INVESTIGATING THE PERFORMANCE OF BLACK SOLDIER FLY LARVAE (*HERMETIA ILLUCENS*) IN FECAL MATTER CO-DIGESTION FOR OPTIMUM PROTEIN

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Investigating the Performance of Black Soldier Fly Larvae (*Hermetia illucens*) in Fecal Matter Co-digestion for Optimum Protein

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

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LIST OF ABBREVIATIONS AND ACRONYMS

BSF	Black Soldier Fly
BSFL	Black Soldier Fly Larvae
CP	Crude Protein
DoE	Design of Experiment
DM	Dry Mass
FCR	Feed Conversion Rate
FS	Fecal Sludge
FSM	Fecal Sludge Management
JKUAT	Jomo Kenyatta University of Agriculture and Technology
MUST	Meru University of Science and Technology
OSS	On-Site Sanitation
SOBEE	School of Biosystems and Environmental Engineering
SRI	Sanitation Research Institute
SWEED	Soil, Water and Environmental Engineering Department
SDGs	Sustainable Development Goals
SFD	Shit Flow Diagrams
SOP	Standard Operating Procedures
TKN	Total Kjedahl Nitrogen
UDDTs	Urine Diverting Dehydrating Toilets
VIPL	Ventilated Improved Pit Latrines
R	Residue/Frass weight
W	Initial Feed weight
WR	Waste Reduction

ABSTRACT

Poor hygiene and limited access to safe sanitation, and large-scale open defecation, contribute to poor health, undermine economic growth, and pollute the environment. Thus, fecal waste management is an immediate and serious environmental problem facing urban municipalities, peri-urban and rural areas in low and middle-income countries. Residents in rural and urban informal settlements such as slums and refugee camps rely on On-Site Sanitation (OSS) technologies. Poor disposal of the fecal sludge results in environmental pollution and outbreak of diseases thus endangering education, productivity and life quality of the residents. However, fecal waste is a valuable resource that contains nutrients and energy value that are beneficial to human beings and the environment if reintegrated into the value chain. The Black Soldier Fly Larvae (BSFL) can act as an ecological engineer by co-digesting the fecal matter, adding value to it, reducing the volume and ultimately contributing to safe disposal of the end products. This study characterized the feed substrates which included urine-diverting dry toilets (UDDT) fecal matter from Kunene Primary School and kitchen waste from Meru University of Science and Technology cafeteria (1:0, 1:1, 2:1, 4:1 and 0:1) for their nutritive content. The effect of the co-digested substrates on waste weight reduction, *Hermetia illucens'* larval weight gain, and crude protein content during co-digestion was also evaluated. Samples of larvae were collected after every 2 days for larval determination and protein content analysis using the Kjeldahl method of nitrogen determination. The waste reduction index (WRI) was determined after 50% pupation. The larvae grew on all substrates yielding 33–39% dry matter (DM) protein content and larval weight ranging from 1.1 to 1.7 g per five larvae. Results indicate that a 1:1 co-digestion ratio resulted in the highest WRI, DM crude protein content (39%), and larval weight. It was also noted that waste reduction efficiency, growth performance, and protein content of BSFL were greatly influenced by the characteristics of the rearing substrate provided. This study used the circular economy-based approach which provides a win–win situation to sanitation provision and environmental management while realizing products with potential for livelihood improvement. The findings provide significant insights for process scale up.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Provision of sustainable sanitation is a global long-standing challenge, particularly in low-and-middle income countries. According to UNICEF & WHO (2020), 4.2 billion people, use sanitation services that leave human waste untreated, threatening human and environmental health. An estimated 673 million people have no toilets at all and practice open defecation, while nearly 698 million school-age children lacked basic sanitation services at their school (UNICEF & WHO, 2020). The most common sanitation concept in urban formal settlements is 'end-of-the-pipe-technology' in which a small volume of excreta is flushed with large volume of water and the mixture conveyed to wastewater treatment site (Maurya, 2012), yet water is a scarce resource. In addition, sewered sanitation systems are costly to install, maintain and employ complicated sewer network. More so, sewered systems result in loss of valuable and non-ending sources of nutrients, energy and fertilizer.

In low- and middle-income communities around the world, 13 per cent of the global population (0.9 billion people) used toilets or latrines where excreta were disposed of in situ (WHO & UNICEF., 2017). In Kenya particularly, only 12% of the national population have access to sewerage services, and approximately 5% of sewage is effectively treated (Mansour, Oyaya & Owor, 2017) due to failures of the sewerage system and inadequate wastewater treatment processes. Onsite sanitation (OSS) is commonly used in the peri-urban settlements and approximately 5.6 million people practice open defecation (Njuguna & Muruka, 2017), which exposes them to sanitation related illnesses. Therefore, effective treatment and management of human fecal waste is of great importance to prevent serious environmental and health effects. In addition,

fecal waste is a valuable resource that contains nutrients and energy value which are beneficial to human beings and the environment if re-integrated into the value chain. Thus, capturing the inherent value of fecal waste could alleviate the environmental constraints to a greater extent and simultaneously provide food, feed and other products of commercial interest.

Moreover, there is an urgent need not only to manage fecal waste, but also to add value to it. This study was carried out at the Meru University of Science and Technology Sanitation Research Centre (MUST SRI). MUST SRI is an ongoing project funded by the Newton-Utafiti fund which has identified the opportunity in fecal waste value addition using the Black Soldier Fly Larvae (BSFL). This project utilized the Urine Diverting Dehydrating Toilets (UDDT) technology for the containment of fecal matter in Kunene Primary School. The fecal waste was then fed to BSFL in the SRI production area for conversion to larval biomass for fish feed. However, the protein content of these larval biomass has not yet been determined.

Research focusing on the use of BSF larvae to manage biowaste such as municipal organic waste, swine, chicken and cattle manure has previously been done. For instance, in a study undertaken by Zheng et al. (2011), 1200 BSFL converted approximately 1248.6 g of fresh dairy manure into 273.4 g dry residue in 21 days. Bioconversion of organic waste into larval biomass had significant potential in production of high value products with simultaneous waste valorization (Surendra, Olivier, Tomberlin, Jha & Khanal, 2016). Besides, BSFL biowaste treatment offers environmentally friendly alternative with very low direct Green House Gas emissions and high reduction of global warming potential (Mertenat, Diener & Zurbrügg, 2019). Thus, the application of BSF larvae is emerging to be very a efficient green technology in bio-waste management.

In Kenya, Sanergy has been using Black Soldier Fly (BSF) systems to treat and upcycle organic waste such as manure, agricultural waste, food waste, and human sludge into

high protein (approximately 35%) and fat (approximately 30%) content of the harvested biomass, used as animal feed (IST-UTS and SNV, 2021). Shumo et al. (2019) reared BSFL on chicken manure (CM), brewers' spent grain (SG) and kitchen waste (KW) and reported a range of 33-41% crude protein. However, information pertaining to the optimization of protein from co-digested fecal waste using BSFL is not readily available in literature thus forming the basis of this particular study. Thus, returning the resource value of fecal matter into the economy through the BSFL reflects a paradigm shift towards a circular economy which focuses on closing loops through resource recovery (Lohri, Diener, Zabaleta, Mertenat & Zurbrugg, 2017).

As mentioned, fecal matter (FM) is a potential feed substrate to the BSFL. However, data on the optimum protein attainable from fecal matter is not readily available. Thus, co-digestion of FM with kitchen waste could improve the efficiency of the BSFL treatment process and protein production, by improving the organic load and nutrient availability, while lowering the effect of inhibitory compounds by dilution (Anjum et al., 2012).

1.2 Statement of the Problem

Sanitation is a human right and vital to health, child development, and social and economic progress. The world is alarmingly off-track to deliver sanitation for all by 2030. With only 10 years left before 2030, the rate at which sanitation coverage is increasing will need to quadruple to achieve SDG target 6.2 (UNICEF & WHO, 2020). Regionally, more than 60% of the human population in Africa have no access to improved sanitation and 40% of the rural population practice open defecation (Lalander et al., 2013). Locally, Sheet Flow Diagram (SFD) Thinking SFD Creation Process And Impacts - Case of Nairobi, Kenya, (2018) highlighted that onsite sanitation facilities contributed to 31% combined fecal sludge, 15% uncontained fecal sludge and 4% open defecation. The SFD graphic shows that 52% of unsafely managed excreta in the city originates from areas relying on onsite sanitation. Thus, the unsafely managed fecal

matter and sludge results in environmental pollution and immediate health consequences of inadequate sanitation, such as cholera outbreaks. Therefore, there is need for a shift from viewing fecal matter as a waste product, but a resource. Nutrient recovery from fecal matter can be achieved through the use of cost-effective bio-resource based technologies such as urinary diverting dehydrating toilets (UDDT). Developing countries, Kenya included have a dire need for high-quality and affordable alternative protein sources for animal protein. Consequently, the few available animal proteins are not available to the reach of many due to the high prices (Nyakeri et al., 2017). As a result, insufficient protein consumption is a persistent problem. A study done in Western Kenya by Nyakeri et al. (2017) reported that BSFL yielded 40% crude protein which could be a cheap and sustainable protein source for animal feed. This study investigated optimum protein that can be achieved from the co-digestion of UDDT fecal matter with kitchen waste which can result in sustainable nutrient recovery while mitigating health, environmental and economic impacts.

1.3 Objectives

1.3.1 Main Objective

The main objective of this study was to investigate the optimum protein attainable from black soldier fly larvae (BSFL) co-digestion of fecal matter.

1.3.2 Specific Objectives

The specific objectives of this study were to:

a) Characterize fresh fecal and kitchen waste used as feed into their chemical and elemental composition.
b) Determine performance of Black Soldier Fly Larvae (BSFL) in the conversion of blended fecal and kitchen waste.

c) Evaluate the optimum protein content as a factor of larval weight, feed type and Black Soldier Fly Larvae (BSFL) age.

1.4 Research Questions

1) How does the chemical and elemental composition of untreated food and fecal waste vary?
2) What is the effect of feed formulation on the waste reduction index, feed conversion and the bioconversion rate of BSFL?
3) What is the effect of the feed substrate, BSFL age and larval weight on the optimum protein?

1.5 Justification

Reviewed literature indicates that BSFL has been used successfully to reduce both organic solid waste (Gold et al., 2020; Lalander et al., 2019; Nguyen et al., 2015; Oonincx et al., 2015) and fecal sludge (Lalander et al., 2019; Banks et al., 2014; Lalander, Diener, Zurbrügg, et al., 2013). Nyakeri et al. (2019) and Rehman et al. (2017) observed that mixing human and cow manure with banana peels and soybean curd residue (food wastes and food production by-products) increased BSF larval weight compared to the individual wastes. However, BSFL conversion of co-digested UDDT fecal matter remains limited in literature. Furthermore, previous studies have reported the range of crude protein content of harvested larval biomass after bioconversion (Gold et al., 2020; Nyakeri et al., 2017; Nguyen et al., 2015; Diener et al., 2009). Nonetheless, the crude protein content achievable from the BSFL treatment of co-digested fecal matter is limited in literature. Therefore, the study investigated the optimum crude protein content achievable from the BSF treatment of co-digested fecal matter.

1.6 Scope and Limitations of the Study

Characteristics of fecal matter vary depending on the feeding habits of the society. The study used UDDT fecal matter from Kunene Primary School, a rural public school in Meru with rural setting food-based diets. Mixed kitchen waste was obtained from MUST cafeteria. Waste reduction, bioconversion and feed conversion rates were determined for each feed formulated. The study was confined to the larval and prepupal stages of the BSF since the fly only feeds during the larval stage and reserves enough energy for its growth and development.

Collection, treatment and valorization of fecal waste for nutrient recovery is a relatively new research area. Therefore, inadequate previous studies in the research area were experienced. Secondly, the Modified Gompertz model used requires a relatively large number of data points (at least 10 data points). Lastly, since the study was self-sponsored, financial challenges which caused some delays were experienced especially during laboratory analysis.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Overview

Fecal matter is part of human nature and everyday life meaning that it is an unavoidable evil that should be carefully handled for a healthy and sustainable environment. As a way of managing our environment, access to a proper sanitary environment is a global priority which plays a critical role in promoting human health, wellbeing, livelihoods and dignity while protecting degradation of the ecosystems. Sanitation generally entails the separation of human excreta hygienically from human, animal and insect contact (Rose et al., 2015). Every adult human being produces 130 g of feces and 1.4 litres of urine per day (Rose et al., 2015). The economic, social and health benefits of good sanitation include: higher productivity, better performance at school and work, lower medical costs and minimal downtime (Gross & Günther, 2014). However, more than 4.5 billion people globally are living without access to safely managed sanitation services and approximately one billion people lack basic access to sanitation facilities (Andersson, Otoo & Nolasco, 2018). Moreover, rapid population growth and urbanization, particularly in developing countries has far outpaced municipalities' capacity to provide adequate sanitation to the urban dwellers despite sanitation being vital for sustainable development (Owusu, 2010).

Children, girls and women are the worst victims of poor sanitation facilities since their quality of life, safety and health are severely compromised (Kumar, 2017). According to records from WHO (2015), one in five children die from diarrhea diseases and their medical consequences are much higher than death rates of HIV aids and malaria. In addition, these diseases jeopardize education, human productivity and quality of life for dwellers. Therefore, management of fecal matter that might end up in the environment

is an important need that should be addressed since it plays an essential role in the overall management of global sanitation related challenges.

In 2015, the global community approved 17 SDGs and 169 global targets which were designed to be integrated, indivisible and aimed to balance the economic, social, and environmental dimensions of sustainable development (United Nations, 2018). The 2030 agenda further seeks to address gender equality, empowerment of women and human rights (World Health Organization WHO and the United Nations Children's Fund, 2017). For instance, goal 6 aims at "ensuring availability and sustainable management of water and sanitation for all." Fresh water, in sufficient quality and quantity, is vital for all aspects of life and sustainable development. Water resources are embedded in all forms of development such as food security, health promotion and poverty reduction, in sustaining economic growth in agriculture, industry and energy generation, and in maintaining healthy ecosystems.

Target 6.2 aims at achieving access to adequate and equitable sanitation for all and target 6.3 seeks to improve water quality by reducing pollution, halving the proportion of untreated wastewater, and globally increasing recycling and safe reuse by 2030 (Andersson et al., 2018). These targets emphasize on ending open defecation, which is a major risk to public health and is closely associated with extreme poverty (United Nations, 2018). However, the emphasis does not address treatment and disposal of fecal waste from on-site sanitation (OSS) systems such as latrines and septic tanks. As a result, the fecal waste from OSS is directly discarded into water bodies or nearby fields (Rose et al., 2015), ending up in our water bodies during the rainy seasons. These practices contribute to pollution of both surface and groundwater, contamination of agricultural produce and spreading of water-borne diseases such as diarrhoea, typhoid, cholera, amoeba infections and helminthiasis (Lalander et al., 2013). Thus, technologies

focusing on resource recovery from human excreta are important in supporting the achievement of the SDGs.

At national level, article 43 (b) of the Kenyan constitution declares sanitation as a basic human right whereas article 42 guarantees the right to a clean and healthy environment. However, sanitation, as one of the sectors in Kenya has remained a low investment priority area due to institutional fragmentation. This leads to lack of coordination in carrying out sanitation investments (Mansour et al., 2017). For instance, Shit Flow Diagrams (SFDs) produced for Kisumu and Nakuru towns showed that 419,072 and 369,839 people respectively were dependent on onsite sanitation (Mansour et al., 2017). This indicates that over 65% of excreta produced in these two cities ends up in the environment untreated due to inefficient treatment technologies. According to SFD Thinking SFD creation process and impacts - Case of Nairobi, Kenya, (2018) as graphically presented in Figure 2.1, 66% of the fecal sludge produced within Nairobi Municipality is unsafely managed.

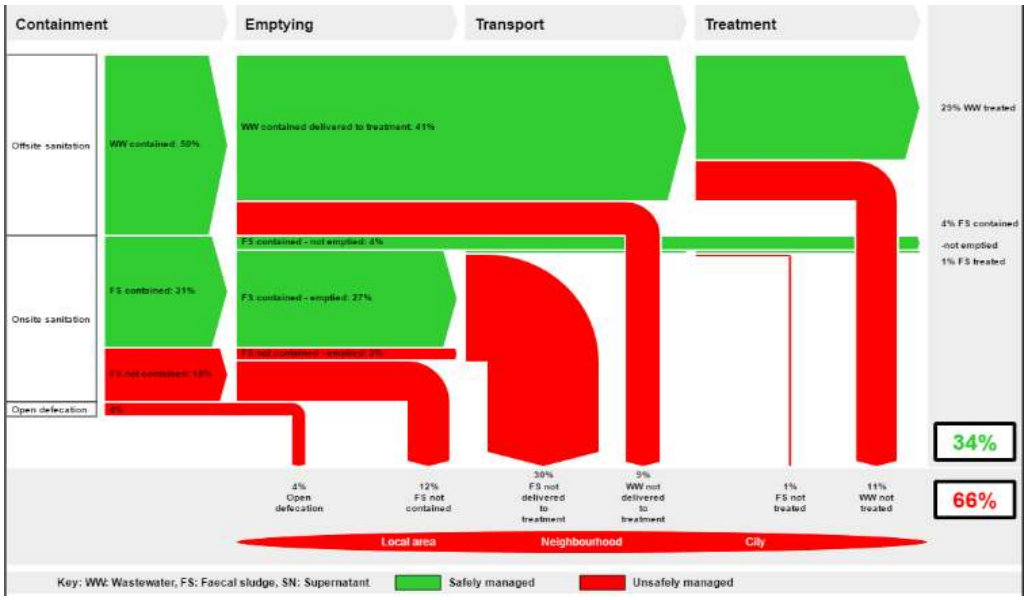


Figure 2.1: Sheet Flow Diagram for Nairobi City (*SFD Thinking SFD creation process and impacts - Case of Nairobi, Kenya*, 2018)

In addition, the revised Kenya Environmental Sanitation and Hygiene Policy 2016-2020 and National Open Defecation Free Kenya 2020 Campaign Framework commits the government to ending open defecation in Kenya by 2020 through toilet provision (UNICEF, 2018). However, collection, treatment and valorization of fecal waste for nutrient recovery is limited in literature.

2.1.1 Fecal Waste Management

Fecal waste management comprises storage, collection, transportation, treatment and safe disposal of FS (UNICEF & WHO, 2020; Gensch et al., 2018; Peal, Heymans, Hawkins, Evans & Blackett, 2014). Effective management of fecal waste involves policies, interactions and transactions among different people and institutions at each point in the service chain (Strande, 2014). However, this is not the practice in low-and-middle income countries. Drainage and sewerage facilities in most developing countries are under-developed, unplanned, and inadequate. More so, sewerage-connected toilets

need high amounts of running water and a regular supply for waste disposal which is expensive especially in slums, schools and emergency camps. Sewered systems are unfeasible in developing countries due to high installation and maintenance costs (Banks et al., 2014), require expertise to operate and maintain, consume enormous amounts of energy and resources while still leaving a lot of emissions to eco-environments (Hu, Fan, Wang, Qu & Zhu, 2016). In addition, the expansion and development of the operational conventional sewer networks does not keep pace with the growing population and rapid urban expansion (Strande, 2014). Moreover, the constructed sewer-lines and wastewater treatment plants in low-income countries frequently fail resulting in poor fecal sludge management (FSM).

