3. REVIEW

The table below summarizes the research on polyethylene biodegradation as a plastic-type that is commonly used as food packaging.

Table 2. Biodegradation of Polyethylene Materials

Sr. No	Type of plastic and Size/Weight	Types of microbial population	Analysis Methods	Source of the microbes used	Major findings/ conclusions/inferences	Name of the microbes/enzymes responsible	Reference
1.	Polythene bags and plastic cups were cut in small strips (undefined size)	Pure culture and *In-situ* soil population (consortium)	Species Identification (Morphology and Biochemical test); Soil Parameter test (pH, Temp, Alkalinity, Organic Matter, Chlorides, Moisture content); Dry weight loss	Five soil location: medicinal garden soil (A), sewage soil (B), energy park soil (C), sludge area soil (D), agricultural soil (E)	The experiment undergo in one month, and the highest weight loss was achieved by *Aspergillus niger* (12.5%) and *Proteus vulgaris* (12.5%) The degradation process is faster in the laboratory condition than *In-situ* treatment in the soil because the use of single strain microorganism	F1(*Aspergillus niger*), F2(*Aspergillus nidulance*), F3(*Aspergillus flavus*), F4 (*Aspergillus glaucus*), F5(*Penicillium*), B1(*Pseudomonas*), B2(*Bacillus subtilis*), B3(*Staphylococcus aureus*), B4(*Streptococcus lactis*), B5(*Proteus vulgaris*), B6 (*Micrococcus luteus*)	(Priyanka & Archana, 2011[b])
2.	LDPE sheet (2 x 2 cm with similar weight) and powder (0.50 g/100 ml)	Single culture population of fungi	Biodegradation analysis (Colonization test and Strum Test); Polyethylene	Sea water sample was collected from Kovalam coast-off the Bay of Bengal, 500 m	The Sturm test result from *Aspergillus sp* was 83% followed by *Aspergillus versicolor* was 77% for a week of incubation. *Aspergillus* sp was grow rapidly in 7th days of incubation and only	*Aspergillus versicolor* *Aspergillus sp.*	(R. Pramila, 2011)

			Surface Observation (Light Microscope and SEM)	away from shore at the depth of 5 cm	increase for 22%, while *A. versicolor* was growing rapidly (95%) only after 7th days of incubation from the total 17 days of colonization test.		
3.	LDPE films (commercially used NTUC plastic bag) was cut in 5 x 1 cm	Single culture population of bacteria	Biodegradability Test (Weight Loss, Tensile Strenght, Extention at Break, FTIR-ATR, GC-MS, SEM); Planktonic and Biofilm Growth (CFU)	Pure culture from ATCC	After 120 days of the incubation period in rotary shaker, the highest degradation rate according to the test result: 20% of weight reduction, 80% of carbonyl index reduction, intense growth of biofilm, and more significant micro cracks was achieved by B1 isolate as *Pseudomonas aeruginosa* (PAO1). The longer incubation time would elevate the biofilm formation on the surface and increasing the production of biodegradation enzyme, which decreasing the CI continuously along the incubation time.	(B1) *Pseudomonas aeruginosa* PAO1 (ATCC 15729), (B2) *Pseudomonas aeruginosa* (ATCC 15692), (B3) *Pseudomonas putida* (KT2440 ATCC 47054) and (B4) *Pseudomonas syringae* (DC3000 ATCC 10862)	(Kyaw *et al*., 2012)
4.	Two types of HDPE and one type of LDPE plastic bags	Bacteria consortium	Mean weight loss	Three sites of the mangrove soil sample in Suva, Fiji Islands	Nearly 4 % of weight loss in HDPE class 3 after eight weeks. The decrease of melting point, bonding strength, and delta H in HDPE class 3 are predicted because of the additives. Lower biodegradability of LDPE class 1 and HDPE class 2 is assumed due to the lack of additives in polymer compound.	*Bacillus, Micrococcus, Listeria, Staphylococcus, and Vibrio*	(Kumar *et al*., 2007)

5.	Polyethylene carry bags and cups	The consortium of bacterial and fungi in compost soil	Weight loss and reduction in tensile strength	There are two types of sources: First was naturally buried polyethylene carry bags and cups in the municipal compost yard of Kavali town. The second was the polyethylene strips that intentionally buried in compost soil with the municipal solid waste	The weight loss percentage HDPE was only 3.68%, while LDPE was 11.01% in the 12 months of incubation. The tensile strength was reduced by 18.48% for HDPE and 12.55% for LDPE.	Following were predominant fungi (*Aspergillus niger, A. ornatus, A. nidulans, A. cremeus, A. flavus, A. candidus and A. glaucus*) and bacteria (*Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Diplococcus* sp., *Micrococcus* sp., *Pseudomonas* sp. *and Moraxella* sp)	(Vijaya & Mallikarjuna Reddy, 2008)
6.	Polyethylene bag wastes (pure water sachets)	The consortium of bacteria and fungi species	Percentage of weight loss	Soil samples in the refuse dump	After 16 weeks of incubation and chemical treatment to polymer, it only resulted in a 1.98% weight loss.	*Pseudomonas aeruginosa, Pseudomonas putida, Bacillus subtilis and Aspergillus niger*	(Nwachukwu *et al.*, 2010)
7.	Plastic cups and polythene bags (both was in 1 cm diameter for the lab work)	Pure culture and consortium (*In-situ* method)	Biodegradation Analysis (Dry weight loss); Microbial Identification (Biochemical	The mangrove soil sample was from two zones along the Vellar estuary, India. First, colonized with	*Pseudomonas* and *Moraxella* sp. were the most effective bacteria for degrading 20.54% ± 0.13 of polythene and 8.16% ± 0.65 of plastic, respectively, in one month period.	**Gram-positive bacteria**: *Bacillus* sp., *Staphylococcus* sp., *Diplococcus* sp., and *Micrococcus* sp.; **Gram-negative bacteria:** *Moraxella* sp., and	(Kathiresan, 2003a)

