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Valorizing Black Soldier Fly Frass via Anaerobic Co-Digestion: Biogas Potential, Microbial Safety, and Heavy Metal Dynamics

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Abstract

Insect frass was evaluated for its potential in biogas production, and the effect of anaerobic digestion (AD) on its microbiological quality, both alone and in co-digestion with poultry droppings and cow dung, with respect to biogas yield, process stability, and the safety of the resulting digestate. Biogas production was highest in the frass-poultry co-digestion system (8.2 mL/g VS) at pH 6.3, followed by frass alone (7.5 mL/g VS) at pH 6.7, whereas the inclusion of cow dung reduced yields. The microbiological study of frass indicated that the initial colony-forming units (CFU/ml) of frass were >3

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× 10^4 on both Salmonella-Shigella and nutrient agars, and 8.0 × 10^3 on MacConkey and 9.0 × 10^3 eosin methylene blue (EMB) agars. After anaerobic digestion, CFU/ml values reduced to 5 × 10^6 on nutrient agar, with no growth found on MacConkey, Salmonella-Shigella agar, and eosin methylene blue agar. Heavy metal study showed a decrease in cobalt, nickel, copper, and lead concentrations after AD, although zinc levels increased from 2.99 to 3.01 mg/L, exceeding WHO safety guidelines (1.0 mg/L). These findings demonstrate that insect frass is a viable feedstock for biogas generation, particularly when co-digested with nitrogen-rich materials, and that AD contributes to pathogen reduction and metal immobilization for most elements. However, zinc accumulation poses a potential environmental risk, necessitating further treatment or monitoring if the digestate is to be used agriculturally.

Keywords: Insect-Frass, Co-Digestion, Heavy Metals, Microbial Safety, Circular Economy.

INTRODUCTION

In Western countries, the use of insects as food and feed has grown during the past ten years (Kim *et al.,* 2019). The continuing protein transition in Europe highlights the potential for using insects in food and feed. Four insect species are presently permitted as novel foods in the European Union (EU) (Commission Implementing Regulation (EU) 2017/2470), while eight insect species are permitted for feed (Commission Regulation (EU) 142/2011) (European Commission, 2011, 2017). The insect industry is growing very fast, and this has led to the generation of large quantity of insect rearing by-product like frass (van Huis *et al*., 2021). Insect faeces, shed-off exoskeletons of the insects (exuviae) and substrates residual are the major component of frass (Barragán-Fonseca *et al*., 2022).

Frass, a waste product of insect farming, is made up of insect feces, substrate remnants, and exoskeleton fragments. It contains both insect-derived components like chitin and plant elements like cellulose and lignin.

The mass breeding of insects for food and feed is currently the focus of more specialized companies. As a result, these insects produce enormous amounts of frass, which can pose a major threat to the environment. To prevent this, many applications for frass have been suggested, such as the development of biochar, a solid byproduct of biomass pyrolysis (Yang *et al*., 2019; He *et al*., 2020).

While frass is primarily utilized as fertilizer (Beesigamukama *et al*., 2021), research on its potential as a substrate for anaerobic digestion to produce biomethane is still in its early stages (Dal Magro *et al*., 2024). Anaerobic digestion not only produces energy from biomass but also supports nutrient cycling (Chuenchart *et al*., 2020). Even though anaerobic digestion is a well-established process, research is still being done to increase its efficiency and add additional substrates (Bulak et al., 2023).

Frass fertilizer costs between $0.30 and $0.35 per kilogram, whereas dried black soldier fly (BSF) larvae cost between $1.10 and $1.40 per kilogram. As the insect production industry continues to increase, post-production residues such as frass are also expected. This growth is seen in the industry's projected global market value, which is forecast to rise from approximately $3.2 billion to $17.6 billion by 2032 (http://www.statista.com, 2024; Tanga *et al*., 2021).

