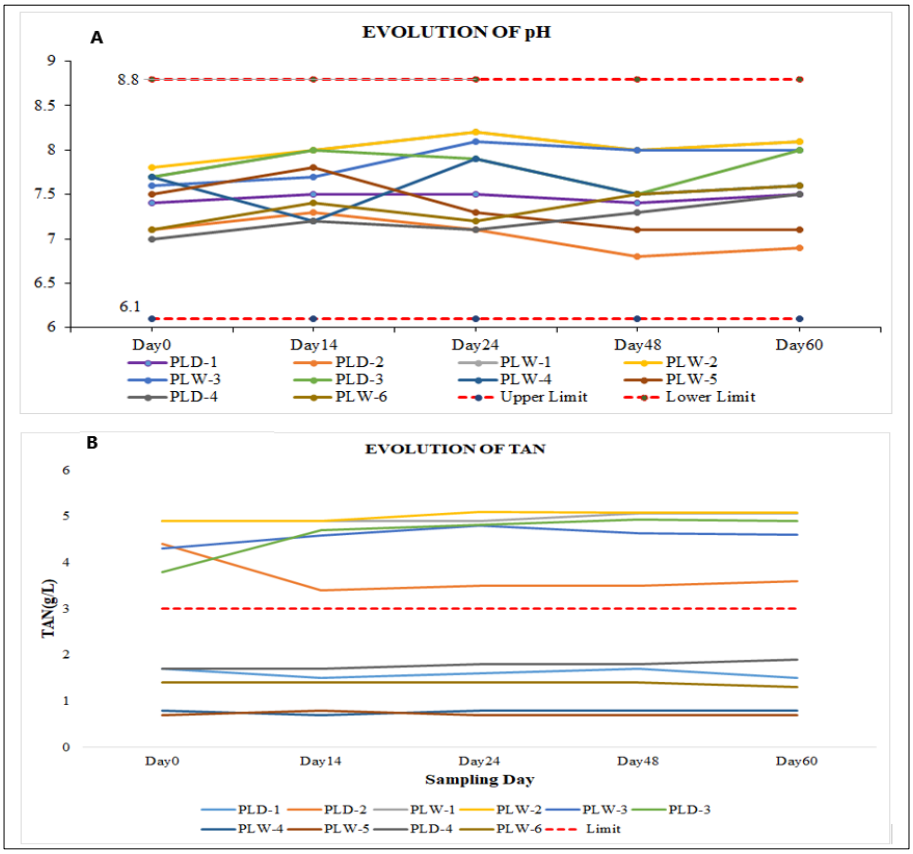

Figure 4-15: Changes in (a) pH and (b) TAN during Anaerobic Digestion of FS Samples (the red dotted line shows the limits favourable for Anaerobic Digestion)

Further, the influence of TS concentration on anaerobic digestion was also investigated by comparing the VS removal or reduction among the liquid and dry FS Samples. VS removal is an indicator of treatment efficiency of anaerobic digestion (Tanimu *et al.*, 2014). No statistically significant difference was observed in the reduction or removal of VS among the dry and liquid FS samples as shown in Figure 4-16 below. However, VS removal efficiency was slightly higher in the dry FS as compared to the liquid FS samples. Generally, the results showed that TS concentration had no significant effect/ influence on the anaerobic digestion of FS samples in this study.

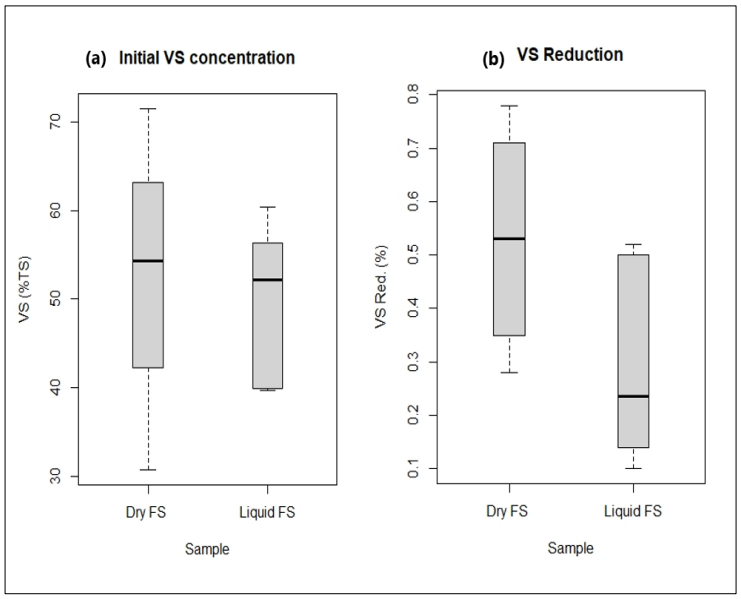

Figure 4-16: Effect of TS concentration on VS removal efficiency

4.2.4.3 Effect of Anaerobic Digestion on Stabilization and Dewatering Characteristics

Figure 4-17 (a) and (b) show that the stabilization of the FS samples measured as SOUR and VS/TS ratio reduced as the sludge underwent anaerobic digestion. It was also observed that the variability for the SOUR reduced while that for the VS/TS ratio increased. There was a significant difference in the median grouped SOUR and VS/TS ratio values for both liquid and dry FS samples before and after the stabilization processes. The Wilcoxon test indicated that the difference were statistically significant at 95 percent confidence interval for SOUR (w=96; p=.0005828). However, when it comes to the VS/TS ratio, a statistically significant difference was observed for the liquid FS samples (p=.04595) only. In addition, the results showed that most of the liquid and dry FS samples became stabilized after 60 days of anaerobic digestion when evaluated using the SOUR and VS/TS ratio stability indices.

The BOD/COD ratio showed a different behaviour from the other metrics of stabilization. As shown in Figure 4-17 (c), the BOD/COD ratio for most of the liquid FS samples remained almost unchanged while that for the dry FS samples increased at the end of the anaerobic digestion process. It was also observed that the variability for the BOD/COD ratio decreased for both types of FS at the end of the anaerobic digestion process. In addition, the results showed that none of FS samples became

stabilized after 60 days of anaerobic digestion when evaluated using the stability index for BOD/COD ratio index which was not expected.

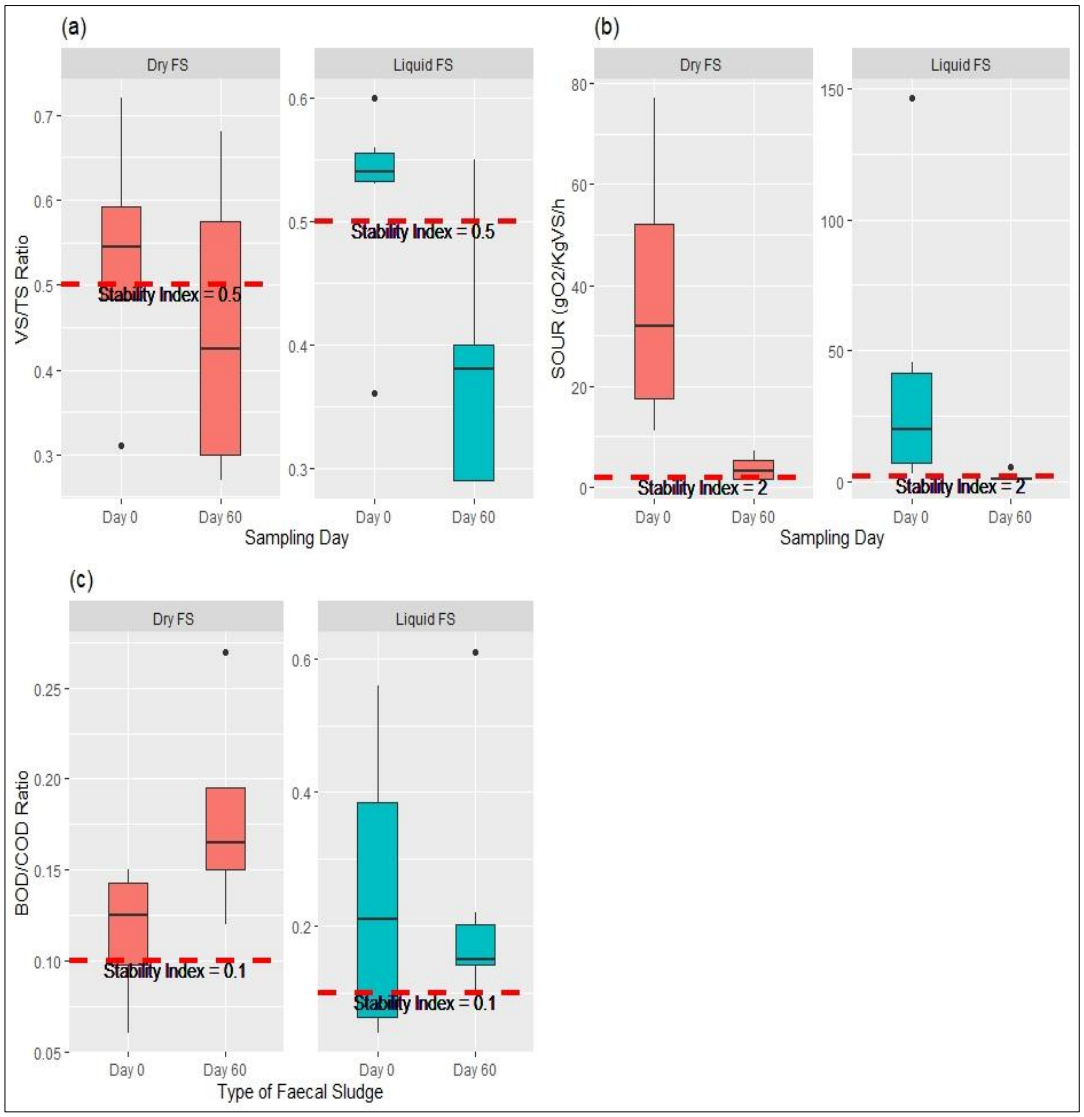

Figure 4-17: Box Plots showing the effect of anaerobic digestion on Metrics of Stabilization (a) VS/TS Ratio, (b) SOUR (c) BOD/COD Ratio

Illustrated in Figure 4-18 are the changes in CST with anaerobic digestion for the grouped liquid and dry FS samples as well as the individual samples. The results indicated that the CST (normalized) for both the liquid and dry FS samples decreased after the anaerobic digestion process. The Wilcoxon test indicated the decrease was statistically significant (i.e. w=79.5; p=.01906) for both types of FS samples. Generally, the CST(s) for the samples decreased consistently over the entire period of anaerobic digestion with an exception of four liquid samples where it increased at day

14 and then decreased thereafter (Figure 4-18(b)). It was also observed that the variability of CST for the samples reduced at the end of the anaerobic digestion process.

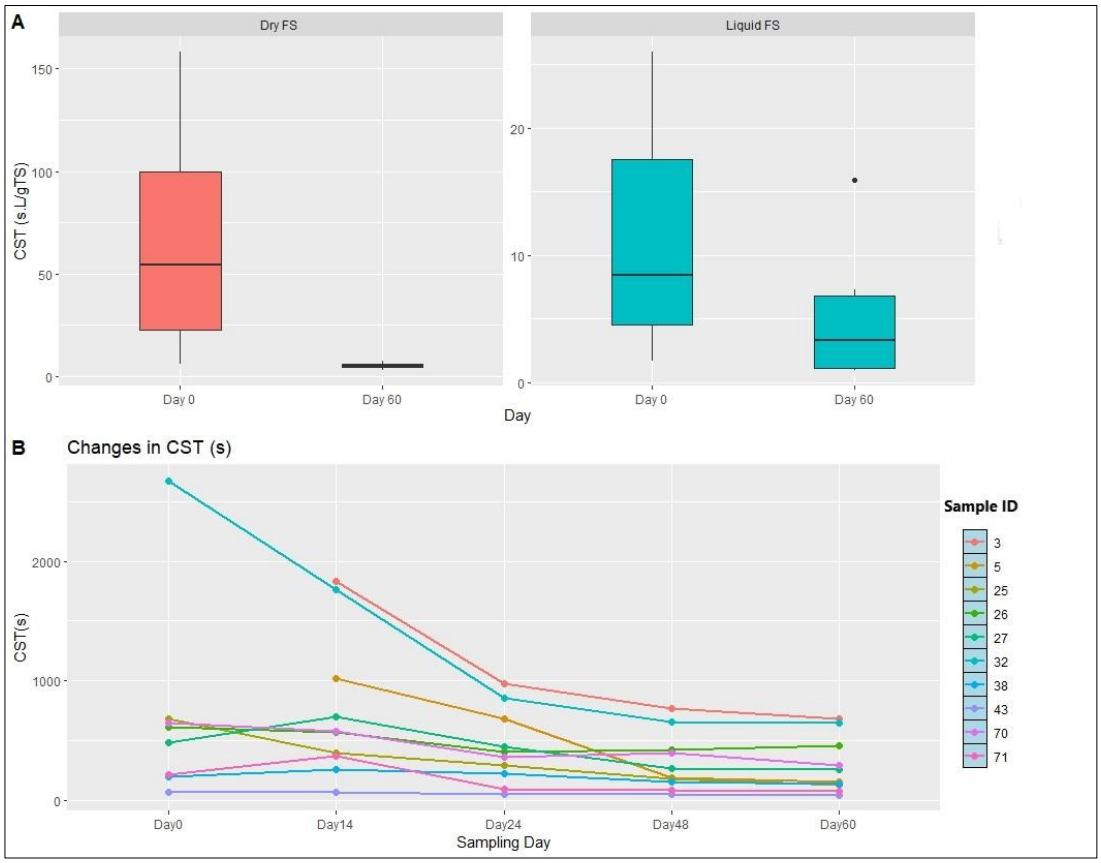

Figure 4-18: Change in Dewatering with Anaerobic Digestion (a) Box for initial and Final Normalized CST (b) CST(s) for individual Samples.

4.2.4.4 Correlation between Dewatering and Stabilization of Anaerobically Digested Sludge

The trend shown in the dewatering performance (CST normalized) before and after the stabilization process was correlated to that shown by SOUR and VS/TS ratio (metrics of stabilization). Results of spearman correlation test indicated statistically significant strong positive correlation between SOUR and CST (R = 0.78 p=.000005) as shown in Figure 4-18 below. This implies that CST of FS would increase with an increase in SOUR. When it comes to the VS/TS ratio, the correlation was found to be weak and not statistically significant (R=0.19, p=.43). These correlations were similar to those observed with the field samples as described in section 4.2.3. However, an improvement in the correlation between SOUR and CST (normalized) was observed with anaerobic digestion.

