Treatment and Reuse Potential of Urine and Faecal Fractions from Urine Diversion Dehydrating Toilets in eThekwini Municipality

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

The eThekwini Municipality, Durban, has implemented around 80 000 *Urine Dehydrating Diversion Toilets* (UDDTs) outside the waterborne network as a practical alternative to pit latrine technologies and as a solution for providing safe, hygienic, and dignified sanitation in urban and peri-urban areas. The major advantage of UDDTs over conventional pit latrines is that it allows source separation at the pedestal interface of faeces and urine. With mixing of faeces and urine significantly reduced, it allows for a drier sludge with less volume than would be expected from conventional latrines to be emptied and disposed of. Source separation allows for the recovery and recycling of nutrients from urine as potential fertiliser substitutes while the mainly organic-origin faecal matter has the potential to be beneficiated via various digestion pathways. This study had the dual purpose of i) investigating the ability of *Black Soldier Fly Larvae* (BSFL) to digest sludge from UDDTs' with and without other organic substrate additives and ii) investigate the presence of certain microbial pathogens in urine-derived struvite, optimise the process for struvite recovery coupled with microbial pathogen deactivation and highlight the health risks associated with the use of these struvite.

For the BSFL component of this study, the effect of three different additives; *Food Waste* (FW), *Poultry Feed* (P) and *Bagasse* (B) on the sludge digestion by BSFL was investigated. BSFL treatment showed a potential for waste reduction, with a reduction of volatile solids recorded in treated samples compared to the control; however there was no reduction in bagasse-supplemented sludge relative to the other amended and unamended sludge. Treatments 2 (75% UDDT sludge+25% Food waste), 4 (75%UDDT sludge+25%Poultry feed) and 5 (50%UDDT sludge+50% Poultry feed) met the South African recommended guidelines for reuse of wastewater as fertiliser in agriculture of 34% reduction in the volatiles solids. Nutrients contents (TKN, nitrogen, phosphate and potassium) increased following the 10 day BSFL-digestion of the sludge with higher nutrient content in the control than in the digested feed. The pH of the sludge (BSFL-digested and control) become more acidic. Results of pathogen removal from the sludge after BSFL digestion was inconclusive since Ascaris, Faecal Coliforms, *E. coli* and total coliforms were absent in both the digested feed and in the control. Other factors such as pH and presence of ammonia have been reported to contribute to pathogen removal in sludge samples. Analysis of the BSFL indicates the absence of helminth eggs before and after feeding on the different feed additives, despite the detection of helminth eggs in the feeds of all treatments. This may mean that the larvae did not consume the ascaris eggs while feeding, even though the ascaris eggs viability were observed to decrease in the feed after digestion The treated sludge did not meet the microbial quality guidelines for sludge to be used as fertiliser in agriculture.

For the urine-derived component of this study, experiments revealed the presence of certain

microbial pathogens in urine-derived struvite and highlighted the health risks associated with the use of these struvite. Reference was also made to literature to ascertain other pathogens that have been implicated in urine-derived struvite and their associated health risk. The presence of these health related microorganisms in struvite is a concern since it is capable of causing food poisoning if residues remain on struvite-fertilised crops, particularly crops consumed raw. However, it was shown that optimising struvite processing temperatures has the potential to inactivate the pathogens in the urine-derived struvite, albeit at different levels. Through this research, guidelines for the processing conditions of urine and applications of the urine-derived struvite were provided.

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1. INTRODUCTION

1.1 BACKGROUND

Faecal sludge contains nutrients that can be recycled and be used in agriculture to supplement synthetic nitrogen and phosphorus fertilisers. Synthetic nitrogen is manufactured from fossil fuel and phosphorus comes from a mining rock, which is non-renewable. However, improper use of faecal sludge in agriculture can result in environmental pollution (Niwagaba, 2007). Untreated faecal sludge has a high oxygen demand as caused by the presence of readily degradable organic matter. Discharging untreated faecal sludge to the environment can cause oxygen depletion in the water surfaces. Hence, the need for proper treatment and digestion prior to application.

Black soldier fly (*Hermatia illucens*) have recently emerged as a biological means for management of faecal sludge from a range of sources (Bank *et al*., 2014). They feed on animal manure, fresh human faeces and municipal organic matter. They have a unique composition of gut microbiota, which enables them to feed on different food types. They are also able to consume a large amount of organic matter in a short period of time and have been shown to convert excreta into a residue that is suitable for use as an organic fertiliser while reducing pathogens (Diener *et al*., 2011and Bank *et al*., 2014). Black soldier fly adults lay eggs near food sources then die. The eggs hatch and the larvae crawl into the source of food to feed while getting enough resources to develop through several larval stages. It takes 2-4 weeks for the final larval form, the prepupae, to emerge. Prepupae are rich in protein and lipid. At the prepupal stage, when larva reach pupa stage they crawl out of their food sources where they can be harvested and protein and lipid be extracted. The high protein content means the harvested larvae can be cleaned, dried and processed to make for animal feed, and it has been suggested that the lipid can be used for biodiesel (Diener *et al*., 2009). There is therefore potential to use *Black Soldier Fly Larvae* (BSFL) for processing organic wastes to yield high value products.

Specifically, in the case of processing *Urine Diverting Dehydrating Toilet* (UDDT) faecal sludge, the sludge could be mixed with organic wastes (food waste and molasses) and used as a food source for BFSL. Although BSFL have been demonstrated to feed directly on fresh faeces (Diener *et al*., 2014), the drier state of UDDT faecal sludge, together with the high sand content, mean that a readily digestible organic feed must be mixed with UDDT faecal sludge to make it a suitable substrate for use by BSFL.

Technology to be selected for treating faecal sludge must ensure that the residue does not pose risk to both human health and the environment. Legislation for regulating treatment, discharge, and disposal for faecal sludge must be established. Current wastewater treatment legislation is mostly applied to faecal sludge treatment. This is disadvantageous since the

nature of faecal sludge and that of wastewater are very different. For example, concentrations of total solids, organic matter and helminth eggs in faecal sludge are higher than those of wastewater by a factor of ten (Niwagaba, 2007). Contact with faecal sludge can pose a health risk to humans since it contains many pathogens. Adequate treatment of the faecal sludge is therefore required; with the choice of the selected treatment dependent on the end user or disposal option (Niwagaba, 2007). This project is aimed at investigating the potential of BSFL for effective digestion of faecal sludge from UDDTs for possible application as fertiliser for agricultural purposes. Re-use of faecal sludge in agriculture will require testing parameters such as: pathogens, nutrients, solids concentration, *Chemical Oxygen Demand* (COD), *Biochemical Oxygen Demand* (BOD) and metals (Niwagaba, 2007).

Human excreta presents rich nutritional value that can be easily recycled (Wohlsager *et al*., 2010). One most studied way of meeting the elevating demands of fertiliser is using nutrients recovered from human excreta. The recovery of nutrients from human excreta will simultaneously reduce pollution effects resulting from untreated wastewater pollution, unsafe excreta disposal and excessive use of chemical fertiliser (Sherteileib, 2014). Traditionally, in other countries like Japan, the re-use of human excreta began as early as the 12th century. Using human excreta as a source of nutrient has the potential to reduce food shortages in developing countries where they cannot afford chemical fertilisers. Urine contains high levels of nutrients, nitrogen, phosphorus and potassium that are essential for plant growth (Makaya *et al*., 2014). Urine contains 80% phosphorus (P) and 90% nitrogen (N) (Manfred, 2011) and recycling nutrients from urine is more profitable when compared to faeces (Makaya *et al*., 2014).

Urine could contribute 10%, 20% and 29% of N, P, and potassium (K), respectively in crop production. Furthermore, the content of metals in urine is very low (Jönsson *et al*., 2000). Maurer and co-workers (2006) showed that chemically produced fertiliser contains cadmium concentrations of 36g/Kg, which is higher than that can be found in urine. Besides advantages already mentioned for urine reuse it also serves as a better strategy to remove organic micro-pollutants from human metabolism. Currently, the application of urine-based fertilisers has received increasing attention because of the ever-increasing chemical fertiliser demands; however, treatment options have received less attention in developing countries.

The sanitation concept of separating and collecting urine is currently being used as an alternative for recovering urine for nutrient recycling (Jonssen *et al*., 2000). Separation and collection of urine to be used as a fertiliser is currently being promoted through urine separating toilets and latrines (Hoglund *et al*., 2002). When collected separately, the two fractions, i.e. urine and faeces, constitute nutrient resources in relatively undiluted form that can be easily recycled

(Jonssen *et al*., 2000). Urine diversion toilets are the best investigated systems, as they are designed to collect urine and faeces separately (Makaya *et al*., 2014) with a toilet bowl divided into a front part collecting urine while the rear part collects faeces (Hoglund *et al*., 2002). Source separation of human excreta can result in creation of unhygienic conditions as human excreta is known to contain pathogens (Karak, 2011). The introduction of urine-derived struvite for agricultural purposes can introduce transmission routes for pathogenic organisms which needs management (Bischel *et al*., 2015). This is currently the main/major concern with the use of urine and struvite as a fertiliser (Makaya *et al*., 2014).

In recent years, UDDTs have been provided in peri-urban and rural areas in Durban, South Africa to improve sanitation generating huge amounts of urine that are stored in tanks. The eThekwini municipality provides UDDTs to approximately 80 000 households in the rural and semi-rural promoting sanitation and nutrient recovery (Tilley *et al.,* 2008). Urine collected from UDDTs are different from fresh urine as they contain high ammonia concentrations, a high pH value and a strong, pungent smell caused by degradation of urea and other organic substances. Due to cross contamination with faeces however, the urine may also contain pathogens as well as high residues of antibiotics, which is a cause for public health concern (Uddert *et al*., 2015). Hence, the urine is required to be treated before being disposed of to prevent ecological degradation of ecosystem.

Struvite ($MgNH_4PO_4.6H_2O$) can be produced from urine and used as a fertiliser in agriculture. Nitrogen and phosphorus are two major nutrients found in urine both of which are essential plant macronutrients. Struvite can easily be precipitated from urine due to the high pH value, high ammonia and phosphate concentrations requiring only a magnesium source to be added to precipitate nearly all phosphate as struvite making it an efficient process for phosphorus recovery (Wilsenach *et al*., 2007; Uddert *et al*., 2015). Struvite precipitation only takes a few minutes if a very soluble magnesium source is used (Etter *et al*., 2011). Other benefits of struvite include volume and weight of carrying urine is reduced, long-term storage of nutrient, slow nutrient release and good availability of phosphorus. However, the high cost of reactor setup, urine collection, socio-cultural availability are some of the hurdles that needs to be addressed for commercial extraction of struvite from urine.

In cases where no pathogen inactivation occurs during the collection and storage period, viruses and bacteria may pose an unacceptably greater risk (Hoglund *et al*., 2002). Therefore, establishing sustainable treatment methods for urine before application as a fertiliser is necessary to prevent the spread of disease. Pathogens such as bacteriophages, *Salmonella, Enterococcus spp.*, *E. coli,* eggs of helminths have been reported to be present in struvite extracted from urine contaminated with these pathogens (Uddert *et al*., 2015; Bischel *et al*.,

2015).

1.2 RATIONALE

UDDTs were proposed to be a solution to improvement of sanitation while conserving water. Urine and faeces are separated; the urine can be leached into the soil or harvested for further processing and the volume of faecal matter to be collected and disposed of significantly less than a mixed pit latrine. However, management and processing of faecal sludge poses a challenge to local authorities, if users are unwilling or unable to manage sludge at the household level which involved burial on-site, a practice which has been subsequently been halted due to user complaints of handling faecal matter (Roma *et al.*, 2013) . This poses a challenge in eThekwini Municipality, where approximately 80 000 UDDTs have been installed and where users are now demanding a free emptying service similar to that made available to householders served by conventional pit latrines.

1.2 AIMS AND OBJECTIVES

The overall aim of this study was to better understand the microbial risk associated with the beneficiation of faecal sludge from UDDTs using BSFL technology and Struvite Reactors. The benefits associated with reuse will be balanced against health risks associated with all stages of faecal sludge and urine handling and processing. The specific project objectives are as follows:

1. To optimise, as far as practical, the conditions which promote the ability of BSFL to digest UDDT faecal sludge in a manner suitable for up-scaling to field conditions.

2. To optimise, as far as practical, the conditions which maximize the growth conditions of BSFL grown on mixed UDDT and a readily bioavailable organic substrate.

3. To measure levels of health-related organisms in UDDT faecal sludge, BSFL and BSFL digested residual sludge, and to assess the health implications for householders, farmers and /or workers using processed by-products.

4. To monitor the fate of the pathogen indicators during urine processing, including measuring levels in struvite and struvite-depleted urine. This includes developing an understanding of how these respond to drying of struvite under different combinations of temperature and relative humidity.

5. To develop broad health-based guidelines on how UDDT faecal sludge and source separated urine can be processed safely for beneficiation.

2. METHODOLOGY

This chapter has two sections that provide the methodology used for BSFL and struvite production, respectively.

2.1 TREATMENT OF UDDT FAECAL SLUDGE BY BSFL

This section provides details of the methodology used to evaluate the treatment of BSFL on UDDT sludge and its combination with other organic substrates.

2.1.1 Sample Collection and Preparation

UDDT sludge and food waste were collected from the Isipingo BSFL treatment plant, south of the city of Durban. Bagasse, a dry pulpy organic fibrous residue left over juice extraction, was collected from Eston sugarcane mill while poultry feed was bought from a local pets and aquarium store. Both UDDT and food waste samples were collected in closed containers and stored in the cold room at 4°C. Two-day old BSFL were obtained from AgriProtein in Cape Town and they were fed 90% food waste and 10% poultry feed for 15 days in the nursery at a temperature of 28°C and relative humidity of 60%. All samples were immediately stored at 4°C after collection.

