Emissions of gases during bio-conversion of agro-waste by black soldier fly larvae

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ABSTRACT

This laboratory-scale study was designed to investigate the emissions of carbon dioxide (CO_2), ammonia (NH_3), and hydrogen sulfide (H_2S) gases during the bio-conversion of agro-waste by black soldier fly larvae (BSFL) for 14 days. The study included three experimental treatments: a control group without waste and BSFL (T0, lab background), treatment 1 containing waste with BSFL (T1), and treatment 2 containing only waste (without BSFL, T2). Process efficiency was measured by waste reduction and bio-conversion rate. Gas emissions from the process were collected using the static chamber method and determined using the gas absorption method. The results in the treatment T1 showed a notable BSFL survival rate of 99.7%, indicating a favorable condition for BSFL growth. The waste reduction rate in the T1 treatment (74.3%) was approximately two times higher than that of T2 (38.7%), indicating the ability of BSFL to decompose organic wastes efficiently. The pH and moisture content of the waste were monitored throughout the 14-day trial for both T1 and T2, and similar trends were observed. Compared to T0, gas emissions from T1 and T2 were higher. Furthermore, the CO_2 and H_2S emissions in T1 were higher than those in T2, while NH_3 levels released in T2 were relatively higher than in T1. The preliminary results presented here could be the basis of future studies on gas emission via BSFL treatment of agro-waste.

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1. Introduction

In 2022, agricultural production in Vietnam generated a large amount of organic waste, about 159 million tons, of which crop residues account for 56.6%, livestock by-products 39%, aquatic production 0.6%, and forestry 3.8% (Nguyen & Cao, 2024). Composting has become an adaptable method for converting agricultural wastes or by-products into organic fertilizer. However, the composting process also releases greenhouse gas (GHG), such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), sulfur compounds (such as hydrogen sulfide, H_2S), and ammonia (NH_3). According to Barrington et al. (2002), the organic carbon (C) loss rates during composting were 13.6% - 51.3%, with almost all the C (70%) being emitted into the air as CH_4 and CO_2. Moreover, about 9.6% - 74% of the initial total nitrogen level is lost through composting, primarily by NH_3 volatilization (Barrington et al., 2002; Jiang et al., 2013; Yang et al., 2015). The volatilization of C- and N-containing gases into the atmosphere not only decreases the quality of the final organic fertilizers but also causes secondary atmospheric pollution (Yang et al., 2015). While H_2S was the most abundant compound, with 39.0 - 43.0% of the total five volatile sulfur compounds generated during the composting of municipal solid waste (Zhang et al., 2013).

For implementing circular economy principles, insect-based bioconversion using black soldier fly larvae (*Hermetia illucens*) (BSFL) is one of the sustainable approaches to managing organic waste (Wang & Shelomi, 2017; Huis, 2020). These larvae can decompose a wide range of organic waste and convert it into high-quality biomass for livestock and aquaculture. Industrial-scale BSF farm requires less land area and water than livestock, resulting in a lower water footprint (Wang & Shelomi, 2017; Huis, 2020; Coudron et al., 2024). Besides, it generates fewer GHG, H_2S, and lower NH_3 emissions than any conventional pasture (Wang & Shelomi, 2017; Michishita et al., 2023).

Since 2022, BSF has been allowed to be raised in Vietnam according to the Decree No. 46/2022/ND-CP (GV, 2022). Although there are various advantages to BSFL production, its environmental impact, such as CO_2, H_2S, and NH_3 emissions, has recently become a critical consideration. When the industry of manufacturing BSF grows, information on direct CO_2, H_2S, and NH_3 releases from BSFL bioconversion is required. To date, the Vietnamese literature needs more data on the CO_2, H_2S, and NH_3 emitted during composting agro-waste by BSFL. Therefore, this study aimed to compare CO_2, H_2S, and NH_3 emissions from composting with and without BSFL and the effects of inoculation with BSFL on the efficiency and rate of agro-waste treatment.

