The extraction and isolation of polyethylene-based plastic-degrading bacteria from Iloilo City Engineered Sanitary Landfill, Mandurriao, Iloilo City

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Abstract

Plastics are known for being a durable material while still maintaining a low cost of production. It is an important material for commercial use all over the world. However, due to the lack of a reliable method of disposal, the risk of plastic pollution is steadily increasing throughout the years. Bioremediation is a promising method in this aspect as it is an eco-friendly way of dealing with this problem. Thus, this study aimed to extract and isolate bacteria from Iloilo City Engineered Sanitary Landfill and determine their biodegradation capability on low density polyethylene, high density polyethyelene, and polyethylene terephthalate. Bacteria were extracted from randomly chosen sites and two strains were identified, EMBP2A and MSAP2A. There were three set-ups done in triplicates: EMBP2A cultivated with LDPE, HDPE, and PET, MSAP2A cultivated with the same plastics, and the control set-up with the bacteria-free medium. After 10 days of incubation, Strain EMBP2A had decreased the weight of an HDPE plastic to a high percentage of 0.437% and PET to 0.147%. Strain MSAP2A had achieved the highest dry weight loss of 0.495% for PET.

Keywords: *bioremediation, strain, LDPE, PET, HDPE*

Introduction. Plastic has been widely used in many of the products present today due to the low cost of production and high durability of this material. However, due to the uncontrolled increase in the production rate [1], it slowly became a problem which led to pollution on water and land [2]. Several methods of disposal are currently being utilized such as landfilling and incineration; however, due to the environmental problems posed by these methods, it is necessary to look for an alternative method which is both environmentally friendly and still is efficient in the disposal of plastics. One promising field currently being studied is bioremediation [3].

To be able to understand bioremediation more, it is necessary to define the terms that were commonly used in this study. The terms are plastic and biodegradation. Based on the study by Andrady and Neal [4], plastic is a versatile material made up of polymers that is usually utilized for commercial use. Plastics can be categorized into different types using a code. Based on the system established by the Society of the Plastics Industry [5], there are seven codes in classifying plastic: 1 for Polyethylene Terephthalate (PET), 2 for High Density Polyethylene (HDPE), 3 for Polyvinyl Chloride (V), 4 for Low Density Polyethylene (LDPE), 5 for Polypropylene (PP), 6 for Polystyrene (PS), and 7 for others. This system is usually used by consumers and recyclers for identification and segregation. Biodegradation is a method of degradation that uses microorganisms to breakdown polymers in the plastic either through metabolic or enzymatic action [6]. There is a study done to assess the biodegradation capability of microbes extracted from a dumpsite. Bolo et al. [7] extracted bacteria from the Payatas Dumpsite, Quezon City, Philippines, and out of the four isolates, *Pseudomonas stutzeri* is the most well-known plastic degrader and has the highest efficiency among them.

There are studies that identified the microbes associated with dumpsites. Based on the study by Williams and Hakam [8], the species isolated from four dumpsites in Port Harcourt Metropolis, Nigeria, were *Bacillus spp., E. coli, Klebsiella spp., Proteus spp., Pseudomonas spp., Staphylococcus aureus,* and *Streptococcus spp.* There are studies that identified the microbes that have the capability to degrade polymers. Bhardwaj et al. [9] researched about the microbial populations that are associated with plastic degradation. Based on their findings, the microbes that are known for their biodegradation capability are *Rhizopus delemar, Firmicutes, Protobacteria, Penicillium, Rhizopus arrizus,* and *Pseudomonas stutzeri.*

There are several methods available for the assessment of the biodegradation capability of the microbes. The study by Bolo et al. [7] used two methods, namely the Fyrite gas analyzer and scanning electron microscopy. Shovitri et al. [10] used a different method wherein the dry weight loss of the plastic is used to confirm plastic degradation. There are also methods for the incubation of bacteria with the plastic samples. Singh et al. [11], Kathiresan [12], and Sowmya et al. [13] used the conical flask method, while Mahdiyah et al. [3] and Shovitri et al. [10] used the soil burial method. Although the field has been studied extensively, there is a lack of research regarding this field here in the Philippines, a

