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Characterization of Polypropylene Synthetic Plastic-Degrading Bacteria from Seawater Samples and Their Decomposition Profiles

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Abstract:
Background*: The increase in the amount of landfill in Indonesia has reached 175,000 tonnes / day or the equivalent of 64 million tones / year. In the health sector, synthetic plastics are used as materials for packaging medicine bottles and infusion bottles. Polypropylene synthetic plastics are very slow to be degraded, so they become a major problem in environmental pollution. This study aims to determine the types of bacteria and the ability of these bacteria isolates to degrade polypropylene plastic.*
Materials and Methods*: The method used in this research included macroscopic and microscopic characterization of bacterial isolates, biochemical tests, and biodegradation test for polypropylene synthetic plastics during the incubation period of 1 week, 2 weeks, 3 weeks, and 4 weeks using a shaker incubator. The result of this study obtained 4 bacterial isolates that were able to degraded polypropylene plastics from seawater samples in Padang city, West Sumatra, Indonesia.*
Results*: Based on macroscopic characteristic, the results of the isolation of polypropylene plastic bacteria from seawater samples in Padang city were bacteria isolates with the code ILT-14. These bacterial are included in the group of gram-negative bacteria. Molecular identification was carried out in the biotechnology laboratory of LIPI using the 16S rRNA gene determination method. The result showed that isolate ILT-14 has similarities with Stenotropomonas maltophilia. The percentage of polypropylene plastic decomposers for 30 days was 10.8 %. FTIR analysis showed the difference in the percent value of the transmission of carbon groups and decreased aromatic groups. When compared with non-degraded plastics there was a decrease in percent. Scanning Electron Microscopy (SEM) analysis showed that ILR-14 polypropylene plastic isolates was able to degrade the complex polymers into monomer forms.*
Conclusion: *ILT-14 bacteria isolate was Stenotropomonas maltophilia.*
Key Words*: isolation, biodegradation, microorganism, polypropylene plastic*

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I. Introduction

In Indonesia, lately the use of synthetic plastics is increasingly popular among the public because it has many advantages and is practically used in the pharmaceutical field. The use of synthetic plastics including as a material for making infusion bottles, bottles for liquid preparations, eye drop packaging and food and beverage packaging[1].

Biodegradation is a method that can solve a problem that occurs in the environment between physical and chemical degradation methods or other plastic structures[2]. Plastic biodegradation has been extensively studied over the past three decades. Currently, enzymatic degradation is a popular method used to treat environmental plastic waste. This method is carried out through biodegradation by microorganisms that produce enzymes that able to degrade plastics without causing danger to the surrounding environment[3].

The rate of plastics biodegradation by bacteria is influenced by several factors. These factors are humidity, types of microorganisms, temperature, pH, polymer types, and polymer thickness[4]. The biodegradation conditions including pH, temperature, nutrients, minerals, oxygen, and humidity must be adjusted to the types of microorganisms that were used[5]. In this research, isolation of bacteria from seawater samples and screening of polypropylene plastic-degrading bacteria will be carried out.

II. Material and Methods

Materials

The material used in this study were $AgNO_3$ (Merck®), aquadest (Bratachem®), alcohol antiseptic 70% (Bratachem®), Nutrient Broth (NB) (Merck®), Nutrient Agar (NA) (Merck®), sodium hypochlorite, crystal violet, lugol, safranin, ethanol 96% and H_2O_2.

Collection of Polypropylene Plastic Sample

Polypropylene plastic samples were taken from plastics on the seawater in Padang city. Polypropylene plastic was cut with sterile scissors, then put into a sterile bottle.

Isolation and Purification of Polypropylene Plastic Bacteria

Polypropylene plastic samples were weighed as much as 20 g then made up to 100 ml by adding sterile aquadest in Erlenmeyer, then it was stirred until homogeneous. Then series dilution was done until 10^{-8}. Then from the series dilution, as much as 1 ml was pipetted into the petridish and poured into the mineral and polypropylene plastic medium using the pour plate technique. Next, it was incubated at 27°C for 1 to 3 x 24 hours. The growing bacterial colonies were purified using the streak plate method on NA medium[6].

Macroscopic and Microscopic Identification of Bacterial Isolates

Identification of bacterial isolates was done by observing the macroscopic and microscopic characteristics of the bacteria. Macroscopic observations were carried out by directly observing the characteristics of the bacterial isolate colonies including: color, shape, edges and elevation of the colony[7]. Microscopic observation was carried out by gram staining method. DNA isolation was carried out at the center of the LIPI Biotechnology Testing Laboratory.

