Characterisation of faecal sludge from urine diversion toilets: impact on black soldier fly larvae growth

by

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ABSTRACT

The area managed by eThekwini Municipality in KwaZulu-Natal, South Africa, was extended in 2002 to include approximately 80 000 households without basic sanitation. The municipality adopted urine diversion dehydrating toilets (UDDT) as a dry on-site sanitation option. The adoption of the UDDTs over other sanitation methods was due to UDDTs being cost effective, the geographical location of these households and the impracticality of providing a sewer system. The UDDTs offered waterless sanitation in a water-constrained environment and the pedestal separated faeces and urine at source, making it possible to handle the treatment of the two excreta streams separately. These UDDTs overcame the problem faced during the desludging of ventilated improved pit (VIP) toilets as UDDT vaults are smaller and allow for easier manual emptying because of the lower moisture content of the faecal sludge (FS). It was the responsibility of the household to empty the vaults once they were full, bury the FS on the property and plant a tree to mark the burial site. The urine was directed to a soakaway located near the unit.

In 2017, the eThekwini Water and Sanitation (EWS) unit implemented an UDDT emptying campaign due to the householders' dissatisfaction over emptying UDDT sludge and the possibility of exposure to FS sludge after burial on the household property. In addition, there was growing concerns over health risks due to potentially pathogenic sludge. A solution was required to treat the waste from the UDDTs, and through funding from the Bill & Melinda Gates Foundation, a project was initiated to investigate the feasibility of using the black soldier fly (BSF) larvae technology. Under this project, a full-scale BSF larvae processing plant was established in Durban, South Africa, with the aim of treating 20 tons of UDDT FS per day. The BSF larvae have been shown to be consumers of a wide range of decomposing organic matter, including kitchen waste, human waste, animal waste, restaurant waste, and vegetable waste.

In 2017, there was very little data available on the characteristics of UDDT FS and the impact of UDDT FS on the bioconversion process using BSF larvae. A study was therefore initiated to determine the bioconversion and waste reduction capabilities using the BSF larvae.

This study was undertaken by monitoring and determining different material flows in batch reactors fed on two types of substrates: (i) UDDT sludge, and (ii) a mix of UDDT sludge and primary sludge (PS) by performing mass balances. Due to the unavailability of data from the full-scale plant and problems experienced with commissioning components of the plant, small-scale studies were conducted under the same conditions as at the full-scale plant, and the data from these studies was then used to predict the overall material flows of the treatment plant. The small-scale studies were performed in sheds at the BSF larvae plant under uncontrolled environmental conditions, and data was generated from either on-site measurements, or through laboratory analysis of the substrates before and after treatment. This data was then extrapolated to generate material flows for the full-scale BSF larvae waste management plant.

The mass balances were carried out on a dry matter basis, wet matter basis, volatile solids, and ash. The growth of larvae was monitored every three days over a period of 13 days. Laboratory analyses were carried out on the UDDT sludge, and a mix of UDDT sludge and PS to analyse the difference in the composition before and after treatment with the BSF larvae. Laboratory analyses included total solids, volatile solids, ash, chemical oxygen demand, calorific value, carbon to nitrogen ratio, and protein content.

The change in depth of the substrates during the small-scale trial was measured to determine the reduction in volume due to treatment with the BSF larvae. Humidity and temperature fluctuations were also monitored in the sheds to investigate the effect of changes in these conditions on the process.

Due to poor waste management services in the areas where UDDTs are installed, users of UDDTs generally dispose of other wastes (termed trash) in the toilets, including metal objects. The presence and concentration of heavy metals such as Pb, Cd, As, Cr, Cu and Ni was determined through laboratory analyses of the BSF larvae and remaining substrate (residue) after 13 days of treatment, as the presence of these metals can affect the market potential of the BSF larvae and residue.

The outcomes from this project showed that the use of BSF larvae has potential as a process for FS management with resource recovery. Treatment by BSF larvae reduced the mass of UDDT sludge by 28% wet basis in the small-scale trial operating in an

uncontrolled and low maintained system and achieved a bioconversion of 6% wet basis. The study also showed that bioconversion could be increased by co-digestion of UDDT sludge with PS as it increased from 6% to 9% with the addition of 15% mass of PS. It was also found that the characteristics of the UDDT sludge directly affected the characteristics of the BSF larvae, which can potentially influence the market value of the BSF larvae. For example, UDDT sludge had a high ash content which resulted in an increase in the ash content of the BSF larvae fed on UDDT sludge. Furthermore, the bioconversion process was shown to be sensitive to temperature and humidity as changes in these conditions affected the relative waste reduction and bioconversion rates.

Outcomes from this study indicate that the bioconversion and waste reduction of the UDDT sludge could be improved by co-digestion with an organic substrate and using an environment with controlled temperature and humidity.

PREFACE

The experimental work described in this dissertation was carried out at the University of KwaZulu-Natal in the College of Agriculture, Engineering and Science (CAES) at the Department of Chemical Engineering, Howard College Campus, Durban, from 2016 to December 2021, under the supervision of the late Professor Christopher Buckley and Ms. Susan Mercer.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION 1 - PLAGIARISM

I, Ellen Tafadzwa Mutsakatira, declare that.

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other person's data, pictures, graphs, or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other person's writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted then:
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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and references sections.

Signed: 

DECLARATION 2 – CONFERENCES, PRESENTATIONS, AND PUBLICATIONS

Several postern, oral presentations and publications were delivered during this study:

1. **Mutsakatira, E.T,** Buckley, C.A, Mercer, S.J. 2019. Preliminary Mass Balance on the Black Soldier Fly Larvae Facility in Durban, College of Agricultural Engineering and Science (CAES) Research and Innovation Day. [Poster]. Westville, UKZN, Durban, 26 October 2017
2. **Mutsakatira, E.T,** Buckley, C.A, Mercer, S.J: Preliminary Mass Balance at A Black Soldier Fly Larvae Faecal Sludge Treatment Facility in Durban, South Africa, [Poster and oral presentation] *International Young Water Professionals Conference, Cape Town South Africa, 10 to 13 December 2017*
3. **Mutsakatira, E.,** Buckley, C.A., and Mercer, S.J., 2018, July. Potential use of the black soldier fly larvae in faecal sludge management: a study in Durban, South Africa. In *Proceedings of the 41st WEDC International Conference "Transformation towards sustainable and resilient wash service", Egerton University, Nakuru, Kenya* (pp. 9-13).
4. Peguero, D.A., **Mutsakatira, E.T**., Buckley, C.A., Foutch, G.L. and Bischel, H.N., 2021. Evaluating the microbial safety of heat-treated fecal sludge for black soldier fly larvae production in South Africa. *Environmental Engineering Science*, *38*(5), pp.331-339.

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- My friends: Tapuwa, Lisa, Noma, Vivian, and Daniela, thank you for the encouragement.
- To everyone who contributed in any way to make this project successful, I am grateful.

DEDICATION

I dedicate this to the late Professor Christopher Buckley; I will never forget the impact you made in my life and thank you for pushing me to get out of my comfort zone.

GLOSSARY OF TERMS

Absorbent	A substance that can soak up liquids or water
Biochar	Charcoal produced by pyrolysis of biomass in the absence of oxygen
Bioconversion	The conversion of organic materials such as animal waste, food waste, vegetable waste or human waste by insects into valuable products, including larval biomass
Bulking agent	A substance used to improve structure and porosity or to lower moisture content
Calorific value	The amount of heat energy released by a sample
Deep row entrenchment	Involves the burial of faecal sludge into trenches and covered with soil, eliminating odours, and safely containing human pathogens in the sludge. A tree with high nutrient consumption rates can be planted.
Dry basis	A measure of how much solids are in a substance, excluding water.
Excreta	Waste (faeces and urine) discharged from the human body
Faecal sludge	Raw or partially digested, a slurry or semisolid, and results from the collection, storage, or treatment of combinations of excreta and blackwater, with or without greywater
Grow-out	The place used to process UDDT sludge with BSF larvae
Improved sanitation	Systems that are designed to separate human excreta from the user and the excreta managed in a safe way
Instars	The developmental stage of arthropods, such as insects, between each moult
Layer's mash	Feed formulated for chickens that are laying table eggs
Mass transfer	The net mass movement from one location to another or the travel of individual chemical species from high-concentration regions to low-concentration regions.

Neonates	Newly hatched insect eggs
On-site sanitation	A sanitation system in which excreta and wastewater are collected, stored and/or treated in the place where they are generated.
Prepupae	The sixth instar of the Black soldier fly larvae when it stops feeding to initiate pupation and the exoskeleton darkens
Public private partnership	A legal agreement between two or more public and private sectors of a long-term nature
Service level agreement	A legal document between a service provider and a client which specifies the services
Substrate	A material from which the black soldier fly grows or obtains its nourishment
Superstructure	The part of a toilet entirely above its foundation
Trash	Solid waste or unwanted or unusable materials usually discarded after their primary use
Urine diversion dehydrating toilet	A waterless toilet that has a pedestal designed to separates urine and faeces at the source
Vault	Permanent waterless storage section located beneath the pedestal of a urine diversion dehydrating toilet to store human excreta and which is emptied regularly by a local waste removal company or household owners.
Ventilated improved pit latrine	A type of toilet which collects human faeces in a hole constructed underground and the superstructure has an additional vertical vent pipe with a fly screen
Waste reduction	The reduction of the organic wastes by the black soldier fly larvae
Wet basis	A measure of how much solid and water are in a substance.

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LIST OF ABBREVIATIONS

BMGF	Bill & Melinda Gates Foundation
BSF	Black Soldier Fly
COD	Chemical oxygen demand
CSMA	Chemical Specialties Manufacturers' Association fly larval medium
DM	Dry matter
FS	Faecal Sludge
LaDePa	Latrine Dehydration and Pasteurization
PRG	Pollution Research Group
PS	Primary Sludge
TS	Total Solids
UDDT	Urine Diversion Dehydrating Toilet
UKZN	University of KwaZulu-Natal
VIP	Ventilated Improved Latrine
VS	Volatile Solids
WASH R&D	Water, Sanitation & Hygiene Research & Development Centre

1 INTRODUCTION

Lack of access to safely managed water and sanitation remains a growing challenge to service delivery in most developing countries. The World Health Organisation (W.H.O, 2017) estimates that 36% of the global population still lacks access to basic sanitation facilities, and 2 billion people have no access. South Africa faces similar challenges, where 230 thousand households, particularly in underserved rural and peri-urban areas, have no access to basic sanitation (SA, 2021). Although there is an increase in the percentage of people in South Africa with access to improved sanitation, there is a need to look for options for safely managing faecal sludge (FS) as 38.1% of the population of South Africa are served by on-site sanitation service (SA, 2021). On a global level, poorly managed sanitation services are linked to transmission of diseases like diarrhoea which is estimated to cause 432 000 deaths annually (W.H.O, 2017) .

The eThekwini municipality is a metropolitan area in South Africa which was created in 2000 to serve the people in and around the City of Durban. In 2002, the boundaries were extended to include areas not previously served by the eThekwini municipality (Municipality, 2014), resulting in the eThekwini Water and Sanitation (EWS), a division of eThekwini Municipality responsible for the provision of water and sanitation services, inheriting approximately 60 000 households without basic sanitation services. The combination of the cholera outbreak in 2000 in Kwazulu-Natal (KZN) which resulted in 105 000 cases and 220 fatalities by July 2021(Hoque and Zeleke, 2005) and the millennium development goals for water and sanitation drove the installation of approximately 80 000 urine diversion dehydrating toilets (UDDTs) in the unserved areas (Gounden et al., 2006).

As highlighted by Gounden et al., (2006), UDDTs were installed by EWS as the on-site sanitation solution as they addressed the following challenges:

- It was costly to install sewerage infrastructure and the terrain made it impractical.
- Ventilated Improved pit (VIP) latrines were not sustainable due to the challenges of desludging the pits (difficulty of access and the disposal of solid wastes in VIPs). Furthermore, the cost of emptying VIPs was high.

- UDDTs, if properly used, would allow for resource recovery or safe disposal of the faecal sludge (FS).
- UDDTs allowed for households to empty the vaults once full, hence there was no need for the municipality to empty vaults.
- There was low risk of environmental contamination as it was expected that there was break down of FS waste in the vaults before being buried on site.

The UDDTs offer waterless sanitation in a water-constrained environment and a specifically designed pedestal allows for separation of faeces and urine, making it possible to handle the two waste streams separately (Gounden et al., 2006, Austin, 2006). The UDDT has two vaults with one vault being used at a time. Once the first vault is full, the pedestal is moved and placed over the second vault; this allows the FS to dehydrate in sealed vault while the other vault fills up (Buckley et al., 2008b). According to Buckley et al. (2008b), UDDT design specification was based on the concept that there would be pathogen deactivation after approximately one year, and hence the householders could manually empty the vaults, bury on-site and plant a tree to use the nutrients in the FS. However, Austin, (2006) highlighted that pathogen deactivation based on storage time would vary across UDDTs as it was influenced by environmental conditions, operational factors, and user habits.

The proposed management of the FS from UDDTs was on-site burial and the planting of a tree to extract nutrients from the FS. UDDT vaults are smaller than VIP vaults, allowing easier manual emptying because of the lower moisture content of the FS.

Although, the design of the UDDTs were beneficial there were unresolved social and health related issues. Studies by Roma et al. (2013) and Mkhize et al. (2017) showed that there was growing user dissatisfaction as users aspired to own a flush toilet and to not handle their own FS. Furthermore, research showed there was the possibility that the FS could be pathogenic even after storage, resulting in environmental and health concerns associated with handling this sludge (Austin, 2001, Buckley et al., 2008b, Trönnberg et al., 2010). The eThekwini Municipality therefore decided to provide an emptying service to households once every two years and to transport UDDT FS that could not be buried on-site to a central waste treatment facility.

The use of Black Soldier Fly (BSF) larvae technology to treat the FS was selected as the most suitable treatment option, as it would allow the management of FS from UDDTs to follow a circular economy approach. This approach envisioned the removal and transportation of UDDTs FS to a central processing plant to produce valuable products that could potentially be upscaled. The benefits of using this approach was creation of employment, business opportunities and reduction of costs associated with emptying and transporting UDDTs by the sale of potential valuable products (Alcock et al., 2016a).

In 2014, proposals were requested from different cities by the Bill & Melinda Gates Foundation (BMGF) and the Department for International Fund in the UK for projects aimed at assessing different types of partnerships, including service level contracts and incentivised contracts to deliver sanitation services. EWS identified the emptying of UDDTs and the treatment of the FS using BSF larvae technology as a case study to test these types of partnerships with the main objectives of improving sanitation services for the poor and marginalised communities while reducing the costs to the municipality. In addition, the partnerships would create employment and opportunities for small businesses (Alcock et al., 2016a).

Khanyisa Projects, together with the Water, Sanitation & Hygiene Research & Development (WASH R&D) Centre (formerly the Pollution Research Group (PRG)) at the University of KwaZulu-Natal (UKZN), applied for this funding, which was awarded in 2015. This funding covered the capital expenditure or plant infrastructure for the construction of the BSF larvae facility, as well as funding for social assessments, laboratory analyses, project management and for undertaking business modelling to determine the economic feasibility of treating FS from UDDTs using BSF larvae. The BSF larvae facility was built and designed to treat up to 20 tonnes wet mass of FS per day to ascertain the viability of this process at full-scale.

A service level agreement (SLA) was implemented between the eThekwini municipality and a private operator, The Biocycle. The Biocycle (a subsidiary of Insect Technology Group) would be responsible for operating the BSF larvae facility which would be established at Isipingo Wastewater Treatment Works in Durban. Under this partnership, The Biocycle and would be paid a gate fee by the municipality (a public entity) to

process the sludge. The profit from this partnership, based on total income (gate fee and income from the sale of any end products) less all operational costs, would be shared equally between the partners (Alcock et al., 2016a).

The creation of this SLA was important to ensure a sustainable business operation to reduce the costs of processing and disposing of UDDT sludge. Burying the FS on-site was not seen as a long-term solution due to impending environmental legislation prohibiting it, and disposal of FS at a hazardous landfill site is expensive due to disposal costs and transport.

The BSF larvae are voracious consumers of a wide range of decomposing organic matter such as kitchen waste (Nguyen et al., 2015), human waste, animal waste (Sheppard et al., 1994, Li et al., 2011b, Myers et al., 2008), restaurant waste, decomposing matter and vegetable waste (Alvarez, 2012). The BSF larvae are high in protein and fat, making them a potential animal feedstock (Makkar et al., 2014). The remaining residue has potential for reuse in agriculture (Singh et al., 2017).

There is limited data available on UDDT sludge characteristics and its impact on the bioconversion rate using BSF larvae. The WASH R&D Centre provided scientific and academic support on the use of the BSF larvae to treat UDDT sludge in terms of characterising the properties of the FS, the BSF larvae and the residue

This is a monitoring study which investigated the bioconversion and waste reduction capabilities of using the BSF larvae to treat UDDT sludge using a mass balance approach. The outcomes of the study will contribute towards a greater understanding of this technology and its application for FS treatment.

1.1 Problem statement and justification

UDDTs were not being emptied by all users and becoming full and unsafe, and there were clear health and safety issues (Buckley et al., 2008b, Trönnberg et al., 2010) with user emptying due to the presence of pathogenic sludge and householder's dissatisfaction that the municipality was not providing a free emptying service as was provided to households with VIP latrines (Mkhize et al., 2017, Roma et al., 2013). In response, EWS made it a policy to provide a free emptying service every 2 years.

However, emptying and disposal of FS is costly, so the most cost-effective and efficient option needed to be found.

Burial on site is one of the most cost-effective options as no transport is required but there are limitations due to space constraints and growing environmental concerns. Another potentially viable solution is a circular economy approach through processing FS into valuable products where the sale of these end products could offset emptying and transport costs. This project aims to demonstrate if the BSF larvae technology is a viable solution for processing FS while potentially generating valuable products.

1.2 Aim

This study aimed to assess the applicability of BSF larvae technology in processing UDDT sludge and a mixture of UDDT sludge and primary sludge (PS) by performing mass balances.

1.3 Objectives

The objectives of this study were:

- To determine the physical and chemical properties of FS from UDDTs to assess if they are within the range for FS management using the BSF larvae technology
- To carry out a mass balance and determine the factors which affect the mass balance when processing UDDT sludge at the facility.
- To determine the effects of UDDT sludge composition on the bioconversion and waste reduction capabilities of BSF larvae using mass balances.
- To determine factors which affect mass balances when using UDDT as a substrate.
- To determine the effect of different substrates on the mass balance.
- To determine the growth and composition of the BSF larvae using UDDT sludge as a feed.

1.4 Study limitations and duration

The study took approximately two years to complete the experimental work (August 2016 to December 2018). During the time of study, the BSF facility was going through the commissioning and improvement phase. This included the installation of new equipment and frequent changes by the operator to the initial concept for the plant. This

impacted the study as no reliable data was available from the full-scale operation hence modifications were done to do pilot studies under similar conditions as the full-scale plant.

2 LITERATURE REVIEW

This chapter focuses on a literature review of UDDTs and the use of BSF larvae as an innovative sanitation solution to manage UDDT sludge in the eThekwini Municipality. It aims to understand factors in the literature associated with the sludge generated by UDDT and its overall management. The study also considers any previous studies of similar BSF larvae systems to identify any useful techniques and the knowledge gap that needs to be addressed.

2.1 On-site sanitation in eThekwini Municipality

In eThekwini Municipality, on-site sanitation is largely provided through UDDTs and VIP latrines (SA, 2021). In South Africa, the government considers VIPs and UDDTs as an acceptable basic sanitation level (Gounden et al., 2006). UDDTS and VIPs are both forms of pit latrines that allow for the collection of faeces, urine and anal cleansing materials and sometimes include the addition of grey water and solid waste (Buckley et al., 2008b, Buckley et al., 2008a). However for a pit latrine to qualify as VIPs or UDDTs it should (i) prevent contact between the user and the waste (ii) have a vent pipe installed with a fly screen, (iii) have a slab that should withstand the construction of a superstructure without collapse and (iv) guarantee privacy and dignity to the user (Buckley et al., 2008a).

According to Gounden et al. (2006), the UDDTs were rolled out to try and solve the challenges associated with VIPs such as the high cost to empty the VIPs, while maintaining a basic sanitation level. UDDTs allowed householders to empty the FS themselves as the vaults were smaller compared to VIPs vaults and easy to access. Furthermore, UDDTs allowed for resource recovery of nutrients as faeces and urine were separated at the source (Gounden et al., 2006, Buckley et al., 2008b).

2.2 Urine dehydrating diversion toilets in eThekwini

A UDDT is a double vaulted dry toilet with urine diverted to a soak-away located near the unit. A pedestal is located above one of the vaults, into which faeces, anal cleansing material, and a covering material (e.g., soil) are dropped. The main benefit of UDDTs is that it allows for nutrient recovery as streams can be handled separately (Kvarnström et al., 2006).

2.2.1 Components of the urine dehydrating diversion toilet

This section will describe the different UDDTs components which include the vaults and superstructure.

2.2.1.1 UDDT vaults

UDDTs have smaller capacity vaults than VIP latrines which reduces the amount of sludge to be handled. Figure 2.1 shows a UDDT superstructure with two small vaults. The recommended design of the vaults is 70 litres per person per year. The vault panels are made of a lightweight material to allow for easy access during emptying. In addition, the panels prevent flies from entering the vaults while allowing adequate stormwater drainage. It was estimated that one UDDT vault would take one year to fill up based on a five-member household where only faeces and recommended covering material is added without the addition of liquids and solid waste.

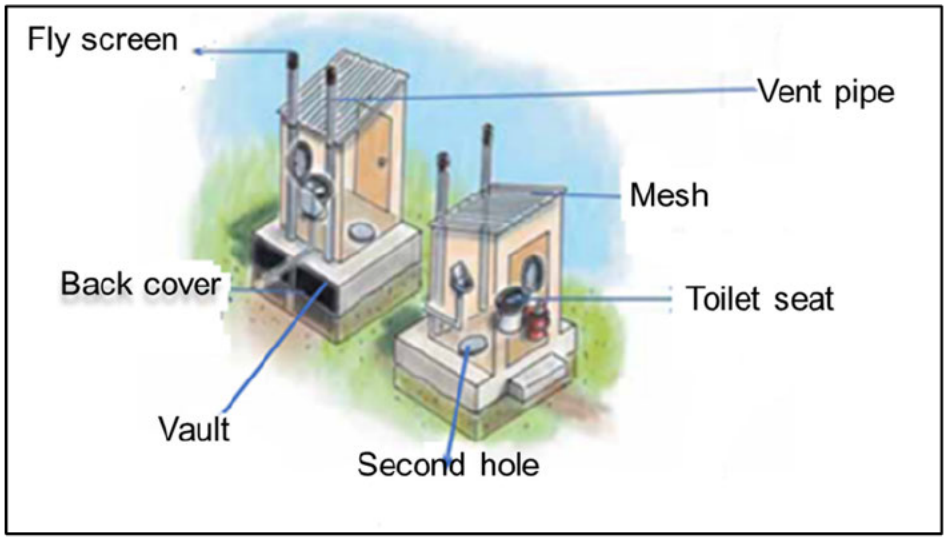

Figure 2.1: The UDDT showing all the components
Adapted from Mkhize et al., (2017)

2.2.1.2 Superstructure

The superstructure includes vents on both vaults with a fly screen which aids with keeping out flies and helps to remove odour from the toilet.

2.2.1.3 Operation of UDDT

The UDDTs separate urine and faeces at source by a specifically designed pedestal. This separation allows the faeces to dry within the vault, reducing odour and flies (Roma et al., 2013, Jimenez et al., 2006). Urine is then diverted to a soakaway and penetrates the soil (Roma et al., 2013, Trimmer, 2015). The faeces and anal cleansing matter are collected in the vault (Roma et al., 2013, Roma et al., 2011), and an alkaline moisture removing substance like ash and sand is added after defecating as a covering material (Trimmer, 2015).

On average, a vault will take approximately one year to fill, depending on the number of people living in a household and user habits. The plastic pedestal is then moved onto the second vault (Buckley et al., 2008b, Strande et al., 2014, Roma et al., 2013). Once the second vault is full, the contents of the first vault would have stabilised due to the drying process and decomposition (Roma et al., 2013, Buckley et al., 2008b). The contents are deemed stable and safe to handle and can then be manually emptied by the household owner or by a contractor and buried.

2.2.2 Contents of UDDT

The contents of UDDT vaults consist of human excreta and anal cleansing matter but may include trash depending on user habits.

2.2.2.1 Human excreta

Human excreta are composed of faeces and urine. According to Vinnerås (2002), typical excreta produced per person is 550 kg/yr of urine and 51.5 kg/yr of faeces as wet mass. However, different parameters affect the physical and chemical properties and quantity of faeces or urine. These are mainly the type and amount of food the users consume, environmental conditions, occupation, intake of water, and the user's health (Niwagaba, 2007, Rose et al., 2015). Healthy people with similar diets have a similar composition (chemical and physical) of human excreta.

Urine consists predominantly of water (approximately 93-96%), and the nutrients found in urine are water-soluble (Rose et al., 2015). A significantly higher portion of nutrients is found in the urine when compared to faecal matter. A few pathogenic organisms such

as *Schistosoma haematobium, Salmonella typhi, Salmonella paratyphi* ,and *Leptospira interrogans* can be found in urine. (Trimmer, 2015, Rose et al., 2015).

The main components of faeces is water, nutrients, residual food and bacteria; they can also include pathogenic viruses (Rose et al., 2015). Research has been carried out to quantify the nutrient composition of human excreta by several researchers. Users consuming food with low fibre tend to have smaller quantities of faeces (Rose et al., 2015). Trimmer (2015) and Rose et al. (2015) suggested that the fresh faeces have a pH range of 6.6 -7.0 with a moisture content of 80% - 83% (g of water/g of wet sample). Table 2.1 shows the physiochemical properties and biodegradability of fresh faeces

Table 2.1: Physio-chemical properties and biodegradability of fresh faeces

Property	**Faeces**
Total COD/mg COD g^{-1}	322
Moisture content/g H_2O g^{-1}	0.79
Ash/g g^{-1}	0.04
Total solids/ g g^{-1}	0.21
Volatile solids/g g^{-1}	0.2
Biodegradable COD/mg COD g^{-1}	112

*adapted from(Rose et al., 2015)

2.2.2.2 Absorbent agent

Austin and Cloete (2008) suggest that an absorbent (or covering material) be added after defecating. Ash, soil, sodium hydroxide (NaOH), straw or wood shavings are the most common absorbent agents. The type of absorbent agent used depends on availability in the area. In Durban, sandy soil is usually used as an absorbent and the recommended amount to be added is a cupful. A bucket is usually placed in the UDDT superstructure with the absorbent (Austin, 2006).

The addition of absorbent to faecal matter facilitates the dehydration process by reducing the water content to less than 25% and controls the odour and flies (Austin, 2006, Trimmer, 2015). Depending on the type of absorbent used, the alkaline properties of the absorbent can cause a rise in pH of the faecal matter, which also aids in pathogen deactivation (Austin and Cloete, 2008, Trimmer, 2015, Kvarnström et al., 2006).

The type of absorbent used will determine the rate of pathogen deactivation. Other absorbents can aid the mass of water through the faecal matter by making the vault contents porous, for example, straw or wood shavings.

2.2.2.3 Household wastes

In communities where there is no active solid waste collection system, households often use UDDTs to dispose of their solid waste (termed trash). This results in the vaults filling up more quickly and interferes with the dehydration and degradation processes that occur in the vaults. Zuma (2015) sampled one active and one standing vault of a UDDT and found household wastes that included feminine products, textiles, and lightweight materials.

2.2.3 Processes occurring in UDDTs

There are several processes taking place within the vaults of the UDDT. This section provides an overview of these processes.

2.2.3.1 Dehydration, fly control and odour elimination

The separation of faeces and urine at source aids in ensuring low moisture content. The addition of absorbent after defecating, aids in the faecal matter dehydration process, prevents flies, and reduces odour (Niwagaba, 2009). In addition, dry and hot climates promote faecal matter dehydration to low moisture content, assisting in the pathogen inactivation, hence the ambient conditions could influence the rate of degradation. Other studies (Austin, 2006, Chien et al., 2001, Niwagaba et al., 2009, Endale et al., 2012) have shown that the selection of an absorbing material can also influence the reduction of pathogens, for instance, wood ash which has a high pH of approximately 10.

2.2.3.2 Faecal sludge Degradation

Two phases occur during the FS degradation in the vaults i.e., the filling phase and the standing phase. During the filling phase, some faecal matter is subjected to aerobic degradation due to air contact causing the reduction of organic matter and moisture (Buckley et al., 2008b). Once all the faecal matter is covered with sand or ash; degradation and evaporation stop due to the absence of air. The characteristics of the

UDDT sludge were found to be influenced by the amount of sand added and contributed to the variability (Buckley et al., 2008b).

During the standing phase, aerobic degradation and evaporation continues in the top layer of the FS in the vault, with slight changes in the characteristics occurring underneath the surface. Buckley et al. (2008b) developed a simple model to explain the processes occurring in the UDDT during the filling of the vault. The model predicted that drying occurs on exposed FS before the absorbent is added, resulting in uniform moisture content; however, it was found that the materials in the UDDT are heterogeneous, and the degradation characteristics are highly influenced by the absorbent (sand) which is added to the vault.

2.2.4 Operational factors affecting characteristics of FS from UDDTs

The location, type of on-site sanitation system, environmental conditions, emptying frequency and user habits play a crucial role in determining FS characteristics (Strande et al., 2014). UDDTs are user sensitive and largely depend on the behaviour of users for their successful operation (Austin, 2006).

