UNESCO-IHE
Institute for Water Education

Sanitising faecal sludge with ammonia (from urea) in the context of emergency situations

María Eliette González Pérez

MSc. Thesis MWI-SE 2014-16

April 2014

Camp for displaced people in Democratic Republic of Congo (Photo: © Christian Als, 2008)

Sanitising faecal sludge with ammonia (from urea) in the context of emergency situations

Master of Science Thesis
by
María Eliette González Pérez

Supervisors
Prof. D. Brdjanovic, PhD, MSc (UNESCO-IHE)

Mentors
C.M. Hooijmans, PhD, MSc (UNESCO-IHE)
J.L.C.M. van de Vossenberg, PhD, MSc (UNESCO-IHE)

Examination committee
Prof. D. Brdjanovic, PhD, MSc (UNESCO-IHE)
C.M. Hooijmans, PhD, MSc (UNESCO-IHE)
J.L.C.M. van de Vossenberg, PhD, MSc (UNESCO-IHE)
J. Spit, MSc (WASTE)

This research is done for the partial fulfilment of requirements for the Master of Science degree at the UNESCO-IHE Institute for Water Education, Delft, the Netherlands

Delft
April 2014

Abstract

Sanitising faecal sludge with ammonia (from urea) in emergency situations

In an emergency situation, the collection, treatment and disposal of the human excreta and waste in a safe way is one of the biggest challenges to face, regardless the type of disaster. When it comes to faecal sludge management, the traditional desludging methods can often not be appropriate in an acute emergency phase. Consequently, relief organisations aim to apply new and simple approaches and modular technologies that are effective in terms of cost and time.

For this thesis the ammonia-based sanitation by the addition of urea was studied as an innovative approach for faecal sludge treatment. The research was designed to assess the required conditions and factors affecting the formation of the sanitising agent ammonia (NH_3).

The research evaluated, at a laboratory scale, the application of urea to black water collected from vacuum toilets in The Netherlands. This was followed by scaled-up experiments on faecal sludge in Blantyre, Malawi, to evaluate the adaptability and up-scalability of the urea treatment in the context of emergency sanitation.

The urea amendment increased the pH and the ammonia concentrations in the treated faecal material, contributing to the formation of NH_3. The addition of 1% (w/w) urea concentration to faecal sludge was sufficient to enhance the inactivation of *E. coli*, *Salmonella* and total coliforms to produce a sanitised sludge. The disinfection time of the faecal treatment, measured as less than <1000 *E. coli*/100 mL, can be achieved in less than 1 week at temperatures ranging from 20ºC to 30ºC. A larger than 3 $\log_{10}$ reduction of viable *E. coli*, *Salmonella* and total coliforms in faecal sludge was reached in less than 4 days with ammonia concentrations above 10 g/L.

The results indicated that urea treatment is a reliable and simple approach that allows obtaining safe (faecal) sludge for disposal or reuse, in terms of the studied microorganisms.

Keywords: Sanitation, ammonia, urea, faecal sludge, black water, emergency sanitation

Department of Environmental Engineering and Water Technology, UNESCO-IHE, P.O. Box 3015, 2601 DA Delft, The Netherlands

Acknowledgements

I am forever grateful to my sponsor NUFFIC, for granting me the opportunity to overcome myself and to live this unforgettable and marvellous overseas experience in The Netherlands.

I want to express my gratitude to my supervisor, Prof. Damir Brdjanovic, PhD, MSc, for his encouragement and support during theses 18 months; for his inspirational words of wisdom and motivation in every single lecture. *"...be critical".* I will never forget.

I also would like to deeply thank my two wonderful mentors: Tineke Hooijmans, PhD, MSc and Jack van de Vossenberg, PhD, MSc for the useful comments, remarks and engagement through the learning process of this master thesis.

Furthermore I would like to thank my external examiner Jan Spit, MSc, for introducing this very interesting topic and line of research, as well for the support on the way. Likewise, I want to give special thanks to Katie Anderson, MSc, from WASTE, for being an outstanding supporter and colleague during the field work in Malawi.

In addition, many special thanks to the laboratory staff, especially to Fred Kruis and Peter Heerings, for their unconditional support and motivation during the laboratory sessions.

To the many lecturers and staff members from UNESCO-IHE: who have been essential in the education of this renewed young water professional, enabling me to achieve this Master of Science.

I would like to thank my beloved family and my loved one, who have supported me throughout the entire process, both keeping me harmonious and helping me putting the pieces together. I am -grateful for your love.

Last but not least, a very special thanks to my dear friends: those who were by my side helping disinterestedly when I needed the most.

This report is on the treatment of faecal sludge in emergency situations on the request of WASTE Advisers as part of the Emergency Sanitation Project (ESP) and the S(P) EEDKITS Project. The ESP project is funded by the US Office for Foreign Disaster Assistance (OFDA) and is a consortium of the International Federation of Red Cross and Red Crescent Societies (IFRC), WASTE and Oxfam GB. S(P)EEDKITS has received funding from the European Union seventh Framework Programme (FP7/2007-2013) under grant agreement No 284931.

Table of Contents

List of Figures

List of Tables

Abbreviations

BOD	Biological Oxygen Demand
CFU	Colony-forming Unit
COD	Chemical Oxygen Demand
DS	Dry Solids
E. coli	*Escherichia coli*
FS	Faecal Sludge
FSM	Faecal Sludge Management
HAV	Hepatitis A Virus
JMP	Joint Monitoring Programme
MDG	Millennium Development Goals
MPN	Most Probable Number
MWK	Malawian Kwacha
ND	Non detectable
O&M	Operation & Maintenance
OSS	On-site Sanitation Systems
PAHO	Pan-American World Health Organization
RPM	Revolutions per minute
SS	Suspended Solids
spp.	Sub-species
TAN	Total ammonia nitrogen
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
TS	Total Solids
TSS	Total Suspended Solids
TVS	Total Volatile Solids
UASB	Up Flow Anaerobic Sludge Blanket
UDDT	Urine Diverting Dry Toilet
UV-VIS	Ultraviolet-visible (spectrophotometry)
VIP	Ventilated Improved Pit Latrine
VS	Volatile Solids
VSS	Volatile Suspended Solids
WHO	World Health Organization

List of Symbols

≈	Almost equal to
%	Percentage
C	Carbon
$CO(NH_2)_2$	Urea
€	Euro
K	Potassium
kg	Kilogram
L	Litre
mg	Milligram
mL	Millilitre
N	Nitrogen
NH_3	Ammonia
NH_3-N	Ammonia-Nitrogen
NH_4^+	Ammonium ion
NH_4^+-N	Ammonium-Nitrogen
ºC	Degree Celsius
OH^-	Hydroxide ion
P	Phosphorus
pH	Power of Hydrogen
ppm	Parts per million
T	Temperature
w/w	weight/weight

CHAPTER 1

Introduction

The lack of access to safe drinking-water, sanitary facilities for excreta disposal and hygiene behaviours are one of the many problems that developing countries deal with the burden disease reduction caused by those factors. According to information from the Joint Monitoring Programme (JMP), by 2011 the world's sanitation coverage in urban and rural areas was 64%, yet in some African countries the coverage barely reaches 50%. It is particularly stated that 1.2 billion people defecate in the open, representing 15% of the world population (WHO/UNICEF JMP for Water Supply and Sanitation 2013).

Furthermore, it forecasted that in 2015, 2.5 billion people will use unimproved sanitation facilities, meaning that the MDG target of reducing the sanitation gap will be missed by a half billion people. Unquestionably, the main consequences of inadequate access to safe drinking water and lack of sanitation facilities causes serious health issues and environmental pollution. Figure 1-1 reflects the sanitation situation worldwide.

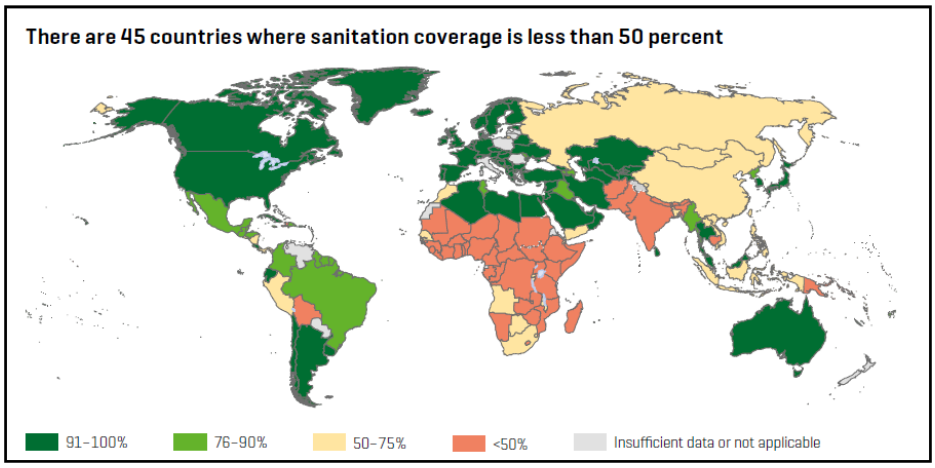


Figure 1-1 Global sanitation trends in 2011 (WHO/UNICEF JMP for Water Supply and Sanitation 2013)

Nonetheless, the provision of sanitation and toilet facilities does not solve the stated problem and avoid the transmission of diseases. The conventional centralised sanitation approaches have proven not to improve the sanitation conditions in developing countries, considering that 90% of the generated and collected wastewater is arbitrarily discharged without any adequate treatment (Langergraber and Muellegger 2005). Hence, exposing humans to the pathogens present in the faecal matter and harming the environment. Furthermore, the requirements of piped water supply and sewage system can be O&M demanding and very costly, which is usually more than four times that of on-site alternatives) and cannot be afforded by developing nations (WHO 1992). On top of that, the traditional sanitation concepts are built on the premises that human excreta and wastewater are wastes that should be disposed, believing that the environment will be able to safely assimilate them.

In contrast with centralised sanitation paradigm, on-site sanitation systems (OSS) deal with excreta where it is disposed, providing a hygienic and satisfactory solution for communities. Considering this advantage, OSS are being widely promoted as they play an important role for increasing the access to improved sanitation. These decentralised alternatives are particularly implemented in rural and peri-urban dwellings in developing countries, where space and population density are main issues. The Figure 1-2 shows that the majority of the developing regions are mostly covered with on-site sanitation facilities.

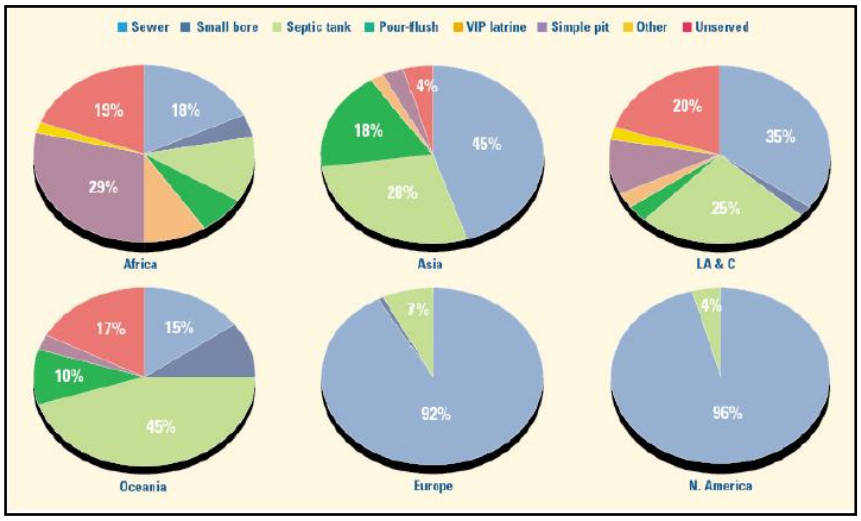


Figure 1-2 Sanitation breakdowns by world region.(Supply et al. 2003)

Notwithstanding, OSS also face major issues regarding to the management of the generated by-products, as it is the faecal sludge (FS). Strauss et, al., 2002 (cited in Eawag/Sandec 2008) states that this stage has been neglected in the sanitation systems due to other priorities and considering it involves a number of challenges, i.e. occupational health risks due to manipulation during vault emptying, inadequate faecal sludge stream treatment and indiscriminate disposal. If human excreta are not managed properly, the risks of food- and feed- borne diseases transmission are high. Consequently, it is very important to encourage and develop simple yet effective initiatives aimed to improved faecal sludge treatment schemes, especially in West African countries, South East Asia, Latin American and the Caribbean (Eawag/Sandec 2008).

If the Faecal Sludge Management (FSM) already represents a challenge itself, when emergency situations occur, the provision of adequate sanitation results an even greater defy. Considering that the technical options for emergency excreta treatment are limited and there is lack of sufficient capacity and logistics. A notable example of it is the recent and ongoing cholera outbreak in Haiti, which took place after the 2010 earthquake and has killed nearly 8,000 people (PAHO/WHO 2013). In view of extreme humanitarian response situations like this, the selected methods for treatment and disposal for a safe faecal sludge should be simple, low-cost effective, reliable and efficient.

One of the most common sources of infection by excreta-related pathogens for both humans and animals is the ingestion of contaminated food and water. Therefore, is extremely important to mitigate the risk of spreading diseases by applying simple methods as treatment. The earlier the treatment is applied, the less the risk of disease transmission there is. Within some of the well-known and used faecal treatment there are:

- ✓ Chemical sanitation with alkali: using ash or lime to increase the pH and temperature

- ✓ Drying faeces: to reduce the moisture content
- ✓ Composting: not a sanitising method *per se*, yet it generates high temperatures that removes pathogens
- ✓ Anaerobic digestion: creates thermophilic conditions (>45ºC). At mesophilic conditions (20 - 35 ºC), high ammonia concentration (7 g N/L) and pH of 8 improve pathogenic reduction, as Ottoson, 2008 states, [cited in (Nordin 2010)].
- ✓ Incineration of faeces: to generate alkaline ashes used to enhance soil's buffering capacity. Though is not a viable treatment option due to its high moisture content (10%), according to Niwagaba et al., [cited in (Nordin 2010)].
- ✓ Chemical sanitation with Ammonia: Ammonia Nitrogen (NH_3-N), whether it comes from urine or added urea, gives alkaline pH, pushing the NH_3 formation. The treatment is strongly dependant on pH, temperature and storage conditions (Ottoson et al. 2008)..

According to Semenov et al., 2007; Sidhu et al., 2001, cited in (Nordin 2010), the inactivation of pathogens in the environment depends on synergy of several factors like temperature, pH, moisture content, nutrient competition and carbon content. However, the level of inactivation may be uncertain and slow. In general, the best established methods for excreta disinfection are based on temperature, pH, moisture and ammonia.

In this thesis, the biochemical treatment with ammonia (from urea) in faecal sludge was be studied. This interesting and innovative treatment option to handle urine and faeces has been studied by the University of Agricultural Sciences (SLU) in Uppsala, Sweden, in cooperation with the Sustainable Sanitation Design foundation. The tests have demonstrated that ammonia (NH_3) is a very powerful disinfectant chemical that is able to inactive pathogens such as viruses (Emmoth et al., 2011), bacteria (Vinnerås et al. 2008) protozoa (Jenkins et al., 1998) and helminth eggs (Nordin et al., 2009;Pecson and Nelson, 2005), cited in (Fidjeland et al. 2013). However, the carried out researches have been focused in the context of ecological sanitation, for the reuse of excreta in agriculture.

In this regard, further studies of the ammonia effect over faecal sludge are needed so there is more knowledge on pathogens' die-off kinetics and the required conditions to successfully inactivate microorganisms, all this within the context of emergency situations.

1.1. RESEARCH PROBLEM

The people directly affected when a disaster takes place are often in extreme conditions and are at high health risk because of the collapse of the sanitation infrastructure. However, not only the provision of good sanitation is a challenge itself but to find and develop innovative, safe, effective and sustainable faecal sludge management strategies to improve the health conditions of exposed populations.

The main problem for safe sludge handling is the lack of rapid installation kits or equipment, as well as effective, efficient, affordable and low-tech methods for treating and disposing faecal sludge in such a way that doesn't cause health risk to population, especially in emergency situations. On top of that, the lack of skilled manpower that is able to design, operate and maintain sanitation systems, especially when an emergency strikes is another challenge in this field.

On the other hand, knowing the fertilising properties of nutrients present in human excreta, the re-use of sludge for agricultural purposes could be one of the activities for faecal sludge post-treatment. However the manipulation or either the application of human excreta, as fertiliser for food production, can pose serious

risks to people's health. Thus, by sanitising the faecal sludge, all successive activities done in faecal sludge management can be performed in a safe manner.

1.2. PURPOSE OF THE STUDY

This study was undertaken in the frame of safe faecal sludge management and emergency sanitation. Different organisations and projects are putting efforts together in the search of suitable approaches for faecal sludge treatment in emergency situations. The Ministry of Foreign Affairs of Netherlands supports the researches done by the Netherlands Red Cross, the S*P*eedkits project and WASTE on defining the requirements for a safe sludge handling in emergency situations.

The purpose of this research is to generate more knowledge on the sanitising effect of the urea, i.e. the inactivation of pathogens present in the faecal sludge. Even though the treatment promises to be quite simple to operate, one of the crucial findings is to know the sufficient dosage, storage time and necessary environmental conditions, in order to finally achieve the inactivation of pathogenic microorganisms.

Furthermore, the investigation pretends to find out the reliability, efficiency and feasibility of this interesting approach not only in controlled laboratory conditions but also in a FSM pilot facility in Malawi. The main objective of the field testing study is to determine if the faecal sludge disinfection approach with urea can be reproduced and applied to treat waste from latrines in a bigger scale in Malawi.

CHAPTER 2

Objectives

The general objective of this thesis was to assess the efficiency of urea treatment as a method for disinfecting faecal sludge, applicable under emergency conditions, in order to obtain safe sludge that does not pose health risks to exposed populations.

The specific objectives were:

- To determine the minimum necessary dosage of ammonia to be mixed with faecal sludge to obtain WHO guideline level of <1000 *E. coli*/100 mL
- To evaluate how the sludge composition, ambient temperature, contact time and pH affect the pathogen inactivation.
- To assess if the process of urea application is efficient and effective, in terms of time and costs, for faecal sludge management in emergency situations.

CHAPTER 3

Background

3.1. Emergency situations

Emergencies, conflicts and disasters happen frequently, including natural disasters, chemical or radiological incidents, complex emergencies, and deliberate events. A substantial fraction of the disease burden derived from these events is attributable to environmental risk factors (WHO 2013).

An emergency situation can be defined as an immediate, urgent, and critical situation of a temporary nature, regardless of its cause, which may seriously endanger or threaten the lives, health, or safety of individuals.