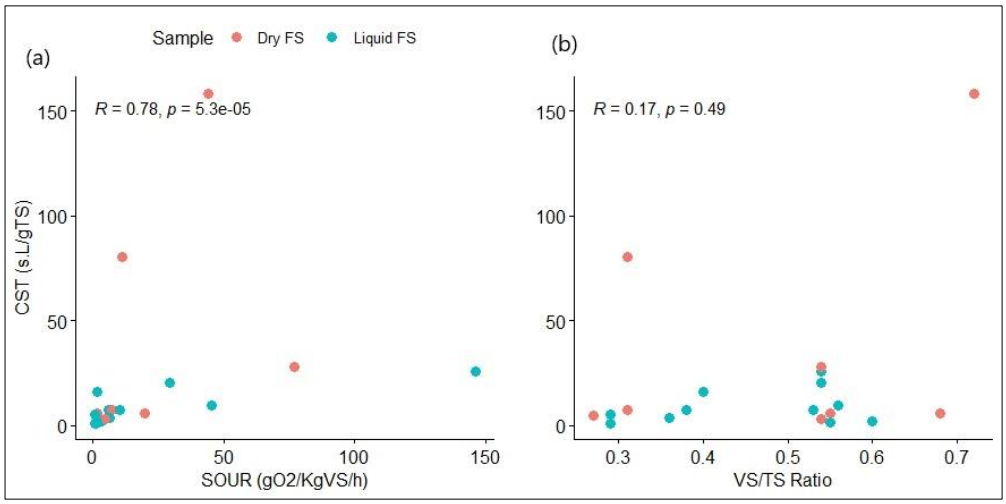

Figure 4-19: Scatter Plot Showing Correlation between CST and (a) SOUR (b) VS/TS Ratio for Anaerobically Digested Samples

On the contrary the BOD/COD ratio was negatively correlated to CST. However, the correlation was weak and not statistically significant (R=-0.3, p=.2). This trend was similar to that observed with the field FS samples where the correlation between the CST and BOD/COD ratio was also negative (see Figure 4-20).

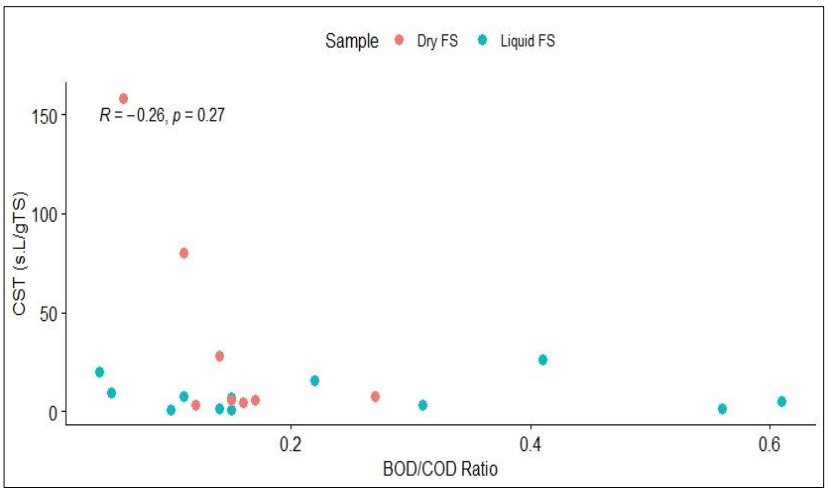

Figure 4-20: Scatter Plot Showing Correlation between CST and BOD/COD Ratio for Anaerobically Digested Samples

4.3 Chapter Summary

This chapter Presented findings in line with the set research questions/hypotheses. Findings on methods and criteria for measuring FS stabilization were elaborated. The chapter then presented results on differences in FS stabilization based on sludge age and type of containment (i.e. wet vs dry onsite containment facilities) before finally presenting results on the relationships between dewatering and stabilization. Results

in anaerobic digestion of FS and the effect of intrinsic physical-chemical characteristics were also presented. The next Chapter discusses the results presented in this chapter in order to respond to the research questions and hypotheses.

CHAPTER FIVE: DISCUSSION

5.1 Introduction

The previous chapter presented findings of the study in line with the research questions and hypotheses of the study. This chapter discusses the results linking them to the respective research questions and hypotheses. As the aim of the study was to determine how to measure FS stabilization using rapid and low costs methods in order to contribute to the enhancement of predicting its dewatering performance, the chapter discusses the results from this context. It therefore first discusses the results on the literature search for methods for measuring FS stabilization before discussing the laboratory tests and experimental results on stabilization and its relation to dewatering. The chapter then delves into effects of anaerobic digestion on the dewatering and stabilization of FS as well as the differences based on type of FS/ or onsite containment. It is concluded with a discussion on the effects of physical chemical characteristics on anaerobic stabilization of FS.

5.2 Literature Search on Methods for Measuring Stabilization

An overview of the methods and criteria used to measure or quantify the level of stabilization of organic substrates was presented in 4.2.1 based on the findings from the literature search. A total of 14 methods were identified that can be applied to measure FS stabilization. The results of the desk study revealed that these methods have mostly been applied in studies that focused on measuring the stabilization of wastewater sludge and composts. Only two of the methods were found to have been applied in FS studies. However, their application was not with the primary aim of measuring stabilization. These were TS/VS ratio and BOD/COD ratio whose determination is based on common physico-chemical parameters of FS (i.e. BOD, COD , VS and TS) that form part of most FS characterization studies that have been conducted by many researchers (Niwagaba, *et al.*, 2014; Velkushanova *et al.*, 2021).

Overall, the methods were summarized into three major categories which included: 1) Microbial Activity Methods; 2) Elemental Composition; and 3) Surrogate Methods. Based on the results of the screening using the decision matrix (Appendix 1), the five best methods (out of the 14 identified) that scored above 80 percent were selected and subjected to further evaluation through lab experiments and tests to determine their suitability to be applied to measure FS stabilization. The five methods included:

VS/TS and BOD/COD Ratio from the elemental composition category as well as SOUR, BMP, and DHA which are all from the Microbial Activity category of methods. The literature review revealed that all the five methods selected were cheap and easy to perform in the local laboratory. Further stability indices (i.e. an evaluation criteria) for the five methods that were required to define a sample as stabilized or not stabilized were identified from literature as presented in Table 4-2 of section 4.2.1. The BMP method despite meeting the criteria, is a very lengthy method (i.e. takes couple of weeks or months to be completed) which makes it not appropriate for rapid application and quick decision making. However, it was the only method that was performed under anaerobic conditions which is the main process for FS stabilization during the time it is stored in containment systems such as pit latrines and septic tanks as reported by other researchers (Van Eekert *et al.,* 2019; Nwaneri, *et al.,* 2008). It was important in this study to understand the ability of various types of FS to undergo stabilization under anaerobic conditions. Anaerobic digestion is the most common biological mechanism that is used for FS stabilization in practice through technologies such as bio digesters and settling thickening tanks (Tayler, 2018; Ronteltap, *et al.,* 2014).

5.3 Physical Chemical Characteristics of Faecal Sludge Samples

Tables 5-1 and 5-2 summarizes the physical chemical characteristics of the samples used in this study and compared with literature values of FS and wastewater sludge. The results of the physical chemical characteristics were in the range of values reported by other studies for septic tank and dry pit latrine sludge from Lusaka (Ward *et al.*, 2021; Tembo, 2019) and other studies conducted in Sub Saharan Africa (Table 9 and 10 below). However, in the current study, the values of BOD_5 were higher than those reported for Lusaka FS by one study conducted by Tembo (2019) which ranged from 0.5 g/L to 3.57 g/L. This can be because the method used in this study (i.e. membrane electrode method) was different from that which used in the study by Tembo (2019) i.e. Winkler method. Nonetheless, the BOD values were similar to those reported in other countries in sub–Saharan Africa. Studies in Kenya and Uganda by Gudda *et al.* (2017) and Awere *et al.* (2020) reported BOD_5 values for FS from dry pit latrines in the ranges from 11 g/L to 35.9 g/L. The normalized CST values for the FS samples from wet containment facilities were in the range of values reported by Gold *et al.* (2018) for lined pit latrines and septic tank sludge. However, the

normalized median CST value for FS from dry containment facilities was about 8 times higher than that reported by Gold *et al.* (2018) for unlined pit latrine sludge from Uganda. This can be explained by the variability of FS from one location to another.

Table 5-1: Results of Physical chemical, Stabilization and Dewatering Analysis of Dry FS Samples.

	Physico-chemical Parameters						Stabilization Metrics			Dewatering Performance
	pH	**EC (mS/cm)**	**COD (g/L)**	**BOD (g/L)**	**VS (%TS)**	**TS (%ds)**	**BOD/COD Ratio**	**VS/TS Ratio**	**SOUR (gO_2/kg VS/hr)**	**CST (s.L/gTS)**
Mean	7.3	13.8	152.5	12.3	55	14.8	0.08	0.6	31.9	65.2
Median	7.5	12.9	130.2	12.6	54.8	15.8	0.08	0.6	21.9	79.2
SD	0.5	5.3	61.9	1.1	16.5	4.9	0.03	0.2	26.2	46.7
N	10	10	11	10	11	11	10	11	10	11
Literature Values[4]										
Means	7.64-7.8[a,b]	12.1-14.2[a,b]	112.8-122.6[a,b,f]	1-24.6[c,e,f]	43.2-56.4[a,b]	14.7-17.9[a,b]	0.008 – 0.26[c,e,f]	0.5[g]	3-19.8[h*,i*]	10[b+]
Medians	7.66-7.8[a,b]	11.2-14.5[a,b]	108-127.2[a,b,f]	22.7[f]	52 – 59[a,b]	14.8[a,b]	0.21[f]	-	-	9[b+]

(a) Ward et al. 2021, (b) Gold et al. (2018), (c) Awere et al. (2020), (d) Bassan et al. (2013), (e) Tembo (2019), (f) Gudda et al. (2017), (g) Van Eekert et al., (2019), (h) Sánchez et al., (2006), (i) Lasaridi and Stentiford, (1998)

[4] All Literature values are for FS collected from partially and unlined dry pit latrines.

[b+] Value for FS from unlined pit latrine only

[h*,i*] Wastewater sludge values.

Table 5-2: Results of Physical chemical, Stabilization and Dewatering Analysis of Liquid Samples.

	Physico-chemical Parameters						**Stabilization Metrics**			**Dewatering Performance**
	pH	**EC (mS/cm)**	**COD (g/L)**	**BOD (g/L)**	**VS (%TS)**	**TS (%ds)**	**BOD/COD Ratio**	**VS/TS Ratio**	**SOUR (gO_2/kg VS/hr)**	**CST (s.L/gTS)**
Mean	7.7	14.8	78.4	7.2	50.3	2.3	0.2	0.5	12.7	12.4
Median	7.7	15.8	36.6	6.9	52.1	2.4	0.3	0.5	7.2	9.4
SD	0.4	8.6	77.5	4.4	6.0	1.2	0.2	0.09	14.3	9.2
N	10	10	9	10	11	11	8	11	10	11
Literature Values										
Means	7.4-7.8[a,b]	2.3-14.6[a,b]	7.6-72.1[a,b,d]	1.45[d+]	53.5-73.2[a,b,d]	1.1-4.8[a,b]	0.19[d+]	-	3-19.8[h*,i*]	11-63[a]
Medians	7.3-7.8[a,b]	1.3-12.4[a,b]	9.8-53.3[a,b]	-	51.8-75.5[a,b]	1.1-2[a,b]	-	-	-	13-56[a]

(a) Ward et al. 2021, (b) Gold et al. (2018), (d) Bassan et al. (2013), (h) Sánchez et al., (2006), (i) Lasaridi and Stentiford, (1998)

The FS samples that were used in Part 1 of the study comprised fresh human excreta, pit latrine sludge and septic tank sludge. The physico-chemical characterization results of the pit latrine and septic tank samples as shown in Table 4-3 were also in the same ranges of literature values presented in Tables 5-1 and 5-2. The values of the physico-chemical characteristics of fresh excreta reported in this study (Table 4-3) were similar to the results reported by other studies that characterized the physical, chemical and biological properties of fresh human excreta. Specifically, the average VS content of the fresh excreta of 84 percent and compares very well with the results reported by Nwaneri *et al.,* (2008) and Roseet at al. (2015) which ranged from 84- 92 percent Similarly the average COD values for the fresh excreta was 87.6g/L which was higher than the range of values reported by Rose, *et al.* (2015). This is possible since the characteristics of fresh human excreta also vary depending on the age, health and diet of the individuals (Lopez, *et al.,* 2002). The COD concentration of the fresh excreta in this study was less than that for the pit latrine sludge samples which was contrary to the results reported by Van Eekert *et al.* (2019) and Nwaneri, *et al.* (2008). This result can be attributed to the practice of adding chemicals and other foreign materials (mostly from cleaning and bathing activities) to pit latrines which is common in Lusaka as reported by Tembo (2019). This practice alters the natural characteristics of FS quality and most likely results in an artificial increase in the COD of the FS as found in this study. In their study, Van Eekert *et al.* (2019) also reported that one sample recorded a COD content which was higher than the fresh excreta and they attributed this to addition of chemical and other foreign materials to the pit latrine where this sample was collected.

5.4 Part 1: Methods for Measuring Faecal Sludge Stabilization

5.4.1 Evaluation and Performance of BOD/COD Ratio

The BOD/COD ratio results for FS in Part 1 of the study (Figure 4-2) ranged from 0.11 to 0.18 which was about 14 times higher than the average of 0.008 reported for Lusaka FS by Tembo (2019). This difference is as a result of the low BOD values for FS that were reported by Tembo (2019) as compared to those reported in this study. Nonetheless, the values in this study were within the ranges for pit latrine sludge in Ouagadougou (0.14 – 0.17) as reported by Bassan *et al.* (2013). On the contrary the

results are lower than those reported by Awere *et al.* (2020) for FS collected from VIP latrines (BOD/COD ratio of 0.34) and Appiah-Effah *et al.* (2020) for FS collected from public toilets (BOD/COD ratio of 0.3) which was reported to be largely fresh and not stabilized. When it comes to the fresh excreta, the BOD/COD ratio averaged 0.45 which was higher than the results for FS collected from onsite containment facilities reported in this study as well as those reported by the other researchers. The BOD/ COD ratio of 0.45 is an indication that the fresh excreta contains largely undigested organic matter that is slowly biodegradable (Zavala, *et al.,* 2002; Bakare *et al.,* 2012; Appiah-Effah *et al.,* 2020). The presence of slowly degradable organic matter in the fresh excreta was an indication that the microorganisms will require a much longer time to degrade the organic matter in the sludge (Awere *et al.,* 2020).