Preparation of Bacteria Isolates Stock

All isolated pure bacterial cultures were inoculated on NA slants and stored at 4°C as the collection of bacterial isolate.

Polypropylene Plastics Screening by Bacterial Isolates using Shake Flask Experiment

The 5% (v/v) bacterial isolate inoculum was inserted into the mineral medium added with a aseptically sterile polypropylene synthetic plastic film[8]. Mineral medium was shaken with a Rotary shaker incubator (Bigger digital®) at 130 rpm agitation and 37°C temperature for 30 days, sampling was done every 7 days for the weight reduction of polypropylene plastic films[9].

Preparation of Polypropylene Plastic Samples

Thin film of polypropylene plastic was made by cutting the polypropylene plastic packaging with a size of 1.5 cm x 1.5 cm. Then the thin film was washed with 70% alcohol and sterile distilled water. Next it was given UV light with a wavelength of 365 nm for 15 minutes.

Determination of Plastic Polymers Dry Weight which has been Degraded by Marine Bacteria Isolates

Dry weight determination of the remaining polymers of polypropylene plastic films that have been degraded by bacteria was done by taking plastic films, then washing them with 70% alcohol, rinsing it with sterile aquadest and drying at 80°C until the constant weight was achieved. After drying, synthetic plastic films were weighed[10]. The percentage of plastic weight reduction obtained was calculated using the following formula:

$$\% \text{ Plastic Weight Reduction } = \frac{R1-R2}{R1} X\ 100\%$$

Description: R_1 = Initial Weight of Plastic Film (g)
R_2 = Final Weight of Plastic Film (g)

III. Results

In this study, the total colony of bacterial isolates were calculated and screening for polypropylene plastic-degrading bacteria was done. From the isolation stage of polypropylene synthetic plastic-degrading bacteria from seawater samples in Padang City using NA medium, some bacterial isolates were found (Table 1).

Table 1. The total bacterial isolate that were found on the isolation stage of polypropylene synthetic plastic-degrading bacteria from seawater samples in Padang City using NA medium

No.	Sample Type	Sample Point	Total Bacteria Colonies	Total Colony (CFU/gr)
1.	Seawater isolate samples	4	121	1,21 x 18^{8} CFU/ml

Table 2. Screening Observation of Bacteria Growing on Plastic Medium

No	Source of Isolate	Isolate Code	Total Colonies	Description
1.	Seawater	ILT-13	11	Small bacteria colony
		ILT-14	50	Small bacteria colony
		ILT-15	27	Big bacteria colony
		ILT-16	25	Big bacteria colony

Description : ILT = Seawater Isolated

From the screening results of polypropylene plastic-degrading bacterial isolates, as much as 4 bacterial isolates were grew on the modified media and were observed on the 5th day. This result was supported by the opinion[11] which stated that the time span of 5 days was needed by bacteria isolates to adapt to the new environment.

Biodegradation test for polypropylene plastic were carried out according to the method[11], the results can be seen on Figure 1.

