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Sanitizing Faecal Sludge using Lactic Acid Bacteria in Emergency

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Master of Science Thesis
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Abstract

Providing safe excreta collection and disposal in emergency situations has remained one of the most urgent priorities in the disaster relief effort. This is because of the high risk to human health that exposed and unsanitized human excreta pose. With an increase in the frequency and intensity of natural disasters in recent years, humanitarian aid and related organisations worldwide are currently exploring sustainable low cost sanitation technologies and methods that promote safe excreta collection and disposal in disaster and emergency situations.

The objective of the research was to evaluate the sanitizing effect of lactic acid and other metabolites produced by Lactic Acid Bacteria (LABs) on human excreta by exploiting and promoting the Lactic Acid Fermentation (LAF) process. The research further explored the pre-conditions required (such as sugar and inoculum concentrations) and the suitability of Yakult, a probiotic milk product, being used as the microbial inoculant to the treatment process. Molasses was selected and used as the sugar additive for the promotion of the LAF process and *Escherichia coli* as the indicator organism.

Laboratory-scale batch LAF experiments were conducted at the UNESCO-IHE Institute of Water Education laboratory facility in Delft, The Netherlands using black water collected from a treatment plant in Sneek, northern Netherlands. This was followed by field tests carried out in Blantyre, Malawi to assess the up-scalability of the laboratory-scale experiments.

WHO guidelines (2006) were employed to assess the level of sanitizing of the LAF process on faecal sludge.

Optimal concentrations for molasses and inoculum used were established to be 10% w/w concentration in faecal sludge for both parameters respectively. This 10% w/w molasses concentration translated to a total sugar (glucose & fructose) concentration of 1.5-2.0g/L. In the laboratory, sanitization of faecal sludge was achieved in 9-15 days. Field investigations revealed shorter sanitization times of 7-9 days; the variation being attributed to a difference in the alkalinity levels of the faecal sludge. A lactic acid concentration of 20-30g/L was established as one that triggers the sanitization effect in the faecal sludge with corresponding pH values of 3.8-4.2 being achieved at sanitization. A suppression of faecal sludge odor was noted as an important deliverable of the treatment process and an attempt was made to establish the odor threshold number during the treatment process. However the results of the threshold odor number (TON) proved to be inconclusive.

Results of the research thus indicated the potential of the Lactic Acid Fermentation (LAF) process in the treatment of faecal sludge in both emergency situations and also as a treatment option for onsite sanitation facilities such as pit latrines. Further investigations into the kinetics of the lactic acid bacteria with respect to their interaction with different environmental conditions were recommended.

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Abbreviations

CFU	Colony forming unit
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EM-DAT	Emergency Events Database
FS	Faecal Sludge
LAB	Lactic Acid Bacteria
LAF	Lactic Acid Fermentation
LPS	Lipopolysaccharides
OSS	On-site Sanitation Systems
RNA	Ribonucleic acid
TS	Total Solids
TTA	Total Titrable Acid
UDDT	Urine Diverting and Dehydration Toilets
vcc	Viable cell count
VS	Volatile Solids
WHO	World Health Organisation

List of Symbols

°C	Degrees Celsius
K_d	natural die-off rate constant
K_{td}	Treatment die-off rate constant

CHAPTER 1

Introduction

Natural and man-made disasters such as hurricanes, floods, earthquakes and wars, political unrest, nuclear disasters respectively, often lead to emergency situations. These emergency situations are characterised for example by, population displacements, widespread destruction of infrastructure and disruption of basic services that support people's livelihood. Usually the impacts thereof are often sudden and catastrophic and can range from a lack of food and outbreak of diseases, to a lack of basic shelter and ultimately to loss of human life.

In most instances, the trend as with regards to responding to these disaster situations, has usually given priority to the provision of medical care, food and water supply to the affected areas. Matters pertaining to the treatment and disposal of human excreta have usually received secondary attention. This is in spite of inadequate sanitation facilities and poor hygiene practice being the cause of most common diseases occurring in emergency situations (Harvey Bastable, Andy.,, 2007). Connolly et al., (2004) further observe that diarrhoeal diseases are a major cause of morbidity and mortality and account for around 40% of all deaths in the acute phase of emergency situations. They further link diarrhoeal diseases to inadequate quality and quantity of water, substandard and insufficient sanitation facilities, overcrowding, poor hygiene, and scarcity of soap.

It is thus imperative that for the response to emergency situations to be all encompassing, the aspect of treatment and safe disposal of human excreta must be accorded the importance it deserves. It should thus be rendered a key element of any disaster response strategy and should be given the same priority as that of the provision of medical care, food and water supply. The importance of safe excreta disposal cannot be overemphasized. According to Davis and Lambert, (2002) as cited in Bastable, Gb, & Reed, (2004), diseases transmitted via the faeco-oral route, such as diarrhea, have been shown to account for 40% of all childhood deaths in an emergency.

Franceys (1992) has shown that the introduction of safe excreta disposal can greatly reduce the incidence of excreta-related communicable diseases such as cholera, typhoid, dysentery (including shigellosis), diarrhea, hookworm, schistose omiasis and filariasis helminth infestations. This position has further been supported by Cluster (2006) who points out that the most important preventive measure to ensure communicable diseases are prevented following natural disasters, is by ensuring the uninterrupted provision of safe drinking water and adequate sanitation needs.
Bastable et al., (2004) have however acknowledged that many humanitarian aid agencies are aware of these facts and wish to give a greater emphasis to excreta disposal.

1.1. Problem Statement

Providing good faecal sludge management in emergency situation has remained a challenge for all the various actors involved in the humanitarian effort following a disaster. There is usually large scale disruption of infrastructure, water quality becomes compromised, there is poor sanitation, and massive numbers of the population are displaced into temporary crowded shelters.

The situation is even more compounded when providing this humanitarian effort in developing countries where backbone sanitation infrastructure is inadequate and in some cases virtually nonexistent. Also, because of other competing requirements such as food and medical services during emergencies, development of suitable treatment and disposal methods of large quantities of human excreta in (post) emergency settings has been often neglected by the various players involved. This has often led to inappropriate disposal and treatment solutions such as the creation of unsanitary defecation fields and landfills which often have adverse environmental and health implications as highlighted in an internal report on *Requirements for Faecal Sludge Treatment and disposal in emergency situations* compiled by WASTE`, Netherlands Red Cross, Aldus Bouwinnovatie in consultation with International Federation of Red Cross and Red Crescent Societies, Oxfam UK (2012).

1.2. Significance of Research

Lactic acid is an important compound because of its inhibiting capabilities on a wide range of microorganisms. This capability that lactic acid poses has been used for a long time now in the food preservation industry. Helander et al., (1997) suggests that in its undissociated form, lactic acid poses high antimicrobial activity with an inhibitory effect of 10-600 times stronger than that of its dissociate forms. Lactic acid can be produced cheaply by fermentation of simple sugars using lactic acid bacteria, a fairly large group of bacterial species which is readily found in foodstuff and in the natural environment. In addition to producing lactic acid, lactic acid bacteria also produce other metabolites, which are antimicrobial in nature (Prescott et al., 1959). Studies have shown that in the presence of chelating agents, the inhibitory activity of metabolites produced by lactic acid bacteria can be extended to pathogenic organisms such as *Escherichia coli* (Belfiore et al., 2007). *Escherichia coli* were used as the indicator organism for the research. Also worth noting is that, live strains of lactic acid bacteria can be found in Yakult, a readily available probiotic diary product that is employed in this research.

Because of the factors outlined above, it follows then that lactic acid fermentation using the lactic acid bacteria strain found in Yakult could thus be exploited as a cost effective and efficient faecal sludge treatment technology option. Its use can not only be applied in emergency situations but also for onsite treatment systems that do not effectively treat the faecal sludge otherwise.

1.3. Research Questions

1.3.1. Main Research Question

Is the lactic acid fermentation process, using lactic acid bacteria obtainable in Yakult, a probiotic dairy product, a feasible treatment option for faecal sludge? If it is, can the process be extended to treat faecal sludge in emergency situations?

1.3.2. Sub Research Questions

The following sub research questions are considered in the research:

- Can lactic acid bacteria found in Yakult proliferate in different media?
- What are the (pre-) conditions required to promote lactic acid fermentation in faecal sludge using Yakult?
- What is the sanitizing effect of lactic acid?
- What conditions are necessary to use lactic acid successfully to disinfect faecal sludge?
- Is disinfection using lactic acid bacteria suitable for emergencies;

1.4. Hypothesis

It is hypothesised that lactic acid fermentation process can be used as a low cost but yet effective method for treating faecal sludge and that the process can be extended to treat faecal sludge in emergency situation. Because of the live *Lactobacillus casei* Shirota strain (YAKULT Australia, nd) readily found in the fermented milk drink, Yakult; it is hypothesised that the synergetic effect of lactic acid produced from the fermentation of simple sugars and the antimicrobials produced by the *Lactobacillus* bacteria, can be exploited in the treatment of faecal sludge.

1.5. Objectives and Proposed Work

Whereas substantial progress has been made in developing suitable technologies for wastewater treatment in developing countries over the past decades, very little progress has been made in addressing the management and treatment of sludge's from on-site sanitation facilities by problem holders or researchers.

It will been seen from the literature review presented in chapter two, that pit latrines, a form of on-site sanitation systems (OSS) are the most common form of excreta disposal in low income areas of developing countries and also for emergency situations. This situation is unlikely to change anytime soon because of the huge costs associated with the alternative conventional wastewater systems. It is thus imperative that robust, low cost and yet effective methods and technologies for the treatment of the faecal sludge are employed.

The capacity of Lactic Acid Bacteria (LAB) to lower pH and also the antimicrobial potential of the other metabolites (bacteriocins) they produce have been greatly exploited in the food preservation industry. It is this very capacity that the LAB poses that could potentialy be exploited in the treatment of faecal sludge.

1.5.1. General Objective

The general objective of the research is to investigate and evaluate the suitability of the LAF process using a LAB species obtained from Yakult, a fermented milk drink, as a means for treatment of faecal sludge and to extend its applicability to emergency situations.

1.5.2. Specific Objectives

The specific objectives of the study include:

- To prepare sludge that will mimic faecal sludge using black water from a wastewater treatment facility. This will be done by ensuring the physical characteristics are close to that of faecal sludge and if need be, spiking it with indicator organisms if not present, so as to make it representable to real faecal sludge.
- To establish the preconditions required for the production of lactic acid during the fermentation of the faecal sludge mixture using Yakult as the inoculant media.

- To analyse and monitor the LAF process by carrying out measurements of relevant parameters
- To establish a relationship between the amount of lactic acid produced and the sanitizing effect
- To extend the findings of the laboratory experiments to field studies on fresh faecal sludge in Malawi having an emergency sanitation concept
- To evaluate whether LAF process can be used to treat odours

1.6. Report Outline

Chapter 2 of the report consists of the literature review.
Chapter 3 gives a detailed outline of the methodology that was followed in carrying out this research both in the laboratory and in the field. Chapter 4 presents the results of the research in a chronological manner and provides a concise discussion thereof. The final Chapter, Chapter 5 draws up a conclusion to the research and provides recommendations based on the findings of the research.

CHAPTER 2

Literature Review

This chapter aims to provide a general idea of emergency and emergency situations and also give a general overview in the trend of natural disasters in the recent past. It further provides the challenges and the current norms as regards to managing emergency situation. Faecal sludge management is thus introduced as one of the major challenges that are faced by the various acters in the humanitarian effort. A general description of the faecal sludge pathogens which are of importance to public health is then given. This is followed by a brief and concise outline of the 4 pathogens groups found in faecal sludge and their respective indicator organisms. An introduction to Lactic acid is made, what it is, how it is produced and its benefits. Thereafter, an overview of lactic acid bacteria is given followed by an outline of the metabolic pathways indicating how lactic acid is produced by fermentation. An account of the sanitizing mechanisms using lactic acid and antimicrobials produced by the lactic acid bacteria also made. The chapter closes by looking at Yakult, the probiotic milk drink that was used in this research followed by a brief background of the area where the field work was conducted at the end.

2.1. Emergency

Münch et al., (2006), suggests that emergency is a situation that is caused by natural or manmade disasters, which affects people's lives, the infrastructure that supports them and their natural environments. Natural disasters are disasters that are caused by forces of nature. These may include but not limited to earthquakes, floods, hurricanes and volcanic eruptions. Whereas, manmade disasters are those that are as a result of man-made hazards such as nuclear accidents, fires and can also be as a result of civil unrest, terrorism and wars.

2.1.1. Natural Disasters

Natural Disasters can be categorised as being hydro-meteorological, geophysical or biological in nature. Examples of hydro-meteorological disasters include: floods, wave surges, storms, droughts, landslides and avalanches. Geophysical disasters will include earthquakes, tsunamis and volcanic eruptions; whereas biological disasters will include epidemics and insect infestations (Survey 2010).

According to Cluster, (2006), natural disasters that have a sudden onset and broad impact can create many factors that when combined, tend to increase the risk of morbidity and mortality resulting from communicable diseases. The potential impact of communicable diseases is often presumed to be very high in the chaos that follows natural disasters.

Usually, large numbers of people are forced to seek temporary shelter in camps. In most cases the situation in these camps is characterized by crowded conditions with **inadequate sanitation** and waste management, inadequate sources of water, scarcity of food, malnutrition, and a reduced level of immunity, which all contribute in escalating the devastation(Connolly et al. 2004).

Increase in incidence and intensity of natural disasters

According to the Emergency Events Database (EM-DAT) maintained by the Centre for Research on the Epidemiology of Disasters., 1988) (CRED) at the Catholic University of Louvain, Belgium (http://www.emdat.be/), there has been an increase in the incidence of natural disaster in the last four decades(see **Figure 2.1**below)

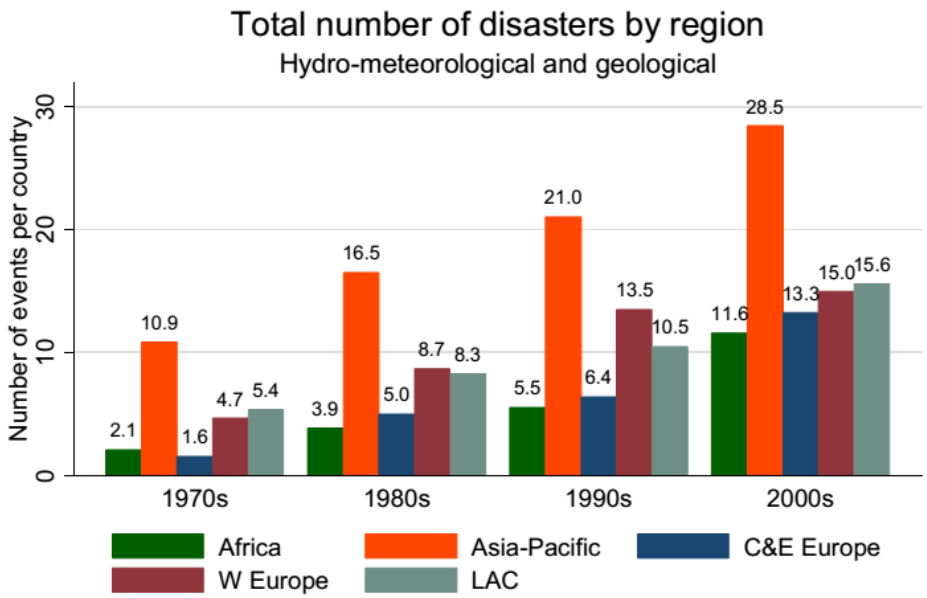


Figure 2.1 *Incidence of Natural disaster by region, 1970-2008*

Source: Emergency Events Database (EM-DAT, 2005)

This increase in the incidence of natural disaster has a clear implication on the morbidity and mortality as a result of communicable diseases.

2.1.2. Phases of an Emergency

Davis and Lambert (2002) as cited in Bastable et al., (2004) defines 5 stages for an emergency. These 5 stages include:

- Immediate emergency (1-2 weeks) **Phase 1**
- Stabilization (0.5- 2 months) **Phase 2**
- Recovery (several months)
- Settlement (perhaps years)
- Resolution

For the purposes of this research, phases 1 and 2 shall be considered as the emergency period with which the research seeks to investigate the application of lactic acid treatment for faecal sludge.

In Phase 1 of an emergency, it is important that rapid interventions are deployed to provide among others, basic facilities to contain and separate so as to mitigate outbreaks of communicable diseases. According to

Bastable et al., (2004), the risk of major epidemics and mortality rates is often high in this 1st phase. The objective of any adopted excreta disposal programme is to achieve or surpass the Sphere minimum standards.

The Sphere minimum standards is an initiative that was launched in 1997 by a group of humanitarian NGOs who formulated a Humanitarian Charter and identified Minimum Standards that are to be attained in disaster assistance in key sectors that included water supply and sanitation, food aid, nutrition, shelter and medical services. This gave birth to the first Sphere handbook which was published in 2000 (Sphere Project. 2003).
It thus follows that the Humanitarian Charter together with the Minimum Standards form the basis of an operational framework for accountability in disaster assistance efforts.

Phase 2 of an emergency is characterised by stability of the situation and more sustainable interventions can be implemented for the longer-term. It is under this phase that community structures may start to reassemble and also morbidity and mortality rates start to fall. This phase may apply to all subsequent phases after the immediate emergency phase and may last several months. Interventions under this phase are designed for the longer term -term use. However, it is more economical to apply flexible short term interventions for the immediate emergency phase that can easily be converted for longer term use.

2.2. Faecal Sludge

Faecal sludge (FS) can be said to include sludges of various consistencies accumulating in and withdrawn from on-site sanitation systems such as septic tanks, aqua privies, pit latrines and un-sewered public toilets (Ingallinella AM, et al., (2002).

The problems and challenges associated with the management of faecal sludge are spread across all the components of the faecal sludge stream which include pit/vault emptying, haulage, storage or treatment, and reuse or disposal (Strauss, et al, 2002).

Table 2.1 highlights some of the challenges and problems associated with faecal sludge management

Table 2.1 Current FS Management Practices – Causes, Problems and Consequences

FS management component and aspect	Causes	Problems	Consequences
Emptying + collection			
• Technical	• Limited or no accessibility to pits • Inappropriate emptying equipment • Manual, non-mechanised emptying	• Overflowing pits • Emptying frequency often very low • Informal or emergency emptying of pits and indiscriminate disposal of FS	At neighbourhood level, mainly • Health hazards from openly dumped FS and through use of contaminated water • Eye and nose sores • Non-functionality of infrequently emptied septic tanks → solids carry-over
• Institutional / financial	• Poor service management • Users' low affordability for pit emptying • Lack of information (e.g. on how septic tanks work)		
Haulage			
• Technical	• Traffic congestion • Lack of suitable disposal or treatment sites at short distance from the area of FS collection	• Collectors dump FS in an uncontrolled manner at the shortest possible distance from where FS was collected	At district or municipal level, mainly: • Pollution of surface and (shallow) groundwater • Eye and nose sores • Health hazards from use of contaminated surface water (e.g. for vegetable irrigation)
• Institutional	• Lack of urban planning → lack of suitable disposal or treatment sites at short distance from the area of FS collection • Lack of involvement of private sector service providers • Lack of suitable incentive and sanctions structure		
• Financial/economic	• Collectors minimising haulage distance and time		
Treatment			
• Technical	• Lack of proven and appropriate treatment options	• FS is used or dumped untreated	At district or municipal level, mainly: • Health hazards through use of contaminated water sources and water pollution
• Financial / economic	• Where FS treatment exists: private collectors / entrepreneurs avoid the paying of treatment fees		
• Institutional / financial	• Lack of political will to invest in treatment • Lack of effective cost recovery • Lack of urban planning • Lack of information	• Non-availability of suitable treatment sites • Use or discharge of untreated FS	

Source: (Strauss, et al, 2002)

It can clearly been seen here that in the event of a disaster, the challenges highlighted above only get compounded thus making faecal sludge management in emergencies an even more challenging undertaking.

2.3. Faecal Sludge Management in Emergency

Dealing with faecal sludge in Emergency situations has remained a challenge in many developing countries that are struck with disasters. Because of other competing requirements such as food and medical services, development of suitable treatment and disposal methods of large quantities of human excreta in (post) emergency settings has often been neglected by the various players involved in the humanitarian effort. This has often led to in appropriate disposal and treatment solutions such as the creation of unsanitary defecation fields and landfills which often have adverse environmental and health implications. This has

been highlighted in an internal report on *Requirements: Faecal Sludge Treatment and disposal in emergency situations* compiled by WASTE`, Netherlands Red Cross, Aldus Bouwinnovatie in consultation with International Federation of Red Cross and Red Crescent Societies, Oxfam UK (2012).

2.4. WHO Guidelines on wastewater, excreta and grey water

To render faecal sludge sanitized, the following World Health Organization (WHO) guidelines apply: The guideline stipulates a benchmark of <1000 *E. coli* number/g total solids in 100ml as a benchmark for the treatment of faeces and faecal sludge. As such this research adopted this benchmark as a measure for the level of sanitization. See Table 2.3 below.

Table 2.2 Guideline values for verification monitoring in large-scale treatment systems of grey water, excreta and faecal sludge for use in agriculture

	Helminth eggs (number per gram total solids or per litre)	***E. coli* (number per 100 ml)**
Treated faeces and faecal sludge	<1/g total solids	<1000/g total solids
Greywater for use in:		
• Restricted irrigation	<1/litre	$<10^{5}$ [a] Relaxed to $<10^{6}$ when exposure is limited or regrowth is likely
• Unrestricted irrigation of crops eaten raw	<1/litre	$<10^{3}$ Relaxed to $<10^{4}$ for high-growing leaf crops or drip irrigation

[a] These values are acceptable due to the regrowth potential of *E. coli* and other faecal coliforms in greywater.

Source:(World Health Organization 2006)

2.5. Pathogens in Faecal Sludge

Pathogens are simply micro organisms that are capable of infecting a host and causing disease (Bitton 2005). The development of disease depends on various factors, including the degree to which the microorganism has to cause disease, infective dose, the physiological condition of the host and other environmental factors. Some microorganisms are opportunistic pathogens, and are not capable of causing disease to healthy persons but may affect compromised individuals.

Pathogens found in wastewater maybe excreted by persons and animals that are carriers or are infected with disease (Metcalf & Eddy, 2003). It thus follows that this analogy can be extended to faecal sludge. These pathogens are classified into four broad categories which are bacteria, protozoa, helminths, and viruses. However, certain authors (Leclerc et al. 2002) only recognise three groups leaving out the helminth parasites that are generally not studied by microbiologists but are nonetheless of great concern as regards human health (Bitton 2005).

2.5.1. Bacteria

Bacteria constitute a large kingdom of prokaryotic microorganisms characterised by having cells that lack a membrane bound nucleus (karyon). The organisms whose cells do have a nucleus are called eukaryotes (Bitton 2005). Typically these organisms are few micrometres in length (1-2µm), except for filamentous bacteria (usually >100µm) and cynobacteria (5-50µm). Bacteria occur in a wide range of shapes, ranging from cocci (spherical shape) to bacilli (rods) and spirals (Bitton 2005). See **Figure 2.2Error! Reference source not found.** below

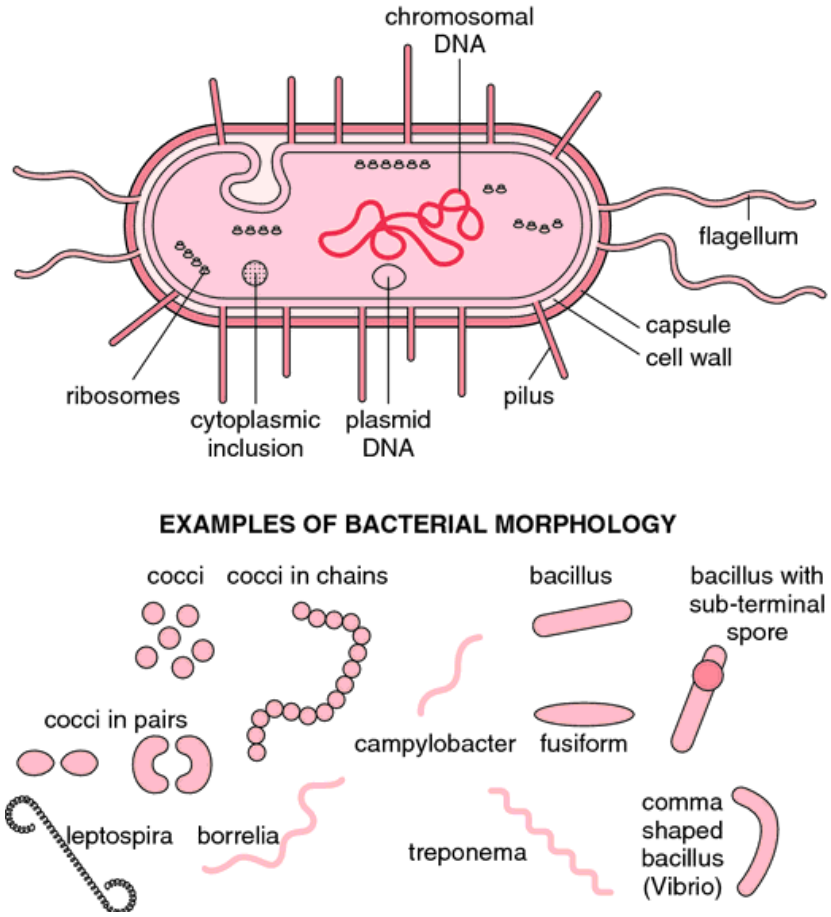


Figure 2.2 *Generalised structure of bacterium*

Source: http://medical-dictionary.thefreedictionary.com/Bacterium

2.5.2. Bacterial Pathogens

Bacterial pathogens are bacterial strains that have the ability to cause disease and thus termed virulent. According to Stanier (1987), this ability to cause disease by these virulent organisms is largely, but not restricted to, the organisms ability to produce compounds or toxins that produce specific harmful effects to the host. These toxins could either be proteins or lipopolysaccharides in nature. The potential for causing disease is thus dependant on the stability of the virulent organism in the environment and also on the dose of the virulent organism necessary for infecting the host and causing disease. As these pathogens exist, at least temporarily, in one or more natural environments, termed reservoirs of infection, Leclerc et al., (2002), categorizes bacterial pathogens as either being enteric (bacteria of the intestines) or bacteria of the aquatic environment.

For humans to get infected, i.e. have growth of microorganisms within host tissue, there has to be a characteristic mode for an infectious disease to be transmitted to humans. Stanier (1987) identifies 3 common modes of infection. These include: (1) the oral-faecal route (ingestion of food or water contaminated by faeces), (2) the respiratory route and (3) direct contact with an infected person or object.
The growth of the microorganism within host tissue can only be achieved if a host-parasite relationship with the human is established. Stanier (1987), suggests that colonization of a surface or tissue invasion of the host depends on specialized molecular structures on the surface of the bacterial cell that bind to specific receptor sites on the host cells. Once this relationship is established, growth of the microorganism now depends on successful competition with the host's normal micro-flora for essential nutrients.
For the purpose of this literature review and research, focus shall thus be on the bacterial pathogens that are associated with the oral-faecal route. **Figure 2.3** shows a schematic of the oral-faecal route.

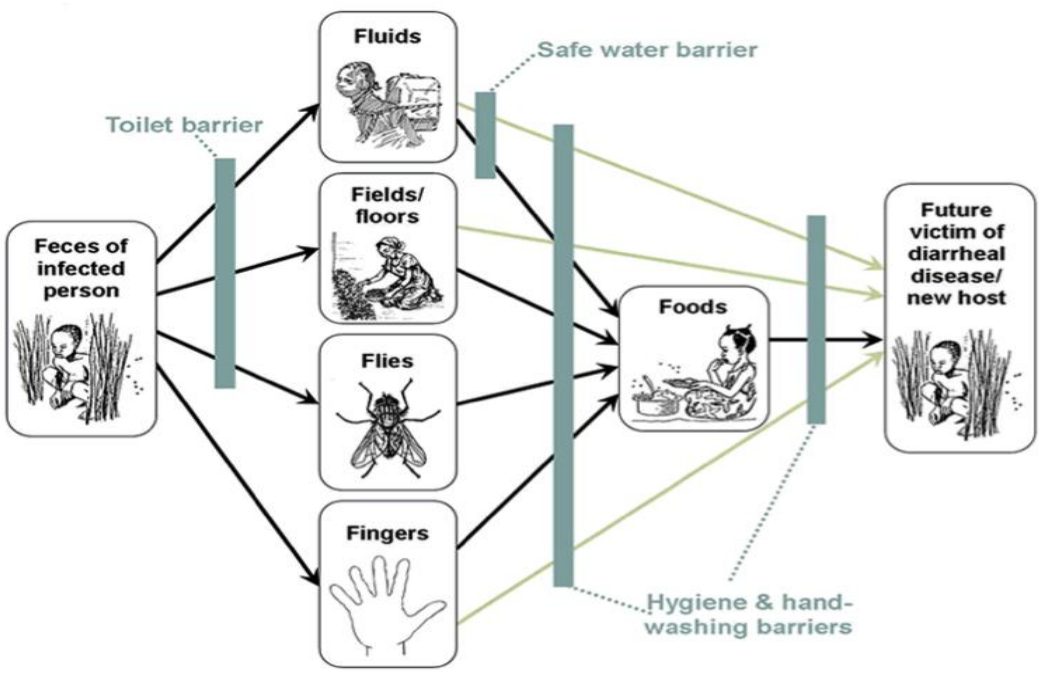


Figure 2.3 *The oral-faecal route: transmission route of faecal-oral diseases*
Source: http://www.lifesaversystems.com/_blog/LIFESAVER_Blog/post/what-is-the-f-diagram/

Faecal matter contains up to 10^{12} bacteria per gram. According to Dean and Lund (1981) as cited in Bitton (2005), this bacterial content in faeces, accounts for approximately 9 percent by weight.

According to Dott and Kampfer (1988) as cited in Bitton (2005) wastewater bacteria are characterized into four groups:

- Gram negative facultative anaerobic bacteria: e.g., *Aeromonas, Pleisiomonas, Enterobactor, Escherichia and Shigella*
- Gram negative aerobic bacteria: e.g., *Pseudomonas, Alcaligenes, Flavobacterium, Acinetobactor.*
- Gram positive spore-forming bacteria: e.g., *Bacillus*
- Non spore-forming gram positive bacteria: e.g., *Arthrobactor, Corynebacterium, Rhodococcus.*

Table **2.3** below gives detail to a selection of oral- faecal pathogens and their transmission routes. As seen from the table, many of the pathogenic organisms are transmitted via multiple transmission routes rather than just one.