Figure 3-1 Factors playing an important role during an emergency situation

The situation might be worsened for a longer period if there is an inadequate relief response. Four factors can be listed as the most critical ones when an emergency situation takes place (European Commission 2013):

a) Health Care

The lack of health care is often a major problem contributing to widespread disease and high mortality rates for common and treatable diseases. In addition, very low quality of medical services, remoteness of the sick individual and lack of economic resource to afford medical assistance.

b) Food and Security

Depending on the type of emergency (war, urban disasters, earthquakes, etc.), the main causes of food insecurity are related to displacements, fear of violence, epidemics, and lack of access to food.

c) Logistics

Due to logistical problems and a lack of infrastructure, the transport of humanitarian goods and personnel by land or air is risky, very difficult and extremely expensive throughout the country or affected area. In very remote regions, the provision of humanitarian aid is even more complex.

d) Water and Sanitation

Access to clean water and sanitation services is a large contributor to health conditions. In emergencies, the health risks are high because of the collapse of the sanitation infrastructure. Therefore, delivering adequate sanitation is quite a challenge and is one of the key survival factors for the people that have been directly affected by the emergency.

In this latter category, the faecal sludge management becomes a great challenge for the humanitarian organisation, considering that the appropriate handling and treatment of large amount of human excreta in (post) emergency conditions has often been neglected due to other priorities, such as food and health care provision (Bouwinnovatie and Societies 2012).

3.2. Faecal sludge

Faecal sludge can be defined as the undigested or partially digested slurry accumulated or treated in pits or vaults of on-site sanitation facilities like pit latrines, septic tanks, vault installations, etc. Compared to conventional sewage characteristics, these liquids are several times more concentrated in suspended and dissolved solids. The FS might be treated separately on co-treated with the sludge produced in municipal wastewater treatment facilities (Eawag/Sandec 2008).

3.2.1. Faecal sludge characteristics

Table 3-1 Parameters used for FS characterisation

Parameter	Description
pH	It is a very important quality parameter for characterisation. Wastewater and FS with extreme pH values are difficult to treat biologically.
Total Solids (TS)	TS are used to evaluate the reuse potential of wastewater and to determine the most adequate treatment operation and process.
Conductivity	By measuring conductivity, the salinity can be assessed. The salt content is a very important parameter for agricultural wastewater/sludge re-use.
Total Volatile Solids (TVS)	The TVS/FS ratio is used to determine the content of organic matter.
Total Kjeldahl Nitrogen	It is the total amount of organic and ammonia nitrogen. Data is required to evaluate the bio-treatability of the wastewater. Insufficient nitrogen may require additional nitrogen to make the waste treatable.
Ammonium (NH_4^+) (AN)	Ammonia nitrogen is found in aqueous solutions as ion (NH_4^+) or as gas (NH_3), depending on solution's pH. In wastewater treatment, about 60-70% of the influent's TKN will be as NH_4-N and the rest as organic N. The total soluble organic nitrogen is the difference between TKN and its NH_4-N concentration (of a filtered sample).
C/N ratio	A balanced C/N ratio is important for aerobic and anaerobic digestions of FS. It is also crucial for the biogas production.
BOD/COD ratio	Typical BOD/COD ratio in raw wastewater is 0.3-0.8. Above 0.5, the waste is considered to be easily treatable by biological processes. Values ≤ 3 mean that the waste may have toxic components or adapted microorganisms and it needs to be previously stabilised.
Faecal Coliforms (MPN)	The presence of specific and/or representative group of microorganisms allows the assessment of wastewater treatment plant performance and the possibility of waste reuse.
Helminth eggs	The term describes the worms collectively. They are one of the principal causes for human diseases. Some representative helminths are *Ascaris lumbricoides* and *Schistosoma mansoni.* It is primarily the eggs stage, rather than larvae, that is present in wastewater. They can be removed in common treatment processes like

	sedimentation, filtration and stabilisation ponds.
Heavy metals	Heavy metals like cadmium, lead and mercury happen in commercial and industrial wastewater. They have to be controlled if the waste is intended to be re-used for agriculture.

[Adapted from: Metcalf & Eddy, 2003, cited in (Eawag/Sandec 2008)]

3.2.2. Faecal sludge production

Figure 3-2 depicts the values on daily per capita volumes and loads collected faecal sludge from septic tanks and pit latrines. These are average numbers that can be used for preliminary planning and design where local data is often missing. Actual quantities may vary from one place to another.

Parameter	Septage [1]	Public toilet sludge [1]	Pit latrine sludge [2]	Fresh excreta
BOD [g/cap•day]	1	16	8	45
TS [g/cap•day]	14	100	90	110
TKN [g/cap•day]	0.8	8	5	10
Volume [l/cap•day]	1	2 (includes water for toilet cleansing)	0.15 – 0.20	1.5 (faeces and urine

[1] Estimates are based on a faecal sludge collection survey conducted in Accra, Ghana.

[2] Figures have been estimated on an assumed decomposition process occurring in pit latrines. According to the frequently observed practice, only the top portions of pit latrines (~ 0.7 ... 1 m) are presumed to be removed by the suction tankers, since the lower portions have often solidified to an extent that does not allow vacuum emptying. Hence, both per capita volumes and characteristics will range higher than in the material which has undergone more extensive decomposition.

Figure 3-2 Daily per capita volumes, BOD, TS, and TKN values of different types of faecal sludge [(Heinss et al., 1998), cited in (Eawag/Sandec 2008)]

3.2.3. Hygienic quality of FS

In most of developing regions, the occurrence of nematode infections caused by helminths is high. *Ascaris* eggs are particularly persistent in the environment. Nematode eggs are indicators to determine the hygienic quality and safety of the biosolids generated during the wastewater treatment in order to obtain a safe sludge. The concentration of helminth eggs in the solids is related to the level of infection in the population from which the faecal sludge or wastewater is collected. In case of re-using the biosolids as soil conditioner and fertiliser, the treatment must reduce to the maximum the helminth egg concentration and their viability, or the solids must be stored long enough to achieve their inactivation. Figure 3-3 shows the concentrations of *Ascaris* and *Trichuris* eggs in raw faecal sludge samples.

	Ascaris [eggs/g TS]	Trichuris [eggs/g TS]
Public toilet sludge		
Sample 1	13	2
Sample 2		9
Septage		
Sample	3	2
Sample 4	94	24
Sample 5	29	15

A study conducted in Bangkok, Thailand, revealed average values for helminth eggs of 6 eggs/g TS in 256 raw septage samples. (Koottatep et al., 2005)

Figure 3-3 Prevalence of *Ascaris* eggs and *Trichuris* eggs in Kumasi's (Ghana) raw faecal sludge (Eawag/Sandec 2008)

In places where nematodes are not prevailing, some other pathogenic microorganisms (i.e. Salmonellae spp.) or bacteriophages may be used instead, as indicators of hygienic quality of faecal sludge.

3.2.4. Faecal sludge characterisation and factors affecting it

The characteristics of FS widely depend to location (household, district, and city). The factors that influence quality and are responsible for the high variability of the FS characteristics are illustrated on Figure 3-4.

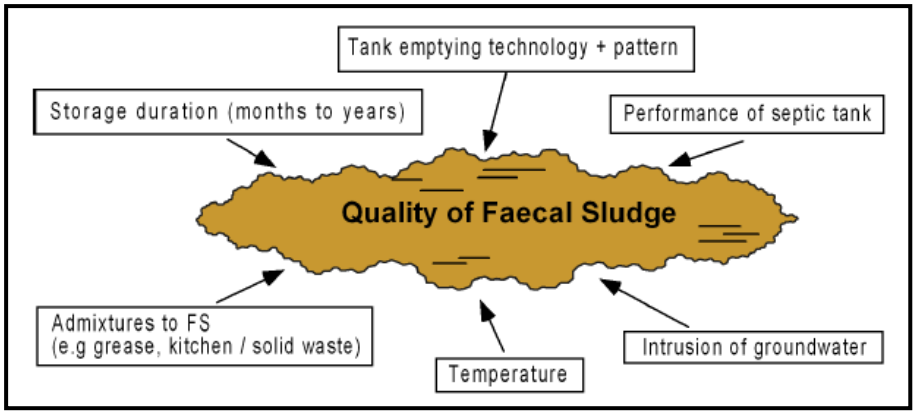


Figure 3-4 Factors influencing characteristics of faecal sludge [Heinss et al., 1998, cited in (Eawag/Sandec 2008)]

In on-site sanitation systems, the faecal sludge is degraded via anaerobic digestion. The organic stability of faecal sludge is dependent on several factors, such as ambient temperature, retention time and presence of inhibiting substances.

One basic distinction can facilitate to group the faecal sludge into two broad categories: high vs. low-strength sludge. Based on several monitoring studies from various regions of the world, the following faecal sludge characteristics can be summarised on Table 3-2. Characteristics of sewage sludge are also displayed for comparative purposes.

Table 3-2 Characteristics of faecal sludge and its comparison with sewage

	Public Toilet Sludge	**Septic Tank Sludge**	**Sewage**
Characterisation	Highly concentrated, mostly fresh FS, stored for few days or weeks	Low concentration, usually stored for several years; more stable than public toilet sludge	Tropical sewage
COD (mg/L)	20,000 - 50,000	<10,000	500 - 2,500
COD/BOD	2:1 - 5:1	5:1 - 10:1	2:1
NH_4-N (mg/L)	2,000 - 5,000	<1,000	30 - 70
TS	≥3.5%	<3%	<1%
SS (mg/L)	≥30,000	≈ 7 ,000	200 - 700
Helminth eggs (n^o/L)	20,000 - 60,000	≈ 4,000	300 - 2,000

[Adapted from Heinss et al., 1998, cited in (Eawag/Sandec 2008)].

In conclusion, the faecal sludge has high concentrations and is a variable product. This means that it cannot be considered and treated as wastewater but calls for specific treatments and design criteria. Consequently, the designed treatment should not be based on general or standard characteristics but rather on results obtained on the particularities of each case.

3.3. Faecal sludge management (FSM)

At the present, the FSM has to cope with several challenges such as: health risks through manual emptying, indiscriminate disposal and complete lack of inadequate treatment (Eawag/Sandec 2008).

a) *Traditional manual emptying*: sludge is evacuated from pits or vaults with buckets, posing serious health hazards for the emptier(s). Also it a risk to public health considering that the sludge is disposed arbitrarily.

b) *Indiscriminate disposal*: long distances between disposal sites and dwellings, difficult access for lorries in poor urban settlements, traffic congestions and high discharging fees for each FS load delivered are the main causes why hundred thousand tons of untreated faecal matter are uncontrollably disposed into urban and peri-urban environments.

c) *Lack of inadequate treatment*: the major risks are the health related impacts. Pathogens present in the FS can contaminate food or water due to unhygienic practices. Contamination may also occur before and/or during, treatment, handling and re-use for agriculture. Besides, FS has impacts on soil because of presence of salts, heavy metals, excess of nutrients, hormones and persistent organic compounds. Moreover, if the excess of nutrients infiltrate to groundwater or are flushed away into surface water, it can lead to consumption of dissolved oxygen in lakes and rivers.

3.3.1. Processing steps in faecal sludge treatment

A sanitation system can be described as a series of possible process steps.

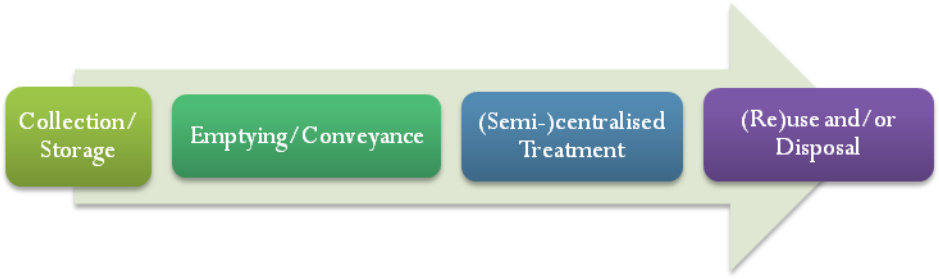


Figure 3-5 FSM in the context of sanitation [Adapted from (Eawag/Sandec 2008)].

Table 3-3 Different collection and storage systems that need regular desludging

On-site Sanitation Systems	(Semi-)centralised Treatment Units
• Single pits • Single Pit VIPs • Settling tanks • Septic tanks • Anaerobic baffled reactors • Anaerobic filters	• Waste stabilisation ponds • Aerated ponds • Trickling filters • UASB • Activated sludge reactor • Anaerobic biogas reactor

The so-called low technologies are the most appropriate for FSM in developing countries. In this regard, the external energy and chemical input should be minimal. As a result, the land requirements for faecal sludge treatment are relatively large.

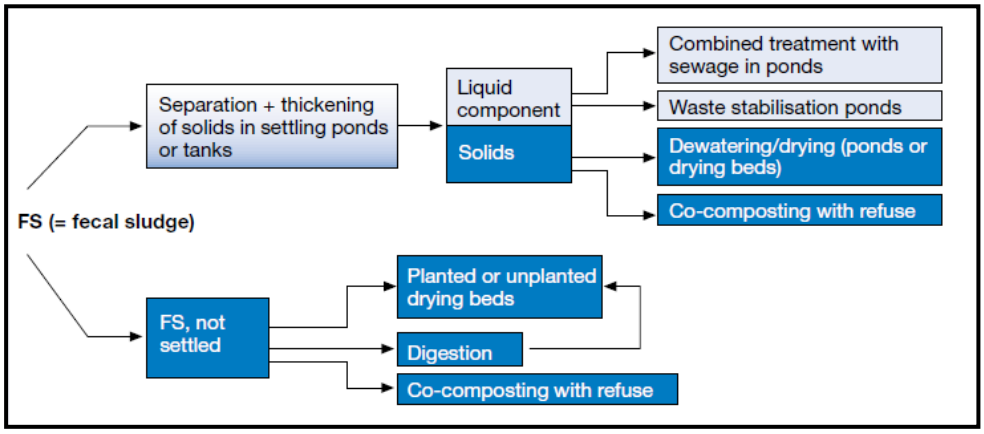


Figure 3-6 Faecal sludge treatment options in developing countries (Strauss, M., Montangero 1999).

Based on the previous faecal sludge characteristics some other aspects should be considered for the faecal sludge treatment train:

- **Solid liquid separation** as a first treatment step, to concentrate the biosolids and helminth eggs. It can be done in sludge drying beds or sedimentation ponds/tanks.

- **Stabilisation** of the undigested sludge on anaerobic reactor or ponds. Stabilised sludge can be directly dewatered and composted.
- **High removal efficiencies of organic matter** (TOC, COD) **and nutrients** (N and P), if main purpose is to reduce the environmental pollution.
- **Limit nutrient losses** to create a stabilised, safe and valuable product for agricultural reuse.

3.4. Type of infectious agents in human excreta

Various infectious agents and parasites are to be found in domestic wastewater as well as in effluents of wastewater treatment plants. Enteric pathogens can be found in high concentrations in the faeces. Figure 3-7 categorises the infectious microorganisms of interest for the public health.

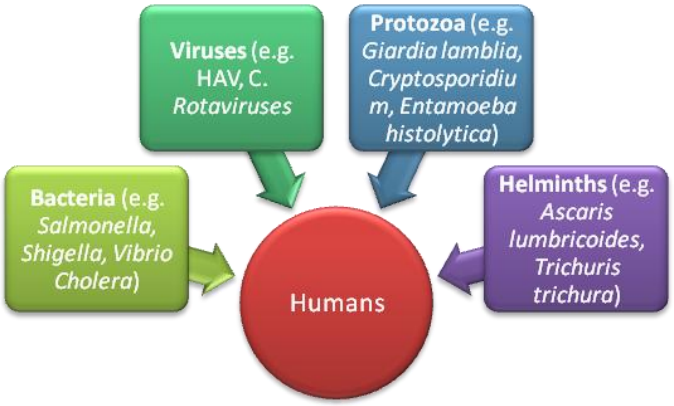


Figure 3-7 Categories of organisms of public health significance (Bitton 2005).

These infectious agents are evaluated on their virulence and capacity to harm or to sicken humans.
the term *virulence* can be defined as "the necessary dosage of an infectious agent to infect the host and cause a disease (Bitton 2005)". The minimal infective dosage varies per microorganism. For instance, for *Salmonella typhi* and enteropathogenic *E. coli* thousands to millions cells (10^6) are necessary to create an infection; whereas for viruses, protozoa and helminths can infect at low doses, only a few units may be sufficient for an individual.

Aside for pathogens present in faeces, urine may contain infectious agents as well. In principle, the urine is relatively harmless and it is considered to be sterile; yet faecal cross-contamination may take place during urine collection. The contamination risks are higher with diarrhoeal diseases and with the misuse of urine-diverting toilets. The occurrence of some pathogens in the urine it rarely caused when very specific microorganisms are present in the blood or when urine is their main route of excretion, as it is with bacteria *Leptospira interrogans* and the helminth *Schistosoma haematobium.* Consequently, faecal pathogens have to be considered if the urine stream wants to be reused [(Wilson & Gaido, 2004; Feachem, 1983), cited in (Nordin 2010)].

Bacteria are responsible for the majority of the gastrointestinal illnesses and many faecal bacteria occur all around the world like *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp. and some strains of *E. coli.* In developing regions where sanitation is poor, *Vibrio cholerae* and *Shigella* spp. are common causes of diarrhoea. it is stated that the faecal matter contains up to 10^{12} bacteria per gram; the content of bacteria in faeces represent 9% (w/w) [Dean and Lund, 1981), cited in (Bitton 2005).

Viruses are also present in waste and wastewater; approximately 140 types of enteric viruses are known. They enter orally to the human body, multiply in the gastrointestinal track and are excreted in large amounts from infected individuals. Viruses are responsible for a broad range of diseases like fever, respiratory diseases, paralysis and gastrointestinal infections, even in developed regions (Bitton 2005). Norovirus, adenovirus, astrovirus and rotavirus are known to cause viral gastroenteritis. The latter is the most common cause of gastroenteritis in children and produces similar incidence rates indistinctly from the region in the world. In adults is less common due to immunity to the virus.

A number of **protozoan** parasites can cause gastroenteritis – most commonly *Giardia* – but *Entamoeba histolytica* and *Cryptosporidium* species have also been implicated. These harmful microorganisms are important causes of morbidity and mortality, even in high come regions and countries. These tree protozoan are zoonotic, meaning they can be transmitted to humans from other species; also even humans can be the reservoir. Protozoan parasites may be particularly difficult to inactivate, considering they produce *oocysts*, which are able to survive outside their hosts under unfavourable environmental conditions.

