

ORGANIC WASTE BIOCONVERSION AND WEIGHT GAIN DYNAMICS OF THE BLACK SOLDIER FLY *Hermetia illucens*

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ABSTRACT

The anaerobic decomposition of urban organic wastes produces significant amounts of environmentally harmful biogases and leachates. In the context of a circular economy, the valuation of these wastes is an alternative for producing environmental and economic benefits. *Hermetia illucens* larvae digest a wide range of organic materials, converting them into high-value protein, oils and organic fertilizer. The bioconversion and weight gain dynamics of larvae fed with household waste (HW), restaurant waste (RW), pig manure (PM) and bovine manure (BM) were measured. By fitting the data to the logistical model, rates of larval weight gain and waste decomposition were calculated. The highest weight gain was obtained with RW ($0.315 \pm 0.090 \text{ g (g}^{-1} \text{ d}^{-1})$), followed by BM ($0.214 \pm 0.054 \text{ g (g}^{-1} \text{ d}^{-1})$), PM ($0.213 \pm 0.064 \text{ g (g}^{-1} \text{ d}^{-1})$), and HW ($0.199 \pm 0.064 \text{ g (g}^{-1} \text{ d}^{-1})$). The larvae decomposed PM, RW, and HW with the same efficiency ($0.72 - 0.85 \text{ g d}^{-1}$) and faster than BM (0.53 g d^{-1}). The data adjustment to the logistical model helped analyze the weight gain dynamics of the larvae, as well as the bioconversion of wastes into biomass and the waste decomposition. This information provides criteria for optimizing organic waste management.

Keywords: Greenhouse gases, ecotechnologies, bioaccumulation, growth rate, decomposition rate.

INTRODUCTION

Agrifood systems make up all the processes involved in the production of food and its use. These processes require inputs and result in the production of food, raw materials, services, economic income, as well as a variety of social and environmental effects (Berkum *et al.*, 2018). Organic waste generated by the processing of crops, manure, and food is a problem with a significant environmental impact. One-third of all food produced for human consumption is estimated to be wasted every year (FAO, 2013). According to UNEP statistics (2016), agrifood systems contribute to the degradation and depletion of natural resources in the world due to inefficient and unsustainable practices. It is estimated that 30 % of the greenhouse gas (GG) emissions worldwide (Berkum *et al.*, 2018) come from such agrifood systems.

Citation: Ramírez-Méndez VA, Guzmán-Plazola RA, Pérez-Panduro A, Sánchez-Escudero J. 2022. Organic waste bioconversion and weight gain dynamics of the black soldier fly *Hermetia illucens*. *Agrociencia*. <https://doi.org/10.47163/agrociencia.v56i8.2635>

Editor in Chief:
Dr. Fernando C. Gómez Merino

Received: April 05, 2022.
Approved: September 22, 2022.
Published in Agrociencia:
January 11, 2023.

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Cities are the main waste-producing centers, due to the concentration of people and their economic activity. In 2012, the global production of municipal solid waste was approximately 1.3 billion Mg per day, with this figure expected to rise to 2.2 billion by 2025 (Bernache-Pérez, 2012; Hoornweg and Bhada-Tata, 2012). The organic fraction of total waste produced is higher in developed nations than in developing countries. This waste is degraded by anaerobic decomposition in open-air landfills, which release methane, CO₂, and leachates into the environment (Acurio *et al.*, 1997; IPCC, 2015).

Diminished agrifood waste could substantially reduce the emissions of greenhouse gases and contribute to the UN's Sustainable Development Goals (IPCC, 2014; UN, 2016). However, changes in consumption patterns and diets would be required, which could be difficult to generalize. The circular economy model, originated in China in 1998 (Yuan *et al.*, 2006), proposes the use of ecotechnologies for the treatment and transformation of agrifood waste.

