

Polyethylene Terephthalate Plastic Degradation Using Soil Bacteria from Mount Jayawijaya, Papua, Indonesia

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Abstract. The purpose of this study was to determine the ability of soil bacterial isolates from Mount Jayawijaya, Papua to degrade polyethylene terephthalate synthetic plastics. Research methods used were macroscopic, microscopic and biochemical characterization of bacterial isolates, polyethylene terephthalate synthetic plastics biodegradation test using dishaker method (Shake Flask Experiment) was performed for 30 days and sampling of plastic weight reduction was done once a week. The characterization result of nine bacterial isolates were Gram positive bacteria and the identification results by the biochemical test showed seven different bacterial species, namely: *Bacillus* sp. 1, *Bacillus* sp. 2, *Bacillus* sp. 3, *Bacillus* sp. 4, *Bacillus* sp. 5, *Bacillus* sp. 6 and *Bacillus* sp. 7. The result of biodegradation test showed six isolates with ability to degrade synthetic plastic while 1 isolate (ITP 6.2) does not have the ability to degrade synthetic plastics. Bacteria isolate that have a high ability to degrade synthetic plastic was ITP 3.4 (3.41%).

Keywords: bacteria, biodegradation, polyethylene terephthalate

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I. Introduction

Plastic is one of the most vital man-made products that has been produced in large quantities and widely used for various purposes in everyday life. Gradually, the demand for this synthetic products is growing rapidly (Ghost et al., 2013). Around 140 million tons of synthetic polymers are produced worldwide each year (Singh et al., 2014). About 35% of plastics produced in developing countries are used for packaging (Veethahavya et al., 2016). In the pharmaceutical field, the use of synthetic plastics includes the material for IV drip bottles, syrup medicine bottles, eye drops packaging also for food and beverage packaging (Kruger et al., 2015).

This kind of plastic has a high stability and cannot enter the degradation cycle in the biosphere. Because its nature that very slowly degraded, this condition became major problem of environmental pollution (Singh et al., 2014). Due to poor waste management and improper recycling, plastic waste has accumulated in the environment in large numbers and became a threat for the earth (Kruger et al., 2015).

Nonbiodegradable plastic is the common used plastic such as polyvinyl chloride, polypropylene, polystyrene, polyethylene terephthalate, polyurethane and polyethylene (Kruger et al., 2015). The most problematic of synthetic plastic is polyethylene (Singh et al., 2014). Polyethylene terephthalate is a kind of plastic consist of fossil-based synthetic polymers (obtained from hydrocarbon and petroleum derivatives), its use in plastic products which caused the buildup in the environment have attracted the attention of people around the world (Yoshida et al., 2016)

Biodegradation is a method that can solve this environmental problem among other physical and chemical degradation methods (Singh et al., 2014). Plastic biodegradation has been widely studied over the past three decades. Nowadays enzymatic degradation is a common method used to overcome plastic waste. This biodegradation method use enzymes produced by microorganisms that can degrade plastics without causing harm to the environment (Bhardwaj et al., 2012). Microorganisms that can degrade plastics are found in more than nine genera, from bacteria and fungi, including *Bacillus megaterium*, *Pseudomonas* sp., *Azotobacter vinelandii*, *Ralstonia eutropha*, *Halomonas* sp. and others (Chee et al., 2010).

Many studies aims to explore polyethylene synthesis polymers degrading-microbe isolated from soil. In previous studies, from the soil of landfill in Padang city was obtained *Pseudomonas* sp. bacteria that were able to degrade polyethylene synthetic plastics by 11.7% within 1 month (Agustien et al., 2016). Hadad et al. (2005) also isolated bacteria from the soil and obtained *Brevibacillus borstelensis* which able to degrade polyethylene with weight reduction of 30% within 1 month.

Most exploration of plastic degrading-bacteria has been carried out from the soil of landfills and mountain, such as from Mount Jayawijaya, located in Mimika Regency, Papua. Mount Jayawijaya is located in the highlands and has extreme weather conditions that indicated the presence of polyethylene terephthalate synthetic plastics degrading-bacteria. This kind of synthetic plastic is extremely hard to break down by the soil environment (Ruslan et al., 2018). The research by Ruslan et al. (2018) obtained bacterial isolate that able to degrade polyethylene plastic type Polyethylene Terephthalate (PET), namely *Bacillus* sp. with a percentage of 4.77% b/b.

