Black Soldier Fly Larvae Frass

Subjects: Green & Sustainable Science & Technology
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Frass is a compost-like material and has the characteristics of immature compost. In a commercial context, black soldier fly larvae (BSFL) frass often refers to a mixture of primarily BSFL faeces, substrate residues, and shed BSFL exoskeletons. Schmitt and de Vries contended that frass is a mixture of uneaten feed materials, insect derivatives, such as skins and faeces, and a microbial population that carries out fermentation.

black soldier fly larvae frass | frass properties | frass nutrient composition | frass physiochemical characteristics

1. Black Soldier Fly Larvae (BSFL) Frass

Researchers use the term residue for the BSFL second by-product, which has the same meaning as the frass. Diener et al. [1] stated that the second by-product of BSFL treatment is residue or BSFL digestate; while, according to Green and Popa [2], Quilliam et al. [3], and Zahn [4], frass is the waste excreted by the larvae of BSFL composting. Frass is a compost-like material [5] and has the characteristics of immature compost [6][7][8]. In a commercial context, BSFL frass often refers to a mixture of primarily BSFL faeces, substrate residues, and shed BSFL exoskeletons [9]. Schmitt and de Vries [10] contended that frass is a mixture of uneaten feed materials, insect derivatives, such as skins and faeces, and a microbial population that carries out fermentation.

1.1. Harvesting BSFL Frass

BSFL frass is usually harvested after 9–23 days of BSFL composting, depending on the type of waste used [6][9][11][12][13]. Alternatively, the frass is harvested when more than 40–90% of larvae have transitioned to the prepupae stage [9][14][15]. The harvest time is crucial in full-scale BSFL treatment operations to ensure maximum larval production and good quality product [16]. Harvested BSFL frass may come in dry or wet compost. Dry BSFL frass is produced mainly as granular dark brown granular texture and has lower water content [7]. Meanwhile, wet BSFL frass is dense, grey in colour, has high moisture content, and has the consistency of thick, moist clay [17]. The good BSFL frass texture will allow easy storage, packaging and transport without further transformation or stabilization [18].

1.2. BSFL Frass Weight

BSFL was introduced as an efficient way to convert biowaste into larvae products and frass while reducing a large volume of waste [19]. One thousand kilograms of food and vegetable substrate in the BSFL treatment process can yield 25.0% (250 kg) BSFL frass and 12.5% (125 kg) BSFL [20]. Salomone et al. [18] reported that BSFL treatment of 30 tonnes of food waste per day generated 33.3% (9990 kg/day) BSFL frass and 7.7% (2310 kg/day) BSFL. Another study by Quilliam et al. [3] found that the bioconversion of brewery waste produced 33.8% (738 kg) BSFL frass and 2.5% (55.1 kg) BSFL from 2182 kg of the substrate, while 858 kg of poultry manure produced 62.9% (540 kg) BSFL frass and 6.4% (54.9 kg) BSFL. In summary, the weight of BSFL frass could exceed 33% of the original substrate weight and the weight generated will be affected by the types of substrates.

2. Physiochemical Characteristics of BSFL Frass

2.1. Temperature

The harvested BSFL frass has a temperature of 24 °C to 27 °C [6][21]. The rapid composting of BSFL occurs in the mesophilic phase; BSFL continues to transform the substrate, while higher air circulation during the BSFL composting process maintains a relatively constant waste temperature (about 30 °C) for optimum waste consumption by BSFL [22]. Rearing BSFL at the optimum temperature improves their ability to reduce *Escherichia coli* [23].

2.2. The pH of the BSFL Frass

Referring to **Table 1**, BSFL frass from food waste range from the lowest of 5.6 in fruit and vegetables and the highest as 8.0 pH value in mixture of food waste, chicken faeces, and sawdust (3:2:1 ratio); and maize straw substrates. The pH of BSFL frass typically ranges between 7.0 and 8.0, which is the optimum range for promoting plant growth [24] and providing a conducive environment for the beneficial bacterial communities in BSFL frass [25].

Table 1. The physiochemical characteristics of BSFL frass from different food waste types.