			and Morphologic al); THB/THF (Colony Counting)	Rhizophora sp. and Vicennia sp. Along the Vellar estuary (11°29' N; 79°46' E; Southeast coast of India).	*Aspergillus glaucus* was more capable than *A. niger* in degrading 7.26% ± 0.51 of plastics, and 28.8% ± 2.40 of polythene. Few microbial species that unable to degrade plastic material (*Bacillus* sp, *Diplococcus* sp., *Aspergillus ornatus, A. cremeus, A. flavus, A. candidus, A. ochraceus, A. nidulans*). These microorganism were not selected for the lab biodegradation trial. The polyethylene bags start to shown the biodegradation result after 6 months of soil incubation, while plastic cup was showing a result after 9 months of soil incubation. Polyethylene degradation was tend to be faster than plastic degradation because polyethylene was 5x thinner than plastic.	*Pseudomonas* sp.; **Fungi**: *Aspergillus niger, A. candidus, A. ornatus, A. nidulans, A. cremeus, A. flavus, A. ocharaceus,* and *A. glaucus*	
8.	LDPE powder from Sigma Aldrich Chemical Co., polyethylene bags, and plastic cups (both was in	Pure culture population of actinomyce tes, bacteria, and fungi	Biodegradati on Analysis (Dry weight loss); Microbial Identification (Biochemical and Morphologic al);	Garbage soil samples (waste disposable site dumped with polythene bag and plastic cup) were collected from sidco, coimbatore,	The degradation of polyethylene bags is higher than in plastic cups. After six months the highest weight loss percentage achieved by Actinomycetes (*Streptomyces* KU8 in 46.16% ± 0.01) followed by bacteria (*Pseudomonas* sp. in 37.09 ± 0.01, and *Bacillus* sp. in 30.64% ± 0.08), then the fungi	*Streptomyces* KU8, *Streptomyces* KU5, *Streptomyces* KU1, *Streptomyces* KU6, *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus* sp., *Aspergillus nidulans* and *A. flavus*	(Usha et al., 2011a)

	1 cm diameter for the lab work)		THB/THF (Colony Counting	Tamil nadu. The soil sample were taken from the 3-5 cm depth and put inside sterile container and then air dried at room temperature.	(*Aspergillus flavus* in 20.96% ± 0.15)		
9.	LDPE carry bag strips and domestic waste mix with plastic (were cut in small strips but undefined size)	Single culture population	Biodegradation analysis (Biomass weight loss, estimation of total Carb and total protein in the culture supernatant); Stability of mutation gene (Gell electrophores is for DNA isolation, and Capillary gel electrophores is for DNA stability analysis)	It source from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh	The induce mutated *Pseudomonas putida* by UV and EMS successfully results in better response for degrading commercial or municipal dump yard due to strain improvement and longer duration. It also shows the better result on degrading plastic material compared to the domestic waste	*Pseudomonas putida*	(Talkad *et al*., 2014)

10.	LDPE films (2 x 2 cm with similar weight) and LDPE powder	Single culture population	Biodegradation analysis (Colonization test and Strum Test); Polyethylene Surface Observation (Light Microscope and SEM)	A soil sample from the municipal solid waste (MSW) landfill area, Pallikaranai, Chennai	Both fungi species can change the polymer structure such as cracks, the formation of pits, sporangia, and spores that grow on the LDPE film surface. The highest CO_2 production (5.9328 g/L) achieved by *Mucor circinelloides*	*Aspergillus flavus*, and *Mucor circinelloides*	(Pramila & Ramesh, 2011)
11.	LDPE films (undefined size but it must be similar weight) and LDPE powder	Single culture population	Bacteria Growth Rate; Biofilm formation ability (BATH test, Bacterial Biomass, Quantification of Biofilm); Biodegradation analysis (Strum Test); Bacteria Identification (16s rRNA sequencing)	A soil sample from the MSW landfill, Palikaranai, Chennai Tamil Nadu, South India	PL-4 isolate shows a low ability of biofilm formation but has the highest doubling time in just 28 minutes. Indicating the biofilm cannot be the only one indicator of the biodegradation rate. PL-2 and PL-3 isolate showed a tremendous result on the biodegradation rate based on the Sturm test result. Acinobacter baumannii (PL-2 and PL-3) was bacteria with the highest number of biodegradation rate according to a Strum Test result, they also the most hydrofob cells compared to two others.	*Brevibacillus parabrevis* (PL-1), *Acinetobacter baumannii* (PL-2, PL-3), *Pseudomonas citronellolis* (PL-4)	(Pramila *et al*., 2012)
12.	The antioxidant-free LDPE-F31N film	Single culture population	Biodegradation Analysis (FTIR, SEM observation);	Soil and LDPE sample from around Nogi Town, Tochigi	Bacteria No 14, 19, 20 are considered to be the highest degradation rate on LDPE film. The positive result of	*Bacillus circulans* (No. 14), *Bacillus brevies* (No. 19), *Bacillus*	(Watanabe et al., 2009a)

	were madeby Nippon Petrochemical Co., Ltd., (5400 in numerical average molecular weight, 8800 in weight average molecular weight). Then the films were crushed to be a powder by freeze shattering.		Bacteria Identification (Bergeys's manual of determinative bacteriology, morphology, and biochemical analysis)	Prefecture, Japan	biodegradation was examined by discerning of bacteria's body marks and traces, also bacteria cells on the LDPE surface, which *Bacillus circulans* had the most noticeable body marks. The data of FTIR shown as Infra red absorption range at some point of carbon chains which showed as a degradation result. The increase absorption of -OH in the 1080 cm^-1 area showed as a noticeable result of biodegradation	*sphaericus* (No. 20), and 17 others	
13.	LDPE mulch films, package films from Yakult, and random LDPE films	Soil consortium population	FTIR, biological optical microscope, and SEM observation	Nogi town, Tochigi prefecture (mulch film from cabbages field, Yakult packaging from house garden soil, and random films from disposal site area)	The result of lactophenol cotton blue staining found great activities from microorganisms by forming a black area around the cavities, indicates that microbes absorb the staining dye.	undefined	(Ohtake *et al.*, 1998)

