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INVESTIGATION OF THE EFFECTIVENESS OF ADDITIVES IN ENHANCING STABILISATION AND SANITISATION OF FAECAL SLUDGE IN EMERGENCY SITUATIONS

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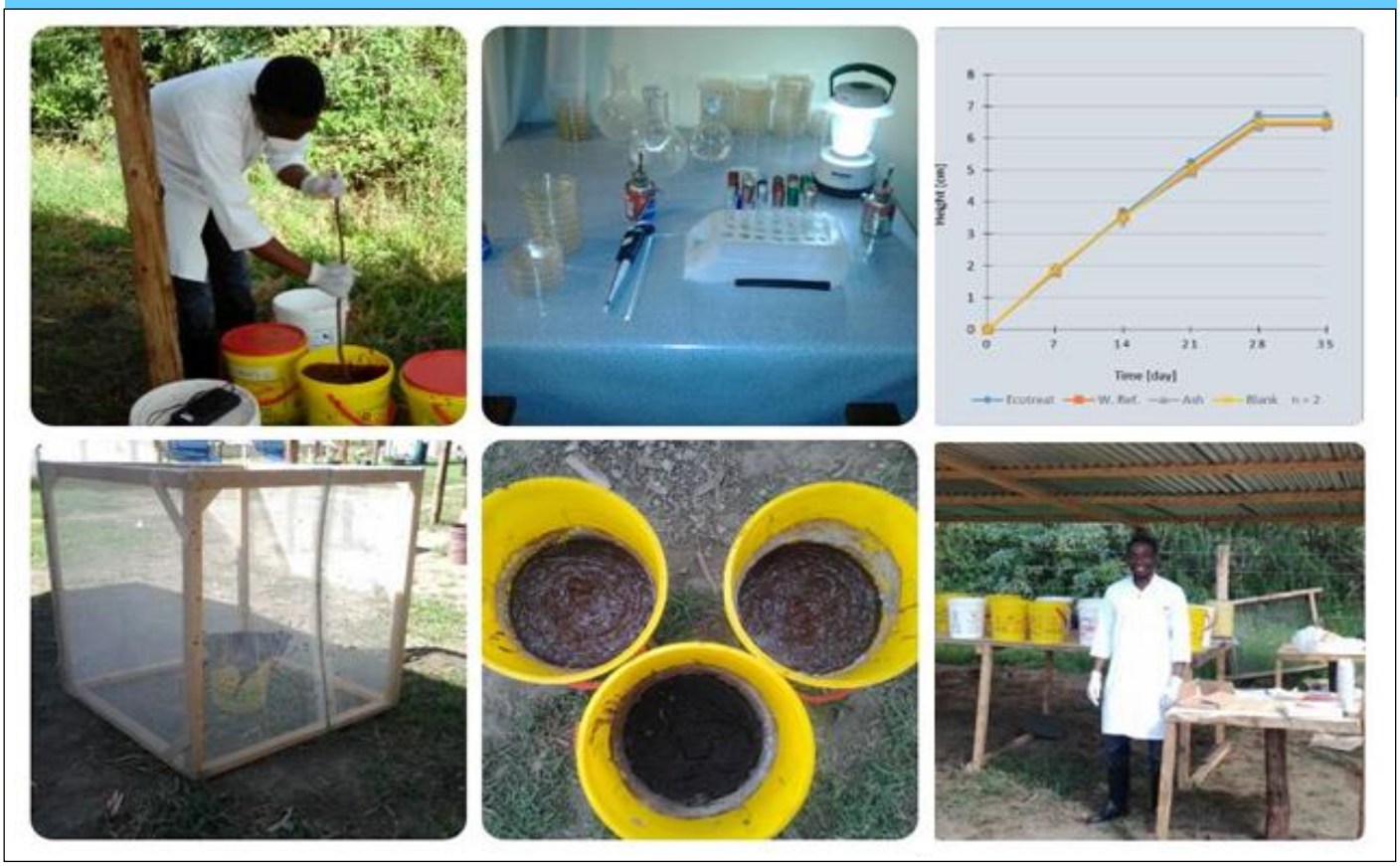

INVESTIGATION OF THE EFFECTIVENESS OF ADDITIVES IN ENHANCING STABILISATION AND SANITISATION OF FAECAL SLUDGE IN EMERGENCY SITUATIONS

Master of Science Thesis
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Abstract

Sanitation is the first barrier against vector transmission from human excreta to people, therefore, the provision of an adequate sanitation system as well as drinking water and hygiene are crucial for survival of a disaster-effected population, especially in the immediate phase of an emergency.
The few sanitation facilities (commonly pit latrines) that are firstly constructed or reconditioned after the disaster have stricken are usually overused thus speeding-up their filling rate. However, several products are sold in the market claiming their ability in slowing the filling rate of pit latrines and reducing pathogen concentration in faecal sludge.
This research aimed to investigate this claim by testing the effect of promising chemical additives Ikati and Soda, and evaluate the effectiveness of other additives in enhancing reduction of volatile solids concentration and *E. coli* removal below 38 % and 10^3 cfu/100 ml, respectively. Moreover, the research also evaluated the effect of additives on odour, fly attraction and volume reduction. Additionally, the study was extended to the evaluation of the application protocols of biological additives.
The laboratory scale experiment was conducted in Naivasha, Kenya, using fresh faecal sludge, in 10 litre plastic container. Two trials were conducted and a total of five additives were tested. In the first trial (batch experiment), the performance of the chemical additives (Ikati and Soda) and biological (Ecotreat and Sannitree) was assessed according to supplier's recommended dosing protocols. On the other hand, the second trial (step-feeding) tested Ecotreat and wood-ash, being Ecotreat dosed based on the Consortium LICE SM dosing protocol. The trials were conducted in 3 and 2 replicates, respectively, including blanks and water reference. Samples were collected before, during and after treatment with the additives, after stirring the content in the reactors for 2 minutes, and the parameters above mentioned were analysed, including temperature, pH and total solids. The analysis were conducted according to standardised procedures, with exception of fly attraction which is not standardised, therefore a specific procedure was developed and implemented. The results of the experiments were compiled and statistically analysed using ANOVA and correlation.

It was found that the pH on the reactors treated with Ikati and Soda rose from 5.4 to 10.0 and 9.4 pH units, respectively. Moreover, an *E. coli* removal from Log 8 to below detection limit was achieved within 2 days. Furthermore, a significant reduction of 17 % and 21 % on VS/TS, 52 and 39 % on height was observed for Ikati and Soda respectively. However, wood-ash imposed a reduction on odour from 2000 to 444 TON units and it attained a reduction of 87 % on fly attraction. On the other hand, no significant differences were found between the biological additives and the controls at the end of the treatment periods for almost all parameters in the two trials.

The results showed that Ikati and Soda are able to rapidly sanitise faecal sludge, and they are likely to achieve stabilisation and volume reduction. Moreover, it was concluded that pH plays an important role on *E. coli* removal. Additionally, it was seen that wood-ash can serve as a good and cheap treatment against odour and flies. On the other hand, no evidences were found to support the claim that biological additives can speed-up degradation rate of faecal sludge, thus extending the life of pit latrines. Similarly, no evidences were obtained to conclude that the Consortium LICE SM dosing protocol improves performance of biological additives.

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Abbreviations

ANOVA	Analysis of variance
ASBR	Anaerobic sequencing batch reactor
BOD	Biochemical oxygen demand
CBA	Cost-benefits analysis
CEA	Cost-effectiveness analysis
CER	Cost-effectiveness ratio
COD	Chemical oxygen demand
FS	Faecal sludge
IDP	Internally Displaced Person
JPM	Joint Monitoring Program
KEWI	Kenya Water Institute
MDGs	Millennium Development Goals
TON	Threshold Odour number
TS	Total solids
UDDT	Urine diverting dehydrating toilet
UNHCR	United Nations High Commissioner for Refugees
UNICEF	United Nations Children's Fund
UNISDR	United Nations International Strategy for Disaster Reduction
VIP latrine	Ventilated improved pit latrine
VS	Volatile solids
WASH	Water, sanitation and hygiene promotion
WHO	World Health Organization

CHAPTER 1

Introduction

In this chapter, a background of sanitation in emergencies is presented, highlighting the challenges of the operation and maintenance of pit latrines during emergencies, due their high filling rate. Manufacturers of additives claim about the ability of their products in reducing the volume of the pit content and the number of pathogens in the faecal sludge, thus ensuring long life of the pit and safe disposal or reuse of the treated sludge. The chapter shows the helpfulness of such a potential solution for faecal sludge management in emergencies, thus presenting the questions the research aims to address and the respective objectives.

1.1. Background

Worldwide, the human being lives under the risk of a disaster, either natural or human made, so that the disaster risk reduction is a general concern for all States (UNISDR, 2015). The magnitude 7.8 earthquake, followed by numerous aftershocks of magnitude 5, in Nepal, in April 2015, where have been reported in 18 May 2015, a death toll of about 8,600 people and over 16,000 injured and the current Syrian crisis where more than 4 million[1] refugees have been registered (up to 04 October 2015), are examples of disasters that led to emergencies with world impact (UNISDR, *et al.*, 2015).

The provision of improved sanitation system is still a challenge worldwide, mainly in developing countries. This challenge is exacerbated during emergency situations, where rapid response in providing an adequate faecal sludge management is required in order to prevent vector transmission from human excreta thus reducing the risk of diseases outbreak and protecting the environment (Daves, *et al.*, 1995, Harvey, 2007, UNHCR, 2007). Furthermore, it is essential to provide an adequate sanitary facility in a humanitarian response for the safety, health, well-being and dignity of people (The Sphere Project, 2011). About 50 % of deaths during the acute phase of an emergency are related to diarrhoeal diseases (Abdallah, *et al.*, 2000). Therefore, the provision of a safe excreta disposal system is as important as the provision of drinking water during emergencies (The Sphere Project, 2011). However, personal and collective hygiene habits such as hand washing, proper cooking of food and cleaning backyard, objects and freshfood, also play an important role in disease prevention (Carr, *et al.*, 2001).

[1] http://data.unhcr.org/syrianrefugees/regional.php

1.2. Problem definition

In emergencies high amounts of faecal sludge (FS) are produced and if these are not properly managed, the chance of disease outbreaks is high. It is important to reduce the amount of FS and the number of pathogens as much as possible. Pit latrines are the most common technology adopted during emergencies. Although a maximum of 20 people per latrine is the target, normally at the initial stage of a humanitarian response there are no adequate sanitation facilities installed, therefore this target is increased to 50 people per pit latrine, and then being gradually reduced as more facilities are placed (The Sphere Project, 2011). In this scenario, the life time of a single pit latrine with a cross-sectional area of 1 m^2 and depth of 3 m will drop from about 2 years to 10 months and the lifetime will keep dropping as the ratio number of users per toilet increases, what is characteristic at the initial phase of installation of a refugee or internally displaced persons (IDP) camp (Harvey, *et al.*, 2002). When the pit is full, it is normally either capped and a new pit is opened, or the pit is emptied and then recommissioned (WIN-SA, 2011). Often, land availability, geological conditions, inexistence of proper disposal site, presence of solid material and lack of proper equipment and personnel can pose a challenge for these two procedures.

A wide range of additives, both biological and chemical, are marketed with a claim of being able to enhance stabilisation and sanitisation of organic matter in pit latrines. Moreover, the products are claimed to reduce the filling rate of pit latrines, and have impact on reduction of odour and fly attraction through the action of either enzymes, microorganisms, nutrients or by chemical processes. However, there is not enough knowledge on how this technology can be applied for faecal sludge management in emergencies, due to the high amount of faecal sludge that is produced. Additionally, factors such as investment, operational and maintenance costs and requirements have to be taken in consideration.

1.3. Significance of Research

Scientific researches have being conducted to assess the veracity of the claim that additives are able to accelerate the degradation process in the pit latrine, and some results showed there is a potential to achieve sanitisation and stabilisation of faecal sludge by the aid of additives (Anderson, *et al.*, 2015, Kemboi, 2015). On the other hand, there is another group that defends that there is not sufficient evidences to conclude that additives are able to accelerate the rate of mass loss on faecal sludge (Bakare, *et al.*, 2012, Bakare, *et al.*, 2015, Foxon, *et al.*, 2009). The discovery of such products could be a breakthrough, not only for application under emergencies, but also at normal living environment (households). The frequency of empting pit latrines would be reduced thus extending their life time and improving sanitation and health status of the population, mainly in developing countries, where provision of proper sanitation system is still a challenge.

In 2015, an UNESCO-IHE MSc student investigated a range of biological and chemical additives to assess their effectiveness within 2 weeks. She found that two chemical additives were able to quickly stabilise and sanitise the sludge, showing higher performance at a lower total solids concentration, while the biological additives were not so good at the removal of *E. coli*, nor that they could stabilise the sludge (Kemboi, 2015). Not all additives could be tested, and time was lacking to test additives over a longer period and to optimise the effect of

the promising chemical additives. Therefore there is a need for further research for testing of new additives over a longer period of time and for optimisation of the effect of the two promising chemical additives. Moreover, the results of this study will contribute to the enrichment of scientific knowledge on the effect of additives on odour, flies and volume reduction.

1.4. Research questions

This research aims to answer the following questions:

- Can additives prolong the life of pit latrines in an emergency situation by slowing down the filling rate?
- Are additives effective in enhance sanitisation and stabilisation of faecal sludge?
- Is the use of additives a cost-effective technology for faecal sludge management?
- Does LICE Consortium SM dosing protocol significantly improve the performance of biological additives?

1.5. General Objective

The general objective of this study is to investigate the effect of different chemical and biological additives in enhancing stabilisation and sanitisation of faecal sludge in other to extend the life period of pit latrines and ensure safe disposal of faecal sludge in emergency situations.

1.6. Specific Objectives

The specific objectives of this research are:

- Assess the effectiveness of different additives in enhancing stabilisation (VS/TS reduction $\geq$ 38 %) of faecal sludge, thereby achieving volume reduction.
- Investigate the effectiveness of different additives in removal of pathogen indicator organisms (*E. coli*) below WHO guideline value for unrestricted agricultural use ($\leq 10^3$ cfu/100 mL), as well as reduction in odour and fly attraction.
- Study the effect of dosing protocol on the performance of biological additives.

1.7. Hypothesis

The hypothesis for effectiveness of chemical additives is:

- Chemical additives are able to rapidly reduce more than 38 % of VS/TS on faecal sludge and simultaneously reduce *E. coli* below 10^3 cfu/100 ml. Therefore, they can be used to treat faecal sludge within the acute phase of an emergency. Moreover, flies and odour are significantly reduced. However, no significant volume reduction is expected.

The hypothesis for efficacy of biological additives is:

- Treatment of faecal sludge with biological additives is an efficient technology to ensure rapid degradation of faecal sludge thus slowing down the filling rate of pit latrines. Furthermore, a VS/TS reduction higher than 38 % as well as flies and non-pleasant odours reduction are expected to occur. This treatment technology can be used to treat faecal sludge within the acute phase of an emergency and its results can be significantly improved by adopting the LICE Consortium SM dosing protocol.

CHAPTER 2

Literature review

This chapter shows the link between disaster, emergency, sanitation and diseases. It highlights the challenge in the operation and maintenance of pit latrines during emergencies and shows the usefulness of additives to address this challenges. Furthermore, it briefly discuss about requirements for sanitation and stabilisation of faecal sludge. Moreover, the chapter debates on the previous researches conducted to assess the effectiveness of additives in slowing the filling rate of pit latrines and reduce the concentrations of pathogen in faecal sludge, highlighting the gaps on the researches thus directing towards the significance and objectives of this research.

2.1. Disaster & Emergency

In the present decade the humanity has been subjected to several extreme events, both natural and human-made. The Haiti earthquake in 2010, Nepal earthquake in 2015 and the actual Syria crisis are examples and all resulted in disasters.

Disaster is defined as an extreme event affecting a significant number of people, surpassing their vulnerabilities, thus resulting in human deaths, injuries, material and financial damage (Wisner, *et al.*, 2002).

According to the Annual Disaster Statistical Review report 2014, more than 300 natural disasters were registered in 2014, which is 60 units less than the average yearly frequency registered within the period of 2004 to 2013. However, it affected about 140.8 million people, caused the death of more than 7,000 people and a total economic damages of about US$ 99.2 billion. The figure 1 below, illustrates the disaster trend from 1990 to 2014. It is important to note that the highest damages were registered in Asia, followed by America and then Europe (Guha-Sapir, *et al.*, 2015). Moreover, an unprecedented number of 59.5 million people were forcibly displaced worldwide, being 19.5 million refugees, 38.2 million internally-displaced persons (IDPs) and 1.8 million asylum-seekers (UNHCR, 2015).

It is common, but not always, that a disaster leads to an emergency. For that to happen, the impact of the disaster should require extraordinary intervention to ensure survival, safety and welfare of a large number of a population (UNICEF, 2007).

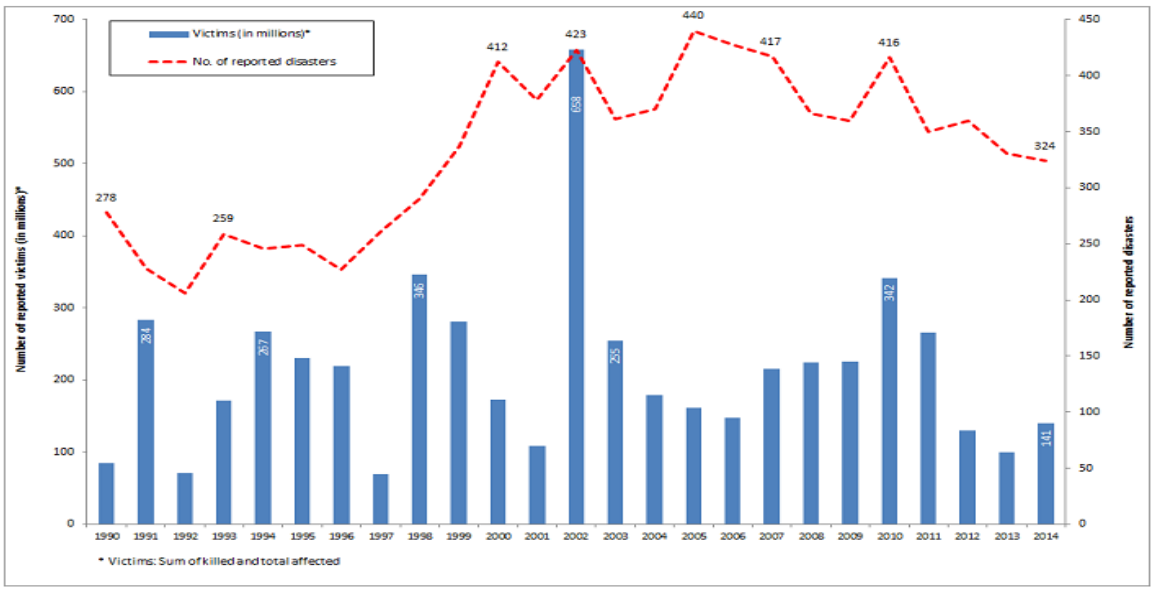


Figure 1: Worldwide disaster trends and victims (1990 – 2014). [Source: Annual disaster statistical review 2014 (Guha-Sapir, et al., 2015)]

2.2. Sanitation

Oxford Dictionary defines sanitation as "*conditions relating to public health, especially the provision of clean drinking water and adequate sewage disposal*".
WHO, however, restricts this definition to "*the provision of facilities and services for the safe disposal of human urine and faeces*". In this research, the term sanitation refer to disposal human excreta.

The access to improved sanitation plays an important role in safeguarding the health status of the population. Although great improvements have been achieved since the establishment of the Millennium Development Goals (MDGs), the access to safe sanition is still a challenge worldwide. Adopted in 2000 and with the vision to 2015, the MDG target on sanitation was to reduce by half the percentage of population without access to improved basic sanitation. According to the Millennium Development Goals Report 2015 about 2.1 billion people worldwide benefited from an improved sanitation service since 1990, representing an increase of fourteen procent into the 54 % of the worldwide population with access to improved sanitation system in 1990 (UN, 2015). Although the coverage rate of improved sanitation facilities has increased, there are still more than 900 million people practising open defecation, out of the 2.4 billion without access to inproved facilities, thus posing a risk to public health (UN, 2015).

2.3. Emergency, diseases and sanitation

During emergencies, massive numbers of people from different places, with different habits and health status are gathered in a temporary camp. It is often that the camps are devoided of basic sanitation systems at the initial phase. Therefore, there is high risk of vector transmission

from the immune vector carriers to those with weaker immunetary system, what would lead to a cycle diseases transmission (Wisner, *et al.*, 2002).
Several species of microorganisms are present in the human excreta, including those that are pathogens. The disease-causing microorganisms can enter the host mainly through contaminated food, water and objects, thus causing diseases such as polio, cholera, typhoid, hepatitis, cryptosporidiosis, ascariasis, and schistosomiasis (Fewtrell, *et al.*, 2001, Wisner, *et al.*, 2002). However diarrhoea, cholera and typhoid are often the main concern among the disaster-affected population, being diarrhoea responsible for over 50 % of deaths during acute phase of an emergency (Abdallah, *et al.*, 2000). This faecal-oral disease incidence risk can be greatly reduced if basic sanitation systems are provided to the disaster-affected population. Furthermore, personal and collective hygiene habits such as hand washing, proper cooking of food and cleaning backyard, objects and fresh food, also play an important role in disease prevention (Carr, *et al.*, 2001). The figure 2 below shows the several routes through which a vector can be transmitted and the possible barriers.

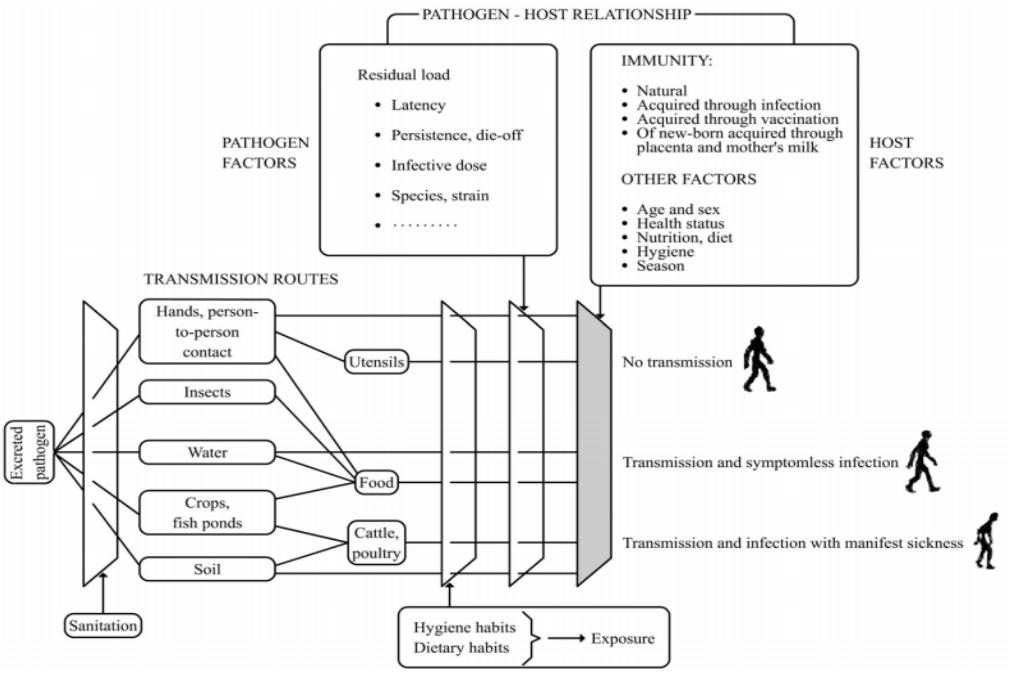


Figure 2: Transmission routes of the faecal-oral vectors. [Source: (Carr, et al., 2001)]

2.3.1. Sanitation program response

With the objective to improve the efficiency of humanitarian response and its accountability in emergency situations, a set of universal minimum standards was created in 1997 by a group of humanitarian response agencies and organisations. This set of minimum standards was then compiled in a book designated as *The Sphere Handbook*, and it focus on four core areas including water supply, sanitation and hygiene promotion (WASH) (The Sphere Project, 2011). Although appointing family toilets as the ideal option for excreta disposal, the Sphere Book set the target of no more than 20 people/toilet. However, the number of existent sanitation facilities at the initial phase of the humanitarian response is normally small, therefore a maximum of 50 people/toilet is allowed at this phase (The Sphere Project, 2011).

Based on that, a *sanitation programme response* to an emergency is normally divided into two distinct phases (Daves, *et al.*, 1995, Harvey, *et al.*, 2002, Wisner, *et al.*, 2002):

2.3.2. Immediate phase (acute emergency phase)

It is the phase immediately after occurrence of the disaster clash and is characterised by high crude mortality rate (above 1/10,000 affected people/day) and instability. The aim of a sanitation programme at this stage is to rapidly act in order to control and contain potential sources of excreta-related diseases for safeguard of the life, health and livelihoods of the disaster-affected population (Harvey, 2007, Harvey, *et al.*, 2002, Wisner, *et al.*, 2002). The duration of this phase can be from several weeks to 3 months (Abdallah, *et al.*, 2000, Harvey, *et al.*, 2002). The sanitation programmes should be designed in order to meet the Sphere minimum standards for excreta disposal, however depending on the nature and magnitude of the emergency, these minimums may not be met in this first phase (Harvey, 2007, The Sphere Project, 2011).

2.3.3. Stabilisation phase (post-emergency phase)

It is the phase that precedes the acute emergency. Wisner *et al.*, (2002) defines the beginning of the post-emergency phase as the time at which the crude mortality rate decreases below 1/10,000 people/day (Wisner, *et al.*, 2002). In this phase, more sustainable and longer term solutions can be implemented as the relief activities are improved and expanded in order to ensure safety against sanitation-related diseases and safeguard of the environment. This period can last for 6 months or even several years, depending on the complexity of the emergency, however the Sphere minimum standards should be fulfilled (Harvey, 2007, The Sphere Project, 2011).

The present research will focus on faecal sludge management during the acute phase of an emergency.

2.4. Faecal sludge treatment

2.4.1. Sanitisation of faecal sludge

Sanitisation of faecal sludge can be defined as the appropriate pathogen reduction that ensures safe handling, disposal and reuse of the treated sludge and effluent liquids, safeguarding the health of the human beings and the receiving environment (Carr, *et al.*, 2001, Strande, *et al.*, 2014). Therefore it is important to know the disposal/end use of the treated sludge in order to determine the level of treatment (Strande, *et al.*, 2014). According to WHO (2006), the use of treated sludge in agriculture vary depending on the level of treatment. For unrestricted irrigation, for example, a treatment level of *E. coli* $\leq 10^3$ cfu/100 ml and helminth ova $\leq$ 1/litre is required. The table 6 below presents the different applications based on the final E. coli concentration.

Table 1: E. coli numbers for different crops (Adapted from: WHO Guidelines for the safe use of wastewater, excreta and greywater- 2006).

Type of irrigation	Option	Required pathogen reduction by treatment (log unit)	Verification monitoring level (*E.coli*/100 ml)	Notes
Unrestricted	T-DO-W	4	$\leq 10^3$	Root crops
	T-DO-W	3	$\leq 10^4$	Leaf crops
	$T\text{-}DI_H$	2	$\leq 10^5$	Drip irrigation of high-growing crops
	$T\text{-}DI_L$	4	$\leq 10^3$	Drip irrigation of high-growing crops
	T	6 or 7	$\leq 10^1$ or $\leq 10^0$	Verification level depends on the requirements of the local regulatory agency
Restricted	T_{LI}	4	≤ 1	Labour-intensive agriculture (protective of adults and children under 15)
	T_{HM}	3		Highly mechanized agriculture
	T-SSI	0.5		Pathogen removal in a septic tank

T = Treatment

DO = die-off

W = Washing of produce

$DI_{H/L}$ = Drip irrigation (H = High crops; L = Low crops)

LI = Labour intensive

HM = Highly mechanized

SSI = Subsurface irrigation

The rich pathogenic biodiversity found in the human excreta (viruses, salmonellae, cholera bacteria, faecal coliforms, protozoan cysts, etc.) makes difficult to evaluate the health risk imposed by individual microorganism, therefore indicator organisms are normally used (Carr, *et al.*, 2001, Horan, 2003). They are organisms adopted as representative for faecal contamination and are used to quantify the contamination of the treated sludge (Horan, 2003). However, some microorganisms that are found in the human excreta, such as total coliforms, have the potential to be of non-faecal source, therefore the selection of the indicator organisms have to be carefully studied (Henze, *et al.*, 2008, Mara, *et al.*, 2010, Sen, *et al.*, 2011).

