

Research Paper**Process performance evaluation of faecal matter treatment via black soldier fly**Valary Oyoo^{a,*}, Joy Nyawira Riungu^a, Prasanta Dey^b, James Gitonga Kirimi^c and Rosemary M. Matheka^d^a Sanitation Research Institute, Meru University of Science and Technology, Meru, Kenya^b Aston Business School, Aston University, Birmingham, The United Kingdom^c Directorate of Livestock and Fisheries Development, Meru County Government, Kenya^d Civil and Environmental Engineering, Meru University of Science and Technology, Kenya

*Corresponding author. E-mail: valary.oyoo@yahoo.com

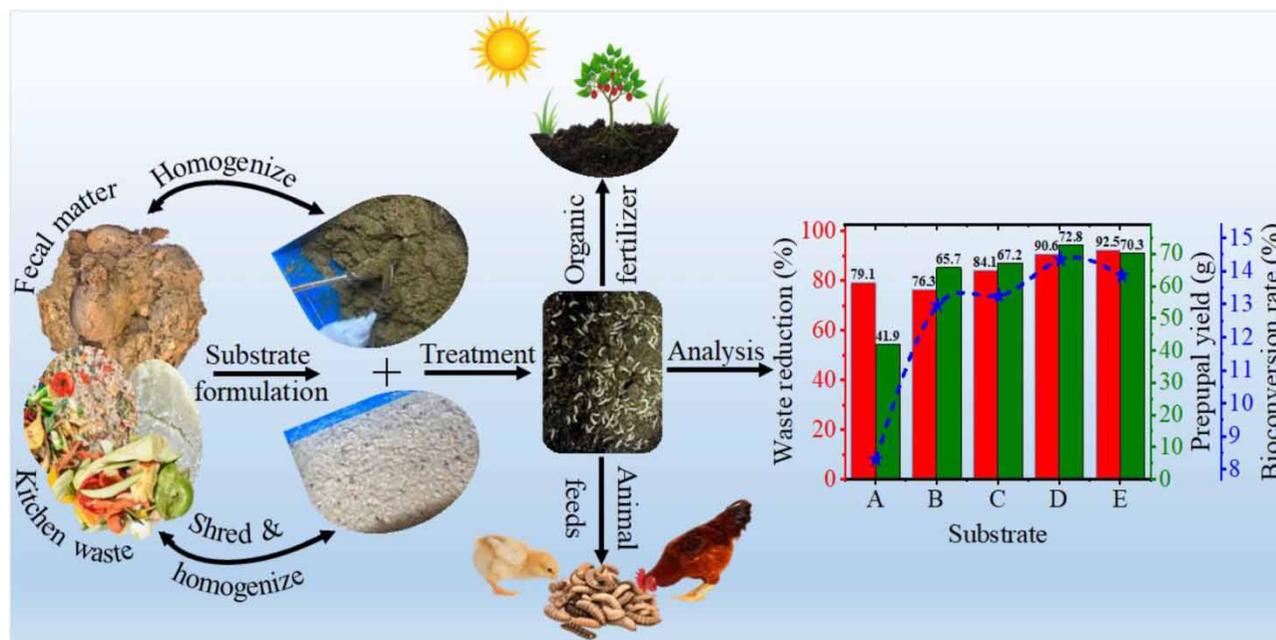
ABSTRACT

Sustainable management of faecal matter is a prevailing global challenge. In this study, we assessed black soldier fly (BSF) process performance during co-treatment of faecal matter using kitchen waste (FM:KW) to formulate five feeding substrates. About 1 kg of each feed substrate was treated utilizing 5 g of 5-day-old BSF larvae after which 100 larvae were randomly picked at 3-day intervals from each treatment to monitor the larval weight gain across the treatment process. Larval days to 50% pupation, mean pupal yield, waste reduction rate (WR), bioconversion rates (BRs), and feed conversion rates (FCRs) were monitored for the process performance. Study results showed that the substrate 1:1 attained the best measures of high WR, waste reduction index (WRI), BR, FCR, and overall pre-pupal yield within a shorter development time. Further, we modelled the BSF larval weight gain using the modified Gompertz model to assess the least time for optimal biomass conversion for animal feed processing. The BSF larvae exhibited an S-shaped growth curve and the modified Gompertz model adequately quantified the BSF larval growth performance. In the future, our methodology will pave the way for effective treatment and valorization of faecal matter from onsite sanitation facilities, manage organic municipal wastes and provide alternative animal feed and bio-fertilizer.

Key words: circular bioeconomy, co-treatment, faecal matter, *Hermetia illucens*, sanitation**HIGHLIGHTS**

- Effect of co-treatment on faecal conversion using black soldier fly is investigated.
- BSFL flourished on substrates despite variations in nutrient composition.
- Co-treatment significantly increased waste reduction and conversion.
- The co-treatment strategy enhanced the performance efficiency of BSF larvae.
- Modified Gompertz model revealed the BSFL optimal harvest time.

GRAPHICAL ABSTRACT



INTRODUCTION

Access to adequate and safe sanitation services for everyone is essential for health promotion, environmental protection and community welfare (Chandana & Rao 2022). However, providing overall access to safe and equitable sanitation amenities, especially in low-wage countries, remains a worldwide challenge. For instance, 4.5 billion individuals globally have no access to adequately maintained sanitation services along the entire value chain, and 892 million people still defecate in the open (Neto & Camkin 2020). Moreover, 2.7 billion inhabitants worldwide depend on onsite facilities for their faecal sludge management (FSM), with the number expected to rise to 150% by 2030 (Chandana & Rao 2022). Onsite sanitation systems, in developing countries, are sustainably resilient and potentially suitable for faecal management, since they adapt more to environmental and demographic changes and can permit the recovery of nutrients. Yet only about 18% of faecal sludge produced from these onsite amenities globally gets treated before disposal or end-use (Weststrate *et al.* 2019); this contributes to underground water pollution, agricultural produce contamination, and the spread of enteric diseases like helminths and diarrhoea. Hence, there is a need to explore alternative green technologies to safely manage faecal waste and enhance the circular economy.

