S.T. Yau High School Science Award (Asia) 2020

Research Report

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Title of Research Report

Biodegradation of Styrofoam by Larvae of *Tenebrio molitor*

Date 31 August, 2020

Biodegradation of Styrofoam by Larvae of *Tenebrio molitor*

[CHAN Ka Man, LAM Ching Yan]

Abstract

Styrofoam is a common type of waste which occupies most of the space in landfills. The amount of discarded Styrofoam keeps rising each year, causing severe environmental problems. Recent study has demonstrated that larvae of *Tenebrio molitor* (mealworms) are able to degrade a variety of polystyrene, including Styrofoam, by consuming it. In this study, the consumption and biodegradation of Styrofoam by mealworms and their gut bacteria were investigated.

Mealworms were fed with Styrofoam under different conditions for ten days. Mealworms fed with small blocks of Styrofoam mixed with bran resulted in the largest percentage decrease in weight of Styrofoam (79.5%) after ten days. The death rate of the control group fed with bran only (12.5%) was lower than the death rates of all the experimental groups of mealworms fed with Styrofoam (ranging from 23.3% to 93.3%).

Polystyrene (PS) films made by dissolving Styrofoam in dichloromethane were added to gut suspension extracted from the guts of mealworms under various conditions. The rate of biodegradation of PS films by gut suspension was found to be the highest when pH=5 and with extra addition of Nutrient Broth (NB) medium.

Two strains, *Exiguobacterium sp.* and *Lactococcus sp.* were isolated from the gut suspension of mealworms which had undergone enrichment for one month with PS films as the sole source of carbon and identified based on their morphology. *Exiguobacterium sp.* was the predominant genus while *Lactococcus sp.* was less abundant.

Keywords: biodegradation, polystyrene, Styrofoam, *Tenebrio molitor*, mealworm, gut bacteria

Acknowledgement

We would like to express our sincere gratitude to our supervising teacher, Ms Ady Wong, for her support and advice given in this competition. We would also like to thank all the staff members at school who have provided us with required materials and accompanied us during the experiments.

Commitments on Academic Honesty and Integrity

We hereby declare that we

1. are fully committed to the principle of honesty, integrity and fair play throughout the competition.
2. actually perform the research work ourselves and thus truly understand the content of the work.
3. observe the common standard of academic integrity adopted by most journals and degree theses.
4. have declared all the assistance and contribution we have received from any personnel, agency, institution, etc. for the research work.
5. undertake to avoid getting in touch with assessment panel members in a way that may lead to direct or indirect conflict of interest.
6. undertake to avoid any interaction with assessment panel members that would undermine the neutrality of the panel member and fairness of the assessment process.
7. observe all rules and regulations of the competition.
8. agree that the decision of YHSA(Asia) is final in all matters related to the competition.

We understand and agree that failure to honour the above commitments may lead to disqualification from the competition and/or removal of reward, if applicable; that any unethical deeds, if found, will be disclosed to the school principal of team member(s) and relevant parties if deemed necessary; and that the decision of YHSA(Asia) is final and no appeal will be accepted.

(Signatures of full team below)

X 

Name of team member: CHAN Ka Man

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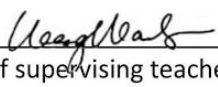
Name of team member: LAM Ching Yan

X 

Name of supervising teacher: WONG Wai Sum

Noted and endorsed by:

(signature) 

Name of school principal:

WUN Chi Wa

Content page

1.Introduction

1.1 Styrofoam waste problems

Styrofoam, or expanded polystyrene foam (EPS), is a specific type of polystyrene known for its low density and high insulating capability. It is commonly used to make a variety of single-use consumer goods, including food containers and packaging materials.

Figure 1: Common Styrofoam products[1]

Being considered as non-biodegradable for over a million years, most Styrofoam waste is destined to be directly discarded in the landfill, where it could remain for a very long time. In Hong Kong, about 30 400 tonnes of waste Styrofoam were disposed of at landfills in 2017 [2]. Due to its lightweight nature, Styrofoam quickly fills up the landfills. Up to this point, by volume, around 30% of solid waste in the landfill space around the world is occupied by Styrofoam [3]. Furthermore, Styrofoam will jeopardize our health if it gets into the food chain. If Styrofoam gets into the ocean, marine animals may accidentally consume it. EPS has been classified by the US National Institutes of Health (NIH) and the International Agency for Research on Cancer (IARC) as a human carcinogen[4]. People who consume fish that has eaten EPS will be prone to cancer. Hence, Styrofoam is a potential hazard to us, to the ecosystem and to the environment.

Figure 2: Styrofoam waste in landfills[5]

[1] Image from Hana Mae Nassar and Lasia Kretzel. (January 1, 2020). *Vancouver Styrofoam container ban goes into effect.* CityNews.
[2] Environmental Protection Department, Statistics Unit. (November 2019). *Monitoring of Solid Waste in Hong Kong Waste Statistics for 2018.*
[3] Collier County. *THE FACTS ON STYROFOAM: REDUCE AND REUSE.*
[4] Michelle Rose Rubio. (September 6, 2018). *Dealing with Polystyrene Wastes.* EcoMENA.
[5] Image from Andrea D. Steffen. (July 15, 2019). *This Eco-Friendly Alternative To Styrofoam Is Made From Plants*. Intelligent Living.

1.2 Current remedial measures to Styrofoam problem

Recycling of Styrofoam is considered as a way to alleviate the problem. However, Styrofoam pellets are the only type of Styrofoam that Styrofoam-recycling companies accept. Even more, recycling of Styrofoam is not cost-effective. As Styrofoam is bulky with 98% of air and only 2% of plastic, it occupies a huge volume and requires remarkably high transporting cost [6]. As a result, Styrofoam has to be compressed using a Styrofoam densifier or compactor before they can be transported, hence increasing the expenses. Besides, Styrofoam waste usually contains impurities on its surface, such as food debris and dirt. Additional cost would be needed in order to get rid of unwanted materials. Processing a single pallet of Styrofoam would result in a net loss of USD$725.85 [7]. It is clear that recycling is not an effective way to reduce Styrofoam waste.

1.3 A novel biological approach to deal with Styrofoam waste

Advancement in the scientific field suggests a method which may nip the problem in the bud. Recent research substantiated that larvae of *Tenebrio molitor*, commonly known as mealworms, are able to degrade Styrofoam and release carbon dioxide[8] as a product. Consumption of Styrofoam by mealworms is at a fairly fast speed. It is found that 100 mealworms could consume between 34 and 39 milligrams of Styrofoam per day[9], while natural decomposition of Styrofoam is estimated to be around 500 years[10].

