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Pathogen inactivation from faecal waste via black soldier fly treatment

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ABSTRACT

Sustainable development goal 6.2 advocates for increasing access to equitable and adequate hygiene and sanitation for all by 2030, and its indicator target is to raise the proportion of the population using safely managed sanitation. On the contrary, it is approximated that only 54% of people globally have access to safely managed sanitation, while 2.4 billion lack access. Recent innovations have however seen the development of low-cost technologies that can be customized depending on the region. The container based sanitation (CBS) is one of the options for safely managing the waste compared to conventional sewerage systems that are expensive. However, the need for emptying and transportation of filled faecal sludge (FS) containers on daily basis, leads to high operational costs and increases the operational exposures risks to faecal pathogens amongst the CBS operators. The study determined the performance efficiency of Black Soldier Fly Larvae in inactivation of E.coli via seeded container based sanitation- Urine diverting dry toilet model. Sample was transported to the lab for analysis of E.coli after every 3 days from Seeded households and samples from Non seeded households in replication. One gram of sample was used in serial dilution up to factor 5.The inoculum was plated in Tryptone Bile Glucuronic (TBX) agar for enumeration according to manufacturer's instructions. Plates with 30-300 colonies were considered viable for counting. The study used statistical analysis IBM SPSS software from the sets of experiments. The E.coli (CFU/ml) mean varied between 7.3±1.3 and 10.1±2.8 for Seeded treatments while non-seed treatments ranged between 7.5±1.6 and 10.4±3.2. A significant variation occurred between the two treatments at $p<0.05$(SH and NH). Findings from this study showed that there was reduction of E.coli coliforms with Bioconversion of organic waste using the BSF technology has been noted to reduce the E.coli load and reducing the volume of the bio-waste. Sanitation policy makers can apply the findings of this study in formulation of policies on safe handling and disposal of faecal matter from onsite sanitation facilities.

Introduction

Sustainable development goal 6.2 advocates for increasing access to equitable and adequate hygiene and sanitation for all by 2030, and its indicator target is to raise the proportion of the population using safely managed sanitation (UNWATER). On the contrary, it is approximated that only 54% of people globally have access to safely managed sanitation, while 2.4 billion lack access (JMP,

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2020; UNICEF & WHO, 2020). In sub-Saharan Africa, it has been estimated that only 7.6% of the population has been connected to sewer systems whereas, in Kenya, 30% of the population has access to improved sanitation (UNICEF & WHO, 2020). The sanitation challenges are expected to exacerbate since the world population is projected to increase to 9.7 billion by 2050 (Leridon, 2020). Previously, Engineers focused on conventional wastewater treatment to address the sanitation needs of the population, in urban areas.

Conventional sewer systems are however not only capital intensive but also have a high operation and maintenance cost, high water requirements, and adequate planning in the layout of the pipe network (Strande *et al.,* 2014). As such, onsite sanitation technologies, such as pit latrines, and septic tanks which were initially thought of as a sanitation solution for rural areas, have been widely adopted in urban and peri-urban areas (Strande *et al.*, 2014).78% of sanitation needs among peri-urban and poor urban settlements in Kenya are met by onsite technologies, whereas conventional sewer systems coverage stands at 12% (Riungu *et al.*, 2021).

Despite the high adoption of onsite systems, there has been a lag in the development of faecal sludge (FS) treatment technologies to handle the faecal waste generated from these systems (Strande *et al.,* 2014). In onsite sanitation technologies and services, faecal matter is stored, treated, and disposed of near the place of generation **(**Jeuland *et al.,* 2013***)***. If not well managed FS generated from on-site systems can lead to high environmental and public health risks, owing to the high pathogenic load in excreta (Riungu, 2021). The poor status of sanitation experienced in developing countries has led to an increase in contribution to infectious diseases such as polio, diarrhoea, cholera, hepatitis A, and typhoid (Ritchie &Roser, 2021).