Parasitic worms or most commonly referred as to **helminths** are not commonly studied by microbiologists. Considering their presence in wastewater and their great implication regarding public health, helminths should be investigated more extensively. Worms like *Ascaris lumbricoides* and *Trichuris* spp. are common in developing regions with poor sanitation practices and infections are acquired by food-borne routes. One particular characteristic of helminth eggs is their tolerance to environmental stresses and to treatments in wastewater treatment plants. Also their concentration varies among seasons.

Table 3-4 Concentration of microorganisms in wastewater (number of microorganisms per 100 mL) (Henze 2008).

Microorganism	High	Low
E. coli	$5 \cdot 10^8$	10^6
Coliforms	10^{13}	10^{11}
Clostridium perfringens	$5 \cdot 10^4$	10^3
Faecal *Streptococcae*	10^8	10^6
Salmonella	300	50
Staphylococcus aureus	10^5	$5 \cdot 10^3$
Coliphages	$5 \cdot 10^5$	10^4
Giardia	10^3	10^2
Roundworms	20	5

3.5. Chemical treatment with ammonia

A very interesting approach for faecal sludge treatment is the pathogen inactivation derived from uncharged ammonia (NH_3), which has demonstrated to be a harmless chemical substance capable to efficiently inactivate bacteria (Vinnerås et al. 2008). (Warren, 1962) states that "the concentration of ammonia might be a beneficial nutrient or a toxic agent to microorganisms. The toxic effects of NH_3 are widely known, whereas NH_4^+ ions can be tolerated by most of microorganisms, even at high concentrations", quoted in (Nordin 2010).

According to (Fidjeland et al. 2013), the sanitising effect of the ammonia is strongly related to the source of the sludge, NH_3 concentration which also depends on the volume of flush water, the faeces/urine mixing

ratio, infiltration and ventilation. Consequently, the designed/selected sanitation system to apply ammonia treatment must ensure to meet the factors previously mentioned. In conclusion, the ammonia treatment should be based on measurements of NH_3 concentrations and pH and not on flush water volume, considering the faecal sludge chemical properties are dependent on some other factors (Fidjeland et al. 2013), as they were already described.

3.5.1. Urea as a source of ammonia

"80% of the nitrogen that is excreted from the human body with the urine is in form of urea [$CO(NH_2)_2$] and 7% as NH_3/NH_4^+" (Lentner & Geigy, 1981), cited in (Nordin 2010). The urea is obtained from urine, which quickly degrades into ammonia and carbonate. By adding urea to faecal material, the same decomposition products are obtained as in urine.

$$CO(NH_2)_2 + 3H_2O \xrightarrow{Urease} 2NH_4^+ + OH^- + HCO \qquad \textbf{Equation 3-1}$$

The urea decomposition results in alkaline pH (around 9), which affects the equilibrium between NH_3/NH_4^+ in aqueous solution, favouring the NH_3 formation. The obtained unionised ammonia (NH_3) is the main sanitising agent from the urine, yet the carbonates (CO_3^{2}) are also suggested to contribute with the pathogens inactivation [(Park & Diez-Gonzalez, 2003), cited in (Nordin 2010). Aqueous ammonia solution can also be added to a substrate, stabilising the pH up to 10; yet only ammonia (NH_3 -NH_4^+) and not carbonates would be added up..

The ammonia gas is highly soluble in water given its polarity and capacity to form hydrogen bonds. The solubility of NH_3 (aq) in liquids depends on the temperature and the ratio can be calculated by Henry's law constant. The dissolved NH_3 gas quantity is directly proportional to the partial pressure of the NH_3 gas above the solution; thereupon the ventilation and head space volume affect the NH_3 gas solute concentration.

$$NH_3(gas) \xrightarrow{H} NH_3(aq) + H_2O(l) \xrightarrow{K_b} NH_4^+(aq) + OH^-(aq) \qquad \textbf{Equation 3-2}$$

The ammonia behaves as weak base when in solution, producing hydroxide ions by the water de-protonation (Equation 3-2). The dissociation constant K_a enables to quantify the relationship between NH_3 (aq)/NH_4^+ (aq). The pK_a of NH_3/NH_4^+ can be calculated by Equation 3-3 [Emerson et al.,1975), cited in (Nordin 2010), where the temperature is in degrees Kelvin.

$$pK_a = 2729.92 / T + 0.090181 \qquad \textbf{Equation 3-3}$$

In addition, the fraction present as free uncharged ammonia (NH_3), expressed as percentage of total ammonia, in the aqueous solution can be also calculated with the following expression:

$$f\,NH_3 = 1 / (10^{pKa - pH} + 1) \qquad \textbf{Equation 3-4}$$

(Nordin 2010) asserts that the fraction of dissolved ammonia NH_3 (aq) is affected by pH or temperature, or even both. The influence or impact of temperature is greater for a moderate alkaline p H (8-10), whereas at

a pH of 11 more than 90% of the ammonia is present as NH_3, regardless of temperature, as it could be appreciated in Figure 3-8.

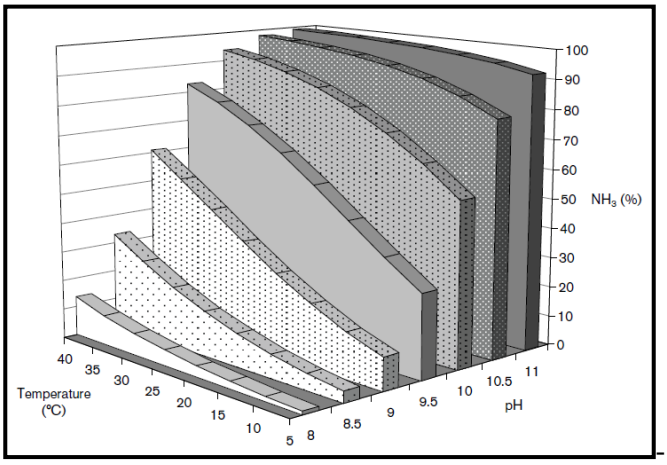


Figure 3-8 Fraction of total ammonia present as NH_3 (aq) at combinations of pH 8-11 and temperatures 5-40ºC (Nordin 2010).

Throughout the literature on ammonia-based sanitation, there are some ambiguities when referring to the term "ammonia" since a differentiation between the chemical species ammonium (NH_4^+) and ammonia (NH_3) is not necessarily done. As well, the analytical methods to measure ammonia in waste and liquid waste can use terminology as NH_3-N (ammonia-nitrogen) or NH_4-N (ammonium-nitrogen) that create some confusion in the nomenclature. In this study, the expression TAN refers to Total Ammonia Nitrogen (NH_4^+ + NH_3) which is the ammonia measured in sludge and/or in wastewater. From here on, when NH_3 is indicated, it refers as uncharged ammonia and its concentration is given in g/L.

3.5.2. Inactivation mechanism

Ammonia molecules are known to be highly soluble in water as well as in lipids. This mechanism may enhance the ammonia transportation over the membranes and other cellular walls by simple diffusion. The effects of the ammonia are the destruction of the membrane potential, as well as the denaturalisation of the bacterial membrane and cell proteins, as it stated by [(Bujozek, 2001), cited in (Nordin 2010)]. In addition, the ammonia gas causes cell damage by a quick alkalinisation of the cytoplasm. in order to maintain an optimum internal pH, the cells have to take protons from the outside but sacrifice potassium ions (K^+) instead, which will eventually lead to the bacterial cell decay.

CHAPTER 4

SAFETY GUIDELINES WHEN WORKING WITH BIOLOGICAL INFECTIOUS MATERIAL

The World Health Organization acknowledges the importance to work safely in laboratories especially when manipulating biological infectious materials. As (future) sanitary engineers, our task is to ensure the environmental quality and public health, meaning that professionals in the sanitation field will be always exposed to biological hazardous materials.

In this regard, it is extremely important that wastewater treatment plants and research laboratories that work with bio-hazardous material namely wastewater and sludge develop safety protocols; adopt and implement safe practices while working with pathogenic microorganisms. This will secure biological assets and will reduce threats to public health during clinical and research work. Consequently, aspects such as personal responsibility and risk assessment need to be stressed out while practising such safety guidelines.

The WHO classifies risk groups according to infective microorganisms, where wastewater treatment plants and its laboratories fall under the Risk Group 2 category (moderate individual risk, low community risk).

"A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited." (WHO 2004)

It is important to take into consideration that the personnel exposed to water-borne pathogens or are in contact with sewage, follow an adequate vaccination programme to reduce the potential risks of water-borne diseases. This recommendation is based on the fact that this study implicated the manipulation and exposure to potentially infectious material present in blackwater and faecal sludge. In this sense, a rigorous vaccination and medication plan was followed for two months, in order to be protected for the working conditions to be encountered in Malawi, Africa. The vaccination programme included doses of Hepatitis A and B; Diphtheria, Tetanus, Poliomyelitis (DTP) vaccine, typhoid fever vaccine and malaria prophylaxis.

In general terms, any technical procedure must be executed carefully to avoid the formation of aerosols and droplets. During the execution of any task, one must use care caution when working with biological infectious material and personal protection equipment must be worn at all times. Besides, the best defence against water-related infections is to practice good hygiene and good housekeeping.

These biosafety guidelines can cover aspects related to collection, transportation, handling, storage and working precautions while manipulating infectious material like wastewater and (faecal) sludge. The information expressed in this section is a combination of standardised safety manuals, lecture from Dr. Leslie Robertson, Biological Safety Office at Delft University of Technology and collected personal experience from the laboratory and field work during this research. Furthermore, what it is expressed in this chapter are only suggestions and they are not intended to be policy and are not all inclusive. The safety measures depend on the particular circumstances and location.

4.1. Collection

- Collection of the potentially infectious material should be done in closed containers that avoid spillages and are easy to handle like jerry cans.
- Devices used for collection such as taps and hoses should avoid splashes and should not create aerosols.

4.2. Transportation

- The infectious material should be transported in adequate and authorised vehicles.
- The car/truck should have proper loading and off-loading tools and devices.
- The containers must be very well secured and fastened, in order to avoid spillages and overturns.
- The driver responsible for the transportation must be trained in handling hazardous material.
- The vehicle should be intensively sanitised after collecting and transporting the material.

4.3. Storage

- All material that is stored must be properly labelled with the following information:
  - To whom it belongs
  - Date
  - Concentration of solution
  - Name of substance
- When storing material for preservation in a cold room, the containers should be isolated or stored in a special area to avoid cross contamination of other samples.
- The storage area must be very well ventilated, illuminated, and spacious, with surfaces easy to clean and sanitised.

4.4. Waste management

Waste is defined as everything that is discarded.

- All contaminated material that cannot be reused or recycled should be decontaminated, sterilised or incinerated within the laboratory, following an approved procedure.
- In case of transporting the waste to another facility, the objects should be packed in a approved manner

4.4.1. Decontamination

Autoclaving is the best method for decontamination of materials. When autoclaving, the material should be contained in special autoclavable colour-coded bags that indicate whether the object should be autoclaved and/or incinerated.

4.4.2. Handling and disposal

- The laboratory must have an identification and separation system for the generated infectious materials. National and international regulations state the following categorisation (WHO 2004):
  1. Non-infectious waste: It can be reused and recycled. It can be classified as household waste.

2. Contaminated (infectious) “sharps”: hypodermic needles, broken glass, knives, and blades. These objects should be treated as infectious and should be disposed in puncture-proof containers with covers.
3. Contaminated material that can be decontaminated by autoclave and reused or recycled afterwards.
4. Contaminated material to be autoclaved and finally disposed.
5. Contaminated material for direct incineration.

4.5. Personal Protection

- Use the correct personal protection equipment, e.g., safety glasses, gloves, laboratory coat, for each job task.
- Laboratory coats or uniforms must be worn all the time for work. Clothing must protect arms and legs and should not be loose-fitting.
- Wearing protecting coats or gowns outside the laboratory, i.e., cafeteria, libraries, offices, meeting rooms; must be strictly prohibited.
- Contaminated clothes must be washed regularly, preferably on-site. It is not advisable to bring contaminated clothes home. Keep the working clothes (and shoes) at working areas. Bringing the contaminated clothes outside work may expose other people to pathogens.
- Personal belongings and street clothing must be stored in lockers and should not be mingled with laboratory garment.
- Selection of gloves depends on type of work involved. Latex gloves must be used for lab procedures when potential infectious material is involved. In addition, special (rubber) gloves must also be used when handling heavier equipment like containers or drums full of sludge or wastewater. After use, gloves must be removed carefully and disposed accordingly.
- Hands must be washed and disinfected properly and constantly with disinfectant soaps/alcohol, especially after working with infectious material and before leaving working areas. Make sure to wash and scrub under nails with a brush.
- When necessary safety glasses, goggles or face shields must be worn to protect eyes and face from splashes, impacting objects, etc.
- Safety protective filter masks should be worn when exposed to bio-aerosols and/or, toxic and corrosive fumes with pungent smells.
- Special (rubber) boots or shoes must be worn all time and they must not be open-toed footwear.
- Contaminated footwear with infectious material must be cleaned thoroughly. A washing area could be designated with washing devices like trays with sanitising soaps and/or chlorine; it could be placed before entering to working areas.
- No eating, drinking, smoking, using cosmetics and handling contact lenses must be allowed in the laboratories.
- Take a shower at the end of the shift.
- If you get sprayed or soaked with sewage or sludge, change clothes, take a shower.
- Usage of laboratory equipment for cooking and refrigeration must be strictly forbidden.
- Stationery items like pens, pencils, notebooks, etc., used during laboratory sessions may be contaminated with infectious material and should be disinfected before leaving the working area, otherwise they should remain within the laboratory facilities.

4.6. Other precautions

- The international biohazard symbol must be displayed on the rooms where pathogenic microorganisms are handled.

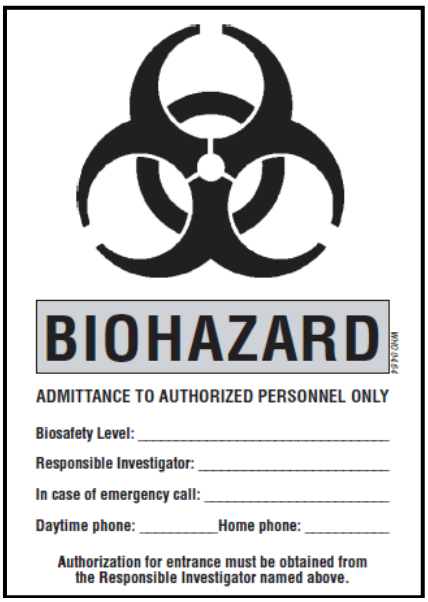


Figure 4-1 Biohazard warning sign for laboratory areas

- Laboratory doors must remain closed to avoid contamination of other rooms.
- Only authorised personnel should be allowed to enter in the designated working areas.
- Use the hood when manipulating chemicals or materials that contain toxic gases or offensive smells.
- Accidents and major spillages should be reported to the laboratory supervisor and a protocol for the clean-up must be followed.
- Written records of incidents should be created or maintained.

Laboratory facilities and design features

- Working areas should be spacious that facilitate the cleansing and maintenance tasks.
- Walls, floors, bench tops and working surfaces should be smooth, impermeable, easy to clean and resistant to chemicals and disinfectants.
- Spaces should be kept neat and clear and not overcrowded with equipment and materials.
- The laboratory working areas must be provided with cleansing tools and chemicals, like paper towel, disinfectant agents (soaps, ethanol 70% and bleach), brush and dust pan, and garbage bins, among others. These items should be close at hand.

Finally, special attention must be given to training and education, since the experience shows that most of the accidents and injuries occur by unsafe work practices or incorrect procedures, combined with poor training and inadequate supervision.

In this regard, it is the responsibility of the facilities and workers to revise their own situation according to the risks they are exposed to and to apply the correct protection measures to guarantee a safe working environment.

CHAPTER 5

Materials and Methods

A two-phase study was carried out to evaluate the sanitising potential of faecal sludge with ammonia obtained from urea. In a laboratory experiment in UNESCO-IHE, Netherlands, black water from vacuum toilets was treated with urea. In a second phase in Blantyre, Malawi the ammonia study was scaled-up to treat faecal sludge from pit ;latrines..

5.1. LABORATORY STUDY IN NETHERLANDS

5.1.1. Wastewater used in the experiments

Due to the non-availability of faecal sludge in the Netherlands, several waste materials were analysed before the sanitising experiment, aiming to find the one with the most similar characteristics to faecal sludge. In a first attempt, primary sludge was collected from Harnaschpolder wastewater treatment plant, where its solids and microbiological content were analysed. The total solids test revealed that the sludge was merely toilet paper, hence it was considered not be representative.

On a second attempt, black water from a demonstration project for decentralised sanitation system was selected for the study. The fresh material was collected from the facilities of the company Landustrie in Sneek, Friesland. The black (toilet) water originated from 32 rental accommodations located in the Lemmerweg-Oost neighbourhood of Sneek. These households were equipped with vacuum toilets that require minimal volume of water for flushing.

The material was collected and transported in 10 L plastic buckets and stored at laboratory's cold room at 4°C with no chemicals addition for preservation. The batch of black water selected for the experiments was characterised in terms of total solids, volatile solids, temperature, conductivity, pH and pathogens concentration.

The laboratory phase in the Netherlands had the main objective to become familiar with safe working techniques while manipulating infectious material. Considering the pathogenic risks of the black water, it was decided to sterilise it and spike it with a non-pathogenic strain of *Escherichia coli* to a concentration of 10^8 CFU/100 mL. This figure represents the average concentration found in domestic wastewater. Inasmuch as the tests with spiked sludge did not give satisfying results, regarding the urea hydrolysis and pH increase, the approach changed and raw blackwater was used instead to run the sanitisation experiments.

5.1.2. Urea treatment

Considering the context of an emergency situation, the approach weight/weight (w/w) was decided to be used since it is a simple and quick way to calculate the amount of urea to add per mass of sludge.

Extra pure pearls of urea [$CO(NH_2)_2$] was added to the black water in concentrations of 1%, 2% and 3% (w/w) based on the wet weight of the sample (Acros Organics, Germany, 98%). In addition, a free-urea reactor was established as a control (0%). Each concentration was tested in a set of duplicates. The

treatment was evaluated by performing two batch tests at constant temperatures of 20°C for 16 days and 30°C for 8 days in 500 mL sealed glass bottles, to prevent the ammonia volatilisation.

