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Valorization of Organic Wastes by Biotransformation with Black Soldier Fly Larvae: Prospecting of Agricultural Fertilizers

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Black soldier fly (BSF) larvae have been studied for the side-products obtained through the biotransformation of organic wastes. During the study, frass, which is a mixture of larval waste and non-degraded residues, was evaluated as an agricultural fertilizer. Biodegradation was carried out in a laboratory-scale reactor for 21 days with temperature and relative humidity control, comparing the process at two constant temperatures of 29 and 31ºC, and maintaining the ambient humidity between 65-80%. Meat, cooked food, fresh food and grass wastes were used, evaluating 4 mixtures with different carbon-to-nitrogen ratios (C:N), each with three replicates and a control substrate, with a feeding rate of 47.62 mg/larvae·day. During biotransformation, substrate pH, moisture and ash were monitored. The results were compared with the colombian technical standard NTC 5167: 2022 "Products for Agricultural Industry", which establishes the quality criteria for fertilizers. The greatest reduction of residues (%DM) at 29 and 31°C was in the 12:1 mixture with 66.89 and 56.56%, respectively. The percentages of nitrogen (N_T), potassium (K_2O) and phosphorus (P_2O_5) satisfied with the 1% allowed by the colombian regulation, with the exception of phosphorus (P_2O_5) in the 14:1 mixture, which is below 0.26% at 29°C and 0.16% at 31°C. The parameters of the physicochemical characterization of the frass in both trials satisfied with the established limits; however, the 10:1 and 8:1 moisture content at 31°C was exceeded by 14.10 and 20.00%, respectively.

1. Introduction

The demographic, economic and industrial expansion of recent times is related to the generation of solid waste, since its origin from households, industries and constructions is increasing (Niu et al., 2023; Shah et al., 2023). More than 2 billion tons of solid waste were produced annually worldwide during 2020, which is 0.79 kg/capita daily (Niu et al., 2023; Shah et al., 2023). The increase in solid waste generation was projected to increase by 73% by 2050 from 2020, reaching 3.88 billion tons (Shah et al., 2023). In developed countries, most of the solid waste is organic (Franceschi F., 2023). There are not enough green technologies, infrastructure and integrated solid waste management systems, and the strategies currently employed in industrialized countries are not yet considered environmentally sustainable (Khanal A., 2023; Franceschi F., 2023). The volume of waste arriving at illegal landfills is increasing (Mandpe et al., 2023). The transmission of infections by biological vectors, the contamination of groundwater by leachates, air pollution due to the generation of by-products such as dioxins, soil contamination and its loss of fertility, and contamination in the food chain, are some of the consequences caused by the wrong treatment of solid waste (Vinti G., 2023).
The use of synthetic fertilizers in the agricultural industry has been a precursor to climate change through the generation of greenhouse gases, acidification and eutrophication of surface waters, contamination of water sources by phosphate concentrations, chemical runoff, loss of biodiversity and carbon sequestration (Mahankale N., 2023; Litskas V., 2023). Although chemical fertilizers increase crop yields, their production has

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endangered global food security, as the nutrients have a low concentration, which is lost through microbial degradation, hydrolysis, leaching and photosynthesis (Rajput V. & Singh A., 2023). However, these products are of vital importance, since 33% of the world's land surface is degraded (Santiago S., 2023).
Black soldier fly (BSF) larvae has been used in recent years in organic solid waste composting processes, because of the compositional characteristics of its frass (a mixture of waste not degraded in the process and larval excrement (Basri et al., 2022)) can be used as organic fertilizer (Boudabbous et al. 2023); it has also been shown that the growth of newly germinated plant species is favored when using this frass (Romano et al., 2023). In this research, the physicochemical characteristics and composition of the frass were compared with the colombian quality standards for the use of fertilizers obtained from organic wastes (NTC 5167:2022).
Through this article, the methodology implemented during the experimentation of the biotransformation process of organic solid domestic waste at 29 and 31ºC is structured. The highest reduction of residues at 29 and 31ºC was with the 12:1 mixture, while the lowest reduction in both treatments was with the 8:1 mixture, which was attributed to the excessive presence of meat residues. The highest amount of nitrogen and potassium in the frass at 29ºC was with the 8:1 mixture, and of potassium was with the 14:1 mixture; however, all macronutrients were within the 1% allowed by NTC: 5167, with the exception of potassium in the 14:1 mix. At 31ºC the macronutrients were found in greater proportion in the 12:1 mixture, although the 14:1 and 8:1 mixtures showed deficiencies of phosphorus and potassium.