The Kenyan Government spends KES 27 billion (USD 365 million) annually (one percent of the national gross domestic product (GPD)) on the treatment of sanitation-related illnesses (MOH, 2016). Among children, diarrheal disease and intestinal worm infestation contribute to at least 40% of deaths among under-five children (MOH, 2010); Riungu, 2021), in addition to 35% of children suffering from moderate to severe stunting (UNICEF, 2013).

Escherichia coli belong to a family of Enterobactericeae and Escherichia genus. It is a gram-negative bacteria and facultative anaerobic. It is normally found in the large intestines of mammals (Gebis, 2019; Gomes et al., 2019). In the laboratory is isolated by growing on a Tryptone Bile Glucuronide (TBX) selective medium and incubated at 37°C for 24 hours where it yields greenish colonies. It's found in the environment where there is faecal contamination which in turn causes infections when food or water gets into contact with faecal matter. Escherichia coli are known to be a normal flora in the human intestines, although it has been associated with causing infections in humans such as diarrhoea.

The infections are a result of faecal contamination with food or drink (Blyton & Gordon, 2017). Poor handling of the faecal matter can lead to environmental contamination by these bacteria as its high concentration is an indicator of contamination. Treatment of the faecal matter is important in reducing the contamination in the environment. The Poor design of treatment plants, pit latrines, conventional sewers, and lack of sanitation facilities are the root causes of the increase of microbes in the environment. The inadequate sanitation facility has resulted in people resorting to open defecation. This has increased threats to health as groundwater is contaminated which leads to high concentrations of E. coli in the environment (Hajam *et al.*, 2023).

Higher E. coli concentrations in faecal sludge are a reliable indicator of faecal contamination and indicate potential health risks that result from inadequate sanitation practices (Kumwenda et al., 2017). Inadequate faecal waste treatment not only contributes to environmental pollution but also increases the possibility of microbial transmission across multiple channels, raising serious public health problems (Nakagiri *et al.*, 2015, 2016). Addressing the issues of faecal sludge management necessitates comprehensive approaches that include improved sanitation infrastructure, effective treatment technologies, and community involvement efforts targeted at encouraging proper sanitation and minimizing faecal contamination pathways.

The presence of Escherichia coli in the environment underscores the urgent need for sustainable sanitation solutions that mitigate microbial risks and safeguard public health (Bain *et al.*, 2014). In addition to direct human health implications, contamination with E. coli can also compromise ecosystem integrity and biodiversity, further exacerbating environmental degradation.

Effective faecal sludge management practices, including proper treatment and disposal methods, are essential for minimizing microbial pollution and mitigating the adverse impacts of faecal contamination on water resources, soil quality, and ecological health. Investing in resilient sanitation infrastructure and promoting behavior change interventions are paramount to achieving universal access to safe and sustainable sanitation services while ensuring the protection of environmental and human health.

Ensuring the microbial safety of the products of sanitation forms the prime objective in sanitation. Viscous heater (VH) has been used as a pasteurizer in resource recovery from sanitation products. The viscous heated faecal sludge inactivates the somatic coliphage, heterotrophic bacteria, total coliform, and Escherichia Coli at a temperature range of 60°C and 80°C for 1-6 min Peguero *et al*. (2021) reported eradication of Salmonella Spp. and Escherichia coli at a temperature of 55 °C in the faecal sludge to non-detectable levels. The solar photo-inactivation of wastewater assessment showed the inactivation of human pathogenic bacteria (Salmonella enteritidis and E. coli O157:H7) (Nahim-Granados et al., 2018).

The Black Soldier Fly (BSF) larvae inactivation efficiency studies show limited information and discrepancy in pathogen inactivation which warranted the present study.

This study is part of a project on evaluating the potential of container-based sanitation as a sanitation option among peri-urban areas. In container-based sanitation (CBS, faecal sludge (FS) is captured in sealable containers. The Containers, once full are replaced with clean containers, allowing the transportation of filled containers to off-site treatment sites (Ferguson *et al.,* 2022; CBSA, 2021).