A total mass of 800 g feed (UDDT Sludge and Additive mixture) for each treatment protocol in the experiment was prepared according to varying ratios as listed in **Table 1**. A mass of 200 g of this feed mix was then transferred to a 1 L container and this was prepared in triplicates. The sludge-additive mixture was inoculated with 200 BSFL, fed at a feeding rate of 100 mg feed (Sludge-Additive mixture) $larvae^{-1}.day^{-1}$ for a period of 10 days. Distilled water was added to the feed mix in each of the treatment protocols in order to increase the moisture accordingly.

2.1.2 Determination of Moisture Content of Faecal Sludge and BSFL Growth Rate

Initial tests were conducted in order to estimate how much water is required to reach the desired moisture content of 70-80%. Crucibles were prepared and dried in an oven for 1 hour at 105°C. Crucibles were removed after an hour and allowed to cool down. Faecal sludge from UDDT, *Food Waste* (FW), *Poultry Feed* (PF) and *Bagasse* (B) was mixed in order to get a well-mixed sample. Samples weighing 100 g were mixed according to different treatments (**Table 1**). Samples of 20 g from each treatment was transferred into a crucible of known mass and this was done in triplicates (60 g in total used). Samples were dried in an oven for 24 hours at 105°C, thereafter removed and allowed to cool for 15 minutes and then weighed out. Different amount of distilled water was added to the remaining 40 g of samples, the amount of water added was based on speculation and the above procedures were repeated.

Table 1. Sample treatments with varying water addition to correct for moisture. Key: B = Bagasse, FW = Food Waste, PF = Poultry Feed, UDDT = Urine Diverting Dehydration Toilet.

Treatment No.	Original samples without water addition (100 g)	Samples with distilled water added (40 g)
Treatment 1	UDDT only	UDDT only + 10 mL
Treatment 2	75UDDT + 25FW	75UDDT + 25FW + 5 mL
Treatment 3	50UDDT + 50FW	50UDDT + 50FW + 10 mL
Treatment 4	75UDDT + 25PF	75UDDT + 25PF + 15 mL
Treatment 5	50UDDT + 50PF	50UDDT + 50PF + 40 mL
Treatment 6	75UDDT + 25B	75UDDT + 25B +30 mL
Treatment 7	50UDDT + 50B	50UDDT + 50B + 40 mL

2.1.3 BSFL Growth Rate Determination

The sludge-additive mixture was inoculated with 100 pre-weighed BFS larvae. The larvae was fed at the feeding rate of 100 mg feed (Sludge-Additive mixture) $larvae^{-1}.day^{-1}$ at a depth of 100 mm for a period of 10 days. At day 0 (first day of the experiment and day 10 (last day of the experiment), 25 randomly picked larvae were weighed to determine their growth rate and sludge digestion. The experiments were conducted in a controlled environment with a temperature of 28°C and relative humidity of 60%.

2.1.4 Establishment of the Best UDDT Faecal Sludge to Organic Matter Ratio

Three different additives: *Food Waste* (FW), *Poultry Feed* (P) and Bagasse (B) were used at different percentages of 0 % (Z), 25% (Y) and 50 % (X) to aid in the digestion of the sludge. The additives were added to various proportions of sludge and a defined amount of BFSL to establish the optimal conditions for sludge digestion as shown in **Table 2**.

Table 2. Different percentage ratio of UDDT sludge to additives used in feed preparation for BSFL digestion.

Additives	Experimental Setup					
Food waste (FW)	0-UDa-FWx	0-UDb-FWy	0-UDc-FWz	1-UDa-FWx	1-UDb-FWy	1-UDc-FWz
Bagasse (BG)	0-UDa-BGx	0-UDb-BGy	0-UDc-BGz	1-UDa-BGx	1-UDb-BGy	1-UDc-BGz
Poultry feed (PF)	0-UDa-PFx	0-UDb-PFy	0-UDc-PFz	1-UDa-PFx	1-UDb-PFy	1-UDc-PFz

Key: UD= UDDT sludge; 0 =No BSFL added, 1 =BSFL added; FWx =50%; FWy =25%; FWz = 0%; BGx= 50%; BGy= 25%; Bgz= 0%; PFx=50%, PFy= 25%; PFz= 0%; UDa= 50%; UDb= 75%, UDc= 100% UD

2.1.5 Analysis of Physico-Chemical Parameters

Physio-chemical analyses of the sludge and of sludge mixed with feed additives were conducted before and after digestion with BSFL following methods previously established by the University of KwaZulu-Natal (UKZN) (Pollution Research Group, UKZN, Standard Operating Procedure:

http://prg.ukzn.ac.za/laboratory-facilities/standard-operating-procedures).

2.1.5.1 Moisture Content, Volatile Solids and Ash Content

After thorough mixing of samples to homogeneity, 20 g (Vml, Wg) from each treatment were transferred into a pre-weighed crucible (W1) and dried in an oven for 24 hours at 105 °C. Samples were then removed the next day and allowed to cool down for 15 minutes before weighing **(**W2**)** and moisture content was calculated by using equation (1).After weighing the samples for moisture content determination; samples of known mass were then heated in a furnace at a temperature of 550°C for 2 hours and cooled at lab temperature then weighed to determine the volatile solids. The lost mass of the samples gave the number of volatiles solids and this was calculated using the equation shown in eq. (2).

$$\text{Moisture content } (\%) = [Wsample - (W2 - W1)] \text{ X } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where W1 = weight of empty crucible (g); W2 = weight of residue after oven (105ºC) (g) and

W Sample = Weight of sample (g). = ((WSample-W2)/W Sample) ×100

$$\text{Volatile solide reduction } (\%) = \frac{(Vi - Vo)}{(Vi - (Vi - Vo))} \text{ X } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where V_i = volatile fraction in feed sludge and V_o = volatile fraction in digested sludge.

2.1.5.2 Measurement of pH value

Well homogenised samples (20 g) was added to 20 mL distilled water in a 50 mL beaker and mixed continuously for 5 min to form a homogenous solution. Samples were allowed to settle for 15 min and the pH of the supernatant was measured with pH meter (Hach sensION™+ MM374) (Lindsay and French, 2005).

2.1.5.3 COD measurement

Samples (1 g) were thoroughly dissolved in 500 mL of distilled water, blended for 2 minutes, and then transferred to 1 L bottle and filled with 500 mL of distilled water. COD was analysed using closed reflux titrimetric method. In this method, a strong acidic dichromate solution was used to digest the diluted sample for 2 h. Silver sulphate was used as a catalyst and mercuric sulphate was used as a masking agent to prevent chloride interference. The dichromate was partially reduced by the oxidizable material present in the sample. The excess dichromate is titrated with ammonium iron (II) sulphate and the COD value calculated from the amount of dichromate. The half-reaction for the reduction of dichromate is: $Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3-} + 7H_2O$. The remaining dichromate is titrated with a standard ammonium iron (II) sulphate solution: $Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ \rightarrow 6Fe^{3+} + 7H_2O + 2Cr^{3+}$. The equivalence point is indicated by the sharp

colour change from blue-green to red as the ferroin indicator undergoes reduction from iron (III) to the iron (II) complex. (http://prg.ukzn.ac.za./laboratory-facilities/standard-operating-procedures.

2.1.5.4 Nutritional Analysis

Samples (1 g) were thoroughly dissolved in 500 mL of distilled water, blended for 2 minutes, and then transferred to 1 L bottle and filled with 500 mL of distilled water. Total Kjeldahl nitrogen determination, which is the sum of nitrogen bound in organic substances, nitrogen in ammonia (NH_3-N) and in ammonium (NH_4^+-N), the test methods from the Pollution Research Group, UKZN, were used (http://prg.ukzn.ac.za./laboratory-facilities/standard-operating-procedures). Nitrogen content was estimated by dividing the protein content by 6.25 (Diener *et al.,* 2009). Phosphate and potassium contents were analysed using Varian 710-ES ICP-OES Analyzer (Palo Alto, CA, USA).

2.1.6 Microbial Analysis of the Faecal Sludge

The population of faecal coliforms, *E. coli*, and total coliforms in the UDDT sludge were determined using standard membrane filtration technique and plating on different selective media. The plates were incubated at respective different incubation temperatures and time. Fifty millilitres of different serially diluted samples were filtered. The filters were placed on different selective media and incubated appropriately. For *E. coli* and total coliforms, Chromocult® Coliform Agar (Merck) was used and the plates were incubated at 35°C for 24 hours. For faecal coliforms, m-FC agar was used and the plates were incubated at 44.5°C for 24 hours. The number of typical colonies were counted and expressed as *Colony Forming Units* per millilitre of sample (CFU/mL).

2.1.7 Enumeration of Helminth Eggs

Samples (10 g) of sludge and digested sludge mixed with feed were analyzed for the presence of helminth eggs using the method described Moodley *et al.* (2008) for sludge, as modified by Pebsworth *et al.* (2012). Composite samples from three replicates from each treatment were emptied into a beaker followed by the addition of ammonium bicarbonate. The mixture was stirred for homogeneity and then poured through a set of sieves (mesh size 20-100 µm). Helminth eggs pass through the larger sieve where they are retained. Sediment was washed off the second sieve and allowed to stand. After the sediments settled, the supernatant was discarded, and the sediments were centrifuged. Thereafter, the resulting supernatant was collected and zinc sulphate resulting in the flotation of helminth eggs present. The supernatant containing eggs were washed with water to remove zinc sulphate. Eggs were transferred to a clean test tube containing some 3 mL of water and centrifuged. The supernatant was discarded while retaining the pellet which contains helminth eggs at the bottom of the test tube. The light

microscope was used for the enumeration of eggs present in the pellet. The eggs were classified as viable or non-viable based on their morphology.

2.2 STRUVITE PRODUCTION FROM SOURCE-SEPARATED URINE AND ANALYSES

This section provides details of the methodology used to evaluate the struvite precipitation from source-separated urine.

2.2.1 Urine Collection

Fresh urine was collected from collection tanks serving households at eNtshongweni, a peripheral informal settlement in Durban. This fresh urine was collected by field teams of the *eThekwini Water and Sanitation* (EWS) unit and was transported to the EWS Newlands Mashu field test facility for immediate sampling and analysis. The collection was conducted in triplicate and samples for microbiological analysis were collected in sterile 50 mL centrifuge tubes. The samples were taken to the laboratories at the School of Life Sciences, UKZN (Westville Campus) for analysis.

2.2.2 Struvite Production

The techniques used were developed for the most efficient phosphorus recovery in a field production setting. A manual 320 L struvite reactor was used to produce struvite from fresh urine at the EWS Newlands Mashu facility (**Figure 1**). The dimensions of the reactor were: diameter 300 mm; height 840 mm; with 160 mm of freeboard. The reactor was fitted with four vertical baffles and two mixing impellers for improved mixing. The mixing shaft was equipped with a bearing ring assembly making the mixing crank free-moving and easy-to-use. A manual valve was used to contain or drain the reactor contents between reaction periods.

During struvite production, industrially produced magnesium was mixed with fresh urine of between 1 to 3 weeks old at the UDDTs collection tanks. The historical average concentration of phosphorus (280 mg P/L) from the same UDDTs was used to prepare a magnesium. The process was conducted manually; 40 L of urine was poured in the reactor adding the magnesium dosing solution (0.5 L) into the top vessel. Mixing was conducted by 60 counts of one second per crank arm revolution. The struvite precipitated was collected in a nylon filter bag by opening the valve that would drain the content into the filter bag and this was repeated four times, resulting in the production of up to 100 g of struvite from 160 L of urine. The struvite was collected onto a circular nylon filtration unit and transported back to the laboratories at the School of Life Sciences, University of KwaZulu-Natal (Westville Campus) for analysis.

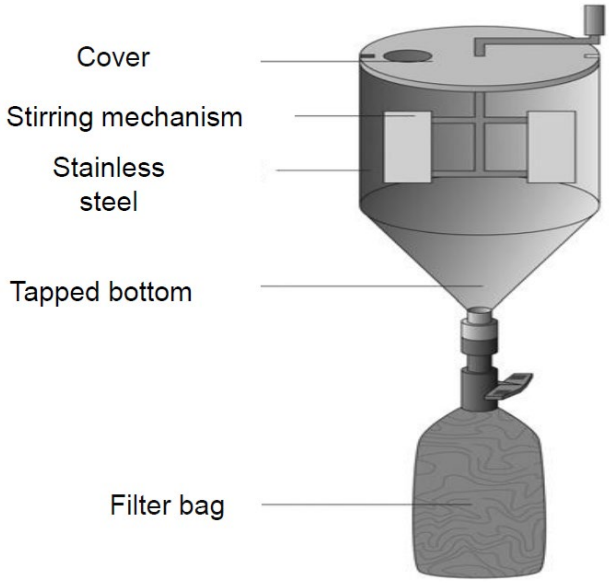

Figure 1. Representation of the composition of the struvite reactor.

2.2.3 Optimisation of Struvite Recovery from Urine

2.2.3.1 Optimisation of molar concentrations

Study on different Mg: P molar ratio (1:1, 3:1 and 5:1) was conducted relative to the initial measured P concentration. This was followed by urine crystallisation process at 10, 30 and 60 minutes stirring time. Thereafter, the recovered P & N concentrations were determined from the obtained struvite at different intervals, and the molar ratio that resulted in higher P & N recovery at a particular stirring time was used as optimal molar ratio for pH optimisation studies. Recovery efficiencies of P & N from struvite were determined by calculating final concentration of respective parameter and compared to the initial concentration in the urine.

2.2.3.2 Optimisation of pH

The urine sample was adjusted to different pH ranging from 7.0 to 11.0 using 1 M HCl or 1 M NaOH. This was followed by crystallisation of the urine sample at optimum reaction time (Mg: P molar ratio optimisation) for 30 minutes prior to filtration. The resultant crystalline filtrates from each pH were then analysed for P & N concentrations. The recovery efficiencies were calculated by measuring the final P & N concentration from the produced struvite and then compared relative to the initial concentration in the urine used.

2.2.3.3 Crystal and micro-elemental analysis of urine-derived struvite

The crystals (0.5 g) obtained from urine crystallisation process was filtered, dried at room temperature overnight and then stored at 4°C for *Scanning Electron Microscopic* (SEM) and *Energy Dispersive X-Ray Spectrometer* (EDS) analyses for elucidation of surface, size and chemical components of the crystals.

