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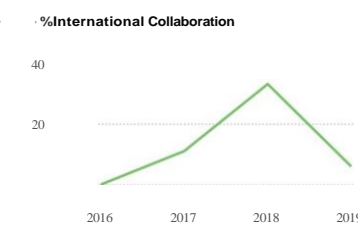
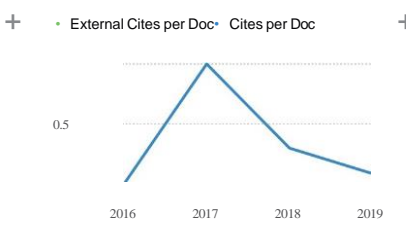
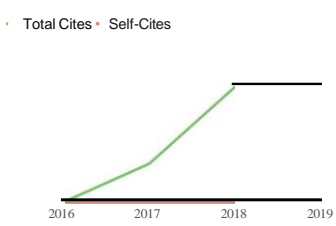
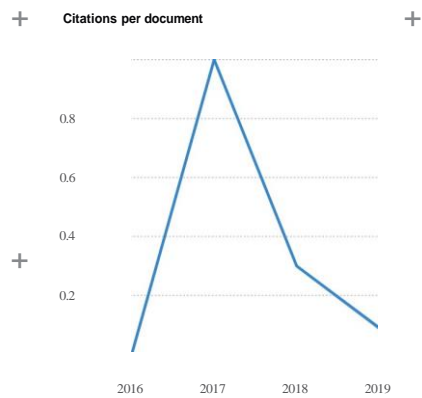
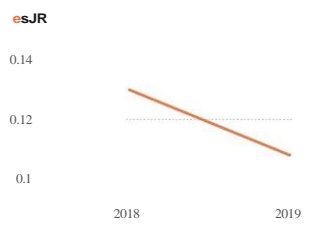
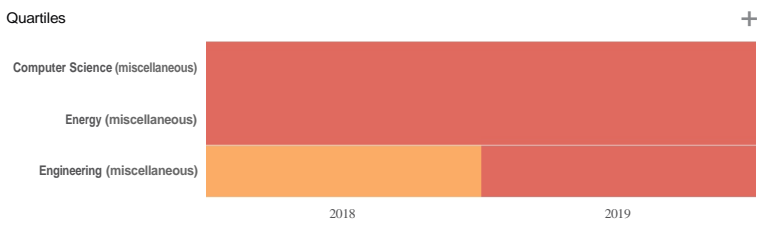
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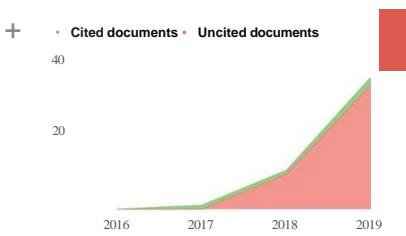
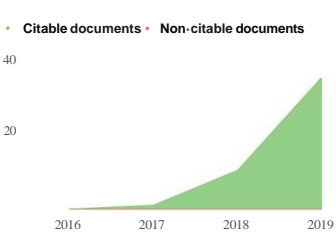
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Potential of Linear Low-Density Polyethylene (LLDPE) Plastic Degradation by Mixed-Cultured Bacteria in Controlled Environment

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Abstract

This preliminary study was aimed to determine the ability of mixed-cultured Pseudomonas aeruginosa and Brevibacterium sp. in degrading Linear Low-Density Polyethylene (LLDPE). This study was started by preparing the plastics into 1x1 cm² cut mechanically. The plastics were placed in a petri dish containing Nutrient Broth (NB) media and mixed-cultured bacteria. The variations of bacterial composition (%) were 10, 50, and 75; variations of temperature (°C) were 25, 30, 35; variations of acidity (pH) were 5, 7, 9; and variations of contact time (Td; days) were 10, 30, 60, and 90. LLDPE plastic degradation was analyzed using gravimetry, Fourier Transform Infra-red (FTIR) and Scanning Electron Microscope (SEM). Both bacteria were resistance or non-sensitive to LLDPE substance as xenobiotic substrate. According to gravimetry and FTIR analysis, the highest removal was at 30°C, pH 7, with bacterial composition of 75% (v/v) incubated for 30 days. Gravimetry analysis result showed a loss of LLDPE plastic weight from 0.1548 gram to 0.1464 gram, or 70.67%. The result was reconfirmed using SEM analysis, which showed morphological changes of LLDPE plastic sample surface. Although the degradation occurred very slow, mixed-cultured bacteria has a potential for capacity increase in degrading LLDPE in further studies, including the addition of co-substrate in continuous culture.

Keywords: *brevibacterium sp., pseudomonas aeruginosa, linear low-density polyethylene, xenobiotic, FTIR*

1. Introduction

Plastic is deliberately produced to be impenetrable by oxygen (O₂), thus requires longer time to decompose or degrade naturally by microorganisms involved in decomposition. Other than, plastic has several advantages, i.e. anti-rust, flexible, strong, light, not easily broken, easy to color, resistance to various chemical substances, insulation against electricity, and low density. One common type of plastic is ethylene monomer long chain polyethylene polymers (C₂H₄) [1], [2]. Other than light, transparent, and economical, polyethylene (PE) plastic has resistance property, thus difficult to degrade naturally, which lead to accumulation in the environment [3].

Plastics buried in soil will hinder or inhibit the process of water absorption by plants, thus interrupting photosynthesis process. The life of animals in the soil is disrupted and even causes death due to entrapment in plastic waste or due to ingestion of plastic waste. These animals are vital components in material and energy flow in the soil, leading to loss of nutrients in agricultural lands [3]. Plastic waste in water can also jeopardize the marine ecosystem, especially affecting food chain in the waters.

Polyethylene can be degraded by UV light at 280-300 nm wavelength for 10 days to form hydroperoxide and disconnect polyethylene long chain to form shorter fragments known as monomers [4]. However, degradation process using UV light has negative

effect, i.e. the greenhouse effect. Burning plastic waste will also cause negative effect, in which air pollution caused by gasses resulting from burning plastic, including Carbon Dioxide (CO₂) and Carbon Monoxide (CO). Therefore, a solution is needed to process plastic waste without endangering environment.