Sanitary collection, treatment and disposal, or reuse of faecal matter (FM) from onsite sanitation facilities like septic tanks and pit latrines remain a daunting task especially in low- and middle-income countries (Diener *et al.* 2011), and the sludge is directly discarded into nearby fields or water bodies. Kitchen waste (KW) management, on the other hand, is another growing environmental concern with about 50–80% accounting for municipal waste (Chaher *et al.* 2020). In the past few decades, its production has significantly escalated with environmental footprints along with increased fast-food consumption and hotel industry development.

The circular economy model ensures safe and hygienic treatment of faecal sludge and also provides a pathway to reclaim the nutrients present back to the matter cycle generated (Patón *et al.* 2022). Current technological developments have seen the fruition of innovative technologies for faecal waste collection (container-based sanitation) and treatment technologies such as anaerobic digestion, lime addition, sun drying, black soldier fly (BSF) and drying beds for the management of onsite accumulated faecal sludge. CBS is a cost-effective bio-resource-based technology that has recently emerged, allowing separate collection of urine and faeces via the Urine Diverting Dehydrating Toilet (UDDT) system. After every toilet use, cover materials like lime, ash or sawdust are added to keep the faeces dry and odour-free (Riungu 2021). However, adding the cover materials to CBS collected faecal is not adequate for pathogen inactivation, thus the need for a post-treatment step before reuse/disposal.

The BSF (*Hermetia illucens*) can convert FM and organic municipal waste into larval biomass (a protein source for animal feed) and bio-fertilizer (for soil conditioning) (Lopes *et al.* 2020). BSF technology promotes valuable waste utilization in reuse and recycling systems and can bridge the gap in FSM. One major challenge of BSF biomass conversion is the often-poor performance efficiency with plenty and readily available organic wastes like faecal waste (Lalander *et al.* 2019). The poor performance on some of the organic side streams like FM has always been linked to the high-fibre constituent with poor biodegradability. Since biomass conversion is vital for effective faecal waste management, solutions to boost performance efficiency and biomass conversion are urgently required for sanitation promotion. Additionally, developing tools for evaluating BSF growth performance is critical in enhancing its production and utility. Hence, mathematical models depicting BSF larval growth performance can provide effective tools to determine the least time for optimal biomass conversion and to advance research on BSF technology. Though various mathematical models exist, only a few studies have experimented and modelled the BSF larval growth performance. For upscaling of BSF technology, further research is essential in bridging the gap of faecal co-treatment and establishing optimal process performance parameters.

An effective black soldier fly larvae (BSFL) treatment for organic waste needs strategies that maximize extant knowledge of the influence of different bio-waste nutritional compositions on the performance of larvae. Comparable to other organic waste treatment alternatives such as composting or anaerobic digestion, co-treatment, that is the treatment of mixed biowastes of various kinds, could reduce variability and increase BSF performance (Gold *et al.* 2018). Notably, mixing several side streams can produce a more balanced and nutritious substrate for larval development. Previously, studies (Rehman *et al.* 2017) observed that co-treating human and cow manure alongside food wastes (such as banana peels and soybean curd residue) improved larval weight in comparison to the independent substrates. FW and KW co-treatment strategy (treating FM alongside KW using BSFL) is, therefore, a potential solution that can achieve a balance in macronutrient composition in the co-treated substrates, hence improving and enhancing biomass conversion and BSFL performance on faecal waste treatment.

In this work, FM is valorized by BSFL through a co-treatment strategy using KW in the ratios 1:0, 4:1, 2:1, 1:1 and 0:1 to determine the best ratio for optimum waste conversion and organic reduction as portrayed in Figure 1. Further, the BSF larval weight gain was evaluated using the modified Gompertz model to determine the optimal harvesting time for animal feed processing. Besides, the methodology permits efficient faecal valorization while allowing for projections of the optimal larval harvest time for animal rearing (Figure 1). Compared to previous research, the co-treatment strategy in this study displays



Figure 1 | FM co-treatment with KW using black soldier fly for a sustainable bioeconomy.

improvement in waste reduction, organic conversion and the overall pre-pupal yield. This methodology can be used in a vast range of organic side streams from municipal waste, food wastes and FM to improve sanitation, especially in the post-COVID-19 era. Thus, BSFL technology through this co-treatment strategy will provide a sustainable solution to the growing faecal menace and organic municipal waste and supplement the conventional sewer systems by providing a novel option for onsite faecal management.

MATERIALS AND METHODS

Fresh human FM was obtained from the container-based facility installed at Meru University of Science and Technology – Sanitation Research Institute (MUST-SRI) while KW constituting equal portions of vegetable, fruit and food waste was obtained from the MUST cafeteria. Five-day-old BSFL were acquired from the MUST-SRI rearing unit.

Fresh human FM and KW collection and processing

A 20-l bucket was used to collect FM in the CBS facility, with approximately 10 g of wheat bran added after every toilet use for odour control. Fresh human faecal was collected daily for 3 days, where used containers were swapped with clean ones. On the third day, the collected faecal from the three containers was mixed in a larger container. The content was further thoroughly homogenized using a rod to achieve a unified sample. About 10 kg of FM was then drawn from the container and further mixed before being supplied into the feeding containers (in portions) for the treatment experiment.

For KW, 10 kg constitutes equal amounts of vegetable waste (kales, tomatoes and cabbage), fruit wastes (avocado, orange and banana) and food waste (ugali, rice, beans, green grams, meat, arrowroots, sweet potatoes and chapatti) were used for the treatment process. The vegetable and fruit wastes were shredded to 1 cm to increase the surface area for BSFL to assimilate. Finally, the KW constituents were homogenized (Figure 2) to remove substrate variation and mimic the pretreatment in BSF treatment systems (Gold *et al.* 2020).

Substrate formulation

Five treatments (in triplicates) were prepared at different mixing ratios of FM to KW as shown in Figure 2. Plastic tins measuring 26 cm × 13 cm × 11 cm were used as treatment chambers. The co-treatment substrates were thoroughly homogenized (Figure 2) to reduce variation and simulate the pretreatment mechanisms used in BSFL systems (Gold *et al.* 2020).



Figure 2 | Substrate processing, formulation, treatment and BSF larval harvesting.