2. Materials and Methods

2.1. Materials

The experiment was conducted at the Research Institute for Biotechnology and Environmental (RIBE), Nong Lam University, Ho Chi Minh City. The BSF larvae used for the trial were taken from the BSF Research Facility of CJ Korea, RIBE. This study used an agro-waste mixture of brewery waste, coconut meal, soy pulp, and water as the substrate for the BSFL throughout the trial according to our rearing protocol (RIBE, 2017). After egg hatching, the BSFLs were fed this mixture for seven days. Seven-day-old larvae (DOL) with an average weight of 13.8 mg were used for the trial.

2.2. Experimental design and methods

In this study, three experimental treatments, including a control group without the addition of waste mixture and BSFL (T0, lab background), treatment 1 containing waste mixture with BSFL (T1, BSFL composting), and treatment 2 containing only waste mixture (T2, without BSFL), were tested in triplicate for 14 days. The control treatment was used to check that no background studied gases were present at the location of the gas measurement.

Based on preliminary trials, 1,000 BSFLs were inoculated into the composting tray with a feeding rate of 500 mg wet weight/larva per day. The larvae were fed on days 1, 2, 4, 7, and 10 of the treatment T1 and T2 by adding 500 g of new substrate to each tray without mixing. The larvae were reared at 28°C, 90% relative humidity, under natural light conditions, which were chosen to mimic the larvae's natural habitat (RIBE, 2017). To ensure optimal larval development, the density, feeding rate and frequency, and environmental conditions were used according to our rearing protocol (RIBE, 2017).

A photo of a composting tray and its associated equipment is shown in Figure 1. The laboratory-scale composting experiment was done in a plastic composting tray (61 × 42 × 15 cm) mounted with an airtight transparent acrylic lid, with a total volume of 53.2 L, for 14 days.

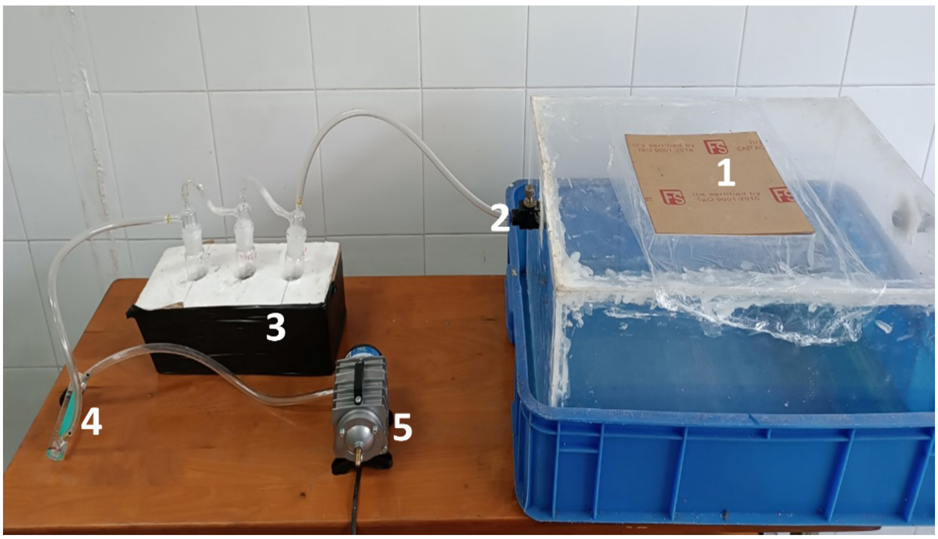

Figure 1. Diagram of a laboratory-scale composting system for measurement of gaseous emissions. (1) Composting tray with acrylic lid, (2) air valve, (3) three consecutive impingers, (4) rotameter, (5) air pump.

2.3. Bioconversion efficiency

Substrate samples were taken from the initial agro-waste mixture and leftover frass at the end of the bioconversion. The number of larvae per tray was determined by manually separating them from their substrate and counting them. After that, the total weight of larvae (*g*, wet weight (ww) and survival rate (%) were recorded per tray. To measure the dry weight (dw), substrates and BSFL were dried at 105°C for 24 h.