This research is a follow-up study which was previously conducted by Iqbal (2018) who obtained nine bacteria that have been characterized and showed the potential to degrade polyethylene terephthalate (PET) plastic using solid media in biodegradation test. However, biodegradation tests using liquid media have not been carried out. Therefore, this study was conducted to test the ability of nine soil bacterial isolates from Mount Jayawijaya, Papua in degrading plastic polyethylene terephthalate (PET) using mineral liquid media. By using this method it is expected that the ability of bacterial isolates in degrading polyethylene terephthalate plastic can be maximized.

II. Materials And Method

Tools and Materials

Tools used in this research were Erlenmeyer (Pyrex[®]), measuring cup (Pyrex[®]), test tube (Pyrex[®]), beakers (Pyrex[®]), stirrer bar, tweezers, spatula, dropper pipette, micro pipette (Transferpette[®]), inoculation needle, digital scale $d = 0,001$ g (Metler Toledo[®]), spiritus burner, object glass, autoclave (All american[®]), oven (Memmert[®]), laminar air flow (Elisa[®]), Rotary shaker incubator (Bigger digital[®]), vortex (Digisystem[®]), microscope, magnetic stirrer hotplate, hot plate (Cimarec[®]), thermometer (Omron[®]), pH meter (Hanna[®]).

The materials used in this research were Nutrient Agar (NA) Medium (Merck[®]), standard solution McFarland 0,5, packaging plastic PET (Polyethylene Terephthalate), alcohol 70% (Brataco[®]), aquadest (Brataco[®]), spiritus (Brataco[®]), Potassium dihydrogen phosphate (Merck[®]), Dipotassium hydrogen phosphate (Merck[®]), Ammonium phosphate (Merck[®]), Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) (Merck[®]), Sodium chloride (Merck[®]), Ferro sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) (Merck[®]), Calcium dichloride dihydrate ($CaCl_2 \cdot 2H_2O$) (Merck[®]), Manganese sulfate hydrate ($MnSO_4 \cdot H_2O$) (Merck[®]), Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) (Merck[®]), Zinc sulphate pentahydrate ($ZnSO_4 \cdot 7H_2O$) (Merck[®]), cotton, gauze, violet crystal, safranin and plastic wrap.

Bacterial Isolates Used

This study used nine isolates of Mount Jayawijaya, Papua soil bacteria that had been isolated by previous researchers with code ITP 3.1, ITP 3.2, ITP 3.3, ITP 3.4, ITP 6.2, ITP 6.3, ITP 10.4, ITP 10.5, ITP 10.6 (Iqbal, 2018).

Preparation of Nutrient Agar Medium

NA Medium (Merck[®]) was made by dissolving 20 grams of nutrient agar powder with 1 liter of aquadest in Erlenmeyer, then it was heated on a hotplate and stirred until homogeneous. The solution then sterilized by autoclave at temperature $121^\circ C$ pressure of 15 lbs for 20 minutes (Cappuccino and Sherman, 2005; Agustien et al., 2016).

Preparation of Mineral Salt Liquid Medium for Degradation of Terephthalate Polyethylene Synthetic Plastic

The mineral salt medium was dissolved in 1 liter of sterile aquadest. The mineral salt medium consists of Potassium dihydrogen phosphate 0.2 gram (Merck[®]), Dipotassium hydrogen phosphate 1 gram (Merck[®]), Ammonium sulfate 1 gram (Merck[®]), Magnesium sulfate heptahydrate 0.5 gram (Merck[®]), Sodium chloride 1 gram (Merck[®]), Ferro sulfate heptahydrate 0.01 gram (Merck[®]), Calcium dichloride dihydrate 0.002 gram (Merck[®]), Manganese sulfate hydrate 0.001 gram (Merck[®]), Copper sulfate pentahydrate 0.001 gram (Merck[®]), Zinc sulphate pentahydrate 0.001 gram (Merck[®]), pH medium was 7.0 (Gnanavelet et al., 2012; Agustien et al., 2016).

Research Procedure

Rejuvenation of Bacterial Isolate Stock from the Soil of Mount Jayawijaya

Pure culture stock of bacterial isolates were inoculated on an slant NA medium, then incubated for 18-24 hours at $35-37^\circ C$ and rejuvenated every day before testing (Das and Kumar, 2015).