14.	Pure LDPE film (Branched low-density (0.92 g cm)3 polyethylene (LDPE) with an average molecular weight of 191 000) and LDPE with a photosensitizer (LDPE-L0235). Both was cut in 3 x 3 cm size	Single culture population	Biodegradation Analysis (Dry Weight Loss, Reduction of Molecular Weight, BATH test, Biofilm Viability, Bacterial Biomass, FTIR-ATR); Microbes Identification (16S rDNA)	The soil in dumping site of the polyethylene production plant of Carmel Olefins, Haifa, Israel	The biodegradation rate was calculated by gravimetric and molecular weight loss, and the result was reduced by 11 and 30%, respectively. The treatment of UV irradiation gives the highest dry weight loss in LDPE-L0235 compared to LDPE (7.8 ± 0.8 % and 6.2 ± 0.3 %). The UV treatment to LDPE-L0235 increasing the biodegradation rate for 39% and when compared to the standard irradiated LDPE only 25% increase was found. Bacillus borstelensis strain 707 has the very low hydrophobicity, it causing the bacteria unable to form a strong bound to the LDPE surface by a biofilm formation. The combination of maximum time of UV radiation and the maximum time of incubation will resulting a maximal result in biodegradation of polyethylene (17% and 34% reduction in gravimetric and molecular weight respectively, which obtained from 120 h of UV irradiation and 90 days of incubation)	*Brevibacillus borstelensis* strain 707	(Hadad et al., 2005b)
15.	Pure LDPE film (Branched low-density	Single culture population	Evaluation of bacterial hydrophobicity (BATH	Soil samples from 15 sites which polyethylene	The bacteria can degrade LDPE up to 8% in just four weeks, and with the addition of mineral oil to the growth, the medium was able	*Rhodococcus ruber* isolate C208	(Gilan *et al.*, 2004)

	(0.92 g cm)3 polyethylene (LDPE) with an average molecular weight of 191 000) and LDPE with a photosensitizer (LDPE-L0235). Both was cut in 3 x 3 cm size		and SAT); Identification of strain C208 (16S rDNA analysis); Biofilm analysis (FDA and SEM); Changes of polyethylene structure (Attenuated Total Reflectance (ATR)-FTIR), Dry weight loss	waste from agricultural use had been buried	to improve the degradation of LDPE by about 50% in 4 weeks of incubation. UV + C208 reduced the carbonyl index by 66% and reduced the terminal double bond indexed about 20%, when compared to UV untreated with C208. Maximal result of weight loss achieved by the mineral-oil-amended medium (50% more than mineral oil free medium) at concentration of 0.05% of mineral oil. Higher concntration of mineral oil had smaller effect on the biodegradability of the polymer		
16.	6% starch-polyethylene-prooxidant degradable plastic	No microbial population	FTIR, tensile strength, percent elongation, strain energy, molecular weight, and number of molecules per sample	The known culture was used, but the source was not mentioned	Those bacteria enzymes performed the veatryl alcohol lignin peroxidase activity. The results are a significant reduction of tensile strength, percent elongation, and strain energy achieved by an active enzyme from *S. setonii* 75Vi2. In contrast with that, only *S. setonii* 75Vi2 that not perform the changes in molecular weight and number of molecules per sample.	An inactive and active extracellular enzyme from *Streptomyces viridosporus* T7A, *S. badius* 252, and *S. setonii* 75Vi2	(Pometto *et al.*, 1992)

17.	Degradable polyethylene (DPE) (degradable by UV and oxidation, PDQ^{TM6}, Willow ridge Plastics, USA)	Single culture and consortium	Weight loss, and GC	Soil sample that was used to buried the polyethylene samples for 2–4 years	The biofilm formation reduced the weight up to 7%, the *P. frequentans* only reduced 0.45–0.50% with or without preheated treatment, and *B. mycoides* only reduced by 0.01%	*Penicillium frequentants*, and *Bacillus mycoides*	(Seneviratne *et al*., 2006)
18.	HDPE and LDPE films contained iron photo-inducer and phenolic antioxidant	Single culture population of bacteria and fungi	ATP, ADP assays, size exclusion chromatography (SEC), optical microscopy and SEM observation, NMR spectrophotometry	Microbes isolate was purchased from American Type Culture Collection, except for *Nocardia asteroids* LAB 911	The highest ability to perform biofilm formation on the polyethylene surfaces was achieved by *R. rhodochorus* and *N. asteroides*	*Rhodococcus rhodochorus* ATC 29672, *Aspergillus flavus* ATCC 26873, *Mortierella alpine* ATCC 36965, *Cladosporium cladosporoides* ATCC 20251, and *Nocardia asteroids* LAB 911	(Koutny *et al*., 2006)
19.	UV treated and non UV treated LDPE film	Consortium population	Sturm test, microbial biomass, microbial count, soil pH measurement, FTIR, X-ray diffraction (XRD)	Soil samples from 11 location taken randomly from landfill which PE wastes had been buried	The presence of selected microorganism inside the soil incubation was more efficient to the biodegradation rate of 29.5% and 15.8% for UV treated, and non UV treated LDPE respectively	*Lysinibacillus xylanilyticus*, and *Aspergillus niger*	(Esmaeili *et al*., 2013)

			analysis, SEM observation				
20.	Pre-treated LDPE by thermal treatment (TT 105^{o}C and 150^{o}C) and accelerated aging treatment (AAT)	Consortium population of four filamentous fungi	Differential scanning calorimetry (DSC), wide-angle X-ray scattering (WAXS), FTIR, SEM observation, GC	The fungi isolate obtained from the American Type Culture Collection	The AAT to LDPE enhances better than TT to LDPE, which can be observed from the result of CO_2 production and the hyphae penetration to the LDPE.	*Aspergillus niger* ATCC 9642, *Gliocladium virens* ATCC 9645, *Penicillium pinophilum* ATCC 11,797, and *Phanerochaete chrysosporium* H289	(Manzur *et al*., 2004)