Substrate characterization

The substrate was dried at a temperature of 105 °C, and moisture content was evaluated in triplicate as the ratio of the substrate's wet weight to dry weight (Meneguz *et al.* 2018). The substrate pH was determined using a Multi HANNA meter by mixing the fresh substrate with distilled water in a 1:10 ratio and analyzed. Fat content was determined through the Soxhlet extraction method (Hewavitharana *et al.* 2020), while crude protein was analyzed via the automated Kjeldahl method based on total nitrogen with a conversion factor of 6.25 (Krul 2019). Carbohydrate content was determined using the Anthrone method (Ludwig & Goldberg 1956).

BSF process performance

About 1 kg of the thoroughly homogenized formulated substrate was supplied into each feeding tin in batch. About 5 g of 5-day-old larvae were transferred into each treatment unit containing the formulated substrate. The treatment experiment duration was determined by the time required for 50% of the BSFL to reach maturity (become pre-pupae). Larval samples were taken randomly at 3-day intervals from the feeding troughs to determine the larval biomass on fresh weight. After BSFL maturity, the larvae were manually separated from the frass (digested and undigested substrate) and weighed to determine the total larval biomass and residue, respectively. After larvae and residue separation for every treatment, the harvested larvae were weighed using an electronic weighing balance readable to 0.01 g. Residual substrates were also compiled from the feeding containers and weighed using an electronic weighing balance with a precision scale of 1 g. The efficacy of BSF to flourish in the different substrates during treatment was assessed to consider both sustainable organic waste reduction and larval biomass production. The mean larval and pre-pupal wet weight, waste reduction rate (WR), waste reduction index (WRI), bioconversion rates (BRs), and feed conversion rates (FCRs) were used to calculate the process performance of BSFL. The WR was measured for every treatment on a wet weight basis of the substrate and residue mass using Equation (1) (Meneguz *et al.* 2018). The BR was measured for every treatment using Equation (2). Higher BRs indicated a good bioconversion efficiency (Gold *et al.* 2020). The WRI measured the average WR per unit time (day) and was calculated using Equation (3). While FCR was calculated using Equation (4).

$$WR = \left(\frac{FC}{W} \right) \times 100 \quad (1)$$

where WR is waste reduction (as a percentage), FC is feed consumed (in grams) and W is initial feed weight (in grams).

$$BR = \left(\frac{LY}{W} \right) \times 100 \quad (2)$$

where BR is the bioconversion rate (expressed in percentage), LY is the larval yield (in grams) and W is the total feed applied (in grams).

$$WRI = \left(\frac{WR}{t} \right) \quad (3)$$

$$FCR = \frac{FC}{r} \quad (4)$$

where WRI is the waste reduction index (as a percentage), WR is the overall degradation, t is the bioconversion time taken (in days), FCR is the feed conversion ratio, FC is the total feed consumed, and r is the larval weight gain, respectively.

Modified Gompertz model

Since the optimal larval biomass weight gained from the different mixed substrates was unknown, the modified Gompertz model represented by Equation (5) was used to simulate the experimental data in MATLAB in order to evaluate fit parameters P_0 , r_{\max} , and t_0

$$P = P_0 \cdot \exp\left(-\exp\left(\left(e \frac{r_{\max}}{P_0}\right) \times (t_0 - t) + 1\right)\right) \quad (5)$$

where P is cumulative larval weight gain (in grams) at treatment time t (in days), P_0 denotes the biomass potential of the substrate (in grams), r_{\max} indicates the maximum conversion rate (in percentage), t_0 is the lag time in days, and e (2.71828) represents the natural logarithm.

Statistical analysis

The experiment results were statistically analyzed using IBM SPSS Statistics (Version 23) by conducting a one-way analysis of variance (ANOVA) at a 95% confidence interval. This was followed by a pairwise Tukey post hoc comparison to determine whether a significant difference occurred in the mean performance parameters and mean nutrient composition in the individual substrates (Lalander *et al.* 2019). A p -value < 0.05 was a considerable indication of a significant variation between the substrates.

RESULTS AND DISCUSSION

Figure 3(a)–3(c) depicts the nutrient composition of the feed substrates A, B, C, D and E with the summary given in Table 1.

A significant variation was observed in the nutritive characteristics (carbohydrates, crude fats and protein content) among the rearing substrates ($p < 0.05$) as shown in Figure 3(a)–3(c). However, significant differences did not occur in the substrates' moisture content ($p > 0.05$). All the substrates exhibited suitable conditions for larval growth, with the crude protein content varying between 22.87% for FM and 9.83% for KW (Table 1). In previous studies (Rose *et al.* 2015; Lalander *et al.* 2019), a relatively higher protein content of 38.8 and 35.5% on human faecal was reported. The high crude protein content in the reported studies was probably from the microbe biomass present in the gut system (Rose *et al.* 2015).

At MUST-SRI, faecal waste was collected from CBS used by students whose diet consists of vegetables and cereals signifying low protein content of the ensuing substrate. In addition, the FM was mixed with wheat bran after every use for odour control and moisture content regulation. Thus, it is hypothesized that the low crude protein content in the FM observed

