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An assessment on the potential of tenebrio molitor used for biodepolymerization of plastics and polystyrene: influencing factors, various feeding cases and gut microbiota

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Abstract: The present study aims to analyze the potential of mealworms (*Tenebrio Monitor*) used for the biodepolymerization of waste papers, plastics and polystyrenes. The various influencing factors were analyzed such as: temperature, light intensity, moisture content and energy sources. The study was conducted with two types of feeding cases that the first was, the waste papers, plastics and polystyrene were fed with oats (Avena sativa) and the above wastes were added directly to the mealworms without any additional energy sources. A similar weight of 100gms of all three wastes has been added with above two mentioned feeding cases to the mealworms. The study was conducted in a larval stage of mealworms for a period of nearly 30 days and 100 mealworms were used for each and every combination of feeding cases. Mealworms have biodepolymerized 40% of the plastic waste and polystyrene within the period of 10-12 days.
Based on the obtained results, it has been found that the gut microbiota and enzymes are the responsible for the biodepolymerization and biodegradation of plastic, polystyrene and paper waste respectively. The present study reveals that mealworms have the potential to survive even after intake of polymer substances, this leads to new pathway for the sustainable management of polymer waste and paper waste.

Keywords: Biodepolymerization, Plastic waste, Polystyrene, styrofoam, mealworms

1. Introduction

Plastics are becoming an avoidable material in our daily life and industries all around the world produces an average yearly amount of 359 million tons in 2018 [1, 2, 14, 15]. The exponential production rate and wider usage of plastic causes severe environmental problems and creates potential threat to ecosystem when it was not properly disposed [3, 4, 16]. The continuous prevailing nature and strong enough to get easily biodegradable tendency makes the plastic waste persistently remain in the nature environment in various form like macro, micro and nano plastics [5, 17, 18]. From the earlier studies it can be said the many insects are having capability to gnawing and intake plastic, in various forms like polyvinyl chloride (PVC), polyethylene (PE), polypropylene (PP) and polystyrene (PS) packaging films [6, 7, 19, 20]. Thereafter, from the extensive literature review, it has been notices that Tenebrio molitor (T. molitor) having the potential to intake styrofoam and polyethylene.

T. molitor plays a vital role in facilitating the ecosystem and having the capacity to spread its potential to various fields of sciences [8]. The treatment methods presently existing in various industries requires huge space, energy and many times causes environmental problems. Therefore Biodegradation of plastic waste using various types of insects are still in its infant stage even at national and international level which really having a sustainable solution, further, it prevents the mixing of plastic waste into various other natural resources and safeguard the environment [9].

The microbiota presents in the gut of the mealworm having a prime part in biodepolymerisation of plastic waste [10]. Nearly, $5{-}6 \times 10^5$ colony forming units (CFU) of microorganisms were present in the gut of the meal worms. The gut microbiotas are very sensitive and it is influenced by food, antibiotics which further having the effects on microbial community. [10]. Therefore, gut microbiota are highly necessary for PS biodegradation, various environmental factors and other inhibiting compounds may have adverse effect on the gut microbiota and its growth. Isolating the gut microorganisms from the meal worms and culturing it for the biodegradation purpose is an alternative approach where it seems to be pioneering attempt towards the plastic biodegradation [11, 12, 13].

The present study aims to analyze the potential of mealworms (*Tenebrio Monitor*) used for the biodepolymerization of plastics and polystyrenes. The various influencing factors were monitored such as: temperature, light intensity, moisture content and energy sources.

2. Materials and Methods

2.1 Experimental Setup

The experimental setup as shown in Figure 1 & 2 for the biodegradation process is as follows: tank and a different set of plastics containers will be adopted. The tank was divided into 4 compartments as in each compartment 200 numbers of mealworm were added. Similarly, in each plastic container, the same amounts of mealworms were added. Tank and plastic containers were kept under dark conditions. Influencing parameters of the mealworms such as temperature, humidity, and light intensity were measured by using humidity and lux meter. Finally, the duration of degradation of plastics were measured at the various life stages of mealworm.

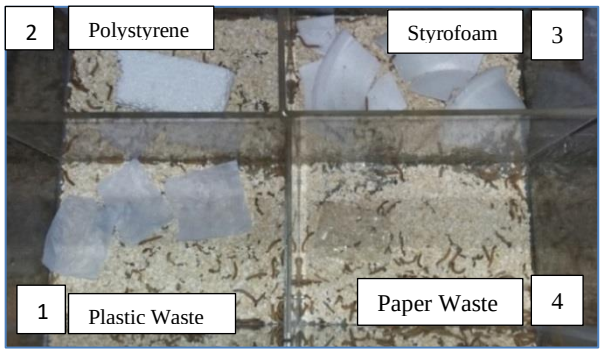

Figure 1. Experimental set up of with nutrient.