Adams *et al.*, (2008) state that a good indicator must always be detectable even when there is only an indication of the presence of the pathogen of concern (Adams, *et al.*, 2008). It should not reproduce in the studied environment and should have similar survival characteristics of that of the pathogen of concern. Moreover, an ideal indicator should be rapidly and easily detectable and at low cost. The coliform bacteria, helminths, Enterococci and faecal Streptococci are some of the most adopted faecal-indicator organisms (Atlas, 1984, Mara, *et al.*, 2010, Sen, *et al.*, 2011).

Indicators of sanitisation

Total coliforms have been adopted as indicator of faecal contamination of waters for long time. They are present in the intestinal tract, and are in abundance in human faeces. However, these organisms can also be found in plants and soils, therefore its detection does not necessarily point to faecal contamination, although it is an indication (Gerardi, *et al.*, 2005). Laboratory procedures have been developed in order to identify those coliforms of faecal origin from those that are non-faecal and this is possible due to the ability of faecal coliforms to produce acidic gases in 24 hours by fermentation of lactose, at 44.5 ^{o}C (APHA, *et al.*, 1999, Henze, *et al.*, 2008). The total coliforms family includes microorganism of genera *Escherichia, Citrobacter, Enterobacter* and *Klebsiella*. Under tropical conditions, the non-faecal coliforms exhibit the same ability of producing gas under 44.5 ^{o}C as the faecal coliforms, what makes the use of coliforms bacteria as indicator organisms unreliable (Strande, *et al.*, 2014).

Escherichia coli, or simply *E. coli*, belongs to the Enterobacteriaceae family and is naturally present in the human gut. It is widely used as indicator organisms to assess faecal contamination in water treatments. Although it is part of the coliform group, *E. coli* has the peculiar ability to produce β-glucuronidase, what differs them from the others coliforms (Ashbolt, *et al.*, 2001). Although it is easily identifiable within the faecal coliforms, they share similar limitations. WHO (2006) established a guideline of *E. coli* $\leq 10^3$ cfu/100 ml for safe use of wastewater, excreta and grey water in agriculture.

Intestinal *Enterococci* are streptococci microorganisms with the ability of growing at pH 9.6, temperature range of 10 to 45 ^{o}C and in a 6.5 % NaCl environment. Apart from that, these microorganisms can be exposed to temperatures of 60 ^{o}C for up to 30 minutes and they are able to reduce 0.1% methylene blue (Ashbolt, *et al.*, 2001). Due to this ability to resist adverse conditions and the fact that they rarely breed in water, faecal *Enterococci* can be considered as better indicator than *E. coli* (Gleeson, *et al.*, 2002).

Helminths are eukaryote microorganisms which include nematodes (round worms), cestodes (flat worms) and trematodes (flukes) commonly found in developing countries. A round shaped worm, *Ascaris lumbricoides* is often adopted as indicator due to the ability of its eggs to resist treatments. Therefore, the inactivation or removal of the *Ascaris* eggs is a strong indication of the efficiency of the treatment process since it ensures that the less resistant microorganisms have been removed. The accurate procedure to evaluate the performance of a treatment in *Ascaris* removal is by counting the viable eggs (Strande, *et al.*, 2014). A guideline of Helminth eggs $\leq$ 1.0 ova/litre has been established by WHO (2006) for safe reuse of wastewater, excreta and grey water.

Although *Enterococci* could be assumed as being better pathogen indicators than *E. coli* as described above, and recognizing that the best would be to investigated both *E. coli* and *Enterococci*, this research will be restricted to investigation of *E. coli* removal only. This fact is due to logistical constraints on the availability of proper laboratory equipment to conduct the analyses for *Enterococci* (or Helminth eggs). Moreover, reduction in odour, VS and fly attraction will also be considered in assessing sanitisation of faecal sludge.

2.4.2. Stabilisation of faecal sludge

Stabilisation of faecal sludge is the processes in which readily degradable matter is converted, resulting in a more stable material consisting of complex molecules with lower oxygen demand which does not adversely affect the disposal in the environment (Andreoli, *et al.*, 2007, Strande, *et al.*, 2014). The typical process consists of biological breakdown of fermentable organics in the presence or absence of oxygen (Rofe, 1994). Moreover, sludge can also be stabilised through non-biological processes and the most common mechanisms are chemical and heat stabilisation (Veenstra, 1999). Total volatile solids (VS), BOD and COD are the commonly adopted indicators of faecal sludge stability (Strande, *et al.*, 2014). However, other factors such as Specific Oxygen Uptake Rate (SOUP), pathogen and odour reduction can also be considered (Eikum, *et al.*, 1977, Kazimierczak, 2012, Strande, *et al.*, 2014). A sludge is considered as being stable when a volatile solids reduction of 38 % or more is achieved (EPA, 1995, EPA, 1993, Kazimierczak, 2012).

Indicators of stability

BOD measures the oxygen requirement for biodegradation of organic matter, but not all oxygen demanding material in faecal sludge is biodegradable, what can affect the reliability of the BOD measurement if not accounted. The measurement of BOD can also be affected by other factors such as particulate size distribution, sampling and analytical procedures such as filtration and dilutions, what makes difficult to replicate the results (APHA, *et al.*, 1999, Ramalho, 2012, Strande, *et al.*, 2014). Moreover, the standard measurement procedure implies an incubation period of 5 days (BOD_5) which can be considered too long waiting period to obtain results of a test (Ramalho, 2012).

On another hand, the COD test is faster (taking 1.5 – 3 hours) and more representative than BOD. It is an important parameter and widely used to assess performance of wastewater treatment process through mass balances (APHA, *et al.*, 1999, Henze, *et al.*, 2008). The measurement of COD is used to determine the majority of organic fraction present in the substrate through oxidation by a strong chemical oxidant (normally potassium dichromate) (Henze, *et al.*, 2008). The standard closed reflux method applied to measure COD has an upper determination limit of 400 mg/l and dilutions are required for higher values as in the case of faecal sludge (APHA, *et al.*, 1999, Strande, *et al.*, 2014). Although it has advantages over BOD, the reliability of the COD measurement can be adversely affected by the presence of suspended solids, and it is extremely important to have an homogeneous sample (Boyles, 1997). Moreover, it is important to note that not all biodegradable material is indeed degraded by microorganisms.

The reduction of total volatile solids (TVS) is widely used as indicator to assess stability of treated sludge, being the simplest and direct method (Andreoli, *et al.*, 2007). A sludge is considered to be stable when a volatile solids reduction greater than or equals to 38 % (EPA, 1995, EPA, 1993, Kazimierczak, 2012).

In this research, the measurement of stability of treated faecal sludge will be based on VS reduction, as well as odour and pathogen reduction.

2.5. Enhancing stabilisation and stabilisation of faecal sludge in emergency

Although there are several additives in the market which are claimed by the manufactures to be able to increase the rate of degradability of faecal sludge and extend the life period of a pit latrine, scientific studies to assess the veracity of this claim are divergent. Recently in 2014, UNESCO-IHE in association with WASTE, conducted laboratory and field trials to evaluate the effectiveness of biological and chemical additives for faecal sludge treatment in the relief and earlier recovery phases of an emergencies. The laboratory experiment was conducted at UNESCO-IHE laboratory in Delft, Netherlands, in 500 ml bottles using black water, while the field trials were conducted in 50 litre plastic bucket in Blantyre, Malawi, using faecal sludge. Lactic acid bacteria, urea, lime and other eleven chemical and biological additives were evaluated (Anderson, *et al.*, 2015, Kemboi, 2015).

2.5.1. Lactic acid treatment

Malambo (2014) evaluated the anti-pathogenic effect the probiotic milk product *Yakult*, containing a strain of the lactic acid bacteria *Lactobacillus casei* Shirota. This technology has been applied in the food industry (Vandenbergh, 1993). The field experiment was conducted in triplicate as a batch process for 9 days, using a 7 years old faecal sludge emptied from a pit latrine. The optimum sugar and inoculum concentrations applied were previously determined in the laboratory experiment. A control unit was set.
As result, it was shown that lactic acid was able to reduce *E. coli* below detectable levels after 7 days, having an average reduction from 1.5 x 10^8 to less than 10^3 cfu/100 ml (more than 5 Log units) and it is suggested that the inactivation of bacteria was caused by the acid environment induced by the treatment (a pH of approximately 4 was recorded). This conclusion is supported by Helander, *et al.* (1997), who explains that the antipathogenic effect of lactic acid is due to its ability to penetrate the cytoplasmic membrane of microorganism when undissociated, reducing the intracellular pH. This, results in disruption of the trans-membrane proton motive force of the lipopolysaccharides molecules of the microorganism and consequently causing microbial die-off (Helander, *et al.*, 1997). Based on this results, the researcher concluded that lactic acid can be used to treat faecal sludge in emergency situation and the treatment cost was estimated to be € 2/m^3 of faecal sludge with an initial cost for pre-culturing of € 33.2.

The high concentration of lactic acid bacteria found at the treated sludge confers it the potential to be used has inoculum culture for subsequent treatment batches, thus minimising cost, however the performance of this technology is dependent on some environmental conditions of faecal sludge, such as temperature and pH (Sheeladevi, 2011, Yuwono, *et al.*, 2008). High temperatures and low pH, (30 – 40 ºC) and (5.5 – 4) respectively, are favourable for growth of lactic acid bacteria (Büyükkileci, *et al.*, 2004, Malambo, 2014, Sheeladevi, 2011).

Although the technology achieved the WHO guideline of *E. coli* removal for unrestricted irrigation of root crops (*E. coli* ≤ 10^3 cfu/100 ml), the environmental dependence could make it unsuitable for application in humanitarian relief camps located at colder regions in the world or for unlined pit latrines which are dug in soils containing carbonates. Furthermore, trained personnel is required for the initial phase of the treatment process (pre-culturing of lactic acid

bacteria), and that may not be available at the earlier stage of a humanitarian response of an emergency. This factor could limit the applicability of the technology. Moreover, the effect of this treatment technology in volume reduction and in another pathogen such as *Enterococcus* was not investigated though *Enterococcus* seemed to be more resistant to some additives treatments than *E. coli* (Kemboi, 2015, Perez, 2014). Besides that, the treatment end product is acidic, what could require further treatment if to be disposed in the environment.

2.5.2. Urea treatment

When urea is added to faecal sludge it will decompose into ammonia and carbonate, in a processes enhanced by the enzyme urease which is present in faeces. The product of this decomposition process has an alkaline pH which leads to ammonia formation. Ammonia then can penetrate the walls and cellular membranes of bacteria by diffusion process enhanced by its high solubility in water and in lipids, causing disruption of the membrane potential, destructing the cells proteins and the bacterial membrane, what leads to pathogens die-off (Bujoczek, 2001). It is important to note that the pathogen removal mechanism of urea treatment is similar to that of lactic acid, investigated by Malambo (2014).

By conducting an experiment in two trials, Perez (2014) investigated the effect of urea on *E. coli*, and other bacteria reported as *Salmonella*, total coliforms and *Enterococcus* removal on faecal sludge. The first trial determined the optimum dosage which were then tested in the second trial to assess the effect of mixing intensity on the performance of the treatment. The experiment showed that *E. coli* reduction, as well as inactivation of *Salmonella* and total coliforms, was achieved below WHO guidelines in 4 days of urea treatment (40 % purity) at a dose of 1 % (w/w). The treatment cost was estimated to be € 16/m^3 of faecal sludge. However Perez (2014) identified that the "*novelty ammonia approach*" had no effect on the white colonies on the plate *(reported as Enterococcus*) during the period of the experiment (3 days), even after doubling the dosage, although it has been reported to achieve such results over longer period (Magri, *et al.*, 2013).

The upscaling of this technology for faecal sludge treatment in emergencies would require existence of further treatment unit such as sludge drying beds since the final product from urea treatment is not stabilised (Perez, 2014). Besides that, temperature plays an important role on decomposition of urea with urease, therefore the wide application of the treatment technology is limited. Moreover, the urea treatment will not be suitable for direct application in pit latrines since an air-tight vessel and a mixer are required to avoid ammonia volatilization and ensure homogenization of the sludge, respectively, for better performance of the treatment (Perez, 2014). Apart from that the toxic nature of ammonia gas, its unpleasant odour and the possible adverse effects of urea (irritation, redness and pain to the skin and eyes) may conflict with the safety criteria for faecal sludge treatment in emergency situations (WASTE, *et al.*, 2012).

2.5.3. Alkaline treatment (lime treatment)

Lime has been traditionally used in wastewater treatment plant as sludge conditioner. Its application in sewage sludge treatment has revealed its additional abilities to reduce odour and pathogenic population in the treated sludge, thus improved its potential reuse in agriculture (Strande, *et al.*, 2014, Veenstra, 1999, Williford, *et al.*, 2007). The alkaline nature of lime confers it the ability to increase the pH of faecal sludge above 12, as well as temperature (due to the exothermic reactions) and ammonia concentration. The combination of this effects is

harmful for microorganism, resulting in pathogens die-off (Pecson, *et al.*, 2005, Polprasert, *et al.*, 1981, Strande, *et al.*, 2014, Wong, *et al.*, 2000).

Nobela (2014) studied the effectiveness of lime treatment for safe disposal of faecal sludge to the environment, in emergencies. Nobela (2014) tested the effect of dosage on the increase of pH to find the optimum dosing range, where then, the anti-pathogenic ability of lime was assessed. *E. coli* and total coliforms were rapidly inactivated to undetectable levels after 5 hours at pH 10, with a lime dosing range of 5 – 12 %, and after less than 1 hour at pH 11 with a lime dosing range of 7 – 17 %, thus complying with WHO guideline for safe reuse/disposal (of $\leq 10^3$ cfu/100 ml). For total coliforms, the reduction below detectable levels was achieved 2 hours and 1 hour, at pH 11.5 and 12 and with lime dosing range of 9 – 19 % and 10 – 24 %, respectively. The treatment cost was estimated to be € 12/m^3 of faecal sludge.

Based on these results, Nobela (2014) recommends the used of lime for faecal sludge in emergency, due to its ability to rapidly inactivate pathogens. However, it has been observed that higher lime dosages are required in order to speed up the pathogen removal effect of lime treatment what could imply high cost. Moreover, the performance of the lime treatment is greatly improved by ensuring homogeneous mixing of faecal sludge and lime in the container, what could require a special apparatus/device if the technology is to be applied. Furthermore, the pH drops after the initial reaction, thus increasing potential regrowth of pathogens (Strande, *et al.*, 2014). Besides that, lime will increase the dry solids content of the sludge, due to its poor solubility in aqueous solution thus increasing the cost of the final treatment step. Apart from that, special safety measures are required when handling lime since it is harmful to the skin, eyes and lungs (Strande, *et al.*, 2014, Veenstra, 1999).

2.5.4. Chemical and biological additives

Kemboi (2015) investigated the effect of different biological and chemical additives on speedy stabilisation and sanitisation of faecal sludge. In total 11 additives were tested, chosen based on the requirements for faecal sludge treatment in emergency situations. After testing the additives for 7 and 14 days, at dosages 1.7 % and 3.4 % (w/w or v/w), the results showed a promising sanitisation and stabilisation effect on faecal sludge treated with chemical additives, especially for Ikati (naturally mined carbonate) and Soda (Sodium carbonate).

Although the performance of the additives was dependent on total solids concentration in faecal sludge, the chemical additives Ikati and Soda were able to achieve *E. coli* removal below 10^3 cfu/100 ml and volatile solids reduction higher than 38 % at lower total solids concentration. In the meantime, no significant reduction in volatile solids and in *E. coli* concentration was found between faecal sludge treated with bio-additives and the controls, what finds support on conclusions from previous studied on additives (Bakare, *et al.*, 2015, Buckley, *et al.*, 2008, Foxon, *et al.*, 2009). It is stated that the sanitising effect of the chemical additives Ikati and Soda was attributed to the carbonate ion which is present on their composition, as suggested by Jarvis et al. (2001), Park et al. (2003), Arthurs et al. (2001) and Diez-Gonzalez et al. (2000) when assessing the carbonate effect on cow manure. However, further researches are required to better understand the anti-pathogenic mechanism of carbonate (Russell, *et al.*, 2001).

Though the additives Ikati and Soda showed promising effect in rapidly treat faecal sludge, the pathogen die-off and the storage period of treated sludge after which reactivation of the *E. coli* may occur, was not investigated. Additionally, not all bio-additives were tested and time was scarce to investigate the effect of biological additives over long time.

It was observed that the performance of the promising chemical additives Ikati and Soda is dependent on the solids concentration of the sludge (Kemboi, 2015). Moreover, it is important to note that the pH of the units treated with additives Ikati and Soda increased above 9, what could have contributed to their treatment performance (Couderc, *et al.*, 2008, Nobela, 2014, Russell, *et al.*, 2001).

2.6. Other researches on effectiveness of additives

(Jere, *et al.*, 1998) investigated the effect of a spore forming non-pathogenic bacteria in speeding up the rate of degradation of the pit latrine sludge, as an alternative to solution to desludging. A dosage of 300 g was applied once a week in four pit latrines, over a period of 4 weeks. The researchers found that the volume of the pit content decreased after the experimental period thus concluding that the bio-organic breakdown compound were able to reduce the filling rate of pit latrines. The conclusion is however not solid due to the fact that no control was used for comparison against the treatment. Moreover, the pressure and mixing factors introduced by the dosing procedure were not taken into account when analysing the performance of the additive (Foxon, *et al.*, 2009).

Bakare *et al.*, (2015) conducted a laboratory and field research to evaluate the effect of pit latrine additives on VIP content. Two unidentified different additives were used. Under laboratory trials, 300 g of VIP sludge was filled in a 300 ml plastic bottle. The test was performed in five replicates, including blanks (water reference and control), over 30 days. For the field experiment, 30 pit latrines were selected being 16 used as treatment units (8 latrines for each additive), 7 as control and another 7 as water reference and trial was conducted during 6 months. Two different approaches were used to assess the effect of the additives in volume reduction. The first method measured the distance between the surface of sludge within the pit and the pedestal of the latrine with aid of a laser tap measure. On the other hand, the second method used a novel approach of stereoscopic digital photograph, which gave a 3D images with spatial coordinates of the surface of the pit content.
Differences between controls and treated units were observed in both laboratory and field trials, however the author concluded that these differences were statistically insignificant, thus cautiously concluding that there was no evidences of the effectiveness of pit latrine additives in reducing the filling rate of pit latrines.

Foxon *et al.*, (2009) evaluated the effectiveness of biological additive when developing a protocol for testing pit latrine additives in the laboratory. Two trials were conducted, using 300 ml honey jar filled with 300 g of faecal sludge samples form VIP latrines and the set-up included controls (water reference and blanks) in 3 or 5 replicates. In the first trial, 7 additives were evaluated under both aerobic and anaerobic conditions, over a period of 46 days. The second trial was conducted in 27 days, testing four new additives together with another three used in the first trial. This experiment was performed only under aerobic conditions, and

controlling the rate of dehydration of the samples by increasing the humidity in the fume cupboard.
The researcher found that biological degradation processes occurred faster in the presence of oxygen as has been previously reported (Henze, *et al.*, 2008, Metcalf & Eddy, 2003). Moreover, it was observed that significant mass loss occurred in all aerobic reactors, however the reduction attained by the different treatments and the controls was found to be of the same statistical magnitude. Therefore, the researcher concluded not to have found evidences that additives can enhance degradation of organic matter in pit latrines.

2.7. Application protocols of biological additives

The diversity of biological additives claimed to reduce the filling rate of pit latrines is proportional to the variety of their different application procedures. Normally, each product has its own dosing protocol which is recommend by the manufacture. The biological additives application protocols ranges from the simple single dose applied every three month, once a month, to daily and even per latrine usage (bio-systems SA, 2015; Sannitree, 2015; SepClean, 2015; BCI Environment, 2015).

Jere *et al.,* (1998) injected non-pathogenic bacteria into a pit using a perforated pressure tube, what could have contributed to the positive results obtained (Foxon, *et al.*, 2009). Taljaard *et al.*, (2003) adopted a dosing ratio of 2:5 (faecal sludge to additive) and the results showed a promising ability of biological additive to reduce the content of pit latrines, however the results could have been influenced by the high dosage adopted (Foxon, *et al.*, 2009).
A marked biological additive Consortium Lice SM, with a dosing protocol of an initial seeding of 1 kg/m^3 total pit-volume in the first day, a starter dose of 500g/m^3 of pit-volume from day 2 to day 6 and a maintenance dose of 25g/person/per day use, starting at day 7 beyond, is claimed to have reduced 100 % of the pit content during a maintenance period of 45 days, in a trial conducted in Chad, (UNHCR, 2015).

There is a potential to enhance the effect of biological additives in reducing the filling rate of pit latrines by adopting an adequate application protocol (Foxon, *et al.*, 2009). Therefore, in this research the LICE Consortium SM application protocol (Figure A-2 in Appendix C) was adopted for evaluation, by applying it in another biological additive. The choice of LICE protocol was based on the impressive results that are claimed to be achieve in previous experiments.

2.8. Additives selected for experiments

The laboratory scale experiment conducted in Naivasha (Kenya) tested a total of 5 additives, being three chemical (Ikati, Soda and Ash) and two biological (Ecotreat and Sannitree). The selection criteria is presented on Section 3.2.2., however the characteristics of the additives are described below.

2.8.1. Chemical additives

Since promising results on treatment of FS had been observed on previous experiment conducted in Malawi with chemical additives Ikati and Soda, therefore the products had been selected for further investigation on the present research (Kemboi, 2015).

Sodium carbonate

Also known as washing soda or soda ash, sodium carbonate (Na_2CO_3) is a sodium salt of carbonic acid (see figure 3). It is a white crystalline powder, odourless and exhibits hygroscopic properties when exposed to air. Apart from that, it is soluble in water and when dissolved it splits into ions. When added to FS sodium carbonate will impose a high pH which together with carbonate is said to impact on die-off of *E. coli* bacteria (Diez-Gonzalez, *et al.*, 2002, Diez-Gonzalez, *et al.*, 2000, Jarvis, *et al.*, 2001, Kemboi, 2015). When Jarvis *et al.* (2001) studied the mechanism of carbonate killing of *E. coli*, he carefully concluded that the divalent metal binding property of carbonate was the key factor responsible for inactivation for *E. coli* by destabilizing the neutral repulsive forces and salt bridges that protect the lipopolysaccharides molecules and or proteins, therefore resulting in inactivation and release of periplasmic proteins from the cells. Besides that, soda ash is also used as conditioner in water treatment, in order to improve coagulation and flocculation processes, however there is lack of information about similar effects on faecal sludge.

Ikati

It is an odourless grey crystalline product obtained from soda ash mining and normally marketed in Hardwares in Kenya by the name of *magadi soda* (see figure 3). The product has been used in villages in Kenya since decades with the claim supported by a cultural believe that it reduces the filling rate of pit latrines. Being a by-product of soda ash, Ikati exhibits similar properties as sodium carbonate and they are expected to produce similar results as observed by Kemboi (2015).

Figure 3: Chemical additives Ikati and Soda (L - R)

Ash

Since decades that wood ash has been used as desiccant for faecal sludge in low income communities all over the world. The practice of cover the sludge with ash after each defecation has been seen as a simple and cheap technology to minimise risk of spreading of disease vector transmission from faeces in poor communities such that the practice has been embraced and defunded by health organizations. Furthermore, the use of ash has become popular among the urine diverting dehydrating toilet (UDDT) users and container-based toilet companies for the reasons mentioned above and also due to its claimed ability to rapidly inactivate odour nuisance thus reducing fly attraction and therefore breaking a disease vector transmission route.

However, no scientific evidences are found to support this claim. Besides that, ash has been said to have the ability to inactivate *E. coli* bacteria by rising the pH of faecal sludge to unsuitable levels for *E. coli* survival (Ivanković, *et al.*, 2014, Niwagaba, *et al.*, 2009, Stenström, *et al.*, 2004). Niwagaba *et al.* (2014), after comparing antibacterial effect of ash and sawdust dosed during the collection phase of source-separated faeces of a UDDT, observed that *E. coli* die-off in the first 7 days of treatment was faster for faecal sludge treated with ash relatively to the one treated with sawdust. Moreover, he observed that the bacteria was still detectable for sawdust treatment while it was not detected in ash treatment after 60 days. This result was attributed to the combination of both desiccation and alkalinity effects imposed by addition of ash (Niwagaba, *et al.*, 2009). However, it is suggested that the antibacterial effect of ash depends on the dose and the type of ash. Ash can be obtained from combustion of different materials, such as firewood and leaves, from wood chips, and from plants (Stenström, *et al.*, 2004).

2.8.2. Biological additives

Two biological additives were used in the research and their selection was mainly based on the availability, however other factors were considered and they are described on section 3.2.2.

Ecotreat

Originally from Kenya, Ecotreat is a biological additive produced by a company called EcoSave. Its culture is made up of a single bacteria strain from *Bacillus* sp. which rapidly multiply. The enzyme secreting bacterium in Ecotreat are facultative, meaning that it can service and be active in both aerobic and anaerobic conditions (Wanjuki, personal communication, Thursday, December 10, 2015 05:04PM).

The product has been reported to improve the performance of anaerobic sequencing batch reactors by preserving healthy microbial population and enhancing the metabolism and rate of digestion of organic matter (Ochieng', 2015). By applying dosages of 0.5 % and 1.0 % of a slaughterhouse effluent and having 0 % as control, reductions of 50 % in TS and 91 % in COD were observed after 8 hours of contact time. The researcher further observed that a reduction of 14 % in COD was achieved at the dosage of 1 %. However, despite the fact that the experiment was conducted with no replicates for comparison of the results (only one reactors per dose), other parameters of interest for the present research were not monitored.

Sannitree

Information regarding Sannitree is scarce, however is known that it is a consortium of microorganisms namely *Nitrosomonas* sp., *Nitrobacter* sp., *Aerobacter* sp., *Bacillus subtilis*, *Cellulomonas* sp., and enzymes such as Protease, Amylase, Hemicellulase, Lactase, Lipase. The product is manufactured in South Africa and sold with a claim of being able to reduce odour in pit toilets, reduce fly population and the pump-out frequency as well as prolong the life of the soakaway.

2.9. Statistical analysis

2.9.1. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) is a statistical procedure employed to assess if two or more groups of data of an experiment differ significantly or not. It compares the difference of the

means among and within the experiments (Hinkelmann, *et al.*, 2008, Montgomery, 2000). ANOVA is a generalisation of the Student's T-test, where the responses of more than two groups of experiments can be compared. One-way ANOVA is applied when comparing the effect of different treatments on a particular response (monitoring parameters). On the other hand, if two independent variables are considered (e.g.: assessing effect of different treatments over time), then the use of two-way ANOVA would be appropriate. Moreover, the test is normally performed adopting a significance level of 5 % and confidence interval of 95 % (Norman, *et al.*, 2008, Plichta, *et al.*, 2009, Seltman, 2012).
As the rule of thumb, the null hypothesis assumes that there is no significant difference between the means of the groups (between treatments). If the calculated p-value is smaller than the adopted level of significance, then there are enough evidences to reject the null hypothesis. The rejection of the null hypothesis is an indication that at least one of the means differ from the others. Moreover, the smaller the p-value, the higher the significance of the difference (Aczel, 1995, Rubin, 2012). Furthermore, when significant difference is found, a post-hoc test is conducted in order to locate the differences. For this propose, the TukeyHSD post-hoc test is normally adopted (Dean, *et al.*, 1999, Hinkelmann, *et al.*, 2008, Seltman, 2012).

2.9.2. Correlation analysis

Correlation analysis is applied to evaluate the strength of the relationship between two variables. A correlation coefficient (r) is determined and it ranges between negative one (-1) and one (1) (Seltman, 2012).