The CBS system allows separate collection and storage of faeces and urine in different containers, in a model further referred to as UDDT. Currently, the CBS operators are in charge of collecting waste, transportation, and treatment of the FS (Mackinnon et al. 2018). The CBS provides an entry point to the circular economy in sanitation. The circular economy promotes the conversion of waste into valuable resources; such as nutrients available in waste are harnessed and thereafter put back into the matter cycle (Riungu, Ronteltap, *et al*., 2019; Simha & Ganesapillai, 2017; Hyun *et al*., 2019; Nagy *et al*., 2019).

Container-based sanitation (CBS) service chains are labor-intensive, with a wide variety of skills needed and safety issues (Russel *et al*., 2015). Daily emptying and transportation to a treatment facility are costly (O'Keefe *et al.,* 2015). The door-to-door collection raises vehicle fuel costs as it relies on regular emptying and servicing of containers by a CBS provider. There is also the employment of drivers for the vehicles used for the transportation of the containers and which increases the operational cost.

The long distance between homes where collection takes place and treatment centres is another cost. Therefore, finding new ways for optimizing the cost is a necessary field of research (Diener *et al.,* 2015). The research is part of a bigger research project investigating ways to enhance pathogen inactivation from faecal matter coming from urine diverting dehydrating toilets (UDDT), at Nchiru a peri-urban settlement.

Materials and Methods

In this study, three urine diverting dry toilet were installed in households within Nchiru. The Urine diverting dry toilets (UDDT) were supplied by Sanergy Kenya, a sanitation enterprise promoting container based sanitation (CBS) within informal slum settlements. UDDT principle enables waste separation at the source, thus faeces and urine are collected via two different 30-liter sealable containers. The experiment investigated the on-site treatment of UDDT-generated faeces using the black soldier fly larvae as follows; Three UDDT systems were installed within three households. Containers for faeces collection were pre-seeded with 5 days old obtained from the MUST Bsf rearing unit larvae before introduction for waste collection. The used containers were collected and exchanged with clean ones after 14 days, which has been reported as the active feeding time for BSF larvae (Matheka *et al.,* 2021).

The experiment investigated also off-site treatment of urine-diverting dry toilet-generated faeces using the Black Soldier Fly technology as follows: Three urine-diverting dry toilet systems were installed at three different households at Nchiru. Daily, the used containers were collected and swapped with clean ones.

The collected faecal wastes were transported to the MUST treatment facility where a treatment experiment was set up. The faecal waste was then fed to 5-day-old larvae in 1.5m × 1.5m × 3m troughs through batch feeding as. The samples were taken after three days for laboratory analysis of *E.coli* for a period of 12 days in two trials.

Analytical procedures

E.coli Enumeration

Immediately after the samples were transported from the field, 20g of faecal waste sample from each household was placed into sterile stool containers. Replication was done by taking three samples from each household. Sample constitution was done by transferring 1g of each household sample into the sterilized falcon tube containing 10 ml of sterilized distilled water and then shaking vigorously to ensure uniform suspension. One millilitre of the sample suspension was transferred into the first test tube with 9ml of sterilized distilled water and shaken to mix making a final volume of 10ml (dilution factor 1). To the second tube containing 9ml of sterilized distilled water (dilution factor 2), 1 ml was taken and added from the first tube.

The process was repeated up to the last tube (5th) denoted dilution factor 5. With 3 replications for each household sample and serial dilution of up to the 5th dilution factor, there were a total of 90 test tubes with inoculums to be plated in Tryptone Bile Glucuronic (TBX) agar for enumeration. T

he sterile plates were placed in a biosafety hood where the media was held, media was dispensed on the plates to allow it to solidify. Once the agar had solidified, 0.1ml of inoculum sample from each test tube was transferred into a petri dish and spread using a sterile glass rod spreader. All the plates were incubated at 37^{0}C for 24 hours and afterwards, the number of colonies counted. Only plates with 30-300 colonies were considered to have viable colonies.