2. Methods and materials

2.1 Egg hatching

Black soldier fly eggs were purchased from Grupo Evolutio SAS, Bogotá D.C. Hatching was carried out in a horizontal incubator for 7 days at a temperature between 26.5 - 27.5°C and humidity between 65 - 75% (Chia et al., 2018), with chicken feed and water as substrate (Barrett et al., 2022). The larvae used for the experiment were those hatched between the second and third day, and after the fifth day no hatchlings were evident (Chia et al., 2018). Larvae and substrate were separated by vibratory sieves. Neonates present in a 0.50g sample of separated larvae were counted and this count was repeated until a maximum standard deviation of 5%. The total number of larvae was calculated by mass balance.

2.2 Food waste preparation

Fresh waste (fruit, vegetable and tuber peels) from Fruver Home (Bogotá D.C.); cooked waste from Bogotá households; meat waste from different restaurants in Bogotá D.C.; and pruning and lawn waste collected from the Universidad de América (Bogotá D.C.) were used. Household waste was used like Barrett et al. (2022). After collection, the waste was homogenized through size reduction in a press mill (Universal, Colombia) (Barrett et al., 2022). As evidenced in Table 1, four mixtures with four different carbon - nitrogen ratios (R C:N 14:1, 12:1, 10:1 and 8:1), each with three replicates (with larvae) and one control substrate (without larvae), were prepared in plastic containers of 209,72 cm^2 surface area. Using 700 g as the basis of calculation in wet mass, the reported quantities were calculated.

Table 1. Amounts of organic residues in the mixtures according to R C:N

Mixture	R C:N	Fresh food waste (g)	Cooked food waste (g)	Grass waste(g)	Meat waste (g)
1	14:1	364.0	266.0	70.0	0.0
2	12:1	275.2	205.3	54.4	165.1
3	10:1	214.2	156.1	41.3	288.4
4	8:1	149.1	109.2	28.7	413.0

2.3 Reactor configuration and measurement variables

The biotransformation process was carried out at Universidad de América, Bogotá D.C., Colombia (4°36′07″N 74°03′43″O). Reactor temperature was set for treatment 1 between 28.5 and 29.5°C and for treatment 2 between 30.5 and 31.5°C (Chia et al., 2018). Relative humidity was set between 65-80% for both treatments (Chia et al., 2018). Batch reactor feed rate was 47.62 mg/larvae·day for the 16 containers (Chia et al., 2018).
On days 1, 3, 6, 8, 10, 10, 13, 15, 17, 20 and 21, the variables of pH, humidity and ash percentage of the substrate were monitored according to the methodology described in NTC 5167: 2022 (ICONTEC, 2022); and the humidity and temperature of the environment were monitored using the digital temperature and humidity controller SHT2000 (Dualtronica, Bogotá D.C., Colombia), a digital temperature and humidity controller SHT2000 (Dualtronica, Bogotá D.C., Colombia), the VN-583 extractor fan (Techman Electronic U.S.A.), the PHX120X120X38 extractor fan (Phoenix AIR) and the double analog timer (EBCHQ), installed in the laboratory-

scale reactor. The instruments used were: potentiometer PRO pH Meter MW101 (Milwaukee, Romania), drying and sterilization oven TE - 393/1 (Tecnal, Brazil) and muffle MM10 - 0150 series (Terrigeno, Medellín, Colombia).