Characterization of Each Bacterial Isolate by Macroscopic, Microscopic and Biochemical Test

Nine bacterial isolates used in this research were characterized based on macroscopic character includes the shape, edge, elevation and color of the bacterial colony. Microscopic observations includes Gram staining of the bacteria and the shape of bacterial cells. The identification of bacterial isolates by biochemical tests includes TSIA (Triple Sugar Iron Test), motility, requirement of oxygen during growth, catalase, oxidase, glucose fermentation, oxidation fermentation. To differentiate species in genus *Bacillus*, some test such as indol, citrate, lactose, glucose, sucrose and mannitol, methyl red, vesche proskauer, arabinose, xylose, nitrate and gelatin tests were performed (Cowan, 1974).

Preparation of Polyethylene Terephthalate Synthetic Plastic Film

Thin film of polyethylene plastic was made by cutting polyethylene terephthalate packaging plastic in the form of a square sheet with a size of 1.5 cm x 1.5 cm as the main carbon source. Subsequently this thin film was weighed, disinfected with 70% alcohol for 30 minutes and transferred to sterile aquadest for 20 minutes, then it was irradiated by UV with wavelength 365 nm for 15 minutes (Das and Kumar, 2015; Pramila and Ramesh, 2011).

Preparation of Bacterial Suspension

Bacterial suspension was prepared by inoculating 1-2 ose of bacterial colonies into saline solution (0.85% NaCl solution) in a sterile test tube. Saline solution was prepared by dissolving 8.5 grams of NaCl (0.85% w / v) with 1 L aquades (Yang et al., 2015). The solution then homogenized with vortex and compared with McFarland 0.5 solution (Kyaw et al., 2012). McFarland 0.5 solution was made from 0.05 ml 1% BaCl₂ with 9.95 ml 1% H₂SO₄ with estimated number of bacteria 1.5 x 10⁸ / mL (Dalynn, 2014).

Biodegradation Test of Polyethylene Terephthalate Synthetic Plastic by Bacterial Isolates using Shake Flask Method

A 5% (v/v) of bacterial isolate inoculum was inserted into a mineral medium which had been added co-metabolites of palm oil and sterile polyethylene terephthalate synthetic plastic films which inserted aseptically (Kyaw et al., 2012). Medium was shaken with rotary shaker incubator (Bigger digital[®]) at agitation of 130 rpm and temperature of 37°C for 30 days. Every 7 days, the decreasing weight of synthetic polyethylene terephthalate synthetic film was sampled (Jumaah, 2017). Control was made from mineral medium without inoculated bacteria.

Determination of Dry Weight of the Remaining Synthetic Plastic Polymers

Determination of dry weight of the remaining polymer of polyethylene terephthalate plastic film that has been degraded by bacteria was done by taking a plastic film then it washed with 70% ethanol then rinsed with sterile aquadest and dried at 80°C until the weight was constant. After drying, synthetic plastic films were weighed (Saminathan et al., 2014; Das and Kumar, 2015). The percentage of the reduction of plastic weight obtained was calculated using the following formula (Saminathan et al., 2014):

$$\% \text{ Plastic Weight Reduction} = \frac{R_1 - R_2}{R_1} \times 100\%$$

Annotation: R₁ = Initial Weight of Plastic Film (gram)

R₂ = Final Weight of Plastic Film (gram)

Data Analysis

Data obtained from the study were analyzed descriptively then presented in the form of tables and graphs.

III. Results And Discussion

Macroscopic and Microscopic Characterization of Bacterial Isolates

The morphological characteristics of soil bacteria from Mount Jayawijaya can be observed macroscopically and microscopically (Gram properties of bacterial isolates and cell shape). The result of bacterial macroscopic and microscopic observations are presented in Table 1.

Table 1. Observation for Macroscopic and Microscopic Characteristics of the Polyethylene Terephthalate Synthetic Plastic Degrading-Bacteria

No	Isolate Code	Macroscopic Characteristic of Bacterial Colony				Microscopic Characteristic		
		Coloration	Shape	Margin	Elevation	Gram	Cell Form	Endospore
1.	ITP 3.1	White	Circular	Entire	Flat	Positive	Bacillus	(+)
2.	ITP 3.2	Yellowish white	Circular	Iregular	Convex	Positive	Bacillus	(+)
3.	ITP 3.3	Yellowish white	Circular	Iregular	Convex	Positive	Bacillus	(+)
4.	ITP 3.4	White	Circular	Entire	Convex	Positive	Bacillus	(+)
5.	ITP 6.2	White	Circular	Iregular	Flat	Positive	Bacillus	(+)
6.	ITP 6.3	Yellowish white	Circular	Iregular	Flat	Positive	Bacillus	(+)
7.	ITP 10.4	White	Circular	Iregular	Convex	Positive	Bacillus	(+)