The Colony Forming units (CFUs) were calculated as:

$$\text{CFUs/1g of original sample} = \frac{\text{colonies counted x dilution factor}^2}{\text{amount of inoculum into each petri dish (0.1ml)}}$$

Statistical analysis

The analysis was done in triplicates, the experiment results were statistically analysed using IBM SPSS (version 23) by conducting a one-way analysis of variance (ANOVA) at a 95% confidence interval. This was followed by a pairwise Turkey post hoc comparison to determine whether a significant difference occurred in the mean between households and within each household (Lalander et al., 2019). A p-value <0.05 was a considerate indication of a significant variation between the households.

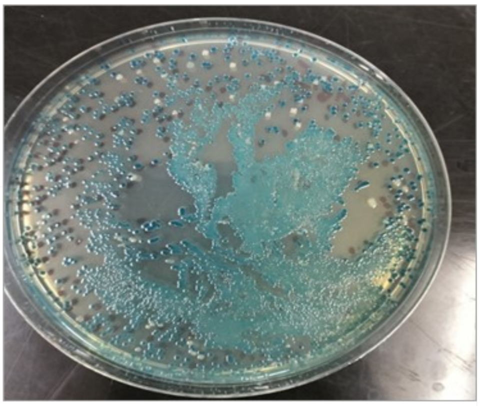

Figure 1: *green colonies of E. coli isolated from TBX agar*

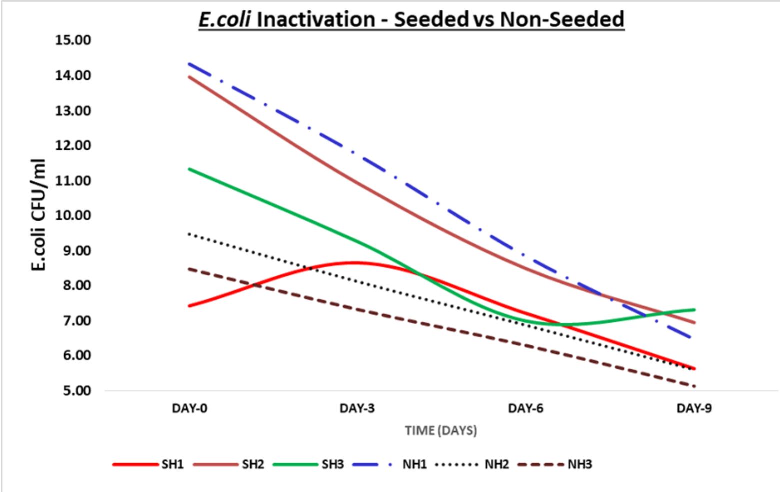

Figure 2: *E. coli Logs distribution by the days for seeded and Non-seeded across households over time in days*

†Mean followed by different superscripts in the same rows varied significantly (p<0.05).

*larval weight measurement was done on a wet weight basis.

Results and Discussion

Figure 1 shows green colonies of *E. coli* isolated from TBX agar. Figures 2 and Table 1 show the distribution of E. coli (CFU/ml) data for seeded and non-seeded treatment systems respectively. The *E. coli* (CFU/ml) inactivation mean varied between 7.3±1.3 and 10.1±2.8 for the substrates subjected to Seeded treatments (Figure 2), while Non-seed treatments recorded a relatively higher mean inactivation range between 7.5±1.6 and 10.4±3.2 .A significant variation occurred between the two treatments at p<0.05(SH and NH) as shown in Table 1. Similarly, when households of the same treatment were compared, for instance, SH1, SH2, and SH3, E. coli inactivation varied significantly Table 3.1. The same trend was recorded within non-Seeded household treatments.