There is a general agreement for a BOD/COD ratio of as low as 0.1 to be used as stability index in the wastewater and composting sector as presented earlier in Table 3 (Mangkoedihardjo, 2006; Borglin *et al.,* 2012). Comparison of the ratios found in this study with the stability index identified from literature revealed that the dry and liquid FS samples (which had a sludge age of three years) can be categorized as stabilized because they both had an average BOD/COD ratio of 0.1 (i.e. no difference was observed between the two types of FS with the same age). In addition the dry FS-VVL sample with a sludge age of six months recorded a ratio of 0.2 which was not very far from the ratios recorded by those with a sludge age of more than three years. Even though the samples with a sludge age of three years were categorized as stabilized (based on the stability index for wastewater sludge and composts), their BOD and COD concentrations which were in the ranges of 5.2 – 16.9g/L and 46.4 – 113.06g/L respectively. The results indicated that the samples were characterized by high organic matter content requiring further stabilization it is discharged into the environment (Awere *et al.,* 2020). Based on this, a BOD/COD ratio for FS of as low as 0.1 does not necessarily mean that a sample is stabilized. Instead, it is as a result of the high COD values as compared to BOD which was an indication of the presence of high proportions of hard biodegradable organic and inorganic matter. In this study, this was attributed to the suspected addition of chemicals and foreign materials to pit latrines which in turn contributed to the low BOD/COD ratio of close to 0.1 (Tembo, 2019). This also applies to industrial wastewater which has very high COD concentrations as compared to BOD as reported by Mangkoedihardjo, (2006). Based

on these results, it is clear that a BOD/COD ratio of 0.1 cannot be used as a stability index for FS samples outrightly. This is because the same ratio can also indicate toxicity i.e. the presence of high concentration of organics in a sample that inhibit the activity of microbes responsible for biological degradation. This principle of BOD/COD ratio of closer to 0.1 as an indicator of toxicity is also presented by Samudro and Mangkoedihardjo, (2010) and Mangkoedihardjo, (2006). Samudro and Mangkoedihardjo, (2010) have defined three zones of BOD/COD ratio that can be used to characterize organic substances as shown in the Table 5-3 below.

Table 5-3: Results of Physical chemical, Stabilization and Dewatering Analysis of Liquid Samples.

Zone	BOD/COD Ratio Limits	Characteristics
Toxic	0 - 0.1	High concentration of hard biodegradable organics in form of COD (e.g. COD> 100g/L, BOD approx. 10g/L)
Biodegradable	0.1 – 1	High concentration of easily biodegradable organics in form of BOD (e.g. COD >100g/L, BOD approx. 50g/L). Levels can include slowly, average and easily biodegradable
Acceptable/ Stable	0 – 0.1	COD and BOD concentration levels within the limits acceptable for discharge into the environment.

Based on Table 5-3, the performance of the BOD/ COD ratio as a measure of FS stabilization in this study can be summarised as follows:

i. The dry and liquid FS samples (with sludge age of three years) which had ratios closer 0.1 can be characterized to be in the toxic zone which means they can undergo further stabilization under favourable conditions.
ii. The dry FS-VVL sample (with a sludge age of 6 months) which had a ratio of 0.2 was characterized to be in the in the slowly biodegradable zone; and
iii. The fresh excreta which had a BOD/COD ratio of 0.45 was in the average to easily biodegradable zone.

The ability of all the samples to be stabilized further was confirmed through the BMP test.

5.4.2 Evaluation and Performance of VS/TS Ratio

The VS/TS ratio results for the dry and liquid FS samples as shown in Figure 4-3 ranged from 0.4 to 0.7 which was within the ranges reported by Van Eekert et al. (2019) for pit latrine sludge (0.1 – 0.9) and Shaw and Dorea, (2021) for septic tank (0.4 – 0.9). When it comes to the fresh excreta the VS/TS ratio averaged at 0.8 which was similar to the average of 0.8 reported by Van Eekert *et al.* (2019). Velkushanova *et al.* (2021) also stated that the VS/TS ratio of FS discharged at treatment facilities typically ranges from 0.43 to 0.73. Generally the VS/TS ratio has been used as an indicator of the fraction of sludge solids that are organic/ biodegradable. The fresh excreta recorded the highest VS/TS ratio (0.8) among all the samples which was an indication that it contained largely organic matter that is biodegradable (Doku, 2002).

As can be seen from the graph in Figure 4-3, the trend shown by the VS/TS ratio was well correlated with age of the FS samples. There is a general agreement for a VS/TS ratio of as low as 0.5 to be used as stability index in the wastewater and composting sector as presented earlier in Table 3 (Cokgor *et al.,* 2012). Comparison of the ratios found in this study with the stability index identified from literature revealed that the dry FS samples (which had a sludge age of three years) can be categorized as stabilized because they had an average VS/TS ratio below 0.5. On the contrary, the liquid FS samples (with also a sludge age of three years) were categorized as not stabilized because they had an average VS/TS ratio of 0.6. The difference between the dry and liquid FS samples of the same sludge age can be due to either the inherent variability in the VS/TS ratio of FS as reported by Van Eekert *et al.* (2019) or differences in the levels of stabilization even among samples with the same age. In this study, the dry

FS-VVL sample with a sludge age of six months recorded a ratio of 0.7 (meaning it was said to be not stabilized) which was greater than the ratios for samples which had a sludge age of more than three years. At the same time its VS/TS ratio was lower than that for the fresh excreta (i.e. 0.8).

Even though the VS/TS ratio results revealed that dry-FS samples can be categorized as stabilized (with reference to the stability index from wastewater and composting sector), Van Eekert *et al.* (2019) reported that pit latrine samples with VS/TS ratio of as low as 0.2 were able to undergo further stabilization invitro under anaerobic conditions. This was also true in this study as was observed with the BMP test. Thus, the difference in the stabilization among the dry and liquid FS samples with the sludge age of three years was as a result of the nature of sludge. In this study, it was observed that the dry-FS samples (with a VS/TS ratio of less than 0.5) had the highest TS concentrations (i.e. ranged from 171 to 201g/L) which resulted in the low ratio as it was not the same case when it come to their respective VS concenrations. The high TS concentration could be due to sand or grit which has been reported to be present especially in FS from unlined dry pit latrines (Tembo, 2019). Based on this, it is clear that VS/TS ratio of 0.5 does not seem to apply when it comes to establishing the stabilization of FS. This is supported by Velkushanova et al., (2021) who also stated that care has to be taken not to directly transfer empirical relations from wastewater, as the VS/TS ratio of FS is heavily influenced by the wide range of inorganic substances in samples. In this regard, the stability index for VS/TS ratio for FS samples can vary from one source to another based on the nature of the sanitation facility and user behavior e.g. the addition of chemicals and other foreign materials such household waste in pit latrine. For unlined and partially line pit latrines, the FS has been reported to have high grit and sand content which influences VS/TS ratio to a greater extent (Tembo, 2019; Velkushanova, *et al.*, 2021).

5.4.3 Evaluation and Performance of DHA

The DHA test measures the level of microbial activity in a sludge sample which is indicated by the activity of the enzyme Dehydrogenase which catalyses the oxidation process of organic matter during stabilization (Chung and Neethling, 1989; Pourakbar *et al.*, 2020; Stier and Fischer 1998). Thus, a high DHA which is indicated by the darker red coloured solution formed at the end of the test is synonymous to high microbial activity and high organic matter content in a sludge sample (Sánchez *et al.*,

2006; Xie *et al.,* 2008). This was the first time the DHA test was being applied to measure stabilization of FS samples. Thus, it was important under this study to determine the optimal test conditions such as dilutions (to achieve adequate concentration of biodegradable organic matter) and concentration of the TTC salt (high concentration can cause toxicity) which was important to achieve optimum performance of the DHA test (Sánchez *et al.,* 2006; Dufour & Colon, 1992). The preliminary DHA experiments showed that at VS concentrations up to 2.5g/L, TTC concentrations above 0.2 percent (w/v) were toxic to the dehydrogenase enzyme and resulted in a reduction in the DHA. Further, DHA was insensitive to TTC concentrations of as high as 0.3 percent (w/v) at VS concentrations exceeding 5g/L. However, at VS concentrations of as high as 15g/L resulted in INT Formazan solutions with optical density above the detection limit of spectrophotometer. This was attributed to high levels of oxidizable material in the samples which resulted in higher levels of microbial activity. Based on these results the optimum VS concentration to conduct the DHA test was found to be between 2.5 – 5g/L and TTC concentration of 0.2 percent (w/v). These results are similar to the findings by Lopez *et.al.* (1986) and Bitton and Koopman, (1982) who carried out similar experiments on activate sludge samples.

The quantitative results for the DHA test were not as expected because the approach to quantify the microbial activity by measuring the optical density (i.e. absorbance at 485nm) of the red formazan solution was not successful. Repeated absorbance measurements/ readings on the same formazan solution (from the same cuvette) changed drastically by either decreasing or increasing each time. A high variability was also observed in replicate measurements of the same samples. This is contrary to the results reported by Chung and Neethling, (1989) who measured DHA of activated sludge and reported the results as absorbance at 490nm. Drastic changes in the absorbance readings can occur if the solution is turbid or if the reading is done when the samples are still hot and have not cooled down to room temperature. In this study the samples were cooled down to room temperature and centrifuged for 10 mins at 1200g to get a clear supernatant before taking the absorbance measurements. Thus, the unstable readings observed in this study were attributed to either a fault with the spectrophotometer or the spectrophotometer needed to be calibrated with a calibration

standard i.e. standard Typhenyl Formazan (TF) compound. The sourcing of the standard was a challenge during the time that the study was being conducted.

Figure 4-5 shows the qualitative results of the DHA test. There was a visible difference in the colour intensity of the formazan solution with the fresh excreta sample giving a red danker colour followed by the VVL and lastly the pit latrine and septic tank samples. The darker red solution produced by the fresh excreta is an indication that the sample had a high concentration of biodegradable organic matter which resulted in high microbial activity as compared to the other samples.

Based on these results, the DHA showed great potential to be used as a method for measuring FS stabilization. However, the test needs to be evaluated further using TF standards to generate calibration curves for spectrophotometer to verify its performance which could not be achieved this this study.

5.4.4 Evaluation and Performance of SOUR

The SOUR test measures the level of aerobic microbial activity in sludge which is indicated by the oxygen uptake rate i.e. a high uptake rate is synonymous to high microbial activity and high organic matter content (EPA, 2001). The literature research revealed that the SOUR test has mostly been applied in the study of stability of activated sludge samples from waste water treatment and composts. In this regard, two methods i.e. Standard method for wastewater 2710B and EPA method 1683 provide guidance on how to perform the SOUR test on activated sludge and biosolids respectively. From these methods, it was clear that the SOUR test is best suited for samples collected from aerobic environments (e.g. activated sludge treatment systems) which are homogenous with suspended solids concentration of not more than 5g/L (or TS less than 2 percent) to ensure adequate mixing during the test (EPA, 2001; APHA, 2004).

The characteristics of FS samples are different from that of waste water in the sense that they are collected from environments that have been reported to be mostly anaerobic in nature (Bourgault, *et al.*, 2019; Van Eekert *et al.*, 2019). Thus inactivation of the aerobic microbial populations (due to FS being stored under anaerobic conditions) present in FS is most likely to affect the oxygen uptake rate during the SOUR test. Aerobic microorganisms have been reported to be present in FS from dry pit latrines and pour flush systems (Torondel *et al.*, 2016; Bryne *et al.*, 2019). In

addition, the majority of FS samples from pit latrines including those in this study have been reported to have high total and suspended solids concentration in the excess of 5g/L (Velkushanova, *et al.*, 2021) which could limit adequate mixing and oxygen transfer rates during the test.

Based on the results of the literature search, this is the first time the SOUR test was being applied on FS samples. Thus, it was important under this study to determine the optimal conditions such as dilutions (to achieve adequate mixing and oxygen transfer rates) and aeration time (to acclimatize the FS samples to an aerobic environment in order to activate the microbes) which was important to obtain maximum performance of the SOUR test. However, it must be noted that because SOUR test conditions are not identical to the conditions in pit latrines and septic tanks, the observed measurements under this study may not be identical of the actual oxygen consumption rate.

The results of the aeration experiments as shown in Figure 4-6 revealed that the maximum SOUR for the fresh excreta was reached after 180 minutes (i.e. three hours) of continuous aeration while that for the pit latrines samples was reached after 60 mins of aeration. Samson and Ekama, (2000) conducted similar aeration tests on anaerobically digested primary waste water sludge and found that maximum SOUR was reached after 5 hrs of aeration. The results indicated that fresh excreta took longer to be acclimatized to the aerobic conditions which can be attributed to the fact that fresh excreta has been reported to be predominantly made of microorganism from the human gut i.e. mostly from the phyla firmicutes and bacteriodetes which have been linked to be more abundant in anaerobic/ facultative environments (Hsieh *et al.*, 2016; Magne *et al.*, 2020; Malele *et al.*, 2018; Chen *et al.*, 2016). On the other hand, Ward *et al.*, (2019) found that FS samples from household containment systems such as pit latrines and septic tanks had a higher proportion of microorganism from the actinobacteria phylum which have been reported to be aerobic (Malele *et al.*, 2018). This could explain the difference in aeration time between the fresh excreta and the pit latrine samples.

With regards to the dilution of FS samples to achieve adequate mixing, samples were diluted to VS concentration of between 2.5 – 5g/L which gave TS of less than 2 percent. The VS concentration between 2.5 – 5g/L was chosen as the optimum range for the SOUR test in this study. This is because the original VS concentrations of the

samples (which ranged from 36 – 80g/L) meant high levels of oxidizable material which resulted in higher levels of microbial activity and rapid rates of oxygen consumption (EPA, 2001). This made it very difficult to measure the DO concentration at regular intervals for at least 15 – 30 seconds. The optimal VS concentration was similar to that chosen for the DHA as VS concentration of 15g/L resulted in formazan solutions with optical densities above the detection limit of the spectrophotometer as shown in section 4.1.2.3. This is supported by Kim et al. (1994), Oviedo et al. (2005) and Sánchez et al. (2006) who reported a strong correlation between SOUR and DHA. The correlation is logical because both methods measure microbial activity, however, instead of measuring rate of oxygen consumption, DHA measures the activity of the enzyme Dehydrogenase which catalyses the oxidation process of organic matter during stabilization (Chung and Neethling, 1989)

As can be seen from the graph in Figure 4-7, the trend shown by the SOUR results was well correlated with the age of the FS samples. There is a general agreement for a SOUR value of 2 gO_2 /kg VS/h to be used as stability index in the wastewater sector as presented earlier in table 4. Comparison of the SOUR values found in part 1 of this study with the stability index identified from literature revealed that none of the samples can be categorized as stabilized. However, there was a difference between the SOUR value for the fresh excreta and those for FS collected from onsite containment facilities. This was an indication that the fresh excreta is less stabilized as compared to the rest of the samples. Similar to the BOD/COD and VS/TS ratio results, there was no significant difference between the SOUR for the dry FS-VVL (which had a sludge age of six months) with that for the dry and liquid FS samples (which had a sludge age of 3 years). Nonetheless, its value was still slightly higher than for the samples with sludge age of above three years. Based on the SOUR results obtained in this part of the study, it is therefore prudent to apply this test to measure the stabilization of FS.

5.4.5 Evaluation and Performance of BMP

The BMP tests provided results of methane production from pit latrine samples in the ranges of 92 to 287 NmL CH_4/gVS, with the highest being that for the VVL sample. These values were similar to the average value of 242.3 NmL CH_4 /gVS reported for FS from Hanoi (Hoai et al., 2018). Among all the samples, the fresh excreta had the highest BMP, while the septic tank sludge had the lowest i.e. 371.5 and 73.3 NmL CH_4/gVS respectively. The BMP for the fresh excreta in this study was similar to that

reported by Lalander et al. (2018) i.e. 338.5 NmL CH_4/gVS. These results are also supported by Van Eekert *et al.* (2019) and Sam, *et al.*, (2022) who reported that anaerobic digestion and biogas production from FS is possible. Further, in this study, the microcrystalline cellulose which was used as a positive control gave a BMP of 358.2 NmL CH_4/gVS which was within the range reported by other studies (Lalander *et al.*, 2018; Hansen *et al.*, 2004). The BMP for the positive control was also within the range recommended to show good performance of the BMP test (Holliger *et al.*, 2016). In addition, the blank (inoculum) had a BMP of 12.84 NmL CH_4/gVS which was within the range recommended to show low endogenous methane yield from the inoculum i.e. < 50 NmL CH_4/gVS (Filer, Ding and Chang, 2019).

