Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review The potential of insect frass for sustainable biogas and biomethane production: A review

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HIGHLIGHTS

G R A P H I C A L A B S T R A C T

- Literature review on biogas and biomethane potential from frass insectbased biomass.
- Frass is a new biomass with interesting bioenergy potential.
- Variability of biogas production depending on substrate and digestion conditions.
- Frass-based biomass has comparable biogas production with traditional manure.
- Frass use for bioenergy production aligns with the principles of circular economy.

ARTICLE INFO

Keywords: Bioenergy production Anaerobic digestion Innovative biomass Insect species Circular economy



ABSTRACT

Insect-based protein production has gained traction in recent years. This has led to the increasing production of frass, the residual substrate from insect farming. As a relatively new substrate with characteristics that are not widely known, its energetic potential still needs to be investigated. In this context, this literature review aims to evaluate the potential of frass as a feedstock for bioenergy production through anaerobic digestion.

From the literature search, 11 studies were selected, and showed a wide range of biogas (44 m³/ton VS to 668 m³/ton VS) and methane (26 m³/ton VS to 502 m³/ton VS) production potentials from insect frass, mostly comparable with traditional biomasses of liquid and solid slurry. Results are influenced by factors such as substrate type, digestion conditions and presence of co-digestion substrates. The need of further investigation on the economic viability has been highlighted, with a focus on the possibility of upgrading biogas to vehicle-grade biomethane.

1. Introduction

Over the past few decades, there has been a significant shift in human dietary habits, due to rapidly increasing incomes, population growth,

and urbanization in the developing world. This led to a rise in the demand for animal-based proteins, which is projected to keep rising by 2050 (Boland et al., 2013). This growing demand is in conflict with the need to reduce the environmental impacts generated by intensive

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https://doi.org/10.1016/j.biortech.2024.131384

Received 20 May 2024; Received in revised form 27 August 2024; Accepted 27 August 2024 Available online 30 August 2024

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farming, which is one of the main drivers of greenhouse gas (GHG) emissions, loss of biodiversity, diseases and consumption of land, food and water (Machovina et al., 2015). For this reason, it is necessary to find new ways of producing large quantities of high-quality proteins while minimizing environmental impact. This necessity has led to the notion of introducing insect farming for the production of protein. In fact, insect farming has been proven to be more environmentally sustainable than traditional livestock farming, particularly when insects are reared on waste (Smetana et al., 2016). Furthermore, the consumption of insects as a source of animal protein is advantageous from several points of view: they require less water, emit relatively less GHG and ammonia, and have lower risks of transmitting zoonoses (Van Huis et al., 2013). Insect-based proteins can be utilized both to reduce the impact of the feed for traditional livestock and, as a stronger solution, as substitutes for animal proteins in human nutrition (Smetana et al., 2016). Nonetheless, insect consumption is not a novel concept in many parts of the world since it is estimated that at least 2 billion people globally eat insects on a regular basis (Van Huis et al., 2013).

In 2017, the European Commission approved the use of seven insect species for the production of animal feed: black soldier fly (*Hermetia illucens*), common housefly (Musca domestica), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes chiaratus*), and field cricket (*Gryllus assimilis*), which became eight in 2021, with the addition of silkworm (*Bombyx mori*) (European Commission 2017; European Commission, 2021). Additionally, the novel foods legislation, Regulation (EU) 2015/2283, which took effect on January 1, 2018, regulated the consumption of insects as human food. To date, there are 4 species of edible insects authorized for human consumption in the EU: *Tenebrio molitor, Locusta migratoria, Acheta domesticus* and *Alphitobius diaperinus* larvae (International Platform of Insects for Food Feed (IPIFF), 2023).

At the same time, rapid population growth and urbanization have resulted in the increased generation of the organic fraction of municipal solid waste (OFMSW), which is anticipated to rise further. By 2050, the worldwide waste generation is predicted to exceed 3.4 billion tons, with biodegradable materials accounting for up to 44 % of total collected waste globally (Lopes et al., 2022). Because the disposal of large amounts of organic waste causes environmental problems globally, like the release of GHG into the atmosphere and soil/water contamination with hazardous substances and nutrients from leachates, many strategies for its management are being investigated, including novel approaches (Hénault-Ethier et al., 2024). In this context, the employment of insects is also beneficial, as demonstrated by the use of black soldier fly larvae (BSFL) for composting (Basri et al., 2022). BSFL can reduce the volume of organic inputs by 54 %, which means that this composting process appears to be a promising alternative to standard approaches like landfill or incineration (Basri et al., 2022; Hénault-Ethier et al., 2024). Additionally, the larval biomass rich in proteins and lipids generated via organic waste management can be used as animal feed (Lopes et al., 2022).

Both insect rearing for protein production and BSFL composting generate a waste known as frass (Lopes et al., 2022). This term comes from the German word *fra* β , which refers to feeding animals in general. The term was later used in English, with the first known use in relation to insect excrement and leftovers dating back to 1854; however, frass has begun to gain attention in the agricultural sector only in the past few years (Hénault-Ethier et al., 2024). Frass can have different appearances, depending on the type of insect and on the type of feed. For example, frass resulting from BSFL composting looks like an immature compost-like material. In general, it is typically composed of feees, substrate residues and exoskeleton remain. From a biochemical point of view, it contains plant-derived molecules such as cellulose, lignin and hemicellulose, and molecules derived from insects such as chitin. Microbial populations may also be present within it (Lopes et al., 2022). Basri et al., 2022). From a legislative point of view, the European

Commission (2021) defines frass as "a mixture of excrements derived from farmed insects, the feeding substrate, parts of farmed insects, dead eggs and with a content of dead farmed insects of not more than 5 % in volume and not less than 3 % in weight".