Table 2.3 Selected faecal-oral pathogens and selected transmission routes

Pathogen	Important reservoir/carrier	Transmission			X in food
		water	food	p-to-p	
Campylobacter jejuni	Variety of animals	+	+	+	+
Enterotoxigenic *E. coli*	Man	+	+	+	+
Enteropathogenic *E. coli*	Man	+	+	+	+
Enteroinvasive *E. coli*	Man	+	+	Ni	+
Enterohaemorrhagic *E. coli*	Man	+	+	+	+
Salmonella typhi	Man	+	+	±	+
Salmonella (non-*typhi*)	Man and animals	±	+	±	+
Shigella	Man	+	+	+	+
Vibrio cholerae O1	Man, marine life?	+	+	±	+
Vibrio cholerae, non O1	Man and animals	+	+	±	
Hepatitis A	Man	+	+	+	–
Norwalk agents	Man	+	+	Ni	–
Rotavirus	Man	+	ni	+	–
Cryptosporidium parvum	Man, animals	+	+	+	–
Entamoeba histolytica	Man	+	+	+	–
Giardia lamblia	Man, animals	+	±	+	–
Ascaris lumbricoides	Man	–	+	–	–

X in food - multiplication in food p-to-p – person-to-person
+ yes ± rare - no ni - no information

Source: *Fewtr*ell et al., (2001)

2.5.3. Protozoa

Protozoa are a diverse group of unicellular eukaryotic organisms whose cells are surrounded by a cytoplasmic membrane covered by a protective structure called a pellicle (Bitton, 2005). Metcalf & Eddy (2003) go further to identify that the majority of the protozoa are aerobic heterotrophs with some that are aero tolerant anaerobes and a few that are anaerobic. Their primary food source are the bacteria. They mostly reproduce by binary fission, although a few species reproduce sexually. They are able to form cysts, or protective coatings, which allow them to survive outside a host and/or under adverse environmental conditions for extended periods of time. Protozoan cysts are also able to protect the organism from disinfection with chlorine(Bitton 2005), and are quite resistant to extreme drying and starvation thus making them difficult to treat when found in faecal matter.

2.5.4. Protozoa pathogens

Pathogenic protozoa's just like pathogenic bacteria, have the ability to cause disease in humans.
Despite the group being highly diverse, Stanier (1987) suggests that there are fewer than 20 species of protozoa that are human pathogens.

The following figure gives examples of pathogenic protozoa, most of which can be found in faecal sludge.

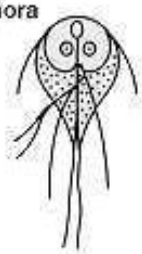
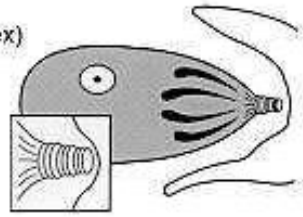
Phylum	Subphylum	Representative Genera	Major Diseases Produced in Human Beings
Sarcomastigophora (with flagella, pseudopodia, or both) 	Mastigophora (flagella)	*Leishmania*	Visceral, cutaneous and mucocutaneous infection
		Trypanosoma	Sleeping sickness Chagas' disease
		Giardia	Diarrhea
		Trichomonas	Vaginitis
	Sarcodina (pseudopodia)	*Entamoeba*	Dysentery, liver abscess
		Dientamoeba	Colitis
		Naegleria and *Acanthamoeba*	Central nervous system and corneal ulcers
		Babesia	Babesiosis
Apicomplexa (apical complex) 		*Plasmodium*	Malaria
		Isospora	Diarrhea
		Sarcocystis	Diarrhea
		Cryptosporidium	Diarrhea
		Toxoplasma	Toxoplasmosis
Microspora		*Enterocytozoon*	Diarrhea
Ciliophora (with cilia) 		*Balantidium*	Dysentery
Unclassified		*Pneumocystis*	Pneumonia

Figure 2.4 *Classification of Parasitic Protozoa and Associated Diseases*

Source: Baron, (1996)

2.5.5. Viruses

Viruses are obligate intracellular parasites that are composed of an infectious nucleic acid encapsulated in a protein coat called a capsid. Viruses do not have any cytoplasm or metabolism of their own and therefore multiply only within a host cell where their nucleic acid directs for their replication, first, of viral macromolecular components which are then later assembled into new viruses (Stanier, 1987; Metcalf & Eddy., 2003).

Classification of viruses is mainly by phenotypic characteristics, such as shape and sizes, nucleic acid type (DNA or RNA), how they replicate, host cells they infect, and also the type of disease they cause. As regards with classification based on the host cells they infect, Bitton, (2005) identifies 3 separate classes which include animal, algae and bacterial phage's.

2.5.6. Viral human pathogens

There are approximately 140 types of enteric viruses excreted by humans that are capable of producing infection (Bitton, 2005). These enteric viruses multiply in the gastrointestinal tract and are released in the faecal matter of infected individuals. Metcalf & Eddy.,, (2003) points out that the Norwalk virus and rotavirus as being the major diarrhoea disease pathogens among the list of the most important human enteric viruses as with regards to public health issues. The others include enteroviruses (polio, echo and cox sackie), reoviruses, caliciviruses, adenoviruses, and hepatitis A virus.

Other than the helminths, viruses have been observed to have the longest survival time in the environment and as such, proving to be of major concern as regards to public health issues. Goyal et al., (1984) demonstrated that even in harsh marine environmental conditions, viruses can survive for a long period of time thus presenting a potential public health problem to humans. They further demonstrated that they were able to isolate viruses even in situations where faecal indicator bacteria were absent hence reaffirming previous arguments on the inadequacy of these bacteria for predicting the virological quality of a given sample and hence faecal contamination.

However, Clesceri et al., (1989) in standards and methods for the examination of water and waste water, indicates that routine examination for enteric viruses is not currently recommended, unless under special circumstances such as disease outbreaks or special research. This is so because the available methodology has important limitation.

Nonetheless, detection and enumeration of viruses is possible using the following methods: *plant leaf local lesion assay*, for the enumeration of plant virions. This method involves applying a suspension of virions previously concentrated from a sample, onto the surface of a leaf together with an abrasive material that tears small holes in the walls of the plant cells. A local infection is thus initiated by each virion that enters a host cell, creating a region that becomes discoloured and easily noticeable; *the plaque assay* is a method commonly used for the enumeration of animal and bacterial viruses. It involves infecting host cells growing in a thin layer on a medium partially solidified by agar. Similarly, the infected cell establishes an infection which makes the infected area (plaque) differ from surrounding cell layer. These plaques can then be made more visible by applying a dye that stains live cells and not those killed by the viruses (Stanier 1987).

2.6. Indicator Organisms

The concept of indicator organisms of faecal contamination was conceived because of issues surrounding the difficulty in isolating and identifying the few pathogenic organisms present in waste and polluted waters. Also the associated costs, time and need for specialized skills of conducting these direct detection tests led to the adoption and use of indicator organisms, which are more numerous and more easily tested for, for target pathogens (Bitton, 2005; Metcalf & Eddy.,, 2003).

Indicator organisms should poses among others, the following characteristics: It should be a member of the intestinal micro flora of warm blooded animals; it should be consistently present when pathogens are present and absent in samples that are not contaminated; it should be present in quantities equal or greater than of the target pathogenic organisms; it should not reproduce outside of the host organism; it should easily be detectable by quick, easy, and inexpensive means; it should have comparable or slightly better resistance than target organisms when subjected to harsh environmental conditions or lethal parameters of sludge treatment (Bitton, 2005; Metcalf & Eddy., 2003; Arthurson, 2008).

The scope of this research limited the investigations to only one of the four categories of faecal sludge pathogens. Therefore, for the remainder of the report, focus will mostly be on bacterial pathogens and their response to the treatment process under investigation.

As with regards to bacterial indicator organisms, there is no universal agreement on which indicator organism is to be used, as no single bacterial meets the criteria to predict the existence of all pathogenic bacteria of interest; however a list of widely used indicator organisms may include total coliforms, faecal coliforms, faecal streptococci, *Escherichia coli, Clostridium perfringens*, bifidobacteria (Bitton, 2005; Arthurson, 2008)

In this research, *Escherichia coli* was used as the indicator organism to monitor and assess the sanitizing effects of the lactic acid fermentation process.

Escherichia coli are gastrointestinal tract bacteria of warm blooded animals belonging to the genre faecal coliforms. They are gram-negative facultative anaerobic bacteria that exist in several strains, many of which are harmless. However, there are quite a number strains that are pathogenic and can cause diarrhoea disease.

A number of methods have been developed to detect *Escherichia coli,* but for the purpose of this research, Chromocult coliform Agar test will be applied. Chromocult coliform Agar (Merk Millipore International, Germany) is a selective and differential chromogenic culture medium intended for the detection, differentiation and enumeration of *Escherichia coli,* and other faecal coliforms from samples within a 24 hour period.

2.7. Lactic Acid

Lactic acid (2-hydroxypropionic acid or 2-hydroxypropanoic acid), CH3-CHOHCOOH is a naturally occurring organic acid. It is a weak acid, which means that it only partially dissociates in water. Lactic acid dissociates in water resulting in ion lactate and H+. This is a reversible reaction and the equilibrium is represented below.

$CH_3CH\ (OH)\ CO2H \rightleftharpoons H^+ + CH_3CH\ (OH)\ CO2$-Ka= 1.38 x 10^{-4}

Depending on the environmental pH, weak acids such as lactic acid are either present as the acid in its undissociated form at low pH or as the ion salt at higher pH. The pH at which 50% of the acid is dissociated is called the pKa, which for lactic acid is 3.86. There are two optical isomers of lactic acid: L(+)-lactic acid and D(–)-lactic acid

According to Helander et al., (1997) weak organic acids such as lactic acid are important compounds because of their inhibiting capabilities on a wide range of microorganisms. In its undissociated form, lactic acid poses high antimicrobial activity. The inhibitory effect of undissociated organic acids is 10-600 times stronger than that of their dissociate forms.

The antimicrobial action of lactic acid has got to do with, but not entirely, its ability in the undissociated form to penetrate the cytoplasmic membrane of micro organisms, resulting in reduced intracellular pH and disruption of the transmembrane proton motive force of the lipopolysaccharides molecules of the outer membrane of the pathogenic organism.

Lactic acid can either be produced by chemical synthesis or fermentation.

The **chemical synthesis** of lactic acid involves the hydrolysis of lactonitrile, a derivative of petrochemicals, by a strong acid to produce a racemic mixture of D-and L-lactic acid. Lactic acid can also be synthesised by other means which include: oxidation of propylene glycol, reaction of acetaldehyde, carbon monoxide, and water at elevated temperatures and pressures, hydrolysis of chloropropionic acid, and nitric acid oxidation of propylene(John et al. 2009)

Lactic acid is also produced by **fermentation**. The process involves the fermentation of sugars or sugar containing hydrolyzates or the single step conversion of starchy or cellulosic wastes by direct conversion using amylolytic lactic acid producing microorganisms. Also by the simultaneous hydrolysis and fermentation with concomitant addition of saccharifying enzymes and Inoculum together(John et al. 2009). Of relevance to this research, is the fermentation process of sugars using lactic acid bacteria, a process which is explained in more detail in the next section.

2.8. Lactic Acid Bacteria

Lactic Acid Bacteria (LAB) are generally a large number of species of bacteria which poses the ability to form relatively significant quantities of lactic acid from carbohydrates(Prescott , et al., 1959). Because of the desirable reactions they catalyze, and also the undesirable activities which they promote, the Gram-positive bacteria of the genera *Lactobacillus, Streptococcus,* and *Leuconostoc* are of special importance in the food and fermentation industries. According to Abdel-Rahman et al., (2013) the optimal growth conditions vary depending on the producers, since these bacteria can grow in the pH range of 3.5-10.0 and temperature of 5-45℃.

LAB are classified under two groups. The fist group, homofermentative LAB, comprises bacteria that convert carbohydrates to lactic acid as the principle end product where as heterofermentative LAB, comprises bacteria which produce in addition to lactic acid, volatile acids and carbon dioxide in relatively high quantity(Prescott , et al., 1959). See table below.

Table 2.4 Homofermentative and heterofermentative Lactic acid bacteria

Characterization	Homofermentative LAB	Heterofermentative LAB
Products	Lactic acid	Lactic acid, ethanol, diacetyl, formate, acetoin or acetic acid, and carbon dioxide
Metabolic pathways	Hexose:Embden–Meyerhof pathway Pentose:pentose phosphate pathway	Hexose: phosphogluconate and phosphoketolase pathway Pentose:phosphoketolase pathway
Theoretical yield of lactic acid to sugars	Hexose: 1.0 g/g (2.0 mol/mol) Pentose: 1.0 g/g (1.67 mol/mol)	Hexose: 0.5 g/g (1.0 mol/mol) Pentose: 0.6 g/g (1.0 mol/mol)
Genera	*Lactococcus*, *Streptococcus*, *Pediococcus*, *Enterococcus*, some *Lactobacillus*	*Leuconostoc*, *Oenococcus*, some *Lactobacillus* species
Availability for commercial lactic acid production	Available due to high selectivity	Not available due to high by-product formation

Source: (Prescott , et al., 1959).

According to Abdel-Rahman et al., (2013), heterofermentation LAB first metabolize 6-carbon monosaccharide's to 5-carbon monosaccharide's and carbon dioxide. The 5-carbon monosaccharide is then cleaved to glyceraldehyde 3-phosphate and acetyl phosphate by phosphoketolase. And through the phosphoketolase pathways the 5-carbon monosaccharide is converted to lactic acid and by-products such as acetic acid and other low molecular mass compounds with a maximum lactic acid yield of 0.6 g lactic acid per gram of 5-carbon monosaccharide. On the other hand, homofermentative LAB possess aldolase enzymes and produce lactic acid as the major end product. They are of interest for commercial scale lactic acid production because of their higher expected lactic acid yield per monosaccharide converted. See the metabolism pathways below for the metabolism described above.

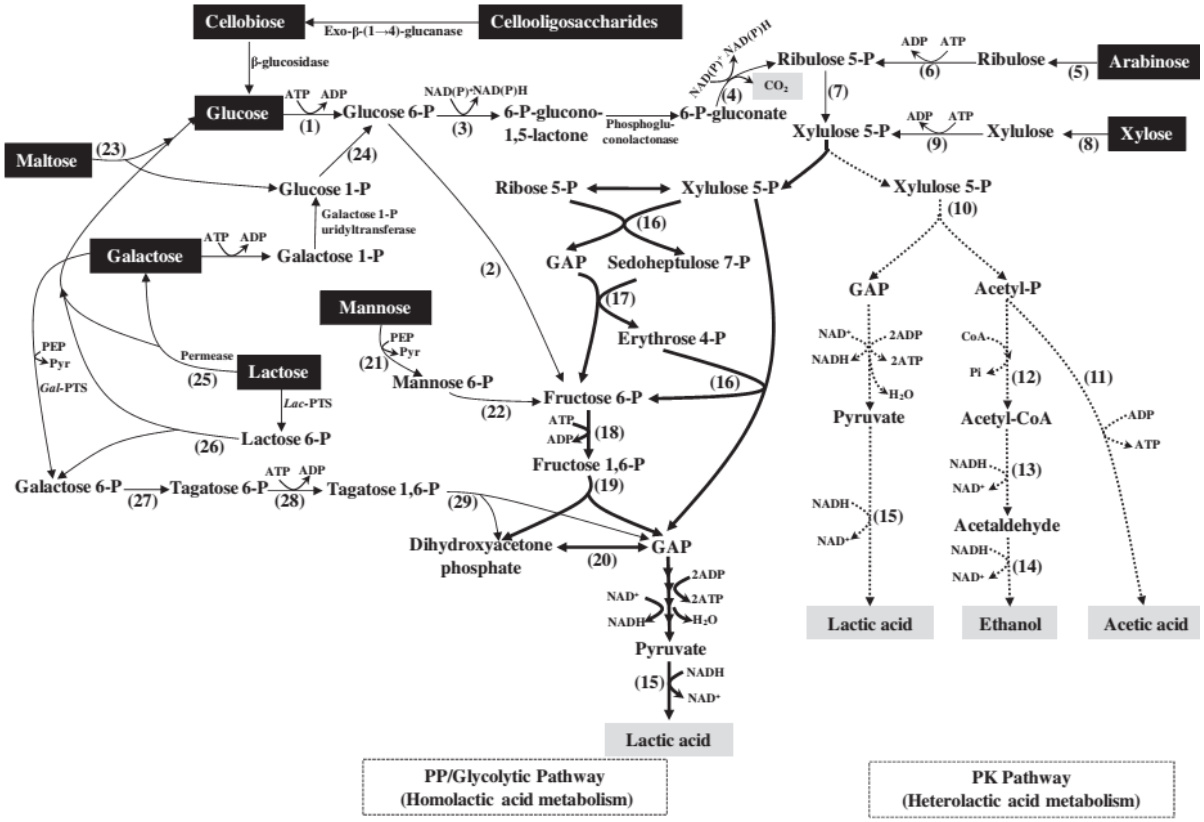


Figure 2.5 *Homolatic and Heterolactic acid Metabolism pathways*

Source:

$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH$ (Homolactic fermentation)
Sugar (glucose) → Lactic Acid + Energy (ATP)

$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$ (Heterolactic fermentation)
Sugar (glucose) → Lactic Acid + Ethanol + Carbon dioxide Energy (ATP)

The LAB are also particularly unique from other bacterial species in that they are capable of surviving without iron (Helander, et al, 1997), an essential element for the growth of all microorganisms.

As a result of this unique capability of surviving without iron and also because of the production of lactic acid and other metabolites, particularly the heterofermentative LAB, which are antimicrobial in nature, LAB thus become perfect candidates whose characteristics can be used as sanitizing agents against pathogens found in faecal sludge

2.9. Yakult

Yakult is a probiotic fermented milk drink that is produced using *Lactobacillus casei* Shirota, a particular strain of lactobacillus which was isolated in 1930 by Dr. Minoru Shirota, the founder of Yakult, and used in the fermented milk drink Yakult since 1935 (Fujimoto et al., 2008). Like the other LAB described earlier, Lactobacillus *casei* Shirota is Gram-positive species which is non-spore-forming, and devoid of

cytochromes. This bacteria strain is equally a preferential nonaerobe but is aerotolerant, acid-tolerant, and strictly fermentative. Yakult is mostly produced in 65ml bottles and the company states that each bottle contains 6.5 billion live Lactobacillus *casei* Shirota bacteria.

2.10.Sanitizing mechanism

Belfiore et al., 2007 suggests that unlike Gram positive bacteria, the inhibition of Gram negative enteric bacteria such as *Escherichia coli* is especially problematic due to their resistance to antimicrobials. The reasons suggested for this resistance is the inability of the antimicrobials to penetrate the protective outer membrane of the Gram negative bacteria made up of glycerophospholipids and lipopolysaccharides (LPS) molecules. Several reports suggests that the synergetic use of chelators (outer membrane disrupting agents) and antimicrobials produced by LAB extends the antimicrobial spectrum to include the Gram negative bacteria as well (Helander et al., 1997; Belfiore et al., 2007). Helander et al., (1997) explains that treatment with chelators such as ethylenediaminetetraacetate (EDTA) results in the removal by chelation of divalent cations from lipopolysaccharides molecules of the outer membrane of the Gram negative bacteria thus permeabilizing it and allowing for antimicrobial action.

However, Alakomi et al., (2005), argues that lactic acid itself, is capable of permeabilizing Gram negative bacteria. He demonstrates that LPS release is substantially observed in a sample of Gram negative bacterial species treated with lactic acid only, even more than in EDTA treated samples.

Having outlined the above, it is worth noting that different LAB species exhibit differences in the extent of inhibitory effects on Gram negative bacteria. Ligocka & Paluszak (2005) demonstrated that the width of growth inhibition zones of pathogenic bacteria varied depending on the species of the LAB used. Some species did not show any growth inhibition of certain pathogen bacteria species at all.

The research will thus seek to exploit the synergetic permeabilizing capability of lactic acid and the antimicrobial effect of metabolites produced by the *Lactobacillus casei* Shirota, species of LAB, *to* inhibit the growth of enteric pathogens found in the faecal sludge.

In the event that favourable conditions that promote the cultivation of this particular species of LAB, are not met and growth inhibiting characteristics are not exhibited, the research will thus consider running parallel experiments with a different strain of LAB, most likely cultivated from sour milk produced locally in Malawi or from other fermented food products. Also selection from a culture of efficient microorganisms (EM) may also be considered to qualify its findings.

WHO guidelines for THE SAFE USE OF WASTEWATER, EXCRETA AND GREYWATER shall applied in making an assessment of the level of sanitisation using the LAF process.

2.11.Field Details

Upon achieving satisfactory results during the first phase of the research at the laboratory facility at UNESCO-IHE Institute for Water Education in the Netherlands, field investigations were conducted in Malawi.

Malawi is a long and narrow, landlocked south-eastern African country located between latitudes $9^0$22', and $17^0$03'S and $33^0$40' and $35^0$55'E with a total territory of 119,140 km^2. Most of Malawi's rapidly growing population which stands at around 13 million lives in rural areas. It has an agro based economy contributing about 30% of GDP and about 85% of total employment. The capital, Lilongwe is the largest city, with Blantyre as the second biggest city

Blantyre is located in the southern part of the country and is considered the economic capital of the country. It has a reasonably well developed system of physical and social infrastructure. However like most developing countries, most of this infrastructure is characterized by problems such as low coverage levels and poor state of maintenance and disrepair.

In terms of sanitation, the city faces acute sanitation challenges with very limited sewer coverage. Many people rely on on-site sanitation facilities such as pit latrines and septic tanks.

Figure 2.6 *Administrative map of Malawi*

Source: http://www.nationsonline.org/oneworld/map/malawi-administrative-map.htm

Malawi was selected as the location for the field investigations because of WASTE's interest and involvement in this research.
WASTE is an international NGO that works towards sustainable improvement of the urban poor's living conditions and the urban environment in general. WASTE is participating in the Speedkits projects and takes the lead amongst other organizations such as the Red Cross Society and MSF in the WASH activities.

The Speedkit project is a European Union initiative that seeks to work with humanitarian organisations, such as the Red Cross Society to among others; develop equipment solutions (kits) that can be used during emergency situations. These kits are to be designed to be easily mobilizable, modular and adaptable, low cost, high-tech in their conception but low-tech in use.
As part of the Speedkit project, pit emptying equipment was being tested to empty Pit-latrines in the Blantyre area. The fresh sludge was being transported by truck to a municipal wastewater treatment plant.

It is because of this readily available source of faecal sludge that it was decided to set up an experimental site at the treatment plant and conduct investigations into 3 different treatment technologies that would feed into the Speedkit project

CHAPTER 3

Methodology

In this chapter, the materials and methods used in carrying out this research are outlined. The research was divided into 4 phases: Literature review, Laboratory-scale LAF experiments, Field research (up scaling) and Result analysis& Report writing.

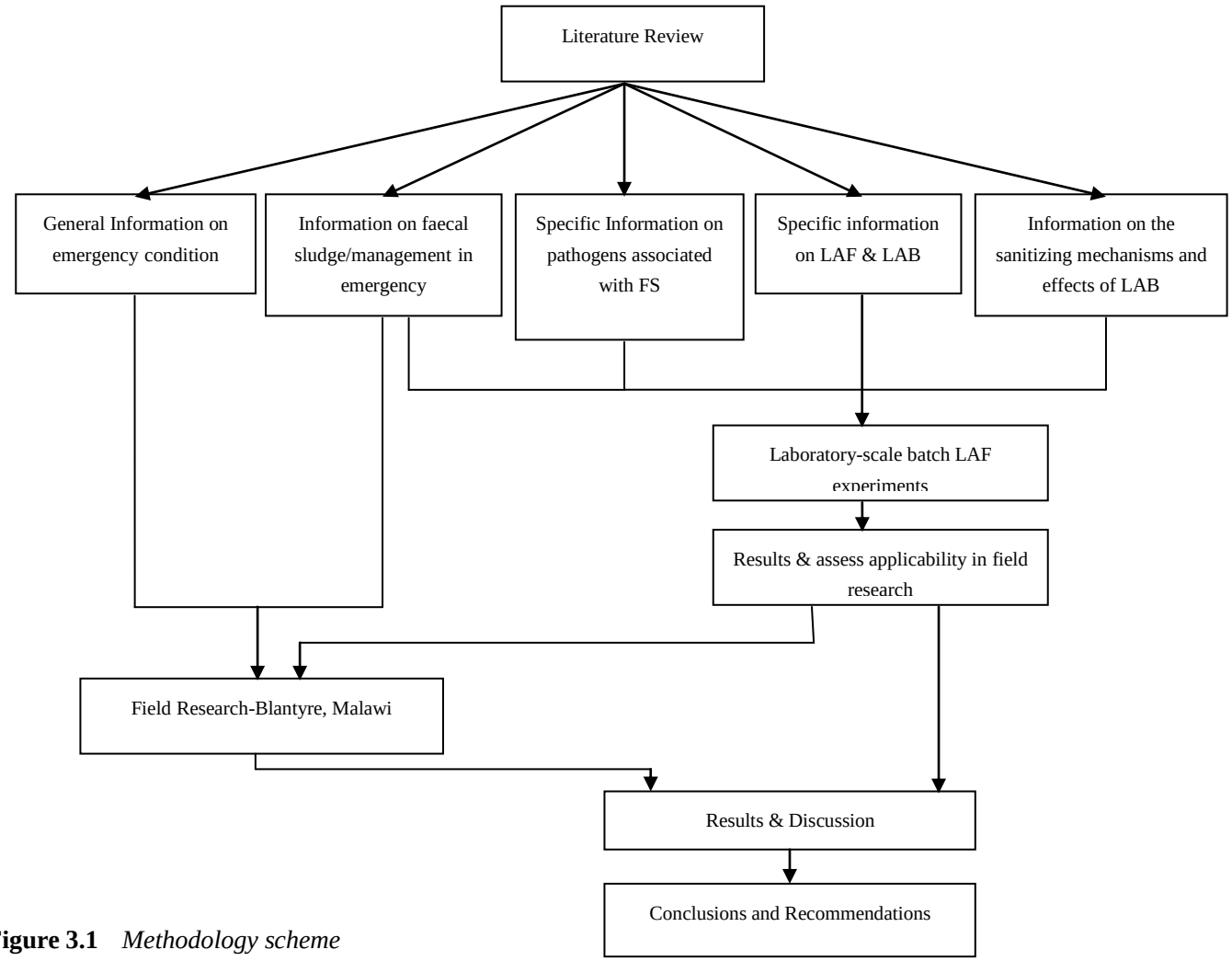


Figure 3.1 *Methodology scheme*

3.1. Literature review

Scholarly material in 6 key areas was gathered, analysed and evaluated throughout the course of the research. The 6 key areas included:

- General information about emergency conditions;
- Faecal sludge and faecal sludge management in emergency;
- Specific Information on pathogens associated with faecal sludge;
- Information on Lactic Acid Fermentation (LAF) and
- Lactic Acid Bacteria (LAB);
- Sanitizing mechanisms and effects of LAB;

3.2. Laboratory Experimentation

Laboratory experiments were conducted at the UNESCO-IHE Institute of Water Education Laboratory, in Delft, The Netherlands. The following materials and methods were used and followed respectively.

3.2.1. Bacteria

Lactobacillus casei Shirota (*L. casei*) found in the fermented milk drink Yakult (Yakult Honsa Co. Ltd, Japan) was used in the LAF experiments. The bacteria species was obtained directly from a sample Yakult packaged in a 65ml bottle. *L. casei* was enumerated using de Man Rogosa Sharpe (MRS) agar in the spread plate method. Using a piston-driven air displacement pipette, 0.1ml of serially diluted samples was placed on the MRS agar plates and incubated at 37 ^{o}C for 3-5 days in an upside position. The *L. casei* Shirota formed whitish colonies on the MRS agar plates. Their numbers were enumerated with the aid of a colony counter.
Before inoculating the *L. casei* into the faecal sludge, a pre-culture was prepared in milk (see section 3.4.3below for details of preparation of the milk pre-culture)

Escherichia coli (*E. coli*) strain ATCC 25922 was used as the indicator organism for the experiments. The *E. coli* strain was obtained from UNESCO-IHE Laboratory. *E. coli* was enumerated using chromocult coliform agar (Merk Millipore International, Germany) in the spread plate method. Using a piston-driven air displacement pipette, 0.1ml of serially diluted sample was placed on the chromocult coliform agar plates and incubated at 37 ^{o}C for 24hrs in an upside down position. *E. coli* formed dark blue colonies on the chromocult agar plates and their numbers enumerated with the aid of a colony counter.

The black water was spiked with 1% v/w concentration of Laboratory *E. coli* stock to obtain a pre-treatment *E. coli* concentration of approximately 10^8 CFU/100ml of black water.

The presentation of the enumeration results of the two bacteria species was made per 100ml of sample and expressed in colony forming units (CFU).

$$\text{Number of bacteria present} = \frac{\text{Colonies counted}}{0.1} \text{ x dilution factor x } 100 \text{ [CFU/100ml]} \quad (3.1)$$

Different trial dilutions for the samples were prepared as the correct dilutions were not immediately known. Dilution water was prepared by dissolving 8g sodium chloride in 1,000ml of demineralised water. The

solution thereof, was autoclaved at 121 ^{o}C for 15 minutes, allowed to cool and then stored in the refrigerator at about 4 ^{o}C.

Figure 3.2 *Yakult, Chromocult coliform & MRS agar plates*

3.2.2. Substrate

As faecal sludge was not readily available, black water from a vacuum toilet treatment facility in Sneek, The Netherlands was used as substrate for the fermentation experiments. The black water was autoclaved, centrifuged and spiked with a strain of Escherichia *coli*. The centrifuging process was able to increase the dry matter content of the black water from around 2% to over 10%. Table 3.1 below shows the characterization of the black water used for the laboratory LAF experiments.

Table 3.1 Black water characterization

No	Description	Total Solids (%)	Volatile Solids (%)	COD (mg/L)	Alkalinity (g/L $CaCO^3$)	pH	Temp (^{o}C)
1	Sneek black water (before centrifuge)	1.5	74		22	6.5	21
2	Sneek black water (after centrifuge/before treatment)	12	79			7.6	22

3.2.3. Sugar Additives

Cane molasses was used as a source for the sugar additives. It was collected from the sugar plant in Roosendaal, The Netherlands. Table **3.2Error! Reference source not found.** below shows the composition of the molasses used for the laboratory experiments

Table 3.2 Composition of Molasses

No	Description	Total Solids (%)	Total Sugars-Glucose & Fructose (mg/L)
1	Cane molasses (as measured at UNESCO-IHE Lab)	87%	200 (see Appendix B)

3.2.4. Fermentation experiments

Miniature batch fermentation reactors were prepared using 200ml glass bottles fitted with a syringe for sample withdrawal. The experiments were conducted for periods ranging between 10-21 days.

Three sets of batch experiments were conducted, two of which were to ascertain optimal experimental parameters and one to investigate the sanitization effect of the LAF process using optimal parameters established in the initial two experiments.

The experiments were conducted at 30 ^{o}C in a temperature controlled room. The initial two experiments were performed in duplicate whereas the third one was performed in triplicate.

Apparatus

- Miniature batch fermentors
- Autoclave
- Steam bath.
- colony counter
- Analytical balance, capable of weighing to 0.1 mg.
- glassware
- 90mm Petri dishes
- piston-driven air displacement pipette (0.1 & 1-5ml)

Materials
Faecal Sludge
Yakult & milk
Molasses

Procedure:

Preparation of Pre-culture (Inoculum media)
Milk Pre-culture

- Miniature batch fermentors were used. The pre-culture in milk was prepared by diluting 0.1ml, 0.2ml, 0.5ml, 1ml of Yakult in pasteurized whole milk to make 100ml pre-culture. A control without any Yakult addition was equally prepared.

- Samples from the control and the pre-culture were collected and plated on MRS agar plates and incubated at 37 degrees Celsius. pH was monitored after 2hrs, 5hrs, 21hrs, 27hrs, 3d ....
- Production of lactic acid was measured using reflectometric lactic acid test kit.