Waste management planning must take this into account (Bulak *et al*., 2020). In comparison to direct composting, this offers options to channel frass into the generation of biogas and

biomethane, with additional advantages such residual heat recovery and the reuse of digestate as fertilizer, producing larger synergies (Dal Magro *et al*., 2024).
Insect frass must be valued to improve the sustainability and economic viability of the insect-rearing sector, which calls for investigation of its possible applications. Numerous uses for frass have been found through research, such as fertilizer in agriculture, biogas production, and pharmaceuticals (Coviello, 2026). However, given the growing emphasis on using agricultural waste in insect rearing, concerns are raised about potential chemical and microbiological risks in frass (Safitri *et al*., 2024). Prior research focused mostly on nutritional elements such as nitrogen, phosphate, potassium, and chitin without sufficiently evaluating safety hazards. If frass-related dangers get into soil and water sources, these risks could affect the ecosystem and food chain. This highlights the necessity of safety assessments before market launch, as required by current legislation (European Commission, 2021).

The increase in food waste generation which is usually managed via landfills, incineration, and anaerobic digestion, constitutes a sizable portion of the world's organic waste. However, black soldier fly (*Hermetia illucens*) larvae, which effectively breaks down a variety of organic wastes and transforms them into valuable biomasses like oils and proteins, has been said to be more sustainable treatment approach that is gaining popularity worldwide (Kim *et al*., 2021). The BSFL also generates waste even after decomposing food waste, and the microbiological safety of this waste generated by this insect is of major concern (Bessa *et al*., 2021).

Total viable counts (TVCs, up to 10.5 log colony forming units (CFU)/g), Enterobacteriaceae (up to 9.3 log CFU/g), lactic acid bacteria (up to 8.7 log CFU/g), bacterial endospores (up to 7.7 log CFU/g), and fungi (up to 9.0 log CFU/g) have all been reported for BSFL and YMW frass obtained from various substrates (Gold *et al*., 2020; Van Looveren *et al*., 2022a). Van Looveren *et al*. (2022a) did not find *Clostridium perfringens* above 1.0 log CFU/g or *Salmonella* in BSFL frass from chicken feed. In contrast, frass from BSFL fed a combination of potato starch, wheat, and protein kibbles showed levels of *Clostridium perfringens* as high as 2.2 log CFU/g in another study (Van Looveren *et al.,* 2022b). Furthermore, *B. cereus* and *Salmonella agona* were found in BSFL frass from a variety of substrates (Wynants *et al*., 2019a). Major bacterial genera discovered in BSFL frass, including pathogenic species from *Bacillus* and *Clostridium*, were listed by Gold *et al*. (2020).

There is little information on chemical and microbiological pollutants in frass, what is presently known suggests that contamination varies depending on the types of contaminants, insect species, and feed substrates. Mycotoxins, dioxins, heavy metals, pesticide residues, and food-borne infections are examples of possible risks (Safitri *et al*., 2024).

The specific aim of this present study is to explore the potential of anaerobic codigestion in treating frass to make it microbiologically and chemically safe for field application and as well production of biogas as a source of renewable energy.

MATERIALS AND METHODS

Preparation for Frass

The black soldier fly's colony was maintained thus; the temperature was maintained between 24 °C and 36 °C. Sixty to seventy-five percent relative humidity was maintained throughout larval stages and 12 to 16 h of light each day during adult mating. Food waste, pap chaff, and chicken manure were used to feed Black Soldier Flies larvae, and they were harvested on day 22. This harvesting was done by sifting the frass from a mixture of larvae and biomass. The frass primarily consists of egesta from the larvae, unfed substrate and chitins in the form of shed exoskeleton as larvae grow from one instar to another (There are 5 instars in the larvae, and they shed their 'skin' every time they evolve into a new instar) (Ortiz et al., 2016).

Biogas Production from Frass
Table 1 shows the summary of the experimental setup of the potential use of frass in biogas production. An improvised 2.5 litres biodigester was used for biogas production. The substrates (insect frass, cow dung, poultry droppings) were mixed with water in 1:1 ratio on a fresh weight basis. The 0.45 kg of inoculum was mixed with 0.45 kg of insect frass, summing up to 0.9 kg. Thereafter, 0.9 L of water was added to make a final volume of 1.8 L, which constitutes seventy percent of the digester volume (2.5 L) (Buraimoh *et al*., 2020).