It is estimated that in Europe the current production of frass already exceeds several thousand tons per year, and that it could reach 1.5 million tons yearly by 2025 (Hénault-Ethier et al., 2024). Therefore, insect-based techniques yield not only a product suitable for food and feed, but also a rising quantity of organic waste, which can potentially be used for a few different purposes. In this way, such methods can fit with the concepts of a circular economy, in which waste from one process becomes a resource in another (Lopes et al., 2022). Frass is currently primarily used as a fertilizer in agriculture, due to the presence of organic compounds and micronutrients, which reduce abiotic stressors in plants while also improving soil structure and microbiota (Hénault-Ethier et al., 2024). The EU regulates its usage as a fertilizer via Regulation (EU) No 142/2011 "on animal by-products and derived products not intended for human consumption", which requires frass to be treated at 70 °C for one hour, in order to be authorized on the markets. In addition to this type of sanitization treatment, the Commission also foresees that frass may also be processed by composting or transformation into biogas; still, the safety of the final product (compost and digestate) should always be compliant with microbiological limits, in order to be utilized as a fertilizer (International Platform for Insects as Food and Feed, 2021).

As suggested by forementioned Regulation, this type of biomass can also be considered as a source for bioenergy production. In general, bioenergy production involves the transformation of organic matter into usable energy, such as heat, electricity, or biofuels. The extraction of energy from biomass can be done via anaerobic digestion (AD). The key organic components of biomass are lignin, cellulose, and hemicellulose, of which frass is largely constituted. AD involves a series of degradation processes driven by microorganisms in the absence of oxygen, resulting in digestate and biogas (Sakthivel et al., 2024). Digestate serves as a biofertilizer in agriculture (Koszel & Lorencowicz, 2015) enhancing process circularity. Biogas, primarily methane (CH₄) (50 %–75 %) and carbon dioxide (CO₂) (25–50 %), with traces of hydrogen sulfide (H₂S) and hydrogen (H₂) (Plugge, 2017; Khan et al., 2017) can be utilized for low-quality energy needs or upgraded to biomethane. The methane content determines biogas' calorific value, while CO2 acts as a contaminant. Upgrading to biomethane enhances energy density, enabling applications like grid injection or vehicle fuel (Ahmed et al., 2021). Additionally, AD generates residual heat that can be utilized for various purposes, such as breeding other insects and, at least partially, for sanitizing frass (Wedwitschka et al., 2023). This results in further synergies from employing frass digestion, as opposed to sole frass composting. In this context, the aim of this review is to evaluate the potential of frass to produce bioenergy via AD, a frontier that has been relatively unexplored for this type of waste.

Abubakar et al. (2023) reviewed recent advancements in insect employment for biogas production, reporting promising potentials and the need for further research on the topic. This work aims to contribute to the expansion of knowledge in this field by comprehensively reporting, to our knowledge, all currently available biogas and biomethane production potentials and applied methodologies. The achieved results could serve as a foundation for further studies and investigations in this field and could facilitate the development of more efficient AD processes. The main novelty is related to the exploration of frass as a viable feedstock for bioenergy production, potentially expanding the range of available biomass sources and enhancing the efficiency and sustainability of AD processes.

2. Materials and methods

The search was carried out using Scopus® database. Considering that some studies may not have used the term "frass" to describe insect

farming waste, the following keywords were combined: 1) "frass" AND "biogas"; 2) "insects", "breeding", "waste" AND "biogas"; 3) "larval" AND "waste" AND "biogas".

A more general search on frass shows that many studies (>1600 on Scopus®; >2900 on Google Scholar®) can be found that mention this particular waste mass, but by adding the chosen keywords, the number is drastically reduced to ~25 studies. Following an evaluation of the titles and abstracts of the obtained results, the selected studies were chosen based on their relevance to the aim of this review. Excluded works were studies, or reviews, on the use of the BSFL composting technique for different purposes, such as the treatment of food waste or digestate and consequent production of proteins, or biodiesel. All relevant studies were published recently, between 2018 and 2024.

3. Results and discussion

Table 1 reports the findings from the analysis of the different reviewed studies. The following aspects were evaluated:

• Type of insect, to observe which species were used for this purpose and the potential differences in terms of biogas quantity and availability of frass.

- Type of frass and/or eventual treatment, to understand if the composition and potential pretreatments of frass affect its biode-gradability and, therefore, the methane yield.
- Insect growth feed, since the feed used for insect rearing influences the nutritional content of the frass.
- Use of substrate in co-digestion, to observe whether the combination of frass with other substrates can enhance the digestion process.
- Inoculum and inoculum:substrate ratio (I/S), to observe the potential influence of the I/S on biogas production, given that the type of inoculum and I/S are responsible for initiating and sustaining the AD process and can ensure optimal microbial activity.

These parameters were all also evaluated as a baseline for future studies.