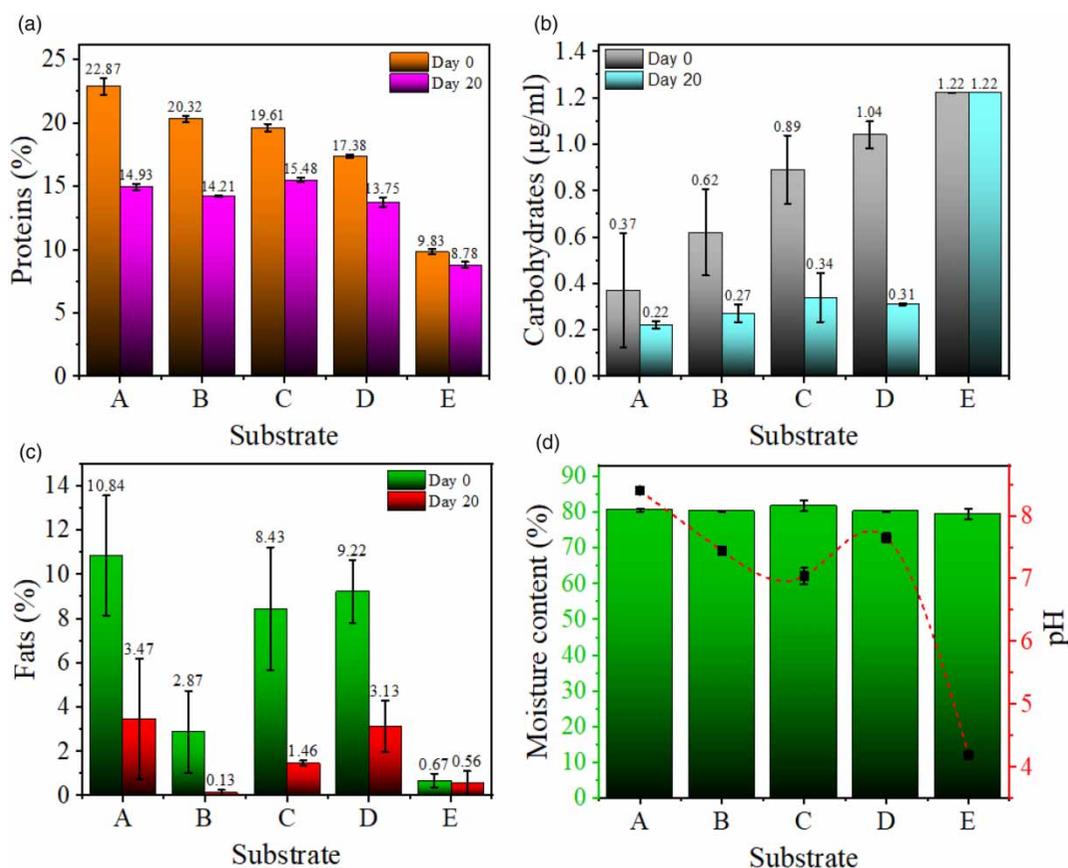


Figure 3 | Substrate characterization: (a) proteins, (b) carbohydrates, (c) fats and (d) pH and moisture content.

Table 1 | Substrate characterization at $p < 0.05$

Substrate	MC%	pH	Fats%	Proteins%	Carbohydrates%
A (1:0)	80.56 ± 0.43 ^a	8.40 ± 0.04 ^e	10.84 ± 2.71 ^b	22.87 ± 0.66 ^d	0.37 ± 0.25 ^a
B (4:1)	80.16 ± 0.06 ^a	7.65 ± 0.07 ^d	2.87 ± 1.86 ^a	20.32 ± 0.25 ^c	0.62 ± 0.19 ^{ab}
C (2:1)	81.71 ± 1.53 ^a	7.04 ± 0.13 ^b	8.43 ± 2.79 ^b	19.61 ± 0.30 ^c	0.89 ± 0.15 ^{bc}
D (1:1)	80.18 ± 0.04 ^a	7.44 ± 0.01 ^c	9.22 ± 1.42 ^b	17.38 ± 0.12 ^b	1.04 ± 0.06 ^c
E (0:1)	79.32 ± 1.47 ^a	4.19 ± 0.00 ^a	0.67 ± 0.31 ^a	9.83 ± 0.18 ^a	1.22 ± 2.69E-15 ^c
<i>p</i> -Value	0.303	0.000	0.000	0.000	0.000

Mean ± standard deviation ($n = 3$). Mean values followed by different superscript letters in the same columns were significantly different ($p < 0.05$). Crude protein, crude fats, and carbohydrates were measured on dry weight.

in the present study was likely due to the influence of dietary differences between the students using the CBS facility or the extreme weather conditions like high temperatures, which may have led to nitrogen volatilization (Rose *et al.* 2015).

On the contrary, KW was rich in carbohydrate concentration ($1.2 \pm 2.69E-15 \mu\text{g/ml}$) but displayed inferior protein and fat content of 9.83 and 0.67% on a dry matter basis, respectively. The initial pH for the FM and the co-treatment substrates in Figure 3(d) ranged between 7.04 and 8.40, which was within the previously reported (Ma *et al.* 2018) pH values (6.0–10.0) for biomass conversion by BSFL. However, KW had an initial pH of 4.19, this could be attributed to the presence of citrus fruits like oranges in the mixture. It was observed that co-treatment of FM and KW in different ratios improved the nutrient composition and the pH of the substrates compared with the FM and KW distinctively.

The overall pre-pupal yield (PpY) and subsequent residue (*R*) are important parameters for BSFL performance efficiency. Hence, the pre-pupal yield and residue were characterized at the end of the treatment process as portrayed in Figure 4(a) and 4(b). Significant variation was observed in both pre-pupal yield and waste residue across all the substrates at ($p < 0.05$) as summarized in Table 2. The pre-pupal yield increased with reducing FM on the formulated substrates while the residue