Other alternative that has been developed in the last 10 years is biotechnological plastic degradation by utilizing microorganisms. Biodegradation process occurs because complex substances are used by microorganisms as nutrient source or energy source for their growth by utilizing enzymes to breakdown complex substances into more simple substances [5], [6]. According to [7], *Microbacterium paraoxydans* and *Pseudomonas aeruginosa* was known to have the ability to breakdown polymers efficiently with removal percentage of 61.0%, while *P. aeruginosa* in growth media can degrade LDPE plastic with 50.5% efficiency in 2 months. Polymers were broken down into smaller polymers by *M. paraoxydans* and *P. aeruginosa* used as carbon source. This degradation process is started by the formation of a biofilm in the polymer surface. The biofilm contains microorganism cells which creates a layer in hydrated matrix from polysaccharides and proteins on the surface of the plastic [8]. Based on [5], there was a decrease of plastic weight incubated in media for 120 days and increase of biofilm cells for the first 40 days of incubation.

Each microorganism has different characteristics, thus the degradation process of one microorganism would be different or varies from other microorganisms. Based on this description, this study is required to test the ability of mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* As biodegradator in the process of LLDPE plastic processing in controlled laboratory scale.

2. Methodology

2.1. Bacteria Cultivation and Preparation of Plastic Samples

The mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* was obtained from the collection of Microbiology Laboratory of Environmental Engineering, Universitas Trisakti. Cultivation of mixed-cultured bacteria was conducted in batch culture in Erlenmeyer flask aerobically, which contained growth media, nutrient broth (NB). Bacterial growth was observed every day. After reaching exponential phase, the mixed culture bacteria were ready to be tested. LLDPE samples were cut mechanically to form 1x1 cm², the smaller the plastic, the higher the surface area, thus higher chance for effective contact with degradation bacteria.

2.2. Sensitivity Test

Before the main study, sensitivity test was conducted to determine the vulnerability of *Pseudomonas aeruginosa* and *Brevibacterium sp.* on LLDPE sample as xenobiotic substance which can inhibit growth or kill bacteria in certain concentration [9]. LLDPE sample was placed in the center of a petri dish containing mixed-cultured bacteria and growth media. Inhibition zone formation surrounding plastic sample showed inhibition of culture growth, thus cannot be used as biodegradator and vice versa.

2.3. Research Design

The dependent variable in this study was linear low-density polyethylene (LLDPE) plastic samples, while the independent variables were composition of mixed-cultured bacteria (%; v/v), temperature (°C), acidity (pH), and contact time (Td; days). Variations in the composition of mixed-cultured bacteria were 10%, 50%, and 75% of total solution volume. Optimum temperature and pH were

determined by variations of tropical temperature (°C) which were 25, 30, and 35 and with pH variations of 5, 7, and 9 which represented acid, neutral, and alkaline. This study was conducted by placing 1% (w/v) LLDPE sample in testing reactor containing growth media from 10%, 50%, 75% (v/v) mixed-cultured bacteria. This study was conducted for 90 days with 3 times repetition performed on contact time (Td; days) 10, 30, 60, and 90. Afterwards, gravimetry, Fourier Transform Infra-red (FTIR) and Scanning Electron Microscope (SEM) analyses were conducted.

2.4. Gravimetric Analysis

Gravimetry was used to determine the percentage of LLDPE plastic sample weight loss. Gravimetry is a quantitative chemical analysis based on the principle of plastic sample measurement before and after degradation. Therefore, the percentage of degraded plastic weight loss can be determined. According to [9], the percentage of degraded plastic weight loss can be determined by the following formula:

$$\% \text{ weight loss} = \frac{W(a)-W(b)}{W(a)} \times 100\% \quad (1)$$

W(a): plastic weight before degradation

W(b): plastic weight after degradation

2.5. Fourier Transform Infra-red (FTIR) Analysis

Plastic content analysis using Fourier Transform Infra-red (FTIR) based on infra-red spectrum was conducted to determine changes in functional groups of LLDPE sample before and after contact to mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* LLDPE Biodegradation can be confirmed within 2500-3000 cm⁻¹ wavenumber, which is the typical peak of PE substance [7].

2.6. Scanning Electron Microscope (SEM) Analysis

This analysis was conducted to determine morphological structure and shape of LLDPE sample surface before and after incubation with mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* The morphology of LLDPE sample which completed biodegradation process by bacterial activity was observed with SEM using 10-3,000,000 magnification, 4-0.4 mm depth of field and 1-10 nm resolution.

3. Results and Discussion

The growth of mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* in liquid media Nutrient Broth (NB) containing LLDPE sample was shown by changes in color. Color changes occurred gradually from transparent to cloudy yellow observed on Day-30, Day-60, and Day 90 showed increased bacterial concentration of *Pseudomonas aeruginosa* and *Brevibacterium sp.* in liquid media Nutrient Broth (NB) (Figure 1).

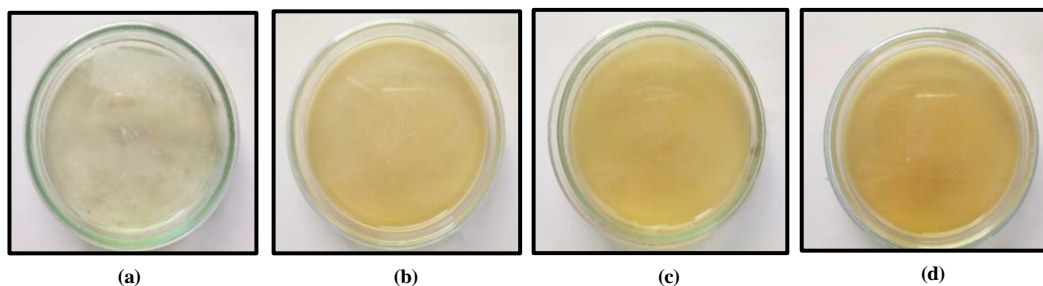


Figure 1. The growth of *Pseudomonas aeruginosa* and *Brevibacterium sp.* surrounding LLDPE sample (a) 0 day, (b) 30 days, (c) 60 days, and (d) 90 days

These color changes revealed that the growth activity of mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* was not disturbed by LLDPE plastic sample with xenobiotic property. These bacteria were suspected to grow by using carbon substance (C) as a nutrient source in growth media contained in LLDPE sample.

The result of sensitivity test showed that there was no inhibition zone surrounding LLDPE sample, thus mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* can be considered resistant or non-sensitive to toxic xenobiotic substance (Figure 2). Therefore, these mixed-cultured bacteria were suspected to have the ability to degrade or decompose LLDPE plastic.

