Potential of bacteria isolated from landfill soil in degrading low density polyethylene plastic

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Abstract. Plastic is an important material and used for many purposes. It is returned to the environment as a waste which is recently considered as the second largest solid waste. The persistency of plastic in the environment has been attracted researchers from a different point of view. The study of the degradation of plastic using bacteria isolated from local landfill soil was conducted. Low density polyethylene (LDPE) plastic was used as tested material. Potential isolates were obtained by culturing the candidates in mineral salt medium broth containing LDPE powder. Two of ten exhibited better growth response in the selection media and were used in degradation study. Results showed that isolate SP2 and SP4 reduced the weight of LDPE film significantly to a weight loss of 10.16% and 12.06%, respectively after four weeks of incubation. Scanning electron micrograph analyses showed the surface of LDPE changed compared to the untreated film. It looked rough and cracked, and bacteria cells attached to the surface was also noticed. Fourier transform infrared spectroscopy analyses confirmed the degradation of LDPE film. These results indicated that bacteria isolated from landfill might play an important role in degrading plastic material in the landfill.

Keywords: degradation of plastic, landfills, low density polyethylene, plastic degrading bacteria.

1. Introduction

Polyethylene has been considered as one of the environmental polluting materials for many decades. It is highly resistant to degradation process. On the sides, the uses of polyethylene have been escalated every year, and the annual rate of production goes over 25 million tons [1]. Besides the global use of polyethylene is also increasing to 12% per year and about 140 million tons of synthetic polymers are produced every year [2]. Low density polyethylene (LDPE) is one of a type of polyethylene and used for manufacturing of various materials and goods. This is due to its characters, such as lightweight, good impact resistance, extremely flexible, easily cleaned, thermoforming performance, meets food handling guidelines, no moisture absorption, and chemical - and corrosion-resistant. Due to its best characteristics, LDPE is widely used for manufacturing packagings and various containers, dispensing bottles, wash bottles, tubing, plastic bags for computer components, and some laboratory equipments. The plastic bag is the most common used LDPE material in house hold.

With increase uses of polyethylene, its accumulation in the environment has been the current concern in environmental view point. Highly hydrophobic and chemically inert makes it very resistant to biodegradation. It takes hundreds and probably thousands of years to decompose completely in the environment. Then, it has been estimated that 5-7% of domestic waste consists of polyethylene [3]. Many efforts to minimize the accumulation of polyethylene have been afforded both in manufacturing process and management of polyethylene products, and it has been obtaining much attention from biodegradation view point. Yang et al. [4, 5], search for efficient disposal treatment of polyethylene by biological means holds certain concern other than the chemical approach and biodegradation and bio recycling are the alternatives. In fact, due to cost factor and loss of mechanical properties, recycling is not economically feasible. Pramila et al. [6] biodegradation is the safest method of polyethylene breakdown. It generates less toxic residue and shows potentials of bio-geo chemical cycling of the substrate.

Several works on microbial degradation of LDPE have been documented in the last two decades, and several microbes have reported as well. Gram positives such as *Bacillus weihenstephanensis*, *Bacillus* spp., *Staphylococcus* spp., *Streptococococcus* spp., *Diplococcus* spp., have reported to degrade LDPE [7, 8]. While Hussein et al. [9] some gram negatives *Pseudomonas fluorescens, Pseudomonas aeruginosa*, and *Acinetobacter ursingii* isolated from plastic contaminated soil could degrade LDPE molecule. *Moraxella* spp., *Burkholderia cepacia* and *Escherichia coli* are also capable of LDPE degradation. Kavitha et al. [10], studies biodegradation of polyethylene using bacteria isolated from oil contaminated soil. Two selected isolates reduce the weight of polyethylene up to 1.3% after 30 days incubation. Zusfahair et al. [11] bacteria isolated from landfill soil in Banyumas reduces the weight of polyethylene by 2.33% after 30 days incubation. The more promising result has also been recorded previously. Nanda and Suhu [12], bacteria isolated from the waste disposal site are capable of degrading polyethylene. *Pseudomonas* shows very high biodegradation ability of polyethylene followed by *Brevibacillus*, and *Rhodococcus* with the weight loss of 40.5%, 37.5%, and 33%, respectively.

Then, this preliminary study is designed to get potential bacterial isolates to degrade polyethylene from landfill soil. This paper describes the ability of selected bacteria in degrading LDPE, and the results are compared to others reported works.