2.4 Sample collection

Manual separation of frass and prepupal larvae was performed for each of the mixtures used. The samples were weighed on an analytical balance BC220M - series 260 (Precisa Instruments AG, Dietikon, Switzerland). In determining the waste reduction efficiency, the mass of the substrates and the mass of the frass per mixture on a dry mass (%DM) were taken into account; its calculation was by Equation 1 (Ganda et al., 2019).

$$Waste\ reduction\ (\%DM) = \frac{initial\ substrate\ mass\ (\%DM) - frass\ mass\ (\%DM)}{initial\ substrate\ mass\ (\%DM)} x\ 100 \qquad \textbf{(1)}$$

The laboratory analysis performed was the characterization and composition of solid organic materials. Moisture, moisture retention, ash and real density were determined by gravimetric techniques; and the variables of cation exchange capacity and organic nitrogen were determined by volumetric techniques. The pH was measured potentiometrically and the R C:N was quantified by mathematical ratio. Total oxidizable organic carbon and phosphorus (P_2O_5) were measured by colorimetry. Potassium (K_2O) content was quantified by Atomic Emission Spectroscopy (AES), calcium (CaO) and magnesium (MgO) content by Atomic Absorption Spectroscopy (AAS), and total nitrogen (N_T) was calculated by summation of nitrogen species.

2.5 Statistical analysis

Two treatments (two temperatures) were performed with four mixtures, each mixture with three replicates and a control substrate. Differences between the treatments in the frass composition of macronutrients and waste reduction percentage were calculated using analysis of variance (ANOVA) in SPSS version 26 software (IBM Corp, Armonk, NY, USA). The significance level was defined as $p \leq 0.05$.

3. Results and discussion

3.1 Waste reduction evaluation

Table 2 shows that at 29°C the percentage reduction of residues was higher than at 31°C. The R C:N 12:1 and 10:1 at 29°C were those where the consumption of residues was higher; however, the R C:N 14:1 and 8:1 also showed a significant reduction. The R C:N 14:1 and 12:1 at 31°C showed a higher reduction than the rest of the R C:N. The frass (%DM) of: the R C:N 14:1 was 54.87 and 66.10g, the R C:N 12:1 was 46.35 and 60.82g, the R C:N 10:1 was 47.90 and 86.44g, and the R C:N 8:1 was 63.02 and 132.82g, respectively for 29 and 31°C. Biomass resulting from control substrates (%DM) was: 79.10, 107.90 and 157.64g for R C:N 14:1, 12:1 and 10:1, respectively, at 29°C; and 81.45, 67.21, 113.01 and 137.65g for R C:N 14:1, 12:1, 10:1 and 8:1, respectively, at 31°C. Residue reduction was greater in the R C:N than in the control substrates, due to larval consumption.

Table 2. Percentage reduction of residues in dry mass at 29 and 31ºC.

R C:N	Waste reduction (%DM): 29ºC	Waste reduction (%DM): 31ºC
14:1	60.81	52.78
Control substrate 14:1	43.51	41.82
12:1	66.89	56.56
Control substrate 12:1	22.94	52.00
10:1	65.78	38.26
Control substrate 10:1	10.32	19.28
8:1	54.98	5.13
Control substrate 8:1	*	1.68

** Result not provided due to alterations in the environment.*
No significant difference between temperatures and R C:N (p≤0.05) in the reduction of frass residues.

The amount of biomass resulting from the process was lower at 29°C than at 31°C, which implies that the larvae consumed more substrate at the first temperature because the R C:N had lower humidity. The R C:N 10:1 and

8:1 at 31°C had high humidity, 44.96 and 56.22% (on average during the 21 days), respectively, which could not be efficiently consumed by the larvae. The development of BSF larvae is affected by the amount of water present in the substrates, as an excessive amount prevents food consumption by the larvae and forces them to remain outside the substrate on the walls of the containers (Naser El Deen et al., 2023). It was observed that the larvae of the R C:N 8:1 remained outside the substrate, which did not allow the consumption of the substrate and that their growth was lower than in the rest of the R C:N.

The composition of the substrate affects larval feeding, because its omnivorous nature allows it to better transform plant proteins than animal proteins (Nyakeri et al., 2019). In this case, the food that prevented waste reduction in the R C:N 8:1 (in both treatments) was meat waste, which was found in higher proportion and therefore did not favor the biotransformation process. The percentage of organic solid waste reduction with BSF larvae is between 60-68% (%DM) (Gold et al., 2018). In this study the R C:N 14:1, 12:1 and 10:1 at 29°C were within this range, while at 31°C the R C:N did not meet this parameter.

