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Biodegradation of plastic with *Tenebrio molitor* insect larvae as an interdisciplinary contribution to environmental biotechnology

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Resumen

La contaminación es el mayor impacto humano en el planeta, es decir, el medio ambiente está formado por los elementos naturales y artificiales, sus actividades físicas, químicas, biológicas, sociales y culturales y sus respectivas relaciones. El objetivo de esta indagación fue analizar la biodegradación de plásticos, a partir de larvas del escarabajo *Tenebrio molitor*, como aporte a la enseñanza de la biotecnología ambiental. Básicamente, la ejecución de este proyecto se basó en 3 etapas. La caracterización de las larvas *Tenebrio molitor*, la crianza de las larvas y la cuantificación de la capacidad para biodegradar el plástico. Los resultados obtenidos fueron, encontraron que la muestra 1 (M1) tuvo una productividad específica de biomasa de 6.6 gramos mientras que la muestra 2 (M2) una productividad específica de Se registraron 2,3 gramos y logró evidenciar un cambio en cuanto al color y la cantidad de las heces. Finalmente, la biodegradación del poliestireno influye en la producción de compost, beneficiando el consumo de plástico.

Palabras clave: Biodegradación, Plástico, Tenebrio molitor, Biotecnología ambiental, larvas.

Biodegradación del plástico con larvas de insecto Tenebrio molitor como contribución interdisciplinar a la biotecnología a la biotecnología medioambiental

Abstract

Pollution is the greatest human impact on the planet, that is, the environment is made up of natural and artificial elements, their physical, chemical, biological, social and cultural activities and their respective relationships. The objective of this investigation was to analyze the biodegradation of plastics, from larvae of the Tenebrio molitor beetle, as a contribution to the teaching of environmental biotechnology. Basically, the execution of this project was based on 3 stages. The characterization of Tenebrio molitor larvae, the rearing of the larvae and the quantification of the capacity to biodegrade plastic. The results obtained were, they found that sample 1 (M1) had a specific biomass productivity of 6.6 grams while sample 2 (M2) a specific productivity of 2.3 grams was recorded and managed to show a change in terms of color and the amount of feces. Finally, the biodegradation of polystyrene influences the production of compost, benefiting the consumption of plastic.

Keywords: Biodegradation, Plastic, Tenebrio molitor, Environmental biotechnology, larvae.

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I. INTRODUCTION

Humans have overused natural resources without taking into account their depletion, impoverishing the land, disappearing forests and species, and reducing hydrological reserves.[1] Pollution is the greatest human impact on the planet. In this sense, "the environment is made up of natural and artificial elements, their physical, chemical, biological, social and cultural activities and their respective relationships", i.e., human beings are intimately related to the impacts on the environment and, therefore, they are responsible for finding solutions to environmental problems, in order to contribute to reducing the strong environmental impacts caused over the years.[2][3]

It must be recognized that today one of the most widely used products with the greatest negative impact on the environment is plastic, because "since its production more than 100 years ago, its material use in society has gradually increased". The production and burning of these products causes climate change. Plastic waste also clogs our waterways, pollutes our oceans, kills wildlife, and enters our food chain."[1]

The intensive use of plastic has created waste that is difficult to manage, since most plastics do not biodegrade, slowly decomposing into smaller pieces until they become microplastics that are difficult to remove from the environment. [1] The research proposed here aims to analyze the biodegradation of plastics, from larvae of the beetle *Tenebrio molitor*, as a contribution to the teaching of environmental biotechnology.[4][5] We sought to take advantage of the mechanism discovered in this beetle to decompose plastic and thus create an innovative alternative for the teaching of biology.[3]

The development of the proposal makes an interdisciplinary contribution to the teaching of environmental biotechnology, since this field can strengthen the biological knowledge of the subjects and can promote an integrated vision of the world to understand and provide alternative solutions to face current environmental problems in a way that not only promotes student learning and understanding, but also reinforces the critical evaluation of environmental problems such as plastic pollution[6][7].

2. Materials and Methods

2.1 Systematic Analysis

The systematic analysis allowed us to compare different methodologies used with variations in the results, however the differences in them can be attributed to external factors that could intervene. Evenly, compare the number of publications about the biodegradation of plastic that is carried out each year. Carrying out a systematic and bibliometric analysis allows us to see the number of publications made on a certain topic and the impact of these.

2.2 Protocol

Biological characterization of Tenebrio molitor

For the development of the project, it must begin with a biological characterization, which will be carried out with the help of bibliography. This will allow us to identify the different stages of growth and development of the larvae, in addition to providing information about the morphological characteristics that *T. molitor* acquires in each of its stages. Morphological characterization will also make it possible to determine the optimal conditions for the correct development of the life cycle of these insects. It is important to carry out an observation process, since it will help establish the correct way to identify the larvae and thus develop an appropriate culture of them. [2]

Breeding of larvae of *Tenebrio molitor*

Offspring should be started with about 200 larvae of *Tenebrio molitor* that have regular diets of wheat, potato and carrot pieces. These individuals should be separated according to their stages in boxes of approximately 10 x 10 cm with regulated conditions of temperature and humidity, these conditions must favor their life cycle and their reproduction process. [2]

Quantification of consumption capacity

To perform the quantification of polystyrene consumption a random design should be made with which larvae with lengths of between 14 to 20 mm approximately will be selected, in each treatment, around 500 mg of expanded polystyrene (soft foams) will be available. Weekly data will be taken. Each week the polystyrene will have to be weighed with a precision balance to determine the amount of consumption made by the larvae. [5]

3. Results

Biological characterization of Tenebrio molitor

This investigation started with commercially obtained larvae. For the identification of the larvae, complementary bibliography was needed to understand the life cycle in which they were found. Then, the larvae were placed in a 10x10 container at a maximum temperature of 24°C, a minimum of 20°C and a humidity of 51.5%. Likewise, the larvae of *Tenebrio molitor* were fed a strict diet of wheat, potato and carrot. It is important to know that the medium where these larvae remained was covered until total darkness was obtained, to avoid light exposure, since the larvae are photosensitive and do not tolerate high light intensities **Figure 1**.