Proposal designs under this model require technical data on the rates of material and energy between the stages and actors in the recycling processes (Fischer-Kowalski and Haberl, 2015). Likewise, production cycles are optimized when using waste to produce goods and services that benefit of other economic areas. In this context, organic and food waste treatment using black soldier flies (BSF) *Hermetia illucens* to produce compost, oils, protein-rich pastes for animal feed, among other products, promises economic and environmental benefits (FAO, 2013; Salomone *et al.*, 2017). The BSF is studied around the world for its ability to process a wide range of organic waste and its high rate of bioconversion into usable biomass (Sheppard, 1983; Tomberlin and Sheppard, 2002; Diener *et al.*, 2009; Holmes *et al.*, 2016). Due to the composition of its intestinal microbiota, larvae digest a large variety of food substrates, including the remains of decomposing plants, manure, flours, municipal organic waste, carcasses, fecal sludge, and others (Üstüner *et al.*, 2003; Banks, 2014). When the larvae reach a pre-pupal stage, they have accumulated protein (36–48 %), fat (31–33 %) and calcium (7.5 %) in highly nutritional forms for animals, making them a desirable alternative for livestock and fishing food production (Arango *et al.*, 2004; Diener *et al.*, 2011).

The quantification of the bioconversion of substrates by BSF larvae is essential to estimate its potential to transform organic residues. The present work reports on the bioconversion dynamics of various organic wastes and weight gain by *H. illucens* until it reaches the pupal stage, under the hypothesis that all substrates are susceptible to decomposition by the larvae and that their bioconversion rates may vary from one substrate to another. *H. illucens* larvae demonstrated the ability to decompose the four substrates evaluated, with variations in decomposition efficiency and bioaccumulation.

MATERIALS AND METHODS

BSF larvae and wastes evaluated

The larvae used were two to three weeks old (fourth and fifth instar) and weighted less than 0.09 g when collected from an insect farm located in Morelos, Mexico. Five

groups of larvae were formed, and each one was assigned a treatment at random: 1) household waste (remains of fruits, vegetables, egg shells and tortilla, all with seven days of decomposition); 2) restaurant waste (fruits, vegetables, legumes, rice, bread, tortilla, cooked egg and meat, all with two days of decomposition); 3) pig manure from a gestating and lactating swine stable; 4) bovine manure obtained from the Colegio de Postgraduados stables; and 5) control. Household and restaurant wastes were ground up and wrung by hand with a piece of cloth, then mixed until its consistency was homogenous. The wastes were refrigerated inside appropriately labelled bags to facilitate their handling and dosifying during the bioassay. The experiment was carried out in 2019.

Experimental procedure

For each treatment, ten BSF larvae were individually confined in Petri dishes and kept in an incubator at 28 ± 0.5 °C and an RH of 67 ± 3.8 %. In addition, the same food was served a group of ten larva-free Petri dishes under the same conditions. Every 48 hours a new serving of food was given (2.5 to 3.0 g) to the larvae in each treatment and the control group. The leftover food was weighed along with the excretions in each dish (final weight) and the larva itself at each change of serving, using an analytical scale (Scientech ZSA 120 with a precision of 1.0 ten-thousandth of a gram). The weights were measured 12 times over the course of 24 days. The apparent consumption was assumed to be the difference in weight between the beginning and end of each period (*initial weight – final weight*), considering the loss of humidity of the substrate due to evaporation. Therefore, the effective consumption in each period was calculated using the data for lost humidity from the control group.

Estimation of rates and statistical analysis

Using the effective consumption data (decomposition) and the weight gained by the larvae, the accumulation curves were obtained for each treatment. The decomposition was calculated using the weight difference in the Petri dish (*initial residue weight*) - (*remaining weight + larval excretions*). The value was calculated for parameter *r*, which represents the rate of weight gain in the larva (Table 1) and residual decomposition.

Table 1. Growth models adjusted to waste decomposition and weight gain by the *Hermetia illucens* larvae.