3.2 Evaluation of frass as organic fertilizer

The chemical composition of the frass is affected by the percentage of each solid residue in the mixtures (Naser El Deen et al., 2023). The content of N_T, P_2O_5 and K_2O (macronutrients) varies according to the nature of the substrate; Tran et al. (2022) in their review indicates that the content of N_T, P_2O_5 and K_2O in frass with BSF larvae fed with household waste is 2.2, 0.5 and 0.7%; while for Basri et al. (2022) the acceptable amounts of these minerals for agricultural applications are 0.6, 0.22 and 0.25%, respectively, although for commercial organic fertilizers this amount should be a minimum of 2.3% for each one.

According to NTC 5167: 2022, the content of these minerals must be at least 1%, which was obtained in all frass treatments, except for P_2O_5 in the R C:N 14:1 in both treatments and in the R C:N 8:1 at 31ºC, and K_2O in the R C:N 8:1 at 31ºC (ICONTEC, 2022). Table 3 indicates that at 29°C the highest N_T and P_2O_5 content was obtained by the R C:N 8:1 frass and at 31°C by the frass of the R C:N 12:1, while the highest K_2O content was obtained by the R C:N 14:1, however, N_T is the mineral that is present in the highest amount in both treatments. Visvini et al. (2022) state that the increase in N_T is due to the exoskeleton of the larvae within the frass, which is characterized as chitin (composed of nitrogen); this increase is also due to the breakdown of proteins in the larvae's gut microbiota, which allows the increase of ammonium in the substrate.

The MgO and CaO content is not specified in NTC 5167:2022. Acosta & Guzmán (2022), reported 0.36% MgO, using wastes similar to those used in this research. Romano et al. (2022) reported 1.81% CaO, using grains, fruits and vegetables as substrates. In this study, the percentages of MgO and CaO differ from those reported by the aforementioned authors.

Table 3. Characterization of macronutrients and secondary nutrients of the frass at 29 and 31ºC.

	29°C								**31°C**							
	Substrate				**Frass**				**Substrate**				**Frass**			
Nutrient	**14:1**	**12:1**	**10:1**	**8:1**	**14:1**	**12:1**	**10:1**	**8:1**	**14:1**	**12:1**	**10:1**	**8:1**	**14:1**	**12:1**	**10:1**	**8:1**
N_T (%)	0.43	0.90	1.17	1.63	1.96	2.61	3.60	4.23	0.47	0.96	1.32	1.69	2.26	3.07	2.63	2.16
P_2O_5 (%)	0.11	0.19	0.24	0.25	0.74	1.21	1.09	1.44	0.12	0.13	0.13	0.13	0.84	1.55	1.24	0.68
K_2O (%)	0.35	0.32	0.29	0.30	2.65	2.56	2.33	1.45	0.36	0.34	0.33	0.32	3.05	2.59	1.57	0.58
CaO (%)	0.13	0.13	0.09	0.11	0.38	0.29	0.25	0.16	0.14	0.11	0.09	0.06	0.73	0.67	0.46	0.12
MgO (%)	0.05	0.04	0.04	0.04	0.27[a]	0.19	0.14	0.11[a]	0.05	0.04	0.04	0.04	0.32[a]	0.23	0.16	0.07[a]

** Results presented on wet basis. Values with the same letter per row represent significant differences ($p \leq 0.05$).*

The physicochemical characteristics of the BSF larvae frass in this study at 29 and 31°C for the R C:N 14:1 and 12:1 had a granular consistency and the R C:N 10:1 and 8:1 had a thick consistency (Basri et al. 2022); the color of the frass obtained was dark brown, which agrees with Maquilón A. (2022) who highlights the dark color of organic fertilizers as a property that allows better absorption of solar radiation. Studt N. (2010) indicates that frass is not suitable for compost if its texture is thick and has excessive moisture, while the moisture requirement according to NTC 5167: 2022 (ICONTEC, 2022) should be ≤25%, thus all R C:N at 29°C are suitable, and at 31°C only R C:N 14:1 and 12:1 (Table 4). According to colombian regulations (ICONTEC, 2022), the pH must be between 4.0 and 9.0, Tanga et al. (2021) quantifies the pH of BSF frass at 6.79 and Basri et al. (2022) records that the pH of the frass of BSF larvae fed with fruits and vegetables is 5.6, so all treatments complied with this parameter.