2.2.4.1 User habits

Household habits and diet influence the variability of the FS characteristics in UDDTs. UDDTs are user sensitive and largely depend on the behaviour of users for their successful operation (Austin, 2006). The total solids vary according to the cleansing method used, and the addition or omission of greywater in the UDDT. Increasing the number of streams entering the UDDT vault, for instance trash, will directly affect the filling rate as will the number of users (Buckley et al., 2008a, Strande et al., 2014). In addition, the FS characteristics are affected by the type of absorbents used, which can be sand or ash, and how much is added by the user.

2.2.4.2 Storage

The duration for which the FS is stored in the UDDT will influence its characteristics due to the stabilisation or digestion of organic matter, which happens during the standing phase. Strande et al. (2014) noted that FS stored for longer periods will have undergone stabilisation due to microorganism activity and environmental conditions resulting in reduced organic load of the FS.

2.2.4.3 Environmental conditions

The environmental conditions, mainly temperature and humidity, directly influence the FS characteristics. The fluctuating temperature during the wet and dry season will affect the rate of degradation or dehydration of the FS. For example, the wet season will result in an increase in relative humidity hence affecting the rate of evaporation and vice versa (Strande et al., 2014).

2.3 Faecal sludge management of UDDT sludge in eThekwini

FS management involves the emptying, transport, treatment and disposal and/ or end-use of FS (Strande et al., 2014). When UDDTs were first introduced as an on-site sanitation solution in eThekwini, it was envisaged that the homeowners would remove the dry FS from the vaults. The UDDT sludge would then be managed by deep row entrenchment where the sludge is buried in a hole, and a tree is planted to use up any organic matter (Buckley et al., 2008b). It was assumed that the drying time of approximately one year in the vaults was sufficient to allow for pathogen die-off, and thus safe to be removed by the household (Austin and Cloete, 2008).

However, research carried out by Buckley et al. (2008b) showed that *Ascaris ova* were identified in FS removed from UDDTs after standing for a year, which raised health and safety concerns around the removal and handling of FS. Due to these concerns, as well as user dissatisfaction around the emptying of UDDTs, alternative methods to handle the sludge needed to be found (Lalander et al., 2013, Roma et al., 2013, Buckley et al., 2008b).

In eThekwini, the available treatment options for managing UDDT sludge were on-site burial, disposal to a wastewater treatment works, using the latrine dehydration and pasteurisation (LaDePa) unit developed for the treatment of VIP sludge, or disposal at a hazardous landfill site (Alcock et al., 2016a). Burial on-site remained the first option if there was land available. Disposal to a wastewater treatment works was not feasible due to the characteristics of the UDDT sludge which could overload the treatment works as had been previously experienced with VIP sludge (Alcock et al., 2016b, Alcock et al., 2016a). Furthermore, processing with LaDePa was impractical due to the low organic content and presence of absorbent which resulted in a drier sludge which was difficult to extrude. Disposal at a hazardous landfill was expensive as a treatment

option due to transport and disposal fees; approximately R900 per tonne (Alcock et al., 2016a). This prompted the municipality to investigate ways in which the emptying of UDDTs could be managed in a similar manner to the management of FS from VIP latrines (Zuma, 2015). Another potential viable solution would be a circular economy approach through processing faecal waste into valuable products where the sale of these could offset emptying and transport costs (Alcock and Grau, 2019)

The BSF larvae technology was considered as a viable option as it was possible to combine the UDDT sludge with organic waste for treatment into valuable products. However, there was no data of the management of UDDT sludge using BSF larvae and this project explored the option.

2.3.1 Black soldier fly larvae in faecal sludge management

This section will highlight the use of BSF larvae in FS management. Also, the BSF larvae characteristics will be explained as well as factors that affect the bioconversion and waste reduction capabilities.

2.3.1.1 Black soldier fly

The BSF (*Hermentia illucens*) is an insect belonging to the order *Diptera* and *Stratiomyidae* family (Brammer and von Dohlen, 2007, Kim et al., 2011). The BSF feeds on various kinds of decaying organic matter. BSF can tolerate a wide range of temperatures but are well suited for tropics and warmer temperate regions (Sheppard et al., 1994).

The lifecycle of the BSF consists of five stages: the egg stage, larvae stage, prepupal stage, pupae stage, and adult stage (Alvarez, 2012). The life cycle is approximately 44 days, but this can vary depending on the environmental conditions (Tomberlin et al., 2009) and feeding substrate (Nguyen et al., 2013). The females lay eggs near the food source, and once they hatch, the larvae crawl into the food source (Sheppard et al., 1994). During the larval stage, they accumulate food resources for the development throughout the instars (Tomberlin et al., 2002, Sheppard et al., 2002). The stages of the lifecycle of the BSF are shown in Figure 2.2.

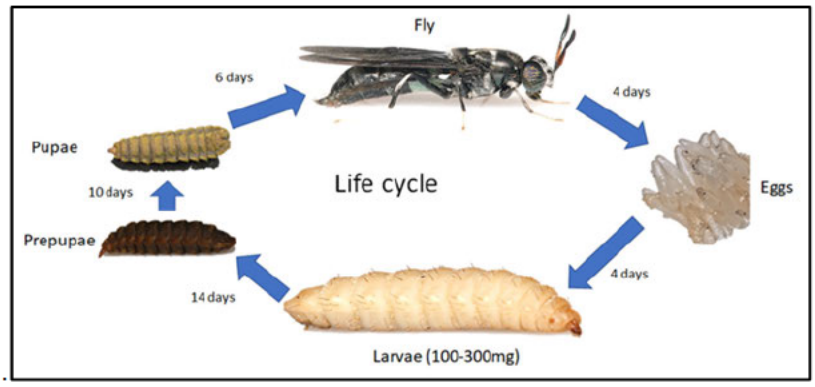

Figure 2.2: The lifecycle of the BSF

(Retrieved from https://www.BioBoosteurope.com/).

2.3.2 Benefits of treating waste with BSF larvae

BSF larvae are used in waste management to reduce the volume or mass of the waste, and the fully grown larvae can be used for different purposes, including as a feedstock for animals.

2.3.2.1 Bioconversion

The high presence of digestive enzymes, including amylase, protease, and lipase, in their gut system, allows the BSF larvae to effectively digest a wide range of wastes (Kim et al., 2011). Furthermore, literature (Banks et al., 2014b, Lalander et al., 2013) shows that the BSF larvae can significantly reduce the organic matter in faecal matter and achieve a high bioconversion (ratio of waste to biomass of larva) rate (Banks et al., 2014b, Lalander et al., 2013). An added advantage of using BSF larvae to treat FS is that it prevents oviposition of houseflies since they will not lay their eggs where the BSF larvae are moderately abundant (Sheppard et al., 1994).

2.3.2.2 Pathogen reduction potential

Pathogenic microorganisms are found in human excreta. The feeding action of the BSF larvae has been shown to facilitate the reduction of bacteria *(Escherichia coli and Salmonella spp)* found in human excreta. However, the BSF larvae have not been observed to deactivate parasites such as *Ascaris sum ova*hence, and therefore, further treatment of the residue or larvae is recommended in *Ascaris sum ova* prevalent areas

(Lalander et al., 2013). In addition, no significant reduction was observed in some bacteria (*Enterococcus spp)* and viruses (*bacteriophage ΦX174*) during the feeding stage (Lalander et al., 2013).

2.3.2.3 Animal feed, fertilizer, and fuel potential

The resulting pre-pupae's nutritional value is approximately 44% dry matter containing 42% protein and 35% fat and could provide a protein source for animal farming (Sheppard et al., 1994). The BSF pre-pupae substituted in fish meal showed that they could have similar nutrients as 100% fishmeal when fed to rainbow trout (St-Hilaire et al., 2007). However, Diener (2010) showed an accumulation of cadmium in pre-pupae and this could limit future use in animal feed production if the FS feedstock contains heavy metals. Table 2.2 shows the different composition of insect meals compared to fish meal and soybean meal in relation to crude protein and crude fat (Diener et al., 2009).The data shows that the BSF has similar nutrient composition with soya meal and fishmeal.

Table 2.2: Composition of insect meals regarding protein and crude fat (% on dry wet basis).

	Protein %	**Crude fat %**
Insect meal		
Black soldier fly (*Hermetia illucens)*		
Pre-pupa	44	33
Larva	42-45	31-35
Pupa	63	15
Larva	38	20
Mealworm (*Tenebrio molitor)*	48-38	29-38
Fishmeal	62-70	8.9-9.3
Soybean meal	43-47	1.5-1.9

*adapted from (Diener et al., 2009)

Li et al. (2011a) showed that the BSF larvae oil is suitable for biodiesel production, increasing the potential of the BSF larvae in waste management and production of cleaner energy. The biodiesel was obtained from the BSF larvae by extracting crude fat

from the BSF larvae using petroleum ether. The extracted crude fat was then converted into diesel by acid-catalysed esterification and alkaline -catalysed transesterification.

The residue (non-digested substrate and excreted products from the BSF larvae) has potential to be used as a soil amendment. Research conducted by Newton et al. (2005) showed the residue could be used in agriculture. However, the highest plant (basil) growth was at low inclusion of the residue (5 - 20%) to clay/sand. It should be noted at higher inclusion, the growth was stunted in comparison with commercially formulated soil which promotes plant growth (Newton et al., 2005).

2.3.2.4 Housefly control

Since the BSF larvae do not feed during the adult stage of their life cycle, they do not transmit diseases (Sheppard et al., 2002). The BSF larvae can also eliminate the housefly oviposition (Sheppard et al., 1994). However, concerns of causing infestation by the fly species' larvae (myasis) assumed to be BSF have been highlighted by Adler and Brancato (1995).

2.3.3 Physiology of BSF

The physiology requirements for successful BSF larvae development are described in this section.

2.3.3.1 Nutrition requirements

The BSF larvae have shown to develop in a variety of wastes (Sheppard et al., 1994, Li et al., 2011b, Myers et al., 2008, St-Hilaire et al., 2007). The Gainesville diet was developed as a diet to feed houseflies and was suggested for mass rearing of the BSF larvae (Sheppard et al., 2002). Poultry feed (15% protein) and Chemical Specialties Manufacturers' Association fly larval medium (CSMA), an artificial media which was developed to feed houseflies have been suggested for rearing BSF larvae (Tomberlin et al., 2002).

Gainesville diet, poultry feed, or CSMA have been used as a control diet by many BSF larvae studies (Gligorescu, 2016, Nguyen et al., 2015, Zhou et al., 2013, Lalander et al., 2019a). Gligorescu et al. (2018) showed the BSF larvae developed faster on Gainesville's diet than on a high protein diet or high carbohydrate diet. Tschirner and Simon (2015) found that BSF larvae feed on poultry feed, developed faster than those

on a protein diet or fibre diet. In another study, the BSF larvae were found to prefer kitchen waste due to the high energy, high carbohydrates, high protein and high fat content (Lalander et al., 2019b, Nguyen et al., 2015).

A comparison of these media is shown in Table 2.3. The data shows that the BSF larvae diet should be between; 15-20% protein, 3-4% fat, 4-16% fibre and 6-14% ash.

Table 2.3: Composition of diet used to rear BSF larvae

Constituents	**Gainesville diet (%)**	**CSMA (%)**	**Poultry feed (%)**
Alfalfa meal	30	27	proprietary information
Wheat bran	50	33	proprietary information
Corn meal	20	-	proprietary information
Brewer's dried grain	-	40	-
Nutrients			
Protein	15.3	19.0	15.0
Fat	3.8	3.0	3.0
Fibre	12.6	20	5.0
Ash	6.3	8.0	13.7
Calcium	4.9	4.0	5.1

* adapted from Tomberlin et al. (2002)

Although fish food diet shared relatively similar high fat, high energy, high protein, and high calories with kitchen waste, the carbohydrate content was undetectable, resulting in longer development time and poor growth (Nguyen et al., 2015, Nguyen et al., 2013). Furthermore, the difference in kitchen waste and fish meal was attributed to the presence of heavy metals in the fish meal, which could have hindered growth (Nguyen et al., 2015). There is a need for waste streams to imitate Gainesville's, CSMA or poultry feed nutritional constituents, for optimal development. However, other constituents of the substrate may be detrimental to BSF larvae growth.

2.3.3.2 Temperature and Humidity requirements

In order to promote growth, the temperature should be maintained between 29-31°C (Barry, 2004, Tomberlin et al., 2002). Relative Humidity should fall between 50 and 70% (Barry, 2004).

2.3.3.3 Moisture content

The ideal moisture of the substrate for BSF larvae bioconversion is 65-85% (Banks, 2014, Cheng et al., 2017). Small prepupal mass difference ranging from 4mg to 6mg was found when using moisture content of 70-75% using food waste (Cheng et al., 2017).

2.3.3.4 pH

An initial substrate pH of between 6.0 to 8.0 is recommended for bioconversion of organic waste using BSF larvae (Ma et al., 2018).

2.3.3.5 Chemical composition of the substrate

The properties and characteristics of the BSF larvae is highly influenced by the chemical composition of the substrate (Meneguz et al., 2018, Spranghers et al., 2016, Tschirner and Simon, 2015). In a study by Meneguz et al. (2018), substrates with high crude protein and high moisture content allowed the BSF larvae to have high crude protein. Spranghers et al. (2016) found the BSF larvae reared in substrates that are energy-dense tend to have high fat content. Also, the fat and ash content of BSF larvae is dependent on the rearing substrate.

2.3.4 Factors affecting bioconversion in a batch system

In FS management, there is a need to evaluate the bioconversion and waste reduction capabilities of the BSF larvae using FS.

The bioconversion process is affected by the larval density (number of larvae per given volume), quantity and quality of the substrate and environmental factors (Paz et al., 2015). The interaction between temperature and food availability can cause either an increase or decrease in larval development and growth.

The larval density has significant effects on the relative growth rates of the larvae. An increase in the larval density results in an increase in competition between BSF larvae

for food and affects the weight gain of the larvae (Paz et al., 2015). Knowledge of the relationship between temperature, larval density, food availability, and their effects on larval growth, waste reduction, and development is essential in waste management using BSF larvae.

The consumption rate (mass of substrate ingested per unit time per larvae) of wastes depends on the life stage of the larvae, the type of substrate consumed, moisture content, temperature and the number of larvae (Diener et al., 2009).

2.3.4.1 Effect of type of faecal sludge on the BSF larvae development

BSF larvae can develop, survive, and reduce the volume of FS (Lalander et al., 2013; Banks et al., 2014). However, different sources of FS such as UTTD sludge, VIP sludge, or PS, could impact larval development and efficiency differently (Lalander et al., 2019b). The study by Lalander et al. (2019a) highlighted that the more stable the sludge, the more negatively it impacts the larval development, as digested sludge resulted in low bioconversion compared to fresh human faeces. This is supported by Banks et al. (2014b), who found a high bioconversion when using fresh human faeces and attributed it to readily available carbon and protein for larval development. Banks (2014) found low bioconversion if VIP latrine sludge was used as it had undergone digestion, hence reducing the availability of nutrients for the BSF larvae. High waste reduction of FS with higher biodegradable content was achieved in studies by Lalander et al. (2019b), however, low waste reduction was experienced when using digested sludge. On this note for a FS management process, it is necessary to have a high waste reduction (reduction of a FS mass) while achieving a good bioconversion (mass of substrate to larval mass).

The characteristics of UDDT sludge at the stage at which the vaults are emptied will affect the BSF larvae development. According to Buckley et al. (2008b), there is not much change in FS characteristics during the filling phase. However, during the standing phase, the characteristics of the UDDT sludge will be mostly non-biodegradable matter.

UDDT sludge in the active stage is more likely to have more organic content suitable for the BSF larvae than in the standing phase. However, FS from UDDTS during emptying is a combination of both vaults (standing and filling phase). Conditions of the

UDDT sludge during the emptying phase needs to be explored for BSF larvae development to see if it is better to have sludge from the filling stage or the standing stage.

2.3.4.2 Effect of substrate quality and quantity on the BSF larvae

The quantity and quality of food consumed directly influences the growth, reproduction, composition, and development of insects (Huffaker et al., 1984, Oonincx et al., 2015, Tschirner and Simon, 2015). Growth needs to assimilate food into biomass, hence the influence of diet quality and food availability (Hilbert, 1995). Varying the composition of the BSF larval diet influences the final composition of the pre-pupae (Burtle et al., 2012, Tschirner and Simon, 2015). Feeding the BSF larvae on different substrates will affect the development of the larvae and the dry mass reduction of the substrate and affect critical production factors, including the yield of BSF larvae (Diener et al., 2011c, Burtle et al., 2012, Tschirner and Simon, 2015). In 2013, Zhou and co-workers showed that the BSF larvae reduce the dry matter and nitrogen content of FS by approximately 40 to 46% and 22 to 56%, respectively, depending on the substrate and strain (genetic variant) of the BSF larvae used.

A high protein diet results in more nitrogen and the dry matter in the substrate being reduced (Zhou et al., 2013, Nguyen et al., 2015). Nitrogen plays a role in all metabolic processes of insects, and it can generally limit the growth and fertility of insects (Huffaker et al., 1984). BSF larvae tend to use a high protein diet and high-fat content more effectively than other diets (Oonincx et al., 2015, Nguyen et al., 2013). However, excess proteins in the diet tend to reduce the larvae survivability (Tschirner and Simon, 2015, Gobbi et al., 2013).

Nguyen et al. (2015a) showed that a diet that has higher fat and energy content tended to produce larvae of greater mass. Gobbi et al. (2013) further showed that meat meal produced more poorly developed larvae than poultry feed. Feed sources shifting to anaerobic conditions impede larval contact with the substrate, thus decreasing the yield of larvae and reducing waste reduction (Diener et al., 2011c).

Lalander et al. (2019a) evaluated the development of BSF larvae on eight urban waste streams to identify substrate properties contributing to the treatment efficiency (waste reduction and bioconversion) and larval development as shown in Table 2.4. The study

showed the more nutritious and balanced the substrate, the higher the bioconversion and the higher the material reduction. In addition, the size of the prepupae and development time is influenced by the substrate characteristics.

Enhancement of the nutritional value of FS by adding market waste reduced the development time of the larvae, and significantly increased the weight gain of the pre-pupae (Diener et al., 2011c).

Table 2.4: Process efficiency and larval development properties of BSF larvae to the final larval stage prepupae (pp) in BSFL composting with different substrates. Values presented are mean ± standard deviation (n=3).

	Bioconversion (%DM*)	**Material reduction (%DM*)**	**Prepupal weight (mg)**	**Time to 50% pp (days)**
Poultry feed	12.8 ± 0.7	84.8±3.6	251±6	16
Dog food	13.4 ± 0.9	60.5±1.5	252±6	18
Food waste	13.9±0.3	55.3±4.1	212±4	19
Fruit and vegetable	4.1±0.2	46.7±3.1	218±4	42
Abattoir waste	15.2±1.6	46.3±2.9	248±3	17
Poultry manure	7.1±0.6	60.0±2.3	164±14	19
Human faeces	11.3±0.3	47.7±1.1	245±5	19
Primary sludge	2.3±0.1	63.3±1.9	137±5	28-32
Undigested sludge	2.2±0.2	49.2±3.7	145±5	46-51
Digested sludge	0.2±0.6	13.2±0.8	70±5	N/A

DM*- dry matter and adapted from Lalander et al. (2019b)

2.3.4.3 Effect of temperature on the BSF larvae development

The ambient temperature significantly regulates the metabolism and the development rate of the BSF larvae (Jarošík et al., 2004). Hilbert (1995) showed that an increase in temperature directly influences final weight of insects and developmental rate. However, after the optimum temperature is reached, an increase in temperature results in the loss of final weight of the insect and lower developmental rate. Insects have upper and lower threshold temperatures, which have different effects on the organism's

development (Dixon et al., 2009). Holmes et al. (2016) suggests that the development of the BSF larvae ceased at temperatures below 19 °C, although lower temperatures can be tolerable to larvae as they can produce heat energy with their writhing movement when feeding (Alvarez, 2012). The BSF larvae's upper-temperature threshold is in the range of 30-36 °C, which affects their development and survivability (Tomberlin et al., 2002). Optimal temperatures of BSF larvae for efficient bioconversion of waste is between 27-31°C (Tomberlin et al., 2002).

Durban's weather is warm and subtropical. The summer months are generally warm and wet, while the winter can be dry to moist. The temperature ranges from a high of 30°C to a low of 11°C during the year. Since the climate conditions vary throughout the year, it is essential to know the larvae's growth rates under uncontrolled conditions to see the viability of treating FS with BSF larvae using a low-cost technology (Alcock and Grau, 2019).

2.3.4.4 Effect of larval density and feeding rate on growth rate of the larvae

Larval density and feeding rate influence the larvae's growth rate, bioconversion, waste reduction, pH, and the temperature of the substrate. The larvae can consume from 25mg to 500mg of organic waste per larva per day (Diener et al., 2011a). A low feeding rate would imply an increased number of larvae per mass of substrate. Increasing the larval density causes a rise in competition between the larvae, hence affecting their larval growth (Paz et al., 2015, Diener et al., 2011c). Excessive food may result in anaerobic conditions due to the undigested substrate. Anaerobic conditions might reduce the pH and reduce the larvae's growth and development by hindering feeding rate (Paz et al., 2015).

High feeding rates tend to lower the waste reduction, growth rate and ph. High larval densities with high metabolic activities elevate the temperature by overcrowding in the substrate. Increasing the temperature may result in the temperature exceeding the optimum temperature (Paz et al., 2015). According to Banks et al. (2014a) the feeding rate required to produce high bioconversion and waste reduction is 100 mg of fresh human faeces per larva per day. However, the BSF larvae feeding rate on UDDT sludge might be higher due to decreased biodegradable content. The BSF larvae process needs to reach a balance between the feeding rate and larval density.

2.3.4.5 Effect of feeding/substrate depth

An increase in depth might limit feed accessibility by the BSF larvae. Furthermore, an increase in substrate depth might create anaerobic conditions, hence hindering waste reduction efficiencies. According to Brits (2017) and Caruso et al. (2014) the substrate's optimal depth was less than 5 cm for increased larval efficiencies.

2.3.4.6 Effect of retention time

The retention time of the BSF larvae on the substrate is influenced by the quality of feed, the feeding rate, and the age at which BSF larvae are added to the substrate. The retention time when using FS as feed varies as it depends on the stage of harvesting. Diener et al. (2011c) found it took 27 days using 167 mg larvae/day to have all BSF larvae transformed to prepupae and achieve a dry waste reduction of 55%. However, Banks et al. (2014b) found using fresh faeces as a substrate resulted in the waste reduction of 46% at a feeding rate of 100 mg larvae/day for 12 days.

2.3.4.7 Effect of moisture content

According to Banks (2014), the substrate's moisture content has an impact on prepupal production. His findings highlighted that a feed moisture content of 75% had a significant impact on bioconversion compared to using feed with a moisture content of 65%. The ideal moisture content for BSF larvae bioconversion when using FS is 65 - 85% (Banks, 2014). Lalander et al. (2020) investigated the effect of high moisture content (76%-97.6%) and found that increasing moisture content decreased bioconversion and material reduction. Also, high moisture content impacted the survivability of the BSF larvae. Using 70-75% moisture content in a controlled environment allows for easy separation of the BSF larvae and residue (Cheng et al., 2017).

2.4 Establishment of the UDDT-FS management programme in eThekwini

The UDDT FS management in eThekwini was established based on the reasons mentioned in Section 2.3. For a successful operation, there was a need to have a clear protocol with regards to UDDT emptying, transportation and disposal and/ or end use of the FS.

2.4.1 Emptying of UDDTs

EWS implemented free FS emptying services of UDDTs to the households in 2017. The emptying service would be conducted every two years and provided through a managing contractor on behalf of EWS (C, 2019). UDDTS were emptied manually as mechanical emptying of UDDTs was challenging due to the presence of sand and trash in the sludge as well as the low moisture content of sludge. Furthermore, the UDDT vault is smaller to allow for easier manual emptying. As described by Christopher, (2019) the emptying and transportation of faecal sludge is shown in Figure 2.3

Figure 2.3: The emptying and transportation of faecal sludge from UDDTs.

2.4.2 Transportation of faecal sludge

The 250 L bins with the UDDT sludge were transported in two ways depending on the area. If space and social dynamics allowed, the first option was to bury the UDDT on-site. The second option, if burial on site was not possible, the sludge from UDDTs within a 40 km radius would be transported to the BSF larvae facility for central processing (Alcock and Grau, 2019, Zuma, 2015).

2.4.3 End-use or disposal

The options for disposing of the UDDT FS were burial on-site with tree planting, transport to a hazardous waste site, or transport to a central faecal waste processing facility (Zuma, 2015, Roma et al., 2011).

The initial modelling of these options showed high costs with both the burial and hazardous waste site disposal option. However, the BSF facility was a viable option for treating UDDT sludge as it would reduce costs by selling the end products (Alcock and Grau, 2019). Therefore, the municipality opted to build a full-scale facility to test the feasibility of using BSF larvae to treat the UDDT sludge (Alcock and Grau, 2019).

2.5 Hypotheses

Equipped with the knowledge that BSF larvae is a potentially efficient tool for the management of FS, it can be an option for UDDT sludge management However, there are fragmented and scare information on its applicability and performance. Furthermore, the municipality projected to reduce costs by reselling the products from a low cost BSF larvae technology but the residue and BSF larvae had to meet the South African regulations.

It can be hypothesised that:

- UDDT sludge contains organic matter necessary for the development of the BSF larvae.
- BSF larvae fed on UDDT sludge meet South African regulations to be used as an animal feed.
- The residue produced after UDDT sludge is used as a substrate for the BSF larvae meets South African regulations to be used as fertilizer or to be disposed of safely.
- BSF larvae thrive in warm and humid conditions and Durban weather is conducive for the development of BSF larvae.

3 EXPERIMENTAL METHODOLOGY

This chapter outlines the research method that was followed in the study to determine the mass balance of the BSF facility. A mass balance approach was developed to monitor the BSF larvae processing plant. It was more feasible to do pilot studies under the same conditions as at the processing plant and to use the data generated to predict the overall material flows of the plant as there was no data available on the full-scale operation. In addition, pilot studies were easier to manually handle than on the full-scale operation.

A two-phased pilot study approach was used to carry out mass balances.

I. Phase 1, the experiments focused on utilisation of UDDT sludge by the BSF larvae and growth of larvae on the UDDT stage.
II. Phase 2, the experiments to determine the mass balances were carried out using UDDT sludge mixed with approximately 15% PS as a substrate.

The data collected would determine the mass of substrate reduced and the BSF larvae produced during the bioconversion process. Direct sampling was used to determine the system characteristics. The mass balances were carried out on a dry matter basis using data on volatile solids, ash, and water content.

Ethical clearance to research the impact of the BSF larvae to treat UDDT sludge was obtained from UKZN Biomedical Research Ethics Committee (Appendix 1). The ethical committee ensured that research is conducted in a responsible and ethically accountable way as human waste and animal specimens were used.

3.1 Research location

The research was carried out at the BSF larvae plant operated by The Biocycle in Durban, KwaZulu-Natal, South Africa (GPS co-ordinates -29.990306, 30.905917). The BSF larvae plant is located inside the Isipingo Wastewater Treatment Plant. The UDDT FS used in this study was collected from UDDTs situated within a 40 km radius of the Isipingo plant.

3.2 BSF larvae production

The breeding of BSF larvae used in this study was carried out at Agri Protein, Philippi, Cape Town at no cost. The newly hatched neonate BSF larvae were shipped to The Biocycle within approximately two days from hatching. The neonates were shipped in layer mash (i.e., poultry feed for laying hens) using tubs containing 0.75 g of neonates .

3.2.1 Nursery operation

Upon arrival, the two-day-old larvae were placed in a controlled environment (i.e., the nursery) on a specific diet before introducing the substrate. Nursery feeding was important to reduce mortality in the first larval stage by promoting growth. The nursery was constructed based on the greenhouse concept. It was made using polycarbonate plastic sheeting with a mesh; the floor was made of concrete, and it had a basic air conditioner system. The BSF larvae were fed in trays, and no measures were used to prevent the BSF larvae crawling out. Temperatures in the nursery ranged between 28°C - 30°C, which is optimum for larval rearing. The temperature was maintained using heat from an air conditioner (Aux air), and the cooling effect achieved by ventilation and air conditioning.

The two-day old BSF larvae were first fed on formulated feed. The formulated feed is a specific diet for the larvae during the early stages and is supplied by Agri Feed who are producers of animal feed. Each nursery box was made up of 0.75 g two-day-old larvae which were fed 3 kg of formulated feed for four days. The nursery feeding rate was 20 mg larvae^{-1} day^{-1} at a depth of between 1 to 2 cm.

Five nursery boxes made up of 3kg of formulated feed and 0,75g of larvae were sampled to quantify the wet mass of the BSF larvae in the nursery boxes. The content of the boxes was manually homogenised using a rod before separation to ensure uniform distribution of six-day-old BSF larvae and the remaining feed substrate. The initial mass of the box (BSF larvae together with food waste) were measured using a Micro Electronic Platform Scale A12E. A representative sample of 10% of the total mass (food waste and BSF larvae) was removed from the box, and the BSF larvae separated from the remaining food waste using an 0.8 cm sieve. Once separated, the mass of the BSF larvae and remaining food waste were weighed separately.

3.3 Experimental plan

This section will describe the materials and the methods used to determine the mass balance to obtain the bioconversion and waste reduction in the two-phased approach. The pilot studies were carried out using the same substrate and conditions as to the full-scale plant.

3.3.1 Phase 1 experimental plan

During Phase 1, the study focused on utilising UDDT sludge by the BSF larvae in 12 batches of approximately 21 kg (wet) of feed. Characterisation of UDDT sludge before and after treatment was carried out. The growth of larvae on the UDDT sludge was monitored over 13 days.

