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Degradability of Plastics in Mangrove Soil from Eco-Mangrove Reserve in Calapan City, Oriental Mindoro Philippines

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ABSTRACT

Mangrove forests are indigenous to tropical as well as subtropical regions worldwide. Mangrove soil is a rich source of plastic-degrading bacteria but no local study has been done to support its potential benefits. This study was conducted to assess the suitability of an improvised nutrient medium for the isolation of plastic-degrading bacteria from mangrove soil obtained from Silonay Eco-Mangrove Reserve, Calapan City, Philippines. Forty-Two (42) Winogradsky column WC), the first 21 WC contain mineral salt medium and the remaining 21 contain sea salt medium. Plastic samples were observed for 36 days by comparing the initial and final weights. WC with mineral salt medium showed the most changes in the final plastic weight while the seawater medium Winogradsky column showed less plastic weight changes. Laboratory test showed that plastic degrading bacteria that were gram-negative were *Citrobacter fruendii, Enterobacter agglomerans, Pseudomonas aeruginosa* and *Arizona spp*. The study showed that mangrove soil in Calapan City, Oriental Mindoro is a rich source of biodegrading bacteria. The use of improvised nutrient medium solves the

unavailability of expensive materials and can be used in exploring the potential use of identified bacteria for future work in biotechnology. Further studies need to be done to investigate other bacterial species aside from identified bacteria that can biodegrade plastics.

Keywords **—** Biological Science, Winogradsky, plastic-degrading bacteria, improvised nutrient medium, Philippines

INTRODUCTION

One of the major environmental threats is the least rate of degradation or non-biodegradability of the organic materials under natural condition, e.g. plastics. The plastics of various forms such as nylon, polycarbonate, polyethylene-terephthalate, polyethylene, polypropylene, and polystyrene, polytetraflouro ethylene, polyurethane and polyvinyl are continuously used in our day-to-day life.

Plastic materials are commonly used in food, clothing, shelter, transportation, construction, medical and recreation industries worldwide. Being sturdy and durable, they resist biodegradation. The very slow decomposition process contributes to pollution (Kathiresan, 2003). Plastic grocery bags can clog sewer pipes, create stagnant water, an ideal breeding ground for mosquitoes which have the potential to spread diseases (EduGreen, 2005; Environmental Literacy Council, 2005; IRIN, 2005a; IRIN, 2005b).

Degradable plastics are plastics designed to undergo a change in their chemical structure under specific environmental conditions resulting to a loss of some properties in a period of time (Gemini, Tennakoon, Weerasekara, & Nandasena, 2006). Oxo-biodegradable plastic is polyolefin plastic, a stable polymer consisting of long chains of ethylene monomers or polyethylene (PE) to which has been added amounts of metal salts to speed up the natural degradation process. The degradation process is shortened from hundreds of years to years and/or months of degradation. With the use of these degradable plastics, pollution may be minimized since it can be degraded in a short period of time.

Environmental degradation of polyethylene (PE) is a combined action of light, heat and microbial activity. Scientists recognize that PE cannot be easily degraded by microorganisms. To hasten biodegradation, PE is blended with starch and then added biodegradable additives and photo-initiators. Despite attempts to enhance the biodegradation of PE mixtures, their biodegradability is

still very low (Tokiwa, Calabia, Ugwu, & Aiba, 2009).

In recent years, there has been renewed interest in the degradation of plastics by bacteria and fungi. Some microorganism such as bacteria, fungi, and actinomycetes are involved in the degradation of both natural and synthetic plastics. Most of these microbes naturally thrive in soil. The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganism responsible for the degradation differs from each other and they have their own ideal growth conditions (Gemini, *et al.,* 2006). Wool and Cole (1991) believe that "the natural environment, whether in laboratory or field tests, with solid or aquatic system, must favor the degradation of readily decayed natural materials such as carbohydrates, lipids and proteins."

This study was conceptualized in search of cheap alternatives to commercial mineral enriched media for isolating plastic-degrading bacteria. Also, this study established baseline data for future research on biodegrading bacteria in mangrove soil.