How to cite this article:
CSE: Canja, JSO, Galan, JC, Hilis, RS, Jolito, FC, III. 2020. The extraction and isolation of polyethylene-based plastic-degrading bacteria from Iloilo City Engineered Sanitary Landfill, Mandurriao, Iloilo City. Publiscience. 3(1):106-110.
APA: Canja, J.S.O., Galan, J.C., Hilis, R.S., & Jolito, F.C. III. (2020). The extraction and isolation of polyethylene-based plastic-degrading bacteria from Iloilo City Engineered Sanitary Landfill, Mandurriao, Iloilo City. *Publiscience,* 3(1):106-110.

country that ranks third among other countries of the world in producing plastic waste dumped into the oceans [14]. It is also necessary to note that the existing studies might have overlooked the use of the plastic swab as a method of collecting bacterial samples. This study aimed to extract and isolate bacteria from Iloilo City Engineered Sanitary Landfill, Mandurriao, Iloilo City, and assess their biodegradation potential on LDPE, HDPE, and PET. It specifically aimed to:

(i) extract plastic swab and soil samples from five randomly selected areas in the dumpsite;

(ii) isolate bacteria from the swab and soil samples extracted from the dumpsite; and

(iii) determine which isolated bacteria has the best bioremediating capability using a formula to compute for the plastic chips' dry weight losses.

Methods. The methods were divided into five main steps namely sample collection, plastic preparation, bacteria culture cultivation, plastic degradation, and data analysis. The steps were done for all nine setups, and the data were collected after 10 days upon the placement of the plastic films in the medium.

Sample collection. The bacteria were extracted from plastic waste, using swab sampling, and from soil samples taken from the dumpsite. The plastic wastes were randomly selected, and a total of five swab samples were collected and sealed inside test tubes. They were then transported to a laboratory in Philippine Science High School - Western Visayas Campus (PSHS-WVC). For the soil, five samples were collected from a depth of 10 - 20 cm, then placed inside sterile containers and kept at a temperature of 4°C [7].

Random sampling was done by having an aerial view of the area (Coordinates: 10°42'34.7"N 122°31'25.8"E) and sectioning it equally into 25 sites. Five randomly selected sites were chosen using a random number generator. Site 1 was located in area 7, site 2 in area 6, site 3 in area 13, site 4 in area 18, and site 5 in area 9.

Plastic preparation. Three different types of plastics (PET, HDPE, LDPE) were cut into strips having dimension of 2cm x 2cm with three replicates for each plastic in each set-up. They were sterilized in 70% ethanol for approximately 30 minutes, washed with distilled water and subsequently dried in an incubator at 60°C for 24 hours. Afterwards, plastics were put into a silica gel-containing desiccator for 24 hours for total water evaporation. Initial dry weight of plastic was measured with an analytical balance [11].

Bacteria culture cultivation. The bacterial samples in the swabs were plated on nutrient agar (NA) medium using streak method of inoculation. Three plates of NA media were utilized for the growth of the bacteria. The plates were incubated at 30°C for 24-48 hours. Colonies with different morphological appearance was subcultured onto fresh NA for the purpose of identification.

As for the soil samples, four grams of each were suspended in 96 ml of sterile distilled water and shaken vigorously for two minutes. These were then heated at 60°C for 60 minutes in a water bath. The mixture was then put to rest to allow the soil particles to settle. It was then plated on nutrient agar using streak method. Incubation was done at 30°C for 24-48 hours. Identification was done using morphological observation and Gram staining, then selective media were used to allow the specific bacteria to grow [12].

Samples were then plated in selective media, eosin methylene blue (EMB) agar and mannitol salt agar (MSA), to further categorize the bacteria. Out of the ten plates, two were selected for incubation based on their morphological properties, one from EMB and the other from MSA.T The strains were given codes for identification.

Plastic degradation. Three set-ups were made, the first being the medium inside the Petri dish where Bacteria 1 was cultivated with the three types of plastic strips (HDPE, LDPE, and PET) having each type of test plastics in triplicates. The second setup was the medium with Bacteria 2, and the same was done for this set-up. The last set-up is the control, which was maintained with polyethylene strips in the microbe-free medium. Pre-weighed strips of sterilized plastics of each type were aseptically transferred to the Petri dish containing mineral salt medium (MSM) and inoculated with the bacteria to be tested.