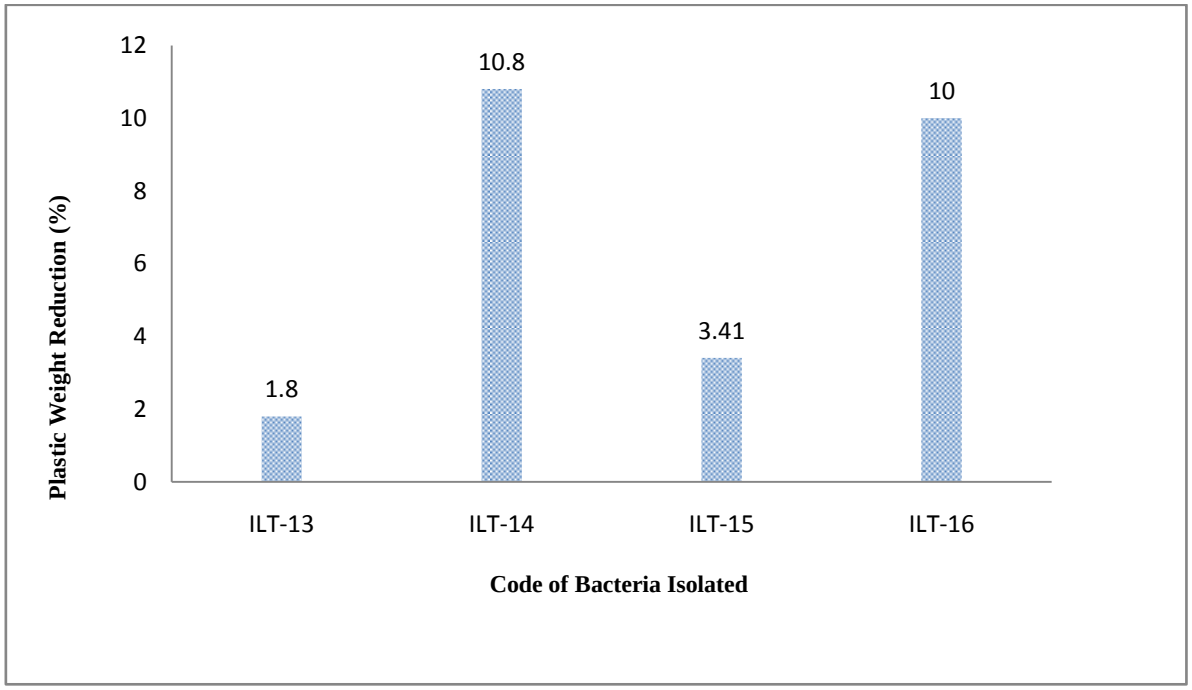
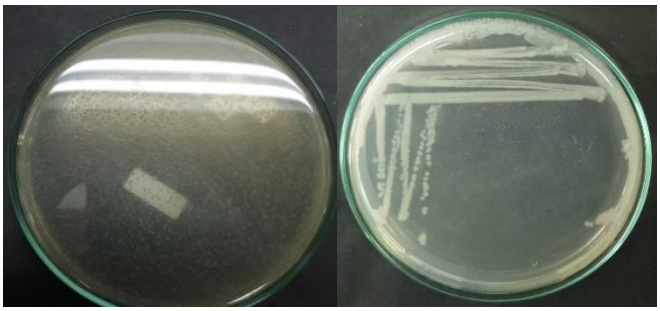


Fig. 1. Biodegradation Test of Polypropylene Plastics by isolated bacteria from Seawater

From Figure 1, it can be seen that 4 bacterial isolates were tested for polypropylene plastic degradation during the 4 week incubation period. There was a reduction in polypropylene plastic weight which has different results for each bacteria. The highest reduction of plastic film weight was found in isolate ILT-14 by 10.8%. Next was isolate ILT-16 by 10%, ILT-15 by 3.41% and ILT-13 by 1.8%. Biodegradation of polypropylene plastics is a very slow process because polypropylene has different properties. This plastic is a semi-crystalline polymer, which is chemically and thermally stable. Biodegradation of plastics can take a long and extreme time depending on the molecular weight of polymer, this process can take up to 1000 years for some types of plastics. In general, biodegradation of plastics by microorganisms is a very slow process, and some microorganisms are unable to degrade certain plastics[12].

In the isolation stage of synthetic plastic-degrading bacteria using mineral and NA medium, a number of bacterial isolates were found (Figure 2).



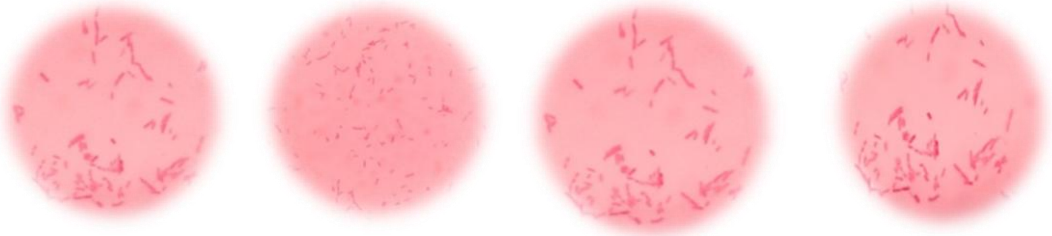
A **B**

Fig. 2. (a) Polypropylene plastic bacteria isolate process on mineral media, (b) polypropylene plastic bacteria after 24 hours incubation on NA medium

Table 3. Macroscopic and Microscopic Observation of Bacteria Isolates

Isolate Code	Macroscopic Observation					Microscopic Observation	
	Coloration	Shape	Margin	Surface	Elevation	Gram Staining	Cell shape
ILT-13	White	Circular	Irregular	Rough	Raised	Negative	Bacilli
ILT-14	White	Circular	Entire	Smooth	Raised	Negative	Bacilli
ILT-15	White	Circular	Entire	Rough	Raised	Negative	Bacilli
ILT-16	White	Circular	Entire	Rough	Raised	Negative	Bacilli