It is clear from literature that organic substrates will still exhibit BMP even after being subjected to biological treatment processes for a few weeks or months. Lalander *et al.* (2018) reported BMP of as high as 188 NmL CH_4/gVS from human faeces after it was subjected to a two-stage treatment process i.e. black soldier fly (BSF) followed by anaerobic digestion. Similarly, Bożym and Siemiątkowski (2020) and Maulini–Duran *et al.* (2013) reported biogas production from composted sewage sludge. Thus, it seems various treatment strategies of sewage, FS and fresh excreta do not exhaust the BMP of the substrates, thus, the use of a gas production in sum in 21 days (GS_{21}) to indicate stability is more logical. According to literature, a GS_{21} value of as low as 20 Nl/Kg TS is said to represent a well stabilized compost sewage sludge (Bożym and Siemiątkowski, 2020). This is because within 21 days, the optimum microbial activity can be achieved but not the actual BMP (Binner *et al.*, 1999). This is supported by the GS_{21} value for the inoculum (which has a low organic matter content) in this study (i.e. 5.44Nl/Kg TS) which was less than 20 Nl/Kg TS and was similar to that reported by Bożym and Siemiątkowski, (2020) for composted sewage sludge (i.e. 4.9Nl/kg TS) after 12 weeks of maturation. Comparison of the GS_{21} values found in this study (as can be seen in Figure 4-9) with the stability index identified from literature revealed that none of the samples can be categorized as stabilized. However, there was a difference between the GS_{21} value for the fresh excreta and those for the FS samples collected from onsite containment facilities. This was an indication that the fresh excreta was less stabilized as compared to the other samples. In addition, a difference was also observed between the GS_{21} values for the dry FS-VVL (255.9Nl/kg TS) which had a sludge age of six months with that for the liquid and dry FS samples (53

and 154Nl/ Kg TS respectively) which had a sludge age of more than three years. These differences were an indication that there might be differences in the levels of stabilization even among the samples with the same age.

5.4.6 Comparative Analysis and Selection of Methods

In all the methods, it was observed that the stabilization results were correlated to the age of the samples i.e. the fresh excreta (with sludge age of 1 day) was the least stabilized followed by the VVL sample (which had a sludge age of six months) and lastly the pit latrine/ septic tank samples (which had sludge of three years). The differences in level of stabilization were more visible between the fresh excreta and the FS samples collected from onsite containment facilities (Figures 4-2, 4-3, 4-7 and 4-9). However, in all the methods except the BMP, the observed differences in stabilization between the FS samples with sludge age of six months and those with sludge age of three years was not as expected, and not in line with the hypothesis stated in section 1.4. This is supported by Ward *et al.* (2022) who saw no differences in stabilization indicators of FS based on time in containment. This can be explained by the assertion that FS undergoes rapid degradation of organic matter in the first few months of being added into the pit latrine, thereafter, the rate of degradation is reduced (Bakare et al., 2012; Van Eekert *et al.,* 2019; Nwaneri, *et al.,* 2008).

Evaluation of the stabilization results against the stability indices for wastewater identified from literature showed difference in terms of performance between methods in the microbial activity category (BMP and SOUR) and the elemental composition category (VS/TS and BOD/COD ratio). Stability indices for BMP (as GS21) and SOUR revealed that none of the samples can be categorized as stabilized which was different to what was observed with the stability indices for VS/TS ratio and BOD/COD ratio. However, it should be noted that the stability indices used in this case were for wastewater sludge and might not be representative for FS. Further evaluation was conducted in the second part of research which included batch stabilization experiments to determine if the stability indices for wastewater sludge are representative for FS as well.

Further, the observed correlations in the trends among the methods (excluding DHA) which was an indication that they can be said to be suitable for measuring FS stabilization. Despite this, a consistent agreement among the methods was not

observed. The corroboration of the BMP results (which follow a biological stabilization process that is predominant in onsite containment systems) supports the suitability of the other methods especially the SOUR which is an aerobic method. The disadvantage of the BMP test is that it takes longer (three to six weeks) to be completed (i.e. time consuming) and is labour intensive. Based on this, the SOUR, VS/TS and COD/BOD ratio methods were preferred for application in the second part of the study. The DHA was not selected due to the issues encountered with the method as stated earlier.

5.5 Part 2: Faecal Sludge Stabilization and its Relation to Dewatering Performance

The relationship between FS stabilization and dewatering performance was assessed by analysing the correlations and trends within the data sets for both untreated FS (i.e. raw sludge collected from containment facilities) and sludge that was subjected to further stabilization under controlled anaerobic conditions in the laboratory. The changes in physical chemical characteristics, dewatering performance and stability during the anaerobic digestion processes were also assessed and are discussed in the subsequent sections. In this part of the study, two sludge models or types were analysed i.e. liquid FS samples collected from wet containment systems (wet pit latrines and septic tanks) and dry FS samples collected from dry containment systems (i.e. dry improved and unimproved pit latrines).

5.5.1 Trends in Stabilization and Dewatering Characteristics of Untreated Samples.

The box plots in Figure 4-10 showed the relationships between type of FS and dewatering performance as well as stabilization. The Figure also showed the variability in the dewatering and stabilization among the FS samples. The variability in the values of stabilization (BOD/COD ratio, VS/TS ratio and SOUR) and dewatering performance (normalized CST) results is consistent with what has been reported in other FS characterization studies (Ward *et al.*, 2019; Semiyaga *et al.*, 2017; Ward *et al.*, 2021; Strande *et al.*, 2018a).

In this study, a statistically significant difference (p=.003) was observed between the normalized CST values for the dry and liquid FS samples (see Figure 4-10 (a)). The dry-FS samples recorded higher normalized CST values (i.e. poor dewatering

performance) as compared to the liquid FS samples. These results are supported by Ward *et al.* (2021), Gold *et al.*, (2018) and Kimwaga and Mayo, (2021) who found that FS samples from dry pit latrines (which can be compare to dry FS in this study) had poorer dewatering performance (i.e. significantly higher CST) as compared to samples from wet containment facilities i.e. septic tanks. When it comes to stabilization, a statistically significant difference (p=.03) was observed between the SOUR values for the dry and liquid FS samples only (Figure 4-10). Dry FS samples recorded higher SOUR values as compared to the liquid FS samples. However, none were categorized as stabilized based on the stability index identified from literature. This was similar to what was observed in Part 1 of this study. A positive medium strength correlation (R=.48, p=.033) was also observed between SOUR and normalized CST, meaning that as FS becomes more stabilized (indicated by a reduction in SOUR) its dewatering performance improves (i.e. CST will also reduce).

No differences were observed in the BOD/COD and VS/TS ratio between the dry and liquid FS samples. Bassan *et al.*, (2013) also found no difference in the BOD/COD ratio of dry pit latrine and septic tank samples. While a positive weak correlation (R=.13, p=.57) was observed between VS/TS ratio and CST normalized, the BOD/COD ratio of the field samples was found to be negatively correlated (i.e. to CST - R=-.42, p=.079) which was strange and not expected. Ward, *et al.*, (2022) also found that another stabilization indicator associated with COD (i.e. pCOD[5]/COD) was negatively correlated to normalized CST while VSS/TSS ratio (volatile suspended solids/ total suspended solids) was positively correlated. The stability indices (i.e. for VS/TS ratio and BOD/COD ratio) identified from literature revealed that the majority of the samples can be categorized as not stabilized, with an exception of the dry FS samples when assessed using the BOD/COD ratio (Figure 4-10 (c)). Based on what was observed in Part 1 of the study, a BOD/COD ratio of closer to 0.1 in FS can in most cases be associated with toxicity rather than stabilization (refer to Table 5-3). This can attributed to the suspected addition of chemicals and foreign materials to pit latrines (Tembo, 2019) which in turn results in low BOD/COD ratio of close to 0.1.

In this study, a difference was also observed in the biodegradable organic matter content (BOD and VS concentrations) between the dry and liquid FS samples (see

[5] Particulate COD is found by subtracting soluble COD from total COD (Ward et al., submitted)

table 6 and 7). Generally the dry FS samples had high VS and BOD concentration as compare to the liquid FS samples. The results for VS compares well to the results reported by Ward *et al.* (2021) and Strande *et al.* (2018) who found that FS samples from dry pit latrines had significantly higher VS content as compared to septic tanks. This observation suggested a difference in the level of stabilization among the two types of FS samples which was well associated with the results for SOUR and normalized CST. The trends and associations observed in the organic matter content (VS and BOD), Stabilization metrics (SOUR and VS/TS ratio) and dewatering performance (CST normalized) suggest that level of stabilization has an effect on the dewatering performance of FS samples. This corroborates with what practitioners have been observing in the field that more stabilized FS is easier to dewater than sludge which is less or not stabilized (Semiyaga *et al.,* 2016; Cofie *et al.,* 2006; Ward *et al.,* 2021;Ward *et al.,* 2019). These observations are further discussed in section 5.3.2 which covers changes in the physical chemical, stabilization and dewatering characteristics of FS with anaerobic digestion.

5.5.2 Anaerobic Digestion of Faecal Sludge

5.5.2.1 Effect of Anaerobic Digestion on Physical Chemical Characteristics

When FS undergoes stabilization, the readily biodegradable organic matter is broken down leaving behind a more stable product with less degradable organics (Bassan, et al., 2014). In this study, the parameters BOD, COD and VS which indicate the concentration of organic matter in the FS were measured before, during and after the anaerobic digestion process. The results revealed that during anaerobic digestion, the organic matter in the FS is broken as can be seen in the reduction in COD, BOD and VS concentrations in Figure 4-13. The average organic matter removal after 60 days of anaerobic digestion for COD, BOD and VS were 68 percent 54 percent and 53 percent respectively for dry FS samples and 53 percent, 61 percent and 29 percent respectively for liquid FS samples. Generally, the average COD and VS removal efficiencies were higher than those reported by Sam, *et al.* (2022) and Ward *et al.* (2022) i.e. 30 percent and 20 percent , respectively. In both studies fresh excreta (a mixture of urine and feces) innoculated with pit latrine sludge was used as a substrate for reactors and the retention time was 49 days which was different from the setup in this study. This could explain the difference in the removal efficiencies. On the other hand, Doku, (2002) conducted anaerobic stabilization of FS in laboratory scale upflow

anaerobic sludge blanket reactors and reported COD and VS removal efficiencies of 71 percent and 74 percent respectively which were higher than the averages reported in this study. The reduction in the organic matter content was correlated with the biogas production from the lab reactors which was vented/ expelled regularly from the lab reactors. In addition, a relationship was observed between the anaerobic digestion and the concentrations of organic matter in both the liquid and dry FS samples. This was revealed through the statistically significant differences between the COD, BOD, and VS concentrations of the samples at time 0 and day 60 as shown in Figure 4-14. This was a clear indication that the organic matter in FS was degraded during the 60 days anaerobic digestion process.

5.5.2.2 Influence of Physical Chemical Characteristics on Anaerobic Digestion of Faecal Sludge.

In this study, TS (which is an indicator of moisture content), pH and EC (converted to ammonia nitrogen) were used to access the influence of intrinsic physical chemical characteristics on anaerobic digestion of FS without inoculation or addition of nutrients. Based on TS concentration, two types of anaerobic digestion can be distinguished i.e. dry digestion characterized by TS of 10 percent and above and wet digestion characterized by TS less than 10 percent (Liotta *et al.*, 2014). In this study, the dry FS samples had TS concentration in range of 9 percent – 19.5 percent (they can be categorized to undergo dry digestion) while the liquid FS samples had TS concentration in the range of 2.4 - 6 percent (they can be categorized to undergo wet digestion). Dry digestion has been reported to be problematic as it results in mixing, mass transfer or diffusion limitations (Bollon *et al.*, 2013; Liotta *et al.*, 2014) which can affect the efficiency/ performance of anaerobic digestion. This is because moisture content is essential for anaerobic digestion processes in that it promotes substrate hydrolysis and enables the transfer of process intermediates and nutrients to bacteria (Liotta *et al.*, 2014; Awere *et al.*, 2020). In this study TS concentration was not observed to have had an influence on the anaerobic digestion of the FS samples because no significant differences were observed in terms of overall COD, BOD and VS removal efficiencies between the liquid FS (wet digestion) and dry FS (dry digestion) as presented and discussed in section 4.3.2.1. Based on literature, it was expected that dry digestion (reactors with dry FS samples) will result in lower organic matter removal efficiencies which was not observed. This is also contrary to the trend

observed in untreated samples where the FS samples from wet containment facilities (liquid FS samples) appeared to be more stabilized than the ones from the dry containment facilities (dry FS samples) (see tables 5-1, 5-2 and Figure 4-10 (d)). The results imply that TS concentration (moisture content) is not the main factor that influence anaerobic digestion/ difference in the level of stabilization between FS from wet and dry containment facilities. Other factors such as mixing (containment contents are not mixed), toxicity, storage time and temperature (which varies from one containment to the other) need to be taken into account as well (Van Eekert *et al.*, 2019; Shaw and Dorea, 2021; Doku, 2002). Nonetheless, the addition of water to FS (increase in moisture content) has been reported to increase or improve the rate of stabilization (Van Eekert *et al.*, 2019; Couderc *et al.*, 2008).

Further, pH values of the samples remained within the range which has been reported by Forbis-Stokes *et al.*, (2016) to be suitable for anaerobic digestion (6.3 – 7.8) as shown in Figure 4-15. pH is known to influence enzymatic activity since enzymes are only active within a given narrow pH range. The methanogenic bacteria is said to perform well within the pH range observed in this study but optimally at 7.0 – 7.2 (Jiunn *et al.*, 1997). Based on this, pH had no effect on the anaerobic digestion of the FS samples in this study. As stated earlier, the EC values were used to predict the TAN concentrations in the samples which was used to assess ammonia inhibition during the anaerobic stabilization process. The prediction revealed that the dry FS samples had TAN concentrations possibly within the tolerable limits for anaerobic digestion (<1.5 g/L) as reported by Zuo *et al.*, (2021). On the contrary, the calculated TAN concentration for the liquid samples in this study were above the limit reported to possibly cause inhibition and toxicity (>3g/L) of anaerobic digestion (Zuo *et al.*, 2021). Despite the higher TAN concentration, the liquid samples were still able to undergo anaerobic digestion in this study (as confirmed by biogas production from the reactors) probably because the FS was stored for a long time under anaerobic conditions and the microbes were already acclimatized to the high TAN conditions (Colón, *et al.*, 2015). Sam, *et al.* (2022) also reported that FS samples with TAN concentration above 1.5g/L were able to under anaerobic digestion though the authors attributed the low biogas production of the samples to possible inhibition and higher TS concentration. The predicted high TAN concentration (>3g/L) in liquid FS is most likely due to the accumulation of urine in wet containment facilities (which are fully

lined) unlike in dry pit latrines (which are unlined or partially lined) where the urine percolates to the ground (Forbis *et al.*, 2016; Ward *et al.*, 2019). Further, there were no differences observed between the pH and EC values of the samples during and after the anaerobic digestion which was an indication of process stability.