In addition, the humidity and pH of the substrate were measured daily using a handheld Humidity - pH meter (Model DM-15, Takemura, Japan) in treatments T1 and T2 after measuring gas emission. To evaluate the efficiency of agro-waste treatment and larval rearing in the experiment, the waste reduction rate and bioconversion rate were also estimated with the following equations (Arabzadeh et al., 2022):

$$\text{The waste reduction rate (\%)} = \frac{M-m}{M} \times 100 \quad (1)$$

$$\text{The bioconversion rate (\%)} = \frac{(B_{end}-B_{ini})}{(N-n)} \times 100 \quad (2)$$

where: M and m are the weight (in g of ww) of substrate added in the tray for 14 days and residues at the end of the experiment, respectively; B_{end} and B_{ini}, are the larval biomass (in g of dw) determined at the end and at the beginning of the experiment, respectively, and N and n are the weight (in g of dw) of substrate added in the tray for 14 days and residues at the end of the experiment, respectively.

2.4. Measurements of gas emissions

During composting, the gas evaporated from the tray was collected daily via a ten mm-silicone tube into three consecutive glass impingers with a fritted nozzle. The gas flow rate was maintained at 0.5 L/min for 10 min using an air pump and a rotameter. The first impinger contained 30 mL of 0.02 N sulfuric acid to absorb NH_3 (VS, 1995). The further impinger was filled with 30 mL of $HgCl_2$/KCl solution (WHO, 1981), and the last one was filled with 30 mL $Ba(OH)_2/BaCl_2$ solution (MOH, 1989) to absorb H_2S and CO_2, respectively. Three impingers were submerged in an ice bath, and sun exposure was avoided during the experiments.

The air samples containing NH_3, H_2S, and CO_2 were analyzed daily. During BSFL composting, the released NH_3, H_2S, and CO_2 were determined by the indophenol method (VS, 1995), the methylene blue method (WHO, 1981), and the absorption method using barium saccharate (MOH, 1989), respectively.

Daily emissions of a substance based on the concentration measurements in the units of milligrams per cubic meter were calculated using the following equation:

$$\text{Gas emission (mg/m}^3\text{)} = \frac{(A-B) \times V \times 24}{1000 \times T} \quad (3)$$

where: A and B are concentrations of the collected gas sample and the lab background air sample (T0) (mg/m^3), respectively, V is the volume of the acrylic lid (L) (V = 53.2 L), 24 is time of the day, 1000 is the conversion factor for converting m^3 to liters, and T is gas accumulation time (hours) (T = 20.5 h).

2.5. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA). Tukey's honestly significant difference test (Tukey's HSD) was used to test the significant differences among treatment means. The statistical analyses' significance was accepted at $\alpha \leq 0.05$ level. All statistical analyses were conducted using the IBM SPSS Statistics for Windows, Version 22 (Armonk, NY: IBM Corp). All data were expressed as the mean ± standard deviation (SD).

3. Results and Discussion

3.1. Composting efficiency

Table 1 shows the bioconversion performance. In this study, 1,000 7-DOLs decomposed a 2,500 g waste mixture for 14 days, resulting in a final weight of residues of 0.643 ± 0.01 kg ww, while the treatment without BSFL just reduced substrate to 1.53 ± 0.03 kg ww. The average waste reduction rates in the treatments T1 and T2 were 74.3% and 38.7%, respectively. The waste reduction rate in the T1 treatment was approximately two times higher than that of T2, implicating that the BSFL effectively bio-converted agro-waste into biomass, and the treatment efficiency using BSFL was better than typical composting.

Mean larval weight increased from 13.79 ± 0.48 g ww (2.84 ± 0.28 g dw) to 136.86 ± 4.08 g ww (43.9 ± 1.3 g dw) with a larval mass gain over time of 895 ± 94% for a trial of 14 days (Table 1). The average bioconversion rate of T1 is 14.33 ± 0.56% (Table 1). The survival rate was 99.7 ± 0.10%, indicating that the substrate used in this study was suitable.