OBJECTIVES OF THE STUDY

The study was conducted to assess the suitability of an improvised enriched medium for the isolation of plastic-degrading bacteria from mangrove soil obtained from Silonay Mangrove Reserve, Calapan City. Specifically, this study was conducted to: 1) Isolate plastic-degrading bacteria from mangrove soil using Winogradsky columns and to a) Describe the colony characteristics of plastic-degrading bacteria isolated from the Winogradsky column using the 2 growth media; b) Determine the staining and morphological characteristics of various plastic-degrading bacteria isolated from the Winogradsky column using the 2 growth media; and c) Establish the identity of the isolated plastic-degrading bacteria based on its biochemical properties; 2. Determine the rate of plastic degradation of the 2 growth media (i.e. mineral salt medium and sea salt medium) from mangrove soil; and 3) Determine the incubation period with the highest rate of plastic degradation of the 2 growth media (i.e. mineral salt medium and sea salt medium) from mangrove soil.

METHODOLOGY

A two by seven (2 X 7) factorial experiment in Completely Randomized Design (CRD) was used in this study. Factor A was the growth media while

factor B was the incubation period. Each treatment was replicated three times. The different treatment combination is presented in Table 1 while the treatments assignments are as follows:

Factor A – Growth Media

A1 – Mineral salt water
A2 – Seawater

Factor B – Incubation period

B1 – 3 days after incubation
B2 – 6 days after incubation
B3 – 12 days after incubation
B4 – 18 days after incubation
B5 – 24 days after incubation
B6 – 30 days after incubation
B7 – 36 days after incubation

Table 1. Treatment combinations which were used in the conduct of the study

GROWTH MEDIA(A)	**INCUBATION PERIOD (B)**						
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**
A1	A1B1	A1B2	A1B3	A1B4	A1B5	A1B6	A1B7
A2	A2B1	A2B2	A2B3	A2B4	A2B5	A2B6	A2B7

Legend: *A1B1 – mineral salt with PE incubated for 3 days; A1B2 - mineral salt with PE incubated for 6 days; A1B3 - mineral salt with PE incubated for 12 days; A1B4 - mineral salt with PE incubated for 18 days; A1B5 - mineral salt with PE incubated for 24 days; A1B6 - mineral salt with PE incubated for 30 days ; A1B7 - mineral salt with PE incubated for 36 days ; A2B1 – sea water with PE incubated for 3 days; A2B2 - sea water with PE incubated for 6 days; A2B3 - sea water with PE incubated for 12 days; A2B4 - sea water with PE incubated for 18 days; A2B4 - sea water with PE incubated for 24 days; A2B4 - sea water with PE incubated for 30 days; A2B4 - sea water with PE incubated for 36 days.*

Preparation of Winogradsky columns

Soil samples and seawater from Silonay mangrove reserve in Calapan City, Oriental Mindoro were collected in a plastic bucket. Forty- two glass (42) containers were dried after washing with soap and water. To prepare the Winogradsky columns, each glass container was filled with mangrove soil up to 2/3 level. Forty-two (42) were labelled according to treatment combination. Seawater (200mL) was then added to each glass container labeled for A2. Mineral Salt Medium (200mL) was also added to each glass containers assigned for A1.

To prepare a carbon-less mineral salt solution (enriched medium) for growing plastic-degrading bacteria, magnesium sulfate (1.25mL), calcium sulfate (0.2grams), ammonium sulfate (1gram) and ferric chloride (1.8mL) were mixed in 250mL distilled water in a plastic soda bottle. The pH of the solution was then adjusted to pH 6.8 to 7.2 by stepwise addition of 0.1N sodium hydroxide guided by a pH meter. Finally, 250mL of the final solution was added to glass containers assigned for A1.

Preparation of Polyethylene (PE) plastic samples

In preparing the plastic samples, forty-two (42) 6cm x 6cm samples were cut out from used grocery or sando bags (Oxo-biodegradable plastics). Each sample was placed in the 42 glass containers. Using a digital precision scale, initial dry weights were obtained and recorded. Wire loops were prepared to serve as plungers to keep the samples immersed in the liquid portion of the Winogradsky columns. Plastic samples were immersed in the liquid portion of the appropriate Winogradsky column using the insulated wire loops. The columns were stored in a cool, dry, partially lit area for thirty-six days (36 days). After the incubation period, the plastic samples were retrieved, washed and air dried. Final weights were obtained using digital precision scale. The seventh plastic sample in each replicated media sample was transferred to a sterile tube and brought to the Bacteriology lab for further studies.