Generally, the results showed that the inherent characteristics of FS samples (in this case TS, TAN/EC and pH) favour anaerobic digestion. It was also clear from the results that FS is able to undergo anaerobic digestion on its own (without addition of inoculum or nutrient additives) and has intrinsic methanogenic activity (confirmed through the production of biogas) which is in agreement with the results reported by Van Eekert *et al.* (2019).

5.5.2.3 Changes in Stabilization and Dewatering Performance with Anaerobic Digestion

The changes observed in the stabilization (SOUR, BOD/COD ratio and VS/TS ratio) and dewatering performance (CST(s) and normalized CST) with anaerobic digestion varied in this study. As shown in Figure 4-18, the dewatering performance of both the liquid and dry FS samples improved with anaerobic digestion. It can be seen from Figure 4-18(a) that both the dry and liquid FS samples were initially characterized with poor dewatering (high normalized CST at day 0) than after anaerobic digestion (low normalized CST at day 60). It can also be seen from the box plots in Figure 4-18(a) that the variability of dewatering performance among the FS samples narrowed at the end of the anaerobic digestion process. Sam, *et al.,* (2022) also reported a reduction in the CST (s) with anaerobic storage for fresh excreta sample (mixture of feces and urine) innoculated with pit latrine sludge. Ward, *et al*. (2022) also observed a change in the CST (s) with anaerobic storage, however, the trend was not consitent as it reduced and increased again after some time. This decrease and increase trend in CST (s) with anaerobic digestion was also observed in four liquid FS samples in this study (see Figure 4-18(b)). This behavior could be attributed to changes in particle size distribution (i.e. reduction and release of smaller particles) during anaerobic digestion which has been reported to control dewatering performance of FS (Ward *et al.,* submitted). Although a consistent reduction in CST (s) was observed with anaerobic digestion in the majority of the FS samples in this study, this trend can not be generalized as it is dependent on the characteristics of a particular sample (which varies widely) e.g. presence of toxic or inhibitory substances which can deter

continous breakdown/ digestion of smaller particles in the sludge resulting in poor dewatering (Ward *et al.*, submitted). The presence/ accumulation of smaller particles in a sludge sample has been associated with poor dewatering in both FS and waste water Sludge(Christensen *et al.*, 2015; Ward *et al.*, submitted).

5.5.2.4 Correlation between Dewatering and Stabilization of Anaerobically Digested Sludge

In this study, a reduction was observed in stabilization of the samples with anaerobic digestion. However, it was not consistent in all the methods as can be seen in Figure 4-18. Overall, the reduction in stabilization was more evident in the SOUR as compared to the other methods. Evaluation of the stabilization results (after the anaerobic digestion process) against the stability indices identified from literature showed that all the FS samples become stabilized after 60 days of being kept under controlled anaerobic conditions when evaluated using the SOUR. This reduction in SOUR after 60 days of anaerobic digestion is an indication of less microbial activity which means the organic matter in the FS samples become stabilized(Sánchez *et al.*, 2006). It was also observed that the variability of the SOUR narrowed at day 60 as compared to what it was before the FS samples underwent anaerobic digestion. The median SOUR for the liquid and dry FS samples after the stabilization process were 1.43 and 3.19 g O_2/Kg VS/hr respectively (the initial median values were 19.89 and 31.87g O_2/Kg) which were closer to the SOUR stability index of 2g O_2/Kg VS/hr for stabilized wastewater sludge and composts. Based on these results, SOUR seemed to be the most a suitable method for measuring FS stabilization and the stability index for wastewater sludge and compost can be applied to FS samples as well.

When it comes to the VS/TS ratio it was observed that the variability increased after anaerobic digestion (which was not expected) especially in the solid FS samples. Leite, *et al.* (2013) also reported an increase in the variability of the VS/TS ratio of thickened secondary wastewater sludge after anaerobic digestion. This can be attributed to the differences in the concentrations of sand and grit (inorganic matter) in the samples which influenced the potential VS reduction in each sample respectively (Duan *et al.*, 2016). Despite this, the median VS/TS ratio for both liquid and dry FS samples reduced after the anaerobic digestion to below 0.45 which was less than the stability index for waste water sludge (i.e. 0.5). Based on these results, it can be concluded that the VS/TS ratio can be used to measure FS stabilization,

however, the TS results must be normalized for sand and grit content in order to reduce the variability. Measuring sand content in addition to VS and TS does add extra measurement complexity to the method, however, this would be possible to do in most laboratories worldwide. Due the gaps highlighted, it could not be verified if the sludge stability index for VS/TS ratio of 0.5 is applicable to FS as well.

In this study, it was expected that the BOD/COD ratio for all the samples would reduce with anaerobic digestion in line with what was observed in the SOUR and VS/TS ratio. Instead of reducing, the BOD/COD ratio of the dry FS samples increased after the anaerobic digestion process. At the same time the ratio for liquid FS samples remained almost unchanged. The different results between the liquid and dry FS samples can be attributed to the initial concentrations of BOD and COD of the respective samples. High initial concentrations of BOD and COD can result in an increase in the BOD/COD ratio with longer anaerobic digestion retention time (Borglin *et al.*, 2012). Based on these results, it can be concluded that the BOD/COD ratio as a measure of stabilization provides inconsistent results which makes it difficult to define stabilization.

The trends shown by dewatering performance (CST normalized) of the FS samples during anaerobic digestion was correlated to that shown by the results of FS stabilization i.e. SOUR and VS/TS Ratio. A reduction was observed in both normalized CST and FS stabilization (SOUR and VS/TS ratio) with anaerobic digestion. However, the correlation was more strongly reflected in the SOUR ($R=0.78$, $p<.05$) than the VS/TS ratio ($R=0.17$, $p=.49$). These associations were also reflected in the undigested FS samples where the dry FS samples which had the worst dewatering performance (high normalized CST) had the highest SOUR and VS/TS ratio as compared to the liquid FS samples (Figure 4-11). Similar to what was observed in the field samples, BOD/COD ratio was negatively correlated to CST even in the digested samples (Figure 4-20). Ward *et. al* (submitted) also reported a positive correlation between the indicators of stabilization (VSS/TSS and C/N ratio) with dewatering performance though the authors did not observed a consistent improvement in dewatering performance with controlled anaerobic digestion (which is contrary to what was observed in this study). Generally these results validate the initial hypothesis that dewatering performance of FS improves with level of stabilization (as indicated by the normalized CST, SOUR and VS/TS ratio results),

however, further research on more types of FS is needed to validate these findings. This is in agreement with what practitioners in the field and other researchers have been observing that more stabilized FS is easier to dewater than fresh FS (Cofie *et al.*, 2006 ; Ward *et al.*, 2019; Ward *et al.*, 2021; Sam *et al.*, 2022).

5.6 Implications of the Study Findings

In this study different methods that can be used to measure FS stabilization were evaluated to determine the best method. The results revealed that SOUR (a respirometry method) and VS/TS ratio (with the recommendation to normalize for sand content) can be applied to measure the level of FS stabilization. The application of the methods also revealed minimal differences in the level of stabilization between FS samples based on sludge age. This implies that time in containment is not the main determinant for level of stabilization, but other factors such as unfavourable conditions for biological digestion in onsite containment facilities deter FS from becoming fully stabilized despite the longer retention times.

The application of SOUR to measure FS stabilization is an indication that micro-organisms present in FS from OSS containment facilities may not be entirely anaerobic. Understanding the stabilization processes happening in OSS containment facilities should consider determining the availability of both anaerobic and aerobic microbes. In addition, despite the differences in the initial characteristics between FS and wastewater, organic matter in both types of substrates is degraded during biological digestion processes resulting in a stabilized sample with similar characteristics in terms of biological activity e.g. the SOUR of stabilized FS samples in this study was comparable to that for stabilized wastewater sludge. This implies that the well-researched knowledge on stabilization of wastewater Sludge(especially through the use of microbial activity parameters) can be transferred to FS to enhance development of methods for measuring FS stabilization.

Further, BOD/COD ratio was not found to be a suitable method to measure FS stabilization and did not fit in the well-known knowledge for measuring stabilization of wastewater sludge and composts. Therefore, practitioners should reconsider the perception that the low BOD/COD ratio for most FS samples (< 0.1) is an indication of FS stabilization. This in most cases is an indication of toxicity (the presence of high concentration of organics as COD in a sample that inhibit the activity of microbes

responsible for biological degradation) which is similar to the situation with industrial wastewater (Samudro and Mangkoedihardjo, 2010).

The ability of FS in this study to undergo anaerobic stabilization on its own without the addition of nutrients or innocula was an indication that FS from OSS containment facilities is not fully stabilized despite the long retention times at containment. It was also an indication that FS contains microorganisms, biodegradable organic matter and physical chemical characteristics that favour anaerobic digestion at optimal controlled conditions. Thus, practitioners should consider that FS can still undergo further stabilization even after the longer storage time (several years) in onsite containments.

Lastly, the associations between anaerobic digestion and improvement in dewatering performance as well as stabilization was an indication that application of a biological stabilization step at treatment before FS dewatering can be beneficial. In addition practitioners can predict the dewatering performance of FS at treatment by determining its level of stabilization using cheap, easy and rapid methods such as SOUR which only take about 30 minutes to be performed. However, this needs to be further investigated to come up with linear models or relationships between metrics of dewatering (e.g. CST) and stabilization indicators (e.g. SOUR). With further research, there is a possibility the SOUR method can be modified into a field based or online measurement method at treatment since it uses a probe with an oxygen sensor.

5.7 Chapter Summary

This chapter discussed the results and findings from this study. The findings revealed that methods for measuring stabilization (e.g. VS/TS ratio, SOUR and BMP) which have been well applied in the wastewater and compost sector can also be applied to measure FS stabilization. However, the SOUR seemed to be the most suitable among all the methods that where evaluated. The issues identified with the other methods such as the BOD/COD ratio (which increased with anaerobic digestion) and the need to further investigate the possible influence of the sand and grit content on the VS/TS ratio were also discussed. The chapter also revealed that there is a relationship between the FS stabilization and dewatering performance. It was discussed that the level of FS stabilization and the dewatering performance improved with anaerobic digestion. Further, aspects on the ability of FS to undergo further anaerobic digestion and the possible influence from its inherent physico-chemical characteristics were also

discussed. The results revealed the physico-chemical characteristics favour FS to be stabilized anaerobically, however, there is a possibility that they affect the rate especially at containment where the conditions are not controlled. The next chapter presents the conclusions and recommendations of the study

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.0 Introduction

This study was aimed at determining how to measure FS stabilization and how it is related to dewatering performance. This chapter presents the conclusions on each of the research objectives of the study as presented in chapter 1. The chapter ends with recommendations based on the research findings as well as future research work.

6.1 Conclusions

The first objective of the research was to evaluate how to determine/ measure the level of FS stabilization by using rapid and low cost methods. It was found that there are many methods (to a tune of fourteen) that have been widely applied in stabilization studies of wastewater sludge and composts, however, none of these have been applied in the study of FS stabilization. It was also established that these methods can be categorized into three groups' i.e. microbial activity, elemental composition and surrogate methods. The majority of the methods in the elemental composition and microbial activity were determined to have a well-defined index or criteria for evaluating whether a particular sludge is stabilized or not. Screening of all the methods revealed gaps and weaknesses in some methods especially those under the surrogate category. At the same time, the majority of methods under the microbial activity and elemental composition had good attributes which made them to be suitable to be adapted to measure FS stabilization. Performance evaluation of the application of the five methods that were selected to measure stabilization of different types of FS (i.e. fresh excreta, septic tank sludge, wet and dry pit latrine sludge) through laboratory experiments and tests revealed that the VS/TS ratio, BMP and SOUR be applied to measure FS. The disadvantage with the BMP is that it takes long to be completed and the VS/TS ratio requires further investigations to determine the influence of grit and sand content on its performance as an indicator of FS stabilization. Thus, the SOUR was the best evaluated method because it is a cheap and rapid method (i.e. it can be performed in less than 30minutes) and it gave consistent stabilization results which were well correlated with dewatering performance (CST). The SOUR also gave results that fit into the stability index defined for wastewater and composts (i.e. SOUR <= 2g O2/Kg VS/hr indicates a well stabilized sample). Based on these results it was

concluded that FS stabilization can easily be measured using SOUR which is a method under the microbial activity category. In addition, a FS sample with SOUR value of as low as 2g O2/Kg VS/hr can be said to be well stabilized.

The second objective was to determine if stabilization of FS is related to dewatering performance. This was done by assessing the differences in stabilization, organic matter content and dewatering performance of different types of untreated and treated FS (FS was subjected to treatment under controlled anaerobic conditions). The results showed differences in the organic matter content (BOD and VS), stabilization (SOUR) and dewatering (CST normalized) among the different types of FS (i.e. fresh excreta, liquid, dry FS and FS with different sludge age). Generally, the dry FS samples (which had high BOD, VS and SOUR) took longer to dewater (high normalized CST) as compared to the liquid FS samples (which had low BOD, VS and SOUR). Further, a reduction was observed in organic matter content (COD, BOD and VS), stabilization (VS/TS ratio and SOUR) and dewatering performance (normalized CST) with controlled anaerobic digestion in all the types of FS samples. Based on these results it was concluded that a positive relationship exists between FS stabilization and dewatering performance. This meant that a more stabilized FS dewaters faster (low normalized CST) than a less stabilized one. This is well collaborated to what other researchers and practitioners have been observing in the field.

Lastly, on the ability of FS to undergo further anaerobic digestion, it was observed that all the samples were able to be digested under controlled anaerobic conditions. It was therefore concluded that the intrinsic physico-chemical characteristics (i.e. organic matter content, pH, EC and TS concentration) of FS favour anaerobic digestion. However, there is a possibility that factors such as TS concentration, EC and pH affect the rate of digestion inside the onsite containment facilities where the conditions are not controlled.