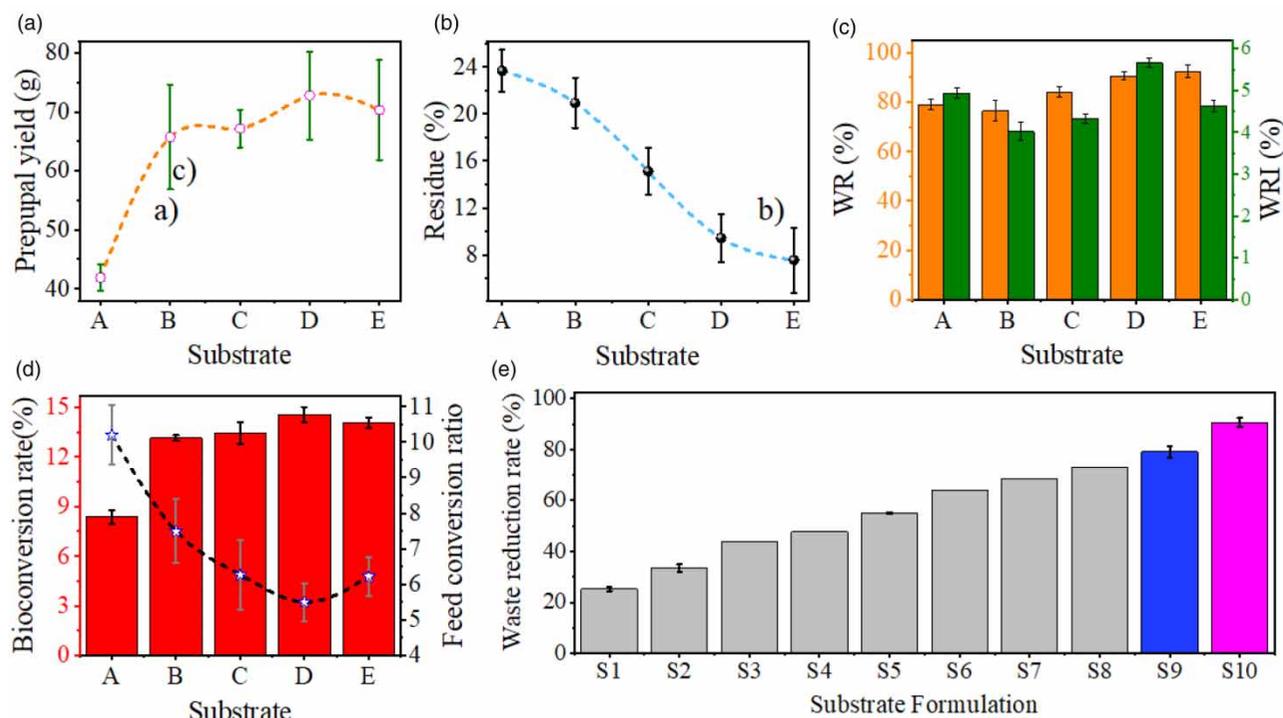


Figure 4 | (a) BSF pre-pupal yield and (b) residue at the end of the treatment process, (c) waste reduction and WRI, (d) bioconversion rate and feed conversion ratio and (e) comparison of waste reduction with previous studies.

Table 2 | Effects of different treatment substrates on WR, WRI, BR, FCR, residue (R) and pre-pupal yield (PpY) of BSFL

Substrate	WR%	WRI%	BR%	FCR	R%	PpY
A (1:0)	79.1 ± 2.1 ^{ab}	4.9 ± 0.1 ^c	8.4 ± 0.4 ^a	10.2 ± 0.8 ^b	23.7 ± 2.1 ^d	41.9 ± 2.2 ^a
B (4:1)	76.3 ± 4.2 ^a	4.0 ± 0.2 ^a	13.2 ± 1.8 ^{ab}	7.5 ± 0.9 ^a	20.9 ± 2.1 ^{cd}	65.7 ± 8.9 ^{ab}
C (2:1)	84.1 ± 2.0 ^{bc}	4.4 ± 0.1 ^b	13.4 ± 0.6 ^{ab}	6.3 ± 1.0 ^a	15.9 ± 2.0 ^{bc}	67.2 ± 3.2 ^{ab}
D (1:1)	90.6 ± 1.8 ^{cd}	5.7 ± 0.1 ^d	14.6 ± 3.5 ^b	5.5 ± 0.8 ^a	9.4 ± 1.8 ^{ab}	72.8 ± 7.5 ^b
E (0:1)	92.5 ± 2.6 ^d	4.6 ± 0.1 ^{bc}	14.1 ± 1.7 ^b	6.2 ± 0.5 ^a	7.4 ± 2.8 ^a	70.3 ± 8.5 ^b
p-Value	0.000	9.4E-07	0.019	0.011	0.000	0.019

Mean ± standard deviation (n = 3). Mean values followed by different superscript letters in the same columns were significantly different ($p < 0.05$). All parameters were measured on wet weight.

percent reduced with reducing faecal. From a previous report, it was confirmed that substrate constituent heterogeneity improves the nutritional quality compared with substrate constituent homogeneity (Tschirner & Simon 2015). Therefore, it is suspected that the addition of KW which was heterogeneous in nature – equal amounts of vegetable wastes, fruits wastes and food wastes – as opposed to the homogeneous faecal waste resulted in improved pre-pupal yield (Figure 4(a)). The poor performance on substrate A as evident in Figure 4(a) and 4(b) (pre-pupal yield 41.88 g and residue 23.69%) could be attributed to the low nutritional composition since the FM was high in fat content, but very low in carbohydrates. It has been established that high-fat content impedes BSFL performance (Fitriana *et al.* 2022) as the larvae accumulate the required body fats for pupation and mature fast and hence fail to fully convert the substrate into body mass resulting in a light-weight and high residual percentage. However, remarkable performance was observed for substrate D, achieving the highest total pre-pupal yield of 72.78 ± 17.5 g with a relatively low residue of 9.44% (Nyakeri *et al.* 2019) because of the balanced nutrient composition.

WR and WRI are significant factors indicating how adequately the feed is degraded and transformed into larval biomass each time.

Statistical variation was observed in the WR and WRI of the different substrates ($p < 0.05$) as shown in Table 2. WR increased with the addition of KW on the formulated substrates as portrayed in Figure 4(c). There was a significantly high WR in substrate E (92.46%) compared with the other substrates A (79.06%), B (76.31%), C (84.13%) and D (90.56%), respectively. It is believed that this could be due to the proper KW feed structure that enabled larval movement through the feed, easing consumption and efficient oxygen supply (Dzepe *et al.* 2021). In a recent study (Chirere *et al.* 2021), a WR of 68 and 85% was reported for human faecal and food waste respectively. In contrast, a previous report (Gold *et al.* 2020) recorded a WR of 39.1 and 48.6% on human faecal. The relatively lower WR in the reported studies could be due to the limited nutritional balance in the used substrates. This implies that the substrate type and nutrient composition affect WR. The BSFL were able to reduce and convert substrate D better than all other substrates, since it had a balanced nutrient composition. This study therefore confirms that the larval waste reduction efficiency can be highly affected by the substrate's energy content (Gold *et al.* 2020).