Poor FSM in developing countries is due to lack of economic motivations for stakeholders within the fecal sludge service chain. Moreover, each individual household has to cater for the cost of emptying and safe disposal of fecal sludge to a treatment plant (Lalander et al., 2013). When a FSM structure is not implemented, the containment structure fills up and the untreated fecal sludge (FS) is disposed directly in the local environment (Strande, 2014). Moreover, safe collection, treatment, and disposal of fecal sludge from on-site sanitation facilities such as pit latrines and septic tanks is often not guaranteed. These practices result in groundwater pollution and contamination of agricultural land (SFD Thinking - Case of Nairobi, Kenya, 2018) as shown in the photo presented as Plate 2.2.

Plate 2.1: Ground Contamination due to Fecal Waste discharge

The final outcome is likely to be an outbreak of diseases such as cholera, diarrhoea, and helminthiasis. To address such problems, many have resorted the development of onsite sanitation technologies that treat human waste directly at or close to its source (Rose et al., 2015) thus producing safe and beneficial end products. Thus, management of feacal waste using the circular economy approach addresses the scarcity of resources such as the depleting nutrients stocks (Lohri et al., 2017).

Circular economy-based approach is a shift from linear use of resources to absolute value creation model that is socially, economically and environmentally positive. In circular economic models, the members and economic actors of the supply chain integrate their resources with each other, so that the business ecosystems can constantly redesign themselves (Fogarassy & Finger, 2020). Circular solutions are essential in tackling the imminent challenges of depleting resources and emerging environmental problems (Fogarassy & Finger, 2020). BSFL have been used as a tool for recycling organic waste to produce feed for aquaculture and livestock (Gariglio et al., 2019, Henry et al., 2015), poultry and pets (Moula et al., 2018), or use to produce bio-energy (Surendra et al., 2016); while generating organic matter that can be used as bio-fertilizer (Setti et al., 2019; Xiao et al., 2018).

Esrey (2001) defined Ecological sanitation (Ecosan) systems as sanitation systems which are designed to recover nutrients and organic matter found in human excreta for safe agricultural reuse. Ecosan is an umbrella term for various sanitation systems using different toilet technologies that consist of confinement, treatment and safe reuse or disposal of human excreta (Dickin, Dagerskog, Jiménez, Andersson & Savadogo, 2018). Costly sewer systems and dependency on the presence of running water can be avoided through Ecosan based technologies. Ecosan should therefore be adopted for the benefit of conserving and protecting environmental and human health, recovering and recycling of nutrients from human excreta.

2.1.2 **On-Site Sanitation Systems (OSS)**

On-site sanitation, also called non-sewered sanitation systems either provide treatment in-situ (such as simple pit latrines) or contain waste that can be transported to off-site treatment (such as septic tanks or emptiable latrines) (UNICEF & WHO, 2020). Globally, 3.1 billion people depend on on-site sanitation technologies (UNICEF & WHO, 2019) and the population is expected to raise to 5 billion by 2030. There is a general prevalence of different on-site sanitation technologies in different geographical regions particularly in emergency camps, rural and peri-urban areas. On-site sanitation facilities include pit latrines with slabs, composting toilets, ventilated improved pit latrines (VIP), pour-flush pit latrines and UDDTs (WHO/UNICEF, 2010).

In developing and transitional countries, pit latrines are common (Rieck, von Müench & Hoffman, 2012) due to the fact that they are relatively affordable, simple and waterless in operation. However, pit latrines result in fecal contamination to water resources due to base flow, especially in flood prone areas and in areas with high-water table. Banks et al. (2014) reported that pit latrines have high life-cycle costs due to fecal sludge emptying and excavation of new pits when emptying facilities are not attainable. In low-and middle-income countries, adequate pit latrine emptying services are not available in many areas and can be expensive. Furthermore, excavation of new pits is

not practical in emergency camps, schools and unplanned settlements where space is a limiting factor (Banks et al., 2014) hence sanitation is compromised. For instance, a report by UNICEF (2018) highlights that in 2015, there were 35 boys per toilet and 29 girls per toilet in Kenyan schools which is above the national standards of 30:1 (boys) and 25:1 (girls), respectively.

To enable achievement of SDG six, there should be development of sustainable, safe, reliable and economically friendly FSM systems (WHO/UNICEF, 2017) that cover the full sanitation service chain as shown in Plate 2.3 after Gensch et al. (2018).

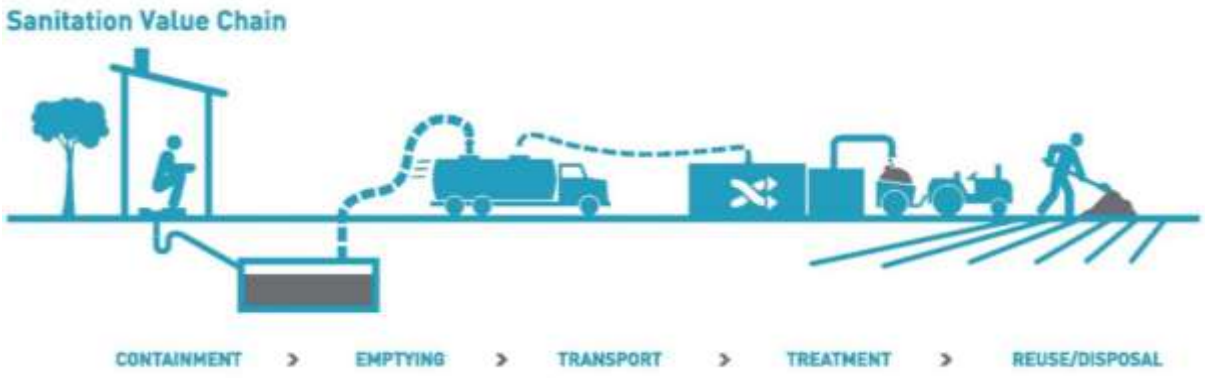


Plate 2.2: Sanitation Value Chain

Urine-Diverting Dry Toilet (UDDT) is one of the toilet technologies that promotes resource recovery from fecal waste using onsite sanitation systems (Dickin et al., 2018). UDDTs are suitable sanitation facilities to use with the aim of closing the nutrient loop through safe reuse of human excreta and sanitized urine (Katukiza et al., 2012). UDDTs fit well into the ecological sanitation concept, especially in densely populated, low lying settlements (Katukiza et al., 2012). UDDTs use the principle of natural separation of urine and feces at the source which is a waterless operation with ventilated containers for fecal storage (Rieck et al., 2012). Urine is separated at the user interface, drained through a piping system and infiltrated into the soil for disposal, or collected, stored and sanitized in containers for use as a fertilizer (Wendland, Deegener & Jorritsma, 2011).

Anal wiping materials and fecal waste are collected into a ventilated container directly below the user interface. After defecation, the user covers the fresh feces with a small volume of dry cover material such as saw dust, rice husks, wood ash, leaves, dry soil, lime, sand, or compost (Rieck et al., 2012) in order to control odour and increase pH, reduce moisture, prevent insect infestation (Wendland et al., 2011), inactivate pathogen (Katukiza et al., 2012) and improve aesthetic for the next user (Rieck et al., 2012). The technology is technically and institutionally appropriate, socially acceptable, economically viable and protective to environment and natural resources (Rieck et al., 2012).

UDDTs allow simple removal, less harmful and safe handling of the fecal waste after filling up of the toilet (Wendland et al., 2011). The risk of surface and ground water contamination is reduced through safe containment of fecal material in ventilated interchangeable water proof containers. This enables post-treatment of the fecal waste using different treatment technologies since the feces are not entirely sanitized. In addition, the toilets can be built in areas which are prone to floods (Wendland et al., 2011) where pit latrines are not appropriate. Moreover, the technology provides opportunities to develop value chains for recycling of human waste for agricultural purposes and allow safe disposal of the by-products. Fecal waste has a high nutritive value and energy potential which can be exploited and re-integrated back into the value chain so as to improve food production thus reducing environmental degradation. Therefore, UDDT is a suitable technology to be used for closing the nutrient loop through the reuse of human excreta in animal feed formulation or biodiesel production.

2.2 Characterization of Rearing Substrates

Characterization is the process of measuring and evaluating the properties of the rearing substrates. Understanding the nature of the physical, biological, and chemical properties of fecal material is necessary for research, design, implementation, and operation of

fecal sludge management solutions (Velkushanova et al., 2021). On-site sanitation (OSS) systems aim at treating human waste at source and provides an affordable and hygienic method of waste disposal. Knowledge of the waste stream entering the system is important for the improvement and development of the systems (Rose et al., 2015).

The elemental characteristics of fecal waste depend on the design and construction of the sanitation facility, the use of the facility, the frequency and method of fecal waste collection. Fecal waste is made up of carbohydrates, proteins, fats, fibre, inorganic materials and bacterial biomass (Penn, Ward, Strande & Maurer, 2018). Health condition and dietary consumption of food and fluids causes variations in the physical and chemical characteristics of fecal waste (Rose et al., 2015). These variables should be analyzed if the generation rate, chemical and physical composition of feces is to be accurately predicted. The reasons for fecal sludge characterization include: selection of the best technology for emptying of sludge from onsite containments, determining loadings for the design and operation of a treatment plant, monitoring of treatment efficiency and pathogen removal, understanding biochemical processes of degradation and nutrient cycling, and evaluating the potential for resource recovery (Velkushanova et al., 2021).

The physico-chemical properties of fecal sludge depend on both the physical and chemical processes within the containment of the fecal matter/sludge. Physico-chemical characteristics include: moisture content, total solids, volatile solids, pH, electrical conductivity, and nutrient content. The moisture content of fecal sludge is highly variable, resulting in uncertainties when expressing different properties based on the total volume or mass. Total solids can be categorized as based on physical properties (suspended and dissolved) and organic content (volatile or fixed). Total solids can be fractionated into total fixed solids and volatile solids by ignition at 550°C. Total fixed solids (ash) are the material left behind after ignition, and are the minerals that do not biodegrade over time. Volatile solids are volatised during ignition at 550°C and are an

indicator of the biodegradability of samples. Organic matter is important for evaluating the level of stabilisation of fecal sludge, biodegradation potential for biological treatment, and impact on receiving environments (Velkushanova et al., 2021).

Fecal sludge has nutrients in organic or inorganic forms. According to Velkushanova et al. (2021), monitoring of the nutrients is important for NH_3 inhibition, adequate nutrients for biological processes, fate in the environment, and potential for valorization as compost. Total Kjeldahl Nitrogen (TKN) is an indicator of the sum of organic nitrogen and NH_3. Other forms of inorganic nitrogen are nitrite and nitrate. The various forms of nitrogen give information on the redox potential of fecal sludge, and level of stabilization in biological processes (Nikiema et al., 2013). Similarly, total phosphorus can either be in organic and inorganic forms.

Determination of pH is important as it can influence biological processes, chemical speciation, and reaction rates (Velkushanova et al., 2021). pH can also act as an indicator of the source of the fecal sludge. The sample preparation method and the process of measuring pH is an important factor, as the method used can alter the pH of the sample. Conductivity is a metric of ions in a solution. Ion concentration is important as high salt concentrations can inhibit biological processes such as in stabilization ponds. Insects have nutritional needs which should be met in the raw feed for their growth and development.

2.3 Life Cycle of Black Soldier Fly and Growth Conditions

BSF can be maintained in a colony and there is an increasing global interest in the mass production of the insect. BSF is a native of the tropical, subtropical and warm temperate zones of America. However, it is now found in different parts of the world through natural and human-mediated dispersal (Banks et al., 2014), especially in the tropical and warmer temperate regions. Diener et al. (2011) reported that BSF are now found between 45^{o}N and 40^{o}S, showing the vast range in-which they occur. In

addition, they are tolerable to temperature extremes by a wide range throughout their life cycle, except during ovipositing.

BSF undergoes five stages in a complete life cycle namely: egg, larval, prepupal, pupal and adult stages (Banks et al., 2014). The larval and pupa stages of the BSF are the longest part of their life cycle whereas their egg and adult stages are relatively shorter (Popa & Green, 2012). The beneficial characteristic of adult BSF is that they do not have functional mouth parts, thus adults do not feed but depend on the fat stored during their larval stage (Tomberlin, Sheppard & Joyce, 2002). Furthermore, adult BSF are not pests since they do not enter into buildings and have a 45-50 days life span (Tomberlin et al., 2002) although the lifespan can be prolonged by food shortage.

Adult BSF are able to mate within two days of emerging from the pupa (Joyce, Sheppard, Kiser, Tomberlin & Sumner, 2009) since they only live for approximately 5-8 days in which they should mate and lay eggs. When mating time comes, they look for secluded bushes where the males choose a partner to mate with, which is achieved through lekking. Lekking is a mating behaviour where males of a species congregate in certain areas and 'call' to the females of the species (Karagodin, Yurina, Bastrakov & Ushakova, 2017).This takes place at a distance from the waste because the female should lay her eggs near a food source where her offspring will easily feed. Each female has the ability to lay clusters of between 500 and 900 eggs (Banks et al., 2014). Female adult lays her eggs in cracks and crevices which are slightly separated from the food source. Within 102 to 105 hours, laid eggs hatch (Tomberlin et al., 2002) but there's need for optimum environmental conditions for this to be achieved.

The larval stage succeeds the egg stage. Hatched larvae craw into the food source (Banks, Gibson & Cameron, 2014) which justifies the importance of the female adults to lay their eggs near a food source. The BSF larvae have a black eye spot and translucent bodies. Banks et al. (2014) highlights that the larvae have a greatly unique

composition of the gut microbiota which enables them to handle a wide range of feeds such as animal manure, human and animal cadavers, palm kernel meal, municipal organic waste, decaying vegetables, fresh fecal waste and pit latrine fecal sludge. The larval stage is the most crucial stage of BSF concerning waste management since it is at this stage that waste is fed upon and converted to valuable products. The larvae are easy to keep and able to develop in a wide range of temperatures (20°C to 45°C) and humidity (45% to 90%) (Karagodin, Yurina, Bastrakov & Ushakova, 2017).

BSFL development time varies depending on the diet, temperature, feeding rate and humidity (Diener et al., 2011). This period could be extended up to four months in case of food shortage. In 1995, Sheppard, Larry, Thompson & Savage found out that BSFL can reduce manure waste by 50% during the larval stages. Most of the pests that consume waste carry bacteria or diseases unlike the BSFL which are capable of inactivating *Salmonella* and *Escherichia coli*. The prepupal stage is the final larval stage of BSF which is important in waste transformation. However, all the other stages are equally important, though they are not directly linked to biomass conversion. This is because growth and developmental anomalies at any stage affects all the stages and thus the food conversion. Prepupae are characterized by changing from white to dark brown in colour and their behavioral migration from the food source to a dark and dry place (Banks et al., 2014). This migration enables their harvesting for either breeding into adults or for processing into biofuel oil and animal feed protein. Banks et al. (2014) mentions that the prepupae can climb inclines of approximately 40 degrees and crawl 100m upwards to find a suitable pupation place. Pupation is the final stage before emergence of adult BSF and takes approximately two weeks inclusive of the prepupal stage. This time period vary depending on feed availability (Sheppard, Larry, Thompson & Savage, 1994). The pupa then develops into adult BSF thus completing their life cycle a process summarized by the schematic diagram shown in Figure 2.3.

Figure 2.2: Lifecycle of a Black Soldier Fly

The use of BSF larvae to consume fecal wastes leads to waste reduction and a larval biomass. In addition, the BSF larvae co-digestion stabilizes waste, reduces emissions of odor, bacterial and fungal growth (Diener, Zurbrügg & Tockner, 2009). The resulting residue can be used in a vermicomposting facility to grow worms, for biogas production in an anaerobic digester, composted or moulded into bio-char for soil stabilization in agriculture (Dortmans, Diener, Verstappen & Zurbrügg, 2017). A study conducted by Caligiani et al. (2018) reported that BSFL was a good source of nutrients like proteins, lipids, and minerals. The high protein and fat content of dried BSF prepupae reinforces its high potential as fly meal in animal feed production.

2.4 BSFL Biowaste Conversion

Rearing of BSF is a sustainable strategy for a value-added bioconversion system. BSF has been suggested as an effective insect for converting many types of organic wastes

such as waste plant tissues, food waste, animal manure and animal offal into insect biomass (Nguyen et al., 2015) which is a useful ingredient for animal feed. Lalander, Diener, Zurbrügg & Vinnerås (2019) found out that BSFL are versatile in their feed preferences and can treat a variety of organic waste streams given that Nitrogen (N) and Total Volatile Solids (TVS) content are high to support the larval growth.

Bioconversion is the upcycling of various waste streams by converting them into larval biomass. Bioconversion of organic waste using the BSF technology have been noted to reduce the microbial load. A study by Lalander, Diener, Zurbrügg et al. (2013) reported a 6 $\log_{10}$ reduction in *Salmonella spp.* in human feces in eight days after using BSFL for treatment of fecal matter from OSS. However, BSFL has minimal effect on *Ascaris ova* (Lalander, Diener, Magri, et al., 2013). In BSF processing facilities, the larvae feed on decomposing organic material, growing from a few millimetres to approximately 2.5 cm while achieving upto 80% waste reduction (Dortmans et al., 2017). The larvae are harvested using a mechanical agitator to separate them from the residue. Previous studies have shown increasing efficiencies with time which indicates that reduction efficiencies will improve through continued research and improved optimization of BSF process parameters and conditions.

A bioconversion rate of between 16-22% for fecal waste was highlighted by Banks et al. (2014) for both batch and continuous feeding. Batch feeding method is where the BSFL are fed a single lump amount of feed at the start of the experiment. BSFL is able to convert various organic waste materials into valuable and less harmful biomass resulting in 65-75% waste reduction (Diener et al., 2011). A study by Banks et al. (2014) reported that BSFL fed on fresh feces through batch feeding produced larger larvae and prepupa than those fed after every 2 days. Nguyen et al. (2015) highlighted that Waste Reduction (WR) in kitchen waste, fish rendering and a mixture of fruits and vegetables was 67.9, 74.2, and 98.9% respectively. This laboratory study indicates that there is great promise for using BSFL as a potential agent for fecal waste management.

2.4.1 Resource Recovery Using Black Soldier Fly Larvae

Resource recovery from waste may result in the development of viable business models for sustainable sanitation solutions (Diener et al., 2014). Innovative sanitation treatment approaches under development aim to recover nutrients from OSS for feed formulation and energy (Diener et al., 2014). Moreover, fecal waste does not have an established market value (Hafford et al., 2018) and there are no seasonal fluctuations in its availability. Therefore, there is need to shift from the "fill and abandon strategy" which involves abandoning the toilets on filling and digging new ones to technologies which enable fecal waste recycling through sustainable production systems. The "fill and abandon strategy" involves abandoning the toilets on filling, removing the top slab and digging new ones. In addition, there is a growing concern, since the nitrogen and phosphate cycle are key factors that have to be maintained within certain levels for the planet to be able to support human existence in the future (Rockström et al., 2009). Thus, stabilization of fecal matter using BSFL would re-integrate the nutrients back to the food chain thus saving on the exploitation of natural resources.