Table 2. Chemicals used for preparing the improvised mineral salt medium

CHEMICALS	CHEMICAL FORMULA	DESCRIPTION	CONCENTRATION	AMOUNT/ VOLUME
Magnesium sulfate	$MgSO_4$	Clear liquid	200 mg per mL = 2.5g per 10 mL	1.25 mL
Calcium sulfate	$CaSO_4$	White crystalline powder	--	0.02 gram

Ammonium sulfate	$(NH_4)_2SO_4$	Off-white powder	--	1 gram
Ferric chloride	$FeCl_3$	Brown-black liquid	0.028 mg per mL	1.8 mL
Sodium hydroxide	NaOH	Solid, whitish pellets	0.1 milliequivalents per liter	4 grams
Hydrochloric acid	HCl	Clear, colorless liquid	0.1 milliequivalents per liter	968 mL distilled water, add 32 mL of muriatic acid

Precautions: *Label properly. Keep out of reach of children. Store in a cool, dry place. Preparation of acid or base solutions must be performed by a knowledgeable adult. To avoid risk of violent chemical reactions,* ***DO NOT ADD OR POUR WATER DIRECTLY TO ACIDS OR BASES NOR MIX ACIDS AND BASES DIRECTLY****. When accidentally placed in contact with skin and eyes, wash with copious amounts of water. See a physician immediately.* ***Purpose of NaOH*** *- for preparing a carbon-less mineral salt solution for growing plastic-degrading bacteria*; ***Purpose of HCl*** *- for adjusting the pH of the enriched medium to achieve pH 7.0±0.2*

Isolation and identification of plastic-degrading bacteria

One hundred milliliters (100 mL) of mineral salt medium was sterilized in an autoclave at 15psi for 20 minutes. Using sterile scissors, a 1cmx1cm –piece was cut from the plastic sample, inoculated in the medium and then incubated at 37 ^{0}C for 2 days. After the incubation period, small amounts of liquid portion of the inoculum were swabbed onto Blood Agar Plate (BAP) and MacConkey plates. The swabbed plates were incubated at 37 ^{0}C for 2 days. After the incubation period, the colony morphologies were observed. Grams staining of the isolates were performed. The isolates were then subjected to biochemical tests to establish the identity of microorganisms. All observations were recorded.

Data Gathering Procedure

Descriptive-quantitative was used in the study. The data were gathered through combined description and quantification of bacteria inoculated and isolated as well as the rate of plastic degradation using the two growth media which were clearly discuss as follows.

1. Description of the bacteria inoculated/isolated by identifying and categorizing different bacterial colonies based on varied appearance and morphology (form and structure).

   a. Colony Shape and size - round, irregular, punctiform (tiny)
   b. Margin edge - entire (smooth), undulate (wavy), lobate (lobed)

c. Elevation - convex, flat, raised
d. Color - color + opaque, translucent, shiny or dull
e. Texture - moist or dry (rough)

2. Rate of plastic degradation (%) using the 2 growth media (mineral salts water and seawater) per incubation period.

Weighing of each plastic film was done in each incubation to compute for the rate of degradation. The degradation rates of each plastic were computed using the formula derived from the research done by Ángeles-López, Gutiérrez-Mayen, Velasco-Pérez, Beltrán-Villavicencio, Vázquez-Morillas, and Cano-Blanco, (2011).

$$rate_{degredation} \qquad \frac{\Delta mass_{plastic}}{t} \qquad \frac{mass_{final} - mass_{initial}}{time_{final} \quad time_{initial}} \text{ x } 100$$

The average degradation was determined and pictures were taken for documentation. In addition, a graph for the rate of plastic degradation was done to determine the highest degradation rate of biodegradable plastic.

The data that were collected, organized, encoded, and analyzed using GLM procedure of the analysis of variance (ANOVA) of the two (2) factor factorial in Completely Randomized Design (CRD). Mean comparison for the significant differences among treatment combination was further analyzed using the Duncan's Multiple Range Test (DMRT) at 1% and 5% level of significance.

RESULTS AND DISCUSSION

Isolation of plastic-degrading bacteria from mangrove soil using Winogradsky columns

a. ***Citrobacter fruendii***

It is a gram-negative bacterium which is a straight rod, occurring both singly and in pairs. It has approximately one (1) micrometer in diameter and 2.0 – 6.0 micrometers in length. It is usually motile by the presence of peritrichous flagella. *Citrobacter spp*. grow readily on ordinary media. At 24 hours, colonies on nutrient agar are generally 2-4 millimeters in diameter, smooth, low, convex and moist. They usually appear translucent or opaque and gray with a shiny surface and an entire edge. Mucoid or rough strains may occur occasionally. Colonies which slowly ferment lactose can resemble Salmonella colonies on enteric media.