6.2 Recommendations

The following are the recommendations based on the study findings:

i. The evaluation of the methods for measuring stabilization revealed that VS/TS ratio and the SOUR can be applied as cheap and rapid methods for measuring FS stabilization. This is the first time that both methods have been applied with the core purpose of determining if a FS sample can be categorized as stabilized

or not. However, the work done under this study was limited to a small sample size collected from containment facilities located Lusaka city. Based on the fact that characteristics of FS can vary widely from one location to another and from one containment to another, it is therefore premature to generalize these findings. It is therefore important that further research on measuring FS stabilization using these methods is conducted to refine and develop these methods further to determine reproducibility and reliability. Some suggested further method development include the need to determine the grit and sand content of the sludge when it comes to the VS/TS ratio. For the SOUR there is need to further develop the method by further investigating the optimum aeration time, dilution factors and the need to develop positive and negative controls to evaluate the method performance.

ii. The results also revealed positive correlations between the stabilization and dewatering performance of FS. It can therefore be recommended that FS must be subjected to a biological stabilization stage at treatment in order to improve its dewatering performance. It also should be possible for practitioners in the field to measure the stabilization of FS using rapid and low cost methods such as the SOUR and at the same time be able to predict its dewatering performance. However, this needs to be further investigated to determine a linear model or relationships between metrics of dewatering (e.g. CST) and stabilization indicators (e.g. SOUR) to make these predictions accurate.

iii. The results for BOD/COD indicated that the method is not a suitable indicator of FS stabilization. Therefore, practitioners should reconsider the perception that the low BOD/COD ratio for most FS samples (< 0.1) is an indication of FS stabilization.

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APPENDICES

Appendix 1: ETHICAL CLEARANCE

A. Ethical Clearance Certificate by Research Sponsors

Eawag
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eawag aquatic research

Ethical Review Application for Minimal Risk Projects Involving Human Subjects

This form is for use as described in Policy Directive 16-09. Eawag researchers who are planning to engage in minimal risk projects involving human subjects are ***strongly encouraged*** to complete relevant training through workshops or e-learning modules (e.g., the Training and Resources in Research Ethics Evaluation program, http://elearning.trree.org/, or the U.S. NIH Protecting Human Research Participants program, https://phrp.nihtraining.com).

This form is intended for use ***only*** for projects that pose ***minimal risk*** to the human subjects involved in the project and/or to Eawag as an institution.[1] Projects are considered to pose minimal risks if the "NO" box is checked for ALL of the following questions:

		YES	NO
1.	Does the project extend beyond observation of participants conducting normal activities in daily life or sport (e.g., playing games, using apps, performing exercises to test strength, flexibility and/or speed)?		X
2.	Does the project involve children or any other participants for whom a third party (e.g., a parent or guardian) would need to grant permission for participation?		X
3.	Does the project involve any participants who are likely to be coerced or otherwise unduly influenced into participation (e.g., prisoners)?		X
4.	Does the project involve the collection of any data and/or opinions that are NOT anonymized?		X
5.	Would the intended use of the anonymized data and/or opinions to be collected in the project allow the identification of individual participants?		X
6.	Does the project involve collection of sensitive personal data and/or information (e.g., regarding health, sexual orientation, ethnicity, or political/religious conviction) that must be protected?		X
7.	Does the project involve collection of sensitive personal data and/or information (e.g., child or domestic abuse, sexual functioning) that is likely to cause emotional stress or discomfort?		X
8.	Does the project involve any conflicts of interest?		X
9.	Does the project pose any obvious reputational and/or legal risks?		X
10.	Does the project involve the use of any sensitive or restricted technology?		X
11.	Do *both* the Director and Deputy Director of Eawag have a conflict of interest (e.g., direct engagement) with the project?		X

IF **ANY** BOXES ARE CHECKED "YES", DO **NOT** COMPLETE THIS FORM.

Date of application	28 August 2019
Name of applicant (PI)	Linda Strande
Name and date of completion of Human Subject Research Training program completed (if applicable)[2]	
Name of supervisor (if needed)[3]	
Department(s) of applicant and supervisor	Sandec

[1] This form is NOT intended to replace any *external* review of ethics *required* by an external agency (e.g., funding agency, international governmental agency, journal, etc.). This form may be replaced by an ethical review application approved by a Swiss project partner organization (e.g., ETH Zurich, EPFL, a Swiss Cantonal University or University of Applied Sciences).
[2] Completing such a course is ***highly*** recommended.
[3] Not need if the applicant is eligible to submit proposals from Eawag (e.g., tenured or tenure-track researcher)

Project title	Faecal Sludge Quantities and Qualities (Q&Q) in Lusaka
Eawag PSP Number	Not yet assigned
Source (or anticipated source) of funding	GIZ
Project period (or anticipated project period)	August – December 2019
Brief project description (highlighting potential ethical issues if any)	Field testing of method to estimate Q&Qs of faecal sludge on a city-wide scale. Samples taken from pit latrines, will be used to make city-wide estimations.

The applicant hereby certifies that the project "Faecal Sludge Quantities and Qualities (Q&Q) in Lusaka" meets the criteria stated above for minimal risk

Linda Strande		28.08.2019
Applicant Name	Signature	Date

The supervisor (if needed) and Department Head hereby confirm that this project meets the criteria stated above for minimal risk

NA		
Supervisor Name	Signature	Date

Christoph Lüthi		30.08.2019
Department Head Name	Signature	Date

If (***and only if***) the applicant or supervisor is a Department Head, then the signature of the Directorate member who is the Coach[4] for that Department is also needed to confirm that this project meets the criteria stated above for minimal risk

NA		
Name of Directorate Member	Signature	Date

Approved for the Eawag Directorate by:

Rik Eggen		2.9.19
Janet Hering or Rik Eggen	Signature	Date

[4] If the Coach of the Department has a conflict of interest, then another member of the Directorate can sign for the Coach.

B. Receipt for Payment for Ethical Clearance

Appendix 2: LABORATORAY STANDARD OPERATING PROCEDURES

DE-HYDROGENASE ACTIVITY (DHA) TEST

Description

This protocol describes how to measure the Dehydrogenase Activity (DHA) for faecal sludge samples. Dehydrogenases are oxidation-reduction enzymes which participate in the transport of electrons from organic substrate to final electron acceptors (e.g. oxygen in aerobic conditions) during the biodegradation process. In aerobic metabolism the terminal electron acceptor is oxygen, thus the rate of oxygen consumption reflects overall dehydrogenase activity (DHA). Various tetrazolium salts which compete with oxygen in the electron transport system (ETS) have been used to measure DHA. These include 2,3,5-triphenyltetrazolium chloride (TTC) and 2-@-iodophenyl)-3- (p-nitrophenyl)-5-phenyltetrazolium chloride (INT. These salts are water soluble and colorless in their oxidized state and are converted into water insoluble, intensely colored formazans under mild reducing conditions. Thus they can be used to measure the metabolic rate organic substrates such as waste water and activated sludge.

Thus, the DHA test assay is based on the reduction of a colorless tetrazolium salt (e.g. triphenyltetrazolium) to a colored solution (INT) by the oxidative effect of the dehydrogenase enzymes and spectrophotometric measurement of the color intensity. High INT Formazan crystal formation indicate high microbial activity and high organic matter content; low INT Formazan crystal formation indicate low microbial activity and low organic matter content.

DHA is reported as absorbance of the colored INT solution.

Required Materials

- Diluted sample (to the required VS concentration 2.5 – 5 g/L)
- 50ml centrifuge tubes
- Centrifuge
- Spectrophotometer
- 5ml and 2ml pipette tips
- Water Bath
- Test tube rack

- Scale
- DO meter
- Reagents
  - 2,3,5-triphenyltetrazolium chloride (TTC)
  - 37% Formalin (formaldehyde)
  - Ethanol

Method

A. Reagent preparation:

2,3,5-triphenyltetrazolium chloride (TTC) 0.2% Solution

To a 100ml flask add

- 50ml distilled water
- 0.2g of TTC powder

Allow the TTC to fully dissolve and dilute to 100ml mark.

B. Calibration:

- ***Oxygen probe*** — Perform a two point calibration of the DO probe following the manufacturer's procedure as detailed in the manual.
- ***Spectrophotometer*** – perform a one point calibration for the spectrophotometer using the prepared blank. This is to zero the spectrophotometer before taking the absorbance readings for the formazan solution.

C. Analysis Procedure:

*****QUALITY CONTROL*** Do triplicate measurements for all samples**

1. Determine the Votile Solids concentration of the samples in g/L
2. Dilute all samples to have a comparable VS concentration in the range of 2.5 – 5g/L (total volume of atleast 100ml)
3. Check the DO concentration and pH of all the samples before performing the test (DO should be close to Zero and pH between 7 – 8)
4. To 50ml centrifuge tubes add:
   - 5ml of sample
   - 1.5 ml of 0.2% TTC solution
5. Place the test tubes in a water bath at 37℃ for 30mins
6. Remove the test tubes from the water bath and add 0.2ml of 37% formalin to fix the reaction
7. Centrifuge the mixture at 3500g for 6 minutes
8. Discard the supernatant (be careful not to resuspend the pellet)
9. Extract or dissolve the formazan crystals by adding 5ml ethanol to the test tubes. The pellet was resuspended by shaking for 15 s and the formazan was extracted for 30 min in darkness at room temperature.
10. Centrifuge the mixture at 3500g for 4 minutes
11. Measure the optical density (i.e. absorbance) of the supernatant in the spectrophotometer at 485nm and record the results in the lab book.

Considerations/ Quality Control

- Always insure that the DO concentration sample is closer to 0 mg/L (if DO concentration is >1mg O_2/L Deoxygenate the sample by purging with hydrogen gas) and pH is between 7 – 8 (if pH is outside the range adjust the pH by adding 10% (w/v) NaOH solution)
- Ensure to zero the spectrophometer using a blank prepared by mixing 5ml of distilled water with 1.5ml of 0.2% TTC solution, 0.2ml of 37% formalin and 5ml of ethanol.

Lab notebook layout

DHA TEST (PHOTOMETRIC METHOD)							
Lab ID	Name of Analyzer	Tube ID	Dilution Factor	Replicate ID	Absorbance		Comments
MT01	Kapanda		1	1	0.771		Error made removed from results
MT01	Kapanda		1	2	0.256		
KM02	Kapanda		1	1	0.698		
KM02	Kapanda		1	2	0.75		
MT03	Kapanda		5	2	0.853		
MT03	Kapanda		5	3	0.367		
HS04	Kapanda		1	1	0.272		
HS04	Kapanda		1	2	0.388		
CH05	Kapanda		1	1	0.098		
CH05	Kapanda		1	2	0.11		
FS06	Kapanda		1	1	1.129		
FS06	Kapanda		1	2	0.852		

Calculations

No calculations in this test.

References/Links

MEWS SOP, “Total Solids and Volatile Solids”, 2019. Lusaka

A. SPECIFIC OXYGEN UPTAKE RATE (SOUR)

Description

This protocol describes how to measure the specific oxygen uptake rate (SOUR) of faecal sludge samples. Microorganisms in sewage sludge use oxygen as they consume organic matter. The level of microbial activity in a sludge sample is indicated by the microorganisms' dissolved oxygen uptake rate. High dissolved oxygen uptake rates indicate high microbial activity and high organic matter content; low oxygen uptake rates indicate low microbial activity and low organic matter content. SOUR describes the amount of dissolved oxygen used by the microorganisms to consume one kilogram of organic matter and is reported as mg/l of oxygen used per kilogram of organic material per hour. The method consists of initially increasing concentration of dissolved oxygen (DO) in the sample by aeration. An aliquot of the sample is placed in a biological oxygen demand (BOD) bottle and kept well mixed. Using an oxygen-sensing probe, DO is recorded periodically (at 30 minutes intervals) over a 15-minute period, or until DO becomes rate-limiting. The oxygen consumption rate is calculated as the absolute value of the slope of the linear portion of the DO versus time curve. The SOUR is then obtained by dividing the oxygen consumption rate by volatile solids concentration of the sample.

SOUR is reported in units of mg O_2/ Kg VS/hr.

Required Materials

- Diluted sample (to the required VS concentration 2.5 – 5 g/L)
- Magnetic stirrer with stirring bar
- 300ml BOD Bottles
- DO meter with probe adapted to BOD bottle
- Oxygen sensitive membrane
- Stop watch

Method

D. Reagent preparation:

No Reagents are required for this test.

E. Calibration:

- ***Oxygen probe*** — Perform a two point calibration of the DO probe following the manufacturer's procedure as detailed in the manual. When performing multiple analyses, calibration must be checked before each analysis against the sample of reagent water of known DO concentration or reagent water with Zero DO concentration (prepared using the zero DO tablets).

F. Analysis Procedure:

*****QUALITY CONTROL*** Do duplicate measurements for at least one sample per test batch.**

12. Determine the Votile Solids concentration of the samples in g/L
13. Dilute all samples to have a comparable VS concentration in the range of 2.5 – 5g/L (total volume of atleast 500ml)
14. Continuosly aerate the diluted samples using a vacuum pump for about 3 – 5 hours.
15. Fill a 300-mL BOD bottle with the aerated sample immediately after aeration to overflowing.
16. Place the BOD bottle on a magneti stirrer with magnetic stir bar
17. Immediately place the DO sensor probe in the BOD bottle making sure the adapter (on the probe) provides a good seal and switch on the magnetic stirrer.
18. Record the initial DO concentration of the sample as quickly as possible (if DO >= 5mg/l, proceeed with the test and if DO < 5mg/l aerate the sample for about 5mins and repeat from step 4)
19. Continue to record the DO concentration in the bottle every 30 seconds for approximately 10-15 minutes (i.e. DO concentration as a function of time). The actual time required depends on the rate of oxygen depletion. Allow sufficient time to get at least 2mg/L DO difference between start and finish of the test. Be sure to record both the DO measurement and the time.
20. At the completion of the test, dump aqueous content from BOD bottle back into the appropriately marked container. Wash BOD bottle and perform the test on the next sample.

Considerations/ Quality Control

- Always insure that the staring or initial DO concentration of the aerated sample is greater than or equal to 5mg/L
- Ensure there are no large visible air bubbles in the BOD bottle after inserting the Oxygen Probe
- Always perform at least one duplicate per batch of SOUR analyses.

1.

Lab notebook layout

Time (S)	Sample DO (mg/l)					
	CH05	HS04	FS06	MT01	MT03	KM02
0	5.29	4.13	5.58	5.26	5.61	5.74
30	4.32	3.44	4.61	4.04	4.77	4.71
60	4.19	2.99	4.1	3.73	3.49	4.53
90	4.1	2.61	3.7	3.49	2.86	4.38
120	4.03	2.3	3.36	3.27	2.4	4.24
150	3.95	2.03	3.06	3.04		4.1

Calculations

- Construct a plot of DO (mg/L) vs. time (s)
- Draw the best straight lines through points
- Calculate the slope or gradient of the best fit line. The slope is the oxygen uptake rate of the sample expressed in the unit's mg O_2/L/hr.

2. ***OUR (mg O_2/L/hr) = mg O_2/L/S x 60s/min x 60min/hour***

3.

- Calculate the Specific Oxygen Uptake Rate (SOUR). The specific oxygen uptake rate (SOUR) can now be calculated as follows:

4. ***SOUR (mg O_2/Kg VS/hr)*** = OUR/ VS

5. Where VS = kg/L

6. OUR = mg O_2/L/hr

References/Links

MEWS SOP, "Total Solids and Volatile Solids", 2019. Lusaka

United States Environmental Protection Agency (EPA), Method 1683 (Specific Oxygen Uptake Rate in Biosolids), 2001.