8.	ITP 10.5	White	Circular	Iregular	Convex	Positive	Bacillus	(+)
9.	ITP 10.6	White	Circular	Iregular	Convex	Positive	Bacillus	(+)

The macroscopic observation of bacterial colony (Table 1) showed nine bacterial isolates has white to yellowish white coloration. Nine isolates has circular colony form with different margin types, which were the entire type indicated in ITP 3.1 and ITP 3.4 and irregular type found in ITP 3.2- ITP 3.3- ITP 6.2- ITP 6.3- ITP 10.4- ITP 10.5 and ITP 10.6. Three bacteria isolates has flat elevation as shown in ITP 3.1-ITP 6.2 and ITP 6.3 while six bacteria isolates has convex elevation as found in ITP 3.2-ITP 3.3-ITP 3.4-ITP 10.4-ITP 10.5 and ITP 10.6. The study by Agustienet al. (2016) obtained 11 bacterial isolates that have macroscopic character of circular colony, five isolates with entire margin, three isolates with curled margins, three isolates with jagged margins and all of these isolates have convex elevation.

Table 1 also showed microscopic observation results in which nine isolates of the bacteria classified as Gram positive with bacillus cell form and positive endospore. These bacterial cells have spores, while the Gram-positive bacterial group that has spores is only two genera namely the genus *Bacillus* and *Clostridium*. The difference in character between these two genera is that the genus *Bacillus* is aerobic (requires oxygen for its growth) while *Clostridium* is anaerobic (does not require oxygen for its growth) (Cowan, 1974).

Biochemical Test of Soil Bacteria from Mount Jayawijaya

The result of biochemical test of polyethylene terephthalate synthetic plastic degrading-bacteria can be seen in Table 2.

Table 2. Biochemical Test Result Observation of Polyethylene Terephthalate Synthetic Plastic Degrading-Bacteria

No	Treatment	Isolate of Plastic-Degrading Bacteria								
		ITP 3.1	ITP 3.2	ITP 3.3	ITP 3.4	ITP 6.2	ITP 6.3	ITP 10.4	ITP 10.5	ITP 10.6
1.	Aerob/ Anaerob	A	A	A	A	A	A	A	A	A
2.	TSIA	M/K	M/K	M/K	M/K	K/K	K/K	M/K	M/K	M/K
3.	Gas	-	-	-	-	-	-	-	-	-
4.	H ₂ S	-	-	-	-	-	-	+	-	+
5.	Catalase	+	+	+	+	+	+	+	+	+
6.	Oxidase	+	-	-	-	-	-	+	+	+
7.	Motility	+	+	+	+	+	+	+	+	+
8.	Indol	-	-	-	-	-	-	-	-	-
9.	Urea	-	-	-	-	-	-	+	-	+
10.	Citrate	-	-	-	-	-	-	-	-	-
11.	Lactose	-	-	-	-	-	-	-	-	-
12.	Glucose	-	-	-	-	-	-	-	-	-
13.	Sucrose	-	-	-	-	-	-	-	-	-
14.	Mannitol	-	-	-	-	+	+	-	-	-
15.	MR	-	-	-	+	-	+	-	+	-
16.	VP	+	+	+	+	+	+	+	+	+
17.	OF	-	-	-	-	-	-	-	O+ F-	-
18.	Arabinose	-	-	-	-	-	-	-	-	-
19.	Xylose	-	-	-	-	-	-	-	-	-
20.	Nitrate	-	-	-	-	-	-	-	-	-
21.	Gelatin	+	+	+	+	+	+	+	+	+
Identification result		Bacillus sp.1	Bacillus sp.2	Bacillus sp.2	Bacillus sp.5	Bacillus sp.6	Bacillus sp.7	Bacillus sp.3	Bacillus sp.4	Bacillus sp.3

The results biochemical identification of bacterial isolates (Table 2) showed that the nine bacterial isolates that have been tested was classified into *Bacillus* bacterial group. The catalase test results showed nine bacterial isolates with positive results in the formation of air bubbles, which indicates that the nine bacterial isolates are aerobic. All the nine bacterial isolates tested showed a change in the media to red-yellow and yellow-yellow which indicates the presence of acid production, so it can be said that these bacteria are not acid resistant. In general, *Bacillus* bacteria are motile, it proved by the positive results of nine bacterial isolates for the motile test. In the oxidase test, four bacteria showed positive results and five bacteria showed negative results, this is in accordance with the character of *Bacillus* bacteria which have different reactions in different bacterial strains. Other biochemical tests were carried out to determine species differences between genera *Bacillus* (Cowan, 1974).