3.1. Biodegradation Analysis

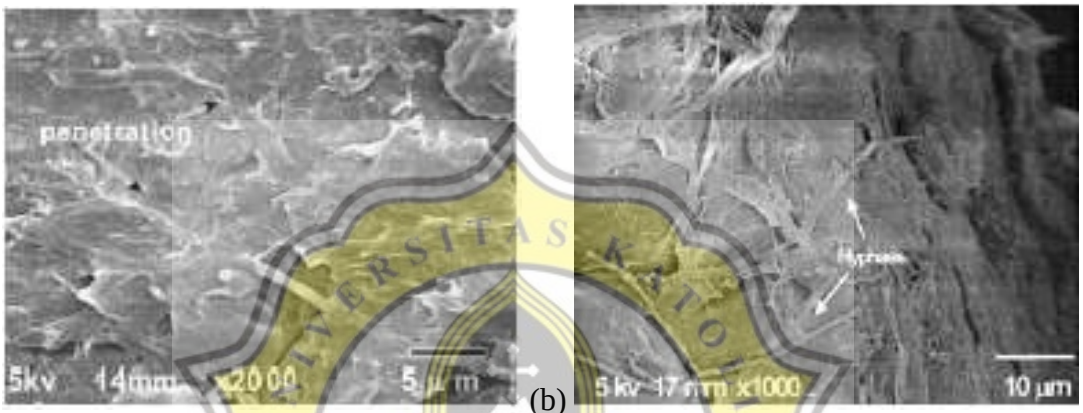
To make a fair comparison of the biodegradation rate among microorganisms that are used for polyethylene biodegradation, it needs to use the same environmental condition and control any factor that might be participating in the biodegradation process. Also, the use of specific microorganisms that able to degrade a specific material will enhance the development of the biofilm on the material surfaces (Gu, 2003). According to table 1, the comparison of effectivity among microorganisms cannot be made because of different conditions between each research that affect the result.

According to Priyanka & Archana (2011[a]), *Aspergillus niger* has the highest weight loss (12.5%) among the other fungi isolates (*A. nidulance* (10%), *A. flavus* (6.81%), *A. glaucus* (8.8%), *Penicillium* (9.3%)) in one month of incubation. Another research from Kathiresan (2003) found out that *Aspergillus glaucus* (28.8% ± 2.40) was more capable than *Aspergillus niger* (17.35% ± 2.00) in degrading polyethylene in one month of incubation. Both of these research were used *A. niger* and *A. glaucus* for polyethylene biodegradation, but both of them also give a different result from the biodegradation process, even though they used the same species. This evidence means that it is not valid to compare one research into another because of the different conditions and factors that might affect the result of weight loss or biodegradation. The study to find suitable microorganisms for polyethylene biodegradation can be done by comparing the characteristic of each microorganism genera that is used in the biodegradation process.

3.2. Biofilm as an Important Factor of Biodegradation

The essential factors that affect the efficiency of biodegradation are the ability of microorganisms to attach on the polyethylene surfaces or create a biofilm so that they can penetrate and release the extracellular enzyme. The higher ability to penetrate or to perform biofilm, then it will be more efficient in the degradation process. Fungal species are considered to be suitable microorganisms to degrade polyethylene (LDPE) because of their ability to create hydrophobic proteins to attach into polymer surface (Kershaw & Talbot, 1998). Other benefits of using fungal species for polyethylene degradation are the

enzymes from fungi are suitable for the insoluble characteristic of LDPE (Shah *et al*., 2008). Then, the growth of fungal species are faster than bacteria species; the ability to penetrate the polymer surface by the extension of the hyphae and fungi are compatible in extreme growth condition such as low pH, low nutrients, and low moisture (Kim & Rhee, 2003). The research from Manzur *et al*., (2004) in Figure 4, also showing the ability of fungi to penetrate LDPE by using the hyphae; (a) AAT treatment showing better penetration of hyphae, and the cavities on the surface are observed, (b) TT/150^{o}C treatment showing hyphae growth on the LDPE surface.

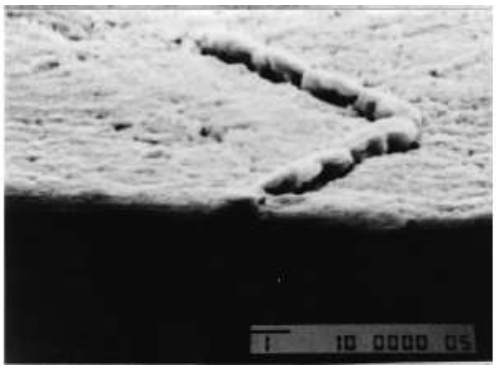
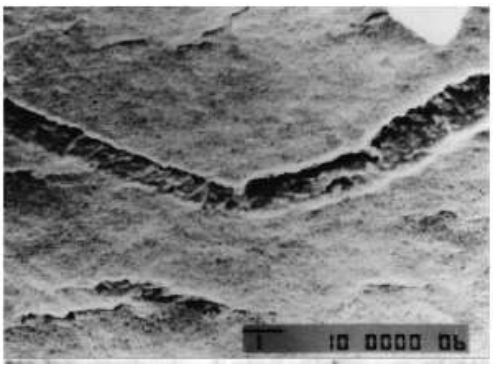


(a) (b)

Figure 18. The SEM observation of LDPE. (a) AAT treatment showing better penetration of hyphae, and the cavities on the surface are observed, (b) TT/150^{o}C treatment showing hyphae growth on the LDPE surface (Manzur *et al*., 2004).

Bacteria species often perform a biofilm to attach to the polyethylene surface and secreting the extracellular enzyme. Once the microorganisms get attached to the polymer surface, it starts performing the biofilm and secreted the extracellular enzyme that helps them to break down the main chain and leads to the formation of low molecular weight fragments. This fragments would be utilized by the microbes as a carbon and energy sources (Usha et al., 2011^{b}). As shown in Figures 5 and 6 shows the forming of body marks was because of the enzyme degradation when the bacteria cells attached to the LDPE film surfaces. The evidence of the microbes can attach to the plastic surfaces and the body marks of bacteria cells that were showing a positive biodegradation process (Watanabe et al., 2009a). The research was used polyethylene sample (pure LDPE-F31N films) formed by the inflation process and without any antioxidant addition. Then, Figure 7 shows the bigger images on the *Pseudomonas aeruginosa* PAO1, *P. aeruginosa* ATCC, *P. putida*, and *P. syringae* biofilm that performs on the polyethylene film surface. Figure

8 shows a result of this biofilm formation on the polyethylene surface; there are several changes on the surface like cavities, pits, and body marks.