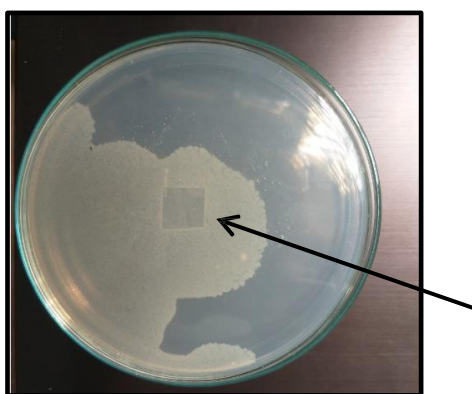


Figure 2. Mixed-cultured bacteria can grow well on Day-48 shown by no inhibition zone surrounding LLDPE sample

3.1. Reduced Weight of LLDPE Sample

Removal efficiency according to reduced weight of LLDPE sample on variations of temperature and pH, and addition of 10%, 50%, and 75% (v/v) mixed-cultured bacteria to a media containing 1% (w/v) LLDPE sample for 30 days of study can be seen in Figure 3. Based on the figure, the addition of 75% (v/v) mixed-cultured bacteria provided removal efficiency of 5.47% at 30°C and pH 7 for 30 days of incubation, proven by reduced sample size from 0.1548 gram to 0.1464 gram. This result showed that LLDPE polymer degradation occurs very slowly. Furthermore, more biodegradator causes more reduction of LLDPE sample weight. Biodegradation process occurs from the breakdown of polymer into monomers or smaller components, then these monomers are mineralized through the process of organic substance change to inorganic. Polymers that are too large to pass through cell membrane should be polymerized to smaller monomers before absorbed and degraded in microbial cells [10], [11]. The study was continued until Day-90, where it is expected to give higher removal result (degradation).

Figure 4 depicted removal efficiency (%; v/v) according to reduced LLDPE sample weight for 90 days at 30°C and pH 7 with the addition of 75% (v/v) mixed-cultured bacteria. Removal efficiency kept increasing to 5.47% on Day-30, however reduced after Day-30 to the end of the study on Day-90. Increased removal efficiency occurred because mixed-culture bacteria performed optimum growth activity or in exponential phase. According to Maier (2008), this phase is marked by fast bacterial growth in current condition. This proved that although degradation

process had occurred by mixed-cultured bacteria as biodegradator by utilizing carbon substance in LLDPE as a nutrient source, the process occurs very slowly. These results were supported by the result of a study [5] which stated that there was a reduced plastic sample weight of 20% incubated in media for 120 days by *Pseudomonas aeruginosa*. Plastic biodegradation process which utilizes microorganism activity is a very slow process, and certain microorganisms are unable to decompose plastic because they do not have enzyme suitable to the substrate or plastic which will be degraded [1], [12].

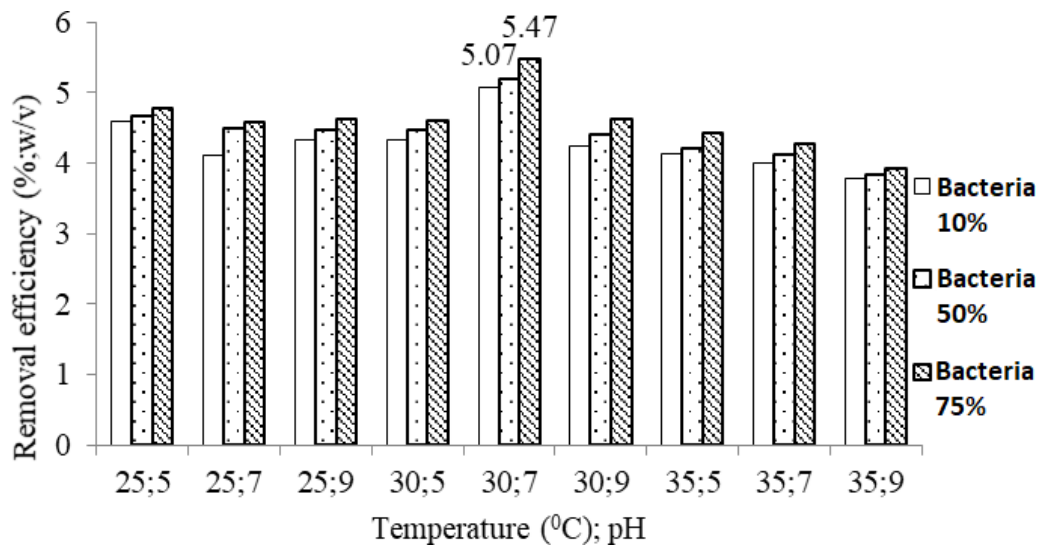


Figure 3. Removal Efficiency (% w/v) on Day-30 on Variations of Temperature and pH with Addition of 10%, 50%, and 75% (v/v) Mixed-Culture Bacteria

Low plastic removal for 30 days at 30°C and pH 7 may also be due to the enzyme contained by mixed-cultured bacteria did not act properly. Enzyme work is highly influenced by environmental condition, which include temperature, pH, oxygen availability, nutrient availability with correct carbon to nitrogen to phosphor ratio (C:N:P). Other than these factors, in order to improve enzyme activity in accelerating LLDPE degradation process, enzyme requires chemical substances in the form of molecules or ions which can improve the biological activity of enzyme, known as co-substrate.

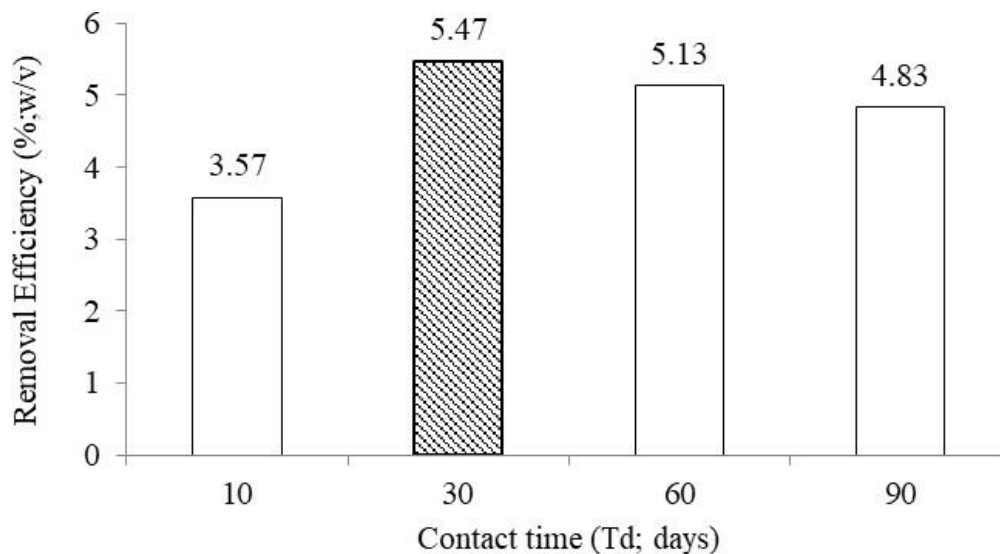


Figure 4. Removal Efficiency (%; w/v) until Day-90

The most commonly used co-substrate is starch. Starch is often used as plastic making material with biodegradable property because starch has several advantages, including abundant amount in the environment, can hold oxygen well, and affordable [11]. The mixture of hydrocarbon polymer and starch is often used to produce high quality plastic film and layers. More starch used in plastic making, the higher the biodegradability. According to [1], [14], biodegradation of a polymer is also affected by physical and chemical characteristics, including the availability of functional group which increases hydrophobic (degradation process in hydrophilic is faster compared to hydrophobic), molecular weight, molecular composition, physical shape and properties of polymer, and polymer density.