Bioconversion by insects is an innovative technology for waste management (Caligiani et al., 2018). Naturally, most insects feed on low-grade biowastes, convert biomass nutrients into their own body biomass which results in significant reduction in the waste quantity and quality. Insects of various species contain large quantities of crude protein (Table 2.1) and fat with high economic value (Liu et al., 2017) which can be used to replace traditional protein sources such as soya beans and fish meal in the poultry and aquaculture compound feed manufacturing industry.

Table 2.1: Comparison of Average Protein Content Among Insects

Insect	Product	Protein Content (%)	Source
BSFL	Larvae	27.1	**Gold et al. (2020)**
		42.2	**Spranghers et al. (2017)**
		40	**Nyakeri et al., (2017)**
Locust and grasshopper	Larvae	14-18	**FAO, (2013); Tiencheu & Womeni, (2017)**
Locust and grasshopper	Adult	13-28	**FAO, (2013); Tiencheu & Womeni, (2017)**
Crickets	Adult	8-25	**FAO, (2013); Tiencheu & Womeni, (2017)**
Termite	Adult	13-28	**FAO, (2013); Tiencheu & Womeni, (2017)**
Housefly	Maggot	54	**FAO, (2013); Tiencheu & Womeni, (2017)**
	larvae	43- 59	**(Cicková et al., 2015)**

Major environmental advantages of insect farming compared to livestock production include: less water and land requirement, lower greenhouse gas emissions, insects have high feed conversion efficiencies and digest low-value organic by-products into high quality larval biomass (Van Huis & Oonincx, 2017). BSF is polyphagous and its gut extracts have high amylase, lipase and protease activities (Kim et al., 2011). As an alternative source of protein, BSFL has superior capabilities compared to other insect scavengers. Thus, it has been employed in sustainable recycling of feces (Lalander, Diener, Zurbrügg, et al., 2013; Diener et al., 2009), animal waste (Sheppard et al.,

1994), and other types of organic waste (Popa & Green, 2012; Diener et al., 2011). Moreover, BSF is not a pest, BSF rearing does not require specific precautionary measures and the larvae reduces the presence of harmful bacteria (Sheppard et al., 1994) in contrast to adult housefly, *Musca domestica*. Thus, the high nutrient content of the BSFL can be employed as the basis of a promising technology to sustain a circular economy.

Caligiani et al. (2018) highlighted that the use of Black Soldier Fly Larvae (BSFL), botanically called *Hermetia illucens* (L.), is a prospective solution for organic waste management. BSFL have been reported to consume and degrade a number of organic materials with material degradation up to 70 % (Diener, Solano, Roa Gutiérrez, Zurbrügg & Tockner, 2011). In addition, previous studies have reported that BSF larvae are capable of converting municipal organic solid waste (MOSW), agricultural waste, fecal waste and excreta from on-site sanitation (OSS) facilities like pit latrines (Diener, Zurbrügg & Tockner, 2009) into larval biomass. For instance, a study by Spranghers et al. (2017) highlighted that BSFL fed on chicken feed, vegetable waste and biogas digestate yielded 41.2%, 39.9% and 42.2% protein content respectively. Food and manufacturing by-product mixes yielded 43.2% protein content (Oonincx, Van Huis, & Van Loon, 2015). However, similar data for fecal waste is scarce and hardly available from literature.

Resource recovery from treatment of fecal waste results in environmentally safe end products which can be used as animal feed ingredient, soil conditioner, solid fuel and feedstock for production of biogas. Moreover, BSF larval biomass has high crude protein and fat content thus closing nutrient loops through reduction of environmental pollution and costs. Selling of the end products can result in financial resources to cater for fecal sludge management costs thus providing sustainable and safe sanitation (Nikiema et al., 2013). In addition, feed-to-protein conversion ratio for BSFL is lower than for swine or cattle (Oonincx et al., 2015) and releases lower ammonia emissions

and minimal greenhouse gases compared to any conventional livestock (Oonincx et al., 2010). Therefore, BSFL treatment of fecal wastes reduces ecological pollution and improves public health through pathogen reduction, stabilization of organic matter and nutrients, and the safe end use or disposal of treated end products.

The digestate processed by fly larvae has a loose granular structure with earthy odor and is suitable for use as an organic fertilizer (Lalander et al., 2016). A post-treatment step for waste decomposed by the black soldier fly larvae is also recommended by Lalander, Diener, Magri, et al. (2013) so as to inactivate pathogenic micro-organisms such as bacteria, viruses, and nematodes before reuse in agriculture. Further treatment of the residue by aerobic composting would lead to faster loss of phytotoxicity and help in the elimination of pathogens due to the thermophilic phase of composting.

Moreover, BSFL fecal waste treatment facilities have low installation and maintenance costs and need no power supply. Furthermore, creating additional value chains and generating a surplus income through the sale of harvested prepupa can strengthen the economic resilience of farmers or small entrepreneurs to natural hazards or market fluctuations (Diener et al., 2011). It enables income generation for small entrepreneurs with little investment. Banks et al. (2014) found out that use of BSFL could be a possible solution to the health problems related to poor sanitation and improper fecal waste management in low- and middle-income countries. Laboratory experiments conducted by Diener et al. (2011) showed that BSFL can significantly reduce sludge biomass.

2.4.2 Benefits of Black Soldier Fly

a) Housefly Control

BSF larvae inhibits oviposition of housefly which is a disease-spreading insect thus reducing the housefly population. It has been documented by Sheppard et al. (1994)

that BSFL colonization of pig and poultry manure had the capacity to reduce common housefly population by 94-100%.

b) Smell/Odor Reduction

Odor reduction is achieved by the voracious appetite of the BSFL making the waste to be processed fast. Furthermore, the larvae excrete dry organic matter and suppress the growth of bacteria (Diener, Zurbrügg, Roa Gutiérrez, Nguyen, Koottatep, Tockner, 2011). With such a combination of characteristics, odors are not given any chance to thrive.

2.4.3 Innovative Fecal Sludge Treatment Technologies

These technologies include: vermicomposting, solar drying, thermal drying and pelletizing, ammonia treatment and resource recovery using BSFL.

a) Vermicomposting

Earthworms are effective in organic waste reduction resulting in organic fertilizer (Rogayan, 2017). Vermi-filter is capable of treating domestic wastewater sludge which has been diluted in a system inoculated with earthworms (Hemalatha, 2012). Interestingly, worms cannot thrive in fresh feces but have to be supported in vermi-compost and layers of soil. Complete coliform removal can be achieved when carried out under proper conditions. However, substrate contamination, need for more skilled laborers and know-how may prevent the widespread application of vermicomposting technology (Mahmood et al., 2021).

b) Ammonia Treatment

Microorganisms such as virus, bacteria and parasites are reduced through ammonia sanitization (Fidjeland, 2015). Pathogen reduction with ammonia is due to the fact that ammonia enters cell membranes, takes up intracellular protons for the formation of

ammonium and acts as a charged ion leading to mal-functioning of the organism (Fidjeland, 2015). This reduces risks for farmers, food consumers and downstream populations. Ammonia treatment is most applicable in areas which use UDDTs.

c)Thermal Drying and Pelletizing

Pelletization involves application of mechanical pressure to increase the density of the material while converting it into pellets (Nikiema, Cofie, Impraim & Adamtey, 2013). Pelletization makes the end products dust free, granular and stable allowing easy storage and transportation. The pellets steadily release nutrients and gradually decrease soil and nutrient losses from agricultural land. The pellets are safe for agricultural use due to the fact that they are free from pathogens and can be used as a dry fuel in industrial combustion.

d) Solar Drying

Solar drying is carried out in greenhouse structures which have transparent covers, walls and concrete basins. Sludge disposed into the basins is processed for approximately 10 to 20 days. Ventilation, temperature and air mixing are controlled in the greenhouse for both continuous and batch operations. Factors that influence evaporation efficiency in solar drying systems are air temperature, solar variation and ventilation rate, initial dry solid content of the sludge and air mixing (Mugauri, Inambao, Septien & Singh, 2018). Efficiency of pathogen reduction is low due to the fact that short wavelength light like the UV is blocked by the greenhouse cover.

2.5 Modelling Process

2.5.1 Background to modelling Process

Mathematical modelling is a useful tool which give clear understanding of biological processes and generates valuable individual predictions. A mathematical model is a

representation of a phenomenon or system that is used to provide insights and predictions about system behavior (Chaturvedi, 2010) by means of variables. Mathematical models allow researchers to investigate the connections of complex regulatory processes and the effect of disruptions of these processes. In addition, computational models help investigators to systematically analyze systems perturbations, develop hypotheses to guide the design of new experimental tests, and ultimately assess the suitability of specific molecules as novel therapeutic target (Fischer, 2008). The study of different biological processes for different living organisms has necessitated development of various mathematical models. These mathematical models address different categories of biological processes, such as metabolic processes, signaling, and regulatory pathways.

Optimization involves finding a set of operating conditions for the process variables that result in the best process performance (Myers, Montgomery & Anderson-cook, 2009). Optimization is used to maximize or minimize the value of a function chosen as the performance index. Optimization was traditionally done by monitoring each specific variable individually and its influence on the response thus one variable was changed at a time and the others remained constant. As a result, optimization was a tedious process due to involvement of multivariable process parameters. In addition, interactive effects between variables were not considered. Therefore, prediction of an overall effect of variables on the particular response was impossible (Bashir et al., 2015). Furthermore, traditional methods increased number of experimental trials leading to an increase in material, time and cost of production. Different methods used in process and product optimization include: One-Factor–at-a time, R model, Response Surface Methodology among others.

2.5.2 Taguchi Methods of Experimental Design

Taguchi approach assists experimenters with limited statistical skills to study and understand how several process parameters affect the process output using limited resources. It is useful in understanding the process and then optimizing the performance of the process using statistical design of experiments (Antony, Warwood, Fernandes & Rowlands, 2001). It provides a systematic approach to better understanding of the process and process parameters that affect the critical process/product characteristics. General steps followed in the Taguchi Method as described by Montgomery (2013) include:

(1) Define the process objective, or more specifically, a target value for a performance measure of the process.

(2) Determine the design parameters affecting the process.

(3) The number of levels that the parameters should be varied at must be specified.

(4) Create orthogonal arrays for the parameter design indicating the number of and conditions for each experiment.

(5) The selection of orthogonal arrays is based on the number of parameters and the levels of variation for each parameter.

(6) Conduct the experiments indicated in the completed array to collect data on the effect on the performance measure.

(7) Complete data analysis to determine the effect of the different parameters on the performance measure.

However, the Taguchi design strategy uses the crossed array approach which results to a very large experiment (Montgomery, 2013). Despite the large number of runs involved, crossed array design does not give any information about interactions between controllable and noise variables.

2.5.3 Modelling with One-Factor-at-a-Time (OFAT)

Optimization can be achieved statistically by using one-factor-at-a-time (OFAT) method. This method involves changing the parameters of a variable while keeping the level of the other variables constant (Zhang et al., 2010). Major disadvantage of this optimization method is that the interaction effects of the variables are not usually considered during the optimization process. Therefore, broad effects of the parameters on the responses are not captured by the OFAT. In addition, the method involves many experimental runs which leads to increase in time of experiment, high cost of materials and the optimization point could be missed.

2.5.4 Modelling with Response Surface Methodology

Response Surface Methodology (RSM) has been developed by researchers for modeling and analyzing engineering problems. RSM is a collection of mathematical and statistical techniques useful for improving, developing, and process optimization (Sayyad, Panda, Javed & Ali, 2007). It is useful for the modeling and analysis of programs in which a response of interest is influenced by several variables and the objective is to optimize this response. RSM has a wide range of applications in designing, development and formulation of new products in addition to existing product improvement (Bashir et al., 2015). RSM is an effective optimization tool due to the fact that it allows simultaneous evaluation of the confirmed factors and their interactions can be identified with less experimental trials. RSM depicts the effect of the independent variables on the dependent variables and generates an empirical

model. For instance, RSM has been applied in: optimization of coagulation-flocculation process of paint wastewater treatment (Kakoi, 2018), optimization of wastewater treatment processes (Bashir et al., 2015) and fluoride adsorption in an aqueous solution by brushite and nutrient the industrial world (Mourabet, El Rhilassi, El Boujaady, Taitai & Bennani-Ziatni, 2017).

Advantages of RSM includes search for relativity between factors, few number of experiments, suitable for multiple variable experiments, finding forecast response and determining most suitable condition (Myers et al., 2009). In order to apply RSM as an optimization tool, some stages (Bashir et al., 2015) have to be followed. These stages include:

(a) selection of the most crucial independent variables and their level on the system through screening studies

(b) the choice of the experimental design and carrying out the experiments according to the selected experimental matrix

(c) the mathematical–statistical management of the obtained experimental data through the fit of a polynomial function

(d) the evaluation of the model's fitness

(e) the verification of the necessity and possibility of performing a displacement in direction to the optimal region; and

(f) obtaining the optimum values for each variable.

The least square technique is vital in fitting a model equation which contains the input variables by ensuring minimal residual error which is measured by the sum of square deviations between estimated and actual responses. This involves calculation of the estimates for the regression coefficients. In addition, calculated coefficients of the

model equation are tested for statistical significance using the following three tests: (a) performed-test for significance of the regression model; (b) test for significance on individual model coefficients; and (c) test for lack-of-fit (Myers, Montgomery & Anderson-cook, 2009). Process performance is evaluated by analyzing the response variable (y) which is dependent on the process variables X_1, X_2, X_3……... X_n which is described by Equation (2.1):

$$y = f(x_1, x_{2,,} x_{3,} \cdots\cdots x_n) + \in \tag{2.1}$$

where y is the response, f is the unknown function of response, X_1, X_2, X_3……. X_n are the input variables which affects the response, n is the number of the independent variables and ϵ is the statistical error that represents other sources of variability not accounted for by f.

RSM models are generally full quadratic equations. The stationary point is either a point of maximum response, minimum response or saddle point and can be determined by including quadratic terms to the polynomial terms as in Equation (2.2) according to Myers, Montgomery & Anderson-cook (2009).

$$y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_i x_i^2 + \sum_{i \neq j=1}^{n} \beta_i x_i x_{ij} + \in \tag{2.2}$$

where β_0 is the value of the fixed response at the center point of the design; β_i, β_{ii} and β_{ij} are the linear, quadratic and interaction effect regression terms, respectively; X_i denotes the level of the independent variable; n is the number of independent variables; and ϵ is random error.

A second order polynomial equation is vital for making predictions and optimizing processes/products. A second order polynomial equation with two process variables is derived as in Equation (2.3):

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \in \quad (2.3)$$

Where y is the predicted response, β_0 is the intercept coefficient, β_1 and β_2 are the linear terms (first order), β_{11} and β_{22} are the quadratic terms (second order) and β_{12} are interaction terms respectively, X_1 is time and X_2 is moisture content which are uncoded process variables. Response surface is represented graphically in a three-dimensional space.

Common Design of Experiment (DoE) methods used include; Box-Behnken Design, Full Factorial Design and Central Composite Design (CCD). A second-order model can be efficiently constructed with CCD which consists of: (i) factorial points, (ii) a central point and (iii) axial points which are at a distance α from the central point (Ayodele & Abdullah, 2018). CCD is applicable for sequential experimentation and provides a reasonable amount of information for testing lack-of-fit while not involving an unusually large number of experimental runs.

The Box-Behnken Design (BBD) applies three levels (−1, 0, +1) for each factor. BBD comprises a specific subset of the factorial combinations from 3^k factorial design. In addition, in a BBD, the experimental points are situated on a hypersphere equally distant from the central point. It is appropriate to evaluate interaction between factors and particularly to study processes without extreme points. In addition, BBD is not appropriate to study factors with more than three levels.

BBD regression model of RSM provides a good explanation of the relationship between the response and the process variables (Bhavsar, Dudhagara & Tank, 2018).

However, RSM is limited since the experimental data are fitted to a polynomial model at second level. It is not correct to assume that all systems with curvature are compatible with a second-order polynomial model (Ayodele & Abdullah, 2018). In addition, RSM is a black-box model where one tries to estimate both the functional relationship between variables and the numerical parameters in those functions, and the estimated values in the model should be verified (Bhavsar et al., 2018).

2.5.5 Modelling with MATLAB

Typically, MATLAB (Mathworks, 2001) is used in: Mathematics and computation, Algorithm development, Modeling, simulation, and prototyping, Data analysis, exploration, and visualization, Scientific and engineering graphics, and Application development, including graphical user interface building (Chaturvedi, 2010). In MATLAB, toolboxes allow learning and application of specialized technology. Toolboxes are comprehensive collections of MATLAB functions (M-files) that extend the MATLAB environment to solve particular classes of problems. Toolboxes are useful in: control systems, simulation, signal processing, fuzzy logic, neural networks, wavelets (Chaturvedi, 2010), optimization, control theory and several other fields of applied science and engineering.

2.5.5.1 Curve Fitting Toolbox in MATLAB

The Curve Fitting Toolbox is a collection of graphical user interfaces (GUIs) and M-file functions built on the matrix laboratory (MATLAB) technical computing environment (Mathworks, 2001). The MATLAB allows easy matrix manipulation, plotting of functions and data, implementation of algorithms, creation of user interfaces and interfacing with programs in other languages according to Rahim & Akif (2015). It also allows quick and easy coding in a very high-level language and it further allows the users to develop their own functions and specialized programs as compared to other models (William, 2011). As such, the curve fitting tool in MATLAB was used in fitting

the Modified Gompertz model for analyzing the kinetics of the substrate degradation in biogas production (Wandera et al., 2018).

The curve fitting toolbox provides graphical tools and command-line functions for fitting curves and surfaces to data. The toolbox allows performance of exploratory data analysis, preprocess and post-process data, compare candidate models, and remove outliers. The toolbox provides parametric and non-parametric data fitting where one can perform a parametric fit using a toolbox library equation or using a custom equation (Mathworks, 2001). Library equations include: sums of Gaussians, weibull, exponentials, polynomials, fourier series, and rationals. Custom equations are equations that are defined to suit specific curve fitting needs such as the Gompertz model used in simulating the Biogas potential from food waste in a continuous two-stage system (Algapani et al., 2017). After creating a fit, the toolbox allows application of a variety of post-processing methods for plotting, interpolation and extrapolation; estimating confidence intervals; and calculating integrals and derivatives.

The toolbox provides a one-term and a two-term exponential model which are applicable when the rate of change of a quantity is proportional to the initial amount of the quantity (Mathworks, 2001). When the coefficient associated with “e” is negative, “y” represents exponential decay. When the coefficient is positive, “y” represents exponential growth. Examples of exponential growth include contagious diseases for which a cure is unavailable, and biological populations whose growth is uninhibited by predation and environmental factors. Algapani et al., (2016) used the curve fitting toolbox to determine the kinetic parameters of hydrolysis, acidogenesis, acetogenesis, and methanogenesis of particular food waste at different temperatures during anaerobic digestion.

