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BIODEGRADATION OF PLASTIC WASTE BY BACTERIA ISOLATED FROM SURABAYA LANDFILLS

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ABSTRACT

Plastic waste is one of the most difficult environmental problems to overcome. Data show Indonesia as the world's second largest contributor to plastic waste. Research on the degradation of plastic waste by microbes become necessary as a safe solution in the handling of plastic waste in Indonesia. Polyethylene (PE) is a stable plastic component polymer, filling 64% of all synthetic plastics. It is mainly used for the manufacture of plastic bags, bottles, and disposable packaging. The purpose of this study was to determine the potency of two bacteria isolated from Surabaya landfills (PD-1 and PD-6) to degrade PE. Biodegradation process was characterized by the percentage of degradation (decrease of PE mass during culture 14 days). Results showed that PD-6 and PD-1 can degrade plastic as much as 10.9% and 4.18%, respectively. Based on this results, it was indicated that both of the bacteria have potential as agents of biodegradation of plastic waste in the environment.

KEY WORDS : Plastic waste, PD-1 isolate, PD-6 isolate, Biodegradation of PE

INTRODUCTION

Every day, there are human activities that produce waste(s). As many as 384 cities in Indonesia produced municipal solid waste (MSW) about 80,235 tons per day (Kardono, 2007). Increasing of solid waste production makes it difficult to handle, so it has become an environmental problem for the city of Surabaya, East Java (Prasetyanti *et al.*, 2014). In Surabaya, as much as 12.45% of the total MSW is plastic, occupying the second largest composition after organic waste (Kardono, 2007). Most of the plastic waste will end in landfill (Barnes *et al.*, 2009), so increasing the amount of waste in cities in Indonesia (2-4% every year) will also cause problems due to limited landfill areas (Wijayanti and Suryani, 2015).

One type of plastic used in various fields is polyethylene (PE) (Gajendiran *et al.*, 2016), including low density polyethylene (LDPE), medium density polyethylene (MDPE), high density polyethylene (HDPE) and linier low density polyethylene (LLDPE) which are used as packaging (Shah *et al.*, 2008). Polyethylene has a long ethylene monomer and is not easily biodegradable (Tokiwa *et al.*, 2009).

Degradation of PE can occur due to collaboration between photo- and thermo-oxidative degradation and the presence of biological activity, such as by microbes (Tokiwa *et al.*, 2009). According to Gajendiran *et al.* (2016) microbial enzymes can accelerate the degradation of LDPE, so that it can help the degradation of polyethylene in the environment.

Various studies have demonstrated the ability of various bacterial isolates that have the ability to degrade PE. Research by Munir *et al.* (2018) showed that SP2 and SP4 bacterial isolates from landfill in Medan, had the potential to degrade LDPE. *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Acinetobacter ursingii* from soil in Baghdad were also known to degrade LDPE (Hussein *et al.*, 2015). Bacterial isolates from garbage dumped areas in Kolenchery, India, were capable to degrade PE (Rosario and Baburaj, 2017).

Previous studies Munir *et al.*, (2018); Hussein *et*

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al., (2015); Rosario and Baburaj, (2017) have shown that bacteria isolated from landfill have the ability to degrade PE. The aim of this study was to determine the ability of bacteria isolated from Surabaya landfill to degrade PE, so that it is expected to help overcome the problem of plastic waste in Surabaya.

MATERIALS AND METHODS

Materials

This research used bacterial cultures that were isolated from Surabaya Landfills (PD-1 and PD-6). PD-1 and PD-6 bacteria were selected because they were hydrocarbonoclastic bacteria (can grow on media containing polyethylen (PE) plastic components). PD-1 and PD-6 bacteria were recultured using Nutrient Agar (NA) (Oxoid) media. Bacteria were grown in liquid Nutrient Broth (NB) media (Oxoid) before being used for PE degradation test. Percentage of PE degradation using selective media consisting of 100 mg of yeast extract, 1 g $(NH_4)_2SO_4$, 200 mg MgSO4.7H_2O, 100 mg NaCl, 20 mg CaCl.2H_2O, 10 mg $FeSO_4$.7H_2O, 0.5 mg $Na_2MoO4.2H_2O$, 0.5 mg $MnSO_4$, 1.6 g K_2HPO_4 and 200 mg KH_2PO_4 at neutral pH. A total of 50 mg sterile PE layer was added to each bottle of culture media. PE was sterilized using 70% alcohol for 30 minutes (Harshvardhan and Jha, 2013).