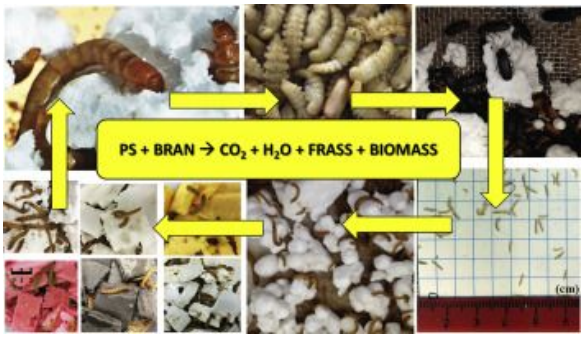


Figure 3: Reactants and products of biodegradation of Styrofoam by mealworms[11]

[6] iSustain Recycling. (January 1, 2018). *Can Polystyrene Be Cost-Effectively Recycled?.*
[7] Jill McCutcheon, Sedona Recycles. (June 17, 2015). *The real cost of recycling foam plastic.* Sedona Red Rock News.
[8] Yu Yang et al. (2015). Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms: Part 1. Chemical and Physical Characterization and Isotopic Tests. Environmental Science & Technology. 49 (20), 12080-12086. DOI: 10.1021/acs.est.5b02661
[9] Environ. Sci. Technol. 2015, 49, 20, 12080–12086
[10] Collier County. *THE FACTS ON STYROFOAM: REDUCE AND REUSE.*
[11] Image from Shan-Shan Yang et al. (January 2018). *Biodegradation of polystyrene wastes in yellow mealworms (larvae of Tenebrio molitor Linnaeus): Factors affecting biodegradation rates and the ability of polystyrene-fed larvae to complete their life cycle.* Chemosphere Volume 191, Pages 979-989

Mealworms are cheap and highly accessible. On average, each mealworm costs around 0.5 HKD. Therefore, biodegradation by mealworms is considered as an economic and environmentally-friendly way to process plastic waste, since mealworms can be reused to serve as a source of protein for fish and birds.

Figure 4: Mealworms consuming Styrofoam (photo was taken in this study)

To conclude, mealworms could make decomposition of Styrofoam within a reasonable time span possible. However, degradation of Styrofoam by mealworms on a large scale is not sustainable as mealworms undergo complete metamorphosis in several months, as shown by Figure 5. It is suggested that the bacteria responsible for the biodegradation extracted from mealworms would be a better option. Nonetheless, bacterial activity is easily affected by various environmental factors, such as pH value. In order to raise the efficiency of biodegradation, the most favourable conditions for the growth of PS-degrading bacteria is another focus.

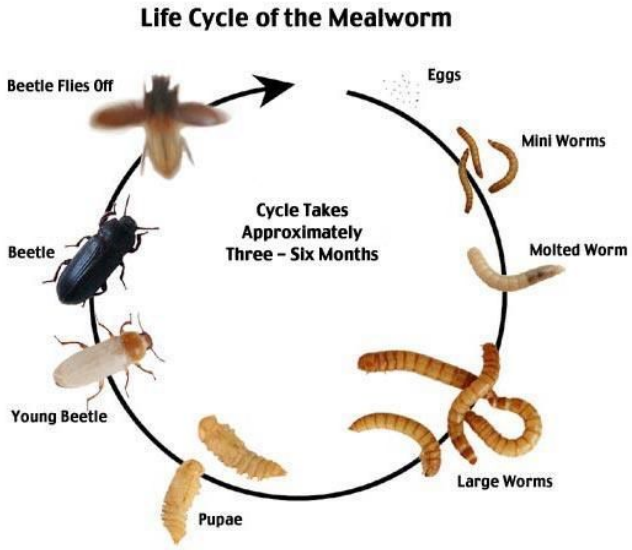


Figure 5: Life cycle of mealworms[12]

[12] Image from Carly Pasell. *Life cycle of mealworm.* blendspace. Retrieved from https://www.tes.com/lessons/VGBjXQ-vhuhUqw/mealworms

2.Objective

In this report, we investigated the biodegradation of Styrofoam by mealworms and their gut bacteria. In addition, the bacteria in gut suspension extracted from mealworms fed with Styrofoam were characterized. The investigation aims at finding out the efficiency of degrading Styrofoam waste by mealworms. We would also like to discuss the possibility of applying the process in practice through investigating the most favorable conditions for biodegradation of Styrofoam, as well as comparing the advantages and shortcomings of each method(mealworms and gut bacteria).

3. Materials and methods

3.1 Biodegradation of Styrofoam by mealworms under different conditions

6 groups of mealworms, 30 specimens each, were subjected to different conditions. The initial mass of Styrofoam in each set-up was approximately the same (0.88g). It was assumed that the consumption rate of Styrofoam was equal to the biodegradation rate of Styrofoam by mealworms.

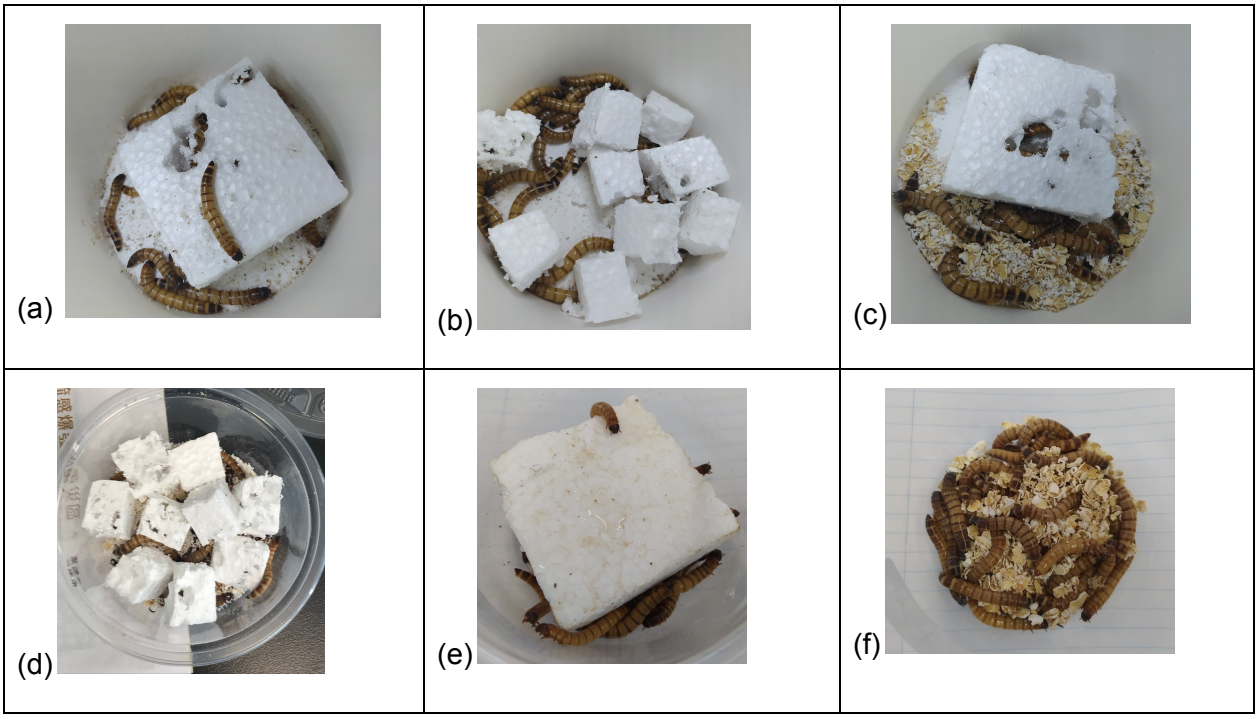


Figure 6: Different groups of mealworms

(a): mealworms fed with Styrofoam

(b): mealworms fed with Styrofoam (cut into 9 smaller pieces)

(c): mealworms fed with Styrofoam mixed with bran (mass ratio of Styrofoam to bran was approximately 2:8)

(d): mealworms fed with Styrofoam (cut into 9 small pieces) mixed with bran (mass ratio of Styrofoam to bran was approximately 2:8)

(e): mealworms fed with gentamicin mixed with bran (30 mg/g) for two days, then fed with Styrofoam only

(f): mealworms fed with bran only

After 10 days, Styrofoam pieces were taken out with forceps and rinsed with distilled water to remove impurities on the surface. The pieces were put under room temperature for one day to ensure the pieces were completely dried. The mass of Styrofoam after 10 days of consumption by mealworms was then measured.