3.2. Fourier Transform Infra-red (FTIR) Analysis

Weight loss according to the composition of mixed-cultured bacteria in gravimetry analysis was confirmed by FTIR analysis. The intensity of functional group of LLDPE sample that did not given mixed-cultured bacteria addition on t0 or as baseline intensity was 0.098 on 2916.37 cm⁻¹ wavenumber. Observation to Day-90 showed that the intensity stayed at 0.098 on 2916.37 cm⁻¹ wavenumber. This showed that there were no changes in functional group intensity in LLDPE plastic without the existence of mixed-cultured bacteria.

The results of FTIR analysis on variations of temperature, pH and addition of mixed-cultured bacteria for 30 days of incubation showed changes of reduced intensity of LLDPE sample functional group on every treatment (Figure 5).

The highest intensity reduction occurred in 75% (v/v) mixed-cultured bacteria addition, at 30°C and pH 7. The intensity changed from 0.098 to 0.028, which obtained 70.67% efficiency of functional group intensity. This proved that a condition 30°C and pH 7 was suitable for the growth of mixed-cultured bacteria, which lead to a more optimal plastic degradation. This study also proved that these mixed-cultured bacteria were included in mesophilic group, which can grow well within 20-45°C and pH range of 5.5-8.0. Greater number of bacteria used as biodegradator also affected degradation process. More bacteria mean more enzyme produced to catalyze more sample, which lead to better degradation. The study was continued until Day-90 with the addition of 75% (v/v) mixed-cultured bacteria at

30°C and pH 7, with the expectation of higher efficiency of functional group intensity.

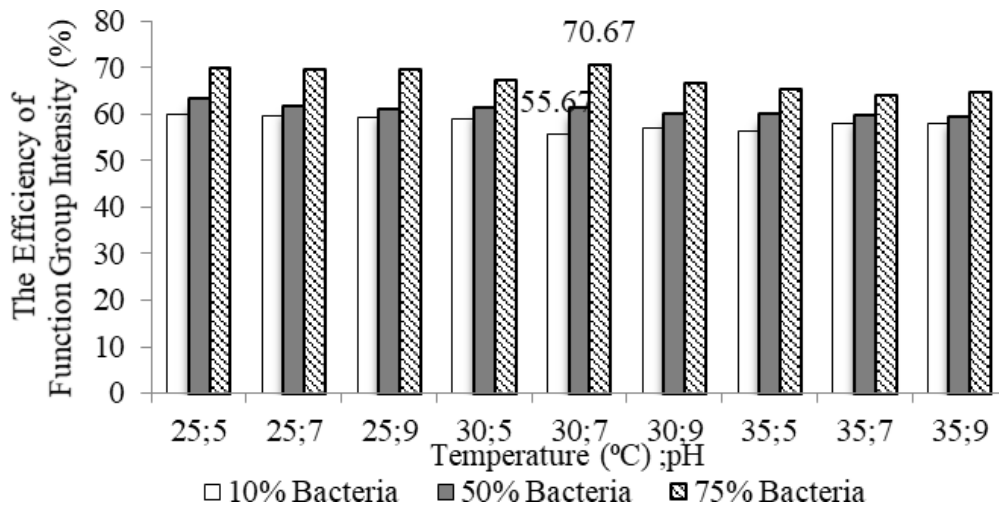


Figure 5. The Efficiency of Functional Group Intensity (%) on Various Treatments for 30 days

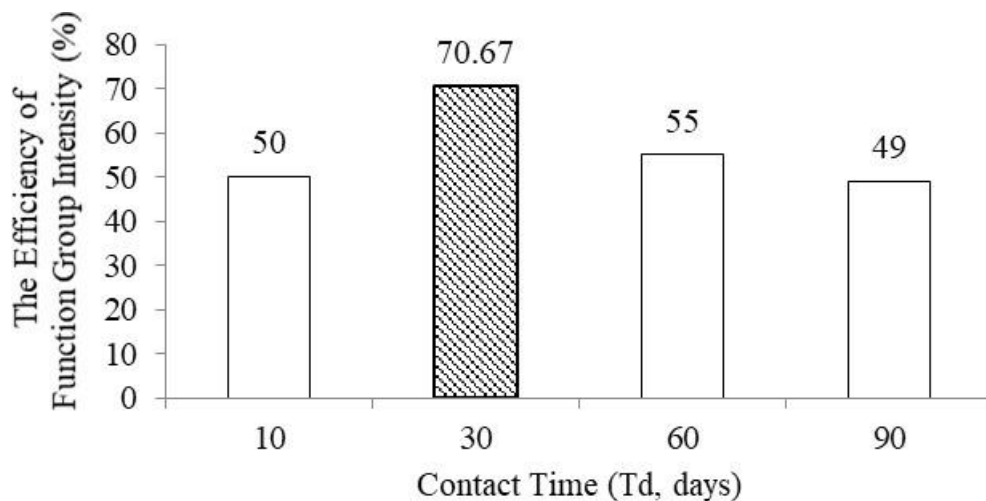


Figure 6. Efficiency of Functional Group Intensity (%) until Day-90

Contrary to expectation, after 30 days of incubation period, the efficiency of functional group removal did not improve until Day-90 (Figure 6). Seventy-five percent (v/v) mixed-cultured bacteria as biodegradator was apparently insufficient to improve degradation efficiency. Although bacteria can still be found on Day-90, the density was reduced 70%. The enzyme produced by mixed-cultured bacteria was seemingly not suitable with LLDPE sample as substrate. The role of enzyme in this process was as a biocatalyst which acted in accelerating chemical reactions without reacting. However, enzyme works specifically, like lock and key, whereas the catalyst substrate should be suitable with the type of enzyme. According to [12], [14], the enzyme produced by bacteria varies in several species of bacteria. *Brevibacillus spp.* is known to degrade polyethylene because it produces protease

enzyme, while *Pseudomonas spp.* produced hydrolase, esterase, and lipase. This was supported by [13], [8] which explained that plastic biodegradation by microorganisms occurred due to hydrolysis or oxidation process by the enzyme owned by microorganism to breakdown polymer substance, aerobically or anaerobically.