B. BIOCHEMICAL OXYGEN DEMAND (BOD5) – MEMBRANE ELECTRODE METHOD

Description

This protocol describes how to measure the five days Biochemical Oxygen Demand (BOD_5) of faecal sludge samples. The BOD_5 is the amount of dissolved oxygen (DO) required for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic materials such as sulphates and ferrous iron. It may also be used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor .The method consists of incubating a sample in a full airtight bottle for five days under specific conditions. Dissolved oxygen is measured initially and after incubation as in 4.2.3. The BOD is computed from difference between initial and final DO concentration.

BOD_5 is reported in units of mg O_2/L.

Required Materials

- Diluted sample
- Reagents:

- Phosphate buffer solution with PH of 7.2.
- Magnesium sulphate solution
- Calcium chloride solution
- Ferric chloride
- Glucose- glutamic acid solution
- Ammonium chloride solution
- Dilution water (use demineralized or distilled water for making sample dilutions).

- Micropipette and tips (5 mL and 2 mL)
- 300ml BOD Bottles
- DO meter.
- Air incubator or water bath adjustable to 20 + 1 oC
- Oxygen sensitive membrane

Method

G. Reagent preparation:

Phosphate buffer solution with PH of 7.2

To a 1000 mL glass bottle add

- 500 mL distilled water
- Dissolve 8.5g KH_2PO_4, 33.4g $Na_2HP_4.7H_20$, and 1.7g NH_4CI in the distilled water.
- Dilute this solution to 1L.
- The PH of this solution should be 7.2 without further adjustment.

**Alternatively, dissolve 42.5g KH_2PO_4 or 54.3g K_2HPO_4 in about 700 mL distilled water. Adjust pH to 7.2 with 30% NaOH and dilute to 1 L.

Magnesium sulphate solution

- Dissolve 22.5g of $MgSO_4.7H_2O$ in distilled water and dilute to 1L.

Calcium chloride solution

- Dissolve 27.5g $CaCI_2$ in distilled water and dilute to 1L.

Ferric chloride

- Dissolve 0.25g $FeCI_2.6H2O$ in distilled water and dilute to 1L

Glucose- glutamic acid solution

** Remember to dry reagent grade glucose and glutamic acid at 103^oC for 1 hour. The solution must be freshly prepared immediately before use.

- Add 150mg glucose and 150mg glutamic acid to distilled water and dilute to 1L.

Ammonium chloride solution

- Dissolve 1.15g NH4CI in about 500ml of distilled water.
- Adjust to PH 7.2 with sodium chloride solution and dilute to 1L

Dilution water

- Place a desired volume of water in a suitable bottle
- Add 1ml of each of the phosphate buffer, $MgSO_4$.7H2O and $FeCI_2$.6H2 solution per liter of water.
- Use dilution water at temperature of 20^oC
- Saturate with DO by aerating with organic free filtered air or rigorous shaking in a partially filled bottle.

H. Calibration:

1. For every batch of BOD test, include a bottle of dilution water as blanks to check the quality of the dilution water. Determine initial and final DO of the blanks (the DO uptake should not be more than 0.2 mg/L and preferably not more than 0.1 mg/L). Discard all dilution water having a DO uptake greater than 0.2 mg/L
2. Periodically check dilution water quality and analytical technique by making BOD measurements on a mixture of 150 mg glucose/L and 150 mg glutamic acid/L as a ‘‘standard’’ check solution. The average 5-d BOD would be 198 mg/L with a standard deviation of 30.5 mg/L.

I. Sample Analysis:

*****QUALITY CONTROL*** Do triplicate measurements for all samples.**

1. Place desired volume of water in a suitable bottle and add 1 mL each of phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$ solutions/L of water (estimate volume of dilution water required based on number of BOD bottles in the batch test). Do not store prepared dilution water for more than 24 h.
2. Check pH of all samples before testing unless previous experience indicates that pH is within the acceptable range i.e between 6.0 and 8.5 (if outside range neutralize the sample with a solution of sulpuric and sodium hydroxide while avoiding sample dilution of more than 0.5%)
3. Sample temperature adjustment—Bring samples to 20 ± 1°C before making dilutions.
4. *Dilution technique*:
   - FS samples have high organics content – make an initial dilution of 1:40 (i.e. measure 25ml of raw FS sample and dilute in a 1000ml lab glass bottle with distilled water) to make waste water.
   - Make several dilutions of diluted sample (with dilution water) that will result in a residual DO of atleast 1 mg/L and a DO uptake of at least 2 mg/L after a 5-d incubation (at least three dilutions e.g. 1:50, 1:25 and 1:10). You can skip this if previous experince.
   - Prepare dilutions either in graduated cylinders or volumetric glassware, and then transfer to BOD bottles.
5. Decanting dilution mixture into a 300ml BOD bottle.

6. Determine initial DO using a calibrated DO meter immediately after filling the BOD bottle with diluted sample and replace any displaced contents with sample dilution to fill the bottle.
7. Stopper tightly, water-seal, and incubate for 5 d at 20°C.
8. After 5 d incubation determine DO in sample dilutions and the blanks.

Considerations/Quality Control

- Ensure there is no air entrained (air bubble) in the sealed BOD bottles before incubation.
- Always include 3 blanks with every batch of BOD analyses.

Lab notebook layout

5 Day Biochemical Oxygen Demand (BOD)

Date of Incubation_____ Time:_____
Date of Reading_____ Time:_____

Notes
Incubation temp. = 20 ° C ± 1 Incubation period = 5 days

Lab No.	Replicate No.	Name Analyser	DF	P (Vol. fraction Sample)	D_1	D_2	Actual (D1 - D2)	BOD (mgO_2/L)	Comment
Blk	1	Kapanda	1	1	5.41	4.95	0.46	0.46	Do uptake > 0.2 (reject results)
PL 1	1	Kapanda	50	0.020	5.32	1.44	3.88	194	
PL 1	1	Kapanda	25	0.040	5.28	1.36	3.92	98	
PL 1	1	Kapanda	10	0.100	5.26	0.88	4.38	43.8	Residual DO less than 1 (discard)
							0	#DIV/0!	
							0	#DIV/0!	
							0	#DIV/0!	
							0	#DIV/0!	
							0	#DIV/0!	
							0	#DIV/0!	
							0	#DIV/0!	
							0	#DIV/0!	
							0	#DIV/0!	

Calculations

$$BOD\ (mg\ O_2\ /L) = D_1 - D_2 / P$$

Where: D1 = DO of diluted sample immediately after preparation, mg/L

D2 = DO of diluted sample after 5 days incubation at 20 o C

P = Decimal volumetric fraction of sample used

References/Links

LWSC SOP, “Biochemical Oxygen Demand”, 2013. Lusaka

American Public Health Association (APHA)/American Water Works Association (AWWA)/Water Environment Federation (WEF) 2005 Standard Methods for the Examination of Water and Wastewater, Washington DC, USA.

Appendix 3: Stabilization Methods Selection Matrix

Decision Matrix for Methods of Measuring Stabilization

Project Title: Method Development for Measuring Faecal Sludge Stabilization and its Relation to Dewatering

Method Attributes **1.** local availability of the test method requirements such as lab equipment, consumables such as reagents; **2.** ability of the test method to be replicated or applied by other researchers through provision of reproducible and consistent results; and **3.** demostrated ability to be applied in measuring biodegradability of sludges from the waste water sector as well as feacal sludge.		**Method of Measuring Stabilization**																											
			Biomethane Potential (BMP)		**VS/ TS Ratio**		**BOD/COD Ratio**		**TOC**		**Carbon/ Nitrogen Ratio**		**Dynamic Respiration Index - DRI**		**Static Respiration Index - SRI**		**Specific Oxygen Uptake Rate - SOUR**		**Dehydrogenase Activity - DHA**		**Esterase Activity - EA**		**Adenosine Triphosphate - ATP**		**Humification (HR, HI, HD)**		**Cellulose and Lignin Content - Acid Detergent Fibre test**		**Cellulase Hydrolysis Test**	
S/N	**Criteria**	Weighting in %	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)	points (max. 10)	assess-ment (2)x(3)
1	**Appropriateness of the method based on findings from literature review**																													
1.1	Robustness	5	8	4	8	4	8	4	8	4	8	4	6	3	6	3	6	3	8	4	8	4	8	4	8	4	8	4	8	4
1.2	Selectivity	5	6	3	6	3	8	4	6	3	4	2	10	5	10	5	8	4	6	3	2	1	6	3	6	3	2	1	8	4
1.3	Defined units of stability index and limit	5	8	4	10	5	10	5	0	0	8	4	8	4	8	4	8	4	8	4	0	0	6	3	10	5	0	0	0	0
1.4	Demostrated ability to measure biodegrability of waste water / or feacal sludges	10	10	10	10	10	8	8	8	8	4	4	6	6	6	6	8	8	10	10	2	2	8	8	0	0	0	0	0	0
Total 1		**25**		21		22		21		15		14		18		18		19		21		7		18		12		5		8
2	**Lab Attributes of the Method**																													
2.1	simplicity of lab techniques and protocal	5	8	4	8	4	8	4	4	2	6	3	2	1	6	3	8	4	10	5	10	5	8	4	8	4	10	5	8	4
2.2	required effort	5	6	3	6	3	6	3	8	4	6	3	4	2	8	4	8	4	10	5	10	5	8	4	6	3	8	4	8	4
2.3	Working range	5	10	5	10	5	8	4	10	5	8	4	10	5	10	5	6	3	6	3	6	3	6	3	8	4	10	5	6	3
2.4	cost	5	10	5	10	5	10	5	8	4	6	3	2	1	4	2	6	3	10	5	10	5	8	4	10	5	10	5	8	4
2.5	local application	10	8	8	10	10	10	10	10	10	4	4	2	2	2	2	10	10	10	10	10	10	6	6	8	8	10	10	10	10
Total 2		**30**		25		27		26		25		17		11		16		24		28		28		21		24		29		25
3	**Application of the Method by Others**																													
3.1	Reproducibility and Repeatability	20	6	12	10	20	10	20	8	16	10	20	8	16	8	16	8	16	10	20	2	4	6	12	8	16	8	16	6	12
3.2	Application in low income settings	25	10	25	10	25	10	25	10	25	8	20	2	5	4	10	10	25	10	25	10	25	8	20	10	25	10	25	10	25
Total 3		**45**		37		45		45		41		40		21		26		41		45		29		32		41		41		37
Grand		**100**		**83**		**94**		**92**		**81**		**71**		**50**		**60**		**84**		**94**		**64**		**71**		**77**		**75**		**70**
Ranking				**5**		**2**		**3**		**6**		**9**		**13**		**12**		**4**		**1**		**11**		**9**		**7**		**8**		**10**

Appendix 4: Laboratory Results (Part 1 of the Study)

A. COD Results

Sample ID	Sample Source	Lab ID	Tube ID	Dilution Factor	Replicate ID	Diluted COD (mg/l)	Sample COD (g/l)	Mean	RSD
Standard	Standard	STD	1	2	1	941	1.88	1.9	0.34%
		STD	2	2	2	946	1.89		
		STD	3	2	3	940	1.88		
101.LSK	Dry Pit Latrine	MT03	4	400	1	231	92.40	104.8	10%
		MT03	5	400	2	280	112.00		
		MT03	6	400	3	275	110.00		
102.LSK	Fresh Excreta	FS06	7	400	1	100	40.00	43.9	10%
		FS06	8	400	2	108	43.20		
		FS06	9	400	3	121	48.40		
103.LSK	Dry Pit Latrine	CHZ01	10	200	1	532	106.40	113.1	10%
		CHZ01	11	200	2	633	126.60		
		CHZ01	12	200	3	531	106.20		
104.LSK	Vertical Vault Latrine	CHZ02	13	200	1	479	95.80	90.6	6%
		CHZ02	14	200	2	428	85.60		
		CHZ02	15	200	3	452	90.40		
105.LSK	Wet Latrine	CHZ03	16	200	1	460	92.00	90.8	1.87%

Sample ID	Sample Source	Lab ID	Tube ID	Dilution Factor	Replicate ID	Diluted COD (mg/l)	Sample COD (g/l)	Mean	RSD
		CHZ03	17	200	2	448	89.60		
106.LSK	Septic Tank	CHZ04	18	200	1	236	47.20	46.4	2.44%
		CHZ04	19	200	2	228	45.60		

B. BOD Results

Sample ID	Source	Lab ID	DF	P (Vol. fraction Sample)	D_1	D_2	Actual (D1 - D2)	BOD (mgO_2/L)	Sample BOD (gO2/L)	Mean	RSD
Blank		Blank	1	1	7.61	7.34	0.27	0.27	0.27		
101.LSK	Dry Pit Latrine	MT03	40	0.004	7.67	6.01	1.66	415	16.6	17.0	3%
		MT03	40	0.004	7.62	5.89	1.73	432.5	17.3		
102.LSK	Fresh Excreta	FS06	40	0.004	7.43	5.47	1.96	490	19.6	20.0	2%
		FS06	40	0.004	7.41	5.38	2.03	507.5	20.3		
103.LSK	Dry Pit Latrine	CHZ01	40	0.02	9.3	3.8	5.5	275	11	12.9	13%
		CHZ01	40	0.02	9.5	2.7	6.8	340	13.6		
		CHZ01	40	0.02	9.4	2.4	7	350	14		
104.LSK	Vertical Vault	CHZ02	40	0.02	9.4	1.5	7.9	395	15.8	16.4	3%
		CHZ02	40	0.02	9.4	1	8.4	420	16.8		
		CHZ02	40	0.02	9.3	1	8.3	415	16.6		
105.LSK		CHZ03	40	0.02	7	1	6	300	12	12.2	2%

Sample ID	Source	Lab ID	DF	P (Vol. fraction Sample)	D_1	D_2	Actual (D1 - D2)	BOD (mgO_2/L)	Sample BOD (gO_2/L)	Mean	RSD
	Wet Pit Latrine	CHZ03	40	0.02	7	0.8	6.2	310	12.4		
106.LSK	Septic Tank	CHZ04	40	0.02	6.8	4.4	2.4	120	4.8	5.2	11%
		CHZ04	40	0.02	6.9	4.1	2.8	140	5.6		

C. VS/TS Results

Sample ID	SOURCE	Lab ID	TS (% wt)	VS (% of TS)	Mean (TS)	RSD (TS)	Mean (VS)	RSD (VS)
101.LSK	Dry Pit Latrine	MT03	20.5	37.4	20.4	1%	37.5	0.4%
		MT03	20.3	37.6				
102.LSK	Fresh Excreta	FS06	4.8	81.3	4.8	2%	81.6	0.4%
		FS06	4.7	81.8				
103.LSK	Dry Pit Latrine	CZ01	14.4	60.8	13.1	15%	60.6	0.5%
		CZ01	11.7	60.4				
104.LSK	Vertical Vault	CZ02	10.8	74.7	10.8	1%	74.6	0.0%
		CZ02	10.9	74.7				
106.LSK	Wet Pit Latrine	CZ04	6.9	55.2	-	-	-	-
105.LSK	Septic Tank	CZ03	18.6	38.8	-	-	-	-

Appendix 5: Laboratory Results (Part 2 of the Study)