BRs indicate the performance efficiency of substrate consumption by the larvae. BR can be described as the mass of the pre-pupal yield per unit mass of the feed consumed. A higher BR is hence desirable when applying BSFL technology to convert waste efficiently. Supplementing FM with KW significantly affected the BR ($p < 0.05$) (Table 2). FM exhibited a BR of 8.38% but when supplemented with KW, the BR improved between 13.15 and 14.56% on a wet weight basis as shown in Figure 4(d). Although the conversion was least for the substrate constituting 100% FM, supplementing with 50% KW improved the conversion rates higher than substrates with 100% KW (Figure 4(d)). The BR for BSFL reared on the FM was relatively lower. Human faeces fundamentally consist of food remains of poor nutritional quality that the digestive system cannot absorb (Nyakeri *et al.* 2019). Thus, it was challenging for the larvae to consume. Supplementing the faecal with the KW balanced the nutrient quality, improving the larval consumption in the co-treated substrates waste. Considerable nutrient improvement through a co-treatment strategy with suitable biowastes such as KW is therefore recommended. From the experiments in this study, supplementing FM along with 50% KW (ratio 1:1) resulted in the most suitable conditions for effective waste conversion with excellent final biomass production. Therefore, the approach can be practically applied to upscale the extant BSF facilities to manage faecal waste from onsite systems.

Further, the FCR shows the proportion of consumed substrate assimilated and hence turns into biomass. When the objective is to maximize biomass production for commercialization, a low feed conversion ratio is essential. The FCR varied between 5.50 and 10.20 in the treatment process as indicated in Figure 4(d). The lowest FCR value was obtained for substrate D (5.50), indicating that supplementing FM with KW at a ratio of 1:1 would yield higher larval biomass. FCR values were high in substrates with high faecal percentages (substrates A and B) as depicted in Figure 4(d), indicating that the substrates were palatable but of low nutrient quality and thus largely excreted and less converted into body mass (Nyakeri *et al.* 2017). Interestingly, substrate D recorded the lowest FCR with a corresponding high BR an indication that it was effectively degraded and also highly transformed into the larval biomass. Additionally, it has been found that the difference in substrate quality affects the rate of substrate degradation and the overall prepupa yield during treatment by the BSFL. Therefore, controlling substrate composition is important to obtain the desirable balance of biomass growth and waste reduction (Miranda *et al.* 2019). The variation of BCR and FCR for treatment substrates was thus estimated at different co-treatment ratios to comprehend and optimize both biomass production and waste reduction.

Finally, WR results were compared with previous studies in literature works as illustrated in Figure 4(e) where (S1–S4 and S8: 100% human faecal, S5: 40% dog food, 40% swine manure and 20% human faeces in the ratio, S6: 11% cow manure, 16% human faeces, 23% mill by-products and 50% vegetable waste, S7: 5% human faeces and 95% food waste, S9: substrate A and S10: substrate D, respectively). It is evident that BSFL reared in substrate mixture 1:1 (S10) of this study surpassed the WR performance of the previous studies (Figure 4(e)) when BSF was reared on pure faecal waste or a mixture of faecal waste with other organic wastes in different ratios (Lalander *et al.* 2013; Banks *et al.* 2014). Additionally, the outstanding performance of the ratio 1:1 makes it an ideal option for organic waste management and mitigates sanitation challenges related to untreated faecal waste.

Figure 5(a)–5(e) portrays the BSF larval weight gain and development time – the time when 50% of the larvae turn into prepupae – under different substrate compositions. The co-treatment strategy of FM with KW displayed a significant impact on the BSF larval development time and larval weight gain. KW produced heavier larvae ($0.3 \text{ g larvae}^{-1}$) though the larvae took longer to develop (20 days) as evident in Figure 5(e). In contrast, FM produced lighter larvae of about $0.18 \text{ g larvae}^{-1}$, yet the larvae developed much faster taking 16 days to mature (Figure 5(a)).

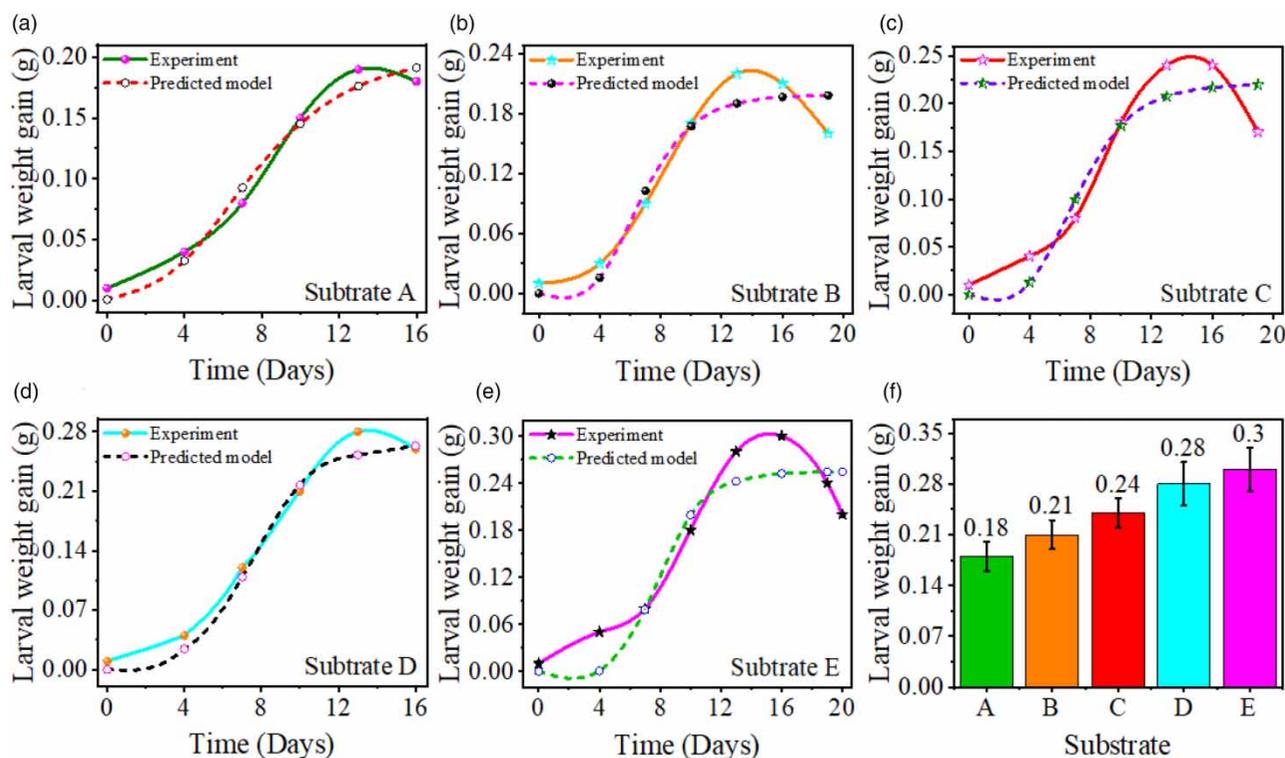


Figure 5 | (a–e) Experimental and model simulation of the larval weight gain and (f) optimal larval weight gain for the substrate compositions.