Figure 5-1 Black water used in experiments

Figure 5-2 Urea treatment set-up

Table 5-1 Mass of black water and added urea used in the laboratory experiments

Urea treatment (% w/w)	Mass of black water (g)	Mass of urea to add (g)
0	500	0.0
1	500	5.1
2	500	10.2
3	500	15.3
TOTAL UREA/BATCH TEST (g)		**30.6**

For the urea addition it was assumed 1 g equals to 1 mL, considering that the material was merely water. The reactors were mixed manually for approximately one minute after the addition of the urea pellets and prior to each sampling, in order to have, as much as possible, homogeneous conditions.

Constant mixing was not considered to be applied in this study, as the black water used for the experiments had low dry matter content. This characteristic facilitates a fast degradation and diffusion of the urea, as this compound is highly soluble in water. The experiments were performed with respect to pH, ammonia concentrations and microorganism reduction.

5.1.3. Solids measurements

The black water samples were dried and ignited for determination of total solids (TS) and volatile solids (VS), respectively. The black water was dried at 105°C for 2 hours for TS and ignited on the furnace at 550°C for another 2 hours. Additionally, the moisture and organic content on the faecal sludge were also calculated. The following set of equations was used for the solids measurements.

$$TS\ \% = 100 \cdot \frac{dry\ sample\ @105°C - weight\ cup}{Wet\ sample - \ weight\ cup}$$ **Equation 5-1**

$$VS\ \% = 100 \cdot \frac{dry\ sample\ @\ 105°C - ignited\ @550°C}{dry\ sample - \ weight\ cup}$$ **Equation 5-2**

$$Moisture\ content\ \% = 100 \cdot \frac{wet\ sample - dry\ sample}{wet\ sample}$$ **Equation 5-3**

$$Organic\ content\ \% = 100 \cdot \frac{dry\ sample - ignited\ sample}{dry\ sample}$$ **Equation 5-4**

5.1.4. pH measurements

pH was monitored with pH electrode, before and after the addition of the urea (WTW, Weilheim, Germany). The reactors were mixed manually for 1 minute before each sampling. For analysis of pH, 10 mL of material were removed from each replicate.

An additional test of pH was done to check if the addition of the urea itself changes the pH of the faecal material on a control (0%) and 1% sample.

5.1.5. Total ammonia-nitrogen concentrations

For the chemical analysis of total ammonia-nitrogen (TAN), 10 mL of the black water were taken from each replicate. The samples were centrifuged at 1200 RPM for 12 minutes, to remove the solids that may interfere with the test method (Rottina 420, Hettich, Germany) In addition, the samples were diluted 1000x in order to obtain the most accurate results possible in the analytical procedure.

The salicylate method was analytical procedure to be used for the ammonia-nitrogen quantification. The samples were analysed with spectrophotometer UV -VIS at 655 nm with 1 cm cells for samples in the range of 0.05 - 2.0 mg NH_3-N/L (DR 600, HACH LANGE, Germany). In the colorimetric method, two reagents: salicylate and cyanurate react with ammonia to create a green-coloured compound. In this method, the colour intensity is proportional to the analyte (ammonia) concentration.

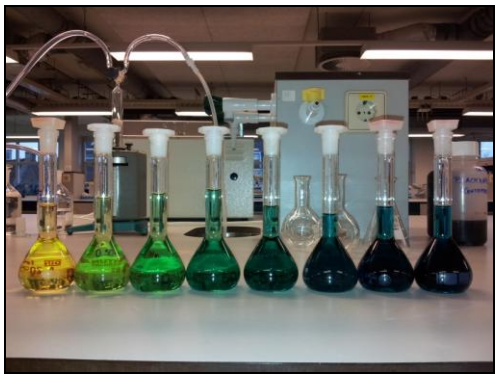


Figure 5-3 Preparation of ammonia-nitrogen calibration curve

5.1.6. Microbiological analysis

Bacteria present in the raw black water were cultured in Chromocult® coliform agar, a culture media that enables easy detection of *E. coli*, *Salmonella* and total coliforms and avoids the need for isolation of pure cultures and confirmatory tests (Merck, Germany). The Pour Plate technique consisted of transferring an aliquot of 0.1 mL from the 10 mL solution into the agar plates. The samples were diluted in saline solution (0.8% NaCl), to avoid osmotic shock in the microorganisms. The samples were prepared into 10-fold series (1:10, 1:100, 1:100, and 1:1000), where each dilution was plated in duplicates. Enumeration of colony forming units (CFU) for *E. coli*, *Salmonella* and total coliforms was made after incubations at 37°C after 24 - 48 hours (APPENDIX B).

The enumeration of the colonies was calculated using the following equation (Lubberding 2013):

$$CFU/100\,mL = \frac{CFU\ per\ plate\ (average) * 1000 * 1}{dilution}$$ **Equation 5-5**

Where 1000 = from 0.1 mL (poured on plate) to 100 mL
Dilution: (1/10, d = 0.1)

5.2. FIELD STUDY IN MALAWI

5.2.1. Faecal sludge used in the experiments

The faecal sludge to be used in the sanitisation study in Malawi was collected from three different locations: household latrines and a local market. However, only one single batch of faecal sludge was used for the urea treatment.

The selected faecal material was a seven-years-old sludge, which and it was collected from a household pit latrine. During the desludging process approximately 200 L of water were used to fluidise the faecal matter; a total of 800 L of faecal sludge were collected from the latrine and an estimated amount of 120 L of rubbish (2 bins) was collected from the pit (APPENDIX C).

Figure 5-4 Fishing of rubbish during desludging of household latrine

Figure 5-5 Filling of drums with collected faecal sludge

5.2.2. Urea treatment

The treatment was evaluated at environment temperature, which was on average 24˚C, using 0%, 1% and 3% urea concentrations (w/w). 2% urea addition was not tested in view of the previous experiments done in the Netherlands, where the results did not show significant difference compared to 1% and 3%.

The sanitisation study started four hours after the collection of sludge. A urea-free storage (0% w/w) represented the reference for the chemical and microbiological treatment. The urea used for the experiments had a purity of 40% and it was sourced from the local agricultural shops. For the urea dosage, the measured density of the sludge was 1.07 g/mL, so the equivalence of 1 kg = 1 L was also applied. For the urea addition, the calculation was done as follows:

$$\text{Urea dosage} = \frac{\text{weight FS x urea conc. (\%)}}{\text{urea purity (\%)}}$$ **Equation 5-6**

50 L drums were filled with 25 - 30 L of faecal sludge and weighed. The reactors were stored on a shelter in the facilities of Zingwangwa wastewater treatment plant in Blantyre as it depicted in Figure 5-7.

Figure 5-6 Addition of urea to faecal sludge

Figure 5-7 Reactors used for the urea treatment

Table 5-2 Weighted faecal sludge and amount of urea used in the experiments in Malawi

Urea treatment (% w/w)	Number of reactors	Mass of FS (kg)	Mass of urea to add (kg)	g urea/kg FS
0	1	31.4	0.00	0.0
1	1	33.8	0.85	25.0
3	1	33.5	2.51	75.0
		TOTAL UREA/BATCH	**3.4**	

One single batch was done for the ammonia treatment, meaning that only one reactor was used per concentration. The reactors were hermetically sealed with an aluminium ring to avoid ammonia losses.

After the addition of urea pellets, the sludge was manually mixed with a stick for 3 minutes approximately. For the sampling, the drums were manually agitated for one minute. The samples were collected in sampling cups (100 mL) from the taps installed at the bottom of the barrels. No mechanical mixing devices were able to be adapted inside the drums.

The treatment ran for a period of 8 days. After the treatment, the sludge was disposed at the wastewater treatment plant.

5.2.3. Total and volatile solids measurements

The faecal sludge samples from the three different batches were analysed at the laboratory facilities of The Polytechnic - University of Malawi. The samples were dried at 105°C for 2 hours for TS and combusted on the furnace at 550°C for 2 hours. The equations used for the solids measurement in the faecal sludge were detailed in section 5.1.3.

Figure 5-8 Total solids measurements in the faecal sludge

5.2.4. pH measurements

pH was measured with pH electrode and probe before the addition of urea (HACH multimeter, Germany). During the treatment, pH was monitored almost every day from the collected samples, where 100 mL of material were removed from each drum.

5.2.5. Ammonia-nitrogen concentrations

For the quantification of the Total Ammonia Nitrogen (TAN = NH_3 + NH_4^+), 100 mL of the sludge were taken from each drum. The samples were diluted 10,000x and 100,000x in order to obtain the most accurate results possible in the analytical procedure.

No centrifugation or filtration technique was used. In this regard, large dilution rates were applied, from the range of 10,000x to 100,000x. The content of ammonia nitrogen was measured spectrophotometrically using ammonia kit reagents [HACH. Low range (0 - 2.50 mg NH_3-N/L), Test 'N Tube. Method 10023]. In this colorimetric method, two reagents: salicylate and cyanurate react with ammonia to create a green-coloured compound; the colour intensity is proportional to the analyte (ammonia) concentration.

5.2.6. Microbiological analysis

The reduction in bacteria *E. coli*, *Salmonella* and total coliforms was monitored by sampling the sludge for its content of pathogens at the start (0 day) of treatment, 4 days, 6 days and 7 days. The first analysis for quantification of *E. coli* and total coliforms amounts in the faecal sludge was done with Brilliance™ *E. coli*/coliforms selective agar; the subsequent microbiological samples were cultured on Chromocult® Coliform Agar (Merck, Germany).

Pour Plate technique which consisted of transferring an aliquot of 0.1 mL into the agar plates. The samples were diluted in saline solution (0.8% NaCl), to avoid osmotic shock in the microorganisms. The samples were prepared into 10-fold series (1:10, 1:100, 1:100, 1:1000 and 1:10,000). Enumeration of colony forming units (CFU) for *E. coli*, *Salmonella* and total coliforms was made after incubating the dishes at 37°C after 24 - 48 hours.

Pathogens reduction kinetics

The reduction of the studied bacteria was estimated based on a first-order exponential decay function $N_t = N_0\, e^{kt}$, where N_0 is the concentration of microorganism at time zero, N is the concentration of active bacteria at time t and k is the first-order rate constant.

The collected data from all the experiments for each urea treatment and temperature were analysed with linear regression, where the microbial concentration was plotted against time on a log scale, according the aforementioned equation. The graphs were made in MS Excel for Windows.

CHAPTER 6

Results

6.1. Ammonia treatment in black water in Netherlands

The laboratory study in the Netherlands was carried out in two batches: a first test performed at the laboratory's ambient temperature of 20°C for 16 days and a second batch test done at a room with fixed temperature of 30°C for 8 days. The tests were carried out from mid November 2013 to mid January 2014 at UNESCO-IHE laboratory facilities.

6.1.1. Black water characteristics

Table 6-1 summarises the results obtained from the parameters that were characterised in the raw black water collected from vacuum toilets. The analyses were made three hours after the black water was collected. The most important parameters for the research purpose were pH (6.9), ammonia-nitrogen (1.45±77 g/L with SD 3.5%) and concentrations of indicator organisms such as *Escherichia coli*, *Salmonella* and total coliforms.

Table 6-1 Physical, chemical and microbiological characteristics of collected black water

PARAMETER	UNIT	VALUE
Temperature	°C	22
pH	pH units	6.9
Conductivity	µS/cm	1,330
Total Solids	%	1.5
Ammonia-nitrogen	g/L	1.5
E. coli	CFU/100 mL	$1.5 \cdot 10^8$
Salmonella	CFU/100 mL	$6.2 \cdot 10^7$
Total coliforms	CFU/100 mL	$2.8 \cdot 10^7$

6.1.2. pH and ammonia concentrations at room temperature (20°C)

Fresh collected black water was used for this batch test at 20°C. The recorded pH in untreated black water (0%) varied from 7.0 to 6.8. Irrespective of the initial low pH of the untreated black water, the addition of 1%, 2% and 3% (w/w) urea resulted in an increase of pH to approximately 9.2 within 3 days, derived from the urea hydrolysis. The raise and stabilisation of the pH in the black water is one of the crucial requisite for the ammonia formation. No decrease in the pH was observed in the treatments. Figure 6-1 and Table 6-2 show the pH recorded values during the first experiment with urea addition.

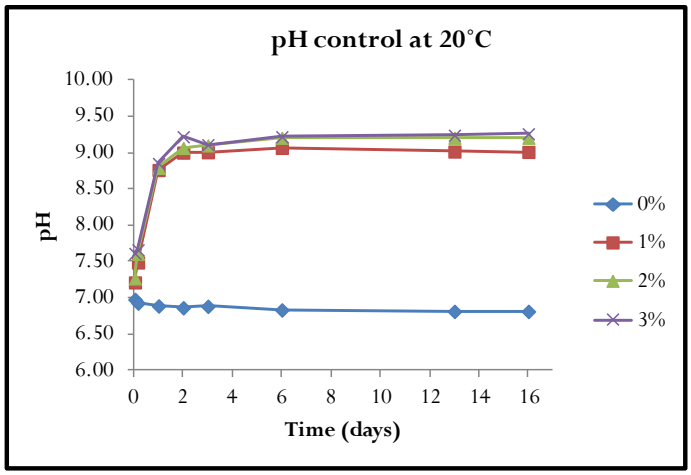


Figure 6-1 Recorded pH during the 16-days of laboratory experiments. Data is given in pH units

Table 6-2 Minimum and maximum recorded pH values during urea treatment in black water

Faecal treatment	pH readings	
	Min	Max
3%	7.6	9.3
2%	7.3	9.2
1%	7.2	9.0
0%	6.8	7.0

The total ammonia nitrogen (TAN) concentrations measured at the end of the batch test were significantly higher compared to the values encountered in the untreated black water of 1.45 g/L. At day 16, higher TAN concentrations were measured in all the treatments (including storage). In the latter, the ammonia concentrations were 1.67 g/L; in 1% reactor was 6.24 g/L; in 2%, 11.5 g/L and in 3%, 15.8 g/L. No trend of decreasing ammonia concentration was observed during the experiment. In samples with low pH > 6.9 with no urea addition, no NH_3 is formed despite the presence of organic ammonia in the faecal material.

Table 6-3 is a summary of all the calculations made on ammonia. The intrinsic ammonia represents the organic ammonia already present in the black water; the ammonia from urea is the calculation of ammonia that is obtained from the urea (assuming 100% degradation), based on stoichiometry; the theoretical total ammonia is the sum of the previous two calculations; TAN represents the total ammonia- nitrogen (NH_4^+ + NH_3). Finally the fraction of free NH_3, it represents the percentage of unionised ammonia from the TAN.

Table 6-3 Calculations of estimated theoretical ammonia together with the measured TAN and the fraction of uncharged ammonia (NH_3), calculated from recorded pH at 20°C)

UREA TREATMENT ON BLACK WATER @ 20°C

AMMONIA BALANCE EXPRESSED IN CONCENTRATION (g/L)						
Urea treatment (%)	Intrinsic ammonia	Ammonia from urea	Theoretical total ammonia (urea + intrinsic)	Measured TAN	Free uncharged ammonia fNH_3 (%)	
					Min	Max
1	1.5	5	6	6	0.8	33
2	1.5	9	11	11	0.9	44
3	1.5	14	15	16	2	47
AMMONIA BALANCE EXPRESSED IN MASS (g)						
Urea treatment (%)	Intrinsic ammonia	Ammonia from urea	Theoretical total ammonia (urea + intrinsic)	Measured TAN	Free uncharged ammonia fNH_3 (%)	
					Min	Max
1	15	3	17	62	0.8	33
2	15	6	20	115	0.9	44
3	15	9	23	158	2	47

6.1.2.1. Microbial analysis

Reduction of *E. coli*, *Salmonella* and total coliforms was studied in relation to pH, temperature and ammonia concentrations. In the first batch test executed at 20°C, the inactivation rates in the untreated black water (0%) showed a natural die-off trend, which it is explained on the batch conditions of the culture. The urea treatment resulted in a faster removal of *E. coli* and *Salmonella* compared to those in the untreated material (0% urea), even at the lowest concentration.

As regards for *E. coli* bacteria, more > 5 $\log_{10}$ units removal was achieved in 6 days with the highest urea concentration (3%); the same log removal was reached yet in 16 days with the lowest concentration (1%) (Figure 6-2). *Salmonella* bacteria seemed to be more sensitive to the treatment and more than 5 $\log_{10}$ units were removed in 6 days only in the three urea treatments. Also; *Salmonella* declined by natural means 4 $\log_{10}$ units during the same time frame (Figure 6-3). On the contrary, the results revealed that total coliforms were more resistant to the ammonia treatments, yet a small but significant reduction of 3 $\log_{10}$ units was noticed in the highest urea concentration of 3% (Figure 6-4).

The non-detectable (ND) or shaded area refers to concentrations of bacteria that are below 3 $\log_{10}$ CFU/100 mL or >1000/100 mL (WHO 2006) and are not able to be enumerated.