Frass was most commonly obtained from the Black Soldier Fly (BSF) (*Hermetia illucens*) (used in 8 studies). Next follows the Silkworm (*Bombyx mori*), which is mentioned twice. The silkworm is also allowed to be used in animal feed, although the breeding of this species has a long history; as the primary producer of silk, it is the domestic insect that has been most commercially exploited (Łochyńska & Frankowski, 2018). Finally, other insects under study are mealworms (*Tenebrio molitor*), crickets (*Gryllus* spp.), and caimani (*Zophobas morio*). The first two are also intended to be utilized in animal nutrition while *T. molitor* was also allowed for human nutrition. The physical attributes,

Table 1

Results of the literature search, reporting the Reference, Insect Species, Frass composition and/or treatment, Insect Feed, Presence of Co-Digestion, Inoculum (+I/S) and BMP $(m^3/ton VS)^*$.

| Reference | Insect | Frass composition/ treatment | Insect feed | Co-digestion | Inoculum (+I/S) | BMP (m ³ /ton VS)* |
|---|---|---|---|---|---|--|
| Łochyńska & Frankowski (2018) | Silkworm (Bombyx mori) | Excrements only | Fresh leaves of white mulberry | No | Digestate | 211.6 |
| 2. Elissen et al., 2019 | Black Soldier Fly (Hermetia illucens) | Hygenized Frass at 60° 24 h $$ | Food industry waste | No | a. Digestate 9:1 and2.3:1b. No inoculum | a. 26–60 b. 166 |
| 3. Wedwitschka et al., 2023 | Black Soldier Fly (Hermetia illucens) | Frass from 6 different feed types | a. Corn silage b. Brewery waste c. Bioet waste. d. Aquatic Plants e. Bran f. 6. Frass from a pilot plant | No | Digestate 3:1 (on VS) | a. 262 b. 259 c. 201 d. 287 e. 250 f. 277 |
| 4. Hol et al., 2022 | Black Soldier Fly (Hermetia illucens) | Frass: a. Hygenized at 60° 24 h b. Non-Hygenized | Agricultural by-products | No | Digestate | a. 226 b. 264 |
| | | Frass: a. Hygenized at 60° 24 h b. Non-Hygenized | Agricultural by-products | Yes, with slurry | Digestate | a. 32 b. 85 |
| 5. Win et al., 2018 | Black Soldier Fly (Hermetia illucens) | Frass ground into <2 mm particles | Chicken feed and food scraps | No | Digestate (2:1 on VS) | 502 |
| 6. Bulak et al., 2020 | a. Mealworm (<i>Tenebrio molitor</i>) b. Cricket (<i>Gryllus</i> <i>spp</i>) c. BSF (<i>Hermetia</i> <i>illucens</i>) | Breeding waste (feces, feed waste and adults remains) | a. Oatmeal and leftover vegetables and fruit Grains, soybeans and alfalfa. Carrot and beetroot scrap | No | Digestate (2:1 on TS) | a. 252 b. 260 c. 208 |
| 7. Bulak et al., 2023 | Caimani (Zophobas morio) | a. Mix of feces and feed waste b. Feces c. Feed waste | Wheat bran and apple pieces | No | Digestate | a. 341.0 b. 349.8 c. 329.8 |
| 8. Lalander et al., 2018 | Black Soldier Fly (Hermetia illucens) | Frass | a. Food wasteb. Fecal material | No | Digestate (3:1 on VS) | a. 322 b. 178.9 |
| 9. Papa et al., 2022 | Black Soldier Fly (Hermetia illucens) | Frass dried and ground to 2 mm | Organic fraction of MSW | No | Digestate (3 g waste to 297 mL of inoculum) | 68–101 |
| 10. Narendra et al., 2022 | Silkworm (Bombyx mori) | Frass | _ | With Cashew fruit nut at different % | Dairy manure + water | 85.9–135.5** |
| 11. Dong et al., 2024 | Black Soldier Fly (Hermetia illucens) | Frass | Swine manure | No Corn straw (24 % tw***) | No No | 67–109 131,95–156,43 |

Note: *BMP=Biochemical Methane Potential VS=Volatile Solids; **calculation based on given data; *** tw = total weight.

consistency, and physiochemical characteristics of insect frass vary greatly across species and production methods. Regarding the insect species reported in this review, crickets, *Z. morio*, and *B. mori* produce frass in pelletized form, while BSF and *T. molitor* produce it in powder form (Beesigamukama et al., 2022). Given the significant variability in composition due to different rearing conditions, it is necessary to investigate the chemical and physical characteristics of each type of frass.

In most studies (eight out of eleven), frass is recovered following insect rearing at laboratory scale, carried out by the authors themselves. In only two of the eleven studies, the frass is recovered by a private pest-control company. Lastly, only one study—that of Dong et al. (2024)—recovered frass from a medium-sized swine farm where BSFL composting was used to treat the swine manure.

Table 1 shows that the composition of frass and treatment methods vary among different studies, with some of the studies lacking information on both aspects. Some authors consider excrement only, while the majority use a mix of feces, feed waste and eventual adult remains. Bulak et al. (2023) perform the analysis both on the mix of feces and feed waste and on the two substrates separately, in order to assess the advisability of waste separation before the process of biogas production. The differences between the average values for biogas potential of the three substrates were low but statistically significant. Furthermore, most authors use frass as it is, but some perform either sanitization, to comply with state law, and drying or grinding to smaller particles, to produce a more homogeneous substrate.