METHODS

Isolates Preparation

Bacterial isolates were streaked on NA, then incubated at room temperature (28±2) ^{0}C for 24 hours. One or two loop full of isolates were also innoculated on NB media, then incubated at room temperature for 24 hours.

Bacterial Adherence to Hydrocarbon (BATH) Test (%)

Cells of bacterial cultures were washed using buffer 16.9 g/L K_2HPO_4 and 7.3 g/L KH_2PO_4. Turbidity of bacterial cells in the buffer were adjust to 0.5 at wavelength 610, and then suspensions (hydrophilic phase) were added by 100 µL hexadecana (hydrophobic phase), then the mixture vortexed for about 3 minutes, incubated for 1 hour. Turbidity of hydrophilic phase was measured using spectrophotometer. The decrease of hydrophilic phase had correlation to hydrophobicity of cells. The percentage value of bound cells in the hydrophobic phase (H) was calculated using the formula below (Rosenberg *et al.*, 1980).

$$N = 1 - \frac{A}{A_0} \times 100\% \quad .. (1)$$

In the formula, H is value of bacterial attachment to hydrocarbons, A is absorbance after addition of the test compound, and A_0 is absorbance before adding test compounds.

PE Degradation Test

PE degradation test was done by inoculating 1 mL of bacterial inoculum from NB media (OD 0.5 at λ = 600 nm) into a culture bottle containing 50 mL of selective media and 50 mg PE. The treatment was incubated at room temperature (28 ± 2) ^{0}C for 14 days with an agitation of 120 rpm.

Degradation Percentage Measurement

Measurement of the percentage of degradation can be determined from the weight changes in the PE substrate that was incubated for 14 days along with the test bacteria. PE was separated from the culture media using filter paper and rinsed with sterile distilled water, then bacterial colonization on the surface of the PE substrate was removed by immersing 2% (v / v) sodium dodecil sulfate (SDS) solution for 4 hours at 50°C (Sivan *et al.*, 2006). PE was dried in an oven overnight with a temperature of 60 °C. Dry PE was weighed using an analytical balance (as the final weight of PE). The percentage of degradation is calculated using the formula below (Hosseini *et al.*, 2010).

$$DE\ (\%) = \frac{W_0 - W_1}{W0} \times 100\% \quad .. (2)$$

In the formula, DE is degradation efficiency (%), W_0 is initial weight, and W1 is final weight.

Total Plate Count (TPC)

Cultures that incubated for 14 days were diluted as needed. Every three last dilutions, the suspensions were inoculated into a Petri dish using the pour plate method, then added 15 mL NA, and homogenized. The culture was incubated for 24 hours at room temperature (28 ± 2) °C. The number of eligible microbial cells (30-300 colonies) is stated in CFU /mL (Ibuot and Bajhaiya, 2013).

pH Measurement

Measurement of the pH of the treatment was carried out at the end of the degradation test treatment using litmus paper on the degradation test treatment bottle.

Data Analysis

All data obtained such as total plate count (TPC) (CFU/mL), PE (%) plastic degradation percentage value, bacterial adherence to hydrocarbone (BATH Test) value, and test bacterial culture pH) were analyzed descriptively.

RESULTS AND DISCUSSION

Bacterial Adherence to Hydrocarbon (BATH) Test

The value of BATH is based on a decrease in bacterial cell turbidity on the water phase (hydrophilic) after adding a PE (hydrophobic) hydrocarbon test compound. A decrease in turbidity in the water phase indicates the attachment of bacterial cells to hydrocarbons. BATH test results are presented in Fig. 1.