Our results agree with the other published results regarding waste reduction, bioconversion, and larval survival rates during agro-waste valorization with BSFL. The BSFL can be reared on various organic wastes, including human and livestock fecal, vegetable, and kitchen wastes. It is well-known that the bio-waste to larvae conversion ratio could vary according to feeding rates, differences in substrate characteristics, and larval densities. In previous studies, the substrate reduction rate of organic wastes ranged between 13.2 and 84.8% (Giannetto et al., 2020; Ebeneezar et al., 2021; Arabzadeh et al., 2022), showing the ability of BSFL to convert organic wastes efficiently. While the biomass conversion ratios were 0.2 - 15.2% (Lalander et al., 2019), 8 - 10% (Giannetto et al., 2020), and 6.80% (Ebeneezar et al., 2021). In the present study, the survival rate in BSFL was found to be similar to or higher than those reported in the previous studies (Oonincx et al., 2015; Lalander et al., 2019; Arabzadeh et al., 2022). It has been stated that the diet properties less affect the survival rate in BSFL (Oonincx et al., 2015; Arabzadeh et al., 2022).

Table 1. Effects of composting with or without BSFL on bioconversion efficiency

Parameters	T1		T2	
	Before	After	Before	After
Waste (kg, ww)	2.5	0.643 ± 0.01	2.5	1.53 ± 0.03
Waste (kg, dw)	0.473	0.188 ± 0.003		
Waste reduction (%)	74.3 ± 0.46		38.7 ± 2.31	
Larvae weight (g, ww)	13.79 ± 0.48	136.86 ± 4.08	-	
Larvae weight (g, dw)	2.84 ± 0.28	43.9 ± 1.3	-s	
Bioconversion rate (%)	14.33 ± 0.56		-	
Number of larvae	1,000	997 ± 3.6	-	
Survival rate (%)	99.7 ± 0.10		-	

3.2. Effects of pH and humidity on gas emissions

The pH and moisture content of the compost substrate were monitored throughout the 14-day composting process for both T1 and T2. The initial pH values were similar between the treatments, starting around 8.0 for T1 and T2 (Figure 2). Throughout the composting process, T1 exhibited a slight increase in pH during the first few days, reaching approximately 8.3 by Day 4. This was followed by a relatively stable period where pH values fluctuated minimally, maintaining a range between 7.8 and 8.3 for the remainder of the process. T2, on the other hand, demonstrated a more gradual and consistent decrease in pH, reaching a value of about 7.5 by the end of the experiment. This decline suggests ongoing microbial activity and organic matter degradation. The pH is a critical factor affecting gaseous emissions and the activity of BSFL and bacteria. The outcomes of this study support the conclusions drawn by Ma et al. (2018) and Meneguz et al. (2018), suggesting that BSFL can change the pH of the substrate to 8.5 - 9.2. Additionally, there is a negative correlation between pH and CO_2 emissions, while a positive correlation exists between pH and NH_3 emissions (Pang et al., 2020a).

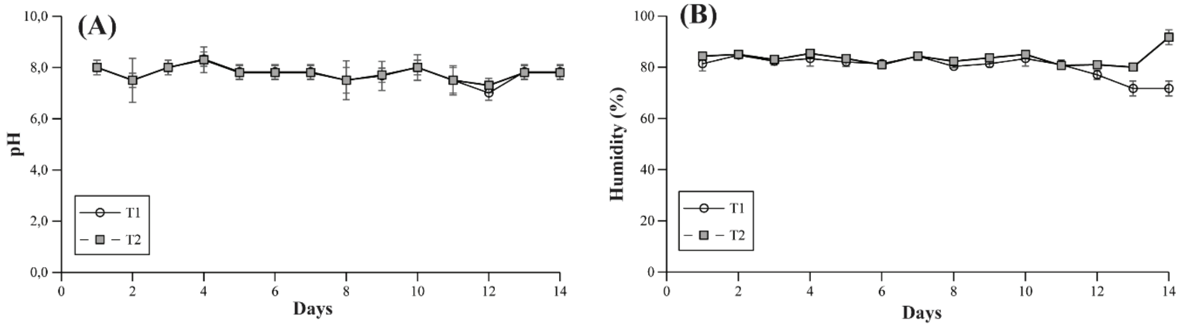

Figure 2. Effects of composting with or without black soldier fly larvae on pH (A) and humidity (B). Error bars represent the standard error of the mean, *n* = 3.