Table 3 showed the results of the macroscopic characteristics observation of four bacterial isolates that can degrade polypropylene plastic. The colony coloration of all four bacterial isolates were white. They have round shapes and raised elevations. The differences of each bacteria colony are characteristic for a particular species. Colony shape, color, shiny or not, smooth or rough surface are characteristics that are needed for the identification of a species. Most bacteria have a whitish, gray, yellowish, to clear color, however some species have a more pronounced color pigment[13].



ILT-13 **ILT-14** **ILT-15** **ILT-16**

Fig. 3. Microscopic Observation of Seawater Bacteria Isolates

Figure 3 showed microscopic characteristic of four seawater bacteria isolates observed under microscope with 1000 magnification. All bacteria isolates were Gram negative bacteria with bacilli cell shape. Commonly, cell shape can be coccus (round), bacilli (rod), comma and spiral. Diplobacilli bacteria are two bacterial cells that are close together. Diplobacilli formed from the splitted pair of bacilli, while streptobacilli has chains form[14].

Table 4. Biochemical Test of Seawater Bacteria Isolates

No	Treatment	Isolate Codes			
		ILT-13	ILT-14	ILT-15	ILT-16
1	Gram staining	-	-	-	-
2	Aerob/anaerob	-	-	-	-
3	TSIA	M	m	M	M
4	Gas	-	-	-	-

5	H_2S	-	+	+	-
6	Catalase	+			
7	Oxidase	+			
8	Mortility	+	-	-	-
9	Indol	-	-	-	+
10	Urea	+	+	+	+
11	Citrate	-	-	+	-
12	Lactose	-	-	-	+
13	Glucose	-	+	+	+
14	Sucrose	-	+	+	+
15	Mannitol	-	+	+	+
16	MR	-	-	-	-
17	VP	-	+	+	+
18	OF	+	-	-	+
19	KCN	+	-	+	+
20	Arginine	-	-	-	-
21	Lysine	-	+	+	+
22	Ornithin	-	-	-	-
23	Phenyllalanin	-	-	-	-
24	Aesculin	-	-	-	-
25	Arabinase	-	-	-	-
26	Raffinose	-	-	-	+
27	Sorbitol	-	-	+	+
28	Trehalase	-	-	+	+
29	Xylose	-	-	+	+
30	Dulcitol			+	+
31	Malanot broth	-	-	-	-
32	Gelatin	+	+	+	+

Result of 16S rRNA Gene Amplification

The results of the electrophoresis showed that the PCR activities have successfully applied the 16S rRNA gene region of polypropylene plastic bacterial isolates from seawater samples (Figure 4).

Electrophoresis Result of PCR 16s rRNA Activity

PCR uses a forward direction 27F AGAGTTGATCCTGGCTGAG primer with a primary 1492 R GTTTACCTTACGACTT primer for the reverse direction. This indicated that the PCR product fragments were the bacterial isolates from seawater samples.

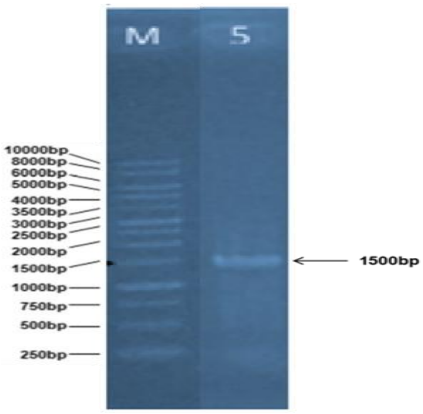


Fig. 4. The results of PCR product amplication of the 16S rRNA gene isolate of seawater bacteria (M = marker, 1kb = 5 = ILT-14)

The 16S rRNA Gene Sequencing Analysis of Polypropylene Plastic Bacterial Isolates

The sequencing results of the of polypropylene plastic bacterial isolates were compared with Gene Bank data using the BLAST program which was carried out online on the NCBI website *hhtp://blast.ncbi.nih.gov/Blast.cgi*. The sequencing data and the results of the BLAST analysis of the polypropylene plastic bacterial isolates can be seen in Table 5.