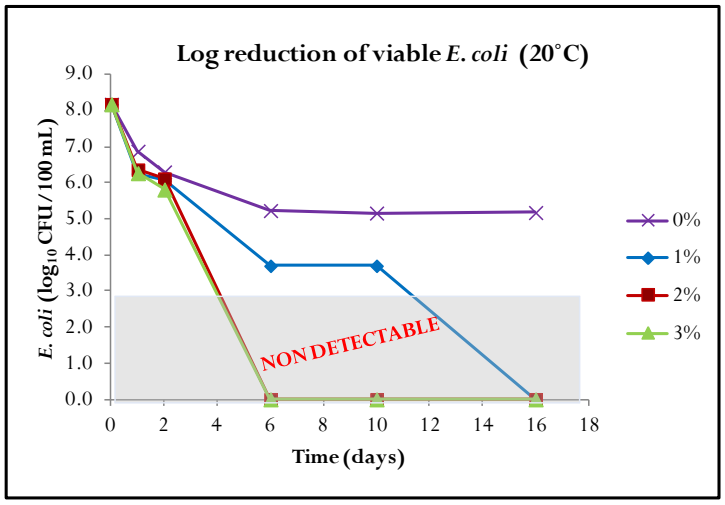


Figure 6-2 *E. coli* in control and urea treated storages in laboratory treatments at 20°C

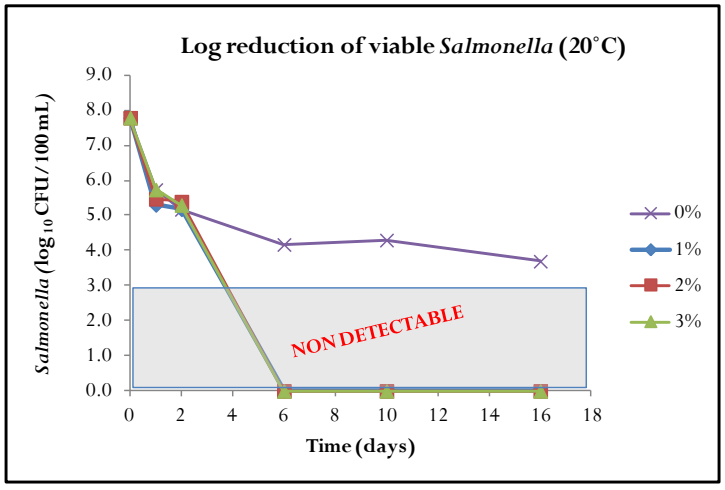


Figure 6-3 *Salmonella* in control and urea treated storages in laboratory treatments at 20°C

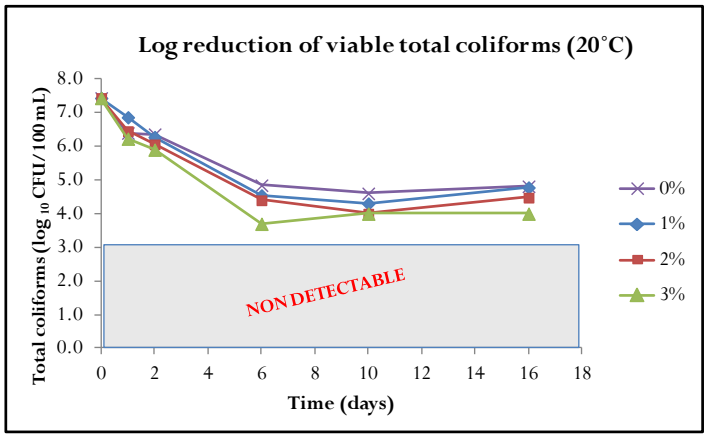
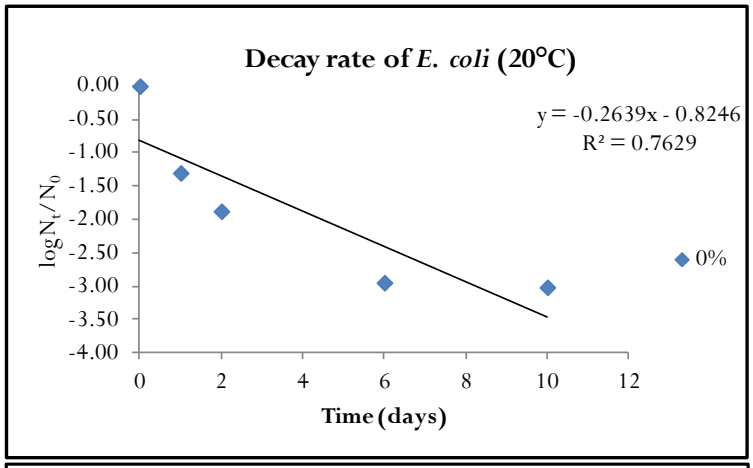


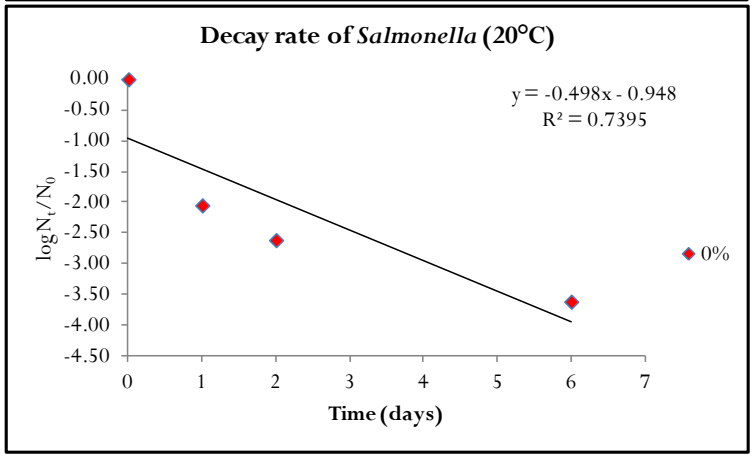
Figure 6-4 Total coliforms in control and urea treated storages in laboratory treatments at 20°C

Reduction rates of studied microorganisms

The inactivation rates of *E. coli*, *Salmonella* and total coliforms in the untreated faecal sludge (0%) were calculated and analysed by linear regression, even though the decay does not follow a clear linear curve.

The expression log N_t/N_0, that represents the negative decay rate ($-K_t$) was plotted against time t (days) (Figure 6-5). The calculations gave results of decay factor of 0.26 d^{-1} for *E. coli*, 0.5 d^{-1} for *Salmonella* and 0.39 d^{-1} for total coliforms, indicating that *Salmonella* bacteria died faster than coliforms and *E. coli* in the untreated storage.





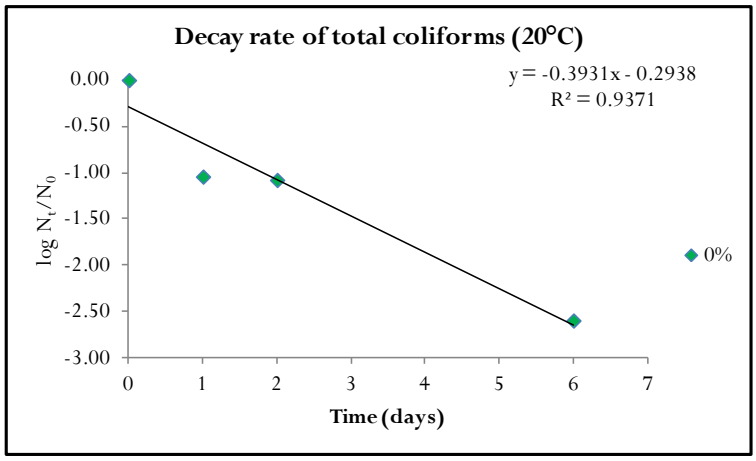


Figure 6-5 Inactivation rates of *E. coli*, *Salmonella* and total coliforms in the untreated storage at 20°C

6.1.3. pH and ammonia concentrations at room temperature 30°C

In the second batch test performed at higher temperature of 30°C The pH in untreated black water (0%) varied insignificantly from 7.09 to 7.66. The addition of 1%, 2% and 3% (w/w) urea increased the pH up to a minimum of 8 within 2 days, derived from the urea hydrolysis. No decrease in the pH and ammonia concentrations was observed in this batch of treatment (Figure 6-6 and Table 6-4).

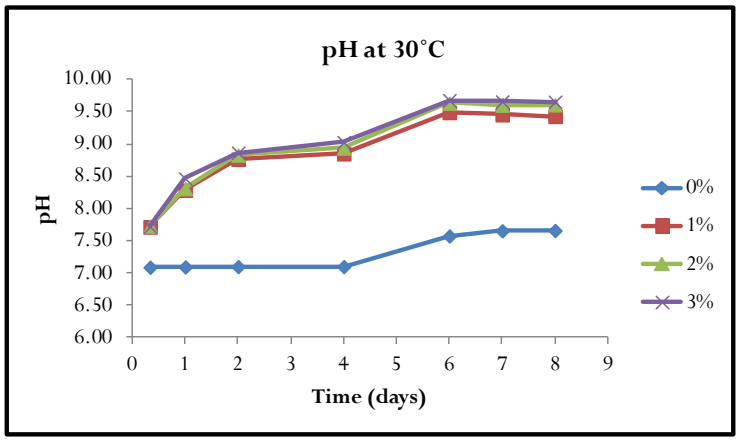


Figure 6-6 Recorded pH during the 8 days of laboratory experiments. Data is given in pH units

Table 6-4 Minimum and maximum recorded pH values during urea treatment in black water

Faecal treatment	pH readings	
	Min	Max
3%	7.72	9.68
2%	7.73	9.64
1%	7.72	9.49
0%	7.09	7.66

The quantified TAN at the end of the second batch test was also significantly higher compared to the collected values from the untreated black water of 0.73 g/L (intrinsic ammonia). At day 8, higher TAN concentrations were measured in all the treatments (including the control). In the latter, the ammonia concentrations increased up to 0.83 g/L at the end of the treatment; in 1% reactor was 6.5 g/L; in 2%, 12.7 and in 3%, 16 g/L. The values of ammonia obtained from urea come from the stoichiometry and the grams of added urea to each treatment from Table 5-1.

At 30ºC, the calculated values of free unionised ammonia (*f* NH_3) resulted higher than those at 20ºC, meaning that a higher percentage of the total was obtained from the total ammonia as temperature and pH increase. For instance, at 3% at 30°C, 82.5% is NH_3 out of the total ammonia-nitrogen (NH_4^+ + NH_3), whereas, for the same urea concentration at 20°C the value is 47.5%, almost half (Table 6-3).

Table 6-5 Calculations of estimated total ammonia NH_{tot} in the black water treatments together with the measured Total Ammonia-Nitrogen (TAN) and the fraction of uncharged ammonia (NH_3), calculated from recorded pH at 20°C (Table 6-4)

UREA TREATMENT ON BLACK WATER @ 30°C

AMMONIA BALANCE EXPRESSED IN CONCENTRATION (g/L)						
Urea treatment (%)	Intrinsic ammonia	Ammonia from urea	Theoretical total ammonia (urea + intrinsic)	Measured TAN	Free uncharged ammonia fNH_3 (%)	
					Min	Max
1	0.7	5	5	7	5	75
2	0.7	9	10	13	5	81
3	0.7	14	15	16	5	82
AMMONIA BALANCE EXPRESSED IN MASS (g)						
Urea treatment (%)	Intrinsic ammonia	Ammonia from urea	Theoretical total ammonia (urea + intrinsic)	Measured TAN	Free uncharged ammonia fNH_3 (%)	
					Min	Max
1	7	3	10	65	5	75
2	7	6	13	127	5	81
3	7	8	16	160	5	82

6.1.3.1. Microbial analysis at 30°C

The microbial count done on ammonia amended showed that all the studied microorganisms were rapidly reduced more than 2 log_{10} units at the second day of treatment; a lower concentration of 1% it took 4 times as long to achieve the same removal figure.

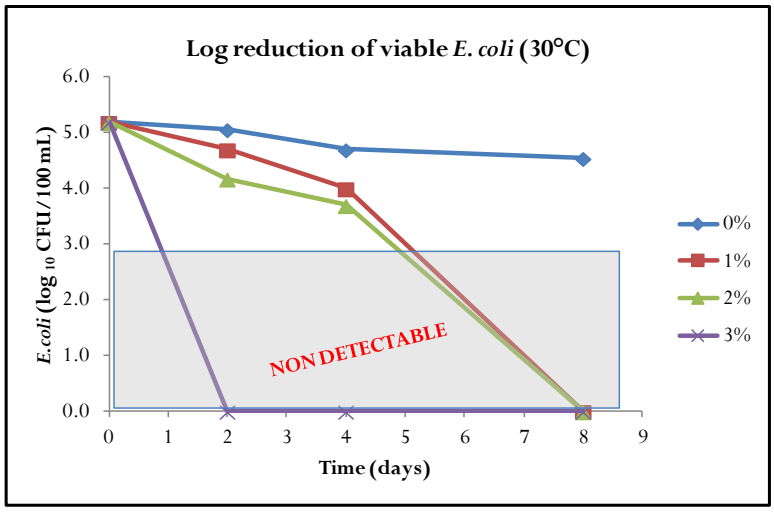


Figure 6-7 *E. coli* in control and urea treated storages in laboratory study at 30°C

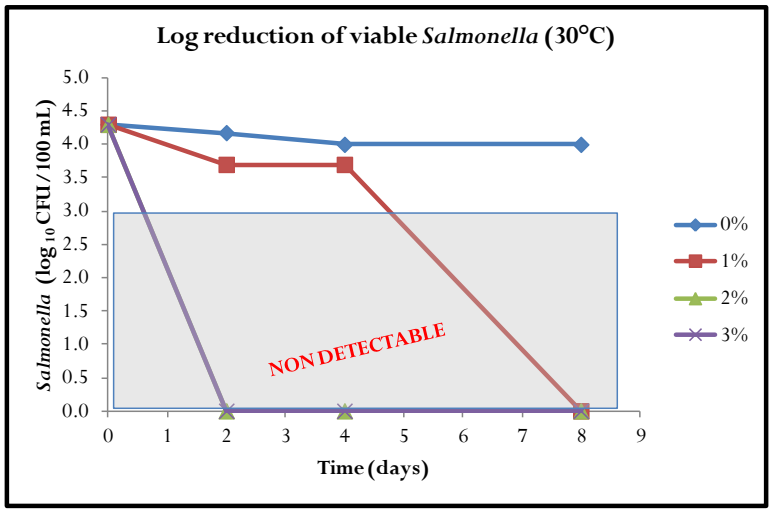


Figure 6-8 *Salmonella* in control and urea treated storages in laboratory study at 30°C

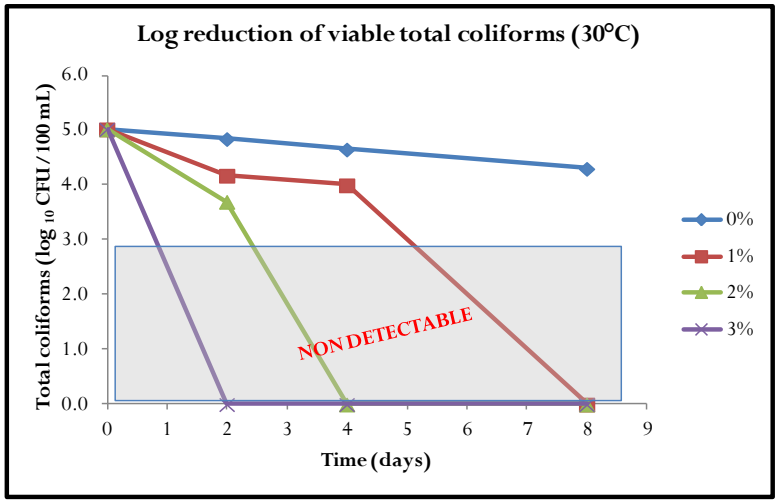
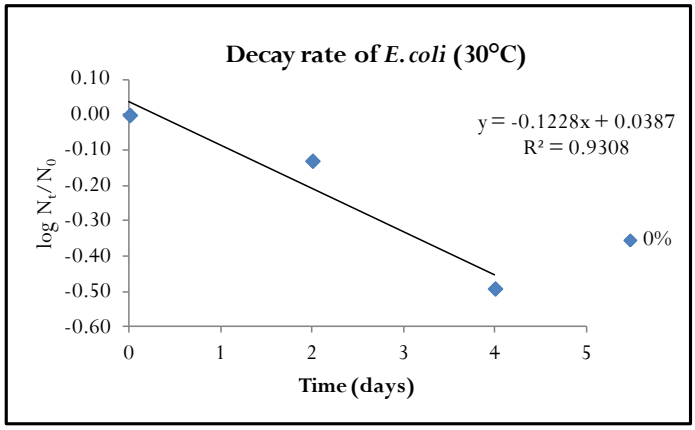


Figure 6-9 Total coliforms in control and urea treated storages in laboratory study at 30°C

Reduction rates of studied microorganisms

The calculations done by linear regression on the treatment at 30°C gave results of decay coefficients *k* of 0.12 d^{-1} for *E. coli*, 0.04 d^{-1} for *Salmonella* and 0.090 d^{-1} for total coliforms, indicating that *E. coli* bacteria got reduced in a faster period compared to coliforms and *Salmonella.*



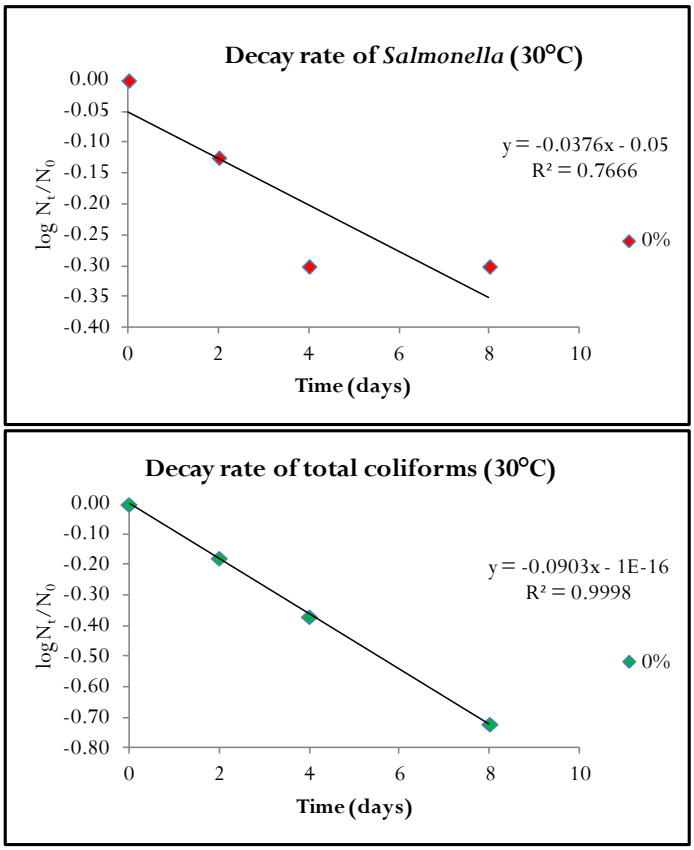


Figure 6-10 Inactivation rates of *E. coli*, *Salmonella* and total coliforms in the untreated storage at 30°C

6.1.4. Urea test in black water

To check if the adding of the urea itself significantly increases the black water pH, an additional test was done with a control (0%) and 1% urea. Before the urea addition to the black water, 1 gram of the chemical compound was dissolved in 5 mL of demi-water and the pH was measured. The pH of the dissolved urea solution was 6.2, neither acidic nor alkaline. Following the same set up for previous batch tests, concentrations of 1% and 0% (w/w) urea concentration were prepared in 500 mL reactors. The pH was measured at time zero and in 15 minutes intervals till the completion of one hour.

The results plotted in Figure 6-11 demonstrated that pH in the urea amended reactor increased 0.97 pH units over 1 hour time, varying from 7.14 to 7.81, which represents a small increase of 9.4%. However, the increase in pH may indicate a fast reduction of the urea to ammonia which was not expected. The pH of the dissolved urea itself was lower than the black water pH (6.2 vs. 7.08/7.14) therefore the urea as chemical compound does not change the pH into alkaline conditions.