Tabla 4. Characterization and composition of solid organic materials in the frass at 29 and 31ºC.

	29°C				31°C			
Parameter	**14:1**	**12:1**	**10:1**	**8:1**	**14:1**	**12:1**	**10:1**	**8:1**
Humidity (%)	10.20	8.58	15.00	23.30	21.40	23.50	39.10	64.50
pH (Unidades)	6.83	5.78	4.92	5.20	7.41	6.31	5.96	5.06
Moisture retention	162.00	196.00	144.00	106.00	213.00	156.00	107.00	40.90
Ashes (%)	25.10	20.90	16.40	11.80	17.60	17.30	10.20	4.52
Cation-exchange capacity (meq/100g)	57.00	63.00	42.10	38.60	57.60	67.30	48.90	17.70
Real Density (g/cm^3)	0.57	0.49	0.53	0.54	0.43	0.54	0.57	0.56
Total Oxidizable Organic Carbon (%)	26.40	29.40	27.60	22.80	27.30	25.60	21.30	11.70
R C:N (Adimensional)	13.00	11.00	7.70	5.40	12.00	8.30	8.10	5.40

** Results presented on wet basis.*

For organic fertilizers, from animal and vegetable wastes in Colombia, the actual density must not exceed 0. 6 g/mL, the ash content must be a maximum of 60%, the total oxidizable organic carbon content must exceed 15%, the cation exchange capacity must be a minimum of 30 meq/100g and moisture retention must be greater than 100% (ICONTEC, 2022), and as shown in Table 4, these variables comply with the NTC 5167-2022 standard, except for moisture retention, cation exchange capacity and total oxidizable organic carbon in the R C:N 8:1 at 31ºC. Basri et al. (2022) affirms that in efficient biotransformation processes the carbon-nitrogen ratio decreases, due to the increase in the amount of nitrogen in the frass, mineralization of organic compounds and loss of carbon dioxide, which occurred in this study (Table 4). According to NTC 5167: 2022 the R C:N should be a maximum of 25:1 (ICONTEC, 2022), a value that was fulfill in all R C:N values. However, Basri et al. (2022) found the R C:N values in the frass between 8:1 and 27:1, which occurred with R C:N 14:1 and 12:1 at 29ºC, and R C:N 14:1, 12:1 and 10:1 at 31ºC.

4. Conclusions

The implementation of meat waste is not suitable for the reduction of organic waste in biotransformation processes with black soldier fly larvae, while cooked and fresh waste are adapted to the nutritional needs of this species and allow an efficient reduction. In controlled laboratory-scale reactors, the relative humidity of the environment should be between 65-80% and the temperature of the medium should not exceed 30ºC. Substrate humidity is a determining variable in the reduction of waste with *Hermetia illucens* larvae, since excessive humidity as in the R C:N 8:1 (46.29 and 56.20% on average during the process) affects the consumption of substrates and the survival of the organisms. To obtain frass with the physicochemical and nutrient characteristics registered in NTC 5167:2022, mixtures composed of animal proteins, vegetable proteins, carbohydrates and lipids in balanced proportions are required, such as R C:N 12:1, with the environmental conditions described in this research. In addition, when moisture is greater than 25% in the frass, drying processes must be included to guarantee maturity and stability in the compost, which increases production costs. Future experimentation should test the effectiveness and performance of frass from black soldier fly larvae as an organic fertilizer directly on agricultural crops and include the characterization and total composition of heavy metals (As, Cd, Cr, Hg and Pb), and a phytotoxic test of the frass. An analysis of the physicochemical parameters and mineral composition on a dry basis should be carried out for comparison with commercial fertilizers. The biotransformation process presented in this paper is viable on an industrial scale in which an automated reactor is designed and tons of organic waste are used to optimize the production of fertilizers.

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