A significant variation occurred between the two treatments at p<0.05(SH and NH). However, when a comparison was done among households of the same treatment, a significant variation was recorded among the treatments themselves within the individual groups for Seeded and Non-seed treatments. (Table 1). For instance, in SH1, Escherichia coli reduced from 7.43 CFU/ml at day 0 to 5.60 CFU/ml at day 9. In NH1, it decreased from 11.74 CFU/ml at day 0 to 6.47 CFU/ml at day 9. The present study is in agreement with Peguero *et al.* (2021), who evaluated the inactivation of in situ Escherichia coli, whereby before the inactivation, there was 9.7 (± 5.74) × 104 CFU/g in wet faecal sludge. After heating the faecal sludge at the 80-degree centigrade set-point for 1 minute, Peguero *et al.* (2021) found 1- to 5-log10 inactivation of Escherichia coli. Similarly, Riungu et al. (2018a) found that the faecal sludge subjected to the viscous heater (VH) had inactivated somatic coliphage, total coliform, and Escherichia coli below the detection limit (1- to 5-log10 inactivation). The inactivation of the Escherichia coli concentrations suggests the effectiveness of container-based systems. This has significant implications for public health and safe faecal waste management.

UDDT-CBS model offers an attractive approach in human waste treatment after which the sludge produced can be treated via post-treatment technology. Besides the reduction in biomass, proper treatment of organic waste includes the sanitization of the material. Antibacterial activity in excretion/secretion of insect larvae is known and has been demonstrated to be effective in maggot

	Seeded			Non-Seeded			P-Values
Parameter	**SH1**	**SH2**	**SH3**	**NH1**	**NH2**	**NH3**	
***E.coli* Inactivation**	7.3±1.3[a]	10.1±2.8[bc]	8.7±1.9[abc]	10.4±3.2[c]	7.5±1.6[bc]	6.8±1.4[a]	0.000

Table 1: *Mean ± standard deviation of E. coli (CFU/ml) and Larval Yield in g/30 BSFL*

therapy for wound debridement (Parnes and Lagan, 2007) and in the inactivation of Enterobacteriaceae such as Salmonella spp. and Escherichia coli (Erickson *et al*., 2004; Liu *et al*., 2008). As an innovative solution for enhancing sanitation in low income urban and peri urban areas, UDDTs-CBS toilets can be offered on a pay-and-use basis in the form of serviced shared facilities where The UDDT principle involves separate collection of faeces and urine (Austin, 2001; Austin &mCloete, 2008; Niwagaba et al., 2009a; Sherpa et al., 2009).

Studies such as Manuela Renna and Laura Gasco's (2018) researched on the effect of rearing substrate on black soldier fly larvae suggest that different feed substrates can have a significant impact on the growth performance, waste reduction efficiency, and chemical composition of black soldier fly larvae. Additionally, Nyakeri et al. (2019) found that an optimal feeding strategy could be used to maximize the biomass production and faecal sludge reduction of black soldier fly larvae. These findings suggest that further research is needed to explore the potential of using different substrates and feeding strategies to increase the efficiency of black soldier fly larvae as a waste reduction method. In addition, further research is needed to determine the safety of using heat-treated faecal sludge for black soldier fly larvae production (Peguero et al., 2021). While studies such as Oonincx et al. (2015) suggest that black soldier fly larvae can efficiently reduce the amount of waste, it is important to ensure that the resulting material is safe for human consumption. Therefore, further research is needed to explore the potential of using different methods to safely reduce the amount of waste and produce a safe, nutrient-rich product.

UDDT-CBSs allow simple removal, less harmful and safe handling of the faecal 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 faecal material in ventilated interchangeable water proof containers. This enables post-treatment of the faecal waste using different treatment technologies since the faeces 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. The technology is technically and institutionally appropriate, socially acceptable, economically viable and protective to environment and natural resources (Rieck *et al*., 2012). SCBS-UDDT services comprise the full sanitation value chain, and therefore meet the requirement for safely managed sanitation according to the WHO standards.