If there is only one reaction that is different, then the species can be categorized differently. Different biochemical tests was used to know the differences in bacteria (Hemraj et al., 2013). From Table 2, the nine bacterial isolates tested produced seven bacterial isolates that showed different characteristics, the seven isolates were then subjected to degradation of polyethylene terephthalatesynthetic plastic and the level of plastic weight reduction was observed.

Testing of Synthetic Polyethylene Polythene Plastic Degradation by Bacterial Isolates

Seven bacterial isolates were tested for the teraphthelate polyethylene synthetic plastic. The tests were carried out for 30 days and every seven days a sample was taken to measure the decreasing weightof synthetic plastic films. The results of the synthetic plastic degradationby bacteria are presented in Table 3.

Tabel3. Data on Weight Loss of Polyethylene Terephthalate Plastic Films Tested in the Media using Incubator Shaker Method

No	Isolate Code	Degradation of polyethylene terephthalate synthetic plastic (%)			
		Week 1	Week 2	Week 3	Week 4
1.	ITP 3.1	0,00	0,85	0,88	1,8
2.	ITP 3.2	0,00	0,98	0,95	1,03
3.	ITP 3.4	0,00	0,00	3,41	3,41
4.	ITP 6.2	0,00	0,00	0,00	0,00
5.	ITP 6.3	0,00	0,00	0,90	1,75
6.	ITP 10.4	0,00	0,83	2,63	2,70
7.	ITP 10.5	0,00	0,00	0,00	0,90

From Table 3 it can be seen the degradation percentage of teraphthelate polyethylene synthetic plastic. The seven bacterial isolates showed different degradation activities. Six isolates were able to degrade synthetic plastic, those were ITP 3.1- ITP 3.2 - ITP 3.4 - ITP 6.3 - ITP 10.4 and ITP 10.5. Only one isolate that does not have the ability to degrade teraphthelate polyethylene synthetic plasticnamely ITP 6.2. Out of the six isolates that are able to degrade plastics, three isolates showed best ability in degrading teraphthelatepolyethylene plastics, which were ITP 3.1- ITP 3.2 and ITP 10.4 (Figure 1).

The difference result in the plastic weight reductionin each type of bacteria was due to differences in the activity of depolymerase enzyme in each bacterial isolate (Agustienet al., 2016). Differences in microbial characteristics including the types of enzymes produced forthe biodegradation process can help polymer degradation (Kavitha et al., 2015). For an efficient biodegradation process the main key is the selection of microorganisms and control of the test conditions. The main focus for degrading polyethylene is centered on the selection of microorganisms for an effective biodegradation process (Abrusciet al., 2011). The most important aspect of biodegradation is the continued growth of microorganisms during the entire biodegradation process (Hadad et al., 2005).

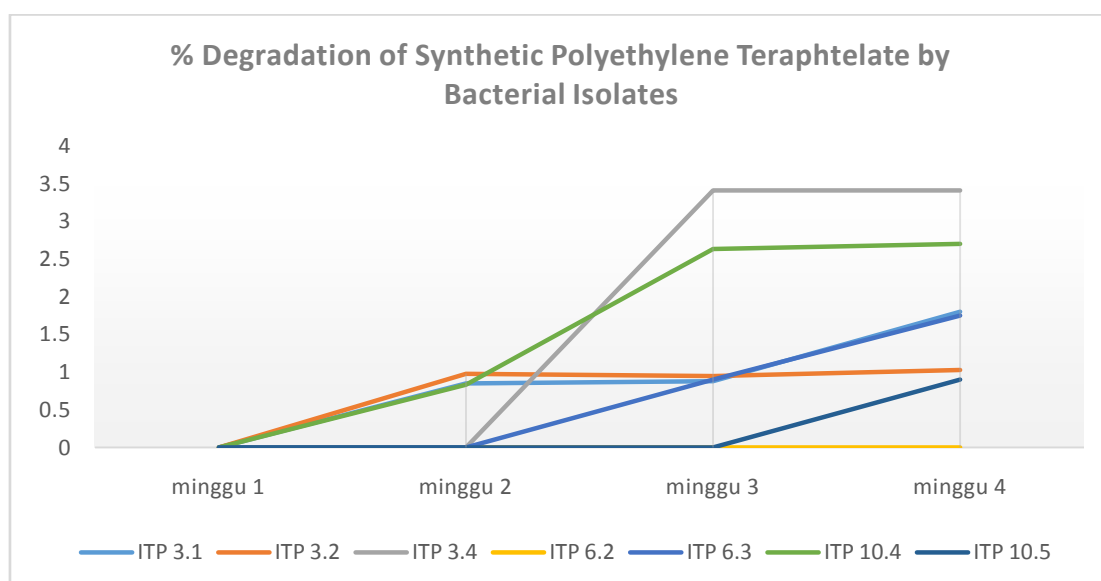


Figure 1. Percentage of Synthetic PolyethyleneTeraphtelate Plastic Degrdatation Using Soil Bacteria from Mount Jayawijaya, Papua.