While treatments T1 and T2 maintained relatively high moisture levels, around 80 - 85%. Throughout the composting process, T1 showed a steady moisture content, with only slight fluctuations, mainly remaining stable at around 80% for the first 11 days but eventually dropping to around 72% by the end of the 14 days. On the other hand, T2 experienced a slight decrease in moisture content over time. However, a significant increase in moisture content was observed on Day 14 in T2, rising sharply to approximately 92%.

The high level of moisture (80 - 85%), as in this study, led to abnormal and improper growth of BSFL. Chen et al. (2019) conducted a quantification of GHG and NH_3 emissions from BSFL raised on pig manure under varying moisture conditions. With the increase in water levels from 45% to 75%, the NH_3 emissions increased for the first six days. The treatment at 75% water level showed the second-lowest level of NH_3 releases. Conversely, the NH_3 emissions from the treatments 85% exhibited a gradual increase during the initial six days, followed by a sharp rise in the subsequent two days (Chen et al., 2019). Meanwhile, it was reported that all treatments of moisture content inoculating BSFL reduced total CO_2 emissions compared to conventional composting, except the 75% treatment during the eight-days (Chen et al., 2019).

3.3. Gas emission during composting

Figure 3 shows the emission pattern of NH_3, H_2S, and CO_2 with composting time in three treatments.

3.3.1.The NH_3 emissions

As presented in Figure 3A, the NH_3 gas produced in the control group, T1 and T2 ranged from 128 - 736 mg/m^3, 414 - 2268 mg/m^3, and 414 - 2541 mg/m^3, respectively. The results showed apparent differences between treatments in NH_3 emission levels during 14 days of monitoring. In the T0, NH_3 concentrations remained low throughout the experimental period, indicating that under our laboratory conditions, NH_3 emissions are minimal. In both composting treatments, NH_3 emissions showed similar trends and were significantly higher than those in T0 ($P < 0.05$). In the first seven days, the NH_3 volatilization rose, then decreased rapidly in the following days and slowly increased until the end of the trial. In general, NH_3 levels released in T2 were relatively higher than in T1. Ammonia emission increased sharply with the substrate added on days 2, 4, 7, and 10, caused by the rapid decomposition of the organic nitrogen compound, and on the 4th and 7th days, the NH_3 production reached its peak. Our research results show that BSFL composting and waste without BSFL significantly increase NH_3 emissions compared to lab background conditions. Additionally, using BSFL in the composting process does not substantially reduce NH_3 emissions compared to composting without BSFL.