Assuming faeces are properly handled throughout the service chain, including treatment and safe disposal/reuse, CBS is likely to be an effective solution for limiting the spread of faecal contamination within household and community environments (Russel *et al.,* (2015).There is a need to encourage sanitation and public health ministries and policy makers to include CBS among their sanitation policy options and to structure financing (e.g. Targeted investment and tariffs, payment by results mechanisms, etc.) and public-private partnerships to support the expansion of CBS-

UDDT services. With expansion of cities at high rates and the number of people living in informal urban settlements expected to grow by more than half by 2030, it is critical that new sanitation technologies and services like SCBS-UDDT be studied and made available to governments and unserved communities.

Conclusion and Recommendation

The experiment was conducted to investigate the inactivation potential for seeded UDDT-CBS model for E.coli where the results showed a decrease in number of coliforms as observed for SH1 log reduction. The performance process of Black Soldier Fly larvae was effective in reducing the *E.coli* load and reducing the volume of the bio-waste. Therefore, the seeded UDDT-CBS technique can be employed to manage, recycle and reduce the microbial load thus ensuring limit in environmental pollution and contamination. Sanitation policy makers can apply the findings of this study in formulation of policies on safe handling and disposal of faecal matter from onsite sanitation facilities. Proper sanitation and hygiene awareness practices should be provided through education to the residents of this area.

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Data Availability Statement

All relevant data are included in the paper or its Supplementary Information.

Competing interests

The authors have declared that no competing interests exist.

References

Bain, R., Cronk, R., Hossain, R., Bonjour, S., Onda, K., Wright, J & Bartram, J. (2014). Global assessment of exposure to fecal contamination through drinking water based on a systematic review. *Tropical Medicine & International Health*, *19*(8), 917-927.

Blyton, M. D. J., & Gordon, D. M. (2017). Genetic Attributes of E. coli Isolates from Chlorinated Drinking Water. PloS One, 12(1), e0169445. https://doi.org/10.1371/journal.pone.0169445

CBSA. (2021). Container-Based Sanitation Alliance About Us. Available from: https://cbsa.global/.

Diener, S., Lalander, C., Zurbrügg, C., &Vinnerås, B. (2015, October). Opportunities and constraints for medium-scale organic waste treatment with fly larvae composting. In *Proceedings of the 15th International waste management and landfill symposium, Cagliari, Sardinia* (pp. 5-9)

Dortmans, B. M. A., Diener, S., Verstappen, B. M., &Zurbrügg, C. (2017). Black soldier flies biowaste processing-a step-by-step guide Eawag: Swiss Federal Institute of Aquatic Science and Technology. *Dübendorf, Switzerland*, *100*.

Englund, M., &Strande, L. (Eds.). (2019). *Fecal Sludge Management: Highlights and Exercises*. Eawag.

Ewald, N., Vidakovic, A., Langeland, M., Kiessling, A., Sampels, S., &Lalander, C. (2020). Fatty acid compositions of black soldier fly larvae (Hermetiaillucens)–Possibilities and limitations for modification through diet. *Waste Management*, *102*, 40-47.

Ferguson, C., Mallory, A., Anciano, F., Russell, K., Valladares, H. D. R. L., Riungu, J., & Parker, A. (2022). A qualitative study on resource barriers facing scaled container-based sanitation service chains. *Journal of Water, Sanitation and Hygiene for Development*, *12*(3), 318-328.

Graf, J., Kayser, O., & Brossard, S. (2014). Designing the next generation of sanitation businesses. *Hystra and the Toilet Board Coalition, Paris, France*.