Low efficiency of LLDPE removal can also be caused by inappropriate culture batch condition, which lead to ineffective contact between mixed-cultured bacteria and sample. Furthermore, the availability of nutrient in growth media kept decreasing until Day-90. One of the factors that affected biodegradation process is a component of a substrate or the size of a substrate. Biodegradation process will occur faster in a simpler substrate making molecules or smaller substrate size. Otherwise, a more complex molecules and larger substrate requires longer time to decompose or degrade [6], [14]. Further studies are needed with the addition of co-substrate in a continuous culture to ensure nutrient availability until the end of observation period.

3.3. Scanning Electron Microscope (SEM) Analysis

LLDPE sample along with 75% (v/v) mixed-cultured bacteria incubated for 30 days at 30°C and pH 7 was reported to have efficiency of 5.47% and 70.67% according to gravimetry and FTIR analysis, respectively. Afterwards, SEM analysis was conducted to confirm whether there were morphological changes in LLDPE plastic sample surface.

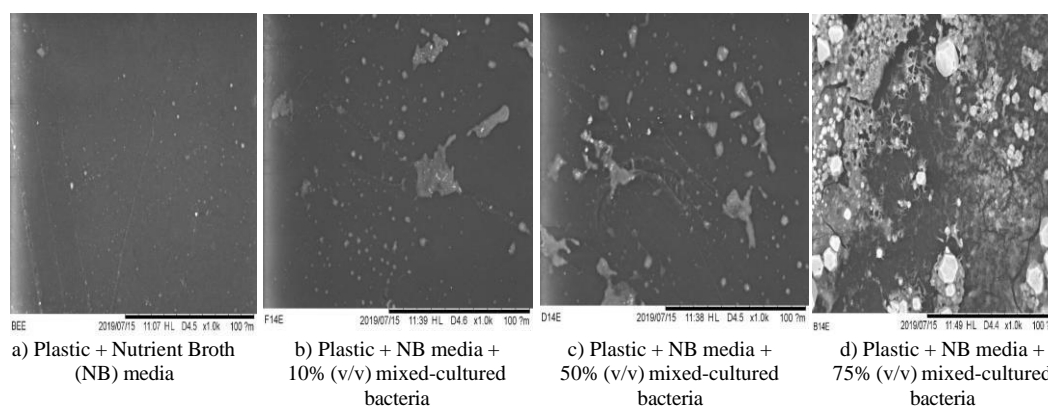


Figure 7. Morphological Changes of LLDPE Sample Surface at 30°C, pH 7

Figure 7 visually depicted morphological changes on LLDPE plastic surface, from smooth, non-porous to damage with fine pores on the surface. Incomplete biodegradation process seemed to occur by mixed-cultured bacteria which were suspected to utilize the plastic as carbon or nutrient source for their growth. The loss of 5.47% plastic weight during 30 days of incubation showed that decomposition or degradation of LLDPE plastic occurred very slowly because morphological changes only occurred on the plastic surface. Physically, the LLDPE sample had not completely broken down on Day-90. Slow biodegradation process may be due to linear low-density polyethylene (LLDPE) is a synthetic polymer consisted of molecules with linear polyethylene with monomer ethylene long chain (C_2H_4) with 0.90-0.94 g/cm^3 density [5], [7]. Other than that, plastic is a substance or substrate foreign to biological system, thus produced resistance due to unsuitability of substrate with available enzyme [9], [10], [14] Biofilm layer consists of microorganism, 80-95% water, inorganic particles, and 85-98% extracellular

polymer substance that came from organic materials. The formation of microorganism cells on polymer surface as biofilm is known as biofouling [8], [12].

4. Conclusion

Mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* in controlled environment on all variations of temperature and pH can degrade LLDPE sample, although the process was very slow. The highest degradation based on removal efficiency from lost weight and efficiency of functional group intensity reduction were each 5.47% and 70.67% and occurred when 75% of mixed-cultured bacteria was added as degradator, and environmental condition was set to 30 °C and pH 7. Degradation did not increase until Day-90. This may be due to reduced nutrients and inappropriate culture batch condition. Therefore, further studies should be conducted with the addition of co-substrate in continuous culture to ensure nutrient availability until the end of observation period.

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Potential of Linear Low-Density Polyethylene (LLDPE) Plastic Degradation by Mixed-Cultured Bacteria in Controlled Environment

by Melati Ferianita Fachrul

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Potential of Linear Low-Density Polyethylene (LLDPE) Plastic Degradation by Mixed-Cultured Bacteria in Controlled Environment

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Abstract

This preliminary study was aimed to determine the ability of mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium* sp. in degrading Linear Low-Density Polyethylene (LLDPE). This study was started by preparing the plastics into $J \times J$ cm² cut mechanically. The plastics were placed in a petri dish containing Nutrient Broth (NB) media and mixed-cultured bacteria. The variations of bacterial composition(%) were JO, 50, and 75; variations of temperature (°C) were 25, 30, 35; variations of acidity (pH) were 5, 7, 9; and **variatfD** of contact time (Td; days) were JO, 30, 60, and 90. LLD PE plastic degradation was analyzed using gravimetry, Fourier Transform Infra-red (FTIR) and Scanning Electron Microscope (SEM). Both bacteria were resistance or non-sensitive to LLDPE substance as xenobiotic substrate. According to gravimetry and FTIR analysis, the highest removal was at 30°C, pH 7, with bac/erial composition of 75% (v/v) incubated for 30 days. Gravimetry analysis result showed a loss of LLDPE plastic weight from 0.1548 gram to 0.1464 gram, or 70.67%. The result was reconfirmed using SEM analysis, which showed morphological changes of LLDPE plastic sample swface. Although the degradation occurred very slow, mixed-cultured bacteria has a potential for capacity increase in degrading LLDPE infurrher srudies, including rhe addition of co-substrate in continuous culture.

Keywords: *brevibacterium* sp., *pseudomonas aeruginosa*, linear low-density polyethylene, xenobiotic, FTIR

1. Introduction

Plastic is deliberately produced to be impenetrable by oxygen (O₂), thus requires longer time to decompose or degrade naturally by microorganisms involved in decomposition. Other than, plastic has several advantages, i.e. anti-rust, flexible, strong, Jjght, not easily broken, easy to color, resistance to various chemical substances, insulation against electricity, and low density. One common type of plastic is ethylene monomer long chain polyethylene polymers (C₂fu) [1], [2]. Other than Light, transparent, and economical, polyethylene (PE) plastic has resistance property, thus difficult to degrade naturally, which lead to accumulation in the environment [3].