Table 5. The 16S rRNA BLAST Result of ILR-14 Seawater Bacteria Isolated

No	Microorganism	% Similarity	Acession No.
1.	*Stenotrophomonas maltophilia* strain	100	CP049956.1
2.	*Stenotrophomonas maltophilia* strain A32	100	MN372320.1
3.	*Bakterium strain* BS1826	100	MK825014.1
4.	*Bakterium strain* BS1619	100	MK824807.1
5.	*Stenotrophomonas maltophilia* strain CStm RA33	100	MH788995.1
6.	*Stenotrophomonas maltophilia* strain LHBAB-1	100	KC858848.1
7.	*Stenotrophomonas maltophilia* strain DZSG-6	100	KC858848.1
8.	*Stenotrophomonas maltophilia* strain DZSG -6	100	KC973235.1
9.	*Stenotrophomonas* sp. strain CW 15-5	100	MH769262.1

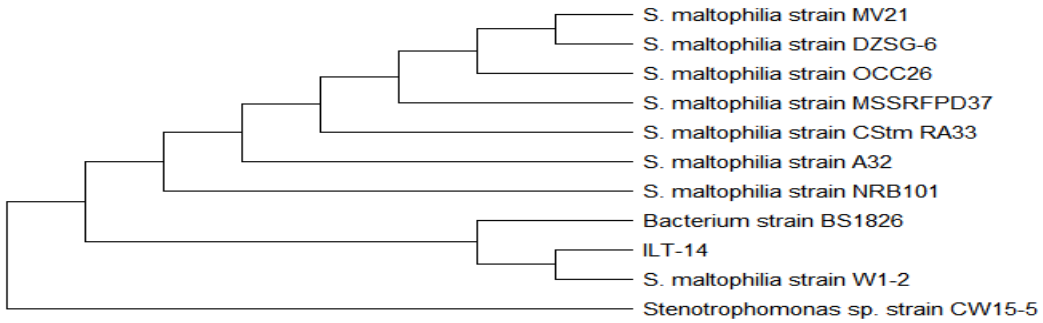
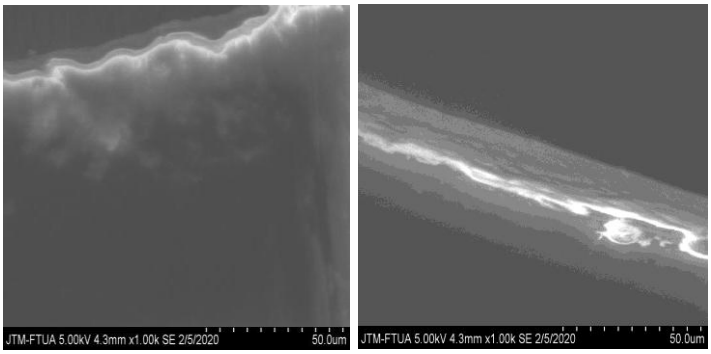


Fig. 5. Phylogenetic Tree of ILT-14 Seawater Bacteria Isolated

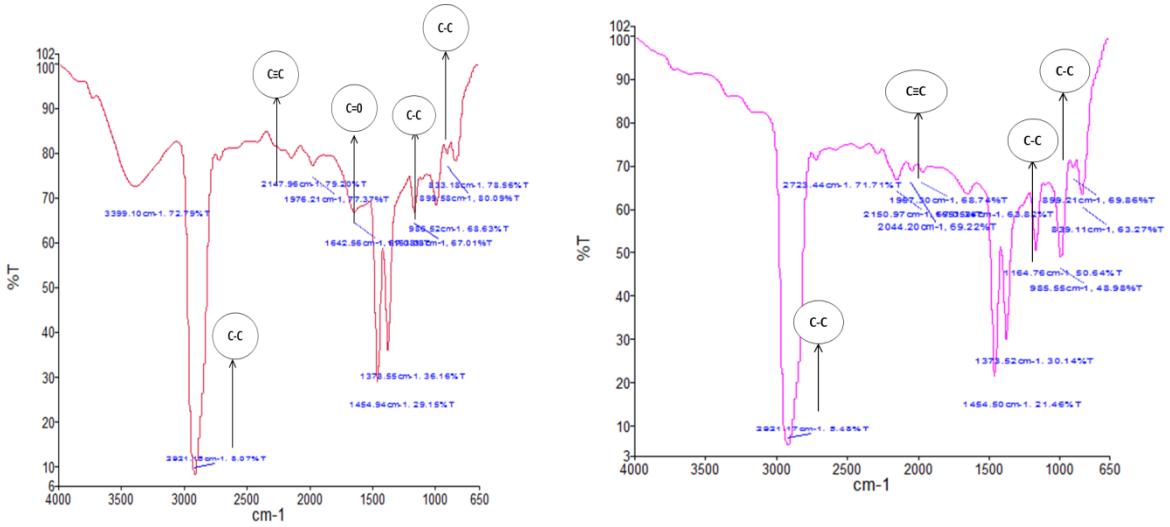
SEM (*Scaning Electron Microscopy*) Characterization