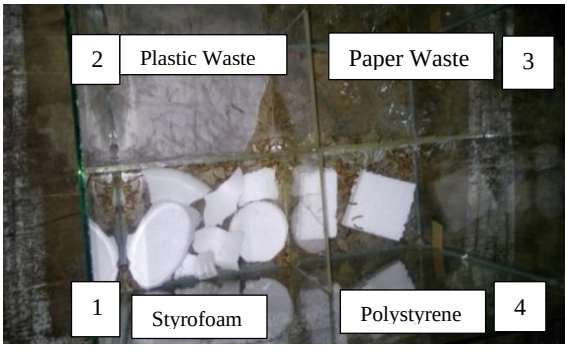

Figure 2. Experimental setup of without nutrient.

2.2 Methodology

Mealworms were stored in a dark place at a temperature of below 35°C because meal worms are highly sensitive to light and prefers dry environment [21]. An incubator was preferred in case of any deviations in the environment. Monitoring parameters as given in Table 2 such as temperature, humidity, and light intensity were monitored on daily basis by using the instruments shown in Figure 3 & 4. The locally available worms which have the ability to degrade plastic were identified. The form of plastics available in the market and their chemical property were studied and furnished in Table 1. Plastic wastes were collected from the several areas of Hyderabad. Oats, slices of apple, carrot and potato were fed to mealworm which enhances the growth conditions during larvae period. Plastic wastes of different micron sizes were added to the mealworm. Maximum efficiency of bio-degradation was found among different micron sizes. Plastic wastes were added in various amount or

dosages and determined the maximum amount of degradation during its total life period. Efficiency of yellow mealworms was compared with two conditions such as: with nutrient and without nutrient.

Figure 3. Humidity meter.

Figure 4. Lux meter.

Table 1. Different plastic wastes and its chemical properties

Sl.No.	Form of Plastic Available in Market	Nature (LDPE / HDPE)	Chemical Compound
1	Plastic Bottle	LDPE	Polyethylene
2	Carry Bags	LDPE	Polyethylene
3	Thermocol	LDPE	Polystyrene
4	Styrofoam	LDPE	Polystyrene

Table 2. Monitoring parameters

Sl.No.	Parameters	Equipment	Model	Efficiency [22]
1	Temperature (°C)	Humidity meter	EC-7643	± 0.5°C
2	Humidity (%RH)	Humidity meter	EC-7643	± 0.5%
3	Light Intensity (Lux)	Lux meter	LX-101A	± 2 Lux

2.3 Identification of Bacteria

The Equipment used in the study of identification of bacteria is such as: Sterilized surgical knife, sterilized loop, sterilized surgical glows, and petri dish. The culturing of bacteria has been done after the preparation of agar medium. Samples of meal worms were taken and it has been bisected using the above-sterilized equipment and it was cut vertically. Semi-liquidised substances were taken for the culturing of bacteria from mealworms gut. Sterilized loop was used to transfer the culture and placed to the agar medium. The above process was done under the biosafety chamber. The culture plates were transferred to the bacteriological incubator to maintain the constant ambient temperature.

The above same procedure was done to the culture medium such as: Blood Agar as shown in Figure. 5. The composition of Blood Agar such as: 0.5% Peptone, 0.3% beef extract/yeast extract, 1.5% agar, 0.5% NaCl, Distilled water, 5% Sheep Blood, pH must be in the range of 7.2 to 7.6. The procedure for the preparation of Blood Agar is as follows: 28 gm of nutrient agar powder was mixed in 1 litre of distilled water; it is stirred for the homogeneous mixture of nutrient medium. The same nutrient medium was autoclaved at 121°C for 15 minutes, after autoclaved it was allowed to reduce the temperature and not to get solidify. The agar was cooled to 45-50°C, and then adds 5% (vol/vol) sterile defibrinated blood was heated to ambient temperature and mixed smoothly to avoid the air bubbles. It is further dispersed into sterile plates when it was liquid.