Tap water with Molasses addition Pre-culture

- Miniature batch fermentors were also used here. The pre-culture in tap water was prepared by diluting 1g, 5g, 10g and 20g of molasses in tap water to make 1000ml pre-culture. A control without molasses addition was equally prepared. 1ml of Yakult was inoculated in all the reactors including the control.
- Samples from the control and the pre-culture were collected and plated on MRS agar plates and incubated at 37 degrees Celsius. pH was monitored after 0d, 1d, 2d, 3d, 6d, 7d ....
- Production of lactic acid was measured using reflectometric lactic acid test kit.

Treatment 1 - Optimal Sugar concentration experiment

- Miniature batch fermentors were used. Experiments were conducted at different sugar concentrations established by the varying the w/w molasses addition to faecal sludge. 0, 5%, 10% and 20% w/w molasses to faecal sludge concentration were prepared in 30g faecal sludge. Experiments were done in duplicate.
- Samples from all the fermentors were collected after 0d, 1d, 4d, 6d, 8d, 12d and 18d to establish the pH, Lactic Acid concentration and the viable cell count of the LAB and *E. coli.*

Treatment 2 - Optimal Inoculum concentration

- Miniature batch fermentors were used. Experiments were conducted at different Inoculum concentrations established by the varying the w/w pre-culture addition to faecal sludge. 0, 1%, 5%, 10% and 20% w/w pre-culture to faecal sludge concentration were prepared in 20g faecal sludge. 10% w/w molasses to treatment mixture was added to all the fermentors. Experiments were done in duplicate.
- Samples from all the fermentors were collected after 0d, 3d, 10d, and 11d to establish the pH and Lactic Acid concentration

Treatment 3 - Treatment with optimal parameters

- Optimal concentration of sugar and Inoculum addition established in the earlier 2 experiments was used. 10% w/w molasses addition and 10% Inoculum addition were used as optimal parameters. The experiment was conducted in triplicate (see **Figure 3.3** below)
- Samples from all the fermentors were collected after 0d, 2d, 4d, 6d, 10d, 15d to establish the pH, Lactic Acid concentration and the viable cell count of the LAB and *E. coli.*

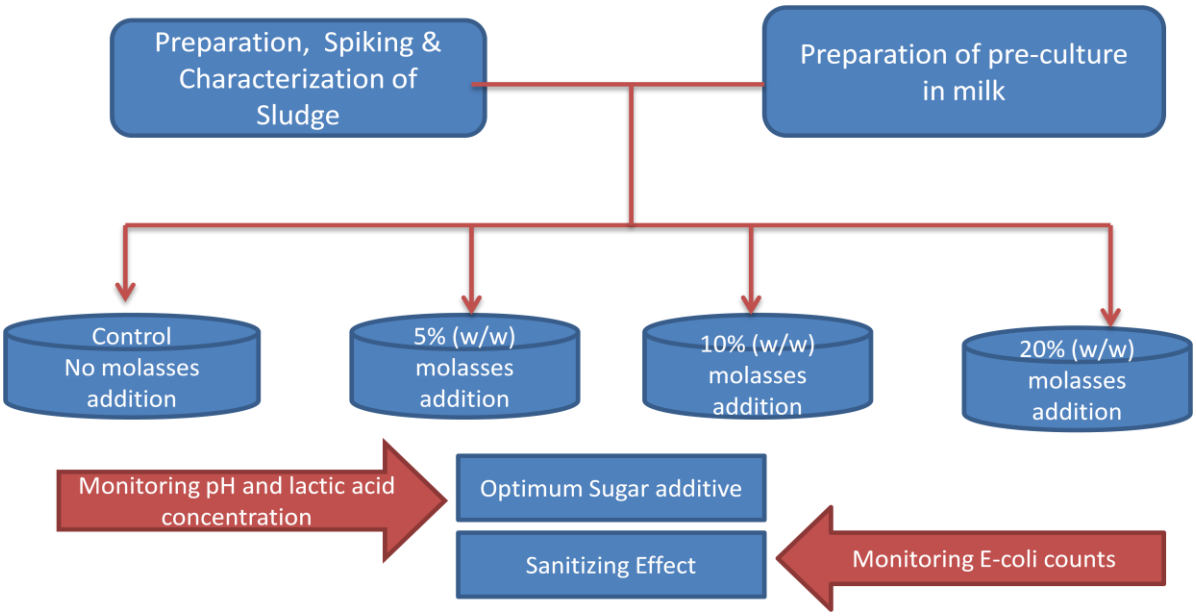


Figure 3.3 *Schematic for laboratory set for optimising sugar experiment*

Figure 3.4 *Lactic acid & Total sugar test kits*

3.3. Analytical methods

- The fermentation process was monitored by measuring pH, concentration of lactic acid and enumeration of lactic acid bacteria.
- The sanitizing effect of the process was monitored and assessed enumeration of the indicator organism, *Escherichia coli*.
- The Volatile Solids (VS) and Total Solids (TS) content before and after the experimentation was assessed following 2540D and 2540E of Standard Methods for the examination of water and wastewater.
- pH was determined by potentiometric measurement using standard pH electrode (4500-H^+ of Standard Methods).
- Total lactic acid concentration was determined reflectometrically using lactic acid test strips (Merk Millipore International, Germany) after appropriate dilution of samples.
- Total sugar (Glucose+Fructose) was measured reflectometrically using total sugar test strips (Merk Millipore International, Germany) after appropriate dilution of samples.
- Reduction of Escherichia *coli* was determined using Chromocult Coliform Agar by spread plating after appropriate dilution of sample in-between appropriate time frames.

Table 3.3 Analytical Methods used for analysing different parameters

No	Parameter/Microorganism	Technique	Method
1	Temperature		SM-2550B
2	pH	Potentiometric	SM-4500-H^+
3	Lactic acid	Reflectometric	
4	Total sugar (Fructose & Glucose)	Reflectometric	
5	Total Solids		SM-2540D
6	Volatile Solids		SM-2540E
7	Odor		SM-2150B
8	*Lactobacillus casei* Shirota	Pour plate	SM-9215
9	*Escherichia coli*	Pour plat	SM-9020

Note on Pour plate method

It should be noted however that, the lowest dilution used for plating was 1:10. Plating of a 1:1 sample was not possible with the methods employed; it follows thus that a dilution of 1:1 could have registered presence of colonies hence the introduction of the concept of detectable limit. Bacterial counts that were under the detection limit (i.e. zero CFU on the agar plate with the least diluted sample) are indicated as 0 log10

As will be seen from all the enumeration graphs in chapter 4, bacterial counts under the detectable limit are indicated as 0 log10.

3.4. Field Research

The field research was conducted at the municipal wastewater treatment plant located in the city of Blantyre, southern part of Malawi.

3.4.1. Experimental Site

Half filled 50L container batch reactors were set at the treatment plant for the purpose of carrying out the lactic acid fermentation experiments.

Measurement and analysis of physical and chemical parameters were conducted at the laboratory facility at the wastewater treatment plant and also at the Laboratory facility at the Polytechnic University.

3.4.2. Faecal Sludge Collection

Faecal sludge was collected from pit latrines around Blantyre using a disludging machine mounted on a 2-ton light truck. This was part of the Speedkit project being implemented by WASTE. The faecal sludge was transported to the municipal wastewater treatment plant in an area called Zingwangwa.

Apparatus

- ROM2 desludging machine complete with fluidizer
- Light Truck
- 50litre plastic containers

Figure 3.5 *ROM2 Desludging machine & 50 litre fermentation reactors*

Procedure

- The sludge in the pit latrine was first of all fished of all foreign materials that are not of faecal matter using a fishing tool, see **Figure 3.6** below. These materials include mostly rags and other foreign materials such as baby dippers, pads etc.
-

Figure 3.6 *Typical Pit latrine & Fishing for rugs before desludging*

- The next step was to fluidize the faecal sludge in cases where it has hardened over a period of time so as to make it suitable for pumping out. Depending of the hardness of the faecal sludge, about 50-150litres of water is used to fluidize the faecal sludge per average sized pit latrine.
- Once fluidized, the faecal sludge is then pumped out using the RAM2 machine.
- When the pit is emptied, the faecal sludge is then transported to the experimental site at the Wastewater treatment plant in Zingwangwa, Blantyre where it is emptied into the 50litre plastic container reactors.
- The reactors are half filled with the faecal sludge to allow for addition of other treatment additives.
- Samples are then collected from the reactors to ascertain the TS, VS and microbiological parameters

Figure 3.7 *Pumping out faecal sludge and emptying in 50 L containers*

3.4.3. Preparation of Pre-culture (Inoculum media)

A pre-culture in milk was prepared as inoculum for the treatment process. A case of Yakult was carried from the Netherlands and milk was sourced locally from a nearby dairy farm. The pre-culture was prepared at least 24-48hrs prior to the start of the treatment.

Apparatus

- 20litre glass container
- 30ml Pipette
- Plastic Funnel
- Bunsen burner

Materials

- 15litres Pasteurized whole milk
- 65ml Yakult

Procedure

- 15L of pasteurized whole milk was measured out into a 20L sterile glass container. The Bunsen burner ensures a sterile environment is maintained as the transfer is being made.

- 30ml of Yakult is transferred into the glass container with the whole milk using a 30ml pipette and the container is closed off using some cotton wool with aluminum foil to allow for escape of CO_2 gas.
- Mixing of the contents is done manually by swirling the 20L glass container for 60 seconds.
- The contents are allowed to stand still at room temperature for at least 48hours.
- After 48 hours, it is anticipated that the LAB have attained exponential growth and the milk mixture has become thick as a result of lactic acid formation and is ready to be used as the Inoculum for the treatment process.

3.4.4. Treatment Experiment- Treatment with optimal parameters

The treatment experiment was conducted in triplicate. One reactor was used a control, making a total of 4 reactors. The faecal sludge was obtained from a pit latrine in Limbe, Blantyre that had been in use for the last 7 years and had since never been emptied.

Apparatus

- 50L Plastic Container Reactors
- Mixer
- 1,000ml measuring cylinder
- 100ml sampling bottles
- Sprayer

Materials

- Faecal Sludge
- Inoculum
- Molasses

Procedure

- Faecal Sludge in all the 4 reactors was weighed using a bathroom scale. This was in the absence of an analytical balance which was not available at the time.
- Optimal concentration of sugar and Inoculum addition established earlier in the laboratory experiments was used. 10% w/w molasses addition and 10% Inoculum addition were used as optimal concentrations.
- 10% w/w molasses was calculated using the weight of the faecal sludge determined in the first step, weighed and added to the 3 treatment reactors.
- 10% w/w Inoculum prepared 48hrs prior was also calculated using the combined weight of the faecal sludge and molasses added in the second step weighed and added to the 3 treatment reactors.
- Mixing of the treatment mixture was done using a power mixer at the beginning of the experiment and prior to every sampling. 3 minutes of mixing in each of the 4 reactors was done.
- Samples from all the reactors were collected after 0d, 2d, 4d, 7d, and 9d to establish the pH, Lactic Acid concentration and the viable cell count of the LAB and *E. coli.*

Figure 3.8 *50L fermentation reactors, mixing equipment & manual mixing*

3.4.5. Odor Tests

The method used for odor test was a standard method under the examination of water and wastewater, method 2150B-Threshold odor test (Clesceri Greenberg, Arnold E., Trussell, R. Rhodes., American Public Health Association., American Water Works Association., Water Pollution Control Federation., 1989)
The principal behind this method is in determining the threshold odor number by diluting a sample with order free water until the least definite perceptive odor is achieved.

The ratio by which the faecal sludge sample had to be diluted with odor-free water for the odor to be just detectable by the odor test is the threshold odor number" (T.O.N.). The total volume of sample and odor-free water used in each test was 200ml.

$$T.O.N = \frac{\mathrm{A} + \mathrm{B}}{\mathrm{A}}$$

A=ml sample and
B=ml odor-free water.

The table below gives the dilutions and corresponding threshold numbers.

Table 3.4 Threshold odor number corresponding to various dilutions

Sample volume (ml) diluted to 200ml	Threshold odor number
200	1
100	2
50	4
25	8
12.5	16
6.3	32
3.1	64
1.6	128
0.8	256

Apparatus

- Steam bath.
- 300ml wide-mouthed Erlenmeyer flasks (recommended 500 ml wide-mouthed Erlenmeyer flasks)
- 90mm Petri dishes
- 100ml sampling bottles
- Pipettes, measuring: 10.0 and 1.0 ml graduated in tenths
- 250ml Measuring cylinder
- Analytical balance
- Odor Test Panel

Materials

Order free dilution water

Conditions for Test

- A selection of 5 persons to make odor tests was made. The panel comprised of colleagues with whom I were working with in the lab and some of the laboratory staff.
- There was need to establish that all the 5 persons did not have a problem with insensitivity and were free from colds; this was established.
- Other conditions for carrying out the test as required by the standard method such as use of scented soaps, perfumes, exposure to smoke or food prior to carrying out the test were all met.
- The test was carried out in the laboratory at the wastewater treatment plant in Zingwangwa. It should however be mentioned that it was not completely possible to eliminate all other external odors such as those emanating from the trickling filters and other plant infrastructure.

Procedure

- Samples of faecal sludge under treatment were collected in 100ml sampling bottles.
- The proper volume of odor-free water was put into the flask first; the sample was then added to the water.
- The dilutions together with the reference were heated to 60°C (±1°C) in a water bath.
- The flask containing the odor-free water was shaken, then the Petri dish lid was removed, and the vapors were sniffed.
- The sample containing the least amount of odor-bearing water was then tested in the same way. When odor was detected in the dilution, more dilute samples were prepared as described above. When odor could not be detected in the first dilution, the above procedure was repeated using the

sample containing the next higher concentration of the odor-bearing water until odor was clearly detected.

- Based on the results obtained in the preliminary test, a set of dilutions using Table 1 as a guide were prepared. One or more blanks were inserted in the series, in the vicinity of the expected threshold, without repeating the pattern.
- It was ensured that the panellist did not know which dilutions were odorous and which were blanks. They smelt each of the flasks in sequence, beginning with the least concentrated sample and comparing with a known flask of odor-free water, until odor was detected with utmost certainty.
- The observations of each tester were then recorded by indicating whether odor is noted (+sign) in each test flask.
- Calculations of panel results to find the most probable average threshold were best accomplished by appropriate statistical method

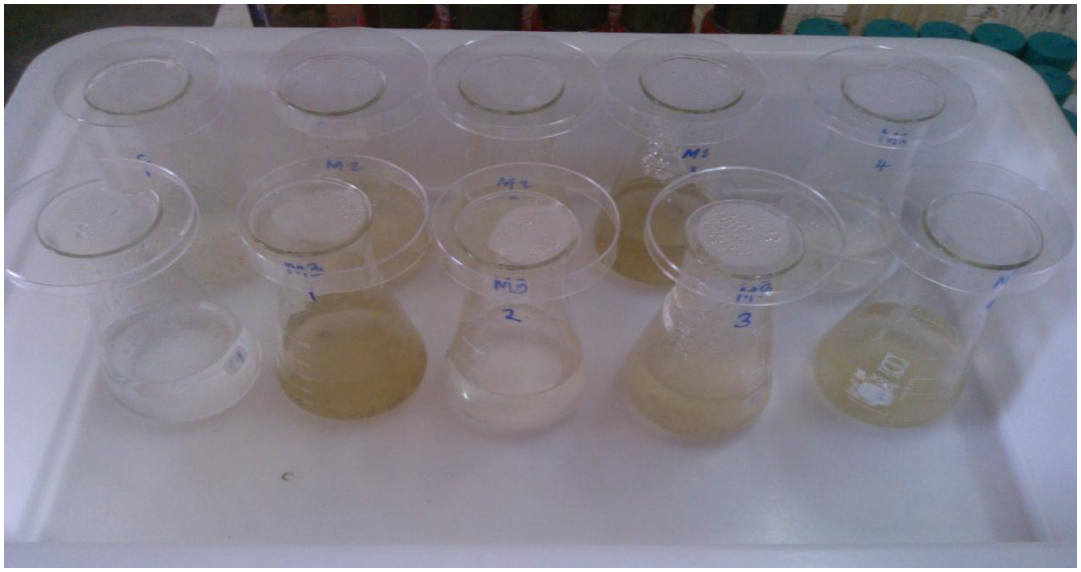


Figure 3.9 *300ml wide-mouthed Erlenmeyer with various dilutions of order bearing water and reference*

CHAPTER 4

Results and Discussion

In this chapter, the results of all the experiments conducted are presented in chronological order and the implication thereof discussed. The chapter begins by presenting the results of the laboratory experiments followed by the results from the field experimental work in Malawi.

The laboratory experiments were conducted to establish the optimal conditions required for lacto-fermentation process to occur and produce a sanitizing effect on a faecal sludge that conforms to WHO standards. The field experiments were conducted as an upscale of the laboratory work so as to examine the practicability of the results obtained from the laboratory. A total of 4 different sets of experiments were conducted.

The first sets of experiments were conducted to evaluate the suitability of two different media for use in the starter culture. A molasses-tap water media and whole milk media were evaluated.
The second set of experiments were conducted to establish the optimal sugar concentration necessary to promote proliferation of lactic acid bacteria once inoculated into the faecal sludge. Different sugar concentrations were evaluated. Thirdly, experiments were conducted to ascertain the concentration of Inoculum in terms of weight per weight with faecal sludge required to ensure and guarantee the lactic acid fermentation process occurs.

Thereafter, using the optimal conditions established in the initial sets of experiments, an experiment was conducted to evaluate the sanitizing effect of the lactic acid bacteria metabolism on faecal sludge using the established optimal conditions.

4.1. Laboratory-scale Experiments

4.1.1. Pre-culture (Inoculum media)

As the lactic acid bacteria strain used in the experiments was obtained from the probiotic fermented milk product Yakult, which comes in 65ml bottles, there was need to find a media that could be used for the initial proliferation of the bacteria (i.e. as some sort of a starter culture) before inoculating into the faecal sludge.

Two different media were selected for this purpose. The first, a solution of tap water (chlorine free) and cane molasses was considered as recommended by WASTE. This was because of its simplicity in preparation and its relative low cost as compared to the second media tested, plain whole milk. Plain whole milk was selected as the second media despite it being relatively more expensive, because it had the advantage of not only containing simple sugars but also proteins and minerals necessary for lactic acid bacteria metabolism.

Molasses pre-culture

The viable cell count (vcc) of the LAB and the pH of the media were the two parameters that were considered in determining the suitability of using the molasses-tap water solution as media for the starter culture in the LAF experiments. 1ml Yakult was added to 0.1%, 0.5%, 1% and 2% w/w molasses concentration solution.

It was noted that the LAB viable cell count (vcc) declined in all the reactors with less than 2% w/w molasses in tap water. By the 2nd day, the LAB vcc numbers had declined appreciably and complete die off was noted in one of the samples with 0.1 % w/w molasses addition. This decline in numbers can be attributed to shock as a result of the new environment and also because the sugar concentration was not adequate to support the fermentation process.

The vcc numbers in all the reactors remained below 9 log units throughout the entire period of the experiment.
Media solutions with a higher molasses concentration were prepared to investigate the response of the LAB and to ascertain whether the sugar concentration was not adequate to support fermentation in the earlier experiments. 1%, 10%, 20% and 40% w/w molasses concentration solutions were prepared. **Figure 4.2** below shows that indeed the LAB responded better to the higher sugar concentration media with all the media solutions except the control registering growth to about 8 log units by the 4th day and up to about 9 log units by the 11 day of the experiments. However these growth numbers were still less by 2 log units when compared to the LAB vcc of the 5th day in the milk media experiment described on page 37.

Figure 4.1 *Miniature batch reactors for Lactic acid bacteria (LAB) proliferation in molasses-tap water*

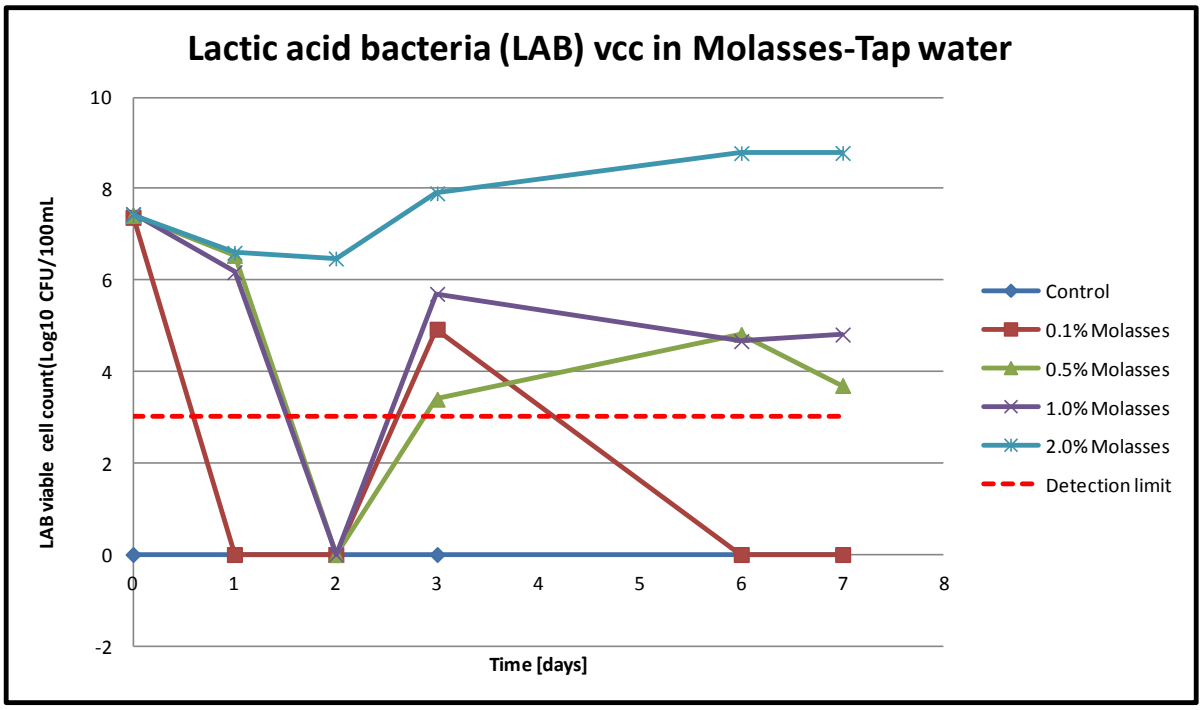


Figure 4.2 *Lactic acid bacteria proliferation in molasses-tap water solution*
(Note: Bacterial counts under the detectable limit are indicated as 0 log10)

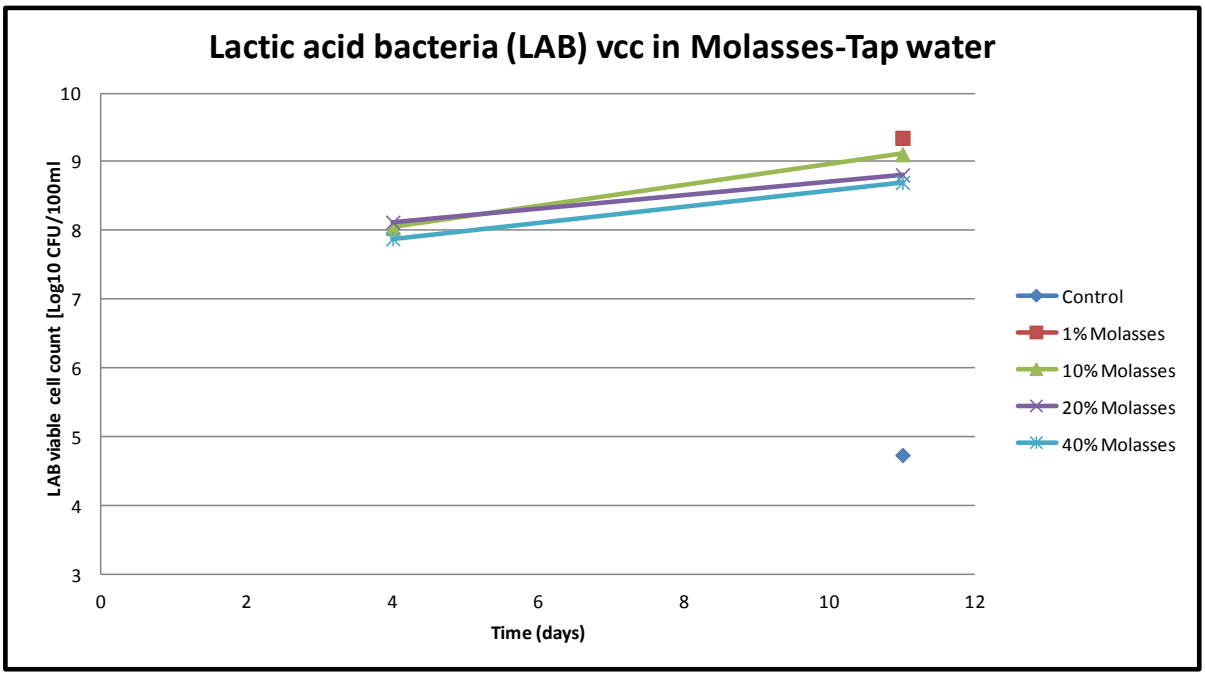


Figure 4.3 *Lactic acid bacteria proliferation in higher conc. of molasses-tap water solution*

Milk pre-culture

Figure 4.4 *Miniature batch reactors for Lactic acid bacteria (LAB) proliferation in milk*

Similarly, viable cell count (vcc) of the LAB and pH of the milk media were considered in determining the suitability of the milk media for use as starter culture. 1% of Yakult concentration was equally used here; but in addition, other Yakult inoculation concentrations were tested.

The curve for the 1.0ml Yakult concentration in **Figure 4.5** below is used for comparison with the molasses-tap water media graph whose Yakult concentration for all the samples was also 1.0ml. The other concentration curves in **Figure 4.5** are used to compare the behaviour of different concentrations in the same media.

The 0.1%, 0.2% and 0.5% Yakult concentrations exhibit very similar trends to the 1.0ml concentration; i.e. an increase in the vcc of LAB of about 4 log units and reaching a maximum on the 4-5 day of the experiment.
Figure 4.5 further illustrates that the maximum vcc of LAB achieved in period 4-5days is independent of the initial Yakult Inoculum concentrations as all the concentration curves tend towards a common maximum value. Negligible increase in the vcc of LAB is noted in the control experiment.

A comparison of the two growth media shows that the milk media better supports growth of the LAB than the molasses-tap water media. A substantial growth of up to 11 log units is noted for the milk media

whereas less than 9 log unit to no growth of LAB was noted for the different sugar concentrations of the molasses-tap water media.

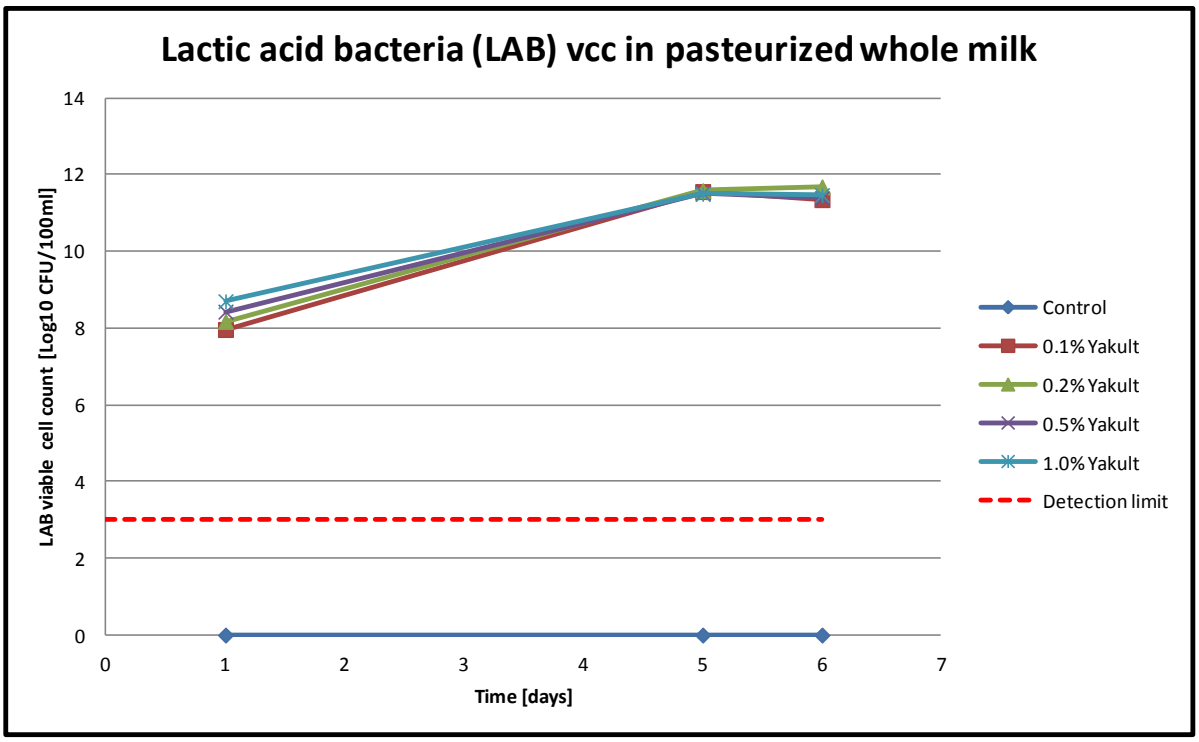


Figure 4.5 *Lactic acid bacteria proliferation in milk*

(Note: Dilutions used for control did not register any colonies; however it is possible that lower dilutions could have)

Figure 4.6 below illustrates the trend in the pH of the milk media over time.

All 3 samples excluding the control exhibited similar trend in the drop of the pH. The control on the other hand exhibited no drop in pH until to the 4th day. The lower pH value of about 3.8 (excluding the control) was attained after 5 days. This corresponds to the period when maximum vcc of LAB is attained. Thus a relationship is established between growth of LAB and the pH of the media. The pH of the control begins to decline after the 4th day. This can be attributed to possible contamination of the control reactor.

4.1.2. Synopsis of Starter culture media experiments

The following synopsis can be drawn:

The milk media better supports the growth of LAB, exhibiting a growth increase of up to an average of $3x10^{11}$CFU/100ml in LAB vcc and a corresponding pH value of below 4 being achieved.

On the other hand, the growth numbers exhibited by the LAB in the molasses-tap water media were not as high as those seen in the milk media with a maximum of below $7x10^{8}$CFU/100ml being registered during the course of the experiment. A difference 3 log units is noted between the two Medias as at the 6th day of the experiment.

Guirard & Snell (1964) put forward that LAB require nutritional requirements such as several B vitamins, ascorbic acid, glucose, acetate etc. It is thus suggested that some of these nutritional requirements may not be available in the molasses-tap water media hence the observation made of lesser growth numbers compared to the milk media.