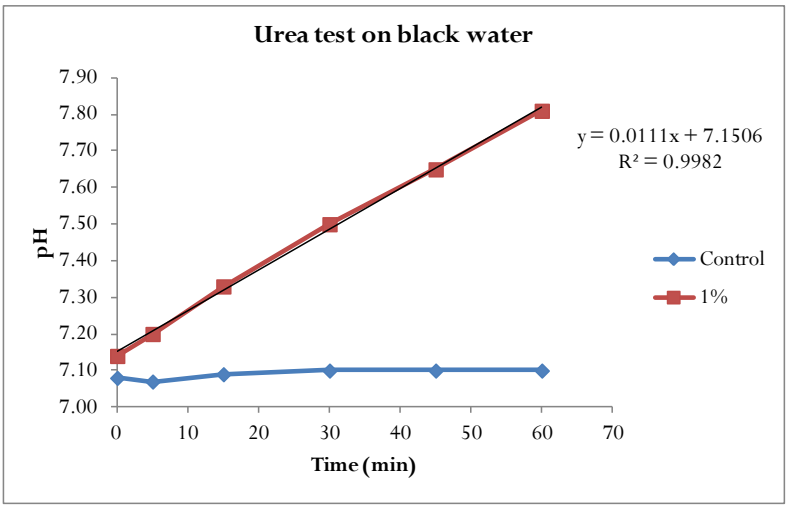


Figure 6-11 Recorded pH values after 1 hour of addition of urea on black water

6.2. Ammonia treatment in faecal sludge in Malawi

6.2.1. Sludge characteristics

During the stay in Malawi, three different batches of faecal sludge were collected during three Fridays in January and February, 2014. The accurate age of the sludge is uncertain. The figures displayed on Table 6-6 are rough estimations made by the contracted desludging company. There was no historical data on the last time the pits were emptied or the pit owner did not know such information. The age suggestion of 1 year, 7 years and 1 month made by desludging personnel was based on the appearance and consistency of the material and the sludge level found in the pit. Regarding the origin of the sludge, the selection and collection of the faecal material follows no specific criterion or routine order for the pit emptying. In addition to the sludge from the market pit latrines, the company in charge of the desludging collects the sludge from pit latrines that have been previously identified by the Red Cross. Hence, the sludge that was able to be collected originated from two private pits and one latrine from the local market in Blantyre.

The physical analyses made on the sludge for temperature, pH, conductivity, total dissolved solids, total solids and volatile solids were done two days after the collection of material, at environment temperature, at the laboratory facilities of the Polytechnic, University of Malawi in Blantyre. This may explain the differences on the results for the recorded temperature, which may be the ambient temperature at which the sludge was exposed on that day at that place and not the temperature of the sludge at the moment of collection.

The mean total solids before the treatment are given in Table 6-6. In general terms, the figures of total solids (5% – 10%) indicate the amount of water present in the sludge samples. The batch of sludge # 1 had an average TS value of 97±3.0 g/L (10±0.30%), whereas batch # 3 collected from the market with an estimated age of 1 month had a concentration of solids of 47±7.16 g/L (5±0.72%). As regards for the organic fraction of the solid material (estimated by weight loss) by burning the sample at 500±30°C, the values of volatile solids point out that around half of the content of solids in the three batches are organics, i.e., in batch #1, 45% of 97g/L is organic material; while that batch #2 contains 55% out of organics if 61±2.7 g/L (APPENDX C). The results obtained from the total and volatile solids measurements show significance variations among themselves. A probable reason of these variations may be the malfunctioning of the oven utilised at the Polytechnic for the drying and combustion of the sludge samples. Moreover, it is assumed that the fluidisation process may have altered the sludge composition and physical characteristic in a significant way.

Batch of faecal sludge # 2 was used for the urea experiments, being one of the reasons why ammonia was measured in that batch only, giving a value of 4.5 g/L. Ammonia concentrations were not measured in batch #1 due to the non-availability of the ammonia kit reagents, caused by the seizure of laboratory equipment from Malawian customs. This unfortunate fact hampered to use the first collected batch of sludge for the ammonia experimentation.

The analysed characteristics of three collected batches of faecal sludge are summarised in on Table 6-6. Although the batch #1 and #3 were not used for ammonia experimentation, the gathered data can help to comprehend better the faecal sludge characteristics.

Table 6-6 Summary of physical/chemical characteristics measured on the collected sludge

		Batch of Faecal Sludge		
PARAMETER	**UNIT**	**1**	**2**	**3**
Collection date		25/01/2014	31/01/2014	07/02/2014
Age of FS		1 year	7 years	1 month
Origin of FS		Household	Household	Market
Temperature	°C	26	21	26
pH	pH units	7.3	7.6	7.3
Conductivity	µS/cm	5,374	8,653	2,600
Total Dissolved Solids	ppm	4,172	7,045	-
Total Solids	%	10	6	5
Volatile Solids	g/L	44	36	28
	%	45	55	59
Moisture content	%	90	94	95
Organic content		45	55	59
Ammonia-nitrogen	mg/L	-	4,500	-

6.2.2. Temperature, pH and ammonia concentrations in sludge treatments

The treatment was carried out at ambient temperature; however the drums were not directly exposed to environmental elements like sunlight and rain. The field study in Blantyre, Malawi, was done during the wetter months: January and February. The treatment was subjected to the weather: 4-6 hours of bright sunshine during the mornings, followed by cloudy and rainy afternoons. The outdoor ambient air temperature during the day could range from 30ºC at midday to a low of 18 ºC during the nights.

Despite the climatic variations, the recorded temperature on the sludge was constant, giving an average value of 24ºC±0.9. The sampling was done during mornings, in which the temperatures ranged from 22 - 25 ºC Table 6-7 display the slightly variant temperature figures recorded on the different urea treatments and in the control. In the latter, the average temperature was 24.1±0.9; in the 1% treatment, the temperature was 24.0±0.9 and in 3% was 24.1±0.9.

Table 6-7 Recorded temperature values in urea treatments, Malawi. Data is given in ºC

Recorded temperature values - Scaled-up experiment			
Day of treatment	**0%**	**1%**	**3%**
0	23.8	23.7	24.2
2	26.0	26.0	26.0
4	24.3	23.9	24.0
6	23.4	23.6	23.8
7	23.3	23.5	23.4
8	23.6	23.5	23.4
AVERAGE	**24.1**	**24.0**	**24.1**
SD	0.9	0.9	0.9
SD %	3.8	3.7	3.7

The pH in the untreated faecal sludge (0% urea) varied from 6.9 to 7.4. Even though there were small variations in the pH measurements, attributed to possible uneven distribution of the sample during the mixing and sampling, the calculated mean pH value of the control was 7.16±0.2 with a standard deviation of 2.25%. As it was stated in the previous section, it is believed that the buffering capacity of the sludge could have been affected by the fluidisation process, where large amounts of water were added to the pit; thus giving neutral pH values of 7 similar to number encountered in drinking water.

After the urea addition the peak pH was recorded at day 2 and it remained stable, as it can been seen in Figure 6-12, meaning that no trend of decrease in pH was observed during the 8-day treatment period. Furthermore, regardless of the initial pH of 7 in the untreated sludge, the urea addition of 1% and 3% (w/w) increased the pH to at least 9. The average pH in the 1% urea reactor was 8.92±0.6 and standard deviation of 6.16%, whereas the 3% treatment had average value of 8.95±0.6 and standard deviation of 8.6%.

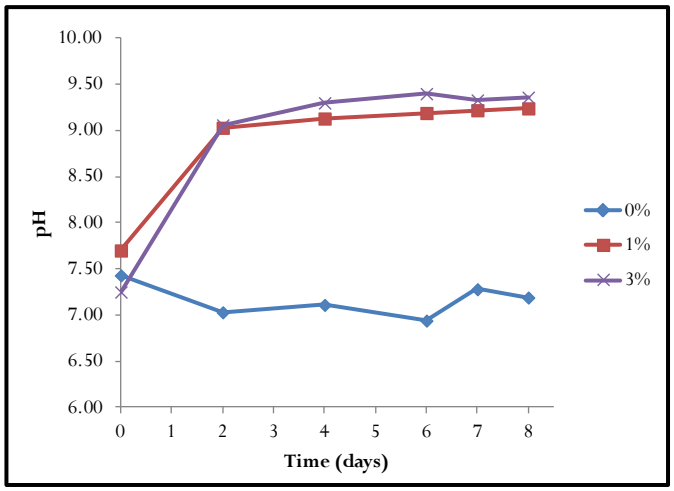


Figure 6-12 Measured pH in the urea treatments at 24°C. Data is given in pH units

The tests demonstrated that the higher the pH, the higher the ammonia concentrations derived from the hydrolysis of urea. For that reason, the ammonia concentrations were measured in the unamended sludge and after the treatment period.

The ammonia analyses made on the liquid fraction of the faecal sludge showed that the ammonia concentrations in the samples were significantly higher compared with the samples where no urea had been added. Figure 6-13 shows the linear increase of the ammonia concentrations in 1% and 3% (w/w) treatment with time, in correspondence with high pH values above 8. The highest value measured at the end of the study was 80 g/L for the 3% (w/w), while the highest value recorded in the 1% (w/w) treatment was 19.1 g/L. It was also determined that the concentrations of the intrinsic ammonia of the sludge in reactor 0% decreased over time, passing from 4.5 to 1.5 g/L. This reduction may be explained by the natural biochemical reactions of nitrification, de-nitrification and ammonification taking place in the reactor under anaerobic conditions.

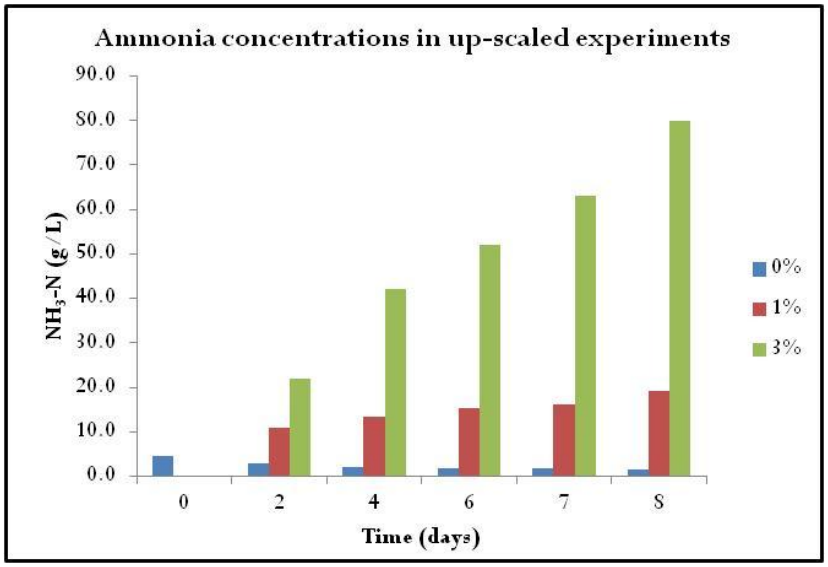


Figure 6-13 Ammonia concentrations measured in the 3 different scaled-up storages for a period of 8 days

In Table 6-8, the intrinsic ammonia refers to the ammonia originally present in the faecal material. Its concentration was measured with the reagent kit before the start of the treatment, giving results of 4.5 g/L. This is the concentration considered as "before treatment". Next, the amounts of ammonia from the added urea (Table 5-2) results on 0.48 kg (for 1%) and 1.42 kg (for 3%) based on the stoichiometry where 1 g of urea corresponds to 0.568 g of ammonia. The sum of the intrinsic ammonia and ammonia from urea gives a theoretical total amount of ammonia (NH_{tot}) concentration of 9.16 g/L (for 1%) and 18.5 g/L (for 3%).

Assuming that all added urea (25 g/kg for 1% and 75 g/kg for 3% treatment) was 100% degraded and no ammonia was lost during the treatment, the concentration of uncharged ammonia (NH_3) could be calculated (based on Equation 3-3 and Equation 3-4). The calculation was made for the average recorded temperature of 24°C and for the minimum and maximum registered pH values for 1% and 3% treatments (APPENDIX D).

The measured TAN for both treatments resulted in very much higher values of ammonia compared to the maxim that can expected according to stoichiometry. For 1%, the maximum concentration that could be obtained was 9.2 g/L. yet the measured TAN was over the double, 19 g/L. The same was observed for 3% concentration. A hypothesis on these great differences could is the biochemical reactions taking place in the reactor under anaerobic conditions, especially ammonification what would increase the ammonia content at the outlet.

Table 6-8 shows the values of ammonia measured in the faecal sludge before and after treatment, ammonia from urea from stoichiometry (based on Equation 3-1), total ammonia which is sum of ammonia present in

the sludge and from urea and free fraction of ammonia (NH_3). The values expressed in terms of mass are based on volume of samples (0.01 L) and concentrations.

Table 6-8 Values of obtained ammonia measurements, theoretical total ammonia and uncharged ammonia (NH_3)

UREA TREATMENT ON FAECAL SLUDGE, MALAWI

AMMONIA BALANCE EXPRESSED IN CONCENTRATION (g/L)						
Urea treatment (%)	Intrinsic ammonia	Ammonia from urea	Theoretical total ammonia (urea + intrinsic)	Measured TAN	Free uncharged ammonia fNH_3 (%)	
					Min	Max
1	4.5	4.7	9.2	19.1	3	53
3	4.5	14.0	18.5	80.0	1	60
AMMONIA BALANCE EXPRESSED IN MASS (kg)						
Urea treatment (%)	Intrinsic ammonia	Ammonia from urea	Theoretical total ammonia (urea + intrinsic)	Measured TAN	Free uncharged ammonia fNH_3 (%)	
					Min	Max
1	4.5	0.5	5.0	19.1	3	53
3	4.5	1.4	5.9	800.0	1	60

During the sampling of the last days of the treatment it was observed that the consistency of the sludge varied between the control and the urea treatments, what lead to do an ammonia test from a sample collected from the top of the 0% reactor and compare it with the ammonia concentrations obtained from a sample taken from the tap at the bottom of the drum. The ammonia concentration from the top was 3.0 g/L vs. 1.5 g/L at the bottom. The sampling was done at a single occasion.

6.2.3. Microbial inactivation

Inactivation rates of *E. coli*, *Salmonella* and total coliforms were analysed in relation to pH, ammonia concentration (from urea amendment) and temperature.

Within the same batch of faecal sludge, the urea treatment resulted in faster inactivation in relation with the untreated faeces. The urea treatment gave clear evidence of bacteria reduction, especially for *E. coli* and *Salmonella*, which were eliminated by day 4 (or even in shorter period). On the contrary, the total coliforms were more resistant to the treatment and were still present at day 4 yet by day 7 they were eliminated in 1% and 3% treatments. Summarised results from CFU counts on day 0, day 4 and day 7 for three treatments: control (0%), 1% and 3% are showed in Table 6-9.

Table 6-9 Average bacteria count, initially, after day 4 and day 7 of treatment at environment temperature.

Studied bacteria	Initially (log_{10} CFU/100 mL)	Day 4 treatment (log_{10} CFU/100 mL)			Day 7 treatment (log_{10} CFU/100 mL)		
		Control (0%)	1%	3%	Control (0%)	1%	3%
E. coli	5.94±0.2	5.11±5.2	0	0	4.85±1.5	0	0
Salmonella	-	-	-	-	4.38±1.7	0	0
Total coliforms	5.24±0.1	4.95±1	4.88±0.6	4.30±0.5	4.31±2.8	0	0

Figure 6-14 and Figure 6-15 show the logarithmic reduction against time for *E. coli* and total coliforms respectively. The curves for control and the urea treatments of 1% and 3% are shown. As it appears on the figures, the values of *E. coli* and total coliforms were below detection on day 4 and 7, respectively, although the log-scale does not allow the expression of zero. In the ammonia-free reactor a natural die-off trend was observed. The anaerobic conditions of the batch reactor reduced the *E. coli* and total coliforms concentrations 1 $\log_{10}$ unit. In addition, no growth of bacteria was observed during the 8-days study.

A logarithmic reduction graph of *Salmonella* was not able to be plotted due to lack of enough measurements. Microbiological data could not be collected from day 1 to 4 due electrical problems in the laboratory that hampered the use incubator during the initial stage of the experiments in Malawi.

The non-detectable (ND) or shaded area refers to concentrations of bacteria that are below 3 $\log_{10}$ CFU/100 mL are not able to be enumerated. Consequently, it can be estimated that the sanitising treatments achieved a pathogen reduction of 6 $\log_{10}$ units for *E. coli* and for 5 log units coliforms; as regards for *Salmonella* it could be only speculated a 6 $\log_{10}$ reduction similar to *E. coli*, based on the assumption that the concentrations of such bacteria were the same in influent, before the urea amendment.

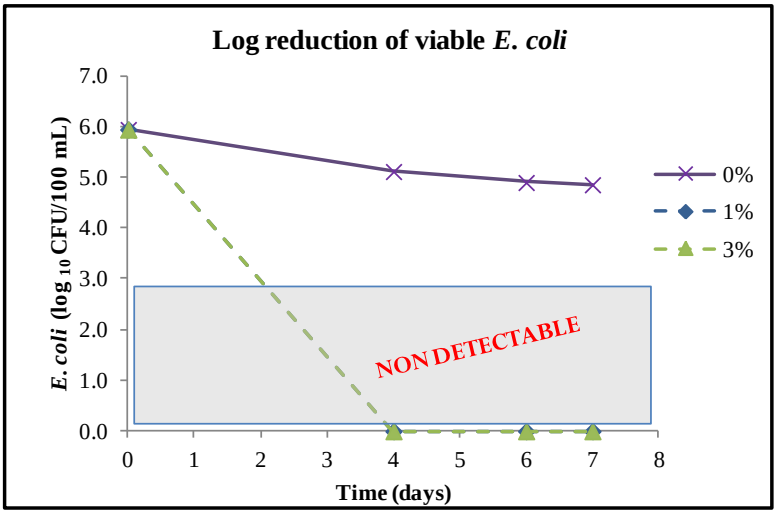


Figure 6-14 *E. coli* in control and urea treated storages in scaled-up treatments

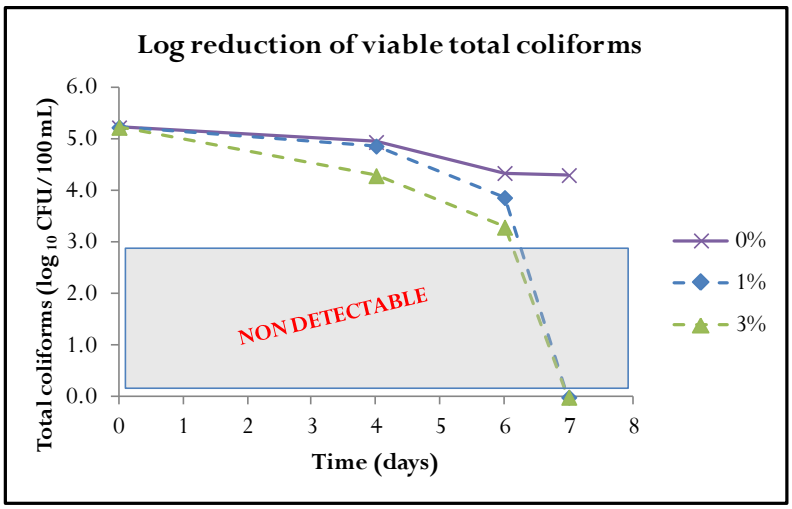


Figure 6-15 Total coliforms in control and urea treated storages in scaled-up treatments

E. coli and total coliforms reduction rates

The inactivation rate of *E. coli* and total coliforms in the untreated faecal sludge (0%) was calculated and analysed by linear regression, where log N_t/N_0 that represents the negative decay rate ($-K_t$) was plotted against time t (days) as is depicted in Figure 6-16 and Figure 6-17, respectively. The calculations gave results of decay factor of 0.16 for *E. coli* and 0.14 for total coliforms, indicating that *E. coli* bacteria died faster than coliforms in the untreated storage.