2.4 MICROBIAL ANALYSIS OF URINE AND STRUVITE AND URINE pH DETERMINATION

2.4.1 Culture Media Preparation

Nutrient broth media was prepared by adding 23 g of the nutrient broth in 1 L of sterile deionized water, mixed and dissolved by heating, autoclaved at 121°C for 15 minutes and left to cool to 50°C for the addition of filtered 1 mL streptomycin. The media was stored in a refrigerator at 4°C. The base agar contained 11 g F agar, 13 g tryptone, 8 g sodium chloride and 1.5 g glucose in 1 L deionized water. The top agar contained 6 g agar (Oxoid), 1 g tryptone, 8 g sodium chloride, and 3 g glucose in 1 L deionized water. The media was autoclaved at 121°C for 15 minutes and cooled to 50°C, after which 100 mL filter-sterilised calcium chloride containing 1 mL streptomycin was added.

2.4.2 Host cell preparation

E. coli was used as host strains for somatic coliphages and bacteriophages were obtained as stock cultures. Initially, these were obtained from the eThekwini Scientific Services microbiology laboratory (*E. coli* 13706 culture) and other two host cultures from Dr Heather Bischel, *Ecole Polytechnique Fédérale de Lausanne* (EPFL) in Switzerland. The stock cultures were prepared by overnight growth so that the bacteria were in log phase. The bacterial host culture (85 µL) was mixed with 15 µL glycerol in 1 mL sterile centrifuge tubes and stored at -80℃ in a freezer. When needed for the detection of phages, the relevant host culture was inoculated into nutrient broth media and incubated at 30℃ overnight.

2.4.3 Plating, incubation and enumeration

Serial dilutions of urine (10^{-1} to 10^{-5}) were prepared prior to analysis, by pipetting 1 mL from the original sample into 9 mL phosphate buffer (PBS; 5 mM $NaH_2PO_4.H_2O$, 10 mM NaCl at pH 7.5). Somatic coliphages and bacteriophages in struvite were enumerated using the method from Decrey and co-workers (2011), modified by using 1 g of struvite instead of 50-100 mg. The struvite was dissolved in 9 mL citrate buffer (pH 5.4), then serial dilutions (10^{-1} to 10^{-3}) were made in phosphate buffer. The presence of somatic coliphages and bacteriophages *øX174* was detected using a double layer agar technique. Sample (1 mL) and *Escherichia coli* bacteriophage MS2 and host culture (0.2 mL were added to 2.5 mL molten top agar and mixed by vortexing for 60 seconds). The mixture was poured onto 90 mm Petri dishes containing solidified base agar. Each dilution was plated in triplicate at incubated at 37°C overnight. The results were recorded by counting the number of plaques formed and calculating the *Plaque Forming Units* per ml or gram (PFU/g or mL). For bacterial analysis, 0.1 mL of the raw sample as well as dilutions 10^{-1} to 10^{-4} was spread-plated in triplicate onto the following selective media for the prescribed incubation period (**Table 3**). After the incubation period, the media was visually inspected for the presence of bacterial colonies. The pH of the urine was determined in triplicate

using a Crison pH-Meter BASIC 20^{+} and the average value was calculated.

Table 3. List of selective media used for the different indicator organisms and the incubation condition

Media:	**Indicator organism**	**Incubation period:**
Chromocult Coliform agar	Total coliforms and *Escherichia coli*	37°C for 24 hours
MFC agar	Faecal coliforms	44.5°C for 24 hours
Kenner Faecal Agar	Enterococci and Faecal Strepococci	35°C for 24 hours
Salmonella Shigella Agar	Salmonella and Shigella	37°C for 24 hours
Nutrient Agar	Total Heterotrophic Bacteria	37°C for 24 hours

2.4.4 Bacterial and phage inactivation during drying

The produced struvite was then divided into 3 equal parts and subjected to drying at different temperatures (25°C, 37°C and 42°C). Samples were taken through the duration of drying experiments; 1 g of the struvite (day 0) and daily during the drying period. Samples were analysed for microbial load as previously described.

In the next chapter (**Chapter 3**), the evaluation of BSFL treatment is presented.

3. BSFL TREATMENT

The various treatment combinations evaluated are listed below:

- Treatment 1: UDDT sludge only
- Treatment 2: 75% UDDT sludge and 25% Food Waste
- Treatment 3: 50% UDDT sludge and 50% Food Waste
- Treatment 4: 75% UDDT sludge and 25% Poultry Feed
- Treatment 5: 50% UDDT sludge and 50% Poultry Feed
- Treatment 6: 75% UDDT sludge and 25% Bagasse
- Treatment 7: 50% UDDT sludge and 50% Bagasse

3.1 MOISTURE CONTENT, VOLATILE SOLIDS AND ASH CONTENT

The descriptive statistics associated with solids testing of UDDT sludge digested under different treatment conditions in the presence or absence of BSFL are reported in **Table 4**. Initially before the digestion, the moisture content of UDDT sludge only (treatment 1) was 70.90%. After digestion by BSFL for a period of 10 days, compared to other treatments, treatment 1 had the lowest moisture content of 58.73% and 66.99% recorded in the control .While the highest moisture content was recorded in treatment 3 at a percentage of 75.39% and in the control of treatment 6 recorded to be 66.99% (**Table 4**). The moisture content gradually increased for all the experimental setup throughout the digesting period (**Table 4**). There was no significant difference between the measured moisture content values between treatments when an ANOVA test was conducted.

The collected UDDT sludge sample had a *Volatile Solids* (VS) value of 33.96% however, after digestion by BSFL a 7.43% reduction was observed while the control without BSFL was reduced by 6.19%. For treatment 2, there were highly reduced volatile solids compared to treatments 1 to 5 at a percentage of 49.71% in the digested feed and to 18.96% in the control. There was no VS reduction recorded in treatments that had bagasse as an additive (treatment 6 and 7). Statistically significant reduction of volatile solids in the presence of BSFL was recorded on treatments 2 (p=0.00), 3 (p=0.04), 4 (p=0.01) and 5 (p=0.00). Whereas in the absence of the BSFL, the significant volatile solids reduction was recorded on treatments 2 (p=0.02), 3 (p=0.04) and 5 (p=0.02).

Table 4. Solids analysis of UDDT sludge digested under different treatment conditions in the presence or absence of BSFL.

Solids	Treatment	1	2	3	4	5	6	7
MC (%)	Initial	70.90 ± 0.23	69.12 ± 0.30	71.74± 0.54	68.73± 0.43	70.26±1.19	70.33 ±2.10	69.79 ± 2.13
	After	58.73±10.21	65.95± 0.26	75.39 ± 0.91	70.57 ±2.23	71.30±2.24	66.28 ±4.20	65.89 ± 5.97
	Control	66.99 ± 3.06	69.06 ± 3.34	72.29 ±0.74	68.65 ±1.83	71.50 ±1	72.59 ±1.01	70.33 ±1.11
VS (%)	Initial	33.96 ±1.05	49.57±0.91	64.92±3.44	55.34±1.18	71.15±2.11	40.81±3.41	46.41±1.84
	After	31.61±1.05	33.11±4.05*	57.56±2.70*	43.90±4.53*	51.39±3.86*	41.57±1.69	47.50±1.60
	Control	31.98±0.80	41.67±0.66*	57.48±1.75*	49.92±1.06	62.04±1.07*	42.55±1.00	45.48±2.13
Ash (%)	Initial	66.04 ±1.05	50.43±0.91	35.08±3.44	44.66±1.18	28.85±2.11	59.19±3.41	53.59±1.84
	After	68.39±1.05	66.89±4.05*	42.44±2.70*	56.10±4.53*	48.61±3.86*	58.61±1.69	52.50±1.60
	Control	68.02±0.80	58.33±0.66*	42.52±1.75*	50.08±1.06	37.96±1.07*	57.45±1.00	54.52±2.13

MC: Moisture content; **VS**: Volatile solids; **1**: UDDT sludge; **2**: 75% UDDT sludge+25% Food Waste; **3**: 50% UDDT sludge+50% Food waste**; 4:** 75%UDDT sludge+25% Poultry Feed; **5**: 50% UDDT sludge+50% Poultry Feed; **6**: 75% UDDT sludge+25% Bagasse; **7**: 75% UDDT sludge+50% Bagasse. Significant relationships ($p<0.05$) are indicated by an asterisk (*)

The ash content of the UDDT sludge only was initially 66.04% and was detected to slightly increase after 10 days of the experiment to a value of 68.39% after digestion and to 68.02% in the control. The ash content in treatment 6 and 7 at initial was similar to the ash content of the digested feed and in the control (**Table 4**). There was a general increase in ash content from the initial compared to the ash content conducted at the end of the experiment. The significant increase in ash content recorded in the presence of BSFL was on treatments 2 (p=0.00), 3 (p=0.04), 4 (p=0.01) and 5 (p=0.00). Whereas in the absence of the BSFL the significant increase of ash content was recorded in treatment 2 (p=0.02) and 5 (p=0.02).

3.2 DETERMINATION OF pH VALUE

The results of pH measurements recorded in the experiment ranged from slightly alkaline to alkaline with the exception of sludge mixed with food waste (**Figure 2**). Statistical analysis revealed significant differences between the means of all the treatments (p=0.01). The initial pH of UDDT sludge only was 8.33 but increased after digestion. The pH value of the UDDT sludge was the highest (8.85) compared to the pH of other sludge-feed mixes used in the treatment before and after digestion. Lowest pH value was recorded in treatment 3 at a value of 6.36 which was further reduced to 4.42 after digestion. For control samples, pH ranged from 5.11 in treatment 4 to 8.84 in treatment 1. A decrease in pH profiles of the digested samples and control was recorded in treatment 2, 3 and 4 while slight increase was recorded in treatment 1 (**Figure** 2).

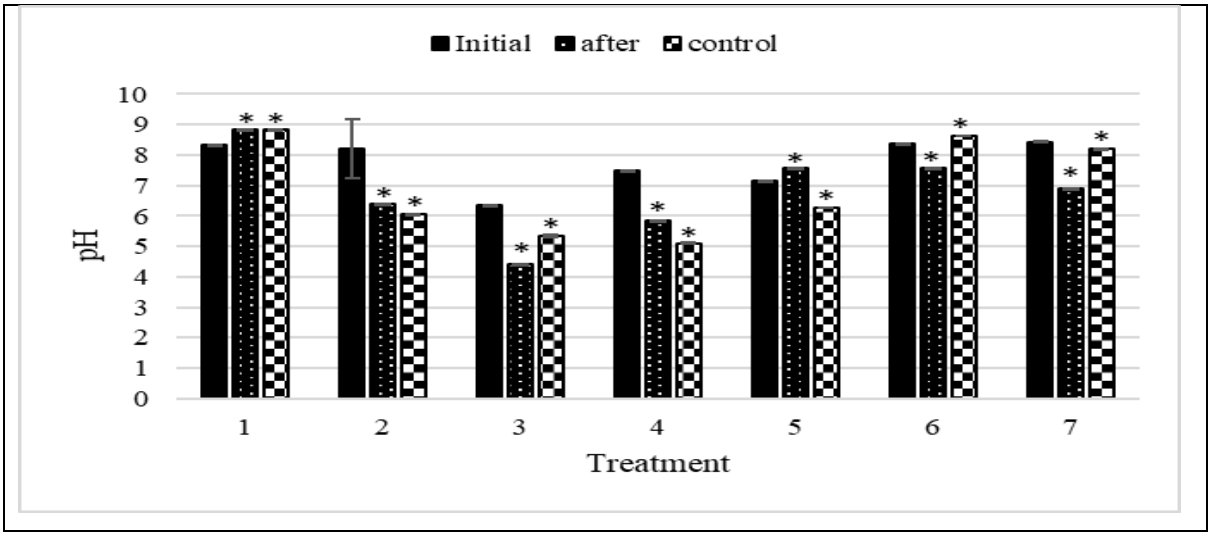

Figure 2. pH profiles of the samples before and after digestion in the presence (after) and absence (control) of BSFL. Key: Treatment 1 (UDDT sludge only), Treatment 2 (75%UDDT+25%Food Waste), Treatment 3 (50%UDDT+50%Food Waste), Treatment 4 (75%UDDT+25%Poultry Feed), Treatment 5 (50%UDDT+50%Poultry Feed), Treatment 6 (75%UDDT+25%bagasse), Treatment 7 (50%UDDT+50%bagasse).

3.3 CHEMICAL OXYGEN DEMAND (COD)

Figure 3 presents the COD profile of the different treatment steps. The COD of the UDDT sludge was 563.1 mg/g (Treatment 1). After digestion, the COD of the UDDT sludge was reduced to 326.7 mg/g, the lowest value among all treatments.

In treatment 3, the mixture of food waste with sludge and subsequent digestion with BSFL saw an increase in COD values. Furthermore, a great increase in COD profile recorded on treatments 3 (p=0.00) for the control and after digestion and on treatment 6 (p=0.02) in the control. Addition of bagasse as a feed additive before digestion (treatment 6) revealed only a slight decrease in COD values (385.53 mg/g). A decrease in the level of COD level was recorded in treatments 1, 2 and 7, after digestion with BSFL whereas a significant decrease was recorded on treatment 1 (p=0.00) in the control and after digestion.