Table 1: Experimental setup for Biogas Production from Frass

S/N	Experiment	Code
1	Frass	F
2	Frass + cowdung	F + C
3	Frass + Poultry Droppings	F + P
4	Frass + Poultry + cowdung	F + P + C

Bacteriological analysis of Frass before and after Anaerobic Digestion
Microbial safety of the frass was determined by taking 1 g of frass sample before and after anaerobic digestion. The samples were serially diluted, and plated on Eosin methylene blue (EMB), Salmonella-Shigella agar (SSA), MacConkey agar (MAC), and nutrient agar (NA). The colony count was done and colony forming unit was calculated (Looveren *et al*., 2022a).

Heavy Metals Analysis
Digestion
A 250 mL conical flask was filled with precisely one gram (1.00 g) of the sample. Each sample was placed in a fume hood with 20 mL of a digestion mixture made up of perchloric acid, nitric acid, and sulfuric acid at a ratio of 1:2:2 (v/v/v). The contents were cooked on a hot plate for about 15 to 20 minutes, or until nearly full digestion was indicated by the development of white vapors (Maria and Csaba, 2002). The flask was left to cool after the digestion was halted. To guarantee that the metals were completely dissolved, 20 mL of distilled water was added after the liquid had cooled and brought to a boil. After cooling the mixture even more, it was filtered using Whatman No. 42 filter paper, it was put into a 50 mL volumetric flask and diluted with distilled water to the appropriate level. The resulting solution was transferred into clean, labeled plastic bottles and preserved for metal determination using 4200 MP-AES or Atomic Absorption Spectrophotometry (AAS).

Instrumental Analysis
The micro plasma atomic emission spectrophotometer (MP-AES 4200), manufactured by Agilent in California, USA, was used for the analysis. It was connected to a computer and ran via the MP-AES software interface. Operational wavelength used are as follows: Cd (228.8 nm), Co (241.7 nm), Cr (425.4 nm), Cu (324.754 nm), Fe (259.9nm), Ni (352.4 nm), Pb (283.3 nm), Hg (437 nm) and Zn (472.215 nm).

The device with the proper pre-set unit was used to directly acquire metal concentrations in digested solid samples. The device was adjusted to reflect the dilution factor if sample dilution was necessary. For solid samples, all concentrations were computed as mg/kg or ppm based on dry weight; for metal concentrations in solution, the value was represented as milligrams per litre (mg/L) or ppm.

Determination of the pH
The hydrogen ion concentration was measured at a day interval using a 3 in 1 multipurpose instrument (QUMOX 2X Soil PH Tester, Digital Plant Soil Moisture Meter with PH Value/Temperature/Moisture).

Determination of C/N Ratio
Carbon-Nitrogen ratio was determined by determining the total Carbon against total Nitrogen. The procedure is explained below;

Method: Walkey-Black Method
Into a 250 ml conical flask (Erlenmeyer) 1.0 g out of the samples was weighed (1.0 g should be used if organic C content is between 1 % and 3%; 2.0 g if it is less than 1%). Ten (10 mL) of 1N $K_2Cr_2O_7$ solution was pipetted into each flask, swirls and gently the samples were dispersed. Thereafter, 20 mL conc. H_2SO_4 was added and swirled and allowed to stand for 30 mins. Hundred millilitres (100 mL) of distilled water was added after standing for 30 minutes. Ten millilitres (10 mL) of O-phosphoric acid was added. Few drops (3-4 drops) of indicator were added and titrated with 1N Ferrous Ammonium Sulphate OR Ferrous Sulphate till the colour changes from green to blue and finally to red (maroon color) which is the end point. The blank titration was done in the same manner, but without the samples. The result was calculated according to this formula;

$$\% \text{ TOC} = \boldsymbol{mL\ FAS\ for\ blank - ml\ FAS\ for\ sample\ \div Weight\ of\ Frass\ in\ g\ \times Normality\ of\ FAS\ \times 0.390}$$