In the considered studies, insects were reared on different types of substrates. Most studies employ waste as feed, with many evaluating BSF frass of larvae raised on food and agricultural waste. This contributes to coupling the concept of BSF composting, foreseen for the treatment of organic waste (Basri et al., 2022), with biogas production. It must be underlined that the use of different insect feed substrates can influence the composition of the frass (i.e. total solids (TS) and volatile solids (VS), nitrogen, protein, fat, and fiber composition), and therefore the results of the analysis in terms of biochemical methane potential (BMP). Among the main features, TS and VS are the most important characteristics to observe. TS represent the solid part of the substrate, while VS represent the organic substance and therefore the share that is degradable by microorganisms, that produce biogas.

Tests for the evaluation of BMP were carried out with similar AD methods, among the eleven reviewed works. All were carried out at laboratory scale in mesophilic conditions (35 °C–37 °C) under stirring, but at different volumes (from 0.3 L to 400 L) and with different retention times (from 16 to 61 days). Digestate is used as inoculum in every work, excluding those of Dong et al. (2024) and Narendra et al. (2022), where no inoculum and manure + water were applied, respectively (Table 1). The inoculum for the start-up of the AD should provide key microorganisms for hydrolysis, acidogenesis, and methanogenesis. Digestate contains a diverse range of microorganisms and N and P elements, and its pH value is typically in the optimal range, making it a suitable inoculum for AD. Different I/S are used, usually 2:1 or 3:1 (TS/ TS). I/S of 2:1, 3:1 and 4:1 have been shown to favor methanogenesis (Li et al., 2022; Sri Bala Kameswari et al., 2012). In this regard, in the work of Elissen et al. (2019), the test with a I/S equal to 9:1 shows a lower biogas production if compared to the test with a I/S equal to 2.3:1.

In most studies, BMP results are normalized to standard conditions of temperature and pressure (273.15 K, 1013.25 kPa); however, this is not clearly specified in the works of Bulak et al. (2020), Bulak et al. (2023), Narendra et al. (2022) and Dong et al. (2024). The BMP results vary from a methane production of 26 m^3 /ton VS (Elissen et al. (2019) up to that of 502 m^3 /ton VS (Win et al., 2018) both from BSF frass. The frass that produced the two extreme values both come from rearing systems using food waste as feed, but the one with the highest value also uses chicken feed; the lowest production is given by a digestion with a higher than usual I/S (9:1) while the highest, with a I/S of 2.3:1. From the study by Hol et al. (2022) it can be learned that the addition of slurry to the

digestion substrate causes a reduction of 67.8 % in BMP (from 264 m³/ ton VS to 85 m³/ton VS for non-sanitized frass), against a reduction in TS of 17.3 % (0.81 g TS to 0.67 g TS). According to the study by Dong et al. (2024), the addition of corn straws (24 % of total weight) results in an increase in average production, from 89.25 m³/ton VS to 140 m³/ton VS, which could be explained by the variation in TS from 14.35 % to 19.49 % and by the different duration (30 vs 61 days). It should also be noted that although this work is the only one that conducted AD tests without an inoculum, it obtained results similar to those of other studies; moreover, in this study, additives, specifically iron oxide (Fe₃O₄) nanoparticles, were added to investigate their impact on biogas production, particularly during the inoculum-free start-up phase of the AD process. The authors reported significantly boosted average biogas yields, with a notable increase in methane production compared to control groups.

To ensure optimal degradation of organic matter by microorganisms and subsequent biogas production, substrates used in AD must adhere to specific physico-chemical parameters. Therefore, the critical parameters that need to be assessed for frass to better understand its performance in AD trials include total solids (TS), volatile solids (VS), pH, carbon-tonitrogen ratio (C/N), and biochemical composition, which are the same parameters for evaluating the AD performance of traditional biomasses (Abbassi-Guendouz et al., 2012; Lamolinara et al., 2022). To determine these parameters, the authors employ the analytical procedures as reported in Table 2. As can be found in the table, apart from 2 studies that did not specify the characterization procedure, all other studies used the gravimetric method for assessing TS and VS, while for the other listed parameters, differences in the selected method can be observed among the studies. To understand the relevance of different analytical techniques in this type of study, we can observe that TS and VS were always performed, while pH and C/N ratio were not performed often. In particular, only 4 studies mention the method for the measurement of pH and 6 studies the method for C/N ratio. The biochemical composition was declared in 6 of the studies, measuring cellulose, hemicellulose, fibre, lipid and protein composition.

Based on TS content, AD can be divided into: dry AD (≥ 15 % TS) and wet AD (<15 %) (Li et al., 2023). Table 3 shows the variability of the TS and VS contents between the different insect frasses. In most of the reviewed studies, the AD process occurred under wet conditions, given the use of digestate (with low TS) as inoculum and the selected I/S ratios. Studies using different biomasses report higher biogas productions under wet AD conditions, with TS percentages between 7 and 10 % (Budiyono et al., 2014; Deepanraj et al., 2015; Ahmadi-Pirlou and Mesri Gundoshmian, 2021). In accordance, only the studies by Narendra et al. (2022) and Dong et al. (2024) used dry operational conditions, and their BMP results showed lower than average values but not too dissimilar to other studies using wet conditions. Apart from this evidence, even though TS influences methane production, among these studies, there does not appear to be a clear connection between the variability of TS of frass and the production of biogas.