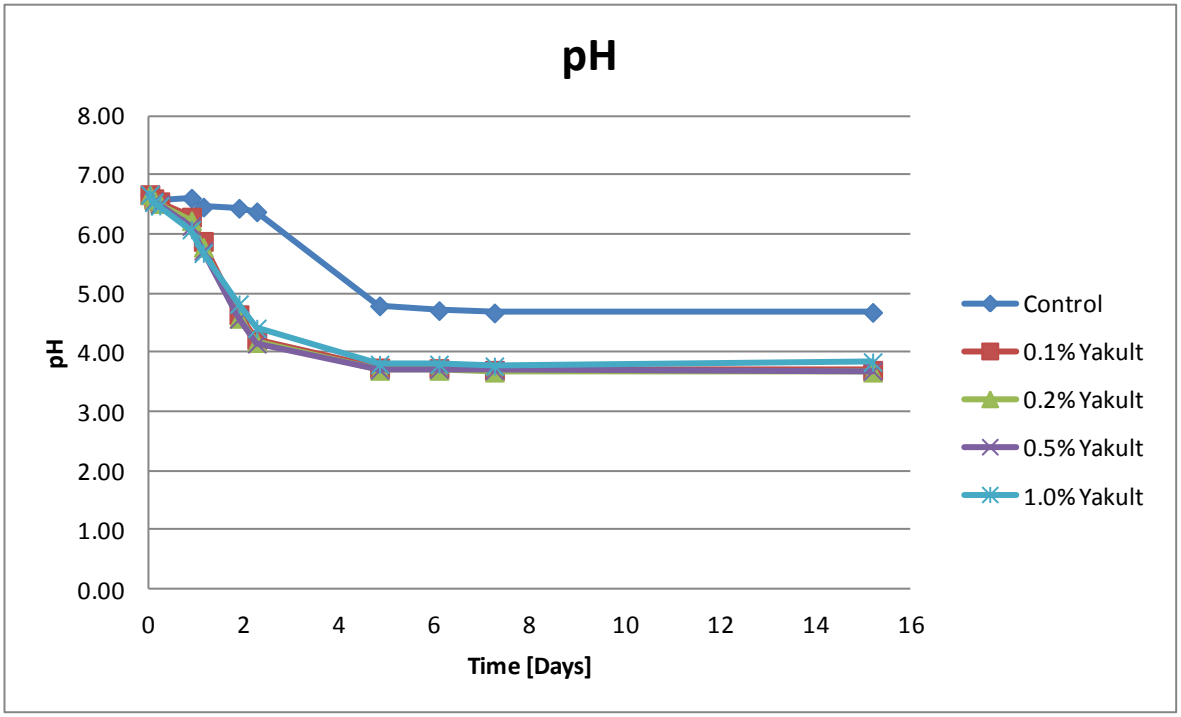


Figure 4.6 *Lactic acid bacteria proliferation in milk*

4.2. Batch Test 1 - Optimal Sugar concentration experiment

Experiments were conducted in duplicate in simple miniature batch fermentation reactors. Different sugar concentrations were applied by varying the w/w molasses concentration in faecal sludge. 0, 5%, 10% and 20% w/w molasses concentration in faecal sludge were investigated.

The experiments were conducted in a temperature controlled room of 30°C. Relevant parameters were analysed from 5ml samples drawn from each of the fermentation reactors at stipulated times during the course of the experiments.

Figure 4.7 *Miniature batch reactors for Optimal sugar concentration experiments*

4.2.1. LAB proliferation

During the initial 24 hours of the experiment, the viable cell count (vcc) of LAB in each of the reactors exhibited some reduction. This can be attributed to the process of the bacteria adapting to a new environment (Ramos et al. 2001)

The viable cell count of the LAB is however seen to increase as at the 4th day of the experiment indicating an adaptation to the new environment and subsequent proliferation. Between day 4 and 6, rapid growth of the LAB is noted.

Faster adaptation and proliferation is noted in samples with molasses concentration greater than 10% w/w in faecal sludge, i.e. samples 2 & 3.
Maximum growth numbers are seen to be realised around the 6th day of the experiment with all samples showing vcc of LAB greater than the initial numbers at inoculation.

From the 6th to the 10th day, the vcc of LAB begins to decline; nonetheless, as at the 18th day, all samples from the 3 reactors with an exception of the control reactor still have vcc of $7x10^{10}$CFU /100ml of sample or more.

Samples from the control reactor (i.e. without any molasses addition) also showed an increase in the vcc of LAB. This could be attributed to a presence of some simple sugars in the faecal sludge. But because no extra sugar was added, the vcc in the control remained lower by 2 to 3 logs units as at the 18th day of the experiment.

Finally, samples from the reactor that had neither molasses addition nor Inoculum added indicated a vcc of LAB of less than 6 long units per 100ml of sample throughout the period of the experiments.

Results from this set of experiments clearly indicated that the LAB are able to proliferate in the treatment mixture of molasses and faecal sludge. Further, the results indicate that a sugar additive is an important requirement necessary to promote the proliferation of LAB in faecal sludge and consequently production of lactic acid and other metabolites.

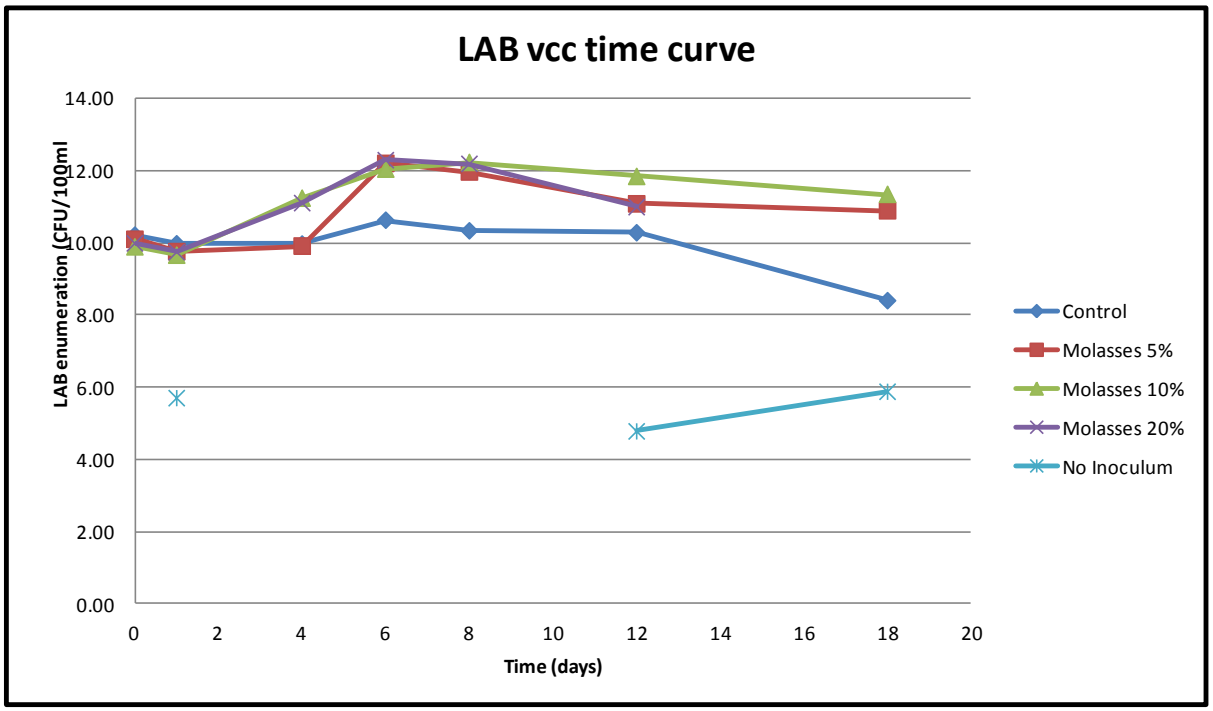


Figure 4.8 *Lactic acid bacteria proliferation in Optimal sugar concentration experiments*

4.2.2. pH values

Measurements of pH were done concurrently with the lactic acid concentrations measurements, plating for vcc determination of LAB and *E. coli*.

Figure 4.9 below illustrates that the pH in the control reactor and in reactors with less than 10% w/w molasses concentration in faecal sludge, exhibit stable or a slight increase in pH.
On the other hand, samples with molasses concentration in faecal sludge greater or equal to 10% w/w, exhibit a decline in pH value with a minimum value of around pH 4 being recorded.

This result places further emphasis on the need to have a w/w molasses concentration in faecal sludge of 10% and above to achieve lower pH concentrations in the treatment mixture.

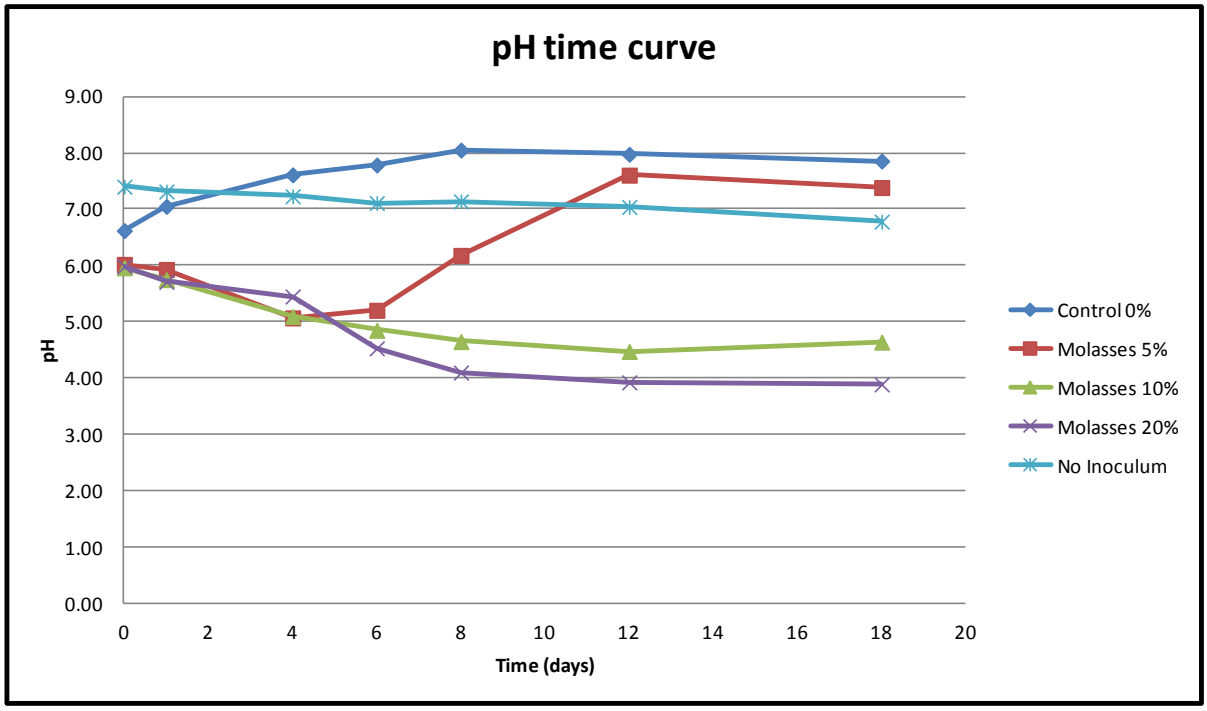


Figure 4.9 *pH measurements in Optimal sugar concentration experiments*

4.2.3. Lactic Acid

Lactic acid concentration was determined reflectometrically using lactic acid test strips after appropriate dilution of samples.

Figure 4.10 below illustrates how the lactic acid concentration varied with time. As can be seen from the figure, the lactic acid concentration in reactors having less than 10% w/w molasses addition is seen to have either remained low or gradually decreased to values in the vicinity of 100mg/L (see appendix B) whereas it is seen to spike to values of over 35 g/L in reactors with a molasses concentration in faecal sludge of greater than or equal to 10% w/w.

Due to a lack of the lactic acid testing kit in the first 5 days of the experiment, no data was collected for the period. However, it can be assumed that the starting concentration of lactic acid in all the reactors was around the same relatively low value since all the reactors were inoculated with the same w/w percentage of starter milk pre-culture of 10%. This assumption can further be qualified by realising that the pH in all the reactors at the start of the experiment was in the same range of between 6.0 and 6.6. (See Appendix B)
It is also interesting to note that the lactic acid concentration in all the reactors is seen to remain stable from the 12th day onwards with samples from reactors with molasses addition greater or equal to 10% w/w, exhibiting high lactic acid concentration of above 38 g/L. Samples from reactors with less than 10% w/w molasses addition, showed very low stable values of lactic acid concentration of 100mg/L for the period preceding the 12th day.

However some inconsistency is noted in the duplicate reactor of the 10% molasses addition reactors. Here, it was observed that the lactic acid concentration actually reduces in the reactor. The reasons that could have contributed to this inconsistency are quite difficult to pinpoint due to that fact that the experiment was

done only in duplicate, hence among other reasons, identifying the result as a possible outlier is not possible. However possibilities could include aspects such as, a lack of homogeneity of the treatment mixture arising from inadequate mixing, other possible human errors such as inconsistency at sampling, during dilution of samples, at plating etc. However, one would argue that, since the vcc of LAB in both the reactor and its duplicate did not vary much so the results should have been relatively similar. Thorough mixing and extra care on handling samples was put into practice in the later experiments so as to minimise such inconsistencies.

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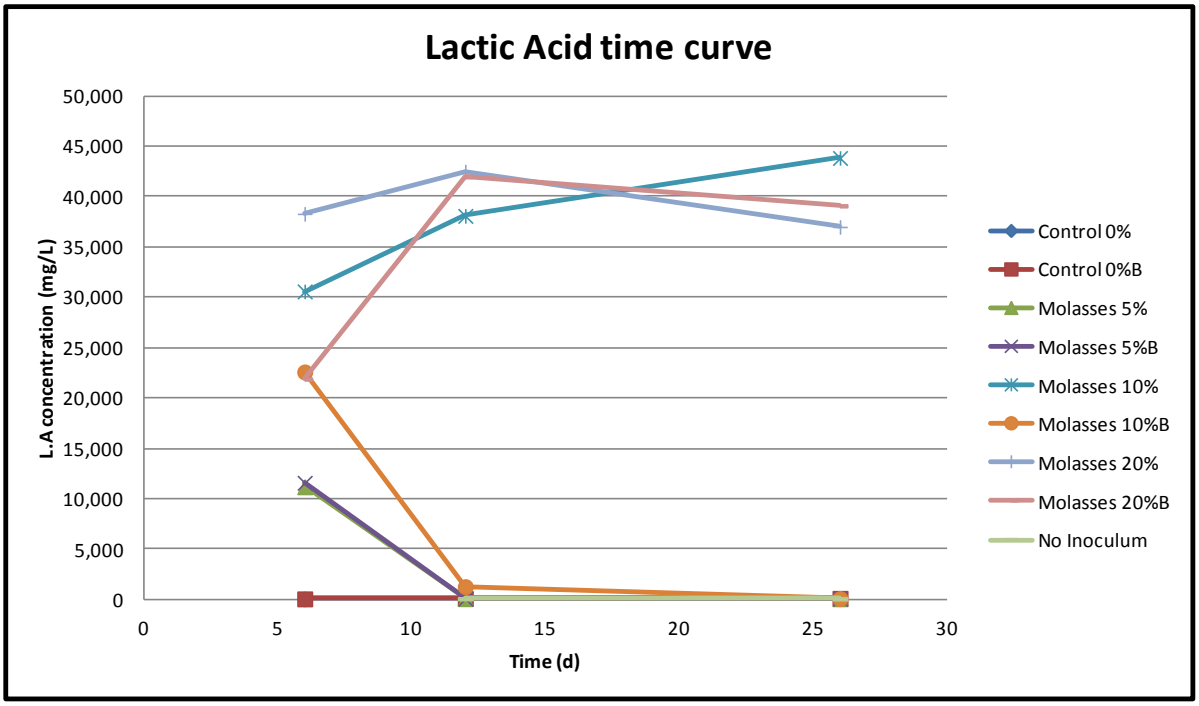

Figure 4.10 *Lactic acid concentration in Optimal sugar concentration experiments*

4.2.4. Sanitization - *E. coli* suppression

The sanitizing effect of the lactic acid fermentation process in this set of experiments was equally checked despite it not being the core objective of the experiments whose focus was only on identifying the optimal sugar concentration necessary for the promotion of the lactic acid fermentation (LAF) process. The sanitizing effect is considered in detail during the 3rd set of experiments whose core objective is to investigate the sanitizing effect of the lactic acid fermentation (LAF) process.

Figure 4.11 below, illustrates how the viable cell count (vcc) of *E. coli* varied with time during the course of the experiment. Average values of the duplicate reactors are not used here because of a discrepancy in the results from the duplicates. Therefore individual results are shown. An increase in the vcc of *E. coli* is noted in all the reactors during the first 6 days of the experiment. Because *E. coli* is a heterotrophic organism, this increase in numbers can be attributed to the increase of the available food substrate in the treatment mixture (i.e. the added molasses). Varma A. & Palsson O.B, (1993.) point out catabolic pathways

that involve glucose conversion in the metabolism of *E. coli* substantiating the ability of *E. coli* to take up glucose.

Also, because the pH of the environment is still close to neutral, no suppression is noticed in these initial days. However, in the period following the 6th day, we notice that the suppression effect begins to manifest in some of the reactors. This period is characterised by appreciable amounts of lactic acid building up in the treatment mixture and a drop in pH to around 4.

The suppression effect is more strongly manifested in samples collected from part of the reactors having 10% w/w or more molasses concentration in faecal sludge.
Between the 12th and 18th day, some samples from the higher molasses concentration reactors exhibit suppression levels that are below detectable limits.

On the other hand, all the samples from the 5% or less w/w molasses concentration reactors exhibited lower *E. coli* suppression. All the samples from these reactors exhibited an *E. coli* viable cell count of above $1x10^8$ CFU/100ml as at the end of the testing period.
The small noticeable reduction in the vcc of *E. coli* in samples collected from the control reactors may be attributed to natural die off as was noted in the *E. coli* proliferation experiments (See Appendix B).

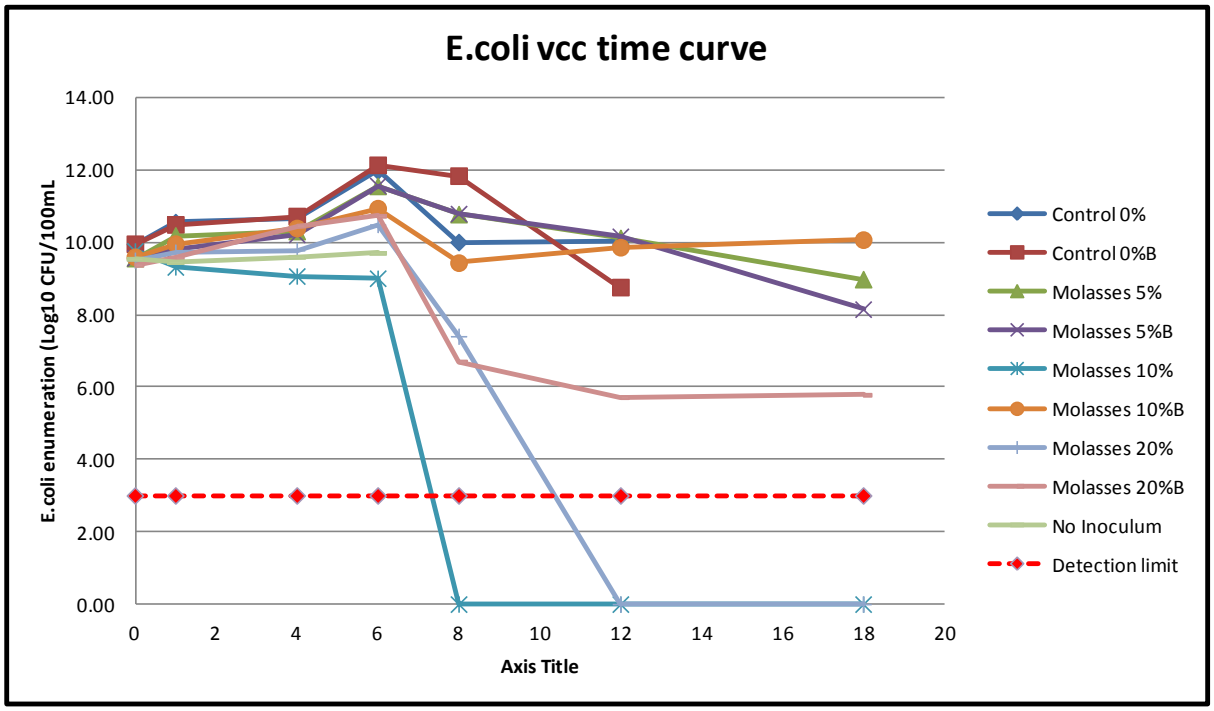

Figure 4.11 *E. coli suppression in Optimal sugar concentration experiments*
(Note: Bacterial counts under the detectable limit are indicated as 0 log10)

4.2.5. Synopsis of Treatment 1 experiments - Optimal Sugar Concentration experiment

The following hypothesis can be put forward following the optimal sugar concentration experiments:

1) Addition of a sugar supplement is required to promote growth of LAB and consequently necessary for the production of considerable amounts of lactic acid and a significant reduction of pH.
2) Only samples with sugar concentration greater than 10% w/w addition of molasses produced significant concentrations of lactic acid with the concentrations of the 20% w/w addition of molasses showing little variability with that of 10%.
3) It thus follows that 10% w/w addition of molasses is selected as the optimal sugar concentration.
4) Homogenization of the reactor mixture of faecal sludge and sugar additive (molasses) and also in the milk pre-culture is required and necessary for consistency in results. The inconsistency in results of samples 2 and 2B may be attributed to a lack of adequate mixing.
5) There exists a strong correlation between Lactic acid concentration build-up and the growth of LAB. This on its own suggests that, the LAB are responsible for the production of the lactic acid observed in the faecal sludge which consequently is responsible for the reduction in pH.

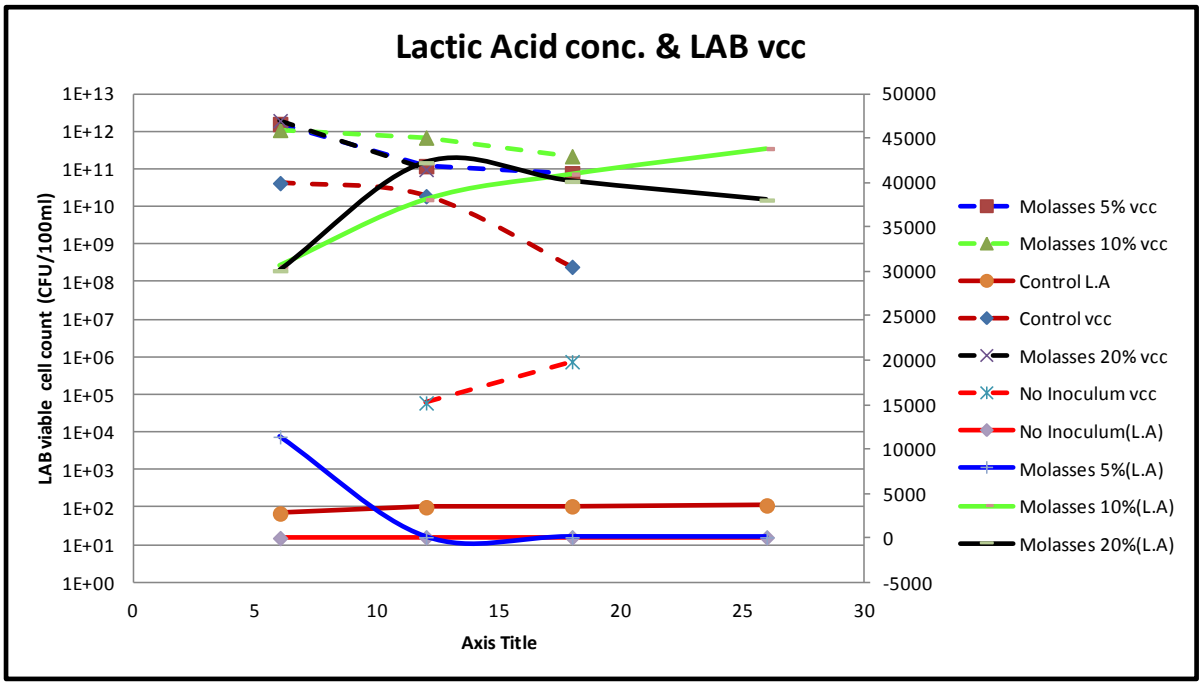

Figure 4.12 *Lactic Acid conc. & LAB vcc correlation in Optimal sugar concentration experiments*

4.3. Batch Test 2 - Optimal Inoculum concentration experiment

The second batch of experiments were conducted so as to investigate the behaviour of different Inoculum concentration on the lacto fermentation process and establish an optimal Inoculum concentration thereof. The experiments were again conducted in duplicate in miniature batch fermentation reactors. Faecal sludge was inoculated with different concentrations of the milk pre-culture by varying the % w/w of the milk pre-culture added to the faecal sludge media. The milk pre-culture was prepared by diluting 1ml of Yakult in pasteurized whole milk to make 1000ml milk pre-culture media (0.1% w/w Yakult). The milk pre-culture was incubated at 37 degrees Celsius for at least 30 hours to allow the bacteria to adapt to the new environment and reach exponential growth(Cho et al. 1996). The different milk pre-culture concentrations that were investigated are 0, 1%, 5%, 10% and 20% w/w of milk pre-culture in the faecal sludge. 10% w/w molasses concentration in faecal sludge was the concentration used for all the reactors. This is following its adoption as the optimum sugar additive concentration from the earlier Optimal Sugar concentration experiments (see section 4.2.5 above).

Relevant parameters were analysed from 5ml samples drawn from each of the fermentation reactors at stipulated times during the course of the experiments.

4.3.1. Indicator parameters monitored

Only pH and Lactic acid concentration were monitored during these sets of experiments. Viable cell count of the LAB was not monitored. This is because of the strong correlation between pH, Lactic acid concentration and the vcc of LAB that was established during the optimal sugar concentrations experiments (see paragraph 4.2.5). As such, monitoring of pH and Lactic acid only was adequate to give an indication of how well the lacto fermentation process was proceeding.

4.3.2. pH

Figure 4.13 below shows the trend in the pH of the batch fermentation reactors over the period of the experiment. The pH of all the samples exhibited a similar trend. There is first a rapid drop in the pH values i.e. between the 1st and 3rd day of the experiment. This is followed by a period with a steady linear drop in the pH up to the 11th day of the experiment.

Interestingly, the pH of the control reactor also exhibited a drop in pH value similar to the other samples that had been inoculated with the milk pre-culture. This drop in pH of the control sample can be attributed to possible contamination of the control sample by the LAB. This was revealed by a random check on the vcc of LAB that indicated high levels of LAB in the control.

The sample with 20% w/w milk pre-culture addition exhibited the lowest pH for all the sampling points. However, the difference in values between the 20% and 10% samples is only -1.8%; which is quite low and insignificant when compared to -6.6% and -9.0% difference with the 5% and 1% milk pre-culture addition respectively.

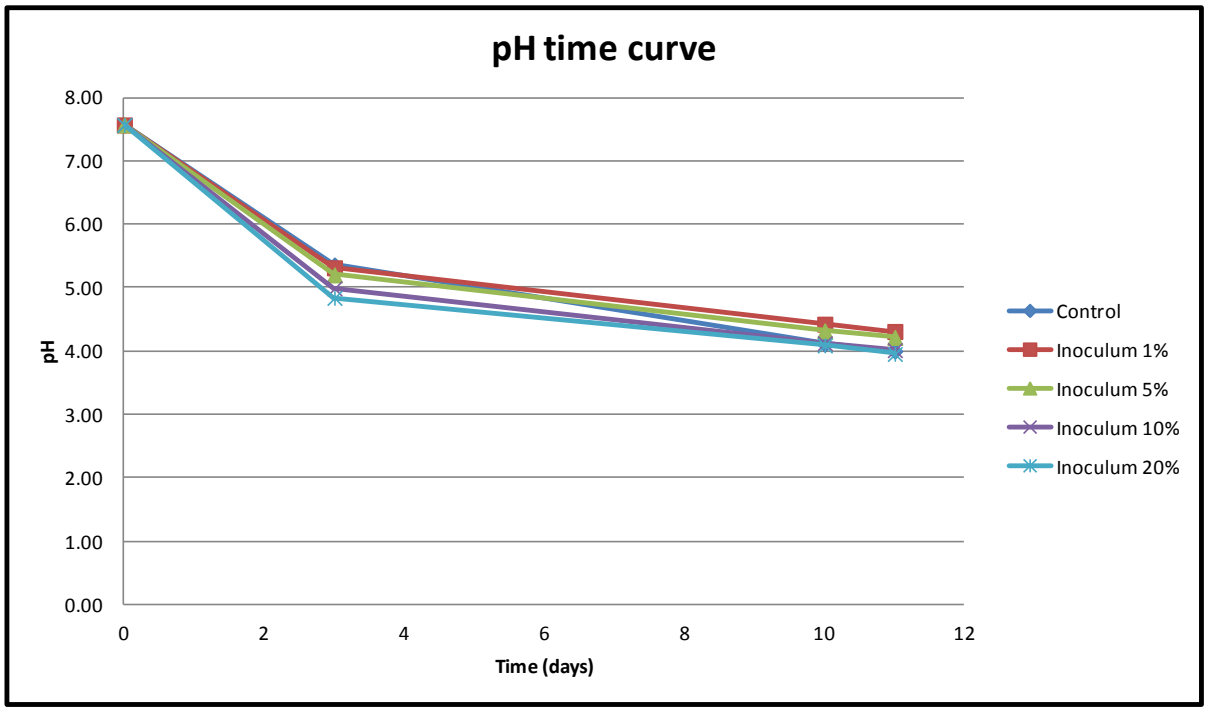

Figure 4.13 *pH measurements in Optimal Inoculum concentration experiments*

4.3.3. Lactic Acid

Figure 4.14 below illustrates how the lactic acid concentration in the reactors varied with time. The lactic acid concentration in all the reactors increased substantially from the initial concentration levels. Just as in the optimal sugar concentration experiments, the maximum lactic acid concentration in all the reactors is noted around the 11-12th day of the experiment with values here being slightly lower and ranging between 21,000mg/L to 28,000mg/L.

The lactic acid concentration in all the reactors seems to level off after attaining a maximum value between 3 and 11 days. This trend is also noted for the optimal sugar experiments conducted earlier. However, the lactic acid concentration in the 20% inoculum concentration reactor is seen to increase more slowly and to continue increasing after the 11-12th day period.

Like was also noted with the pH, the Lactic acid concentration in the control reactor also exhibited a rather unexpected trend where an increase in concentration is noted. The reasons for this rather unexpected behaviour have already been outlined above (see section46).
The reactor with the 10% Inoculum concentration exhibited the highest concentration of lactic acid.

Generally, the effect of Inoculum concentration on the lactic acid fermentation process is not very pronounced. This can be seen from the minimal variation of the maximum lactic acid concentration of the 1%, 5% and 20% reactors with the 10% Inoculum concentration reactor which is less than 20% in all cases.