Triplicates were maintained for each type of plastic and were left on the incubator. After 10 days, the plastic discs were collected and washed thoroughly using distilled water. They were then dried in a hot air oven at 50°C overnight for at least 10 hours and were weighed for final dry weight.

Data Analysis. Dry weight loss percentage is used to indicate the biodegradation rate of plastic during the incubation process. The percentage weight loss was calculated using the formula below

$$Weight\ loss\ \% = \frac{I.W. - F.W.}{I.W.} \times 100$$

where I.W. is the initial dry weight of the plastic strips pre-degradation and F.W. is the final dry weight of the plastic strips post-degradation [13]. The dry weights of the plastic strips after degradation were also subjected to statistical analysis using one-way analysis of variance (ANOVA).

Safety Procedure. The researchers underwent biosafety training before the conduct of the study. Proper personal protective equipment was used during the whole conduct of the study. During the sample collection, boots, gloves, and masks were used to protect the researchers. During the laboratory work, lab gowns, gloves, and masks were used. Proper tools were also used during the inoculation and preparation of the bacteria. Samples were sterilized and dried before being disposed of in the biohazard bin, while excess chemicals, which were unused, were stored in the laboratory.

Results and Discussion. Degradation of plastics by microorganisms has been studied for several years. The present investigation was performed to provide an analysis on the biodegradation potential of bacteria from a local dumpsite using the three commonly used plastics:

PET, HDPE, and LDPE. Biological decomposition of synthetic materials such as plastics can be facilitated by microorganisms in natural environments.

A total of 28 colonies were isolated from plastic waste swab and soil samples taken from Iloilo City Engineered Sanitary Landfill using NA and TSB media. This was done by identifying the morphological appearance of colonies. After facilitating growth in selective media, strains of bacteria were identified. Bacterial strains EMBP2A and MSAP2A as shown in the plates below were subjected to the three types of plastics. These were the bacterial codes used that would indicate the nature of the bacterial strain. Strain EMBP2A signifies that the bacterial sample was collected from plastic swab in site 2 of the sampling area and was cultivated in EMB media. Strain MSAP2A signifies that the bacterial sample was collected from plastic swab in site 2 of the sampling area and was cultivated in MSA media.

Plate 1. Identified bacterial strains EMBP2A and MSAP2A for incubation.

Dry weight loss percentage is used to indicate the biodegradation rate of plastic during the incubation process. The percentages for the three types of plastics are shown below (Figure 1) for the entire biodegradation period of 10 days. It showed that the plastics used have decreased the weight of some plastic strips. A minimal increase in the weight of other plastic strips can also be observed. Mean weight changes were computed and used to compare the effectiveness of biodegradation between the two strains of bacteria. A negative mean indicates that there was a gain in weight instead of a loss. These plastic strips that have been found to increase their weight were undegraded in the incubation time period of 10 days. Strain EMBP2A had decreased the weight of an HDPE plastic to a highest percentage of 0.437% and PET to 0.147%. Strain MSAP2A had achieved a highest dry weight loss of 0.495% for PET.

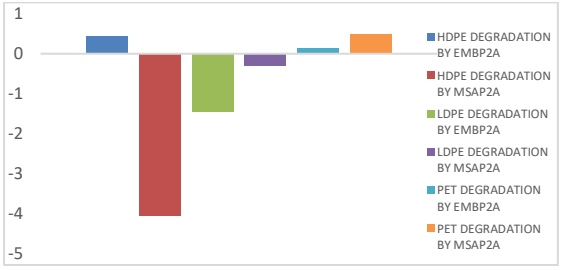

Figure 1. The comparison of the dry weights of HDPE, LDPE, and PET after bacterial degradation by strains EMBP2A and MSAP2A.

Using one-way analysis of variance (ANOVA) with α value set to 0.05, it was found that there was no significant statistical difference between the dry weights as presented in Table 1. Given the two bacterial strains, EMBP2A and MSAP2A, none of them have shown a significant effect on the degradation of any type of plastic used as shown below:

Table 1. P-values of HDPE, LDPE, and PET plastic chips.