Surprisingly, the larvae developed rapidly (16 days) with a steady weight gain when the feed was supplemented with KW reaching about $0.28 \text{ g larvae}^{-1}$ in ratio 1:1 (Figure 5(d)). Substrates B and C recorded up to about 0.21 and $0.24 \text{ g larvae}^{-1}$ respectively within 19 days, indicating that co-treatment enhanced the performance of the individual substrates. Moreover, it was observed that till day 4, the larval weight gain was high for substrate A (Figure 5(a)) compared to substrate E (Figure 5(e)). This disparity could be attributed to the low pH recorded in KW (Figure 3(d)) resulting from the influence of feed combination with the citrus fruits. The low pH conditions in the KW affected the larval performance as the larvae became inactive and slowed down, waiting for microbial activities in the feed to alkalize (Nyakeri *et al.* 2019) so the pH to the optimum range BSF can comfortably operate. However, once alkalized, the larvae steadily gained weight from days 4 to 20 upon which the optimum larval weight gain was recorded (Figure 5(e)). The study suspects that due to the poor nutrient composition in FM, the larvae experienced stunted growth, while the growth was balanced when in KW or feed supplemented with KW. Convincingly, substrate characteristics, nutrient availability and feed accessibility influenced larval development time and the overall larval growth as illustrated in Figure 5(a)–5(f) (Rose *et al.* 2015).

To ascertain these results, the larval weight gain was characterized using the modified Gompertz model with the model parameters shown in Supplementary material, Table S2. The modified Gompertz model (Tyagi & Aboudi 2021) was used to predict the optimal larval weight gain to identify the best larval harvesting time for the different substrate compositions. The BSFL exhibited an S-shaped growth curve and the modified Gompertz model adequately quantified the BSF larval growth performance (Figure 5(a)–5(e)). From these results, t_0 indicated the overall time required by the larvae for material conversion, while r_{\max} demonstrated the ease of substrate conversion. Based on the results indicated in Supplementary material, Table S2, the optimum larval biomass (P_0) of substrate D was high at 0.255, making it the best substrate for larval weight gain. Substrate D resulted in a higher P_0 (0.255) than the substrates with KW (0.226) and FM only (0.199).

This result shows that the kinetic growth factors were dependent on the quality of the rearing substrate. The comparison between the experimental data and model prediction shown in Supplementary material, Table S2 using the R^2 variation outlined was in good agreement. The model extrapolated the maximal larval weight that can be achieved from each substrate in a given time. Substrate A required 3 days ($t_0, -2.6$) for the larva to achieve its optimum biomass, substrate E required 5 days ($t_0, -5.4$) while the co-treated substrate D required 2 days ($t_0, -2.4$) for optimal biomass conversion. Additionally, there was the ease of conversion of the substrate (59%) for the BSFL to its own biomass in substrate D, hence an increase in the larval weight. The co-digested feed indicated a considerable improvement in the larval weight gain and conversion performance compared with the individual substrates. These findings indicate that co-treatment enhanced the substrate dependency by harmonizing both micro and macronutrients in the feeding substrates. This was because the feeding substrate significantly affected the larval weight gain.

CONCLUSION

The process performance of the BSF was successfully studied, and it was found that the substrate valorization can be enhanced by co-treatment of FM using KW. The study results revealed that substrate with 100% FM performed poorly in relation to waste reduction, biomass conversion and pre-pupal yield among all the substrates. Human faeces fundamentally consist of food remains of poor nutritional quality that the digestive tract cannot absorb. Thus, it was challenging for the larvae to consume and convert it to biomass successfully. Supplementing the faecal substrate with the KW balanced the nutrient content thereby improving the larval performance in the co-treated substrates. The findings of this study indicate that supplementing FM with 50% KW resulted in the most suitable conditions for effective BSF performance. Considering the necessity to collaborate reduction efficiency of the main substrate (FM) and proper biomass production (for animal feed), we recommend co-treatment of FM and KW at a ratio of 1:1. The high WR, BR, overall pre-pupal weight and low FCR values attained for this ratio across the treatment process supports this recommendation. From this study results and the ease of KW availability, supplementing FM with KW has the potential for sustainable faecal management and alternative larval protein at both small-scale and industrial levels. Therefore, the BSFL larvae co-treatment approach is a suitable technique that can be employed to manage, recycle and recover FM nutrients to ensure citywide sanitation, limit environmental pollution and promote sustainable economic growth.

ACKNOWLEDGEMENTS

This research was funded by the GCRF Block Grant Funding, Project Ref:28022. The authors would like to thank the Vice Chancellor, Meru University, Kenya, for his valuable support during the study.