The UDDT sludge was manually mixed with water to increase the moisture content and then placed in twelve plastic containers (60 cm x 40 cm x 20 cm) at a depth of 10 cm. The six-day-old BSF larvae mixed with the remaining formulated feed (remaining substrate from the nursery) was added to each plastic container. The number of BSF larvae added to the substrate were based on the neonates in the nursery supplied and counted by Agriprotein, however mortality after the nursery was not recorded. Furthermore, the neonates were supplied by Agriprotein according to intended mass of substrate and the number of BSF larvae was not calculated.

The BSF larvae processed the UDDT sludge for 13 days. After 13 days, the BSF larvae were separated using wet separation from the residue and analyses carried out. Wet separation is a technique used to separate BSF larvae and residue using water. As shown in Figure 3.1 there were 15 sampling points, and the samples were collected in triplicates.

Composite samples were collected so that the change in characteristics after larval growth trials could be determined. All samples were collected in triplicates and stored in 1L containers at 4 °C prior to analysis.

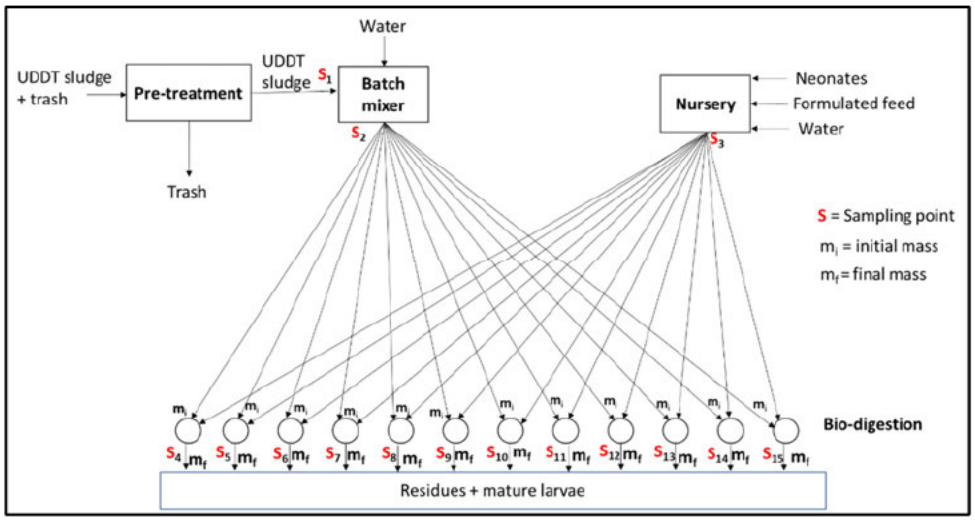

Figure 3.1: A diagrammatic representation of the Phase 1 experimental plan illustrating the sampling points and mass collection

3.3.2 Pre-treatment of the UDDT sludge

The sourced UDDT sludge was manually screened using rakes to remove any trash as shown in Figure 3.2. The trash was loaded into plastic buckets prior to disposal at a landfill.

Figure 3.2: Screening of the UDDT sludge using rakes

UDDT sludge is dry due to the addition of sand as an absorbent and the storage time and contains trash. UDDT sludge (300kg) was mixed with water in a Rolo 2-tonne horizontal paddle concentrate mixer for approximately 30 minutes to increase the moisture content to around 60%. An increase in moisture content promotes larval growth by making the substrate accessible for BSF larval feeding.

3.3.3 BSF Larvae growth trials

The UDDT sludge from the mixer was placed in 12 containers (60 cm x 40 cm x 20 cm) at an evenly layered depth of 10 cm as shown in Figure 3.3. The containers were randomly placed with four per row in the grow-out area of the full-scale plant. The area was termed the grow-out area as this is where the conversion process would take place by the BSF larvae. Monnit™ Wit Wireless Sensors (Monnit Corporation, South Salt Lake, UT, USA) with one temperature probe was placed in the grow-out area to record temperature variations and relative humidity at hourly intervals during the investigation.

An average of 21 kg of UDDT FS, equivalent to the 10 cm layer, was measured using a Micro Electronic Platform Scale A12E scale. The BSF larvae, together with the remaining nursery food waste was layered evenly on top of the UDDT sludge.

The trial containers were placed in the grow-out area in sheds with no environmental controls. The trial containers were layered horizontally on the growing beds and were not covered as shown in Figure 3.3. The trials were operated as a batch process for 13 days for the bioconversion process using a feeding rate of 70 mg larvae^{-1} day^{-1} of UDDT sludge added. The number of neonates were supplied by Agriprotein based on the intended 21kg of UDDT substrate as described in Section 3.2.1.

Figure 3.3: The arrangement of the trial containers in the grow-out area.

The mass of the residue and the BSF larvae were measured before separation on day 13. The mature larvae were separated using wet separation. Wet separation involves the addition of water to make a slurry which allows for the separation of the BSF larvae from the slurry residue due to the different densities. The mixture of mature BSF larvae and the residue was homogenously mixed using rods after the addition of water. The mixture was left overnight to separate the BSF larvae and residue. As BSF larvae are less dense than water; they floated to the top of the mixture and were harvested manually using a sieve. Figure 3.4 shows process flow chart of the experimental procedure for Phase 1.

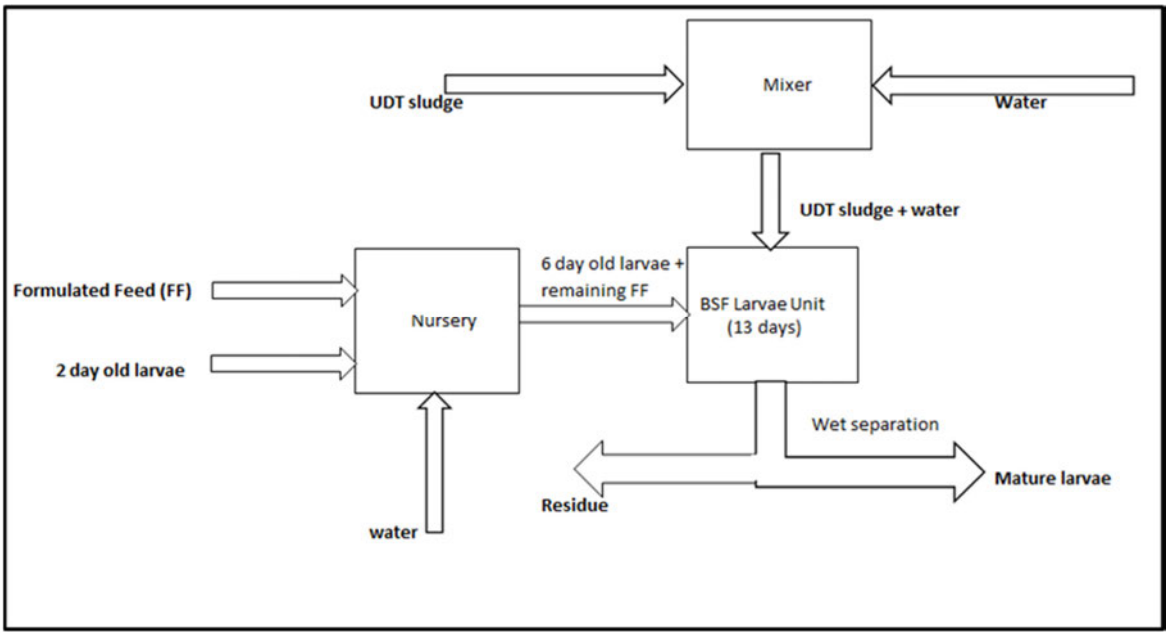

Figure 3.4: Flowchart of the BSF larvae process for Phase 1 experiments.

3.3.4 Growth of larvae

The growth of the BSF larvae was measured in terms of increased BSF wet larval mass every three days. Thirty larval counts were collected to determine larval weight. The BSF larvae were washed and dried with a paper towel before weighing and then returned to their representative containers.

The growth rate was estimated by using a differential equation that assumed constant exponential growth as shown by the Equations 3.1 and 3.2 (Huffaker et al., 1984). (see Figure 4.6).

$$\frac{dw/w}{dt} = \frac{d(\ln w)}{dt} = \frac{\ln(\frac{w_f}{w_i})}{\tau} = k \quad (3.1)$$

Where W_f = final weight of the larvae

W_i = initial weight of the larvae

τ = development time

Since the growth was not exponential, the specific growth rate was estimated it was exponential between measured times.

$$Growth\ rate = \frac{1}{\tau}\ln\frac{w_{(n+1)}}{w_n} \quad (3.2)$$

Where w_n is the nth mass measurement.

3.4 Experimental analysis

All analyses were performed at the WASH R&D Centre laboratory unless otherwise stated. In addition, all analytical procedures followed standard operating procedures listed in Appendix 2.

Characterisation of the UDDT sludge and the BSF larvae was carried out before and after treatment to see the effect of the bioconversion process. The analyses were performed in four categories: chemical analysis, heavy metal analysis, nutrient analysis, and thermal analysis. The samples were analysed in triplicates and the analyses were done before and after the treatment to see the effect of the bioconversion process.

3.4.1 Total Solids/Moisture Content

To determine the mass of total solids (TS) prior to treatment and after the treatment, a representative mass sample of approximately 20 g of UDDT sludge was dried in a hot air oven at 105 °C for 24 h. The remaining residue was cooled and weighed. The residual material indicated the total solids content and was expressed as g/g wet sample. The difference between the mass of total solids and the initial mass of FS is the moisture content of the sample. Volatile solids and ash were measured by heating the UDDT sludge sample at 550 °C for two hours. The mass of volatile solids was

expressed as a ratio of the mass of total solids. Volatile solids quantify the organic matter, and ash quantifies the amount of inorganic matter. Total solids in the feed and BSF larvae were calculated as shown by Equation 3.3.

$$\mathrm{TS}_{\text{Feed or larvae}}(\mathrm{kg}) = [\mathrm{TS}]_{\text{sample}} \times \text{ total feed}_{\text{wet mass}}\ (kg) \tag{3.3}$$

Where $[\mathrm{TS}]_{\text{sample}}$ is expressed in g/g wet sample.

Volatile solids in the feed were calculated as shown by Equation (3.4.

$$VS_{feed\ or\ larvae} = [VS]_{sample} \times TS_{feed\ or\ larve}\ (\mathrm{kg}) \tag{3.4}$$

Where $[\mathrm{VS}]_{\text{sample}}$ is expressed as g of VS/g of TS

Ash content was calculated as shown in Equation (3.5.

$$Ash_{feed\ or\ larvae} = [Ash]_{sample} \times TS_{feed\ or\ larve}\ (\mathrm{kg}) \tag{3.5}$$

Where $[\mathrm{Ash}]_{\text{sample}}$ is expressed as g of ash/g of TS

3.4.2 Feed depth

Feed depth in each of the trial containers was measured using a transparent 15cm ruler. The ruler was inserted into the sludge. Three measurements were taken to find an average reading.

3.4.3 Calorific value

Calorific value is the measure of the energy content in a sample and is affected by different factors such as the level of stabilization, type of on-site sanitation, and sand content. To measure calorific value, the sample of the substrate before and after treatment with BSF larvae was dried using the method described in 3.4.1. After drying, the sample was ground to powder and 0.7g weighed into a capsule. The sample was placed in a Parr 6200 calorimeter which is an oxygen bomb calorimeter in a high-pressure oxygen atmosphere and the calorific value was read automatically from the screen.

3.4.4 Crude protein

For protein analysis, FS samples were homogeneously mixed using a stirring rod and 0.2 + 0.1g measured into a crucible. A Leco Tru Mac CNS was used to determine the Carbon, Nitrogen and Sulphur content in the sample. The total Nitrogen content was used to determine the crude protein using the Jones conversion factor of 6.25 (Jones, 1931). The crude protein was determined using a conversion factor of 4.76 to avoid overestimating the protein content (Janssen et al. (2017). The protein analysis was done on both the substrate and the BSF larvae before and after the BSF larvae process.

3.4.5 Heavy metals and nutrient analysis

The mature BSF larvae and residue were analysed for nutrient and heavy metals content to determine the potential for use as an animal feed. Analyses to determine the elemental nutrient content were conducted. The heavy metal content, which includes cadmium, arsenic and lead was also analysed.

The samples were dried at 105 °C for 24hrs before analysis. Approximately 0.2 g of each sample was digested in 8 mL of aqua regia (HCl/HNO_3, ratio 3:1) for 30 min at 80°C. After cooling down, 15 mL of distilled water was added, and the obtained solution was filtered using a 0.45 µm filter paper to remove all the undigested matter (fibre and sand). Distilled water was added to the solution to get a final solution of 50 ml. Each prepared solution was diluted 100 times in 2% HNO_3 solution, and their metal composition was analysed using a Varian 710-ES ICP-OES Analyzer (Palo Alto, CA, USA).

The results are presented in ppm, and the given metal concentrations are for 50 mL solution and 0.2 g of sample. The heavy metals were analysed in the UDDT substrate and BSF larvae using the method described above and bioaccumulation was calculated as shown in Equation 3.6. (Walker, 1990).

$$Bioaccumulation = \frac{concentration\ of\ heavy\ metal\ in\ BSF\ larvae}{concentration\ in\ the\ substrate} \quad (3.6)$$

3.4.6 Statistical analyses

Data collected from samples were expressed as the mean plus standard deviation. Measured concentrations of different parameters were multiplied by the mass of total substrate to obtain the overall mass of each tested parameters.

3.4.7 Calculations

The calculations used in Phase 1 are explained in the following section. The use of these formulas listed herein allowed for sampling errors to be propagated throughout all calculations.

3.4.7.1 Bioconversion

Bioconversion is defined as the uptake of mass of the BSF larvae relative to the initial substrate mass expressed as a percentage. The greater the bioconversion, the more efficient the BSF larvae are in converting the substrate into larval mass.

Bioconversion in wet basis is calculated as shown in Equations 3.7 and 3.8 (Banks et al., 2014b).

$$\text{Bioconversion (\%)} = \frac{\text{final mass of BSF larvae (kg)}}{\text{Total mass of substrate added (kg)}} \text{ x } 100\% \qquad (3.7)$$

Bioconversion in dry matter was calculated as shown in Equation 3.8:

$$\text{Bioconversion (\%)} = \frac{\text{final mass in dry mass of BSF larvae (kg)}}{\text{Total mass dry substrate added (kg)}} \text{ x } 100\% \qquad (3.8)$$

3.4.7.2 Relative Waste reduction

Waste reduction refers to the mass of substrate reduced during the bioconversion process relative to the initial substrate mass, expressed as a percentage. The amount of waste reduced on a wet basis was calculated in Equation 3.9 (Harnden and Tomberlin, 2016).

$$\text{Relative Waste reduction \%} = \frac{\text{T(kg)} - \text{R(kg)}}{\text{T (kg)}} \text{x } 100\% \qquad (3.9)$$

T is the total substrate given (kg), R is the residual mass remaining after bioconversion, which includes non-digested food and excreted products.

Dry mass reduction is the percentage of substrate consumed by the BSF larvae on a dry matter basis as shown in Equation 3.10 (Diener, 2010). The use of the dry mass reduction allows for a comparison of results from feeds with different moisture contents. It is a more accurate description of consumption of the sludge by the BSF larvae because it is not subject to moisture evaporation.

$$Dry\ mass\ reduction\ (\%) = \frac{(T\ x\ DM_t) - (Rx\ DM_r)}{Tx\ DM_t} \text{ x } 100\% \quad (3.10)$$

T is the total substrate given (kg), R is the residual mass remaining after bioconversion, which includes non-digested food and excreted products. DM_t is the average total solids of the total substrate, and DM_r is the average total solids content of the residual mass.

The reduction rate is calculated as shown in Equation 3.11 (Diener, 2010).

$$Reduction\ rates = \frac{T - R}{Vxd} \quad (3.11)$$

Where T is the total quantity of substrate given (kg), R is the residual mass remaining after bioconversion, which includes non-digested food and excreted products. V is the volume of the sludge (based on feed depth and area of the container), and d is the number of days.

3.4.7.3 Error propagation

Error from sampled data was carried throughout the calculations to illustrate the possible range of each parameter. This is explained in Equation 3.12. Where multiple trials were performed to measure X, the best estimate was the average, $\bar{X}$. The average value was calculated by summing all measured values and then dividing by the number of trials (N).

$$\bar{X} = \frac{\sum_{i=1}^{N} X_i}{N} \quad (3.12)$$

The error associated with the measurement tool was included as it contributed to the degree of error of measured value.

The approach used, which depended on the arithmetic operation and the equations used, are summarised in the following equations.

For addition and subtraction using uncertainties, Equation 3.13 was used:

$$\Delta z = \sqrt{(\Delta x)^2 + (\Delta y)^2} \tag{3.13}$$

Where Δz is the uncertainty of the result and Δx, and Δy is the addition or subtraction terms' uncertainties.

Secondly, for multiplication and division using the uncertainties, Equation 3.14 (Coleman and Steele, 2018) was used:

$$\frac{\Delta z}{z} = \sqrt{(\frac{\Delta x}{x})^2 + (\frac{\Delta y}{y})^2} \tag{3.14}$$

Where Δz is the uncertainty of the result and Δx, and Δy is the uncertainty of the multiplication or division terms, z is either the product or quotient and x, y is the multiplication or division terms.

3.5 Phase 2 experimental plan

The aim of the Phase 2 experimental plan was to determine the mass balance in the processing plant by undertaking three trials lasting 13 days each using BSF larvae fed on UDDT sludge mixed with PS at a depth of 5 cm. The combination of UDDT sludge and PS was such that PS was 14 wt. % of UDDT sludge. It worth noting that in Phase 2 the trash was not removed in order to determine the feasibility of treating UDDT sludge with trash present. The data obtained from the mass balance was used to determine the bioconversion and waste reduction. The data obtained using the pilot trials of approximately 300kg was extrapolated to 1 tonne UDDT sludge to calculate the mass balances, bioconversion, and waste reduction for the full-scale plant. The experimental trials were conducted on a smaller scale to overcome the challenges of manually handling 1.78 tonnes of UDDT sludge, the full-scale plant's daily input. The experimental set up and sampling points are shown in Figure 3.5.

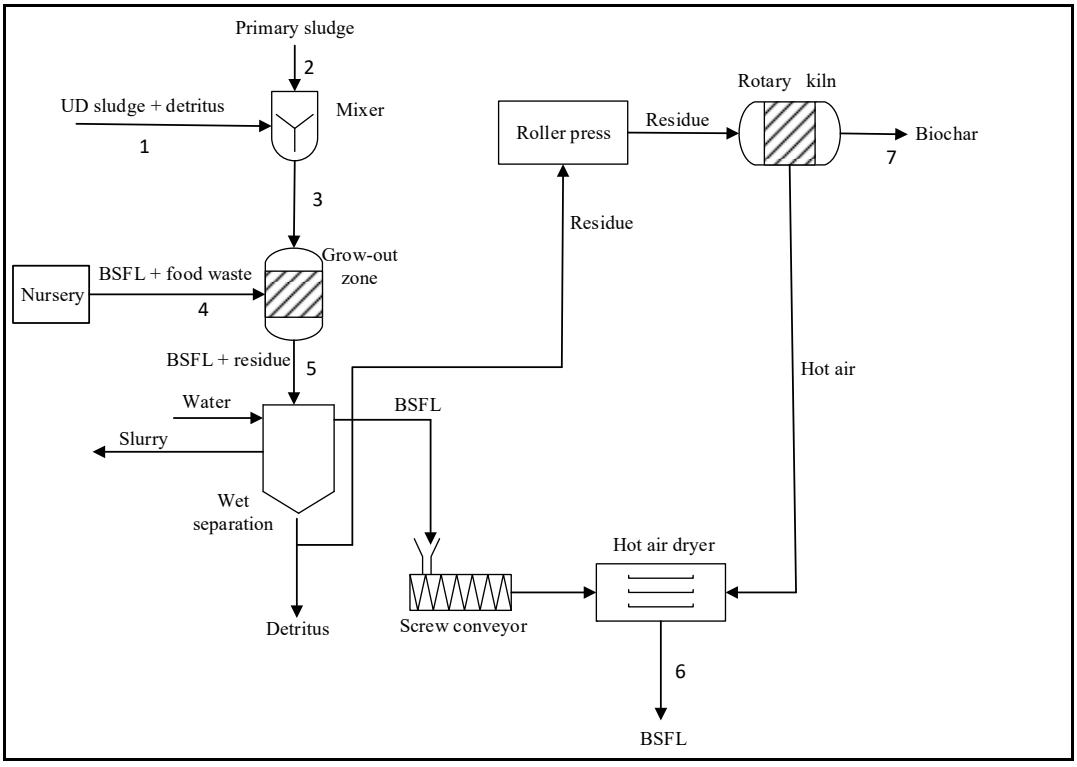

Figure 3.5: Process flow diagram of the BSFL plant showing the sampling points represented by numbers for analysis

Three trials with an area dimension of 5.32 ± 0.46 m^2 were set up on three consecutive days in the growing beds in the grow-out area. The composite sludge (UDDT + PS) was laid on the growing beds at a depth of 5.56 ± 0.64 cm.

Monnit^{TM} Wit Wireless Sensors (Monnit Corporation, South Salt Lake, UT, USA) with one temperature probe were placed in the grow-out area to record temperature variation and relative humidity at hourly intervals during the investigation.

The trials were operated as a batch process for 13 days using a larval feeding rate of 70mg of FS/larvae/day. After 13 days, FS residue and BSF larvae were separated using wet separation method as the residue was not sufficiently dry to allow effective dry separation of BSF larvae and residue. Samples were collected at the beginning and at the end of the bioconversion process to determine the change in physicochemical properties of the UDDT sludge and BSF larvae. The mass added to each trial were weighed at the beginning and at the end of the bioconversion process.

3.5.1 Larvae preparation

Two-day old larvae were fed on post-consumer waste from restaurants and supermarkets. The waste, which included bread, rice, and meat, was supplied by SmartMatta (Durban). SmartMatta is a company that deals with solid waste management in Durban. The two-day-old larvae were fed for five days on a mixture of restaurant food waste and layers mash in the ratio of 90:10, respectively. Layer mash is a formulated feed for laying hens. Layer mash was added to increase the nutrition of the food waste. The restaurant waste was shredded using an industrial meat mincer through an 0.8 cm sieve to reduce particle size and homogenised manually to improve the feeding rate of the BSF neonates. The two-day-old larvae were fed at a ratio of 3g neonates to 9.4kg of food waste and 1kg of layers mash based on a feeding rate of 20 mg larvae^{-1} day^{-1} for four days at a depth of 5 cm.

To quantify the wet mass of the BSF larvae in the nursery boxes, six-day old BSF larvae and remaining feed substrate from the nursery were homogenised using a rod to distribute uniformly. A Micro Electronic Platform Scale A12E scale was used to measure the food waste and the initial mass of the BSF larvae. A representative 10% of the total mass (food waste and BSF larvae) was removed and separated. This 10% mass was then further separated using an 0.8 cm sieve and then weighed separately. This provided the mass of the BSF larvae and food waste used as the inputs to the processing of the UDDT sludge.

3.5.2 Feed preparation

A Rolo 2-tonne horizontal paddle concentrate mixer was used for mixing the UDDT sludge and primary sludge. Electricity was used to drive the frame. Firstly, the mixer homogeneously mixed the UDDT sludge for 45 minutes before sample collection as shown in the schematic diagram in Figure 3.5. Secondly, the primary sludge (PS) was pumped from a graduated tank into the homogenised UDDT sludge. The PS samples were collected during the pumping of PS into the UDDT sludge. The composite samples were mixed in the mixer for approximately 45 minutes before being laid on the growing beds. The composite samples were collected from the growing beds. The samples were collected in triplicates and stored in 1-litre containers at 4 °C before analysis.

3.5.3 Larval growth trials

UDDT sludge and PS were mixed as described in Section 3.5.2. After mixing, the mixer dispensed the feed (composite sludge) into a skid loader Caterpillar and transported to the grow-out section. Three pilot trials were set up in the grow-out area on consecutive days and contained approximately 300 kg of the feed at a depth of 5 cm to mimic the full-scale operation (1.5 tonnes) to monitor the change in characteristics due to the BSF larvae processing. The feed was laid in the grow-out area manually, and evenly levelled using a rake. The feed was weighed using the weighing bridge before being laid on the grow-out area at an average depth of 5 cm. The grow-out strips were an average of 2.42 m x 2.20 m x 0.05 m in dimensions. Six-day-old BSF larvae were added to the feed for the bioconversion process to take place over a period of 13 days. The six-day-old larvae had a feeding rate of 70mg FS/larvae/day for 13 days. The total number of BSF larvae added to each trial were estimated to be 329 670 for each trial.

After 13 days of processing with the BSF larvae, samples of BSF larvae and composite sludge residue were collected. The pH, temperature and feed depth of the trials were noted at the beginning and at the end of the processing time. The temperature probe was placed in the grow-out area to record temperature variations and relative humidity at hourly intervals.

3.5.4 Harvesting process

The BSF Larvae and residue samples (Sample point 5 in Figure 3.5) of the trials were collected at the end of the 13 days and separated manually using a wet separation technique. A Micro Electronic Platform Scale A12E scale was used to measure the mass of the residue, trash, and BSF larvae before separation. Water was used as a solvent to allow for the separation of the residue and BSF larvae. As the residue has a greater specific gravity than water, it settled in the tank, while the BSF larvae floated, allowing separation. The contents of each trial container were homogenised manually using a rod to allow the BSF larvae and residue to separate. The residence time to allow effective separation was found to be 12-14 hrs. The trash was handpicked from the mixture using a fork, and the mass noted. A sieve was used to collect the BSF larvae. Mixing was repeated until all the BSF larvae had been collected. The wet mass of the collected BSF larvae was noted. The wet mass of the residue was calculated.

3.5.5 Processing of the mature larvae and residue

3.5.5.1 Drying

A single shell rotary drum dryer was used to reduce the moisture content and pasteurise the BSF larvae. As shown in Figure 3.5, the collected BSF larvae were added to the processing unit through the screw conveyor and passed through the dryer with an inlet temperature of 150 °C and an outlet temperature of 100 °C. The rotary dryer was indirectly heated as it used exhaust air from the retort kiln which is used for biochar formation to dry the larvae. The exhaust air and the BSF larvae were in co-current flow during the drying process. Temperature probes were located at the dryer entrance and exit, and the temperature readings were read from the control board. The rotary dryer was operated at 15 rpm at a flow rate of 0.03 kg/s. Samples of the dried BSF larvae were collected and weighed as shown by sample point 6 in Figure 3.5.

3.5.5.2 Belt Filter Press

A belt filter press was used to remove water from the residue before being passed to the retort kiln. The belt filter press dewatered the residue by applying pressure and squeezing water out and drained to the sewer drain. Due to the high moisture content of the residue caused by wet separation, it had to be processed twice in the filter press to reach the required moisture content for processing into biochar. Some of the residue was lost due to handling during the pressing process, and it could not be accounted for.

3.5.5.3 Biochar formation

Pyrolysis is the thermal chemical decomposition of organic material at elevated temperatures in the absence of oxygen. Petroleum gas was liquefied using an evaporator and supplied the fuel for the burner for pyrolysis at 500 °C in a retort kiln. Five 48kg cylinders were used to supply the liquid for the burner. The syngas produced from the retort kiln was used to generate heat for the rotary dryer. Temperature probes were located near the burner to record the temperatures. The residue was fed automatically by conveyor. The characteristics of the biochar as shown by sample point 7 in Figure 3.5 were determined in order to compare the change in characteristics with the residue sample point 5 in Figure 3.5

3.5.5.4 Sample collection

Samples were collected at different sampling points as illustrated in Figure 3.5 and explained in the sections above. A summary of these samples is provided in Table 3.1.

Table 3.1: Summary of samples collected in the process

Sample point	Description	Sample mass/kg	Replicates
1	UDDT sludge	1.0	3
2	PS	1.0	3
3	Composite sludge	1.0	3
4	BSF larvae (6 day old)	0.5	3
4	Remaining food waste	1.0	3
5	BSF larvae	0.5	3
5	Residue	1.0	3
6	Dried larvae	1.0	3
7	Biochar	1.0	3

3.5.6 Experimental analysis

All analysis of the samples and calculations were performed using the same procedures as described in Section 3.4.

4 PHASE 1 RESULTS AND DISCUSSION

4.1 Introduction

The previous chapter highlighted the methodology used to obtain data to perform mass balances and presented the analyses carried out. In this chapter, the results obtained will highlight the potential of the BSF larvae to process UDDT sludge. Mass balances were performed on a wet and dry basis, and volatile solids and ash content balance were determined. The growth of larvae was monitored every three days over 13 days. To analyse the nutrient concentration of the substrate and the residue, an evaluation based on calorific value, C/N ratio, and protein content was conducted. The change in depth of the trial was measured to determine volume reduction. Environmental conditions based on humidity and temperature were monitored as they influence BSF larvae growth. To evaluate the level of toxicity of the BSF larvae and residue in terms of heavy metals, the presence, and the concentration of Pb, Cd, As, Cr, Cu and Ni were investigated.

4.2 Environmental conditions

Larvae growth and development are known to be sensitive to temperature and humidity. The BSF larvae thrive in a warmer temperate climate (Brammer and von Dohlen, 2007). It was hypothesised that Durban weather is conducive to growing BSF larvae as it has a yearly average temperature of 24.5°C.

Temperature changes could have negatively impacted the BSF larvae growth as they have a lower temperature threshold between 16°C and 19°C (Holmes et al., 2016). The lower temperature threshold of the BSF larvae was determined when reared on the Gainesville diet at 70% diet (n=24) (Holmes et al., 2016). Low temperature tend to cause the BSF larvae to slow down their metabolism, eat less and develop slower (Dortmans et al., 2017).