(a)  (b) 

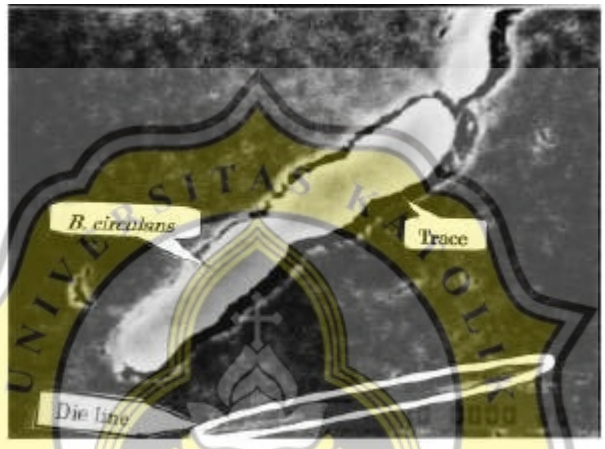
(c) 

Figure 19. SEM images on the LDPE film surface showing a positive biodegradation process by Bacillus circulans (Isolate no. 14). (a) The images of bacteria cells are attached to the LDPE film surface. (b) The body marks left behind after the cell cleaning process. (c) Traces of the biodegradation process around the bacteria cells and extruder die line (Watanabe et al., 2009a).

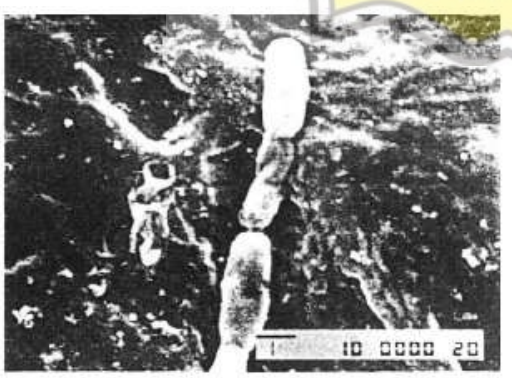
(a)  (b) 

Figure 20. SEM images on the LDPE powder by microbes from the soil in the refuse dump.
(a) Bacteria cells are attached to the LDPE powder before the cell cleaning process. (b) Body marks on the LDPE powder after the cleaning process (Watanabe et al., 2009a).

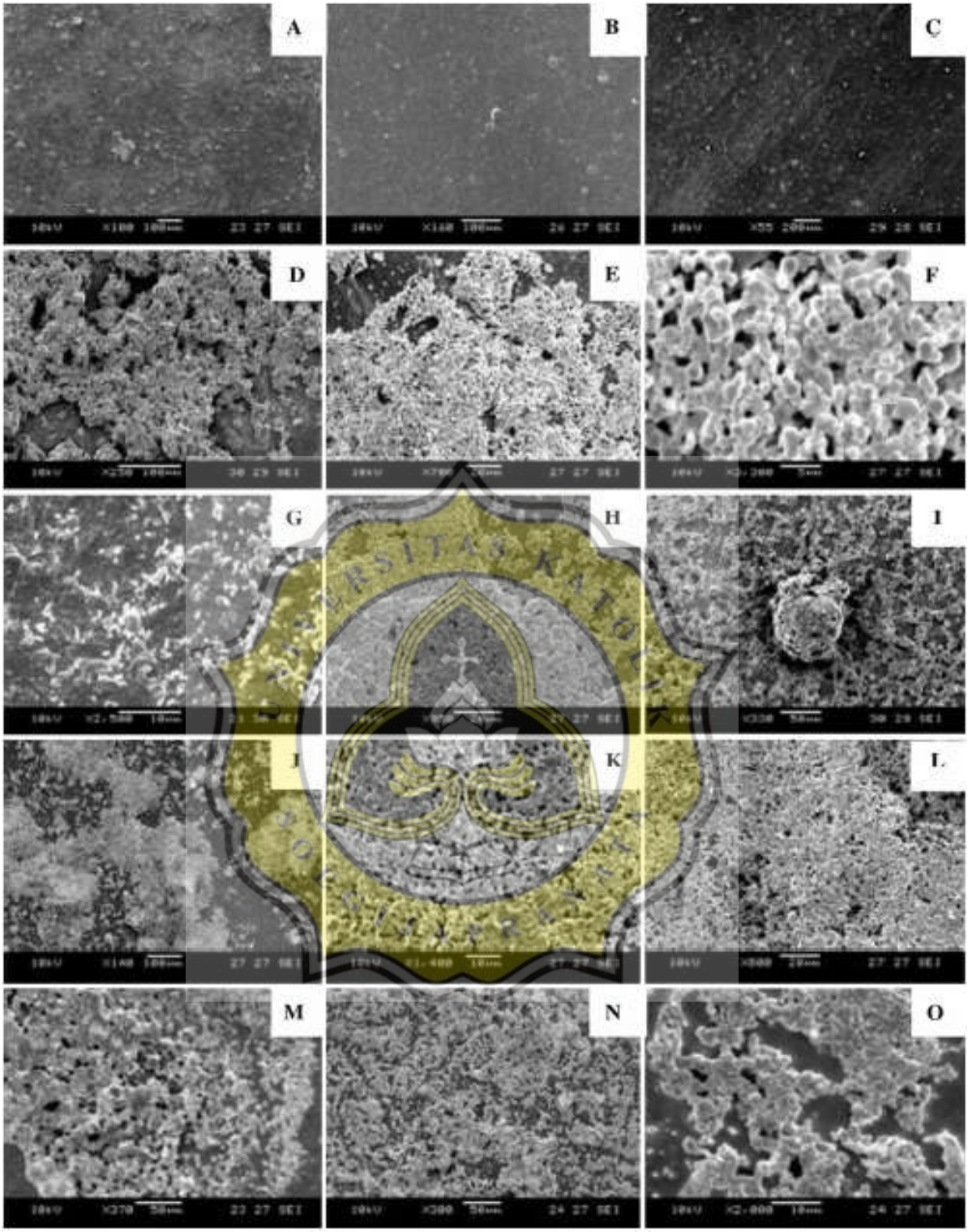


Figure 21. SEM observation on the biofilm formation. (a-c) absence of biofilm formation in control, (d-f) biofilm formation of *Pseudomonas aeruginosa* PAO1, (g-i) *Pseudomonas aeruginosa* ATCC, (j-l) *Pseudomonas putida*, (m-o) *Pseudomonas syringae* after incubation in 40, 80, and 120 days respectively (Kyaw *et al*., 2012).