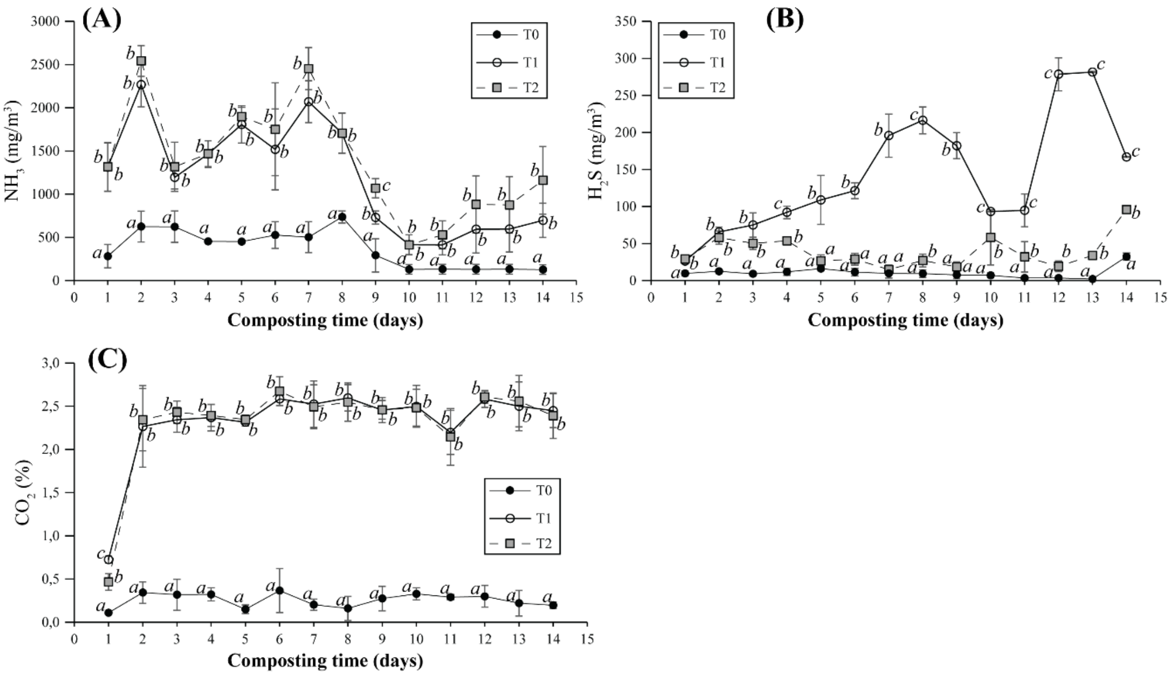

Figure 3. Changes of concentrations of NH_3 (A), H_2S (B), and CO_2 (C) among treatments measured via the static chamber during 14-day composting of agro-waste. Error bars are mean ± SD (n = 3). Different letters above the error bars indicate significant differences at the 0.05 level (ANOVA and Tukey's HSD).

High levels of NH_3 emissions during composting could negatively affect the environment, as NH_3 is a gas that has the potential to pollute the atmosphere and contribute to the formation of acid rain. Lindberg et al. (2022) reported that NH_3 emission in conventional composting was 100-fold higher than in BSFL composting. However, other studies and BSFL mass-production producers have reported high levels of NH_3 emissions in BSFL composting, in the range of 30 - 40% of the input nitrogen (Lalander et al., 2019; Yang, 2019; Lopes et al., 2020).

Nitrogen losses via NH_3 emissions from BSFL composting are one of the critical environmental problems resulting from larval production and affect the quality of the final product as organic fertilizer. Many factors, such as water content, pH, temperatures, and other physicochemical factors, influence ammonia volatilization (Koerkamp, 1994). As discussed above, the high moisture content enables the absorption of NH_3, which hinders microbial activity and NH_3 emission (Guo et al., 2012; Chen et al., 2019). Furthermore, to avoid NH_3 emission, the equilibrium between NH_4^+ and NH_3 should be maintained by keeping an acidic pH and temperatures below 20°C (Koerkamp, 1994). The higher NH_3 emissions obtained in this study might be due to higher pH, as Chen et al. (2019) indicated.

3.3.2. The H_2S emissions

Figure 3B exhibits the H_2S concentration recorded during the composting trial. The results showed apparent differences between treatments in H_2S emission levels. In the control group (T0), H_2S concentration was kept very low and unchanging throughout the trial time, with values ranging from 2 to 32 mg/m³. While H_2S concentration in treatment T1 increased significantly compared to T0 ($P < 0.05$), starting from day 2 and peaking on day 8 with a value of about 220 mg/m³. After that, the H_2S concentration gradually decreased but remained higher than the control group until the end of the study period. In particular, from day 10th to day 14th, H_2S concentration in T1 increases again, especially on days 12th and 13th. For treatment T2, H_2S levels also increased significantly compared to T0, but the emission level was lower than that of T1. H_2S concentration in T2 peaked around day 4th with a value of about 50 mg/m³, then gradually decreased and fluctuated at a lower level than T1 until the end of the study period.

The study results showed that composting, whether composting with or without BSFL, significantly increased H_2S emissions compared to lab background conditions. The increase in H_2S emissions in treatments T1 and T2 can be explained by the decomposition of sulfur-containing organic compounds in the compost, leading to the release of H_2S gas. Notably, the H_2S concentration in T1 was significantly higher than that in T2, showing that the composting process using BSFL can increase the release of H_2S compared to composting without BSFL. This may be due to the vigorous activity of BSFL in decomposing organic matter, leading to faster and greater release of H_2S. However, these high emissions levels can negatively affect the environment and human health, as H_2S is a toxic gas with an unpleasant odor and can cause many health problems if exposed to high concentrations.