Type of Food Waste	pH	C/N Ratio	Moisture (%)	Temperature (°C)	Total Nitrogen as N (%)	Total Phosphorus as P_2O_5 (%)	Total Potassium as K_2O (%)	References
Kitchen waste	7.0	8:1	50	-	-	-	-	[26]
Kitchen waste	7.4	17:1	63	-	-	-	-	[11]
Municipal food waste	7.3–7.4	8:1–9:1	63–65	26.3–26.5	-	-	-	[21]
Household food waste	7.4	17:1	56	-	2.2	0.1	0.1	[27]
Food waste, chicken faeces, and sawdust (3:2:1 ratio)	6.1–8.0	-	72	27.0	1.7	1.1	2.1	[6]
Fruits and vegetables	5.6	27:1	10	-	1.8	-	-	[9]
Maize straw	8.0	-	38	-	0.6	2.5	2.1	[14]
Okara and wheat bran	7.5	8:1	-	-	4.8	1.0	0.9	[28]
Okara and wheat bran	7.7	10:1	-	-	3.2	0.8	0.5	[28] *
Brewery spent grain	7.7	17:1	30	-	2.1	1.2	0.2	[29]

* Frass obtained from post-treatment of thermophilic composting. References without an asterisk indicate referred study was using fresh harvested frass.

2.3. Moisture Content of the BSFL Frass

Referring to **Table 1**, the driest BSFL frass can be seen from brewery spent grain at 30% moisture content, and the wettest at 72% moisture content from the food waste, chicken faeces, and sawdust mixture (3:2:1 ratio). The high moisture content of frass makes harvesting difficult because the frass has a clay-like texture, making it hard to collect the BSFL biomass under the wet frass. One way to harvest the BSFL biomass from the wet frass is by rinsing it under running water [30]. However, this method washes out the frass, which is a waste since the frass can be processed into beneficial products for agricultural use. Using a suitable pre-treated waste in BSFL treatment (processing parameters and feeding strategies) can produce dry frass. It is worth noting that dry frass is considered immature compost since the organic waste must undergo rapid composting for about two weeks which may contains phytotoxins compounds that may inhibit plant growth. Many researchers recommend post-treatment of the dry frass to ensure its maturity and stability [31].

2.4. The C/N Ratio of the BSFL Frass

There are numerous approaches for determining organic fertiliser stability and behaviour in soil, and the soil C/N ratio may reveal vital soil information. As shown in **Table 1**, C/N ratio of BSFL frass derived from different types of food may range from 8:1 to 27:1; kitchen waste range from 8:1 to 17:1, municipal food waste range from 8:1 to 9:1, household food waste at 17:1, fruits and vegetables at 27:1, okara and wheat bran at 8:1 (fresh frass), okara and wheat bran (composted frass) at 10:1, and brewery spent grain at 17:1. In a well-conducted composting process, the C/N will decrease constantly. This is due to the biological mineralization of carbon compounds and loss as CO_2 [32].

2.5. Nutrient Content (NPK) of the BSFL Frass

Most insect frass has a high nutrient content, such as organic N and P [4]. Chen et al. [33] stated that the continuous movement of BSFL could reduce the BSFL frass temperature, which helps retain nitrogen in the BSFL frass and ensure a high nitrogen content in the BSFL frass. The nutrient content of BSFL frass is influenced primarily by the nutrient content of the substrate fed to the BSFL [24]. Referring to **Table 1**, BSFL frass derived from food waste have total nitrogen content range from 0.6 to 4.8, total phosphorus content ranges from 0.8 to 2.5 and total potassium range from 0.2 to 2.1.

As shown in **Table 2**, for agronomic purposes, total nitrogen, total phosphorus, and total potassium are acceptable at the range of greater than 0.6, 0.22, and 0.25, respectively. Commercial organic fertilizer has 2.3% total nitrogen content, 2.3% total phosphorus content, and 2.3% total potassium content. Commercial chemical fertiliser, however, is composed of higher nutrient content: 16% total nitrogen, 16% total phosphorus, and 16% total potassium. If the researchers compare this to the BSFL frass nutrient content in **Table 1**, fresh frass and composted frass from okara and wheat bran substrates have higher total nitrogen content (3.2% and 4.8%) than the commercial organic fertilizer (2.3%). Total nitrogen of BSFL frass from household food waste (2.2%); mixture of food waste, chicken faeces, and sawdust (1.7%); and fruit and vegetables (1.8%) are also close to the value of commercial organic fertilizer. However, total phosphorus and total potassium composition of BSFL frass from household food waste have not passed the preferable range. The lowest total nitrogen (0.6%) of BSFL frass can be seen in maize straw substrates, however, it also has total phosphorus (2.5%) and total potassium (2.1%) content like the commercial organic fertilizer. It is possible to observe that the mixture of two or three types of food waste present the acceptable and consistent value of BSFL frass nutrient content. Food waste, chicken faeces, and sawdust (3:2:1 ratio) has 1.7% (total nitrogen), 1.1% (total phosphorus), and 2.1% (total potassium). Respectively, okara and wheat bran mixture have 3.2–48% (total nitrogen), 0.8% (total phosphorus), and 0.5% (total potassium). Therefore, the mixture of two or different types of substrates may be preferable to produce high nutrient BSFL frass.