2.5.5.2 Performance Evaluation Criteria

The performance of models is evaluated using various statistical measures. The evaluation measures include: correlation coefficient (R), mean absolute error (MAE), mean square error (MSE), Root mean square error (RMSE) and Nash–Sutcliffe Efficiency (NSE). The Curve Fitting Toolbox supports goodness of fit statistics for parametric models using the sum of squares due to error (SSE), R-square, Adjusted R-square, and Root mean squared error (RMSE).

a) **Sum of Squares Due to Error (SSE)**

This statistic measures the total deviation of the response values from the fit to the response values. SSE is also known as the summed square of residuals. A value closer to 0 indicates that the model has a smaller random error component, and that the fit will be more useful for prediction.

b) R-Square

R-square is a measure of the amount of reduction in the variability of y obtained by using the independent variables x_1, x_2….x_n in the model (Montgomery, 2013). It is also called the square of the multiple correlation coefficient or the coefficient of multiple determination. The coefficient of determination, R^2 indicates the degree to which a model explains the observed variation in the dependent variable, relative to the mean. R-square is defined as the ratio of the sum of squares of the regression *(*SSR*)* and the total sum of squares *(*SST) as shown in Equation (2.4).

$$R^2 = \frac{\text{SSR}}{\text{SST}}$$

(2.4)

The R^2 always can take on any value between 0 and 1, where a higher R^2 indicates a better model fit. When interpreting the R^2, higher values indicate that more of the

variation in the dependent variable y is explained by variation in the independent variable x.

c) Degrees of Freedom Adjusted R-Square

The adjusted R^2 is used in selection of regression models and it is usually equal to or less than R^2. The statistic increases if the addition of another independent variable explains a substantial amount of variance and is calculated as shown in Equation (2.5). Adjusted R^2 is only a measure of how much the model explains while controlling for model complexity. It has the particularity of being less subjected to variation than the R^2 when a new term is added to the regression (Myers & Montgomery, & Anderson-cook, 2009). When the adjusted R^2 value is much lower than the R^2 value, it is an indication that the regression equation used may be over-fitted to the sample.

$$Adusted\ R^2 = 1 - (1 - R^2).\frac{(n-1)}{n-k-1}$$

(2.5)

Where:

n= the number of observations and

k= the number of independent variables.

However, the adjusted R-square statistic is a best indicator of the fit quality when comparing two models that are nested — that is, a series of models each of which adds additional coefficients to the previous model.

d) Root Mean Square Error (RMSE)

The Root Mean Square Error (RMSE) (root mean square deviation, RMSD) is a frequently used measure of the difference between values predicted by a model and the values actually observed from the environment that is being modelled (Kanda et al.,

2016). These individual differences are also called residuals, and the RMSE serves to aggregate them into a single measure of predictive power. RMSE provides information on the short-term performance which is a measure of the variation of predicted values around the measured data. The lowest the RMSE, the more accurate the prediction is (Azid et al., 2013).

2.6 Predictive models

Predictive modeling is widely used in many different aspects of micro-biology. Models are used to describe the behavior of micro-organisms under different physical and chemical conditions

(Gibson et al., 1988) such as pH, temperature, and water activity. Actual growth rates for most living things are not constant over the entire growth period since growth increases to a maximum then decreases. According to Zwietering et al. (1990), bacterial growth often shows a phase in which the specific growth rate starts at a value of zero and then accelerates to a maximal value in a certain period of time, resulting in a lag time. In addition, growth curves contain a final phase in which the growth rate decreases and finally reaches zero. Microbial growth period consists of four phases, which are a representative of a growth curve, including an initial stage of little change, a stage of accelerating change, a stage of decreasing change, and a stationary stage (Gibson et al., 1987). Predictive models have been used to estimate parameters such as lag time, generation time, maximum growth rate, and maximum cell concentration of microorganisms under particular conditions (Gibson et al., 1988). The estimates of growth rate, lag time, generation time and time to reach maximum growth rate are then obtained by fitting the equations to the data. Sigmoidal curves have been fitted using different mathematical functions such as logistic, Gompertz, Richards, Schnute, and Stannard (Tjerve & Tjerve, 2017, Zwietering et al., 1990). Although these curves are born in deterministic contexts, they have been generalized to include stochastic effects

aimed at bridging the gaps that often exist between theoretical results and experimental data (Albano et al., 2020). Sigmoidal functions were chosen since actual growth rates of living organisms are not constant over the entire growth period since growth increases to a maximum then decreases.

Commonly used sigmoidal functions are the logistic and Gompertz functions. The Modified Logistics Model (Zwietering et al., 1990) and Modified Gompertz Model (Wandera et al., 2018, Zwietering et al., 1990) are given in Equations (2.6) and (2.7) respectively.

$$P = \frac{P_0}{\left\{1+exp\left[\frac{4r_{max}}{P_0}.(t_0-t)+2\right]\right\}}$$

(2.6)

$$P = P_0.exp\left\{-exp\left[\frac{r_{max}.e}{P_0}.(t_0-t)+1\right]\right\}$$

(2.7)

Where:

P = Expected value (cumulative product volume as a percentage) as a function of time (t) in days.

P_0 = Maximum Product potential of the substrate as a percentage per gram of the sample.

r_{max} = Absolute growth rate as a percentage per day which is the tangent to the curve at the lag time t_0,

t_0 = Lag time in days which falls where $P = P_0.\exp(-e)$ as reported by Tjerve & Tjerve, (2017).

e = Natural logarithm (2.718281828)

t = Time at inflection in days

According to goodness of fit criteria, the Gompertz model has a better fit than the logistic model. The Gompertz model has three parameters, is simple and therefore easy to use. In addition, the three-parameter model is more stable because the parameters are less correlated. The logistic model should be used when the maximum specific grow rate (growth kinetics, μ_{max}) is to be estimated; while the modified Gompertz model can be used to estimate the lag time and maximum biomass productivity (Phukoetphim et al., 2017).

Gibson et al. (1987) determined the growth responses of *Clostridium botulinum* in the model pork slurry system containing 1.5-4.5% sodium chloride at a range of storage temperatures (15-27°C). The growth curves were fitted by both logistic and Gompertz models and estimates of lag time, growth rate and generation time calculated for each fit. Generally, the Gompertz model obtained better fits and was easy to use compared to the logistic function. Similar results are reported in a study by Zwietering et al. (1990) where several sigmoidal functions (logistic, Gompertz, Richards, Schnute, and Stannard) were compared to describe a bacterial (*Lactobacillus plantarum*) growth curve. The difference between a logistic and a Gompertz function is that a logistic function is symmetrical around the point of maximum growth rate, whereas the Gompertz function is asymmetrical since it a more improved version of the logistics model. In addition, the Gompertz model has a more upward increase at the lag phase, reaches a maximum rate of growth at a lower growth level, and has a more gradual

decrease in growth rate (Garthright, 1991). Albano et al. (2020) considered a modified Gompertz diffusion process (including exogenous factors in its infinitesimal moments) to model the effect of anti-proliferative and/or cell death induced therapies in untreated tumor growth. The results indicate that the model was a valuable tool for adjusting the drug administration scheme in the preclinical setting so as to improve the treatment efficacy and optimize the schedule to be proposed to patients in clinical trials.

In biology, the Gompertz model has been used to describe tumor and bacterial growth (Zwietering et al., 1990, Tjerve & Tjerve, 2017), mortality, lifespan, growth of animals and plants. In applied research, the model has been used in renewable energy to improve the performance of agricultural biogas plants (Wandera et al., 2018), simulate the kinetics of thermophilic and hyper thermophilic anaerobic process of particular food waste at different temperatures (Algapani et al., 2016), and simulate biogas potential (Algapani et al., 2017) among others.

2.6 Conceptual Framework

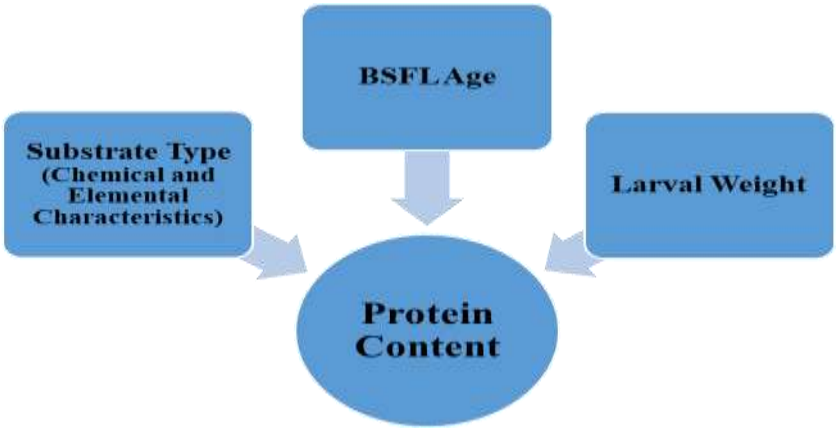


Figure 2.3: Conceptual Framework

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out at Meru University of Science and Technology Sanitation Research Institute (MUST SRI). MUST is located along Meru-Maua road in Nkomo Ward/Location, Tigania West sub-county, Meru county, Kenya. Urinary Diverting Dehydrating Toilets (UDDT) fecal matter was collected from Kunene Primary school, a public primary school, located 200 meters from MUST gate. Kunene Primary school has a total enrolment of 315 pupils. The study area is presented in Figure 3.1.

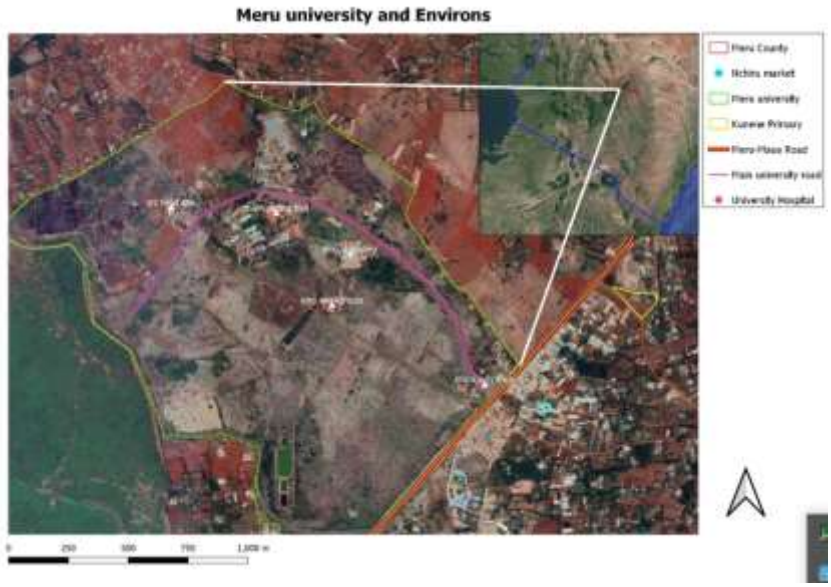


Figure 3.1: A Map Showing the Study Area

3.2 Characterization Fresh Fecal and Kitchen Waste

Rearing substrates for the BSFL were prepared from fresh fecal matter and kitchen waste. Fecal matter was collected from the UDDTs in Kunene Primary School. Using 20 kg capacity portable buckets, the fecal matter was transported to the MUST SRI. Kitchen waste were food leftovers which were collected from the MUST cafeteria. Each waste type was collected on site within 24 hours of production, and transported to the MUST SRI production area. Figure 3.1 presents a process flow diagram from waste collection to treatment point.

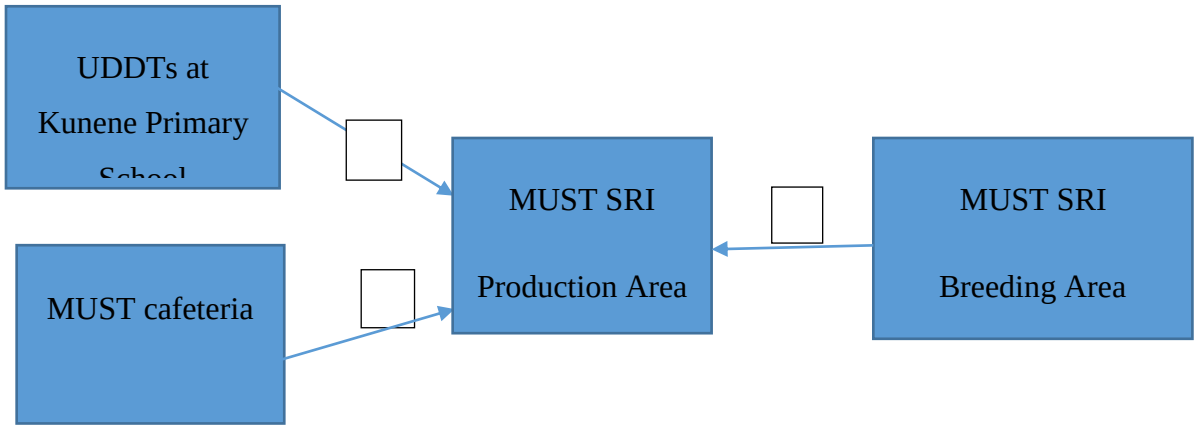


Figure 3.2: Process Flow Diagram

Where:

a - the transportation of fecal waste from the UDDTs,

b - the transportation of kitchen waste and

c - the transportation of 5 days old BSFL.

Rehman et al. (2017) and Diener et al. (2011) formulated BSFL feed substrates using different ratios which guided the choice of the mixing ratios in the study. Five

treatments (three replications per treatment) were prepared with using random mixing ratios of fecal to kitchen waste as detailed in Table 3.1.

Table 3.1: Sample Composition

Treatments	Composition	Mix Ratio
a	Fecal Waste	1:0
b	Fecal Waste + Kitchen Waste	1:1
c	Fecal Waste + Kitchen Waste	2:1
d	Fecal Waste + Kitchen Waste	4:1
e	Kitchen Waste	0:1

The mixtures were homogenized to mimic the pre-treatments used in BSFL treatment facilities (Dortmans et al., 2017). Plastic containers (260 mm*130 mm *110 mm), which were locally available and cheap were used as treatment units (Appendix 1). To save on space, individual containers were stacked upon each other with ventilation frames in-between them to allow free air circulation. Samples collected in triplicate from each treatment unit, dried at 105^0 C and threshed in preparation for laboratory analysis.

Crude protein analysis was based on Total Kjedahl Nitrogen (TKN) method which comprised of three steps including digestion, distillation and titration. Prepared sample were placed on the digestion unit for 3 hours which involved fast heating period for 10 minutes followed with slow heating period for 120 minutes and cooling period for 50-60 minutes. Distillation process applied NaOH solution as excess base that reacted with digested sample and 4% Boric acid as receiving solution. The digested samples and blanks solution were all distillate respectively. Each collected distillate was added with 5 drops of mixed indicator working solution. The distillate was then titrated with 0.1N

HCl standard solution until colour change were observed. The standard protein-nitrogen conversion factor of 6.25 was used in this study (Osman et al., 2019). The crude protein content was calculated as shown in Equation (3.5).

$$\%\ Protein = \frac{(A-B*N*1.4007*6.25)}{Weight\ of\ Sample} * 100$$

(3.1)

Where

A=Volume of 0.1N HCL used in sample titration

B= Volume of 0.1N HCL used in blank titration

N=Normality of HCL

Fat extraction was done using Soxhlet extraction method (Osman et al., 2019, Gopalasatheeskumar 2018). The powdered samples were weighted 3gm on tared filtered paper and place into extraction thimble. This sample were dried inside drying oven for 5 hours at 100^0C. Soxhlet glassware used for fat analysis were dried in drying oven for 1 hour at 100^0C then cool inside desiccator. This glassware was weighted and recorded. Approximately 250 ml of petroleum ether were poured into the prepared glassware and connected to Soxhlet apparatus. Heat the apparatus for 14 hours with flow rate of 150 solvent adjusted approximately at 150 drop/min. The extraction process was stopped after 14 hours and the extract were let to cool down for 30 minutes. The solvent was evaporated using vacuum evaporator until it completely dried. The glassware then transferred to desiccator. The glassware was reweighted to calculate the dried content and the fat content was calculated based on Equation (3.6).

$$\% \, Fat = \frac{Weight \ of \ lipid}{Weight \ of \ sample} * 100$$

(3.2)

Substrates' calcium, iron, copper, potassium, magnesium, sodium, phosphorus, and zinc contents were estimated according to Poitevin (2012) procedures. Carbohydrates content was determined by the method of difference following the FAO (2003) procedures. Fat extraction was done using Soxhlet extraction method (Gopalasatheeskumar 2018). To determine the pH and electrical conductivity (EC), fresh samples mixed with distilled water at a ratio of 1:10 (weight of wet sample/volume of distilled water) were used and the pH and EC of the sample were measured using a Multiparameter Water Quality Meter presented in Appendix 5, (pH Meter) (MK900-CN, China) calibrated in the range of 4.01 to 10.01. The moisture content was determined by fresh sample weight reduction after drying at 105 °C in an oven (Memmert UN 30-240V Universal Oven) for 24 hours (Appendix 3). Ash content was determined gravimetrically after sample incineration at 550 degrees celcius by muffle furnace (Model: JK-SX2-5-12N) presented in Appendix 4. pH, EC, MC, TS and TVS were determined from the MUST Chemistry Laboratory. Laboratory nutrient analysis was done at Kenya Industrial Research and Development Institute (KIRDI), Nairobi.

3.2.1 Data Analysis

Results obtained were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) statistical software for mean, standard deviation and residual errors. Independent sample F-test for equality of means was carried at 95% confidence interval to test for statistical significance of the data.

3.3 Performance of BSFL in the Conversion Process

Six replications of all the samples (shown in Table 3.1), each weighing 1000g of the wastes were prepared. Five-day old BSF larvae was bought from the SRI breeding area. 5 gram of 5 day old larvae was added into each 1000g feed mix at approximately 65 - 85% moisture content. Moisture content was monitored using gravimetric method (Shukla et al., 2014). Plastic boxes (300mm*200mm*60mm) which were locally available were used as the treatment unit as presented in Appendix 1. Batch feeding method, where the BSFL are fed a single lump amount of feed at the start of the experiment was used. Experimental monitoring was done with no additional feed. All treatments were done at room temperature (between 27-30^{o}C). Samples for the first three replicates were monitored during the larval, prepupal and pupal stages of the insect lifecycle. To evaluate the larval performance efficiency in consuming and metabolizing the rearing substrates, the total final biomass (larvae + pupae) and the residual substrates were weighted. For all the samples, data collected was summarized and presented as shown in Table 3.2.

Table 3.2: Determination of BSFL Performance

Variables	Reading
Initial feed weight (W)	
Residue/Frass weight (R)	
Feed consumed (FC=W-R)	
Waste reduction (WR)	
Initial larval weight (IL)	
Final larval weight (FL)	
Larvae yield (LY)	
Bio-conversion (%)	
Feed Conversion Rate (FCR)	

The treatment performance of BSFL in converting waste to larval biomass was estimated by calculating the Waste Reduction (WR), Waste Reduction Index (WRI), bioconversion and Feed Conversion Rate (FCR) on wet mass basis. Waste Reduction

was calculated according to Equation (3.1), which is the ratio of ingested feed (calculated as the difference between weight of total feed and weight of residue (R)) to the weight of the total feed (Diener et al., 2009, Rehman et al., 2017).

$$WR = (FC / W) * 100\% \quad (3.1)$$

Where:

WR = Waste Reduction (as a percentage),

FC = feed consumed (in grams) and

W = Initial feed weight (in grams).