Determination of Total Nitrogen
Method: Kjeldahl Method
An appreciable quantity (1.0 g) of oven dried sample was weighed into a labeled, dry and clean digestion tube. Subsequently, 15 mL of the digested mixture was added to each tube. It was digested at 360 ^{0}C for 2 hours. The solution turned colourless and remained sand white. If solution is still colored, heat for a further 1 hour. The contents were allowed to cool. About 25 mL-distilled water was added and was mixed well until no sediment dissolves. It was allowed to cool. Thereafter the mixture was made up to 50 mL with distilled water and was well mixed. It was allowed to settle so that a clear solution can be taken from the top of the tube for analysis. The Nitrogen was determined using the digest as outlined below;
The digested sample (10- 20 mL) was transferred into an Erlenmeyer flask. One sachet of the Nitraver 5 powder pillow was added. It was shaking thoroughly for 1min and allowed it to stand for 5 minutes or until dark residues were formed. An amber colour was developed. The absorbance was read using DR 2010 spectrophotometer, HACH, USA at 410 nm.

RESULTS
Biogas Yield and pH
The best biogas yield, the day, and the pH of frass, frass plus poultry droppings, frass plus cow dung, and frass plus cow dung and poultry droppings at twenty-one days' retention time. The biogas production was measured based on the water displacement method. The best yield of 8.2 mL/g VS was recorded from the co-digestion of frass and poultry droppings (F+P), followed by the anaerobic digestion of frass alone (**Table 2**).

Table 2: Best biogas yield across all treatment, and the optimum pH

S/N	Experiment	Code	Biogas Yield (mL/g VS)	pH	T ^{0}C	Day
1	Frass	F	7.5	6.7	40	21
2	Frass + cow dung	F + C	4.5	6.7	40	21
3	Frass + Poultry Droppings	F + P	8.2	6.5	40	21
4	Frass + Poultry + cow dung	F + P + C	0.5	7.0	40	13

Bacterial Colony Forming Unit (CFU)

Table 3 shows the colony forming units of frass and it digestates at varied dilution factors. There is no growth at $10^{2,}$ 10^4 and 10^6 dilution factors on MaConkey agar (MAC) and 10^6 dilution factors on Salmonella-Shigella agar (SSA) and Eosin Methylene Blue (EMB) respectively for frass digestate.

Table 3: Bacterial colony forming units in frass and digestate on five culture media

Media Used	Frass (CFU/mL)			Digestate (frass) (CFU/mL)		
	10^2	10^4	10^6	10^2	10^4	10^6
NA	>3.0 x 10^4	> 3 x 10^6	> 3 x 10^8	>3.0 x 10^4	1.2 x 10^6	5 x 10^6
EMB	9.0 x 10^3	2.1 x 10^5	8.0 x 10^6	8.0 x 10^3	1.6 x 10^5	No growth
MAC	8.0 x 10^3	2.0 x 10^5	8 x 10^6	No growth	No growth	No growth
SSA	>3.0 x 10^4	> 3 x 10^6	> 3 x 10^8	1.6 x 10^4	1.02 x 10^6	No growth

Heavy Metals

Table 4 shows the reduction in heavy metals content of frass after anaerobic digestion. It's only Zinc value that did not reduce, rather it increases by 0.02 from 2.99 mg/L to 3.01 mg/L.

Table 4: Heavy metal analysis of the frass and its digestate

Heavy Metals	Unit	Frass	Digestate	WHO limit
Cobalt	mg/L	0.012	0.009	0.01
Nickel	mg/L	0.021	0.017	0.02
Zinc	mg/L	2.99	3.01	1.0
Copper	mg/L	0.471	0.389	1.00 - 2.00
Lead	mg/L	0.041	0.029	0.05 - 1.00

C/N Ratio

The Carbon-Nitrogen ratio results of some selected organic wastes were determined. The result is presented in table 6 below. Poultry droppings have low C/N ratio of 8:1. While cow dung has the highest C/N ratio of 25:1 (table 5).

