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Article

# Study of the Effect of Biodried Material as Feed for *E. foetida* in a Vermicomposting Process

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## Abstract

Agricultural and agro-industrial waste can be valorized through biodrying, a process that uses microbial activity to accelerate water loss to obtain a biodried material (BM) with high calorific value and potential use as a biofuel. This material has the advantage of being easily transported, stored, and preserved until later use. However, its high organic matter content allows it to be used for other purposes. In this study, the use of BM (made from orange peel, grass, mulch, pruning waste, and compost), either alone or mixed with fresh organic waste (FOW) as feed for *Eisenia foetida* in a vermicomposting system was evaluated over a period of 49 days. The proportions of BM used were: 100%, 75%, 50%, 25%, and 0%, with the remainder completed with FOW. During the bioprocess, temperature, humidity, and pH were monitored, and at the end of the experiment, the survival and reproduction of *E. foetida* as well as the quality of the humus obtained were analyzed. In the treatments containing 100% and 75% BM, the worm population decreased by 28.5% and 7.7%, respectively, although the highest number of cocoons (28 and 24 cocoons kg<sub>humus</sub><sup>-1</sup>) was observed in these treatments compared with all others. The humus obtained from all treatments complied with the NMX-FF-109-SCFI-2008 standard, which designates quality grades as Extra, First, and Second. The treatment with 100% BM produced First quality humus, but the treatments with mixtures of BM and FOW produced Extra quality humus. The results support the diversification of BM uses and its incorporation into sustainable bioprocesses such as vermicomposting and the production of new value-added products.

**Keywords:** biodried material; fresh organic waste; *E. foetida*; vermicomposting

## 1. Introduction

Although the productivity of the agricultural and agro-industrial sectors is fundamental to the economy of Mexico, it invariably generates large volumes of residual biomass throughout the production chain. According to the Agricultural Census, Mexico has a cultivated agricultural area of 21,635,876 hectares [1], from which it is estimated that in 2023 approximately 559 million tons of crops (fruits, vegetables, legumes, grains, and flowers) were produced, generating large quantities of residues [2].

Due to the composition of agricultural and agro-industrial waste, their improper management and disposal can lead to sanitary and environmental problems. These may include infections caused by pathogenic microorganisms, respiratory conditions, and the transmission of diseases such as cholera, dengue, and amoebiasis, among others. As a result of the lack of separation and/or treatment

of the organic fraction prior to its deposition at final disposal sites, its decomposition generates greenhouse gases and leachates, in addition to promoting the proliferation of pests and insects [3].

Through the application of appropriate valorization technologies, organic waste, rather than representing a problem, can constitute a strategic opportunity as secondary resources, and their utilization could contribute to reducing water pollution, soil degradation, and greenhouse gas emissions. In addition, the development of infrastructure and knowledge oriented toward the valorization of biowaste not only promotes technological innovation but also supports the creation of green jobs, strengthening circular value chains in the country. Some traditional alternatives for waste utilization, such as composting, vermicomposting, anaerobic digestion, incineration, and, more recently, biodrying, could be applied to the valorization of residues from the agro-industrial sector to obtain economic, environmental, and social benefits.

Biodrying is an aerobic process that removes water from the waste mass by utilizing the metabolic heat generated by microbial activity during the degradation of residues, resulting in a reduction in the weight and volume of the waste and thereby facilitating its transport, storage, and final disposal. As a result of this process, a stabilized biodried material (BM) is obtained, with minimal moisture content and high calorific value, for which potential use as a biofuel has been demonstrated to date [4], although new applications are currently being explored. Because BM is a microbiologically stabilized product with an undegraded organic fraction, this material can be utilized in a vermicomposting process in order to convert it into an organic amendment.

Along this line of research, Contreras-Cisneros et al. [5] evaluated the effect of BM as an organic soil amendment during seed germination and the growth and development of lettuce plants. These authors first assessed the phytotoxicity of aqueous extracts of BM and compared it with the effect of compost extract; the results showed that the BM extract promoted lettuce seed germination even more effectively than the compost extract. Subsequently, they evaluated its effect on the growth and development of lettuce seedlings and on soil properties. In this case, the results showed positive effects on plant growth and development, although they did not surpass the positive effect observed with compost. In addition, the physicochemical properties of the soil improved with the addition of BM as an organic amendment. Biodrying therefore represents a viable alternative to composting, particularly in regions with water scarcity, as it does not require the addition of water and can be completed in a shorter time.

Composting and vermicomposting have been widely demonstrated to be bioprocesses applied to the treatment of organic waste for the production of organic amendments. Vermicomposting is a process in which the metabolic activity of earthworms (*E. foetida*) promotes the transformation of organic matter into a nutrient-rich product capable of improving soil properties and supporting plant development [6]. The production of vermicompost can help reduce the use of synthetic fertilizers; in addition, its application to soils contributes microbial diversity, enzymes, and nutrients, while also reducing the presence of pathogens [7].

The optimal development of a vermicomposting system depends on various environmental and physicochemical factors such as temperature, moisture, and pH; however, the quality and chemical composition of the degradable substrate used is fundamental, since the components of waste mixtures may interfere with the reproduction and survival of earthworms [8].

Based on the above, the objective of this study was to evaluate the survival, growth, and reproduction of *E. foetida* in a vermicomposting system using BM as a substrate, as well as to assess the quality of the humus obtained, with the aim of proposing an alternative application of the biodrying process to diversify the use of the resulting BM.

## 2. Materials and Methods

At the beginning of this study, the composition of the biomaterial (BM) obtained from the biodrying of a mixture of agro-industrial waste (orange bagasse), pruning residues (grass, mulch, and leaf litter), and compost used as an inoculum to initiate biological activity was characterized [9]. The BM used presented a pH of 7.8, a moisture content of 9.7%, organic matter content of 39.42%,

and a water retention capacity of 46.07%, and consisted of 58.82% orange peel, 14.71% grass, 14.71% mulch, 5.88% leaf litter, while the remaining fraction corresponded to small fragments of the mixture.