The following conclusion are drawn from the correlation coefficient (Seltman, 2012, van Belle, *et al.*, 2004):

- The closer to (-1), the stronger the negative relationship
- The closer to (1), the stronger the positive relationship
- The closer to (0), the weaker any relationship

2.10. Cost-effectiveness analysis

Cost-effectiveness analysis (CEA) is an economic evaluation which relates the cost of a treatment (program) to its major benefits (outcomes). It is used in planning as an alternative to cost-benefits analysis (CBA) when comparing different options (different treatments) that provide different levels of a specific outcome. Therefore, it helps to identify the option that will best value the investment money, thus helping in decision making (Cellini, *et al.*, 2010, Edejer, 2003). The major advantage of CEA over CBA is that the outcome should not necessarily be monetary, however it can only analyse one outcome at a time (Compernolle, *et al.*, 2008).
When independent options are compared, their respective cost-effectiveness ratios (CERs) are determined and ranked. The lower the CER, the more cost-effective the option (Phillips, 2009).

$$CER = \frac{Cost\ of\ intervention}{Effect\ produced}$$

Equation 1: Determination of cost-effectiveness ratio

CHAPTER 3

Materials and methods

This chapter describes the material and methods that were applied it this research in order to achieve the objectives. It presents the summary of the methodology used in a form of a scheme, describes the experiments with respective set-ups, dosing protocols and sampling methodology as well as the analytical procedures adopted.

3.1. Methodology

The research was carried out in three main stages, namely: desk study, field work and result analysis & report writing. The methodology is summarised in the figure 4 below:

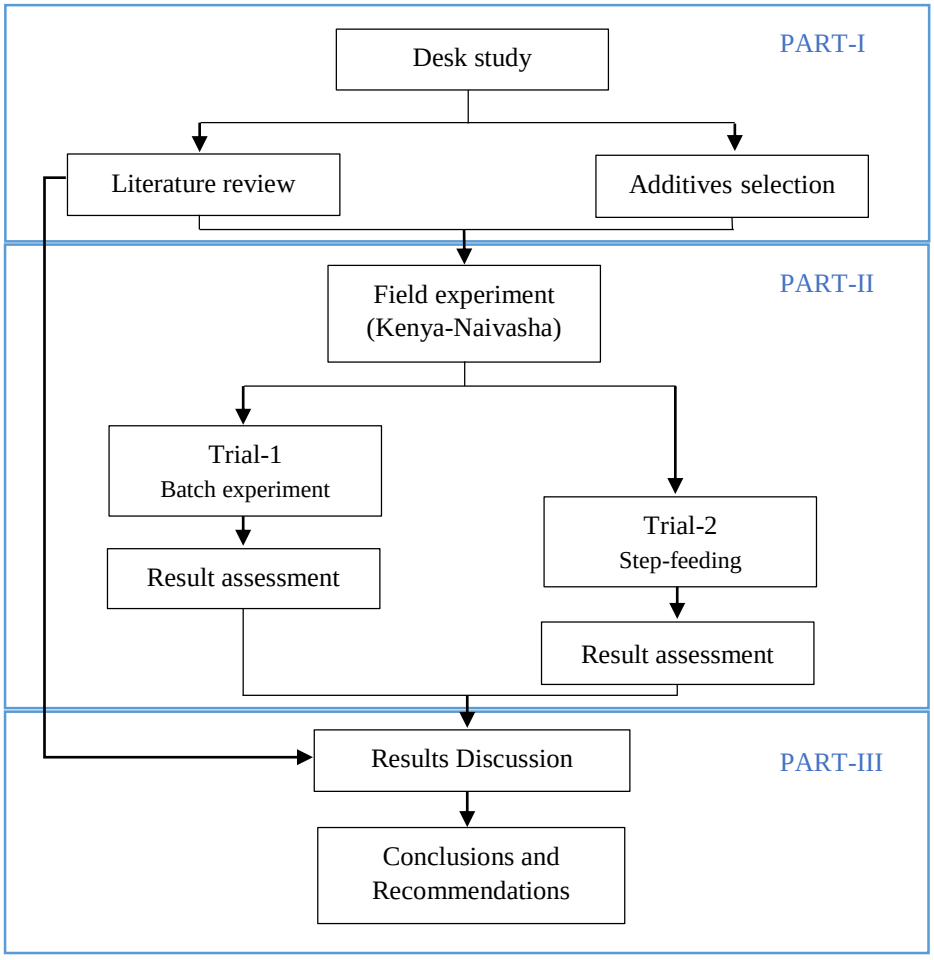


Figure 4: Methodology scheme

3.2. Desk study

3.2.1. Literature review

Literature review was conducted by searching for information that is relevant to the objective. The main focus considered are:

- General information about disasters and emergency trends worldwide.
- Relationship between emergency, disease and sanitation.
- Information about the tested additives.
- Scientific papers on the use of additives for rapid sanitisation, stabilisation and volume reduction of faecal sludge.

3.2.2. Selection of additives

The chemical additives were selected from previous research based on their promising effect on enhancing sanitisation and stabilisation of faecal sludge (Kemboi, 2015). The choice of the biological additive was mainly based on availability and costs. However the aspects considered are presented on table 2 below:

Table 2: Criteria for selection of additives

Requirements	Description
Expected results	Should be clearly stated the ability to slowdown the filling rate of pit latrines and impacts on odour and fly attraction
Dosing protocol	The application procedure should be indicated in the label, clearly described and it should be simple to be easily understood by an ordinary member of a community.
Safety	The product should not be hazardous to people and to the environment.
Storage	Additive can be stored at ambient temperatures for a period not less than 12 months.

A free samples of biological additives Sannitree Bio-Enzyme Granules for Pit Toilets (Figure C-1 in Appendix C) and Ecotreat were provided by the respective suppliers. The products came from South Africa and Kenya, respectively, and they meet the selection criteria above described, therefore they were used in the research.

3.3. Fieldwork: experimental set-up

The experiment was conducted in Naivasha, Kenya, in a sanitation service provider called Sanivation which provides container based UDDT to the customers and then collects the faecal sludge twice a week, which is then transformed into a clean burning alternative charcoal (Figure C-2 in Appendix C). In general, three chemical additives (Ikati, Soda and Ash) and two biological (Sannitree and Ecotreat) were tested, and the experimental protocol consisted of a set of testing units (with addition of additives) and controls (water reference: with addition of only water; and blank: no addition of neither additive either water), tested in a 10 litre plastic containers, using fresh faecal sludge (not old than two weeks) collected from Naivasha Prison. Additionally, the reactors were covered on top with a mosquito net to prevent fly breathing inside the vessel, what would disturb the experiment. Samples for faecal sludge characterisation were collected from each unit after stirring the mixture manually for two minutes, with the aid

of a wooden stick (figure 5). The parameters monitored were: height, TS, VS, pH, temperature, odour, fly attraction, and *E. coli*.

Figure 5: The researcher stirring the reactors before sampling

Two trials were performed:

3.3.1. Batch experiment: Investigating effectiveness of different additives under manufacture's dosing protocols (trial-1)

This experiment aimed to investigate the effectiveness of additives in reducing the volume of faecal sludge in a pit latrine that is already full and four additives were used, namely, Ikati, Soda, Sannitree and Ecotreat. The set-up comprised of a total of eighteen reactors (see figure 6 below), being triplicate for each treatment as well as for the controls (blank and water reference). Each reactor was filled with 2.25 kg of fresh faecal sludge and then the respective dose of the additive was added for each set of three (see table 3 below). It is important to mention that the chemical additive Ikati was sieved using a mosquito net before being applied to remove trash and ensure small and uniform particle size.

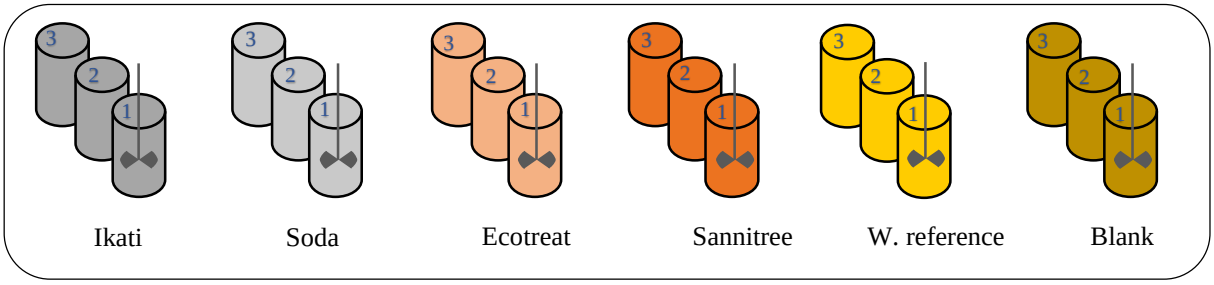


Figure 6: Schematic representation of experimental set-up on trial-1

Dosing protocol of the additives

The adopted dose of 7 % (w/w) for chemical additives Ikati and Soda was determined by doubling the dose adopted by Kemboi (2015), from which the additives showed promising effect in sanitisation and stabilisation of FS. Although Kemboi (2015) observed that the performance of the chemical additives Ikati and Soda was TS dependent, it was adopted a dose based on weight due to applicability reasons, since it is difficult to know on beforehand the TS concentration of the sludge in order to determine the respective dose under an emergency situation. On the other hand, the manufacture's recommended dose was adopted for biological

additives Ecotreat and Sannitree, which were 4.0 and 3.3 %, respectively. The dosing protocol is summarised in table 3 below.

Table 3: Adopted dosing protocol of chemical and biological additives for trial-1

Chemical additives		Dosing protocol			Remarks
	Set-up	Dosage (w/w)	FS quantity	Product dosage	
Name	No.	[%]	[g]	[g]	
Ikati	1.1	7.0	2250	157.5	Single dose
Soda	1.2	7.0	2250	157.5	Single dose

Biological additives	Dosing protocol				Dilution water	Remarks
	Set-up	Recommen. dose	FS	Applied dosage		
Name	No.	[%]	[g]	[g or mL]	[mL]	
Sannitree*	1.3	3.3	2250	8.3	412.5	Single dose
Ecotreat	1.4	4.0	2250	90.0	-	Single dose

*Dosage based on the size of the pit (100 g/3 m³), therefore a multiplication factor was adopted to account for number of users (see Appendix A)

Sampling and response evaluation

A sample of 1 kg was collected from the raw FS at the beginning of the experiment for initial characterization, and subsequent samples of 150 g were collected once a week on each reactor (every 7 days) to evaluate the responses of the treatments and the controls. However, the parameters *E. coli*, temperature, height and pH were intensively monitored in the first week of the experiment, being pH and temperature measured daily and height on days 1, 3, 5 and 7 for all reactors, while *E. coli* was analysed on the first 3 days particularly for Ikati, Soda and Blank.

Figure 7: Experimental set-up of train-1 (10 L buckets in triplicate for each treatment, including the controls)

3.3.2. Step-feeding experiment: Investigating the efficiency of Consortium LICE SM dosing protocol (trial-2)

The main objective of this experiment was to analyse the effectiveness of additives in slowing down the filling rate of pit latrines. Due to resources limitations (faecal sludge), this trial was conducted in duplicate and only two additives were tested, being one biological (Ecotreat) and the other chemical (wood-ash). Besides the treatments, a set of blank and water reference was also included (see figure 8 below). Furthermore, Ash was sieved before application, for the same reasons as it was done with Ikati. The choice of Ecotreat was based on the fact that less assumptions had been made in scaling down the dosage and the product was locally made, therefore it would have been easier to obtain if needed. The experiment had a total duration of 35 days.

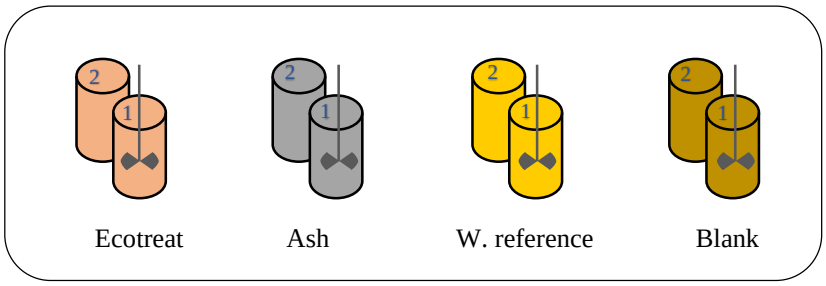


Figure 8: Schematic representation of experimental set-up on trial-2

Dosing protocol of the additives

For this experiment, the biological additive was dosed following the dosing protocol of the biological additive Consortium LICE SM, which has been claimed to produce better results. This protocol consists of initial seeding (day 1), starter dosing (from day 2 to day 6) and maintenance dosing (from day 7 and beyond).

For the chemical additive Ash, a daily use of a pit latrine was simulated assuming that the additive is dosed after every use of the toilet. Therefore the dosage was determined by weighting the amount of ash taken with hands, using a sample size of five persons, and then the arithmetic average was determined (table A-3 in Appendix-A). A result of 44.4±2.1 g/user was obtained and it was similar to the dosage determined by Sanivation (between 40 – 50 g/user) after conducting similar exercise with three of their customers. By following the recommendation from Sanivation, a dose of 20 % (w/w) was adopted. Moreover, custom made scoops were used for daily addition of 75 g of faecal sludge and its respective doses of Ecotreat as per protocol. The dosing protocol is summarised in table 4 below.

Table 4: Adopted dosing protocol of biological (Ecotreat) and chemical (Ash) additives for trial-2

Phase	Day	Ecotreat	FS [g]	Ash	
		Quick priming [mL]		Ratio [%]	Dose [g]
Initial seeding [lice/pit volume]	1	21.3	75.0	20.0	15.0
Starter [lice/pit volume]	2 to 6	3.5	75.0	20.0	15.0
Maintenance	7 to 28	2.1	75.0	20.0	15.0
Testing sanitisation	29 to 35	0.0	0.0	20.0	0.0

Figure 9: A view from inside of the reactors of trial-2 (L-R: Ecotreat, Ash, Water reference and Blank)

Figure 10: Taking measurement from the panel in order to determine the dose of Ash.

Sampling and response evaluation

Samples for characterisation were collected before, during (every 7 days) and after treatment, and the above listed parameters were analysed. However *E. coli* removal was analysed from the second week (day 14) for reactors treated with Ash and for Blank while it was assessed at the end of the experiment for Ecotreat and water reference (days 28 and 35). This schedule is justified by the fact that the experiment protocol required daily addition of fresh faecal sludge throughout the experimental period, meaning addition of bacteria as well, therefore the sanitisation effect was not expected to occur. For this reason the feeding process was stopped after four weeks (28 days) and the reactors were left for additional 7 days in order to investigate the ability of the treatments in reducing *E. coli* concentration.

3.4. Analytical methods

3.4.1. Physical, chemical and biological parameters

The methods, material and apparatus necessary to measure the parameters were adopted according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1999), with exception of fly attraction that has no standard or recommended test and they are summarised in table 5 below. Additionally, it is important to mention that *E. coli*, TS and part of Odour analysis was conducted at a special laboratory installed at Dea's Garden (residential address) while VS analysis was carried out at KEWI, in Nairobi.

Table 5: Parameters and methods of determination

Parameter	Method	Standard Method
Physical		
Total solids and volatile solids	Gravimetric method	SM 2540G
Temperature	Direct measurement (thermometer)	
Volume	Height measurement (tape measure)	
Odour	Threshold odour test	SM 2150
Fly attraction	Flies counting (panel of 5 persons)	
Chemical		
pH	Direct measurement (pH meter)	
Biological		
E.coli	Surface plate method	ISO 9308-1

TS analysis

Samples of 20 to 35 g of FS were added to pre-prepared aluminium cups (dried in the oven for 1 hour at 103-105 ^{o}C and weighted) and then put in the oven at 103-105 ^{o}C overnight. In the next day, the samples were removed from the oven a put in the desiccator to cool and then they were weighted and the value registered. After weighting the samples were put back into the oven at 103-105 ^{o}C for 1 hour then removed to desiccator and weighted again. TS was determined by applying the following formula (APHA, *et al.*, 1999):

$$TS\ [\%] = \frac{(A - B) \times 100}{C - B}$$

Equation 2: Determination of TS

Where:

A – weight of dried residue + dish, mg
B – weight of dish
C – weight of wet sample + dish, mg, and

VS analysis

After conducting TS analysis, the samples were put in the desiccator and transported to KEWI for VS analysis. This analysis consisted in burning the organic matter present in the sample at 530-550 ^{o}C for 1 hour in a muffle furnace and then it was removed to a desiccator before being weighted. It is important to refer that all samples were weighted before being placed into the furnace and again after burning for 1 hour. Afterward, the samples were put back to ignite for further 30 minutes and then weighted after cooling in a desiccator. The VS/TS was then determined using the formula below (APHA, *et al.*, 1999).

$$VS\ [\%] = \frac{(A - D) \times 100}{A - B}$$

Equation 3: Determination of VS/TS

Where:

A – weight of dried residue + dish, mg
B – weight of dish
C – weight of wet sample + dish, mg, and
D – weight of residue + dish after ignition, mg

Figure 11: The researcher conducting VS analysis at KEWI

Temperature and pH analysis

Temperature and pH were determined by direct measurement using a thermometer and a pH meter respectively after stirring the content of the reactors. The devices were inserted to about half of the depth of the sludge and besides that, the accuracy of the pH was checked always before conducting the measurements by measuring the pH of a known sample of distilled water. Additionally, the pH meter was re-calibrated once every three weeks.

Volume

Volume reduction was assessed by monitoring height variation with the aid of a tape-measure. The adopted procedure consisted in measure the depth of FS (from the top of the reactor to the surface of the sludge) and then subtract the total height of the reactor (28 cm) by the measured depth, thus obtaining the height of the sludge in the reactor.

$$h = H - d$$

Equation 4: Determination of height of FS in the reactors

Where:

h – height of FS in the reactor
H – total height of the reactor (= 28 cm)
d – measured depth

E. coli analysis

Chromocult Coliform Agar (CCA) was used as medium for culturing of *E. coli* bacteria, therefore 13.3 g of the powder was dissolved in 500 mL of distilled water in a rounded bottom flask and then heated at 100 ºC in a water bath for at least 90 minutes. A digital temperature logger was used to monitor the temperature inside the water bath. When the Chromocult was totally dissolved, it was put to cool to 50 ºC and then poured on 90 mm petri dishes which were left to dry at room temperature for at least 24 hours before use. Since the concentration of *E. coli* in fresh faecal sludge is high, series of dilutions had to be done in order to obtain clear and countable number of cells in the dishes (within a range of 30 – 300 cfu), therefore the dilution protocol shown in figure 13 below was adopted. Moreover, the dilution water was prepared by dissolving 4.3 g of sodium chloride in 500 ml of distilled water which was then sterilised at 121 ºC for 15 minutes and afterward it was left to cool and stored in the fridge before use.

Figure 12: The researcher conducting E. coli analysis

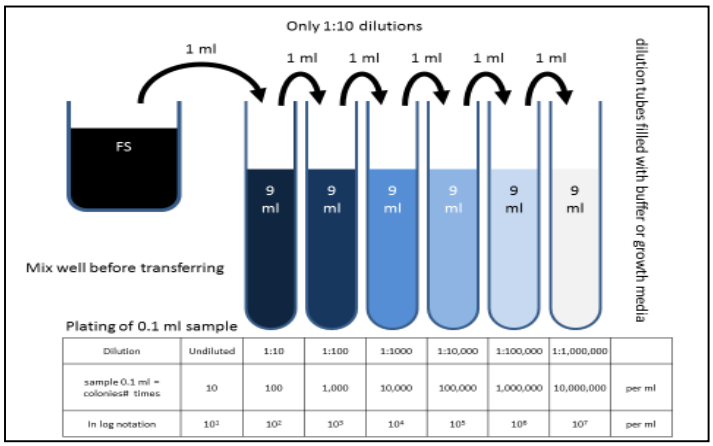


Figure 13: FS dilution protocol adopted for E. coli count and enumeration

Odour

Odour was assessed using the threshold method [SM-2150B] (APHA, *et al.*, 1999). This method consists of determining the threshold odour by sniffing a sample after dilution with an odour-free water. A panel of five and three was used, depending on the availability of personnel. The threshold number (TON) is defined as being the highest dilution ration of the sample with odour-free water at which odour is just noticeable. A series of dilutions was prepared and given to the panel to detect the presence or absence of odour. The samples were distributed from low concentrated to high concentrated, together with separate flasks of odour-free water as reference. The TON was determined form the last dilution beyond which odour was no longer noticeable. Each trial was performed using a total volume of 200 ml of the mixture of sample and odour-free water and the respective TON is determined (APHA, *et al.*, 1999).

It is reported as "no odour observed" when odour is not detected at 200 ml flask without dilution. And TON is equal to 1 when odour is observed in the 200 ml undiluted sample. An example of TON calculation is presented on table 6 below.

$$TON = \frac{A + B}{A}$$

Equation 5: Determination of threshold odour number

Where:

A – volume of sample [ml]

B – volume of odour-free water [ml]

Figure 14: Panellists and the researcher conducting odour analysis

Table 6: Determination of threshold odour number

FS sample (A)	Odour-free water (B)	TON
[mL]	[mL]	[-]
5.00	195.00	40
1.00	199.00	200
0.40	199.60	500
0.20	199.80	1000
0.15	199.85	1333
0.12	199.88	1667
0.11	199.89	1818
0.10	199.90	2000
0.09	199.91	2222

Fly attraction

For fly attraction, no standardised method of assessment was found in the literature. Some experiments have been conducted to assess attract ability of specific products to insects and to particular fly species (Bartlett, 1985, Becher, *et al.*, 2010), however the material and methods applied could not be feasible and appropriate to be implemented for faecal sludge in a field experiment. Therefore the following procedure was developed (Zindoga, 2016)

- A 1.0 m x 1.0 m x 1.0 m (1 m^3) tunnel covered by fly screen on the sides was prepared and used as the reference volume. The tunnel was then placed over the reactors for measurement and left there for 5 minutes. The number of flies that are trapped inside the tunnel was counted by a panel of five persons and the result registered. The final number of flies attracted was determined as the arithmetic average of the results from the panel. The result was then expressed as flies per cubic metre.

Figure 15: Invented apparatus for measurement of fly attraction

3.4.2. Statistical analysis

Descriptive statistical analysis was carried out by determining the mean and standard deviations for all treatments and bar graphs were used to summarise the results. Moreover, inferential statistical analysis was applied to help draw conclusions from the results of the experiment. As part of that, correlation analysis was performed in order to assess the relationship between pH

and *E. coli* removal. Additionally, analysis of variance (ANOVA) was conducted to qualify the magnitude of the effect of treatments by comparing the means.
It is important to mention that the response on odour reduction was not included on statistical analysis due to the fact that only one sample per group was analysed, therefore it is impossible to determine the mean and assess the deviation within the groups. Similarly to odour, the response on fly attraction was not statistically analysed due to the high standard deviations found within the groups. It should be noted that the analytical procedure of these two parameters provide a non-absolute result because of inherent variation in individual olfactory and visual capabilities (APHA, *et al.*, 1999).

3.4.3. Cost-effectiveness analysis

A cost-effectiveness analysis (CEA) was conducted based on the results of the experiments. The performance of the chemical additive Ikati on volume (height) reduction was compared to two conventional methods of managing pit latrine when it is full, namely to empty the pit with using an exhauster truck or construct a new pit latrine. Although only purchasing costs were considered for costing the treatments with additives, it is known that a complete financial evaluation should include factors presented on table 7 below.

Table 7: Aspects to be considered in a detailed cost analysis

Aspect	Description
Investment	Initial cost required for implementation of the treatment technology (e.g. purchasing, infrastructure and equipment)
Operation	Cost related to daily operation of the treatment technology (e.g. routine dosages of the additive, energy requirement for mixing)
Maintenance	Cost required for maintenance of equipment

For CEA, the following assumptions were made (detailed procedure is presented on Appendix-A):

Table 8: Assumptions made for cost-effectiveness analysis model

Nr.	Description	Value	Reference
1	Average FS produced per person per day	350 g	Strande et al., 2014
2	Annual average number of users per pit	20	The Sphere Project, 2011
3	Cost of emptying a pit latrine (an emptying efficiency of 80 % is adopted for exhauster trucks)	€ 65	Based on local observation in Kenya
4	Cost of building a new traditional pit latrine with an impermeable slab, including maintenance per user per year (made often from local materials)	€ 102	(WASHCost, 2012)
5	Cost of building a new pit latrine with a concrete impermeable slab, or VIP type latrine with concrete superstructures, including maintenance per user per year (with ventilation pipe and screen to reduce odours and flies)	€ 505	(WASHCost, 2012)

CHAPTER 4

Results and analysis

This chapter presents the results from the fieldwork conducted in Naivasha (Kenya), starting by presenting the characteristics of the FS used on the experiments, followed by the profiles of the responses being assessed (temperature, pH, *E. coli*, TS, VS, flies, odour and height. It is also found in this chapter the technical challenges faced in the field, which were mainly on faecal sludge collection and on materials and equipment.

4.1. Characterisation of faecal sludge

The experiment was conducted using fresh faecal sludge obtained from Naivasha Prison. It was thick and before dosing into the reactors, the sludge was mixed with urine adopting the ratio 2:1 (urine:FS v/w) and was stirred manually for 5 minutes using a wooden stick. A sample of 1 kg was collected and characteristics are presented on table 8 below:

Table 9: Initial characterisation of faecal sludge

Nr.	Parameter	Unit	FS-1	FS-2	Literature
1	Moisture content	%	78.2	80.3	-
2	Total solids (dry solids)	%	16.2	14.0	$\geq 3.5\%^{****}$
3	Volatile solids (dry solids)	%	73.7	71.7	65^{***}
4	Temperature	^{o}C	18	23	-
5	pH	-	5.4	6.4	$6.55\text{-}9.34^{**}$
6	Odour (TON)	-	1667	2000	-
7	Flies	flies/m^3	63	42	-
8	*E. coli*	cfu/100 mL	8.70×10^8	*****	$1x10^7$ - $1x10^{8*}$

FS-1: faecal sludge used for trial-1
FS-1: faecal sludge used for trial-2
*Nobela (2015)
**Kengne *et al.* (2011) in Strande et al (2014)
***NWSC (2008) in Strande et al (2014)
****Heinss *et al.* (1998) in Strande et al (2014)
*****Not analysed

4.2. Temperature profiles

Temperature was measured daily in the first seven days of the experiment and then once every 7 days for the remaining period. It was observed that the temperature profile did not follow a continuous trend over time, in all treatments. However, the pattern observed on both trial-1 (**raw data on Appendix B-6**) and trial-2 (**raw data on Appendix B-8**) were similar for all treatments throughout the experimental period. Moreover, it was measured an increase of 5 ^{o}C

from the start up to the end of the experiment on trial-1 (see figure 16) while for trial-2 it ranged between 21 ^{o}C and 23 ^{o}C (see figure 17). Despite the similarities on the patterns between experiments, the highest temperature on trial-1 was 23.3 ^{o}C registered on Ikati reactor, while 23.5 ^{o}C was the highest for trial-2, registered on the blank and on woo-ash. Furthermore, it is important to note that no temperatures below the initial (below temperature of raw FS) was registered on trial-1 in all reactors while for trial-2, all treatments presented temperatures below the initial, from day-21 up to the end of the experiment. Additionally, it can be observed that the measured temperatures of the reactors have similar daily pattern of the measured ambient temperature (the data for ambient temperature that was made available from Sanivation included only from day-0 to day-14 of trial-1). However, it can be noted that the average temperature of almost all reactors is about 1 ^{o}C above that of the ambient on day-2 and day-14, while an opposite scenario is observed on day-6.