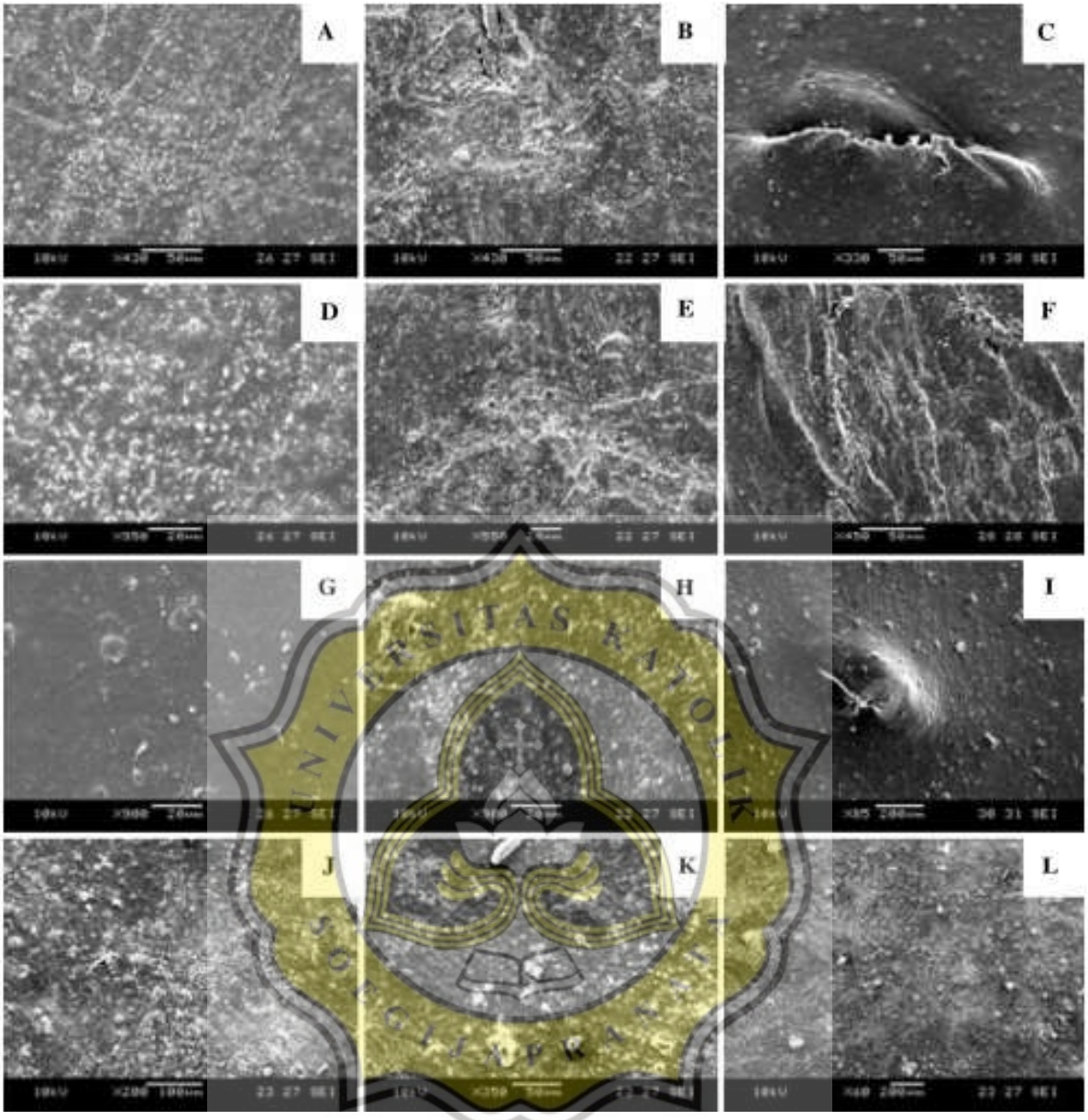


Figure 22. SEM observation on the surface changes of polyethylene film (after washing the biofilm layer with 2% SDS). (a-c) after incubation with *Pseudomonas aeruginosa* PAO1, (d-f) *Pseudomonas aeruginosa* ATCC, (g-i) *Pseudomonas putida*, (j-l) *Pseudomonas syringae* after incubation in 40, 80, and 120 days respectively (Kyaw *et al*., 2012).

Pseudomonas spp. have a slower ability on biofilm and planktonic cell formation when compared to other microorganisms in the biodegradation process (Kyaw *et al*., 2012; Tolker-Nielsen *et al*., 2000). Actinomycetes are the most well-distributed microorganisms in the nature that inhabit the soil, and most of them are well known for the capability to degrade plastic materials (Usha et al., 2011a). The research from

Esmaeili *et al*. (2013) in Figure 9 and Figure 10, showing the SEM observation on fungi and bacteria biofilm that attach to the polyethylene surface.