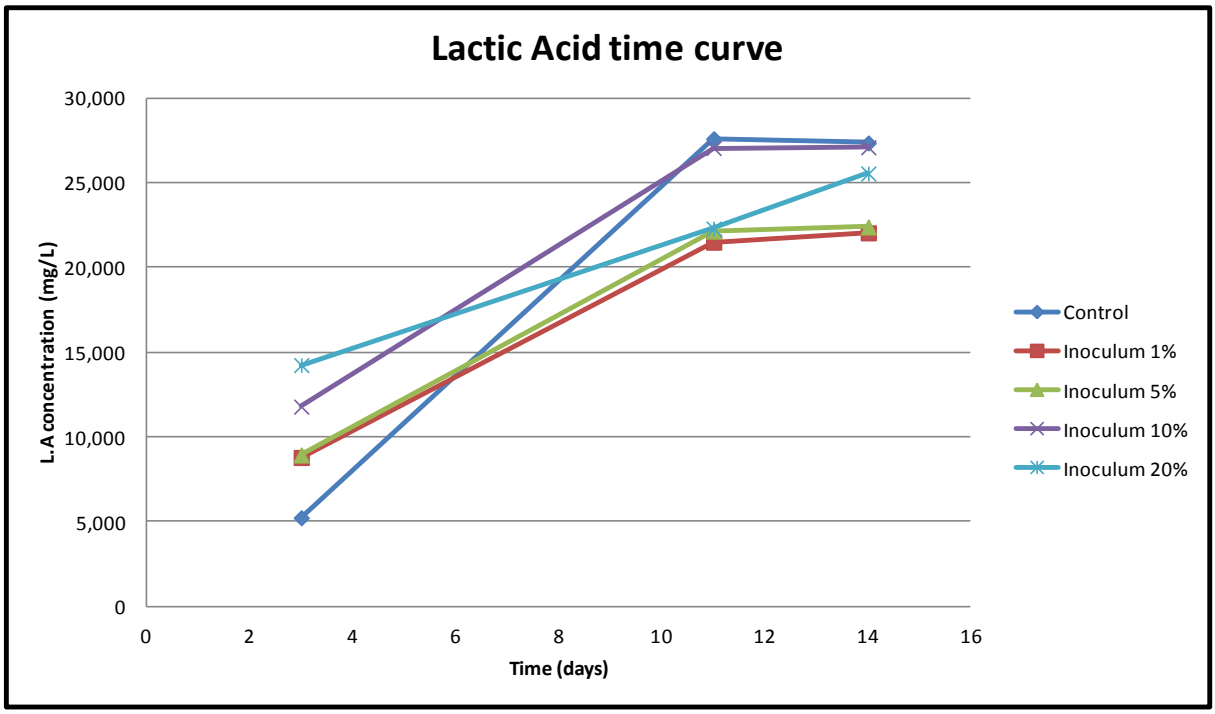

Figure 4.14 *Lactic Acid concentration in Optimal Inoculum concentration experiments*

4.3.4. Synopsis of Treatment 2 experiments - Optimal Inoculum Concentration experiment

The following synopsis can be made for the optimal Inoculum concentration experiments:

1) The concentration of the milk pre-culture does not have a profound effect on the lacto fermentation process. A concentration of 20% w/w milk pre-culture Inoculum produced results that did not vary so much with 1% w/w milk pre-culture Inoculum with a less than 4% variation at maximum concentration.
2) Despite having few data points, slight variations are noted in the rate at which lactic acid concentration increases. The rate is seen to be higher in reactors with higher Inoculum concentration, i.e. it increases in reactors with 1% to 10% w/w milk pre-culture inoculum addition but drops again in the reactor with a concentration of 20% w/w milk pre-culture Inoculum (see **Figure 4.15** below). Because the control was not sterile, it equally exhibited an increase in the lactic acid concentration.

3) Based on the rates of lactic acid concentration increase, 10% milk pre-culture Inoculum addition is selected as the optimum Inoculum concentration, and hence was adopted for the rest of experiments.
4) It should be stated however, that Inoculum concentration as low as 1% w/w milk pre-culture addition could still be used and results would still be within ±20% variation.

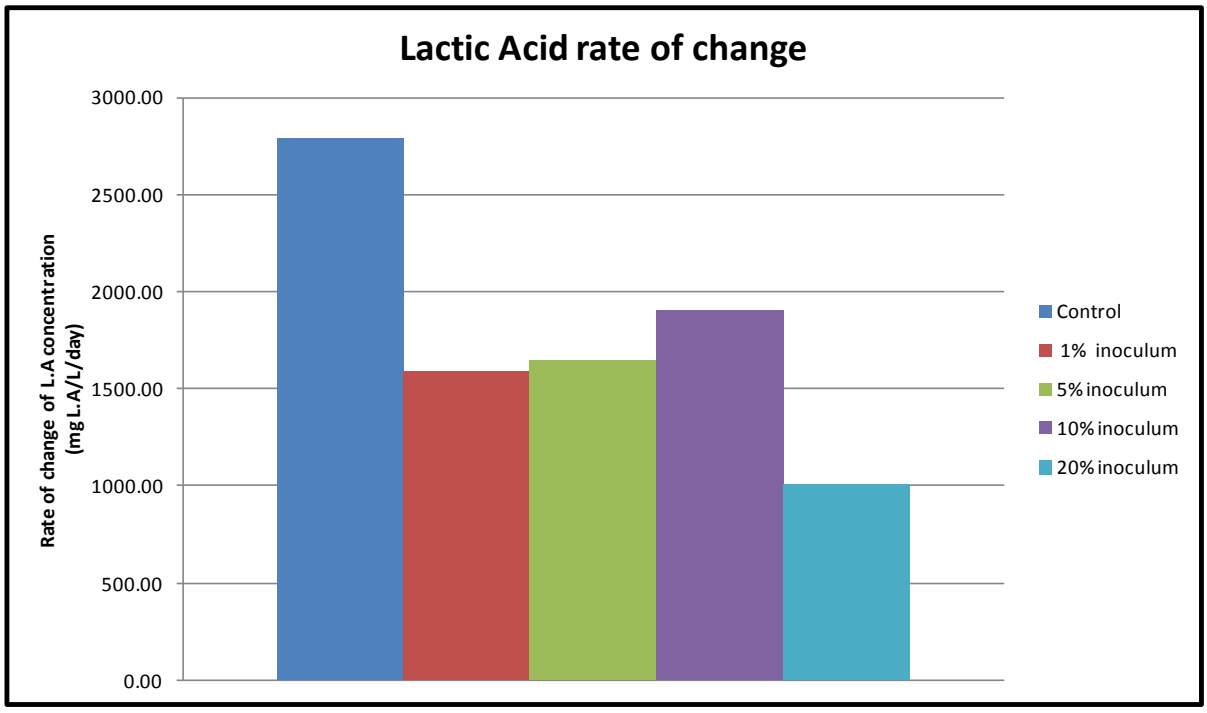

Figure 4.15 *Lactic Acid rate of change in Optimal Inoculum concentration experiments*

4.4. Batch Test 3 - Treatment with optimal parameters

The core objective of this set of experiments was to investigate the sanitizing effect of the lactic acid fermentation process. The experiments were conducted using optimal Inoculum and sugar concentration parameters established in the previous 2 sets of experiments. The experiments were conducted in triplicate so as to improve on the independence of the results. Furthermore, the experiments were conducted in miniature batch fermentation reactors with 10% w/w molasses addition and 10% milk pre-culture Inoculum addition as the applied optimal parameters.

4.4.1. Variables measured and monitored

The parameters monitored during this set of experiments were the viable cell count of *E. coli* and LAB. Lactic acid concentration and pH were equally measured to monitor the lactic acid fermentation process.

4.4.2. LAB proliferation

Greater consistency in the results of the viable cell count (vcc) of the LAB is observed here. This improvement in result consistency is attributed to adoption of more thorough mixing regimes of substrates and ensuring extra care was taken to avoid contamination of individual reactors, and also on handling of samples.

Figure 4.16 below illustrates the growth trend of the LAB. All the 3 treatment reactors exhibit a similar growth trend of rapid growth for the first 3 days of treatment, followed by a less rapid but stable growth period. Due to time limitations, the LAB growth trend was only monitored for a period of 10 days, within which growth was still active without any distinct maximum being attained.

By the 10th day of the experiment the average vcc of LAB had increased by 6 log units from the initial value to an average of $1.2x10^{12}$ CFU/100ml.

Since the first samples were only collected after 48hrs, it is not clear whether a lag phase was experienced during the initial 24hrs of treatment. Nonetheless, what can be noted is the rapid increase of the vcc of the LAB when the entire 48 hrs period is considered in totality.

On the other hand, samples from the control reactor (i.e. without any molasses and Inoculum addition) only showed some slight increase in the vcc of LAB over the entire treatment period. This was as expected, since no molasses or Inoculum was added to the control reactor and also more care was taken to minimize possible contamination from the other reactors that had been inoculated.

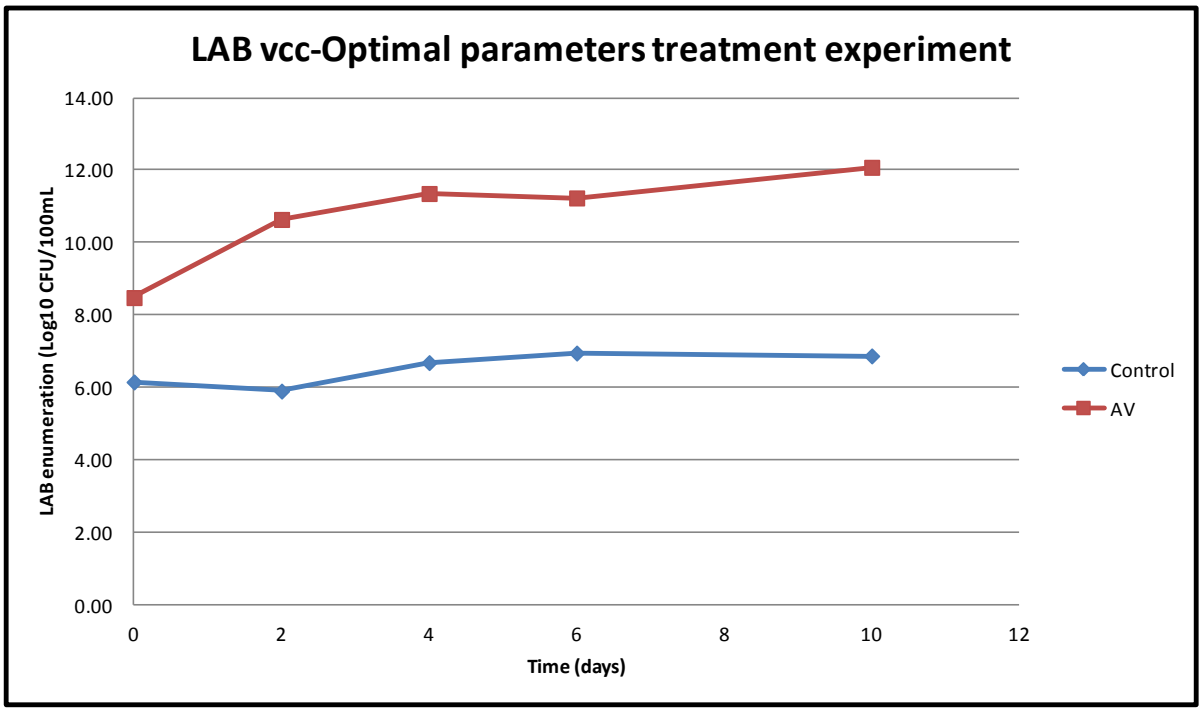

Figure 4.16 *Lactic acid bacteria proliferation in Optimal parameter experiments*

4.4.3. pH

Figure 4.17 below illustrates the average pH value over time in all the 3 treatment reactors and also the pH for the control reactor. As can be noted, the average pH value gradually decreases from its initial value of 6.5 to a stable minimum value of 3.9 after about 12 days.

The pH of the control remained above 6.5 throughout the experimentation period. This is because there was no lacto fermentation process taking place in the control reactor. We were also able to avoid contamination from the other treatment reactor thus upholding the integrity of the experimentation

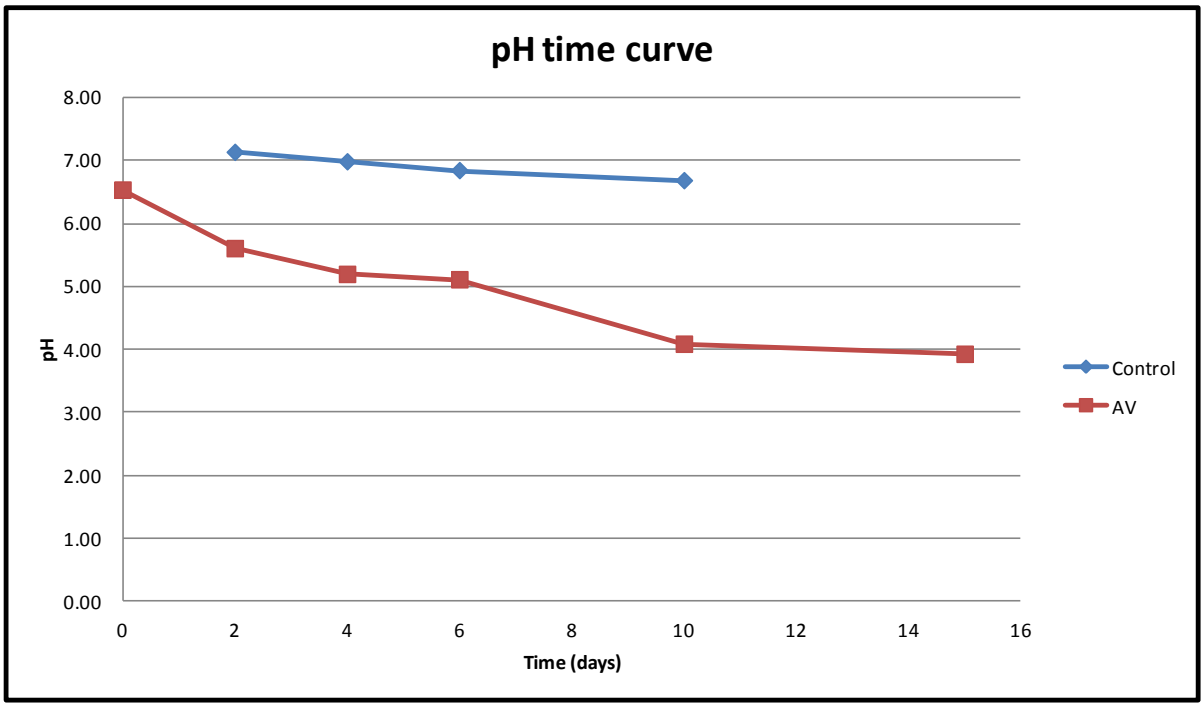

Figure 4.17 *pH measurements in Optimal parameter experiments*

4.4.4. Lactic Acid

Figure 4.18 below illustrates how the average lactic acid concentration varied over time. It can be noted that the lactic acid concentration in the treatment reactors increased substantially with time tending towards a maximum concentration of 39,000mg/L as at the 15th day of the experiment.

Whereas very little lactic acid concentration is noted in the control. Concentration of the lactic acid in the control reactor remained below 50mg/L throughout the course of the experiment. This indicates an over 700 fold increase in the concentration of lactic acid in the treatment reactors as at the 15th day of the experiment.

The results above suggest that adding 10% w/w molasses and milk pre-culture in faecal sludge has the potential to increase the lactic acid concentration over 700 fold. The sanitizing effects thereof are discussed in section 4.4.5 below.

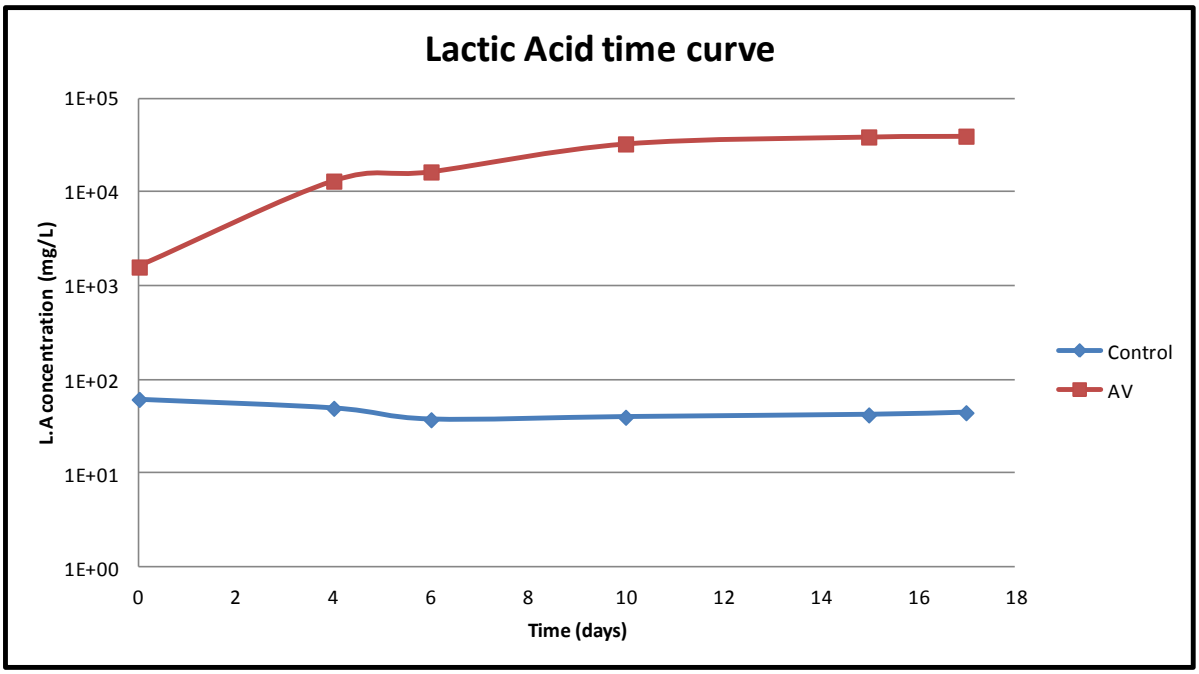

Figure 4.18 *Lactic acid concentration in Optimal parameter experiments*

4.4.5. Sanitization - *E. coli* suppression

The suppression effect on the *E. coli*, our selected indicator organism, was used to determine the level of sanitization or rather sanitization effect of the optimum parameter treatment experiments. As indicated in the literature, WHO guidelines stipulate a health based target *E. coli* number of <1000/g total solids/100ml (World Health Organization 2006).

Figure 4.19 below illustrates the viable cell count number (vcc) of *E. coli* during the course of the experiment. It can be seen that the vcc of the *E. coli* increases during the initial 4-5 days. This increase in vcc numbers can be attributed to an increase of the available food substrate in the treatment mixture (i.e. the added molasses).

Also, because the pH of the faecal sludge environment is still close to neutral during this period, no real threat is posed as yet to the *E. coli* as a result of adversity of the environment.
However, after day 5, we notice that suppression of *E. coli* begins to take effect and is characterised by a rapid decline of the *E. coli* numbers.

During this period, appreciable amounts of lactic acid in the treatment mixture and a drop in pH are observed (see section 4.4.3, 4.4.4 above and **Figure 4.21** below).

By the 15 day of the experiment, we see that the average vcc of the *E. coli* has reduced to levels below the detectable limit and consequently below the recommended WHO (2006) guidelines of <1000CFU/100ml of sample. This indicates a log reduction of approximately 7 log units.

On the other hand, samples from the control reactor, exhibited very minimal reduction in the vcc of *E. coli*; i.e. in the 17 days of the experiment, the vcc of *E. coli* reduced from $3.2x10^9$ CFU/100ml to $5.7x10^8$ CFU/100ml signifying a log reduction of only 1 log unit. This was as expected and the probable reason for this reduction is natural die off of the *E. coli* bacteria.

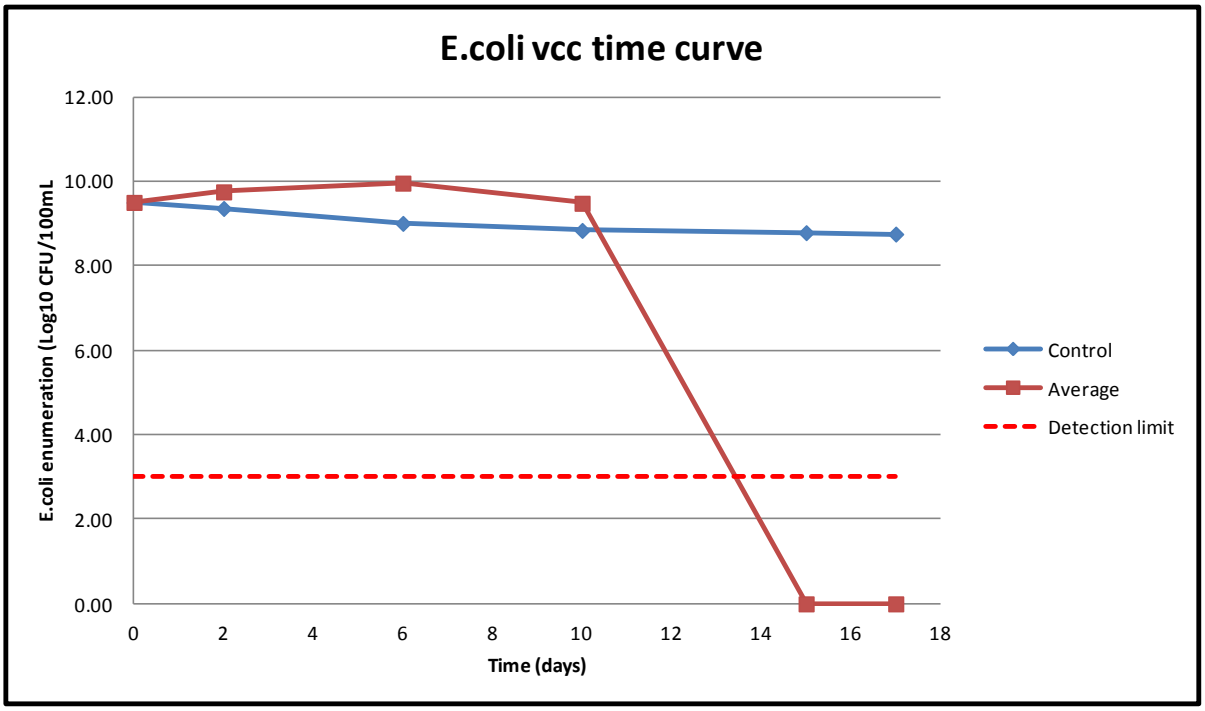

Figure 4.19 *E. coli suppression in Optimal parameter experiments*
(Note: Bacterial counts under the detectable limit are indicated as 0 log10)

The rate at which *E. coli* is suppressed i.e. for the period in which the suppression effect kicks in, is shown in **Figure 4.20** and expressed as a treatment suppression rate constant, K_{td}, and has a value of -1.14.

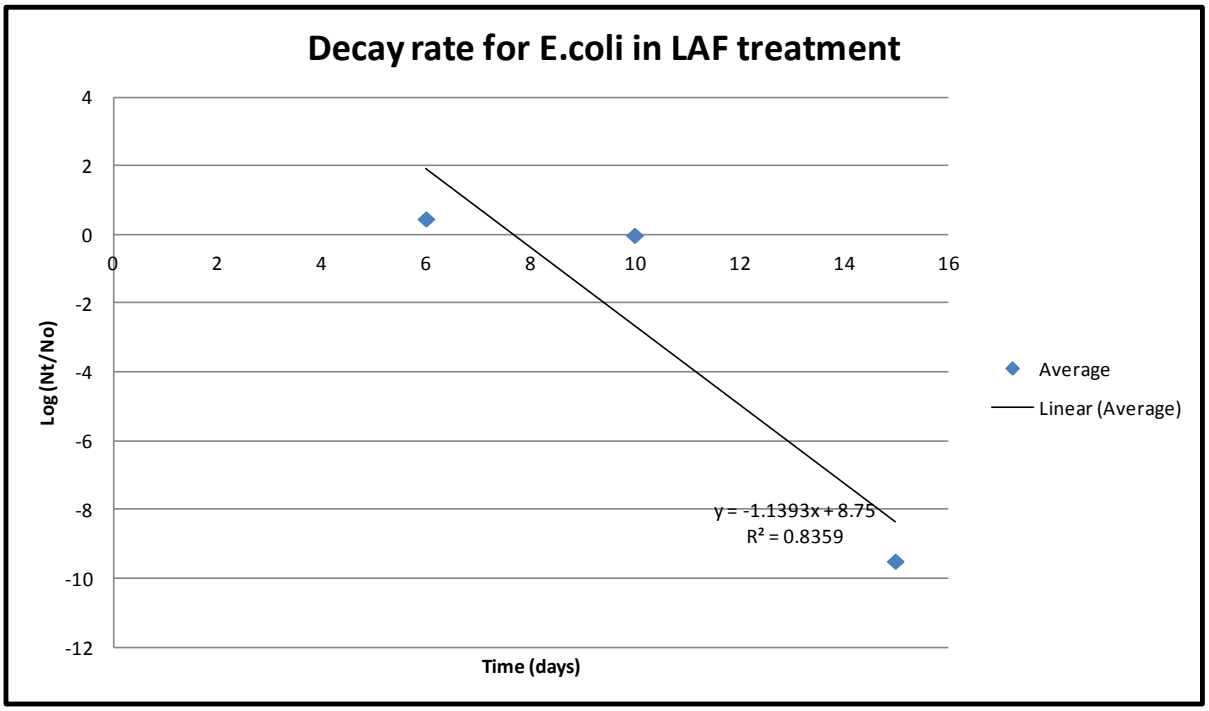

Figure 4.20 *Treatment decay rate for E. coli in Optimal parameter experiments*

4.4.6. Synopsis of Treatment 3 experiments -Treatment with Optimal Parameter experiment

1) Using the 10% w/w molasses and milk pre-culture in faecal sludge concentration, sanitization of faecal sludge (<1000 *E. coli* CFU/100ml) using the Lactic Acid Fermentation (LAF) process is **possible**.
2) A log reduction of approximately 7 log units is achieved as at the 15th day of experimentation.
3) It can be suggested that the sanitization mechanism is triggered when the lactic acid concentration reaches the range of 20g/L to 30g/L (see **Figure 4.21** below).
4) A pH of between 4.3 and 3.8 correlates with the setting in of the suppression effect (see **Figure 4.22** below)

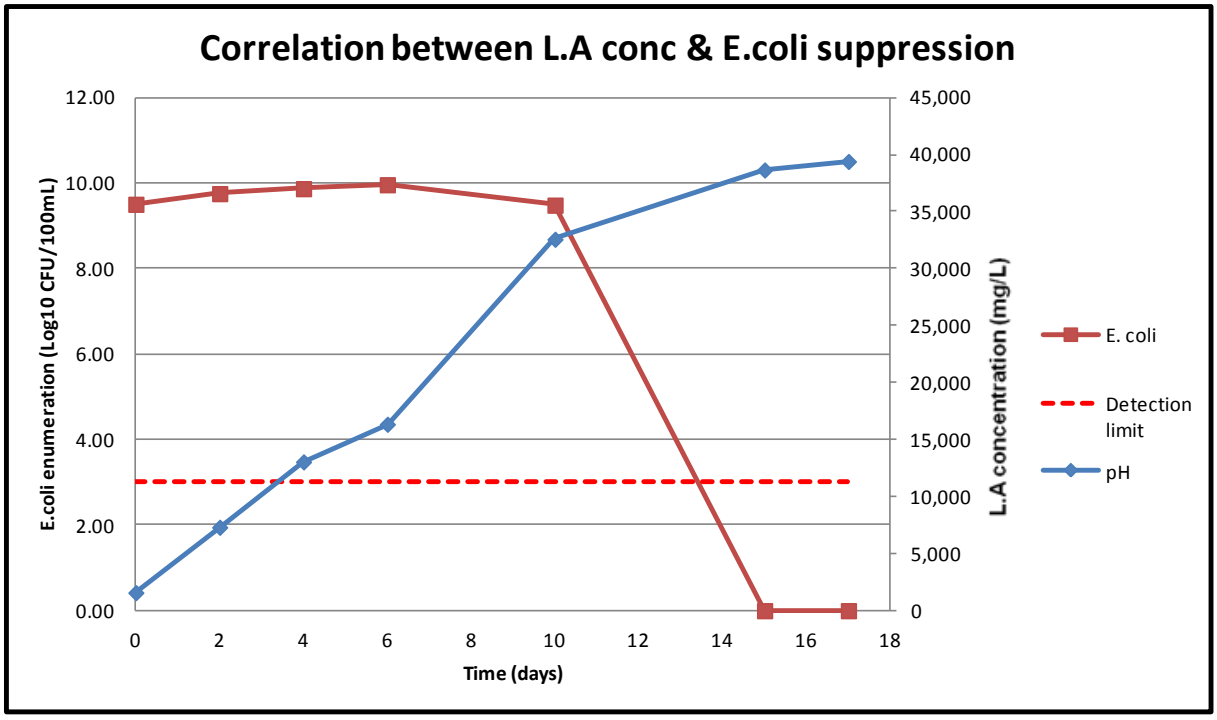

Figure 4.21 *Lactic Acid conc. & E. coli suppression correlation in Optimal parameter experiments*

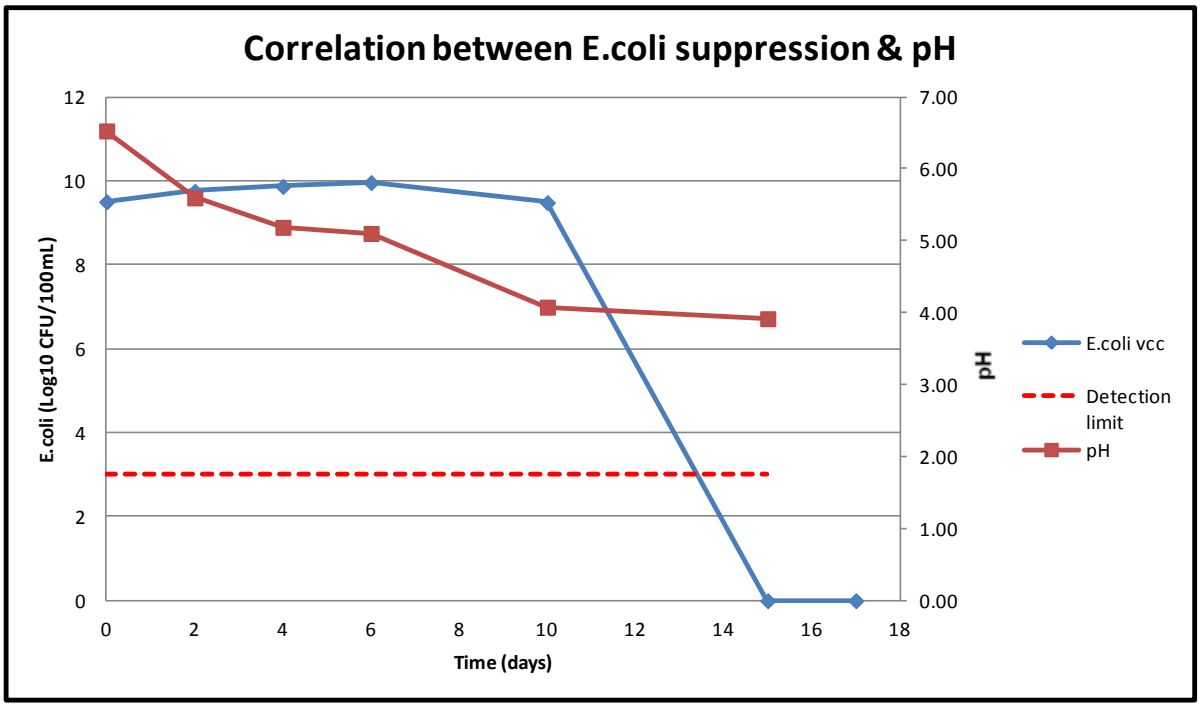

Figure 4.22 *E. coli suppression & pH correlation in Optimal parameter experiments*

4.5. Up-Scale Field Experiments (MALAWI)

As indicated at the beginning of the chapter, the field experiments were conducted as the final component of this research. The experiments were carried out to test the up scalability of the laboratory work and also to test whether the results obtained in the laboratory were replicable. Since the optimal parameters had already been established in the laboratory, only experiment 3 - the optimum parameter experiment was replicated and up scaled in the field. The experiments were conducted in triplicate in half filled 50 litre batch reactors. Further details of the experimental setup are provided in chapter 3 under field methodology. The results of the field experiments are presented starting with the characterisation of the faecal sludge then followed by results of the treatment.