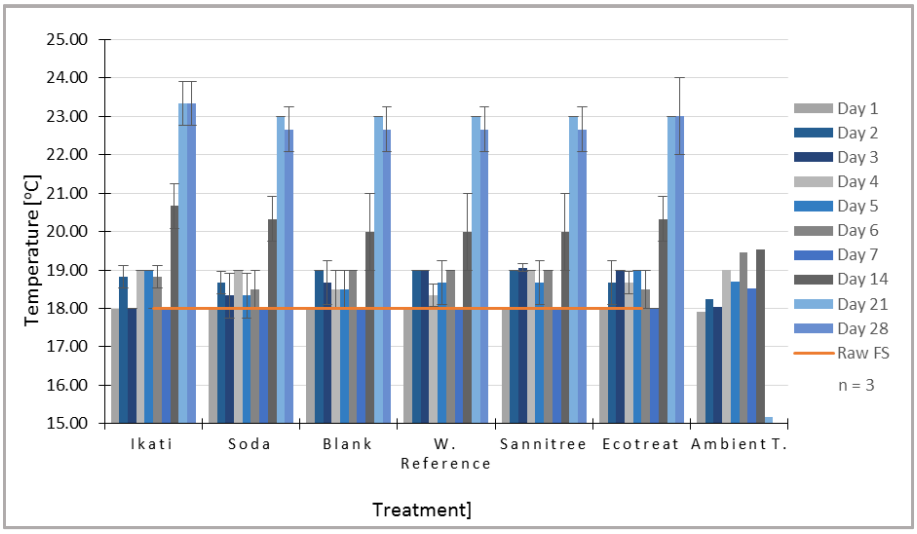


Figure 16: Temperature profile of Trial-1

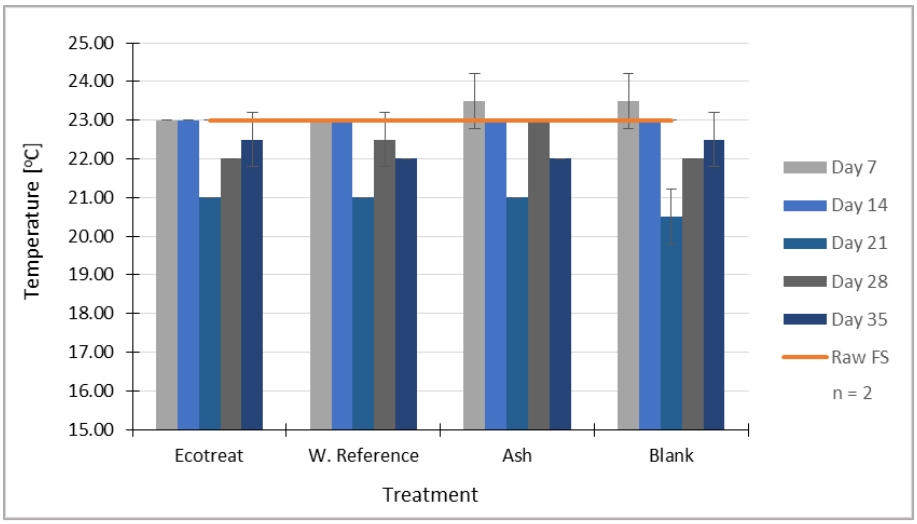


Figure 17: Temperature profile Trial-2

4.3. pH profiles

Similarly to temperature, pH was also measured daily in the first seven days of the experiment and then once every 7 days for the remaining period. The results showed that pH of FS treated with chemical additives Ikati and Soda on trial-1 (**raw data on Appendix B-5**) was high on day-1 and it remained stable at approximately 10 and 9.4 respectively throughout the experimental period (figure 18 below). For the remaining additives, together with the controls (blank and water reference), the pH remained closer to that of the raw faecal sludge (5.40).

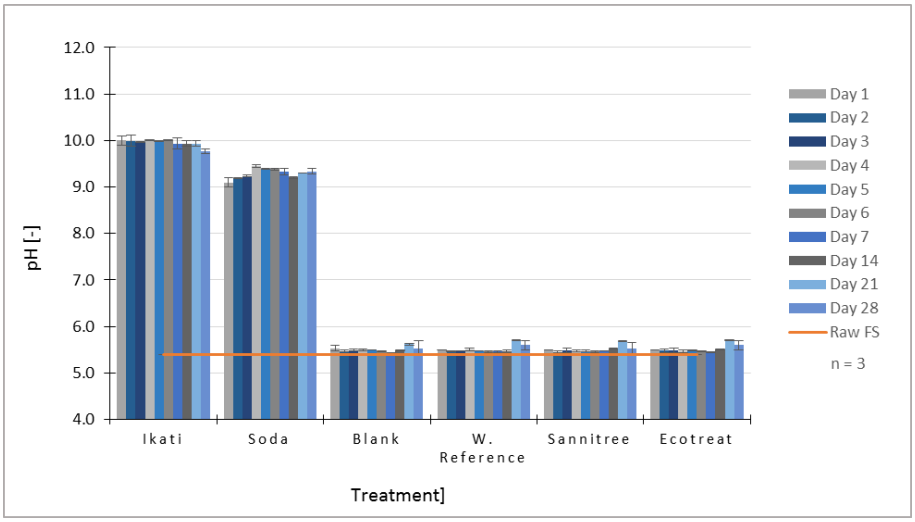


Figure 18: pH profile on Trial-1

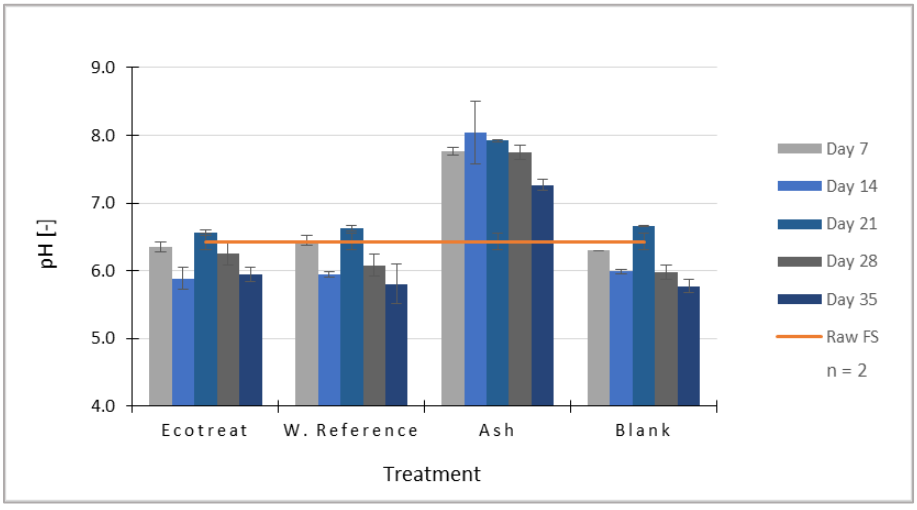


Figure 19: pH profile on Trial-2

For trial-2 (figure 19 – **raw data on Appendix B-7**), a trend similar to that of trial-1 was observed for biological additive Ecotreat and the controls. On the other hand, the highest pH achieved by the chemical additive Ash was 8.05 on day-14, however it dropped 0.3 pH units

from day-14 to day-28. Besides that, it was observed a general drop of pH in all reactors when the feeding process stopped from day 28 to day 35.

4.4. *E. coli* profiles

The *E. coli* concentrations for trial-1 (**raw data on Appendix B-13**) and trial-2 (**raw data on Appendix B-**14) are presented on figures 20 and 21 below, respectively. It can be seen for trial-1 that the chemical additives Ikati and Soda achieved about 4 Log reduction after a contact time of 24 hours, having then the concentration reduced to below detection limit (below 10^3 cfu/100 mL) after 48 hours. No *E. coli* regrowth was observed on Ikati and Soda during the experiment, although 2 x 10^3 cfu/100 ml were detected in one of the triplicate samples of the treatment with Ikati on day 14. The remaining treatments were not able to achieve more than 2 Log unit removal and an increase in *E. coli* concentration was observed on the treatment with Ecotreat from day-21.

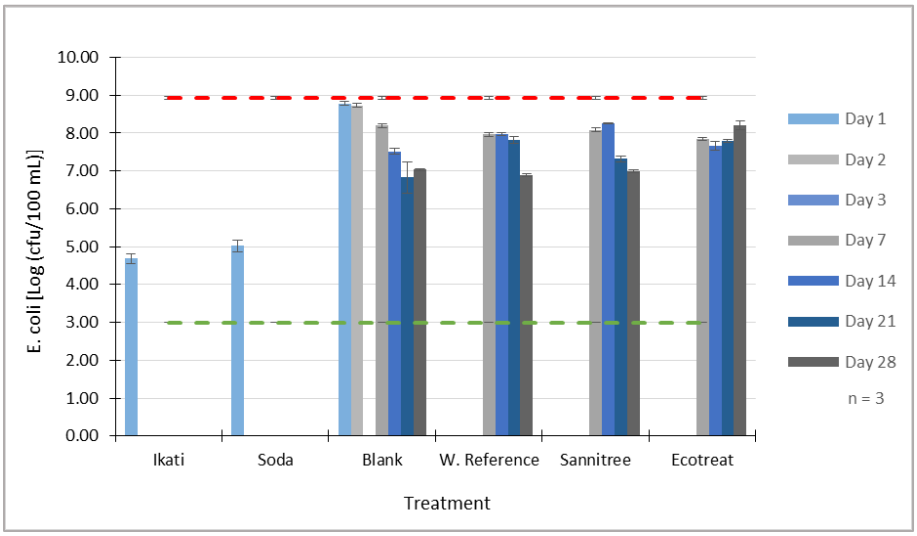


Figure 20: E. coli profile on Trial-1

The *E. coli* concentration on the raw faecal sludge used in trial-2 was adopted to be the same as that of trial-1, since it was determined on the initial characterisation and first analysed on day-14. From the results presented on figure 21 below, it can be seen that none of the treatments was able to reduce *E. coli* concentration below detection limit. Ash imposed a reduction of about 2 Log units, while the remaining treatments led to about 1 Log removal. Moreover, almost no difference in *E. coli* concentration was observed after stop the step-feeding process on day-28 until day-35, with exception of the blank, where it increased about 1 Log unit.

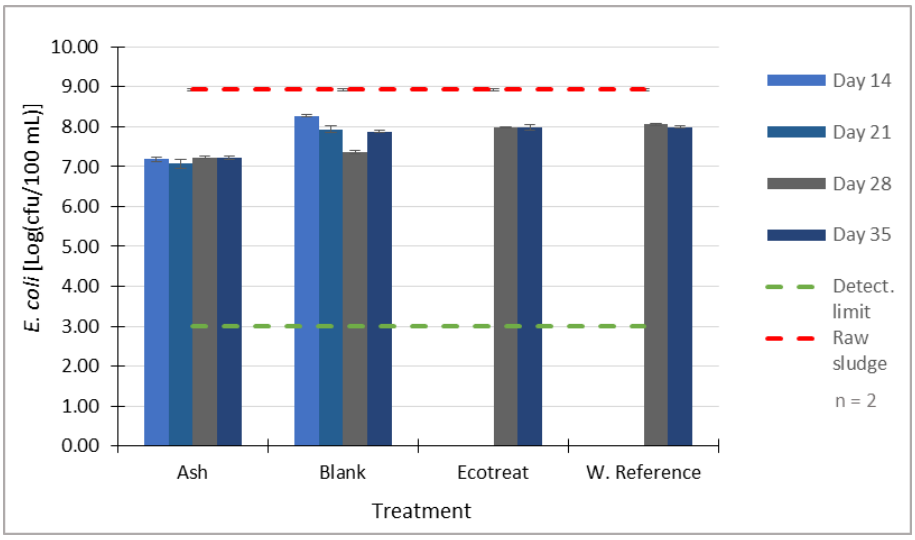


Figure 21: E. coli profile on Trial-2

4.5. TS profiles

The TS response to the treatments on trial-1 is presented on figure 22 below (**raw data on Appendix B-3**). It can be seen that all treatments and the controls (water reference and blank) impacted on solids reduction, especially Ecotreat which achieved a total reduction of 40.4 %. However Ikati and Soda had the particularity of increase solids concentration by 11 % and 7 % respectively in the initial phase, which was then followed by a progressive reduction. Moreover, small differences of 2.1 % and 1.4 % were observed between the TS reduction of FS treated with biological additive Sannitree and that of water reference and blank respectively

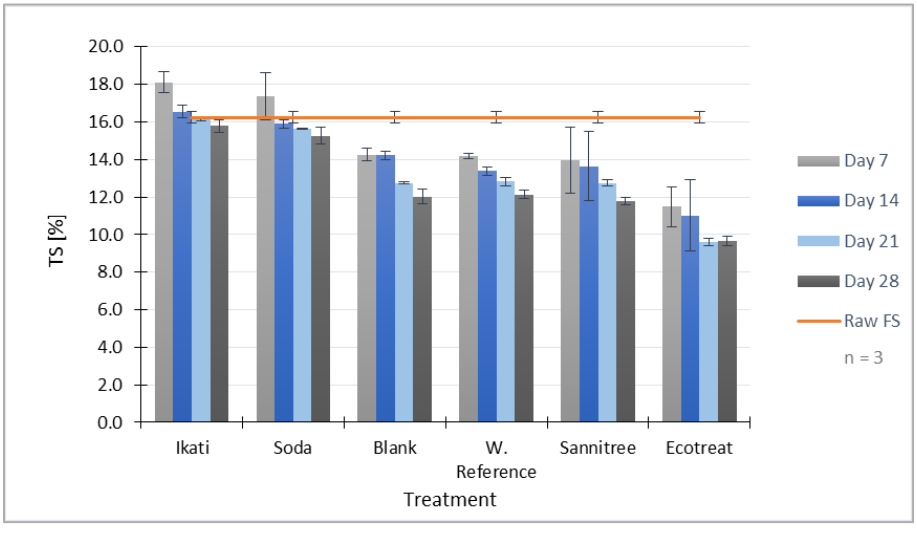


Figure 22: TS profile on Trial-1

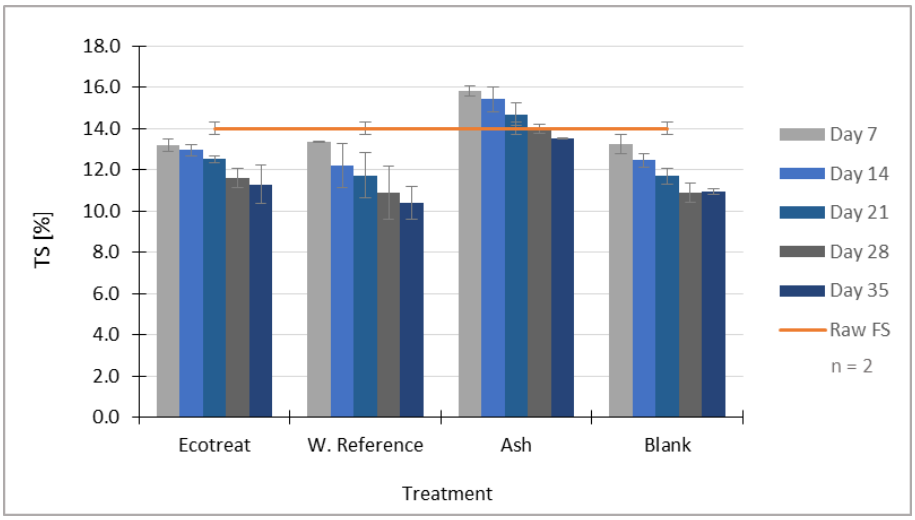


Figure 23: TS profile on Trial-2

For the second trial (figure 23), a trend similar to that of trial-1 was observed (**raw data on Appendix B-4**), being it uniform between treatments throughout the experimental period. Similarly to Soda and Ikati, the chemical additive Ash caused an increase in TS of 12.9 % at the beginning of the treatment. Whereas, the smallest TS measured after 35 days was 10.4 % (ds), determined on the water treatment reactors. In addition to that, it can also be seen that the total TS reduction observed for Ecotreat on experience two was of a small magnitude of that observed on trial-1 after 28 days of treatment, being their difference of about 23 percentile points. On the other hand the difference for water reference and blank between trial-1 and trial-2 was 2.9 and 3.7 percentile points respectively.

4.6. VS/TS profiles

Likewise for TS, it was also observed reduction of volatile solids in all treatments including controls, with special emphasis on chemical additives Ikati and Soda on trial-1 (figure 24 – **raw data on Appendix B-3**). While an average reduction of 3 % was observed for biological additives and the controls on the first seven days, Ikati and Soda showed high reduction of 17 % and 21 % respectively on the same period. However, the VS/TS reductions achieved by the different treatments between consecutive analysis was of similar magnitude for all treatments since day-14 until the end of the experiment. Moreover, Ikati and Soda attained the highest VS/TS reductions of 20.1 and 25.0 % respectively, after 28 days of treatment. Despite the high reductions achieved by Ikati and Soda, none of the treatments achieved volatile solid reduction greater than or equals to 38 %.

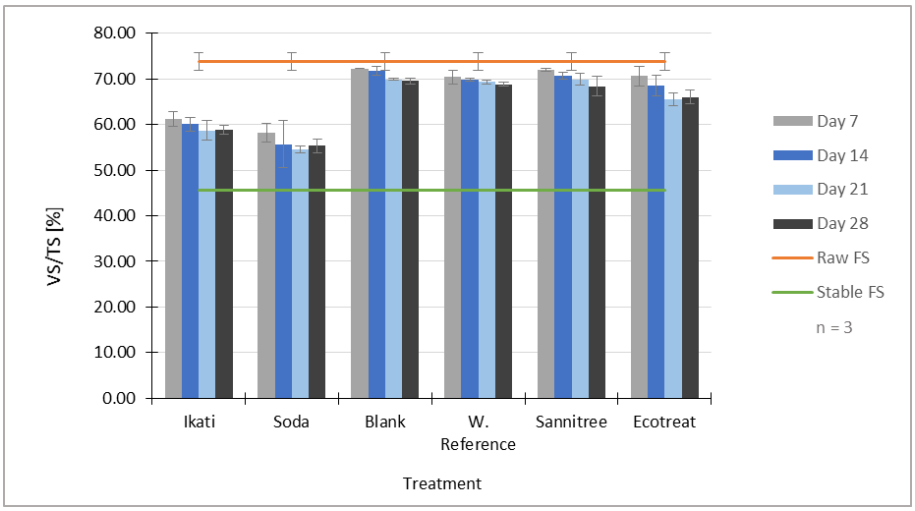


Figure 24: VS/TS profile on Trial-1

For trial-2 (figure 25 – **raw data on Appendix B-4**), the chemical additive Ash behaved in the similar manner as the chemical additives Ikati and Soda in the first trial, showing the highest reduction of 19 % at the end of experiment. The remaining treatments achieved a reduction of 7 % for both Ecotreat and water reference and 5 % for blank. It can also be seen that the volatile solids reduction attained by Ecotreat on trial-2 was smaller than that achieved on trial-1. Similarly to trial-1, none of the treatment achieved the stability index.

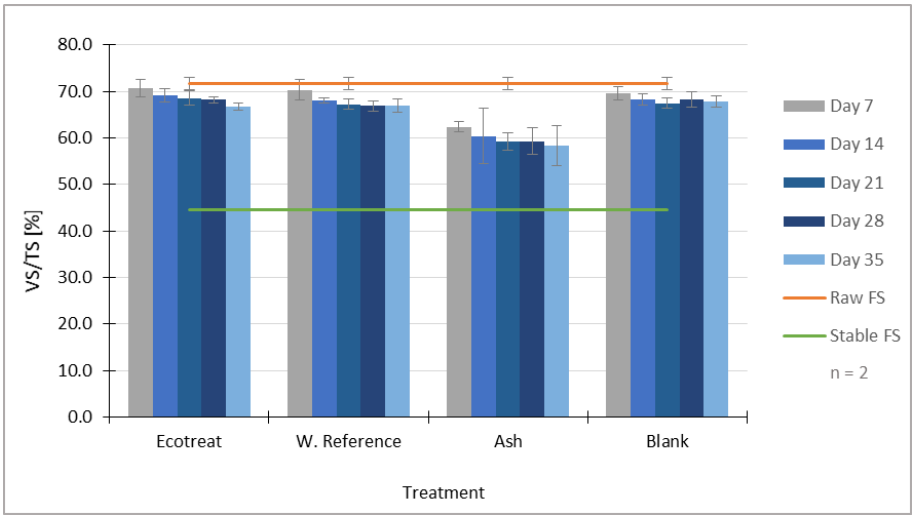


Figure 25: VS/TS profile on Trial-2

4.7. Flies profiles

The results obtained from flies counting in both trial-1 (figure 26 **- raw data on Appendix B-9**) and trial-2 (figure 27 **- raw data on Appendix B-10**) showed inconsistent trend within the treatments throughout the experimental period, however similar trend is found between treatments on trial-1. In the other words, it can be seen for all treatments that the number of flies reduced after the first week, then was followed by an increase on the second week and

then the same tendency was observed on the third and fourth weeks. Despite this inconsistency, the number of flies counted for Ikati and Soda were always smaller than that of the remaining treatments, having achieved a total reduction of about 53 % and 55 % respectively, followed by Ecotreat, with about 44 % reduction. It can also be noted that the number of flies counted on day-21, on the blank suppressed that of the initial characterisation by about 5 %.

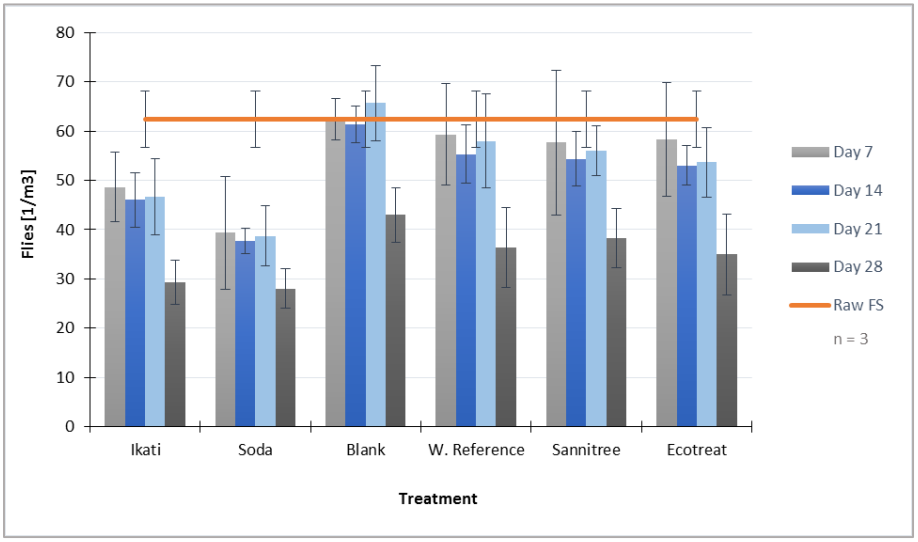


Figure 26: Flies profile on Trial-1

On the other hand, the number of flies counted in almost all treatments of trial-2 exceeded that of the raw FS from the first week until the end of the treatment, with exception of Ash where a reduction of about 87 % (from raw FS) was observed on days-7 and day-35. Moreover, it can be seen that Ash maintained the same pattern throughout the experimental period, having always the smallest number of flies. Apart from that, the blank showed almost a linear reduction from day-14 to day-35, with a weekly average reduction of about 6 %. Furthermore, it can also be observed that fly attraction increased for Ecotreat and water reference by about 14 % and 8 % respectively, after stopping the feeding process on day-28.

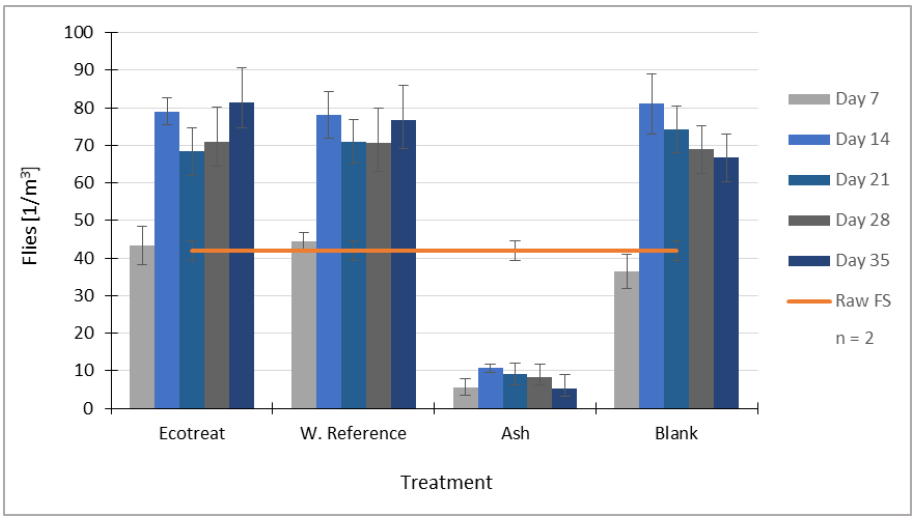


Figure 27: Flies profile on Trial-2

4.8. TON profiles

Similarly to fly attraction, the chemical additives Ikati and Soda on trial-1 (figure 28 - **raw data on Appendix B-11**) and Ash on trial-2 (figure 29 - **raw data on Appendix B-12**) achieved the best performance on odour reduction. On trial-1 for instance, Ikati and Soda were able to reduce the TON value from 1667 to 800 and 850 within seven days, what represents a reduction of 52 % and 49 % respectively, while Ash on trial-2 attained the final reduction of about 78 %. For other treatments however, the measured odour increased by 8 % and 7 % for controls (blank and water reference) and Sannitree on days 21 and 28 respectively, on trial-1. Likewise in trial-1, the determined TON value in trial-2 also increased for blank and water reference by 11 % on day-21 and day-28 respectively, after have been static at the initial TON value in the previous weeks. Moreover, Ecotreat performed better on trial-1 than on trial-2, ending with a total reduction of 20 % and 9 % respectively.

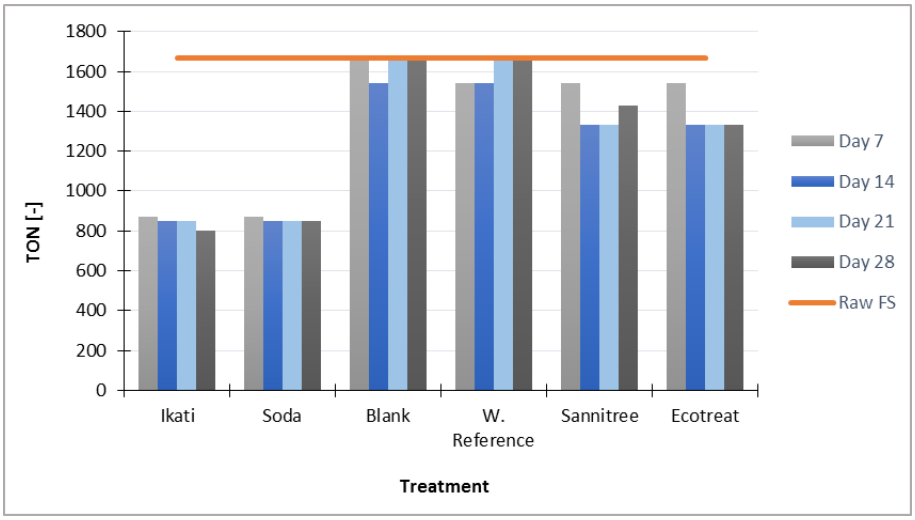


Figure 28: TON profile on Trial-1

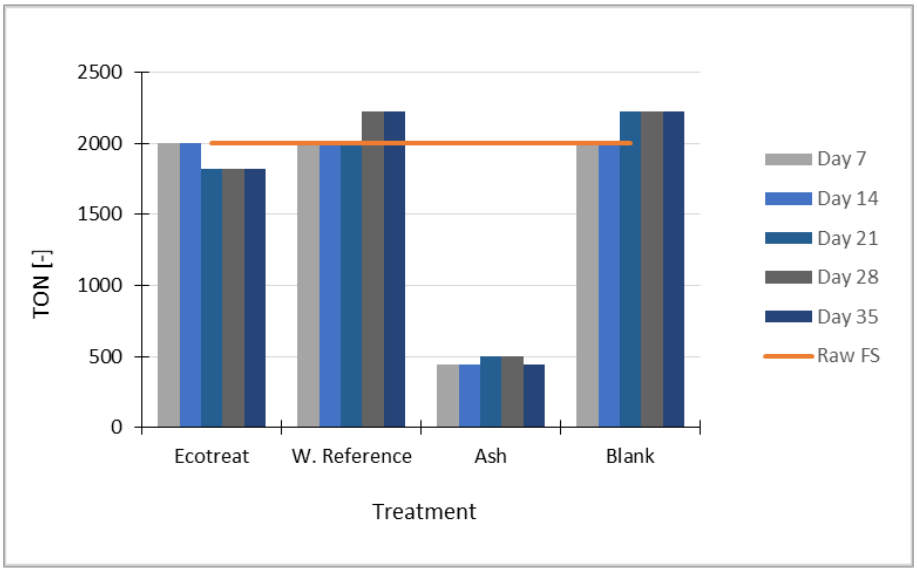


Figure 29: TON profile on Trial-2

4.9. Height profiles

The figure 30 below shows the profile of height by treatments on trial-1 (**raw data on Appendix B-1**). There can be noted changes in the height of sludge heap of all treatments, however no trend over time can be found. Moreover, it can be observed that the treatment with chemical additives Ikati and Soda led to reduction of about 38 % from the initial height, what was observed immediately from day-1. On the contrary, it was observed that the height of sludge heap on the reactors treated with biological additive Ecotreat increased about 20 % on day-7, and remained unchanged at about 10.3 cm up to the end of the experiment. Similarly, an increase on the height of sludge heap was observed on the treatment with Sannitree and on the controls (blank and water reference) on day-14. The observed rising was of about 2.0 cm for both Sannitree and water reference, and of about 2.6 cm for blank.
On the other hand, the results from trial-2 (figure 31 - **raw data on Appendix B-2**) showed a linear increase of the sludge heap height over time for all treatments during the step-feeding period (from day 0 to day 28), with an average slope of 13º. Moreover, the results showed that there was almost no differences between the heights of the different treatments at the end of the step-feeding period (day-28) and those measured at the end of the experiment (day-35). Furthermore, it can also be observed that Ecotreat had a slight positive change of slope from day-7 while, on the other hand the slope of Ash treatment slightly decrease. Furthermore, it was seen that the two additives ended the experiment differing from their respective controls (water reference and blank) in about 4 % and 3 % respectively (for Ecotreat and Ash respectively).