Table 2. The physiochemical characteristics of different types of fertilisers.

Type of Fertilisers	Type of Food Waste	pH	C/N Ratio	Moisture (%)	Temperature (°C)	Total Nitrogen as N (%)	Total Phosphorus as P_2O_5 (%)	Total Potassium as K_2O (%)	References
Compost from windrow composting	Food waste and yard waste	6.8–7.4	17:1–23:1	46–61	26–28	-	-	-	[34]
Compost from composting bin	Cafeteria food waste and yard waste	7.5	-	-	30	0.9	0.8	0.4	[35]
Compost from aerated composting	Household food waste	6.5–7.5	36:1	32–33	32	0.9–1.0	0.6–0.7	0.9–1.0	[36]
Digestate from anaerobic digester	Municipal food waste	8.1	11:1	85	-	9.6	2.4	2.3	[37]
Commercial organic fertiliser		-	-	-	-	2.3	2.3	2.3	[35]
Commercial chemical fertiliser		-	-	-	-	16	16	16	[35]
Preferred for agronomic purposes		6–8	<30:1	30–45	Ambient temperature	>0.6	>0.22	>0.25	[21][28][35][38][39][40]

2.6. Nutrient Composition of Food Waste Attribute to the BSFL Frass Composition

Referring to the study of Palma et al. [41], seven almond by-product (hulls and shells) samples were obtained from California processors, and BSFL frass samples were evaluated for their composition in terms of ammonium, phosphorus, and potassium production. Using the results shown in **Table 3**, the highest protein (67.7 g kg^{-1}) and calcium (2.8 g kg^{-1}) was obtained from sample 5 has contributed to the lowest ammonium (400 ppm) production in BSFL frass and highest phosphorus concentration (825.0 ppm). The lowest protein (40.1 g kg^{-1}), highest fat (31.0 g kg^{-1}), and highest starch content (5.33 g kg^{-1}) obtained from sample 1 was attributed to the considerable amount of ammonium (615.0 ppm), phosphorus (355.0 ppm), and potassium (35.4 ppm). Sample 7 has the lowest fat (14.6 g kg^{-1}), lowest calcium (1.9 g kg^{-1}), and lowest sugar (53.2 g kg^{-1}) and, as a result, has the lowest potassium content (17.9 g kg^{-1}). Sample 4 has the highest amount of sugar (291.3 g kg^{-1}) and has the highest amount of potassium in BSFL frass (44.6 g kg^{-1}). The highest amount of potassium can also be found in sample 3 (44.6 g kg^{-1}). However, the highest ammonium content (5695.9 ppm) and lowest phosphorus content (227.5 ppm) can be found in sample 2, where the sample has the lowest starch (3.57 g kg^{-1}). The results from this research have shown that high protein and calcium in food waste composition will cause a good effect in the BSFL frass composition. The lowest starch composition in food waste, however, has resulted in the highest ammonium and lowest phosphorus content in BSFL frass. Sugar, however, may be attributed to the amount of potassium in the BSFL frass.

Table 3. The composition of food waste attribute to BSFL frass composition.

Sample	Type of Waste	Nutrient Composition [e]					BSFL Frass Composition			
		Protein	Fat	Calcium	Starch	Sugar	NH_4-N [f]	PO_4-P [f]	K [e]	References
1	Pollinator hulls [a,b]	40.1	31.0	2.4	5.33	152.7	615.0	355.0	35.4	[41]
2	Nonpareil Hulls [b,c]	46.3	20.5	2.1	3.57	243.5	5695.0	227.5	38.5	[41]
3	Pollinator Hulls [b,c]	41.0	24.8	2.3	5.03	178.1	755.0	405.0	44.6	[41]
4	Nonpareil Hulls [c,d]	55.3	22.3	2.2	4.23	291.3	5052.5	515.0	44.6	[41]
5	Monterey Hulls [c,d]	67.7	26.5	2.8	4.33	119.2	400	825.0	43.4	[41]
6	Pollinator Hulls [c,d]	40.6	22.9	2.6	4.93	202.1	1420.0	485.0	36.0	[41]
7	Mixed Almond Shells [c,d]	42.6	14.6	1.9	3.65	53.2	2595.0	245.0	17.9	[41]

[a] harvest year: 2016, [b] product origin: Chico region, [c] harvest year: 2017, [d] product origin: Buttonwillow region, [e] quantity in g kg^{-1} dry matter, [f] quantity in ppm.