4.5.1. Characterization of Faecal Sludge

Table 4.1 Characterization of Faecal Sludge (Malawi)

No	Parameter	Unit	Sludge 1	Sludge 2	Sludge 3
1	Age of Sludge	Months/Years	1 year	7 years	1 month
2	Source	N/A	Household	Household	Market
3	pH		7.3	7.6	7.3
4	Temperature	°C	26	21	26
5	TDS	ppm	4,170	7,045	-
6	Total Solids	%	8.6	5.6	4.2
7	Volatile Solids	%	45	55	59
8	Alkalinity (av.)	g/L $CaCO_3$	10	10	10

Sludge 2, i.e. the 7 year sludge was used as substrate for the treatment experiments. There is no particular reason as to why this sludge was selected only that it was readily available at the start of the experiments.

4.5.2. Dry Matter Content of Molasses

Table 4.2 Composition of Molasses

No	Description	Total Solids (%)	Total Sugars-Glucose & Fructose (mg/L)
1	Cane molasses	87%	20,000
2	Cane molasses (RSA)	10%	Not used/discarded

4.5.3. pH

Figure 4.23 below illustrates the average pH values over time in all the 3 treatment reactors and also the pH of the control. As can be noted, the average pH value drops rapidly from the 1st day of the experiments to about the 3rd day; thereafter a gradual levelling off is noted as the pH approaches the value 4. Compared to the pH values obtained during the lab experiments, it is observed that the minimum pH values obtained here were just slightly higher than those observed in the laboratory. The difference noted in the final pH values was about 0.1; i.e. 4.1 and 4.2 for the laboratory and field pH values respectively.

The pH for the control showed a slight drop during the initial 3 days and thereafter levelling off and maintaining a stable pH of just below 7 throughout the rest of the experimentation period. These results suggest that up scalability is possible (see **Figure 4.24** below).

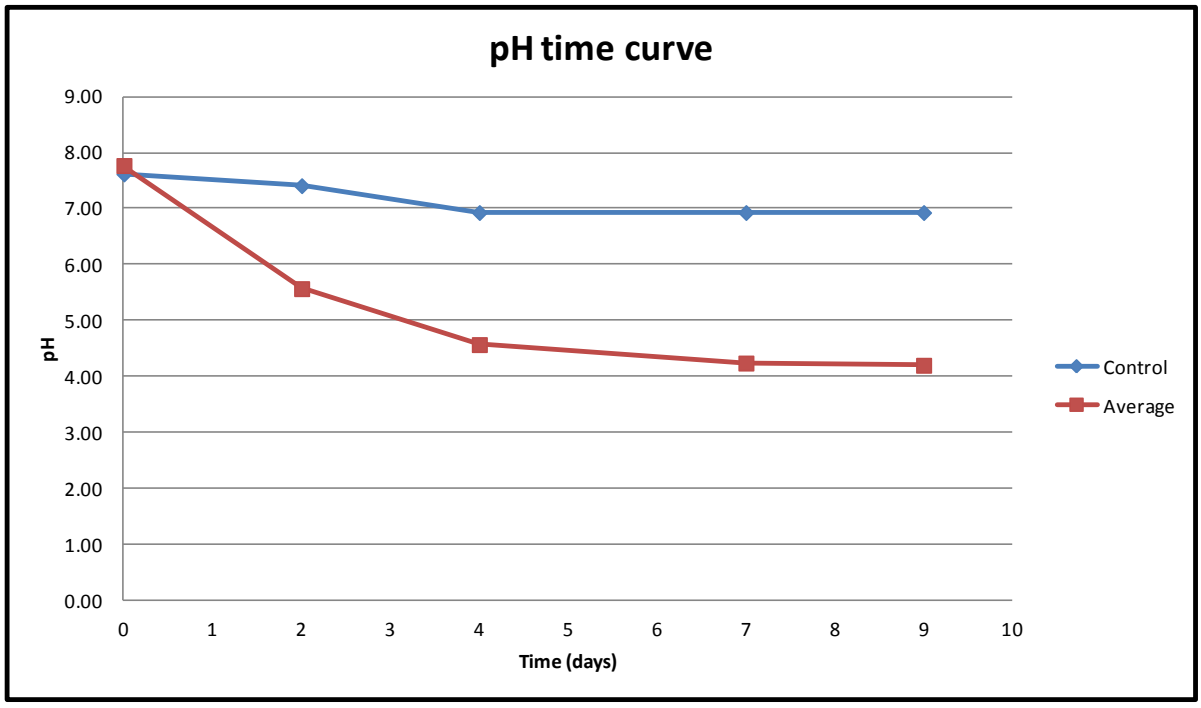

Figure 4.23 *pH measurements in up-scale field experiments*

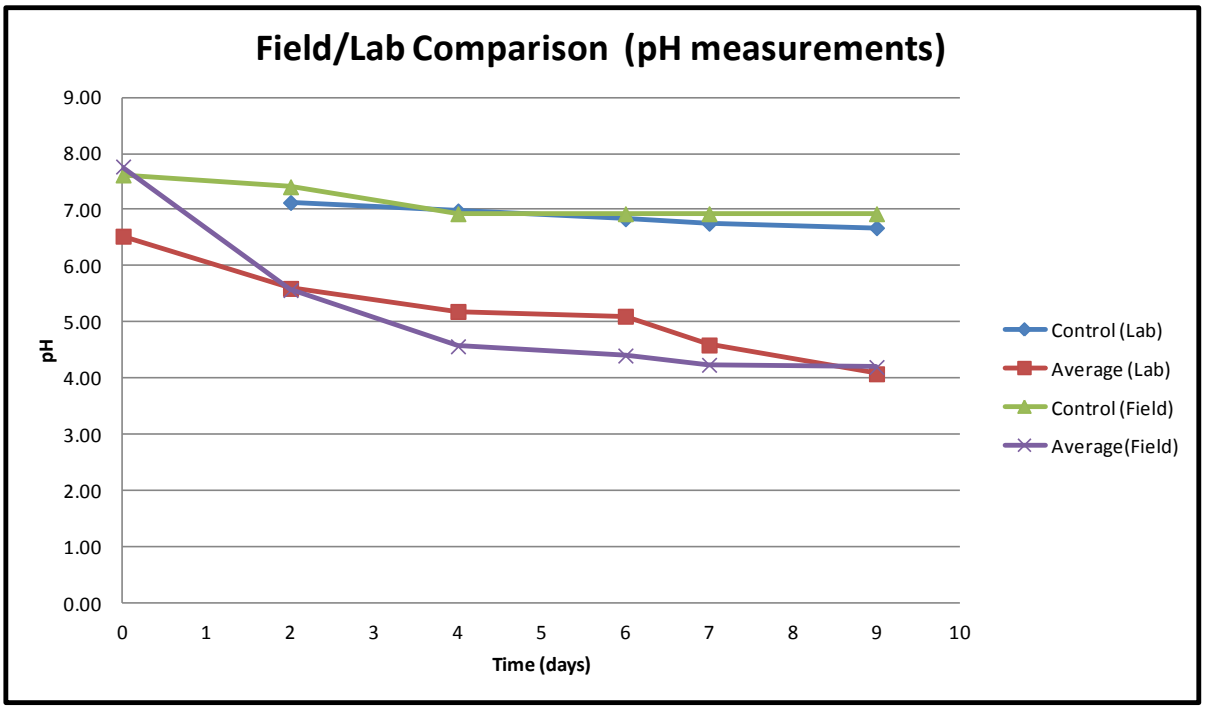

Figure 4.24 *pH measurements comparison - Laboratory/ field experiments*

However, the pH that is noted in the laboratory (see **Figure 4.24** above) remains above pH 5 for a longer period than is observed in the field. It is suggested that the alkalinity of the faecal sludge may contribute to this lag. This is because the alkalinity of the faecal sludge in the laboratory was more than twice that of the field faecal sludge, i.e. 10 and 22g/L respectively. Therefore this trend could be as a result of a higher buffering capacity.

4.5.4. Lactic Acid

Figure 4.25 below illustrates how the average lactic acid concentration varied over time in the treatment reactors and also in the control. All the samples from the treatment reactors exhibited similar trend in the concentration of lactic acid hence average values were adopted. It can be noted that the average lactic acid concentration increases very rapidly in the initial period of 0-4 days.
As from the 7th day onwards, the average lactic acid concentrations reaches and is maintained at values above 46,000mg/L.

Compared to the laboratory results, the rate of increase of the lactic acid concentration in the initial 2 days of the experiment is much higher. Also the maximum stable concentration noted in the field experiments of above 46,000mg/L is equally much higher than the maximum concentration of 40,900mg/L that was achievable in the laboratory scale experiments (see **Figure 4.26** below).
It is therefore suggested that again, the difference in alkalinity of the faecal sludge used in the laboratory and that used in the field could have contributed to the differences in trends outlined above.
The lactic acid concentration from the control reactor remains around its initial low value of between 60-80mg/L and does not increase over the entire treatment period. This further qualifies the suggestion that the production of lactic acid is solely as a result of the molasses and Inoculum added to the treatment reactors.

By the 9th day, which was the last experimental day (due to a limitation of time); the lactic acid concentration had increased almost 700 fold also.

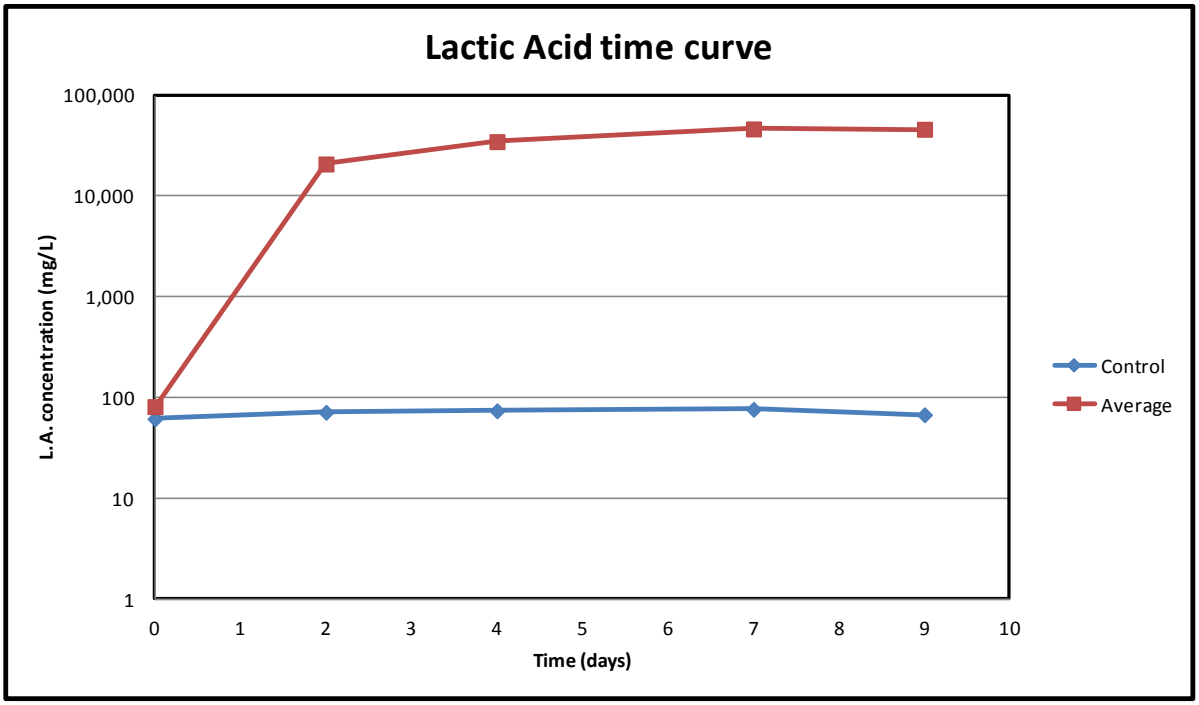

Figure 4.25 *Lactic acid concentration in up-scale field experiments*

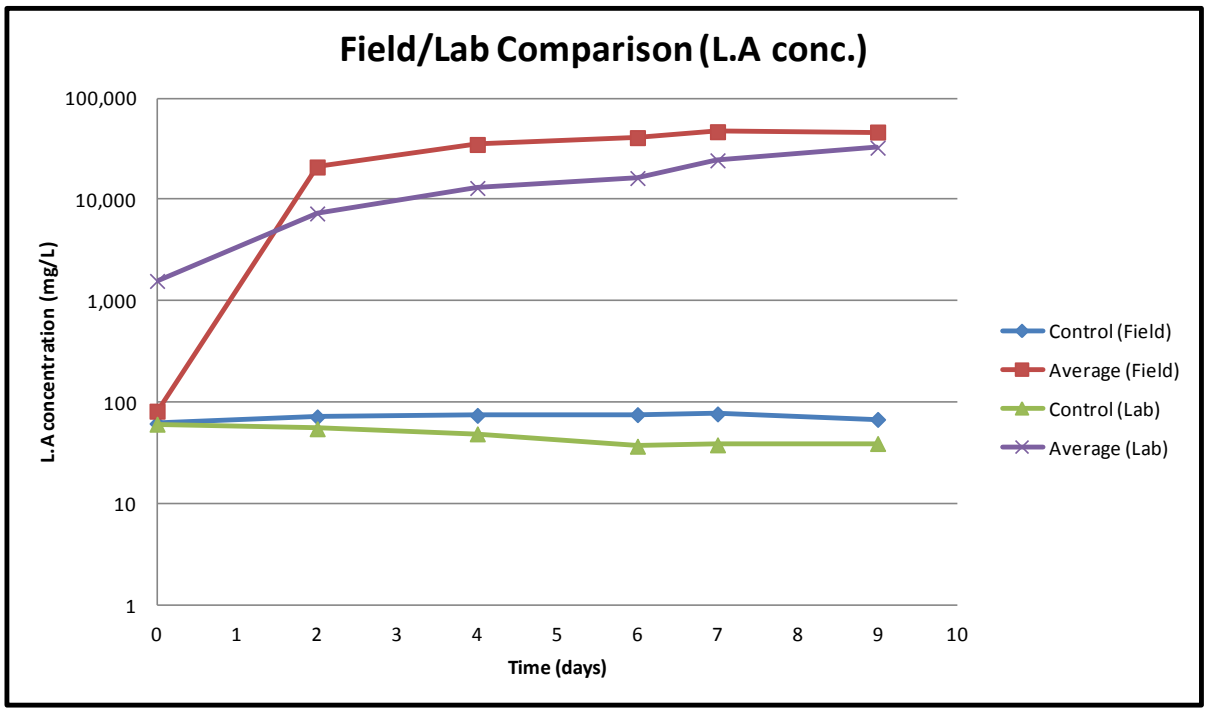

Figure 4.26 *Lactic acid concentration comparison - Laboratory/ field experiments*

4.5.5. Sanitization Effect - *E. coli* proliferation

Similar to the laboratory scale experiments, sanitization of the treatment process here also, is evaluated by considering the suppression of *E. coli,* the selected indicator organism. All the samples from the treatment reactors exhibited similar trend in the viable cell count (vcc) of *E. coli*. The average vcc of the *E. coli* bacteria from the treatment reactors is seen to increase during the initial 0-2 days of treatment experiment. This is attributed to an increase in food substrate made available in the faecal sludge by addition of molasses. Also, because the pH of the faecal sludge environment is still close to neutral during this period, no real threat is posed as yet to the *E. coli* due adversity of the environment. However, a decline in the vcc is seen to set in after the 3rd day of treatment.

It is suggested that this decline in the vcc of *E. coli* is due to the increase in lactic acid concentration and metabolites produced by the lactic acid bacteria. This line of thought is also supported by Schillinger, et al., (1996) and also Shirai et al., (2001).

However, the samples collected from the control reactor indicated no notable increase in the vcc of *E. coli* bacteria in the initial 0-2 days of the experiment. This observation qualifies the suggestion made earlier that the increase in vcc of the *E. coli* in the initial 0-2 days is as a result of the added food substrate.
Instead, a steady and gradual reduction in vcc of the *E. coli* is however noted in the control reactor over the entire period of the experiment. This gradual reduction can be attributed to natural die off.
As at the 7th day of the experiment, suppression of the *E. coli* to below detectable numbers is noted in all the treatment reactors. An average log reduction of more than 5 log units is registered, i.e. from $1.47x10^{8}$ CFU/100ml to $<1.0x10^{3}$CFU/100ml.
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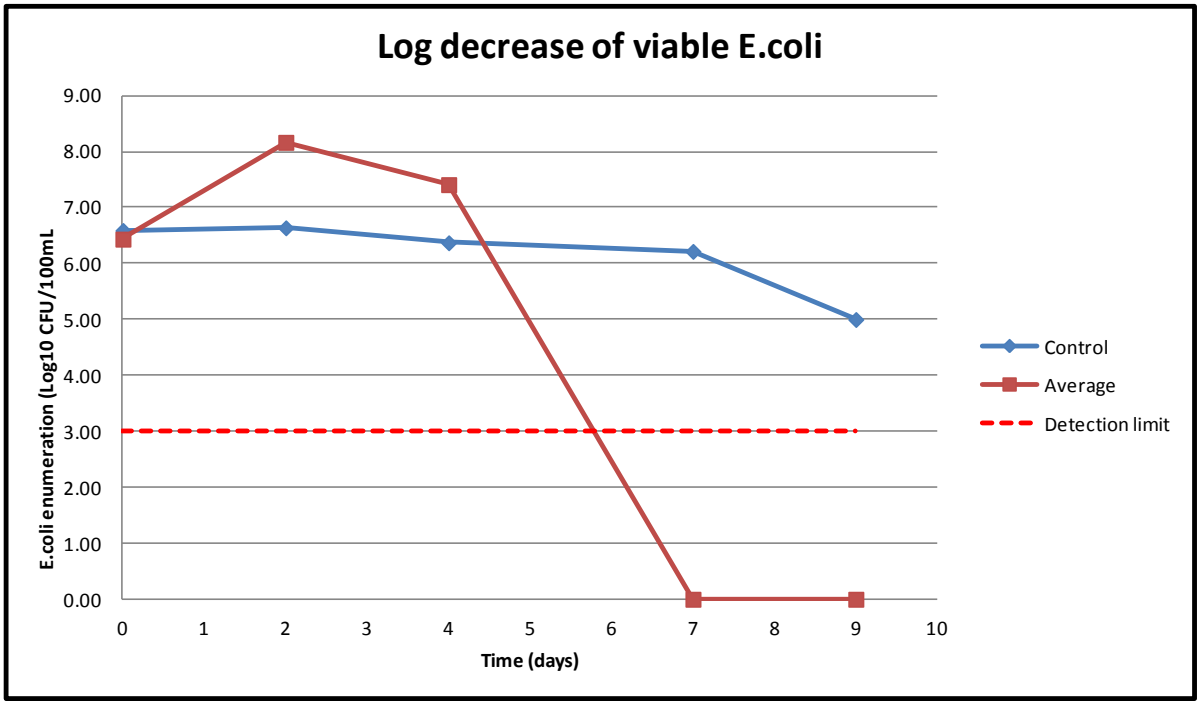

Figure 4.27 *E. coli suppression in up-scale field experiments*
(Note: Bacterial counts under the detectable limit are indicated as 0 log10)

Compared to the laboratory results, what particularly stands out is the sanitization period which appears shorter for the field experiments. It is suggested here also that due to the difference in alkalinity of the faecal sludge used in the laboratory and field investigations, the sanitization period is shorter for the lower alkaline faecal sludge. This is because of the shorter period it takes for the build up of lactic acid concentration, related metabolites and pH in the lower alkaline faecal sludge.

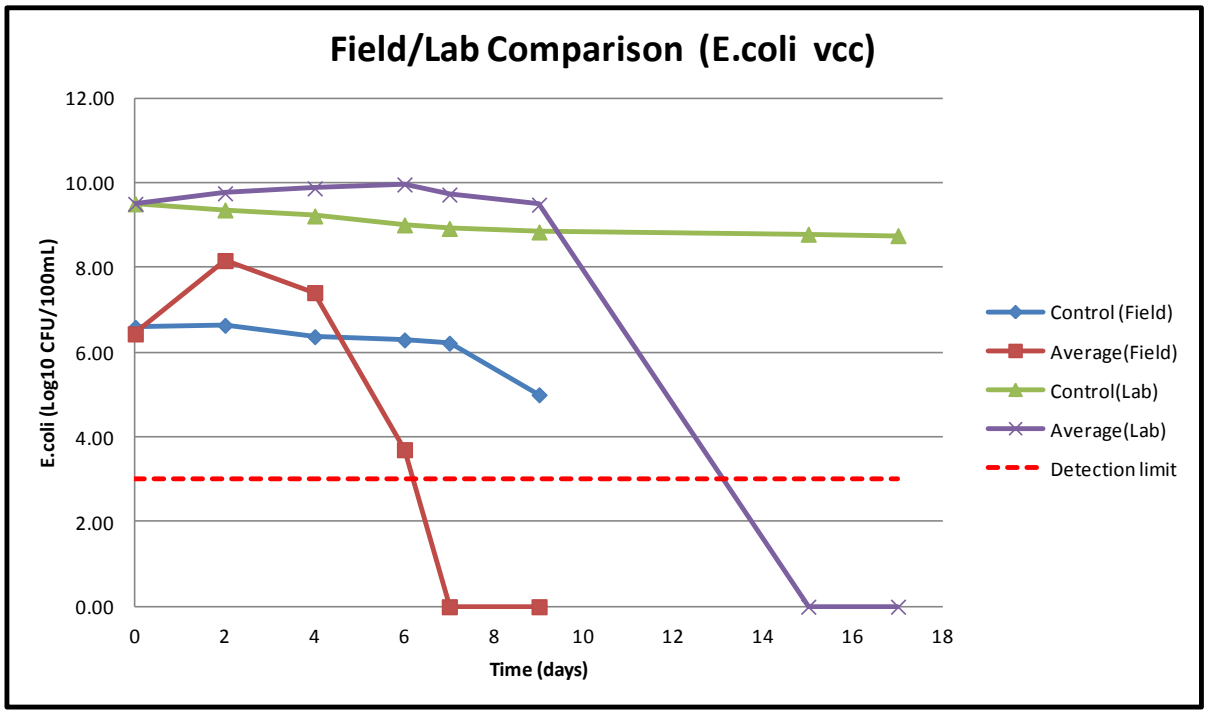

Figure 4.28 *E. coli suppression comparison field/Lab experiments*
(Note: Bacterial counts under the detectable limit are indicated as 0 log10)

Secondly, the treatment suppression rate is seemingly higher for the field experiments than for the laboratory experiments. This can be seen when the absolute values of the treatment suppression rate constant, K_{td}, are compared; i.e. a value of 1.14 and 1.64 for the laboratory and field experiments respectively (see **Figure 4.29** below).

Reasons as to why the field experiments exhibited a higher treatment suppression rate K_{td} may require further research into the interaction of microbial kinetics and environmental factors and conditions.

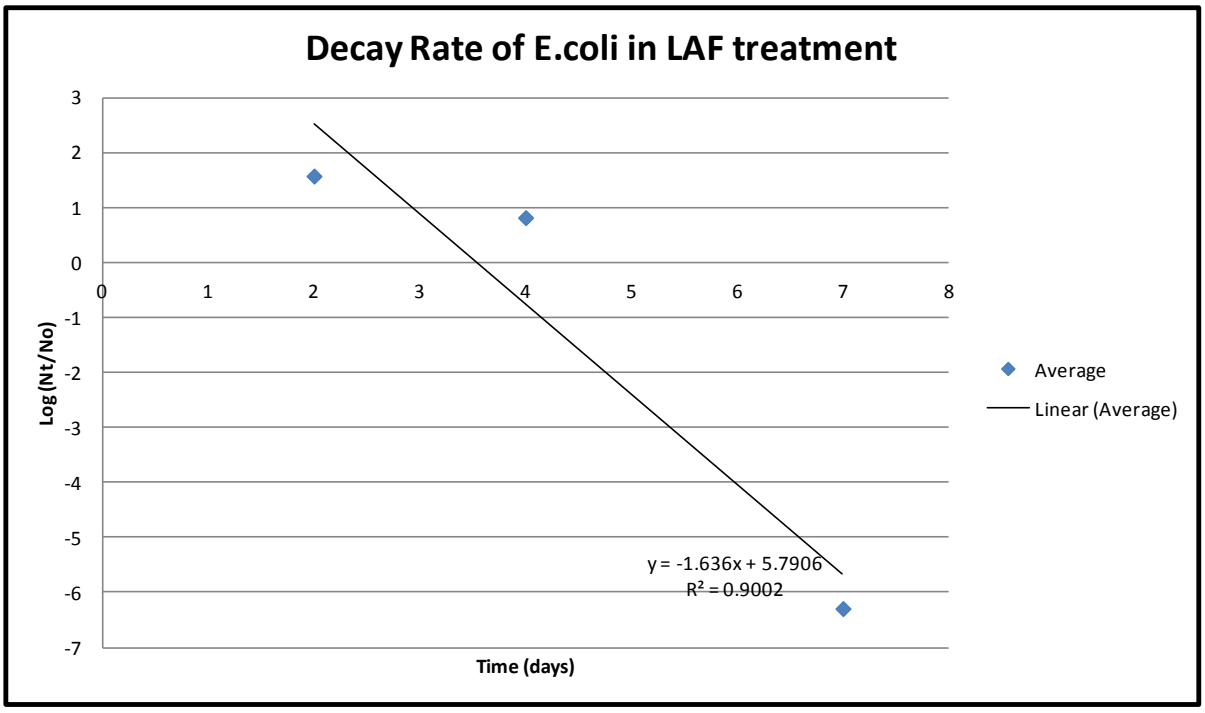

Figure 4.29 *Treatment decay rate for E. coli in up-scale field experiments*

4.5.6. Effect of treatment on Odor

An attempt was made to quantify the effects of the treatment process on odor by way of assigning an odor threshold number. During the laboratory investigations, it was observed that the lactic acid fermentation (LAF) process completely suppressed the odor from the faecal sludge and replaced it with a somewhat sour smell. However, this assessment was only qualitative, hence the need to carry out a quantitative analysis.

An odor panel of 5-6 people was assembled and method 2150B-Threshold odor test (Clesceri Greenberg, Arnold E., Trussell, R. Rhodes., American Public Health Association., American Water Works Association., Water Pollution Control Federation., 1989) was used to quantify the LAF treatments' effect on faecal sludge odor by way of assigning an odor threshold number. The principal behind this method was in determining the threshold odor number by diluting a sample with order free water until the least definite perceptive odor was achieved.

It should be mentioned however that, the procedure of this test was not so precise and reproducible and the results of the test were more or less subjective. Furthermore, a lot of data points were required in order to make the use of statistical tools to analyse the results possible. However, due to time limitations, it was not possible to collect enough data points for statistical analysis and also not enough time to correct the flaws that were noted (see Appendix A). It thus follows that it was not possible to draw concrete conclusions and assign specific threshold numbers to reflect the effect of the treatment on the odor of the faecal sludge.

4.5.7. Effect of treatment on Total Solids

Table **4.3** below indicates the total solids content of the faecal sludge after treatment. The results indicate that the treatment reactors had 3% more total solid content than the control reactor. This could be attributed to the build up of biomass in the faecal sludge as a result of the treatment. Further investigations into this phenomenon should be conducted to ascertain the variations in the biomass and also the growth patterns of the biomass.

Table 4.3 Total Solids concentration in control and treatment reactors after treatment

No	Parameter	Control reactor	Treatment reactors (av)	% change

1	Total Solids (%)	11	14	3

4.5.8. Synopsis of Up-scale field experiments (Treatment with Optimal Parameter experiment)

1) The Optimal parameter experiments conducted in the laboratory were proven to be up-scalable and the results thereof replicable.
2) Complete sanitization was achieved in 7 days, i.e. half the time it took at laboratory scale. Further research into the interaction of microbial kinetics and environmental factors/conditions may be required to explain this phenomenon but a difference in alkalinity of the faecal sludge's is suggested.
3) An *E. coli* log reduction of more than 5 log units was achieved in these field experiments in just a period of 7 days.
4) A higher lactic acid concentration of above 46g/L was also noted by the 7th day at field scale, well above the results obtained at laboratory scale; here also, a difference in alkalinity of the faecal sludge's is suggested.
5) A maximum of 49 g/L was achieved at field scale as opposed to a maximum of 41 g/L obtained at laboratory scale.
6) Similar to the results obtained from the lab experiments, the sanitization mechanism in the field was also triggered when the lactic acid concentration reached the range of 20 g/L to 30 g/L (see **Figure 4.30** below).

From the synopsis above it can be suggested that the alkalinity of the faecal sludge plays a role in the LAF process.

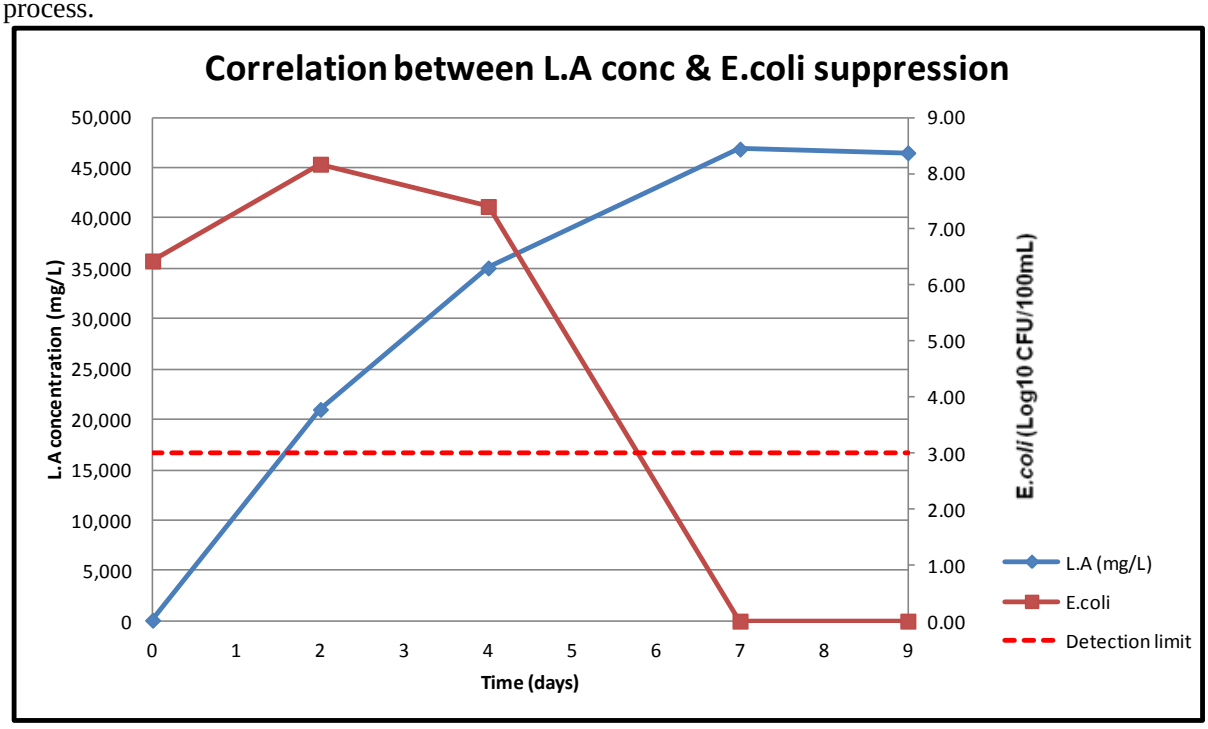

Figure 4.30 *Correlation between L.A conc. & E. coli suppression in up-scale field experiments*
(Note: Bacterial counts under the detectable limit are indicated as 0 log10)

4.6. Process costing for Treatment Method

The ultimate goal of this costing section is to give a broad but fair impression of what the process cost of treatment is likely to be using this treatment method which employs the use of lactic acid bacteria (LAB). This cost estimate only takes into account the operation cost (minus labour) without considering the capital or investments cost. This approach has been selected because it is simple and also because the scope of the research did not cover the aspect of faecal sludge collection and storage and hence assumes the prior step of collection has already been handled. Simple cost functions are made use of here without delving so much into more complex functions such as power functions, etc.