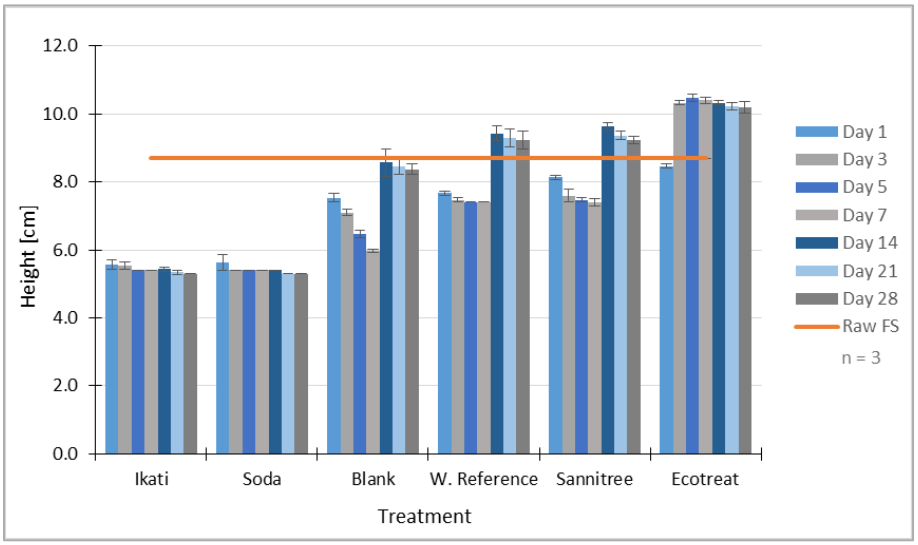


Figure 30: Height profile on Trial-1

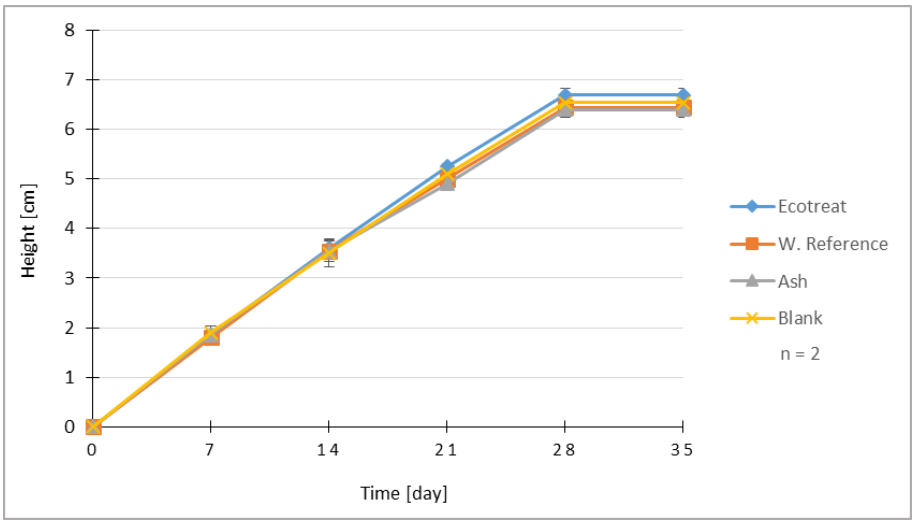


Figure 31: Height profile on Trial-2

4.10. Statistical analysis

4.10.1. Analysis of variance

The performance of the different additives at the end of the treatment period was statistically compared with the controls and between each other using one-way analysis of variance. The test was followed by a post-hoc test when proved the existence of significant difference at 5 % level of significance. Moreover, statistical model (two-way ANOVA) was applied to assess the effect of time on the different treatment responses. Additionally, a correlation analysis between pH and E. coli was performed. The results are presented below.

Analysis of variance of Temperature

The analysis of variances on temperature did not provide sufficient evidences, at 5 % significance level, to conclude that the temperature mean of at least one of the groups of treatments differs from the others in both trial-1 and trial-2. However, the two-way ANOVA test provides sufficient support to affirm that temperature varied over time. Moreover, no statistically significant difference were observed when comparing the temperature means of the different treatments of trial-1 with those of trial-2. Similarly, it was seen that the temperature of the reactors on trial-1 followed the pattern of ambient temperature, therefore no statistically significant difference was found between the two groups of data.

Analysis of variance of pH

The data analysis on pH indicated that the pH of the chemical additives Ikati and Soda (in trial-1) and Ash (on trial-2) at the end of the respective treatment periods, are statistically greater than that of the other treatments on their respective trials. Similarly, it was observed that the treatment period has effect on final pH in both trial-1 and trial-2. Furthermore, the comparison between the two trials showed that the pH attained by of Ash (trial-2) is statistically smaller than that of Ikati and Soda (trial-1).

Analysis of variance on *E. coli*

From the data analysis, it was found enough evidence to conclude that the final *E. coli* concentration of chemical additives Ikati and Soda are significantly smaller than that of the remaining treatments on trial-1. On the other hand, none of the biological additives achieved significant reduction compared to the controls. Additionally, the two-way ANOVA showed that treatment duration has effect on *E. coli* removal.

Likewise trial-1, the analysis of data from trial-2 showed that the *E. coli* reduction achieved by chemical additive Ash is significantly different from that of the remaining treatments. On the other hand, no statistically significant difference was found between reductions achieved by blank, water reference and Ecotreat.

The comparison between the two trials showed that *E. coli* concentration at the end of 28 days of treatment with Ikati and Soda is significantly smaller than that of Ash. However, it was seen that *E. coli* concentration on reactors treated with Ecotreat under the step-feeding dosing protocol is statistically lower than that treated under the manufacture's dosing protocol.

Analysis of variance on TS

The one-way analysis of variance on TS data of trial-1 found sufficient evidences, at 5 % significance level, to conclude that at least one of the six treatment group means was different from the others. Subsequently, the TukeyHSD post-hoc test illustrated that the final TS of both Ikati and Soda is statistically smaller than that of all the remaining treatments, however no evidences were found to conclude that the final TS concentration of the two chemical additives are different. Moreover, it was seen that the final TS concentration of the biological additive Ecotreat is statistically different from that of the controls (water reference and blank) and of Sannitree. Additionally, the two-way ANOVA provided strong evidences to conclude that there is significant effect of treatment duration on final the TS concentration.

In regard to trial-2, the analysis provided enough evidences to affirm that the TS concentration on FS treated with Ash is statistically smaller than that of the controls (blank and water reference). However, the difference between the Ash and Ecotreat was found to be not statistically significant. Similarly to trial-1, sufficient evidences were found to conclude that there is significant effect of treatment duration on final the TS concentration. On the other hand, the comparison between the two trials provided sufficient evidences to conclude that the final TS concentration of Ikati is significantly greater than that of Ash on day 28, although no significant difference was observed between Ikati and Soda as well as between Soda and Ash on day 28. Moreover, the test provided enough evidences to affirm that the final TS concentration of FS treated with Ecotreat under its recommended dosing protocol is greater than that treated under the adopted Consortium LICE dosing protocol. However, it is important to note that the initial TS concentration on trial-1 was greater than that of trial-2.

Analysis of variance on VS/TS

The result from one-way ANOVA test for trial-1 gave enough evidences, at significance level of 5 %, to conclude that the final VS/TS of at least one of the six groups of treatment differ from the others. Therefore, the TukeyHSD post-hoc test was conducted and it showed that the final VS/TS of the chemical additives Ikati and Soda are significantly different from that of the controls, and of the biological additives Ecotreat and Sannitree. However, no enough evidences were found to affirm that the differences between final VS/TS of the biological additives and that of the controls (both water reference and blank) are significant. Furthermore, the difference between final VS/TS of the two chemical additives Ikati and Soda was found to be not

statistically significant. On the other hand, the two-way ANOVA provided enough support to affirm that treatment time, as factor, has effect on VS/TS reduction.
For the second trial, the data did not provide sufficient evidences, at 5 % significance level, to conclude that the mean of the final VS/TS of at least one of the different treatment groups differ from the others. Similarly, no evidences were found to affirm that treatment time has effect on VS/TS reduction.
When the results of the two trials were compared (both at treatment time of 28 days), enough evidences were found, at 5 % significance level, to conclude that at least one of the group means differed from the others. Consequently, the post-hoc test was conducted and it showed that the VS/TS concentration of the treatment with Ash (trial-2) is statistically smaller than that of biological additives Ecotreat and Sannitree (trial-1). However, no sufficient evidences were found to conclude that the VS/TS of the chemical additives Ikati, Soda and Ash are significantly different. Moreover, the analysis showed that there is no sufficient evidences to conclude that the VS/TS of the faecal sludge treated with biological additive Ecotreat under manufacture's recommended protocol differs from that treated with the same additive under Consortium LICE dosing protocol.

Analysis of variance of Height

For trial-1, the TukeyHSD test revealed that the height mean of all groups of treatment differ one from the other. However, it is important to refer that the Tukey HSD test was conducted after have been noticed form one-way ANOVA test, the existence of evidences that the mean of at least treatment group was significant different from that of the others. Furthermore, it was seen that the contact time has effect on the height at the end of treatment period.
Unlike trial-1, the analysis of variances indicated did not provide sufficient evidences to conclude that the height means of the different groups of treatments in trial-2 are significantly different, at 5 %, at significance level. From the comparison between the two trials, it was seen that the height mean of the reactors treated with Ikati and Soda (trial-1) is significantly smaller than that of Ash (trial-2), at 5 % significance level. Additionally, it was observed a statistically significant difference between the height mean of Ecotreat in trial-1 and its mean on trial-2, being the height in trial-2 smaller than that in trial one.

Correlation analysis

The Perason's correlation analysis showed the existence of a strong negative correlation between *E. coli* removal and pH, giving a correlation coefficient of - 0.979, at 95 % confidence interval. When the correlation coefficient (r) is squared (r^2), it results in a coefficient of determination of 0.959, what means that about 96 % of *E. coli* variation can be explained by variation of pH.

4.11. Cost-effectiveness analysis

The costs presented on table 10 below were determined based on the dosages adopted in the experiment and they were calculated per cubic meter of FS to be treated. It is important to refer that only purchasing cost were considered, however a more detailed analysis would be required if the technologies are to be implemented. Additionally, chemical additive ash is not a marketable product, therefore no costs were indicated. The results are summarised on table 9 below.

Table 10: Purchasing cost of the different additives tested in the research

	Additives				
Description	**Ikati**	**Soda**	**Ash**	**Ecotreat**	**Sannitree[1]**
Quantity/m^3 of FS	70 kg	70 kg	200 kg	40 L	330 g
Unit cost	250 KSH	300 KSH	-	350 KSH	0.31 ZAR**
Exchange rate*	0.00860	0.00860	-	0.00860	0.05806
Investment Cost [€]	150.5	180.6	0.00	120.4	6.0

[1]See Appendix-A for detailed calculations
*Source: www.oanda.com, accessed on 21/03/2016 at 01:00am
**Source: https://www.sealwatertech.co.za/bio-enzymes-sachet-pit-toilet?search=sannitree, accessed on 21/03/2016 at 01:00am
KSH – Kenya Shillings (Kenyan currency)
ZAR – South African Rand (South African currency)

Based on the results presented above, Ikati was selected for comparison with conventional solutions applied when a pit latrine is full (to empty the pit latrine or deactivation of the existing and construction of a new pit latrine). The results are presented on the figure 32 below, showing effectiveness in terms of annual cost per percentage of volume reduction (see detailed calculation on Appendix-A, Calculations-2).

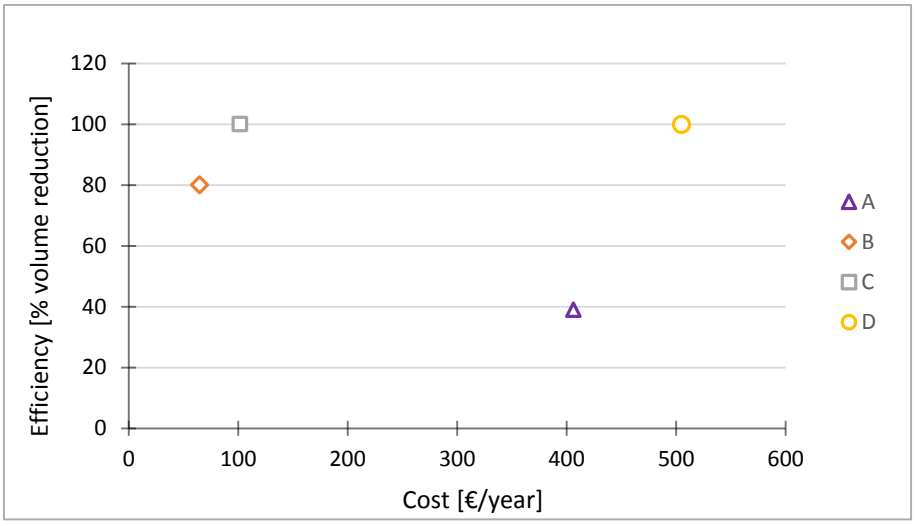


Figure 32: Cost-effectiveness of commercial additives versus conventional options for a full pit latrine

Therefore, the cost-effectiveness ratios (CER) were determined and are presented on table 11 below:

Table 11: Determination of CER for commercial additives versus conventional options for a full pit latrine

Options	**Cost - C (€/year)**	**Effectiveness - E (% volume reduction)**	**C/E (€/year/% reduced)**
A	406	39	10.4
B	65	80	0.8
C	102	100	1.0
D	505	100	5.1

Where:
Option A – Treat the sludge with Ikati.
Option B – Emptying a pit latrine using an exhauster truck.
Option C – Build a new pit latrine (traditional pit latrine with an impermeable slab, made often from local materials).
Option D – Build a new pit latrine (pit latrine with a concrete impermeable slab, or VIP type latrine with concrete superstructures with ventilation pipe and screen to reduce odours and flies).

4.12. Technical challenges

4.12.1. Faecal sludge collection

After arriving at the work place (Naivasha), we came to know that it would not be possible for Sanivation to provide fresh faecal sludge with no additives (ash) for the students to conduct the experiments, as had been initially agreed. That was an unexpected situation which had to be overcame by looking for alternative sources of fresh faecal sludge to start the research.
With the support of Sanivation, almost all sanitation companies (and NGO's) based Kenya were contacted, however none of them gave a positive response since the sludge already contained either ash or sawdust. Additionally, more than 10 pit latrines were visited to verify the quality of the content but it was observed that the sludge was very old and it even contained maggots breathing inside in all visited pits. By knowing that the filling rate of latrines public places is higher, a pit latrine from a school was emptied (figure 33 below), however due to the raining season and the capacity of the pump, the exhauster truck, only sucked the black water from the top of the pit instead of sludge.

Figure 33: Failed attempt of use FS from school's pit latrine; Successful FS collection at Naivasha prison (L;R)

The solution was found at the maximum security Naivasha G.K. Prison, which is the second biggest prison in Kenya, with over 3000 inmates, some of them serving death sentences. With the support of the District administration and the Assistant Prison's Commissioner in Naivasha, four container based toiles hired from Sanivation were installed at the prison and they were supplemented by water containers for hand washing, soap and personal protective equipment for the prisoners (figure 33 above). It is important to mention that the installation of the toilets at the prison was followed by two days of training to the prisoners in how to use the toilets and explanation on the objective of collecting the sludge. This processes was necessary to ensure proper use of the toilets, safeguarding the health of the users and the environment, as well as preserving the toilets that had been hired from Sanivation. Apart from that, sensitisation was

extremely important to break the superstitious thoughts of using the sludge for witchcraft purposes which had stopped the prisoners from using the toilets in the first two days after installation.
The sludge collected from the toilets was then poured into bigger containers and then transported to the experimental site when the container was full. The sludge was thick and before dosing into the reactors it was mixed with urine adopting the ration 2:1 (urine:FS v/w) and then stirred manually for 5 minutes using a wooden stick.

4.12.2. Laboratory and equipment

No laboratory facility was found at the working site (at Sanivation) however the student was in possession of a some equipment and materials such as a pH meter, thermometer, micropipette, glass flasks and laboratory consumables (such as Chromocult Coliform Agar and 90 mm petri dishes) taken from UNESCO-IHE laboratory. Therefore a special laboratory was installed at Dea's Garden (residential address), making use of available resources and some creativity to complete the list of equipment and materials necessary to conduct the required analysis. Some examples are presented in the figures below:

Pan used as water bath for dilution of Chromocult and to heat up samples for TON analysis. A digital temperature logger was used to monitor the temperature inside the water bath.

Figure 34: Water bath used for dilution of Chromocult and to heat up samples for TON analysis

Laboratorial set used when conducting *E. coli* analysis. It includes a rack made from a 5 litres plastic jerrycan, which was used as stand for dilution tubes. Handmade lamps were filled with denatured alcohol (also known as methylated spirit) and its burner flame used to dry the glass spreader. Additionally, a rechargeable lamp was normally used to improve visibility when conducting the analysis. There can also be seen a micropipette, glass spreader and petri-dishes already prepared for analysis.

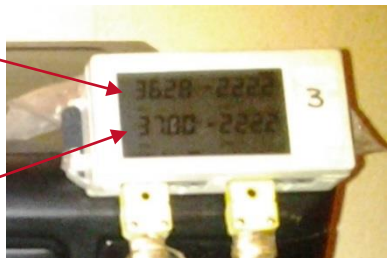
Figure 35: Set-up used for E. coli analysis

An autoclave borrowed from UNHCR Regional Office in Nairobi. Its operation does not require electricity, what makes it appropriate for fieldwork.

Figure 36: Field autoclave used for steralisation of laboratorial material

Temperature on the upper grill of the oven (36.28 ºC) for *E. coli* analysis

Temperature on the lower grill of the oven (37.00 ºC) for *E. coli* analysis



A kitchen oven was used as incubator to incubate the plates with *E. coli* samples at 37 ºC for a period of 24 hours. Initials trials were conducted before starting the analysis in order to find the proper position of the temperature regulator that could give temperature of 37±2 ºC and assess the workability of the technology. Moreover, a digital temperature logger was used to monitor the temperature inside the oven

Figure 37: Incubator used for incubation of E. coli colonies

Temperature on the upper grill of the oven (105.98 ºC) for TS analysis

Temperature on the lower grill of the oven (105.42 ºC) for TS analysis

Similarly to *E. coli* analysis, a kitchen oven was used to dry the samples of TS analysis at 105 ºC. The temperature in the oven was manually set at 105±2 ºC after several trials to fix the regulator at the right position. Additionally, a digital temperature logger was used to monitor the temperature inside the oven

Figure 38: Oven used for TS analysis

The rounded bottom glass flasks were not enough to conduct odour analysis, therefore plastic bottles were used. The bigger bottles were cut at half height and then used to increase the number of flasks.

Figure 39: Flasks used for TON analysis

CHAPTER 5

Discussion

This chapter discuss the findings of the research, relating them to the objective of the study and compares it with what has been reported in previous researches as well as with related literature.

5.1. Initial characterisation of faecal sludge

Almost all measured parameters are within the range of those reported in literature, although higher VS was measured (Strande, *et al.*, 2014). However, the high concentration of volatile solids is a consequence of the freshness of the sludge (4 days old) as it can also be noted on Nobela (2014), Malambo (2014) and Perez (2014) when comparing VS of sludge from public toilet (from a market) and from household. Furthermore, based on TS and VS concentrations the sludge can be categorised as high strength faecal sludge (Henze, *et al.*, 2008). Moreover, it is also important to note that the FS characteristic is affected by several factors such as ambient temperature and moisture, the way it is collected and the storage period (Strande, *et al.*, 2014).

5.2. Effect of the additives on temperature

It was seen that none of the additives had significant impacted on the temperature of faecal sludge. This result suggests that the exothermic effect of chemical additives Ikati, Soda and Ash on FS is considerably smaller than that reported by (Andreasen, 2001) for alkaline treatment with quick lime, however similar results were reported by Kemboi (2015) for Ikati and Soda. Additionally, the fact that reactors were not located in a closed place made it impossible to have control over room temperature, what could have reduced the possible influence of ambient temperature on the reactors and improved the assessment of the effect of the additives on temperature, since no significant difference was found between ambient temperature and the temperature in the reactors

5.3. Effect of the additives on pH

From the results can be seen that Ikati imposed higher pH than Soda thus contradicting to what was observed by Kemboi (2015) with two adopted doses of 1.7 % and 3.4 % (w/w). On the other hand, when the dosages are expressed in terms of TS, it is noted that the adopted dose for chemical additives Ikati and Soda (7 % w/w) falls in between those adopted by Kemboi (2015) of 1.7 % w/w (124 % additive/TS) and 3.4 % w/w (20.4 % additive/TS) however higher pH was achieved. This observation suggests that the reported correlation between pH and TS was no observed (Counts, *et al.*, 1975, Kemboi, 2015). Nevertheless, it is important to note that the

way FS respond to pH variations depends also on its buffer capacity as observed by Nobela (2015).
For the chemical additive wood-ash, the achieved pH was lower than that reported by Austin (2002), Schönning *et al.,* (2014) and Ivanković *et al.,* (2014), however similar values have been reported by some container-based UDDT enterprises which use Ash as desiccant, such as Sanivation. Furthermore, Schönning et al, (2014) suggests that the ability of Ash to rise pH of FS is strongly dependent on the source of ash and the amount dosed.

5.4. Effect of the additives on *E. coli*

The main goal of alkaline treatments is to rise pH to unsuitable levels for microorganisms breeding (Strande *et al.,* 2014). Although none of the additives was able to rise pH above 12, the chemical additives Ikati and Soda increased it to maximums of 10.0 and 9.4 respectively and achieved a significant *E. coli* reduction (to below detection level). This performance is a result of the combination between high pH and carbonate ion that is present on their composition, which has been shown to have sanitising effect (Arthurs *et al.,* 2001; Diez-Gonzalez *et al.,* 2000; Park *et al.,* 2003). Additionally, it also indicates that *E. coli* die-off can occur at a pH range of 8.5 to 12 as reported in previous studies (Kemboi, 2015, Ouali, *et al.*, 2014).
Similarly to what was observed by Kemboi (2015), biological additives were not able to reduce *E. coli* concentration below detection limit. Moreover the concentration at the end of the treatment with Ecotreat on trial-1 is of the same Log scale as that of raw faecal sludge, suggesting to occurrence of *E. coli* multiplication.
Furthermore, it is important to note that the fact that smaller Log reduction was achieved by Ash and by the biological additives when compared to that attained by Ikati and Soda reinforces the concept that high pH favours *E. coli* removal, which was also demonstrated by the correlation analysis (Nobela, 2014, Ouali, *et al.*, 2014, Strande, *et al.*, 2014). Additionally, it is extremely important to mention that the surface plate method for *E. coli* counting and enumeration is sensitive to several factors such as the surrounding environment where the analysis is conducted, the representability of the sample (1.0 ml), the efficiency of the mixing with dilution water, and the accuracy of the manual counting of forming colonies. Those factors can affect the precision of the test.

5.5. Effect of the additives on TS and VS/TS reduction

Similarly to what was observed by Kemboi (2015), it was seen in the two trials that the chemical additives (Ikati and Soda on trial-1 and Ash on trial-2) which were added as powder, increased the solids content on the sludge. This effect has also been said to occur in lime treatments (Counts, *et al.*, 1975, Strande, *et al.*, 2014, Veenstra, 1999). Although Soda and Ikati are known to be soluble in water, the finding suggests that the solubility of these two products in FS is lower and it occurs in a slower rate than in water. On the other hand, the significant VS/TS reduction attained by the chemical additives Ikati, Soda and Ash could be attributed to the process known as alkaline hydrolyses which has been reported to have effect on reduction of volatile solids by disintegration and solubilisation of organic matter and destruction of volatile solids in biodegradation processes of sludge under aerobic and anaerobic conditions (Bina, *et al.*, 2004, Cassini, *et al.*, 2006, Li, *et al.*, 2015).

As for Ecotreat, the observed reduction in TS indicates that the biodegradable particulate fraction were biologically hydrolysed into more readily accessible compounds, as also observed by Ochieng', (2015) altought on slaughterhouse wastewater.

5.6. Effect of the additives on reduction of fly attraction

The quantification of fly attraction on faecal sludge has been firstly introduced simultaneously in the present study and by Muathe (2016), therefore innovative assessment approaches were developed and applied. The novel fly measurement method developed in this research showed that the chemical additives had higher effect on reduction of fly attraction, with more emphasis on Ash. This result is similar to that obtained for odour reduction, what suggests to a possible relationship between these two parameters as observed by Hewitt (2011). Additionally, it has been reported that domestic flies have preference for moist and fermenting mediums for breathing and their presence is influenced by ambient temperature (Hewitt, 2012, Hewitt, 2011). Furthermore, the fly measurement method showed to be efficient in providing a qualitative indication of fly attraction although the results are not so precise and reproducible.

5.7. Effect of the additives on odour reduction

Proliferation of nuisance odour from pit latrines is one of the main factors that stop people from use sanitation facilities all over the world. Even with that tremendous effect on improvement of access to sanitation facilities, this parameter is normally not quantified in researches on faecal sludge treatment, therefore there is lack of information for comparison.

From the experiments can be seen that high TON reduction occurred on reactors treated with Ash, followed by other chemical additives Ikati and Soda. For chemical additives Ikati and Soda, the effect on odour reduction can be attributed to the rise of pH, which then reduces microbial activity that causes putrification thus resulting in reduction of emission of odorous gasses (Strande, *et al.*, 2014). However, for Ash, the effect can be mainly attributed the dosing protocol combined with its desiccant properties. The dosing protocol (dose of Ash every time after using the toilet) increases the contact area and volume of the sludge with the additive, what enhances desiccation thus reducing microbial activity what then can lead to reduction of odour.

Additionally, it was observed during the experiment that nuisance smell (odour) increased always after mixing the content of the reactors. This observation shows the importance of to make distinction between apparent odour and measured odour, being the apparent odour the one detected without interfering on the sludge and the tested odour would be the one determined by TON test.

Regarding the analytical procedure, it can be said that the precision of the method showed to be low and the results are hardly reproducible due to the different sense of smell of human beings (APHA, *et al.*, 1999). Apart from it, can be said that the procedure is not so user friendly by requiring the panel to sniff non-pleasant odours.