Figure 1 showed the velocity of bacterial activity in degrading the synthetic terephthalate polyethylene plastic which was carried out for four weeks. This research results showed that the ability to degrading synthetic plastic was different for each bacteria. Seven bacterial isolates used at first week observation did not show any reduction in plastic weight. This was due to the limited adaptation of the bacteria toward edible carbon source, because the only carbon source in the media was plastic film of synthetic terephthalate polyethylene. During the incubation period of 2 weeks and 3 weeks there was a reduction in the plastic weight by the bacteria ITP 3.1, 3.2, 3.4, 6.3 and 10.4. This is because the bacteria start eating synthetic plastic, this ability is related to the enzyme activity produced by the bacteria. Agustien et al. (2016) stated that the polymer biodegradation process by the microbes is related to the ability of microbes to adapt to new substrates. At the incubation period of 4 weeks, six bacteria isolates seemed to have been able to adapt to polyethylene terephthalate plastic. So, it can be concluded that the ability of bacterial isolates to degrade polyethylene terephthalate plastic can be observed started from 4th week.

Microorganisms are able to stick to the surface of polymers, as long as they are hydrophilic. Organisms that are able to stick to the surface are able to grow using polymers as their carbon source. This is because the bacteria are in a state of lacking nutritional sources, so it using plastic as a source of nutrition to support its growth. In the primary degradation stage, extracellular enzymes produced by organisms caused the main chain to divide, resulted in the formation of low molecular weight fragments, such as oligomers, dimers, or monomers. These low molecular weight compounds will later be used by microbes as a source of carbon and energy. Small oligomers can also diffuse into the organism and assimilate the internal environment (Alshehriet al., 2017). The comparative data obtained by the line equation that illustrates the relationship between time and percentage reduction in residual weight of terephthalate polyethylene plastic can be seen in Table 4.

Table 4. Comparative Data on Biodegradation Rate of Terephthalate Polyethylene Plastics

No.	Bacterial Isolate Code	Equation	k (%/week)	T _{1/2} (Week)	T _{~100%} (Week)
1	ITP 3.1	$y = -0,448x + 100.19$	0,448	112,03	223,6
2	ITP3.2	$y = -0,303x + 100.01$	0,301	166,1	332,2
3	ITP 3.4	$y = -1,023x + 100$	1,023	48,8	97,7
4	ITP 6.2	$y=100$	0	0	0
5	ITP 6.3	$y = -0,44x + 100.35$	0,44	114,4	228
6	ITP 10.4	$y = -0,803x + 100.37$	0,803	62,7	124,9
7	ITP 10.5	$y = -0,18x + 100.18$	0,18	278	556

Table 4 showed the linear regression curve of the reduction of plastic weight from polyethylene film. This linear regression curve is used to characterize the degradation profile of this plastic film (Majid et al., 2002). The constant rate value, which was derived from the gradient on the slope for the terephthalate polyethylene film can be seen in Table 4. Terephthalate polyethylene has biodegradation rate of 0.448% per week for ITP 3.1, while for ITP 3.2 was 0.301% per week, ITP 3.4 was 1.023% per week, ITP 6.2 was 0% per week, ITP 6.3 was 0.44% per week, ITP 10.4 was 0.803% per week and ITP 10.5 was 0.18% per week. According to Majid et al. (2002) biodegradation rate is influenced by microbe population, temperature, pH, humidity and the nature of degraded plastic.

Data in Table 3 and Table 4 showed the weight loss of polyethylene terephthalate plastic films was very small and slow. Polyethylene is a stable polymer consisting of long chains of ethylene monomers which cannot be degraded easily by microorganisms. Biodegradation in polyethylene is a very slow process (Alshehriet al., 2017). Polyethylene terephthalate has different properties and this plastic is a semi-crystalline polymer, which chemically and thermally stable. Biodegradation of plastics can take a long and extreme time depending on the molecular weight of the polymer, this process can take 1000 years for some types of plastic. In general, biodegradation of plastics by microorganisms is a very slow process, and some microorganisms cannot degrade certain plastics (Singh et al., 2014). The small weight reduction of polyethylene terephthalate plastic film as shown in Table 3, was also caused by the ability of bacterial isolates from the Mount Jayawijaya soil that were used to decompose plastics.