The number of mealworms alive after 10 days was also counted to calculate the death rate of mealworms during the period of investigation.

$$\text{Death rate} = (\text{Number of dead mealworms}/30) \times 100\%$$

3.2 Characterization of PS-degrading gut bacteria in mealworms

3.2.1 Preparation of liquid carbon-free basal medium (LCFBM)

LCFBM was used for the enrichment of PS-degrading microorganisms. It was prepared with distilled water and contained by mass 0.2% NaH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.02% KH_2PO_4, 0.1% yeast extract, according to the composition described previously (Kitamoto et al., 2011 and Aneta K.Urbanek el al., 2020). The prepared medium was sterilized in a pressure cooker at 12 psi for 20 minutes.

3.2.2 Preparation of polystyrene films (PS films)

PS films were prepared for microbial degradation. Styrofoam was dissolved in dichloromethane at 0.03g/mL. The solution was then spread on Petri dishes. The plates were incubated at room temperature for one day. The PS films formed were taken off after one day and cut into 3x3 mm pieces. The films were rinsed with ethanol, followed by distilled water, and dried again prior to use.

3.2.3 Preparation of gut suspension

50 mealworms fed with Styrofoam as the sole diet for 12 days were collected. The surface of mealworms were sterilized by immersion in ethanol for 1 minute and then rinsed with distilled water. Guts were drawn out and pooled in a 10 mL centrifuge tube containing 5 mL of distilled water. After being shaken on a vortex mixer for 5 minutes, the gut tissues were carefully removed with a pipet and the gut suspension remained in the tube.

Figure 7: gut mixture before vortexing

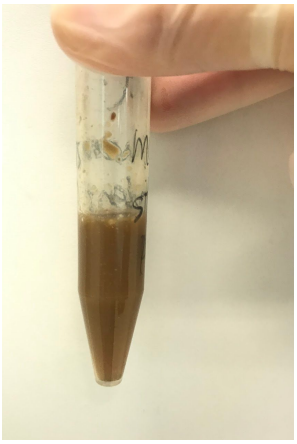


Figure 8: gut suspension after vortexing and removal of gut tissues

The prepared gut suspension was then transferred to a 250mL conical flask, which contained 80 mL of LCFBM and 1 g of PS films. The flask was incubated at room temperature for one month for enrichment of bacteria.

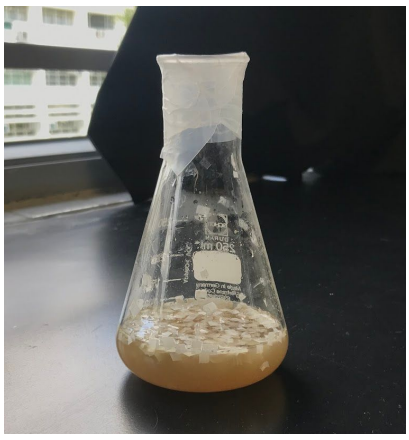


Figure 9: Set-up for enrichment of bacteria

3.2.4 Isolation of gut bacteria

50 µL of the enrichment prepared in the previous step was spread across plates with Lysogeny Broth (LB) agar. After incubation of plates at room temperature for 24 hours, colonies were picked and spread to other LB plates with sterile inoculation loop, where they were kept until pure colonies of isolates were obtained on the basis of observations of morphologies of colonies formed on the plate.

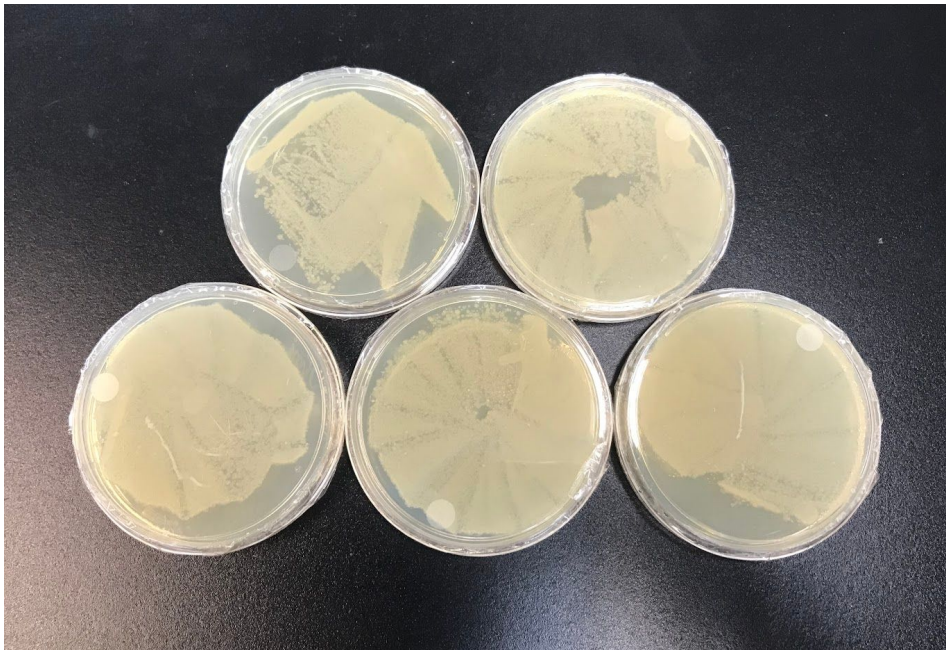


Figure 10: Bacteria grown on LB agar spread with 50 µL gut suspension after incubation of 24 hours (reverse)

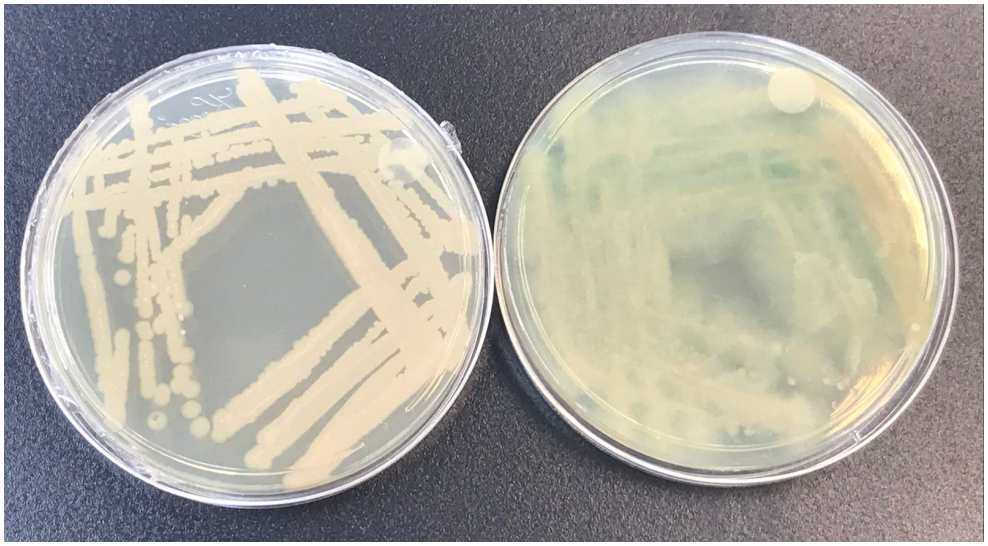


Figure 11: Bacteria grown on LB agar streaked with colony picked from plates in Figure 10 after incubation of 24 hours (reverse)

3.2.5 Characterization of gut bacteria

Samples were identified based on colonial and morphological characters. Gram staining method was used.