5.8. Effect of the additives on height reduction

It was observed that Ikati a Soda significantly reduced the sludge heap height when compared to both initial height and to other treatments and controls. This effect suggests that both products

may improve settleability of FS as have been document for lime, however a more detailed investigation with appropriate tests (e.g. Jar test) should be required to assess this potential ability (Deneux-Mustin, *et al.*, 2001, Henze, *et al.*, 2008, Marani, *et al.*, 2004, Qasim, 1998). Furthermore, the rising sludge observed on the remaining reactors during trial-1, and was also observed Kemboi (2015), could be motivated by the compact layer that is formed on the surface of the reactor, which does not allow the gases that are formed through biological reactions to be released to the atmosphere, causing the sludge to rise. This conclusion was made after observing that the height of the sludge heap reduced after stirring the content in the reactors.
Despite the rising sludge, the experiment showed that both controls (water reference and blank) performed better than the biological additives in the two trials as also observed by Kemboi (2015) Buckley *et al.*, (2008), Foxon *et al.*, (2009) and Bakare *et al.*, (2015). However, a field observation showed that although the measured height on water reference was smaller than that of Ecotreat, the sludge treated with Ecotreat appeared to be more watery. Moreover, the fact that no significant differences was observed in the height profile of the treatments during trial-2 can suggests that the Consortium LICE SM dosing protocol did not improve the performance of the biological additive, however it is important to note that the rising sludge phenomenon did not occur under this protocol.
Regarding the methodology used to assess volume reduction, it was seen to be a simple and precise method, since it can easily be related to volume by simple mathematical models when the average surface area is known. Additionally, similar technic has been applied by Bakare *et al.*, (2015) when investigating the effect of additives on VIP latrine sludge, however making use of electronic devices.

5.9. Statistical analysis

Although the assumption of ANOVA test were not fully satisfied, the test was adopted since contradictory results were obtained from its alternative non-parametric Kruskall-Wallis rank sum test (e.g. cases of post-hoc test which did not report significant difference between groups, after having enough evidences from the analysis of variance test to reject the null hypothesis which states that the mean of the groups are the same). Additionally, it has been reported that ANOVA is more powerful than Kruskall-Wallis rank sum test when analysing data with small sample size (Motulsky, 2013). Moreover, on Kruskall-Wallis test the observations are ranked from the smaller to the highest and then the rank sums of the different groups are compared. Therefore, some information from the data is lost by replacing the observations by the ranks, thus making the test less powerful than ANOVA (McDonald, 2009).

5.10. Cost-effectiveness analysis

The results showed that the dose of additives into pit latrines is the less cost-effective option to achieve volume reduction when compared to conventional solutions. However, it is said that the choice of the best option should not only rely on the cost-effectiveness ratio since there are other factors that in practice should be taken in consideration (Compernolle, *et al.*, 2008). In this specific case, there are several aspects to be considered for emptying of a pit latrine such as availability of proper equipment and personnel, access road for exhauster truck and proper disposal site. Similarly, the construction of a new pit latrine will depend on factors such as availability of space and materials, and soil conditions (rocky soils are difficult to dig).

Moreover, the cost of the services and that of the additives vary with location, even within the same country, therefore the optimisation of the effect of promising additives can still provide a feasible alternative to conventional solutions for faecal sludge management in both emergency and normal (non-emergency) situations. Additionally, additives can provide other advantages such as *E. coli* removal, odour and flies reduction, which are not accounted by analysing only the cost-effectiveness ratio.

CHAPTER 6

Conclusion

This chapter presents the conclusions drawn to the research, based on the results, observations and supported by the statistical analysis as wells as by the literature review. The chapter is structured in the way to answer the questions that guided the research. Furthermore, a summarised comparison between promising chemical additives Ikati, Soda, wood-ash and lime is presented in the form of a table.

6.1. General conclusion

Based on the findings of this study, the general conclusion that can be drawn is that the chemical additives are more likely to enhance stabilisation and stabilisation of faecal as well as reduce odour, volume and fly attraction. While, on the other hand, the research did not find evidences to support the claim that biological additives are able to reduce the filling rate of pit latrines.
Besides that, the study showed that the use of wood-ash could be a cheap and efficient treatment technology for reduction of odour and fly attraction, therefore its application could be recommended as a first provisory action to break vector transmission route of faecal oral diseases in the acute phase of emergency situations.

6.2. Volume reduction

From the results of this study, it can be concluded that chemical additives Ikati and Soda can, potentially, reduce the height of faecal sludge heap thus extending the life of pit latrines, however the effect of mixing should not be neglected. On the other hand, no evidences were found to conclude that the use of biological additives can enhance biodegradation of faecal sludge thus slowing down the filling rate of pit latrines.

6.3. Stabilisation and sanitisation of faecal sludge

6.3.1. Stabilisation of faecal sludge

The study showed that none of the additives attained volatile solids reduction greater than or equals to 38 %, which is the recommended baseline for stabilisation (EPA, 1995, EPA, 1993, Kazimierczak, 2012). However, the chemical additives Ikati, Soda and Ash were able to enhance stabilisation of faecal sludge and they are likely to achieve the guideline interval for stabilisation, with more emphasis on Ikati and Soda. Moreover, although significant total solids reduction was achieved with Ecotreat, the experiment showed that biological additives had no significant effect on stabilisation of faecal sludge, what reinforces the findings from previous studies (Bakare, *et al.*, 2012, Bakare, *et al.*, 2015, Buckley, *et al.*, 2008, Foxon, *et al.*, 2009, Kemboi, 2015).

6.3.2. Sanitisation of faecal sludge

E. coli removal

Based on the results of this study and on what has been previously reported, it is concluded that chemical additives Ikati and Soda are able to enhance *E. coli* die-off on faecal sludge (Kemboi, 2015) and provide a final product that can be safely use in agriculture for unrestricted irrigation of root crops (WHO, 2006). Similar effect is likely to be attained using chemical additive Ash as reported in previous studies (Ivanković, *et al.*, 2014, Niwagaba, *et al.*, 2009, Stenström, *et al.*, 2004). Additionally, it provided sufficient evidences to conclude that pH plays an important role on microbial die-off, as previously reported (Nobela, 2014, Ouali, *et al.*, 2014, Strande, *et al.*, 2014). On the other hand, it has been shown that biological additives are not susceptible to enhance *E. coli* die-off bellow WHO guideline value for unrestricted irrigation of root crops (*E. coli* $\leq 10^3$ cfu/100 ml) on faecal sludge, as previously observed by Kemboi (2014).

Reduction of fly attraction

The research showed that wood-ash has strong abilities to rapidly reduce fly attraction on faecal sludge when applied every time after each use of toilet. Moreover, similar effect is likely to be achieved with chemical additives Ikati and Sofa if applied under similar dosing protocol. On the other hand, it shows that biological additives do not have significant effect on fly attraction.

Reduction of odour

In the same manner, wood-ash has demonstrated to be able to attain significant effect on odour reduction within short contact time, when applied every time after each use of toilet. Similarly, the chemical additive Ikati and Soda may achieve similar effect under the same dosing protocol. Furthermore, it was shown that biological additives had no significant effect on odour reduction.

6.4. Cost-effectiveness of additives technologies

Under the adopted dosages and based on the results of the experiment, it is concluded that the chemical additives are more cost-effective than the biological, although operational and maintenance costs were not considered on the analysis. However, conventional solutions (emptying the pit latrine or decommissioning of the full pit latrine and building of a new one) showed to be more cost-effective than the use of commercial additives, when considering only a single benefit of volume reduction.

6.5. Efficiency of Consortium LICE SM dosing protocol

The research did not find evidences that Consortium LICE SM dosing protocol is able to improve performance of biological additives on faecal sludge treatment and on volume reduction.

6.6. Comparison between Ikati, Soda, wood-ash and lime

The table 12 below presents the different attributes of the chemical additives Ikati, Soda, wood-ash and lime for comparison. The values indicated for lime were obtained from previous research conducted by Nobela (2014).

Table 12: Comparison between different chemical treatment technologies

		Treatment			
Nr.	**Aspect**	**Ikati**	**Soda**	**Wood-ash***	**Lime**
1	Treatment mechanism	Alkaline treatment	Alkaline treatment	Alkaline treatment	Alkaline treatment
2	Highest pH	10.0	9.4	8.1	12.4
3	*E. coli* Log removal	8 (below detection limit)	8 (below detection limit)	2	6-7 (below detection limits)
4	Treatment time	2 days	2 days	-	5-120 minutes
5	VS/TS reduction	20 %	25 %	19 %	**
6	Odour reduction	52 %	49 %	78 %	**
7	Flies reduction	53 %	55 %	88 %	**
8	Volume reduction	39 %	39 %	0 %	**
9	Drawbacks of technology	• Mixing for homogenisation • Increases TS	• Mixing for homogenisation • Increases TS	• Reduces caloric value of the sludge • Increases TS	• Mixing for homogenisation • Increases TS
10	Post-treatment	Drying beds	Drying beds	-	Drying beds
11	End-use of the sludge	Fertiliser	Fertiliser	-	Fertiliser
12	Effect on humans	**	[1]Sodium carbonate has no or a low skin irritation potential but it is considered irritating to the eyes	**	[2]Considered harmful to the skin, eyes and lungs
13	Effect on the environment	Not toxic	Not toxic	Not toxic	Not toxic
14	Treatment cost/m^3	150.5 €	180.6 €	0.00 €	**

-The treatment technology did not achieved sanitisation of faecal sludge, therefore treatment time and end-use of the sludge are not indicated.
*Determined under step-feeding dosing protocol
**Not reported
[1]Source: (OECD, *et al.*, October 2002)
[2]Source: Strande *et al.*, (2014)

CHAPTER 7

Recommendations

This chapter presents suggestions for future researches, which were developed based on the findings and the conclusion drawn in this study.

The study recommends the following:

1. Further research should be conducted in order to optimise the effect of promising chemical additives Ikati, Soda and wood-ash both in quantity and time, to attain stabilisation, sanitisation of faecal sludge, volume reduction and cost effectiveness. The study should include a comparison of the effect of the additives under batch and step-feeding dosing protocols.

2. The potential ability of the chemical additives Ikati and Soda in improving settleability of faecal sludge should be further investigated, since it could provide an alternative for conventional technologies.

3. Further study on the effect of different desiccants (such as sawdust) on reduction of odour and fly attraction should be conducted and the results compared with that achieved by wood-ash.

4. If to be conducted, further researches on the claimed ability of biological additive in enhancing volume reduction should include conditions where water is allowed to flow/percolate out to simulate the situation of pit latrines which are normally not lined.

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Appendices

Appendix A: Materials and methods

Table A-1: Dosing protocol for chemical and biological additives on trial-1

Chemical additives		Dosing protocol			Remarks
	Set-up	Dosage (w/w)	FS quantity	Product dosage	
Name	No.	[%]	[g]	[g]	
Ikati	1.1	7.0	2250	157.5	Single dose
Soda	1.2	7.0	2250	157.5	Single dose

Biological additives	Dosing protocol				Dilution water	Remarks
	Set-up	Recommen. dose	FS	Applied dosage		
Name	No.	[%]	[g]	[g or mL]	[mL]	
Sannitree*	1.3	3.3	2250	8.3	412.5	Single dose
Ecotreat	1.4	4.0	2250	90.0	-	Single dose

*Dosage based on the size of the pit (100 g/3 m³), therefore a multiplication factor was adopted to account for number of users (see details below)

Determined by assuming:

Ref.	Assumptions:	
1	Volume of pit latrine [L]	3000
2	Volume of experimental container [L]	25
3	FS produced per person per day [g/p/d]	750
4	No. of users per toilet [No.]	12

Table A-2: Dosing protocol adopted for biological additive Ecotreat (based on dosing protocol of additive LICE)

Phase	Day	Dosage of additive LICE			Ecotreat	FS [g]	Ash	
		Recommended [g]	Ration [g/L]	Dose - Quick priming	Quick priming [mL]		Ratio [%]	Dose [g]
Initial seeding [lice/pit volume]	1	3000	1.00	25.0	21.3	75.0	20.0	15.0
Starter [lice/pit volume]	2 to 6	500	0.17	4.2	3.5	75.0	20.0	15.0
Maintenance	7 to 28	25	0.10	2.5	2.1	75.0	20.0	15.0
Testing sanitisation	29 to 35				0.0	0.0	20.0	0.0

Table A-3: Measurement of the quantity of ash taken by hand by a panel of five people

Nr.	Name	Ash taken [g]
1	Justine Muathe	42
2	Evans Mahuana	47
3	Protas O. Mussumba	44
4	John Munene	46
5	Marcos Zindoga	43
Average		**44.4**
Std. deviation		**2.07**

Calculations A-1: Determination of the dosage and cost of biological additive Sannitree

Determination of the dosage of biological additive Sannitree:

1. According to manufacturer's protocol, 100 g of the additive is poured into the pit once every 30 days. Adopting a standard pit volume of 3 m^3, then the ratio additive/pit volume is:

$$dose_0 = \frac{100\ g\ of\ additive}{3\ m^3\ of\ pit} = 33.3\ g\ additive/m^3\ of\ pit$$

2. Since the experiment had to be conducted in small scale, the dose was then scaled down to the reactor sizes. A reactor size of 25 L was adopted to scale down the dosage.

$$dose_1 = 33.3\ g\ additive/m^3\ \times \frac{25}{1000}\ m^3\ = 0.825\ g\ additive$$

3. The additive is normally used in households, where only few number of people use the toilet. Therefore, a factor was assigned to the dose to account for the high usage of pit latrines during acute phase of an emergency. For that, a ratio of 50 users/pit toilet during acute phase of an emergency and an average family size of 5 people/household, were adopted (Bongaarts, 2001, The Sphere Project, 2011). Then a multiplication factor *f* was determined:

$$f = \frac{50}{5} = 10$$

4. Then, the adopted dose was determined:

$$dose_{adopted} = 0.825\ g\ additive \times 10 = 8.25\ g\ of\ additive$$

5. Dilution water: 100 g of additive is diluted into 5 litres of water.

$$Dilution\ water = \frac{5\ l\ water}{100\ g\ additive} \times 8.25\ g\ additive = 0.4125\ l = 412.5\ ml$$

For cost analysis (determined based on the adopted dose):

1. Quantity needed for treatment of 1 m^3 of faecal sludge:

$$Additive = \frac{8.25\ g\ additive\ \times 1000\ L\ of\ FS}{25\ L\ of\ FS} = 330\ g\ of\ additive$$

2. The cost is then calculated based on the price of its commercial packages (110 g additive is sold at 34.2 ZAR).

$$Cost_{ZAR} = \frac{330\ g\ additive\ \times 34.2\ ZAR}{110\ g\ additive} = 102.6\ ZAR$$

3. Exchanging to Euro (exchange rate of 0.05806)

$$Cost_{Euro} = 102.6 \times 0.05806 = 6.0\ Euro$$

Calculations A-2: Detailed calculations for cost-effectiveness analysis

Table A-4: Assumptions made for cost-effectiveness analysis

Nr.	Description	Value	Reference
1	Density of faecal sludge	1000 kg/ m^3	
2	Volume of a pit latrine	3 m^3	
3	Average FS produced per person per day	350 g	Strande et al., 2014
4	Annual average number of users per pit	20	The Sphere Project, 2013
5	Cost of emptying a pit latrine of	€ 65	Based on local observation in Kenya
6	Cost of building a new traditional pit latrine with an impermeable slab, including maintenance per user per year (made often from local materials)	€ 102*	(WASHCost, 2012)
7	Cost of building a new pit latrine with a concrete impermeable slab, or VIP type latrine with concrete superstructures, including maintenance per user per year (with ventilation pipe and screen to reduce odours and flies)	€ 505*	(WASHCost, 2012)

* Values adjusted with inflation rate.

1. Amount of faecal sludge produced per year:

$$FS = \frac{350\ g}{person \times day} \times \frac{10^{-6}\ m^3}{g} \times 20\ persons \times 365\ days/year = 2.6\ m^3/year$$

It means that the filling period of a pit latrine will be almost a year. Therefore, for the present analysis a filling period of one year is adopted and assuming that a pit latrine is full when it has 90 % of its capacity filled with faecal sludge.

$$FS_{adopted} = \frac{90}{100} \times 3\ m^3 = 2.7\ m^3$$

2. Cost of treatment of FS with Ikati per year:

$$Cost_{Ikati} = 150.5\ €/m^3 \times 2.7\ m^3/year = 406\ €/year$$

3. Cost of building and maintenance of pit latrines

Table A-5: Capital and recurrent expenditure benchmarks for sanitation services [Adapted from (WASHCost, 2012)]

Cost component	Latrine type in area of intervention	Cost ranges [min-max] in US$*
Total capital expenditure **(per latrine)**	Traditional pit latrine with an impermeable slab (made often from local materials)	7-26
	Pit latrine with a concrete impermeable slab, or VIP type latrine with concrete superstructures (with ventilation pipe and screen to reduce odours and flies)	36-358
	Pour-flush or septic-tank latrine, often with a concrete or brick-lined pit/ tank with sealed impermeable slab, including a flushable pan	92-358
Total recurrent expenditure **(per person, per year)**	Traditional pit latrines with an impermeable slab (often made from local materials)	1.5-4.0
	VIP type latrines	2.5-8.5
	Pour-flush or septic-tank latrines	3.5-11.5

*Values determined in 2011

3.1 For traditional pit latrine with an impermeable slab:

$$Cost_{total} = Cost_{constr.} + Cost_{maint.}$$

$$Cost_{total} = 26\frac{\$}{year} + 4\frac{\$}{person \times year} \times 20\ persons = 106.0\ \$/year$$

By applying the inflation rate[2] and exchanging the currency[3] to Euro, the cost is 102 €/year.

3.2 For traditional pit latrine with a concrete impermeable slab, or VIP latrine:

$$Cost_{total} = Cost_{constr.} + Cost_{maint.}$$

$$Cost_{total} = 358\frac{\$}{year} + 8.5\frac{\$}{person \times year} \times 20\ persons = 528\ \$/year$$

By applying the inflation rate[2] and exchanging the currency[3] to Euro, the cost is 505 €/year.

[2] http://www.saving.org/inflation/ accessed on 18/04/2016 at 02:07 am
[3] https://www.oanda.com/currency/converter/ accessed on 18/04/2016 at 02:07 am

Appendix B: Raw data

Table B-1: Results from height measurement on trial-1

Sampling day	TREATMENT		Height	Volume
	Treat. Number	Treatment	Height	[cm^3]
0	-	Raw FS	8.7	3,773.5
			8.7	3,773.5
			8.7	3,773.5
1	1	Ikati	5.4	2,342.2
	2	Soda	5.5	2,385.5
	3	Blank	7.4	3,209.6
	4	W. ref.	7.6	3,296.4
	5	Sannitree	8.2	3,556.6
	6	Ecotreat	8.4	3,643.4
	1	Ikati	5.7	2,472.3
	2	Soda	5.9	2,559.0
	3	Blank	7.6	3,296.4
	4	W. ref.	7.7	3,339.8
	5	Sannitree	8.1	3,513.3
	6	Ecotreat	8.5	3,686.8
	1	Ikati	5.6	2,428.9
	2	Soda	5.5	2,385.5
	3	Blank	7.6	3,296.4
	4	W. ref.	7.7	3,339.8
	5	Sannitree	8.1	3,513.3
	6	Ecotreat	8.5	3,686.8
3	1	Ikati	5.4	2,342.2
	2	Soda	5.4	2,342.2
	3	Blank	7.2	3,122.9
	4	W. ref.	7.4	3,209.6
	5	Sannitree	7.5	3,253.0
	6	Ecotreat	10.4	4,510.9
	1	Ikati	5.6	2,428.9
	2	Soda	5.4	2,342.2
	3	Blank	7.0	3,036.2
	4	W. ref.	7.5	3,253.0
	5	Sannitree	7.5	3,253.0
	6	Ecotreat	10.3	4,467.5
	1	Ikati	5.6	2,428.9
	2	Soda	5.4	2,342.2
	3	Blank	7.1	3,079.5
	4	W. ref.	7.5	3,253.0
	5	Sannitree	7.8	3,383.1
	6	Ecotreat	10.3	4,467.5
5	1	Ikati	5.4	2,342.2
	2	Soda	5.4	2,342.2
	3	Blank	6.4	2,775.9
	4	W. ref.	7.4	3,209.6
	5	Sannitree	7.4	3,209.6
	6	Ecotreat	10.6	4,597.6
	1	Ikati	5.4	2,342.2
	2	Soda	5.4	2,342.2
	3	Blank	6.6	2,862.7
	4	W. ref.	7.4	3,209.6
	5	Sannitree	7.5	3,253.0
	6	Ecotreat	10.4	4,510.9
	1	Ikati	5.4	2,342.2
	2	Soda	5.4	2,342.2
	3	Blank	6.4	2,775.9
	4	W. ref.	7.4	3,209.6
	5	Sannitree	7.5	3,253.0
	6	Ecotreat	10.4	4,510.9
7	1	Ikati	5.4	2,342.2
	2	Soda	5.4	2,342.2
	3	Blank	6.0	2,602.4
	4	W. ref.	7.4	3,209.6
	5	Sannitree	7.3	3,166.3
	6	Ecotreat	10.4	4,510.9
	1	Ikati	5.4	2,342.2
	2	Soda	5.4	2,342.2
	3	Blank	6.0	2,602.4
	4	W. ref.	7.4	3,209.6
	5	Sannitree	7.4	3,209.6
	6	Ecotreat	10.5	4,554.2
	1	Ikati	5.4	2,342.2
	2	Soda	5.4	2,342.2
	3	Blank	5.9	2,559.0
	4	W. ref.	7.4	3,209.6
	5	Sannitree	7.5	3,253.0
	6	Ecotreat	10.3	4,467.5

Sampling day	TREATMENT		Height	Volume
	Treat. Number	Treatment	Height	[cm^3]
14	1	Ikati	5.4	2,342.2
	2	Soda	5.4	2,342.2
	3	Blank	8.2	3,556.6
	4	W. ref.	9.3	4,033.7
	5	Sannitree	9.7	4,207.2
	6	Ecotreat	10.3	4,467.5
	1	Ikati	5.5	2,385.5
	2	Soda	5.4	2,342.2
	3	Blank	8.5	3,686.8
	4	W. ref.	9.3	4,033.7
	5	Sannitree	9.7	4,207.2
	6	Ecotreat	10.4	4,510.9
	1	Ikati	5.4	2,342.2
	2	Soda	5.4	2,342.2
	3	Blank	9.0	3,903.6
	4	W. ref.	9.7	4,207.2
	5	Sannitree	9.5	4,120.5
	6	Ecotreat	10.3	4,467.5
21	1	Ikati	5.3	2,298.8
	2	Soda	5.3	2,298.8
	3	Blank	8.2	3,556.6
	4	W. ref.	9.1	3,947.0
	5	Sannitree	9.3	4,033.7
	6	Ecotreat	10.1	4,380.7
	1	Ikati	5.3	2,298.8
	2	Soda	5.3	2,298.8
	3	Blank	8.5	3,686.8
	4	W. ref.	9.2	3,990.4
	5	Sannitree	9.5	4,120.5
	6	Ecotreat	10.3	4,467.5
	1	Ikati	5.4	2,342.2
	2	Soda	5.3	2,298.8
	3	Blank	8.7	3,773.5
	4	W. ref.	9.6	4,163.9
	5	Sannitree	9.3	4,033.7
	6	Ecotreat	10.3	4,467.5
28	1	Ikati	5.3	2,298.8
	2	Soda	5.3	2,298.8
	3	Blank	8.2	3,556.6
	4	W. ref.	9.0	3,903.6
	5	Sannitree	9.3	4,033.7
	6	Ecotreat	10.0	4,337.4
	1	Ikati	5.3	2,298.8
	2	Soda	5.3	2,298.8
	3	Blank	8.4	3,643.4
	4	W. ref.	9.2	3,990.4
	5	Sannitree	9.3	4,033.7
	6	Ecotreat	10.3	4,467.5
	1	Ikati	5.3	2,298.8
	2	Soda	5.3	2,298.8
	3	Blank	8.5	3,686.8
	4	W. ref.	9.5	4,120.5
	5	Sannitree	9.1	3,947.0
	6	Ecotreat	10.3	4,467.5

Table B-2: Results from height measurement on trial-2

Sampling day	Treat. Number	Treatment	Weight g	Depth cm	Height [cm]	Volume [cm^3]	Sampling day	Treat. Number	Treatment	Weight g	Depth cm	Height [cm]	Volume [cm^3]
0	1	Ecotreat	0	28	0.0	0.00	21	1	Ecotreat	1850	22.7	5.3	2298.80
	2	W. Ref.	0	28	0.0	0.00		2	W. Ref.	1700	22.9	5.1	2212.05
	3	Ash	0	28	0.0	0.00		3	Ash	2000	23.1	4.9	2125.31
	4	Blank	0	28	0.0	0.00		4	Blank	1650	23	5.0	2168.68
	1	Ecotreat	0	28	0.0	0.00		1	Ecotreat	1850	22.8	5.2	2255.43
	2	W. Ref.	0	28	0.0	0.00		2	W. Ref.	1750	23.1	4.9	2125.31
	3	Ash	0	28	0.0	0.00		3	Ash	1950	23.1	4.9	2125.31
	4	Blank	0	28	0.0	0.00		4	Blank	1650	22.8	5.2	2255.43
7	1	Ecotreat	650	26.2	1.8	780.73	28	1	Ecotreat	2250	21.4	6.6	2862.66
	2	W. Ref.	640	26.1	1.9	824.10		2	W. Ref.	2200	21.7	6.3	2732.54
	3	Ash	750	26.2	1.8	780.73		3	Ash	2615	21.6	6.4	2775.91
	4	Blank	600	26.2	1.8	780.73		4	Blank	2100	21.5	6.5	2819.28
	1	Ecotreat	650	26.1	1.9	824.10		1	Ecotreat	2260	21.2	6.8	2949.41
	2	W. Ref.	660	26.3	1.7	737.35		2	W. Ref.	2210	21.4	6.6	2862.66
	3	Ash	750	26.1	1.9	824.10		3	Ash	2615	21.6	6.4	2775.91
	4	Blank	600	26.0	2.0	867.47		4	Blank	2150	21.4	6.6	2862.66
14	1	Ecotreat	1250	24.3	3.7	1604.82	35	1	Ecotreat	2250	21.4	6.6	2862.66
	2	W. Ref.	1200	24.3	3.7	1604.82		2	W. Ref.	2180	21.7	6.3	2732.54
	3	Ash	1410	24.4	3.6	1561.45		3	Ash	2600	21.6	6.4	2775.91
	4	Blank	1150	24.7	3.3	1431.33		4	Blank	2100	21.5	6.5	2819.28
	1	Ecotreat	1250	24.5	3.5	1518.08		1	Ecotreat	2260	21.2	6.8	2949.41
	2	W. Ref.	1150	24.6	3.4	1474.70		2	W. Ref.	2200	21.4	6.6	2862.66
	3	Ash	1350	24.5	3.5	1518.08		3	Ash	2610	21.6	6.4	2775.91
	4	Blank	1150	24.3	3.7	1604.82		4	Blank	2150	21.4	6.6	2862.66