C. freundii is responsible for reducing nitrate to nitric oxide to nitrogen gas in the environment. This conversion is an important and crucial stage in the nitrogen cycle and also help in recycling of nitrogen. *Citrobacter freundii* has also been investigated for biodegradation of tannic acid which is usually present in the water tends to coat anything else that is placed in the water, metals, plastics, or even viruses. Thus, it helps in degrading some layers of plastics (Yirka, 2013).

Table 3. Macroscopic Appearance of *Citrobacter fruendii*

CRITERIA	CHARACTERISTICS
Gram Stains:	Gram-negative.
Morphology:	Straight rods, occurring singly and in pairs.
Size:	1 micrometer in diameter by 2.0-6.0 micrometers in length.
Motility:	Motile
Capsules:	None.
Spores:	None.

b. *Enterobacter agglomerans*

It is a gram-negative bacteria and approximately 0.6-1.0 micrometers in diameter and 1.2-3.0 micrometers in length. In general, the strains from environmental sources grow better at 20-30 0C, whereas strains from clinical sources grow better at 37 0C. Colonial morphology differs greatly among Enterobacter spp. ranging from smooth, irregularly round to rough "cauliflower" type colonies.

Enterobacter has a wide range of applications, from aiding in plant growth to degrading polyethylene. It has the ability to degrade polyethylene which is the most common plastic and has long been classified as one of the main pollutants of the planet and found in the gut of worms dubbed plastic-eating worms (J. Yang, Y. Yang, Wu, Zhao & Jiang, 2014). This bacterium is the main contributor to the ability of these worms to break down polyethylene. Research in this particular area has been limited, but could offer a solution to the growing problem of increased plastic deposition on the planet. Enterobacter also works alongside with other bacteria in the gut of these worms to break down the plastic, similar to the gut bacteria found in humans. This discovery offers another benefit of Enterobacter which can help to limit the expansion of plastic waste in the future.

Table 4. Macroscopic Appearance of *Enterobacter agglomerans*

CRITERIA	CHARACTERISTICS
Gram Stains:	Gram-negative.
Morphology:	Straight rods
Size:	Diameter of 0.6-1.0 micrometers Length of 1.2-3.0 micrometers
Motility:	Some are motile by four to six peritrichous flagella.
Spores:	None.

c. ***Pseudomonas spp.***

This kind of bacteria is a gram-negative which is straight or slightly curved rods, but is not helical. Its size is 0.5-1.0 micrometers in diameter and 1.5-5.0 micrometers in length. Some colonies produce water-soluble pigment pyoverdin or pyocyanin that fluoresce white to beige under UV light. Other non-flourescent soluble and insoluble, pigments exist. They do not produce prosthecae and are not surrounded by sheaths.

Nanda, Sahu, and Abraham (2010) reported that *Pseudomonas spp.* are predominant in nature and are often found to survive in nutrient deficient ecosystems with their versatile metabolism. Hence, they would be able to utilize polyethylene amended in the nutrient medium as carbon and energy source when basal nutrients in the medium are exhausted. In addition, Kathiresan (2003) also reported that *Pseudomonas spp.* degraded the plastic up to 8.16% and 20.5% of degradation was observed anaerobically.

Table 5. Macroscopic Appearance of *Pseudomonas spp.*

CRITERIA	CHARACTERISTICS
Gram Stains:	Gram-negative
Morphology:	Straight or slightly curved rods, but not helical.
Size:	Diameter of 0.5-1.0 micrometers Length of 1.5-5.0 micrometers.
Motility:	Almost all are motile but some are non-motile.
Capsules:	None.
Spores:	None.

Based on the results of the grams staining, colony morphology and biochemical tests, the microorganism identified and isolated from the improvised nutrient Winogradsky column (i.e. mineral salt medium and sea salt medium)

was *Citrobacter fruendii, Enterobacter agglomerans, Pseudomonas aeruginosa* and *Arizona spp*.

These results can be associated that mangrove soil can be used as medium for plastic degradation. This result conforms to the findings of Kathiresan (2003) and Kathiresan and Bingham (2001) who mentioned and discussed that mangrove soil is known to be a rich source of plastic biodegrading bacteria.

The use of growth media in mangrove soil gives the opportunity to isolate and culture them in the laboratory. The results of the present study may open opportunities for future work in the field of biotechnology. With the limitations in the availability of expensive materials, local home-made version can be an attractive option in exploring the potential use of *Citrobacter fruendii, Enterobacter agglomerans, Pseudomonas aeruginosa* and *Arizona spp*. as plastic degrading agent. In addition, it may now be possible to propagate biodegrading strains of the said bacteria in the laboratory particularly the possible genetic engineering of *Citrobacter fruendii, Enterobacter agglomerans, Pseudomonas aeruginosa* and *Arizona spp* and other bacteria that specifically degrade plastics.