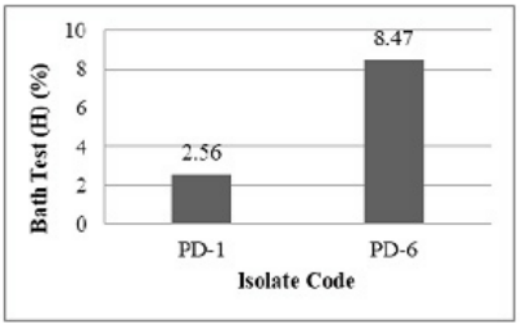


Fig. 1. Value of Bacterial Adherence to Hydrocarbon (H)

The results show that the BATH (H) values in both isolates have different values. PD-6 isolates have a higher H value compared to PD-1 which is equal to 8.47%. According to Arutchelvi *et al.* (2008) the stages of polymer biodegradation from the polyolefins group can be preceded by the attachment of microorganisms on the polymer surface followed by the growth of microorganisms as a form of use of polymers as the main carbon. Furthermore, polymer degradation is occured and continued by further degradation until the polymer substrate is degraded all.

BATH test results are based on observations of changes in turbidity in the water phase (hydrophilic). Decreasing of turbidity is an indication of the ability of bacteria in adhering to the hydrocarbon phase (Pramila *et al.*, 2012). The values of H isolates PD-1 and PD-6 are different. It shows that the hydrophobicity value of the cell wall surface of each bacterium is different. The difference in H values is related to the effectiveness of biofilm formation on hydrophobic surfaces (Pramila et al., 2012).

PE Degradation Percentage (%)

The degradation percentage of PE was obtained from the ratio between the weight of PE residues and the initial weight of PE (Fig. 2). This study used an initial weight of PE of 0.05 g which was incubated for 14 days. After the incubation period ends, treatment cultures show differences with control cultures. Treatment cultures showed the presence of test bacterial growth characterized by turbidity in the culture medium (Fig. 3). The formation of foam in the treatment culture also showed the presence of biosurfactant production by the test bacteria (Fig. 3). Control treatments showed a percentage of 0.63%, while PD-1 and PD-6 isolates reached 4.18% and 10.9%.

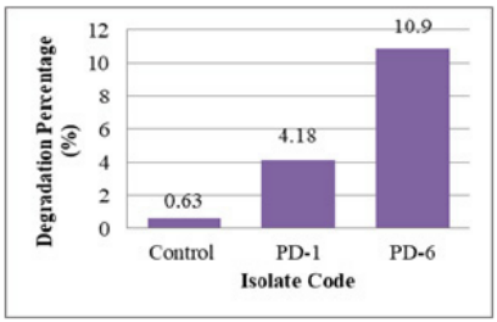


Fig.2. PE Degradation percentage by bacterial isolates.

The principle of the biodegradation test is to measure the weight of the polymer before and after treatment, to obtain the difference in weight or weight of the residue. Bacterial activity can be seen by the increase turbidity of the culture and the appearance of foam at the end of the incubation (Fig. 3). Isolates of PD-1 and PD-6 showed differences in the percentage of degradation, but when compared with the control treatment, both showed better values, respectively 4.18% and 10.9%. Biodegradation depends on the characteristics of the polymer, type of organism and pretreatment on the polymer (Shah, 2008).

Total Plate Count (TPC)

The results of the calculation of the number of microbes are presented in Table 1. The initial number of bacteria was calculated based on the results of the detection of bacterial suspensions at OD = 0.5 with λ = 600 nm. At the end of the incubation period, both isolates showed a decrease in the number of microbes. The decrease in the TPC value indicates a change in conditions in the culture. The decrease in the number of bacteria probable due to the inhibition of bacterial growth due to the use of polymers or intermediate products from the degradation process that is toxic to bacteria. This can occur when the TPC has decreased while the percentage of degradation has increased. Similar to the results of this study which showed the number of bacteria decreases along with the increase in the percentage of PE degradation.