Microscope slides were immersed in 75% ethanol and dried prior to use. The slides were then sterilized with flame and left to chill. Pure colony of bacteria on LB plate was picked and smeared onto a slide, which contained a drop of distilled water. Air-dried, heat-fixed smear of cells were flooded for 1 minute with crystal violet staining reagent. The slide was washed by immersion in distilled water. The slide was flooded with Gram's iodine. After one minute, the slide was washed by immersion in distilled water.The slide was then flooded with 95% ethanol (a decolorizing agent), followed by safranin after 30 seconds. After one minute, the slide was washed by immersion in distilled water until no color appeared in the effluent and then blot dry with absorbent paper.

The results of the staining procedure were observed under oil immersion using a Bright field microscope.

3.3 Biodegradation of Styrofoam by gut bacteria under different conditions

The method used to compare the rate of biodegradation of PS films under different circumstances was inspired by the simple colorimetric method described previously (T. K. Meng et al., 2018), with modifications due to absence of colorimeter and related equipment in the school laboratory.

3.3.1 Preparation of PS films containing Ca^{2+} ions

Styrofoam was dissolved in dichloromethane at 0.03g/mL. The solution was mixed with calcium nitrate (dissolved in distilled water at 10% weight per volume) in ratio of 3:1 by volume. After that, the solution was spread on Petri dishes. The plates were incubated at room temperature for one day. Then, the PS films formed were taken off after one day and cut into 5x5 mm pieces. The films were rinsed with ethanol, followed by distilled water, and dried again prior to use.

3.3.2 Investigation of biodegradation rate

Gut suspension prepared in step 2.2.3 was used. 1 mL gut suspension was added to a test tube containing five 5x5mm pieces of PS film. 10 set-ups, including 3 controls which did not contain gut suspension, were prepared. The pH value was adjusted by citric acid and sodium hydroxide. Nutrient Broth (NB) medium was added as an extra supply of nutrient.

(i) 1 mL gut suspension (pH=3) + 5x5mm PS films
(ii) 1 mL gut suspension (pH=5) + 5x5mm PS films
(iii) 1 mL gut suspension (pH=7) + 5x5mm PS films
(iv) 1 mL gut suspension (pH=9) + 5x5mm PS films
(v) 1 mL gut suspension + 1 mL NB medium + 5x5mm PS films
(vi) 1 mL gut suspension + 5x5mm PS films
(vii) 1 mL gut suspension + 5x5 mm PS films (each film was cut into four smaller pieces)
(viii) 1 mL LCFBM + 5x5mm PS films
(ix) 1 mL NB medium + 5x5mm PS films
(x) 1 mL distilled water + 5x5mm PS films

The test tubes were sealed with parafilm and incubated at room temperature for one week to avoid the liquid from drying.

Calcium test kit©Colombo was used with reference to the manual given to compare the concentration of calcium ions in the above setups. Firstly, 1 mL of sample solution was transferred from the test tube to another clean tube with a pipette and then diluted to 5 mL with distilled water. Five drops of sodium hydroxide solution was added to the tube to allow any magnesium ions present to precipitate. A small scoop of Patton-Reeder indicator was then added to the tube. After addition of the indicator, the tube was swirled so as to dissolve the indicator. The solution appeared purple. Finally, EDTA solution was added to the tube until the colour of solution changed from purple to blue. The more the EDTA solution added, the higher the concentration of calcium ions in the sample.

Figure 12: colour of sample solution before end point

Figure 13: colour of sample solution at end point

4.Results

4.1 Biodegradation of Styrofoam by mealworms

4.1.1 Weight change of Styrofoam

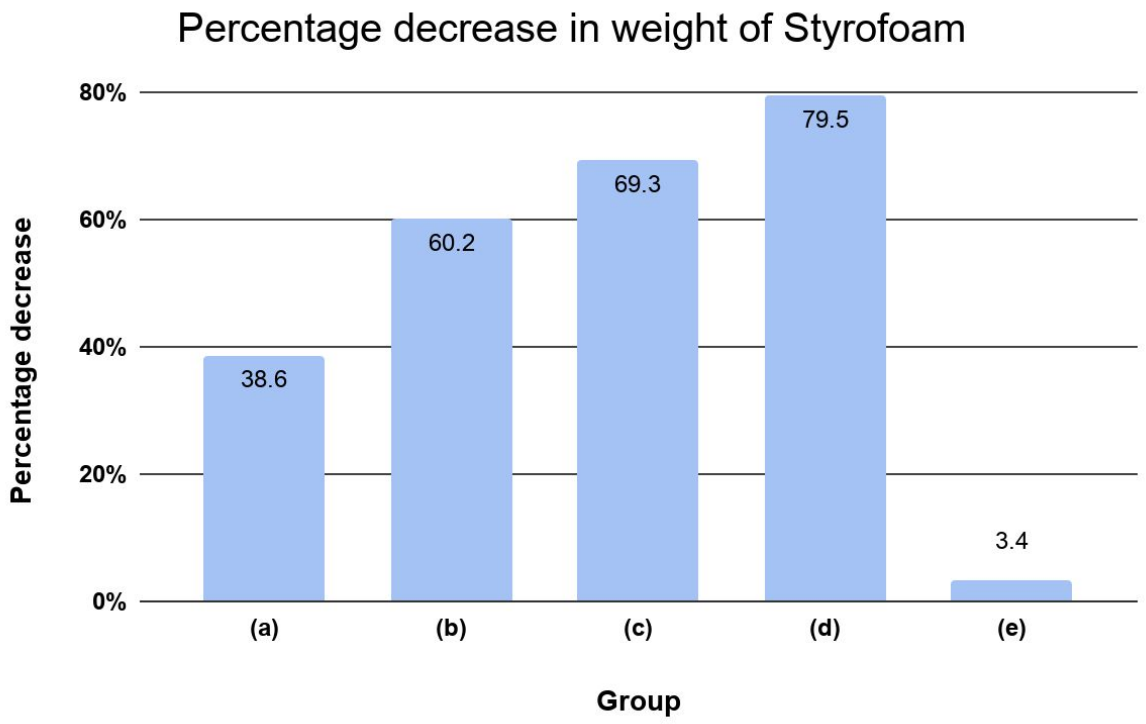


Figure 14: Percentage decrease in weight of Styrofoam (if any) after 10 days

(a): mealworms fed with Styrofoam (b): mealworms fed with 9 small Styrofoam pieces (c): mealworms fed with Styrofoam mixed with bran (d): mealworms fed with 9 small Styrofoam pieces mixed with bran (e): mealworms fed with gentamicin mixed with bran followed by solely Styrofoam

Percentage decrease of Styrofoam varied a lot among different conditions.

Least decrease was observed in group (e), which was fed with gentamicin followed by a large block of Styrofoam. The weight only decreased by 3.4%.

Group (d) had the largest decrease in Styrofoam weight, where 79.5% of Styrofoam was broken down by mealworms. Group (d), which was fed with 9 small Styrofoam pieces mixed with bran,

performed well in the experiment with the decrease in weight being nearly 10% higher than Group (c) that came second with a percentage decrease of 69.3%.

Two obvious trends were observed. To start with, extra supply of bran resulted in larger decrease in weight of Styrofoam. Either fed with a large block or small pieces of Styrofoam, mealworms supplied with extra bran were able to consume more Styrofoam during the period of investigation and thus a larger decrease in weight of Styrofoam was recorded. When mealworms were given one large block of Styrofoam, supply of bran was able to raise the percentage decrease in weight of Styrofoam by 30.7%. When mealworms were given nine smaller blocks of Styrofoam, supply of bran only raised the percentage decrease in weight of Styrofoam by 19.3%.