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

Hajam, Y. A., Kumar, R., & Kumar, A. (2023). Environmental waste management strategies and vermi transformation for sustainable development. Environmental Challenges, 13, 100747. https://doi.org/10.1016/J.ENVC.2023.100747

Hyun, C., Burt, Z., Crider, Y., Nelson, K. L., Prasad, C. S. S., Rayasam, S. D. G., et al. (2019). Sanitation for low-income regions: a cross-disciplinary review. Annu. Rev. Environ. Resource. 44, 287–318. doi: 10.1146/annurev-environ-101718-033327

Jeuland, M. A., Fuente, D. E., Ozdemir, S., Allaire, M. C., and Whittington, D. (2013). The long-term dynamics of mortality benefit from improved water and sanitation in less developed countries. PLoS ONE 8: e74804. doi: 10.1371/journal.pone.0074804

Kumwenda, S., Msefula, C., Kadewa, W., Ngwira, B., & Morse, T. (2017). Estimating the Health Risk Associated with the Use of Ecological Sanitation Toilets in Malawi. Journal of Environmental and Public Health, 2017. https://doi.org/10.1155/2017/3931802

Lalander, C., Diener, S., Magri, M. E., Zurbrügg, C., Lindström, A., &Vinnerås, B. (2013). Fecal sludge management with the larvae of the black soldier fly (Hermetiaillucens)—From a hygiene aspect. *Science of the Total Environment*, *458*, 312-318.

Liu, Q., Chmely, S. C., &Abdoulmoumine, N. (2017). Biomass treatment strategies for thermochemical conversion. *Energy & Fuels*, *31*(4), 3525-3536.

Liu, X., Chen, X., Wang, H., Yang, Q., ur Rehman, K., Li, W., ... & Zheng, L. (2017). Dynamic changes in nutrient composition throughout the entire life cycle of the black soldier fly. *PLoS One*, *12*(8), e0182601.

Liu, Z., Minor, M., Morel, P. C., &Najar-Rodriguez, A. J. (2018). Bioconversion of three organic wastes by black soldier fly (Diptera: Stratiomyidae) larvae. *Environmental Entomology*, *47*(6), 1609-1617.

Lützhøft, H. C. H., Boe, K., Fang, C., &Angelidaki, I. (2014). Comparison of VFA titration procedures used for monitoring the biogas process. *Water Research*, *54*, 262-272.

Mallory, A., Mdee, A., Agol, D., Hyde-Smith, L., Kiogora, D., Riungu, J., & Parker, A. (2022).The potential for scaling up container-based sanitation in informal settlements in Kenya. *Journal of International Development*.

Mansour, G., & Esseku, H. (2017). Situation analysis of the urban sanitation sector in Ghana. *Urban Sanitation Research Initiative Ghana: Accra, Ghana*.

Manuela Renna, and Laura Gasco. (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: 5776–84. https://doi.org/10.1002/jsfa.9127

Matheka, R. M., Raude, J. M., Murunga, S. I., Riungu, J. N., &Wandera, S. M. (2021). Effect of fecal matter co-digestion with kitchen waste on Hermetiaillucens' larval weight and protein content.*Journal of Water, Sanitation and Hygiene for Development*, *11*(5), 746-757.

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 (Hermetiaillucens) larvae. *Journal of the Science of Food and Agriculture*, *98*(15), 5776-5784.

MOH. (2014). State of Sanitation in Meru County. www.wsp.org

Nagy, J., Mikola, A., Pradhan, S. K., and Zseni, A. (2019). The utilization of struvite produced from human urine in agriculture as a natural fertilizer: a review. Period.Polytech. Chem. Eng. 63, 478–484. doi: 10.3311/PPch.12689

Nakagiri, A., Kulabako, R. N., Nyenje, P. M., Tumuhairwe, J. B., Niwagaba, C. B., & Kansiime, F. (2015). Performance of pit latrines in urban poor areas: A case of Kampala, Uganda. Habitat International, 49, 529–537. https://doi.org/10.1016/J.HABITATINT.2015.07.005

Nakagiri, A., Niwagaba, C. B., Nyenje, P. M., Kulabako, R. N., Tumuhairwe, J. B., & Kansiime, F. (2016). Are pit latrines in urban areas of Sub-Saharan Africa performing? A review of usage, filling, insects and odour nuisances. BMC Public Health, 16(1). https://doi.org/10.1186/S12889-016-2772-Z

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 fecal sludge reduction. *Journal of Insects as Food and Feed*, *5*(3), 201-213.