2. Method

2.1. Isolation of bacterial candidates and preparation of LDPE powder

Bacterial candidates were isolated from local landfill soil in Medan using a clean shovel. The soil was collected from three different sites (of the plastic accumulated site) and mixed thoroughly. One gram of sample was inoculated to 20 ml of Nutrient Broth (NB) media and incubated overnight under shaking condition (120 rpm). A serial dilution was prepared with NaCl 0.9% solution, 0.1 ml of sample was plated on Nutrient Agar (NA). Cultures were incubated at room temperature (26 ± 2 ° C) overnight. The growing colonies were then transferred to a new NA plate to obtain pure isolates. The isolates were kept at 4° C for further study. LDPE powder was prepared by dissolving the LDPE beads in xylene with continuous stirring and boiled for 15 minutes. The clump LDPE was rinsed with 96% ethanol and dried completely in an oven at 50° C. The LDPE was cut into small pieces and mashed with blander to get its powder form.

2.2. Screening of bacterial isolates and biodegradation test of LDPE

Potential of isolates in degrading LDPE was screened in Mineral Salt Medium Broth (MSMB) containing LDPE powder. Glucose (0.5%) was also supplemented to the media to initiate the growth. Two ml of bacterial suspension (with turbidity of 0.5 McFarland) was inoculated to 50 ml of MSMB. Culture was incubated at room temperature (26 ± 2 ° C) at rotary shaker (120 rpm) for 4 weeks. The growth of isolates was monitored every week through measurement of optical density (OD) value and

total plate count [9]. Biodegdaration test of LDPE by selected isolates was done in broth medium. Two bacterial isolates showing good growth responses based on OD and CFU values were used for the test. Five ml of bacterial suspension (with turbidity of 0.5 McFarland) were inoculated into 45 ml MSMB. A surface sterilized LDPE sheet (1.5 cm x 1.5 cm) was inoculated into each culture flask, cultures were prepared in triplicates. Cultures were incubated at room temperature (26 ± 2 ° C) at rotary shaker (120 rpm) for 4 weeks [13].

2.3. Analyses of LDPE degradation

The ability of selected isolates in degradation of LDPE film was evaluated by weight loss, scanning electron micrograph (SEM), and Fourier transform infrared spectroscopy (FT-IR Spectroscopy). Analyses of weight loss of each treated sheet were measured using analytical balances. The weight loss of LDPE was calculated by the following formula and the results obtained were compared with LDPE weight control.

$$\text{Weight loss (\%)} = \frac{\text{(Initial weight - Final weight) X 100}}{\text{Initial weight}} \quad (1)$$

The surface morphology of LDPE film was observed using Scanning Electron Microscopy (SEM). LDPE slabs placed on the sample holder and scanned at 5000 × magnification. The untreated film was also analyzed for comparison. Changes and formation of functional group of degraded LDPE were studied through FTIR analyses. Degradation was confirmed using untreated LDPE.

3. Results and Discussion

3.1. The growth of bacteria in LDPE containing media

Low density polyethylene degrading bacteria were isolated from local landfill soil. The sample was inoculated first in LB media and transferred to NA. Ten isolates, showing different characters by morphological appearances such as types, elevation, color, and the edge of the colonies, were obtained. Each isolate was labeled a specific code from SP 1 to SP 10. Most of them showed white to yellow colonies with majority of them were flat. The initial screening of LDPE degrading potential of isolates was done in medium containing LDPE as the only carbon sources. The growth of isolates was monitored by measuring the absorbance of cultures (OD 600 nm) and the total plate count of culture every week for four one month. Results showed that three isolates (SP2, SP3, and SP4) exhibited good growth until the end of cultivation, until four weeks. While seven others were unable to survive until week-4, and some of these were not detected from week-2. Only three isolates SP2, SP3, and SP4, exhibited the growth from week-3 to the end of cultivation. Results as shown on Table 1, indicates that isolates SP2, SP3, and SP4 could utilize LDPE molecule existed in the medium to support the growth. Two of them showing best growth response (SP2 and SP4) were used for further study.

Table 1. The growth of isolates in MSMB media containing LDPE powder

Code of Isolate	Absorbance at OD 600 nm			CFU/ml		
	Initial	Week-1	Week-4	Initial	Week-1	Week-4
SP1	0.125	0.137	-	19×10^6	22×10^6	-
SP2	0.121	0.240	0.490	22×10^6	37×10^6	150×10^6
SP3	0.111	0.120	0.259	17×10^6	41×10^6	103×10^6
SP4	0.120	0.200	0.504	20×10^6	33×10^6	200×10^6
SP5	0.121	0.133	-	21×10^6	27×10^6	-
SP6	0.113	0.130	-	19×10^6	25×10^6	-
SP7	0.113	0.123	-	20×10^6	29×10^6	-
SP8	0.120	0.127	-	23×10^6	20×10^6	-
SP9	0.130	0.137	-	22×10^6	30×10^6	-
SP10	0.123	0.132	-	16×10^6	25×10^6	-

Results as in Table 1 showed that the absorbance of culture was relatively in line with the number of cell or CFU per ml culture. It was also recorded for data of week-2 and week-3 (not shown in Tabel 1). The reduction of growth and the inability of some isolates to survive indicated that isolates could not utilize LDPE molecule supplemented media. The bacteria used glucose (0.5%) until week-2 and some to week-3 or until it was used up and did not show the growth response afterward. On the other hand, SP2, SP3, and SP4 were assumed to have an ability to utilize LDPE, as indicated by the value of absorbance of culture and the CFU per ml. Based on this result, it indicates that the number of bacteria capable of degrading LDPE in the environment is limited. Hussein et al. [9] previously used the same procedure in the screening of bacteria from plastic contaminated soil. He also obtained three bacterial isolates with higher growth response in a plastic powder containing media.