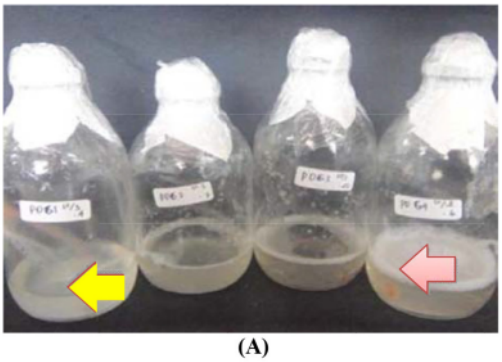
The decrease in the number of bacteria can also occur because the presence of bacterial adhesion on the test substrate, so it is present in lower concentration in agitation phase. This may occur because in the biodegradation mechanism of polymer polyofelin begins with the attachment of microorganisms on the polymer surface followed by the growth of these microorganisms (Arutchelvi *et al.*, 2008).

Table 1. Calculation result of the number of microbes in the treatment.

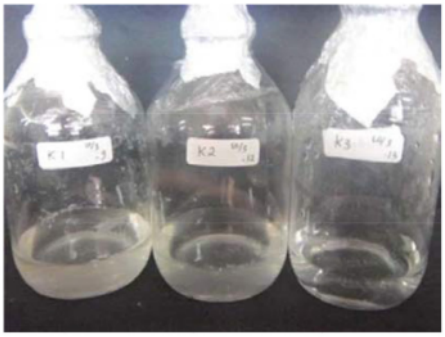
Isolate Code	Total Plate Count (CFU/mL)	
	Early Incubation	After Incubation
PD-1	4.0×10^9	2.5×10^8
PD-6	1.5×10^{12}	5.6×10^6
PD-1	4.0×10^9	2.5×10^8
PD-6	1.5×10^{12}	5.6×10^6

pH Value of the Culture

The pH of the culture is presented in Fig. 4. The pH of the culture at the beginning of the incubation period was made homogeneous (pH 7). pH decreases at the end of each incubation period. Culture pH is one of the factors that influence microbial growth and as an indication of polymer biodegradation process (Scragg, 1999). Decreasing pH in the biodegradation process of PE indirectly indicates that new functional groups have formed in the culture that can reduce the pH of the culture. The decrease in the pH of the culture in the results of this study shows the formation of acidic



(A)



(B)

Fig. 3. Quantitative test result of PD-6 (A) and control (B). Bacterial growth were showed by the presence of turbidity in the culture (yellow arrow) and foam formation (red arrow).

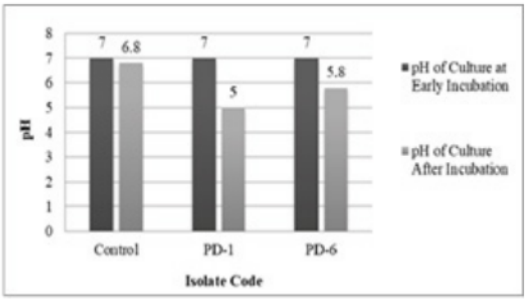


Fig. 4. pH value of each isolate in early and after incubation.

compounds as new chemical compounds as a result of the metabolic process of the test bacteria. According to Harsvardhan and Jha (2013), the initial mechanism of biodegradation of high molecular weight polymers is oxidation or hydrolysis of the polymer by enzymes which then form functional groups that can increase the hydrophilicity of the polymer. This causes the ease of bacteria in

accessing and assimilating the polymer.

CONCLUSIONS

Results showed that PD-6 and PD-1 can degrade plastic as much as 10.9% and 4.18%, respectively. Based on this results, it was indicated that both of the bacteria have potential as agents of biodegradation of plastic waste in the environment.

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