Model	Formula
Exponential	$y = y_0 * e^{(r * t)}$
Logistic	$y = K / (1 + ((k - y_0) / y_0) * e^{(-r * t)})$
Gompertz	$y = e^{(-\log(y_0)) * e^{(-r * t)}}$
Monomolecular	$y = 1 - (K - y_0) * e^{(-r * t)}$

r: growth rate; *y*₀: initial weight; *t*: days; *k*: maximum capacity of weight gain and decomposition.

The weight loss data for the pre-pupae were adjusted to the equation $y = t / (A + (B * t))$, where A indicates the intensity of reduction in fresh weight and B the speed of weight stabilization of the larva before it pupates. Using the MODEL procedure in the SAS v. 9.3 statistical program (The SAS Institute, Cary, North Carolina), the adjustment was made to determine the parameter values (r , y_0 , A and B). Likewise, its descriptive statistics (means and variances) were calculated, along with the normality and variance homogeneity tests, the ANOVAs or Wilcoxon and Kruskal-Wallis tests (as appropriate), as well as Tukey's multiple comparisons, with $\alpha = 0.05$ in all cases.

RESULTS AND DISCUSSION

The larvae fed with restaurant waste (RW) reached a maximum average weight of 333.2 ± 73.8 mg; household waste (HW) had a weight of 293.2 ± 61.5 mg; bovine manure (BM), 270.7 ± 43.2 mg; and for pig manure (PM), 246.8 ± 51.6 mg. The only statistically significant differences were between RW and PM (Tukey, $p = 0.05$). The *H. illucens* larvae reached a fresh weight higher than those reported in other studies, where they were fed with different substrates and reached a weight of 69 to 263 mg per larva (Diener *et al.*, 2009; Ferdousi *et al.*, 2020; Jucker *et al.*, 2017; Oonincx *et al.*, 2015a; Oonincx *et al.*, 2015b; Nguyen *et al.*, 2013; Nguyen *et al.*, 2015).

Weight gain dynamic in individual larvae

Out of the four mathematical models evaluated, the logistic growth model had the best adjustment. Its determination coefficient (R^2) was the highest and had high significance in its parameter hypothesis tests (y_0 and r) (Data not shown). Taking the highest values observed as a basis, the adjustment of the model was compared using values of $K = 0.4$ and $K = 0.5$, obtaining a better adjustment with $k = 0.4$, where K represents the limit or highest possible weight (g) after which the larva stops gaining weight reaching the pre-pupa stage. The ANOVA performed on the r values indicated significant differences between treatments. The highest value of this parameter was obtained with the RW treatment, followed by BM, PM, and finally, HW (Table 2).

Table 2. Parameters of the Logistic model[†] estimated for the dynamics of fresh weight gain for *Hermetia illucens* larvae with different food sources. Initial weight of larvae < 0.090 g.

Food source	Parameter	Estimated parameter average [‡]	Parameter	Estimated parameter average [§]	Range of values of R^2
HW	r	0.199 ± 0.064 b	y_0	0.088 ± 0.026	0.681 – 0.992
RW	r	0.315 ± 0.090 a	y_0	0.080 ± 0.030	0.882 – 0.988
PM	r	0.213 ± 0.064 b	y_0	0.081 ± 0.020	0.901 – 0.987
BM	r	0.214 ± 0.054 b	y_0	0.076 ± 0.013	0.899 – 0.979

[†]Estimated for a value $k = 0.4$ g. HW: household waste; R: restaurant waste; PM: pig manure; BM: bovine manure. [‡]All the averages of the estimators are adjusted to a normal distribution, according to the Shapiro-Wilk test. r is given in grams of fresh weight ($\text{g} \cdot \text{day}^{-1}$). [§]Means with the same letter are statistically equal (Tukey $P \alpha = 0.05$; $F = 5.37$, $\text{Prob} > F = 0.0041$).