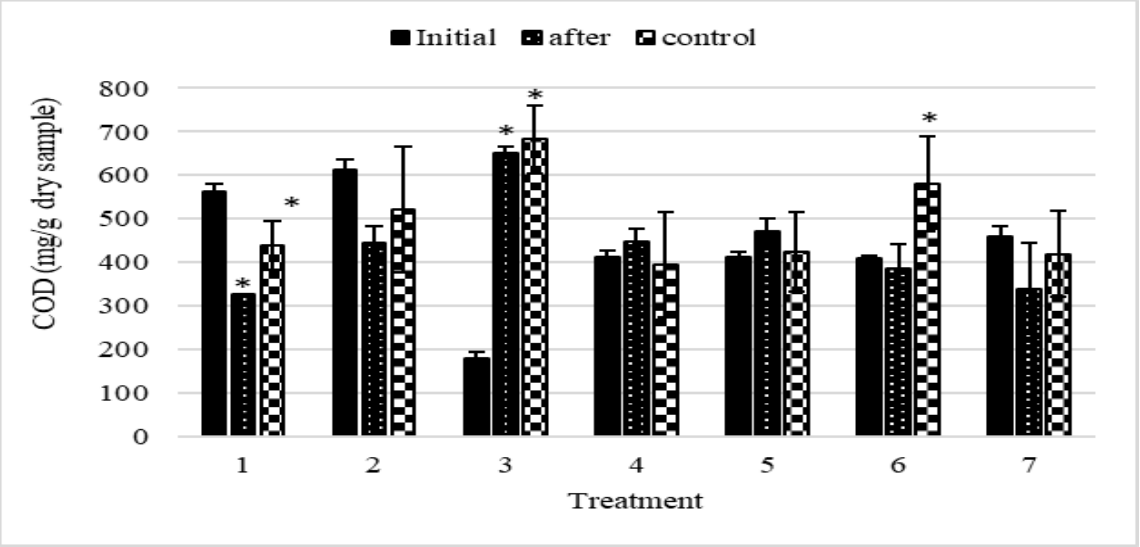

Figure 3. Profile of COD for the different treatment conditions before and after digestion. Key: Treatment 1 (UDDT sludge only), Treatment 2 (75%UDDT+25%Food Waste), Treatment 3 (50%UDDT+50%Food Waste), Treatment 4 (75%UDDT+25%Poultry Feed), Treatment 5 (50%UDDT+50%Poultry Feed), Treatment 6 (75%UDDT+25%bagasse), Treatment 7 (50%UDDT+50%bagasse).

3.4 NUTRITIONAL PROFILES OF UDDT SLUDGE AND UDDT SLUDGE WITH ADDITVES

3.4.1 Total Kjeldahl Nitrogen (TKN)

The sample with UDDT sludge only had TKN of 1.87% which decreased following 10 days of the experiment to 1.75% in the digested sludge and to 1.76% in the control (**Figure 4**). After digestion, the highest percentage of TKN was recorded in treatment 3 at a value of 2.59% while a lowest TKN percentage was recorded in treatment 7 at a value of 1.01%. For control, the highest TKN percentage was recorded in treatment 3 at a value of 2.47% with a lowest TKN recorded in treatment 7 at a value of 1.41%. The significant increase relative to the initial was recorded on treatment 2 the control (p=0.03).

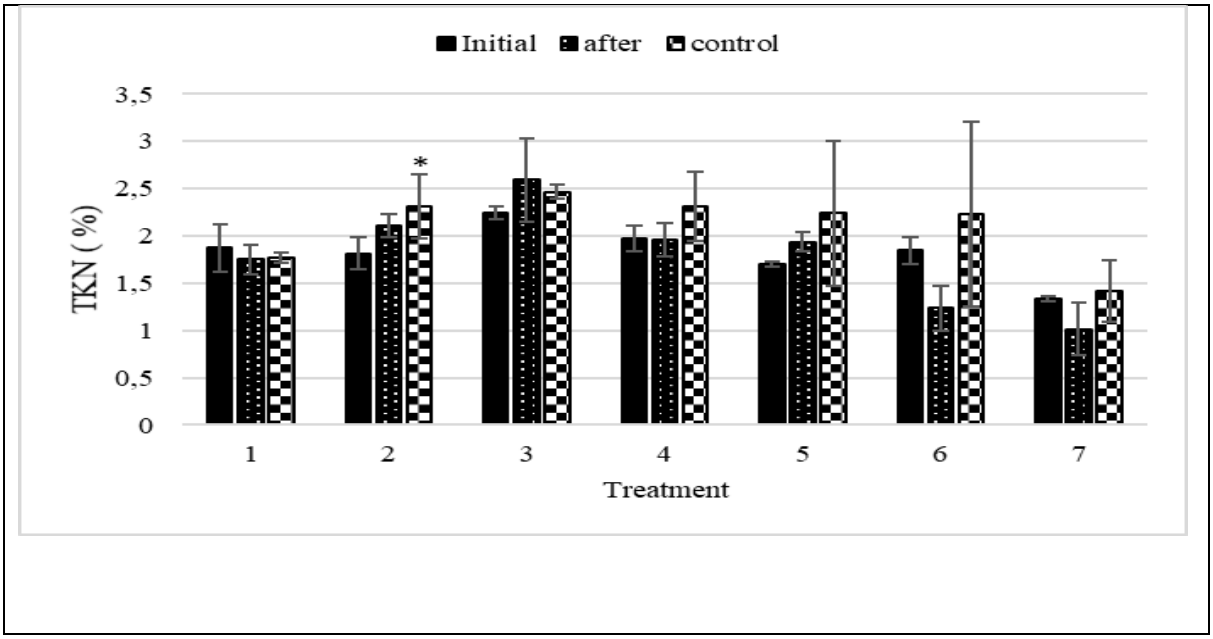

Figure 4. Total Kjedahl Nitrogen in the faecal sludge samples before and after digestion in the presence (after) and absence (control) of BSFL. Key: Treatment 1 (UDDT sludge only), Treatment 2 (75%UDDT+25%Food Waste), Treatment 3 (50%UDDT+50%Food Waste), Treatment 4 (75%UDDT+25%Poultry Feed), Treatment 5 (50%UDDT+50%Poultry Feed), Treatment 6 (75%UDDT+25%bagasse), Treatment 7 (50%UDDT+50%bagasse).

3.4.2 Nitrogen

The sample with UDDT sludge only had 1.36% of Nitrogen at initial which slightly increased to 1.42% in the digested sludge and to 1.51% in the control (**Figure 5**). An addition of food waste to UDDT sludge by 50% (treatment 3) resulted in the increase of nitrogen by approximately 60%. Treatment 6 and 7 which had bagasse as an additive showed low nitrogen in the initial and at the end of the experiment. After digestion the highest nitrogen content was recorded in treatment 3 recorded at a value of 2.51% while the lowest nitrogen content of 1.18% was recorded in treatment 7. For control, the highest nitrogen content was recorded in treatment 5 at a value of 2.73% and the lowest value of 1.24% recorded in treatment 7. For all the treatments, nitrogen content was higher in the control compared to the initial and digested feed.

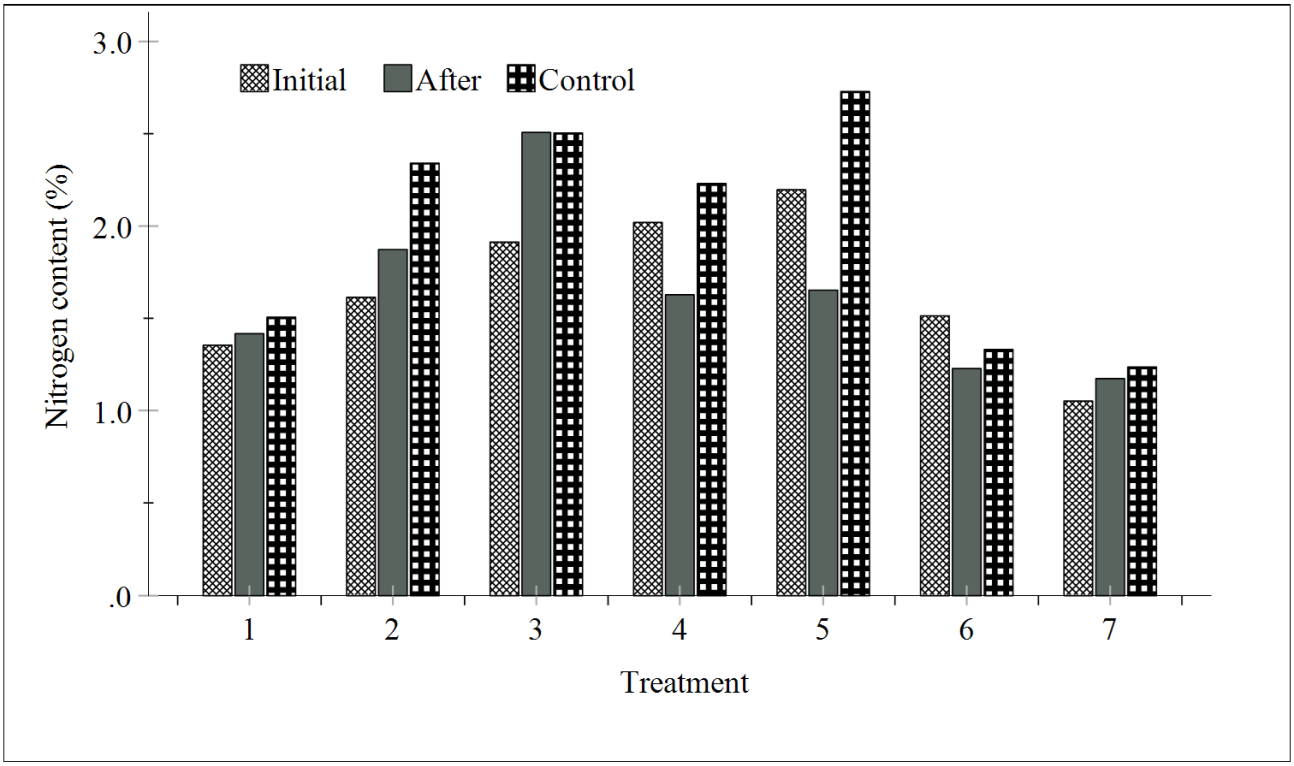

Figure 5. Percentage nitrogen concentration in the faecal sludge samples before and after digestion in the presence (after) and absence (control) of BSFL. Key: Treatment 1 (UDDT sludge only), Treatment 2 (75%UDDT+25%Food Waste), Treatment 3 (50%UDDT+50%Food Waste), Treatment 4 (75%UDDT+25%Poultry Feed), Treatment 5 (50%UDDT+50%Poultry Feed), Treatment 6 (75%UDDT+25%bagasse), Treatment 7 (50%UDDT+50%bagasse).

3.4.3 Phosphate

The sample with UDDT sludge only had phosphate content of 1.31% which slightly increased after digestion to 1.42% and highly increased in the control to 6.11% (**Figure 6**). After digestion, the highest phosphate content was recorded in treatment 5 at a value of 2.60% while a lowest content was recorded in treatment 7 at a value of 0.92%. In the control, the highest content was recorded in the sample with UDDT sludge only at a value of 6.11% while a lowest content was recorded in treatment 7 at a value of 0.87%. The significant statistical difference was recorded in treatment 1 (control), 2, 3, 4, 5 (after) and 7 at a p-value 0.00. Treatment 3 after digestion was significantly different with a p-value 0.02 and the control of treatment 5 was significantly different by a p-value 0.04.

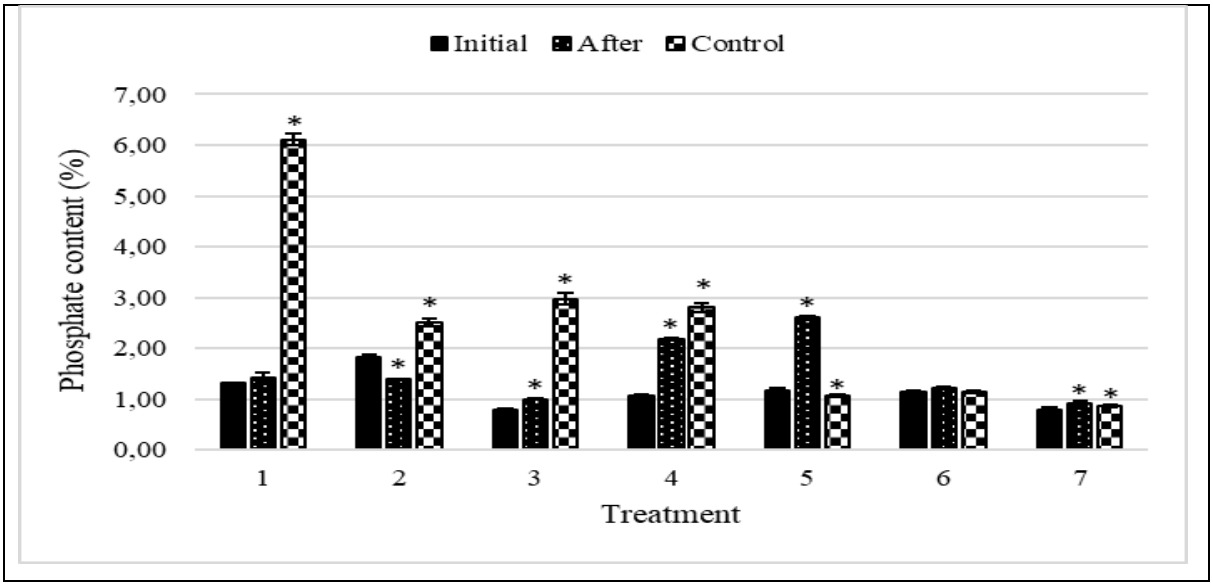

Figure 6. Percentage phosphate concentration in the faecal sludge samples before and after digestion in the presence (after) and absence (control) of BSFL. Key: Treatment 1 (UDDT sludge only), Treatment 2 (75%UDDT+25%Food Waste), Treatment 3 (50%UDDT+50%Food Waste), Treatment 4 (75%UDDT+25%Poultry Feed), Treatment 5 (50%UDDT+50%Poultry Feed), Treatment 6 (75%UDDT+25%bagasse), Treatment 7 (50%UDDT+50%bagasse).

3.4.4 Potassium

The sample with UDDT sludge only had the potassium concentration of 0.91% which increased after digestion to 1.14% and to 1.38% in the control. The highest potassium content after digestion was recorded in treatment 5 at a value of 1.21% with the lowest content recorded in treatment 7 at a value of 0.44%. In the control, Treatment 4 had the highest potassium content than other treatments at a value of 1.57% while the lowest potassium content was recorded in treatment 6 at a value of 0.71%. Treatments 1/3(control)/5(after)/6 and 7 control had statistical significance difference relative to the initial at a p-value 0.00. Treatment 2 after digestion was different from the initial at a p-value 0.01, whereas treatment 3 significantly differed from initial at p vale 0.02 and the control of treatment 5 significantly differed from the initial at p-value 0.03.