Rate of plastic degradation in the 2 growth media (i.e. mineral salt medium and sea salt medium) from mangrove soil

A. Plastic Weight as influenced by Type of Medium

Plastic weight prior to the incubation was determined to facilitate the easy determination of the plastic degradability rate in each incubation period.

In addition results of the analysis were also presented in Table 4 and Table 5. Results revealed that the plastic weight before incubating the plastic in the two media are comparable ($P>0.05$).

Likewise, the weight of the plastics after 36 days of incubation was also recorded and found out that the growth medium did not significantly ($P>0.05$) affect the final plastics weight (Table 4 and Table 6) after incubation. This result implies that since the incubated and isolated bacteria in the two test media were found to be similar, then it can be deduced that both medium has the ability to degrade some layers of the plastic used in the study (i.e. due to the decrease in plastic weight after incubation).

However, though similar, the lower plastic weight of samples using mineral salt medium (i.e. improvised nutrient medium) after incubation cannot be taken for granted. Results can be associated with the fact that the biodegradation of plastics continues actively under different soil conditions according to their properties, because the microorganism responsible for the degradation differs

from each other and they have their own ideal growth conditions (Gemini *et al.,* 2006) that indeed affects the plastic degradability using the two test medium.

Table 6. Plastic weight (mg) as affected by two (2) Growth media

Medium	Plastic Weight Before Incubation	Plastic Weight After Incubation
Mineral Salt Medium	51.048[a]	44.667[a]
Sea Salt Medium	51.905[a]	47.524[a]

Legend: Means within column with the same superscript are comparable (P>0.05).

Table 7. ANOVA for plastic weight before incubation

Source of variation	DF	SS	MS	F value	Pr>F
Model	14	923.809524	65.986395	1.21[ns]	0.3206
Media	1	85.7142857	85.7142857	1.58[ns]	0.2198
Time	6	257.1428571	42.8571429	0.79[ns]	0.5864
Media*Time	6	580.9523810	96.8253968	1.78[ns]	0.1405
Error	27	1466.666667	54.320988		
Corrected Total	41	2390.476190			

Table 8. ANOVA for plastic weight after incubation

Source of variation	DF	SS	MS	F value	Pr>F
Model	14	1646.511905	117.607993	2.61[*]	0.0158
Media	1	85.714286	85.714286	1.90[ns]	0.1792
Time	6	1007.619048	167.936508	3.73[**]	0.0079
Media*Time	6	550.285714	91.714286	2.03[ns]	0.0955
Error	27	1217.107143	45.072042		
Corrected Total	41	2863.619048			

B. Plastic Weight as influenced by Incubation Period

The plastic weight in each incubation period was also determined and analyzed and was presented in Table 9.

Results revealed that the plastic weight at incubation period of 3, 6, 12, 18, and 24 days has significantly higher (P<0.01) compared to the weight of plastics incubated at 30 and 36 days (Table 10). These results suggest that the incubation period can be correlated to the plastic weight wherein the longer the incubation period resulted to the increase in the degradability of the plastic material, thus reduces the final weight.

These findings are in line with the findings of Orhan, Hrenović, and Büyükgüngör (2004) who reported that incubation period indeed plays a vital role in the degradability rate of different plastic materials. In addition, Gemini et al., (2006); Kathiresan (2003); and Kathiresan and Bingham (2001) also discussed the mechanisms which play significant role in the degradability of plastic materials such as physical damage due to the microorganisms and the biochemical effects from the extracellular materials produced by the microbial activity. Moreover, the rate of degradation is also affected by environmental factors such as moisture, temperature and biological activity (Orhan *et al.,* 2004).

Table 9. Plastic Weight (mg) as affected by different incubation period

Incubation Period	Mean
3 Days	51.667[a]
6 Days	50.000[a]
12 Days	51.500[a]
18 Days	45.500[ab]
24 Days	45.333[ab]
30 Days	39.500[b]
36 Days	39.167[b]

Legend: Means within column with different superscript are significantly different (P>0.01).