The K value, derived from the logistic growth model, helps define that BSF larvae can gain up to 0.4 g in weight. More than identifying a minimum weight required to initiate the pupa formation and metamorphosis processes suggested by Nijhout and Williams (1974), the K value helps identify when the larvae have reached their maximum gain of biomass, which is critical for its economic use as a food and/or energy resource.

The general fresh weight gain dynamics in *H. illucens* larvae in each treatment helps define the point at which the larvae reach their maximum daily weight gain capacity (Figure 1A), which occurs in $K/2$ (0.2 g). From that point forward, the daily gain in biomass in the larva gradually decreases until it reaches zero in the pre-pupa stage. The larvae fed with RW reached a maximum weight gain capacity of 0.061 g d⁻¹ two

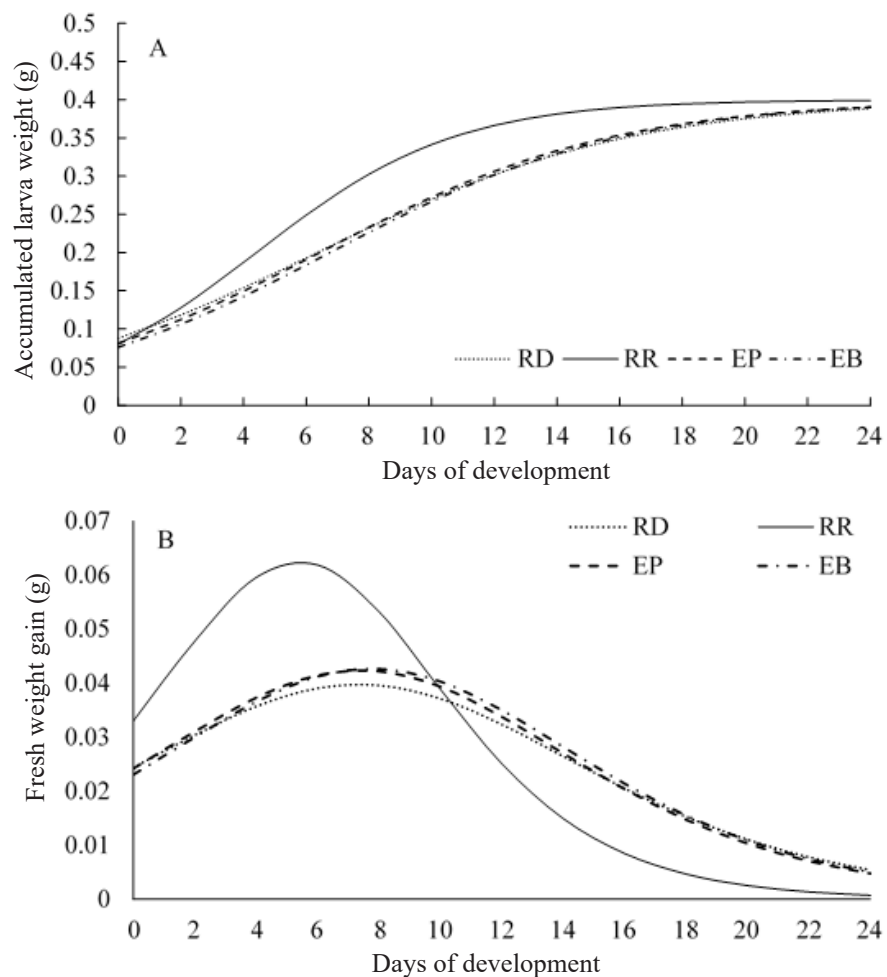


Figure 1. Estimation of the general dynamics of biomass accumulation (A) and daily weight gain (B) in the *Hermetia illucens* larvae, according to the logistic model $y = k / (1 + ((k - y_0) / y_0) * e^{-r * t})$, in four treatments. HW: household waste; R: restaurant waste; PM: pig manure; BM: bovine manure. Initial larval weight < 0.090 g. For the parameter values, see Table 2.

days before the larvae fed with other residues (HW, PM and BM), which reached a maximum weight gain capacity around 0.040 g d^{-1} . In addition, the larvae fed with RW reached their maximum gain five days before the larvae fed with other treatments (HW, PM and BM) (Figure 1B), indicating that they reach their maximum weight several days before they do with the other wastes.