To determine whether BM could have a negative effect on earthworm growth and reproduction, an acute toxicity bioassay was performed using a filter paper contact test with the biodried material extract (BME) for 48 h. The bioassay was conducted in triplicate, following the procedure described in OECD guideline No. 207 [10]. Prior to the bioassay, the BME was obtained using the modified ASTM D3987-85 method established in NOM-141-SEMARNAT-2003 [11]. Briefly, after 24 h of agitation (150 rpm) followed by settling of a mixture of 100 g of BM (dry basis) in 1000 mL of distilled water, the filtrate (8–12  $\mu\text{m}$  pore size) was obtained, with a final BME concentration of 0.1 g mL<sup>-1</sup>. From the BME, five dilutions were prepared for the exposure of *E. foetida* to different concentrations across five treatments (BME1, BME2, BME3, BME4, and BME5), as shown in Table 1. A positive control (C+) containing 2-chloroacetamide (0.013 g mL<sup>-1</sup>) was included as a reference substance, along with a negative control (C-) consisting of distilled water.

**Table 1.** Concentrations of the biodried material extract (BME) used in the acute toxicity bioassay with *Eisenia foetida*.

Treatment	Concentration	
	% BME	g mL <sup>-1</sup> (d.b.)*
BME1	100	0.1
BME2	75	0.075
BME3	50	0.05
BME4	25	0.025
BME5	10	0.01

\*d.b.: dry basis.

Subsequently, a feasibility test was conducted to evaluate the use of BM in a vermicomposting system with *E. foetida*, using BM at different concentrations (100%, 75%, 50%, and 25%) as feed or substrate in experimental units with dimensions of 14 cm × 31 cm × 16 cm, in triplicate. The experimental units consisted of a 5 cm soil layer (1.36 kg) with a moisture content of 60% and a water retention capacity of 31.83%, 130 clitellated earthworms to achieve a density of 2500 worms m<sup>-2</sup> [12], and finally a 0.15 kg substrate layer, incorporated weekly. The substrate preparation added weekly to each treatment consisted of a mixture of BM and FOW, as shown in Table 2. The FOW used consisted of 50% lettuce residues and 50% carrot bagasse.

Prior to the feeding trial, the FOW were conditioned to promote their degradation for 5 days at room temperature in order to facilitate the subsequent absorption of nutrients by the earthworms, while the BM was conditioned for one week by adding the necessary amount of water to obtain a substrate moisture content of 65%. The vermicomposting process lasted 49 days, during which water was sprayed every three days to maintain adequate moisture throughout the process.

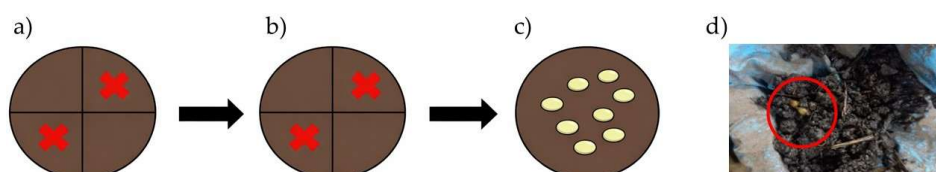
**Table 2.** Weekly Composition of the substrate.

Treatment Conc. BM	Composition (kg)*		Moisture Content (%)
	BM (kg)	FOW (kg)	
100%	0.150	0	62 ± 0.26
75%	0.113	0.037	70.9 ± 0.2
50%	0.075	0.075	70.4 ± 0.2
25%	0.037	0.113	82.6 ± 0.27
0%	0	0.150	85.3 ± 0.17

\* Amount prepared per experimental unit. Experiment performed in triplicate.

During the vermicomposting process, temperature, moisture, and pH were monitored. After 49 days of processing, the survival of *E. foetida* was evaluated according to the number of earthworms

initially introduced (130 worms) in each experimental unit. The reproduction was evaluated based on the number of adult individuals, hatchlings, and cocoons of *E. foetida* manually sorted into humus samples obtained using the quartering method (Figure 1), according to NOM-021-RECNAT-2000 [13], from 1 kg humus samples. Firstly, the humus sample was disposed in a circular disk, divided into four equal sections and the opposite quarters were discarded and the remaining two sections were mixed (Figure 1a). In a next step, the process was repeated with the new mixed reduced sample and again a reduced sample was obtained from the mix of opposite quarters (Figure 1b). Finally, the homogenized sample is used for physicochemical analysis and count of earthworm cocoons (Figure 1c). The cocoons identified visually in the samples (Figure 1d) were quantified and any juvenile earthworms that presented a brownish coloration and an approximate length of 2 cm were considered as offspring and counted.



**Figure 1.** Quartering method used to obtain a representative humus sample for cocoon counting. a) First step; b) Second step; c) Reduced sample; d) Cocoons identified. Sections marked with red crosses indicate the portions discarded during each quartering step, according to NOM-021-RECNAT 2000 [13].

Finally, after the 49-day period had elapsed, the humus produced up to that point in all treatments was characterized in accordance with NMX-FF-109-SCFI-2008 [14], which establishes the criteria that earthworm humus must meet for commercialization within Mexican territory. Table 3 presents the physicochemical parameters considered by the standard for humus characterization.

Statistical analyses were conducted in Python using pandas, SciPy, and statsmodels, with significance evaluated at  $\alpha = 0.05$ .

Differences in worm survival among treatments were evaluated using Fisher's exact test, as some treatments exhibited complete survival, resulting in perfect separation that violates the assumptions of standard binomial models.