Table 4.4 Cost estimate for LAF treatment/m^3 of faecal sludge treated

No	Description	unit	Quantity	Unit Cost	Total Cost	Comment
A	**CAPITAL COSTS**					Not included
	N/A					
B	**OPEX**					
B1	**Preparation of pre-culture & sludge additives**					
	Milk requirements/m^3 of sludge	litres	100	0.3	€ 30.00	Assumed a one off cost (treated faecal sludge can be used as inoculum for successive treatments)
	Yakult required/m^3 of sludge	litres	0.2	6	€ 1.20	Assumed a one off cost (treated faecal sludge can be used as inoculum for successive treatments)
	Molasses required/m^3 of sludge	litres	100	0.02	€ 2.00	
	Subtotal of Item B1				**€ 33.20**	
B2	**Energy requirements**					
	Mixing/m^3 of sludge	KWh/ m^3				
C	**RESULTING COST**					
	Total Initial Cost/m^3				**€ 33.20**	
	Running Cost/m^3 (minus Yakult & milk cost)				**€ 2.00**	

Table **4.4** above indicates an initial treatment process cost of €33/m^3. This cost includes the cost of the rich starter milk pre-culture which represents 94% of the total treatment process cost per m^3. However, it is expected that once the initial inoculation has been made using the rich starter milk pre-culture, treated faecal sludge rich in lactic acid bacteria can be used for subsequent inoculations. It is recommended however, that investigations into the use of treated faecal sludge, rich in lactic acid bacteria (LAB), be conducted to test this hypothesis.

It thus follows that the running treatment process cost of €2/m^3 is thus taken as the long term treatment cost of faecal sludge using LAB.

4.7. Comparison with Ammonia and lime treatment

Research into two other faecal sludge treatment options were conducted in parallel with the lactic acid bacteria treatment research, i.e. treatment with quick lime and treatment with ammonia. Table **4.5** below summarizes the results of the 3 treatment options.
The obvious advantage of the lactic acid bacteria treatment option is that because it is a biological treatment; it has an economic advantage both in terms of capital cost and operation cost. A snapshot of the operational costs of the treatment has been given in the previous section. It should be stated that there still exists potential to reduce these operational costs even further. Biological methods aimed at increasing the simple sugars content of the molasses by way of hydrolysis of the sucrose present are open for exploitation. This would entail a substantial reduction in the molasses requirements of the treatment process.

Another advantage of the lactic acid bacteria treatment option is that the products of the fermentation process are non toxic. It thus follows that disposing of the treated faecal sludge, that contains LAB and substantial amounts of lactic acid would not pose an immediate danger to the environment. The U.S. Environmental Protection Agency has passed lactic acid as non toxic to humans and the environment (United States. Biopesticides and Pollution Prevention Division., 2009). However, further investigation into the intense use of lactic acid treated faecal sludges in agriculture need to be made.

Also, it is feasible to integrate the lactic acid bacteria treatment technology into the existing sanitation technological options currently being applied in emergency situation. This is advantageous in that there is less need to design new technological options (hardware) to target the integration of the LAB treatment technology aimed at enhancing the sanitization of the faecal sludge. This is as opposed to treatments such as ammonia treatment that would require the design of air tight compartments that would minimise the escape of ammonia during treatment.

The major disadvantage of the lactic acid treatment technology is the need for a sugar source to promote the fermentation process. The cost of molasses has been noted as the major cost driver of the simple costing done in Section 4.6 above.

Table 4.5 Comparison with ammonia and lime treatment

No	Attribute	LAB treatment	NH_4 treatment	Lime treatment
1	Sanitization time	7-15 days	8 days	5-120 minutes
2	*E. coli* log removal	6-7 (below detectable limits)	6-7 (below detectable limits)	6-7 (below detectable limits)
3	Odor suppression	Yes (sour smell)	Smell of ammonia	Yes
4	End pH	3.8-4.2	9	12.4
5	Effect on environment	Non toxic	corrosive	Non toxic
6	Effect on ground water	Non contaminant	Non contaminant	Non contaminant
7	Energy requirements	10 kW/L	?	?
8	Sludge disposal after treatment	Drying bed	Fertilizer/drying bed	Sanitary landfill/drying beds
9	Re-use of FS	Yes-Agriculture	Yes-High in N	No
10	Chemical use	Sugar additive required	Urea	Quick and hydrated lime
11	Technology	Biological treatment	Chemical treatment	Chemical treatment
12	Treatment cost	€ $2/m^3$	?	?
13	Problems/shortfalls	Temperature dependant (30-40 °C optimum)	Homogenous mixing required	Homogenous mixing required
14	O&M	Minimal	Minimal	Minimal
15	Robustness of technology	Yes (mixing required)	Yes	Yes
16	Integration with existing emergency technical option	Yes - (Highly recommended)	Yes - care must be taken (NH_4 is toxic)	Yes

CHAPTER 5

Conclusion and Recommendations

This chapter seeks to draw conclusions to this research. It further seeks to answer the questions the research undertook to explore and relate these answers to the objectives undertaken by the research. Recommendations are made as with regards to the way forward of the technology and further prospects of study.

5.1. Conclusion

5.1.1. Sanitization of faecal sludge using lactic acid bacteria

The research undertook to establish the feasibility of sanitizing faecal sludge using a strain of lactic acid bacteria (*Lactobacillus casei* Shirota) found in the probiotic milk product, Yakult; and establish whether the treatment could be applied in emergency situations. The research has concluded that the treatment of faecal sludge using the *Lactobacillus casei* Shirota strain is feasible. Its application as a faecal sludge treatment option can be exploited and its use can be extended to emergency situations.

5.1.2. Lactic acid bacteria sanitization in emergency context

Sanitizing faecal sludge using lactic acid bacteria can be applied at all the stages of an emergency situation. For example, in the 1st Phase of an emergency, the field manual for excreta disposal in emergencies prescribes as a first option for excreta disposal, installation of trench latrines were rapid response is required and limited space is available for shallow trenches (Harvey Bastable, Andy.,, 2007). However, the constraint in the application of trench latrines has been that they are unsuitable in places where the water table is high, in rocky or unstable soils, often create odor problems and usually associated with poor cleaning and maintenance. From the results of this research, it is suggested that most of these constraints can be resolved by treating the faecal sludge using lactic acid bacteria. It has been observed that this treatment process has excellent sanitizing capabilities and also eliminates odors from the faecal sludge. Thus its use can minimize the risk to pollution of ground water in situations where the ground water table is high and also can be a solution to the odor problem associated with trench latrines.

In the 2nd phase of an emergency, sanitizing faecal sludge using lactic acid bacteria can easily be integrated into the existing technical options prescribed by the field manual for excreta disposal in emergencies (Harvey Bastable, Andy.,, 2007). And because of its relatively low running cost, the technique can be quite attractive considering the benefits that come along with it.

Technical options prescribed for use during the 2nd phase of emergencies such as simple latrines, ventilated improved pit (VIP) latrines, ecological sanitation (Eco-San) options such as terra preta which already explores this option, can all integrate lactic acid bacteria sanitization to enhance performance of the respective technology options. Problems associated with the risk to pollution of ground water and problems to do with odor can all be minimised by integrating this technology. It can further be integrated with newer technological options being developed such as the "raised latrines" being developed under the WASTE

S(P)EEDKITS project to reduce odors and pathological risks to persons operating the facilities (i.e. persons handling the emptying of the facilities).

In relation to the Sphere minimum standards, sanitizing faecal sludge using lactic acid bacteria fits in well into complimenting standards 1 & 2 whose guidelines among others, stipulate the need to ensure the environment is free from contamination by human faeces and also the need to maintaining and cleaning of the toilet facilities (Sphere Project. 2003). The technology complements these standards in that the use of lactic acid for sanitizing faecal sludge poses limited or no risk to the environment. This is according to the environmental assessment of lactic acid by the U.S Environmental Protection Agency, (United States. Biopesticides and Pollution Prevention Division., 2009) that established that l-lactic acid satisfied all non-target toxicology data requirements and as such considered to be non toxic to the environment. Also since the technology sanitizes the faecal sludge, risk to persons maintaining and cleaning the toilet facilities is reduced.

5.1.3. Aspects about the technology

The following specific aspects about this treatment technology were established in accordance to the set out objectives.

Starter culture

It was established that a milk media was more suitable for use in preparation of the starter culture. This was because of the rapid proliferation of the lactic acid bacteria (LAB) that was noted in milk. A molasses-tap water media can also be used for preparation of the starter culture although proliferation of the LAB was less satisfactory. A 0.2% Yakult concentration in milk was established as the optimal ratio of Yakult to milk on preparation of the starter culture. A period of 48hours was required for the LAB to attain maximum growth and render the starter culture ready for inoculation. 10% w/w concentration of starter culture to faecal sludge was selected as the optimal inoculum concentration. Nonetheless, lesser concentration of 1% equally produced satisfactory results.

It is suggested that LAB treated faecal sludge can be used to inoculate subsequent treatments. This is because of the high concentration of LAB that is observed at complete sanitization. Further investigations into this hypothesis need to be conducted however, as this alone would greatly reduce the cost of applying the technology and improve on the prospects of its applicability.

Sugar Additive

An optimal molasses concentration in faecal sludge of 10% w/w was established that ensures the lactic acid fermentation (LAF) process proceeds optimally. This 10% w/w molasses concentration, translated to be in the range of 1.5-2.0g/L total sugar (glucose & fructose) concentration. This goes to say, other simple sugar sources such as fruit waste can be used as long as the target concentration of 1.5-2.0g/L simple sugars in faecal sludge is attained to guarantee an optimal LAF process.

5.2. Recommendations

The following recommendations are made:

Way forward

Investigations into the use of LAB treated faecal sludge as inoculum for subsequent treatments should be conducted. This is because of the high concentration of LAB that is observed at complete sanitization that can be exploited. Furthermore, this is particularly important because it has the potential of substantially reducing the process cost of treatment from €33/m^3 to €2/m^3 of faecal sludge.

Further investigations into the microbial kinetics of LAB and how they are affected by external environmental factors should equally be conducted. This will enable us to understand how external factors such as alkalinity of the faecal sludge affect the LAF process thereby opening up prospects of further improvements to the process.

A few studies have been conducted to investigate the efficiency of different strains of LAB on the LAF process; it would particularly be interesting to conduct similar investigations to explore the efficiency of the different LAB strains such as those found in Sauerkraut (sour cabbage) and other readily available food sources.

Further investigations into stabilization of the faecal sludge after this treatment should equally be conducted. The COD, Nitrogen and Phosphorous contents should be investigated so as to pave way for the potential re-use of the treated faecal sludge in agriculture both as a nutrient source and also as soil nourishment.

Lastly, it should be emphasized that these further investigations into the use of lactic acid bacteria in sanitizing faecal sludge which would eventually lead into the integration of this technology into the current technological options in emergencies is highly recommended.

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Appendices

Appendix A Threshold odor number (TON)

Table 5.1 List of SWAT input parameters

DATE	PANELIST	C				M1				M2				M3			
3/2/2014	**Odor threshold number**	**256**	**32**	**64**	**128**	**128**	**256**	**32**	**64**	**4096**	**512**	**1024**	**2048**	**2048**	**4096**	**512**	**1024**
	Happiness Nobela (Researcher)					−	+	+	+	+	+	+	±	+	−	+	+
	Elliette González Pérez (Researcher)																
	Steven (Lab technician)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Simenti (Driver)	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+
	Rashid (Lab technician)	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
11/2/2014	**Odor threshold number**	**4096**	**1024**	**2048**	**512**	**512**	**1024**	**2048**	**4096**	**1024**	**2048**	**512**	**4096**	**512**	**2048**	**4096**	**1024**
	Happiness Nobela (Researcher)									+	+	+	+	+	+	+	+
	Elliette González Pérez (Researcher)	±	+	+	+	+	+	+	+	+	+	+	±	+	−	+	−
	Steven (Lab technician)									−	+	−	+	+	+	+	+
	Simenti (Driver)								−	+	+	+	−	+	−	+	+
	Rashid (Lab technician)									+	+	+	−	+	−	+	+
	Bizi (Lab technician)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Appendix B Data Tables & Graphs

Appendix B1: Alkalinity test.

vol	pH	vol	pH	vol	pH	vol	pH	vol	pH	vol	pH	vol	pH	vol	pH	vol	pH
0.0	8.21	0.8	7.13	1.6	6.55	3.0	6.21	4.6	5.95	7.5	5.71	11.5	5.10	15.5	4.61	19.5	4.27
0.1	8.07	0.9	7.04	1.7	6.55	3.2	6.17	4.8	5.93	8.0	5.61	12.0	5.00	16.0	4.62	20.0	4.21
0.2	7.97	1.0	6.93	1.8	6.52	3.4	6.13	5.0	5.89	8.5	5.47	12.5	4.92	16.5	4.63	20.5	4.26
0.3	7.84	1.1	6.85	1.9	6.46	3.6	6.10	5.2	5.85	9.0	5.43	13.0	4.85	17.0	4.58	21.0	4.15
0.4	7.71	1.2	6.80	2.2	6.39	3.8	6.06	5.6	5.82	9.5	5.40	13.5	4.79	17.5	4.54	21.5	4.04
0.5	7.59	1.3	6.75	2.4	6.34	4.0	6.04	6.0	5.74	10.0	5.20	14.0	4.72	18.0	4.51	22.0	3.91
0.6	7.44	1.4	6.68	2.6	6.29	4.2	6.02	6.5	5.73	10.5	5.32	14.5	4.67	18.5	4.44	22.5	3.77
0.7	7.26	1.5	6.61	2.8	6.24	4.4	5.96	7.0	5.63	11.0	5.21	15.0	4.64	19.0	4.37	23.0	3.65

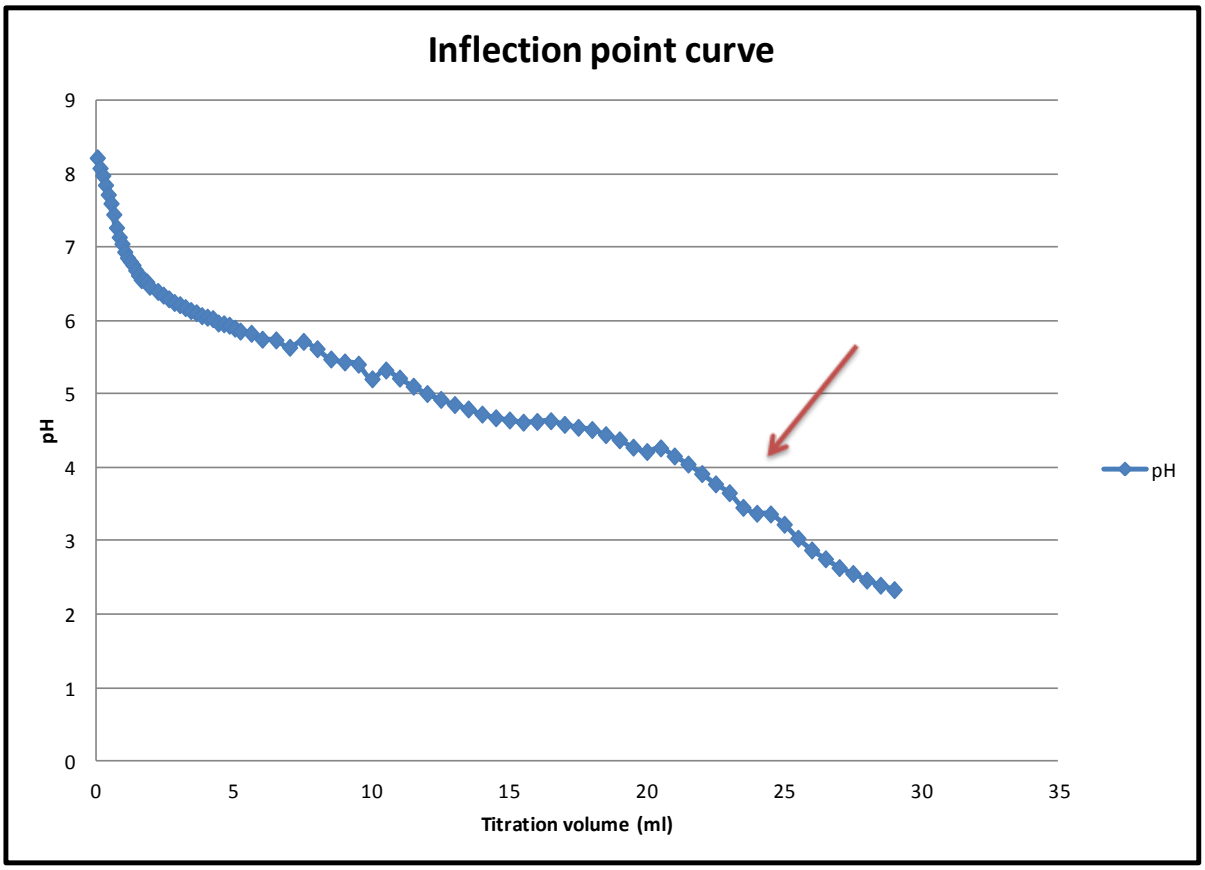

Figure 5.1 *Inflection point graph*

Alkalinity as mg/L HCO $= \frac{V*N*1000}{mL\ sample} *61$

$\ldots = \frac{11.2*0.2*1000}{4.88} * 61$

…

….=28,000 mg/L HCO3≡ 22,960 mg/L CaCO3

Alkalinity as mg/L HCO $= \frac{V*N*1000}{mL\ sample} *61$

$\ldots = \frac{11.2*0.2*1000}{4.88} * 61$

…

….=28,000 mg/L HCO3≡ 22,960 mg/L CaCO3

Appendix B2: Natural die off of *Escherichia coli.*

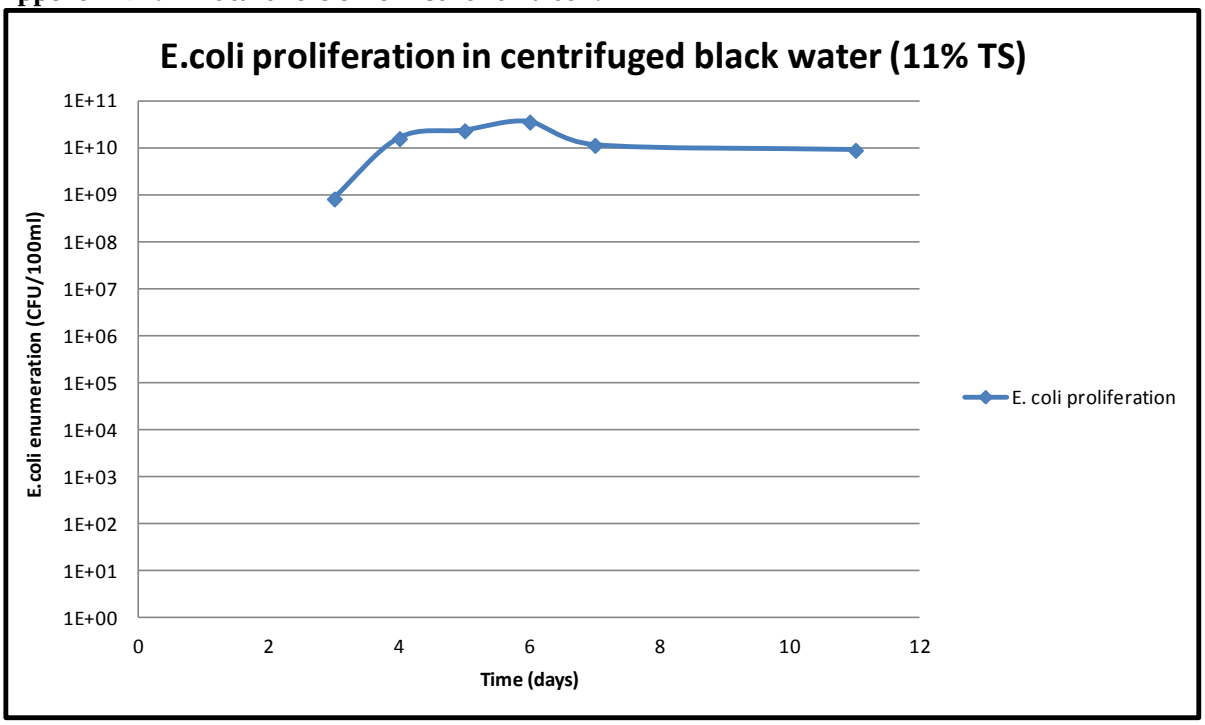

Figure 5.2 *E. coli proliferation depicting natural die off*

***Escherichia coli* Natural die off constant K_d.-0.08**

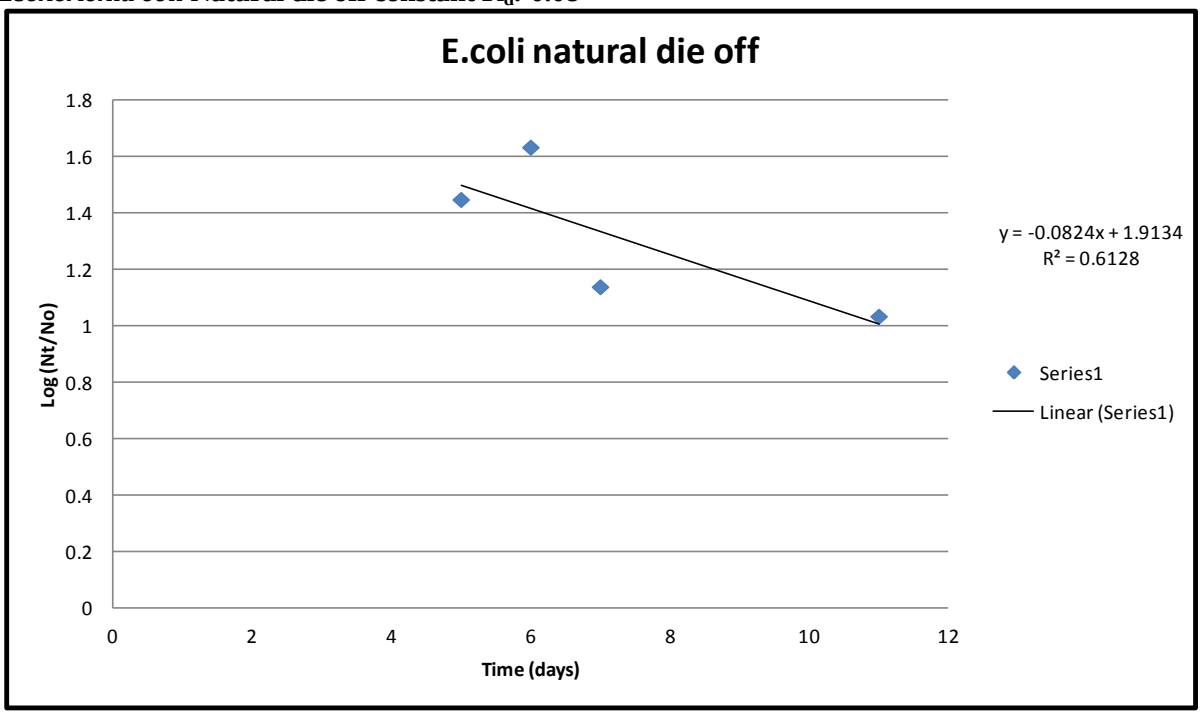

Figure 5.3 *Natural die of constant K_d*

Appendix B3: Summery of Lactic acid bacteria (LAB) proliferation in milk

Days	**Control**	**0.1% Yakult**	**0.2% Yakult**	**0.5% Yakult**	**1.0% Yakult**
1	0.00E+00	9.25E+07	1.50E+08	2.70E+08	5.05E+08
5	0.00E+00	3.65E+11	3.85E+11	3.30E+11	3.15E+11
6	0.00E+00	2.25E+11	4.85E+11	2.45E+11	2.95E+11
Log Scale					
Days	**Control**	**0.1% Yakult**	**0.2% Yakult**	**0.5% Yakult**	**1.0% Yakult**
1	0.00E+00	7.97E+00	8.18E+00	8.43E+00	8.70E+00
5	0.00E+00	1.16E+01	1.16E+01	1.15E+01	1.15E+01
6	0.00E+00	1.14E+01	1.17E+01	1.14E+01	1.15E+01

Summery of Lactic acid bacteria (LAB) proliferation in molasses added tap water

Days	**Control**	**0.1% Molasses**	**0.5% Molasses**	**1.0% Molasses**	**2.0% Molasses**
0	0.00E+00	2.38E+07	2.64E+07	2.82E+07	2.71E+07
1	0.00E+00	0.00E+00	3.50E+06	1.50E+06	4.00E+06
2	0.00E+00	0.00E+00	0.00E+00	0.00E+00	3.00E+06
3	0.00E+00	8.50E+04	2.50E+03	5.05E+05	8.00E+07
6	0.00E+00	0.00E+00	6.55E+04	4.65E+04	6.05E+08
7	0.00E+00	0.00E+00	5.00E+03	6.50E+04	6.10E+08
Log scale					
Days	**Control**	**0.1% Molasses**	**0.5% Molasses**	**1.0% Molasses**	**2.0% Molasses**
0	0.00	7.38	7.42	7.45	7.43
1	0.00	0.00	6.54	6.18	6.60
2	0.00	0.00	0.00	0.00	6.48
3	0.00	4.93	3.40	5.70	7.90
6	0.00	0.00	4.82	4.67	8.78
7	0.00	0.00	3.70	4.81	8.79

Summary of LAB proliferation in more concentrated molasses added tap water

Days	**Control**	**1% Molasses**	**10% Molasses**	**20% Molasses**	**40% Molasses**
4	#DIV/0!	#DIV/0!	1.11E+08	1.33E+08	7.65E+07
11	5.45E+04	2.25E+09	1.30E+09	6.50E+08	5.04E+08
Log scale					
Days	**Control**	**1% Molasses**	**10% Molasses**	**20% Molasses**	**40% Molasses**
4			8.04	8.12	7.88
11	4.74	9.35	9.11	8.81	8.70

Appendix B4 - *E. coli* suppression, LAB proliferation, pH and Lactic acid concentration

Plate count data for LAB proliferation - Batch Test 1 - Optimal sugar concentration experiment

Lactobacillus

Date			Plate: 12/12/2013		Count: 13/12/2013			Plate: 13/12/2013		Count:16/12/2013			Plate: 16/12/2013		Count: 24/12/2013			Plate: 18/12/2013		Count: 24/12/2013			Plate: 20/12/13		Count: 03/01/2014			Plate: 24/12/13		Count: 03/01/2014			Plate: 30/12/13		Count: 03/01/2014		
Days			0					1					4					6					8					12					18				
Sample	Details of sample		Count/plat	Av. Count	Dilution (1:	Enumerati	Av. Enume	Count/plat	Av. Count	Dilution (1:	Enumerati	Av. Enume	Count/pla	Av. Count	Dilution (1:	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:r	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:	Enumeration/100ml		Count/pla	Av. Count	Dilution (1:r	Enumeration/100ml		Count/pla	Av. Count	Dilution (1:r	Enumeration/100ml	
Control 0%	Molasses addi	0.00	185	182	100,000	1.82E+10	1.63E+10	112	91	100,000	9.05E+09	9.40E+09	91	83	100,000	8.30E+09	9.25E+09	29	30	1,000,000	3.00E+10	4.23E+10	Too many	#DIV/0!	100,000	N/A	#DIV/0!	0	0	10,000,000	N/A	#DIV/0!	1	1	1,000,000	5.00E+08	2.50E+08
	Faecal sludge	30.00	179					69					75					31					Too many					0					0				
Control 0%B	Molasses addi	0.00	148	144	100,000	1.44E+10		102	98	100,000	9.75E+09		90	102	100,000	1.02E+10		63	55	1,000,000	5.45E+10			#DIV/0!	100,000	N/A		0	0	10,000,000	N/A		0	0	1,000,000	0.00E+00	
	Faecal sludge	30.00	140					93					114					46										0					0				
Molasses 5%	Molasses addi	1.50	98	116	100,000	1.16E+10	1.25E+10	50	44	100,000	4.35E+09	5.70E+09	42	50	100,000	5.00E+09	8.15E+09	184	184	10,000,000	1.84E+12	1.58E+12	Too many	#DIV/0!	1,000,000	N/A	#DIV/0!	18	14	10,000,000	1.35E+11	1.20E+11	76	79	1,000,000	7.90E+10	7.73E+10
	Faecal sludge	30.00	133					37					58										Too many					9					82				
Molasses 5%B	Molasses addi	1.50	142	135	100,000	1.35E+10		61	71	100,000	7.05E+09		126	113	100,000	1.13E+10		178	132	10,000,000	1.32E+12		Too many	#DIV/0!	1,000,000	N/A		11	11	10,000,000	1.05E+11		90	76	1,000,000	7.55E+10	
	Faecal sludge	30.00	128					80					100					86					Too many					10					61				
Molasses 10%	Molasses addi	3.00	71	67	100,000	6.70E+09	7.75E+09	35	36	100,000	3.55E+09	4.55E+09	178	173	1,000,000	1.73E+11	1.73E+11	clustered	#DIV/0!	10,000,000	#DIV/0!	#DIV/0!	246	246	10,000,000	2.46E+12	1.69E+12	49	51	10,000,000	5.10E+11	6.90E+11	18	18	10,000,000	1.80E+11	2.20E+11
	Faecal sludge	30.00	63					36					167					clustered										53					18				
Molasses 10%B	Molasses addi	3.00	90	88	100,000	8.80E+09		60	56	100,000	5.55E+09		fungi grow	#DIV/0!	1,000,000	#DIV/0!		clustered	#DIV/0!	10,000,000	#DIV/0!		80	92	10,000,000	9.15E+11		75	87	10,000,000	8.70E+11		26	26	10,000,000	2.60E+11	
	Faecal sludge	30.00	86					51					fungi grow					clustered					103					99					26				
Molasses 20%	Molasses addi	6.00	107	97	100,000	9.65E+09	9.65E+09	23	27	100,000	2.65E+09	5.53E+09	126	126	1,000,000	1.26E+11	1.26E+11	clustered	#DIV/0!	10,000,000	#DIV/0!	1.94E+12	171	171	10,000,000	1.71E+12	1.53E+12	2	2	10,000,000	2.00E+10	9.75E+10	0	0	10,000,000	N/A	#DIV/0!
	Faecal sludge	30.00	86					30					fungi grow					clustered					170					2					0				
Molasses 20%B	Molasses addi	6.00		#DIV/0!	100,000	#DIV/0!		81	84	100,000	8.40E+09		fungi grow	#DIV/0!	1,000,000	#DIV/0!		194	194	10,000,000	1.94E+12		145	136	10,000,000	1.36E+12		18	18	10,000,000	1.75E+11		0	0	10,000,000	N/A	
	Faecal sludge	30.00						87					fungi grow					clustered					126					17					0				
No Inoculum	Molasses addi	0.00		#DIV/0!	100,000	#DIV/0!		8	5	100	5.00E+05	5.00E+05	0	0	1,000,000	N/A	N/A	0	0	1,000,000	N/A	N/A	No data	#DIV/0!		N/A		6	6	10	6.00E+04	6.00E+04	9	8	100	7.50E+05	7.50E+05
	Faecal sludge	30.00						2					0					0					No data					6					6				