To find the bacteria, again two more nutrient medium such as: MacConkey Agar as shown in Figure 6 and Nutrient Agar medium as shown in Figure 7 were used and prepared as per the microbiological protocol and followed the same procedure adopted to Blood Agar. The compositions and procedure were not disclosed here due to the limitation in pages for publications. MacConkey Agar was used to find the bacteria present in the excreta of meal worm. Figure 8 shows the bacteria culture from crushed mealworm with saline and Figure 9 represents the culture preparation from crushed bacteria for Aspect Ratio (10^{-3} to 10^{-6})

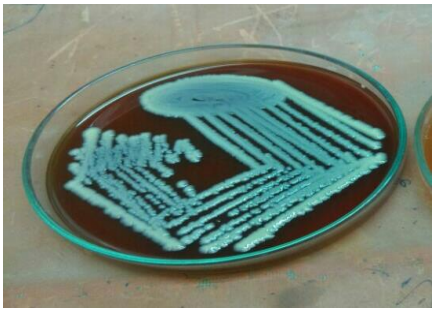

Figure 5. Bacteria colony formed in Blood Agar isolated from mealworms gut.

Figure 6. Bacteria colony formed in MacConkey Agar isolated from mealworm excreta.

Figure 7. Media preparation.

Figure 8. Bacteria Culture from crushed mealworm with saline.

Figure 9. Culture Preparation from crushed bacteria for Aspect Ratio (10^{-3} to 10^{-6}).

3. Result and Discussion

The experimental results for degradation of yellow mealworms are discussed. The efficiency of with nutrient and without nutrient for degradation of plastic waste such as Styrofoam, carry bags and plastic bottles of different experimental setup have been discussed. The following sample figures 10 and 11 shows the intake of Styrofoam and plastic waste by meal worms.

Figure 10. Styrofoam degradation.

Figure 11. Plastic waste degradation.

3.1 Comparative study on styrofoam with nutrient and without nutrient

The biodegradation of Styrofoam study was also conducted. The efficiency of biodegradation of Styrofoam with nutrient as shown in Figure 12 was found 79.35% for a period of 30 days, whereas, it was 47.86% for without nutrient. Therefore, it is observed from the obtained results that the biodegradation of Styrofoam with nutrient shows very high reduction efficiency when compared with Styrofoam without nutrient.

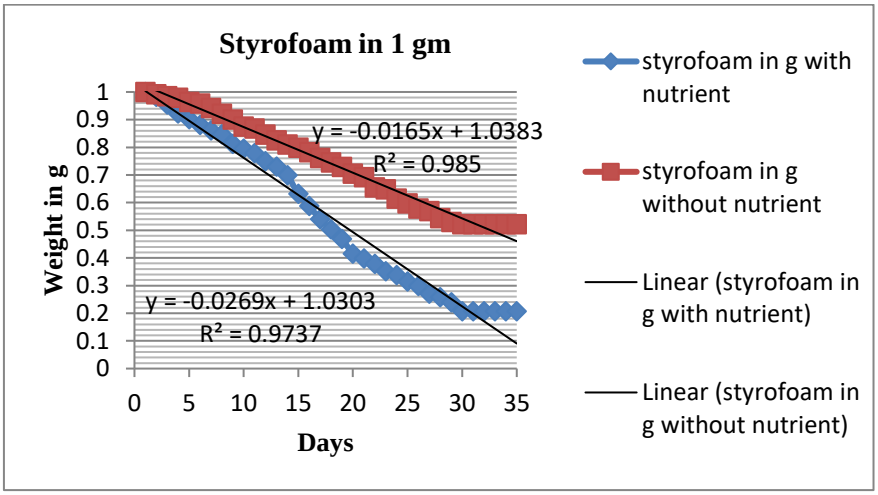

Figure 12. Reduction in weight of Styrofoam with nutrient and without a nutrient.

3.2 Comparative study on carry bags with nutrient and without nutrient

The biodegradation of carry bag study was also conducted. The efficiency of biodegradation of carry bag with nutrient as shown in Figure 13 was found 46.99% for a period of 30 days, whereas, it was 40.22% for without nutrient. Therefore, it is observed from the obtained results that the biodegradation of carry bag with nutrient shows nearly equal reduction efficiency when compared with carry bag without nutrient.