**Table 3.** Physicochemical specifications for the characterization of earthworm humus.

Characteristics	Value
Total Nitrogen	From 1 to 4% (Dry basis: d.b.)
Organic Matter	From 20% to 50% (d.b.)
C/N ratio	$\leq 20$
Moisture*	From 20 to 40% (Wet basis: w.b.)
pH**	From 5.5 to 8.5
Conductivity	$\leq 4 \text{ dS m}^{-1}$
Bulk density on a dry mass basis	0.40 to 0.90 $\text{g mL}^{-1}$ (w.b.)

\* Moisture in worm humus can reach values up to 60% depending on the composition of the substrate used. \*\* A pH of 7 is preferred. Material from tropical regions may have a pH lower than 7, and materials from arid zones may have a pH higher than 7.

Reproductive responses (cocoon production and offspring counts) were analyzed using generalized linear models (GLM) with treatment as the explanatory factor. Cocoon counts were modeled using a Poisson distribution, while offspring counts were analyzed with a negative binomial distribution to account for overdispersion, followed by all pairwise contrasts with Holm correction for multiple comparisons.

The chemical properties of the resulting vermicompost (organic matter and total nitrogen) were analyzed using one-way analysis of variance (ANOVA) with treatment as the fixed factor. When significant differences were detected, Tukey's honestly significant difference (HSD) test was used for pairwise comparisons.

The C/N ratio was calculated from the mean carbon and nitrogen concentrations for each treatment. Variability was estimated by propagating the measurement variance from the unaveraged triplicate carbon and nitrogen determinations. Because the ratio corresponds to treatment-level measurements rather than independent biological replicates, C/N values were interpreted descriptively rather than subjected to inferential statistical testing. Results are reported as mean  $\pm$  standard deviation, and treatments sharing the same letter in Tukey comparisons are not significantly different ( $p \geq 0.05$ ).

### 3. Results and Discussion

#### 3.1. Acute Toxicity Bioassay of BME

The results of the acute toxicity bioassay of BME in earthworms showed that only the highest extract concentration in treatment BME1 ( $0.1 \text{ g mL}^{-1}$ ) caused an immediate toxic effect, with 33.3% mortality of the exposed earthworms. In contrast, no mortality was observed in the other dilutions with lower extract concentrations (BME2 to BME5) (Table 4).

**Table 4.** Survival percentage determined by the acute contact toxicity bioassay of *E. foetida* exposed to different concentrations of biodried material ( $n = 3$ ).

Treatment	BME concentration ( $\text{g mL}^{-1}$ ) (d.b.)	% Survival
BME 1	0.1	66.67
BME 2	0.075	100
BME 3	0.05	100
BME 4	0.025	100
BME5	0.01	100
Control -	(-)	100
Control +	(+)	0

It should be noted that, during the aqueous extraction of a solid substrate, components present in the material (soluble salts, polar organic compounds, and other substances resulting from the decomposition of organic matter) are leached into a known volume of liquid, resulting in a sample representative of the readily extractable components of the solid material. Thus, the immediate availability of compounds in the BME allows the toxic potential of the bioavailable components of BM, absorbed through the earthworm's skin, to be assessed using the acute contact toxicity test.

The results obtained from the acute bioassay constitute a warning signal of BM toxicity; however, it cannot be assumed whether its use as a sole substrate represents a risk to *E. foetida* in a vermicomposting system. This underscores the need to determine whether, under realistic conditions, BM can be used as a feed substrate without posing a risk to soil fauna.

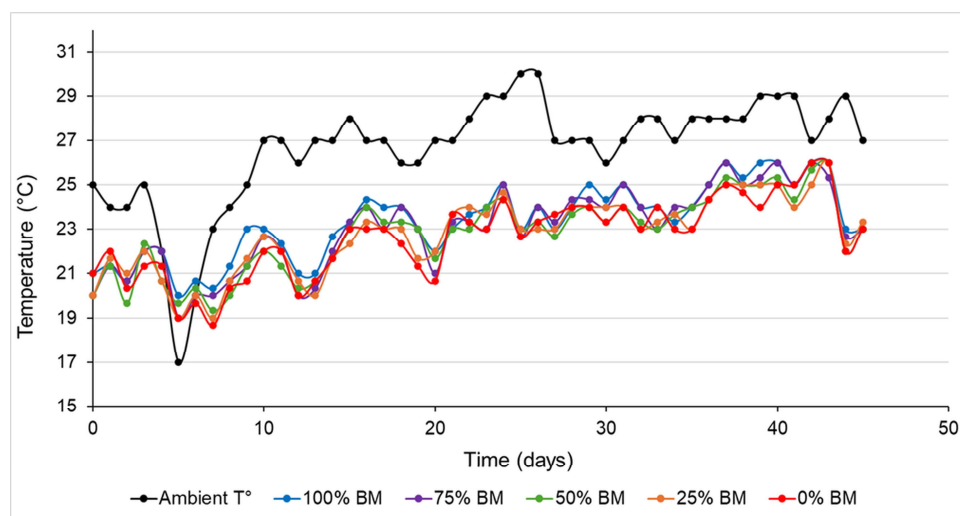
In contrast to the acute contact toxicity test, toxicity assays in solid substrate provide a more realistic assessment of the material's effects on earthworm survival, as the organisms are exposed to its components not only through the dermal route but also via ingestion. However, in solid-substrate assays, soil properties such as pH, organic matter content, and texture can significantly modulate the effect of the substrate. In particular, in BM, toxic components may adsorb onto organic particles, becoming less bioavailable than in BME, where they are fully dissolved and more accessible to the earthworms.

### 3.2. Monitoring of the Vermicomposting System Using BM as Substrate

#### 3.2.1. Temperature

During the vermicomposting process, temperature was measured daily, and day-to-day variations corresponded to changes in ambient temperature. As shown in Figure 2, a slight increase in temperature was observed, which did not exceed 25 °C by the end of the process, with no statistically significant differences among treatments ( $p > 0.05$ ). In other words, the percentage variation in BM content as a feed substrate did not significantly influence the system temperature.