In contrast to available data on GHG and NH_3 emissions via organic waste valorization using BSFL, the published articles provide limited information regarding sulfur compound emissions. Michishita et al. (2023) found that the presence of BSFL led to a notable reduction in the emission of smelly sulfur compounds like dimethyl disulfide and dimethyl trisulfide, which are commonly generated as a result of methionine and cysteine breakdown by *Lactobacillus* and *Enterococcus* bacteria. These findings were very dissimilar from those of our study. The discrepancy might be due to the different experimental conditions.

H_2S is a well-known malodorous gas that is commonly produced in the process of composting organic waste. Several factors, such as moisture content, aeration, and porosity of composts, can be controlled while composting to reduce the generation and release of odor smells (Zhang et al., 2013). The ideal moisture content for developing the composting process was 40 - 60%

(Zhang et al., 2013). Nonetheless, the substrate's moisture level in this study (72 - 85%) was higher than the recommended range, as indicated above. Moreover, the odor gas formation in BSFL composting is typically attributed to poor oxygen transfer from insufficient aeration.

3.3.3. The CO_2 emissions

Figure 3C shows the CO_2 concentration (%) during the experiment. During 14 days of monitoring, the results showed a clear difference in CO_2 emission levels between treatments. The concentration of CO_2 in the control group remained very low, ranging from 0 to 0.4% throughout the study period. Levels of CO_2 in T1 and T2 increased sharply from the first day and finally became relatively stable at approximately 2.5% for the remainder of the study period. These were significantly higher emission levels than the control group, demonstrating the organic decomposition activity in both treatments.

Carbon dioxide is the most significant GHG and is the primary emission generated during the rearing process of BSFL from organic materials (Boakye-Yiadom et al., 2022). Several studies have assessed the CO_2 emissions generated during the rearing of BSFL with different substrates (Pang et al., 2020a; Pang et al., 2020b; Lindberg et al., 2022). Most research indicates that CO_2 levels demonstrated low emissions in the early days, which gradually increased, culminating in peak emission rates during the mid-treatment phase, and a steady decrease in the later stages can be attributed to the stabilization of the substrate and a decline in the metabolic activities of both the bacteria and BSFL (Chen et al., 2019; Pang et al., 2020a).

Compared to these works, our result showed different CO_2 emission patterns. It could be due to a difference in the feeding regime (amount of feed, feeding frequency). In our study, 1,000 7-DOLs were fed five times with a feeding rate of 500 mg ww/larva per day (a feed amount of 2,5 kg ww). In the study of Chen et al. (2019), 450 3-DOLs were fed one time with a feed amount of 160 g dw, while in the study of Pang et al. (2020a; 2020b) 1,600 - 1,800 3-DOLs were fed one time with a feed amount of 1 - 1,2 kg ww.

Several factors, such as the C/N ratio, moisture content, pH, and batch feeding time, may influence the rate of CO_2 emissions during the composting process of BSFL (Boakye-Yiadom et al., 2022). Pang et al. (2020b) reported that with an increasing C/N ratio of the substrate, the CO_2 emissions in BSFL composting were increased. As stated above, an increase in substrate moisture content was negatively associated with emissions of CO_2 (Chen et al., 2019). For pH effects, the emissions of CO_2 were found to be minimal under extreme pH conditions of 3.0 and 11.0, while they peaked at the optimal pH levels of 5.0 and 7.0 (Pang et al., 2020a). Zhang et al. (2021) observed that an increase in batch feeding frequency led to increased CO_2 emissions.

4. Conclusions

The waste reduction rate in the BSFL treatment (74.3%) was approximately twice as high as without BSFL (38.7%). The pH and moisture content of both treatments showed a similar trend. The CO_2 and H_2S emissions in T1 were higher than those in T2, while NH_3 levels released in T2 (414 - 2541 mg/m^3) were relatively higher than in T1 (414 - 2268 mg/m^3). Temperature affects the metabolic activities of BSFL and the microorganisms and gas emissions during composting. Therefore, temperature monitoring should be considered in future studies. Management and control of these gas emissions are necessary to ensure that the composting process does not adversely affect the environment and public health. This suggests that further research is needed to determine the optimal conditions to maximize the environmental benefits of using BSFL in composting.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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