3.2. Characters of selected isolates

Simple characteristic analysis of isolate SP2 and SP4 was performed to have basic features of the isolates, the results are shown in the following table.

Table 2. Characteristic of selected isolates

Isolate	Gram	Morphology/ Cell arrangement	Biochemical test				
			Amylase	Citrate	H_2S*	Motility	Catalase
SP2	Positive	streptoccus	+	+	Y/R	+	-
SP4	Positive	streptobacil	+	-	Y/R	+	-

*Yellow at *slant* / Red at *butt.*

Both isolates were gram positive. SP2 was coccus, while SP4 was basil, both were arranged in strepto form. Physiological characters of isolates were very much similar, except in utilization of citrate as shown in Table 2. Some works on the identification of bacteria degrading LDPE have previously been reported; some isolates were gram positive and some gram negative. Deepika and Jaya [7] and Mukherjee & Chatterjee [8] found some LDPE degrading gram positives *Bacillus weihenstephanensis*, *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Diplococcus* spp. While Hussein et al. [9] identified some gram negatives *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Acinetobacter ursingii* isolated from plastic contaminated soil could degrade LDPE molecule. Some other gram negatives such as *Moraxella* spp., *Burkholderia cepacia*, and *Escherichia coli* have also been reported to degrade LDPE.

3.3. Degradation of LDPE

After four weeks of the incubation period, the ability of isolates in degrading LDPE film was measured by weight loss. Results are shown in Table 3. The low density polyethylene incubated with SP2 and SP4 showed a reduction of weight up to 10.16% and 12.06%, respectively after four weeks.

Table 3. Weight loss of LDPE film after 4 weeks

No.	Treatment	Initial weight (g)	Final weight (g)	Weight loss (%)
1	Control	0.0059	0.0059	0
2	SP2	0.0059	0.0053	10.16
3	SP4	0.0058	0.0051	12.06

Weight reduction of LDPE after four weeks incubation with isolate SP2 and SP4 compared to control (incubation without isolate) indicated that film was degraded by tested bacteria. Reduction of weight of LDPE film is due to the enzymatic analyses of LDPE polymer. Then, the bacteria utilized LDPE molecule as the carbon source to support the growth. The rate of weight loss during of LDPE film found in this study was amazingly high and much higher compared other reported works. Kavitha et al. [10] bacteria isolated from contaminated soil reduced LDPE weight by 1.2% to 1.3% after incubation for 30 days under laboratory condition. Zusfahair et al. [11] found that the longer incubation period, the higher weight loss would be. Bacteria he isolated from landfill soil in Banyumas enabled to degrade polyethylene to a weight loss of 2.33% after 30 days of incubation. Furthermore, a quite higher weight loss of LDPE film was obtained using fungi isolated from the same landfill soil with this current work. The selected fungi (RH03 and RH06) reduced the weight of LDPE film from 5.13% and 6.63%, respectively after 45 days of cultivation. The results are being presented somewhere.

In parallel with weight loss analyses, the number of bacterial cells attaching to the film or forming biofilm was counted. The bacteria were released from LDPE film using glass sand and plated on plate count agar. Results indicated the number of SP2 and SP4 cell on surface film was 1.41×10^7 and 1.36×10^9 cfu/film. The higher number of the SP4 cell than that of SP2 in line with weight loss rate, which was also higher for SP 4 as described above. Thus it is inferred that the higher the number of bacteria forming a biofilm, the more active biodegradation process was. Then, since plastic does not contain soluble and do not absorb water, it is assumed that formation of biofilm is very essential in degrading LDPE. Alex [14] reported the formation of biofilm on the surface of plastic is commonly found during degradation studies.

3.4. Micrograph analyses

Scanning electron micrograph confirmed that LDPE film was degraded. Tested isolates attaching to the surface of the film caused degradation. Figure 1 shows morphological surface of the film treated with selected isolates (Sp2 and SP4) for 30 days in MSMB. The surface of the film as shown in B and C was damaged. Several changes, such formation of holes, wrinkles, cracks, and rough, were observed. It was quite different from the surface of control/untreated film as shown in A.