The decay rates for the 1% and 3% urea treatments were not able to be plotted for all studied bacteria since not enough data could be collected. In addition, the bacteria count results by day four and onwards were zero, which hampers the graph construction.

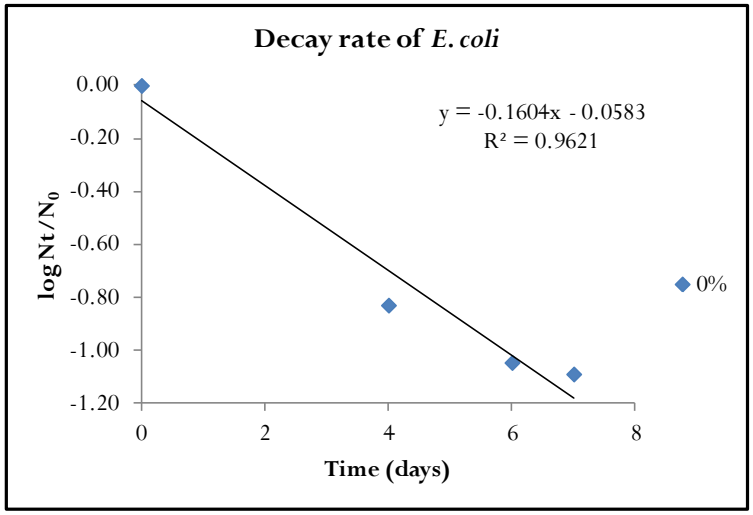


Figure 6-16 Inactivation rate of *E. coli* in the untreated storage

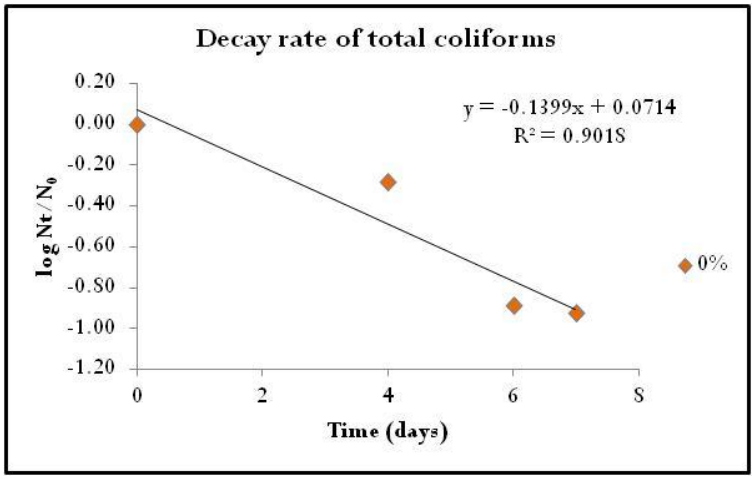


Figure 6-17 Inactivation rate of total coliforms in the untreated storage

CHAPTER 7

Discussion

7.1. Sludge characteristics

Two types of faecal materials with different characteristics were used in this study: black water from vacuum toilets during the laboratory phase in Netherlands and faecal sludge from households pit latrines during the scaled-up experiment in Malawi. When a comparison between the two materials is made, it is determined that the pH of the faecal sludge is slightly higher compared to black water. In the same way, the total solids percentage and the ammonia concentrations were also high in the sludge, whereas the concentrations of the studied bacteria present in the sludge where lower than those found in the toilet water, considering that the sludge had undergone bacterial decomposition for over a year(s).

These differences in the compositions of these materials could be explain on their origins; the fresh black water was collected from vacuum toilets, while the faecal sludge collected from pit latrines was estimated to be one year old and above. In addition to that, the collection system influences and determine greatly the characteristics of the sludge and black water, meaning that vacuum systems are not ventilated, use low flush water volumes while pit latrines are ventilated and do not use water for flushing. Consequently, the faecal sludge from the pit latrine may have high ammonia concentration, even though most of the ammonia could have been volatilised due to the ventilation. Moreover, the content of ammonia in the human waste (mainly urine) and the chemical characteristics of the faecal material are also dependent on the diet regimes and may vary greatly from one region to another, as it in this case, where a comparison between materials from Netherlands, Europe to Malawi, Africa is made.

The sludge characterisation of the faecal sludge collected from the three different pit latrines in Malawi varies from one to another, which it is believed to be influenced by the fluidisation process, where large amounts of water are added to the latrine in the dependence of the sludge level in the pit, the sludge permeability and moisture content. The pit-emptying process has large uncertainties, considering that is not a standardised procedure. It would mostly depend on the pit-emptier assessment if the sludge has been fluidised it enough to be pumped out of the pit into the vacuum tanker.

Figure 7-1 Desludging lorry in Blantyre, Malawi

Figure 7-2 Spray nozzle used for faecal sludge fluidisation

The observed desludging process is done through fluidisation and a vacuum system (Figure 7-1). To prevent clogging of the system, the rubbish is fished out before of the fluidisation. Next, pressurised clean water is injected into the pit with a spray nozzle (Figure 7-2).

7.2. pH, temperature and ammonia nitrogen in the sanitation study

The effects of pH and temperature have been studied on the ammonia formation derived from the urea hydrolysis. The breakdown of the urea molecules, by faecal bacteria, increases the pH above 9, favouring the ammonia formation. This mechanism was observed during the urea treatment carried out during the laboratory phase with black water and the pilot test done with faecal sludge in Malawi. The following Figure 7-3 shows the proportional relation between the pH and ammonia formation against time, using 1% (w/w) urea concentration on the faecal sludge in Malawi.

The urea addition increased the pH to at least 8, reaching stable values above 9. The measured TAN increased along with the pH due to the ammonia formation. Between day 6 and 8, the determined TAN rose from 15 g/L to 20 g/L which could be explained by the fact that the pathogens were already inactivated by day 7 and take up of the ammonia compound was no longer occurring. In addition to these findings, the pH figures and ammonia concentrations evaluated in all the tested urea treatments done in black water and in faecal sludge treatments during this study were similar to values presented in other researches by Nordin (2010) and Fidjeland *et al.* (2013).

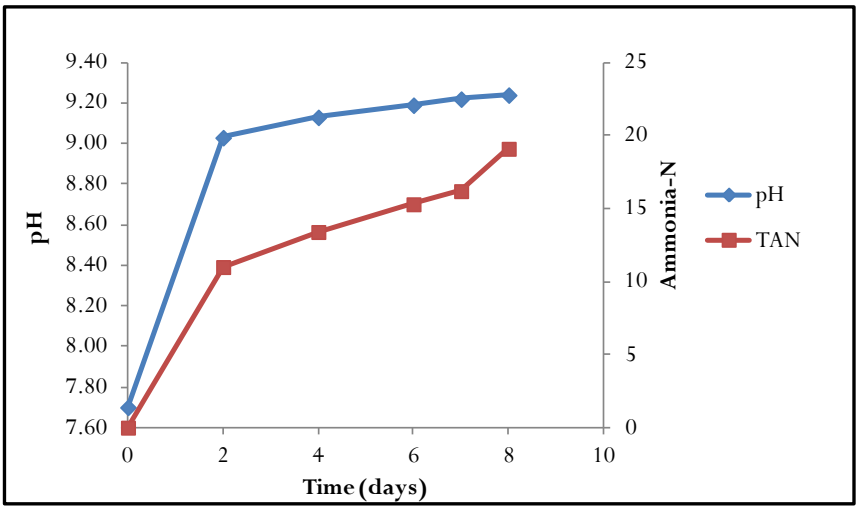


Figure 7-3 Ammonia concentrations for 1% urea treatment in combination with time and pH in scaled up experiment for 8 days in Malawi

As regards for temperature, in some samples its influence over the ammonia increase was not too significative, as it was observed between the two batch tests performed in the laboratory study at fixed 20°C and 30°C, despite other researches done by Fidjeland *et al* 2013 and Nordin 2010 demonstrate the opposite. It is stated that the higher the pH and/or temperature, the higher the free ammonia concentrations.

The difficulty on the assessment of the temperature effects on the ammonia formation relies on the difference in the ammonia concentrations of the black water before each test. The measured TAN for batch test at 30ºC was 0.73 g/L vs. 1.45 g/L in batch test at 20ºC; hence the obtained concentrations of ammonia at 30°C cannot be higher than those encountered at 20°C. The lower values of ammonia (0.73 g/L) are explained on the fact that this material was not fresh but it had been stored at 4°C for one month approximately; even at low temperature, biochemical activity in the wastewater samples could have been taken place.

7.3. Reduction rates of microorganisms

0%, 1%, 2% and 3% (w/w) urea treatments were performed using black water and faecal sludge in Netherlands and Malawi, respectively (2% was excluded in Malawi), showing results that ammonia concentrations can reduce in a significant way the pathogenic content of faecal materials, even at the lowest urea concentrations. The inactivation rates showed a positive correlation with increasing ammonia concentrations and pH for all studied microorganisms. During the conditions tested, the pH alone did not caused the inactivation of microorganisms but it played a crucial indirect role for the ammonia formation, a chemical compound known to be toxic for organisms (Warren 1957).

For all the temperatures and all evaluated bacteria, the inactivation time for the untreated material (0%) required more than 8 days, which it was not studied further due to time constraints. This longer reduction time could be influenced by low pH values and small presence of ammonia concentrations measured in the faecal materials, especially black water (Table 6-5). Moreover, in some samples the effects of the temperature could not be fully appreciated and understood, i.e., the reduction time required (<8 days) is higher at 20°C than at 30°C, which is <4 days. Nevertheless, all the urea treatments, at the 3 studied temperatures targeted 5$\log_{10}$ unit reduction (or more) within a short period of time of less than 8 days.

Table 7-1 Treatment time in days required to achieve a 5 $\log_{10}$ reduction in *E. coli*, *Salmonella* and total coliforms in faecal material during urea treatment (1-3% w/w) at temperatures 30, 24, 20°C

Temperature	Microorganisn	Urea treatment		
		3%	2%	1%
30°C (NL)	*E. coli*	2	<8	<8
	Salmonella	2	2	<8
	Total coliforms	2	4	<8
24°C (MWI)	*E. coli*	<4	-	<4
	Salmonella	<6	-	<6
	Total coliforms	1 week	-	1 week
20°C (NL)	*E. coli*	<6	<6	<4
	Salmonella	<4	<4	<4
	Total coliforms	4	<6	<6

- Not studied.

Escherichia coli and total coliforms are among the most commonly used indicators for faecal contamination (Bitton 2005). The coliforms were studied as an indicator for pathogen reduction but the plating results showed they persisted a little longer in the treated storages as the urea treatment were also lower. However, at 30°C the 3% urea treatment achieved a $5\log_{10}$ reduction within 2 days only. The slow reduction of the coliforms could be a combination of resistance to ammonia and exposure time to the toxic compound. The laboratory experiments revealed that *Salmonella* can be inactivated in less than 4 days at 20°C and only in 2 days at higher temperature of 30°C, with the highest urea treatment (3%).

Overall, the results demonstrated that at 30, 24, 20°C, all studied bacteria were reduced up to a health target established by the World Health Organization of $6\log_{10}$ (WHO 2006).

7.4. Technical challenges in Malawi

Despite the urea treatment on the faecal sludge in Malawi resulted in a good reduction in the indicator organisms of *E. coli*, *Salmonella* and total coliforms, the obtained results could have been seriously compromised due to a series of unfortunate events related to logistics, material procurement and laboratory conditions. In this sense, the seizure of laboratory materials and equipment, delayed the experiment schedule for more than one week, narrowing it down to 10 days only. Additionally, the temporary substitution of the original Chromocult® agar media for a different chromogenic culture media (Brilliance™ *E. coli*/coliforms selective agar), did not allow measuring the initial concentrations of *Salmonella* in the sludge, which explains the very little data that could be collected for this pathogen. In addition to that, the malfunctioning of the laboratory apparatus, along with the poor conditions of the laboratory's electrical installation, caused constant power failures, affecting the performance of the equipment and limiting their usage; hence the results obtained for microbial analyses might have been jeopardised due to the constant shut down of the incubator.

The most significant event was the power cut of 4 days that prevented the use of the incubator, which it explains the critical 4-day gap appearing in the microbial analyses at the beginning of the treatment. The lack of data made difficult the calculation of the decay rates in the different urea treatments, especially for *Salmonella*.

On the other hand, in order the ammonia-based treatment to be successful, the treatment must be performed in a closed container so ammonia losses do not occur. This specific requirement complicated the option of adapting a mixing device inside the drums. Therefore, by having no proper mixing conditions in the reactor, the faecal material was not distributed evenly and the concentrations of ammonia and microorganism, leading to the risk of having pouches with untreated material and possible re-growth of

pathogens. Furthermore, even the sampling was a challenge, considering the amount of rubbish inside the reactor that constantly clogged the installed taps.

7.5. Urea treatment implications

The provided services in an emergency situation should have the objective of supplying a healthy environment for the affected population. This is achieved by isolating and inactivating the disease-carrying organisms present in the human excreta. In addition, emergency sanitation is limited with resource availability and time constraints. The ammonia-based treatment is an innovative approach for inactivation of pathogens present in faecal material.

In this research, urea treatment proved to be efficient for disinfection of *E. coli*, *Salmonella* and total coliforms within 8 days (<6 $\log_{10}$) in faecal sludge and black water, complying .the health-based target of 6log10 defined by the World Health Organization In this sense, ammonia treatment (derived from urea) could be adapted and applied to faecal sludge treatment in emergencies situations.

7.5.1. Applicability of approach in emergency situations

Considering that adding large amount of water alters the properties of the sludge, in the ammonia based treatment, the fluidisation process is not preferred as the faecal material should be concentrated as much as possible. The higher the ammonia concentrations, the better results can be achieved in the pathogen inactivation.

The level of treatment of the faecal sludge would depend on the targeted microorganism and the final disposal of the sludge. The strategy of the treatment should be designed essentially on the intrinsic characteristics of the faecal sludge, as it shown in Figure 7-4. Besides this, the approach can be designed based on the temperature and required sanitising time. The latter can be regulated by the amount of urea to be added, thus the higher the urea concentration, the faster the inactivation of the pathogenic organisms would be.

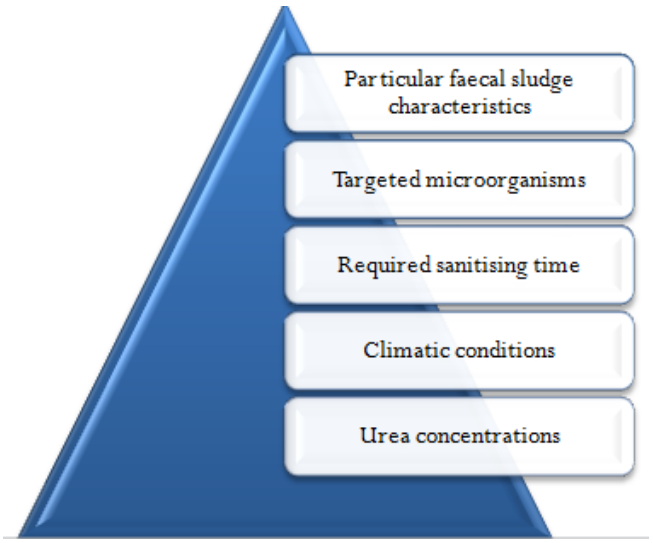


Figure 7-4 Hierarchal relationship for urea-based sanitation approach design

7.5.2. Adaptability to on-site treatment

The urea treatment approach has potential to be applied on site. If the pit latrine is lined and another pit is available, the full pit latrine itself could function as storage. The mass of the sludge can be calculated from the volume of pit and sludge level. The added urea must be mixed thoroughly with the faeces and the pit should be covered to avoid ammonia losses.

Raised latrines could also be considered as storage. Urea or ammonia solutions could be added to the full collecting vault. The main constraint of a raised latrine is its ventilation system, which make the ammonia gas to escape.

For instance, vacuum toilets and pour flush latrines may be the most suitable devices for the ammonia treatment due to the low volumes of flushing water. The main limitation of pit latrines and ventilated improved latrines In contrast, the pit latrines and the ventilated pit latrines (VIPs), even they are diluted with flush water, they may be not ideal for this approach since they are not provided with air tight storage to avoid the ammonia losses.

Urine can be selected as a source of ammonia. By separating the streams of urine and faeces in urine diverting dry toilets (UDDT), the ammonia obtained from the urine can be used to potentially sanitise the human faeces, thus reducing the urea dosage.

In case that the previously described option is not feasible and the sludge should be transported to a storage facility, the sanitation treatment could be applied in the collection container by adding urea to the full container and keeping it tightly closed. The use of mechanised desludging and handling of excreta significantly reduce the exposure of the raw bio-infectious material to the environment and to humans.

7.5.3. Sanitised faecal sludge as fertiliser

As it had been demonstrated in this study, the ammonia sanitation of faecal material increased the free ammonia fraction. This total ammonia-nitrogen is not consumed or lost during the chemical treatment. Therefore an additional effect of the faecal sludge derived from the urea treatment is its high fertilising value. Nutrients derived from human waste (urine + faeces) such as organic carbon, potassium, phosphorus and nitrogen are essential for plant growth and micro-biological life in the soil. Hence, the obtained safe sludge could be reused in agriculture as fertilizer. Nonetheless, it should be considered that the higher the urea dosage in the treatments, the higher the nitrogen concentrations which could be too high for sludge reuse.