Table B-3: Results from TS and VS analysis on trial-1

Day	Sampling day	Treat. Number	Name	Replicate	Dry weight and dish	Weight of empty dish	Weight of wet sample and dish	Weight of ash and dish	TS	VS/TS
					[g]	[g]	[g]	[g]	[%]	[%]
15/12/2015	0	-	Raw Sludge	A	6.66	2.00	30.12	3.21	16.56	74.01
				B	6.54	2.00	30.52	3.29	15.93	71.61
				C	6.60	2.00	30.31	3.13	16.26	75.46
22/12/2015	7	1	Ikati	A	6.97	2.00	30.43	3.87	17.48	62.38
		2	Soda	A	6.63	2.00	30.29	4.03	16.35	56.11
		3	Blank	A	5.98	2.00	30.30	3.10	14.06	72.35
		4	W. Ref.	A	6.01	2.00	30.14	3.16	14.26	71.10
		5	Sannitree	A	6.42	2.00	30.08	3.22	15.74	72.40
		6	Ecotreat	A	5.39	2.00	30.01	3.07	12.10	68.44
		1	Ikati	B	7.14	2.00	29.94	3.95	18.40	62.06
		2	Soda	B	7.32	2.00	30.33	4.12	18.78	60.15
		3	Blank	B	6.14	2.00	30.25	3.15	14.65	72.22
		4	W. Ref.	B	5.93	2.00	30.03	3.23	14.02	68.70
		5	Sannitree	B	5.42	2.00	29.90	2.96	12.26	71.93
		6	Ecotreat	B	4.86	2.00	29.83	2.78	10.28	72.73
		1	Ikati	C	7.20	2.00	30.17	4.11	18.46	59.42
		2	Soda	C	6.74	2.00	29.97	3.98	16.95	58.23
		3	Control	C	5.96	2.00	30.14	3.10	14.07	72.22
		4	W. Ref.	C	6.03	2.00	30.22	3.15	14.28	71.46
		5	Sannitree	C	5.90	2.00	30.02	3.10	13.92	71.79
		6	Ecotreat	C	5.39	2.00	29.97	2.99	12.12	70.80
29/12/2015	14	1	Ikati	A	6.74	2.00	30.00	3.96	16.93	58.65
		2	Soda	A	6.64	2.00	30.00	4.04	16.57	56.03
		3	Control	A	5.92	2.00	30.00	3.12	14.00	71.43
		4	W. Ref.	A	5.94	2.00	30.00	3.18	14.07	70.05
		5	Sannitree	A	5.33	2.00	30.00	2.97	11.89	70.87
		6	Ecotreat	A	5.35	2.00	30.00	3.02	11.96	69.55
		1	Ikati	B	6.61	2.00	30.00	3.77	16.46	61.61
		2	Soda	B	6.72	2.00	30.00	3.86	16.86	60.59
		3	Control	B	6.01	2.00	30.00	3.09	14.32	72.82
		4	W. Ref.	B	6.03	2.00	30.00	3.22	14.39	69.73
		5	Sannitree	B	6.06	2.00	30.00	3.17	14.50	71.18
		6	Ecotreat	B	4.60	2.00	30.00	2.78	9.29	70.00
		1	Ikati	C	6.55	2.00	30.00	3.83	16.25	59.78
		2	Soda	C	5.99	2.00	30.00	3.98	14.25	50.38
		3	Control	C	6.02	2.00	30.00	3.17	14.36	70.90
		4	W. Ref.	C	5.28	2.00	30.00	3.00	11.71	69.51
		5	Sannitree	C	6.07	2.00	30.00	3.23	14.54	69.78
		6	Ecotreat	C	5.31	2.00	30.00	3.13	11.82	65.86
05/01/2016	21	1	Ikati	A	6.49	2.00	30.00	3.89	16.04	57.91
		2	Soda	A	6.32	2.00	30.00	4.00	15.43	53.70
		3	Control	A	5.61	2.00	30.00	3.08	12.89	70.08
		4	W. Ref.	A	5.65	2.00	30.00	3.12	13.04	69.32
		5	Sannitree	A	5.55	2.00	30.00	3.06	12.68	70.14
		6	Ecotreat	A	4.63	2.00	30.00	2.87	9.39	66.92
		1	Ikati	B	6.52	2.00	30.00	3.76	16.14	61.06
		2	Soda	B	6.33	2.00	30.00	3.95	15.46	54.97
		3	Control	B	5.64	2.00	30.00	3.10	13.00	69.78
		4	W. Ref.	B	5.56	2.00	30.00	3.08	12.71	69.66
		5	Sannitree	B	5.48	2.00	30.00	3.10	12.43	68.39
		6	Ecotreat	B	4.71	2.00	30.00	2.97	9.68	64.21
		1	Ikati	C	6.55	2.00	30.00	3.96	16.25	56.92
		2	Soda	C	6.47	2.00	30.00	4.02	15.96	54.81
		3	Control	C	5.47	2.00	30.00	3.05	12.39	69.74
		4	W. Ref.	C	5.56	2.00	30.00	3.11	12.71	68.82
		5	Sannitree	C	5.68	2.00	30.00	3.07	13.14	70.92
		6	Ecotreat	C	4.72	2.00	30.00	2.94	9.71	65.44
13/01/2016	28	1	Ikati	A	6.31	2.00	30.00	3.76	15.39	59.16
		2	Soda	A	6.14	2.00	30.00	3.81	14.79	56.28
		3	Control	A	5.27	2.00	30.00	2.97	11.68	70.34
		4	W. Ref.	A	5.36	2.00	30.00	3.04	12.00	69.05
		5	Sannitree	A	5.35	2.00	30.00	3.07	11.96	68.06
		6	Ecotreat	A	4.68	2.00	30.00	2.93	9.57	65.30
		1	Ikati	B	6.46	2.00	30.00	3.88	15.93	57.85
		2	Soda	B	6.33	2.00	30.00	3.90	15.46	56.12
		3	Control	B	5.49	2.00	30.00	3.08	12.46	69.05
		4	W. Ref.	B	5.37	2.00	30.00	3.04	12.04	69.14
		5	Sannitree	B	5.24	2.00	30.00	3.09	11.57	66.36
		6	Ecotreat	B	4.66	2.00	30.00	2.93	9.50	65.04
		1	Ikati	C	6.49	2.00	30.00	3.81	16.04	59.69
		2	Soda	C	6.36	2.00	30.00	4.03	15.57	53.44
		3	Control	C	5.34	2.00	30.00	3.03	11.93	69.16
		4	W. Ref.	C	5.47	2.00	30.00	3.10	12.39	68.30
		5	Sannitree	C	5.32	2.00	30.00	2.97	11.86	70.78
		6	Ecotreat	C	4.79	2.00	30.00	2.90	9.96	67.74

Table B-4: Results from TS and VS analysis on trial-2

Day	Sampling day	Treat. Number	Name	Replicate	Dry weight and dish	Weight of empty dish	Weight of wet sample and dish	Weight of ash and dish	TS	VS/TS
					[g]	[g]	[g]	[g]	[%]	[%]
06/01/2016	0	-	Raw Sludge	A	5.86	2.00	30.21	3.11	13.68	71.24
				B	6.02	2.00	30.07	3.08	14.32	73.13
				C	5.94	2.00	30.10	3.15	14.02	70.81
13/01/2016	7	1	Ecotreat	A	5.66	2.00	30.17	3.02	13.0	72.13
		2	W. Ref.	A	5.69	2.00	29.62	3.15	13.4	68.83
		3	Ash	A	6.37	2.00	29.93	3.61	15.6	63.16
		4	Blank	A	5.62	2.00	30.05	3.06	12.9	70.72
		1	Ecotreat	B	5.80	2.00	30.33	3.16	13.4	69.47
		2	W. Ref.	B	5.77	2.00	30.14	3.06	13.4	71.88
		3	Ash	B	6.49	2.00	30.07	3.72	16.0	61.69
		4	Blank	B	5.86	2.00	30.41	3.21	13.6	68.65
20/01/2016	14	1	Ecotreat	A	5.66	2.00	29.83	3.09	13.2	70.22
		2	W. Ref.	A	5.62	2.00	29.96	3.17	12.9	67.68
		3	Ash	A	6.47	2.00	30.18	3.58	15.9	64.65
		4	Blank	A	5.54	2.00	29.92	3.15	12.7	67.38
		1	Ecotreat	B	5.58	2.00	30.08	3.14	12.7	68.16
		2	W. Ref.	B	5.23	2.00	30.26	3.02	11.4	68.42
		3	Ash	B	6.22	2.00	30.12	3.85	15.0	56.16
		4	Blank	B	5.44	2.00	30.13	3.06	12.2	69.19
27/01/2016	21	1	Ecotreat	B	5.92	2.00	33.00	3.19	12.6	69.64
		2	W. Ref.	B	6.00	2.00	34.00	3.34	12.5	66.50
		3	Ash	B	6.52	2.00	32.00	3.78	15.1	60.62
		4	Blank	B	5.47	2.00	31.00	3.15	12.0	66.72
		1	Ecotreat	C	5.84	2.00	33.00	3.25	12.4	67.45
		2	W. Ref.	C	5.51	2.00	34.00	3.12	11.0	68.09
		3	Ash	C	6.44	2.00	33.00	3.87	14.3	57.88
		4	Blank	C	5.31	2.00	31.00	3.05	11.4	68.28
26/01/2016	28	1	Ecotreat	B	4.27	2.00	21.00	2.71	11.9	68.69
		2	W. Ref.	B	4.71	2.00	25.00	2.92	11.8	66.05
		3	Ash	B	4.77	2.00	22.00	3.07	13.9	61.37
		4	Blank	B	4.13	2.00	21.00	2.70	11.2	67.14
		1	Ecotreat	C	4.14	2.00	21.00	2.69	11.3	67.75
		2	W. Ref.	C	4.29	2.00	25.00	2.74	10.0	67.69
		3	Ash	C	4.83	2.00	22.00	3.21	14.2	57.24
		4	Blank	C	3.90	2.00	20.00	2.58	10.6	69.52
05/02/2016	35	1	Ecotreat	B	4.39	2.00	22.00	2.78	12.0	67.36
		2	W. Ref.	B	4.08	2.00	21.00	2.71	10.9	65.87
		3	Ash	B	4.57	2.00	21.00	2.99	13.5	61.48
		4	Blank	B	4.21	2.00	22.00	2.73	11.1	66.97
		1	Ecotreat	C	4.13	2.00	22.00	2.72	10.7	66.20
		2	W. Ref.	C	3.87	2.00	21.00	2.60	9.8	67.91
		3	Ash	C	4.44	2.00	20.00	3.09	13.6	55.33
		4	Blank	C	4.17	2.00	22.00	2.68	10.9	68.66

Table B-5: Results from pH measurement on trial-1

Day	TREATMENT Treat. Number	TREATMENT Name	pH [-]
Raw FS	1	Ikati	5.40
	2	Soda	5.40
	3	Control	5.40
1	1	Ikati	10.10
	2	Soda	9.00
	3	Control	5.60
	4	W. ref.	5.50
	5	Sannitree	5.50
	6	Ecotreat	5.50
	1	Ikati	9.90
	2	Soda	9.20
	3	Control	5.50
	4	W. ref.	5.50
	5	Sannitree	5.50
	6	Ecotreat	5.50
	1	Ikati	10.00
	2	Soda	9.10
	3	Control	5.50
	4	W. ref.	5.50
	5	Sannitree	5.50
	6	Ecotreat	5.50
2	1	Ikati	9.90
	2	Soda	9.17
	3	Control	5.49
	4	W. ref.	5.45
	5	Sannitree	5.47
	6	Ecotreat	5.49
	1	Ikati	9.95
	2	Soda	9.19
	3	Control	5.47
	4	W. ref.	5.47
	5	Sannitree	5.44
	6	Ecotreat	5.51
	1	Ikati	10.13
	2	Soda	9.20
	3	Control	5.46
	4	W. ref.	5.47
	5	Sannitree	5.45
	6	Ecotreat	5.44
3	1	Ikati	9.95
	2	Soda	9.24
	3	Control	5.51
	4	W. ref.	5.47
	5	Sannitree	5.46
	6	Ecotreat	5.50
	1	Ikati	9.97
	2	Soda	9.22
	3	Control	5.48
	4	W. ref.	5.48
	5	Sannitree	5.55
	6	Ecotreat	5.52
	1	Ikati	9.98
	2	Soda	9.25
	3	Control	5.48
	4	W. ref.	5.48
	5	Sannitree	5.47
	6	Ecotreat	5.43
4	1	Ikati	10.02
	2	Soda	9.42
	3	Control	5.50
	4	W. ref.	5.47
	5	Sannitree	5.45
	6	Ecotreat	5.45
	1	Ikati	9.99
	2	Soda	9.47
	3	Control	5.51
	4	W. ref.	5.52
	5	Sannitree	5.49
	6	Ecotreat	5.45
	1	Ikati	10.00
	2	Soda	9.45
	3	Control	5.48
	4	W. ref.	5.52
	5	Sannitree	5.47
	6	Ecotreat	5.49
5	1	Ikati	10.00
	2	Soda	9.39
	3	Control	5.50
	4	W. ref.	5.48
	5	Sannitree	5.48
	6	Ecotreat	5.49
	1	Ikati	9.98
	2	Soda	9.37
	3	Control	5.49
	4	W. ref.	5.47
	5	Sannitree	5.48
	6	Ecotreat	5.49
	1	Ikati	9.98
	2	Soda	9.40
	3	Control	5.50
	4	W. ref.	5.47
	5	Sannitree	5.45
	6	Ecotreat	5.47
6	1	Ikati	10.01
	2	Soda	9.37
	3	Control	5.45
	4	W. ref.	5.47
	5	Sannitree	5.48
	6	Ecotreat	5.47
	1	Ikati	10.02
	2	Soda	9.40
	3	Control	5.47
	4	W. ref.	5.45
	5	Sannitree	5.45
	6	Ecotreat	5.46
	1	Ikati	9.99
	2	Soda	9.37
	3	Control	5.46
	4	W. ref.	5.45
	5	Sannitree	5.44
	6	Ecotreat	5.45
7	1	Ikati	9.80
	2	Soda	9.30
	3	Control	5.41
	4	W. ref.	5.46
	5	Sannitree	5.48
	6	Ecotreat	5.46
	1	Ikati	10.00
	2	Soda	9.40
	3	Control	5.40
	4	W. ref.	5.48
	5	Sannitree	5.47
	6	Ecotreat	5.44
	1	Ikati	10.01
	2	Soda	9.27
	3	Control	5.43
	4	W. ref.	5.44
	5	Sannitree	5.48
	6	Ecotreat	5.44
14	1	Ikati	9.88
	2	Soda	9.20
	3	Control	5.49
	4	W. ref.	5.46
	5	Sannitree	5.52
	6	Ecotreat	5.51
	1	Ikati	9.99
	2	Soda	9.19
	3	Control	5.50
	4	W. ref.	5.49
	5	Sannitree	5.53
	6	Ecotreat	5.49
	1	Ikati	9.94
	2	Soda	9.23
	3	Control	5.48
	4	W. ref.	5.46
	5	Sannitree	5.52
	6	Ecotreat	5.51
21	1	Ikati	9.90
	2	Soda	9.30
	3	Control	5.60
	4	W. ref.	5.70
	5	Sannitree	5.68
	6	Ecotreat	5.70
	1	Ikati	9.89
	2	Soda	9.31
	3	Control	5.64
	4	W. ref.	5.70
	5	Sannitree	5.70
	6	Ecotreat	5.70
	1	Ikati	10.00
	2	Soda	9.30
	3	Control	5.62
	4	W. ref.	5.72
	5	Sannitree	5.68
	6	Ecotreat	5.71
28	1	Ikati	9.80
	2	Soda	9.40
	3	Control	5.40
	4	W. ref.	5.60
	5	Sannitree	5.40
	6	Ecotreat	5.50
	1	Ikati	9.70
	2	Soda	9.30
	3	Control	5.50
	4	W. ref.	5.50
	5	Sannitree	5.60
	6	Ecotreat	5.70
	1	Ikati	9.80
	2	Soda	9.30
	3	Control	5.70
	4	W. ref.	5.70
	5	Sannitree	5.60
	6	Ecotreat	5.60

Table B-6: Results from temperature measurement on trial-1

Day	TREATMENT Treat. Number	TREATMENT Name	Temperature [°C]
Raw FS	1	Ikati	18.00
	2	Soda	18.00
	3	Control	18.00
1	1	Ikati	18.00
	2	Soda	18.00
	3	Control	18.00
	4	W. ref.	18.00
	5	Sannitree	18.00
	6	Ecotreat	18.00
	1	Ikati	18.00
	2	Soda	18.00
	3	Control	18.00
	4	W. ref.	18.00
	5	Sannitree	18.00
	6	Ecotreat	18.00
	1	Ikati	18.00
	2	Soda	18.00
	3	Control	18.00
	4	W. ref.	18.00
	5	Sannitree	18.00
	6	Ecotreat	18.00
2	1	Ikati	19.00
	2	Soda	18.50
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	18.00
	1	Ikati	18.50
	2	Soda	18.50
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	19.00
	1	Ikati	19.00
	2	Soda	19.00
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	19.00
3	1	Ikati	18.00
	2	Soda	18.00
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.19
	6	Ecotreat	19.00
	1	Ikati	18.00
	2	Soda	18.00
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	19.00
	1	Ikati	18.00
	2	Soda	19.00
	3	Control	18.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	19.00
4	1	Ikati	19.00
	2	Soda	19.00
	3	Control	18.00
	4	W. ref.	18.50
	5	Sannitree	19.00
	6	Ecotreat	18.50
	1	Ikati	19.00
	2	Soda	19.00
	3	Control	19.00
	4	W. ref.	18.00
	5	Sannitree	19.00
	6	Ecotreat	18.50
	1	Ikati	19.00
	2	Soda	19.00
	3	Control	18.50
	4	W. ref.	18.50
	5	Sannitree	19.00
	6	Ecotreat	19.00
5	1	Ikati	19.00
	2	Soda	19.00
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	18.00
	6	Ecotreat	19.00
	1	Ikati	19.00
	2	Soda	18.00
	3	Control	18.50
	4	W. ref.	18.00
	5	Sannitree	19.00
	6	Ecotreat	19.00
	1	Ikati	19.00
	2	Soda	18.00
	3	Control	18.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	19.00
6	1	Ikati	19.00
	2	Soda	19.00
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	18.50
	1	Ikati	19.00
	2	Soda	18.00
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	18.00
	1	Ikati	18.50
	2	Soda	18.50
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	19.00
7	1	Ikati	18.00
	2	Soda	18.00
	3	Control	19.00
	4	W. ref.	18.00
	5	Sannitree	18.50
	6	Ecotreat	19.00
	1	Ikati	19.00
	2	Soda	19.00
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	19.00
	1	Ikati	19.00
	2	Soda	19.00
	3	Control	19.00
	4	W. ref.	19.00
	5	Sannitree	19.00
	6	Ecotreat	19.00
14	1	Ikati	21.00
	2	Soda	20.00
	3	Control	20.00
	4	W. ref.	19.00
	5	Sannitree	21.00
	6	Ecotreat	20.00
	1	Ikati	20.00
	2	Soda	20.00
	3	Control	19.00
	4	W. ref.	21.00
	5	Sannitree	20.00
	6	Ecotreat	21.00
	1	Ikati	21.00
	2	Soda	21.00
	3	Control	21.00
	4	W. ref.	20.00
	5	Sannitree	19.00
	6	Ecotreat	20.00
21	1	Ikati	24.00
	2	Soda	23.00
	3	Control	23.00
	4	W. ref.	23.00
	5	Sannitree	23.00
	6	Ecotreat	23.00
	1	Ikati	23.00
	2	Soda	23.00
	3	Control	23.00
	4	W. ref.	23.00
	5	Sannitree	23.00
	6	Ecotreat	23.00
	1	Ikati	23.00
	2	Soda	23.00
	3	Control	23.00
	4	W. ref.	23.00
	5	Sannitree	23.00
	6	Ecotreat	23.00
28	1	Ikati	24.00
	2	Soda	23.00
	3	Control	23.00
	4	W. ref.	22.00
	5	Sannitree	23.00
	6	Ecotreat	24.00
	1	Ikati	23.00
	2	Soda	22.00
	3	Control	23.00
	4	W. ref.	23.00
	5	Sannitree	22.00
	6	Ecotreat	22.00
	1	Ikati	23.00
	2	Soda	23.00
	3	Control	22.00
	4	W. ref.	23.00
	5	Sannitree	23.00
	6	Ecotreat	23.00

Table B-7: Results from pH measurement on trial-2

Day	Sampling day	Treat. Number	Name	Replicate	pH [-]
06/01/2016	0	-	Raw Sludge	A	6.5
				B	6.3
				C	6.5
13/01/2016	7	1	Ecotreat	A	6.4
		2	W. Ref.	A	6.5
		3	Ash	A	7.72
		4	Blank	A	6.3
		1	Ecotreat	B	6.3
		2	W. Ref.	B	6.4
		3	Ash	B	7.81
		4	Blank	B	6.3
20/01/2016	14	1	Ecotreat	A	5.77
		2	W. Ref.	A	5.92
		3	Ash	A	8.37
		4	Blank	A	5.97
		1	Ecotreat	B	6
		2	W. Ref.	B	5.97
		3	Ash	B	7.72
		4	Blank	B	6.01
27/01/2016	21	1	Ecotreat	B	6.59
		2	W. Ref.	B	6.6
		3	Ash	B	7.91
		4	Blank	B	6.65
		1	Ecotreat	C	6.53
		2	W. Ref.	C	6.66
		3	Ash	C	7.93
		4	Blank	C	6.67
26/01/2016	28	1	Ecotreat	B	6.37
		2	W. Ref.	B	6.2
		3	Ash	B	7.82
		4	Blank	B	5.91
		1	Ecotreat	C	6.13
		2	W. Ref.	C	5.97
		3	Ash	C	7.68
		4	Blank	C	6.06
05/02/2016	35	1	Ecotreat	B	5.87
		2	W. Ref.	B	6.01
		3	Ash	B	7.21
		4	Blank	B	5.84
		1	Ecotreat	C	6.02
		2	W. Ref.	C	5.6
		3	Ash	C	7.33
		4	Blank	C	5.71

Table B-8: Results from temperature measurement on trial-2

Day	Sampling day	Treat. Number	Name	Replicate	Temperature [°C]
06/01/2016	0	-	Raw Sludge	A	23
				B	23
				C	23
13/01/2016	7	1	Ecotreat	A	23
		2	W. Ref.	A	23
		3	Ash	A	23
		4	Blank	A	24
		1	Ecotreat	B	23
		2	W. Ref.	B	23
		3	Ash	B	24
		4	Blank	B	23
20/01/2016	14	1	Ecotreat	A	23
		2	W. Ref.	A	23
		3	Ash	A	23
		4	Blank	A	23
		1	Ecotreat	B	23
		2	W. Ref.	B	23
		3	Ash	B	23
		4	Blank	B	23
27/01/2016	21	1	Ecotreat	B	21
		2	W. Ref.	B	21
		3	Ash	B	21
		4	Blank	B	20
		1	Ecotreat	C	21
		2	W. Ref.	C	21
		3	Ash	C	21
		4	Blank	C	21
26/01/2016	28	1	Ecotreat	B	22
		2	W. Ref.	B	22
		3	Ash	B	23
		4	Blank	B	22
		1	Ecotreat	C	22
		2	W. Ref.	C	23
		3	Ash	C	23
		4	Blank	C	22
05/02/2016	35	1	Ecotreat	B	23
		2	W. Ref.	B	22
		3	Ash	B	22
		4	Blank	B	23
		1	Ecotreat	C	22
		2	W. Ref.	C	22
		3	Ash	C	22
		4	Blank	C	22

Table B-9: Results from flies count on trial-2

		Raw sludge	Treatment			
	Pannelist		**Ecotreat**	**W. Reference**	**Ash**	**Blank**
Day 0	1	43	43	43	43	43
	2	39	39	39	39	39
	3	44	44	44	44	44
	Average	42	42	42	42	42
	St. Deviation	2.6	2.6	2.6	2.6	2.6
Day 7	1		38	47	8	36
	2		44	42	4	32
	3		48	44	5	41
	Average		43	44	6	36
	St. Deviation		5.0	2.5	2.1	4.5
Day 14	1		82	71	12	78
	2		80	83	10	75
	3		75	80	10	90
	Average		79	78	11	81
	St. Deviation		3.6	6.2	1.2	7.9
Day 21	1		67	73	13	82
	2		71	77	11	67
	3		64	62	7	69
	4		78	69	9	78
	5		62	74	6	75
	Average		68	71	9	74
	St. Deviation		6.3	5.8	2.9	6.2
Day 28	1		73	68	8	74
	2		79	81	5	62
	3		61	63	12	71
	Average		71	71	8	69
	St. Deviation		9.2	9.3	3.5	6.2
Day 35	1		83	77	6	67
	2		74	69	7	73
	3		87	84	3	60
	Average		81	77	5	67
	St. Deviation		6.7	7.5	2.1	6.5

Table B-10: Results from flies count on trial-1

Day	Pannellist	Treatment																															
		Raw sludge					Ikati					Soda					Blank					W. Reference					Sannitree					Ecotreat				
		1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD	1	2	3	Mean	SD
Day 0	1	60	57	60	59	1.73																													
	2	60	75	70	68	7.64																													
	3	50	65	65	60	8.66																													
	4	55	80	60	65	13.23																													
	5	60	65	55	60	5.00																													
	Average	57	68	62	62	5.7																													
	St. Deviation	4.5	9.1	5.7																															
Day 7	1						50	60	50	53	5.8	50	35	55	47	10.41	60	70	60	63	5.77	50	75	50	58	14.43	65	40	55	53	12.58	50	70	65	62	10.41
	2						40	65	55	53	12.6	50	30	50	43	11.55	65	70	65	67	2.89	50	75	55	60	13.23	75	45	65	62	15.28	50	65	60	58	7.64
	3						40	45	55	47	7.6	35	20	55	37	17.56	55	65	65	62	5.77	55	70	60	62	7.64	75	45	65	62	15.28	45	70	60	58	12.58
	4						35	50	50	45	8.7	30	25	50	35	13.23	55	60	60	58	2.89	60	75	60	65	8.66	70	40	55	55	15.00	45	75	50	57	16.07
	5						40	55	40	45	8.7	30	30	45	35	8.66	60	70	55	62	7.64	45	60	50	52	7.64	70	40	60	57	15.28	45	70	55	57	12.58
	Average						41	55	50	49	7.1	39	28	51	39	11.5	59	67	61	62	4.2	52	71	55	59	10.2	71	42	60	58	14.6	47	70	58	58	11.5
	St. Deviation						5.5	7.9	6.1			10.2	5.7	4.2			4.2	4.5	4.2			5.7	6.5	5.0			4.2	2.7	5.0			2.7	3.5	5.7		
Day 14	1						35	55	50	47	10.4	45	40	30	38	7.6	55	55	60	57	2.9	60	55	60	58	2.9	65	60	45	57	10.4	40	50	55	48	7.64
	2						45	50	45	47	2.9	35	45	40	40	5.0	60	70	70	67	5.8	55	55	65	58	5.8	60	55	55	57	2.9	50	65	50	55	8.66
	3						45	40	50	45	5.0	35	40	35	37	2.9	65	65	70	67	2.9	50	45	65	53	10.4	60	55	50	55	5.0	55	60	65	60	5.00
	4						40	45	55	47	7.6	40	35	35	37	2.9	55	65	55	58	5.8	55	50	60	55	5.0	55	50	45	50	5.0	55	50	45	50	5.00
	5						35	45	55	45	10.0	35	40	35	37	2.9	50	60	65	58	7.6	45	50	60	52	7.6	50	65	45	53	10.4	45	60	50	52	7.64
	Average						40	47	51	46	5.6	38	40	35	38	2.5	57	63	64	61	3.8	53	51	62	55	5.9	58	57	48	54	5.5	49	57	53	53	4.0
	St. Deviation						5.0	5.7	4.2			4.5	3.5	3.5			5.7	5.7	6.5			5.7	4.2	2.7			5.7	5.7	4.5			6.5	6.7	7.6		
Day 21	1						40	55	55	50	8.7	45	35	45	42	5.8	75	60	75	70	8.7	50	70	55	58	10.4	65	60	50	58	7.6	55	55	55	55	0.0
	2						45	50	50	48	2.9	45	35	50	43	7.6	70	55	65	63	7.6	50	65	60	58	7.6	65	60	55	60	5.0	55	60	40	52	10.4
	3						35	60	50	48	12.6	35	30	45	37	7.6	70	65	70	68	2.9	45	75	60	60	15.0	60	55	55	57	2.9	55	60	45	53	7.6
	4						35	50	45	43	7.6	40	30	40	37	5.8	75	55	70	67	10.4	55	60	55	57	2.9	55	50	45	50	5.0	50	65	45	53	10.4
	5						35	50	45	43	7.6	35	30	40	35	5.0	65	50	65	60	8.7	45	70	55	57	12.6	60	55	50	55	5.0	50	65	50	55	8.7
	Average						38	53	49	47	7.8	40	32	44	39	6.1	71	57	69	66	7.6	49	68	57	58	9.5	61	56	51	56	5.0	53	61	47	54	7.0
	St. Deviation						4.5	4.5	4.2			5.0	2.7	4.2			4.2	5.7	4.2			4.2	5.7	2.7			4.2	4.2	4.2			2.7	4.2	5.7		
Day 28	1						30	30	30	30	0.0	35	30	30	32	2.9	45	45	40	43	2.9	35	35	40	37	2.9	35	40	40	38	2.9	30	35	40	35	5.0
	2						25	35	30	30	5.0	35	25	25	28	5.8	40	45	40	42	2.9	35	30	45	37	7.6	30	50	45	42	10.4	25	35	50	37	12.6
	3						25	35	25	28	5.8	30	25	20	25	5.0	45	50	35	43	7.6	35	30	50	38	10.4	35	45	40	40	5.0	25	40	40	35	8.7
	4						20	35	25	27	7.6	30	30	20	27	5.8	40	50	35	42	7.6	30	25	50	35	13.2	30	45	35	37	7.6	30	35	45	37	7.6
	5						25	35	35	32	5.8	30	30	25	28	2.9	40	55	40	45	8.7	40	25	40	35	8.7	30	40	35	35	5.0	20	40	35	32	10.4
	Average						25	34	29	29	4.5	32	28	24	28	4.0	42	49	38	43	5.6	35	29	45	36	8.1	32	44	39	38	6.0	26	37	42	35	8.2
	St. Deviation						3.5	2.2	4.2			2.7	2.7	4.2			2.7	4.2	2.7			3.5	4.2	5.0			2.7	4.2	4.2			4.2	2.7	5.7		