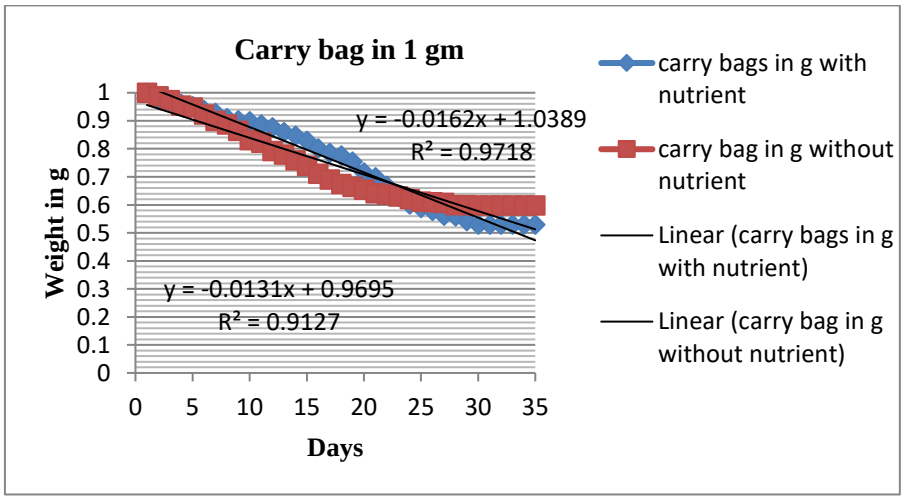

Figure 13. Reduction in weight of Carry bag with nutrient and without nutrient.

3.3 Comparative study on the aplastic bottle with nutrient and without nutrient

The efficiency of biodegradation of plastic bottle with nutrient as shown in Figure 14 was found 24.39% for a period of 30 days, whereas, it was 16.18% for without nutrient. Therefore, it is observed from the obtained results that the biodegradation of plastic bottle with nutrient shows slightly higher reduction efficiency when compared with an aplastic bottle without nutrient.

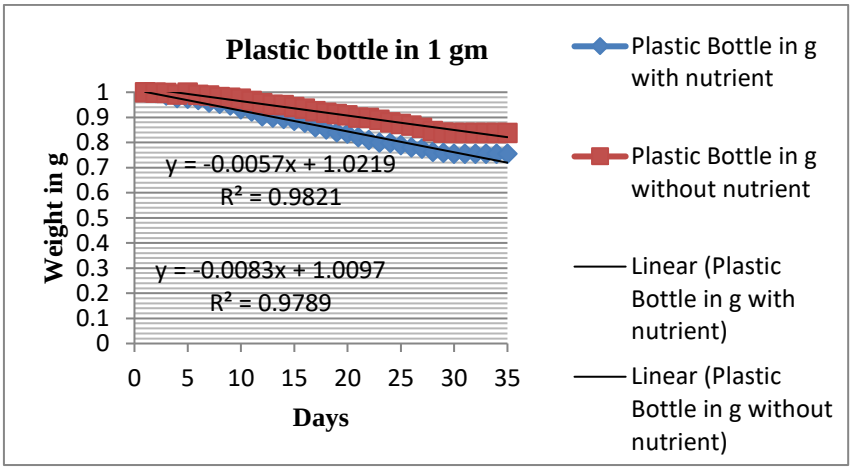

Figure 14. Reduction in weight of plastic bottle with nutrient and without nutrient.

4. Conclusions

It has been found that the bacteria which are responsible for biodegradation of plastic waste are Klebsiella oxytoca and another one is Bacillus Subtilis. These are the possible bacteria present in the mealworm gut and it has digested and degraded the plastic wastes. Comparing the efficiency of mealworms with respect to the cases such as: with and without nutrient, the results show that efficiency is more for with nutrient. It has been found that styrofoam (with nutrient) is comparatively having higher biodegrading reduction efficiency than the other waste used in the study. Based on the obtained results, the ranking of biodegradability of plastic waste are Styrofoam >Carry bags > Plastic bottle.

The higher the addition of an amount of plastic wastes shows the higher polymer load to the mealworms which results in lower biodegradability. From the obtained data, it has been found that mealworms have higher ability to biodegrade the low-density polyethylene when compared with high-density polyethylene. Based on the obtained results, it has been found that the environmental parameters such as: temperature, humidity and light intensity having a higher influence over on mealworm intake capacity and growth. The 50% biodegradation of thermocol with nutrient was achieved in 15 days as same as, from 50% to highest reduction efficiency was achieved in another 15 days. Further, the same pattern was observed in Styrofoam. The total duration for biodegradation was ended in 30 days, further; the biodegradation efficiency was noted for another 5 days, where, there was no intake happened. Therefore, from the 31stday, it has been found that there is a decrease in biodegradation efficiency due to the end of the larva (II stage) and it has started to convert to pupa (III stage).

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