The advantages of urea treatment can be listed as follow:

- Simple and easy approach
- Urea is low-cost and easily available material.
- Cost-effective approach
- Urea is not hazardous. Pellets can be stored for long periods
- Short sanitising time
- Low treatment pH (compared to lime)
- Not required high skilled operators
- Nutrient value for reuse of treated biowaste.
- Reduce vector attraction

The disadvantages of urea treatment can be listed as follow:

- Urea is not classified as a hazardous product. However, it may cause irritation, redness and pain to the skin and eyes. Safe handling and storage precaution of urea is required.
- The ammonia gas is toxic and has an unpleasant pungent smell.
- Proper sealing of urea treatment container is limiting the application of the treatment in the public raise latrine.

7.5.4. Urea treatment costs

The following costs are based on the urea dosages applied in the ammonia treatment on the faecal sludge in Malawi. The calculations were done based on the following information:

€ 1.00	MWK 570	
1 Bag urea	5 kg	
Cost/bag	MWK 2,000	€ 3.51
Cost kg urea	€ 0.70	

Table 7-2 Urea treatment costs in faecal sludge, Malawi

FIELD WORK MALAWI					
Urea treatment (% w/w)	**Number of reactors**	**Mass of FS (kg)**	**Mass of urea to add (kg)**	**g urea/kg FS**	**Cost of treatment (€)/kg FS**
0	1	31.4	0.00	-	-
1	1	33.8	0.85	25.0	0.015
3	1	33.5	2.51	75.0	0.132
	TOTAL UREA/BATCH		**3.4**		

CHAPTER 8

Conclusions and Recommendations

The addition of 1% (w/w) urea concentrations to faecal sludge at ambient temperature of 24ºC is sufficient to produce a safe sludge. *Escherichia coli, Salmonella* and total coliforms were inactivated by the achieved ammonia concentration in the evaluated mixes of sludge. The sanitising time of the faecal treatment, measured as <1000 *E. coli*/100 mL, can be achieved in less than 1 week. A >3 $\log_{10}$ reduction of viable *E. coli, Salmonella* and total coliforms in faecal sludge was reached with ammonia concentrations above 10 g/L

The final stabilised pH ≈9 depended on the characteristics of the sludge and the ammonia formation, which is the crucial element for the microorganism inactivation.

The efficiency of the sludge treatment can be affected by factors as the collection system and desludging process.

There is potential to sanitise faecal material with ammonia obtained from urea at full scale and apply it in emergency situations. The approach demonstrated to be effective, with short sanitising periods and simple technology involved. Besides the disinfecting effects, the urea treatment increased the nutrient and fertiliser value of the sludge for reuse.

The treatment recommendations should be formulated for the specific characteristics of the human waste to be treated/used and not only based on environmental conditions and flushing water volumes.

Recommendations and future research

The results obtained in this study indicated the potential sanitising effect of the ammonia from urea on faecal material. In order to overcome the challenges faced during this study, the novelty ammonia approach needs further research for its implementation, assessment and operation.

- Plan accordingly in advance before implementing a scaled-up experiment
- (Re) design or adjust the laboratory facilities to work with biological infectious material
- In order to obtain a safe (faecal) sludge, the inactivation of other pathogenic (micro)organisms present in the faecal material such as viruses, bacteriophages, protozoa and helminths ought to be studied.
- Study the on-site sanitation effects of ammonia in different collecting systems namely, dry toilets, pit latrines, pour flush latrines.
- Development of mixing tecnhiques and/or devices to be adapated inside the storage under anaerobic conditions.
- Evaluate urine as source of ammonia to disinfect faecal material in the context of ecological sanitation.
- Study the disinfecting potential of intrinsic ammonia from the urine and/or faecal slugde
- Appropriate mixing urea with the faecal sludge needs to be developed

References

A. Nordin, K. Nyberg BV (2009) Inactivation of Ascaris Eggs in Source-Separated Urine and Feces by Ammonia at Ambient Temperatures Inactivation of Ascaris Eggs in Source-Separated Urine and Feces by Ammonia at Ambient Temperatures . doi: 10.1128/AEM.01250-08

Bitton G (2005) Wastewater Microbiology, Third Edit. John Wiley & Sons, Inc., New Jersey

Bouwinnovatie A, Societies RC (2012) Requirements Faecal Sludge Treatment and disposal in emergency situations.

Eawag/Sandec (2008) Faecal Sludge Management (FSM). Düberdorf, Switzerland

European Commission (2013) Echo factsheet The Democratic Republic of Congo. 186:2–4.

Fidjeland J, Magri ME, Jönsson H, et al. (2013) The potential for self-sanitisation of faecal sludge by intrinsic ammonia. Water Res 46:1–10. doi: 10.1016/j.watres.2013.07.024

Henze M (2008) Biological wastewater treatment: principles, modelling and design.

Langergraber G, Muellegger E (2005) Ecological Sanitation--a way to solve global sanitation problems? Environ Int 31:433–44. doi: 10.1016/j.envint.2004.08.006

Lubberding HJ (2013) Microbiology for ES and MWI Laboratory Notes.

Nordin A (2010) Ammonia Sanitisation of Human Excreta Treatment Technology for Production of Fertiliser.

Ottoson J, Nordin a, von Rosen D, Vinnerås B (2008) Salmonella reduction in manure by the addition of urea and ammonia. Bioresour Technol 99:1610–5. doi: 10.1016/j.biortech.2007.04.009

PAHO/WHO (2013) As Cholera Death Toll Climbs in Post-Earthquake Haiti, New Studies Show Disease Emerging as a Driver of Progress in Public Health. http://www.paho.org. Accessed 9 Oct 2013

Strauss, M., Montangero A (1999) Cities without Sewers. 15–16.

Supply W, Council S, UNICEF (2003) Global water supply and sanitation assessment: 2000 report.

Vinnerås B, Nordin A, Niwagaba C, Nyberg K (2008) Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate. Water Res 42:4067–74. doi: 10.1016/j.watres.2008.06.014

Warren KS (1957) The differential toxicity of ammonium salts. 497–501.

WHO (1992) A Guide to the Development of on-Site Sanitation. World Health Organization

WHO (2013) Environmental Health in Emergencies. http://www.who.int/environmental_health_emergencies/en/. Accessed 5 Oct 2013

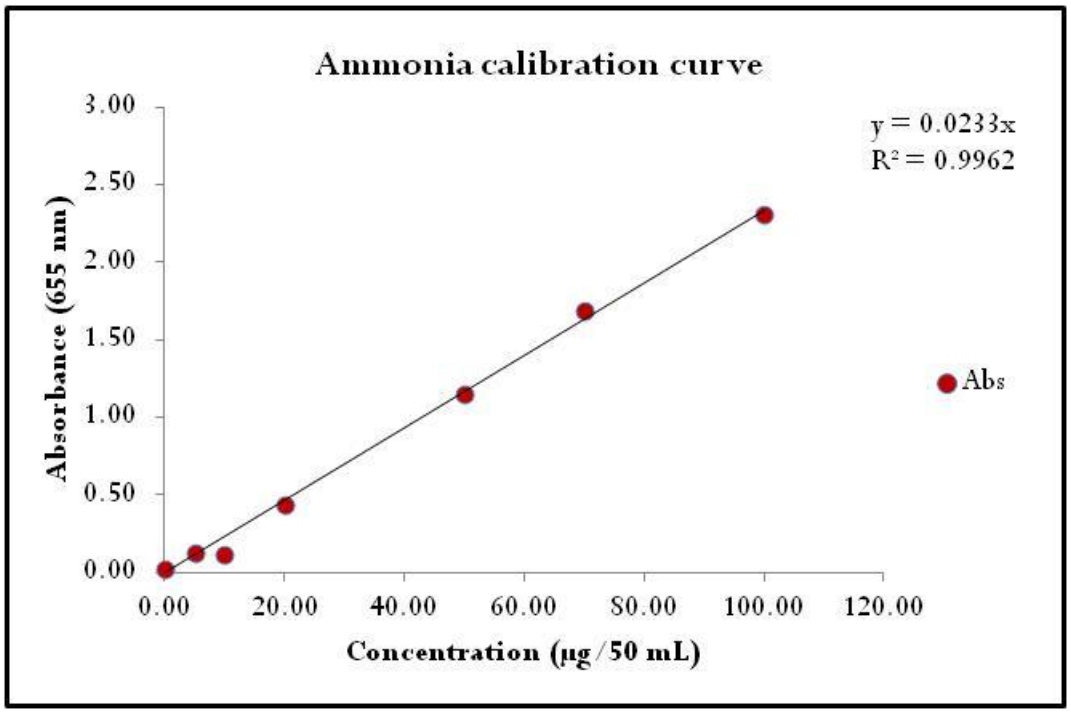
WHO (2004) Laboratory biosafety manual. Geneva

WHO (2006) Guidelines for the Safe Use of Wastewater, Excreta and Greywater. I:

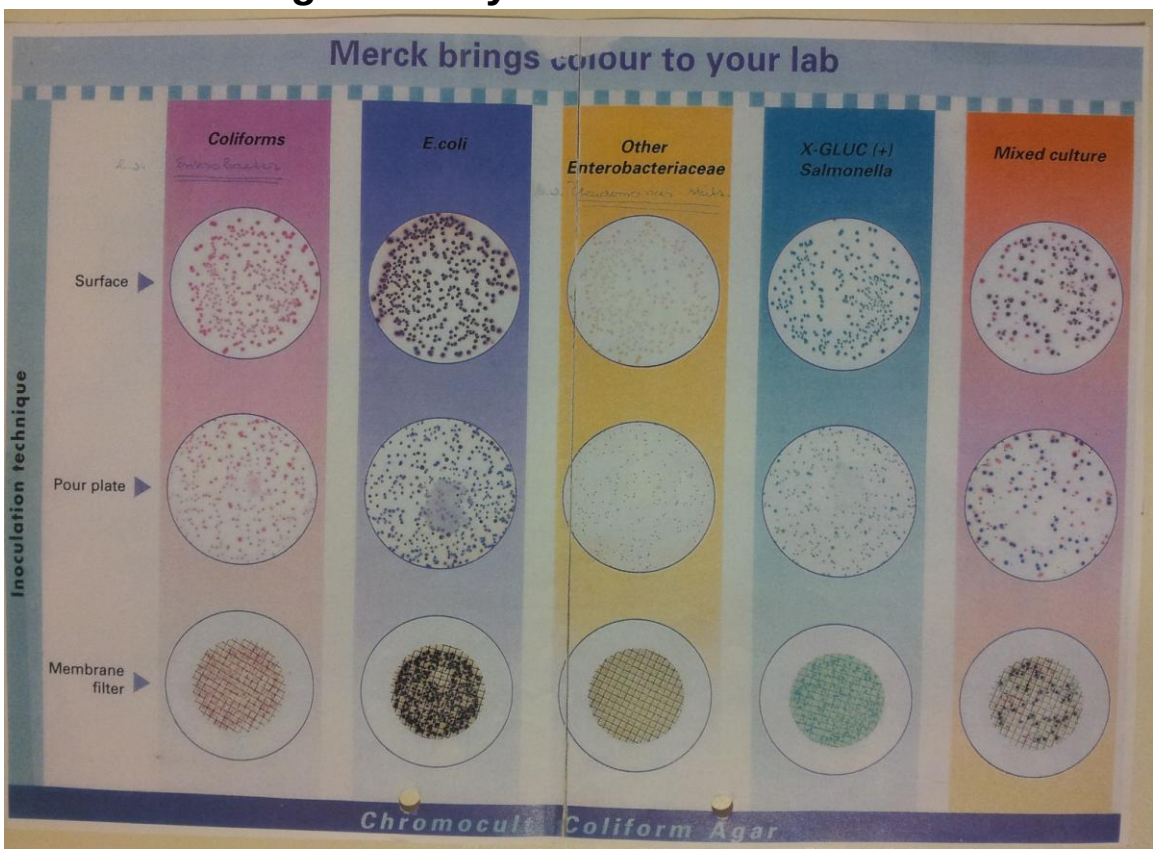
WHO/UNICEF JMP for Water Supply and Sanitation (2013) Progress on Sanitation and Drinking Water - 2013 Update.

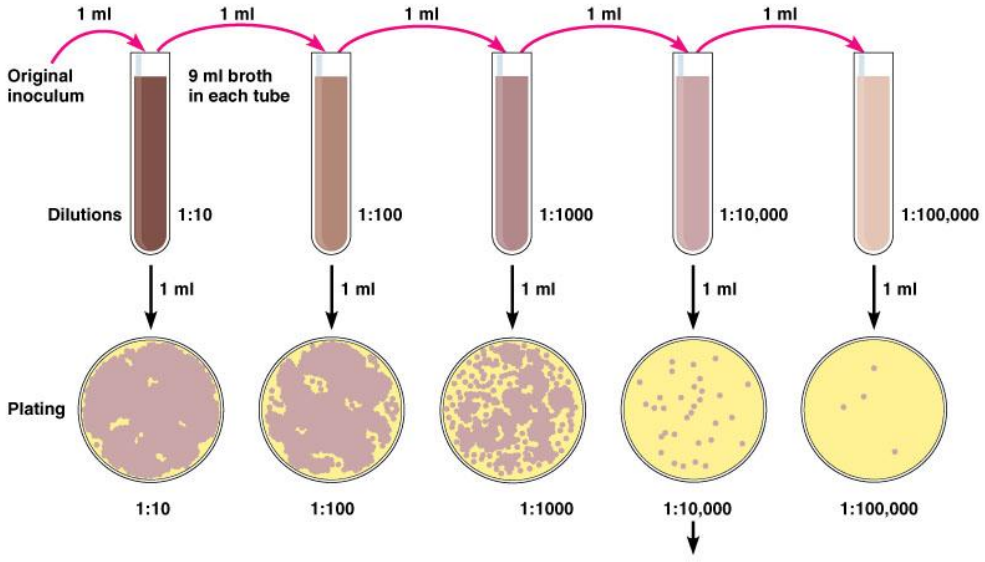
Appendices

Appendix A Calibration curve for Ammonia-Nitrogen measurements



Appendix B Culture media and inoculation technique used for microbiological analyses





Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000/ml in sample.)

Appendix C Summary of desludging process in Malawi

Date of collection	25/01/2014	31/01/2014	07/02/2014
Batch of sludge nº	1	2	3
Name/Locality	Bangwe township - Blantyre	Bangwe township - Blantyre	Bangwe Market - Student sludge
Type of Facility	Latrine	Latrine	Latrine
Number of Facilities	1	1	1
Sludge level (m)	0.4	0.5	0.7
Desludging equipment	ROM2	ROM2	ROM2
Volume of water for fluidisation (L)	200	200	180
Rubbish fished out (L)	70	50	40
ROMs/IBC filled (L)	2	2	2
Total volume sludge removed (L)	1600	1600	1600
Comments	Latrines full of rubbish such as: clothes, HIV vials, bottles, beer cans; even a phone charger		

Appendix D Calculations of total solids, volatile solids organic and moisture content in faecal sludge, Malawi.

BATCH Faecal Sludge 1

Collected 25/01/2014
1 year old

	B	D	A	C	TS %	TS (g/L)	VS %	VS (g/L)	Moisture content %	Organic Content %
I	2.2703	19.2017	3.9713	3.1705	10.05	100.46	47.08	47.30	89.95	47.08
II	2.2351	19.9506	3.885	3.1706	9.31	93.13	43.30	40.33	90.69	43.30
III	2.2464	22.9081	4.2418	3.3404	9.66	96.57	45.17	43.63	90.34	45.17
			AVERAGE		10	97	45	44	90	45
				SD	0.30	2.99	1.54	2.85	0.30	1.54
				SD %	3.10	3.10	3.41	6.51	0.33	3.41

BATCH Faecal Sludge 2

Collected: 31/01/2014
7 years old

	B	D	A	C	TS %	TS (g/L)	VS %	VS (g/L)	Moisture content %	Organic Content %
I	2.3004	24.4746	3.7431	2.9694	6.51	65.06	53.63	34.89	93.49	53.63
II	2.2618	23.6163	3.6559	2.8822	6.53	65.28	55.50	36.23	93.47	55.50
III	2.2355	29.0685	3.6815		5.39	53.89		0.00	94.61	
			AVERAGE		6	61	55	36	94	55
				SD	0.22	2.17	0.93	0.67	0.22	0.93
				SD %	3.54	3.54	1.71	1.88	0.23	1.71

BATCH Faecal Sludge 3

Collected: 07/02/2014
1 month old

	B	D	A	C	TS %	TS (g/L)	VS %	VS (g/L)	Moisture content %	Organic Content %
I	2.294	19.009	3.1947	2.6193	5.39	53.89	63.88	34.42	94.61	63.88
II	2.2705	27.3412	3.1957	2.7331	3.69	36.90	50.00	18.45	96.31	50.00
III	2.2866	24.3741	3.3733	2.697	4.92	49.20	62.23	30.62	95.08	62.23
			AVERAGE		5	47	59	28	95	59
				SD	0.72	7.16	6.19	6.81	0.72	6.19
				SD %	15.35	15.35	10.55	24.48	0.75	10.55

Appendix E Ammonia measurements and calculations

Date of reading	Day of treatment	0%	1%	3%
04/02/2014	0	7.43	7.70	7.25
06/02/2014	2	7.03	9.03	9.06
08/20/2014	4	7.11	9.13	9.30
10/02/2014	6	6.94	9.19	9.40
11/02/2014	7	7.28	9.22	9.33
12/02/2014	8	7.19	9.24	9.36
	AVERAGE	**7.16**	**8.92**	**8.95**
	SD	0.1612	0.5492	0.7681
	SD %	2.2505	6.1584	8.5818

Date of test	Day of treatment	Urea treatment	Dilution factor	Measured NH_3-N	NH_3-N (mg/L)	NH_3-N (g/L)	Observations
BEFORE UREA TREATMENT							
04/02/2014		-	10,000	0.45	4500	4.5	Raw Sludge
AFTER UREA TREATMENT							
06/02/2014	2	0%	10,000	0.28	2,800	2.8	
		1%		1.10	11,000	11.0	
		3%	100,000	0.22	22,000	22.0	
08/02/2014	4	0%	10,000	0.22	2,200	2.2	
		1%		1.34	13,400	13.4	
		3%	100,000	0.42	42,000	42.0	
10/02/2014	6	0%	10,000	0.18	1,800	1.8	
		1%		1.53	15,300	15.3	
		3%	100,000	0.52	52,000	52.0	
11/02/2014	7	0%	10,000	0.17	1,700	1.7	
		1%		1.62	16,200	16.2	
		3%	100,000	0.63	63,000	63.0	
12/02/2014	8	0%	10,000	0.15	1,500	1.5	0% collected from bottom. Consistency: very liquid (leachate)
		1%		1.91	19,100	19.1	
		3%	100,000	0.80	80,000	80.0	
12/02/2014	8	0%	10,000	0.30	3,000	176	0% collected from top. Consistecy: Thick