Table B-11: Results from TON analysis on trial-1

Sampling day	Treatment	Raw sludge						Ikati						Soda						Control					W. Reference					Sannitree					Ecotreat				
	TON (serial dilutions)	200	400	1000	1333	1667	2000	400	667	800	850	870	1000	400	667	800	850	870	1000	400	1000	1333	1539	1667	400	1000	1333	1539	1667	400	1000	1333	1429	1539	1667	400	1000	1333	1539	1667
0	Justine Muathe (Student)	+	+	+	+	-	-																																	
	Protas Mussumba (Security)	+	+	+	-	-	-																																	
	Paul Manda (Operator)	+	+	+	-	-	-																																	
	Hassan Juma (Carpenter)	+	+	+	+	+	-																																	
	Evans Mahuana (Security)	+	+	+	+	+	-																																	
	TON (determined)					1667																																		
7	Justine Muathe (Student)							+	+	-	-	-		+	+	+	-	-		+	+	-	-	-	+	+	+	-		+	+	+	+	-		+	+	+	-	
	Protas Mussumba (Security)							+	+	+	+	-		+	+	+	+	-		+	+	+	+	+																
	Paul Manda (Operator)							+	+	+	+	+		+	+	+	+	-		+	+	+	+	-																
	Hassan Juma (Carpenter)							+	+	-	-	-		+	+	+	+	-		+	+	+	+	-																
	Evans Mahuana (Security)							+	+	+	+	+		+	+	+	+	+		+	+	+	+	-																
	Joseph Garaiga (House manager)																								+	+	-	-		+	+	+	+	-		+	+	+	+	
	Daniel Ngarima (House security)																								+	+	+	+		+	+	-	-	-		+	+	-	-	
	TON (determined)											870						870						1667				1539						1539					1539		
14	Justine Muathe (Student)							+	+	-	-			+	+	+	-			+	+	-	-		+	+	+	+		+	+	+				+	+	-		
	Protas Mussumba (Security)							+	+	+	+			+	+	+	-			+	+	-	-		+	+	+	+		+	+	-				+	+	+		
	Paul Manda (Operator)							+	+	+	+			+	+	+	+			+	+	+	+		+	+	+	+		+	+	-				+	+	+		
	Hassan Juma (Carpenter)							+	+	+	-			+	+	+	+			+	+	+	-		+	+	+	-		+	+	-				+	+	-		
	John Munene (security)							+	+	+	-			+	+	+	-			+	+	+	-		+	+	+	-		+	+	-				+	+	-		
	TON (determined)										850						850						1539				1539						1333					1333		
21	Justine Muathe (Student)							+	+	-	-			+	+	+	-			+	+	-	-	-	+	+	+	+	-	+	+	+				+	+	-		
	Protas Mussumba (Security)							+	+	+	+			+	+	+	-			+	+	+	-	-	+	+	+	+	+	+	+	-				+	+	+		
	Paul Manda (Operator)							+	+	+	-			+	+	+	-			+	+	+	+	+	+	+	+	+	-	+	+	+				+	+	-		
	John Munene (security)							+	+	-	-			+	+	+	+			+	+	+	+	-	+	+	+	-	-	+	+	-				+	+	-		
	Evans Mahuana (Security)							+	+	+	-			+	+	+	-			+	+	+	+	-	+	+	+	+	+	+	+	-				+	+	-		
	TON (determined)										850						850						1539	1667			1539	1667					1333					1333		
28	Justine Muathe (Student)							+	+	-	-			+	+	+	-			+	+	+	-	-	+	+	+	+	-	+	+	+	-			+	+	-		
	Marcos Zindoga (Student)							+	+	-	-			+	+	+	-			+	+	+	+	+	+	+	+	+	+	+	+	-	-			+	+	+		
	John Munene (Ex- Security)							+	+	-	-			+	+	+	+			+	+	+	+	-	+	+	+	+	-	+	+	+	+			+	+	-		
	Joseph Askari (Home security)							+	+	+	+			+	+	+	-			+	+	+	+	-	+	+	+	+	+	+	+	-	-			+	+	-		
	Evans Askari (Home security)							+	+	+	-			+	+	+	-			+	+	+	+	-	+	+	+	+	-	+	+	+	-			+	+	+		
	TON (determined)									800							850							1667					1667				1429					1333		

Table B-12: Results from TON analysis on trial-2

Sampling day	Treatment	Raw sludge							Ecotreat						W. Reference						Ash							Blank					
	TON (serial dilutions)	200	400	1000	1333	1667	2000		400	1000	1333	1667	1818	2000	400	1000	1333	1667	2000	2222	250	300	350	400	450	444	500	400	1000	1333	1667	2000	2222
0	Justine Muathe (Student)	+	+	+	+	+	-	2000																								
	Mumoh (Security main-gate)	+	+	+	+	-	-	1677																								
	Evans Mahuana (Security)	+	+	+	+	-	-	1677																								
	John Munene (security)	+	+	+	+	+	+	2222																								
	Marcos Zindoga (Student)	+	+	+	+	+	-	2000																								
	TON (determined)						2000	1903.593																								
7	Justine Muathe (Student)								+	+	+	+	+	-	+	+	+	+	-		+	+	+	+	+	-		+	+	+	+	-	-
	Protas Mussumba (Security)								+	+	+	+	-	-	+	+	+	+	+		+	+	+	+	+	-		+	+	+	+	+	+
	Evans Mahuana (Security)								+	+	+	+	+	-	+	+	+	+	+		+	+	+	+	+	+		+	+	+	+	+	-
	John Munene (Ex-security)								+	+	+	+	+	+	+	+	+	-	-		+	+	+	+	-	-		+	+	+	+	+	-
	Marcos Zindoga (Student)								+	+	+	+	+	+	+	+	+	-	-		+	+	+	-	-	-		+	+	+	+	+	-
	TON (determined)													2000					2000						444								2000
14	Justine Muathe (Student)								+	+	+	+	+	-	+	+	+	+	+		+	+	+	+	+	+		+	+	+	+	-	-
	Protas Mussumba (Security)								+	+	+	+	-	-	+	+	+	+	+		+	+	+	+	-	-		+	+	+	+	-	-
	Mumoh (Security)								+	+	+	+	+	-	+	+	+	+	-		+	+	+	+	-	-		+	+	+	+	+	-
	John Munene (Ex-security)								+	+	+	+	+	-	+	+	+	-	-		+	+	+	+	+	-		+	+	+	+	+	-
	Marcos Zindoga (Student)								+	+	+	+	+	+	+	+	+	+	-		+	+	+	+	+	-		+	+	+	+	+	-
	TON (determined)													2000					2000						444								2000
21	Justine Muathe (Student)								+	+	+	+	+		+	+	+	+	-		+	+	+	+	+	-	+	+	+	+	+	-	-
	Protas Mussumba (Security)								+	+	+	+	-		+	+	+	+	+		+	+	+	+	+	+	-	+	+	+	+	+	+
	Marcos Zindoga (Student)								+	+	+	+	+		+	+	+	+	-		+	+	+	+	-	-	-	+	+	+	+	+	-
	John Munene (security)								+	+	+	+	-		+	+	+	-	-		+	+	+	+	+	+	-	+	+	+	+	+	-
	Evans Mahuana (Security)								+	+	+	+	-		+	+	+	-	-		+	+	+	+	-	-	-	+	+	+	+	+	+
	TON (determined)												1818						2000								500						2222
28	Justine Muathe (Student)								+	+	+	+	-		+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-
	Marcos Zindoga (Student)								+	+	+	+	-		+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-
	John Munene (Ex- Security)								+	+	+	+	+		+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-
	Joseph Askari (Home security)								+	+	+	+	+		+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+
	Evans Askari (Home security)								+	+	+	+	-		+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+
	TON (determined)												1818							2222							444						2222
35	Justine Muathe (Student)								+	+	+	+	-		+	+	+	+	+	-	+	+	+	+	+	-		+	+	+	+	-	-
	Marcos Zindoga (Student)								+	+	+	+	-		+	+	+	+	+	+	+	+	+	+	+	-		+	+	+	+	+	+
	John Munene (Ex- Security)								+	+	+	+	+		+	+	+	+	+	-	+	+	+	+	+	+		+	+	+	+	-	-
	Joseph Askari (Home security)								+	+	+	+	-		+	+	+	+	+	-	+	+	+	+	-	-		+	+	+	+	+	-
	Evans Askari (Home security)								+	+	+	+	-		+	+	+	+	+	+	+	+	+	+	-	-		+	+	+	+	+	-
	TON (determined)												1818							2222						444							2222

Table B-13: Results from E. coli analysis on trial-1

Day	Dilution	Raw FS			Mean	SD	Ikati			Mean	SD	Soda			Mean	SD	Blank			Mean	SD	Water Reference			Mean	SD	Sannitree			Mean	SD	Ecotreat			Mean	SD	Detection Limit
		Sample 1	Sampler 2	Sample 3	cfu/100 ml		Reactor 1	Reactor 2	Reactor 3	cfu/100 ml		Reactor 1	Reactor 2	Reactor 3	cfu/100 ml		Reactor 1	Reactor 2	Reactor 3	cfu/100 ml		Reactor 1	Reactor 2	Reactor 3	cfu/100 ml		Reactor 1	Reactor 2	Reactor 3	cfu/100 ml		Reactor 1	Reactor 2	Reactor 3	cfu/100 ml		
0	1.00E+00																																		1.00E+03
	1.00E+01																																		1.00E+03
	1.00E+02	vercrowde	vercrowde	vercrowded																															1.00E+03
	1.00E+03	271	133	196																															1.00E+03
	1.00E+04	79	93	85	8.57E+08																														1.00E+03
	1.00E+05	3	1	12																															1.00E+03
	1.00E+06	0	0	0																															1.00E+03
	cfu/100 ml	7.90E+08	9.30E+08	8.50E+08																															1.00E+03
	Log(cfu/100ml)	8.90	8.97	8.93	8.93	0.04																													1.00E+03
1	1.00E+00																																		1.00E+03
	1.00E+01						4	7	4	5.00E+04	1.73	12	7	14	1.10E+05																					1.00E+03
	1.00E+02						0	0	0			0	0	0																						1.00E+03
	1.00E+03						0	0	0			0	0	0																						1.00E+03
	1.00E+04																70	55	58	6.10E+08																1.00E+03
	1.00E+05																14	26	12																	1.00E+03
	cfu/100 ml						4.00E+04	7.00E+04	4.00E+04			1.20E+05	7.00E+04	1.40E+05			7.00E+08	5.50E+08	5.80E+08																	1.00E+03
	Log(cfu/100ml)						4.60	4.85	4.60	4.68	0.14	5.08	4.85	5.15	5.02	0.16	8.85	8.74	8.76	8.78	0.06															1.00E+03
2	1.00E+00						0	0	0	0.00E+00		0	0	0	0	0.00E+00																					1.00E+03
	1.00E+01						0	0	0			0	0	0																						1.00E+03
	1.00E+02						0	0	0			0	0	0																						1.00E+03
	1.00E+03																																		1.00E+03
	1.00E+04																62	48	52	5.40E+08																1.00E+03
	1.00E+05																11	15	7																	1.00E+03
	cfu/100 ml						0.00E+00	0	0			0	0	0			6.20E+08	4.80E+08	5.20E+08																	1.00E+03
	Log(cfu/100ml)						#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	8.79	8.68	8.72	8.73	0.06															1.00E+03
3	1.00E+00						0	0	0	0.00E+00		0	0	0	0																						1.00E+03
	1.00E+01						0	0	0			0	0	0																						1.00E+03
	1.00E+02						0	0	0			0	0	0																						1.00E+03
	1.00E+03																																		1.00E+03
	1.00E+04																																		1.00E+03
	1.00E+05																																		1.00E+03
	cfu/100 ml						0	0	0			0	0	0																						1.00E+03
	Log(cfu/100ml)						#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!															1.00E+03
7	1.00E+00						0	0	0	0.00E+00		0	0	0	0	0.00E+00																					1.00E+03
	1.00E+01						0	0	0			0	0	0																						1.00E+03
	1.00E+02						0	0	0			0	0	0								overcrowde	vercrowde	vercrowded			261	vercrowde	vercrowded			124	69	221			1.00E+03
	1.00E+03																154	136	182	1.57E+08		105	83	91	9.30E+07		138	116	111	1.22E+08		63	77	73	7.10E+07		1.00E+03
	1.00E+04																32	14	9			9	21	13			42	14	21			12	3	8			1.00E+03
	1.00E+05																7	3	11			0	0	0			0	0	0			0	0	0			1.00E+03
	1.00E+06																0	0	0			0	0	0			0	0	0			0	0	0			1.00E+03
	cfu/100 ml						0.00E+00	0	0			0	0	0			1.54E+08	1.36E+08	1.82E+08			1.05E+08	8.30E+07	9.10E+07			1.38E+08	1.16E+08	1.11E+08			6.30E+07	7.70E+07	7.30E+07			1.00E+03
	Log(cfu/100ml)						#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	8.19	8.13	8.26	8.19	0.06	8.02	7.92	7.96	7.97	0.05	8.14	8.06	8.05	8.08	0.05	7.80	7.89	7.86	7.85	0.05	1.00E+03
14	1.00E+00						2	0	0	6.67E+02		0	0	0	0																						1.00E+03
	1.00E+01						0	0	0	0.00E+00		0	0	0																						1.00E+03
	1.00E+02						0	0	0			0	0	0																						1.00E+03
	1.00E+03																31	41	29	3.37E+07		87	105	89	9.37E+07		172	188	181	1.80E+08		58	51	35	4.80E+07		1.00E+03
	1.00E+04																6	1	0			8	22	16			48	37	33			10	2	7			1.00E+03
	cfu/100 ml						2.00E+03	0.00E+00	0.00E+00			0.00E+00	0.00E+00	0.00E+00			3.10E+07	4.10E+07	2.90E+07			8.70E+07	1.05E+08	8.90E+07			1.72E+08	1.88E+08	1.81E+08			5.80E+07	5.10E+07	3.50E+07			1.00E+03
	Log(cfu/100ml)						3.30	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	7.49	7.61	7.46	7.52	0.08	7.94	8.02	7.95	7.97	0.04	8.24	8.27	8.26	8.26	0.02	7.76	7.71	7.54	7.67	0.11	1.00E+03
21	1.00E+00						0	0	0	0		0	0	0	0	0.00E+00																					1.00E+03
	1.00E+01						0	0	0			0	0	0																						1.00E+03
	1.00E+02						0	0	0			0	0	0			23	142	96	8.70E+06																1.00E+03
	1.00E+03																14	18	7			53	78	71	6.73E+07		18	20	26	2.13E+07		68	58	61	6.23E+07		1.00E+03
	1.00E+04																0	0	0			16	0	0			0	0	0			3	0	11			1.00E+03
	cfu/100 ml						0.00E+00	0.00E+00	0.00E+00			0.00E+00	0.00E+00	0.00E+00			2.30E+06	1.42E+07	9.60E+06			5.30E+07	7.80E+07	7.10E+07			1.80E+07	2.00E+07	2.60E+07			6.80E+07	5.80E+07	6.10E+07			1.00E+03
	Log(cfu/100ml)						#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	6.36	7.15	6.98	6.83	0.42	7.72	7.89	7.85	7.82	0.09	7.26	7.30	7.41	7.32	0.08	7.83	7.76	7.79	7.79	0.04	1.00E+03
28	1.00E+00						0	0	0	0		0	0	0	0	0.00E+00																					1.00E+03
	1.00E+01						0	0	0			0	0	0																						1.00E+03
	1.00E+02						0	0	0			0	0	0			103	109	112	1.08E+07		79	71	84	7.80E+06		105	87	101	9.77E+06		overcrowde	vercrowde	vercrowded			1.00E+03
	1.00E+03																17	12	8			4	14	2			7	4	11			216	135	142	1.64E+08		1.00E+03
	1.00E+04																0	0	0			0	0	0			0	0	0			17	41	26			1.00E+03
	cfu/100 ml						0.00E+00	0.00E+00	0.00E+00			0.00E+00	0.00E+00	0.00E+00			1.03E+07	1.09E+07	1.12E+07			7.90E+06	7.10E+06	8.40E+06			1.05E+07	8.70E+06	1.01E+07			2.16E+08	1.35E+08	1.42E+08			1.00E+03
	Log(cfu/100ml)						#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	#NUM!	7.01	7.04	7.05	7.03	0.02	6.90	6.85	6.92	6.89	0.04	7.02	6.94	7.00	6.99	0.04	8.33	8.13	8.15	8.21	0.11	1.00E+03

0 Not detected
Not analysed
hight deviation
Adopted as final result

Table B-14: Results from E. coli analysis on trial-2

	Dilution	Ecotreat		Mean	Std. Dev.	W. Reference		Mean	Std. Dev.	Ash		Mean	Std. Dev.	Blank		Mean	Std. Dev.	Detection Limit
Day		Sample 1	Sample 2	[cfu/100 mL]		Sample 1	Sample 2	[cfu/100 mL]		Sample 1	Sample 2	[cfu/100 mL]		Sample 1	Sample 2	[cfu/100 mL]		
14	1.00E+00									n.v.	n.v.							1.00E+03
	1.00E+01									overcrowded	overcrowded							1.00E+03
	1.00E+02									170	142	1.56E+07						1.00E+03
	1.00E+03													178	197	1.88E+08		1.00E+03
	1.00E+04													46	42			1.00E+03
	cfu/100 ml									1.70E+07	1.42E+07			1.78E+08	1.97E+08			1.00E+03
	Log(cfu/100 ml)									7.23	7.15	7.19	0.06	8.25	8.29	8.27	0.03	1.00E+03
21	1.00E+00																	1.00E+03
	1.00E+01																	1.00E+03
	1.00E+02									142	98	1.20E+07		overcrowded	overcrowded			1.00E+03
	1.00E+03									17	25			75	101	8.80E+07		1.00E+03
	1.00E+04									8	5			7	4			1.00E+03
	cfu/100 ml									1.42E+07	9.80E+06			7.50E+07	1.01E+08			1.00E+03
	Log(cfu/100 ml)									7.15	6.99	7.07	0.11	7.88	8.00	7.94	0.09	1.00E+03
28	1.00E+00																	1.00E+03
	1.00E+01																	1.00E+03
	1.00E+02	overcrowded	261			overcrowded	overcrowded			164	182	1.73E+07		212	247	2.30E+07		1.00E+03
	1.00E+03	101	97	9.90E+07		109	121	1.15E+08		42	29			72	67			1.00E+03
	1.00E+04	10	14			23	32			3	7			20	5			1.00E+03
	cfu/100 ml	1.01E+08	9.70E+07	2.83E+06		1.09E+08	1.21E+08			1.64E+07	1.82E+07			2.12E+07	2.47E+07			1.00E+03
	Log(cfu/100 ml)	8.00	7.99	8.00	0.01	8.04	8.08	8.06	0.03	7.21	7.26	7.24	0.03	7.33	7.39	7.36	0.05	1.00E+03
35	1.00E+00																	1.00E+03
	1.00E+01																	1.00E+03
	1.00E+02	202	overcrowded			overcrowded	overcrowded			179	155	1.67E+07		overcrowded	overcrowded			1.00E+03
	1.00E+03	88	108	9.80E+07		96	102	9.90E+07		11	17			81	73	7.70E+07		1.00E+03
	1.00E+04	17	6			41	27			0	0			16	22			1.00E+03
	cfu/100 ml	8.80E+07	1.08E+08			9.60E+07	1.02E+08			1.79E+07	1.55E+07			8.10E+07	7.30E+07			1.00E+03
	Log(cfu/100 ml)	7.94	8.03	7.99	0.06	7.98	8.01	8.00	0.02	7.25	7.19	7.22	0.04	7.91	7.86	7.89	0.03	1.00E+03

Appendix C: Datasheet of the additives

Figure C-1 Datasheet of biological additive Sannitree Bio-Enzyme Granules for Pit Latrines [http://www.sannitree.co.za/our-products/pit-toilet-maintenance-bio-enzyme-granules/ (Accessed in September 2015)]

BIO-ENZYME GRANULES FOR PIT TOILETS™

Double Action Bio-Enzyme Granules are a blend of freeze-dried bacteria and enzymes specially formulated to rapidly digest organic waste, reduce bad odours and the fly population found in and around pit toilets and latrines.

It has no significant impact on beneficial insects or organic material.

Enquire about this product

How does it work?

This double action forumlae is specially formulated to rapidly digest organic waste. It also contains an active ingredient which attacks the larvae of flies, preventing them from shedding their skins. As this is an essential part of the larvae's growth and development, this double action product effectively stops the larvae from turning into flies.

The benefits

- ✓ Rapidly digests organic waste assuring easy pump-outs
- ✓ Attacks and neutralises fly larvae
- ✓ Reduces bad smells
- ✓ Non-hazardous to people, animals and water bodies
- ✓ Can be beneficial when discharged after pumping to sewage treatment works

What's it good for?

– Pit toilet maintenance & fly control

BUT DON'T TAKE OUR WORD FOR IT…

I would like to take this opportunity to thank u for this great product its affordable and working miracles my toilet is now spotless I have recommended it to my neighbours also.I give it a 100% rating! Read more

Brenda, Private

ACTIVE INGREDIENTS

Bacteria: Nitrosomanas sp. Nitrobacter sp. Aerobacter sp. Bacillus subtilis, Cellulomonas sp.

Enzymes: Protease, Amalyse, Hemicellulase, Lactase, Lipase

DOSAGE
Mix 100 grams into a 5 litre bucket of water. Allow to stand for 10 minutes, stir and empty into pit.

Repeat treatment monthly.

PACKAGING
100 gram sachets – 10 per tray

100 gram sachets – 250 per carton

STORAGE
12 months. Keep in a cool, dry place

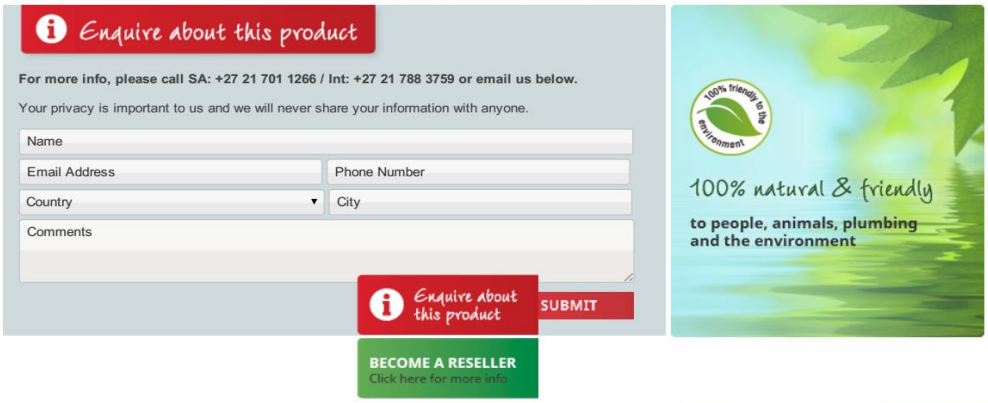

Figure C-2 Dosing protocol of biological additive Consortium Lice SM [Source: BCI Environment, 2015]

Procedures and Precautions

Doses are indicative and are calculated using the total volume of the pit, and the maximum of number of users per day

The objective of seeding is to prime the pit with the new microorganisms as fast as possible, in order to rapidly accommodate the faecal sludge to be treated.

It is important to emphasize that procedures and recommendations have been made as a guideline to insure the most efficient use of the product provided by the manufacturer, taking into account all possible environmental constraints of the pit latrine.

However, the operator can choose to adjust the dosing as soon as the first seeding batch accommodation has been established in the faecal sludge of the pit

The following dosing procedure is for a maximum volume of 3m3. Beyond this volume and up to 10m3, the quantity of product added should not exceed 3 Kg/m3. Two examples are given depending on whether the pit is empty or filled above 50%.

Phase 1 initial seeding:

- Wait 2-3 days so that the bottom of the pit is completely covered with excreta to at least 2 to 3cm depth.
- Dosing: 1Kg/m3 total volume pit at DAY 1 - a pit of 3m3 therefore requires a 3kg dose of product. Beyond this volume and up to 10m3, the quantity should not be more than 3Kg/m3
- The SM Consortium Lice product has to be diluted in warm water, stirred and then poured in its entirety into the pit. This is to prevent wastage of the product that would, if used in powder form, float around the pit and adhere to the walls instead of the excreta.

Phase 3 Maintenance:

- Dosing: 25gr / starting at Day 7.
- Example with a latrine designed for 50 people per day: 5 persons x 25gr = 1,25Kg/month. This amount would then be diluted in warm water, stirred and poured into the pit DAY 7 to DAY 30.
- For the second month, maintenance from Day 1 to Day 30 is still 25gr x number of daily users.

Phase 2 Starter:

- Dosing 500gr/pit over a period of 5 days at DAY 2, DAY 3, DAY 4, DAY 5 and DAY 6.
- This would therefore require 2.5Kg of product for a pit volume of 3m3 MAX).
- Dilute 500gr of the product in warm water, stir and then pour all of the solution into the pit.

CAUTION:

The number of users per month of the pit must be carefully considered in order to ensure effective dosing and to avoid under-dosing.
It is necessary to double the dose when latrine users are taking antibiotics.

Special care must be taken to avoid the introduction of other bactericidal products such as bleach or other products containing chlorine, ammonia, and other types of disinfectants. Introduction of bactericidal products before or after using SM Consortium Lice will reduce its effectiveness.

Usage Instructions

- Mix the powder with warm water.
- Leave the mixture to rest for 10 to 15 minutes.
- Add the entire mixture directly to the pit.

Precautions

Bacteria, belonging to **Group 1** according to AFNOR X 42-211, are **microorganisms that are classified unlikely to cause disease in humans.** They should be considered as non-pathogenic under the normal use of the product.

As a general rule, the following must be avoided:

- Inhalation, and ingestion.
- Contact with eyes, open wounds, broken skin or mucous membranes and upper respiratory tract.
- The use of this product should be strictly limited to the purpose for which it was intended.
- The user's attention is drawn to the possible risks that may be incurred when a product is used for purposes other than those for which it has been designed.
- Only staff fully trained in the use of SM Consortium Lice should be allowed to handle the product.
- When handling the product the use gloves is mandatory, the operator must also wear a dust mask when handling the powder to avoid breathing in the product.
- In case of contact with the skin - immediately wash off any product with soap and water.
- The container used to dose or dilute the powder should be strictly reserved for this purpose or discarded immediately after use.
- The product must be kept out of the reach of children.
- The product must be kept away from food and beverages, including animal feed.

Storage Conditions

- Store in a cool, dry place.
- For professional use: contains a biological accelerator.
- Carefully close the packaging after using the product.

Packaging

- 5 kg pails
- 25 kg bags

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