This is a favorable outcome, as temperature increases during vermicomposting, particularly within the thermophilic range ( $>40$  °C), can adversely affect the development of *E. foetida*. Earthworms can develop within a temperature range of 15–25 °C, which ensures their survival, growth, and capacity to convert the substrate into humus [15], a process during which changes in pH also occur.



**Figure 2.** Temperature profile during the vermicomposting process (n = 3).

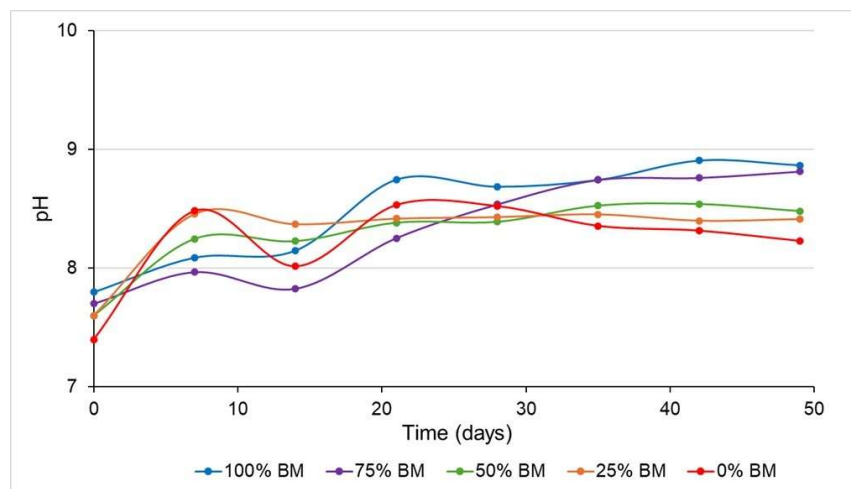
#### 3.2.2. pH

During vermicomposting, pH variations commonly occur over a broad range, from slightly acidic to slightly alkaline (pH 6–8), largely depending on substrate composition [16], [17] and on the predominant microbial populations at each stage of the process [18]. According to several studies, increases in pH may be associated with the formation of ammonium ions ( $\text{NH}_4^+$ ) by ammonifying bacteria during the degradation of nitrogenous organic substrates [19], whereas decreases may result from the nitrification of ammoniacal compounds into nitrates ( $\text{NO}_3^-$ ) and nitrites ( $\text{NO}_2^-$ ), as well as from  $\text{CO}_2$  production and the formation of organic acids due to the combined activity of earthworms and acidogenic microbial communities [20].

In this study, pH evolution during vermicomposting showed that, although the substrates initially exhibited slightly alkaline pH values (7.4–7.8), these progressively increased to a range of 8.23–8.88 by the end of the process. The first sharp increase in pH occurred during the first week, followed by a slight decrease on day 14, which did not alter the overall increasing trend through to the end of the process (Figure 3).

Although the results of this study are not consistent with those reported by Mago et al. [21] and Karapantzou et al. [22], who observed a continuous decrease in pH toward neutrality and slight acidity even after 90 and 120 days, some similarity is found with the results reported by De Medina-Salas et al. [16] for a vermicomposting process using a mixture of orange peel waste, eggshells, and other plant residues. In that study, the waste underwent a six-week pre-composting stage to prevent temperature increases that could negatively affect the performance of *E. foetida* during

vermicomposting. During pre-composting, pH increased from 5.83 to 6.09, and once transferred to the vermicomposting system, values remained within a neutral to slightly alkaline range over seven weeks without a subsequent decrease.



**Figure 3.** pH profile during the vermicomposting process (n = 3).

pH is a key factor determining the outcome of waste decomposition, and there is an interdependent relationship between pH and microbial communities. Although *E. foetida* can tolerate a pH range of 5.0–8.0, maintaining a favorable environment between 7.0 and 8.0 [23] ensures optimal activity in the mineralization and stabilization of residues during the process. Abrupt increases with sustained values above 8.5 are typically detrimental to vermicomposting performance. In the process evaluated in this study, the pH of all treatments remained within the alkaline range (pH 8.0–9.0), which may be attributed to the consumption of organic acids. Treatments containing 100% and 75% BM exhibited critical pH values above 8.5 for a substantial portion of the process, representing unfavorable conditions for *E. foetida* activity, and ultimately reached the highest pH values.

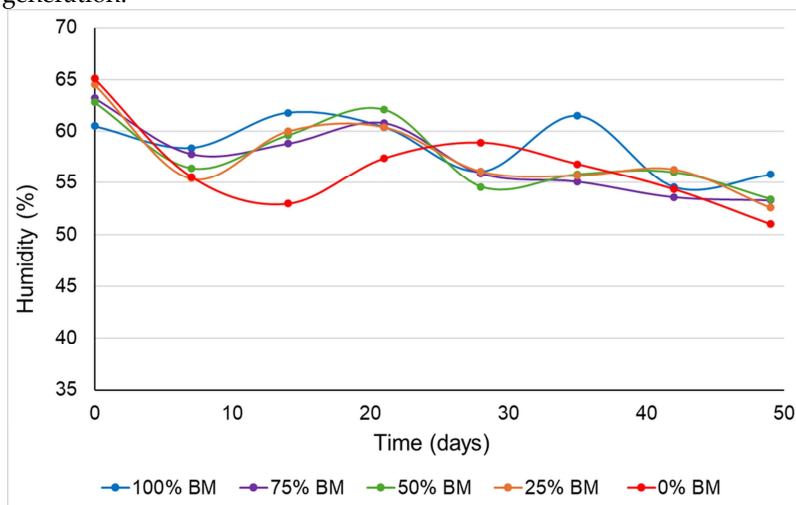
Some authors report that high pH values (as observed in this study) are consistent with the maturation and stabilization stage of the organic substrate, during which microbial communities act in conjunction with *E. foetida* to transform the waste in the vermicomposting process. Karapantzou et al. [22] evaluated physicochemical parameter dynamics during the vermicomposting of winery waste and analyzed the associated bacterial community succession. Similarly, Velásquez-Chávez et al. [18] demonstrated a direct correlation between pH changes and bacterial succession during vermicomposting of cattle manure.