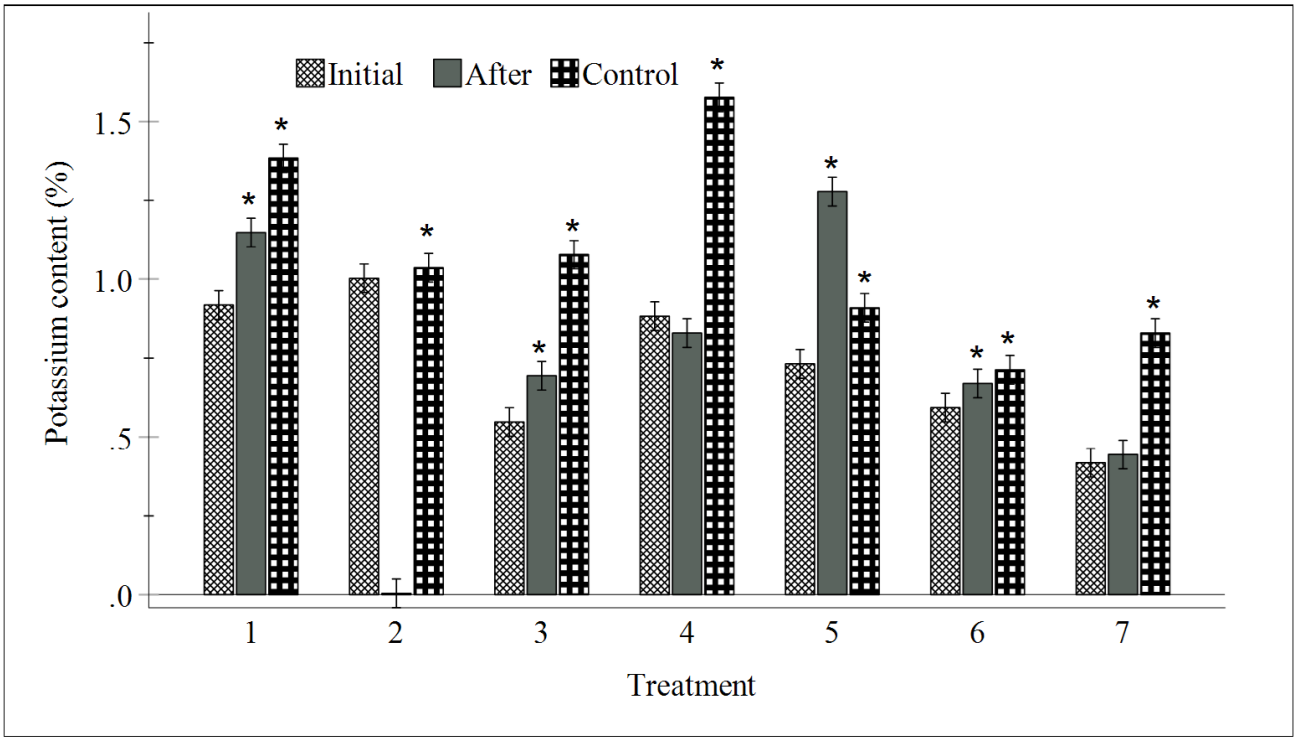

Figure 7. Percentage potassium concentration in the faecal sludge samples before and after digestion in the presence (after) and absence (control) of BSFL. Key: Treatment 1 (UDDT sludge only), Treatment 2 (75%UDDT+25%Food Waste), Treatment 3 (50%UDDT+50%Food Waste), Treatment 4 (75%UDDT+25%Poultry Feed), Treatment 5 (50%UDDT+50%Poultry Feed), Treatment 6 (75%UDDT+25%bagasse), Treatment 7 (50%UDDT+50%bagasse).

3.5 LARVAE GROWTH PROFILE

Treatment 5 which had an equal ratio of UDDT sludge to poultry feed gave BSFL with the highest mass compared to all other treatments recorded at a value of 209.34 mg/g/larvae. The BSFL fed on treatment with equal ratio of UDDT to bagasse had the lowest biomass recorded at a value of 25.69 mg/g/larvae. The BSFL fed on UDDT sludge only also showed low biomass (27.36 mg/g/larvae) compared to when BSFL were fed UDDT sludge supplemented with food waste (treatment 2 and 3) or poultry feed (treatment 4 and 5). Treatments 1, 6 and 7 which had low biomass of BSFL also showed a low percentage reduction of volatile solids or no reduction at all. Whereas, treatments 2, 3, 4 and 5 which had high larvae growth had a high reduction of the volatiles solids ranging from 26 to 57% (**Figure 8**).

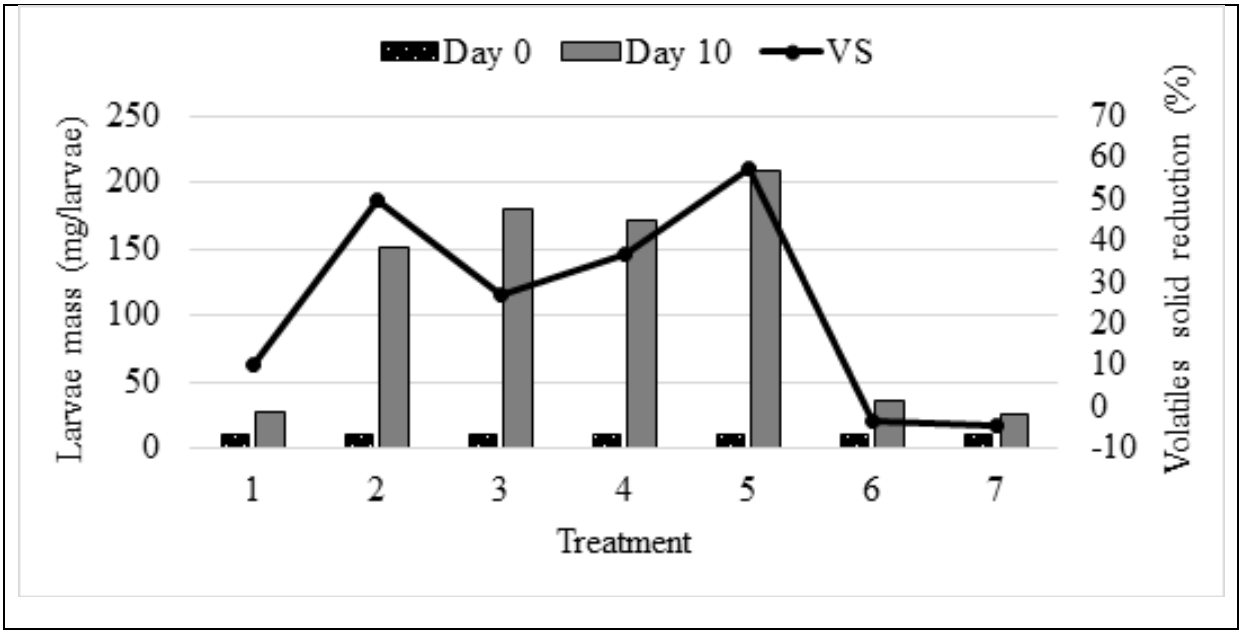

Figure 8: Larval growth (biomass) on different feed combination and volatile solids reduction before and after 10 days of feeding. Key: Treatment 1 (UDDT sludge only), Treatment 2 (75%UDDT+25%Food Waste), Treatment 3 (50%UDDT+50%Food Waste), Treatment 4 (75%UDDT+25%Poultry Feed), Treatment 5 (50%UDDT+50%Poultry Feed), Treatment 6 (75%UDDT+25%bagasse), Treatment 7 (50%UDDT+50%bagasse), VS (Volatile Solids).

3.6 MICROBIAL PROFILE OF UDDT SLUDGE AND UDDT SLUDGE WITH ADDITIVES

Table 5 shows the results of the microbial analysis. Samples from UDDT sludge only had the highest microbial load, increasing the ratio of UDDT to additive ratio increased the microbial load in the initial stage of all treatments. In treatment 1 (UDDT sludge only), faecal coliforms were removed by 99.99% after 10 days of the experiment in the feed digested by the larvae (after), while 100% removal was observed in the control without the addition of the larvae. Similar trends were observed in treatments 2, 6 and 7. In treatment 3, the removal (100%) of faecal coliforms was recorded in the feed digested by the larvae and there was no removal observed in the control. However, in treatment 4 the removal of faecal coliforms also produced the greatest concentration of faecal coliforms (86.52%) in the feed digested by the larvae, with an increase in a load of faecal coliforms in the control feed. Treatment 5 had a 40.90% removal of faecal coliforms in the feed digested by the larvae and 99.09% removal in the control, respectively.

Table 5. Faecal coliforms, *E. coli* and Total coliforms load (103) in the UDDT sludge samples before and after digestion in the presence and absence of BSFL.

IO	T	1	2	3	4	5	6	7
FC	I	7561.21±486.1	7560.67±618.2	4139.8±150.1	1486.82±158.3	554.72±66.6	1600.97±71.5	2036.01±23.4
	A	0.36±0.17*	–*	–	200.49±4.81*	339.75±36.9*	–*	6.16±0.00*
	C	–*	88.88±11.42*	158449.8±133224.10*	223319.03±0.1*	5.26±2.48*	–*	–*
EC	I.	6066.15±510.4	9001.57±10007.4	2936.8±300.24	1694.66±587.85	393.34±33.3	780.26±21.45	1837.38±117.05
	A	–*	27.90±2.07*	1.22±0.57*	2293.73±120.14	1620.33±320.3*	0.44±0.2*	10.70±1.03*
	C	–*	16968.03±5256.4*	40965.9±765.7*	70505.00±35642*	50.88±7.4	38.31±2.6*	–*
TC	I	11135.60±777.6	10312.95±160.8	5661.29±250.1	2222.24±791.3	529.50±40.4	2089.69±285.9	2797.45±210.68
	A	60.33±2.05*	2011.49±103.82*	105,84±15.80	10687.10±696.82	10628.01±641*	41.36±0.21*	78.12±11.40*
	C	–*	13412.82±228.5*	95105.98±21693*	108150.21±117756	735.15±47.14	51.08±20.64*	–*

IO= Indicator organism; FC = *Faecal coliforms*; E.C = *E. coli*; TC=*Total coliform*; T = Treatment, C= Control; I = Initial; A = after; Significant relationships relative to the initial (p<0.05) are indicated by an asterisk (*); (**–**) indicate absence of the colony. Treatment 1 (UDDT sludge only), Treatment 2 (75%UDDT+25%Food Waste), Treatment 3 (50%UDDT+50%Food Waste), Treatment 4 (75%UDDT+25%Poultry Feed), Treatment 5 (50%UDDT+50%Poultry Feed), Treatment 6 (75%UDDT+25%bagasse), Treatment 7 (50%UDDT+50%bagasse).

The removal of total coliforms was high in samples with UDDT sludge only (digested feed = 99.23%; control = 100%), treatment 6 (digested feed = 97.75%; control = 97.74%) and treatment 7 (digested feed = 96.85%; control = 100%). In treatment 2 and 3, the removal of total coliforms was observed in the digested feed while no removal was recorded in the control. The total coliform population was found to increase by 4.81- and 20.07-fold in the BSFL digested samples of treatments 4 and 5, respectively, with increased load, also observed in the control samples.

In samples from UDDT sludge only, *E. coli* was removed by a percentage of 100% in the digested feed and in the control. In treatment 2 and 3, the removal of *E. coli* was observed in the digested feed ranging from 99.66% to 99.96% respectively. Whereas no removal was observed in the control. However, *E. coli* load was not reduced in treatment 4 both in the digested feed and in the control. In treatment 5, *E. coli* was removed by a percentage of 87.61% in the control, whereas an increased *E. coli* population was observed in the digested feed. Treatment 6 had removal of *E. coli* (95.46%) in the control and 99.94% in the digested feed. A similar trend was observed in treatment 7 with 99.34% removal of *E. coli* in the digested feed and 100% removal in the control.

Overall, treatment 1, 6 and 7 effectively removed faecal coliforms, *E. coli* and total coliforms when compared to other treatments with percentage removal ranging from 95.46 to 100%. Treatment 2 had a percentage faecal coliforms removal of 98.82% for control and 100% for digested feed. The removal of *E. coli* and total coliforms was observed in the digested feed and not in the control in treatment 2 and 3. Faecal coliforms, *E. coli* and total coliforms in treatment 3 were removed in the digested feed and not in the control. In treatment 4, there was no removal of total coliforms and *E. coli* in the digested feed and control. However, 87.31% faecal coliforms (after) removal was recorded.

3.7 PROFILE OF ASCARIS EGGS IN FAECAL SLUDGE SAMPLES BEFORE AND AFTER DIGESTION

As shown in **Figure 9**, the viability of Ascaris in samples of UDDT sludge only was recorded to be 60% at initial, which decreased to 29% in the digested sludge and to 25% in the control. The removal of Ascaris viable eggs ranged between 2-35% and 4-35% for the faecal sludge digested in the presence and absence of BSFL, respectively. In the BSFL digested sludge, the highest removal of 35% and 31% was observed in treatments 7 and 1, respectively, while the lowest removal of 2% was observed in treatment 5. Similarly, in the control samples, the highest removal of 35% and the lowest removal of 4% was observed in treatments 1 and 5, respectively.