Control **ILT-14**

Fig. 6. Appearance of the SEM results of polypropylene, polypropylene control plastic before biodegradation test and ILR-14 polypropylene plastic after degradation at 20.00x magnification.

Characterization of functional groups Using Fourier Transform Infra Red (FT-IR)



Control **ILT-14**

Fig. 7. FTIR graph of polypropylene plastic control before biodegradation test and ILT-14 after biodegradation test

Figure 7 showed the result of FTIR against polypropylene that has been degraded by bacteria during the 30 day incubation period. This analysis aimed to determine the changes in functional groups during the biodegradation process. From these data, it can be seen that only a shift in wave numbers occurs.

IV. Discussion

Polypropylene plastic-degrading bacterial isolates have been isolated from seawater samples in Padang City, West Sumatra, Indonesia. Polypropylene plastic was first isolated on mineral media added with polypropylene plastic powder so that bacteria other than polypropylene plastic bacteria cannot grow. From the results obtained, there was bacterial growth in this modified mineral media, the bacteria isolate was gram-negative as shown in Figure 1-3 and Figure 5-6 [16,17].

The isolation of polypropylene plastic bacteria from seawater aimed to obtain potential bacteria with ability to degrading polypropylene plastic as shown in Figure 1. In the literature this kind of bacteria are : bacterial strains such as *Bacillus megaterium, Pseudomonas* sp., *Azotobacter vinelandii, Ralstonia eutropha, Halomonas* sp.[18,19,20]. These bacteria can be used effectively as a microbe that has the potential to degrading synthetic plastics. In this study, the researchers conducted a polypropylene plastic bidegradation test using inoculums ILR-13, ILR-14, ILR-15, ILR-16 bacteria isolates 5% (v/v). The isolates were inoculated into a mineral medium with addition of a sterile polypropylene synthetic plastic film[21] . Mineral medium was sterilized with a Rotary shaker incubator (Bigger digital®) at 130 rpm agitation and 37^{o}C temperature for 30 days. Every 7 days sampling was done for the weight reduction of polypropylene plastic film[22].

Seawater isolate bacteria were able to degrade polypropylene plastic with a reduction in plastic weight percentage during an incubation time of 30 days. The highest percentage of plastic weight reduction was 10.8% on ILT-14 isolate, 10% on ILT-16, 3.41% on ILT-15 and 1.8% on ILT-13. This indicated that the seawater sample bacteria isolates has an ability to degrade polypropylene plastic. Biodegradation is regulated by several factors including polymer characteristics, type of organism, pre-treatment conditions, polymer characteristics, mobility, and molecular weight[23]. Enzyme activity is influenced by the proteins present in bacterial cells during the enzyme biosynthesis process. Protein is the most important factor for breaking down polymer materials in the environment. The broken down polymer will be used as nutrition for microorganisms, therefore its regulation is related to endocellular catabolic enzyme control[24].

As shown in Figure 6, the SEM results of polypropylene plastic is an illustration to confirm that the tested bacterial isolates were able to break down complex polypropylene polymers into monomer forms. The indentations and cracks further confirm that the brittleness brought to the plastic sheet on sheet testing by the bacterial cultures. However, comparison with the control sheet prior to testing reinforces the theory of degradation because the plastic surface does not appear to have any damage[25].

Figure 7 showed that the FTIR spectra to determine the changes in functional groups during the biodegradation process. From these data, it can be seen that only a shift in wave numbers occurs. In addition, the characterization results are supported by previous study which stated that there is an O-H group at a wavelength of 3419.34 cm^{-1} and C-O at 1400.81 cm^{-1}

V. Conclusion

Bacteria ILR-14 isolated is one of the high potential bacterial isolate was found from three isolates of polypropylene plastic-degrading bacteria from sea water in Padang City, West Sumatra, Indonesia. Based on molecular identification of ILR-4 isolated bacteria using 16S rRNA gene observed that ILR-14 was 99% similar to *Stenotrophomonas maltophilia* strain W1-2.

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