It is important to note that the substrate used in this study (BM) is derived from a controlled bio-oxidation and dehydration process, in which diverse microbial communities contributed to temperature increases essential for biodrying to a moisture level insufficient to sustain metabolic activity. Although BM is microbiologically stabilized due to low water activity ( $a_w$ ), microbial communities (bacteria, yeasts, and fungi) remain in a latent state [4], becoming active once the material is subjected to the conditions of a new bioprocess such as vermicomposting.

### 3.2.3. Moisture

Along with pH, moisture content was determined every 7 days throughout the vermicomposting process, two days after the addition of the feed substrate, once moisture had been homogenized. According to Haukka [15], the optimal substrate moisture for *E. foetida* ranges from 70% to 80%. However, in the present study, the system was managed within a tolerance range rather than for maximum productivity, as the objective was not to obtain biomass (earthworms or cocoons) or high-quality compost, but to maintain a culture under low-moisture conditions. Moisture levels ranged from 55% to 65%, corresponding to the lower limit of the tolerable range (Figure 4). In all

treatments, system moisture resulted from the addition of feed substrate with constant water content (Table 2) and from spraying with 25 mL of water every third day during the process, with no losses due to leachate generation.



**Figure 4.** Moisture profile during the vermicomposting process (n = 3).

Moisture monitoring is important because reduced growth rates in *E. foetida* are likely under low-moisture conditions, which can delay reproduction, as moisture limitations may affect the development of the clitellum, a structure essential for reproduction [24].

It is important to note that the conventional vermicomposting process, as reported by De Medina-Salas [16], involves subjecting waste to a pre-composting stage that may last approximately 45 days and results in a non-stabilized material with high moisture content (80.29%) and acidic pH prior to vermicomposting. In contrast, in the process evaluated in this study, the substrate material originates from a biodrying process, which has been reported in several studies [25, 26], with a duration of 25–30 days, sufficient to obtain a stabilized material with low moisture content and slightly alkaline pH, as described by Orozco-Álvarez et al. [25]. An additional advantage of this approach is that no leachates are generated, either during the initial biodrying stage [27] or during vermicomposting.

### 3.2.4. Survival and Reproduction of *Eisenia foetida*

The results for survival and reproduction of *E. foetida* under the different treatments, evaluated after 49 days of processing, are presented in Table 5. The highest mortality (28.5%) was observed in experimental units fed with 100% BM, followed by the 75% BM treatment with 7.7% mortality, with no escape of individuals recorded. For BM concentrations below 75%, survival was 100%.

**Table 5.** Survival and reproduction of *E. foetida* after the vermicomposting process (n = 3).

Treatment	Live earthworms		Survival (%)	Reproduction (cocoons kg <sub>humus</sub> <sup>-1</sup> )	Offspring (individuals kg <sub>humus</sub> <sup>-1</sup> )*
	Initial	Final			
100% BM		93 ± 6.24	71.5 ± 4.80	28.0 ± 12.16	10.7 ± 6.12 <sup>a</sup>
75% BM		120 ± 6.55	92.3 ± 5.04	23.3 ± 3.06	23.3 ± 5.04 <sup>a</sup>
50% BM	130	130	100	13.3 ± 3.06	23.3 ± 5.02 <sup>a</sup>
25% BM		130	100	15.3 ± 8.08	61.3 ± 33.84 <sup>b</sup>
0% BM		130	100	19.3 ± 5.04	59.3 ± 20.04 <sup>b</sup>

\*Different letters indicate significant differences among treatments (negative binomial GLM, pairwise contrasts, Holm-adjusted p < 0.05).

The generation of cocoons in the experimental units was determined in a 1 kg sample of humus (cocoons  $\text{kg}^{-1}$  humus) as an index of fecundity of earthworms in a vermicomposting system with BM as feedstock. Feeding *E. foetida* exclusively with BM reduced adult earthworm survival; however, the number of cocoons recorded under these conditions was higher than in treatments with lower BM concentrations (50%, 25%, and 0% BM). It should be noted that, during the counting of surviving earthworms, juveniles were observed in treatments with fewer cocoons (50%, 25%, and 0% BM), and their abundance and small size made accurate quantification difficult).

Although moisture was maintained below 80%, the treatments with lower BM concentrations promoted the growth and reproduction of *E. foetida*. This can be interpreted as a relevant finding indicating the robustness of the system when BM is used as a feedstock, where moisture gradually stabilized during the process, allowing *E. foetida* to adapt to system conditions.

Furthermore, these results suggest that the complementary mixture with FOW supplied readily assimilable nutrients and supported the adaptation of *E. foetida*, thereby promoting cocoon hatching. Although cocoon production did not differ significantly among treatments (Poisson GLM,  $p > 0.05$ ), with observed values ranging from 13.4 to 28 cocoons  $\text{kg}^{-1}$  humus, offspring production was significantly influenced by BM concentration (negative binomial GLM,  $p = 0.046$ ). Specifically, offspring production declined with increasing BM concentration, with treatments at 50% through 100% BM yielding significantly fewer offspring (23.3 and 10.7 offspring  $\text{kg}^{-1}$  humus, respectively) than the 0% and 25% BM treatments (59.3 and 61.3 offspring  $\text{kg}^{-1}$  humus), suggesting that BM concentrations above 25% create less favorable conditions for hatching.