Another factor that can influence the performance of AD is the composition of the microbial community. There are not many studies regarding frass, and in general, there is very little information about the effect of different substrate combinations on the methanogen community, due to the fact that each anaerobic digester has its own unique microbial community (Kushkevych et al., 2018). In the inoculum-free AD process of BSF frass conducted by Dong et al. (2024), it was found that species such as Methanocorpusculum, Methanosarcina, and Methanomassiliicoccus play crucial roles in the AD reactor, contributing to the optimal microbial diversity of methanogenic archaea. Moreover, Methanoculleus and Methanosarcina showed a positive correlation with biogas production. Recent studies have highlighted that Methanosarcina spp. is relatively robust and capable of withstanding the inhibitory effects of humic acid and ammonia on methane production (Mutungwazi et al., 2021). Methanogenic archaea are highly sensitive to environmental changes such as temperature, oxygen, and pH levels, and they have slow

| Referen | ce | TS and VS | pH | C/N | Biochemical Composition |
|-------------------------|------------------------------|--|--|--|---|
| 1. Łoch Fran (201 | iyńska & kowski 8) | Gravimetrically by following standard procedures (PN-92/ P-50092) | Determined according to standard PB.40 ed. 7: 2010 | N and C contents were determined according to PN-EN 15104:2011 and PN-EN 15289:2011 standards | Cellulose according to Seifert using a mixture of acetylacetone and dioxane, Lignine according to Tappi using concentrated sulfuric acid, Holocellulose using sodium chlorite. |
| 2. Eliss 2019 | en et al., | Not specified | Not specified | Not specified | Not performed |
| 3. Wed et al. | witschka ., 2023 | Gravimetrically by following standard procedures (EN 12880:20004; EN 12879:20004) | Not performed | Not performed | Weender feed analysis |
| 4. Holo 5. Win | et al., 2022 et al., 2018 | Not specified Gravimetrically by following standard procedures (APHA 2540B 2540E) | Not specified Using a Mettler Toledo meter at room temperature (22 ± 1 °C) calibrated with buffers at pH¼ 4.0, 7.0, and 10.0. | Not performed Not performed | Not performed - Weender feed analysis - Hemicellulose, cellulose and lignin - based on the ANKOM Technology Method 5 for acid detergent fiber in feeds, Method 9 for acid detergent lignin and method 6 for neutral detergent fiber |
| 6. Bula | k et al., 2020 | Gravimetrically by following standard procedures (EN 12880:20004; EN 12879:20004) | Potentiometrically by HQ 400 multi- purpose machine in the supernatant obtained by mixing the waste with distilled water | Total C and total N were determined by elemental analysis using a Thermo Scientific Flash 2000 Organic Elemental Analyzer | Protein calculated on the basis of total N using a 6.25 multiplier. Crude lipids analyzed with the use of Soxtec Avanti Raw fibers determined by sequen tial acid-base extraction with hot 1.25 % H₂SO₄ and 1.25 % NaOH on Fibertec 2010 Carbohydrate calculated by subtracting the content of all other components from TS. |
| 7. Bula | k et al., 2023 | Gravimetrically by following standard procedures (EN 12880:20004; EN 12879:20004) | Potentiometrically by HQ 400 multi- purpose machine in the supernatant obtained by mixing the waste with distilled water | Total C and total N were determined by elemental analysis using a Thermo Scientific Flash 2000 Organic Elemental Analyzer | |
| 8. Lalaı 2018 | nder et al., 3 | Gravimetrically by following standard procedures (EN 12880:20004; EN 12879:20004) | Not performed | Total N was determined according to the method described in Lalander et al. (2015). Total C was estimated as described by Haug, 1980 | Not performed |
| 9. Papa | ı et al., 2022 | Gravimetrically by following standard procedures (EN 12880:20004; EN 12879:20004) | Not specified | Total N was measured by elemental analysis using a Flash EA1112, Thermo Scientific Total C was Detected from by Van Bemmelen factor of 1.724. | Lipids Detected by Bligh and Dyer's method. Protein, detected from elemental analysis Hemicellulose Cellulose Lignin Total Fiber Lignocellulose detected by Van Soest method |
| 10. Nar 202 | rendra et al., 22 | Gravimetrically by following standard procedures (APHA 2540B 2540E) | Not specified | Not specified | Not performed |
| 11. Dor 202 | ng et al., 24 | Gravimetrically by following standard procedures (APHA 2540B 2540E) | Not performed | Total C and N were measured with Total carbon analyzer (multi N/C 2100). | Not performed |

growth rates, with generation times ranging from 5 to 16 days compared to the average 1 to 2 days for other bacteria in AD processes. Therefore, it is important to identify and promote beneficial microbial species like Methanosarcina, which are more tolerant to specific inhibitors of the acetoclastic pathway, such as ammonia, in order to maximize biomethane production from frass (Mutungwazi et al., 2021). It will therefore be interesting to deepen the knowledge of the relationships between frass of different species, their microbiological composition and the progress of AD reactions and related microbial communities, also in relation to the sanitization of frass.