Table 5: C/N ratio of selected organic wastes

Substrate	C/N RATIO
Poultry droppings	8:1
Cowdung	25:1
Frass	9:1

DISCUSSION

The present study evaluated the potential of insect frass as a substrate for anaerobic digestion (AD), with a focus on biogas production efficiency, microbiological safety, and chemical safety in terms of heavy metal content. The results indicate that insect frass holds promise as a viable feedstock for biogas generation, particularly when co-digested with nitrogen-rich materials, and that the AD process contributes to the reduction of pathogenic bacteria and most heavy metals. In this study, when a 2.5 L digester was employed, the highest biogas yield (8.2 mL/g VS) was achieved from the co-digestion of frass with poultry droppings (F+P), followed by frass alone (7.5 mL/g VS) (Table 2). However, the inclusion of cow dung (F+C) resulted in lower biogas production, and the ternary mixture (F+P+C) demonstrated severe inhibition, yielding only 0.5 mL/g VS of biogas. According to Liu *et al*. (2026), the lower yield in the ternary mixture could be due to the cellulosic content of the cow dung, which would slow down the degradability process. And this can lead to a prolonged acidogenic stage, which in turn suppresses methanogen growth. Furthermore, accumulation of ammonia has been reported to inhibit methanogens.

The high production of biogas observed in the frass–poultry droppings synergy may be attributed to balanced carbon-to-nitrogen (C: N) ratios, higher readily degradable organic matter, and enhanced microbial activity. The C/N ratios of frass and poultry droppings as shown in table 5 are 9:1 and 8:1, respectively. So, the highest biogas yield in frass and poultry droppings codigestion could be due to the easy degradability of the substrate. This agreed with the work of Tumusiime *et al*. (2022) on optimization of substrate mixing ratios for wet anaerobic digestion of selected organic waste streams for productive biogas systems. Poultry droppings are known to be rich in nitrogen and easily biodegradable compounds, which can stimulate methanogenic activity when appropriately balanced with carbon-rich substrates such as insect frass. Similar findings were reported by Zhan (2023) and Oluwafemi *et al*. (2023), who observed improved biogas yields when poultry manure was co-digested with organic wastes due to enhanced nutrient synergy and buffering capacity.

The lower yield from F+C may be due to an unfavorable C/N balance or the presence of lignocellulosic compounds in cow dung that are more difficult to break down in the conditions that were tested. The significant inhibition seen in the ternary mixture is probably due to too much ammonia or volatile fatty acids (VFA), which can stop methanogenesis. This is a well-known problem in overloaded co-digestion systems (Yang *et al*., 2025). The frass + cow dung treatment produced less biogas (4.5 mL/g VS) than the other treatments. This could be because the lignocellulosic parts of cow dung break down more slowly, which makes it harder for methanogens to get to the substrate (Stefan and Hellen, 2022).

Li *et al*. (2020) stated that cow dung usually has a lower potential for methane unless it is treated beforehand or mixed with substrates that break down easily. The low amount of biogas produced by the ternary substrate mixture (F + P + C) could be because the substrates are overloaded, ammonia is stopping the process, or microbes are competing, which makes the process unstable. When mixed with other substrates, poultry droppings can raise the levels of free ammonia, which is harmful to methanogenic archaea. Yang *et al*. (2025) have also reported similar inhibitory effects in multi-substrate anaerobic digestion systems. The ideal pH range seen in productive treatments (6.5–7.0) matches what is known about the physiological preferences of acetoclastic and hydrogenotrophic methanogens. This further supports the idea that AD of insect frass can be kept within a stable operational window (Li *et al*., 2022). Angelidaki *et al*. (2018) also said that stable biogas systems usually work best when the pH is between 6.5 and 7.5.