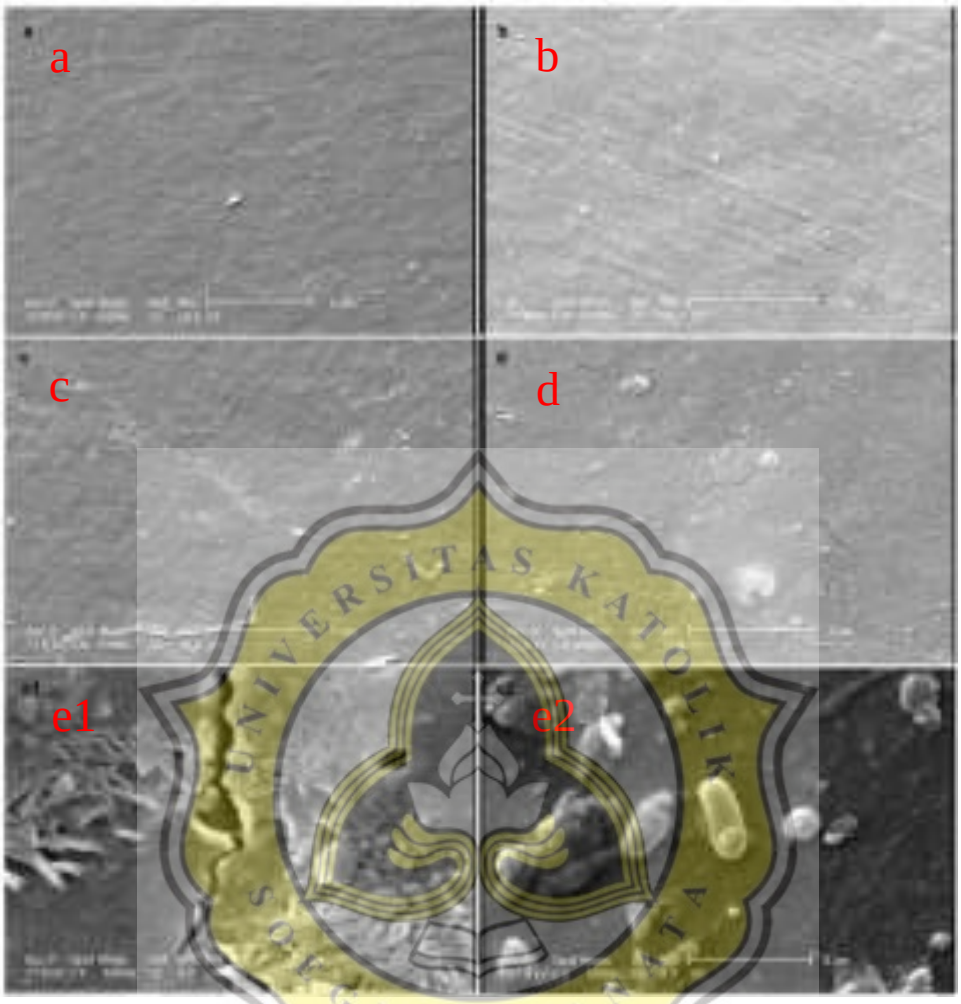


Figure 23.The SEM observation on pure LDPE films before and after 126 days of incubation in soil by different treatments. (a) Blank LDPE film (no UV treated and incubation), (b) UV treated LDPE film without incubation, (c) non-UV treated LDPE incubated in soil without specific microorganism, (d) UV treated LDPE incubated in soil without specific microorganism. (e) Non-UV treated LDPE incubated in soil with specific microorganisms: (e1) penetration of hyphae into LDPE matrix; (e2) biofilm formation on LDPE surface; (e3 and e4) pits and cavities are observed on the LDPE surface (Esmaeili *et al*., 2013).

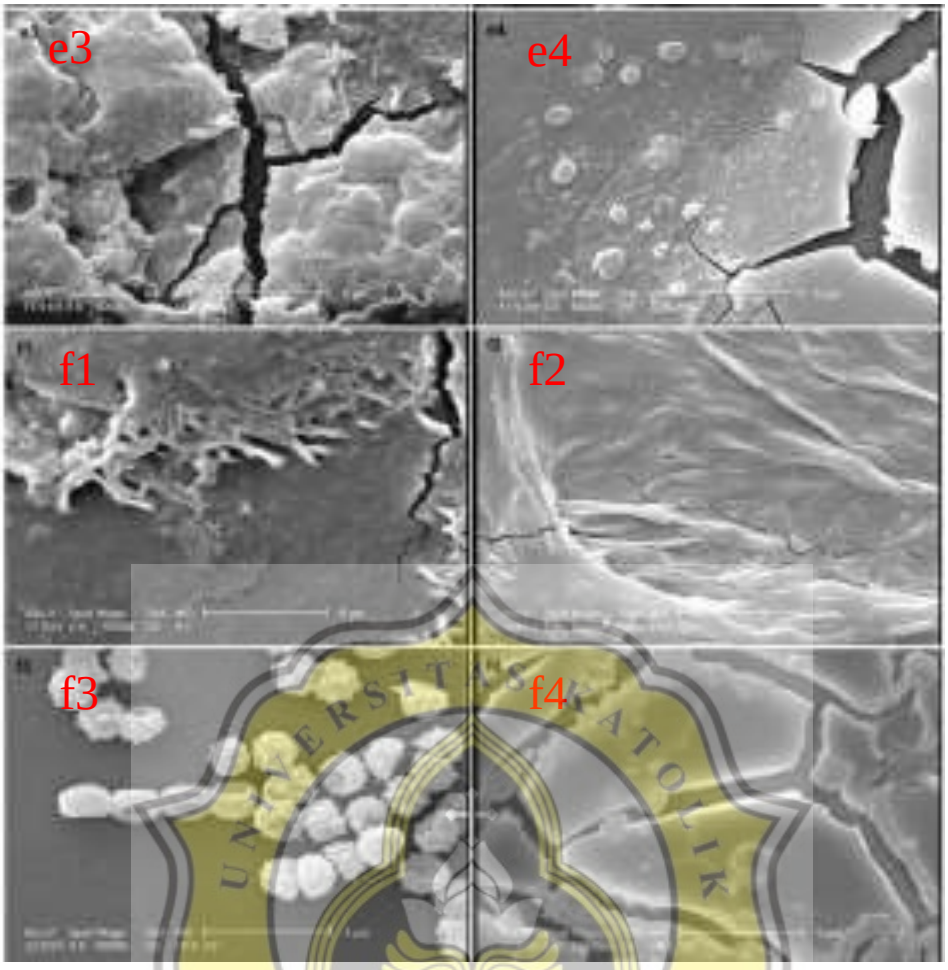


Figure 24.The SEM observation on pure LDPE films before and after 126 days of incubation in soil by different treatments. (f) UV treated LDPE incubated in soil with specific microorganisms: (f1 and f2) penetration of hyphae on the LDPE matrix; (f3) bacterial biofilm formation on the LDPE surface; (f4) pits and cavities are observed (Esmaeili *et al*., 2013).