Figure 1. T. molitor growth stages.

After 15 days, it became evident that the larvae presented morphological changes, changing from larvae to pupae. Their size varied between 1.5 cm and 1.8 cm. At this point, bibliographic references were used to identify and determine the stages of growth and development of Tenebrio molitor. It was possible to identify that the organism undergoes metamorphosis to change its state from egg, larva, adult. This stage of characterization allows us to know in depth this organism and the behaviors it carries out during the whole process.

The approximate weight of *Tenebrio molitor* larvae is (75-80 mg). Before starting with the experimental diets, the larvae must be subjected to a starvation period of 48h. According to the cited bibliography, 5 experimental diets were compared to evaluate the survival rate of tenebrio molitor larvae and the plastic mass lost.

Biodegradation capacity of plastics

The larvae of *Tenebrio molitor* were evaluated for their capacity to degrade different types of plastics such as: flex foam and plastic bags supplied as food for 30 days, counts were made during this time, the larvae showed greater degradation capacity. Weekly controls were carried out in the different groups.

Biomass variation

For the determination of the biomass variation of the larvae of T. molitor, Álvarez and Botache, as detailed in **Table 1**, found that sample 1 (M1) had a specific biomass productivity of 6.6 grams while sample 2 (M2) a specific productivity of 2.3 grams was recorded. In sample 3 (M3) it presented a specific productivity of 0.1 grams and, finally, in sample 4 (M4) a specific biomass productivity of 11 grams was evidenced. The study inferred, from the results presented, that the consumption of low-density polyethylene and expanded polyethylene do not have a significant effect on the development and growth of Tenebrio molitor, since the variation in biomass occurs with an increase in weight.

Table 1. Specific biomass productivity.

Sample	Average starting weight	Average finishing weight	Biomass produced	Time (months)	Substrate consumed (g)	Biomass specific productivity
M1 (25 larvas)	0,01	0,025	0,015	2	0,1	6,6
M2 (30 larvas)	0,02	0,09	0,07	2	0,16	2,3
M3 (25 larvas)	0,01	0,08	0,07	2	0,01	0,1
M4 (30 larvas)	0,013	0,015	0,002	2	0,022	11

Stool productivity

To measure the percentage of feces production, it is necessary to evaluate the relationship between the substrate consumed by the insect and the amount of feces produced, in which it is necessary to show the presence of elements not assimilated by the larvae of Tenebrio molitor. Alvarez and Botache (2019) collected and evidenced a change in the color and quantity of the feces. The feces of samples 1 and 2 (expanded polystyrene consumption) were collected and added to be compared with the sum of the feces collected from samples 3 and 4 as shown in Table 2.

Table 2. Percentage of specific feces productivity

	Feces produced (g)	Time(months)	Consumed substrate(g)	Specific stool productivity	Percentage of stool productivity
expanded polystyrene M1 & M2	2	2	0,26	0,13	13
Low density polystyrene M3 & M4	1	2	0,032	0,03	3

4. Discussion

Chong Chen in 2003 first reported the consumption of microplastics by mealworms. It was found that these worms could eat extruded polystyrene, in 2015 it was noted that polystyrene foam could be degraded and mineralized by mealworms and bacterial strains could also be isolated from their guts. Research in 2018 evidenced that factors such as temperature, type of polystyrene waste and nutrition affect the survival of larvae and thus the rate of biodegradation. [19]

In 2017, the degradation of polyethylene film by mealworms was reported, also the gut microbiota of the worms was analyzed, revealing three dominant bacterial genera: alcaligenes, brevundimonas and myroides. The studies indicated that Styrofoam and polyethylene film could be degraded by these maggots, offering an alternative to the worrisome global plastic pollution. [19]

The results indicate that *Tenebrio molitor* larvae can be an alternative for PE degradation, since these worms can consume polystyrene causing holes in the surface of this material, reducing its volume and weight. It should be noted that although the degradation capacity of these larvae proved to be effective, the amount of larvae is not directly proportional to the amount of polystyrene consumed. This depends on the gastrointestinal bacteria that *Tenebrio molitor* larvae possess. The waste produced by the larvae is evidence of the biodegradation produced by the metabolism of the larvae. [19]

An important element is diet, as it will contribute to both the nutrition of the individual and the success of polystyrene biodegradation. According to [19] *Tenebrio molitor* larvae can consume polystyrene as part of a strict diet. It is also highly resistant and can live on food containing up to 1% water and in low humidity conditions. Even though they lose mass, they conserve water in their metabolism in order to compensate for metabolic water by evaporation. It is important to justify cannibalism in these species and also photosensitivity since they are determining factors for the development of larvae and their degrading capacity. In 2018 Sarmiento explained that these organisms seek to avoid light in all stages of their development, with a high preference for dark places. On the other hand, if there is no proper nutrition, larvae tend to resort to cannibalism to maintain constant water levels, therefore, as there are fewer larvae due to cannibalism, this could influence the biodegradation process [19].

5. Conclusions

The biological characteristics of **Tenebrio molitor** can be determined by environmental factors such as: temperature, humidity and handling of organisms. These play an important role in the development and growth of this organism in the larvae, accelerating or delaying its life cycle.

The biodegradation of polystyrene influences the production of compost, benefiting the consumption of plastic.

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