Field experiments showed fluctuations in temperature and humidity in the grow-out area as shown in Figure 4.1 (raw data presented in Appendix3: Table A.1). The temperature ranged from 14 °C to 35 °C, and the humidity ranged from 48% to 90%. Temperature and humidity fluctuations in the grow-out area might be due to the lack of insulation of

the grow-out and hence is influenced by the ambient conditions (Climate-data.org, 2021).

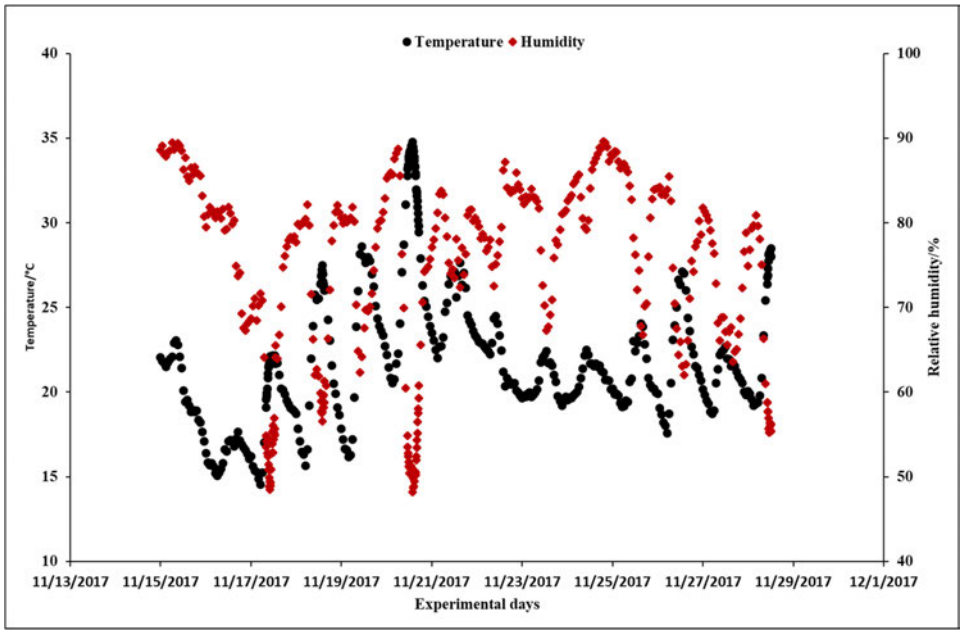

Figure 4.1: Temperature and relative humidity readings over the experimental duration

According to Dortmans et al. (2017), the ideal temperature for the BSF larvae technology is between 28 °C and 32 °C. The maximum developmental temperature threshold for BSF larvae when reared on a grain diet is between 30 and 36 °C, with optimal rearing at 27 °C relative to development time and mortality rates (Tomberlin et al., 2009). Hot temperatures tend to force the BSF larva to crawl away from food, looking for cooler areas, (Dortmans et al., 2017).

Gligorescu (2016) pointed out larval metabolism increased with temperature, and showed that metabolic rate was not influenced only by the feed given, but that there was an interaction between temperature and feed.

Fluctuation in the temperatures resulted in a change in humidity as shown in Figure 4.1. Holmes et al. (2012) determined the effect of relative humidity on egg eclosion and adult emergences. The study highlighted that as humidity reduced, it increased mortality and slowed development. This could be due to dehydration.

The grow-out area was made from bricks and concrete, and although the roof was made from green plastic to absorb heat, it was not sufficient to maintain warmth. A change in

the construction of the grow-out area to improve energy efficiency would help to stabilise environmental conditions.

The substrate was observed to have a crusty top layer as the experiment progressed, which could be to dry air (low humidity) removing moisture from the UDDT sludge.

Brown et al. (2004) define metabolism as the biological processing of energy and material by an organism from the environment. The material and energy are transformed into accessible forms and allocated for survival, growth, reproduction and excreta recycled back into the environment. Clarke and Fraser (2004) study showed that the kinetic energy of cells drive the metabolic rate, and hence an increase in temperature results in an increased metabolic rate.

Based on this assessment, it can be concluded that the environmental conditions in the grow-out area was not favourable for larval development.

4.3 Distribution of measured parameters across different containers

The distribution of the wet mass, dry solids, volatile solids, and ash content from the twelve containers is shown in Figure 4.2 (raw data is presented Appendix 3: Table A.4). Even though the containers were randomly placed during the experimental setup, the data was arranged using the highest initial wet mass to the lowest initial mass and renumbered to allow ease of showing the data.

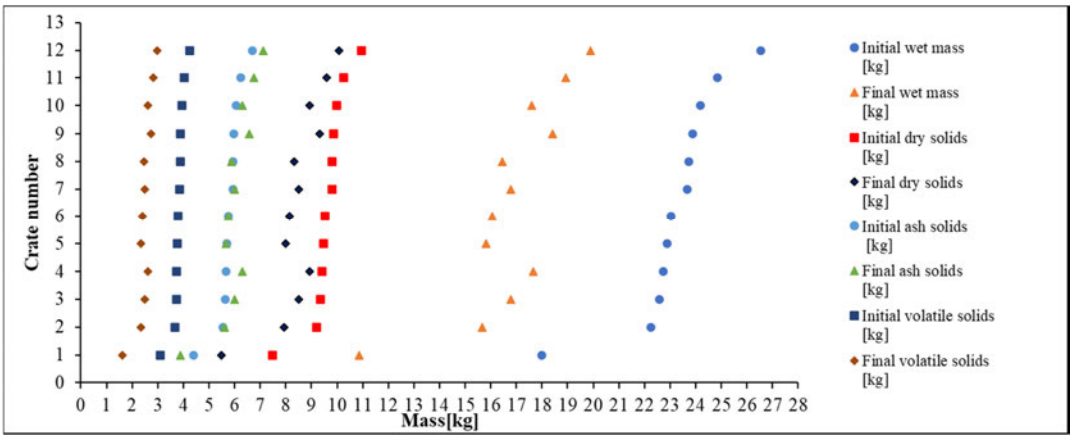

Figure 4.2: The change of wet mass, dry mass, volatile solids, and ash content in different containers (n=12).

The general trend observed across all containers is that moisture is the most abundant component. Water was added to increase the moisture content of the UDDT sludge for accessibility by the BSF larvae (Banks, 2014). Volatile solids (the biodegradable component) are the least abundant component in all containers, ranging from 3.10 kg to 4.25 kg. However, the ash content (non-biodegradable component) does not vary in the initial substrate (5.79 ± 0.52 kg) and the final substrate (5.99 ± 0.77 kg). Furthermore, the ash content is nearly twice that of the volatile content. Similar observations were reported by Austin (2006).

4.4 Change in physicochemical properties of the UDDT sludge

A comparison of the physicochemical parameters after the BSF larvae process is presented in Table 4.1. As can be seen, the VS content decreased during the BSF larvae process, from 0.33 ± 0.01 gVS/gTS in the inflow material to 0.29±0.01g VS/g TS in the outflow material.

Table 4.1: Comparison of the physicochemical parameters in UDDT sludge before and after BSF larvae

		Before UDDT sludge		**After UDDT sludge**	
		Average	**n**	**Average**	**n**
Moisture Content	g of water/ g of wet mass	0.60 ± 0.02	3	0.49 ± 0.3	12
Volatile Solids	g of VS/g of TS	0.33 ± 0.01	3	0.29 ± 0.01	12
Ash	g of ash/ g of TS	0.67 ± 0.01	3	0.71 ± 0.3	12
Feed depth	Cm	10	12	7	6
C/N ratio		12	3	11	12
Protein	%	8	3	9±2	12
Calorific value	MJ/kg of TS	7.23±0.02	3	5.44±0.92	12

[a] refers to the protein on a dry basis

Moisture content significantly reduced from 60% in the UDDT sludge to 40% after 13 days of larval processing. This is supported by, Dortmans (2015) who recorded an increase in total solids from 20% to 50% in a plug flow continuous system. However, in this study, moisture content reduction could be attributed to larval processing and

environmental factors as the vessels were not covered to avoid impacts of the environment. Although the moisture content was low, it didn't allow for sieving the larvae from the residue as the BSF larvae would stick to the residue.

The ash content was not significantly different in the inflow material and the outflow material, while the ash content was nearly two times the volatile solids in the substrate. The available limited biodegradable nutrients were removed and assimilated into larval biomass, as shown by the VS content decrease.

The substrate's feed depth changed from an average of 10 cm to an average of 7 cm.

Carbon and nitrogen are the two main elements in organic wastes, and their proportion is used to predict the decomposition of the substrate. In general, the optimum C:N ratio is in the range of 15-30:1 for the decomposition process (ur Rehman et al., 2017). The C: N rate decreased from 12:1 to 11:1. The initial range of the C: N was very low in comparison to that recommended for optimum decomposition.

Protein content was calculated using the Jones Factor of 6.25 (Jones, 1931), and the UDDT sludge had a low protein content of 8 % on a dry basis. The protein content increased to 9 % in the residual UDDT sludge on a dry basis. The calorific value of the UDDT sludge reduced from 7.23 MJ/kg to 5.44 MJ/kg. The increase in protein and decrease in calorific value could be attributed to experimental uncertainty.

It was noted the remaining nursery feed, which was added into the UDDT sludge with the larvae, could have increased parameters available for the BSF larvae as it had some nutritional value, as shown in Table 4.2.

Table 4.2: The characteristics of the remaining formulate feed from the nursery

	Units	**Average**
Total mass	Kg	2.14
Moisture	g of water/g of wet mass	0.51
Volatile Solids	g of VS/g of TS	0.92
Ash	g of ash /g of TS	0.08
C:N ratio		17

The study found that there was a high ash content and a low volatile solid content in the UDDT sludge (Table 4.1). UDDT sludge was also found to be low in protein.

The moisture content of the UDDT sludge was found to be less than that of fresh faeces. This is to be expected due to the design of the UDDT, which separates urine from faecal material at source and collects the FS in a vault which promotes drying and dehydration of the sludge over time (Buckley et al., 2008b).

When the faecal matter is deposited into the vault, and sand added by the user as a covering material, it combines with the sand, resulting in a highly non-homogeneous mixture (Buckley et al., 2008b, Zuma, 2015). The amount of sand added to the vault is unknown (Zuma, 2015). When the mixture is in contact with air, some faecal matter is removed by aerobic degradation resulting in reduced volatile solids and moisture content, which was supported by the analyses carried out in this study (Buckley et al., 2008b). A study carried out by Buckley et al. (2008b) provided highly variable results as to the composition of the sludge sampled from UDDTs, which implied that the way in which faeces and sand is introduced to the vault varies depending on the user.

Table 4.3 presents the results from the study conducted by Buckley et al. (2008b) compared to the results from this study. The difference in the results could be due to the UDDT sludge used in this study being a mix from both UDDT vaults (i.e. fresh and old sludge) while Buckley et al. (2008b) only analysed UDDT sludge from the standing vault. Furthermore, the variation in the results from Buckley et al. (2008) is due to analysis data from several different toilets whereas in this study the UDDT sludge from the toilets was homogenised before analysis.

The VS reduction results of 4% found in this study (33% to 29%) are similar to a study by Dortmans (2015) who found that VS reduced from 86% to 82%, as measured at different points on two plug flow continuous system at two feeding rates (40 mg dry food/larva/day and 60 mg dry food/larva/day) while using a mixture of 95% of food waste and 5% fresh human faeces.

Table 4.3: Comparison of UDDT sludge properties from Buckley et al. (2008b) to this study

Property	**Range [% mass]**	**Average [% mass]**	**This study (average) [% mass]**
Moisture content	7 – 31	14	60
TS	69 – 93	86	33
Inorganic solids	58 – 92	82	67
Organic solids	1.5 – 11	3.7	33
COD (g/g)	0.006 - 0.28	0.07	-

The results showed that the moisture content was significantly reduced from 60% in the UDDT sludge to 40% in the residue after 13 days of larval processing as shown in Dortmans (2015) supported these findings as they recorded a decrease in moisture content from 80% to 50% in a plug flow continuous system. In this study, as the environmental conditions were not controlled, moisture content reduction could be attributed to larval processing and environmental factors.

C:N ratio is used as an indication of the suitability of a substrate for decomposition. In this study, there was no pronounced reduction of the C:N ratio in the UDDT sludge as it reduced from 12:1 to 11:1. A study by ur Rehman et al. (2017) recommended a C:N ratio of 14 and a pH of 7.8 to enhance bioconversion and aid in the digestion of material that BSF larvae can utilise. The results of this study showed the C:N ratio was lower than that recommended due to low nitrogen and carbon content. Furthermore, UDDT users are wipers, and they use tissue paper, mealie cobs, leaves etc. which are high in cellulose and not easily digested by the BSF larvae.

The study by ur Rehman et al. (2017) also found that the C:N ratio of the substrate decreased when using different ratios of dairy manure and poultry manure as feed for BSF larvae. Lalander et al. (2018) found similar results of a decreased C:N ratio using food waste and fresh faeces when using BSF larvae. The insignificant reduction found in this study could suggest the amount of available C in the UDDT sludge was low as not all C is available for digestion by BSF larvae.

4.5 Material conversion and nutrient flows

Knowledge of mass balances of inputs and outputs during the BSF larvae processing is essential to understand the process needed for optimising the design and operation of the process. Dry matter balance is necessary for calculating nutrient component changes on a mass balance basis.

Mass losses were expected to occur due to water losses from evaporation and metabolism, carbon losses through CO_2 evolution, and nutrient losses through denitrification and volatilization. Therefore, the balances were conducted on a dry matter, volatile solids, and ash content basis. Metabolism and/or evaporation flows was not determined by experimentation but through the mass balance calculations. The composition and mass of the gases emitted were not determined. It should be noted that further investigation needs to be carried out to determine the mass losses to validate the mass balances.

As the process operated in a batch mode, it allowed for the easy measurement of the different parameters. The total flow of nutrients during the BSF larvae processing was estimated using the average values from the 12 replicates as shown in Figure 4.3 (raw data presented in Appendix 3: Table A.3 derived using data from Table 4.2). The flow balances were calculated by multiplying the concentration of the inflow and outflow components with the corresponding mass.

In addition to uptake by the BSF larvae during feeding, the VS also reduced due to loss into the environment. Furthermore, the uptake relative to the total VS was found to be 4.7%.

Of the total 23 kg of the substrate (UDDT sludge and remaining formulated feed) that went into BSF larvae processing (FS plus BSF larvae and residual formulated feed), around 5.12 kg was used lost due to evaporation and /or metabolism, and approximately 1.3 kg (6%) was turned into larval biomass (Figure 4.3).

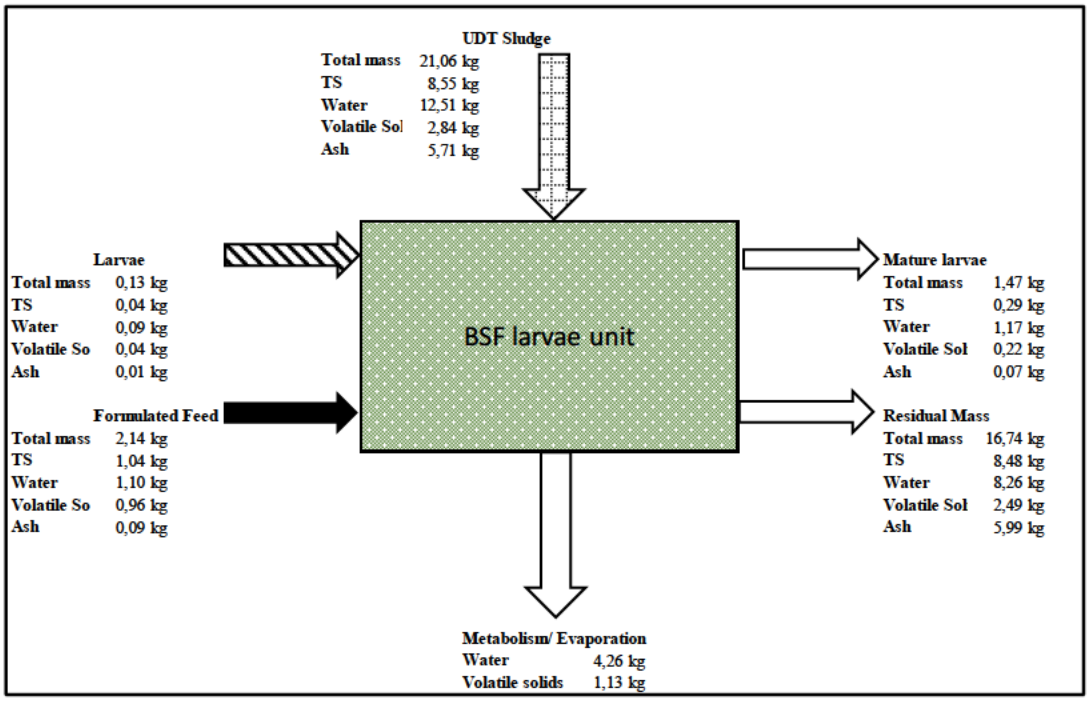

Figure 4.3: The total mass balance of material and nutrients in the 13-day batch process BSF larvae treatment averaged from 12 replicate units.

The relative waste reduction for all containers was an average of 28 ± 5% (ww), 12 ± 6% (TS basis) and 35 ± 5% (VS basis) as shown in Table 4.4. This study's bioconversion was found to be 6% (ww) and 3% (dry basis).

Overall volume reduction in wet basis was 2.07±0.62 kg m^{-3} d^{-1} while on dry basis it 0.362.07±0.62 kg m^{-3} d^{-1} which shows that moistly moisture was lost either due to environment or metabolism by the BSF larvae. Variability of the data could be due to the non-homogenous nature of the feed. Nevertheless, the ash balance is within 5% which also accounts for the discrepancy in TS.

Table 4.4: The mean and standard deviation for the calculated parameters based on the mass balance and measured TS/VS data

Parameter	n	Mean	SD
Relative waste reduction/w/w%	12	28.13	4.56
Relative waste reduction/TS%	12	11.98	5.70
Relative waste reduction/VS%	12	34.72	4.91
Bioconversion rate/w/w%	12	6.37	1.28
Bioconversion rate/TS%	12	3.08	0.62
*Overall wet reduction rates/ kg m^{-3} d^{-1}	12	2.07	0.22
*Overall dry reduction rates/ kg m^{-3} d^{-1}	12	0.36	0.14

*see equation 3.11 (section 3.4.7.3)

4.6 Factors affecting waste reduction and bioconversion of UDDT sludge

The relative waste reduction for all containers was an average of 28 ± 5% (ww), 12 ± 6% (TS basis) and 35 ± 5% (VS basis) as shown in Table 4.4. The bioconversion in this study was low 6% (ww) and 3% (dry basis) in comparison with other studies, which recorded a bioconversion of 19% on a dry basis (Dortmans, 2015). However, Amatya (2009) found a similar dry bioconversion of 4% using cow manure as the substrate.

The waste consumption rate is influenced by the moisture content of the feed and environmental conditions. The ideal moisture of the substrate for BSF larvae bioconversion is 65-85% (Banks, 2014, Cheng et al., 2017). The temperature should be maintained between 29-31°C (Barry, 2004, Tomberlin et al., 2002). Relative Humidity should fall between 50 and 70% (Barry, 2004).

The moisture content (60%) in this study was lower compared to the recommended moisture content. As shown in Figure 4.1 these conditions varied throughout the study and could have resulted in the low rate of waste reduction. In addition, the low relative waste reduction could have been influenced by the low nutritional value of the UDDT sludge and difficulty in digesting UDDT sludge.

4.6.1 Type of substrate

The BSF larvae can decompose different types of organic wastes (Diener et al., 2011c). Relative waste reduction was low compared to what has been reported previously of

more than 70% reduction (dry basis) (Dortmans, 2015). However, Amatya (2009) reported a similar relative waste reduction of 22% using cow manure. The quality and quantity of substrate affect the development of the BSF larvae. Gobbi et al. (2013) found out BSF larvae fed on hen meal from vegetable origin resulted in higher dry waste reduction (70%) and shorter development time (15 days) than those fed on meat meal from animal origin, which had 30 days. In addition, the nutritional content of the substrate influences the mortality of the BSF larvae, with meat meal having a higher mortality (60%) than hen meal (5%) (Gobbi et al., 2013). UDDT sludge has a low nutritional value of 0.33 g VS/g of dry sample which shows that there is limited accessible substrate for the BSF larvae. Unbalanced diets were found to diminish food intake, leading to slow larval growth (Gligorescu et al., 2018). The combined sludge from all the layers in a pit latrine reported a wet reduction of approximately 50% (Banks, 2014).

In this study, UDDT sludge was sourced from both standing and active vaults. The age of the UDDT sludge ranged from 1 to 2 years or more, depending on when the toilet was installed. A waste reduction of less than 50% was achieved This could be due to the sand added to reduce odour and dehydrate the FS, which then becomes part of the constituent of UDDT sludge and reduces the nutritional value (Austin, 2006). Methods of increasing the waste reduction of UDDT sludge should be explored, such as the use of additives which offer the same purpose as the sand, but with a nutritional benefit. Buckley et al. (2008b) showed the added sand (inorganic) was the primary component in the UDDT sludge, which is detrimental to BSF larvae growth. This therefore indicates the need to remove the sand from the UDDT sludge before treatment by BSF larvae, or to use an organic component to dehydrate the faecal matter. Furthermore, Buckley et al. (2008b) mentioned that there was no clear mechanisms as to how the added sand assisted in the dehydration of the faecal matter.

CNS elemental analysis was carried out on the UDDT sludge for characterisation and determination of the nutrient value. The per cent nitrogen was used to estimate the protein content using the Jones factor of 6.25 Jones (1931) and the protein in the sludge was found to be 9.69 ± 0.85%. This is comparable to the protein content of pit latrine sludge of 9%, but it was not specified from which layer it was estimated (Banks, 2014).

In this study BSF larvae bioconversion was 6% (g live BSF larvae /g wet mass added) and 3% (g TS in BSF larvae/g TS in the feed). The carbon content of UDDT sludge is 14.98 ± 0.92%. However, not all carbon in the diet can be utilized by the BSF larvae.

Lalander et al. (2013) and Banks et al. (2014) found the waste reduction on a dry basis to be 73% and 55% respectively using fresh faeces as a substrate, which could be due to readily available carbon in the faeces. There was a dry material reduction of 66.4 to 78.9% in other feed substrates, with the highest waste reduction observed at higher feeding rates in food waste (Diener et al., 2011a).

Similar to micro-organisms, the substrate's physical nature affects the substrate's mass transfer to the BSF larvae. If the substrate is too dry, the system will encounter problems due to the viscosity and osmotic pressure. The texture of the substrate influences how the BSF larvae consume it. There is a need for some structure to the substrate to allow the BSF larvae to consume the feed (Barry, 2004). UDDT sludge is sticky, which could have contributed to low bioconversion and waste reduction.

4.6.2 Bioconversion time

The duration of the experimental trials could be a contributing factor to low bioconversion and relative waste reduction. The BSF larvae development times vary according to the availability of nutrients from 2 weeks and two months. The BSF larvae must acquire a minimum weight to be able to sustain their lifecycle. In this study, the development time was 21days, 13days of which was spent feeding on a low nutritional substrate. Diener (2010) found that the larvae which had limited feed took longer to reach prepupae than those which had abundant food, although the size was similar.

4.6.3 Environmental factors

Temperature changes could have negatively impacted the BSF larvae growth as they have a lower temperature threshold of between 16°C and 19°C as determined when reared on the Gainesville diet at 70% diet (n=24) (Holmes et al., 2016). Low temperature tends to cause the BSF larvae to slow down their metabolism, eat less and develop slower (Dortmans et al., 2017).

According to Dortmans et al. (2017), the ideal temperature for the BSF larvae technology is between 28 °C and 32 °C. The maximum developmental temperature

threshold for BSF larvae when reared on a grain diet is between 30 and 36 °C, with optimal rearing at 27 °C relative to development time and mortality rates (Tomberlin et al., 2009). Hot temperatures tend to force the BSF larva to crawl away from food, looking for cooler areas, (Dortmans et al., 2017).

The recorded changes in temperature (14 ºC to 35 °C) during these trials may have impacted on the development of the BSF larvae as these were outside of the optimal range. Gligorescu (2016) pointed out larval metabolism increased with temperature, but also showed that metabolic rate was not influenced by the feed given, but that there was an interaction between temperature and feed given.

Fluctuation in the temperatures resulted in a change in humidity as shown in *Figure 4.1*. Holmes et al. (2012) determined the effect of relative humidity on egg eclosion and adult emergences. The study highlighted that as humidity reduced, it increased mortality and slowed development. This could be due to dehydration.

Based on this assessment, it can be concluded that the environmental conditions in the grow-out area were not favourable for larval development.

4.6.4 Age of larvae used

It is difficult to assess the impact of the age of BSF larvae on waste reduction as the age of the larvae used in this study differed to other studies. For example Banks (2014) used 18 day old BSF larvae, which were kept in an inert material that prevented them from gaining weight until they were fed on fresh faeces and pit latrine sludge. This study used 6-day old larvae fed on formulated food waste for development before being transferred to the UDDT sludge.

4.6.5 Feeding rates and feeding depth

The feeding rate of the BSF larvae influences the amount of UDDT sludge which can be reduced. The feeding depth of the substrate will affect the maximum volume of sludge that can be treated at the plant. In this study, the feeding rate was 70 mg larvae^{-1}day^{-1} at a depth of 10 cm, which resulted in a dry bioconversion of 3% and a relative waste reduction of 28%. These dry bioconversion results are similar to those in a study by Banks (2014), who found a dry bioconversion of 3.5% when using a feeding rate of 65 mg larvae^{-1} day^{-1} on pit latrine sludge. Furthermore, their research showed that

bioconversion increased with an increased feeding rate (Banks, 2014). This implies a competition of feed at low bioconversion, resulting in low accumulation of mass in the BSF larvae.

The low waste reduction (28%) found in this study could be due to the moisture content of UDDT sludge (60%) and the texture of the sludge which could have restricted the larval movement. This is consistent with other research that showed that a low moisture content and feeding rate resulted in low dry bioconversion and relative waste reduction (Banks, 2014).

In this study, the feeding rate was 70 mg larvae^{-1} day^{-1} and resulted in a bioconversion of 6%. Banks et al. (2014b) recommended a feeding rate of 100 mg larvae^{-1} day^{-1} which resulted in a bioconversion of 22.9% using fresh faeces. This implies that a higher feeding rate results in a higher bioconversion, however this rate could also be affected by the type of FS.

Although increasing the feeding rate results in a higher bioconversion, it can result in a lower waste reduction as the some of the substrate might not be consumed. This is supported by Diener et al. (2009) and Manurung et al. (2016) who found that increasing the feeding rates lowered the waste reduction capabilities of the BSF larvae.

The feeding depth of the substrate will determine the amount of UDDT sludge that can be treated at a given time. The plant was designed to treat 20 tonnes of UDDT sludge per day at a depth of 10 cm. However, the results showed that the depth of 10 cm could not have been accessible to the BSF larvae as the waste reduction and bioconversion were low. A study by Brits (2017) showed that the BSF larvae had difficulties accessing feed at depths higher than 10 cm and that the BSF larval mass decreased with an increase in feed depth. This could have contributed to the low larval mass found. Furthermore, the BSF larvae seemed to find UDDT sludge at 10 cm challenging to access, and this could be due to the stickiness of the UDDT sludge.

Upscaling production at the current 10 cm depth carries many disadvantages, and there is a need to understand UDDT sludge feed accessibilities at different depth.

4.6.6 Effect of moisture content

The moisture content of the substrate allows the BSF larvae to assimilate the required nutrients for their growth. The moisture content of 60% of the UDDT could have hindered the bioconversion and relative waste reduction capabilities of the BSF larvae as it could have dried up over time hindering the BSF larvae consumption. Banks (2014) found that bioconversion was low at a moisture content of 65%, and that increasing the moisture content of FS increased bioconversion; however, the waste reduction was negatively impacted. Although the BSF larvae can treat UDDT sludge at varying moisture contents, there is a need to find a moisture content that results in a balance between bioconversion and waste reduction.

4.7 Nutritional composition of BSF larvae reared on UDDT sludge

A comparison of the characteristics of the initial larvae and mature larvae was undertaken to highlight the effect of UDDT sludge on the composition of the mature BSF larvae. The results are provided in Figure 4.4 and Table 4.5. There was an increase in mass from an average of 0.13 kg to an average of 1.47 kg per container. Total solids in BSF larvae decreased from 0.33 ± 0.05 g of TS/g of wet sample to 0.20 ± 0.050g of TS/g of the wet sample. Also, there was a decrease in volatile solids content from 0.85 ± 0.05g of VS/g of TS to 0.75 ± 0.01g of VS /g of TS. However, ash content accumulation in the mature BSF larvae from 0.15 ± 0.05g of ash/g of TS to 0.25g of ash /g of TS.

Table 4.5: Comparison of the physicochemical parameters of the BSF larvae before and after the bioconversion of UDDT sludge.