This pattern aligns with the findings of Piña-Guzmán et al. [28], who reported that BM application over 21 days did not negatively affect *E. foetida* survival, with rates reaching 99.74% even at 100% BM. Building on this evidence, the present study extended the duration of the bioprocess to 49 days to further assess whether prolonged exposure influences both survival and reproductive outcomes.

The results indicate that prolonged exposure of *E. foetida* to high BM concentrations may negatively affect survival and, consequently, reproduction, as it limits cocoon hatching. Survival varied significantly among treatments (Fisher's exact test,  $p < 0.001$ ), with the 100% BM treatment exhibiting lower survival than all other treatments, and the 75% BM treatment showing reduced survival compared to the 0–50% BM treatments. Gobi and Gunasekaran [29] reported that weight loss, population decline, and minimal or absent cocoon production and hatching in *E. foetida* are negative effects associated with physiological resistance to toxic compounds, which implies an energy trade-off that reduces resources available for reproduction and cocoon hatching. It is possible that certain toxic compounds present in orange peel (e.g., *d*-limonene) were not completely degraded during the biodrying process and therefore adversely affected the survival, reproduction, or cocoon hatching of *E. foetida* [30].

### 3.2.5. Characterization of Vermicompost (Earthworm Humus)

Finally, after 49 days of vermicomposting, the quality of the humus produced by *E. foetida* under the different treatments was evaluated, focusing primarily on the physicochemical parameters established by NMX-FF-109-SCFI-2008 [14]. Table 6 presents the evaluated parameters and the corresponding results.

**Table 3.** Physicochemical specifications for the characterization of earthworm humus.

Parameter	NMX-FF-109-SCFI-2008 [14]	Treatment				
		100% BM	75% BM	50% BM	25% BM	0% BM
pH	5.5-8.5	8.5 ± 0.19	8.5 ± 0.04	8.1 ± 0.06	8.4 ± 0.02	8.2 ± 0.01
Moisture*	20-40%	51.7 ± 1.7	52 ± 0.9	44.6 ± 2.1	45.7 ± 4.4	39.2 ± 1.5
Electrical conductivity (dS m <sup>-1</sup> )	≤4	0.052	0.042	0.042	0.049	0.052
Bulk Density (g ml <sup>-1</sup> )	0.40-0.90	0.68	0.61	0.65	0.67	0.68
Organic matter (%w.b.)**	20-50%	11.83 ± 0.4 <sup>a</sup>	23.42 ± 1 <sup>b</sup>	22.87 ± 2 <sup>b</sup>	25.78 ± 0.7 <sup>b</sup>	23.58 ± 0.9 <sup>b</sup>
Total Nitrogen (%)	1-4%	1.34 ± 0.09	1.13 ± 0.07	1.27 ± 0.1	1.13 ± 0.09	1.37 ± 0.07
C/N Ratio***	≤20	5.21 ± 0.42	12.49 ± 1.00	11.24 ± 1.39	13.47 ± 1.16	10.55 ± 0.70

\* Some plant-derived materials have a higher hygroscopic capacity than those produced from animal-derived residues; therefore, for this case, moisture values up to 60% are acceptable. \*\* Different letters indicate significant differences among treatments (one-way ANOVA, Tukey's HSD,  $p < 0.05$ ). \*\*\* Carbon-to-nitrogen (C/N) ratio calculated from mean carbon and nitrogen concentrations. Uncertainty represents propagated measurement error from triplicate C and N determinations.

According to the results shown in Table 6, the physicochemical parameters varied among treatments. Regarding moisture, treatments with higher BM content (100% and 75%) exhibited higher moisture levels in the produced humus (51.7% and 52%, respectively) compared to treatments with lower BM content. It is noteworthy that the BM used in the vermicomposting process exhibited a water retention capacity of 46.07%, indicating that the transformation of BM and FOW into humus improves the moisture retention of the final product. Similarly, total nitrogen was affected by treatment (one-way ANOVA,  $F(4, 10) = 4.37$ ,  $p = 0.027$ ,  $\eta^2 = 0.636$ ); however, Tukey's HSD post hoc test did not identify significant pairwise differences, likely due to its more conservative nature relative to the overall ANOVA.

The pH of the humus was close to the upper limit established by the standard; however, the application of alkaline amendments to soil can be beneficial, as crops generally respond favorably to pH conditions ranging from slightly acidic to alkaline [22].

For humus derived from treatments with BM concentrations below 100%, the organic matter (OM) content was high relative to the values established in NOM-021-RECNAT-2000 [13], while remaining within the range specified by NMX-FF-109-SCFI-2008 [14]. OM content varied significantly among treatments (one-way ANOVA,  $F(4, 10) = 62.44$ ,  $p = 4.91 \times 10^{-7}$ ,  $\eta^2 = 0.962$ ), with Tukey's HSD post hoc analysis indicating that the 100% BM treatment (11.83 ± 0.4) had significantly lower OM than all other treatments (23.98–25.78%, CI ranges overlapping), which did not differ from one another. In vermicomposting systems, humus is generally expected to contain 20–40% organic matter to be suitable as a soil amendment. This indicates that the compost has undergone maturation and, when applied, can enhance water retention, maintain soil moisture, support microbial nutrient availability, and improve soil structure and aeration. In this study, considering biodrying as a pretreatment process for organic waste, the humus from the 100% BM treatment exhibited the lowest OM content (11.83%), consistent with findings by Usta and Guven [31], who reported lower organic matter content in vermicompost derived from pre-composted pruning and garden waste.