There is a clear difference between the dynamics of weight gain in larvae fed with RW in comparison to the other three treatments (Figure 1), which could be attributed to this type of organic residue having a greater diversity of components and, presumably, a higher protein and lipid content than fruit and vegetable waste (Gold *et al.*, 2018). Even if restaurant waste contains no animal residues, they provide a greater amount of raw protein and specific amino acids than the fruit and vegetable waste, according to Spranghers *et al.* (2016). Under optimal environmental conditions and with sufficient RW availability, larvae raised with such wastes would be expected to reach a fresh weight of 0.4 g.

Using the values determined when adjusting the data to the logistic model, the weight gain capacity of the larvae before its establishment in the experiment was projected (Figure 2A). Under the same considerations, the general fresh weight gain dynamics of larvae during their entire development, feeding on the same wastes and in the same environmental conditions, were displayed in a graph (Figure 2B). The values of day 18 correspond with the values of day 0 in the previous graphs (Figure 1), confirming that the larvae were between 15 and 20 days old.

According to the logistic model, *H. illucens* larvae's daily weight gain capacity is not static, but rather dynamic and varies with time. That is, depending on the larva's actual size and weight or, in other words, on its physiological ability to gain weight depending as it ages. According to the projection based on the logistic model's parameters, under optimal environmental conditions, the larvae would develop completely in approximately 42 days, when it reaches the pre-pupa and pupal stages (Nguyen *et al.*, 2013). In addition, the larvae would reach a usable optimum weight approximately one month after eclosion (RW: 32 d; HW, PM and BM: 37 d).

Weight decline and stabilization in individual larvae

According to the observations, when the larvae reach the sixth instar (pre-pupa), their development stops to allow pupa formation. In this period, the fresh weight of the larvae undergoes a sharp decline, followed by a stabilization that lasts until the pupa is formed. Although the model used to describe this dynamic displays a high R^2 value (Table 2), none of its parameters could be accurately estimated, nor did its estimator display a normal distribution according to the Shapiro-Wilk test (Figure 3).

In the RW treatment, a higher value was obtained for *A* and a slightly lower one for *B* in comparison to the other treatments, indicating that the weight loss in larvae was lower in this treatment and the speed of stabilization did not differ significantly (Table 2). The general dynamics of larval weight decline and stabilization for each treatment are also noticeable, with day two corresponding to the start of the decline (Figure 3).