DATA AVAILABILITY STATEMENT

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

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

- Amrul, N. F., Kabir Ahmad, I., Ahmad Basri, N. E., Suja, F., Abdul Jalil, N. A. & Azman, N. A. 2022 A review of organic waste treatment using black soldier fly (*Hermetia illucens*). *Sustainability* **14** (8). <https://doi.org/10.3390/su14084565>.
- Andersson, K., Otoo, M. & Nolasco, M. 2017 Innovative sanitation approaches could address multiple development challenges. *Water Science and Technology* **77** (4), 855–858. <https://doi.org/10.2166/wst.2017.600>.
- Armah, F. A., Ekumah, B., Yawson, D. O., Odoi, J. O., Afitiri, A.-R. & Nyieku, F. E. 2018 Access to improved water and sanitation in sub-Saharan Africa in a quarter century. *Heliyon* **4** (11), e00931. <https://doi.org/10.1016/j.heliyon.2018.e00931>.
- 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 & International Health* **19** (1), 14–22. <https://doi.org/10.1111/tmi.12228>.
- Carrard, N., Jayathilake, N. & Willetts, J. 2021 Life-cycle costs of a resource-oriented sanitation system and implications for advancing a circular economy approach to sanitation. *Journal of Cleaner Production* **307**, 127135. <https://doi.org/10.1016/j.jclepro.2021.127135>.
- Chaher, N. E., Hemidat, S., Thabit, Q., Chakchouk, M., Nassour, A., Hamdi, M. & Nelles, M. 2020 Potential of sustainable concept for handling organic waste in Tunisia. *Sustainability* **12** (19). <https://doi.org/10.3390/su12198167>.
- Chandana, N. & Rao, B. 2022 A critical review on sludge management from onsite sanitation systems: a knowledge to be revised in the current situation. *Environmental Research* **203**, 111812. <https://doi.org/10.1016/j.envres.2021.111812>.
- Chirere, T. E. S., Khalil, S. & Lalander, C. 2021 Fertiliser effect on Swiss chard of black soldier fly larvae-frass compost made from food waste and faeces. *Journal of Insects as Food and Feed* **7** (4), 457–469.
- Diener, S., 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>.
- Dzepe, D., Nana, P., Kuitche, H. M., Kimpara, J. M., Magatsing, O., Tchuinkam, T. & Djouaka, R. 2021 Feeding strategies for small-scale rearing black soldier fly larvae (*Hermetia illucens*) as organic waste recycler. *SN Applied Sciences* **3** (2), 252. <https://doi.org/10.1007/s42452-020-04039-5>.
- Fitriana, E. L., Laconi, E. B., Astuti, D. A. & Jayanegara, A. 2022 Effects of various organic substrates on growth performance and nutrient composition of black soldier fly larvae: a meta-analysis. *Bioresource Technology Reports* **18**, 101061. <https://doi.org/10.1016/j.biteb.2022.101061>.
- Gold, M., Tomberlin, J., 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>.
- Gold, M., Cassar, C. M., 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>.
- Hewavitharana, G. G., Perera, D. N., Navaratne, S. B. & Wickramasinghe, I. 2020 Extraction methods of fat from food samples and preparation of fatty acid methyl esters for gas chromatography: a review. *Arabian Journal of Chemistry* **13** (8), 6865–6875. <https://doi.org/10.1016/j.arabjc.2020.06.039>.
- Krul, E. S. 2019 Calculation of nitrogen-to-protein conversion factors: a review with a focus on soy protein. *Journal of the American Oil Chemists' Society* **96** (4), 339–364. <https://doi.org/10.1002/aocs.12196>.
- Lalander, C., 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, C. H., Fidjeland, J., Diener, S., Eriksson, S. & Vinnerås, B. 2015 High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling. *Agronomy for Sustainable Development* **35** (1), 261–271. <https://doi.org/10.1007/s13593-014-0235-4>.
- 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>.
- Lopes, I. G., Lalander, C., Vidotti, R. M. & Vinnerås, B. 2020 Using *Hermetia illucens* larvae to process biowaste from aquaculture production. *Journal of Cleaner Production* **251**. <https://doi.org/10.1016/j.jclepro.2019.119753>.
- Ludwig, T. G. & Goldberg, H. J. V. 1956 The Anthrone method for the determination of carbohydrates in foods and in oral rinsing. *Journal of Dental Research* **35** (1), 90–94. <https://doi.org/10.1177/00220345560350012301>.

- Ma, J., Lei, Y., Rehman, K. u., Yu, Z., Zhang, J., Li, W., Li, Q., Tomberlin, J. K. & Zheng, L. 2018 Dynamic effects of initial pH of substrate on biological growth and Metamorphosis of black soldier fly (Diptera: Stratiomyidae). *Environmental Entomology* **47** (1), 159–165. <https://doi.org/10.1093/ee/nvx186>.
- 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. *Wiley Online Library* **98** (15), 5776–5784. <https://doi.org/10.1002/jsfa.9127>.
- Miranda, C. D., Cammack, J. A. & Tomberlin, J. K. 2019 Interspecific competition between the house Fly, *Musca domestica* L. (Diptera: Muscidae) and black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) when reared on poultry manure. *Insects* **10** (12). <https://doi.org/10.3390/insects10120440>.
- Neto, S. & Camkin, J. 2020 What rights and whose responsibilities in water ? Revisiting the purpose and reassessing the value of water services tariffs. *Utilities Policy* **63**, 101016. <https://doi.org/10.1016/j.jup.2020.101016>.
- 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>.
- Nyakeri, E. M., Ogola, H. J. O., Ayieko, M. A. & Amimo, F. A. 2017 Valorisation of organic waste material: growth performance of wild black soldier fly larvae (*Hermetia illucens*) reared on different organic wastes. *Journal of Insects as Food and Feed* **3** (3), 193–202.
- 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.
- Odey, E. A., Li, Z., Zhou, X. & Kalakodio, L. 2017 Fecal sludge management in developing urban centers: a review on the collection, treatment, and composting. *Environmental Science and Pollution Research* **24** (30), 23441–23452. <https://doi.org/10.1007/s11356-017-0151-7>.
- Patón, D., García-Gómez, J. C., Loring, J. & Torres, A. 2022 Composting the invasive toxic seaweed *Rugulopteryx okamurae* using five invertebrate species, and a mini-review on composting Macroalgae. *Waste and Biomass Valorization*. <https://doi.org/10.1007/s12649-022-01849-z>.
- Rehman, K. u., 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* **154**, 366–373. <https://doi.org/10.1016/j.jclepro.2017.04.019>.
- Riungu, J. N. 2021 *Off-site Enhanced Biogas Production with Concomitant Pathogen Removal from Faecal Matter*. CRC Press.
- 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>.
- Tschirner, M. & Simon, A. 2015 Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. *Journal of Insects as Food and Feed* **1** (4), 249–259.
- Tyagi, V. K. & Aboudi, K. 2021 *Clean Energy and Resources Recovery: Biomass Waste Based Biorefineries*, Vol. 1. Elsevier.
- Weststrate, J., Gianoli, A., Eshuis, J., Dijkstra, G., Cossa, I. J. & Rusca, M. 2019 The regulation of onsite sanitation in Maputo, Mozambique. *Utilities Policy* **61**, 100968. <https://doi.org/10.1016/j.jup.2019.100968>.

First received 8 January 2023; accepted in revised form 4 May 2023. Available online 18 May 2023