Waste Reduction Index (WRI)was calculated from Equation (3.3) using the overall degradation, D from Equation (3.2) divided by the number of days used by the larvae to reduce the given amount of waste (Meneguz et al., 2018). High WRI values were indicators of good reduction efficiency.

$$D = (W - R) / W \quad (3.2)$$

$$WRI = (D / t) * 100\% \quad (3.3)$$

Where:

WRI = Waste Reduction Index (as a percentage),

W = Total feed applied (in grams) during bioconversion time t, defined as the moment when 50 % of larvae have developed to pre-pupae (in days) (Diener et al., 2009),

R = Residue left after bioconversion (non-digested substrate + excretion products) (in grams),

D = Overall degradation.

Bioconversion rate (BR) is the conversion of organic materials like fecal waste into usable products. Larval yield was determined from the difference between final and initial larval yield (Diener et al., 2009). The bioconversion rate was calculated using Equation (3.4), which is the ratio of prepupal weight gain biomass on wet basis (g) to the total amount of biowaste initially added (g) on wet basis (Gold et al., 2020, Lalander et al., 2019, Rehman et al., 2017).

$$BR = (LY / W) * 100\% \quad (3.4)$$

Where;

BR = Bioconversion rate (as a percentage),

LY = Larvae yield (in grams) and

W = Total feed applied (in grams).

3.3.1 Data Analysis

The statistical analysis was performed by SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The results of all experiments were analyzed by using one-way analysis of variance (ANOVA), followed by Tukey's HSD (honestly significant differences) for post-hoc testing to compare the significance (p-values) between the means of different groups. $P < 0.05$ was considered to indicate a significant difference between the values under comparison.

3.4 Optimum Protein Content as a Factor of Age, Larval Weight and Feed Type

3.4.1 Experimental Design

The growth of BSF is not a continuous process since growth and development have an end. The model fitted used depended on the experimental data. Therefore, the data collected for both larval weight and protein content was divided into two sets. One set of data was fitted to calibrate the model and the second set of data was fitted to validate the model. The kinetic parameters and the statistical indicator for regression goodness (R^2) was obtained directly from the software.

3.4.2 Data Collection

Three replications of all the samples (shown in Table 3.1), each 1000g of the wastes were prepared. Five-day old BSF larvae was bought from the SRI breeding area. 5 gram of 7 day old larvae was added into each 1000g feed mix at approximately 65-85% moisture content. Three sample replications per treatment were collected. Five larvae were sampled randomly from each treatment unit and their weight determined in triplicate. For laboratory nitrogen determination, samples were collected, cleaned with distilled water, dipped in boiling water, and oven-dried at 105 degrees Celsius for 12 hours. Dried samples were threshed (for particle size reduction) and transferred to the laboratory for Nitrogen determination using Kjeldahl method. A conversion factor of 6.25 was used for crude protein determination (Osman et al., 2019 & FAO, 2003). The data was recorded as shown in Table 3.3.

Table 3.3: Protein Analysis / Larval weight

Treatments	Larval weight/Protein Content per day														
	7i	7ii	7iii	9i	9ii	9iii	11i	11ii	11iii	13i	13ii	13iii	15i	15ii	15iii
a															
b															
c															
d															
e															

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Characteristic Composition of Fecal and Kitchen Waste

Results of laboratory analysis of the composition is summarized and presented in Table 4.1.

Table 4.1: Characteristics of Feed Substrates (mean ± standard deviation)

Treatment/ Parameter	a	b	c	d	e	P value
Crude protein (%)	41.5±0.04	35.31±0.06	40.51±0.02	40.26±0.03	25.94±0.19	6.94E-19
Crude fat (%)	10.77±0.02	9.62±0.19	10.41±0.001	11.09±0.00	7.80±0.19	3.14E-12
Calcium (mg/l)	23.78±0.07	22.81±0.15	22.54±0.02	22.9±0.51	22.4±0.08	0.000316
Phosphorus	1.69±0.01	8.36±0.07	8.39±0.01	6.7±0.01	0.62±0.02	1.09E-20
Copper (mg/l)	3.38±0.06	2.49±0.03	2.22±0.1	1.42±0.16	1.83±0.11	4.28E-09
Zinc (mg/l)	0.48±0.01	0.12±0.01	0.12±0.003	0.16±0.01	0.15±0.02	1.6E-12
Carbohydrates	36.51±0.6	41.34±0.41	36.93±0.53	35.74±0.86	49.05±1.15	4.76E-09
pH	8.74±0.01	7.21±0.01	7.71±0.004	7.93±0.01	4.18±0.01	3.66E-26
Moisture Content (%)	83.37±0.15	82.15±0.29	81.52±0.71	82.45±0.04	80.37±0.52	7.03E-05
Total solids (%)	16.6±0.15	17.85±0.28	18.48±0.71	17.55±0.04	19.63±0.52	6.94E-05
Total Volatile solids (TVS-%)	57.87±1.2	78.66±2.16	81.3±4.93	87.83±2.2	89.1±1.55	$4.75*10^{-7}$
Electrical conductivity (µS)	471.67±2.08	672.33±1.15	695.67±1.53	657±2	805.33±2.08	1.39E-18
Sodium (mg/l)	31.38±0.42	44.51±0.58	34.51±0.23	32.29±0.13	35.77±0.21	$1.05*10^{-13}$
Potassium (mg/l)	20.21±0.17	18.12±0.21	19.29±0.14	16.64±0.31	14.57±0.19	$1.35*10^{-10}$
Iron (mg/l)	6.55±0.35	5.01±0.09	4.26±0.35	3.1±0.17	1.44±0.05	$1.87*10^{-9}$

Where the substrates where fecal matter to kitchen waste in the ratios, a = 1:0; b = 1:1; c =2:1; d= 4:1, and e = 0:1.

The nutrient composition varied significantly among the feed substrates ("a" to "e") with the mean and standard deviation presented in Table 4.1. Mineral concentrations of calcium, phosphorus, magnesium, potassium, copper, zinc, sulphur, sodium, and iron in the feeding substrates had significant ($p<0.05$) variations.

Fecal matter was low in organic matter which is indicated by the Total Volatile Solids (TVS) at 57.8% compared to kitchen waste at 89.1%. Unlike undigested kitchen waste, low TVS in fecal matter was likely due to digestion and absorption of food in the human digestive system for human growth and development. The variations in fecal moisture content and total solids (TS) are attributed to differences in feeding habits particularly the fibre intake. Degradable fibre stimulates growth of bacterial biomass and non-degradable fibre absorbs more water in the colon. The TS for fecal matter is within the range (15-30%) reported by Maurya (2012). Fecal matter had high ash content compared to the other feed substrates which is attributed to the presence of more inert materials. The mean moisture content (MC) for fecal matter was within the range of 63-86% by weight as reported by Rose et al. (2015). Variations in the MC of feces is attributed to the differences in fiber intake as non-degradable fiber absorbs more water in the colon (Eastwood, 1973). Purkayastha et al. (2017) reported that the BSF mouth showed well-developed mandibular-maxillary complex that had similar characteristics of scavengers. Thus, the high dietary moisture content of the rearing substrates made it easy for the fly larvae to feed.

For fecal matter and the co-digested substrates, the initial pH ranged between 7.21 and 8.74. In addition, the low buffer capacity of kitchen waste (pH=4.2) was offset by the better buffer capacity of fecal matter (pH=8.74) after blending which suit the palatability of BSFL. These findings are within the pH range of 6.0-10.0 reported by Ma et al. (2017) and Liu et al. (2017) for BSFL bioconversion of organic waste. Conversely, Rose et al. (2015) reported a range of mean pH values of 5.3–7.5 for fecal matter. However, the pH of fecal matter varies between different individuals consuming

the same diet, and with time (Silvester et al., 1997). Thus, co-digestion balanced pH, nutritive and organic composition of the co-conversion substrates compared to the individual substrates for bioconversion.

The substrates showed large variability in protein content, which was highest in fecal matter and the co-digested substrates and lowest in kitchen waste. In contrast to kitchen waste, the protein in the fecal matter was from gut biomass (Rose et al., 2015). Naturally, the dietary feed intake would influence the fecal crude protein content. However, the findings of 41.5% DM crude protein for fecal matter in this study compare closely with 35.5% and 38.8% DM crude protein reported by Lalander et al. (2019) and Rose et al. (2015) respectively. Nevertheless, a suitable rearing substrate should contain at least 20% crude protein content to be considered a source of protein in a feeding diet (Munguti et al., 2006). Thus, all rearing substrates were suitable for the BSF larval growth due to the fact that the crude protein content of the substrates varied between 25% for pure kitchen waste and 41% for fecal waste on dry matter (DM) basis. Nitrogen is essential for growth, reproduction and survival due to its fundamental role in protein synthesis (Elser et al., 2000). Huberty & Denno (2006) studied the consequences of nitrogen limitation on phloem-feeding plant hoppers, *Prokelisia dolus* and *P. marginata.* The plant-hoppers raised on plants with an enriched nitrogen signature grew to a larger size, exhibited greater survival, and developed more rapidly compared to those raised on nitrogen-deficient plants. Similarly, the BSFL grown on nitrogen rich substrates resulted in heavier larval weight.

Living organisms require phosphorus during their growth and development so as to build their proteins, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) (Woods et al., 2003) a cell. Ribosomal RNA (rRNA) is important to growth as it makes up 50–60% of the ribosome, the growth machinery of the cell (Visanuvimol & Bertram, 2011). Because RNA is almost 10% phosphorus by weight, differences in the availability of phosphorus in the diet may explain the link between phosphorus availability and growth

rate (Elser et al., 2000). Growth requires a high investment in ribosomes for protein synthesis. According to Elser et al., (2000), ribosomes are extremely rich in phosphorus, thus a consistent positive association should be expected between growth rate, RNA concentration, and percent phosphorus. Visanuvimol & Bertram (2011) investigated how dietary phosphorus availability influenced invertebrate growth, development time, consumption, condition, and lifespan using juvenile European house crickets, *Acheta domesticus.* The study revealed that crickets reared on high phosphorus diets gained more weight and contained more nitrogen and phosphorus in their bodies at death than crickets reared on low phosphorus diets. Likewise, BSFL reared on substrates having low phosphorus content resulted in low larval weight and protein content. Therefore, high N and P content are essential for fast growth, development and increase protein content in BSF.

Generally, the individual substrates are nutritionally unbalanced. A study by Silva et al. (2005) reported that insects require considerable amounts of potassium, phosphorus, magnesium and small amounts of calcium, sodium and chlorides during their development. In insect's physiology, mineral ions are important for three major processes: structure formation (Mg), enzyme activation (K, Mg, Fe, Co, Mn), and trigger and control mechanisms (Na, Ca, K) (Silva et al., 2005). Similar to other organic waste stabilization methods such as composting and anaerobic digestion (Li et al., 2009), the treatment of co-digested fecal matter reduced variability and increased the process performance efficiency. Specifically, co-digestion provided a more nutritious and balanced feed for larval growth.

4.2 The Performance Efficiency of the Bioconversion Process

The results of the analysis are presented in Table 4.2.

Table 4.2: Effect of different substrates on *Hermetia illucens* waste reduction, prepupal yield, bioconversion, Feed Conversion Rate (FCR) and Waste Reduction Index (WRI).

Sample	Residue, (g)	Waste reduction, (%)	Prepupal yield, (g)	Bio-conversion, (%)	FCR	WRI
a	167.0	83.3 (1.8)	127 (12.3)^{a}	12.7 ^{a}	6.6^{a}	3.5^{a}
b	133.7	86.6 (1)	226 (13.2)^{b}	**22.6 ^{b}**	**3.9^{b}**	**3.6^{b}**
c	164.0	85.3 (1.6)	220 (15.9)^{b}	22.0^{b}	3.9^{b}	3.7^{ab}
d	153.0	84.7 (0.4)	157.3 (8.5)^{a}	15.7^{a}	5.4^{a}	3.5^{ab}
e	85.0	91.5 (1.3)	146 (7.8)^{a}	14.6^{a}	6.3^{a}	2.9^{c}

Values are in mean (SD): n=3, values bearing different superscripted alphabets differ from each other at $P < 0.05$.

4.2.1 Waste Reduction

The BSFL performed significantly different in terms of reducing and recovering dry matter when fed with different substrates (P=0.001) as presented in Appendix 7. Waste reduction was significantly higher in the substrate "e" (91.5± 1.3%) than in substrates "a" (83.3±1.8%), "b" (86.6±1.5%), "c" (85.3±1.5%) and "d" (84.3±0.4%) on wet basis. However, the larval development period was longer (32 days) in substrate "e" compared 24 days in the other substrates. Generally, the WR achieved in this study was

comparable and even in some cases better than the values reported from previous studies on BSFL treatment using different substrates. Details of findings from various authors is summarized and presented in Table 4.3.

Table 4.3: Comparison of Waste Reduction (WR)

Feeding Substrate	Waste Reduction, (%)	Source
Fecal sludge	73.0	Lalander, Diener, Zurbrügg, et al. (2013)
Fresh human feces	54.0	Banks et al. (2014)
Kitchen waste	67.9	Nguyen et al. (2015)
Fish rendering	74.2	
Mixture of fruits and vegetables	98.9	
Chicken feed	41.8	Diener et al. (2011)
Fecal sludge	54.7	
Municipal organic waste	68.0	

Fecal matter recorded the lowest waste reduction rates in this study. This could be due to its low energy content since it is a waste product of human digestion. Nguyen et al. (2015) reported that substrate energy content affected its reduction efficiency by the BSFL. However, the findings from this research are comparable with the results reported by Mahmood et al. (2021) where BSFL was used to handle household biowaste resulting in a waste reduction range of 77.0–96.1%. In a study by Nyakeri et al. (2019), waste reduction levels ranging from 54 to 92.5% was obtained. This shows that the fly larvae were able to feed and significantly reduce the substrates across all treatments.

However, the quality of the rearing substrates led to variations within and between the treatments.

The waste reduction increased with availability of nutrients in the substrate (Table 4.1) which is in agreement with the findings by Gold et al. (2020) and Banks et al. (2014). Further, these findings agree with the results reported by Diener et al. (2011b) where BSF larval yield and waste reduction in conversion of market waste (MW), municipal organic waste (MOW), fecal sludge (FS), chicken feed and MW: FS in the ratio of 1:1 was investigated. The combination of MW: FS (1:1) promised to be a good combination for both BSF larval biomass production and efficient waste reduction.

4.2.2 Waste Reduction Index (WRI)

Average WRI significantly differed across the different substrates ($P < 0.001$) as presented in Appendix 12, with the highest WRI observed in the substrate "b", followed by "c", "d", "a", and "e" respectively (Table 4.2). In addition, results presented in Table 4.2 for effect of different substrates on *Hermetia illucens* performance efficiency shows that the WRI was highest in the mixed co-conversion substrates than in the individual substrates. For kitchen waste, the WRI was 2.9 which is low compared to 5.9 reported by Mahmood et al. (2021). In another study, Bava et al. (2019) determined the WRI using okara, maize distiller, brewer's grains, and a hen diet as the rearing substrates. WRI indexes ranged from a minimum of 3.0 for brewer's grain larvae to a maximum of 4.90 ± 0.07 for okara larvae which is comparable to the current study. This indicates that formulation increased larval performance which is similar to results reported by Gold et al. (2020). However, the time taken from larvae to prepupae is prolonged when larvae are reared on substrates lacking certain nutrients, mainly protein, which results in prolonged cultivation period. The feeding period is prolonged till the nutrients inside the larvae meet the requirements for development and metamorphosis at which stage the larvae does not feed.

4.2.3 Larval yield

There was a statistically significant difference between the larval yield in the feed substrates ($P<0.05$). The larval yield was highest in substrate “b” and lowest in “a”. All the co-conversion mixtures showed a significant increase in the larval production from the fecal matter (Table 4.2). In another study, Nyakeri et al. (2019) reported similar results where BSFL gave a better larval yield on a mixture of fecal sludge and food waste compared to fecal sludge alone. Also, Rehman et al. (2017b) demonstrated increase in reduction efficiency, final larval weight, and biowaste conversion efficiency after co-composting 40% dairy manure with 60% chicken manure. Co-digestion increases yield due to better buffer capacity and nutrient balance which impacts biological growth through establishment of a positive synergism. In addition, the observed improved larval yield may be attributable to improved nutrient quality due to supplementing fecal matter with kitchen waste.

4.2.4 Feed Conversion Rate (FCR)

There was a significance difference in FCR among the different feed substrates as shown in Appendix 10. The FCR was positively affected by the co-conversion mixtures compared to the individual feed substrates “a” and “e”. The lowest most efficient FCR was 3.8 for substrates “b” and “c” respectively which were both co-digested substrates. In this study, co-digestion had beneficial effects on the FCR compared to the individual substrates. In a previous study, Nyakeri et al. (2019) reported that co-composting 30% fecal sludge with other biowastes improved the waste reduction and biomass conversion efficiency of BSFL compared with composting fecal sludge only. Lalander et al. (2019) demonstrated that co-composting fruit & vegetable waste (low in protein content) with abattoir waste (rich in protein) increased the biomass conversion efficiency from 4% DM (individual substrate) to 14% DM (1:1 fruit & vegetable waste: abattoir waste). Comparing with livestock species, the fly larvae resulted in higher FCR which is partially attributed to the insects’ poikilothermic (cold-blooded) nature; implying that

their metabolism is not used to maintain their body temperature (Ramos-Elorduy, 2008).

4.2.5 Bioconversion Rate (BCR)

There were significant differences in BCR among the five rearing substrates (P=0.000) as presented in Table 4.2. From Table 4.2, the bioconversion rate was higher in mixed feeding substrates than in the pure substrates used which is attributed to better nutrient balance in the mixed substrates. A bioconversion rate of between 16-22% for fecal waste was highlighted by Banks et al. (2014) for both batch and continuous feeding. Gold et al. (2020) reported a bioconversion rate with the range of 15-23% DM for canteen waste, Mahmood et al. (2021) reported 12.9% DM for kitchen waste in comparison to 14.6% DM for kitchen waste in this research. For fecal matter, the bioconversion rate was 19–23% DM as reported by Gold et al., (2020) which is higher than 12.7% DM in the present study. From this study, the bioconversion rate for the co-digested substrates, ranged between 15.7-22.6% DM which closely compares with results from a study by Gold et al. (2020). In another study by Rehman et al. (2017), it was observed that the BCR in BSFL composting was higher on soybean curd residue than on dairy manure, while the composite of the two substrates yielded even higher bioconversion rate. A high bioconversion rate indicates a good bioconversion efficiency. Thus, co-digestion of fecal matter improved the BSFL performance efficiency.

These findings indicate that carbohydrates, protein and fats are essential components of BSFL diet. Larvae fed with high protein content and low carbohydrate content had the shortest pupation period. Larvae fed with low protein and high carbohydrate content substrates had the longest pupation period, were light in weight and developed to pupa and adult which agrees with findings by Barragan-Fonseca et al. (2017). Thus, larvae fed on a diet rich in carbohydrates and lower in protein seem to flourish as long as they receive enough dietary protein to fulfill basic biochemical requirements for growth and

development. It was also observed that substrates with high protein and fat content (Table 4.1) resulted in a shorter larval development time than the low protein substrates which is similar to results from a study by Oonincx et al. (2015a). In addition, low fat and protein content caused larvae to have longer developmental times due to the fact that the BSFL stores most of its nutrients necessary to complete the development during the larval stage. Conversely, Simon et al. (2011) reported that feeding substrates with a higher quantity of protein increase the development period of some predatory fly species. The ratio of nitrogen:phosphorus influence growth rate. The RNA concentration, growth rate and the percent phosphorus are associated. The study shows a positive correlation between phosphorus levels and larval growth rate. Similarly, Huberty & Denno (2006) found out that reduced availability of phosphorus in the diet decrease growth rates in caterpillars and plant hoppers. From the study, co-digesting fecal matter with kitchen waste that has higher nutrient fractions improved the BSFL performance efficiency on the substrates. Thus, formulating different types of bio-waste has the potential to increase process performance of BSFL as reported by Gold et al. (2018) since the formulations are more nutritively balanced.