In addition, Styrofoam with higher surface area to volume ratio caused a larger percentage decrease in weight of Styrofoam. In this investigation, mealworms were given the same mass of Styrofoam, either in form of a whole block or 9 small pieces. It was found that mealworms fed with small pieces of Styrofoam consumed more Styrofoam compared with those fed with a whole block. Under supply of bran, feeding mealworms with smaller blocks of Styrofoam could raise the percentage decrease in weight of Styrofoam by 10.2%. Meanwhile, for mealworms fed with Styrofoam as the sole diet, an increase in surface area of Styrofoam blocks raised the percentage decrease in weight by 21.6%.

4.1.2 Death rate of mealworms

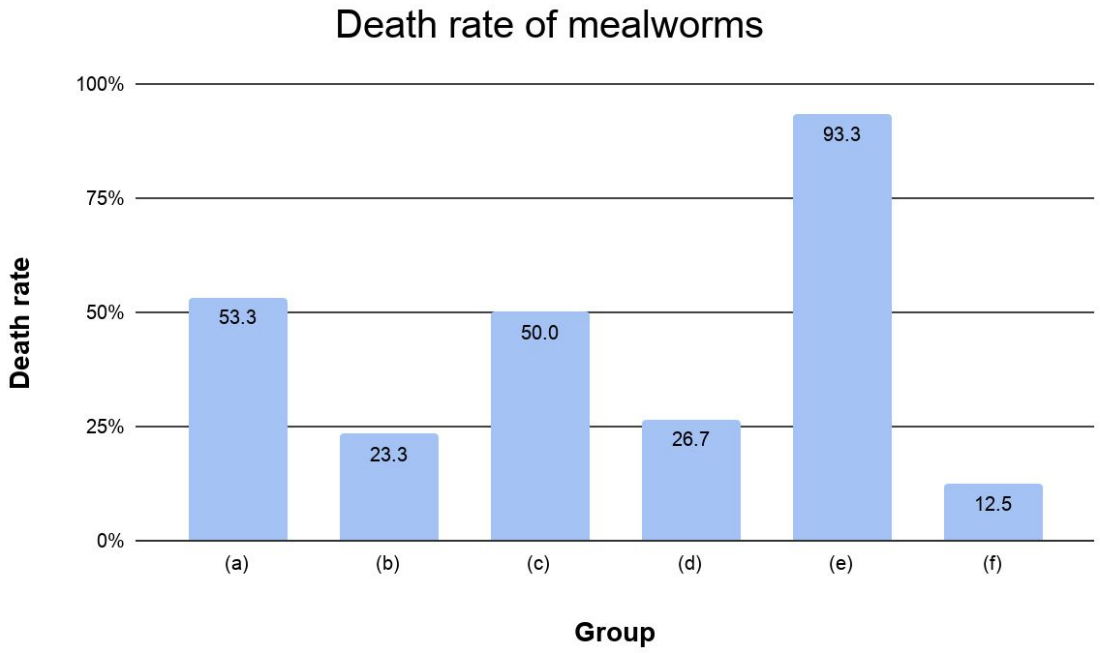


Figure 15: Death rate of mealworms after 10 days

(a): mealworms fed with Styrofoam (b): mealworms fed with 9 small Styrofoam pieces (c): mealworms fed with Styrofoam mixed with bran (d): mealworms fed with 9 small Styrofoam pieces mixed with bran (e): mealworms fed with gentamicin mixed with bran followed by solely Styrofoam (f): mealworms fed with bran

Experimental group (e) fed with gentamicin followed by Styrofoam resulted in the greatest death rate (93.3%) among all the groups. Nearly all mealworms died in group (e). From the results obtained, the control group fed with bran appeared to have the lowest death rate (12.5%).

In general, mealworms fed with small pieces of Styrofoam had a lower death rate than those fed with one larger block of Styrofoam did, with or without addition of bran. The death rate of mealworms fed with a large piece of Styrofoam doubled the death rate of those fed with Styrofoam cut into smaller sizes.

Addition of bran did not have a drastic effect on the death rate of mealworms. According to Figure 15, experimental group (a) fed with a large Styrofoam block had a similar death rate (~50%) with experimental group (c), which was fed with a large Styrofoam block and bran. Same goes for experimental group (b) fed with small pieces of Styrofoam and experimental

group (d) fed with small pieces of Styrofoam plus bran. Both groups showed a similar death rate of mealworms, which was around 25%.

4.2 Characterization of gut bacteria isolated from mealworms

Two different bacteria were observed and identified based on cultural and morphological characters.

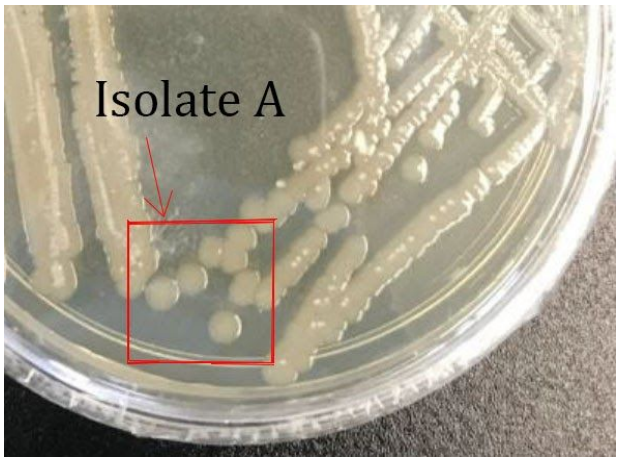


Figure 16: Colony of isolate A

Figure 17: Colony of isolate B

Isolate	Name of bacteria
A	*Exiguobacterium sp.*
B	*Lactococcus sp.*

Table 1: Identification of bacterial isolates

Morphological characteristics of the identified bacteria were given.

Exiguobacterium sp.

Circular colony which was 1-5 mm in diameter. The colony was shiny and yellowish, with convex elevation. Small, rod-shaped and gram positive.

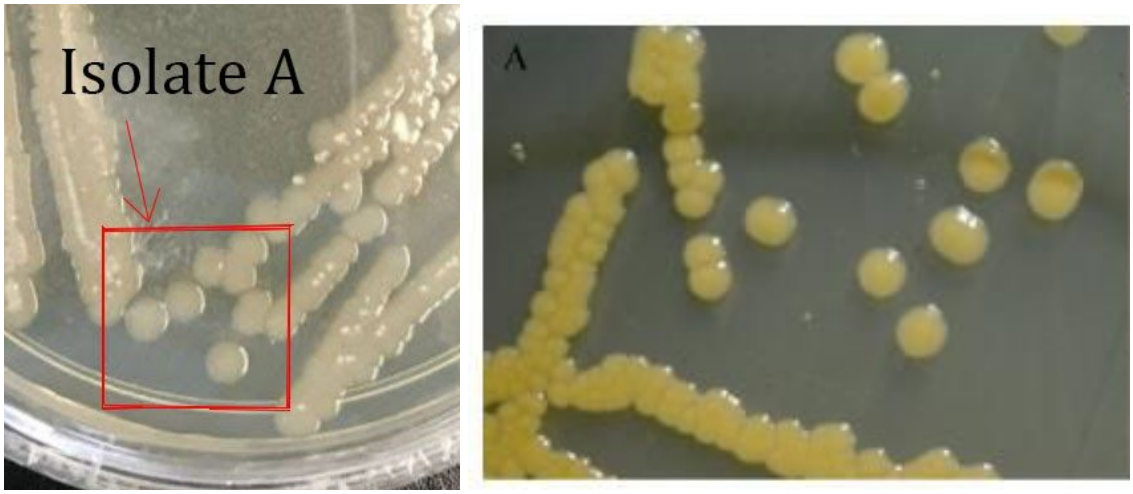


Figure 18: Colony of isolate A (left) and *Exiguobacterium undae* in the TSB solid medium[13] (right)