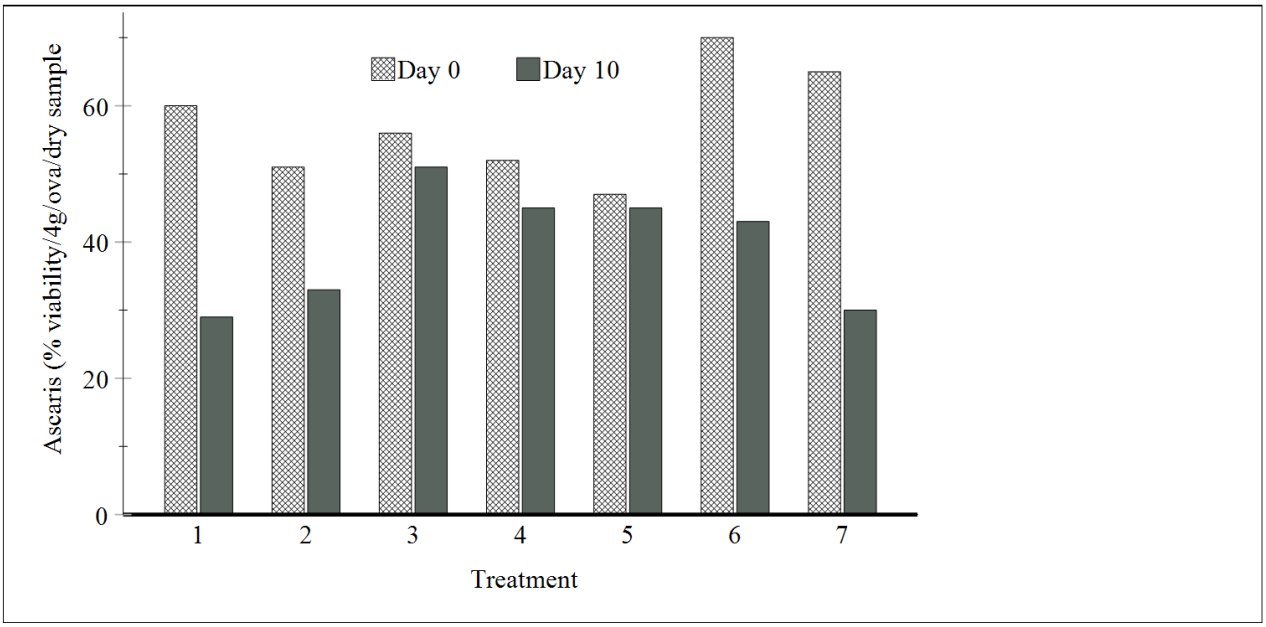

Figure 9: Percentage viable Ascaris eggs in the faecal sludge samples before and after digestion in the presence and absence of BSFL. Key: Treatment 1 (UDDT sludge only), Treatment 2 (75%UDDT+25%Food Waste), Treatment 3 (50%UDDT+50%Food Waste), Treatment 4 (75%UDDT+25%Poultry Feed), Treatment 5 (50%UDDT+50%Poultry Feed), Treatment 6 (75%UDDT+25%bagasse), Treatment 7 (50%UDDT+50%bagasse)

3.8 CONCLUSIONS

Findings from the BSFL experiments concluded that the BSFL treatment of the UDDT faecal sludge for 10 days did not influence the ash and moisture content on the feed. Volatile solids were reduced both in the presence and absence of the larvae. The presence and the absence of the larvae had no effect on the COD, pH and nutrients, but the observed difference was based on the type of feed. The viability of Ascaris also decreased both in the presence and absence of the larvae, showing that other than the presence of the larvae, other factors played a role. *Faecal coliforms* were reduced both in the presence and absence of the larvae, but *E. coli* and *total coliforms* reduced in the presence of the larvae and based on existing literature; it was assumed that pH played a role in decreasing *E. coli* load.

BSFL treatment may not be adequate to completely digest the faecal sludge for subsequent re-use. Reliable and inexpensive methods, such as ammonia sanitization or the use chemical treatment such as chlorine, lime and phosphoric acid, is recommended to further sanitize treated manure while also increasing the product agronomic value. Overall, the best ratio for producing a safe product is using 100% UDDT (treatment 1) and 75% UDDT+25% food waste (treatment 2). Food waste was the best additive compared to bagasse and poultry feed. The use of food waste as an additive to increase the digestion of faecal sludge can also solve a challenge faced in managing pollution caused by improper disposal of food waste.

4. OPTIMISATION OF STRUVITE RECOVERY FROM URINE & MICROBIAL ANALYSIS

This chapter presents the findings from the optimisation of struvite recovery from urine and the microbial analysis of produced struvite.

4.1 OPTIMIZATION OF PARAMETERS AFFECTING P & N RECOVERY FROM URINE

In the process of determining the concentration of P recovered from struvite at different molar ratios (1:1, 3:1, 5:1), there was an increase in P recovery for each molar ratio as the mixing time increases from 10 to 60 min, with highest P recovery recorded at 5:1 molar ratio showing (87.3 ± 2 mg/L) after 60 min. Conversely, 1:1 molar ratio at mixing time of 10 min showed low P recovery (68.1 ± 0.81 mg/L). Similar trend was recorded in the case of N recovery from the struvite, with enhanced N recovery observed at 5:1 molar ratio after 60 min (**Figure 10**).

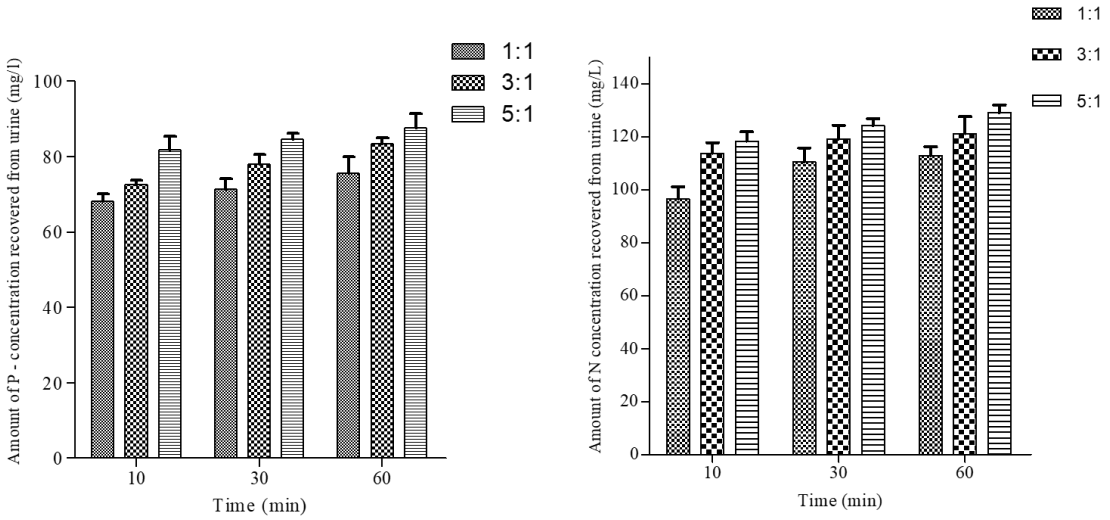

Figure 10. Phosphorus and Nitrogen recovery from urine at different Mg: P concentrations.

There was an increase in P and N recovery from the struvite as the pH increases from 7.0 to 11.0, with maximum P recovered of 94.6 ± 1.2 mg/L (96%) observed at pH 11.0. In the case of N, highest recovery (136.3 ± 102 mg/L) was recorded at pH 11.0 while low N yield of 122.6 ± 1.4 mg/L was recorded at pH 7.0 (**Figure 11**).

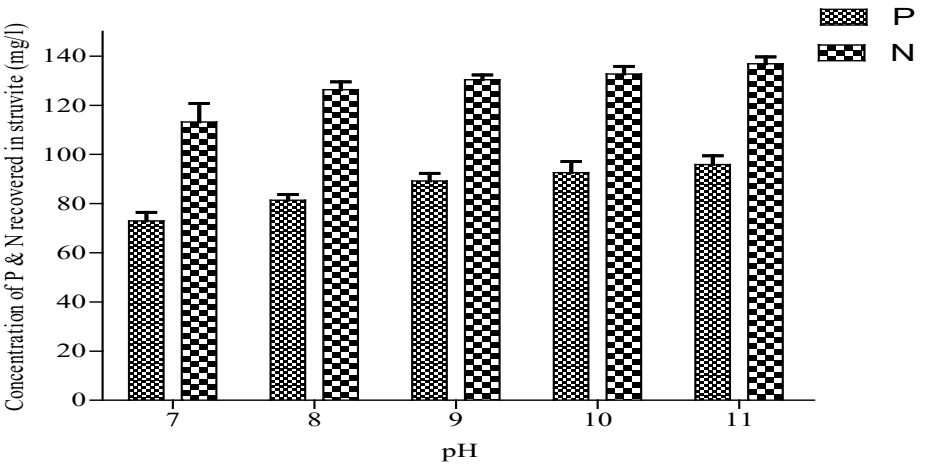

Figure 11. Phosphorus and Nitrogen recovery from urine at different pH values.

4.2 MICROSCOPIC ANALYSES OF CRYSTALS

The struvite particle morphology was elucidated at different molar ratios and mixing times. Similar trends were observed at different molar ratios and mixing times. At 1:1 molar ratio and10 minutes, the crystals appeared rectangular cubic in comparison to 3:1 treatment having an intermediate rectangular X shape. Whereas, at 5:1 molar ratio, the particles were completely X-shaped (**Figure 12**).

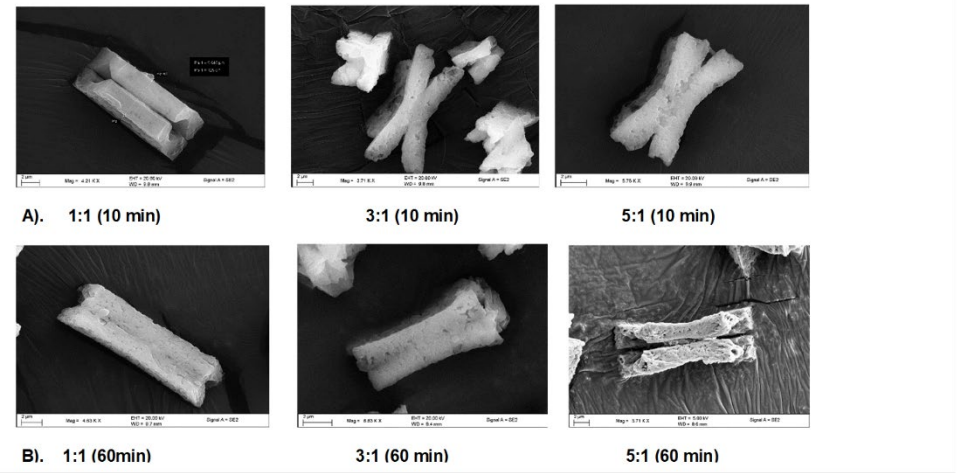

Figure 12. Scanning electron microscope images showing particle morphology of struvite produced under different molar ratios of Mg:P at A) 10 minutes and B) 60 minute treatments.

The SEM ESD (Energy Dispersive X-Ray Spectrometer) results obtained from the same struvite particles revealed a high composition of macro-elements both at 10 and 60 min mixing time (**Table 6**). Mostly observed elements include C & O. The particles also contained elements such as Na, Cl, Cu, Ca, Al and S, but at low amount.

Table 6. EDS Analysis of Mass Percent Chemical Composition in Struvite Crystals at Different Molar Ratios.

	1:1		3:1		5:1	
	10	**60**	**10**	**60**	**10**	**60**
C	37.14	48.12	39.77	38.31	47.85	38.11
O	43.80	39.15	41.39	41.72	39.34	46.55
Na	0.10	0.14	0.54	0.33	-	-
Cl	0.03	0.06	0.45	0.20	0.08	0.17
Cu	0.15	0.11	0.20	0.15	0.09	-
Ca	0.07	0.16	0.10	0.28	0.07	0.05
Al	-	-	-	-	-	-
S	0.01	0.02	0.48	0.17	0.32	-

4.3 ENUMERATION OF PHAGE AND BACTERIA IN URINE-DERIVED STRUVITE

The high availability of nutrients in urine indicates the potential of urine to contribute to food security by using urine-derived struvite as a fertiliser (Pronk and Kone, 2009). Etter *et al*. (2011) has shown that it is economical feasible to provide struvite and the associated plant nutrients to nearby communities who cannot afford chemical fertilisers. However, since source-separated urine is often cross-contaminated with small amounts of faeces, the health risks associated with the use of urine-derived struvite must be considered. This study made use of somatic coliphages and two bacteriophages (*MS2* and ØX*174*) as microbial indicators to investigate the potential health risks associated with urine-derived struvite.

Both bacteriophages (MS2, Phi174x) and somatic coliphage (*E. coli* 13706) tested in this study were found in the struvite produced from urine, with a count of 1.11 x 10^3, 1.35 x 10^3 and 2.08 x 10^3 of MS2, Phi174x and *E. coli* 13706, respectively (**Figure 13**). The results obtained revealed that the struvite contained bacterial and viral contaminants, which might cause health risks. Somatic coliphages occurred at the highest level, compared to bacteriophages *MS2* and Ø*X174*. However, the two bacteriophages are considered to be more representative of human enteric viruses in their occurrence. The high recovery of these organisms in the urine-derived struvite is a cause for concern.

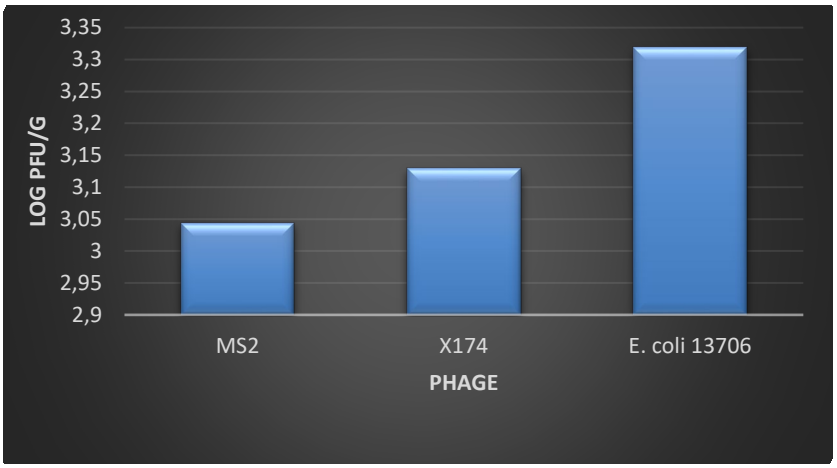

Figure 13. Coliphage/Bacteriophage count in struvite produced from the urine (PFU/g).