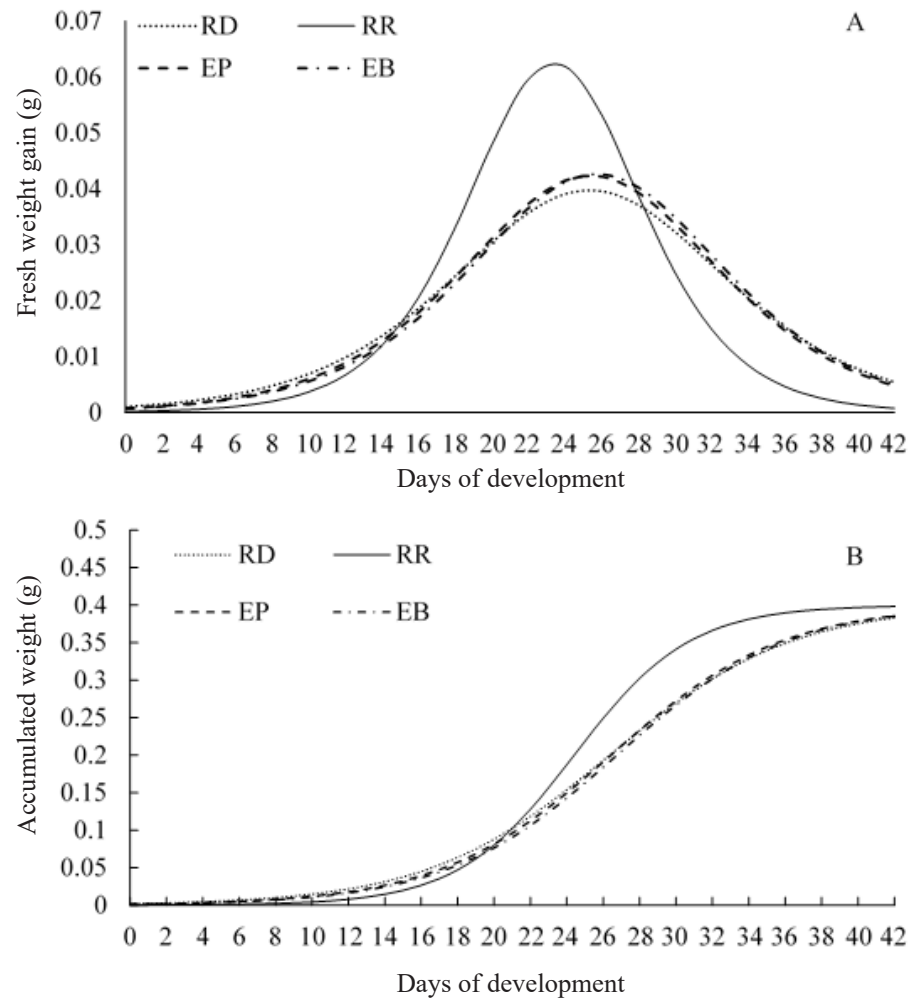


Figure 2. Projection of the daily weight gain dynamics (A) and accumulation of total weight (B) during the whole larval stage of *H. illucens*, using the parameters estimated by the logistic model $y = k / (1 + ((k - y_0) / y_0) * e^{-r * t})$. HW: household weight; RW: restaurant waste; PM: pig manure; BM: bovine manure.

The weight decline and stabilization model show the efficiency that exists in the use of food during larval development when the nourishment comes from a fresher, more diverse source, ensuring a greater conservation of fresh weight when going from a larva to pre-pupa. Although the reduction is between 10 and 20 %, it takes place only 2 d after the larva reaches its maximum weight.

Decomposition of organic wastes by individual larvae

The logistic growth model displayed a better adjustment to the consumption data of the different substrates. It had the highest R^2 , as well as the highest significance in the test of its parameters' hypotheses (y_0 and r) (data not shown). This model was

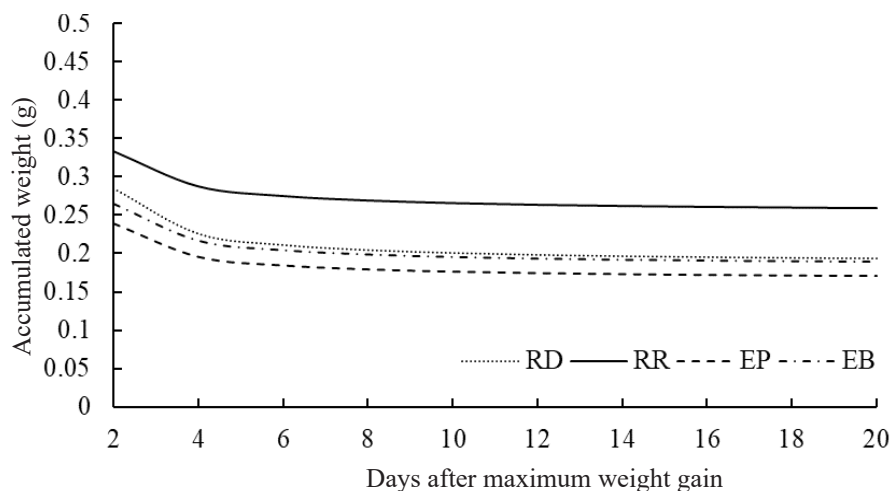


Figure 3. General dynamic of weight decline in *H. illucens* larvae with four treatments using the model $y = t / (A + (B * t))$. HW: household waste; RW: restaurant waste; PM: pig manure; BM: bovine manure.