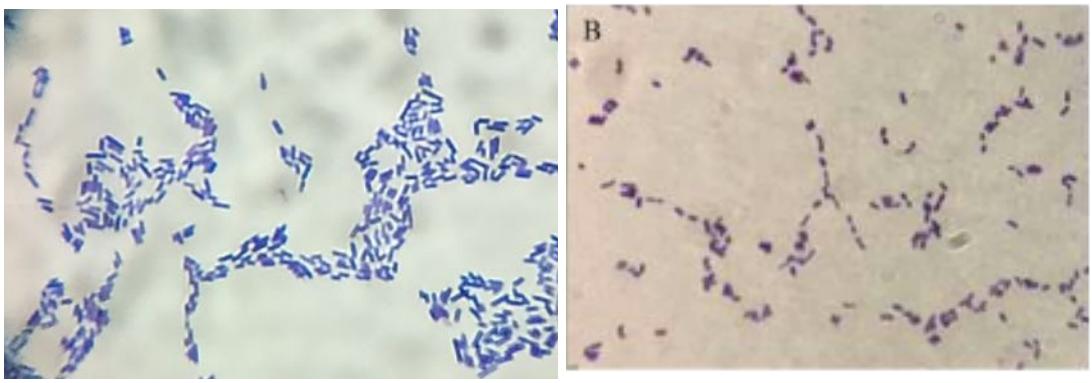


Figure 19: Gram's dye micrograph of isolate A (1000X) (left) and *Exiguobacterium undae*[14] (1000X) (right)

[13] Image from MA Yuan-Yuan, CHEN Xiang-Xiang, LI Min, WANG Jing, ZHANG Xiu, YANG Guo-Ping. Degradation of cinnamic acid by *Exiguobacterium sp*. Strain[J]. Microbiology China, 2017, 44(9): 2079-2088. (Figure 1A)
[14] Image from MA Yuan-Yuan, CHEN Xiang-Xiang, LI Min, WANG Jing, ZHANG Xiu, YANG Guo-Ping. Degradation of cinnamic acid by *Exiguobacterium sp.* Strain[J]. Microbiology China, 2017, 44(9): 2079-2088. (Figure 1B)

Lactococcus sp.

Punctiform colony with round shape and smooth edges. The colony was whitish or creamy coloured, with convex elevation. Coccoid and gram positive.Occurs in chains.

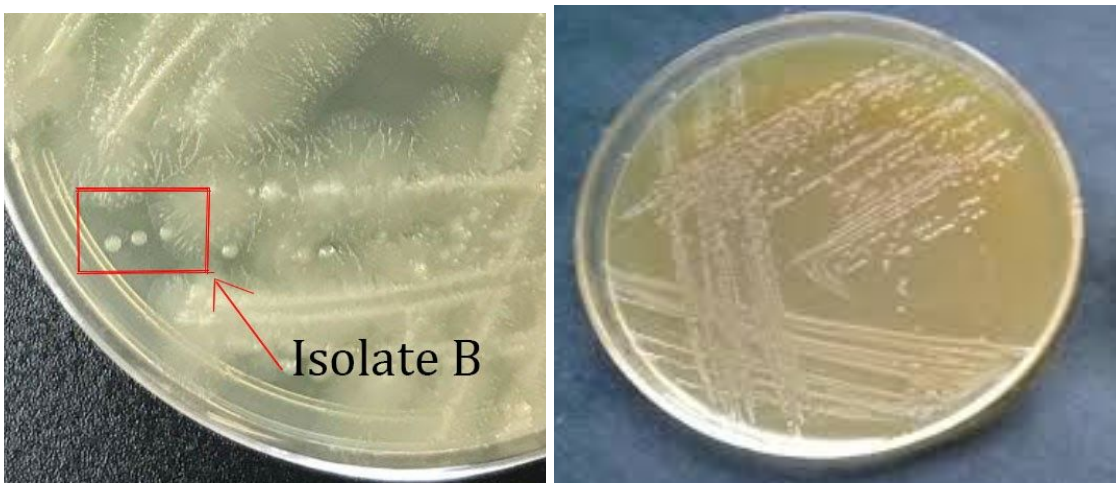


Figure 20: Colony of isolate B (left) and *Lactococcus lactis* on MRS media[15] (right)

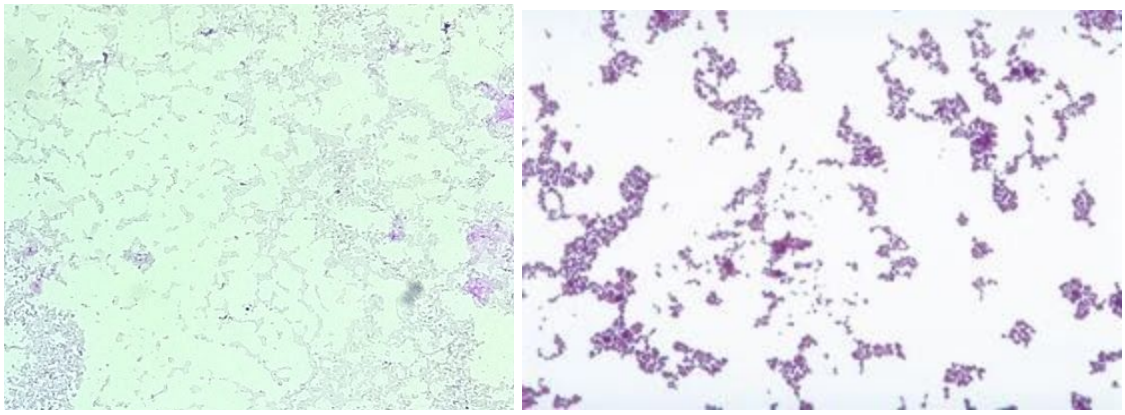


Figure 21: Gram's dye micrograph of isolate B (400X) (left) and *Lactococcus lactis*[16] (right)

[15] Image from P.K. Nagalakshmi, R. Sumathi, K. Kanimozhi and T. Sivakumar. Isolation of bacteriocin nisin producing Lactococcus lactis from dairy products. J. Acad. Indus. Res. Vol. 1(10) March 2013. (Plate 1)
[16] Image from Ward's Science. Ward's® Live *Lactococcus lactis* Culture. Retrieved from https://www.wardsci.com/store/product/8878855/ward-s-live-i-lactococcus-lactis-i-culture

4.3 Biodegradation of Styrofoam by gut bacteria

Condition	Volume of EDTA solution added (μL)	Condition	Volume of EDTA solution added (μL)
(i)	10	(vi)	10
(ii)	20	(vii)	20
(iii)	10	(viii)	Negligible
(iv)	10	(ix)	Negligible
(v)	10	(x)	Negligible

Table 2: Result of biodegradation of PS-film under various conditions

(i): 1 mL gut suspension (pH=3) + 5x5mm PS films (ii): 1 mL gut suspension (pH=5) + 5x5mm PS films (iii): 1 mL gut suspension (pH=7) + 5x5mm PS films (iv): 1 mL gut suspension (pH=9) + 5x5mm PS films (v): 1 mL gut suspension + 1 mL NB medium + 5x5mm PS films (vi): 1 mL gut suspension + 5x5mm PS films (vii): 1 mL gut suspension + 5x5 mm PS films (each film was cut into four smaller pieces) (viii): 1 mL LCFBM + 5x5mm PS films (ix): 1 mL NB medium + 5x5mm PS films (x): 1 mL distilled water + 5x5mm PS films

*Note that the more the EDTA solution added, the higher the concentration of calcium ions in the sample.