Urine-derived struvite was subjected to drying at different temperature conditions to ascertain the effect of temperature in pathogen load removal. The surviving microorganisms concentration was determined and result presented in **Figure 14** and **Figure 15**. A decreased concentration of bacteria and bacteriophages was observed at the different drying temperatures. A progressive increase in pathogen destruction was also observed with increase in the drying temperature. For example, MS2 population was observed to be reduced by 53.3%, 69.29% and 77.15% after 5 days of drying at 25ºC, 37ºC and 42ºC,respectively (**Figure 14** and **Table 7**). Similarly, 56%, 66.59% and 76.76% reduction of Phi174x was observed after 5 days of drying at 25ºC 37ºC and 42ºC, while *E. coli* 13706 population was reduced by 71.2%, 72.78% and 82.09% after 5 days drying of drying at 25ºC, 37ºC and 42ºC (**Figure 14**).

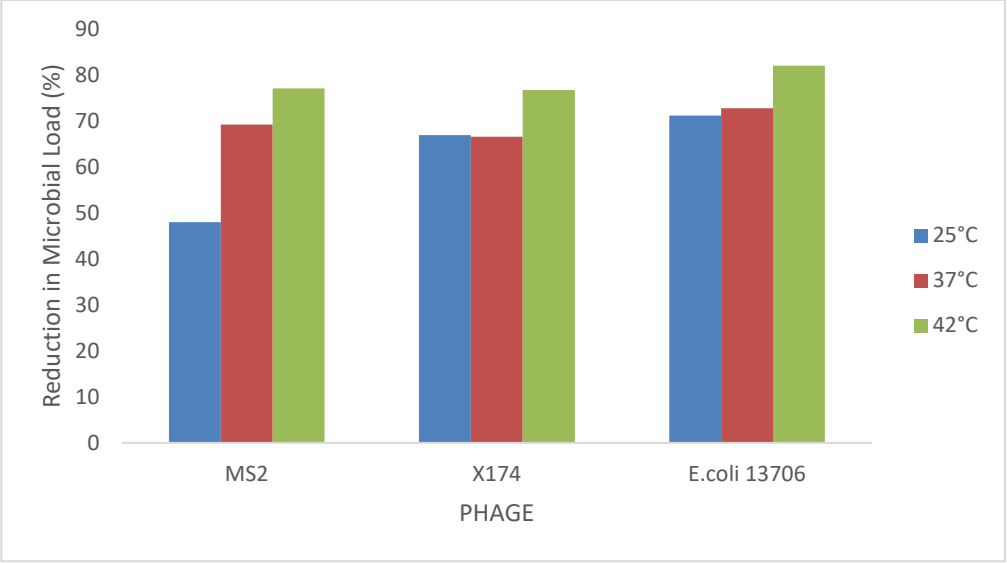

Figure 14. Microbial reduction (%) in struvite at different drying temperatures.

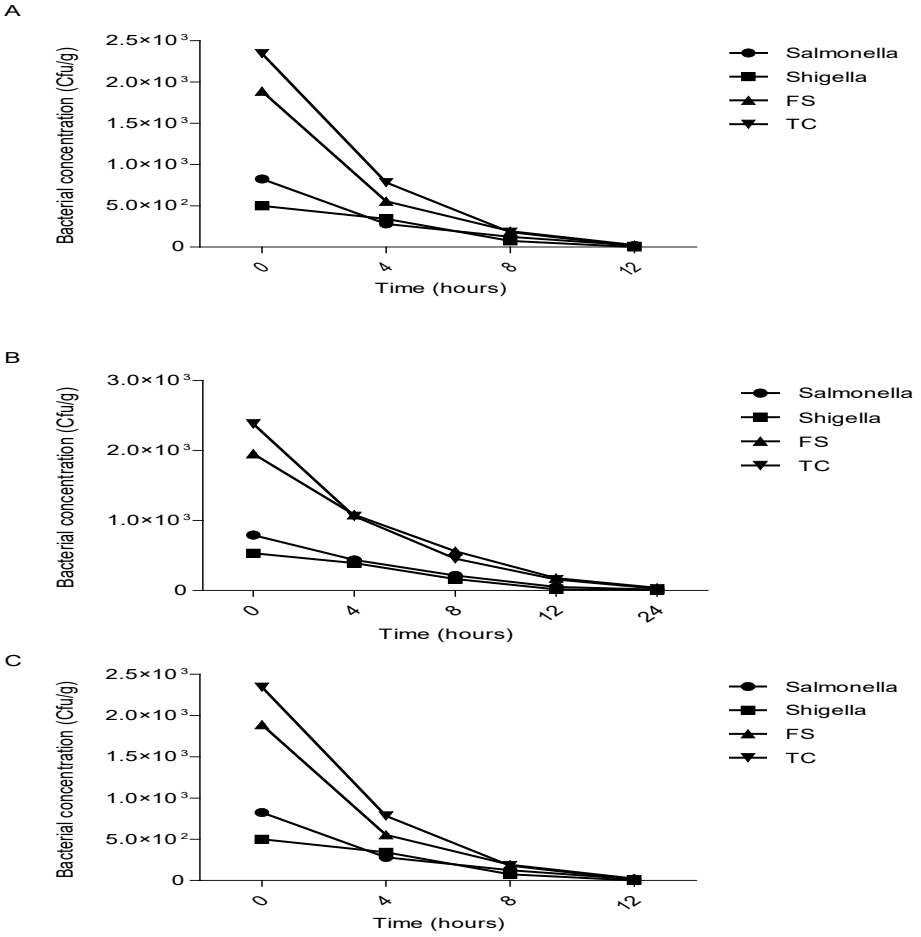

Figure 15. Inactivation of bacteria during drying of struvite at (a) 25ºC, (b) 37ºC and (c) 42ºC.

Table 7. Bacteriophage (MS2, Phi174) and Coliphage (*E. coli* 13706) count (PFU/g) in urine-produced struvite under different drying temperatures.

	MS2		**Phi174**		***E. coli* 13706**	
Drying Temperature (ºC)	Day 0	Day 5	Day 0	Day 5	Day 0	Day 5
25	1107	580	1347	593	2083	600
37	1107	340	1347	450	2083	567
42	1107	253	1347	313	2083	373

Temperature has an effect on the inactivation rate of microorganisms. During struvite drying inactivation was slow at lower temperatures and fast at high temperatures. Hoglund *et al*. (2002) supported this by reporting that lower temperatures creates conditions for the survival of microorganisms stating that the elimination of pathogens at high temperatures was more beneficial. Results obtained in this study are similar to those of Decrey *et al*. (2011) who also investigated the fate of pathogens in urine and struvite. Similar trend was recorded in the number

of bacteria as the heating time increases over days, but no significant inactivation was recorded as the temperature increases from 25 to 42°C. There was high and low number of *Salmonella* and *Shigella*, respectively in the struvite prior to inactivation. *Salmonella* were more resistant to heat in comparison to other temperatures (**Figure 15**).

4.4 MICROORGANISMS IMPLICATED IN URINE-DERIVED STRUVITE

Urine-derived struvite is often source separated from faeces in which cross contamination can occur through improper use of UDDTs. The use of urine-derived struvite possesses a significant pathogenic risk due to the occurrence of several microorganisms that have been implicated in the source separated urine such as: *Escherichia coli, Salmonella, Enterococcus,* Helminth ova such as Ascaris, *Bacteriophages, Rotavirus* and faecal sterols (Simha and Ganesapillai 2017). Most of the pathogens found in urine are a result of cross contamination where displaced faecal matter enters the urine part of the bowl in UDDTs (Hoglund *et al*., 2006). Faecal matter is set to contain a known large number of pathogens particularly bacteria (*Salmonella* sp, *Shigella* sp etc.), bacteriophages (MS2, Coliphages) and protozoa (Manfred, 2011). A list of microorganisms reported to be implicated in urine-derived struvite and the associated health implications is presented in **Table 8**.

Table 8. Health Implications of Organisms in Urine-Derived Struvite.

S/N	Microorganism	Health Implication	References
1	*Escherichia coli*	E. coli is known to cause gastro intestinal illness, infections of the ear, eye, skin and respiratory tracts. The most common symptoms are associated with diarrhea, vomiting, nausea, stomach cramps and low grade fever.	Gaffield *et al.,* 2003
2	*Salmonella*	Salmonella causes Typhoid/paratyphoid fever with symptoms such as headache, cough, fever, malaise, anorexia, bradycardia, They are also the causal agent for diarrhoea and abdominal cramps	Schönning and Stenström, 2004
3	*Enterococcus spp.*	*Enterococcus spp. e.g Enterococcus feacalis* and *Enterococcus faecium* which are the most prevalent are known to cause Vancomycin resistant enterococci (VRE) infections.	DePerio *et al*., 2006
4	*Helminth ova*	Ascaris generally have few or no symptoms which includes wheezing; coughing; fever; enteritis; pulmonary eosinophilia	Schönning and Stenström, 2004
6	*Rotavirus*	Viruses are widely implicated in the urine derived struvites due to their low infective dose and resistance to treatment (in activation) in the stored urine. It causes Gastroenteritis and it is the leading cause of diarrheal disease in children.	Seheri *et al*., 2012 Mans *et al*., 2010 Hoglund *et al.*, 2002

4.5 GUIDELINES FOR USE OF URINE, URINE-DERIVED STRUVITE AND TREATED SLUDGE

Depending on the types of crops to be cultivated using urine-derived struvite, different storage temperatures and storage time are recommended based on the estimated pathogen content as detailed in **Table 9**. The required standard for treated sludge in terms of microbial and pollutant level is shown in **Table 10**. Various application techniques have also been recommended:

Application to crops with space between plants*:* For application to a single plant, a furrow is prepared beside or around (10 cm) the plant. Apply the urine-derived struvite, and close the furrow or hole. This is followed by watering to avoid toxicity effects. Alternatively, the struvite may also be applied after a good rainfall of at least 15 mm.

Application to crops planted densely*:* For a densely planted crops, the struvite may be diluted at least 200% (2:1), and then apply in a uniform manner immediately followed by abundant watering of leaves.

Table 9: Recommended storage times for urine [(a)] based on estimated pathogen content [(b)] and recommended crop for larger systems [(c)] (WHO, 2006).

Storage temp (ºC)	Storage time	Possible pathogens in the urine mixture after storage	Recommended crops
4	≥1 months	Viruses, protozoa	Food and fodder crops that are to be processed
4	≥6 months	Viruses	Food crops that are to be processed, folder crops [(d)]
20	≥1 months	Viruses	Food crops that are to be processed, folder crops [(d)]
20	≥6 months	Probably none	All crops [(e)]

[(a)] Urine or urine water. When diluted it is assumed that the urine mixture has at least pH 8.8 and a nitrogen concentration of at least 1 g/L.

[(b)] Gram positive bacteria and spore-forming bacteria are not included in the underlying risk assessments, but are not normally recognized for causing any human infection of concern.

[(c)] A larger systems in this case is a system where the urine mixture is used to fertilise crops that will be consumed by individuals other than members of the house from which the urine was collected.

[(d)] Not grasslands for production of fodder.

[(e)] For food crops that are consumed raw, it is recommended that the urine be applied at least 1 month before harvesting and that it be incorporated into the ground if the edible parts grow above the soil surface.

Table 10: Sludge classification criteria[A,B]: Microbiological and Pollutant Class.

Microbiological Class A	**Target Value/ Maximum Permissible Value**
Faecal coliform (CFU/g_{dry}	<1000 / 10 000
Helminth(Viable ova/ g_{dry})	<0.25 / 1 ova/4g_{dry})
Compliance requirements	90% compliance; The 10% samples that exceed the Target value may not exceed this value
Pollutant Class A (mg/kg)	
Arsenic (As)	<40
Cadmium (Cd)	<40
Chromium (Cr)	<1200
Copper (Cu)	<1500
Lead (Pb)	<300
Mercury (Hg)	<15
Nickel (Ni)	<420
Zinc (Zn)	<2800
Compliance requirements	A 90% compliance is required to comply with the requirements of a pollutant class. The compliance will therefore only be evident once 10 sample results are available.

A - JE Herselman, LW Burger and P Moodley (2009). *Guidelines for the Utilisation and Disposal of Wastewater Sludge: Requirements for thermal sludge management practices and for commercial products containing sludge*, Volume 5; B - HG Snyman and JE Herselman (2006a). *Guidelines for the Utilisation and Disposal of Wastewater Sludge: Selection of Management Options*, Volume 1.

4.6 CONCLUSIONS

This study investigated the presence of certain microbial pathogens in urine-derived struvite and highlighted the health risks associated with the use of these struvite. Reference was also made to literature to ascertain other pathogens that have been implicated in urine-derived struvite and their associated health risk. The presence of these health related microorganisms in struvite is a concern since it is capable of causing food poisoning if residues remain on struvite-fertilised crops, particularly crops consumed raw. However, different drying temperatures inactivated the pathogens in the urine-derived struvite, albeit at different levels. Guidelines for the processing conditions of urine and applications of the urine-derived struvite were also provided.

5. CONCLUSIONS & RECOMMENDATIONS

This study had two broad objectives: to evaluate BSFL as a technology for UDDT sludge volume reduction and to investigate the presence of certain microbial pathogens in urine-derived struvite, optimise struvite production with subsequent reduction in microbial pathogens and highlight the health risks associated with the use of urine-derived struvite.