4.3 Predictive Modelling of Larval Weight and Optimum Protein

4.3.1 Larval Weight

The simulated results of the kinetic parameters of the larval weight per 5 larvae are shown in Table 4.3.

Table 4.3: Kinetic Parameters of Larval Weight per 5 Larvae of the Experiment by Modified Gompertz Model

Parameter	a (1:0)	b (1:1)	c (2:1)	d (4:1)	e (0:1)
P_0	1.303	1.698	1.526	1.502	1.115
r_{max}	0.153	0.138	0.144	0.130	0.075
t_0	2.408	0.000	1.117	0.026	0.585
R^2	0.989	0.987	0.969	0.986	0.989

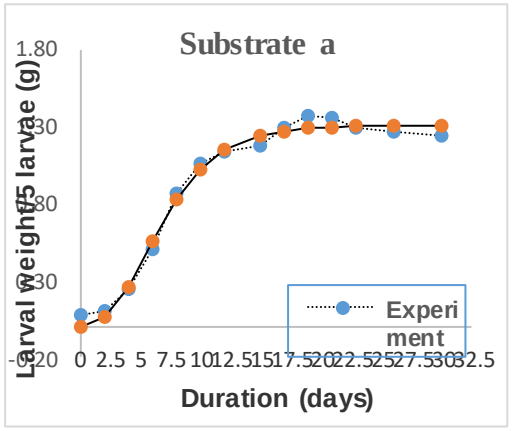
Where:

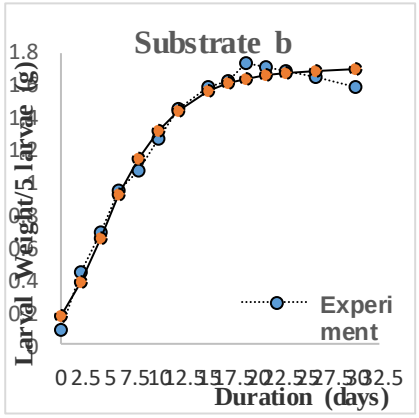
P_0 = Maximum Protein potential of the substrate as a percentage per gram of the sample.

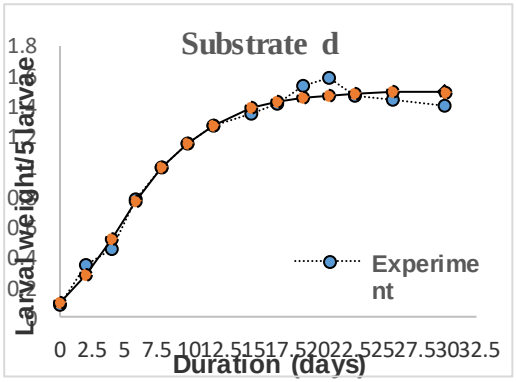
r_{max} = Absolute growth rate as a percentage per day which is the tangent to the curve at the lag time t_0,

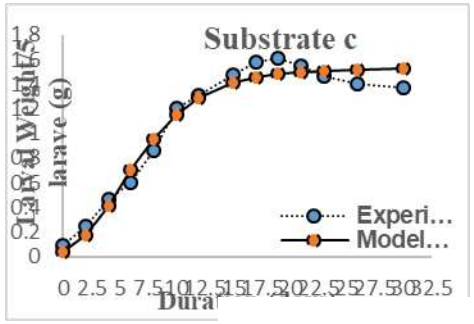
t_0 = Lag time in days which falls where $P = P_0.\exp(-e)$ as reported by Tjerve & Tjerve, (2017).

The p value = $1.23*10^{-24}$ < P=0.05 indicates that different substrates affected the BSF larval weight. Table 4.3 shows that the P_0 of mixed co-conversion substrates was higher compared to the individual FM and KW substrates since the kinematics factors are dependent on the substrate used. The P_0 of substrate "c" was close to that of substrate "d" and significantly higher than that of substrates "a" and "e". Substrate "b" resulted in the highest weight gain (P_0) and a significantly higher prepupal yield. The steep slope for substrate "b" (Figure 4.1) indicates that less time was utilized by the larvae to attain its maximum larval weight compared to the other substrates.









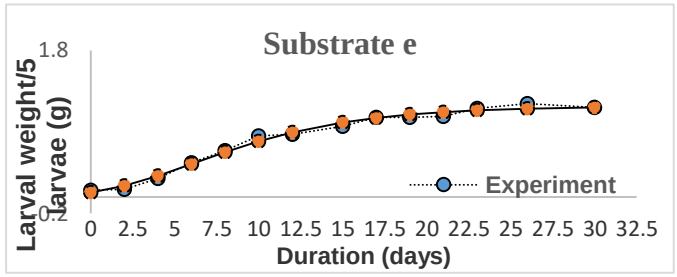


Figure 4.1(a-e): Model Simulation of the Larval Weight

Generally, co-digested substrates resulted in improved larval weight compared to the individual substrates. It was predicted that kitchen waste (had low protein and high carbohydrates results earlier presented in Table 4.1) resulted in the lowest larval weight compared to fecal matter and the co-digested substrates. The larval growth and development time are influenced by factors such as feed availability (Diener et al., 2009), nutrient availability and feed characteristics (Rose et al., 2015). During the larval stage, BSFL consumes a large quantity of food as a reserve for the adult stage. The weight of larvae highly depends on the substrate quality and quantity. In the kitchen waste, a slow growth of the BSFL was observed. This was attributed to the presence of fats, grease and oil covering the kitchen waste, leading to the difficulty for the BSFL to digest and convert the greasy waste into its body weight. Besides, the other substrates were generally free from oil, grease and fat thus favoring the physiological growth of BSFL. Hence, the BSFL consumed less time for growing and developing into prepupae (Spranghers et al., 2017). Similar to anaerobic digestion (Baek et al., 2020), the treatment of a mixture of several substrates increased the BSFL performance and reduce variability. In addition, Nyakeri et al. (2019) and Rehman et al. (2017) observed that mixing human and cow manure with banana peels and soybean curd residue (food wastes and food production by-products) increased larval weight compared to the individual wastes. Thus, mixing substrates can provide a more nutritious and balanced feed for larval growth.

The substrate characteristics (Table 4.1) affect the BSFL growth rate, development and positively correlates with survival rate and larval length (Gobbi et al., 2013). The larval weight is key in growth and development of the fly since the prepupal weight affects the growth, survival, and biological traits related to reproduction of adult flies (Liu et al., 2008). In addition, lower prepupal weight hampers the sustainability of bioconversion process as the adults produced have a lower reproduction ability (Gobbi et al., 2013). Jucker et al. (2017) and Loaiza et al. (2008) found out that substrates that had low

protein and high carbohydrate content resulted in poor larval development. Similarly, weight gain was marginally lower in the low-Protein:high carbohydrate substrates used in this study.

A study by Gold et al. (2018) reported that microbes differ among biowastes and are influential in biowaste decomposition and BSF larval development. BSF larval weight gain is also affected by potential larval dependence on bacteria such as *Bacillus subtilis* which has the ability to digest protein and provide organic phosphorus as food (Liu et al., 2008). For example, a study by Yu et al. (2011) reported that bacteria, *Bacillus subtilis* isolated from the BSF larval gut promoted the growth and development of the conspecific larvae by fermenting the feeding substrate. In addition, microbial communities and numbers could have contributed to the variations in BSFL treatment performance.

The larvae easily converted the feed to its own body mass thus increasing the larval weight. Mixed co-conversion substrates showed a significantly improved larval weight and performance in comparison to the individual substrates. This is attributed to the sufficiently high TVS and protein content in the co-conversion mixtures which support both larval growth and development. It was noted that larval weights were significantly higher where the initial pH ranged between 7.2 and 8.7, and lowest when the initial pH was 4.8. The pH of the substrate greatly affects the activities of bacteria and particular acid-producing microbial populations (Zheng et al., 2017) thus affecting the insect gut microbiome which promotes larval weight gain, growth, and development. A former study by Popa & Green (2012) reported that BSFL neutralized the acidity of compost leachate which may be due to the gut microorganisms responsible for the production of organic acids. Thus, the prolonged lag phase in the kitchen waste (pH=4.8) was due to the alkalization of the substrate caused by the release of ammonia and ammonium ions(Alidadi et al., 2016). This indicates that initial pH significantly affects larval

weight gain and biological growth rate which is contrary to a study done by (Meneguz et al., 2018). Thus, co-digestion of fecal matter increased both the alkalinity capacity and production of larvae, while reducing the fats concentration. Inhibition of the fats was beneficial for gut microbiota development which play a vital role in nutrient biodegradation for larval development. For the kitchen waste which had low pH, the larvae adjusted through alkalization of the substrate caused by the release of ammonium ions and ammonia (Alidadi et al., 2016).

Comparing the larval weight (Table 4.3) and waste reduction results of the substrates (Table 4.2) demonstrates that higher waste reduction did not necessarily result in higher larval weight. Nevertheless, substrate dry matter reduction gives an indication of how sufficiently the substrate is degraded and converted to larval biomass. Adopting BSFL technology for fecal matter management is suitable for safe and sustainable disposal of fecal matter from onsite sanitation systems while reducing of environmental pollution and degradation.

4.3.2 Crude Protein Content

From the study, different substrates have different protein content potential (P_0) as shown in Table 4.4.

Table 4.4: Kinetic Parameters of Protein Content of the Experiment by Modified Gompertz Model

Parameter	a(1:0)	b (1:1)	c (2:1)	d (4:1)	e (0:1)
P_0	32.97	38.71	33.57	35.27	36.95
r_{max}	1.67	1.78	1.83	1.69	2.24
t_0	0	0	0	0	0
R^2	0.9828	0.9853	0.9652	0.9826	0.9914

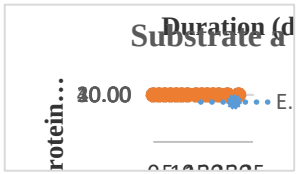
Where:

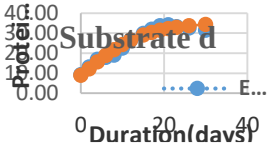
P_0 = Maximum Protein potential of the substrate as a percentage per gram of the sample.

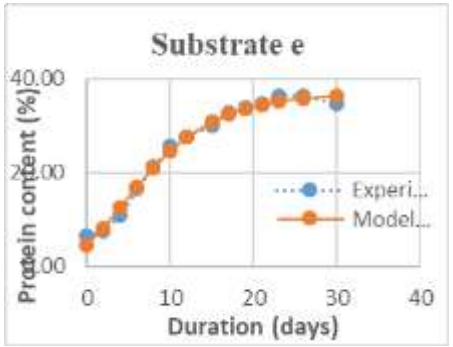
r_{max} = Absolute growth rate as a percentage per day which is the tangent to the curve at the lag time t_0,

t_0 = Lag time in days which falls where $P = P_0.\exp(-e)$ as reported by Tjerve & Tjerve, (2017).

The model was used to predict the maximum crude protein potential and the time required to attain the maximum conversion rate for the different substrates. From Table 4.4, t_0=0 indicates that the larvae had accumulated some protein before the start of the experiment and r_{max} shows the ease of conversion of the feed by the BSFL. Changes in protein content over time during the BSFL treatment is shown in Figure (4.2). A lag phase of two days was observed across all the substrates which suggested that two days were required for the larvae to adapt to the medium.







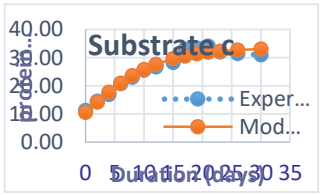


Figure 4.2(a-e): Model Simulation of Crude Protein Content

Thereafter, an increase in crude protein content from 13.5%, 9.9%, 14.2%, 12.8% and 8.1% to 31.9%, 36.2%, 32.8%. 33.8% and 35 .9% for substrates a, b, c, d and e respectively was observed from the second day to day 26. The exponential phase of the protein content coincided with the active feeding stage of the BSFL. It is at this stage that the larvae store enough reserves for use during reproduction and adult stage. The protein content decreased after the exponential phase corresponding to the gradual pupation and shedding off of the larvae and the beginning of the stationary phase. The results show that the modified Gompertz equation fitted the experimental data very well with coefficients of determination exceeding 0.96 with Equation (4.1) giving the optimal conditions for protein content.

$$P = 38.71.exp\left\{-exp\left[\frac{1.78.e}{38.71}.(-t)+1\right]\right\}$$

(4.1)

This demonstrates that the model can be used well to describe the BSFL protein content. Unlike kitchen waste (Table 4.1), fecal matter is low in TVS content. Therefore, co-digestion increased the TVS in the formulated substrates resulting in improved feed conversion for larval growth. From the study, FW contained the highest crude protein content and lowest TVS and yet resulted in the lowest larval crude protein. The larvae accummulated substancial protein quantity for growth while consuming minimal enegy which resulted in low biomass protein content. The study shows that the BSF larvae's protein content vary depending on the type of waste used as food source and the stage at which the larvae are harvested. The findings indicate that the BSFL protein content ranged between 33 and 39% on DM basis which is within the range of 32% to 46% reported by Diener, Zurbrügg, and Tockner (2009) and Spranghers et al. (2017) on BSFL composting.

Fecal matter co-digestion helped to optimize the nutrient balance of the rearing substrates, enhance waste reduction, and larvae growth. This study shows that the larval weight and protein content of BSF larvae are significantly affected by the characteristics of the growing substrate provided. The results show that blending fecal matter with raw kitchen waste fortified the nutritional contents of larval feeding substrates. Similarly, Raksasat et al. (2020) reported that despite the fact that BSFL can ingest various decay materials, some organic wastes such as sewage sludge or lignocellulosic wastes such as waste coconut endosperm are destitute of decent nutrients that could retard the BSFL growth. Thus, co-digestion improved the reliability of the substrates by balancing both macro and micro-nutrients in the rearing subtrates since the nutritional composition of BSFL is highly influenced by the rearing sustrate. The larvae served effectively in the dual roles of high-protein biomass production and waste minimization. From the study, the results suggest that co-conversion ratio of 1:1 was more appropriate for both larval biomass production and waste treatment. This reveals that BSFL can be reliable for feacal waste management and the treatment can be improved through formulating different organic wastes depending on their initial nutritive characteristics. However, the characteristics of fecal matter are dependent on the source (Rose et al., 2015), which can affect an industrial-scale recycling facility.

Thus, the study shows that BSFL co-digestion can be used for fecal waste recycling and management for nutrient re-recovery and re-integration into the food chain and bio-fertilizer production. From this study, BSFL has been used for nutrient recovery from co-digested fecal matter which may constitute a missing link in circular economy design for environment management and resource recovery. Hence reduced environmental pollution, improved sanitation and sustainable economic growth.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From this study, the following conclusions were drawn:

1. The nutritive characteristics of feeding substrates ranged from 25.9-41.5% for crude protein content, 35.7-49.1% for carbohydrate content, 57.9-89.1% for total volatile solids, 4.2-8.7 pH, 0.6-8.4 for phosphorus, 22.4-23.8 for calcium and 1.4-6.6 for iron.
2. A range of 3.53-3.7, 3.9-5.39, 15.7-22.6% was attained for WRI, FCR and BCR respectively for the co-digested substrates. Thus, fecal matter co-digestion improved the performance of BSFL for increased larval biomass production.
3. Optimum protein content attained was 38.71% which was achieved at 50% co-digestion of fecal matter.

5.2 Recommendations

a) Recommendations from the study

i. Characterize the harvested larval biomass into the chemical and elemental characteristics so as to compare the substrate and product chemical and elemental characteristics.

ii. To investigate the effect of the human diet on growth and development of BSFL.

b) Other Recommendations

a) Further research is necessary to assess:
    i. the potential microbial contamination and the food/feed safety risks linked to the use of fecal matter as feed for BSFL in the harvested products and by-products.
    ii. the mass/material balance from the rearing substrates, across the treatment process and in the harvested larvae, residue, shells and the dead fly.
b) Sanitation policy makers in Kenya can apply the findings of this study in the formulation of policies on safe handling and disposal of fecal matter from on-site sanitation facilities.

REFERENCES

Albano, G., Giorno, V., Román-Román, P., Román-Román, S., Serrano-Pérez, J. J., & Torres-Ruiz, F. (2020). Inference on an heteroscedastic Gompertz tumor growth model. *Mathematical Biosciences*, *328*(108428). https://doi.org/10.1016/j.mbs.2020.108428

Algapani, D. E., Qiao, W., di Pumpo, F., Bianchi, D., Wandera, S. M., Adani, F., & Dong, R. (2017). Long-term bio-H2 and bio-CH4 production from food waste in a continuous two-stage system: Energy efficiency and conversion pathways. *Bioresource Technology*, *248*, 204–213. https://doi.org/10.1016/j.biortech.2017.05.164

Algapani, D. E., Qiao, W., Su, M., di Pumpo, F., Wandera, S. M., Adani, F., & Dong, R. (2016). Bio-hydrolysis and bio-hydrogen production from food waste by thermophilic and hyperthermophilic anaerobic process. *Bioresource Technology*, *216*, 768–777. https://doi.org/10.1016/j.biortech.2016.06.016

Alidadi, H., Hosseinzadeh, A., Najafpoor, A. A., Esmaili, H., Zanganeh, J., Takabi, D. M., & Piranloo, F. G. (2016). Waste recycling by vermicomposting: Maturity and quality assessment via dehydrogenase enzyme activity, lignin, water soluble carbon, nitrogen, phosphorous and other indicators. *Journal of Environmental Management*, *182*, 134–140. https://doi.org/10.1016/j.jenvman.2016.07.025

Andersson, K., Otoo, M., & Nolasco, M. (2018). Innovative sanitation approaches could address multiple development challenges. *Water Science and Technology*, *77*(4), 855–858. https://doi.org/10.2166/wst.2017.600

Anjum, M., Khalid, A., Mahmood, T., & Arshad, M. (2012). Anaerobic co-digestion of municipal solid organic waste with melon residues to enhance biodegradability and

biogas production. *Journal of Material Cycles and Waste Management*, *14*(4), 388–395. https://doi.org/10.1007/s10163-012-0082-9

Antony, J., Warwood, S., Fernandes, K., & Rowlands, H. (2001). Process optimisation using Taguchi methods of experimental design. *Work Study*, *50*(2), 51–58

.Ayodele, B. V., & Abdullah, S. (2018). An Overview of Response Surface Methodology Approach to Optimization of Hydrogen and Syngas Production by Catalytic Reforming of Greenhouse Gases (CH4 and CO2). In *Statistical Approaches With Emphasis on Design of Experiments Applied to Chemical Processes methane*. (pp. 65–78). IntechOpen.