The C/N ratio is an important parameter in vermicomposting, as it reflects the rate of organic matter decomposition, indicating both the loss of organic carbon through mineralization and the relative increase in nitrogen during organic matter degradation [32]. Ratios below 15 are indicative of mature and stable vermicompost. In this study, the C/N ratio ranged from 5.21 (100% BM) to 13.47 (25% BM), with all values well below the ≤20 threshold established by NMX-FF-109-SCFI-2008. The 100% BM treatment exhibited a markedly lower C/N ratio (5.21 ± 0.42) compared to the other treatments, which ranged from 10.55 ± 0.70 (0% BM) to 13.47 ± 1.16 (25% BM), with their uncertainty intervals not overlapping suggesting differences. These results confirm that the humus reached

maturity and can be safely applied as an organic amendment without posing risks to plants or negatively affecting soil quality

#### 4. Conclusions

This study demonstrated that the use of biodried material (BM) in a vermicomposting process is viable when combined with fresh organic waste at proportions below 75% BM. Under these conditions, the survival and reproduction of *E. foetida* were supported, with appropriate pH and temperature conditions and moisture levels ranging from 55% to 65%, allowing the bioprocess to proceed effectively and yielding high-quality humus without leachate generation. In contrast, BM used as the sole substrate exhibited slight toxicity, as it reduced the physiological capacity of earthworms for survival and reproduction.

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#### Abbreviations

The following abbreviations are used in this manuscript:

BM	Biodried Material
FOW	Fresh Organic Waste
BME	Biodried Material Extract
OM	Organic Matter
d.b.	Dry basis
w.b.	Wet basis

#### References

1. Instituto Nacional de Estadística y Geografía (INEGI). *Censo Agropecuario 2022: Resultados definitivos*; INEGI: Ciudad de México, México, 2023. Available online: [https://www.inegi.org.mx/contenidos/programas/ca/2022/doc/ca2022\\_rdNAL.pdf](https://www.inegi.org.mx/contenidos/programas/ca/2022/doc/ca2022_rdNAL.pdf)
2. González-Cárabes, R.; López-Sosa, L.B.; López-Mercado, J.; Rutiaga-Quiñones, J.G.; Reynoso-Marín, F.J.; Pintor-Ibarra, L.F.; Ascencio de la Cruz, L.Á.; Morales-Máximo, M.; Aguilera-Mandujano, A.; Hernández-Trujillo, S.L. Exploring the energy potential of agricultural and agroindustrial residues in Michoacán: Characterization to determine the feasibility of solid biofuels. *Energy Convers. Manag.* **X** **2026**, *30*, 101626. <https://doi.org/10.1016/j.ecmx.2026.101626>
3. Díaz-Molina, N.A.; Sosa-Olivier, J.A.; Laines-Canepa, J.R.; Silvan, R.S.; Figueiras-Jaramillo, D.A. Evaluation of the bioenergy potential of agricultural and agroindustrial waste generated in southeastern Mexico. *AIMS Energy* **2024**, *12*, 984–1009. <https://doi.org/10.3934/energy.2024046>

4. Orozco-Álvarez, C.; Gervacio-Hernández, A.; Moreno-Rivera, M.L.; Piña-Guzmán, B.; Robles-Martínez, F. Microbiological and physicochemical characterization during biodrying of organic solid waste. *Processes* **2025**, *13*, 78. <https://doi.org/10.3390/pr13010078>
5. Contreras-Cisneros, R.M.; Robles-Martínez, F.; Franco-Hernández, M.O.; Piña-Guzmán, A.B. Use of biodried organic waste as a soil amendment: Positive effects on germination and growth of lettuce (*Lactuca sativa* L., var. Buttercrunch) as a model crop. *Processes* **2025**, *13*, 2285. <https://doi.org/10.3390/pr13072285>
6. Windarto, S.; Rachmawati, D.; Amalia, R.; Putri, D.A. Vermicompost as a sustainable biofertilizer enhances growth and antioxidant properties of the edible seaweed *Caulerpa racemosa*. *Biocatal. Agric. Biotechnol.* **2026**, *73*, 103965. <https://doi.org/10.1016/j.bcab.2026.103965>
7. Raza, S.T.; Wu, J.; Rene, E.R.; Ali, Z.; Chen, Z. Reuse of agricultural wastes, manure, and biochar as an organic amendment: A review on its implications for vermicomposting technology. *J. Clean. Prod.* **2022**, *360*, 132200. <https://doi.org/10.1016/j.jclepro.2022.132200>
8. Román, P.; Martínez, M.M.; Pantoja, A. *Manual de Compostaje del Agricultor: Experiencias en América Latina*; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2013.
9. Escobar-Sánchez, O.E. *Aplicación de material biosecado como alternativa al uso de composta como enmienda orgánica en el suelo*. Tesis de Maestría en Ciencias en Bioprocesos, Instituto Politécnico Nacional, México, 2025.
10. Testón, R.J.; Santiyán, M.M. Desarrollo del ensayo de toxicidad estandarizado OECD No. 207 en lombriz de tierra expuesta al pesticida organofosforado dimetoato. **2019**, *36*.
11. DOF. *Norma Oficial Mexicana NOM-141-SEMARNAT-2003*; Diario Oficial de la Federación: Ciudad de México, México, 2004.
12. SADER. *Manuales prácticos para la elaboración de bioinsumos 14: Humus de lombriz*; Secretaría de Agricultura y Desarrollo Rural: Ciudad de México, México, 2022. Available online: <https://www.gob.mx/produccionparaelbienestar>
13. DOF. *Norma Oficial Mexicana NOM-021-RECNAT-2000*; Diario Oficial de la Federación: Ciudad de México, México, 2002.
14. DOF. *Norma Mexicana NMX-FF-109-SCFI-2008*; Diario Oficial de la Federación: Ciudad de México, México, 2008.
15. Haukka, J.K. Growth and survival of *Eisenia fetida* (Sav.) (Oligochaeta: Lumbricidae) in relation to temperature, moisture and presence of *Enchytraeus albidus* (Henle). *Biol. Fertil. Soils* **1987**, *3*, 157–162.
16. De Medina Salas, L.; Giraldi Díaz, M.R.; Castillo González, E.; Morales Mendoza, L.E. Valorization of orange peel waste using precomposting and vermicomposting processes. *Sustainability* **2020**, *12*, 7626. <https://doi.org/10.3390/su12187626>
17. Dube, B.; Chimdessa, D.; Sori, G. Preparation and characterization of vermicompost nutrients made from different sources of organic materials. *Sci. J. Anal. Chem.* **2025**, *13*, 16–24. <https://doi.org/10.11648/j.sjac.20251301.13>
18. Velásquez-Chávez, T.E.; Sáenz-Mata, J.; Quezada-Rivera, J.J.; Palacio-Rodríguez, R.; Muro-Pérez, G.; Servín-Prieto, A.J.; Hernández-López, M.; Preciado-Rangel, P.; Salazar-Ramírez, M.T.; Ontiveros-Chacón, J.C.; García-De la Peña C. Bacterial and physicochemical dynamics during the vermicomposting of bovine manure: A comparative analysis of the *Eisenia fetida* gut and compost matrix. *Microbiol. Res.* **2025**, *16*, 177. <https://doi.org/10.3390/microbiolres16080177>
19. Rékási, M.; Mazsu, M.; Draskovits, E.; Bernhardt, B.; Szabó, A.; Rivier, P.A.; Farkas, C.; Borsányi, B.; Pirkó, B.; Molnár, S.; *et al.* Comparing the agrochemical properties of compost and vermicomposts produced from municipal sewage sludge digestate. *Bioresour. Technol.* **2019**, *291*, 121861. <https://doi.org/10.1016/j.biortech.2019.121861>
20. Ananthavalli, R.; Ramadas, V.; John Paul, J.A.; Karunai Selvi, B.; Karmegam, N. Seaweeds as bioresources for vermicompost production using the earthworm *Perionyx excavatus* (Perrier). *Bioresour. Technol.* **2019**, *275*, 394–401. <https://doi.org/10.1016/j.biortech.2018.12.091>
21. Mago, M.; Gupta, R.; Yadav, A.; Kumar Garg, V. Sustainable treatment and nutrient recovery from leafy waste through vermicomposting. *Bioresour. Technol.* **2022**, *347*, 126390. <https://doi.org/10.1016/j.biortech.2021.126390>