5.1 BSFL TREATMENT

There is a need for novel faecal sludge treatment technologies that can reduce pollution volumes while obtaining beneficial value from sludge conversion. BSFL technology has been considered as a potential solution to dealing with faecal sludges in developing countries. This study assessed the effectiveness of BSFL in digesting faecal sludge from UDDTs in eThekwini Municipality, Durban. BSFL technology has shown to offer several economic benefits including its use in the production of animal feeds or its transformation into biodiesel and other oils of economic value (Sheppard *et al*., 2002; Newton *et al*., 2005; Liu *et al*., 2008; Diener *et al*., 2011; Zheng *et al*., 2012; Lalander *et al*., 2013; Banks 2014; Lalander *et al*., 2019). BSFL technology has also been shown to reduce pathogens in chicken and dairy manure (Liu *et al*., 2008), and human faeces (Lalander *et al*., 2013), and are known to feed and grow on fresh human faeces (Lalander *et al*., 2013; Banks 2014). However, several questions have to be answered before BSFL technology can be used effectively in faecal sludge management, including determining the efficiency of BSFL for faecal sludge digestion and prepupal biomass production while feeding on sludge-additive mixtures under different key rearing parameters. It is also an important to determine variations in the physical and chemical characteristics of the different components of UDDT sludge, and what effects this has on BSFL growth.

This study has demonstrated the ability of BSFL to propagate successfully on UDDT sludge. This means that larvae are capable of successfully developing on UDDT sludge. This also implies that BSFL could be consuming UDDT faecal sludge with a range of nutritional contents, while developing into valuable prepupae. However, results from the current study suggest that BSFL alone cannot treat faecal matter to be used as a soil conditioner. Other chemical methods to further sanitise treated sludge, while also increasing the agricultural products, show more economic benefit such as ammonia sanitisation or the use of chemical treatment such as chlorine, lime and phosphoric acid. This study has shown that BSFL are effective at reducing faecal matter quantities. Furthermore, bioconversion rates of faecal sludge into prepupal biomass are more efficient when reared on a sludge-additive mixture than when fed with sludge alone.

The digestion of faecal sludge from the UDDTs by BSFL was investigated and the results

presented in **Chapter 3**. BSFL treatment showed a potential for waste reduction, with a reduction of volatile solids recorded in treated samples compared to the control; however there was no reduction in bagasse-supplemented sludge relative to the other amended and unamended sludge. Treatments 2 (75% UDDT sludge+25% Food waste), 4 (75%UDDT sludge+25%Poultry feed) and 5 (50%UDDT sludge+50% Poultry feed) met the South African recommended guidelines for reuse of wastewater as fertiliser in agriculture of 34% reduction in the volatiles solids (Snyman and Herselman, 2006b). Nutrients contents (TKN, nitrogen, phosphate and potassium) increased following the 10 day BSFL-digestion of the sludge with higher nutrient content in the control than in the digested feed. The pH of the sludge (BSFL-digested and control) become more acidic. Results of pathogen removal from the sludge after BSFL digestion was inconclusive since Ascaris, faecal coliforms, *E. coli* and total coliforms were absent in both the digested feed and in the control. Other factors such as pH and presence of ammonia have been reported to contribute to pathogen removal in sludge samples (Vinnerås, 2007; Karak and Bhattacharyya, 2011). Analysis of the BSFL indicates the absence of helminth eggs before and after feeding on the different feed additives, despite the detection of helminth eggs in the feeds of all treatments. This may mean that the larvae did not consume the ascaris eggs while feeding, even though the ascaris eggs viability were observed to decrease in the feed after digestion The treated sludge did not meet the microbial quality guidelines for sludge to be used as fertiliser in agriculture (Snyman and Herselman, 2006b).

5.2 URINE-DERIVED STRUVITE PRODUCTION

This study investigated the presence of certain microbial pathogens in urine-derived struvite and highlighted the health risks associated with the use of these struvite. Reference was also made to literature to ascertain other pathogens that have been implicated in urine-derived struvite and their associated health risk. The presence of these health related microorganisms in struvite is a concern since it is capable of causing food poisoning if residues remain on struvite-fertilized crops, particularly crops consumed raw. However, different drying temperatures inactivated the pathogens in the urine-derived struvite, albeit at different levels. Guidelines for the processing conditions of urine and applications of the urine-derived struvite were also provided.

5.3 RECOMMENDATIONS

It is recommended that UDDT be supplemented with food wastes (25% or 50%) to ensure optimal growth of BSFL and adequate digestion of the sludge, prior to application in agricultural practices. Careful consideration should be given to the recommended guidelines before the application of the BSFL digested sludge to prevent transference of pathogens into the crops, especially edible crops.

It is recommended that depending on the source of the urine, the population and type of pathogens therein, it must be stored for the appropriate time at the recommended temperature to ensure inactivation of pathogens that might be transferred into the struvite. Temperature of 42°C is recommended for the drying of urine-derived struvite as this temperature was found to be optimal for pathogen inactivation with no consequential effect on nutritional composition and concentration. Furthermore, adherence to the guidelines regulating the application of urine-derived struvite should be ensured to prevent transfer of pathogens into the crops, especially edible crops.

6. LIST OF REFERENCES

BANKS, I. J., GIBSON, W. T. and CAMERON, M. M. 2014. Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. Tropical Medicine and International health, 19**,** 14-22.

BISCHEL, H.N., DUYGAN, B.D.Ö, STRANDE, L., MCARDELL, C.S., UDERT, K.M. AND KOHN, T. 2015. Pathogens and pharmaceuticals in source-separated urine in eThekwini, South Africa. Water Research, 85, 57-65.

CORMIER, J. AND JONES, M. 2014. A double layer assay using plate techniques for enumeration of bacteriophage *MS2*. Journal of Virology Methods 196, 86-92.

DECREY, L., UDERT, K.M., TILLEY, E., PECSON B.M. AND KOHN T. 2011. Fate of pathogen indicator phage *ø174*X and *Ascaris suum* eggs during the production of struvite fertilizer from source separated urine. Water Research, 45, 4960-4972.

DE PERIO, M.A., YARNOLD, P.R., WARREN, J. AND NOSKIN, J.A. 2006. Risk factors and outcomes associated with non-Enterococcus faecalis, non-Enterococcus faecium enterococcal bacteremia. Infection Control and Hospital Epidemiology, 27, 28-33.

DIENER, S., ZURBRÜGG, C. and TOCKNER, K. 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Management and Research, 27**,** 603-610.

DIENER, S., SOLANO, N. M. S., GUTIÉRREZ, F. R., ZURBRÜGG, C. and TOCKNER, K. 2011. Biological treatment of municipal organic waste using black soldier fly larvae. Waste and Biomass Valorization, 2**,** 357-363.

DIENER, S., SEMIYAGA, S., NIWAGABA, C.B., MUSPRATT, A.M., GNING, J.B., MBÉGUÉRÉ, M. AND STRANDE, L. 2014. A value proposition: Resource recovery from faecal sludge – Can it be the driver for improved sanitation? Resources, Conservation and Recycling, 88, 32-38.

ETTER, B., TILLEY, E., KHADKA, R. AND URDET, K.M. 2011. Low cost struvite production using source-separated urine in Nepal. Water Research, 45, 852-862.

KARAK, T. and BHATTACHARYYA, P. 2011. Human urine as a source of alternative natural fertilizer in agriculture: A flight of fancy or an achievable reality. Resources, Conservation and Recycling, 55, 400-408.

GAFFIELD, S., GOO, R., RICHARDS, L. AND JACKSON, R. 2003. Public health effects of inadequately managed stormwater runoff. American Journal of Public Health, 93, 1527-1533.

HERSELMAN, J.E., BURGER, P.W. AND MOODLEY, P. 2006. Guidelines for the utilisation and disposal of wastewater sludge: Requirements for thermal sludge management practices and for commercial products containing sludge: Volume 5. WRC Report No. TT 351/09, Water Research Commission, Pretoria.

HOGLUND, C., STENSTROM, T.A., ASHBOLT, N. 2002. Microbial risk assessment of € source-separated urine used in agriculture. Waste Management Research, 20, 150-161.

JÖNSSON, H., VINNERÅS, B., HÖGLUND, C., STENSTRÖM, T.A., DALHAMMAR, G. AND KIRCHMANN, H. 2000. Recycling source separated human urine. (Källsorterad humanurin). VA-Forsk Report 2000-1, VAV AB, Stockholm, Sweden. (In Swedish, English summary).

KARAK T AND P BHATTACHARYYA. 2011. Human urine as a source of alternative natural fertilizer in agriculture: A flight of fancy or an achievable reality. Resource, Conservation and Recycling 55, 400-408.

LALANDER, C., DIENER, S., MAGRI, M. E., ZURBRÜGG, C., LINDSTRÖM, A. and VINNERÅS, B. 2013. Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens)—From a hygiene aspect. Science of the Total Environment, 458**,** 312-318.

LALANDER, C., DIENER, S., ZURBRÜGG, C. and VINNERÅS, B. 2019. Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (Hermetia illucens). Journal of Cleaner Production, 208**,** 211-219.

LINDSAY, E.A. AND FRENCH, K. 2005. Litterfall and nitrogen cycling following invasion by Chrysanthemoides monilifera ssp. rotundata in coastal Australia. Journal of Applied Ecology, 42, 556-566.

LIU, Q., TOMBERLIN, J. K., BRADY, J. A., SANFORD, M. R. and YU, Z. 2008. Black soldier fly (Diptera: Stratiomyidae) larvae reduce Escherichia coli in dairy manure. Environmental entomology, 37**,** 1525-1530.

MAKHAYA, J.M., SOMDA, M.K., SAVADOGO, A., DIANOU, D., BARRO N. AND TRAÒRE, A.S. 2014. Survival of enteric bacteria in source separated human urine used as fertilizer: Effects of temperature and ammonia. Africa Journal of Environmental Science and Technology, 8, 511-520.

MANFRED, S. 2011. Bacterial inactivation in struvite recovered from urine in South Africa. Master Thesis, École polytechnique fédérale de Lausanne, 1-60.

MANS, J., DE VILLIERS, J.C., DU PLESSIS, N.M., AVENANT, T., TAYLOR, M.B. 2010. Emerging norovirus GII. 4 2008 variant detected in hospitalised paediatric patients in South Africa. Journal of Clinical Virology, 49, 258-264.

MAURER, M., PRONK W. AND LARSEN, T.A. 2006. Treatment processes for source-separated urine. Water Research 40, 3151-3166.

MOODLEY, P., ARCHER, C., HAWKSWORTH, D. and LEIBACH, L. 2008. Standard Methods for the Recovery and Enumeration of Helminth Ova in Wastewater, Sludge, Compost and Urine-diversion Waste in South Africa. Report to the Water Research Commission, WRC Report No. TT322/08, Water Research Commission, Pretoria.

NEWTON, L., SHEPPARD, C., WATSON, D. W., BURTLE, G. and DOVE, R. 2005. Using the black soldier fly, Hermetia illucens, as a value-added tool for the management of swine manure. Animal and Poultry Waste Management Center, North Carolina State University, Raleigh, NC, 17.

NIWAGABA, C. 2007. Human Excreta Treatment Technologies: Prerequisites, Constraints and Performance. Department of Biometry and Engineering, Swedish University of Agricultural Sciences.

PEBSWORTH, P. A., ARCHER, C. E., APPLETON, C. C. and HUFFMAN, M. A. 2012. Parasite transmission risk from geophagic and foraging behavior in chacma baboons. American Journal of Primatology, 74**,** 940-947.

PRONK, W. AND KONE, D. 2009. Options for urine treatment in developing countries. Desalination 248, 360–368.

ROMA, E., PHILP, K., BUCKLEY, C., XULU, S. and SCOTT, D. 2013. User perceptions of urine diversion dehydration toilets: Experiences from a cross-sectional study in eThekwini Municipality. Water SA, 39**,** 305-312.

SCHERTENLEIB A. 2014. Inactivation of pathogens in urine nitrification reactors. Master Thesis.

École polytechnique fédérale de Lausanne.

SCHÖNNING, C. AND STENSTRÖM T.A. 2004. Guidelines on the safe use of urine and faeces in ecological sanitation systems. EcoSanRes Programme and Stockholm Environment Institute, Stockholm, Sweden.

SEHERI LM, PAGE N, MAWELA MPB, MPHAHLELE MJ, STEELE D (2012). Rotavirus vaccination within the South African expanded programme on immunisation. Vaccine, 3, C14-20.

SHEPPARD, D.C., TOMBERLIN, J.K, JOYCE, J.A., KISER, B.C., SUMNER, S.M. 2002. Rearing Methods for the Black Soldier Fly (Diptera: Stratiomyidae), Journal of Medical Entomology, 39, 695–698, https://doi.org/10.1603/0022-2585-39.4.695.

SNYMAN, H. G. and HERSELMAN, J. 2006a. Guidelines for the utilisation and disposal of wastewater sludge: Selection of management options: Volume 1. WRC Report No. TT 261/06 Water Research Commission, Pretoria.

SNYMAN, H. G. and HERSELMAN, J. 2006b. Guidelines for the utilisation and disposal of wastewater sludge: Requirements for the agricultural use of wastewater sludge: Volume 2. WRC Report No. TT 262/06 Water Research Commission, Pretoria.

TILLEY, E., LUTHI, C., MOREL, A., ZURBRUGG, C. & SCHERTLENLEIB, R. 2008. Compendium of sanitation systems and technologies. Dubendorg, Switzerland: Eawag: Swiss Federal Institute of Aquatic Science and Technology.

UDERT, K.M., BUCKLEY, C.A., WÄCHTER, M., MCARDELL, C.S., KOHN, T., STRANDE, L., ZÖLLIG, H., FUMASOLI, A., OBERSON, A. AND ETTER, B. 2015. Technologies for the treatment of source-separated urine in the eThekwini Municipality. Water SA, 41, 212-221.

VINNERÅS, B. 2007. Comparison of composting, storage and urea treatment for sanitising of faecal matter and manure. Bioresource Technology, 98**,** 3317-3321.

WILSENACH, J.A., SCHUURBIERS, C.A.H. AND VAN LOOSDRECHT, M.C.M. 2007. Phosphate and potassium recovery from source separated urine through struvite precipitation. Water Research, 41, 458-466.

WOHLSAGER, S., CLEMENS, J., NGUYET, P.T., RECHENBURG A. AND ARNOLD, U. 2010. Urine – A Valuable Fertilizer with Low Risk after Storage in the Tropics. Water Environment Research, 82, 840-846.