2.7. Heavy Metals in BSFL Frass

BSFL is one of the microorganisms which biologically accumulate heavy metals in their tissues, leaving their frass with low concentration of heavy metals. Previous studies show that large quantities of mercury have been added to the BSFL feedstock to be observed in a 13-day experiment and resulting in low mercury levels in the BSFL frass and were noted to be below the European Union's (EU's) threshold values of 0.7–10 mg Hg/kg [6]. Assessment of the heavy metal contents in BSFL frass showed considerable removal of As, Cd, Pb, and Fe [21]. The result showed 92–98% (0.0002–0.0008 mg/kg) removal of As, 99% to 100% (0.00029–0.00170 mg/kg) of Cd, and 80% to 90% (0.001–0.002 mg/kg) of Pb. Although Fe in BSFL frass content has a removal of 31% to 69% (1.70–2.56 mg/kg) from the initial substrate and Ni has shown no observable removal (0.01–0.03 mg/kg) in the experiment, both values still comply with maximum allowable level for heavy metals in organic fertilizer [21]. Salomone et al. [18] has also measured the concentrations of toxic and essential metals in the BSFL frass fed with food waste substrates and found that the concentration of toxic and essential metals was below the limits stated in the Italian regulation for fertiliser. BSFL frass has low concentrations of heavy metals because of the ability of BSFL to reduce and accumulate various forms of heavy metals in the BSFL treatment process. However, there is concern about heavy metals accumulated in BSFL biomass that may contains cadmium, lead, mercury, zinc, and arsenic from the biowaste, and the amount of the heavy metals absorbed might exceed the maximum allowable amounts of the regulations on animal feed [16].

2.8. Maturity and Stability of BSFL Frass

Early studies on BSFL frass have always focused on only NPK and micronutrients content to analyse BSFL frass as organic fertiliser. However, upon testing BSFL frass on several types of plants, stunted plant growth and reduced biomass production in the plants was observed. Therefore, there is concern on checking the maturity and stability of BSFL frass to identify the possible application. **Table 1** shows a compost level preferred for agronomic purposes. According to Diaz et al. [32], to mimic fertiliser analysis, compost analysis has concentrated on NPK and micronutrient contents while compost quality and maturity are controlled using chemical parameters, such as pH, ammonia, C/N, as well as plant growth, germination tests, and microbial tests.

Within a short period of BSFL rapid composting (two weeks to a month), organic wastes fed by the BSFL may not be properly composted [27][28]. The BSFL composting process must also stop when the larvae reach the prepupae stage, as a result, producing impartial compost and immature compost. Referring to Diaz et al. [32], maturity of a compost can be referred to a compost's level of phytotoxicity. Immature compost basically has a high level of phytotoxicity and will tend to have more growth inhibitors for plants than a mature compost. Phytotoxins, such as heavy metals, phenolic components, organic acids, and salt accumulation, are common in immature compost [42]. Meanwhile, the stability of a compost can be identified when one that is no longer undergoing rapid decomposition and whose nutrients are tightly bonded; unstable compost, on the other hand, may either release nutrients into the soil owing to additional decomposition or tie up nitrogen from the soil [32].

Temperature is an essential factor in determining whether decomposition proceeds at the mesophilic or thermophilic level, or even reaches the maturity level to generate natural plant fertiliser [34]. According to **Table 1**, BSFL frass temperature range from 24 °C to 27 °C, if compared to the temperature of other compost, as shown in **Table 2**, compost from windrow composting (26–28 °C), composting bin (30 °C), and aerated composting (32 °C); all reach an ambient temperature and is considered to have entered maturation phase [35][38]. As suggested by Cooperband [43], the optimum temperatures for bacterial decomposition are at 21–49 °C.

The pH value of compost is worth measuring since it can be used to track the decomposition process. At the beginning of the composting process, the pH value of compost, which indicates acid content, is a result of accumulated acid generation and acid decomposition to produce CO_2 and heat. The acidic condition is caused by the microbial breakdown of organic materials and the generation of organic acids. The pH value of compost will increase over time as the acids were consumed. The pH rise can also be explained by ammonia generation via ammonification and organic nitrogen mineralization via microbial activity. The mineralization of nitrogen molecules, such as nitrates, nitrites, and other organic acids is also involved in the process [35]. According to **Table 1**, the pH of BSFL frass from food waste ranges from 5.6 to 8.0 and **Table 2** shows the pH value of compost from windrow composting (6.8), compost bin (7.5), aerated composting (6.5–7.5), and digestate from anaerobic digestate (8.1) are all close to the pH value of mature compost 6–8 [39].