Results as in Figure 1 shows the changes of film surface after 30 days treated with selected isolates, and it confirms that SP2 and SP4 were able to degrade LDPE molecule. The surface of the film incubated with no isolate (control) was remain smooth. Besides bacterial cells attaching to surface of film was also detected as shown in D. Changes of surface morphology of LDPE using SEM was also previously detected by Kavitha et al. [10] using several magnifications. He found changes of film surface such as holes and erossion of one month treated film. Formation of microbial film or attaching bacterial cell on the surface of treated film found in this study was very important in degradation of LDPE, and it is assumed that the degradation started from surrounding microbial film. Gnanavel et al. [15] biodegradation of plastic occurred through erosion of surface; the extracelluler enzyme systems could not penetrate plastic molecule, it worked only on the surface. The degradation of plastic could occur through chain degradation/de-polymerization and random degradation/reverse of the poly condensation process.

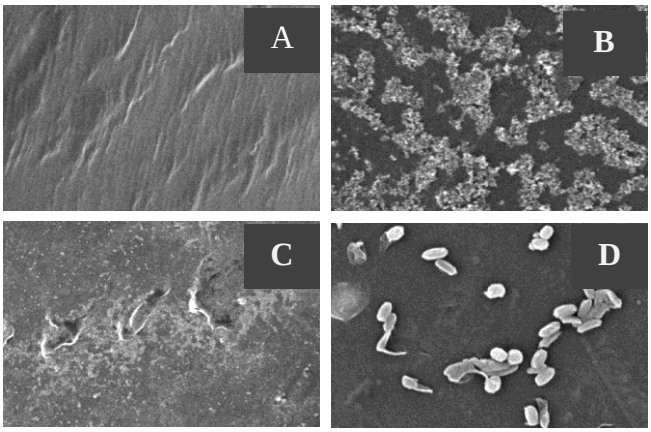

Figure 1. Scanning electron micrographs of treated LDPE film after 30 days of incubation (5000x magnification). (A) control film (incubated with no isolate); treated with SP2; (C) treated with SP4; (D) bacterial cell on the surface of the film.

Analyses of functional group of polyethylene after degradation test showed there were no changes of aliphatic (C-C) spectrum both in control and treated LDPE films (2943.37 and 2870.08 cm^{-1}). However, there was a reduction of wave number spectrum of the methylene group (-CH2) compared to the non-treated polyethylene. Sastrohamidjojo [16] methylene and methylene group could be measured by analyses absorbance of from 1465 to 1370 cm^{-1}. Al Ashraf [17] peaks detected from 1462 to 719 cm-1 could be C-H bending deformation, CH3 symmetric deformation, twisting deformation and rocking deformation. In this study, wave number of methylene group was 1462.04 cm^{-1} for control

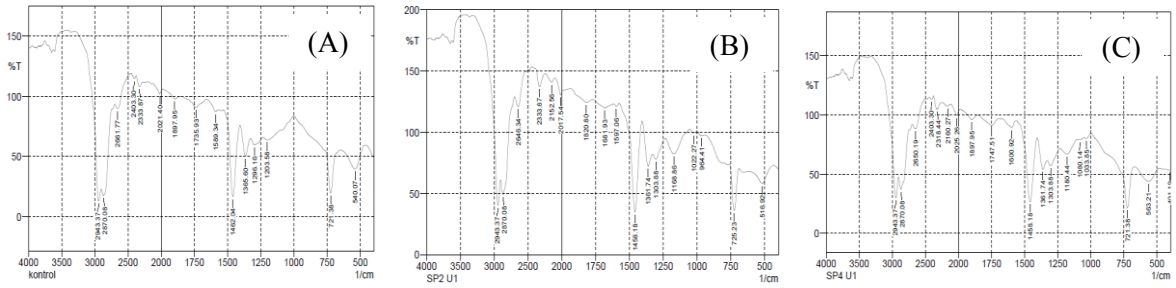

Figure 2. FTIR spectrum of LDPE film after incubated for four weeks. (A) control or untreated film; (B) treated with isolate SP2; and (C) with SP4.

reduced to 1458.18 cm^{-1} for both SP2 and SP4 treated film. Reduction of the wave number of polyethylene indicated that LDPE film had been depolymerized or degraded and the bacterial isolates used liberated methylene for the growth. Gu et al. [18] depolymerization of a higher molecule to simple compound was very important before it can be absorbed and utilized by microbes.

In conclusion, it has been widely known that polyethylene plastics are resistant to degradation and it takes very long time to degrade completely in the environment. Bacterial isolates (SP2 and SP4) obtained from local landfill soil in this study have higher ability in degrading LDPE. Furthermore, it is assumed that the existence of certain bacteria in the landfill plays an important role in degrading plastic.

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