Plastics buried in soil will hinder or inhibit the process of water absorption by plants, thus interrupting photosynthesis **procesmhe** life of animals in the soil is disrupted and even causes death due to entrapment in plastic waste or due to ingestion of plastic waste. These animals are vital components in material and energy flow in the soil, learung to loss of nutrients in agricultural lands [3]. Plastic waste in water can also jeopardize the marine ecosystem, especially affecting food chrun in the waters.

Polyethylene can be degraded by UV light at 280-300 nm wavelength for 10 days to form hydroperoxide and disconnect polyethylene long cha.in to form shorter fragments known as monomers [4]. However, degradation process using UV light has negative

effect, i.e. the greenhouse effect. Burning plastic waste will also cause negative effect, in which air pollution caused by gasses resulting from burning plastic, including Carbon Dioxide (CO₂) and Carbon Monoxide (CO). Therefore, a solution is needed to process plastic waste without endangering environment.

Other alternative that has been developed in the last 10 years is biotechnological plastic degradation by utilizing microorganisms. Biodegradation process occurs because complex substances are used by microorganisms as nutrient source or energy source for their growth by utilizing enzymes to breakdown complex substances into more simple substances [51, [6]. According to [71, *Microbacterium paraoxydans* and *Pseudomonas aeruginosa* was known to have the ability to breakdown polymers efficiently with removal percentage of 61.0%, while *P. aeruginosa* in growth media can degrade LOPE plastic with 50.5% efficiency in 2 months. Polymers were broken down into smaller polymers by *M. paraoxydans* and *P. aeruginosa* used as carbon source. This degradation process is started by the formation of a biofilm in the polymer surface. The biofilm contains microorganism cells which creates a layer in hydrated matrix from polysaccharides and proteins on the surface of the plastic [8]. Based on [51, there was a decrease of plastic weight incubated in media for 120 days and increase of biofilm cells for the first 40 days of incubation.

Each microorganism has different characteristics, thus the degradation process of one **microorganism** would be different or varies from other microorganisms. Based on this description, this study is required to test the ability of mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* As biodegradator in the process of LLDPE plastic processing in controlled laboratory scale.

2. Methodology

2.1. Bacteria Cultivation and Preparation of Plastic Samples

FIJThe mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* was obtained from the collection of Microbiology Laboratory of Environmental Engineering, Universitas Trisakti. Cultivation of mixed-cultured bacteria was conducted in batch culture in Erlenmeyer flask aerobically, which contained growth media, nutrient broth (NB). Bacterial growth was observed every day. After reaching exponential phase, the mixed culture bacteria were ready to be tested. LLDPE samples were cut mechanically to form 1x1 cm², the smaller the plastic, the higher the surface area, thus higher chance for effective contact with degradation bacteria.

2.2. Sensitivity Test

Before the main study, sensitivity test was conducted to determine the vulnerability of *Pseudomonas aeruginosa* and *Brevibacterium sp.* on LLDPE sample as xenobiotic substance which can inhibit growth or kill bacteria in certain concentration [9]. LLDPE sample was placed in the center of a petri dish containing mixed-cultured bacteria and growth media. Inhibition zone formation surrounding plastic sample showed inhibition of culture growth, thus cannot be used as biodegradator and vice versa.

2.3. Research Design

The dependent variable in this study was linear low-density polyethylene (LLDPE) plastic samples, while the independent variables were composition of mixed-cultured bacteria (%; v/v), temperature (°C), acidity (pH), and contact time (Td; days). Variations in the composition of mixed-cultured bacteria were 10%, 50%, and 75% of total solution volume. Optimum temperature and pH were

determined by variations of tropical temperature (°C) which were 25, 30, and 35 and with pH variations of 5, 7, and 9 which represented acid, neutral, and alkaline. This study was conducted by placing 1% (w/v) LLDPE sample in testing reactor containing growth media from 10%, 50%, 75% (v/v) mixed-cultured bacteria. This study was conducted for 90 days with 3 times **repetitio8rformed** on contact time (Td; days) 10, 30, 60, and 90. Afterwards, gravimetry, Fourier Transform Infra-red (**FfIR**) and Scanning Electron Microscope (**SEM**) analyses were conducted.

2.4. Gravimetric Analysis

Gravimetry was used to determine the percentage of LLDPE plastic sample weight loss. Gravimetry is a quantitative chemical analysis based on the principle of plastic sample **meaDment** before and after degradation. Therefore, the percentage of degraded plastic **Bight** loss can be determined. According to [9], the percentage of degraded plastic weight loss can be determined by the following formula:

$$\% \text{ weight loss} = \frac{W(a)-W(b)}{W(a)} \times 100\% \quad (1)$$

W(a): plastic weight before degradation

W(b): plastic weight after degradation

2.5. Fourier Transform **Infra-rrTIR**) Analysis

Plastic content analysis using Fourier Transform Infra-red (FfIR) based on infra-red spectrum was conducted to determine changes in functional groups of LLDPE sample before and after contact to mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* LLDPE Biodegradation can be confirmed within 2500-3000 cm-1 wavenumber, which is the typical peak of PE substance [7].

2.6. Scanning Electron Microscope (SEM) Analysis

This analysis was conducted to determine morphological structure and shape of LLDPE sample surface before and after incubation with mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* The morphology of LLDPE sample which completed biodegradation process by bacterial activity was observed with SEM using 10-3,000,000 magnification, 4-0.4 mm depth of field and 1-10 nm resolution.

3. Results and Discussion

The growth of mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* in liquid media Nutrient Broth (NB) containing LLDPE sample was shown by changes in color. Color changes occurred gradually from transparent to cloudy yellow observed on Day-30, Day-60, and Day 90 showed increased bacterial concentration **■** *Pseudomonas aeruginosa* and *Brevibacterhun sp.* in liquid media Nutrient Broth (NB) (Figure 1).

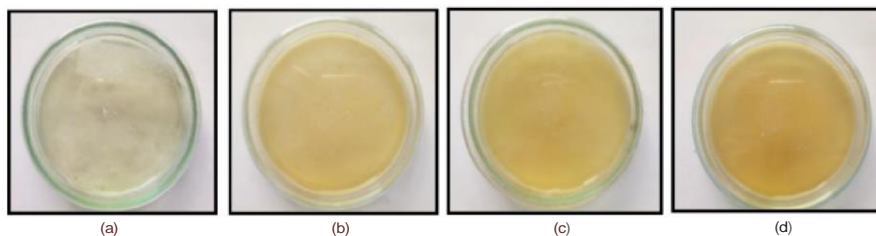


Figure 1. The growth of *Pseudomonas aeruginosa* and *Brevibacterium sp.* surrounding LLDPE sample (a) 0 day, (b) 30 days, (c) 60 days, and (d) 90 days

These color changes revealed that the growth activity of mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* was not disturbed by LLDPE plastic sample with xenobiotic property. These bacteria were suspected to grow by using carbon substance (C) as a nutrient source in growth media contained in LLDPE sample.