The molecular weight of this polymer has a range between 30,000 to 80,000 g /mol. The high molecular weight of widely used plastics caused this kind of plastic is difficult to biodegrade at a high level since the microbial species that can metabolize polymers are rarely present in nature. In previous studies, several microbial strains that could break down polyethylene were identified (Sivan, 2011).

Overall, polyethylene degradation is combined by bio and photo degradation processes. First, by abiotic oxidation (UV exposure) or heat treatment the essential abiotic precursors are obtained, followed by selected

thermophilic microorganisms that can degrade oxidation products of low molar mass (Alshehrei et al., 2017). In concentrated liquid microbial culture under labor conditions, Actinomycetes and Rhodococcus ruber strain C208 produced an 8% reduction in the dry weight of polyolefins during the 30-day incubation time (Gilan et al., 2004).

IV. Conclusions

The conclusions of this study are as follows:

1. Nine bacterial isolates that were characterized were Gram positive bacteria and the identification based on biochemical test showed seven different bacterial species, namely: Bacillus sp. 1, Bacillus sp. 2, Bacillus sp. 3, Bacillus sp.4, Bacillus sp. 5, Bacillus sp. 6 and Bacillus sp.7.
2. Seven bacterial isolates that can degrade polyethylene terephthalate synthetic plastic were obtained, those were ITP 3.4 by 3.41%, ITP 10.4 by 2.7%, ITP 3.1 by 1.8%, ITP 6.3 by 1.75%, ITP 3.2 by 1.04% and ITP 10.5 by 0.9%.