A. TS and VS Results

Sample ID	SOURCE	Lab ID	TS (% wt)	VS (% of TS)	Mean (TS)	RSD (TS)	Mean (VS)	RSD (VS)
01A.LSK.2021.08.31	Dry PL	1	17.7	46.0	17.5	2%	46.15	0%
		1	17.2	46.3				
		1	17.5	46.2				
01B.LSK.2021.08.31	Dry PL	2	19.6	30.9				
01C.LSK.2021.08.31	Dry PL	3	19.8	30.7				
02.LSK.2021.08.31	Septic Tank	4	2.4	45.5				
03.LSK.2021.09.01	Dry PL	5	10.9	71.5				
04.LSK.2021.09.01	Dry PL	6	15.9	80.6				
05.LSK.2021.09.01	Dry PL	7	19.5	42.6				
06.LSK.2021.09.01	Dry PL	8	15.1	70.4	15	1%	70.47	0%
		8	15.1	70.6				
		8	14.8	70.4				
07.LSK.2021.09.01	Dry PL	9	6.8	66.3				
08.LSK.2021.09.01		10	19.2	57.3				
16.LSK.2021.09.02	Dry PL	19	0.7	47.1	0.7	3%	45.25	4%

Sample ID	SOURCE	Lab ID	TS (% wt)	VS (% of TS)	Mean (TS)	RSD (TS)	Mean (VS)	RSD (VS)
		19	0.7	44.8				
		19	0.7	43.8				
21.LSK.2021.09.03	Wet PL	25	2.4	50.6				
		25	2.7	52.4				
		25	2.5	50.6				
22.LSK.2021.09.03	Wet PL	26	2.7	53.7				
23.LSK.2021.09.03	Wet PL	27	4.8	56.4				
24.LSK.2021.09.03	Wet PL	28	1.6	56.9				
25.LSK.2021.09.03	Wet PL	29	1.5	40.1				
26C.LSK.2021.09.03	Dry PL	32	9.0	53.7				
31.LSK.2021.09.06	Wet PL	38	5.4	38.3	5.53	3%	36.86	3%
		38	5.5	36.2				
		38	5.7	36.1				
35.LSK.2021.09.06	Septic Tank	42	5.5	49.8				
36.LSK.2021.09.06	Septic Tank	43	3.9	60.4				
63.LSk.2021.10.04	Dry PL	70	9.2	54.8				
64.LSk.2021.10.04	Wet PL	74	2.5	53.1				

B. COD Results

Sample ID	SOURCE	Lab ID	Tube ID	Dilution Factor	Diluted COD (mg/l)	Sample COD (g/l)	Mean	RSD
01B.LSK.2021.08.31	Dry PL	1	1	200	744	148.8	146.2	3%
		1	2	200	743	148.6		
		1	3	200	706	141.2		
01B.LSK.2021.08.31	Dry PL	2	4	200	651	130.2		
01C.LSK.2021.08.31	Dry PL	3	5	200	531	106.2		
02.LSK.2021.08.31	Septic Tank	4	6	200	93	18.6		
03.LSK.2021.09.01	Dry PL	5	7	200	1008	201.6		
04.LSK.2021.09.01	Dry PL	6	8	200	1115	223		
05.LSK.2021.09.01	Dry PL	7	9	200	640	128		
06.LSK.2021.09.01	Dry PL	8	10	200	1321	264.2		
07.LSK.2021.09.01	Dry PL	9	11	200	539	107.8		
08.LSK.2021.09.01	Dry PL	10	13	200	926	185.2	206.9	15%
		10	14	200	1143	228.6		
16.LSK.2021.09.02	Dry PL	19	25	200	under range	#VALUE!		
21.LSK.2021.09.03	Wet PL	25	31	200	80	16	12.2	28%
		25	32	200	46	9.2		
		25	33	200	57	11.4		
22.LSK.2021.09.03	Wet PL	26	34	200	1009	201.8		
23.LSK.2021.09.03	Wet PL	27	35	200	747	149.4		

Sample ID	SOURCE	Lab ID	Tube ID	Dilution Factor	Diluted COD (mg/l)	Sample COD (g/l)	Mean	RSD
24.LSK.2021.09.03	Wet PL	28	36	200	915	183		
25.LSK.2021.09.03	Wet PL	29	37	200	14	2.8		
26C.LSK.2021.09.03	Dry PL	32	40	200	449	89.8		
31.LSK.2021.09.06	Wet PL	38	48	200	112	22.4		
35.LSK.2021.09.06	Septic Tank	42	52	200	183	36.6		
36.LSK.2021.09.06	Septic Tank	43	53	200	81	16.2	16.06	4%
		43	54	200	83	16.6		
		43	55	200	77	15.4		
63.LSk.2021.10.04	Dry PL	70	56	100	732	73.2		
64.LSk.2021.10.04	Wet PL	74	57	100	657	65.7		
Standard		STD	S	2	1081	2.2		

C. BOD Results

Sample ID	Source	Lab ID	DF	P (Vol. fraction Sample)	D_1	D_2	Actual (D1 - D2)	BOD (mgO_2/L)	Sample BOD (gO2/L)
01A.LSK.2021.08.31	Dry PL	1	40	0.02	7.3	1.1	6.2	310	12.4
01B.LSK.2021.08.31	Dry PL	2	40	0.02	7.2	1.2	6	300	12
01C.LSK.2021.08.31	Dry PL	3	40	0.02	7.3	1.4	5.9	295	11.8

02.LSK.2021.08.31	Septic Tank	4	40	0.02	7.3	5.5	1.8	90	3.6
03.LSK.2021.09.01	Dry PL	5	40	0.02	7.2	0.8	6.4	320	12.8
04.LSK.2021.09.01	Dry PL	6	40	0.02	7.3	0.8	6.5	325	13
05.LSK.2021.09.01	Dry PL	7	40	0.02	7.3	0.8	6.5	325	13
06.LSK.2021.09.01	Dry PL	8	40	0.02	7.3	0.7	6.6	330	13.2
07.LSK.2021.09.01	Dry PL	9	40	0.02	7.1	2.4	4.7	235	9.4
08.LSK.2021.09.01	Dry PL	10	40	0.02	7.1	0.8	6.3	315	12.6
16.LSK.2021.09.02	Dry PL	19	40	0.02	7.3	6.1	1.2	60	2.4
21.LSK.2021.09.03	Wet PL	25	40	0.02	7.2	4.7	2.5	125	5
22.LSK.2021.09.03	Wet PL	26	40	0.02	7.2	3.6	3.6	180	7.2
23.LSK.2021.09.03	Wet PL	27	40	0.02	7.1	3.7	3.4	170	6.8
24.LSK.2021.09.03	Wet PL	28	40	0.02	7.2	4.4	2.8	140	5.6
25.LSK.2021.09.03	Wet PL	29	40	0.02	7.2	5.5	1.7	85	3.4
26C.LSK.2021.09.03	Dry PL	32	40	0.02	7.2	0.9	6.3	315	12.6
31.LSK.2021.09.06	Wet PL	38	40	0.02	7.2	3.7	3.5	175	7
35.LSK.2021.09.06	Septic Tank	42	40	0.02	7.2	3.5	3.7	185	7.4
36.LSK.2021.09.06	Septic Tank	43	40	0.02	7.2	2.7	4.5	225	9
BLK		BLK	1	1	6.38	5.99	0.39	0.39	0.39

D. CST Results

Sample ID	Source	Lab id	Head 1	Head 2	Head 3	Head 4	Average CST(s)	Adjusted CST (s)	Normalized CST (s.L/gTS)	comment
Distilled water			6.6	6.2	7		6.6			
01A.LSK.2021.08.31	Dry PL	1	>3 hours	>3 hours	>3 hours	>3 hours	13107.6	13101	99.3	Didn't dewater easily
01B.LSK.2021.08.31	Dry PL	2	>3 hours	>3 hours	>3 hours	>3 hours	13107.6	13101	86.9	Didn't dewater easily
01C.LSK.2021.08.31	Dry PL	3	>3 hours	>3 hours	>3 hours	>3 hours	13107.6	13101	80.2	Didn't dewater easily
02.LSK.2021.08.31	Septic Tank	4	105.9	95.9	106.5	75.3	95.9	89.3	3.8	
03.LSK.2021.09.01	Dry PL	5	>3 hours	>3 hours	>3 hours	>3 hours	13107.6	13101	158.2	Didn't dewater easily
04.LSK.2021.09.01	Dry PL	6	>3 hours	>3 hours	>3 hours	>3 hours	13107.6	13101	89.2	Didn't dewater easily
05.LSK.2021.09.01	Dry PL	7	2000.5	2084.1	2127.8	1886.2	2024.65	2018.05	11.1	Thick Sludge
06.LSK.2021.09.01	Dry PL	8	>3 hours	>3 hours	>3 hours	>3 hours	13107.6	13101	79.2	Thick Sludge

Sample ID	Source	Lab id	Head 1	Head 2	Head 3	Head 4	Average CST(s)	Adjusted CST (s)	Normalized CST (s.L/gTS)	comment
07.LSK.2021.09.01	Dry PL	9	888.8	961.3	927.1	914.6	922.95	916.35	13.2	Mildly thick
08.LSK.2021.09.01	Dry PL	10	>3 hours	>3 hours	>3 hours	>3 hours	13107.6	13101	65.9	Didn't dewater easily
16.LSK.2021.09.02	Dry PL	19	76.9	62.6	70.4	55.2	66.275	59.675	9.9	Liquid
21.LSK.2021.09.03	Wet PL	25	748.5	633.6	713.2	661.1	689.1	682.5	26.0	Liquid
22.LSK.2021.09.03	Wet PL	26	585.1	662.4	599.9	630.1	619.375	612.775	20.3	Liquid
23.LSK.2021.09.03	Wet PL	27	448.9	507.4	512	469.2	484.375	477.775	9.3	Liquid
24.LSK.2021.09.03	Wet PL	28	353.4	422.4	529	481.8	446.65	440.05	24.8	Liquid
25.LSK.2021.09.03	Wet PL	29	369.4	369.4	381	392.9	378.175	371.575	22.5	Liquid
26C.LSK.2021.09.03	Dry PL	32	2716.9	2582.8	2921.3	2508	2682.25	2675.65	28.1	Mildly thick
31.LSK.2021.09.06	Wet PL	38	216.6	206.7	209.2	185.6	204.525	197.925	3.5	Liquid
35.LSK.2021.09.06	Septic Tank	42	469.9	514.7	571.1	434.8	497.625	491.025	7.5	Liquid
36.LSK.2021.09.06	Septic Tank	43	71.2	72.7	78.9	73.7	74.125	67.525	1.7	Liquid
63.LSk.2021.10.04	Dry PL	70	650.5	695.8	605.2		650.5	644.8	6.0	
64.LSk.2021.10.04	Wet PL	74	166.1	234.3	252.4		217.6	211.9	7.6	
FS 06	Fresh Excreta	FS06	>3 hours	>3 hours	>3 hours	>3 hours	13107.6	13101	269.7	Didn't dewater easily

E. Selected SOUR Results

Time	01A.LSK.2021.08.31	01B.LSK.2021.08.31	01C.LSK.2021.08.31	02.LSK.2021.08.31	03.LSK.2021.09.01	05.LSK.2021.09.01	06.LSK.2021.09.01
0	6.57	6.27	5.58	5.46	5.94	6.30	5.64
30	6.04	6.17	5.54	5.25	5.64	5.52	5.16
60	5.91	6.07	5.5	5.08	5.43	5.29	5.11
90	5.82	6.01	5.46	4.94	5.30	5.11	5.05
120	5.72	5.98	5.42	4.77	5.16	4.91	5.00
150	5.65	5.93	5.38	4.63	5.04	4.74	4.95
180	5.57	5.88	5.34	4.49	4.91	4.55	4.90
210	5.50	5.84	5.30	4.35	4.78	4.37	4.84
240	5.42	5.80	5.25	4.21	4.65	4.17	4.79
270	5.35	5.75	5.22	4.07	4.53	4.01	4.73
300	5.28	5.71	5.17	3.93	4.39	3.82	4.67
330	5.21	5.66	5.13	3.78	4.12	3.63	4.61
360	5.13	5.62	5.09	3.64	4.00	3.42	4.56
390	5.06	5.57	5.04	3.50	3.85	3.23	4.50
410	4.98	5.51	5.00	3.36	3.71	3.04	4.44
440	4.91	5.47	4.96	3.23	3.56	2.86	4.38
470	4.84	5.42	4.90	3.09	3.42	2.66	4.32
490	4.77	5.37	4.86	2.96	3.28	2.46	4.26

Time	01A.LSK.2021.08.31	01B.LSK.2021.08.31	01C.LSK.2021.08.31	02.LSK.2021.08.31	03.LSK.2021.09.01	05.LSK.2021.09.01	06.LSK.2021.09.01
520	4.69	5.32	4.82	2.82	3.14	2.26	4.20
550	4.62	5.28	4.76	2.69	3.00	2.08	4.14
580	4.53	5.23	4.71	2.56	2.85	1.88	4.08
610	4.47	5.18	4.67	2.43	2.71	1.68	4.03
640	4.39	5.13	4.63	2.29	2.55	1.49	3.96
670	4.31	5.08	4.58	2.16	2.40		3.90
690	4.24	5.03	4.53	2.03	2.26		3.83
720	4.16	4.99	4.49	1.91	2.11		3.77
750	4.08	4.94	4.44	1.77	1.96		3.71
780	4.00	4.89	4.40	1.66	1.80		3.65
810	3.92	4.84	4.35				3.59
840	3.85	4.79	4.30				3.52
870	3.77	4.74	4.26				3.46
Slope	-0.002747268	-0.00168126	-0.001547967	-0.004834111	-0.005151856	-0.006820657	-0.002134073
VS (g/L)	5	5	5	10.77	5	5	5
Original VS (g/L)	60.91	46.63	50.16	10.77	59.24	77.51	116.54
DF	12.18	9.33	10.03	1	11.85	15.50	23.31
OUR (mg O2/h)	9.890166031	6.052536867	5.572681208	17.40279913	18.54667987	24.55436677	7.682662929

Time	01A.LSK.2021.08.31	01B.LSK.2021.08.31	01C.LSK.2021.08.31	02.LSK.2021.08.31	03.LSK.2021.09.01	05.LSK.2021.09.01	06.LSK.2021.09.01
SOUR (mg O2/Kg VS/h)	1978.033206	1210.507373	1114.536242	1615.858786	3709.335973	4910.873354	1536.532586
SOUR (mg O2/Kg VS/h) - Original	24,095.74	11,290.40	11,181.03	1,615.86	43,944.50	76,123.45	35,816.57

Appendix 6: PUBLICATIONS FROM THIS RESEARCH

Title of Publication	Journal	Status
1. Assessment of the Influence of Intrinsic Physical-Chemical Characteristics on Anaerobic Stabilization of Faecal Sludge	Journal of Natural and Applied Sciences (JONAS)	Submitted (See proof of submission attached)
2. Metrics for Stabilization of Faecal Sludge and Its Relation to Dewatering Performance	Journal of Water Sanitation and Hygiene for Development (WASHDev)	Manuscript to be submitted by 30th May 2023.