Diaz et al. [32] recommend that at the end of the composting process, the water content should be quite low (about 30%) to prevent any further biological activity in the stabilized material. Although, from a production perspective, the moisture level of finished composts should be between 35% and 45%. If the moisture level falls below 35%, a lot of dust will be created when these composts are transported or spread to fields [40]. In **Table 1**, moisture content of BSFL frass from kitchen waste ranges from 50 to 63%, municipal food waste ranges from 63 to 65%, household food waste at 56%, and mixture of food waste, chicken faeces, and sawdust (3:2:1 ratio) at 72%, fruit and vegetables at 10%, maize straw at 38% and brewery spent grain at 30%. BSFL frass ranges more than 45% from kitchen waste, municipal food waste, household food waste and mixture of food waste, chicken faeces, and sawdust (3:2:1 ratio) in anaerobic conditions showing the condition of BSFL frass is not mature enough. BSFL frass derived from fruit and vegetables is at 10% which is not suitable for agronomic purposes and may lead to hydrophobicity and be difficult to rewet. BSFL frass from maize straw and brewery-spent grain are in the acceptable range for mature compost showing stabilized characteristics for agronomic purposes. As shown in **Table 2**, moisture content of compost from aerated composting is around the range for stabilized compost at 32–33%. Compost from windrow composting contains 46–61% moisture and digestate from anaerobic contains the highest moisture (85%) compared to BSFL frass and other composting techniques.

There are numerous approaches for determining organic fertiliser stability and behaviour in soil, and the soil C/N ratio may reveal vital soil information. Compost with a C/N ratio less than 20:1 is beneficial to plants because the organic nitrogen has mineralized to inorganic nitrogen, which is then available for plant absorption. However, compost with a C/N ratio greater than 30:1, is more likely to immobilise nitrogen for plant uptake [21]. Referring to **Table 1**, all the BSFL frass derived from food waste range from 8:1 to 27:1 which have a C/N value lower than 30:1, proving the stability of BSFL frass in compost. **Table 2** shows the C/N ratio for the conventional organic waste treatment methods, where the C/N ratio of the compost from windrow composting, digestate from the anaerobic digester, and BSFL frass are within the preferred limit for agronomic purposes, such as fertiliser and soil amendment. However, the C/N ratio for the compost from the aerated composting technique is greater than 30:1 [36], which may cause nitrogen immobilisation. Therefore, it is prudent to evaluate the waste characteristics for C/N ratio and load the waste into the reactor to maintain the required C/N ratio for good quality compost.

Although the C/N ratio can provide some information into the stability and behaviour of compost, germination tests on plant growth can provide more detailed performance data. Song et al. [28], has stated that BSFL rapid composting is insufficient for removing phytotoxins from the okara and wheat bran waste because a seed germination test has indicated that the presence of phytotoxins, and Fourier-transform infrared spectroscopy (FTIR) examination revealed that fresh harvested frass had the greatest phenol compounds compared the other two types of BSFL frass which has undergo post-treatment in aerated composting and natural composting. Kawasaki et al. [27] has also claimed that BSFL frass could be an incomplete compost due to its chemical composition and microbial test. Higher ammonium nitrogen and lower nitrate nitrogen was present in BSFL frass derived from household food waste. This significant amount of nitrogen is typical of biologically unstable compost and has the same characteristics as poultry manure compost. A microbial test to analyse plant pathogens in BSFL frass in the household food waste has demonstrated the high relative abundance of Xanthomonadaceae, which contains a genus that causes disease in plants. Alattar et al. [17] has published one of the first studies on the stability of BSFL frass derived from kitchen waste as a soil amendment. The research involved analysing maize growth for 10 weeks and resulted in stunted maize growth, which the authors have ascribed to the high concentration of ammonia and presence of phytotoxins in BSFL frass, despite no analysis of phytotoxicity compounds being done.

In summary, BSFL frass derived from food waste may comply with the preferable range of mature compost suitable for agronomic purposes: pH (6–8), ambient temperature, and C/N ratio lower than 30:1. However, BSFL frass derived from food waste mostly have higher than 45% moisture content which greatly affect to the immaturity of compost. In depth analysis, such as maturity tests, germination tests, and microbial test on BSFL frass is important before applying BSF frass as a soil amendment and using it as an organic fertiliser. Germination tests and microbial tests of BSFL frass derived from food waste have shown that BSFL frass is an immature compost, therefore there is the need for post-treatment of BSFL frass [27][28].

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