Table 10. Plastic Weight as affected by different incubation period

Winogradsky Column	Incubation Period (Days)	Weight In Milligrams					
		Replication 1		Replication 2		Replication 3	
		Initial	Final	Intial	Final	Intial	Final
MINERAL SALT WATER (A1)	3	50	50	50	50	60	60
	6	40	40	40	40	50	50
	12	70	66	60	56	50	47
	18	50	46	60	54	50	46
	24	60	53	50	44	60	53
	30	50	43	40	34	50	43
	36	50	42	50	40	50	41

	3	60	60	40	40	50	50
	6	60	60	50	50	60	60
SALT WATER MEDIUM (A2)	12	60	56	40	37	50	47
	18	50	46	40	36	50	45
	24	50	44	50	44	40	34
	30	40	34	50	42	50	41
	36	40	33	40	32	60	47

Incubation period with the highest rate of plastic degradation of the 2 growth media (i.e. mineral salt medium and sea salt medium) from mangrove soil.

The incubation period with the highest rate of plastic degradation of the two growth media from mangrove soil was shown in Table 11.

Result shows that the highest degradation rate in percentage was recorded at 12 days of incubation period regardless of growth media. In mineral salt medium, the highest rate of plastic degradation is 30.55%, while in sea salt medium, the highest rate of plastic degradation is 27.78%. These results suggest that in the 12th day of incubation period, the growth of bacteria increases and, therefore, their activity on degradation increases. As the period of incubation increases, the growth rate of bacteria slows down, therefore, the rate of degradation decreases since some composition of plastics like waxes, lignin, phthalates, bisphenol A, polyphenols, polybrominated diphenyl ethers, tetrabromobisphenol A and other lowly digestible components takes time to degrade (Talsness, Andrade, Kuriyama, Taylor, & Vom Saal, 2009).

In addition, from the four popular ways as discussed by several researchers (Gemini et al., 2006; Orhan *et al.,* 2004; Albertsson, Andersson, S. O., & Kalsson, 1986) where plastic may be degraded (i.e. chemical, thermal-, photo-, and bio-degradation), biodegradation using different bacterial growth media was tested to be the most effective strategy to degrade different components of plastics and was also proven in previous studies conducted.

With these results, the dissemination of this information to different localities that have immediate access along seashore with available mangrove soil (i.e. that was proven to contain different bacteria that helps degrade plastics) can possibly improve its utilization as bacterial growth medium to fasten the degradability of plastic and to reduce water and air pollution in the province.

Table 11. Degradability rate (%) of plastic from the 2 growth media at the different incubation period

	Degradability Rate (%)	
Incubation Period (Days)	**Mineral Salt Medium**	**Sea Salt Medium**
12	30.55	27.78
18	27.78	27.22
24	25.92	25.96
30	24.99	25.56
36	22.22	24.07

CONCLUSIONS

The isolated bacteria found in the growth media were native to the site of plastics disposal and showed a considerable rate of degradability under natural conditions, yet they also displayed biodegradation in laboratory conditions on synthetic media (i.e. mineral salt medium and sea salt medium). This result provides opportunities that these microbes can be used in both natural and artificial conditions for the degradation of the plastic material.

The growth media did not significantly affect the weight of plastic before and after incubation. On the contrary, the period of incubation greatly affects the degradation process of the plastic material.

The highest rate of degradation was observed at 12 days of incubation period and found out that as the incubation period increases, the growth rate of bacteria slows down, therefore, the rate of degradation decreases.

The bacteria *Citrobacter fruendii, Enterobacter agglomerans, Pseudomonas aeruginosa* and *Arizona spp.* that are found in the mangrove soil are best grown on the mineral salt medium than sea salt medium. In addition, it may now be possible to propagate biodegrading strains of the said bacteria in the laboratory particularly, the possible genetic engineering of *Citrobacter fruendii, Enterobacter agglomerans, Pseudomonas aeruginosa* and *Arizona spp* and other bacteria that specifically degrade plastics.

TRANSLATIONAL RESEARCH

The findings of the study may be best translated into various media of communication for information dissemination, if not, further awareness campaign. Further studies can be done on the effect of biodegrading bacteria

present in the mangrove soil in terms of how fast the degradation rate of specific bacteria. Comparison of the different mangrove soils found in the different area of Calapan City that may help future studies in determining the best biodegrading bacteria that might be grown synthetically to aid in the plastic and air pollution in the province. Lastly, further studies can be done on the effects of the life span, growth and activity of the bacteria *Citrobacter fruendii, Enterobacter agglomerans, Pseudomonas aeruginosa* and *Arizona spp.* which can be found in mangrove soil in terms of the degradation rate of plastics.

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