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References

- [1]. Abrusci C, Pablos JL, Corrales T, López-marín J, Marín I, Catalina F. 2011. Biodegradation of photo-degraded mulching films based on polyethylenes and stearates of calcium and iron as pro-oxidant additives. *Int Biodeterior Biodegradation*, **65**.
- [2]. Agustien A, Jannah M, Djamaan A. 2016. Screening Polyethylene Synthetic Plastic Degrading-Bacteria from Soil. *Der Pharm Lett*, **8(7)**:183–7.
- [3]. Alshehrei F. 2017. Biodegradation of Synthetic and Natural Plastic by Microorganisms. *J Appl Environ Microbiol* , **5(1)**:8–19.
- [4]. Bhardwaj H, Gupta R, Tiwari A. 2012. Microbial Population Associated with Plastic Degradation, **1(5)**:1–4.
- [5]. Cappuccino JG and Sherman, N. 2014. *Microbiology : A Laboratory Manual*. 10th ed. New York: Pearson.
- [6]. Chee J, Yoga S, Lau N, Ling S, Abed RMM. 2010. Bacterially Produced Polyhydroxyalkanoate (PHA): Converting Renewable Resources into Bioplastic. *Curr Res Technol Educ Top Appl Microbiol Microb Biotechnol*.
- [7]. Cowan ST. 1974. *Manual for the Identification of Medical Bacteria*^{2nd}. Cambridge University Press
- [8]. Dalynn Biological. 2014. Mc Farland Standard. In Catalogue No TM50- TM60 p.2.
- [9]. Das MP, Kumar S. 2015. An Approach to Low-Density Polyethylene Biodegradation by *Bacillus amyloliquefaciens*. *Journal Biotech*, **3**, 5:81–6.
- [10]. Ghosh SK, Pal S, Ray S. 2013. Study of Microbes Having Potentiality for Biodegradation of Plastics. *Environ Sci Pollut Res*.
- [11]. Gilan I, Hadar Y, Sivan A. 2004. Colonization, Biofilm Formation and Biodegradation of Polyethylene by a Strain of *Rhodococcus ruber*. *Appl Microb Cell Physiol*, **65**:97–104.
- [12]. Gnanavel G, Mohana VP, Valli J, Kannadasan T. 2012. Degradation of Plastics Using Microorganisms. *Int J Pharm Chem Sci*, **1(3)**:1040–3.
- [13]. Hadad D, Geresh S, Sivan A. 2005. Biodegradation of Polyethylene by the Thermophilic Bacterium *Brevibacillus borstelensis*. *J Appl Microbiol*, **98**:1093–100.
- [14]. Hemraj, V., Diksha, S., Avneet G. . 2013. Review on Commonly Used Biochemical Test for Bacteria. *Innovare J Life Sci*, **1(1)**:1–7.
- [15]. Iqbal M. 2018. Skrinng Bakteri Pendegradasi Plastik Polietilen dan Polistiren dari Tanah Gunung, Jayawijaya. Skripsi. Universitas Andalas, Padang.
- [16]. Jamil SUU, Zada S. 2017. Biodegradation of Polyethylene By Bacterial Strains Isolated From Kashmir Cave, Buner, Pakistan. *J Cave Karst Stud*, **79**:73–80.
- [17]. Jumaah OS. 2017. Screening Of Plastic Degrading Bacteria from Dumped Soil Area. *J Environ Sci Toxicol Food Technol*, **11(5)**:93–8.
- [18]. Kavitha. R, Anju K., Mohanan and Bhuvanewari V. 2015. Biosynthesis Of Copolymer Poly (3-Hydroxybutyrate-Co-3-Hydroxyvalerate) From Palm Oil and N-Pentanol In a 10L Bioreactor. *Int J Plant, Anim Environ Sci*.
- [19]. Krueger MC, Harms H, Schlosser D. 2015. Prospects for Microbiological Solutions to Environmental Pollution with Plastics. *Appl Microbiol Biotechnol*, **99**:8857–74.
- [20]. Kyaw BM, Champakalakshmi R, Sakharkar MK, Lim C. 2012. Biodegradation of Low Density Polythene (LDPE) by *Pseudomonas* Species. *Indian J Microbiol*, **52(3)**:411–9.
- [21]. Majid MIA, Ismail J, Few LL, Tan CF. 2002. The Degradation Kinetics of Poly (3-hydroxybutyrate) Under Non-Aqueous and Aqueous Conditions. **38**:837–9.
- [22]. Pramila R, Ramesh KV. 2011. Biodegradation of Low Density Polyethylene (LDPE) by Fungi Isolated from Marine Water – a SEM Analysis. *African J Microbiol Res*, **5(28)**:5013–8.
- [23]. Ruslan R, Permatadewi A, Djama. 2018. Isolation and Characterization of Polystyrene-Degrading Bacteria *Bacillus* sp. ITP 10.1.1 from Soil Sample of Jayawijaya Mountains, Papua, Indonesia. *Int Res J Pharm*, **9(10)**:85–9.
- [24]. Ruslan R, Permatadewi A, Djamaan A. 2018. Characterization of *Bacillus* sp. ITP 10.2.1 as Degrading-Bacteria of Polyethylene Terephthalate (PET) Synthetic Plastic. *Int Res J Pharm*, **9(11)**:56–9.
- [25]. Saminathan P, SriPriya A, Nalini K, Sivakumar T, Thangapandian V. 2014. Biodegradation of Plastics by *Pseudomonas putida* Isolated from Garden Soil Samples. *J Adv Bot Zool*, **1(3)**:1–4.
- [26]. Singh J, Gupta KC. 2014. Screening and Identification of Low density Polyethylene (LDPE) Degrading Soil Fungi Isolated from Polythene Polluted Sites around Gwalior city (M.P.). *Int J Curr Microbiol Appl Sci*, **3(6)**:443–8.
- [27]. Sivan A. 2006. Biofilm Development of the Polyethylene-Degrading Bacterium *Rhodococcus ruber*. *Appl Microb Physiol*, **72**:346–52.
- [28]. Sivan A. 2011. New Perspectives in Plastic Biodegradation. *Curr Opin Biotechnol*, **22**:422–6.
- [29]. Veethahavya KS, Rajath BS, Noobia S, B MK. 2016. Biodegradation of Low Density Polyethylene in Aqueous Media. *Procedia Environ Sci*, **35**:709–13.

- [30]. Webb HK, Arnott J, Crawford RJ, Ivanova EP. 2012. Plastic Degradation and Its Environmental Implications with Special Reference to Poly(ethylene terephthalate). *Polymers (Basel)*, **5**:1–18.
- [31]. Yang Y, Yang J, Wu W, Zhao J, Song Y, Gao L. 2015. Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms: Part 2. Role of Gut Microorganisms. *Environ Sci Technol*, **49**:12087–93.
- [32]. Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y. 2016. A Bacterium that Degrades and Assimilates Poly(ethylene terephthalate). **351**(6278):1–5.

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