22. Karapantzou, I.; Mitropoulou, G.; Prapa, I.; Papanikolaou, D.; Charovas, V.; Kourkoutas, Y. Physicochemical changes and microbiome associations during vermicomposting of winery waste. *Sustainability* **2023**, *15*, 7484. <https://doi.org/10.3390/su15097484>
23. Pérez-Godínez, E.A.; Lagunes-Zarate, J.; Corona-Hernández, J.; Barajas-Aceves, M. Growth and reproductive potential of *Eisenia foetida* (Sav) on various zoo animal dungs after two methods of pre-composting followed by vermicomposting. *Waste Manag.* **2017**, *64*, 67–78. <https://doi.org/10.1016/j.wasman.2017.03.036>
24. Reinecke, A.J.; Venter, J.M. Moisture preferences, growth and reproduction of the compost worm *Eisenia foetida*. *Biol. Fertil. Soils* **1987**, *3*, 135–141.
25. Orozco-Álvarez, C.; Díaz-Megchun, J.; Macías-Hernández, M.J.; Robles-Martínez, F. Efecto de la frecuencia de volteo en el biosecado de residuos sólidos orgánicos. *Rev. Int. Contam. Ambient.* **2019**, *35*, 979–989. <https://doi.org/10.20937/RICA.2019.35.04.16>
26. Contreras-Cisneros, R.M.; Orozco-Álvarez, C.; Piña-Guzmán, A.B.; Ballesteros-Vásquez, L.C.; Molina-Escobar, L.; Alcántara-García, S.S.; Robles-Martínez, F. The relationship of moisture and temperature to the concentration of O<sub>2</sub> and CO<sub>2</sub> during biodrying in semi-static piles. *Processes* **2021**, *9*, 520. <https://doi.org/10.3390/pr9030520>
27. Yang, B.; Zhang, L.; Jahng, D. Importance of initial moisture content and bulking agent for biodrying sewage sludge. *Dry. Technol.* **2014**, *32*, 135–144. <https://doi.org/10.1080/07373937.2013.795586>
28. Piña-Guzmán, A.B.; Robles-Martínez, F.; Sarmiento-Vargas, M.A.; Melo-Vargas, K.G. Uso potencial de residuos orgánicos biosecados como alimento para lombriz de tierra (*Eisenia foetida*). In *Memorias del XXXVI Congreso Interamericano de Ingeniería Sanitaria y Ambiental*; Guayaquil, Ecuador, 28–31 October 2018.
29. Gobi, M.; Gunasekaran, P. Effect of butachlor herbicide on earthworm *Eisenia foetida*: Its histological perspicuity. *Appl. Environ. Soil Sci.* **2010**, *2010*, 850758. <https://doi.org/10.1155/2010/850758>
30. Karr, L.; Drewes, C.; Coats, J. Toxic effects of d-limonene in the earthworm *Eisenia foetida* (Savigny). *Pestic. Biochem. Physiol.* **1990**, 175–186.
31. Usta, A.N.; Guven, H. Vermicomposting organic waste with *Eisenia foetida* using a continuous flow-through reactor: Investigating five distinct waste mixtures. *J. Environ. Chem. Eng.* **2024**, *12*, 114384. <https://doi.org/10.1016/j.jece.2024.114384>
32. Nazeri, R.; Esmailzadeh, L.; Karimi Torshizi, M.A.; Seidavi, A.; Zangeronimo, M.G. Use of earthworm meal with vermi-humus in diet for laying quail. *Pesqui. Agropecu. Bras.* **2021**, *56*, e02453. <https://doi.org/10.1590/S1678-3921.PAB2021.V56.02453>

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