evaluated with the values of $K = 5$, $K = 5.5$, and $K = 6$, with $K = 5.5$ providing the best adjustment. K represents the highest possible waste decomposition rate per larva in g of fresh weight. Despite the variance between the means of the statistic r being homogenous according to the Bartlett test, the residuals of the analysis did not display any normality (Table 3). Therefore, Wilcoxon rank-sum non-parametric tests were carried out, which gave evidence to show that the parameter EB had a lower sum of scores, and the Kruskal-Wallis test, which gave a highly significant value for $P = 0.0013$ (Table 4).

The substrate decomposition dynamics by the larvae that were given PM, RW and HW were very similar and higher than the capacity of the larvae that were given BM (Figure 4A). According to the logistic model, the greatest capacity of the larvae to decompose

Table 3. Parameters of the logistic model[†] estimated for the dynamic of decomposition (fresh weight) of food from different sources by the *Hermetia illucens* larvae.

Food source	Parameter	Estimated parameter average [‡]	Parameter	Estimated parameter average	Range of values of R^2
HW	r	0.264 ± 0.053 ab	y_0	0.456 ± 0.067	0.853 – 0.981
RW	r	0.299 ± 0.067 a	y_0	0.401 ± 0.097	0.871 – 0.964
PM	r	0.316 ± 0.046 a	y_0	0.434 ± 0.063	0.843 – 0.948
BM	r	0.194 ± 0.062 b	y_0	0.459 ± 0.130	0.732 – 0.959

[†]Estimated for a value of $k = 5.5$ g. HW: Household waste; RW: Restaurant waste; PM: pig manure; BM: bovine manure; r : grams of waste decomposed (g day)⁻¹. [‡]Means with the same letter are statistically equal (Tukey $P \alpha = 0.05$).

Table 4. Comparison test for the medians of the rate r of the logistic model[†], estimated for the dynamic of degradation in fresh weight of wastes from different sources, by the *Hermetia illucens* larvae.

Food source	Parameter	Estimated parameter median [‡]	Coefficient of variation	Final Wilcoxon score [§]
HW	r	0.257	20.15	158
RW	r	0.278	22.49	203
PM	r	0.287	14.63	236
BM	r	0.175	32.03	69

[†]Estimated for a value of $k = 5.5$ g. HW: Household waste; RW: Restaurant waste; PM: pig manure; BM: bovine manure; [‡] r : grams of waste decomposed (g day)⁻¹. [§]Highly significant difference between treatments, according to the Kruskal-Wallis Pr test = 0.0013.