		Initial BSF larvae	**Mature BSF larvae**
Total Solids	g TS/g of wet sample	0.33 ± 0.05	0.20 ± 0.01
Volatile solids	g VS/g of TS	0.85 ± 0.05	0.75 ± 0.01
Ash	g ash/g of TS	0.15 ± 0.05	0.25 ± 0.01

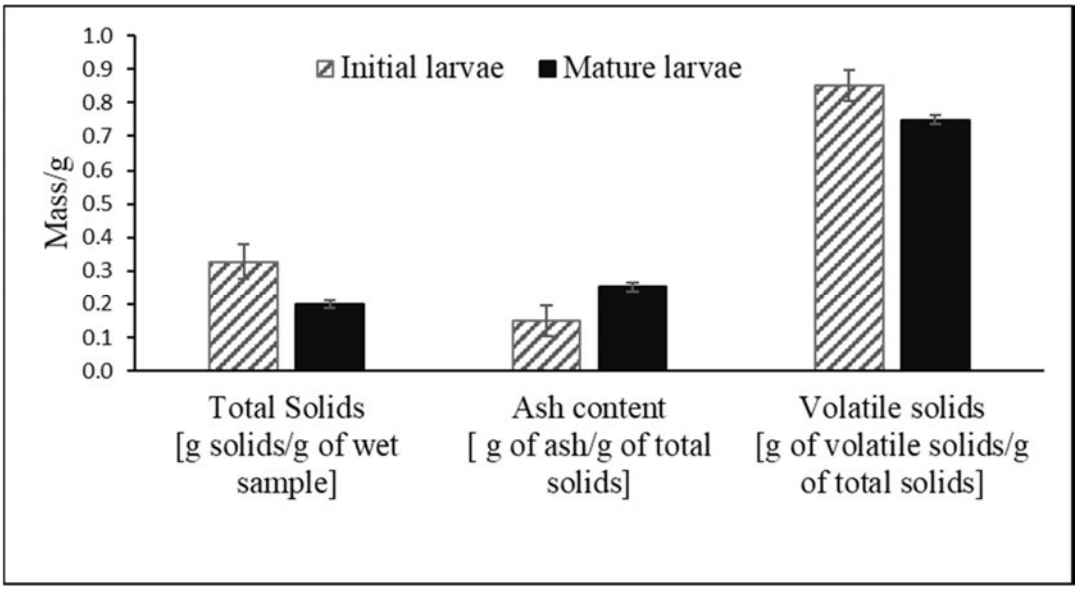

Figure 4.4: Comparison of the physicochemical parameters of the BSF larvae before and after the bioconversion of UDDT sludge

4.8 Larval growth when reared on UDDT sludge

The growth performance of the BSF larvae is crucial for improving its production. The growth of BSF larvae is strongly influenced by the substrate used and its characteristics (Lalander et al., 2019b).

Samples of 30 BSF larvae were collected after 3, 6, 9, 11 and 13 rearing days to measure growth. Generally, there was an increase in the wet mass per larvae from an initial 5.95 mg to 85.54 mg on day 13. There was rapid growth from day zero to day four which was 5.95 mg to 44.93 mg (wet basis). The larvae's growth gradually increased at a slower rate from day 4 to day 13, as shown in Figure 4.5 (raw data presented in Appendix 3: Table A.2). The development of BSF larvae exhibited a logarithmic growth curve. Dortmans (2015) found the average mass of BSF larvae is 224 mg which was higher than what was found in this study (86 mg), and this could be due to different substrates used in the studies (UDDT sludge and a mixture of food waste and fresh human faeces respectively). It therefore appears that the nutritional value of the substrate directly influences the BSF larval mass.

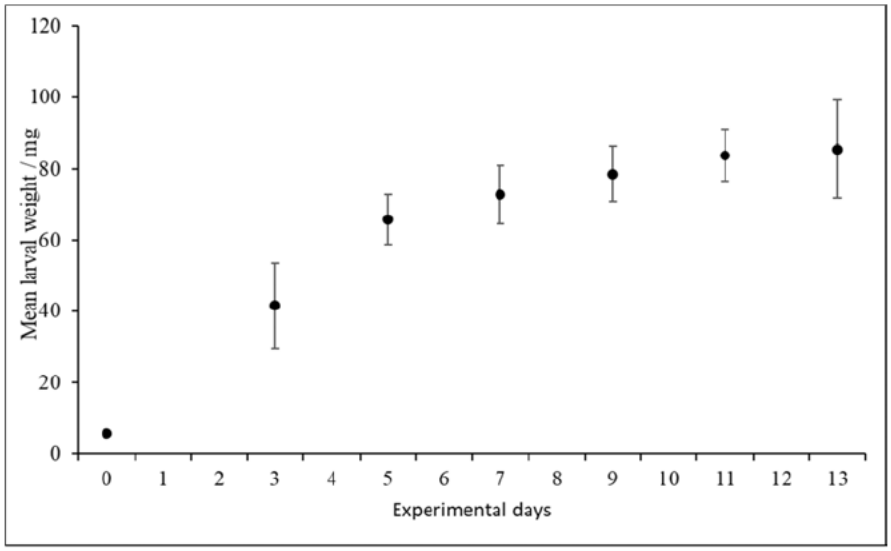

Figure 4.5: The growth of BSF in a period of 13 days.

As shown in Figure 4.5, the growth rate of the BSF larvae was calculated to be 6.12 mg day^{1}. The specific growth rate increased with increasing larval age and body mass until day three, then it abruptly decreased until day seven as shown in Figure 4.6. From day seven, it changes slightly until day 13 when it is almost zero, showing there was not any further significant increase in mass.

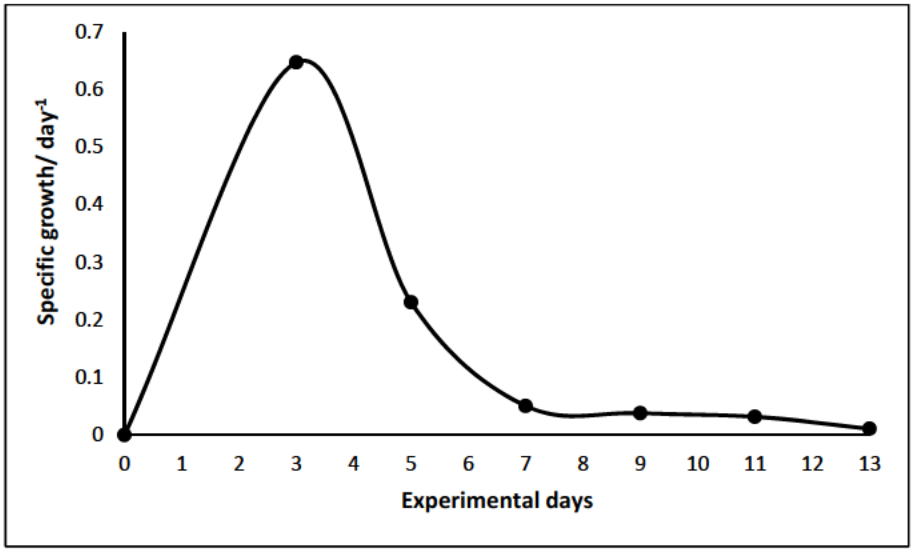

Figure 4.6: Specific growth rate of BSF larvae fed on UDDT sludge (see equation 3.2 in section 3.3.4)

4.9 Factors affecting the growth and composition of the BSF larvae

Research has shown that the growth of the BSF larvae is affected by substrate type, feeding rate used and moisture content of the substrate(Lalander et al., 2019b). Furthermore, the substrate characteristics are a critical parameter in larval growth, including nutritional and physical appearance(Nguyen et al., 2015).

The weight of BSF larvae found in this study is similar to the mass of BSF larvae (89 mg) fed on chicken feed at 12.5 mg larvae^{-1} day^{-1} (Diener, 2010). Furthermore, Diener et al. (2011b) found the prepupal dry mass of BSF larvae fed on pure FS was 18 mg/larvae, which was similar to the 17 mg/larvae found in this study.

There was an interesting pattern in the larval growth with time. There was an exponential growth at the beginning of the trial with a drop off in growth as the experiment progressed. A similar trend was observed by other studies (Caruso et al., 2014, Cheng et al., 2017) using different substrates. This phenomenon can be explained by the natural lifecycle of the BSF larvae, and it is therefore important to harvest the larvae at the optimum growth stage in this lifecycle.

Several factors could have influenced the low larval mass when UDDT sludge was used as a substrate which are discussed in the following sections.

4.9.1 Low quality of the UDDT sludge

The low larval mass of the BSF larvae could be attributed to the low nutritional value of 0.33 ± 0.01 gVS/gTS of the UDDT sludge. Shortage of food availability results in the BSF larvae feeding until they have obtained the minimum energy reserve required to go to the next stage (Diener, 2010). Based on Table 4.1 UDDT sludge used in this study is a low protein diet. Barry (2004) found that low protein/carbohydrate diets tend to produce smaller BSF larva and prepupae and have longer development time. This study supported that of Barry (2004) as the BSF larva had to consume more UDDT sludge to meet the minimum development requirements. However, the UDDT sludge seemed to be difficult to access at lower depths. In comparison, studies that used FS from pit latrines as a substrate resulted in a dry prepupal weight of 45mg/larvae Banks (2014) which is higher than what was found in this study of 17mg/larvae. Diener et al. (2011c) found prepupal dry weight of 18mg/larvae using septic tanks sludge which is

similar to this study, and the similarities can be attributed to both sludges being more degraded and containing less nutrients for larval growth. This is supported by a study by Lalander et al. (2019b) which highlighted that the fresh faeces as a substrate had a higher larval mass than digested sludge; the mass of the prepupal mass (70mg/larvae) of anaerobically digested sludge was similar to this study (89mg/larvae).

The diet recommended by Barry (2004) is a high protein, high fat diet as it resulted in rapid development and larger BSF larvae or prepupae. However, the drawbacks of this diet are low waste reductions. There is a possibility of enhancing the nutritional value of the UDDT sludge by mixing it with other nutritious waste streams to reach a balance between rapid development and high waste reduction.

4.9.2 Moisture content of the substrate

The moisture content of 60% could have inhibited the growth as Banks (2014) found that at a lower moisture content (65%), the development of larvae was lower than at higher moisture content (85%) using pit latrine sludge. This is supported by Cheng et al. (2017) who reported faster larval growth at a higher moisture content (80%) compared to a lower moisture content (70%). However, they concluded that there was no significant difference in larval mass with different moisture contents. Furthermore Cheng et al. (2017), found that at the higher moisture content (80%), the BSF larvae quickly reached their maximum larval weight with post-consumer food waste and pre-consumer food waste substrates. This shows that higher moisture content is desirable as it results in a shorter growth time. However, there should be a balance between shorter growth time and ease of sieving of the residue to remove the BSF larvae, as Cheng et al. (2017) showed it was difficult to sieve out the BSF larvae from the residue at higher moisture content. Hence there is a need to find a moisture content which promotes both larval growth and ease of sieving.

4.9.3 Feeding rate of the larvae and larvae per unit area

Research has shown that increasing feeding rates increases larvae per unit area (Diener, 2010, Banks, 2014). However, this might also lead to an increase in unprocessed UDDT sludge. Interestingly Diener (2010) found that at lower feeding rates, the BSF larvae could attain a similar larval weight with BSF larvae fed at a higher feeding rate. However, they needed a more extended development time. This suggests that

increasing the bioconversion time for the BSF larvae to process the UDDT sludge could have resulted in an increase in larval weight. The growth rate of BSFL on UDDT sludge is similar to the growth rate of BSFL to when pit sludge was used as a substrate (Banks, 2014). The difference in these studies could be attributed to the sludge used, the age of the BSF larvae harvested, and the moisture content of the substrate.

Larval per unit area (number of larvae per unit area of substrate) can influence the growth of the BSF larvae; studies suggest between 8-10 larvae/cm^2 without affecting their survival rate (Caruso et al., 2014). The larvae per unit area used in this study, based on 70 $mglarvae^{-1}day^{-1}$, is approximately 4 larvae/cm^2 and could be lower. This is due to neonates being packaged from Cape town based on the intended amount of substrate and shipped by road to Durban as there is a possibility of mortality during transit.

4.9.4 Feeding depth of the substrate

It was found that the 10 cm depth of the substrate used in this study affected the larval mass. Brits (2017) found that the larval weight decreased with an increase in feed depth of 5 cm and more. The low larval weight could be due to limited feed accessibility due to low temperatures, anaerobic conditions, and stickiness of the substrate at increased depth.

4.9.5 Environmental conditions

Gligorescu (2016) and Harnden and Tomberlin (2016), found that larval growth was influenced by diet and temperature. Their findings showed that larval growth was best at above 27 °C when BSF larvae where reared on a balanced diet, while the smallest growth was found at below 25 °C using the same diet. In this study, the temperature varied (Figure 4.1) which impacted the BSF larval growth. Gligorescu (2016), (Dortmans et al., 2017) found slower development time when environmental conditions and quality of feed are unfavourable. In addition, an increase in larval development time could be due to the time needed to assimilate the nutrients necessary to achieve the minimum weight for pupation (Gobbi et al., 2013).

The BSF larvae in this study had lower ash content (25%) in comparison with BSF prepupae reared on pit latrine sludge (41%) (Banks, 2014). This difference could be

due to the age of the BSF larvae. In this study, the BSF larvae were analysed after 13 days while Banks (2014) did not state the number of days found that the quality of the substrate given to the BSF larvae impact the characteristics of the BSF larvae.

The moisture content of the feed (60%) was lower than the desired range (70-80%) for BSF larvae as found by Dortmans et al., (2017). In addition, Banks (2014) supports this finding as his study demonstrated that using FS from pit latrines using a moisture content of 65% resulted in prepupal dry weight of a mean of 45 mg. This prepupal weight is far lower than what was found in this study of 86mg, and the difference could be due the age of the FS and the feeding regiment. In the study by Banks (2014), the FS was added every seven days until 50% of the larvae were prepupae and the age of the FS was unknown. In this study, the UDDT sludge used was greater than two years old and the nutrients available could have degraded over time. The UDDT sludge was also only added once at the beginning of the 13-day trial.

Banks (2014) highlighted that a low FS moisture content of less than 65%, resulted in low larval mass, leading to low bioconversion. A 1:1 combination of FS and municipal waste resulted in an improved larval mass compared to 100% FS (Diener et al., 2011c).

4.9.6 Heavy metal analysis of harvested BSF larvae and residual substrate

Heavy metals are not biodegradable. Determining the concentration of heavy metals in BSF larvae reared on UDDT sludge is essential when considering the use of BSF larvae as a source of animal protein. The presence of heavy metals in either the BSF larvae or residue might accumulate via the food chain when used as an animal feed or as a soil amendment for growing commercial vegetables respectively.

The results from this study showed the presence of heavy metals in the UDDT sludge. The concentration of heavy metals was predicted to reduce in the UDDT sludge during processing due to BSF larvae uptake.

Table 4.6 shows the analytical results of the harvested larvae and the corresponding residual substrate after rearing on UDDT sludge (Raw data presented in Appendix 3: Table A3, Table A4 and Table A5).

Table 4.6: Heavy metal analysis of the initial substrate, BSF larvae and residual substrate on a dry basis.

Heavy metals	*Initial substrate [mg/kg]	Residual substrate [mg/kg]	*Initial BSF larvae [mg/kg]	Larvae [mg/kg]
Lead	65.6	72 ± 10	77.04	76 ± 8
Cadmium	0.7	0.4 ± 0.1	0.3	0.4± 0.3
Arsenic	0.0	0.3± 0.5	BDL	1 ± 1
Chromium	36	25 ± 4	26	15± 2
Copper	74.2	69± 14	40.27	48 ± 9
Nickel	23.5	21 ± 5	7.20	22± 10
Zinc	170.6	225 ± 61	40.27	244 ± 39

*The initial substrate and BSF larvae were externally analysed and only one result was provided for each determinant.

Figure 4.7 shows that, within the 90% confidence level of the measured errors, the BSF larvae seem to adapt the same concentration as the substrate. All elements seem insignificantly different from the initial substrate and residue except for zinc which increases in the BSF larvae after feeding on the substrate. However, there is a discrepancy with chromium which decreased in the both the residue and BSF larvae, this could be due to the initial substrate being analysed in a different laboratory and only one result available. There is need to do more analysis on the fate of heavy metal during larval feeding as the standard deviation are quite high to reach a conclusive decision.

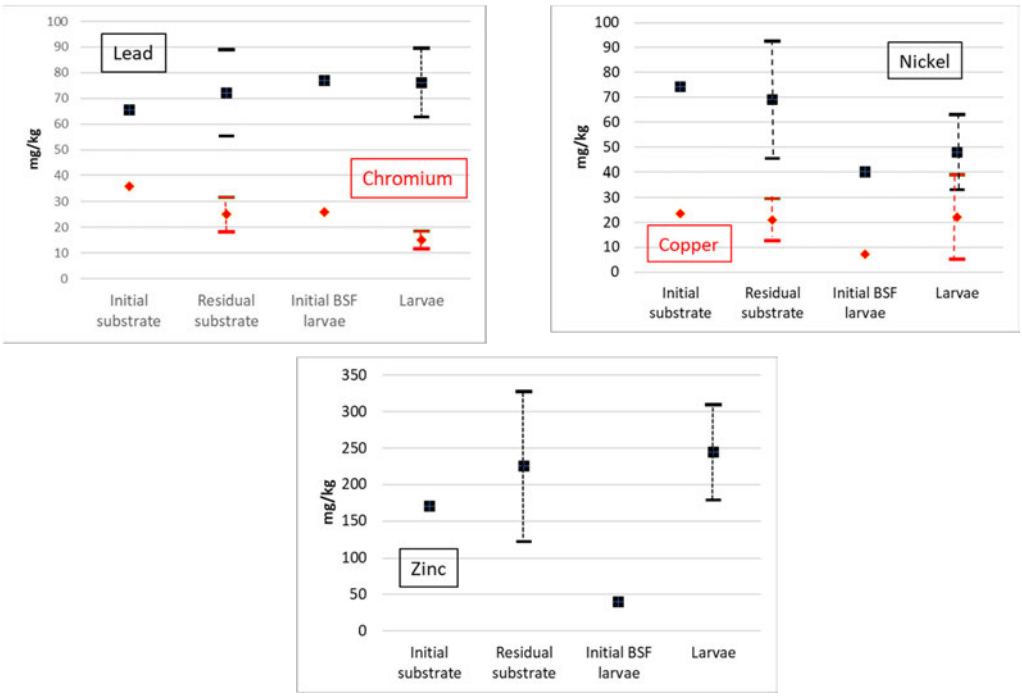

Figure 4.7: The mean concentration of heavy metals (lead, chromium, copper and zinc) on the substrate and BSF larvae during feeding (error bars = 90% confidence intervals based on three samples).

The bioaccumulation factor (BAF, i.e. mean concentration in the organism divided by the mean concentration in the substrate) was calculated to give a BAF of for cadmium of 0.56 ± 0.43 and 1.15 ± 0.11 for lead (Walker, 1990).

As shown in Table 4.5 heavy metals were detected in the collected UDDT sludge. Since heavy metals are inorganic materials, their concentration could be pronounced in degraded UDDT sludge. Furthermore, there is a possibility that the presence of heavy metals is due to the trash, which was found in the UDDT sludge, which included metal objects. The heavy metal contamination can be removed at the source through modifying user behaviour to avoid contamination (Bassan et al., 2013, Banks, 2014).

The heavy metal analysis results from this study were compared to those from a study by Banks (2014) who characterised heavy metals in pit latrine sludge from rural and semi-rural areas of the Western and Eastern provinces of Cape Town, South Africa. Arsenic concentrations in the BSF larvae were similar in both studies, which was

0.7mg^{-1} kg^{-1} in Banks (2014) and 0.95 mg $^{-1}$g in this study. However, a higher concentration of lead (66mg/kg) in BSF larvae reared on UDDT sludge was found compared with BSF larvae reared on pit latrine sludge, with a mean of 7.4 mg/kg. This could be due to the type of trash deposited in UDDT sludge. Overall, the UDDT sludge had lower concentrations of heavy metals than sludge from pit latrines.

A guideline was developed by the South African Water Research Commission (WRC) to assist in categorising sludge intended for agricultural use. The use of UDDT sludge residue has potential to be used as a soil conditioner, however the presence of toxic compounds such as heavy metals and pathogens can restrict this potential reuse.

The UDDT residue results were compared to the limits suggested for use of wastewater sludge in agriculture in Table 4.7. This comparison suggests that the sludge residue should be classified as pollutant "a", and could be used for agricultural use without concerns for heavy metal pollution (Snyman, 2010). However, due to the limitations of this study, there is a need to verify this result by doing an extensive study of UDDT sludge characteristics.

Table 4.7: Comparison of pollutant limits for the agricultural use of wastewater sludge in South Africa (Snyman, 2010) compared to UDDT sludge residue.

Heavy metals	**Pollutant class**			
	a **[mg/kg]**	**b** **[mg/kg]**	**c** **[mg/kg]**	**UDDT sludge residue** **[mg/kg]**
Lead	< 300	300 – 840	> 840	72.15 ± 10.34
Cadmium	< 40	40 – 85	> 85	0.35 ± 0.13
Arsenic	< 40	40 – 75	> 75	0.29 ± 0.54
Chromium	< 1200	1200 – 3000	> 3000	25.29 ± 4.23
Copper	< 1500	1500 – 4300	> 4300	69.47 ± 13.83
Nickel	< 420	420	> 420	20.70 ± 5.25
Zinc	< 2800	2800 – 7500	> 7500	224.69 ± 61.20

Determining the concentration of heavy metals in BSF larvae fed on FS is essential when considering the BSF larvae as a protein source. Table 4.8 provides the regulated concentrations of heavy metals as per the South African regulations compared to those found in the BSF larvae fed on UDDT sludge for use as animal feed.

Table 4.8: Regulated concentration of heavy metals in BSF larvae fed on UDDT sludge on a dry basis.

Heavy metals	**UDDT BSF Larvae/mg kg^{-1} ***	**Maximum content/mg kg^{-1} #**
Lead	75.63 ± 7.53	10
Cadmium	0.36 ± 0.28	2
Arsenic	0.95 ± 1.47	2
Chromium	14.96 ± 2.11	-
Copper	47.63 ± 8.31	25
Nickel	21.83 ± 10.08	50
Zinc	243.97 ± 38.67	90

*this study, #Adapted from Agriculture (2006)

According to Table 4.8 the concentration of lead and zinc in BSF larvae fed on UDDT sludge are too high for animal feed and might inhibit the potential of the larvae to be used as animal feed. Nevertheless, there is a possibility of avoiding contamination of the UDDT sludge at source by educating people as to the correct disposal of waste, or by having effective waste collection programmes in the communities to avoid the disposal of waste in the UDDT vaults.

5 PHASE 2 RESULTS AND DISCUSSION

The previous chapter discussed the feasibility of using UDDT sludge as a substrate for BSF larvae. The results highlighted that UDDT sludge alone was a poor substrate. In this chapter, the results obtained will highlight how using PS as an additive to UDDT sludge can improve the performance of the BSF larvae. In addition, in Phase 2, the moisture content of the substrate was increased, as from the discussions in the previous chapter, the low moisture content of the UDDT sludge could have contributed to the low bioconversion and waste reduction rates. The feeding depth was also lowered to determine the impact on performance. To evaluate the ability of the BSF larvae to process UDDT sludge mixed with PS, mass balances were performed over monitored batch trials. In addition, the rearing feed and trash was not removed.

As in Phase 1, mass balances were performed on a wet and dry basis for volatile solids and ash content. To analyse the nutrient concentration of the substrate and the residue, an evaluation based on total solids, volatile solids, COD, and pH was undertaken. Humidity and temperature were monitored as they influence the growth of BSF larvae.

5.1 Environmental conditions

As expected from Phase 1 the field experiments showed that the temperature and humidity in the grow-out area varied during the duration of the experiment. The temperatures ranged between 19.2 °C and 40.5 °C. Humidity ranged from 43.77% to 97.77%, as shown in Figure 5.1 (raw data is presented in Appendix 3:Table A.6). As the temperature increased, the humidity decreased.

During the trials, a heavy storm was experienced in Durban, resulting in leaking gutters in the grow-out area and the flooding of two trials. However, care was taken not to sample from the flooded parts.

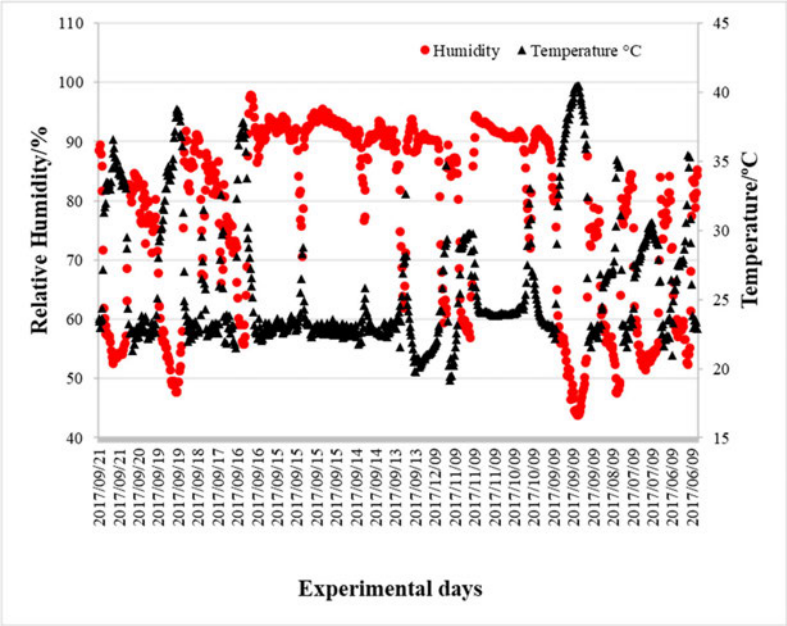

Figure 5.1: Variation of temperature and humidity in the grow-out area during the experimental period for Phase 2.

5.2 Physical and chemical characteristics of the feed and remaining food waste

The physical and chemical characteristics of the UDDT, PS and composite (UDDT mixed with PS) substrates are provided in A comparison was done to evaluate the effect of adding PS to UDDT sludge. The results showed that the moisture content and volatile solids of UDDT sludge were lower than that of PS, but comparable to that in the composite for all three trials. Furthermore, it was observed that the composite sludge had a slightly higher moisture content and volatile solids than UDDT sludge due to the addition of PS. The increase in volatile solids in composite sludge correlated with the increase in the COD.

PS had a higher content of volatile solids compared to UDDT sludge. Furthermore, the low solids content of the PS resulted in a slight increase of volatile solids of composite sludge. The pH of the composite sludge was unaffected by addition of PS to the UDDT sludge.

The characteristic of UDDT sludge in phase 2 (n=3) was similar to what was found during Phase 1 (n=12). The UDDT sludge was found to have high ash and low volatile solids. The similarity in both phases could be expected since the UDDT sludge was collected from the same area. As mentioned in Section in 4.6.1, the sand added to the UDDTs has an influence on the characteristics of the sludge as it contributes to the high ash content. According to

Rose et al. (2015) fresh faeces have an ash content of 0.11g ash/g wet sample. Strande et al. (2014) and Rose et al. (2015) showed that the characteristics of FS and faeces respectively are influenced by environmental conditions, location and user habits; this explains the minor difference between UDDT sludge used in both phases.

The results found in this study was within the range found by Zuma (2015), who observed similar characteristics. However, the reported values in Zuma (2015) were based on both active and standing vaults. These comparisons are shown in Table 5.1.

Table 5.1: A comparison of the characteristics of the UDDT sludge

Characteristic	**Units**	**Phase 1 Range**	**Phase 2 Range**	**Zuma (2015)**
Moisture content	g of water/ g of wet mass	0.59 - 0.60	0.62 - 0.66	0.04 - 0.85
Volatile Solids	g of VS/g of TS	0.32 - 0.33	0.34 - 0.38	9.4×10^{-3} - 0.85
Ash	g of ash/ g of TS	0.67 - 0.68	0.62 - 0.68	
COD	mg COD/g dry sample	-	0.30 - 0.38	8.6×10^{-3} - 1.21
pH		-	7.7 - 7.9	5.3 - 9.1

Zuma (2015) found that UDDTs that were not effectively separating urine at source had FS with a pH as high as 9.1. The pH reported in this study might indicate that UDDT sludge used in this study were collected from UDDTs that were not being used properly and urine was entering the vault. The physicochemical properties of the different types of sludge are listed in Table 5.2.

Table 5.2: Summary of the physiochemical characteristics of the UDDT sludge, PS, and composite sludge in trials 1, 2 and 3 (all measurements done in triplicates.

Analysis	**Units**	**UDDT sludge**			**PS**			**Composite Sludge**		
		Trial 1	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
		Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Total Solids	[g TS/ g wet sample]	0.38±0.01	0.37±0.01	0.33±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.35±0.00	0.32±0.00	0.30±0.01
Moisture Content	[g water / g wet sample]	0.62±0.01	0.63±0.01	0.66±0.01	0.99±0.01	0.99±0.01	0.99±0.01	0.65±0.00	0.68±0.00	0.70±0.01
Volatile Solids	[g VS/g TS]	0.35±0.01	0.34±0.00	0.38±0.01	0.75±0.07	0.76±0.02	0.75±0.02	0.36±0.00	0.33±0.00	0.40±0.02
Ash Content	[g ash/ g TS]	0.65±0.01	0.66±0.00	0.62±0.01	0.25±0.07	0.24±0.03	0.25±0.02	0.64±0.00	0.67±0.00	0.60±0.02
COD	[mg COD/g dry sample]	0.3846	0.3225	0.3045	3.6411	0.9304	0.0325	0.4153	0.2854	0.4561
pH		7.9	7.9	7.7	5.6	6.2	5.3	7.9	7.4	7.7

The remaining food waste from the nursery was characterised to determine if all organic content had been consumed in the nursery. The results are provided in Table 5.3 and showed there were still volatile solids (0.91g VS/g of TS) present in the remaining food waste. This showed that the nursery diet was more than enough for the allocated number of days.