Among all the conditions, the biodegradation rate of PS films was found to be the highest when there was addition of NB medium and when pH value = 5, since higher concentration of calcium ions was present under condition (ii) and (vii), indicated by a larger volume of EDTA solution added. For other conditions (except the control set-ups), the biodegradation rate was found to be similar yet lower than that in condition (ii) and (vii). Results of the control set-ups of the experiment (conditions (viii), (ix) and (x)) were indicated as "negligible", which meant the concentration of calcium ion was too low to be measured by the kit.

5.Discussion

5.1 Implications of the experimental result

5.1.1 Biodegradation of Styrofoam by mealworms

In this study, mealworms were fed with Styrofoam under different conditions for ten days. Percentage decrease in weight of Styrofoam and death rate of mealworms were recorded and analyzed to find out the effect of various conditions on the biodegradation rate by mealworms.

To begin with, it is confirmed that the biodegradation of Styrofoam by mealworms is, to a large extent, due to the activity of gut bacteria. Experimental group (e) of mealworms fed with gentamicin, followed by a sole diet of Styrofoam, resulted in the least percentage decrease in weight of Styrofoam (3.4%) and the highest death rate of mealworms (93.3%). Gentamicin is an antibiotic that suppresses the activity of bacteria. After consuming gentamicin, mealworms were unable to digest Styrofoam, as shown by the result. It is thus suggested that the activity of bacteria in mealworms is the major contributor to the process of biodegradation.

Besides, addition of bran was found to aid the biodegradation of Styrofoam. Addition of bran resulted in a faster rate of biodegradation (i.e. larger decrease in the weight of Styrofoam), in comparison to experimental groups without addition of bran. It might be intuitive to think that addition of bran can raise the survival rate of mealworms, thus more mealworms are available for consumption of Styrofoam. Nevertheless, the result of investigation suggests otherwise. The death rate of the mealworms fed with Styrofoam mixed with bran was similar to those fed with Styrofoam as the sole diet. Mixed diet did not reduce the death rate, yet it successfully raised the consumption rate. It is thought that the addition of bran facilitates the activity of PS-degrading bacteria, rather than absorption of nutrients by mealworms. The exact principle behind is still unclear.

Feeding mealworms with smaller pieces of Styrofoam is found to be an effective way to raise both the biodegradation rate of Styrofoam and survival rate of mealworms. It is deduced that since the surface area to volume ratio of small pieces of Styrofoam is higher than that of a

whole block, more room and space are available for mealworms to bite on. Furthermore, the small pieces of Styrofoam are more dispersed in the tray containing mealworms, which reduces competition for food. These are possible reasons behind a higher biodegradation rate of Styrofoam and survival rate of mealworms in experimental groups fed with small pieces of Styrofoam.

Last but not least, Styrofoam is suggested to be a less favorable diet for mealworms. In this study, the death rate of the control group fed with bran only (12.5%) was lower than the death rates of all the experimental groups of mealworms fed with Styrofoam by 10.8% to 80.3%. Despite their ability to consume Styrofoam, mealworms would have their mortality rate raised if being fed with Styrofoam as a diet for a long time. The increase in mortality rate cannot be compensated by extra addition of bran. Thus, Styrofoam as a diet is said to be less favourable for mealworms.

5.1.2 Characterisation of gut bacteria isolated from mealworms

Two bacteria, *Exiguobacterium sp.* and *Lactococcus sp.,* were isolated from the gut suspension of mealworms given polystyrene as the sole diet. The result is consistent with previous studies (Yu Yang et al., 2015 and Aneta K.Urbanek et al., 2020). Based on observation of colonial morphology, it was found that the abundance of *Exiguobacterium sp.* was higher than that of *Lactococcus sp.*. The result implies that *Exiguobacterium sp.* was able to survive and grow better with polystyrene as the only source of carbon. In view of this, it is suggested that *Exiguobacterium sp.* played a fairly important role in degradation of Styrofoam. Although the abundance of *Lactococcus sp.* was observed to be comparatively lower, it may be due to competition from *Exiguobacterium sp.*. Hence, the biodegradation rate of polystyrene by the isolated strains should be studied individually to give more information on the ability of each strain in degradation of polystyrene.

5.1.3 Biodegradation of Styrofoam by gut bacteria

Most calcium ions were released from the PS-film when pH=5 and with the addition of extra nutrients (NB medium). It was concluded that 5 is the optimum pH value for the growth of bacteria in gut suspension.

On the other hand, addition of supplementary nutrients also helped raising the biodegradation rate. The result was consistent with the result in 4.1.1 (weight change of Styrofoam recorded after feeding mealworms with Styrofoam under different conditions for ten days), where addition of bran was found to increase the consumption rate of Styrofoam by mealworms. It is thus suggested that supply of extra nutrients facilitates the activity of PS-degrading gut bacteria, and thus aids the biodegradation of polystyrene.

5.2 Possible improvements

5.2.1 Biodegradation of Styrofoam by mealworms under different conditions

Throughout this experiment, it is found that compared with the control group, mealworms fed with Styrofoam showed higher death rate. With a fairly high death rate, the biodegradation rate of Styrofoam will be hindered and thus the obtained result may not be able to reflect the actual rate. It is also found that the death rate of mealworms fed with small Styrofoam pieces is lower than those fed with one block of Styrofoam. As a result, it is suggested that several blocks of Styrofoam might be placed instead of only one to reduce competition of mealworms. Also, a bigger tray can be provided to avoid overcrowding of mealworms. In addition, metabolic waste of mealworms should be disposed from time to time to maintain good hygiene conditions. Discarding excretory waste regularly also increases the accuracy when measuring the weight change of Styrofoam, since there is less chance for the waste to stick onto the surface of Styrofoam.

Furthermore, to ensure Styrofoam is actually consumed and digested by mealworms, instead of merely being broken down into pieces mechanically, the weight of mealworms can be measured before and after the investigation. Mealworms fed with normal diet (bran, oatmeal, etc.) and starved mealworms can act as control groups for comparison, so as to conclude if Styrofoam can serve as a fairly good source of nutrients for mealworms. In addition to Styrofoam, further investigation can be done by feeding mealworms with different types of plastics. This is to explore the range of plastic waste that may potentially be treated by mealworms.

5.2.2 Characterization of PS-degrading gut bacteria from mealworms

The result of characterization of PS-degrading bacteria in this report is not reliable since the bacteria were identified based on their morphology due to lack of equipment. Besides, our method only allows identification up to genus level. Metagenomic analysis should be performed if possible to raise the reliability of the result, as well as accurate characterization to species level.

5.2.3 Biodegradation of Styrofoam by gut bacteria under different conditions

In this project, the effect of various environmental factors on the rate of biodegradation by gut bacteria of mealworms was investigated. Gut suspension was used this time as we would like to study the overall effect of all strains in the gut. This time, two strains were isolated and identified. Nonetheless, isolation of bacteria can be repeated for several times with mealworms from different regions, so as to seek for a variety of potentially PS-degrading strains. Further investigation can then be carried out using isolated strains, with reference to the result of characterization of gut bacteria. The effectiveness of polystyrene degradation by individual strain can be studied so as to sort out the most effective strain. On the other hand, different combinations of strains may also be used to investigate if there is any synergistic or antagonistic effect among the strains. The effect of other environmental factors on the growth of gut bacteria, such as temperature, can also be investigated. By this, a specific set of bacterial strains and conditions with the highest efficiency in biodegradation of polystyrene might be found, which provides a potential way to process massive plastic waste.