According to the SEM evidence, fungi will be effective in creating cavities and pits compared to bacteria biofilm because of the penetration ability of fungi to the polyethylene matrix. In contrast, bacteria cells are only able to perform a biofilm on the surface. Amazingly, the research from (Seneviratne *et al*., 2006) found that the biofilm formation of consortium contains the right combination between fungi and bacteria species that can degrade polyethylene faster. The biofilm contains *Penicillium frequentans* and *Bacillus mycoides* perform more significant weight loss (7%) compare to single culture application (0.45 – 0.5% and 0.01% respectively) in the biodegradation process (Figure 11).

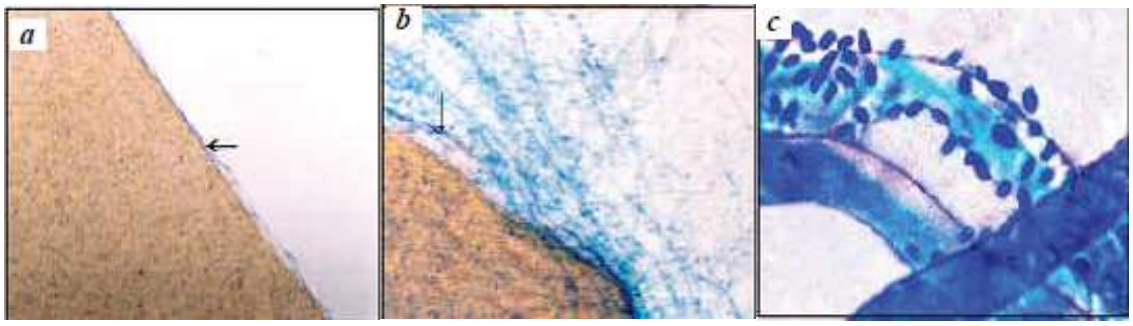


Figure 25. Light microscope observation on *Penicillium-Bacillus* biofilm. (a) The edge mark with an arrow is a piece of degradable polyethylene, (b) colonization of *Penicillium frequentans* on the plastic surface, (c) biofilm formation of fungal filaments by *Bacillus mycoides* (Seneviratne *et al*., 2006).

In this research, *P. frequentans* perform a network of mycelium on the polyethylene surface, then colonized by *Bacillus mycoides*. One type of enzyme that able to degrade by oxidase hydrocarbon chain is alkane monooxygenases, and it can be found in *Bacillus* spp. The mechanism of this fungi-bacteria consortium is by initiate the attachment to the polyethylene surface by filamentous fungi that capable of attaching on the hydrophobic surface by the formation of hydrophobic proteins. Then, the growth and penetration of hyphae will help to transport bacterium into the inside of the polyethylene matrix. This application of fungal-bacterial biofilms will effectively increase the biodegradation rate because the enzymatic reaction from bacteria can be done not only on the polyethylene surface (Seneviratne *et al*., 2006).

3.3. Growth Media, Nutrition, Environmental Factor, and Incubation Time as Supporting Factor in Biodegradation Process

The stability of environmental condition also play as a key role in the biodegradation process. The research from Kathiresan, (2003b) and Priyanka & Archana, (2011b) has been revealed that the biodegradation process with controlled environment condition in laboratory will perform a higher result in biodegradation compared to the biodegradation process that occur in nature environment. Growth media that contain the right amount of inorganic salts and trace elements will perform a better result in the biodegradation process. Most of the growth media that used in the plastic biodegradation test at laboratory only contain of trace elements and essential inorganic salts to support the growth of microorganisms, and use the polyethylene as a sole carbon source. The research from

Watanabe et al., (2009b) showed that change the liquid medium in every 2 weeks along the incubation period was able to prevent the weakened of bacteria's degradative ability, which usually happen because the lack of inorganic salts at the middle to the last period of incubation. Another research from Hadad et al., (2005a) using mannitol and potassium nitrate in their growth medium to check the biodegradation ability of *Bacillus borstelensis* strain 707 if the extra carbon source was added to the medium. The result was shown that *B. borstelensis* was able to biodegrade polyethylene even in the presence of other carbon source like mannitol.

The range of incubation time in the right environmental condition will affect to the biodegradation process result. Hadad et al., (2005a) showed a result that the combination of maximum time of UV radiation and the maximum time of incubation will resulting a maximal result in biodegradation of polyethylene (17% and 34% reduction in gravimetric and molecular weight respectively, which obtained from 120 hours of UV irradiation and 90 days of incubation). This high result of biodegradation was able to achieve because the biodegradation process has been done in the controlled environment. The biodegradation process that happen in nature environment will produce a small result even in the long time period of incubation. The research from Kathiresan, (2003a) which perform in the laboratory and natural environmental condition (soil burial in 2 mangrove zone), shown that the biodegradation of polyethylene was start after 6 (1.98 %) and 9 months (4.21 %) of soil incubation and the biodegradation of plastic was start only after 9 months of soil incubation (0.25 %). Besides of that, the result from laboratory incubation showed a high result in short period of incubation, *Pseudomonas* and *Moraxella* sp. were the most effective bacteria for degrading 20.54% ± 0.13 of polythene and 8.16% ± 0.65 of plastic, respectively, in one month period. *Aspergillus glaucus* was more capable than *A. niger* in degrading 7.26% ± 0.51 of plastics, and 28.8% ± 2.40 of polythene. Laboratory condition and the use of pure culture was more effective for biodegradation than In-situ in the mangrove soil population.