The result of sensitivity test showed that there was no inhibition zone surrounding LLDPE sample, thus mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* can be considered resistant or non-sensitive to toxic xenobiotic substance (Figure 2). Therefore, these mixed-cultured bacteria were suspected to have the ability to degrade or decompose LLDPE plastic.

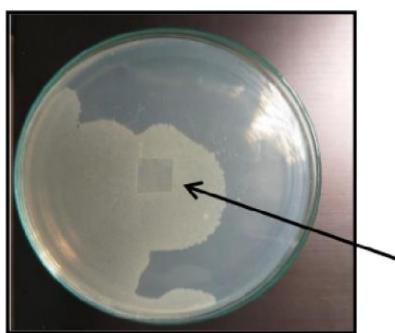


Figure 2. Mixed-cultured bacteria can grow well on Day-48 shown by no inhibition zone surrounding LLDPE sample

3.1. Reduced Weight of LLDPE Sample

Removal efficiency according to reduced weight of LLDPE sample on variations of temperature and pH, and addition of 10%, 50%, and 75% (v/v) mixed-cultured bacteria to a media containing 1% (w/v) LLDPE sample for 30 days of study can be seen in Figure 3. Based on the figure, the addition of 75% (v/v) mixed-cultured bacteria provided removal efficiency of 5.47% at 30°C and pH 7 for 30 days of incubation, proven by reduced sample size from 0.1548 gram to 0.1464 gram. This result showed that LLDPE polymer degradation occurs very slowly. Furthermore, more biodegradator causes more reduction of LLDPE sample weight. Biodegradation process occurs from the breakdown of polymer into monomers or smaller components, then these monomers are mineralized through the process of organic substance change to inorganic. Polymers that are too large to pass through cell membrane should be polymerized to smaller monomers before absorbed and degraded in microbial cells [10], [11]. The study was continued until Day-90, where it is expected to give higher removal result (degradation).

Figure 4 depicted removal efficiency (%; v/v) according to reduced LLDPE sample weight for 90 days at 30°C and pH 7 with the addition of 75% (v/v) mixed-cultured bacteria. Removal efficiency kept increasing to 5.47% on Day-30, however reduced after Day-30 to the end of the study on Day-90. Increased removal efficiency occurred because mixed-culture bacteria performed optimum growth activity or in exponential phase. According to Maier (2008), this phase is marked by fast bacterial growth in current condition. This proved that although degradation

process had occurred by mixed-cultured bacteria as biodegradator by utilizing carbon substance in LLDPE as a nutrient source, the process occurs very slowly. These results were supported by the result of a study [5] which stated that there was a reduced plastic sample weight of 20% incubated in media for 120 days by *Pseudomonas aeruginosa*. Plastic biodegradation process which utilizes microorganism activity is a very slow process, and certain microorganisms are unable to decompose plastic because they do not have enzyme suitable to the substrate or plastic which will be degraded [1], [12].

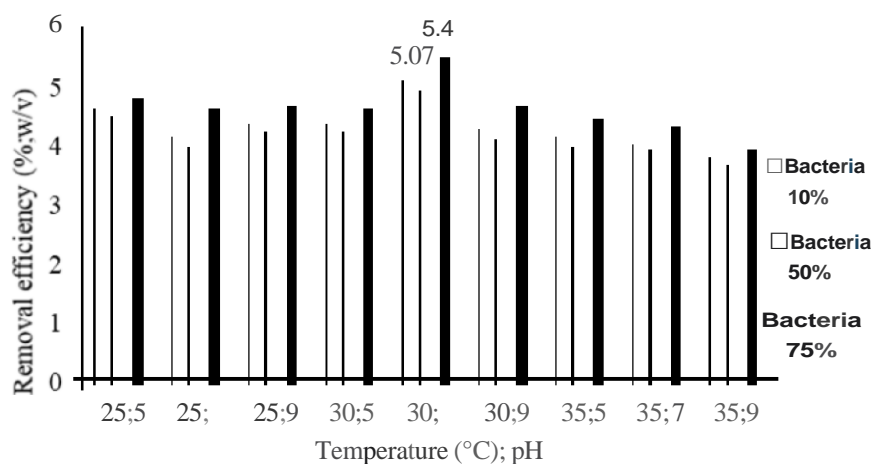


Figure 3. Removal Efficiency(% w/v) on Day-30 on Variations of Temperature and pH with Addition of 10%, 50%, and 75% (v/v) Mixed-Culture Bacteria

Low plastic removal for 30 days at 30°C and pH 7 may also be due to the enzyme contained by mixed-cultured bacteria did not act properly. Enzyme work is highly influenced by environmental condition, which include temperature, pH, oxygen availability, nutrient availability with correct carbon to nitrogen to phosphor ratio (C:N:P). Other than these factors, in order to improve enzyme activity in accelerating LLDPE degradation process, enzyme requires chemical substances in the form of molecules or ions which can improve the biological activity of enzyme, known as co-substrate.

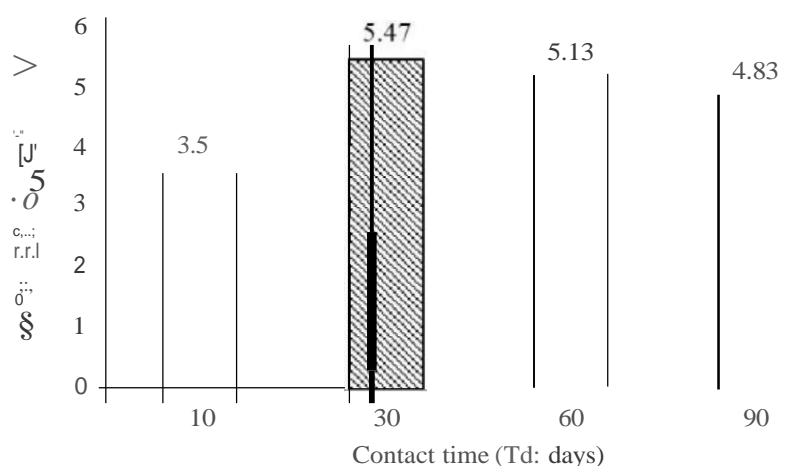


Figure 4. Removal Efficiency(%; w/v) until Day-90

The most commonly used co-substrate is starch. Starch is often used as plastic making material with biodegradable property because starch has several advantages, including abundant amount in the environment, can hold oxygen well, and affordable [II]. The mixture of hydrocarbon polymer and starch is often used to produce high quality plastic film and layers. More starch used in plastic making, the higher the **biodegradability**. According to [II, [141], biodegradation of a polymer is also affected by physical and chemical characteristics, including the availability of functional group which increases hydrophobic (degradation process in hydrophilic is faster compared to hydrophobic), molecular weight, molecular composition, physical shape and properties of polymer, and polymer density.