Table 5.3: Summary of the physiochemical characteristics of the remaining food waste substrate in trials 1, 2 and 3 (all measurements done in triplicates)

	Trial	**Total Solids**	**Moisture Content**	**Volatile Solids**	**Ash Content**
		g dry / g wet sample]	**[g water / g wet sample]**	**[g VS/g TS]**	**[g VS/ g TS]**
		Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Food waste	Trial 1	0.56 ± 0.01	0.44 ± 0.01	0.90 ± 0.00	0.10 ± 0.00
	Trial 2	0.49 ± 0.01	0.51 ± 0.01	0.93 ± 0.01	0.07 ± 0.01
	Trial 3	0.51 ± 0.01	0.49 ± 0.01	0.90 ± 0.00	0.10 ± 0.00

n=3

5.3 Change in physical and chemical characteristics of the substrate during the bioconversion process.

All trials showed a decrease in substrate depth from an average of 5.1 cm to 3.4 cm after processing Table 5.4 shows the change in characteristics of the substrate before and after processing with the BSF larvae in the trials. The moisture content in all the trials reduced from an average of 0.66 g water /g wet sample to a 0.57 g water/g wet sample. Due to the reduction in the sludge volume during feeding by the BSF larvae and a reduction in moisture content, there is an increase in the solids, as shown in Table 5.4.

Table 5.4: Change in characteristics of the substrate during processing

Analysis		**Initial Substrate**			**Final Substrate**		
	Units	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
		Mean ±SD	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**	**Mean ± SD**	**Mean ± SD**
Moisture content	g water/g wet sample	0.65 ± 0.00	0.68 ± 0.00	0.70 ± 0.01	0.55 ± 0.03	0.60 ± 0.03	0.63 ± 0.00
Total Solids	g TS/g wet sample	0.35 ± 0.00	0.32 ± 0.00	0.30 ± 0.01	0.45 ± 0.03	0.40 ± 0.01	0.37 ± 0.00
Volatile Solids	G VS/g TS	0.36 ± 0.00	0.33 ± 0.00	0.40 ± 0.02	0.35 ± 0.03	0.33 ± 0.02	0.40 ± 0.02
Ash Content	g ash /g TS	0.64 ± 0.00	0.67 ± 0.00	0.60 ± 0.02	0.65 ± 0.03	0.67 ± 0.02	0.60 ± 0.02
COD	mg COD/g dry sample	0.42	0.29	0.46	0.37	0.36	0.41
pH	-	7.9	7.4	7.7		7.1	6.7
Feed depth	cm	4.7	5.3	5.2	3.3	3.5	3.5

5.4 Change in characteristics of the BSF larvae after processing the composite sludge

The characteristics of the BSF larvae were also analysed to note any changes during the processing of composite sludge. These results are shown in Table 5.5. There is an increase in the moisture content of the BSF larvae in all trials. This increase in the moisture content could be due to the water from the heavy rainfall and leaking gutters entering the trials. There is a general trend of a decrease in volatile solids (g of VS/ g of TS) and an increase in ash content (g of ash/g of TS). This shows that while feeding, the BSF larvae consume both the organic and inorganic matter in the sludge. This is supported by the results found in Phase 1 where there was a decrease in volatile solids during feeding and an increase in ash content. The potential to use BSF larvae for animal feeding might be reduced as it would contain inorganic content.

Table 5.5: Change in characteristics of BSF larvae during the processing of the composite sludge

Analysis		**BSF LARVAE (Day 1)**			**BSF LARVAE (Day 13)**		
	Units	**Trial 1 [mean]**	**Trial 2 [mean]**	**Trial 3 [mean]**	**Trial 1 [mean]**	**Trial 2 [mean]**	**Trial 3 [mean]**
Moisture content	g water /g wet sample	0.66	0.68	0.70	0.75	0.72	0.76
Total Solids	g TS/g wet sample	0.33	0.31	0.30	0.25	0.28	0.24
Volatile Solids	g VS/g dry sample	0.91	0.92	0.91	0.75	0.79	0.81
Ash Content	g ash/g dry sample	0.09	0.08	0.09	0.24	0.20	0.18

5.5 Characteristics of the BSF larvae after drying

The BSF larvae from one of the trials was able to undergo drying in the rotary drier, and the change in characteristics of the BSF larvae was noted. The rotary drier had an inlet temperature of 150 °C and an outlet temperature of 100 °C. The summary of the change in characteristics is shown in Table 5.6. The drying process reduced the moisture content of the BSF larvae from 0.74 g water/g wet sample to 0.70 g water/g wet sample.

In addition, drying achieved a relative reduction of 35% on a mass basis as shown in Table 5.6. The dryer was operated at 20 rpm. This shows that the residence time in the dryer was not sufficient to allow complete drying. The dryer was not jacketed to reduce the loss of heat from the dryer, which also affected the minimal loss of water from the BSF larvae. However, the drying process did not affect the volatile content and ash content of the larvae.

Table 5.6: Effects of the drying process on the BSF larvae from trial 1

	Units	Initial BSF larvae Mean ± SD	Dried BSF larvae Mean ± SD
Mass	Kg	20.85	13.65
Moisture content	g water /g wet sample	0.74±0.00	0.70±0.00
Total Solids	g TS/g wet sample	0.26±0.00	0.30±0.00
Volatile Solids	g VS/g dry sample	0.76±0.00	0.76±0.01
Ash Content	g ash/g dry sample	0.24±0.00	0.24±0.00

5.6 Material conversion and nutrient flows

A mass balance was performed on the mixer and grow-out area to determine the waste reduction and bioconversion using composite sludge. All the data used in the mass balance was found experimentally in Phase 2. The UDDT sludge masses were

measured using a weighing bridge, and the data recorded before adding to the mixer. The PS was added from a graduated tank, and the density of the PS was assumed to be 1 000 kg /m^3 (the same as water) because the percentage of solids was less than 1%. The mass balance in this study used the same method as described in Section 4.5 and the results are shown in Table 5.7 (raw data presented in Appendix 3: Table A.7, Table A8, Table A9 and Table A.10).

Table 5.7: Mass balance results from the Phase 2 trials

	Initial BSFL	**Final BSFL**	**Initial Feed***		**Remaining food waste from nursery**		**Residue**	
	Wet basis [kg]	**Wet basis [kg]**	**Wet basis [kg]**	**Dry basis [kg]**	**Wet basis [kg]**	**Dry basis [kg]**	**Wet basis [kg]**	**Dry basis [kg]**
Trial 1	7.21	20.85	215	69	29	16	179	81
Trial 2	9.03	26.30	227	67	32	16	121	49
Trial 3	5.10	23.35	272	74	36	18	233	86

***** The initial feed includes UDDT sludge and PS and excludes the trash removed

The data from these 300 kg trials shown in Table 5.7 was then adjusted to reflect a feed basis of 1 tonne of UDDT sludge. This recalculation was carried out due to different masses being used in the mixer and the grow-out area and a common basis was required. Furthermore, this recalculation offered information regarding the space requirements for the plant as the plant was designed to handle 20 tonnes at 10 cm depth. In addition, the data used in the mass balance was based on trial 2, as trials 1 and 3 were affected by the flooding resulting in poor data. *Figure 5.2* shows the mass balance for trial 2 on a 1 tonne of UDDT sludge basis.

In order to construct this mass balance, the following assumptions made were:

- The amount of trash remains constant throughout the process, and the BSFL do not use it as a feed source.
- The UDDT sludge is homogenously mixed

- During the growth phase of the BSF larvae, the mass balance error could not be calculated, because it takes into metabolism and evaporation which were not measured experimentally.

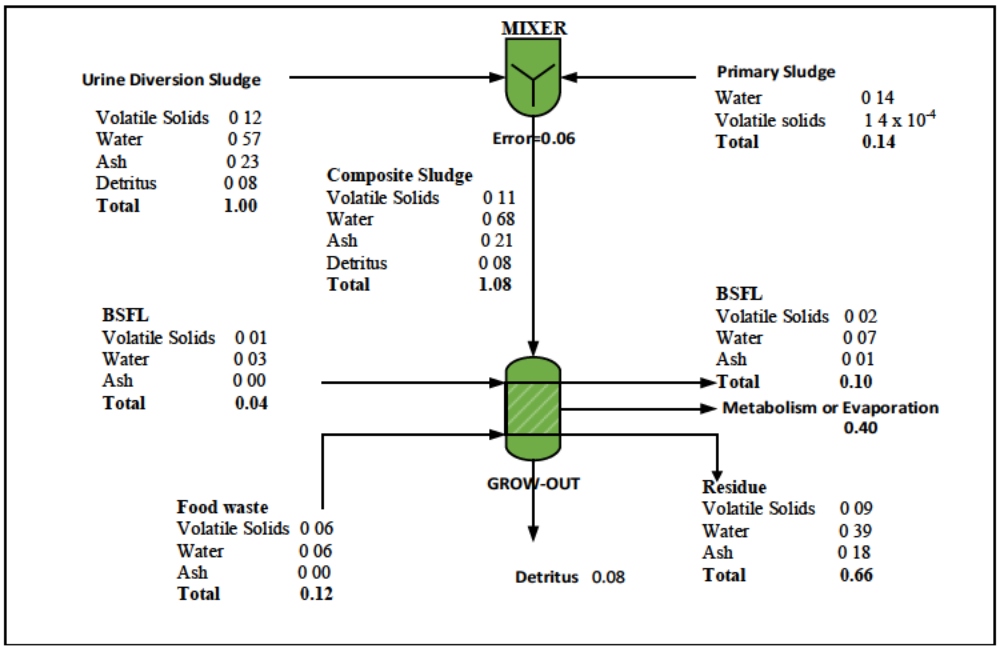

Figure 5.2: Mass balance for trial 2 based on 1 tonne (wet basis) of UDDT sludge

The bioconversion yield of wet waste to larvae based on Figure 5.2 is 9% (mass larvae/mass wet feed) and 8% on a dry basis. It should be noted that bioconversion was calculated including the remaining food waste incorporated in the system. Also, trash was excluded in the calculations as it not consumed. The relative waste reduction is 58% on a wet basis and 36% on a dry basis. Based on the inflow streams (UDDT + PS + Food waste), it was found that 6% of the VS was incorporated into larval mass, and 33% of the total mass was lost due to metabolism or evaporation.

The overall reduction rates were 10.62 kg/ (m^3 d) on a wet basis and 2.62 kg/ (m^3 d) on a dry basis. This showed that moisture was mainly lost due to environmental conditions and metabolism by larvae when compared to total solids as reduction on a wet basis is higher than on a dry basis.

The addition of PS to the UDDT sludge resulted in a bioconversion (mass of BSF larvae/total feed added) of 9% on a wet basis and 8% on a dry basis, which is an increase from a 6% wet basis and 3% dry basis found in Phase 1 (see Table 5.8). The

relative waste reduction in Phase 2 was also found to be greater than in Phase 1. However, it is worth noting that remaining food waste in the UDDT sludge from the nursery as it was 10% of the substrate could have increased the waste reduction and bioconversion rates as it was mixture of food waste and layers mash while in Phase 1 the amount of formulated feed was significantly reduced.

Table 5.8: Comparison of bioconversion and waste reduction for Phase 1 and Phase 2

Phase	**Substrate**	**Bioconversion [%]**		**Relative waste reduction [%]**	
		wet	**dry**	**wet**	**dry**
1	UDDT sludge	6	3	28	12
2	UDDT sludge + PS	9	8	53	36

The difference in these two phases can be attributed to the addition of PS in Phase 2, which increased the organic content as is shown in *Table 5.8*. Due to the high moisture content of PS (99%), there is a need to dewater the PS prior to addition to the UDDT sludge to increase the organic content, thereby increasing the bioconversion rate. The addition of PS increased the moisture content of the UDDT sludge to approximately 70%, which is higher than 60% in Phase 1. Banks (2014) found higher bioconversion rates using a moisture content of 75% than with a moisture content of 65%. The optimum moisture content is around 70 -75% as Lalander et al. (2020) found an increase of feed moisture content greater than 75% resulted in a decrease in bioconversion and waste reduction.

The difference in feeding depth between the two phases could also have contributed to the difference in bioconversion and waste reduction. In Phase 1, the substrate was used at 10 cm depth, and in Phase 2, this was reduced to 5 cm. Dortmans et al. (2017) and Brits (2017) showed that the feed depth should be no more than 5 cm, as the larvae tend to have difficulty processing all the feed and the bottom layer is unprocessed. This is supported by Clarke and Fraser (2004), as their study showed that metabolic rate is usually measured as the consumption of oxygen. Based on this, an increase in feed depth results in the creation of anaerobic conditions which are not favourable for BSF larvae feeding.

6 CONCLUSIONS AND RECOMMENDATIONS

This chapter presents the conclusions derived in this study and reflects on the application of BSF larvae as a management tool for the treatment of UDDT sludge. Based on the respective findings, various suggestions and recommendations have been constructed and are presented here.

6.1 Conclusions

The BSF larvae showed potential to be used as a FS management solution with resource recovery. The BSF larvae managed to reduce the UDDT sludge by 28% wet basis and bioconversion of 6% wet basis on a pilot-scale operation operating in an uncontrolled and low maintained system. The study showed that bioconversion can be increased by increasing moisture content and reducing the feed depth as it increased from 6% to 9%. PS has potential to increase the organic content as highlighted by its characteristics. The values found in this study are comparable to those found in literature, which were conducted at a laboratory scale and in a controlled system.

The use of PS increased the moisture content of the UDDT sludge from 60% to 70% is feasible for BSF larvae development. Although, the beneficial effect of PS was not conclusively determined in this study, it had a higher nutritional value that can be explored further to increase the nutritional value of the UDDT sludge.

The characteristics of the UDDT sludge is affected by the user habits, environment, duration of storage and diet of the community. The addition of sand after use lowers the organic content available for the BSF larvae development. The replacement of sand used in UDDTs with an organic additive could improve the BSF larvae process. It is also worth noting that the UDDT sludge used in these trials is more than 2 years old, thereby impacting on the low nutritional value.

The low bioconversion and relative waste reduction observed can be linked to several factors which were not optimised in the study. However, increasing the nutritional value, reducing the substrate depth, and increasing moisture content of the substrate had a positive impact on the bioconversion and waste reduction capabilities.

The nutritional quality of the different substrates affects the body mass and/or size of BSF larvae. It was also noted that the chemical composition of the substrate influences

the composition of the resulting BSF larvae as ash and heavy metals accumulated in the BSF larvae.

The BSF larvae process is affected by temperature and humidity as it affects the relative waste reduction and bioconversion rates and these conditions varied throughout the study. The plant still needs to be controlled, and adjustments made to increase its efficiency. The plant was designed on existing drying beds and cannot control temperatures and humidity. Durban climate was assumed to be humid and hot to allow for the development of the BSF larvae. However, the temperature data during the bioconversion process showed that the temperature and humidity fluctuated between 19.17 °C to 40.47 °C and 43.77% to 97.77% respectively. This results in different bioconversion and waste reduction during different seasons. The BSF larvae require an optimum temperature of 28°C – 32 °C (Tomberlin et al., 2002).

The UDDT residue results were compared to the limits provided for use of wastewater sludge in agriculture which suggested that the residual sludge should be classified as pollutant "a", and could be used for agricultural use without concerns for heavy metal pollution (Snyman, 2010). However, due to the limitations of this study, there is a need to verify this result by doing an extensive study of UDDT sludge characteristics.

This study revealed that lead and zinc do accumulate in larvae or prepupae. The concentration of lead and zinc in BSF larvae fed on UDDT sludge are too high for animal feed and might inhibit the potential of the larvae to be used as animal feed. In the case of lead and zinc, concerns about the use of prepupae in animal feed are less critical as they can be removed at the source through modifying user behaviour to avoid contamination. The waste treatment technology using BSF larvae may contribute to reducing the burden of an animal protein shortage in the animal feed market and provide new income opportunities for small entrepreneurs in low and middle-income countries.

Although, BSF larvae can treat UDDT sludge, the available nutrients in the UDDT sludge need to be supplemented by an organic substrate as most of the organic content has stabilized. The residue produced could be used as a fertilizer without heavy metal concerns, though further work needs to be done in this area. Even though heavy metals may accumulate in the BSF larvae their concentrations were within permissible limits

for the application of BSF as an animal feed. Finally, the inconsistent weather conditions in Durban are not ideal for the development of the BSF larvae.

6.2 Recommendations

Future research can focus on two main aspects: the biological factors of the BSF larvae and the design and operation of the treatment facility. There is a need to optimize the bioconversion process using UDDT sludge. Exploring either removal of sand in the UDDT sludge, or substitution with an organic additive like PS to increase the organic content of the UDDT sludge, can be beneficial to the bioconversion process.

PS had a high nutritional value that can be explored further to increase the nutritional value of the UDDT sludge. PS has the advantage of being close to the facility and free of charge, thereby cutting down operational costs that can be incurred by sourcing a highly nutritious substrate. BSF larvae adapt to survival mode rather than weight gain if there are low nutrients, as shown by the BSF larvae's low growth.

The research was not conducted in an energy-efficient setting; hence there is a need to control the environment with inexpensive options.

In addition, the floor is made of concrete, absorbing most of the heat and making the UDDT sludge cool, which is not ideal for BSF larvae development.

There is a need for a drainage system to drain excess water after bioconversion as it was noticed the sludge was still wet. The gutters were leaking and ruining the trials, and the plant was not designed to handle any harsh weather conditions.

Processing units were not effective as per design; hence losses were observed. Research needs to focus on the processing equipment on a large scale. For example, the belt press failed to press the water out of the residue before the pre-dryer. There was a significant loss of heat on the retort kiln as it is not jacketed. The flue gas was to be used to dry the BSF larvae in the rotary dryer but proved to be inefficient as only 4% of the moisture was removed. In addition, the rotary dryer was not jacked, contributing to the inefficiency of the drying process of the BSF larvae. There were no temperature probes to show the temperature of the flue gas.

This study did not focus on the fate of pathogens and organic pollutants which are present in UDDT sludge, and which might hinder the use of the UDDT sludge for

agriculture purposes or BSF larvae as animal feed, and more research to evaluate these concerns needs to be undertaken.

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APPENDIX 1: ETHICAL APPROVAL

UNIVERSITY OF
KWAZULU-NATAL
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15 January 2018

Ms ET Mutsakatira (216076873)
School of Chemical Engineering
College of Engineering
216076873@stu.ukzn.ac.za

Dear Ms Mutsakatira

Protocol: Characterization of faecal sludge from urine diversion toilets-impact on black soldier fly larvae
Degree: MSc
BREC Ref No: EXM033/18

I refer to your application to BREC received on 23 November 2017 and wish to advise you that exemption of ethics review has been granted for this study.

This exemption will be **noted** at the next Biomedical Research Ethics Committee meeting to be held on **13 February 2018.**

Yours sincerely

[redacted]
Prof Joyce Tsoka-Gwegweni
Chair: Biomedical Research Ethics Committee

cc postgraduate administrator

APPENDIX 2: STANDARD OPERATING PROCEDURES

UNIVERSITY OF KWAZULU-NATAL	*Standard Operating Procedure* PRG pollution research group	**Effective Date:** 20 June 2013	**Version:** ***003***
		Reviewed: 1 Nov 2017	
SOP_Chem_012 Chemical Analysis_ pH Analysis of Faecal Sludge			Page # ***1of 5***

Standard Operation Procedure – pH of Faecal Sludge

1. Scope and Application

- This method is an electrometric procedure for measuring pH in soils and waste samples.
- Wastes may be solids, sludge, or non-aqueous liquids.
- If water is present, it must constitute less than 20% of the total volume of the sample.

2. Apparatus and Glassware

- pH meter with means for temperature compensation.
- Glass electrode.
- Reference electrode- A silver-silver chloride or other reference electrode of constant potential may be used.
- 50 ml beaker.
- Thermometer and/ or temperature sensor for automatic compensation.
- Analytical balance- capable of weighing 0.1 g.

3. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- After the analysis, clean bottles and beakers with clear water keep it for drying.
- Dispose the used gloves after completion of analysis.
- Clean hands using antiseptic soap.
- Disinfect hands after washing with soap.
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

4. Interferences

- Samples with very low or very high pH may give incorrect readings on the meter.
- For samples with a true pH of >10, the measured pH may be incorrectly low.
- This error can be minimized by using a low-sodium-error electrode.
- Strong acid solutions, with a true pH of <1, may give incorrectly high pH measurements.
- Errors will occur when the electrodes become coated.
- If an electrode becomes coated with an oily material that will not rinse free, the electrode can:

- be cleaned with an ultrasonic bath, or,
- be washed with detergent, rinsed several times with water, placed in 1:10 HCl so that the lower third of the electrode is submerged, and then thoroughly rinsed with water, or,
- be cleaned per the manufacturer's instructions.

5. Procedure

Sample Preparation

- To 20 g of waste sample in a 50 ml beaker, add 20 ml of distilled water, cover, and continuously stir the suspension for 5 min.
- Additional dilutions are allowed if working with hygroscopic wastes and salts or other problematic matrices.
- Let the waste suspension stand for about 15 min to allow most of the suspended waste to settle out from the suspension or filter or centrifuge off aqueous phase for pH measurement.
- **NOTE:** If the waste is hygroscopic and absorbs all the reagent water, begin the experiment again using 20 g of waste and 40 ml of reagent water.
- **NOTE:** If the supernatant is multiphasic, decant the oily phase and measure the pH of the aqueous phase. The electrode may need to be cleaned if it becomes coated with an oily material.

Measurement of pH

- Adjust the electrodes in the clamps of the electrode holder so that, upon lowering the electrodes into the beaker, the glass electrode will be immersed just deep enough into the clear supernatant to establish good electrical contact through the ground glass joint or the fibre-capillary hole.
- Insert the electrode into the sample solution in this manner. For combination electrodes, immerse just below the suspension.
- If the sample temperature differs by more than 2°C from the buffer solution, the measured pH values must be corrected.

6. Results

- Report the results as "waste pH" measured in water at __°C" where "__°C" is the temperature at which the test was conducted.

Tips for new probes in extended dry storage

- Stir the probe in pH buffer 7 solution to dislodge air bubbles.
- Before use, soak new probes or probes that are stored for an extended time in pH 7 buffer for at least 5 minutes.
- Remove reference gel from the probe sensor and the inner surface of the rubber dust cap.
- Calibrate the probe. First with pH 7, then with a second buffer (usually pH 4 or pH 10) and then a third buffer, if necessary.
- Check the calibration. Put the probe into the pH 7 buffer. If the reading is incorrect, the probe is not hydrated. Soak the probe for 5 minutes in pH 7 and repeat the re-calibrate.
- Store the probe dry with the sensor cap on.

- For semi-solids use, gently twist the probe to make sufficient contact with the sample to the sensor.

Clean the pH probe

Before the probe is cleaned:

- For use in dairy, cheese or meat applications, soak the probe in Pepsin Cleaning Solution for 15 minutes.
- Do not use sharp metal objects (needle, pin, etc.) to clean the sensor surface. Scratching the surface of the sensor may cause permanent damage to the probe.
    1. Use a soft-bristle toothbrush to clean the sensor with soap and water.
    2. Make sure all the debris is removed from the sensor surface.
    3. Rinse the probe fully and re-calibrate.

Maximize probe life

- Rinse the probe fully after any calibration, measurement and cleaning.
- Add sensor cap to the end of the probe when not in operation.
- Cool samples to room temperature.
- When semi-solids are tested, make sure solid objects (i.e., bone or gristle) do not scratch the sensor.
- Use new buffers and rinse solution.
- Do not use the probe in environments that will damage the epoxy materials used in the probe tip.
- Keep the probe away from acetone, toluene, methylene chloride, xylene and other strong organic solvents.
- Avoid environments with static electricity. Electrostatic discharge (ESD) can permanently destroy the probe.
- Avoid use at temperatures more than 60°C. Thermal cycling can decrease the life of the probe.
- Do not let oil, fat, food particles, starch, protein or other materials stay on the probe after use.
- Prevent damage to the silicon chip sensor.
- Do not use the probe in hydrofluoric acid or abrasive samples or other environments that can cause damage to the silicon chip sensor.

7. References

http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/9045d.pdf

APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof C.A. Buckley

Signature: ……

Date: ……

Author: Merlien Reddy

Signature: ……

Date: ……

UNIVERSITY OF KWAZULU-NATAL	*Standard Operating Procedure* PRG pollution research group	**Effective Date:** 14 April 2015	**Version:** ***004***
		Reviewed: 1 Nov 2017	
SOP_Chem_003 Chemical Analysis_ Chemical Oxygen Demand Open Reflux			Page # ***1 of 6***

Standard Operation Procedure – Chemical Oxygen Demand Open Reflux, Titrimetric Method

1. Scope and Application

- Chemical Oxygen Demand (COD) measures the oxygen equivalent of that portion of the organic matter in a sample that is easily oxidised by a strong chemical oxidant.
- It is an important and common parameter to measure the amount of organic compounds in streams, industrial waste and in operational control of wastewater treatment plants. It is also applicable for measurements on human excreta.
- The procedure described hereafter is applicable to low COD values 40-400mg/L with sample dilution and high COD values 40-3600mg/L with minimal sample preparation.

2. Summary

- The sample is digested for two (2) hours in a strongly acidic dichromate solution, using silver sulphate as a catalyst and mercuric sulphate as a masking agent to prevent chloride interference. The dichromate is 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 reddish brown as the Ferroin indicator undergoes reduction from iron (III) to the iron (II) complex.

3. Apparatus and Glassware

- Heating Block
- Condensers
- 250 ml Erlenmeyer flasks

- 10ml pipette
- 15ml and 5ml automatic dispensing pipette
- Retort stands
- Tubing
- Water source

4. Interferences

- Difficulties caused by the presence of chlorides in the sample are overcome by the addition of mercuric sulphate to samples before digesting. The chloride ion is then eliminated from the reaction by forming a soluble mercuric chloride complex.

5. Collection, Preservation and Storage

- Collect samples in 1L plastic buckets.
- Preferably, analyse samples immediately after sampling.
- Store samples at 4 °C or freeze dry samples.
- Preserve wastewater samples by acidifying with concentrated sulphuric acid to pH 2 and faecal samples by freeze drying or freezing.
- Determine COD on well- homogenised samples.

6. Safety Precautions

- Handle concentrated sulphuric acid with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- Wear face shield and protect hands from heat produced when contents of the vessels are mixed.
- After the analysis, clean bottles and beakers with water then dry.
- Dispose used gloves after completion of analysis.
- Clean hands using antiseptic soap.
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

7. Sample Preparation –Faecal Sludge

40-400mg/L COD

1. Weigh out 2.0000g of well-mixed faecal sludge sample.
2. Blend the weighed sample with 500ml of distilled water in a 1L blender for 30 seconds on the highest speed.
3. Add 250ml distilled water and blend on highest speed until the sample is homogenised (this could range from 30 to 60 seconds).

4. Transfer the blended mixture into a 1L volumetric flask.
5. Add 200ml of blender washings into the flask and top up to 1L with distilled water.
6. Transfer the 1L solution to a plastic bottle and store at 4 °C.

40-3600mg/L COD

7. Dilute 0.1g of well-mixed faecal sludge sample with 5ml of distilled water in the digestion vessel.

8. Reagents

Standard Potassium Dichromate $K_2Cr_6O_7$ Digestion Solution: 0.0417M

- Add 12.2588g $K_2Cr_6O_7$, previously dried at 105 °C for 2hrs to approximately 500ml distilled water.
- Dissolve and cool to room temperature before diluting to 1L.

Sulphuric Acid H_2SO_4 /Silver Sulphate Reagent Ag_2SO_4 (COD Reagent)

- Add **26g of silver sulphate** crystals or powder to 2.5L of concentrated sulphuric acid using a magnetic stirrer. Shake well and leave for 2days for dissolution.

Ferroin Indicator 2 drops

- Dissolve 1.485g 1:10 phenentroline monohydrate and 0.695g ferrous sulphate ($FeSO_4.7H_2O$) in distilled water and dilute to100ml.

Mercuric Sulphate $HgSO_4$

- Used to remove chlorides, which give a higher COD result. 0.04g crystal or powder.

Ferrous Ammonium Sulphate Fe $(NH_4)_2$ $(SO_4)_2.6H_2O$: 0.25M

- Dissolve 98g Fe $(NH_4)_2$ $(SO_4)_2.6H_2O$ in distilled water.
- Add 20ml concentrated Sulphuric acid H_2SO_4 and dilute to 1L.
- Standardize daily against **Standard Potassium Dichromate $K_2Cr_6O_7$ Digestion Solution**

9. Calibration

- Prepare a standard K2Cr2O7 solution daily to correct any variation in the concentration of the Ferrous Ammonium Sulphate.
- Prepare a blank with each set of samples consisting of 5ml distilled water in place of sample together with all the reagents and digest together with samples.

Standard Preparation

- Add 3ml of standard K2Cr2O7 digestion solution to 5 ml of distilled water. Add 7ml COD reagent and cool it down. Titrate with FAS titrant using 2 drops of Ferroin indicator.
- Quality Control: Potassium hydrogen Phthalate (KHP)

- Lightly crush and then dry out KHP to a constant weight at 120°C. Dissolve 0.0425g in distilled water and then dilute to 250ml. This solution has a theoretical COD of 200mg/L. Solution is stable if refrigerated, for a period of 3 months in the absence of biological growth.
- Dissolve 425mg in a litre. KHP has a theoretical COD of 1.176mgO2/mg and this solution has a theoretical COD of 500ug O2/ml. 0.425g = 500mg/L.

10. Procedure

Sample Preparation for Digestion

- Add app 0.04g (2 match heads) of Mercuric sulphate to a dry 250ml Erlenmeyer flask.
- Add 5 glass beads.
- Add 10ml sample (if 2ml sample used add 8ml distilled water).
- Add 10ml distilled water to another flask (blank).
- Add 5ml potassium dichromate.
- Add 15ml sulphuric acid reagent (with silver sulphate).
- Tilted flask away from user and pour acid down the wall of the flask. (Sample turns green if it is too conc, a lower volume must be used).

Heating Block

- Switch the heating block on one hour prior to testing. The temperature of the solution in the boiling tubes must be stable at 145ºC. Check stability during analysis by immersing a suitable thermometer into the sample, but not touching the sides of the tube. Adjust the digestion temperature as necessary.
- Carefully attach flask to the jacket condenser. Flasks must be level on heating pad.
- Digest samples for 2hrs.Ensure a swift water flow rate in the condensers.
- Cool samples with the condensers still in position.
- Pour approx. 80ml of distilled water through the top opening of each of the condensers into the sample mixture.