Azid, A., Juahir, H., Latif, M. T., Zain, S. M., & Osman, M. R. (2013). Feed-Forward Artificial Neural Network Model for Air Pollutant Index Prediction in the Southern Region of Peninsular Malaysia. *Journal of Environmental Protection*, *04*, 1–10. https://doi.org/10.4236/jep.2013.412a1001

Baek, G., Kim, D., Kim, J., Kim, H., & Lee, C. (2020). Treatment of cattle manure by anaerobic co-digestion with food waste and pig manure: Methane yield and synergistic effect. *International Journal of Environmental Research and Public Health*, *17*(13), 1–13. https://doi.org/10.3390/ijerph17134737

Banks, I. J., Gibson, W. T., & Cameron, M. M. (2014). Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. *Tropical Medicine and International Health*, *19*(1), 14–22. https://doi.org/10.1111/tmi.12228

Barragan-Fonseca, K. B., Dicke, M., & van Loon, J. J. A. (2017). Nutritional value of the black soldier fly (Hermetia illucens L.) and its suitability as animal feed - a review. *Journal of Insects as Food and Feed*, *3*(2), 105–120. https://doi.org/10.3920/JIFF2016.0055

Bashir, M. J. K., Amr, S. S. A., Aziz, S. Q., Aun, N. C., Sethupathi, S., Technology, G., Tunku, U., & Rahman, A. (2015). Wastewater Treatment Processes Optimization Using Response Surface Methodology (RSM) Compared with Conventional Methods : Review and Comparative Study Department of Environmental Engineering , Faculty of Engineering School of Civil Engineering , Enginee. *Middle-East Journal of Scientific Research*, *23*(2), 244–252. https://doi.org/10.5829/idosi.mejsr.2015.23.02.52

Bava, L., Jucker, C., Gislon, G., Lupi, D., Savoldelli, S., Zucali, M., & Colombini, S. (2019). Rearing of Hermetia Illucens on By-products: Influence on Growth, waste Reduction and Environmental Impact. *Animals*, *9*(289).

Bhavsar, S., Dudhagara, P., & Tank, S. (2018). R software package based statistical optimization of process components to simultaneously enhance the bacterial growth , laccase production and textile dye decolorization with cytotoxicity study. *PLOS ONE*, *13*(5). https://doi.org/10.1371/journal.pone.0195795

Caligiani, A., Marseglia, A., Leni, G., Baldassarre, S., Maistrello, L., Dossena, A., & Sforza, S. (2018). Composition of black soldier fly prepupae and systematic approaches for extraction and fractionation of proteins, lipids and chitin. *Food Research International*, *105*(December 2017), 812–820. https://doi.org/10.1016/j.foodres.2017.12.012

Chaturvedi, D. K. (2010). *Modelling and Simulation Systems Using MATLAB and Simulink*.

Cicková, H., Newton, G. L., Lacy, R. C., Kozánek, M., & Helena, C. (2015). The use of fly larvae for organic waste treatment. *Waste Management*, *35*, 68–80. https://doi.org/10.1016/j.wasman.2014.09.026

Dickin, S., Dagerskog, L., Jiménez, A., Andersson, K., & Savadogo, K. (2018).

Understanding sustained use of ecological sanitation in rural Burkina Faso. *Science of the Total Environment*, *613–614*, 140–148. https://doi.org/10.1016/j.scitotenv.2017.08.251

Diener, S., Zurbrügg, C., Roa Gutiérrez, F., Nguyen Dang Hong Morel, A., Koottatep, T., Tockner, K. (2011). Black soldier fly larvae for organic waste treatment–prospects and constraints. *Proceedings of the WasteSafe 2011 – 2nd International Conference on Solid Waste Management in the Developing Countries*, *52*(2), 978–984.

Diener, S., Zurbrügg, C., Roa Gutiérrez, F., Nguyen, Dang Hong Morel A., Koottatep, T., & Tockner, K. (2011). Black soldier fly larvae for organic waste treatment–prospects and constraints. *Proceedings of the WasteSafe 2011 – 2nd International Conference on Solid Waste Management in the Developing Countries*, *52*, 1–8.

Diener, Stefan, Semiyaga, S., Niwagaba, C. B., Muspratt, A. M., Gning, J. B., Mbéguéré, M., Ennin, J. E., Zurbrugg, C., & Strande, L. (2014). A value proposition: Resource recovery from faecal sludge - Can it be the driver for improved sanitation? *Resources, Conservation and Recycling*, *88*, 32–38. https://doi.org/10.1016/j.resconrec.2014.04.005

Diener, Stefan, Studt Solano, N. M., Roa Gutiérrez, F., Zurbrügg, C., & Tockner, K. (2011). Biological treatment of municipal organic waste using black soldier fly larvae. *Waste and Biomass Valorization*, *2*(4), 357–363. https://doi.org/10.1007/s12649-011-9079-1

Diener, Stefan, Zurbrügg, C., & Tockner, K. (2009). Conversion of organic material by black soldier fly larvae: Establishing optimal feeding rates. *Waste Management and Research*, *27*(6), 603–610. https://doi.org/10.1177/0734242X09103838

Dortmans, B., Diener, S., Verstappen, B., & Zurbrügg, C. (2017). *Black Soldier Fly*

Biowaste Processing -A Step-by-Step Guide. Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf,. https://doi.org/10.1117/12.464354

Eastwood, M. A. (1973). Vegetable fibre: its physical properties. *Proc.Nutr.Soc.*, 137–143.

Elser, J. J., Sterner, R. W., Gorokhova, E., Fagan, W. F., Markow, T. A., Cotner, J. B., Harrison, J. F., Hobbie, S. E., Odell, G. M., & Weider, L. W. (2000). Biological stoichiometry from genes to ecosystems. *Ecology Letters*, *3*(6), 540–550. https://doi.org/10.1046/j.1461-0248.2000.00185.x

Esrey, S. A. (2001). Towards a recycling society : ecological sanitation – closing the loop to food security. *Water Science and Technology*, *43*(4), 177–188.

FAO. (2003). *Food and Agriculture Organization of the United Nations*. https://doi.org/10.1111/j.1475-4932.1946.tb01187.x

Fidjeland, J. (2015). *Sanitisation of Faecal Sludge by Ammonia Treatment Technology for Safe Reuse in Agriculture*.

Fischer, H. P. (2008). Mathematical modeling of complex biological systems: From parts lists to understanding systems behavior. *Alcohol Research and Health*, *31*(1), 49–59.

Fogarassy, C., & Finger, D. (2020). Theoretical and practical approaches of circular economy for business models and technological solutions. *Resources*, *9*(6), 1–9. https://doi.org/10.3390/resources9060076

Food and Argriculture Organization of the United Nations. (2013). Edible insects: Future prospects for food and feed security. In *Food and Agriculture Organization of the United Nations* (Vol. 171).

Gariglio, M., Dabbou, S., Crispo, M., Biasato, I., Gai, F., Gasco, L., Piacente, F., Odetti, P., Bergagna, S., Plach, I., Valle, E., Colombino, E., Capucchio, M. T., & Schiavone, A. (2019). Effects of the Dietary Inclusion of Partially Defatted Black Soldier Fly (Hermetia illucens) Meal on the Blood Chemistry and Tissue (Spleen, Liver, Thymus, and Bursa of Fabricius) Histology of Muscovy Ducks (Cairina moschata domestica). *Animals*, *9*(307), 1–13.

Garthright, W. E. (1991). Refinements in the prediction of microbial growth curves. *Food Microbiology*, *8*, 239–248.

Gensch, R., Jennings, A., Renggli, S., & Reymond, P. (2018). *Compendium of Sanitation Technologies in Emergencies* (1st Editio). German WASH Network (GWN), Swiss Federal Institute of Aquatic Science and Technology (Eawag), Global WASH Cluster (GWC) and Sustainable Sanitation Alliance (SuSanA). http://www.bmz.de/de/zentrales_downloadarchiv/Presse/emergency-sanitation-compendium.pdf

Gibson, A. M., Bratchell, N., & Roberts, T. A. (1988). Predicting microbial growth : growth responses of salmonellae in a laboratory medium as affected by pH , sodium chloride and storage temperature. *International Journal OfFoodMicrobiology*, *6*, 155–178.

Gibson, M., Bratchell, N., & Roberts, T. A. (1987). The effect of sodium chloride and temperature on the rate and extent of growth of Clostvidiurn botulinurn type A in pasteurized pork slurry. *Journal of Applied Bacteriology 1987, 62*, 479–490.

Gobbi, P., Martínez-Sánchez, A., & Rojo, S. (2013). The effects of larval diet on adult life-history traits of the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). *European Journal of Entomology*, *110*(3), 461–468. https://doi.org/10.14411/eje.2013.061

Gold, M., Marie, C., Zurbrügg, C., Kreuzer, M., Boulos, S., Diener, S., & Mathys, A. (2020). Biowaste treatment with black soldier fly larvae : Increasing performance through the formulation of biowastes based on protein and carbohydrates. *Waste Management*, *102*, 319–329. https://doi.org/10.1016/j.wasman.2019.10.036

Gold, M., Tomberlin, J. K., Diener, S., Zurbrügg, C., & Mathys, A. (2018). Decomposition of biowaste macronutrients , microbes , and chemicals in black soldier fly larval treatment : A review. *Waste Management*, *82*, 302–318. https://doi.org/10.1016/j.wasman.2018.10.022

Gopalasatheeskumar, K. (2018). Significant Role of Soxhlet Extraction Process in Phytochemical Research. *Mintage Journal of Pharmaceutical & Medical Sciences Vol*, *7*, 43–47.

Gross, E., & Günther, I. (2014). Why do households invest in sanitation in rural Benin: Health, wealth, or prestige? *Water Resources Research*, *50*(10), 8314–8329. https://doi.org/10.1002/2014WR015899

Hafford, L. M., Ward, B. J., Weimer, A. W., & Linden, K. (2018). Fecal sludge as a fuel: Characterization, cofire limits, and evaluation of quality improvement measures. *Water Science and Technology*, *78*(12), 2437–2448. https://doi.org/10.2166/wst.2019.005

Hemalatha, B. (2012). Vermicomposting of fruit waste and industrial sludge. *International Journal of Advanced Engineering Technology*. http://www.technicaljournalsonline.com/ijeat/VOL III/IJAET VOL III ISSUE II APRIL JUNE 2012/14 IJAET Vol III Issue II 2012.pdf

Henry, M., Gasco, L., Piccolo, G., & Fountoulaki, E. (2015). Review on the use of insects in the diet of farmed fish : Past and future. *Animal Feed Science and Technology*, *203*(January 2018), 1–22.

https://doi.org/10.1016/j.anifeedsci.2015.03.001

Hu, M., Fan, B., Wang, H., Qu, B., & Zhu, S. (2016). Constructing the ecological sanitation : a review on technology and methods. *Journal of Cleaner Production*, 1–21. https://doi.org/10.1016/j.jclepro.2016.03.012

Huberty, A. F., & Denno, R. F. (2006). Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. *Oecologia*, *149*, 444–455. https://doi.org/10.1007/s00442-006-0462-8

IST-UTS and SNV. (2021). Black soldier fly (waste) treatment. In *Treatment technologies in practice: On-the-ground experiences of faecal sludge and wastewater treatment*.

Joyce, J. A., Sheppard, D. C., Kiser, B. C., Tomberlin, J. K., & Sumner, S. M. (2009). Rearing Methods for the Black Soldier Fly (Diptera: Stratiomyidae): Table 1. *Journal of Medical Entomology*, *39*(4), 695–698. https://doi.org/10.1603/0022-2585-39.4.695

Jucker, C., Erba, D., Leonardi, M. G., Lupi, D., & Savoldelli, S. (2017). Assessment of Vegetable and Fruit Substrates as Potential Rearing Media for Hermetia illucens (Diptera: Stratiomyidae) Larvae. *Environmental Entomology*, *46*(6), 1415–1423. https://doi.org/10.1093/ee/nvx154

Kakoi, B. K. (2018). *Pollutant Removal from Water Using Plant Materials*.

Kanda, E. K., Kipkorir, E. C., & JKosgei, J. R. (2016). Dissolved oxygen modelling using artificial neural network: a case of River Nzoia, Lake Victoria basin, Kenya. *Journal of Water Security*, *2*. https://doi.org/10.15544/jws.2016.004

Karagodin, V. P., Yurina, O. V., Bastrakov, A. I., & Ushakova, N. A. (2017). Studying development of hermetia illucens fly larvae cultivated on high cellulose plant

substrates. *Agricultural Engineering International: CIGR Journal*, *2017*(2), 283–288.

Katukiza, A. Y., Ronteltap, M., Niwagaba, C. B., Foppen, J. W. A., Kansiime, F., & Lens, P. N. L. (2012). Sustainable sanitation technology options for urban slums. *Biotechnology Advances*, *30*, 964–978. https://doi.org/10.1016/j.biotechadv.2012.02.007

Kim, W., Bae, S., Park, K., Lee, S., Choi, Y., Han, S., & Koh, Y. (2011). Biochemical characterization of digestive enzymes in the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). *Journal of Asia-Pacific Entomology*, *14*(1), 11–14. https://doi.org/10.1016/j.aspen.2010.11.003

Kumar, A. (2017). Beyond toilets and targets: sanitation mission in India. *Development in Practice*, *27*(3), 408–413. https://doi.org/10.1080/09614524.2017.1290050

Lalander, C., Diener, S., Zurbrügg, C., & Vinnerås, B. (2019). Effects of feedstock on larval development and process efficiency in waste treatment with black soldier fly (Hermetia illucens). *Journal of Cleaner Production*, *208*, 211–219. https://doi.org/10.1016/j.jclepro.2018.10.017

Lalander, C. H., Fidjeland, J., Diener, S., Eriksson, S., & Vinnerås, B. (2016). High waste-to-biomass conversion and efficient Salmonella spp . reduction using black soldier fly for waste recycling. *Agron. Sustain. Dev.*, *35*(261). https://doi.org/10.1007/s13593

Lalander, Cecilia, Diener, S., Magri, M. E., Zurbrügg, C., Lindström, A., & Vinnerås, B. (2013). Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens) — From a hygiene aspect. *Science of The Total Environment*, *458–460*, 312–318. https://doi.org/10.1016/j.scitotenv.2013.04.033

Lalander, Cecilia, Diener, S., Zurbrügg, C., Magri, M. E., Lindström, A., & Vinnerås, B. (2013). Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens) — From a hygiene aspect. *Science of The Total Environment*, *458–460*, 312–318. https://doi.org/10.1016/j.scitotenv.2013.04.033

Li, R., Chen, S., & Li, X. (2009). Anaerobic co-digestion of kitchen waste and cattle manure for methane production. *Energy Sources, Part A: Recovery, Utilization and Environmental Effects*, *31*(20), 1848–1856. https://doi.org/10.1080/15567030802606038

Liland, N. S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C. G., Waagbø, R., Torstensen, B. E., & Lock, E. J. (2017). Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae by feeding seaweed-enriched media. *PLoS ONE*, *12*(8). https://doi.org/10.1371/journal.pone.0183188

Liu, Q., Tomberlin, J. K., Brady, J. A., Sanford, M. R., & Yu, Z. (2008). Black soldier fly (Diptera: Stratiomyidae) larvae reduce Escherichia coli in dairy manure. *Environmental Entomology*, *37*(6), 1525–1530. https://doi.org/10.1603/0046-225X-37.6.1525

Liu, X., Chen, X., Wang, H., Yang, Q., Rehman, K., Li, W., Cai, M., Li, Q., Mazza, L., Zhang, J., Yu, Z., & Zheng, L. (2017). Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. *PLOS ONE*, *12*(8). https://doi.org/10.1371/journal.pone.0182601

Loaiza, V., Jonas, J. L., & Joern, A. (2008). Does dietary P affect feeding and performance in the mixed-feeding grasshopper (Acrididae) Melanoplus bivitattus? *Environmental Entomology*, *37*(2), 333–339. https://doi.org/10.1603/0046-225X(2008)37[333:DDPAFA]2.0.CO;2

Lohri, C. R., Diener, S., Zabaleta, I., Mertenat, A., & Zurbrugg, C. (2017). Treatment

technologies for urban solid biowaste to create value products : a review with focus on low- and middle- income settings. *Rev Environ Sci Biotechnol*, *16*, 81–130. https://doi.org/10.1007/s11157-017-9422-5

Ma, J., Lei, Y., Rehman, K. U., Yu, Z., Zhang, J., Li, W., Li, Q., Tomberlin, J. K., & Zheng, L. (2017). Dynamic Effects of Initial pH of Substrate on Biological Growth and Metamorphosis of Black Soldier Fly (Diptera: Stratiomyidae). *Environmental Entomology*, *XX*(X), 1–7. https://doi.org/10.1093/ee/nvx186

Mahmood, S., Zurbrügg, C., Tabinda, A. B., Ali, A., & Ashraf, A. (2021). Sustainable Waste Management at Household Level with Black Soldier Fly Larvae (Hermetia illucens). *Sustainability 2021*, *13*(9722), 1–18. https://doi.org/https: //doi.org/10.3390/su13179722

Mansour, G., Oyaya, C., & Owor, M. (2017). *Situation analysis of the urban sanitation sector in Kenya*.

Mathworks. (2001). Curve Fitting Toolbox User's Guide. In *MATLAB Manual*. papers2://publication/uuid/1AB1E427-49D2-43BA-BE5C-B64EE88F3947

Maurya, N. S. (2012). Is human excreta a waste ? *Int. J. Environmental Technology and Management*, *15*, 3–6. https://doi.org/10.1504/IJETM.2012.049231

Meneguz, M., Schiavone, A., Gai, F., Dama, A., Lussiana, C., Renna, M., & Gasco, L. (2018). Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (Hermetia illucens) larvae. *Journal of the Science of Food and Agriculture*, *98*, 5776–5784. https://doi.org/10.1002/jsfa.9127

Mertenat, A., Diener, S., & Zurbrügg, C. (2019). Black Soldier Fly biowaste treatment – Assessment of global warming potential. *Waste Management*, *84*.

https://doi.org/10.1016/j.wasman.2018.11.040

Montgomery, D. C. (2013). *Design and Analysis of Experiments* (8th ed.). John Wiley & Sons, Inc.

Moula, N., Scippo, M. L., Douny, C., Degand, G., Dawans, E., Cabaraux, J. F., Hornick, J. L., Medigo, R. C., Leroy, P., Francis, F., & Detilleux, J. (2018). Performances of local poultry breed fed black soldier fly larvae reared on horse manure. *Animal Nutrition*, *4*(1), 73–78. https://doi.org/10.1016/j.aninu.2017.10.002

Mourabet, M., El Rhilassi, A., El Boujaady, H., Taitai, A., & Bennani-Ziatni, M. (2017). Use of response surface methodology for optimization of fluoride adsorption in an aqueous solution by Brushite. *Arabian Journal of Chemistry*, *10*, S3292–S3302. https://doi.org/10.1016/j.arabjc.2013.12.028

Mugauri, T. R., Inambao, F., Septien, S., & Singh, A. (2018). *Exploration of the Use of Solar Thermal Energy for Faecal Sludge Drying*. *13*(10), 7598–7603.

Munguti, J. M., Liti, D. M., Waidbacher, H., Straif, M., & Zollitsch, W. (2006). Proximate composition of selected potential feedstuffs for Nile tilapia (Oreochromis niloticus Linnaeus) production in Kenya. *ResearchGate*, *57*(1–4), 131–141.