Table 4 shows the details of the production of biomethane, biogas and its quality. In regard to the biogas values, these vary in the range from 44 m³/ton VS to 668 m³/ton VS. Knowing the BMP, it is therefore possible to obtain the % of CH₄ present within the biogas. The quality of the biogas, and therefore its calorific value, are dictated by its methane content, which in turn depends on the starting substrate characteristics (i.e. TS, VS, nitrogen, protein, fat, and fiber composition) and on the process pH (Abdel-Hadi, 2008). Initial pH can also influence methane

production; Kheiredine et al. (2014) demonstrated that optimal biomethane production occurs with substrates in the neutral pH range, specifically in the range of 6.7–7.5. The insect frass from the studies for which it was evaluated, have a pH value that varies from a minimum of 6.0 to a maximum of 8.2 (Table 3), therefore mostly close to the optimal value. As can be seen in the work by Bulak et al. (2020) (n°6), the percentage of methane is higher for the cricket frass, which had a starting pH of 6.18 \pm 0.04, than for that of BSF which had a starting pH further from the neutral one, of 8.19 \pm 0.06, therefore starting pH may have contributed to this difference. For the forementioned reason, in addition to the quantity of produced biogas (m³/ton VS), it is also necessary to evaluate its percentage of methane. Table 4 shows percentage values of methane content from the eleven different works, which range from 50.3 % to 75 %, with an average of 57 %.

As can be seen from Table 5, the biogas and biomethane production values of frass are, on average, comparable with those of other types of manure from the studies by Kafle and Chen (2016) and Amon et al. (2007). In particular, frass demonstrates production potential superior

Physiochemical parameters of insect frass (total solids (TS) (%), volatile solids (VS) (%TS) and pH). The number of article (N°) refers to the same as in Table 1.

| Reference | Insect | Frass composition /treatiment | Feed | TS (%) | VS (%TS) | pH |
|---|--|---|----------------------------------|-----------|-----------|---------|
| Łochyńska & Frankowski (2018) | Silkworm (Bombyx mori) | Excrements only | Fresh leaves of white mulberry | 31.9 | 80.0 | 7.4–8.2 |
| 2. Elissen et al., 2019 | Black Soldier Fly (Hermetia illucens) | Hygenized Frass at $60^\circ~24~h$ | Food industry waste | 63 | 90 | 7.1 |
| 3. Wedwitschka et al., 2023 | Black Soldier Fly | Frass from 6 different feed types | a. Corn silage | a. 7.3 | a. 81.1 | - |
| | (Hermetia illucens) | | b. Brewery waste | b. 2.6 | b. 51.2 | |
| | | | c. Bioet waste. | c. 9.6 | c. 94.1 | |
| | | | d. Aquatic Plants | d. 12.9 | d. 94.5 | |
| | | | e. Bran | e. 12.4 | e. 85.7 | |
| | | | 6. Frass from a pilot plant | f. 84.1 | f. 91.0 | |
| 4. Hol et al., 2022 | Black Soldier Fly | Frass: | Agricultural by-products | a. 76.6 | a. 89.6 | - |
| | (Hermetia illucens) | Hygenized at 60° 24 h Non-Hygenized | | b. 82.8 | b. 89.8 | |
| 5. Win et al., 2018 | Black Soldier Fly (Hermetia illucens) | Frass ground into $<2 \text{ mm}$ particles | Chicken feed and food scraps | 22.6 | 93.3 | 8.1 |
| 6. Bulak et al., 2020 | 1. Mealworm (Tenebrio | Breeding waste (feces, feed waste and | a. Oatmealand leftover | a. 2.5 | a. 69.7 | a. 6.0 |
| | molitor) | adults remains) | vegetables and fruit | b. 2.2 | b. 73.2 | b. 6.2 |
| | 2. Cricket (Gryllus spp) | | b. Grains, soybeans and alfalfa. | c. 3.1 | c. 70.8 | c. 8.2 |
| | 3. BSF (Hermetia illucens) | | c. Carrot and beetroot scrap | | | |
| 7. Bulak et al., 2023 | Caimani (Zophobas morio) | a. Mix of feces and feed waste | Wheat bran and apple pieces | a. 87.0 | a. 93.9 | a. 6.8 |
| | | b. Feces | | b. 86.8 | b. 93.4 | b. 6.7 |
| | | c. Feed waste | | c. 87.9 | c. 95.2 | c. 6.6 |
| 8. Lalander et al., 2018 | Black Soldier Fly | Frass | a. Food waste | a. 51.7 | a. 85.4 | - |
| | (Hermetia illucens) | | b. Faecal material | b. 67.1 | b. 79.8 | |
| 9. Papa et al., 2022 | Black Soldier Fly | Frass dried and ground to 2 mm | Organic fraction of MSW | 45.7-50.3 | 73.8-80.2 | 7.7-8.2 |
| | (Hermetia illucens) | | | | | |
| 10. Narendra et al., 2022 | Silkworm (Bombyx mori) | Frass | _ | 31.9 | 90 | 6.2–7.9 |
| 11. Dong et al., 2024 | Black Soldier Fly (Hermetia illucens) | Frass | Swine manure | 71.78 | 93.57 | _ |

to that of dairy manure, horse manure and goat manure and in line with that of chicken manure and swine manure. Insect frass is also comparable with the results from Pavi et al. (2017), where two types of organic waste are considered. In contrast, food industry waste in the study by Kafle et al. (2013), demonstrates superior biogas production potential compared to different types of manure, including frass. This can be useful from a co-digestion perspective. In this regard, Wedwitschka et al. (2023) observed, during AD tests, an increase in the ammonium concentration, likely due to the degradation of protein compounds within frass. This can result in the slowing down of the biogas production process, causing a rise in fermentation acids, a drop in pH, ultimately reducing methane formation (Liu et al., 2008). They therefore suggested to perform the AD of insect frass with co-substrates with a lower nitrogen content, as a solution. Bulak et al. (2020), have a similar suggestion, proposing to mix the frass waste with substrates rich in carbohydrates in order to improve the properties of the final feedstock. Lalander et al. (2018) suggest that frass with high TS concentration would necessitate the dilution with co-substrates having a lower TS concentration to effectively operate in continuously stirred tank reactors. Hol et al. (2022) found that non-hygenized frass produced more biogas than hygenized frass (264 m³/ton VS vs 226 m³/ton VS). As a result, they propose to start a dialog with legislators on the transportation of nonhygenized insect frass to an off-site digester, in order to avoid compromising production potential unnecessarily. Another suggestion comes from Bulak et al. (2023), who demonstrated that the eventual separation of feces and food waste of the frass does not bring benefits to the process, given that the BMP of their analyses varied only 3 % between the two components.