Sample Titration

- Titrate the excess dichromate in the digest mixture with standard ferrous ammonium sulphate using 3 drops of ferroin indicator.
- Titrate from a sharp green/orange to red brown end-point.
- Take reading.

11. Waste Disposal

- Collect waste in a 2.5L bottle for Waste Tech collection.

12. Calculation and Data Analysis

$$COD\ (mg\ O_2/L) = \frac{(Blank - Titration) \times molarity\ of\ FAS \times 8000}{Sample\ (ml)}$$

Where:

8000 = Milliequivalent weight of oxygen × 1000 ml/L

$$Molarity\ of\ FAS = \frac{Volume\ 0.0167M\ K_2Cr_2O_7\ Solution\ Titrated\ (ml)}{Volume\ FAS\ used\ in\ titration\ (ml)} \times 0.10$$

$$COD\ (mg\ O_2/L) = \frac{(Blank - Titration) \times molarity\ of\ FAS \times 8000}{Sample\ (ml)} \times \frac{V}{M}$$

$$COD\ in\ Wet\ Sample\ (g\ O_2/g) = \frac{COD\ (mg\ O_2/L)}{1000}$$

$$COD\ in\ Dry\ Sample\ (g\ O_2/g) = \frac{COD\ in\ Wet\ Sample\ (g\ O_2/g)}{Total\ Solids\ (g/g)}$$

Where:

M = Mass of sludge used in sample preparation (g)
V = Total volume (L)

13. Data Quality

Measurement	mg/l COD
Standard Deviation (mg/l COD)	
Confidence Interval (mg/l COD)	

14. References

- Standards Methods for the Examination of Water and Wastewater, 18th Edition, p. 5-19, Methods 5220 C. Closed Reflux, Titrimetric Method (1992).
- Milestone Ethos One Operator Manual MA 133, Rev00/2010.

APPROVAL OF STANDARD OPERATING PROCEDURE

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Signature: ……
Date: ……..

Author: Merlien Reddy

Signature: ……..
Date: ……..

UNIVERSITY OF KWAZULU-NATAL	*Standard Operating Procedure* PRG pollution research group	**Effective Date:** 31 May 2016	**Version:** ***003***
		Reviewed: May 2017	
SOP_Chem_013 Chemical Analysis_ Solids			Page # ***1of 7***

Standard Operation Procedure – Solids

Introduction

Solids refer to matter suspended or dissolved in water, wastewater and faecal sludge. Solids may affect water or effluent quality adversely in a number of ways. Solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory wastewater effluent limitations.

Total Solids are a term applied to material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature. Total solids include **Total Suspended Solids**, the potion of solids retained by a filter and **Total Dissolved Solids**, the portion that passes through the filter of 2.0µm or smaller. **Fixed Solids** is the term applied to residue of total, suspended or dissolved solids after heating to dryness for a specified time at a specified temperature. The weight loss on ignition is called **Volatile Solids**.

Total Solids Dried at 103˚C - 105˚C

1. Scope and Field of Application

- Total Solids are determined in a wide variety of liquid and semi-liquid materials.
- These include portable waters, domestic and industrial waters, polluted waters and faecal sludge produced from treatment processes. It is of particular importance for the efficient operation of a treatment plant.
- A known volume of well-mixed sample is evaporated to dryness in a porcelain crucible in a hot air oven at 105°C; the solids remaining are cooled and weighed.
- The residual material in the crucible is classified as total solids, and may consist of organic, inorganic, dissolved, suspended or volatile matter.

1. Apparatus

- 50ml capacity evaporating porcelain crucibles
- Desiccator
- Drying oven
- Analytical Balance

2. Reagents

- None

3. Interferences

- Highly mineralised water with a significant concentration of calcium, magnesium, chloride and sulphate may be hygroscopic and require prolonged drying, proper desiccation and rapid weighing.
- Exclude large, floating particles from the sample if it is determined that their inclusion is not desired in the final result.
- Disperse visible floating oil and grease with a blender before withdrawing sample portion for analysis because excessive residue in the dish may form a water-trapping crust.

4. Sampling

- Mix the sample well to suspend solids uniformly.
- Remove the test portion rapidly before any settling of solid matter occurs.
- Use a measuring cylinder and not a pipette for sludge and wastewater samples.
- Use a crucible for faeces.

- Use a volume or mass of sample to ensure a measurable residue- limit sample to no more than 200mg residue.
- Suitable aliquots: Liquid samples 100ml, Sludge -30ml, Faeces 10-20g

5. Safety Precautions

- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- Wear gloves suitable for withstanding high temperatures when removing crucibles from the oven.
- After the analysis, clean bottles and beakers with clear water keep it for drying.
- Dispose the used gloves after completion of analysis.
- Clean your hands using antiseptic soap.
- Disinfect hands after washing with soap.
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

6. Calibration

- Check the temperature throughout the oven area by placing a calibrated thermometer on each shelf, after 30 min, check temperature at each level against oven setting.
- Adjust oven setting if necessary.
- If temperatures are uneven on the shelves, check insulation.

7. Procedure

Prepare Crucible

- If volatile solids are to be measured, ignite clean crucible at 550°C for 1 hour in the furnace. If only total solids are to be measured, heat clean crucible to 103-105°C for 1 hour. Store and cool dish in a desiccator until needed. Weigh immediately before use…….W1g.

Sample Analysis

- Measure out appropriate volume (30ml) /minimum mass (10-20g) that will yield a residue between 2.5 and 200mg of a mixed sample using correct volume measuring cylinder or analytical balance…..Vml…Wg. Transfer quantitatively to the weighed crucible, rinsing the cylinder with small volumes of distilled water to dislodge heavy particles. Add washings to the crucible.
- Place in hot oven at 103-105°C for 24 hours.
- ***Dry sample for at least 1hr in an oven 103-105°C, to desiccator to balance temperature and weigh. Repeat cycle of drying, cooling and weighing until a constant weight is obtained, or until weight change is less than 4% of previous weight or 0.5mg, whichever is less.***
- Remove the next day and cool for 15 minutes and weigh…..W2g

8. Calculation

$$Total\ Solids\ in\ Sample\ (mg/l) = \frac{(W_2 - W_1)g \times 100\ 000}{V_{sample}\ (ml)}$$

$$Total\ Solids\ in\ Wet\ Sample\ (g/g) = \frac{(W_2 - W_1)g}{W_{sample}\ (g)}$$

$$Moisture\ Content\ (g) = W_{sample}(g) - [(W_2 - W_1)]g$$

Total Suspended Solids Dried at 103-105°C

1. Scope and Field of Application

Suspended solids are useful determinants in the analysis of polluted, re-use and wastewaters. It is used to evaluate the strength of domestic/industrial wastewaters and to determine the efficiency of treatment units, such as settling tanks, biological filters, and the activated sludge. Use of glass fibre filter pads is preferred to crucibles because of the saving in filtration time and the only prior preparation necessary is drying in an oven for 30mins at 105°C.

A measured volume of well shaken is vacuum filtered through a dried pre-weighed 110mm diameter glass fibre filter. The filters and residue is dried to a constant weight at 103-105°C.

The increase in weight of the filter represents the total suspended solids.

2. Apparatus

- Four- place Analytical balance
- 110mm diameter funnel and flask
- Vacuum pump

3. Reagents

- Nil

4. Interferences

- Exclude isolated large floating particles.
- Samples high in dissolved solids must be washed adequately.
- Loss in mass of the rinsed glass fiber filters must be taken into the final calculation.
- The larger the sample, the smaller the factor applied in the calculation, but avoid prolonged filtrations.

5. Sampling

- Take the sample at a point of turbulence to ensure that it is truly representative.
- Mix sample thoroughly and remove test portion rapidly before segregation occurs.
- Use appropriate volume measuring cylinder and not pipettes.

6. Safety Precautions

- Exercise care when using glassware, vacuum pumps and ovens.
- Good housekeeping and cleanliness are essential for obtaining accurate results.

7. Sample Preparation – Faecal Sludge

- Weigh out between 1.8g and 2g of well-mixed faecal sludge sample.
- Place the weighed out sample into a blender with 250ml of distilled water.
- Blend for 30 seconds.
- Transfer the blended mixture into a volumetric flask and top up to 1L with distilled water.
- Transfer the 1L solution to a plastic bottle and store in the cold room.

8. Calibration

- The analytical balance and ovens are checked and serviced weekly.

9. Procedure

Dry Filter Paper

- Use 110mm glass fiber filter paper Whatman No 4(20-25µm) for sludge.
- Use 20ml sample on a 40mm, 0.45µm glass fibre filter for wastewater and urine (change to a 1µm pore size if the dried residue is more than 200mg).
- Use a smaller pore size if the dried residue is lower than 2.5mg.
- Mark the filter paper with a pen.
- Place papers on the stainless steel mess of appropriate size.
- Position on top shelf in oven at 105°C for 30 min.
- If volatile solids are to be measured, ignite at 550°C for 15 min in a furnace.
- Transfer to desiccator.
- Cool for 20 mins before weighing.

Weigh Filter Paper

- Transfer filter paper rapidly to balance.
- Note mass (W1) grams, to fourth decimal place.

Prepare for Analysis

- Place filter pare into a 110mm diameter funnel, with the marking on the lower side.
- Measure out appropriate volume to yield between 2.5 and 200mg dried residue of well-mixed sample.
- Place funnel into flask with side arm attached to a vacuum pump.
- Apply pump.
- Wet paper with distilled water to seal edges of the paper to surface of the funnel.
- Pour sample onto the filter paper, keeping sample in the middle of the paper.
- When filtration is complete. Remove paper by placing the end of a small thin spatula along the edge of the filter paper and lift slowly.
- Remove the paper with a pair of tweezers, taking care not to tear the paper.
- Fold paper twice to form a triangle enclosing sample residue. This seals the residue in the filter paper and protects it from contact with air.

Dry and Weigh

- Place triangles on a stainless steel mess.
- Place in oven at 105°C for 2 hours.
- Remove from oven and place in desiccator.
- Cool to room temperature.
- Weigh after 20 mins, as rapidly as possible.
- Note mass (W2) grams.

10. Calculation

$$Total\ Suspended\ Solids\ (g/ml) = \frac{(W_2 - W_1)}{V_{sample}(ml)}$$

$$Total\ Suspended\ Solids\ in\ Wet\ Sample\ (g/g) = TSS\ (g/ml) \times DF$$

$$Total\ Suspended\ Solids\ in\ Dry\ Sample\ (g/g) = \frac{TSS_{wet\ sample}}{Total\ Solids\ (g/g)}$$

W = weight of filter paper before oven (105°C) (g)
W_2 = weight of residue + filter paper after oven (105°C) (g)
DF = Dilution Factor

Fixed and Volatile Solids Ignited at 550°C

1. Introduction

The residue from the above methods is ignited to constant weight at 550°C. The remaining solids represent the fixed total, dissolved or suspended solids while the weight lost on ignition is the volatile solids. The determination is useful in control of wastewater treatment plant operation because it offers a rough estimate of the amount of organic matter present in the solid fraction of wastewater, activated sludge and industrial wastes.

2. Apparatus

- Muffle Furnace
- As above

3. Interferences

- Negative errors in the volatile solids may be produced by loss of volatile matter during the drying.

4. Procedure

- Ignite residue from the total solids to constant weight in a muffle furnace at a temperature of 550°C.
- Have furnace up to temperature before inserting sample.
- Usually 2 hours for VIP and sludge samples, 15-20 min for wastewater (200mg residue).
- Let the crucible cool partially in air until most of the heat has dissipated.
- Transfer to a desiccator for final cooling. Do no overload the desiccator.
- Weigh dish as soon as it has cooled to balance temperature.

5. Calculation

$$Volatile\ Solids\ in\ Wet\ Sample\ (g/g) = \frac{(B - C)}{W_{sample}(g)}$$

$$Volatile\ Solids\ in\ Dry\ Sample\ (g/g) = \frac{VS_{wet\ sample}}{Total\ Solids(g/g)}$$

$$Fixed\ Solids\ in\ Wet\ Sample(g/g) = \frac{(C - D)}{W_{sample}(g)}$$

$$Fixed\ Solids\ in\ Dry\ Sample(g/g) = \frac{FS_{wet\ sample}}{Total\ Solids(g/g)}$$

B = weight of residue + crucible before ignition 550°C (g)
C = weight of residue + crucible after ignition -550°C (g)
D = weight of crucible (g)

6. References

Standards Methods for the Examination of Water and Wastewater, 18th Edition, p. 2-62, Methods 2540 Solids, A,B,C,D,E(1992).

APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof C.A. Buckley Signature: ……
Date: ……

Author: Merlien Reddy Signature: ……
Date: ……

UNIVERSITY OF KWAZULU-NATAL	*Standard Operating Procedure* PRG pollution research group	**Effective Date:** 20 June 2013	**Version:** ***001***
		Reviewed:	
SOP_Chem26_Carbon Nitrogen Sulphur (CNS) Analysis (LECO TruMac CNS)			Page #: ***1 of 41***

Standard Operation Procedure – Carbon Nitrogen Sulphur Analysis

1. Scope and Application

The LECO TruMac CNS determines carbon, nitrogen and sulphur macro combustion determinator that utilizes a pure oxygen environment in a ceramic horizontal furnace operating at a high temperature (1350^{O}C), with large ceramic boats for the macro sample combustion process. A combustion gas collection and handling system using helium carrier gas and a thermal conductivity cell for the detection of nitrogen are also utilized.

2. Sample preparation

- No sample dilution required.
- Ensure that the sample is well mixed and 0.2g of a uniform consistency is weighed out into the crucible.
- Use liners for samples with high moisture content

3. Gas Settings

Gas Checks-Spanner no.27 for Affrox
HiQ pure Oxygen – 35psi (241Kpa)
Baseline helium – 35psi (241Kpa)
Compressed air – 40 psi 276 Kpa)
Flow Meters
P-Purge(4.2 L/min)
L-Lance(1.8 L/min)
A-Analyser(3.5 L/min)
Arm Gauge- cup seal and radial seal (black) pressure to seal furnace -10psi

4. Daily Checks

- **Username: leco Password: leco**
- Check gas pressures then open gasses
- Check brown furnace door seals-wash with soap and dry. Place back to front.
- Check furnace temp(1350 degrees celcius)

- System check. Go to diagnostics
- Leak check(oxygen and helium(1263 Hg) independently

5. Start Up

a. Switch instrument and auto loader on
b. Switch computer and Leco software
c. Go to diagnostics and increase furnace temp in increments to 1350°C.
d. The ballast and furnace readings will display on the bottom right of the screen
e. The self-check sequence will start(autoloader and arm)
f. Switch on gasses- stipulated in 3.
g. Furnace temp check
h. System check
i. Leak check
j. Seal check

6. Instrument

- Load sample in autosampler
- Enter sample details onto software
- Press Analyze

7. Calibration Samples

- Nitrogen %: 6.48 ± 0.09
- Carbon % : 72.48 ± 0.25
- Sulphur % : 7.47 ± 0.05

8. Analysis Parameters

Furnace Temperature	1350ºC
TE Cooler Temperature	5°C
Dehydration Time	0 seconds
Purge Cycles	2 seconds

Element Parameters

Baseline Delay Time	6 seconds	Minimum Analysis Time	35 seconds
Comparator Level	100.00		
Endline Time	2 seconds		
Conversion Factor	1.00		
Significant Digits	5		
TC Baseline Time	10 seconds		

Burn Profile

Burn Cycle	Lance	Purge	FlowTime
1	Off	On	5 seconds
2	On	On	5 seconds
3	On	Off	END

Ballast Parameters

Equilibrate Time	30 seconds
Note Filled Timeout	300 seconds

Aliquot Loop

Equilibrate Pressure Time	4 seconds	High Precision	Yes
High Speed	No		

9. Procedure

1. Prepare instrument for operation as outlined in 4 above.
2. Condition the system by analysing 3-5 blanks (crucible is not required).
3. Determine blank.
    - Enter 1.0000 g mass into Sample Login (F3) using Blank as the sample name.
    - Transfer crucible to the appropriate position of the autoloader.
    - Repeat steps 3a through 3c a minimum of three times.
    - Initiate the analysis sequence (F5).
    - Set the blank following the procedure outlined in the operator's instruction manual.
4. Calibrate.
    - Note: Multi-point (fractional weight or multiple calibration samples) may be used to calibrate if desired.
    - A TruMac can be calibrated using several replicates of a single mass range (0.1 g) of Ammonium Nitrate utilizing a single standard calibration. This is a cost-effective and simple process.
    - The calibration can be verified by analyzing different compounds such as Ammonium
    - Sulfate (0.1 g) and/or Urea (0.1 g).
5. Analyze Samples (powder/granular).
    - Weigh ~0.1 g sample into a Crucible; enter mass and sample identification into Sample Login (F3).
    - Transfer crucible to the appropriate position of the autoloader.
    - Repeat steps 5a through 5c for each sample to be analyzed.
    - Initiate the analysis sequence (F5).
6. Analyze Samples (liquid).
    - Place a Nickel Boat Liner into a Crucible.
    - Weigh ~0.1 to 0.15 g of liquid fertilizer into the Nickel Boat Liner; enter mass and sample identification into Sample Login (F3).
    - Transfer crucible to the appropriate position of the autoloader.
    - Repeat steps 6a through 6c for each liquid sample to be analyzed.
    - Initiate the analysis sequence (F5).

10. Shut Down

k. Go to diagnostics- set furnace temp to 200 degrees celcius standby.
l. Export data to excel and save under documents.
m. Switch off Leco software and computer.
n. Switch off the autoloader.
o. Leave the instrument on if used daily.

APPROVAL OF STANDARD OPERATING PROCEDURE

PRG Head: Prof C.A. Buckley

Signature: .....
Date: .....

Author: Merlien Reddy

Signature: .....
Date: .....

APPENDIX 3: RAW DATA FOR PHASE 1 AND 2

Table A 1: Temperature and humidity data for phase 1

Date	Relative Humidity (%)	Temperature (°C)
21/09/2017	88.49	23.65
21/09/2017	88.47	23.46
21/09/2017	89.39	22.94
21/09/2017	87.91	23.54
21/09/2017	85.79	23.65
21/09/2017	81.69	24.36
21/09/2017	71.62	27.2
21/09/2017	61.88	31.3
21/09/2017	57.2	33.42
21/09/2017	52.47	35.59
21/09/2017	53.47	35.03
21/09/2017	54.2	33.23
21/09/2017	57.17	33.05
21/09/2017	63.05	29.49
21/09/2017	68.5	28.71
21/09/2017	82.68	24.35
21/09/2017	82.1	23.24
21/09/2017	82.77	22.54
21/09/2017	81.81	22.6
21/09/2017	80.84	22.73
21/09/2017	79.73	22.68
21/09/2017	81.5	21.28
21/09/2017	82.24	22.78
20/09/2017	84.57	21.75
20/09/2017	84.01	22.26
20/09/2017	84.12	22.26
20/09/2017	82.23	23.01
20/09/2017	83.66	22.62
20/09/2017	83.03	22.3

Table A 1: Temperature and humidity data for phase 1 (continued)

20/09/2017	82.5	22.31
20/09/2017	77.93	23.13
20/09/2017	76.48	22.84
20/09/2017	76.87	23.42
20/09/2017	76.15	23.83
20/09/2017	80.4	23.13
20/09/2017	82.71	22.93
20/09/2017	81.34	22.78
20/09/2017	78.93	22.71
20/09/2017	72.82	23.75
20/09/2017	76.2	23.44
20/09/2017	80.15	22.62
20/09/2017	79.73	22.24
20/09/2017	77.28	22.19
20/09/2017	77.62	22.67
20/09/2017	77.06	22.54
20/09/2017	76.92	22.35
20/09/2017	71.17	23.34
19/09/2017	74.86	23.18
19/09/2017	76.99	22.99
19/09/2017	76.78	23.21
19/09/2017	80.3	22.65
19/09/2017	77.23	23.24
19/09/2017	75.27	23.92
19/09/2017	71.58	25.12
19/09/2017	67.76	28.04
19/09/2017	62.16	29.26
19/09/2017	62.16	29.26
19/09/2017	58.2	31.62
19/09/2017	53.42	34.63
19/09/2017	48.93	35.16
19/09/2017	49.39	38.66

Table A 1: Temperature and humidity data for phase 1 (continued)

19/09/2017	57.94	36.14
19/09/2017	75.36	31.31
19/09/2017	86.61	27.04
19/09/2017	90.16	23.42
19/09/2017	85.79	21.97
19/09/2017	82.36	22.75
19/09/2017	82.92	23.18
19/09/2017	81.69	23.53
19/09/2017	86.26	22.68
19/09/2017	85.52	22.42
18/09/2017	85.94	23.31
18/09/2017	85.91	22.79
18/09/2017	88.34	22.46
18/09/2017	88.87	23.14
18/09/2017	90.24	22.62
18/09/2017	87.88	22.87
18/09/2017	80.24	23.89
18/09/2017	74.88	26.53
18/09/2017	67.56	29.61
18/09/2017	70.24	27.75
18/09/2017	67.25	31.49
18/09/2017	77.47	26.18
18/09/2017	81.82	25.79
18/09/2017	85.83	24.33
18/09/2017	88.1	22.9
18/09/2017	87.23	22.34
18/09/2017	84.93	22.51
18/09/2017	83.43	22.65
18/09/2017	83.73	22.87
18/09/2017	84.15	22.73
18/09/2017	83.57	22.56
18/09/2017	85.62	22.59

Table A 1: Temperature and humidity data for phase 1 (continued)

18/09/2017	86.04	23.21
17/09/2017	81.2	23.24
17/09/2017	84.2	23.01
17/09/2017	84.78	23.13
17/09/2017	81.08	23.33
17/09/2017	81.91	22.74
17/09/2017	82.52	23.24
17/09/2017	86.65	22.54
17/09/2017	81.34	23.27
17/09/2017	76.16	25.99
17/09/2017	71.4	27.92
17/09/2017	66.15	32.56
17/09/2017	69.95	29.68
17/09/2017	68.36	29.98
17/09/2017	75.53	25.83
17/09/2017	82.77	23.77
17/09/2017	80.7	21.94
17/09/2017	76.01	23.43
17/09/2017	74.11	23.9
17/09/2017	77.32	21.82
17/09/2017	74.71	23.64
17/09/2017	76.58	23
17/09/2017	72.98	23.83
17/09/2017	76	22.55
17/09/2017	72.43	22.92
16/09/2017	73.31	23.03
16/09/2017	75.73	23
16/09/2017	72.26	23.14
16/09/2017	71.14	21.98
16/09/2017	72.15	21.98
16/09/2017	73.29	21.74
16/09/2017	72.77	21.5

Table A 1: Temperature and humidity data for phase 1 (continued)

16/09/2017	72.16	28.09
16/09/2017	66.3	32.33
16/09/2017	63.58	33.75
16/09/2017	59.08	37.06
16/09/2017	57.05	36.99
16/09/2017	64.03	35.72
16/09/2017	68.95	33.78
16/09/2017	87.52	30.26
16/09/2017	97.63	25.99
16/09/2017	91.53	22.57
16/09/2017	86.4	22.59
16/09/2017	90.61	22.2
16/09/2017	87.45	23.61
16/09/2017	90	22.38
16/09/2017	90.58	23.28
16/09/2017	94.03	22.46
16/09/2017	93.2	23.21
15/09/2017	92.64	22.94
15/09/2017	94.05	22.63
15/09/2017	92.17	22.88
15/09/2017	91.64	23.48
15/09/2017	91.88	22.89
15/09/2017	91.88	22.89
15/09/2017	88.57	23.2
15/09/2017	84.14	23.44
15/09/2017	81.16	23.74
15/09/2017	81.6	24.06
15/09/2017	76.75	25.51
15/09/2017	75.66	26.54
15/09/2017	70.52	28.32
15/09/2017	78.73	28.75
15/09/2017	89.11	24.91

Table A 1: Temperature and humidity data for phase 1 (continued)

15/09/2017	92.47	23.17
15/09/2017	94.42	22.74
15/09/2017	94.21	23.14
15/09/2017	95.23	22.58
15/09/2017	93.28	23.07
15/09/2017	93	23.08
15/09/2017	93.01	22.77
15/09/2017	92.64	22.8
15/09/2017	92.15	22.49
15/09/2017	91.55	22.86
14/09/2017	90.51	22.78
14/09/2017	89.55	22.9
14/09/2017	91.32	21.9
14/09/2017	85.78	23.38
14/09/2017	87.05	22.61
14/09/2017	88.92	21.98
14/09/2017	83.79	23.6
14/09/2017	82.88	22.94
14/09/2017	76.59	24.56
14/09/2017	77.22	25.82
14/09/2017	81.74	25.14
14/09/2017	87.31	24.12
14/09/2017	87.02	23.86
14/09/2017	90.97	23.34
14/09/2017	90.71	22.74
14/09/2017	92.7	22.8
14/09/2017	91.34	22.44
14/09/2017	91.16	22.56
14/09/2017	90.53	22.32
14/09/2017	91.39	22.68
14/09/2017	91.04	23.06
14/09/2017	91.91	23.05

Table A 1: Temperature and humidity data for phase 1 (continued)

14/09/2017	88.42	23.08
13/09/2017	88.52	23.41
13/09/2017	89.78	22.93
13/09/2017	85.18	23.57
13/09/2017	85.98	23.61
13/09/2017	85.96	23.69
13/09/2017	85.96	24.13
13/09/2017	81.73	24.57
13/09/2017	74.72	21.6
13/09/2017	72.3	23.47
13/09/2017	68.73	25.23
13/09/2017	63.83	26.88
13/09/2017	61.9	28.13
13/09/2017	62.55	27.62
13/09/2017	65.6	27.75
13/09/2017	71.19	32.68
13/09/2017	88.95	28.15
13/09/2017	91.55	23.65
13/09/2017	91.86	20.91
13/09/2017	88.23	20.49
13/09/2017	88.42	19.84
13/09/2017	88.55	19.79
13/09/2017	90.26	20.49
13/09/2017	90.03	20.12
13/09/2017	90.4	21.03
09/12/2017	90.22	21.43
09/12/2017	90.26	22.02
09/12/2017	89.77	22.64
09/12/2017	88.83	22.93
09/12/2017	86.6	23.09
09/12/2017	80.74	24.2
09/12/2017	72.57	24.16

Table A 1: Temperature and humidity data for phase 1 (continued)

09/12/2017	67.89	24.16
09/12/2017	65.08	25.86
09/12/2017	59.42	27.2
09/12/2017	61.93	28.38
09/12/2017	63.28	28.92
09/12/2017	61.83	29.11
09/12/2017	60.9	34.69
09/12/2017	85.56	29.4
09/12/2017	89.42	23.43
09/12/2017	86.72	20.24
09/12/2017	85.31	19.46
09/12/2017	85.57	19.17
09/12/2017	84.15	20.76
09/12/2017	86.53	19.5
09/12/2017	84.88	20.38
09/12/2017	87.13	20.21
09/12/2017	87.2	20.52
09/11/2017	87.01	20.98
09/11/2017	87.23	21.59
09/11/2017	86.93	22.11
09/11/2017	86.49	22.55
09/11/2017	84.64	23.18
09/11/2017	80.20	24.75
09/11/2017	68.61	28.8
09/11/2017	73.13	25.44
09/11/2017	63.42	26.84
09/11/2017	61.29	28.68
09/11/2017	58.59	29.41
09/11/2017	56.84	29.77
09/11/2017	63.77	28.48
09/11/2017	63.89	26.78
09/11/2017	65.97	26

Table A 1: Temperature and humidity data for phase 1 (continued)

09/11/2017	85.87	29.79
09/11/2017	94.09	24.9
09/11/2017	93.18	24.07
09/11/2017	93.12	24.16
09/11/2017	92.22	23.92
09/11/2017	91.55	23.81
09/11/2017	91.63	24.04
09/11/2017	90.7	23.92
09/11/2017	90.64	23.98
09/10/2017	90.61	23.99
09/10/2017	91.7	24.42
09/10/2017	90.58	24.3
09/10/2017	88.71	24.56
09/10/2017	88.14	24.98
09/10/2017	85.52	25.35
09/10/2017	82.01	26.22
09/10/2017	79.68	27.5
09/10/2017	78.74	28.94
09/10/2017	76.59	30.44
09/10/2017	73.7	31.95
09/10/2017	72.06	33.04
09/10/2017	77	30.82
09/10/2017	81.34	29.12
09/10/2017	88.58	27.06
09/10/2017	91.89	24.7
09/10/2017	90.88	23.44
09/10/2017	90.22	23.23
09/10/2017	89.67	23.03
09/10/2017	89.39	23.03
09/10/2017	88.84	23.18
09/10/2017	88.07	23
09/10/2017	86.97	23.08

Table A 1: Temperature and humidity data for phase 1 (continued

09/09/2017	84.02	23.17
09/09/2017	82.28	22.56
09/09/2017	80.63	22.57
09/09/2017	79.93	22.62
09/09/2017	81.98	22.19
09/09/2017	80.27	22.9
09/09/2017	75.5	24.68
09/09/2017	64.91	29.03
09/09/2017	57.39	33.34
09/09/2017	56.33	36.18
09/09/2017	51.67	38.03
09/09/2017	46.61	39.57
09/09/2017	43.75	40.43
09/09/2017	46.95	39.15
09/09/2017	53.3	36.24
09/09/2017	63.74	32.47
09/09/2017	87.52	26.55
09/09/2017	80.12	21.99
09/09/2017	75.25	22.17
09/09/2017	72.68	22.44
09/09/2017	75.35	21.57
09/09/2017	72.31	23.1
09/09/2017	75.61	22.67
09/09/2017	78.88	22.6
09/08/2017	73.94	22.67
09/08/2017	74.1	22.48
09/08/2017	74.66	22.34
09/08/2017	74.94	22.72
09/08/2017	74.1	23.16
09/08/2017	78.49	22.21
09/08/2017	76.33	22.92
09/08/2017	65.57	26.84