	HDPE	LDPE	PET
P-value	0.0631	0.853511	0.9077

Microorganisms play an important role in the biodegradation of materials, including synthetic polymers, such as plastic, in natural environments. During degradation, enzymes from the microorganisms break down the polymers into smaller molecules of short chains that can pass through the microbes' semi-permeable outer membranes, allowing the plastics to be used as carbon and energy sources [6].

In the current study, three types of plastics were used to measure the degradation percentage, which were high density polyethylene, low density polyethylene, and polyethylene terephthalate. These were chosen because they are the most commonly used plastics, and they degrade at a slow rate in the natural environment, estimated in decades, which causes serious environmental problems [14]. A decrease in the mean weight of some plastics indicates that the bacteria can have the potential to use plastic as an alternative carbon and energy source. Strain EMBP2A had decreased the weight of an HDPE plastic to a high percentage of 0.437% and PET to 0.147%. Strain MSAP2A had achieved the highest dry weight loss of 0.495% for PET. Kathiresan and Bingham [15], which have reported in their study that biodegradation using bacteria is ranging from 0.56 to 8.16% for plastics. Their study, however, performed the degradation process in a duration of 90 days. An increase with the weight for some plastic strips could be correlated with its exposure to external factors that could possibly influence the weight. According to Gajendiran et al. [16], due to the adherence of microbes utilizing the polymers, plastic samples could increase in weight.

There was no significant statistical difference found between the dry weights of the plastic strips before and after they were subjected to degradation. Degradation is a naturally occurring phenomena and it could take hundreds of years for a plastic to break down completely. Biodegradation utilizes the capability of microorganisms with the enzymes they produce to enhance the biodegradation process. It would require a considerable amount of time for the bacteria to consume the plastics to achieve a significant decrease in the weight of plastics after they are consumed. This can be observed in studies like that of Singh et al. [6] where they tested the biodegradation capability of bacteria isolated in soil for 40 days; Kathiresan and Bingham [14] where they performed this process in a duration of 90 days; and lastly, Usha et al. [13], where they found out that *Pseudomonas sp.* was able to degrade 28.42% of plastics in a period of six months.

The results of this work were also in accordance with earlier research studies done by Sowmya et al. [1], in which they reported that some bacteria were able to grow on minimal medium containing polyethylene as sole carbon source. This showed its capability to utilize

plastic as a carbon source and to degrade polyethylene. Degradation of plastic was carried out between the two bacterial strains, EMBP2A and MSAP2A. The bacteria were able to degrade PET the most among the three types of plastics. But because of a limited period, in a 10-day course, results showed a nonsignificant biodegradation of the three types of plastic using the two bacterial strains.

Limitations. The study only covers the testing of dry weight loss to confirm degradation without the support of visual testing using scanning electron microscope (SEM). Bacteria were also tested only up to the morphological level based on the growth of the colony. The incubation of the plastic with bacteria only lasted for 10 days due to the time constraint.

Conclusion. Bacteria capable of degrading plastics were isolated from Iloilo City Engineered Sanitary Landfill. In a course of 10 days, in some setups, the bacteria used were able to utilize the plastics as their sole carbon source during the incubation period. Some could degrade the plastics; however, the dry weight loss percentage among all the setups was not significant. The nature of the degradation process also played a major role for the minimal dry weight loss percentage.

Recommendations. It is recommended to replicate the study with focus on reducing external factors that may affect the degradation process of the plastic (e.g. temperature, humidity, etc.). Extending the duration of the experiment and observing the intervals are also recommended to further improve the study. Addition of parameters to be observed to confirm degradation is also advised to further improve the quality of the study. It is also recommended to extend the incubation time for future studies because the biodegradation process could be more extensive the longer the bacteria could feed on the plastics. Future researchers of similar fields can also utilize a scanning electron microscope to observe the surface of the plastics and to see if there is significant physical change on the microscopic level. Implementation of the project on a larger scale can also improve the quality of the data gathered as more accurate representation of the data can be observed.

Acknowledgement. The researchers would like to acknowledge the help of the guards and workers especially to the head of operations, Engr. Ernest Navida from the Iloilo City Engineered Sanitary Landfill for their guidance during the conduct of the data gathering.

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