Nyoka, R., Foote, A. D., Woods, E., Lokey, H., O'Reilly, C. E., Magumba, F., ...& Morris, J. F. (2017). Sanitation practices and perceptions in Kakuma refugee camp, Kenya: Comparing the status quo with a novel service-based approach. *PLoS One*, *12*(7), e0180864.

O'Keefe, M., Lüthi, C., Tumwebaze, I. K., and Tobias, R. (2015). Opportunities and limits to market-driven sanitation services: evidence from urban informal settlements in East Africa. *Environ. Urban* 27, 421–440. doi: 10.1177/0956247815581758

Oonincx, D. G., Van Broekhoven, S., Van Huis, A., & van Loon, J. J. (2015). Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. *PloS one*, *10*(12), e0144601.

O'Reilly, K., Dhanju, R., &Goel, A. (2017). Exploring "the remote" and "the rural": Open defecation and latrine use in Uttarakhand, India. *World Development*, *93*, 193-205.

Oyoo, V. A., Riungu, J. N., Matheka, R. M., Dey, P., & Kirimi, J. G. (2023). Process performance evaluation of faecal matter treatment via black soldier fly. Journal of Water Sanitation and Hygiene for Development,13(6),441–452. https://doi.org/10.2166/WASHDEV.2023.010/1244435/WASHDEV0130441.PDF

Peguero, D. A., Mutsakatira, E. T., Buckley, C. A., Foutch, G. L., &Bischel, H. N. (2021). Evaluating the microbial safety of heat-treated fecal sludge for black soldier fly larvae production in South Africa. *Environmental engineering science*, *38*(5), 331-339.

Purser, B. J., Thai, S. M., Fritz, T., Esteves, S. R., Dinsdale, R. M., & Guwy, A. J. (2014). An improved titration model reducing overestimation of total volatile fatty acids in anaerobic digestion of energy crop, animal slurry, and food waste. *Water Research*, *61*, 162-170.

Riungu, J., Ronteltap, M., & van Lier, J. B. (2018).Build-up and impact of volatile fatty acids on E. coli and A. lumbricoides during co-digestion of urine diverting dehydrating toilet (UDDT-F) feces.*Journal of environmental management*, *215*, 22-31.

Riungu, N. J. (2021). Off-Site Enhanced Biogas Production with Concomitant Pathogen Removal from Faecal Matter Joy Nyawira Riungu. Delft University of Technology.

Russel, K. C., Tilmans, S., Kramer, S., Sklar, R., Tillias, D., and Davis, J. (2015). Impacts of a container-based, household toilet and waste collection service intervention in Cap Haitien, Haiti. Environ. Urban 27, 525–540

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.

Simha, P., and Ganesapillai, M. (2017). Ecological sanitation and nutrient recovery from human urine: How far have we come? A review. Sustain. Environ. Res. 27, 107–116. doi: 10.1016/j.serj.2016.12.001

Strande, L., &Brdjanovic, D. (Eds.). (2014). *Fecal sludge management: Systems approach for implementation and operation*. IWA publishing.

Tilmans, S., Russel, K., Sklar, R., Page, L., Kramer, S., and Davis, J. (2015). Container-based sanitation: assessing costs and effectiveness of excreta management in Cap Haitien, Haiti. Environ. Urban. 27, 89–104. doi: 10.1177/0956247815572746

Tilmans, S., Russel, K., Sklar, R., Page, L., Kramer, S., and Davis, J. (2015). Container-based sanitation: assessing costs and effectiveness of excreta management in Cap Haitien, Haiti. Environ. Urban. 27, 89–104. doi: 10.1177/0956247815572746

VanRiper, F. R. (2021). The Viability of Container-Based Sanitation as a Tool for Sanitation Coverage in Urban Haiti.

Water and Sanitation Programme. (2014). *State of sanitation in Meru County*. Devolution Hub. Retrieved February 3, 2022, from https://devolutionhub.or.ke/resource/state-of-sanitation-in-meru-county