3.1. Economic feasibility

The literature regarding the economic feasibility of producing biogas from insect excrement is scarce. Lalander et al. (2018) carried out an economic evaluation of the value of products generated per ton of waste treated with four different strategies (thermophilic composting, BSF composting, AD, and BSF composting followed by AD). For strategies containing AD, two different scenarios were taken into consideration: the use of biogas for electricity production or the upgrading of biogas to vehicle fuel. The highest value products were obtained using BSF treatment followed by AD, especially when the biogas was upgraded to vehicle grade. Although BSF + AD yielded the highest value products, this does not imply it is the most economically viable strategy, as the study did not consider treatment costs. Estimating the cost of BSF treatment is challenging due to its novelty and lack of available information. Similarly, the costs of upgrading biogas to vehicle-grade gas vary depending on scale, purity requirements, and local conditions. Given the limited availability of studies on this matter, further investigation with economic assessments considering production costs is necessary.

3.2. Future perspectives

Large-scale insect farming as an alternative to traditional protein production for food and feed has significantly expanded in recent years and is expected to continue to grow in the foreseeable future (Steinrücken et al., 2024). As a result, the availability of frass as a waste resource is expected to increase, together with the primary material (Hénault-Ethier et al., 2024). To gauge the daily accumulation of waste in this industry, research has shown that 1700 larvae of yellow mealworms (Tenebrio molitor) can consume 220 g of food, resulting in an insect biomass production of 4 g and 180 g of frass and residues (Poveda, 2021). In 2020, it was estimated that globally, 1 trillion to 1.2 trillion insects of different species are cultivated annually for food and animal feed purposes (Rowe, 2020). Moreover, the industry, particularly for aquaculture feed production and the pet food sector, is rapidly expanding (IPIFF, 2021a,b). In addition, the growing amount of generated insect frass, currently lacks a widely established area of application, apart from being used at small scale as a sustainable organic fertilizer (Steinrücken et al., 2024). Consequently, the use of insect frass as feedstock for the production of biogas and biomethane offers promising prospects towards environmental sustainability and opens up new possibilities in the bioenergy sector (Adetunji et al., 2023). In particular, synergies can be created from the combination of biogas plant and insect farming or from the integration of insect farming in existing biogas

Biogas potential, biomethane potential and CH₄ content (%) from reviewed studies.

| Reference | Frass type* | Biogas potential (m ³ /ton VS) | Biomethane potential (m ³ /ton VS) | CH ₄ content (%) |
|----------------------------------|---|--|---|-----------------------------|
| 1. Łochyńska & Frankowski (2018) | Silkworm (Bombyx mori) | 419.82 | 211.59 | 50.4 |
| 2. Elissen et al., 2019 | Black Soldier Fly | 44–104 | 26–60 | 59 |
| | (Hermetia illucens) – With digestate inoculum | | | |
| | Black Soldier Fly | 285 | 166 | 58 |
| | (Hermetia illucens) – No inoculum | | | |
| 3. Wedwitschka et al., 2023 | Black Soldier Fly | - | 262 | _ |
| | (Hermetia illucens) – Corn silage | | | |
| | Black Soldier Fly | | 259 | |
| | (Hermetia illucens) – Brewery waste | | | |
| | Black Soldier Fly | | 201 | |
| | (Hermetia illucens) – Bioet waste. | | | |
| | Black Soldier Fly | | 287 | |
| | (Hermetia illucens) – Aquatic Plants | | | |
| | Black Soldier Fly | | 250 | |
| | (Hermetia illucens) – Bran | | | |
| | Black Soldier Fly | | 277 | |
| | (Hermetia illucens) – Frass from a pilot plant | | | |
| 4. Hol et al., 2022 | Black Soldier Fly | 409 | 226 | 55 |
| | (Hermetia illucens) – Hygenized Frass | | | |
| | Black Soldier Fly | 464 | 264 | 57 |
| | (Hermetia illucens)- Frass | | | |
| | Black Soldier Fly | 56–146 | 32–85 | 56–58 |
| | (Hermetia illucens) – With slurry | | | |
| 5. Win et al., 2018 | Black Soldier Fly | - | 502 | - |
| | (Hermetia illucens) | | | |
| 6. Bulak et al., 2020 | Mealworm (Tenebrio molitor) | 451.1 | 252.6 | 56.3 |
| | Cricket (Gryllus spp) | 447.4 | 258.8 | 57.9 |
| | Black Soldier Fly | 412.5 | 207.9 | 50.3 |
| | (Hermetia illucens) | | | |
| 7. Bulak et al., 2023 | Caimani (Zophobas morio) – Mix | 654.4 | 341 | 52 |
| | Caimani (Zophobas morio) – Feces | 668.4 | 349.8 | 52 |
| | Caimani (Zophobas morio) – Food Waste | 641.2 | 329.8 | 51 |
| 8. Lalander et al., 2018 | Black Soldier Fly | - | 322.6 | 61.4 |
| | (Hermetia illucens) – Food Waste | | | |
| | Black Soldier Fly | | 178.9 | 55.2 |
| | (Hermetia illucens) – Faecal Material | | | |
| 9. Papa et al., 2022 | Black Soldier Fly | 263–305 | 68–101 | 62–63 |
| | (Hermetia illucens) | | | |
| 10. Narendra et al., 2022** | Silkworm (Bombyx mori) – Additioned with Cashew fruit nut | 119.3–183.1 | 85.9–135.5 | 72–75 |
| 11. Dong et al., 2024 | Black Soldier Fly | 151.85-238.15 | 67–109 | 42–47,2 |
| | (Hermetia illucens) | | | |
| | Black Soldier Fly | 241.51-285.98 | 131,95–156,43 | 50,43–57,94 |
| | (Hermetia illucens) additioned with corn straw | | | |