Summary of LAB proliferation - Batch Test 1 - Optimal sugar concentration experiment

Time (days)	Control (CFU/100ml)	Molasses5% (CFU/100ml)	Molasses 10% (CFU/100ml)	Molasses 20% (CFU/100ml)	No Inoculum (CFU/100ml)
0	1.63E+10	1.25E+10	7.75E+09	9.65E+09	0.00E+00
1	9.40E+09	5.70E+09	4.55E+09	5.53E+09	5.00E+05
4	9.25E+09	8.15E+09	1.73E+11	1.26E+11	
6	4.23E+10	1.58E+12	1.10E+12	1.94E+12	
8	2.15E+10	9.16E+11	1.69E+12	1.53E+12	
12	1.90E+10	1.20E+11	6.90E+11	9.75E+10	6.00E+04
18	2.50E+08	7.73E+10	2.20E+11		7.50E+05

Date: 12/12/2013
Sample: Treatment 1 - Sugar additive concentration experiments
Pre-culture addition (w/w): 10% and 0% in sample E
Pre-culture age: 1 day
Molasses addition (w/w): 0, 5%, 10% & 20%

Plate count data for *E. coli* suppression - Batch Test 1 - Optimal Sugar concentration experiment

Date			Plate: 12/12/2013		Count: 13/12/2013			Plate: 13/12/2014		Count:16/12/2014			Plate: 16/12/2015		Count: 17/12/2015			Plate: 18/12/2015		Count: 19/12/2015			Plate: 20/12/2015		Count: 27/12/2015			Plate: 24/12/2015		Count: 27/12/2015			Plate: 30/12/2015		Count:31/12/2015		
Days			0					1					4					6					8					12					18				
Sample	Details of sample		Count/plat	Av. Count	Dilution (1:	Enumerati	Av. Enume	Count/plat	Av. Count	Dilution (1:	Enumerati	Av. Enume	Count/pla	Av. Count	Dilution (1:	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:r	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:	Enumeration/100ml		Count/pla	Av. Count	Dilution (1:r	Enumeration/100ml		Count/pla	Av. Count	Dilution (1:r	Enumeration/100ml	
Control 0%	Molasses addi	0.00	89	88	100,000	8.75E+09	8.85E+09	323	355	100,000	3.55E+10	3.55E+10	473	473	100,000	4.73E+10	4.98E+10	95	95	10,000,000	9.45E+11		1	1	10,000,000	1.00E+10		101	104	100,000	1.04E+10		No data	#DIV/0!	100,000	#DIV/0!	
	Faecal sludge	30.00	86					387										94					1					107					No data				
Control 0%B	Molasses addi	0.00	81	90	100,000	8.95E+09		Too much	#DIV/0!	100,000	#DIV/0!		525	522	100,000	5.22E+10		147	136	10,000,000	1.36E+12			#DIV/0!	10,000,000	#DIV/0!		67	57	10,000	5.70E+08		No data	#DIV/0!	10,000	#DIV/0!	
	Faecal sludge	30.00	98					Too much					519					124										47					No data				
Molasses 5%	Molasses addi	1.50	42	36	100,000	3.60E+09	3.63E+09	153	153	100,000	1.53E+10	1.09E+10	205	201	100,000	2.01E+10	1.82E+10	36	36	10,000,000	3.55E+11		58	59	1,000,000	5.90E+10		130	130	100,000	1.30E+10		98	95	10,000	9.45E+08	
	Faecal sludge	30.00	30					153					196					35					60					130					91				
Molasses 5%B	Molasses addi	1.50	31	37	100,000	3.65E+09		69	66	100,000	6.55E+09		167	163	100,000	1.63E+10		33	37	10,000,000	3.65E+11		60	61	1,000,000	6.10E+10		143	144	100,000	1.44E+10		15	14	10,000	1.40E+08	
	Faecal sludge	30.00	42					62					158					40					62					144					13				
Molasses 10%	Molasses addi	3.00	62	57	100,000	5.70E+09	4.80E+09	20	21	100,000	2.05E+09	5.53E+09	11	12	100,000	1.15E+09	1.28E+10	1	1	1,000,000	1.00E+09		0	0	1,000	0.00E+00		0	0	10	0.00E+00		0	0	10	0.00E+00	
	Faecal sludge	30.00	52					21					12					1					0					0					0				
Molasses 10%B	Molasses addi	3.00	41	39	100,000	3.90E+09		95	90	100,000	9.00E+09		228	244	100,000	2.44E+10		89	86	1,000,000	8.60E+10		250	270	10,000	2.70E+09		Too many	#DIV/0!	10,000	#DIV/0!		105	117	100,000	1.17E+10	
	Faecal sludge	30.00	37					85					259					83					289					Too many					128				
Molasses 20%	Molasses addi	6.00	32	32	100,000	3.15E+09	2.75E+09	69	53	100,000	5.25E+09	4.48E+09	64	59	100,000	5.90E+09	5.90E+09	37	31	1,000,000	3.05E+10		4	3	10,000	2.50E+07		0	0	10	0.00E+00		0	0	10	0.00E+00	
	Faecal sludge	30.00	31					36					54					24					1					0					0				
Molasses 20%B	Molasses addi	6.00	18	24	100,000	2.35E+09		40	37	100,000	3.70E+09		Too many	#DIV/0!	100,000	#DIV/0!		52	53	1,000,000	5.30E+10		1	1	10,000	5.00E+06		47	52	10	5.20E+05		60	61	10	6.05E+05	
	Faecal sludge	30.00	29					34					Too many					54					0					57					61				
No Inoculum	Molasses addi	0.00	34	35	100,000	3.45E+09	3.45E+09	28	28	100,000	2.80E+09	2.80E+09		#DIV/0!	100,000	#DIV/0!		1	1	10,000,000	5.00E+09			#DIV/0!	3	#DIV/0!			#DIV/0!	3	#DIV/0!		0	0	100,000	0.00E+00	
	Faecal sludge	30.00	35					28										0															0				

Summary of *E. coli* suppression - Batch Test 1 - Optimal sugar concentration experiment

Days	Control 0% (CFU/100ml)	Control 0%B (CFU/100ml)	Molasses 5% (CFU/100ml)	Molasses 5%B (CFU/100ml)	Molasses 10% (CFU/100ml)	Molasses 10%B (CFU/100ml)	Molasses 20% (CFU/100ml)	Molasses 20%B (CFU/100ml)	No Inoculum (CFU/100ml)
0	8.75E+09	8.95E+09	3.60E+09	3.65E+09	5.70E+09	3.90E+09	3.15E+09	2.35E+09	3.45E+09
1	3.55E+10	3.06E+10	1.53E+10	6.55E+09	2.05E+09	9.00E+09	5.25E+09	3.70E+09	2.80E+09
4	4.73E+10	5.22E+10	2.01E+10	1.63E+10	1.15E+09	2.44E+10	5.90E+09	2.84E+10	3.90E+09
6	9.45E+11	1.36E+12	3.55E+11	3.65E+11	1.00E+09	8.60E+10	3.05E+10	5.30E+10	5.00E+09
8	1.00E+10	6.81E+11	5.90E+10	6.10E+10	1.00E+01	2.70E+09	2.50E+07	5.00E+06	
12	1.04E+10	5.70E+08	1.30E+10	1.44E+10	1.00E+00	7.20E+09	4.00E+00	5.20E+05	
18			9.45E+08	1.40E+08	1.00E+00	1.17E+10	1.00E+00	6.05E+05	

Date: 12/12/2013
Sample: Treatment 1 - Sugar additive concentration experiments
Parameter: *E. coli* count
Pre-culture addition (w/w): 10% and 0% in sample E

Molasses addition (w/w): 0, 5%, 10% & 20%

pH measurements - Batch Test 1 - Optimal sugar concentration experiment

Time (days)	Control 0%	Control 0%B	Molasses 5%	Molasses 5%B	Molasses 10%	Molasses 10%B	Molasses 20%	Molasses 20%B	No Inoculum
0	6.60	6.64	6.04	6.00	5.95	5.97	5.99	5.98	7.4
1	6.94	7.16	5.96	5.90	5.75	5.75	5.67	5.75	
4	7.51	7.71	5.10	5.04	5.07	5.12	5.45		7.23
6	7.72	7.85	5.17	5.24	4.85	4.85	4.33	4.73	7.11
8	8.03	8.07	6.16	6.20	4.54	4.76	4.09	4.11	7.14
12	7.92	8.03	7.66	7.55	4.13	4.82	3.94	3.91	7.04
18	7.73	7.98	7.30	7.47	4.00	5.28	3.89	3.89	6.78

Lactic acid measurements - Batch Test 1 - Optimal sugar concentration experiment

Time (days)	**Control 0% (mg/L)**	**Control 0%B (mg/L)**	**Molasses 5% (mg/L)**	**Molasses 5%B (mg/L)**	**Molasses 10% (mg/L)**	**Molasses 10%B (mg/L)**	**Molasses 20% (mg/L)**	**Molasses 20%B (mg/L)**	**No Inoculum (mg/L)**
0									
6	66	71	11,200	11,600	30,600	22,600	38,300	21,900	
12	97	104	99	96	38,100	1,280	42,500	42,000	84
26	103	122	83	78	43,850	99	37,000	39,100	75

Date: 12/12/2013
Sample: Treatment 1 - Sugar additive concentration experiments
Parameter: pH and Lactic acid measurements
Pre-culture addition (w/w): 10% and 0% in sample E
Pre-culture age: 1 day
Molasses addition (w/w): 0, 5%, 10% & 20%

pH measurements - Batch Test 2 - Inoculum concentration experiment

Time (days)	Control	Control B	Inoculum 1%	Inoculum 1%B	Inoculum 5%	Inoculum 5%B	Inoculum 10%	Inoculum 10%B	Inoculum 20%	Inoculum 20%B
0	7.57	7.57	7.57	7.57	7.57	7.57	7.57	7.57	7.57	7.57
3	5.36	5.34	5.31	5.32	5.20	5.21	4.99	4.99	4.84	4.84
10	4.11	4.16	4.40	4.45	4.30	4.36	4.08	4.17	4.09	4.09
11	4.00	4.05	4.30	4.32	4.21	4.23	3.97	4.06	3.95	3.96

Lactic acid measurements - Batch Test 2 - Inoculum concentration experiment

Time (days)	Control (mg/L)	Control B (mg/L)	Inoculum 1% (mg/L)	Inoculum 1%B (mg/L)	Inoculum 5% (mg/L)	Inoculum 5%B (mg/L)	Inoculum 10% (mg/L)	Inoculum 10%B (mg/L)	Inoculum 20% (mg/L)	Inoculum 20%B (mg/L)
0										
3	4,640	5,850	9,000	8,600	8,600	9,300	12,800	10,800	13,000	15500
11	28,000	27,200	22,900	20,100	21,700	22,600	27,700	26,400	22,200	22400
14	27,200	27,500	23,300	20,800	20,600	24,200	25,700	28,500	26,500	24600

Start Date: 20/12/2013
Sample: Treatment 2 - Inoculum concentration experiments
Parameter: *E. coli* count
Molasses addition (w/w): 10%
Inoculum addition (w/w): 0%, 1%, 5%, 10% and 20%
Inoculum age: 2 day
Initial pH in Inoculum: 4.0
Initial Inoculum L.A concentration: 14,100 mg/L
Initial LAB count in inoculum: 5.1E+09
Initial Faecal Sludge L.A concentration: 77 mg/L
Initial *E. coli* count in sludge: 3.2E+07
Initial LAB count in sludge: 4.3E+02
Initial pH in Sludge: 7.6

Plate count data for LAB proliferation - Batch Test 3 - Treatment with optimal parameters experiment

Date			Plate: 23/12/2013		Count: 24/12/2013			Plate: 25/12/2013		Count: 03/01/2014			Plate: 27/12/2013		Count: 03/01/2014			Plate: 29/12/2013		Count: 03/01/2014			Plate: 02/01/14		Count: 08/01/2014		
Days			0					2					4					6					10				
Sample	Details of sample		Count/plate	Av. Count	Dilution (1:r	Enumeratio	Av. Enume	Count/pla	Av. Count	Dilution (:	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:r	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:	Enumeration/100ml	
Control	Molasses ad	0.00	169	143	10	1.43E+06		4	8	100	8.00E+05		0	0	10,000,000	0.00E+00		11	9	1,000	9.00E+06		84	75	100	7.50E+06	
	Inoculum ad	0.00	117					12					0					7					66				
Sample M1	Molasses ad	10%				3.07E+08		47	45	1,000,000	4.45E+10		18	18	10,000,000	1.80E+11		15	15	10,000,000	1.45E+11		135	129	10,000,000	1.29E+12	
	Inoculum ad	10%						42					18					14					122				
Sample M2	Molasses ad	10%				3.07E+08		48	42	1,000,000	4.20E+10		24	24	10,000,000	2.40E+11		19	18	10,000,000	1.75E+11		78	96	10,000,000	9.60E+11	
	Inoculum ad	10%						36					24					16					114				
Sample M3	Molasses ad	10%				3.07E+08		48	46	1,000,000	4.60E+10		27	27	10,000,000	2.70E+11		23	20	10,000,000	2.00E+11		138	141	10,000,000	1.41E+12	
	Inoculum ad	10%						44					27					17					143				

Summary of LAB proliferation - Batch Test 3 - Treatment with optimal parameters experiment

Days	Control (mg/L)	Sample M1 (mg/L)	Sample M2 (mg/L)	Sample M3 (mg/L)	Average (mg/L)
0	1.43E+06	3.07E+08	3.07E+08	3.07E+08	3.07E+08
2	8.00E+05	4.45E+10	4.20E+10	4.60E+10	4.42E+10
4	4.90E+06	1.80E+11	2.40E+11	2.70E+11	2.30E+11
6	9.00E+06	1.45E+11	1.75E+11	2.00E+11	1.73E+11
10	7.50E+06	1.29E+12	9.60E+11	1.41E+12	1.22E+12

Start Date: 23/12/2013
Sample: Treatment 3 - Optimised parameter treatment experiments
Parameter: LAB count
Molasses addition (w/w): 10%
Inoculum addition (w/w): 10%
Inoculum age: 3 days (65hrs)
Initial pH in Inoculum: 3.8
Initial Inoculum L.A concentration: 7,900 mg/L
Initial LAB count in inoculum: 5.100E+09
Initial L.A concentration of treatment sample with 10% molasses and inoculum concentration: 1,580mg/L
Initial *E. coli* count in 100ml of sludge: 3.167E+09
Initial LAB count in 100ml treatment sample: 1.430E+06
Initial pH in Sludge: 6.5
Initial pH of treatment sample with 10% molasses and inoculum concentration: 6.53

Plate count data for *E. coli* suppression - Batch Test 3 - Treatment with optimal parameters experiment

Date			Plate: 23/12/2013		Count: 24/12/2013			Plate: 25/12/2013		Count: 03/01/2014			Plate: 27/12/2013		Count: 03/01/2014			Plate: 29/12/2013		Count: 03/01/2014			Plate: 02/01/14		Count: 03/01/2014			Plate: 7/1/2014		Count: 08/01/2014			Plate: 9/1/2014		Count: 10/01/2014		
Days			0					2					4					6					10					15					17				
Sample	Details of sample		Count/plate	Av. Count	Dilution (1:n	Enumeratio	Av. Enume	Count/pla	Av. Count	Dilution (1	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:n	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:	Enumeration/100ml		Count/pla	Av. Count	Dilution (1:n	Enumeration/100ml		Count/pla	Av. Count	Dilution (1:n	Enumeration/100ml	
Control	Molasses ad	0.00	30	32	100,000	3.20E+09		22	23	100,000	2.30E+09			#DIV/0!	100,000	#DIV/0!		109	102	10,000	1.02E+09		71	70	10,000	6.95E+08		63	62	10,000	6.15E+08		61	57	10,000	5.70E+08	5.70E+08
	Inoculum ad	0.00	34					24										95					68					60					53				
Sample M1	Molasses ad	10%	30	32	100,000	3.20E+09		45	43	100,000	4.30E+09		49	46	100,000	4.55E+09		105	107	100,000	1.07E+10		94	89	100,000	8.90E+09		133	129	10,000	1.29E+09		47	41	10,000	4.05E+08	4.05E+08
	Inoculum ad	10%	34					41					42					109					84					124					34				
Sample M2	Molasses ad	10%	30	32	100,000	3.20E+09		45	53	100,000	5.25E+09		68	64	100,000	6.40E+09		82	83	100,000	8.25E+09		1	1	1,000	1.00E+06		0	0	10	0.00E+00		0	0	10	0.00E+00	0.00E+00
	Inoculum ad	10%	34					60					60					83					1					0					0				
Sample M3	Molasses ad	10%	30	32	100,000	3.20E+09		78	75	100,000	7.50E+09		70	73	100,000	7.30E+09		82	86	100,000	8.55E+09		27	27	10,000	2.65E+08		0	0	10	0.00E+00		0	0	10	0.00E+00	0.00E+00
	Inoculum ad	10%	34					72					76					89					26					0					0				

Summary of *E. coli* suppression - Batch Test 3 - Treatment with optimal parameters experiment

Days	Control (CFU/100ml)	SampleM1 (CFU/100ml)	SampleM2 (CFU/100ml)	SampleM3 (CFU/100ml)	AVERAGE (CFU/100ml)	Q_{calc}	Q_{table}
0	3.20E+09	3.20E+09	3.20E+09	3.20E+09	3.20E+09		
2	2.30E+09	4.30E+09	5.25E+09	7.50E+09	5.68E+09	-0.297	0.970
6	1.02E+09	1.07E+10	8.25E+09	8.55E+09	9.17E+09	0.878	0.970
10	6.95E+08	8.90E+09	1.00E+06	2.65E+08	3.06E+09	0.710	0.970
15	6.15E+08	1.29E+08 (Outlier)	1.00E+00	1.00E+00	1.00E+00	1.000	0.970
17	5.70E+08	4.85E+09 (Outlier)	5.00E-01	5.00E-01	5.00E-01	1.000	0.970

Outliers	n	Q_{table}
	3	0.970
	4	0.892
	5	0.710

Qcalc =(suspected value - nearest value)/(max. value - min. value)

If Qcalc > Qtable, then suspected value is an outlier

Start Date: 23/12/2013
Sample: Treatment 3 - Optimised parameter treatment experiments
Parameter: LAB count
Molasses addition (w/w): 10%
Inoculum addition (w/w): 10%
Inoculum age: 3 days (65hrs)
Initial pH in Inoculum: 3.8
Initial Inoculum L.A concentration: 7,900 mg/L
Initial LAB count in inoculum: 5.1E+09
Initial L.A concentration of treatment sample with 10% molasses and inoculum concentration: 1,580mg/L
Initial *E. coli* count in sludge: 3.2E+09
Initial LAB count in 100ml treatment sample: 1.4E+06
Initial pH in Sludge: 6.5

Lactic acid measurements - Batch Test 3 - Treatment with optimal parameters experiment

Time (days)	Control	Sample M1	Sample M2	Sample M3	AV
0		6.53	6.53	6.53	6.53
2	7.13	5.56	5.60	5.60	5.60
4	6.98	5.20	5.20	5.18	5.19
6	6.84	5.32	5.08	5.13	5.11
10	6.68	4.44	3.86	4.30	4.08
15		4.15	3.86	3.98	3.92

pH measurements - Batch Test 3 - Treatment with optimal parameters experiment

Time (days)	Control (mg/L)	Sample M1 (mg/L)	Sample M2 (mg/L)	Sample M3 (mg/L)	Average (mg/L)
0	61	1,580	1,580	1,580	1,580
4	49	15,000	11,900	14,200	13,050
6	37	11,433	17,100	15,600	16,350
10	39	20,750	37,050	28,100	32,575
15	42	32,800	37,900	39,400	38,650
17	44	29,350	40,900	37,900	39,400

Start Date: 23/12/2013
Sample: Treatment 3 - Optimised parameter treatment experiments
Parameter: LAB count
Molasses addition (w/w): 10%
Inoculum addition (w/w): 10%
Inoculum age: 3 days (65hrs)
Initial pH in Inoculum: 3.8
Initial Inoculum L.A concentration: 7,900 mg/L
Initial LAB count in inoculum: 5.1E+09
Initial L.A concentration of treatment sample with 10% molasses and inoculum concentration: 1,580mg/L
Initial *E. coli* count in sludge: 3.2E+09
Initial LAB count in 100ml treatment sample: 1.4E+06
Initial pH in Sludge: 6.5

Plate count data for *E. coli* suppression - Field Test (MALAWI) - Treatment with optimal parameters experiment

Date			Plate: 03/02/2014	Count: 12/02/2014			Plate: 05/02/2014	Count: 12/02/2014			Plate: 07/02/2014	Count: 12/02/2014			Plate: 10/02/2014	Count: 11/02/2014			Plate: 12/02/2014	Count: 13/02/2014		
Days			0				2				4				7				9			
Sample	Details of sample		Count/plate	Av. Count	Dilution (1:n)	Enumeratio	Av. Enume	Count/pla	Av. Count	Dilution (1	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:r	Enumerat	Av. Enum	Count/pla	Av. Count	Dilution (1:	Enumeration/100ml
Control	Molasses ad	0.00	38	39	100	3.90E+06		46	44	100	4.35E+06		28	24	100	2.35E+06		15	17	100	1.65E+06		1	1	100	1.00E+05	
	Inoculum ac	0.00	40					41					19					18					1				
Sample M1	Molasses ad	10%	4	5	1,000	5.00E+06		132	135	1,000	1.35E+08		16	10	1,000	9.50E+06		0	0	10	0.00E+00		0	0	100,000	0.00E+00	
	Inoculum ac	10%	6					138					3					0					0				
Sample M2	Molasses ad	10%	23	22	100	2.20E+06		165	163	1,000	1.63E+08		5	7	100	6.50E+05		0	0	10	0.00E+00		0	0	1,000	0.00E+00	
	Inoculum ac	10%	21					160					8					0					0				
Sample M3	Molasses ad	10%	1	1	1,000	1.00E+06		130	143	1,000	1.43E+08		76	67	1,000	6.70E+07		0	0	10	0.00E+00		0	0	10,000	0.00E+00	
	Inoculum ac	10%	1					155					58					0					0				

Summary of *E. coli* suppression - Field Test (MALAWI) - Treatment with optimal parameters experiment

Days	Control (CFU/100ml)	Sample M1 (CFU/100ml)	Sample M2 (CFU/100ml)	Sample M3 (CFU/100ml)	Average (CFU/100ml)
0	3.90E+06	5.00E+06	2.20E+06	1.00E+06	2.73E+06
2	4.35E+06	1.35E+08	1.63E+08	1.43E+08	1.47E+08
4	2.35E+06	9.50E+06	6.50E+05	6.70E+07	2.57E+07
7	1.65E+06	2.00E+00	2.00E+00	2.00E+00	2.00E+00
9	1.00E+05	1.00E+00	1.00E+00	1.00E+00	1.00E+00
Log Scale					
Days	Control	Sample M1	Sample M2	Sample M3	Average
0	6.59	6.70	6.34	6.00	6.44
2	6.64	8.13	8.21	8.15	8.17
4	6.37	6.98	5.81	7.83	7.41
7	6.22	0.30	0.30	0.30	0.30
9	5.00	0.00	0.00	0.00	0.00

Start Date: 3/2/2014
Sample: Treatment 4 - Upscale of treatment experiments using optimised parameter
Parameter: LAB count
Molasses addition (w/w): 10%
Inoculum addition (w/w): 10%
Inoculum age: 3 days (65hrs)
Initial pH in Inoculum: 3.8
Initial Inoculum L.A concentration: 16,500 mg/L
Initial LAB count in inoculum: 5.100E+09
Initial L.A concentration of 10% molasses and inoculum concentration: 82 mg/L
Initial *E. coli* count in 100ml of sludge: 4.400E+06
Initial LAB count in 100ml treatment sample: 1.650E+08
Initial pH in Sludge: 7.7

Lactic acid measurements - Field Test (MALAWI) - Treatment with optimal parameters experiment

Time (days)	Control (mg/L)	Sample M1 (mg/L)	Sample M2 (mg/L)	Sample M3 (mg/L)	Average (mg/L)
0	62	85	83	79	82
2	72	21,250	20,150	21,650	21,017
4	75	37,750	28,900	38,550	35,067
7	77	48,000	45,350	47,250	46,867
9	68	46,650	43,700	49,150	46,500

pH measurements - Field Test (MALAWI) - Treatment with optimal parameters experiment

Time (days)	Control	Sample M1	Sample M2	Sample M3	Average
0	7.62	7.47	7.96	7.88	7.77
2	7.41	5.56	5.57	5.58	5.57
4	6.93	4.60	4.58	4.53	4.57
7	6.93	4.25	4.26	4.21	4.24
9	6.93	4.19	4.22	4.19	4.20

Start Date: 3/2/2014
Sample: Field Test (MALAWI) - Treatment with optimal parameters experiment
Parameter: pH
Molasses addition (w/w): 10%
Inoculum addition (w/w): 10%
Inoculum age: 3 days (65hrs)
Initial pH in Inoculum: 3.8
Initial Inoculum L.A concentration: 16,500 mg/L
Initial LAB count in inoculum: 5.100E+09
Initial L.A concentration of 10% molasses and inoculum concentration: 82 mg/L
Initial *E. coli* count in 100ml of sludge: 4.400E+06
Initial LAB count in 100ml treatment sample: 1.650E+08

Initial pH in Sludge: 7.7

Appendix B4: Faecal sludge characterization

Total Solids & Volatile solids of Primary sludge from -Harnaschpolder ASWWTP 14/11/2013

Description	**Sample 1**	**Sample2**	**Sample3**
Weight of cup	2.13	2.13	2.13
Weight of cup + Wet sample	27.12	27.12	27.11
Weight of cup + Dry sample (105℃-2hrs)	3.74	3.61	3.56
Weight of cup + Ashed sample (520℃-3hrs)	2.47	2.45	2.42
Total Solids (TS)	6.4%	5.9%	5.7%
Average Total Solids (TS_{av})	**6.0%**		
Volatile Solids (VS)	78.9%	78.4%	79.7%
Average Volatile Solids (VS_{av})	**79.0%**		

Total Solids & Volatile solids of Old Black water from - Sneek 18/11/2013

Description	**Sample 1**	**Sample2**	**Sample3**
Weight of cup	2.09	2.06	2.09
Weight of cup + Wet sample	28.59	28.66	35.72
Weight of cup + Dry sample (105℃-2hrs)	2.345	2.33	2.49
Weight of cup + Ashed sample (520℃-3hrs)	2.17	2.15	2.21
Total Solids (TS)	0.96%	1.00%	1.17%
Average Total Solids (TS)	**1.0%**		
Volatile Solids (VS)	68.6%	67.9%	69.4%
Average VS	**68.6%**		

Total Solids & Volatile solids of Fresh Black water from - Sneek 26/11/2013

Description	**Sample 1**	**Sample2**	**Sample3**
Weight of cup	2.10	2.08	2.10
Weight of cup + Wet sample	30.46	34.61	30.59
Weight of cup + Dry sample (105℃-2hrs)	2.522	2.57	2.52
Weight of cup + Ashed sample (520℃-3hrs)	2.21	2.20	2.21
Total Solids (TS)	1.50%	1.49%	1.47%
Average Total Solids (TS)	**1.5%**		
Volatile Solids (VS)	74.2%	74.5%	73.3%
Average VS	**74.0%**		

Total Solids & Volatile solids of Centrifuges Fresh Black water from - Sneek 29/11/2013

Description	**Sample 1**	**Sample2**	**Sample3**
Weight of cup	2.11	2.10	2.10
Weight of cup + Wet sample	11.18	9.91	11.18
Weight of cup + Dry sample (105℃-2hrs)	3.18	3.05	3.29
Weight of cup + Ashed sample (520℃-3hrs)	2.32	2.30	2.33
Total Solids (TS)	11.83%	12.07%	13.07%
Average Total Solids (TS)	**12.3%**		
Volatile Solids (VS)	79.7%	78.6%	80.6%
Average VS	**79.6%**		

Total Solids & Volatile solids of Pit latrine faecal sludge (MALAWI) 25/01/2014

Description	**Sample 1**	**Sample2**	**Sample3**
Weight of cup	2.27	2.24	2.25
Weight of cup + Wet sample	21.48	22.19	25.15
Weight of cup + Dry sample (105℃-2hrs)	3.97	3.89	4.24
Weight of cup + Ashed sample (520℃-3hrs)	3.17	3.17	3.34
Total Solids (TS)	8.85%	8.27%	8.71%
Average Total Solids (TS)	**8.6%**		
Volatile Solids (VS)	47.1%	43.3%	45.2%
Average VS	**45.2%**		

Total Solids & Volatile solids of Pit latrine faecal sludge (MALAWI) 31/01/2014

Description	**Sample 1**	**Sample2**	**Sample3**
Weight of cup	2.30	2.26	2.24
Weight of cup + Wet sample	26.78	25.88	31.30
Weight of cup + Dry sample (105℃-2hrs)	3.74	3.66	3.68
Weight of cup + Ashed sample (520℃-3hrs)	2.97	2.88	
Total Solids (TS)	5.89%	5.90%	4.97%
Average Total Solids (TS)	**5.6%**		
Volatile Solids (VS)	53.6%	55.5%	
Average VS	**54.6%**		

Total Solids & Volatile solids after treatment faecal sludge (MALAWI) 31/01/2014-12/02/2014

Description	**C1**	**C2**	**C3**
Weight of cup	2.24	2.25	2.24
Weight of cup + Wet sample	7.92	7.89	7.12
Weight of cup + Dry sample (105℃-2hrs)	2.84	2.85	2.74
Total Solids (TS)	0.11	0.11	0.10
Average TS	**10.6%**		

Appendix B5: Dry matter content of cane molasses

Total Solids molasses from -Roosendaal

Description	**Sample 1**	**Sample2**	**Sample3**
Weight of cup	2.11	2.10	2.12
Weight of cup + Wet sample	17.29	16.88	16.96
Weight of cup + Dry sample (105℃-2hrs)	15.355	14.98	15.14
Total Solids (TS)	87.25%	87.10%	87.70%
Average Total Solids (TS)	**87.3%**		

Total Solids molasses from -Malawi

Description	**Sample 1**	**Sample2**	**Sample3**
Weight of cup	2.09	2.09	2.11
Weight of cup + Wet sample	10.99	13.61	12.90
Weight of cup + Dry sample (105℃-2hrs)	9.099	11.20	10.64
Total Solids (TS)	78.80%	79.03%	79.11%
Average Total Solids (TS)	**79.0%**		