In this study, the biodegradation rate of **PS film** by gut bacteria, instead of that of **Styrofoam**, was investigated since concentration of calcium ions in the films served as the dependent variable in the experiment. Further research should be conducted to investigate if biodegradation of Styrofoam without being processed can be done by gut bacteria. It would help further assess the feasibility of applying gut bacteria in real life as a solution to Styrofoam waste problem.

This time, to monitor the biodegradation of PS films, a method involving detection of calcium ion concentration was used. However, it is suggested that the simple colorimetric method described

previously(T. K. Meng et al., 2018) is a better alternative and should be conducted if possible. Our method requires longer time for calcium ions to accumulate to a level sufficient to be detected by the reagents. Furthermore, the process of biodegradation is not readily observed since the solution of calcium nitrate is colorless. The colorimetric method should be able to overcome these problems. Firstly, the pale blue color of the suspension indicates the PS films are being digested by the strain in the suspension. The color change is easily seen by eyes. We can study the result with a colorimeter as soon as we observe the color change. Secondly, since a colorimeter is capable of detecting the concentration of dye in the suspension even at very low concentrations, the time of investigation can be greatly reduced since it is not necessary to allow the strains to digest the films for a very long time.

5.3 Feasibility of application in real life

From the experiments conducted, it is observed that using mealworms or gut suspension to degrade Styrofoam both have advantages and downsides.

In terms of efficiency, biodegradation by mealworms is relatively convenient as extra steps such as extraction of gut suspension are not needed. In addition, mealworms are able to bite through a large piece of Styrofoam and break it down into smaller pieces, or even powder. By that, the surface area of Styrofoam for biodegradation is increased greatly, which has been proven to facilitate biodegradation. The volume of Styrofoam waste can also be highly reduced since it contains mostly air. As a result, mealworms may be an ideal solution to the problem of bulky Styrofoam waste occupying too much space in landfills.

However, several problems have been encountered when mealworms were used to degrade Styrofoam. Firstly, water must be given periodically and good ventilation should be maintained, otherwise the death rate of mealworms will be high. Another noticeable issue is the accumulation of waste. Excretory products of mealworms give out a pungent smell, which may cause hygiene problems if not handled properly. Apart from that, the biodegradation by mealworms requires constant replenishment of new larvae due to occasional deaths of larvae and the fact that the larvae of *Tenebrio molitor* undergo complete metamorphosis. Larvae will emerge as an adult beetle after 3 to 30 days (depends on living conditions). Further research

should be conducted to confirm if adult *Tenebrio molitor* can carry out biodegradation of Styrofoam just like larvae do. If not, the efficiency of biodegradation will significantly drop when larvae turn into adult beetles.

On the other hand, biodegradation of Styrofoam by gut bacteria faces other obstacles if it is to be used in practice. Unlike mealworms that are able to break down the bulky Styrofoam through biting, degradation by bacterial suspension requires processing of Styrofoam beforehand. Air in Styrofoam, which occupies 95% volume, ought to be removed through dissolution or densification to increase the efficiency of degradation. Another problem is that the degradation of polystyrene by gut bacteria is not really rapid. Most of the PS films (1 g) added for enrichment of gut bacteria remained after one month.

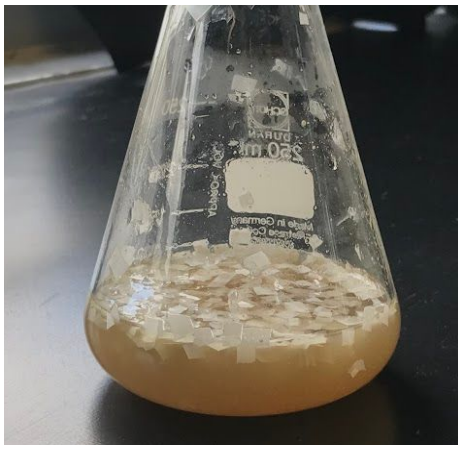


Figure 22: PS films in bacterial enrichment

Figure 23: PS films in bacterial enrichment after one month

Nonetheless, bacteria can overcome the problem of low sustainability encountered by mealworms. To begin with, regular water supply is not required for bacterial suspension. Also, it will not produce smelly waste products. Besides, in light of the high reproduction rate of bacteria, we need not to replenish bacteria frequently. As a result, biodegradation of Styrofoam by bacterial suspension can be conducted over a long period of time with less manpower and supervision.

Summing up, comparing the two of the methods to degrade Styrofoam, the advantages of biodegradation by gut bacteria outweigh biodegradation by mealworms.

Bacteria can reproduce rapidly under moderate conditions. Replenishment is seldom needed in the process of biodegradation by gut bacteria, which would be more cost-effective. Meanwhile, this investigation suggests that Styrofoam will result in a higher death rate of mealworms, compared with those fed with normal diet. Furthermore, the life cycle of mealworms only lasts for several months. As a result, there would be constant loss of mealworms during biodegradation and replenishment is required periodically to maintain a high rate of biodegradation. Another problem is that more manpower is required for biodegradation of Styrofoam by mealworms, since we would need to deal with the pungent excretory products of mealworms, as well as the corpses of mealworms so as to maintain a satisfactory hygiene condition.

It is thus suggested that biodegradation by gut bacteria is a more sustainable and cost-effective choice for biodegradation of Styrofoam. However, the extremely slow rate of biodegradation is still the major obstacle faced by this method. Biodegradation of Styrofoam by bacteria is not likely to be a promising remedial measure that can solve Styrofoam waste problem, unless further research is done to increase the biodegradation rate to a sufficiently high level.

6. Conclusion

All in all, mealworms were able to digest polystyrene. The rates of consumption of Styrofoam by mealworms differ under various conditions. Feeding mealworms with Styrofoam cut into small pieces successfully increased the survival rate of mealworms and consumption rate of Styrofoam. Besides, providing additional nutrients other than simply Styrofoam could raise the biodegradation rate of Styrofoam. Yet, it was inevitable that the death rate of mealworms would be higher when they were given Styrofoam as a part of diet, in comparison with the control group fed with bran.

Gut bacteria of mealworms are deemed as a more appealing alternative for biodegradation of Styrofoam. As proven in this study, gut bacteria play a crucial role in the process of biodegradation. This time, the rate of biodegradation of polystyrene films by gut suspension extracted from mealworms under different conditions was investigated. The rate was found to be the highest when pH value of the medium is 5 and with addition of extra NB medium, suggesting that growth of PS-degrading bacteria was favored under these conditions. Furthermore, two bacteria, *Exiguobacterium sp.* and *Lactococcus sp.*, were isolated from the guts of mealworms fed with polystyrene films. The relatively high abundance of *Exiguobacterium sp.* suggested that it contributed more in degradation of polystyrene.

To sum up, both mealworms and their gut bacteria were able to digest or degrade polystyrene. The advantages and shortcomings of each method were discussed. Mealworms are able to break down large pieces of plastic waste through biting. Yet, the manpower required for handling mealworms and the hygiene issue raised are concerned. Another issue is that *Tenebrio molitor* exists in the form of larva (i.e. mealworms) only for 3 to 30 days. The sustainability of biodegradation by mealworms is thus doubted. Bacteria extracted from guts of mealworms seem to be able to overcome the mentioned obstacles faced by mealworms. Therefore, comparing the two methods, biodegradation of Styrofoam by bacterial suspension is deduced to be more feasible to be brought into practice. Nonetheless, the slow biodegradation rate of polystyrene by bacteria as observed in this study ought to be improved by conducting further study. Otherwise, this method would only allow small-scaled degradation of polystyrene, which could not help with the accumulation of massive Styrofoam waste.

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