Microbiological analysis (Table 3) demonstrated elevated bacterial counts in raw frass cultivated on Nutrient Agar (NA), Eosin Methylene Blue (EMB), MacConkey Agar (MAC), and Salmonella-Shigella Agar (SSA), suggesting the existence of varied microbial populations, including potential enteric bacteria. This result aligns with the research conducted by Gold *et al*. (2021) and Kinyua *et al*. (2022), which indicated that insect frass frequently harbors residual gut microbiota and environmental microorganisms. However, after anaerobic digestion, there was a noticeable drop in the number of microbes, especially on selective media like MacConkey Agar (MAC), Salmonella-Shigella Agar (SSA), and eosin methylene blue (EMB), where no growth was seen at higher dilution levels. This shows that anaerobic digestion works well to get rid of pathogenic and indicator organisms, which makes the digestate safer for the environment. The decrease in pathogenic bacteria following anaerobic digestion supports the results of Manyi-Loh and Lues (2022) and Awasthi *et al*. (2023), who showed that biogas digestion greatly reduces coliforms, Salmonella spp., and other enteric pathogens because of long exposure to anaerobic conditions, volatile fatty acids, and microbial antagonism. The colonial morphology seen in different media backs up the idea that there are mixed bacterial populations in raw frass, such as lactose fermenters and non-fermenters. However, digestate samples showed less diversity. This microbial reduction is critical for safe agricultural application of digestate, especially as soil amendments or biofertilizers.

Heavy metal analysis (Table 4) showed a general reduction in metal concentrations following anaerobic digestion, except for zinc, which increased slightly from 2.99 mg/L to 3.01 mg/L (exceeding the World Health Organization's (WHO) limit of 1.0 mg/L).

The reduction in cobalt, nickel, copper, and lead suggests that anaerobic digestion may promote metal immobilization, adsorption to biomass, or precipitation into less bioavailable forms. Similar reductions in heavy metal bioavailability after anaerobic digestion have been reported by Al Seadi *et al*. (2020) and Li *et al*. (2025), who attributed this effect to sulfide formation, microbial biosorption, and complexation with organic matter in digesters. The slight increase in zinc concentration may be due to mineralization of organic-bound zinc during digestion, releasing it into the liquid phase. Comparable trends were observed by Montusiewicz *et al*. (2020), who reported that zinc often behaves differently from other metals due to its essential biological role and weaker binding to organic complexes.

Zinc accumulation in insect frass is a recognized issue, as insects bioaccumulate this element, particularly in exoskeletal structures (van der Fels Klerx *et al*., 2020). The observed increase may result from concentrating effects due to water loss during digestion or from the mobilization of previously bound zinc fractions. Given Zinc's phytotoxicity at elevated levels and its potential to disrupt soil microbial communities, this finding underscores the necessity of monitoring and possibly treating frass digestate before land application. Strategies such as composting after digestion, phytoremediation, or chemical stabilization could be explored to mitigate zinc bioavailability. Therefore, the integration of insect farming with anaerobic digestion represents a promising pathway within the circular bioeconomy, converting organic waste into valuable products: insects for feed, frass for energy, and digestate for soil improvement. This study supports the technical feasibility of such integration, demonstrating that frass can serve as an effective AD substrate, especially when co-digested with poultry manure to enhance biogas output.

CONCLUSION

Frass, a waste product of insects, is apt as feedstock for anaerobic digestion, with the generation of biogas optimized via co-digestion with poultry manure. The level of the pathogenic bacteria and heavy metal concentration was reduced, which improved the safety of the by-product of anaerobic digestion and digestate. But the presence of Zinc beyond the world health organization (WHO) limits calls for a need to further treat digestate before field application. The anaerobic digestion of insect frass with appropriate digestate management can contribute to sustainable waste valorization, production of renewable energy, and nutrient loops closure within the bioeconomy systems.

This finding emphasizes the need to monitor and perhaps treat frass digestate prior to land application, given zinc's phytotoxicity at increased levels and tendency to disturb soil microbial populations. To reduce zinc bioavailability, techniques such as chemical stabilization, phytoremediation, and composting after digestion could be investigated.

This study supports the integration of insect farming and anaerobic digestion as a sustainable strategy for waste valorization, energy recovery, and nutrient recycling in alignment with the Sustainable Development Goals (SDGs) 3, 7, and 13, promoting positive health, clean energy, and climate action.

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