Myers, R. H., & Montgomery, Douglas C. Anderson-cook, C. M. (2009). *Response surface methodology. Process and Product O Optimization Using Designed Experiments* (S. W. E. David J. Balding, Noel A. C. Cressie, Garrett M. Fitzmaurice, Iain M. Johnstone, Geert Molenberghs, David W. Scott, Adrian F. M. Smith, Ruey S. Tsay (ed.); 3rd ed.). John Wiley & Sons, Inc.,.

Nguyen, T. T. X., Tomberlin, J. K., & Vanlaerhoven, S. (2015). Ability of Black

Soldier Fly (Diptera: Stratiomyidae) Larvae to Recycle Food Waste. *Environmental Entomology*, *44*(2), 406–410. https://doi.org/10.1093/ee/nvv002

Nikiema, J., Cofie, O., Impraim, R., & Adamtey, N. (2013). Processing of Fecal Sludge to Fertilizer Pellets Using a Low-Cost Technology in Ghana. *Environment and Pollution*, *2*(4). https://doi.org/10.5539/ep.v2n4p70

Njuguna, J., & Muruka, C. (2017). Open Defecation in Newly Created Kenyan Counties: A Situational Analysis. *Journal of Health Care for the Poor and Underserved*, *28*(1), 71–78. https://doi.org/10.1353/hpu.2017.0009

Nyakeri, E. M., Ayieko, M. A., Amimo, F. A., Salum, H., & Ogola, H. J. O. (2019). An optimal feeding strategy for black soldier fly larvae biomass production and faecal sludge reduction. *Journal of Insects as Food and Feed*, *5*(3), 201–213. https://doi.org/10.3920/JIFF2018.0017

Nyakeri, E. M., Ogola, H. J., Ayieko, M. A., & Amimo, F. A. (2017). An open system for farming black soldier fly larvae as a source of proteins for smallscale poultry and fish production. *Journal of Insects as Food and Feed*, *3*(1), 51–56. https://doi.org/10.3920/JIFF2016.0030

Oonincx, D. ., Van Huis, A., & Van Loon, J. . (2015). Nutrient utilisation by black soldier flies fed with chicken , pig , or cow manure. *Journal of Insects as Food and Feed*, *1*(2), 131–139. https://doi.org/10.3920/JIFF2014.0023

Oonincx, D. G. A. B., Itterbeeck, J. Van, Heetkamp, M. J. W., Brand, H. Van Den, Loon, J. J. A. Van, & Huis, A. Van. (2010). An Exploration on Greenhouse Gas and Ammonia Production by Insect Species Suitable for Animal or Human Consumption. *PLOS ONE*, *5*(12), 1–7. https://doi.org/10.1371/journal.pone.0014445

Oonincx, D. G. A. B., Van Broekhoven, S., Van Huis, A., & Van Loon, J. J. A. (n.d.). Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PLoS ONE*, *10*(12). https://doi.org/10.1371/journal.pone.0144601

Osman, N. S., Khamil, A. M. I., & Sapawe, N. (2019). Proximate Analysis of Animal Feed Pellet Formulated from Sunflower Shell Waste. *Materials Today: Proceedings*, *19*, 1796–1802. https://doi.org/10.1016/j.matpr.2019.11.218

Owusu, G. (2010). Social effects of poor sanitation and waste management on poor urban communities: A neighborhood-specific study of Sabon Zongo, Accra. *Journal of Urbanism*, *3*(2), 145–160. https://doi.org/10.1080/17549175.2010.502001

Peal, A., Evans, B., Blackett, I., Hawkins, P., & Heymans, C. (2014). Fecal sludge management: A comparative analysis of 12 cities. *Journal of Water Sanitation and Hygiene for Development*, *4*(4), 563–575. https://doi.org/10.2166/washdev.2014.026

Penn, R., Ward, B. J., Strande, L., & Maurer, M. (2018). Review of synthetic human faeces and faecal sludge for sanitation and wastewater research. *Water Rsearch*, *132*, 222–240. https://doi.org/10.1016/j.watres.2017.12.063

Phukoetphim, N., Salakkam, A., Laopaiboon, P., & Laopaiboon, L. (2017). Kinetic models for batch ethanol production from sweet sorghum juice under normal and high gravity fermentations: Logistic and modified Gompertz models. *Journal of Biotechnology*, *243*, 69–75. https://doi.org/10.1016/j.jbiotec.2016.12.012

Poitevin, E. (2012). Determination of calcium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, and zinc in fortified food products by microwave digestion and inductively coupled plasma-optical emission spectrometry: Single-

laboratory validation and ring tri. *Journal of AOAC International*, *92*(5). https://doi.org/10.5740/jaoacint.CS2011_14

Popa, R., & Green, T. R. (2012). Using Black Soldier Fly Larvae for Processing Organic Leachates. *Journal of Economic Entomology*, *105*(2), 374–378. https://doi.org/10.1603/ec11192

Purkayastha, D., Sarkar, S., Roy, P., & Kazmi, A. A. (2017). Isolation and morphological study of ecologically-important insect "Hermetia illucens" collected from Roorkee compost plant. *Pollution*, *3*(3), 453–459. https://doi.org/10.7508/pj.2017.03

Rahim, A., & Akif, A. (2015). Optimal Artificial Neural Network Modeling of Sedimentation yield and Runoff in high flow season of Indus River at Besham Qila for Terbela Dam. *International Journal of Science and Research (IJSR)*, *4*(1), 479–483. https://www.ijsr.net/archive/v4i1/SUB15119.pdf

Raksasat, R., Lim, J. W., Kiatkittipong, W., Kiatkittipong, K., Ho, Y. C., Lam, M. K., Font-Palma, C., Mohd Zaid, H. F., & Cheng, C. K. (2020). A review of organic waste enrichment for inducing palatability of black soldier fly larvae: Wastes to valuable resources. *Environmental Pollution*, *267*(115488). https://doi.org/10.1016/j.envpol.2020.115488

Ramos-Elorduy, J. (2008). Energy supplied by edible insects from Mexico and their nutritional and ecological importance. *Ecology of Food and Nutrition*, *47*(3), 280–297. https://doi.org/10.1080/03670240701805074

Rehman, K. ur, Rehman, A., Cai, M., Zheng, L., Xiao, X., Somroo, A. A., Wang, H., Li, W., Yu, Z., & Zhang, J. (2017). Conversion of mixtures of dairy manure and soybean curd residue by black soldier fly larvae (Hermetia illucens L.). *Journal of Cleaner Production*. https://doi.org/10.1016/j.jclepro.2017.04.019

Rieck, C., von Müench, E., & Hoffman, H. (2012). Technology Review of Urine-Diverting Dry Toilets (UDDTs). *Deutsche Gesellschaft Für Internationale Zusammenarbeit (GIZ) GmbH*, 1–60. papers2://publication/uuid/A119E662-AA96-4E2A-A116-7577686E2B61

Rockström, J., Steffen, W., Noone, K., Persson, A., Chapin, F., Lambin, E., TM, L., Scheffer, M., Folke, C., Schellnhuber, H., Nykvist, B. de W. C., Hughes, S, van der L., H, R., S, S., PK, S., R, C., U, S., M, F., … Fa, F. J. (2009). A safe operating space for humanity Identifying. *Nature*, *461*(7263), 472–475. https://doi.org/10.1073/pnas.1014402108

Rogayan, J. D. (2017). *Vermiculture and Vermicomposting*. https://doi.org/10.13140/RG.2.2.15582.64320

Rose, C., Parker, A., Jefferson, B., & Cartmell, E. (2015). The characterization of feces and urine: A review of the literature to inform advanced treatment technology. *Critical Reviews in Environmental Science and Technology*, *45*(17), 1827–1879. https://doi.org/10.1080/10643389.2014.1000761

Sayyad, S. A., Panda, B. P., Javed, S., & Ali, M. (2007). Optimization of nutrient parameters for lovastatin production by Monascus purpureus MTCC 369 under submerged fermentation using response surface methodology. *Appl Microbiol Biotechnol*, *73*(March 2019), 1054–1058. https://doi.org/10.1007/s00253-006-0577-1

Setti, L., Francia, E., Pulvirenti, A., Gigliano, S., Zaccardelli, M., Pane, C., Caradonia, F., Bortolini, S., Maistrello, L., & Ronga, D. (2019). Use of black soldier fly (Hermetia illucens (L .), Diptera : Stratiomyidae) larvae processing residue in peat-based growing media. *Waste Management*, *95*, 278–288. https://doi.org/10.1016/j.wasman.2019.06.017

SFD Thinking SFD creation process and impacts - Case of Nairobi, Kenya, (2018).

Sheppard, D. C., Larry, G., Thompson, S. A., & Savage, S. (1994). A value added manure Management system using the Black Soldier Fly. *Bioresource Technology*, *50*, 275–279.

Shukla, A., Panchal, H., Mishra, M., Patel, P. R., Srivastava, H. S., Patel, P., & Shukla, A. K. (2014). Soil Moisture Estimation using Gravimetric Technique and FDR Probe Technique : A Comparative Analysis. *AIJRFANS*, *8*(1), 89–92.

Shumo, M., Osuga, I. M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K. M., Subramanian, S., Ekesi, S., van Huis, A., & Borgemeister, C. (2019). The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. In *Scientific Reports* (Vol. 9, Issue 1). https://doi.org/10.1038/s41598-019-46603-z

Silva, A. D. A. E., Varanda, E. M., & Primavesi, A. C. (2005). Effect of the inherent variation in the mineral concentration of alfalfa cultivars on aphid populations. *Bragantia Campinas*, *64*(2), 233–239. https://doi.org/10.1590/s0006-87052005000200010

Silvester, K. R., Bingham, S. A., Pollock, J. R. A., Cummings, J. H., & O'Neill, I. K. (1997). Effect of meat and resistant starch on fecal excretion of apparent N- nitroso compounds and ammonia from the human large bowel. *Nutrition and Cancer*, *29*(1), 13–23. https://doi.org/10.1080/01635589709514596

Simon, P. P., Krüger, R. F., & Ribeiro, P. B. (2011). Influence of diets on the rearing of predatory flies of housefly larvae. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*, *63*(6), 1414–1420. https://doi.org/10.1590/S0102-09352011000600019

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer,

B., Michiels, J., Eeckhout, M., De Clercq, P., & De Smet, S. (2017). Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates. *Journal of the Science of Food and Agriculture*, *97*, 2594–2600. https://doi.org/10.1002/jsfa.8081

Strande, L. (2014). The global situation. In L. Strande, M. Ronteltap, & D. Brdjanovic (Eds.), *Faecal Sludge Management* (pp. 1–14). IWA Publishing. http://www.sswm.info/sites/default/files/reference_attachments/EAWAG SANDEC 2008 Module 5 FSM Lecture.pdf

Surendra, K. C., Olivier, R., Tomberlin, J. K., Jha, R., & Khanal, S. K. (2016). Bioconversion of organic wastes into biodiesel and animal feed via insect farming. *Renewable Energy*, *98*, 197–202. https://doi.org/10.1016/j.renene.2016.03.022

Tiencheu, B., & Womeni, H. (2017). Entomophagy : Insects as Food. In *Insect Physiology and Ecology*. https://doi.org/10.5772/67384

Tjerve, K., & Tjerve, E. (2017). The use of Gompertz models in growth analyses , and new Gompertz-model approach : An addition to the Unified-Richards family. *PLoS ONE*, *12*(6), 1–17. https://doi.org//doi.org/10.1371/journal. pone.0178691

Tomberlin, J. K., Sheppard, D. C., & Joyce, J. A. (2002). Selected Life-History Traits of Black Soldier Flies (Diptera: Stratiomyidae) Reared on Three Artificial Diets. *Annals of the Entomological Society of America*, *95*(3), 379–386. https://doi.org/10.1603/0013-8746(2002)095[0379:slhtob]2.0.co;2

UNICEF. (2018). *Situation Analysis of Children and Women in Kenya 2017*.

UNICEF & WHO. (2019). *Progress on household drinking water, sanitation and hygiene, 2000-2017*. https://washdata.org/sites/default/files/documents/reports/2019-07/jmp-2019-wash-

households.pdf

UNICEF & WHO. (2020). *State of the World's Sanitation: An urgent call to transform sanitation for better health, environments, economies and societies*.

United Nations. (2018). *Sustainable Development Goal 6 Synthesis Report on Water and Sanitation 2018*.

van Huis, A., & Oonincx, D. G. A. B. (2017). The environmental sustainability of insects as food and feed. A review. In *Agronomy for Sustainable Development* (Vol. 37, Issue 5). Agronomy for Sustainable Development. https://doi.org/10.1007/s13593-017-0452-8

Velkushanova, K., Strande, L., Ronteltap, M., Koottatep, T., Brdjanovic, D., & Buckley, C. (2021). *Methods for Feacal sludge analysis*.

Visanuvimol, L., & Bertram, S. M. (2011). How dietary phosphorus availability during development influences condition and life history traits of the cricket, Acheta domesticas. *Journal of Insect Science*, *11*(63), 1–17. https://doi.org/10.1673/031.011.6301

Wandera, S. M., Qiao, W., Algapani, D. E., Bi, S., Yin, D., Qi, X., Liu, Y., Dach, J., & Dong, R. (2018). Searching for possibilities to improve the performance of full scale agricultural biogas plants. *Renewable Energy*, *116*, 720–727. https://doi.org/10.1016/j.renene.2017.09.087

Wendland, C., Deegener, S., & Jorritsma, F. (2011). *Sustainable Sanitation Practice Experiences with urine diverting dry toilets (UDDTs) for households, schools and kindergarten in Eastern Europe, the Caucasus and Central Asia (EECCA) This paper summarizes the experiences of WECF and partners from implemen*. http://www.wecf.eu/download/2011/February/SSP-06_Jan2011_16-221.pdf

WHO & UNICEF. (2017). Progress on drinking water, sanitation and hygiene: 2017 update and SDG baselines. In *World Health Organization*. http://www.who.int/iris/handle/10665/258617

William, J. P. (2011). *Introduction to Matlab for Engineers, III ed* (3rd ed.). http://home.hit.no/~hansha/documents/matlab/resources/matlab_intro_lie.pdf

Woods, H. A., Makino, W., Cotner, J. B., Hobbie, S. E., Harrison, J. F., Acharya, K., & Elser, J. J. (2003). Temperature and the chemical composition of poikilothermic organisms. *Functional Ecology*, *17*(2), 237–245. https://doi.org/10.1046/j.1365-2435.2003.00724.x

World Health Organization (WHO) and the United Nations Children's Fund, (UNICEF). (2017). *Progress on Drinking Water , Sanitation and Hygiene*.

Xiao, X., Mazza, L., Yu, Y., Cai, M., Zheng, L., Tomberlin, J. K., Yu, J., Huis, A. Van, Yu, Z., & Fasulo, S. (2018). Ef fi cient co-conversion process of chicken manure into protein feed and organic fertilizer by Hermetia illucens L . (Diptera : Stratiomyidae) larvae and functional bacteria. *Journal of Environmental Management*, *217*, 668–676. https://doi.org/10.1016/j.jenvman.2018.03.122

Yu, G., Cheng, P., Chen, Y., Li, Y., Yang, Z., Chen, Y., & Tomberlin, J. K. (2011). Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae. *Environmental Entomology*, *40*(1), 30–35. https://doi.org/10.1603/EN10126

Zhang, X., Zhong, J., Fu, W., Li, Z., Zhong, J., Yang, J., Tan, H., & Xiao, L. (2010). Response surface methodology used for statistical optimization of jiean- peptide production by Bacillus subtilis. *Electronic Journal of Biotechnology*, *13*(4). https://doi.org/10.2225/vol13-issue4-fulltext-5

Zheng, H., Powell, J. E., Steele, M. I., Dietrich, C., & Moran, N. A. (2017). Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(18), 4775–4780. https://doi.org/10.1073/pnas.1701819114

Zheng, L., Tomberlin, J. K., Li, Q., Qiu, N., Yu, Z., & Cai, H. (2011). Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. *Waste Management*, *31*(6), 1316–1320. https://doi.org/10.1016/j.wasman.2011.01.005

Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & Van't Riet, K. (1990). Modeling of the Bacterial Growth Curve. *Applied and Environmental Microbiology*, *56*(6), 1875–1881.

APPENDICES

Appendix I: Treatment Units

Appendix II: Bioconversion products

Appendix III: Samples in the oven

Appendix IV: Muffle furnace

Appendix V: Multiparameter Water Quality Meter

Appendix VI: Waste reduction mean, standard deviation and standard errors

Descriptives

score

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
1	3	83.3000	1.80831	1.04403	78.8079	87.7921	81.40
2	3	86.6333	2.45425	1.41696	80.5366	92.7300	84.10
3	3	85.3333	1.55027	.89505	81.4823	89.1844	83.80
4	3	84.7000	.43589	.25166	83.6172	85.7828	84.40
5	3	91.5000	1.30000	.75056	88.2706	94.7294	90.20
Total	15	86.2933	3.23032	.83406	84.5044	88.0822	81.40

Appendix VII: ONE WAY ANOVA

ANOVA

score

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	118.936	4	29.734	10.950	.001
Within Groups	27.153	10	2.715		
Total	146.089	14			

Appendix VIII: Tukey HSD

Multiple Comparisons

Dependent Variable: score

Tukey HSD

(I) waste	(J) waste	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-3.33333	1.34544	.172	-7.7613	1.0946
	3	-2.03333	1.34544	.578	-6.4613	2.3946

	4	-1.40000	1.34544	.831	-5.8280	3.0280
	5	-8.20000*	1.34544	.001	-12.6280	-3.7720
2	1	3.33333	1.34544	.172	-1.0946	7.7613
	3	1.30000	1.34544	.864	-3.1280	5.7280
	4	1.93333	1.34544	.620	-2.4946	6.3613
	5	-4.86667*	1.34544	.030	-9.2946	-.4387
3	1	2.03333	1.34544	.578	-2.3946	6.4613
	2	-1.30000	1.34544	.864	-5.7280	3.1280
	4	.63333	1.34544	.988	-3.7946	5.0613
	5	-6.16667*	1.34544	.007	-10.5946	-1.7387
4	1	1.40000	1.34544	.831	-3.0280	5.8280
	2	-1.93333	1.34544	.620	-6.3613	2.4946
	3	-.63333	1.34544	.988	-5.0613	3.7946
	5	-6.80000*	1.34544	.004	-11.2280	-2.3720
5	1	8.20000*	1.34544	.001	3.7720	12.6280
	2	4.86667*	1.34544	.030	.4387	9.2946
	3	6.16667*	1.34544	.007	1.7387	10.5946
	4	6.80000*	1.34544	.004	2.3720	11.2280

Appendix IX: Bioconversion Rate

ANOVA

score

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	242.483	4	60.621	18.535	.000
Within Groups	32.707	10	3.271		
Total	275.189	14			

Appendix X: Feed Conversion Rate

ANOVA

score

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.912	4	4.978	22.683	.000
Within Groups	2.195	10	.219		
Total	22.106	14			

Appendix XI: Larval yield

ANOVA

score

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24248.267	4	6062.067	18.535	.000
Within Groups	3270.667	10	327.067		
Total	27518.933	14			

Appendix XII: Waste Reduction Index

ANOVA

score

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.146	4	.286	64.012	.000
Within Groups	.045	10	.004		
Total	1.191	14			