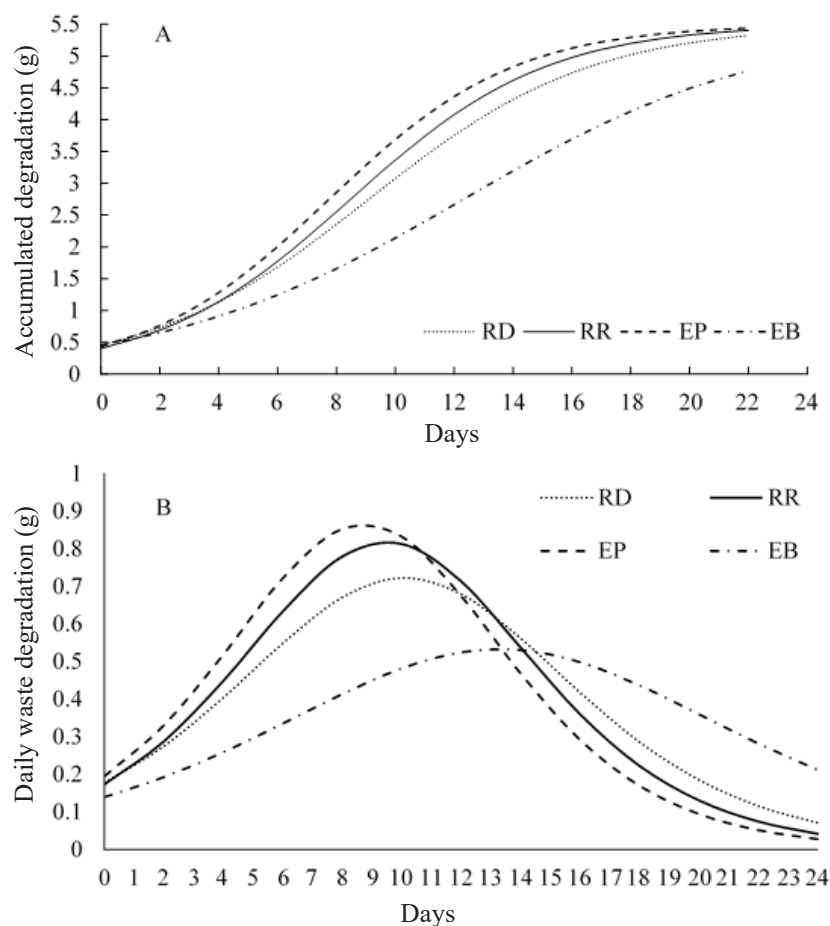


Figure 4. Dynamic of total decomposition (A) and daily decomposition (B) of waste by *H. illucens* larvae. The data were adjusted to a logistic model $y = k / (1 + ((k - y_0) / y_0) * e^{(-r * t)})$. HW: household waste; RR: restaurant waste; PM: pig manure; BM: bovine manure.

the wastes takes place when the value of $K/2$ is reached on day 8 of our observations, which corresponds to an accumulated consumption of 2.75 g. Degradation continues at a decreasing rate until it reaches values near 0, when the larva has completed its development and the pre-pupa stage begins. In the PM treatment, the larvae achieve their highest decomposition rate (0.85 g d^{-1}), one day before those in HW (0.83 g d^{-1}) and RW (0.72 g d^{-1}) treatments. Under the BM treatment, they achieve their highest decomposition rate (0.53 g d^{-1}) 5 and 4 days after the other treatments (Figure 4B). The low decomposition rate observed in the BM is attributed to its high cellulose, hemicellulose and lignin contents, as suggested by Rehman *et al.*, (2017) and Triplehorn and Johnson (2005).

CONCLUSIONS

The *H. illucens* larvae were able to decompose the four types of waste evaluated. The adjusted models helped analyze the rates of substrate conversion into biomass. The greatest efficiency in the accumulation of body mass was observed in the waste with the greatest diversity of components, such as those produced in restaurants [$0.315 \pm 0.090 \text{ g (g} \cdot \text{d}^{-1})$]. With this type of waste, larvae reached an optimum usable weight (0.4 g) several days earlier than when feeding with the other waste forms, suggesting a greater bioconversion efficiency. The organic household, restaurant and pig manure wastes were decomposed by the *H. illucens* larvae at similar rates ($0.72\text{--}0.85 \text{ g d}^{-1}$), although the bovine manure was decomposed with less efficiency (0.53 g d^{-1}), suggesting that its treatment with these larvae may not be the best option. When these larvae reach the pre-pupal stage, their weight decreases, although it is still unclear whether this weight loss only represents the loss of water (as in the case of lepidoptera) or also a loss of body biomass. The information obtained is useful in order to consider the management and handling of this species in industrial bioconversion processes, since it presents an approach to the productive of larvae as a resource.

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