Table A 1: Temperature and humidity data for phase 1 (continued)

09/08/2017	60.81	23.16
09/08/2017	59.01	24.53
09/08/2017	61.43	25.94
09/08/2017	56.4	26.73
09/08/2017	57.02	26.87
09/08/2017	50.1	30.32
09/08/2017	49.35	34.56
09/08/2017	64.09	31.11
09/08/2017	80.34	27.19
09/08/2017	76.7	23.23
09/08/2017	75.99	23.1
09/08/2017	78.73	21.96
09/08/2017	77.44	23.18
09/08/2017	78.7	23.1
09/08/2017	79.57	22.61
09/08/2017	78.18	22.42
09/07/2017	80.62	21.57
09/07/2017	81.76	22.16
09/07/2017	83.34	23.04
09/07/2017	83.73	23.6
09/07/2017	84.46	23.72
09/07/2017	82.24	23.24
09/07/2017	75.42	24.36
09/07/2017	68.95	27.48
09/07/2017	62.18	26.62
09/07/2017	54.3	28.18
09/07/2017	52.15	29.01
09/07/2017	53.98	30.31
09/07/2017	54.16	29.8
09/07/2017	56.04	28.97
09/07/2017	61.04	27.94
09/07/2017	70.04	26.33

Table A 1: Temperature and humidity data for phase 1 (continued

09/07/2017	83.98	26.35
09/07/2017	80.31	23.04
09/07/2017	77.74	22.49
09/07/2017	73.62	23.02
09/07/2017	73.55	23.25
09/07/2017	76.16	21.64
09/07/2017	75.87	22.25
09/07/2017	76.45	22.13
09/06/2017	77.99	22.23
09/06/2017	79.55	22.38
09/06/2017	76.78	23.54
09/06/2017	81.29	22.4
09/06/2017	81.29	23.68
09/06/2017	84.15	22.24
09/06/2017	80.14	24.39
09/06/2017	71.81	26.41
09/06/2017	72.14	20.98
09/06/2017	66.31	23.28
09/06/2017	58.05	26.38
09/06/2017	58.12	27.76
09/06/2017	56.63	28.64
09/06/2017	55.25	35.3
09/06/2017	61.39	30.82
09/06/2017	68.15	29.11
09/06/2017	83.56	23.81
09/06/2017	80.94	22.97
09/06/2017	83.11	22.99
09/06/2017	80.43	23.52
09/06/2017	78.79	23.57
09/06/2017	81.37	23.37
09/06/2017	84.33	22.9
09/06/2017	85.27	22.89

Table A 2: The growth of the BSF larvae over the 13-day period

	Growth of BSF Larvae in g per 30 larvae						
	Day 0	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
1	0.1787	1.3464	1.9876	2.3955	2.4506	2.5063	2.098
2	0.1787	1.767	2.0466	2.2301	2.4359	2.5687	2.0477
3	0.1787	0.9841	2.0564	2.3496	2.569	2.7758	2.766
4	0.1787	1.5819	2.0234	2.4812	2.5151	2.5704	2.9494
5	0.1787	1.7264	2.2018	2.1315	2.3584	2.649	2.3431
6	0.1787	1.1032	1.4583	1.6345	1.872	2.0482	2.1387
7	0.1787	1.1026	2.13	2.4053	2.4645	2.5322	2.7982
8	0.1787	1.0446	2.0703	2.1594	2.4159	2.6621	2.6827
9	0.1787	0.7033	2.13	2.3798	2.6801	2.7158	3.3498
10	0.1787	0.8649	1.9701	2.0163	2.3574	2.5715	2.955
11	0.1787	1.0485	1.6776	1.93	1.9944	2.2862	2.3094
12	0.1787	1.6838	1.9634	2.1192	2.1823	2.2547	2.3572

Table A 3: Change in characteristics of the trials

Trial	Initial wet mass [kg]	Initial dry mass [kg]	Initial volatile solids [kg]	Final wet mass [kg]	Final ash solids [kg]	Final volatile solids [kg]
1	20.64	8.38	2.79	15.75	8.25	2.13
2	21.54	8.75	2.91	18.2	9.12	2.77
3	21.75	8.83	2.94	19.6	11.21	2.63
4	20.45	8.30	2.76	18.2	9.01	2.73
5	20.75	8.42	2.80	17.75	8.63	2.73
6	21.61	8.77	2.92	18.1	9.74	2.26
7	15.85	6.44	2.14	12.1	6.12	1.84
8	22.05	8.95	2.98	19.55	9.27	3.17
9	20.10	8.16	2.71	17.1	8.99	2.43
10	17.71	7.19	2.39	19.2	9.64	2.86
11	20.64	8.38	2.79	20.35	9.85	3.05
12	24.40	9.91	3.29	20.8	10.48	3.24
13	20.91	8.49	2.82	17.5	8.58	2.64

Table A 3: Heavy metal analysis for Phase 1 substrates

Sample description	**Pb 220.353 [mg/kg]**	**Cd 214.439 [mg/kg]**	**As 193.696 [mg/kg]**	**Zn 213.857 [mg/kg]**	**Cr 267.716 [mg/kg]**	**Ni 221.648 [mg/kg]**	**Cu 213.598 [mg/kg]**
Formulated feed	96.50	0.42	12.26	282.68	54.80	BDL*	80.41
UDDT sludge before mixing	65.59	0.65	0.012	170.63	36.01	23.54	74.1454
UD after mixing	62.09	0.33	BDL*	204.65	20.03	18.81	59.56
Starting larvae	77.04	0.32	BDL*	261.21	12.41	7.20	40.27

**BDL refers to below detection limit.*

Table A 4: Heavy metal analysis data for mature BSF larvae for Phase 1

Sample description	**Pb 220.353 [mg/kg]**	**Cd 214.439 [mg/kg]**	**As 193.696 [mg/Kg]**	**Zn 213.857 [mg/kg]**	**Cr 267.716 [mg/kg]**	**Ni 221.648 [mg/kg]**	**Cu 213.598 [mg/kg]**
BSF larvae	72.30	0.51	0.95	239.89	13.88	32.66	59.07
	77.33	0.11	BDL*	223.18	15.68	10.89	37.08
	71.19	0.45	1.65	221.65	14.54	16.83	46.76
	84.26	0.29	BDL*	260.44	14.58	41.17	54.43
	84.49	0.49	BDL*	273.54	18.43	13.17	39.29
	66.89	0.10	BDL*	219.40	13.31	26.51	43.13
	64.64	0.029	0.46	189.97	11.59	13.03	41.80
	83.96	0.93	4.56	323.66	17.69	20.34	59.46

Table A 5: Heavy metal analysis data for residue for Phase 1

Sample description	Pb 220.353 [mg/kg]	Cd 214.439 [mg/kg]	As 193.696 [mg/kg]	Zn 213.857 [mg/kg]	Cr 267.716 [mg/kg]	Ni 221.648 [mg/kg]	Cu 213.598 [mg/kg]
UDDT residue	66.41	0.30	BDL	220.86	23.69	17.63	80.07
	68.77	0.38	0.89	166.90	17.16	17.86	51.98
	69.07	0.16	BDL	198.29	27.54	20.10	75.21
	76.20	0.56	BDL	198.37	26.23	20.27	73.18
	74.05	0.50	BDL	213.95	24.52	17.28	73.08
	87.06	0.42	BDL	280.08	26.42	18.36	67.14
	87.73	0.25	1.21	268.24	30.14	27.15	87.95
	69.92	0.17	1.61	226.15	25.72	19.07	82.43
	64.97	0.33	BDL	206.60	25.30	25.38	72.62
	78.51	0.41	BDL	207.37	24.25	23.12	66.48
	45.19	0.15	BDL	131.19	16.96	11.45	35.49
	72.83	0.52	0.12	395.57	32.58	33.32	81.35
	77.22	0.34	BDL	207.47	28.23	18.14	56.08

Table A 6: Temperature and humidity data for Phase 2

Date	Relative Humidity (%)	Temperature (°C)
28/11/2017	56.17	28
28/11/2017	55.37	28.49
28/11/2017	56.24	28.07
28/11/2017	55.43	28.4
28/11/2017	56.44	28.13
28/11/2017	55.94	28.21
28/11/2017	55.75	28.17
28/11/2017	55.23	28.21
28/11/2017	55.64	28.11
28/11/2017	56.91	27.74
28/11/2017	57.72	27.32
28/11/2017	57.65	26.88
28/11/2017	58.8	26.77
28/11/2017	58.77	26.37
28/11/2017	61.02	25.43
28/11/2017	66.34	23.33
28/11/2017	75.03	20.82
28/11/2017	78.06	19.79
28/11/2017	79.66	19.38
28/11/2017	80.87	19.32
28/11/2017	79.73	19.22
28/11/2017	79.33	19.59
28/11/2017	76.81	19.9
28/11/2017	74.89	20.05
27/11/2017	78.85	19.82
27/11/2017	76.58	20.01
27/11/2017	72.3	20.55
27/11/2017	68.72	20.75
27/11/2017	66.83	20.91
27/11/2017	65.05	21.19

Table A 6: Temperature and humidity data for phase 2(continued)

27/11/2017	64.77	21.53
27/11/2017	63.58	21.71
27/11/2017	67.65	21.54
27/11/2017	67.15	21.94
27/11/2017	65.59	21.96
27/11/2017	67.13	22.68
27/11/2017	68.85	22.48
27/11/2017	68.87	22.39
27/11/2017	66.09	22.2
27/11/2017	68.16	21.36
27/11/2017	72.82	20.55
27/11/2017	76.38	18.89
27/11/2017	77.58	18.72
27/11/2017	79.13	18.84
27/11/2017	80.29	19.3
27/11/2017	80.91	19.49
27/11/2017	81.34	19.84
27/11/2017	81.8	20.17
26/11/2017	78.6	20.7
26/11/2017	80.2	21
26/11/2017	77.77	21.44
26/11/2017	77.18	21.55
26/11/2017	74.26	22.25
26/11/2017	75.48	22.67
26/11/2017	71.02	23.61
26/11/2017	66.1	24.39
26/11/2017	63.29	26
26/11/2017	62.02	27.01
26/11/2017	63.03	27.12
26/11/2017	65.98	26.35
26/11/2017	64.4	26.65
26/11/2017	67.54	25.01

Table A 6: Temperature and humidity data for phase 2(continued)

26/11/2017	70.46	23.93
26/11/2017	74.65	23.09
26/11/2017	82.58	20.52
26/11/2017	85.46	18.73
26/11/2017	83.94	17.58
26/11/2017	83.28	18.03
26/11/2017	83.49	18.22
26/11/2017	83.34	18.7
26/11/2017	84.25	19.04
26/11/2017	84.09	19.91
25/11/2017	84.01	19.99
25/11/2017	83.94	20.19
25/11/2017	82.8	20.25
25/11/2017	80.6	20.42
25/11/2017	76.03	20.84
25/11/2017	70.49	21.96
25/11/2017	70.18	22.82
25/11/2017	66.79	23.86
25/11/2017	67.77	24.05
25/11/2017	74.36	23.3
25/11/2017	72.08	22.92
25/11/2017	76.26	22.41
25/11/2017	78.2	23.03
25/11/2017	82.76	20.78
25/11/2017	84.39	20.62
25/11/2017	85.97	19.43
25/11/2017	86.5	19.51
25/11/2017	86.95	19.16
25/11/2017	86.51	19.14
25/11/2017	86.41	19.36
25/11/2017	87.25	19.78
25/11/2017	88.36	19.92

Table A 6: Temperature and humidity data for phase 2(continued)

25/11/2017	88.47	19.86
25/11/2017	87.91	20.2
24/11/2017	88.08	20.18
24/11/2017	87.26	20.69
24/11/2017	88.97	20.72
24/11/2017	89.49	20.72
24/11/2017	89.63	21.17
24/11/2017	88.84	21.22
24/11/2017	88.88	21.56
24/11/2017	88.16	21.5
24/11/2017	87.6	21.68
24/11/2017	87.07	21.54
24/11/2017	86.26	21.68
24/11/2017	84.1	21.68
24/11/2017	80.3	22.2
24/11/2017	79.17	22.51
24/11/2017	79.47	22.15
24/11/2017	80.55	21.39
24/11/2017	83.07	20.82
24/11/2017	85.73	20.33
24/11/2017	85.37	20.03
24/11/2017	84.89	19.9
24/11/2017	84.59	19.78
24/11/2017	83.27	19.65
24/11/2017	82.89	19.7
24/11/2017	82.57	19.55
23/11/2017	81.47	19.72
23/11/2017	81.16	19.47
23/11/2017	81.06	19.22
23/11/2017	79.22	19.47
23/11/2017	77.4	19.76
23/11/2017	77.96	20.62

Table A 6: Temperature and humidity data for phase 2(continued)

23/11/2017	75.87	21.02
23/11/2017	70.93	21.57
23/11/2017	69.14	21.77
23/11/2017	67.69	21.72
23/11/2017	67.25	22.41
23/11/2017	70.22	22.2
23/11/2017	72.63	22.05
23/11/2017	76.73	21.75
23/11/2017	81.71	20.67
23/11/2017	82.53	20.16
23/11/2017	82.98	19.99
23/11/2017	83.03	19.82
23/11/2017	83.99	19.72
23/11/2017	83.07	19.99
23/11/2017	82.72	19.9
23/11/2017	83.08	19.73
23/11/2017	82.2	19.8
23/11/2017	82.89	19.64
22/11/2017	83.94	19.78
22/11/2017	84.49	19.98
22/11/2017	85.9	20.1
22/11/2017	83.88	20.53
22/11/2017	83.84	20.48
22/11/2017	83.59	20.46
22/11/2017	84.02	20.63
22/11/2017	84.15	20.82
22/11/2017	87.17	20.34
22/11/2017	86.21	21.2
22/11/2017	79.47	22.47
22/11/2017	77.79	23.34
22/11/2017	76.19	24.04
22/11/2017	75.1	24.5

Table A 6: Temperature and humidity data for phase 2(continued)

22/11/2017	72.55	24.33
22/11/2017	74.85	22.92
22/11/2017	78.03	22.25
22/11/2017	77.12	22.41
22/11/2017	76.71	22.53
22/11/2017	78.22	22.67
22/11/2017	78.68	22.87
22/11/2017	78.17	22.92
22/11/2017	79.54	22.99
22/11/2017	80.15	23.19
21/11/2017	80.56	23.36
21/11/2017	80.22	23.61
21/11/2017	81.53	23.97
21/11/2017	81.48	24.19
21/11/2017	80.86	24.52
21/11/2017	76.34	26.16
21/11/2017	73.89	27.05
21/11/2017	77.06	26.35
21/11/2017	72.36	27.63
21/11/2017	75.57	26.86
21/11/2017	78.09	25.6
21/11/2017	73.51	27.12
21/11/2017	74.56	27.12
21/11/2017	73.98	26.85
21/11/2017	75.24	26.37
21/11/2017	78.37	25.28
21/11/2017	80.6	24.75
21/11/2017	83.3	23.23
21/11/2017	83.8	22.7
21/11/2017	83.43	22.67
21/11/2017	81.21	22.02
21/11/2017	79.34	22.48

Table A 6: Temperature and humidity data for phase 2(continued)

21/11/2017	78.03	23.05
21/11/2017	77.12	23.5
20/11/2017	75.69	23.9
20/11/2017	74.85	24.46
20/11/2017	74.56	25.05
20/11/2017	74.23	25.39
20/11/2017	70.59	26.32
20/11/2017	65.55	27.9
20/11/2017	60.74	29.43
20/11/2017	59.32	29.82
20/11/2017	58.07	30.14
20/11/2017	57.53	30.57
20/11/2017	56.46	30.94
20/11/2017	55.14	31.3
20/11/2017	54.33	31.6
20/11/2017	53.43	31.82
20/11/2017	52.38	31.93
20/11/2017	51.96	31.97
20/11/2017	50.48	32.78
20/11/2017	50.17	32.93
20/11/2017	50.65	33.3
20/11/2017	50.41	33.34
20/11/2017	50.41	33.34
20/11/2017	50.19	33.65
20/11/2017	49.47	33.9
20/11/2017	50.15	33.85
20/11/2017	48.9	34.21
20/11/2017	48.72	34.4
20/11/2017	49.82	34.49
20/11/2017	50.03	34.39
20/11/2017	48.19	34.78
20/11/2017	50.42	34.56

Table A 6: Temperature and humidity data for phase 2(continued)

20/11/2017	50.56	34.57
20/11/2017	51.25	34.51
20/11/2017	50.93	34.52
20/11/2017	50.41	34.25
20/11/2017	50.96	34.13
20/11/2017	51.36	34.27
20/11/2017	51.14	33.88
20/11/2017	51.15	33.78
20/11/2017	50.41	34.13
20/11/2017	51.24	33.93
20/11/2017	51.76	33.75
20/11/2017	52.77	33.42
20/11/2017	52.41	33.3
20/11/2017	53.5	33.2
20/11/2017	54.82	32.78
20/11/2017	60.49	31.08
20/11/2017	69.94	28.72
20/11/2017	76.29	27.07
20/11/2017	85.55	24.04
20/11/2017	88.74	22.28
20/11/2017	88.19	21.68
20/11/2017	87.55	20.77
20/11/2017	85.72	20.54
20/11/2017	85.91	20.84
20/11/2017	85.65	21.45
20/11/2017	85.26	22.2
19/11/2017	82.87	22.71
19/11/2017	81.24	23.34
19/11/2017	80.31	23.6
19/11/2017	80.13	23.91
19/11/2017	79.37	24.36
19/11/2017	77.11	25.09

Table A 6: Temperature and humidity data for phase 2(continued)

19/11/2017	74.39	26.23
19/11/2017	71.61	26.97
19/11/2017	70.08	27.76
19/11/2017	69.54	27.95
19/11/2017	69.75	27.62
19/11/2017	67.55	27.96
19/11/2017	64.17	28.59
19/11/2017	62.3	28.15
19/11/2017	64.85	25.97
19/11/2017	70.32	23.86
19/11/2017	80.17	19.69
19/11/2017	81.88	17.22
19/11/2017	80.57	16.28
19/11/2017	80.28	16.17
19/11/2017	80.05	16.57
19/11/2017	80.56	16.64
19/11/2017	79.93	17.22
19/11/2017	80.62	17.82
18/11/2017	81.22	18.64
18/11/2017	82.08	19.1
18/11/2017	81.26	19.91
18/11/2017	79.69	20.49
18/11/2017	77.89	21.57
18/11/2017	72.08	23.04
18/11/2017	66.31	24.26
18/11/2017	60.77	26.06
18/11/2017	61.26	26
18/11/2017	58.79	26
18/11/2017	58.15	26.37
18/11/2017	59.65	26.2
18/11/2017	59.48	26.54
18/11/2017	58.18	26.97

Table A 6: Temperature and humidity data for phase 2(continued)

18/11/2017	57.69	27.15
18/11/2017	56.59	27.48
18/11/2017	57.53	27.22
18/11/2017	57.74	26.96
18/11/2017	57.71	26.86
18/11/2017	58.94	26.46
18/11/2017	59.88	26.37
18/11/2017	61.9	25.54
18/11/2017	62.7	25.46
18/11/2017	62.03	25.75
18/11/2017	66.25	23.91
18/11/2017	71.6	21.96
18/11/2017	79.73	19.19
18/11/2017	82.15	16.63
18/11/2017	80.45	15.67
18/11/2017	79.97	16.31
18/11/2017	79.95	16.48
18/11/2017	79.64	17.09
18/11/2017	79.86	17.84
18/11/2017	79.86	17.84
18/11/2017	77.74	18.73
17/11/2017	78.38	18.88
17/11/2017	78.02	18.99
17/11/2017	78.32	19.07
17/11/2017	77.95	19.28
17/11/2017	77.2	19.5
17/11/2017	76.08	19.86
17/11/2017	74.76	20.12
17/11/2017	70.06	20.21
17/11/2017	66.77	21.02
17/11/2017	63.96	21.72
17/11/2017	65.53	21.69

Table A 6: Temperature and humidity data for phase 2(continued)

17/11/2017	64.13	21.91
17/11/2017	54.93	22.18
17/11/2017	55.66	22.03
17/11/2017	55.13	21.99
17/11/2017	56.93	21.94
17/11/2017	55.51	21.77
17/11/2017	54.4	22.09
17/11/2017	56.06	22.17
17/11/2017	52.89	22.01
17/11/2017	53.91	22.14
17/11/2017	55.27	21.85
17/11/2017	54.06	21.69
17/11/2017	52.81	21.87
17/11/2017	52.81	21.87
17/11/2017	53.94	21.99
17/11/2017	50.86	21.79
17/11/2017	49.28	22.05
17/11/2017	48.91	22.13
17/11/2017	48.47	21.75
17/11/2017	49.45	21.75
17/11/2017	49.93	21.64
17/11/2017	48.9	21.67
17/11/2017	50.86	21.53
17/11/2017	51.47	21.46
17/11/2017	52.82	21.09
17/11/2017	52.44	20.82
17/11/2017	52.77	20.22
17/11/2017	54.71	19.94
17/11/2017	54.21	19.75
17/11/2017	54.88	19.58
17/11/2017	53.56	19.09
17/11/2017	64.12	17.02

Table A 6: Temperature and humidity data for phase 2(continued)

17/11/2017	70.81	15.24
17/11/2017	71.66	14.55
17/11/2017	70.26	14.87
17/11/2017	68.43	15.28
17/11/2017	71.04	15.36
17/11/2017	70.2	15.62
17/11/2017	68.69	16.22
16/11/2017	68.38	16.07
16/11/2017	68.07	16.35
16/11/2017	67.3	16.57
16/11/2017	67.6	16.72
16/11/2017	69.29	16.91
16/11/2017	74.06	17.14
16/11/2017	74.06	17.14
16/11/2017	73.75	17.64
16/11/2017	74.89	17.26
16/11/2017	80.31	16.79
16/11/2017	79.84	17.09
16/11/2017	81.11	17.18
16/11/2017	81.82	17.09
16/11/2017	79.26	16.52
16/11/2017	79.15	16.63
16/11/2017	81.66	15.79
16/11/2017	80.52	15.48
16/11/2017	81.24	15.26
16/11/2017	81.37	15.06
16/11/2017	80.6	15.22
16/11/2017	81.11	15.62
16/11/2017	81.53	15.8
16/11/2017	81.87	15.7
16/11/2017	80.95	15.85
16/11/2017	79.47	16.39

Table A 6: Temperature and humidity data for phase 2 (continued)

15/11/2017	80.74	17.09
15/11/2017	83.22	17.64
15/11/2017	85.52	18.2
15/11/2017	85.75	18.34
15/11/2017	85.94	18.91
15/11/2017	86.56	18.83
15/11/2017	85.72	18.95
15/11/2017	86.48	18.84
15/11/2017	84.96	19.19
15/11/2017	85.45	19.55
15/11/2017	87.71	19.43
15/11/2017	86.32	20.09
15/11/2017	88.52	21.43
15/11/2017	89.12	22.08
15/11/2017	89.39	22.79
15/11/2017	88.94	23.05
15/11/2017	88.69	22.92
15/11/2017	89.45	22.1
15/11/2017	88.53	21.94
15/11/2017	88.35	21.77
15/11/2017	87.83	21.49
15/11/2017	88.04	21.71
15/11/2017	89.13	21.82
15/11/2017	88.61	22.04

Table A 7: Stream mass and moisture content analysis for phase 2

Sample Point	Sample Description	Stream mass [wet basis kg]	Sample analysis (n=3)	Moisture Content [g H20/g wet sample]		
				Trial 1	Trial 2	Trial 3
1	Urine diversion sludge	1780	1	0.6235	0.6284	0.6739
1		1780	2	0.6255	0.6216	0.6588
1		1840	3	0.6093	0.6330	0.6549
2	Primary sludge	267	1	0.9848	0.9806	0.9884
2		250	2	0.9971	0.9917	0.9859
2		200	3	0.9977	0.9969	0.9855
3*	UD+ PS	260	1	0.6579	0.6765	0.7088
3*		280	2	0.6511	0.6759	0.7088
3*		320	3	0.6093	0.6758	0.6895
4	Food waste	28.84	1	0.4395	0.5088	0.4921
4		32.21	2	0.4363	0.4984	0.4966
4		35.7	3	0.4477	0.5093	0.4931
4	Larvae	7.21	1	0.6627	0.6829	0.7008
4		9.025	2	0.6638	0.6822	0.7066
4		5.1	3	0.6638	0.6851	0.6962
5	Residue	216	1	0.5639	0.5846	0.6103
5		168.75	2	0.5771	0.6086	0.6250
5		256.15	3	0.5124	0.6158	0.6315
5	Larvae	20.85	1	0.7417	0.7252	0.7582
5		26.3	2	0.7469	0.7249	0.7632
5		23.35	3	0.7454	0.7212	0.7563
5	Trash	15.75	1			
5		21	2			
5		11.5S5	3			

6	Dried larvae	13.65	1	0.7011
6		-	2	0.7020
6		-	3	0.7021

Table A 8: Total solids analysis for phase 2

Sample Point	Sample Description	Sample analysis (n=3)	Total Solids [g dry/g wet sample]		
			Trial 1	Trial 2	Trial 3
1	Urine diversion sludge	1	0.3765	0.3716	0.3261
1		2	0.3745	0.3784	0.3412
1		3	0.3907	0.3670	0.3451
2	Primary sludge	1	0.0152	0.0194	0.0116
2		2	0.0029	0.0083	0.0141
2		3	0.0023	0.0031	0.0145
3*	UD+ PS	1	0.3421	0.3235	0.2912
3*		2	0.3489	0.3241	0.2986
3*		3	0.3362	0.3242	0.3105
4	Food waste	1	0.5605	0.4912	0.5079
4		2	0.5637	0.5016	0.5034
4		3	0.5523	0.4907	0.5069
4	Larvae	1	0.3373	0.3171	0.2992
4		2	0.3362	0.3178	0.2934
4		3	0.3362	0.3149	0.3038
5	Residue	1	0.4361	0.4154	0.3750
5		2	0.4229	0.3914	0.3750
5		3	0.4876	0.3842	0.3685
5	Larvae	1	0.2583	0.2748	0.2418
5		2	0.2531	0.2751	0.2368
5		3	0.2546	0.2788	0.2437
6	Dried larvae	1	0.2989		
6		2	0.2980		
6		3	0.2979		

Table A 9: Volatile solids analysis for phase 2

Sample Point	Sample Description	Sample analysis (n=3)	Volatile Content [g VS/g dry sample]		
			Trial 1	**Trial 2**	**Trial 3**
1	Urine diversion sludge	1	0.3575	0.3376	0.3889
1		2	0.3570	0.3362	0.3804
1		3	0.3349	0.3411	0.3634
2	Primary sludge	1	0.8257	0.7855	0.7282
2		2	0.7002	0.7686	0.7497
2		3	0.7271	0.7318	0.7632
3*	UD+ PS	1	0.3560	0.3288	0.4144
3*		2	0.3549	0.3289	0.3898
3*		3	0.3486	0.3411	0.3813
4	Food waste	1	0.9034	0.9210	0.9002
4		2	0.9044	0.9322	0.9068
4		3	0.9056	0.9066	0.9066
4	Larvae	1	0.9097	0.9153	0.9119
4		2	0.9107	0.9165	0.9127
4		3	0.9108	0.9147	0.9123
5	Residue	1	0.3596	0.3138	0.3788
5		2	0.3679	0.3316	0.4113
5		3	0.3117	0.3528	0.4084
5	Larvae	1	0.7595	0.7981	0.8199
5		2	0.7579	0.7884	0.8091
5		3	0.7604	0.7856	0.8091
6	Dried larvae	1	0.7620		
6		2	0.7646		
6		3	0.7468		

Table A 10: Ash content analysis for phase 2

Sample Point	Sample Description	Ash Content [g ash/g dry sample]		
		Trial 1	**Trial 2**	**Trial 3**
1	Urine diversion sludge	0.6425	0.6624	0.6111
1		0.6430	0.6638	0.6196
1		0.6651	0.6589	0.6366
2	Primary sludge	0.1743	0.2145	0.2718
2		0.2998	0.2314	0.2503
2		0.2729	0.2682	0.2368
3*	UD + PS	0.6440	0.6712	0.5856
3*		0.6451	0.6711	0.6102
3*		0.6514	0.6577	0.6187
4	Food waste	0.0966	0.0790	0.0998
4		0.0893	0.0678	0.0932
4		0.0944	0.0739	0.0934
4	Larvae	0.0903	0.0847	0.0881
4		0.0893	0.0835	0.0873
4		0.0892	0.0853	0.0877
5	Residue	0.6404	0.6862	0.6212
5		0.6321	0.6684	0.5887
5		0.6883	0.6472	0.5916
5	Larvae	0.2405	0.2019	0.1801
5		0.2421	0.2116	0.1796
5		0.2396	0.2144	0.1909
6	Dried larvae	0.2380		
6		0.2354		
6		0.2532		

Table A 11: pH and feed depth analysis for phase 2

SP	Description	pH			feed depth/[mm]		
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1	UDDT	7.8	7.9	7.6			
1		8	7.87	7.57			
1		8	8.23	7.91			
2	PS	5.91	5.59	5.38			
2		6.52	5.63	5.33			
2		6.47	5.65	5.32			
3*	UD+ PS	7.9	8.33	7.88	40	55	45
3*		7.92	8.11	8.18	45	50	50
3*		7.91	8.11	7.44	55	55	60
5	Residue	7.8	7.16	6.62	35	35	30
5		7.92	7.13	6.75	30	40	40
5		7.95	6.97	6.89	35	30	35
5	Larvae						