Note: * "Frass type" describes Insect Species, Feed and Co-digestion substrate, when present, from Table 1 and 2; ** calculation based on given data.

plants. For example, biogas production generates exhaust heat which could be used and optimized for further insect farming. Additionally, digested residues could also serve as a base for other insects feed (Wedwitschka et al., 2023). In fact, there is limited information on the digestate resulting from the AD of frass. Bulak et al. (2020) found that the digestates obtained after the fermentation of Z. morio post-breeding waste were similar in terms of TS, VS, ash, total C, C:N ratio, protein content, and VFA compared to the initial feedstock (waste + inoculum). The pH values in all digestates were slightly basic, indicating that acidification and potential inhibition of methanogenesis during the monosubstrate fermentation of Z. morio wastes did not occur. The significant variation in phenols in the tested post-ferments does not pose a threat to the stability of the fermentation process, as it is lower than the inhibitory concentrations reported in the literature. Having limited information available, it will be necessary to deepen the knowledge regarding digestate resulting from frass AD.

Concerning bioenergy production, advancements in biogas upgrading technologies, can allow frass-derived energy to be integrated into existing energy infrastructures, including natural gas grids and transportation systems. This can help reduce the carbon footprint of these industries, aligning with global efforts to contrast climate change, and helps reduce the environmental impacts caused by traditional or improper management of solid waste, such as emission of toxic pollutants (e.g., dioxins), GHG, and the damaging of soil quality (Adetunji et al., 2023; Guo et al., 2024).

Moreover, research efforts aimed at optimizing frass utilization pathways for AD are essential for realizing its full potential. Some suggestions for future experiments can be drawn from the reviewed literature. Some of these studies recommend enhancing the composition of frass through co-digestion with other substrates, carefully considering nitrogen content to avoid process instability, and exploring different methods to research on the economic feasibility of biogas production from insect frass. Further research could be directed towards understanding how the total solids content can influence the AD process of this type of waste, determining which type of co-digestion substrate may be most suitable to enhance biomethane production, and the characterization of digestate produced from frass AD.

4. Conclusions

This literature review addresses the production of biogas and biomethane from insect "frass", hence valorizing this waste material. Studies show a wide range of biogas and methane production potentials, with some values comparable with traditional organic wastes (e.g. manure). However, results are influenced by factors such as substrate type and digestion conditions.

Biogas and biomethane potentials and CH₄ content (%) from other literature works, for comparison.

| Reference | Biomass | Biogas potential (m ³ /ton VS) | Biomethane potential (m ³ / ton VS) | CH ₄ content (%) |
|------------------------|---|--|--|-----------------------------------|
| Kafle and | Dairy cow manure | 295 | 204 | 69.1 |
| Chen | Horse manure | 222 | 155 | 70.1 |
| (2016) | Goat manure | 242 | 159 | 65.8 |
| | Chicken manure | 425 | 259 | 61.1 |
| | Swine manure | 495 | 323 | 65.3 |
| Kafle et al. (2013) | Brewery grain waste | 508 | 316 | 62.2 |
| | Bread waste | 575 | 306 | 53.1 |
| | Pacific saury fish waste | 635 | 435 | 68.6 |
| | Mackerel fish waste | 736 | 526 | 79 |
| | Cuttle fish waste | 777 | 543 | 84 |
| Amon et al. | Dairy cow manure | 208.2 | 136.5 | 65.6 |
| (2007) | Dairy cow manure | 267.7 | 159.2 | 59.5 |
| Pavi et al. (2017) | Organic fraction of municipal solid waste (OFMSW) | 215.0 | 164.5 | 76.5 |
| | Fruit and vegetable waste (FVW) | 350.0 | 275.9 | 78.7 |

Studies recommend optimizing the composition of frass through codigestion with other substrates, considering nitrogen content to avoid process instability, and exploring different methods to enhance the economic feasibility of biogas production from insect frass. Overall, frass utilization for bioenergy production shows promising potentials and aligns with the principles of circular economy.

CRediT authorship contribution statement

Adele Dal Magro: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. Daniela Lovarelli: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. Jacopo Bacenetti: Writing – review & editing, Methodology, Conceptualization. Marcella Guarino: Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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A.D. Magro et al.

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