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3.2. Fourier Transform Infra-red (FTIR) Analysis

Weight loss according to the composition of mixed-cultured bacteria gravimetry analysis was confirmed by FTIR analysis. The intensity of functional group of LLDPE sample that did not given mixed-cultured bacteria addition on t0 or as baseline intensity was 0.098 on 2916.37 cm⁻¹ wavenumber. Observation to Day-90 showed that the intensity stayed at 0.098 on 2916.37 cm⁻¹ wavenumber. This showed that there were no changes in functional group intensity in LLDPE plastic without the existence of mixed-cultured bacteria.

The results of FTIR analysis on variations of temperature, pH and addition of mixed-cultured bacteria for 30 days of incubation showed changes of reduced intensity of LLDPE sample functional group on every treatment (Figure 5).

The highest intensity reduction occurred in 75% (v/v) mixed-cultured bacteria addition, at 30°C and pH 7. The intensity changed from 0.098 to 0.028, which obtained 70.67% efficiency of functional group intensity. This proved that a condition 30°C and pH 7 was suitable for the growth of mixed-cultured bacteria, which lead to a more optimal plastic degradation. This study also proved that these mixed-cultured bacteria were included in mesophilic group, which can grow well within 20-45°C and pH range of 5.5-8.0. Greater number of bacteria used as biodegradator also affected degradation process. More bacteria mean more enzyme produced to catalyze more sample, which lead to better degradation. The study was continued until Day-90 with the addition of 75% (v/v) mixed-cultured bacteria at

30°C and pH 7, with the expectation of higher efficiency of functional group intensity.

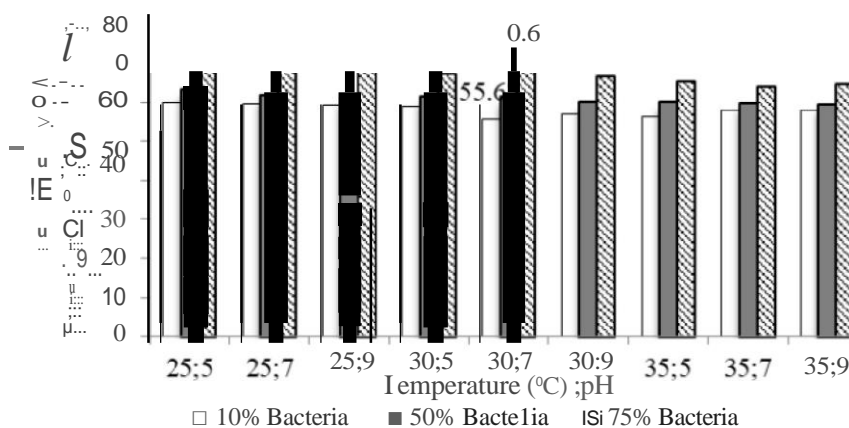


Figure 5. The Efficiency of Functional Group Intensity (%) on Various Treatments for 30 days

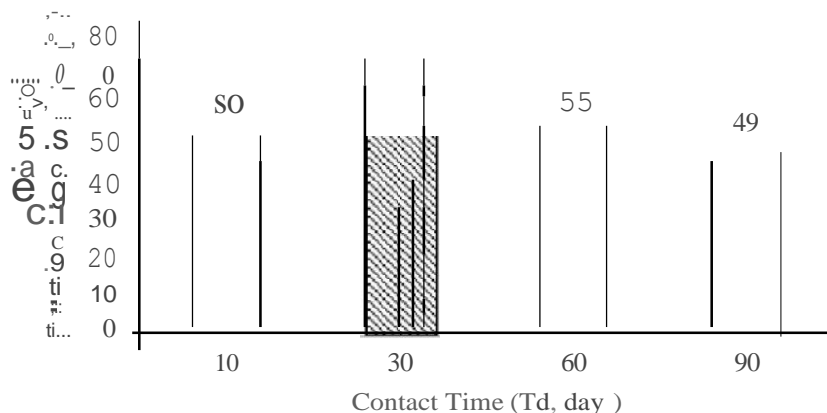


Figure 6. Efficiency of Functional Group Intensity (%) until Day-90

Contrary to expectation, after 30 days of incubation period, the efficiency of functional group removal did not improve until Day-90 (Figure 6). Seventy-five percent (v/v) mixed-cultured bacteria as biodegradator was apparently insufficient to improve degradation efficiency. Although bacteria can still be found on Day-90, the density was reduced 70%. The enzyme produced by mixed-cultured bacteria was seemingly not suitable with LLDPE sample as substrate. The role of enzyme in this process was as a biocatalyst which acted in accelerating chemical reactions without reacting. However, enzyme works specifically, like lock and key, whereas the catalyst substrate should be suitable with the type of enzyme. According to [12], [14], the enzyme produced by bacteria varies in several species of bacteria. *Brevibacillus spp.* is known to degrade polyethylene because it produces protease

enzyme, while *Pseudomonas spp.* produced hydrolase, esterase, and lipase. This was supported by [13], [8] which explained that plastic biodegradation by microorganisms occurred due to hydrolysis or oxidation process by the enzyme owned by microorganism to breakdown polymer substance, aerobically or anaerobically.

Low efficiency of LLDPE removal can also be caused by inappropriate culture batch condition, which lead to ineffective contact between mixed-cultured bacteria and sample. Furthermore, the availability of nutrient in growth media kept decreasing until Day-90. One of the factors that affected biodegradation process is a component of a substrate or the size of a substrate. Biodegradation process will occur faster in a simpler substrate making molecules or smaller substrate size. Otherwise, a more complex molecules and larger substrate requires longer time to decompose or degrade [6], [14]. Further studies are needed with the addition of co-substrate in a continuous culture to ensure nutrient availability until the end of observation period.

3.3. Scanning Electron Microscope (SEM) Analysis

LLDPE sample along with 75% (v/v) mixed-cultured bacteria incubated for 30 days at 30°C and pH 7 was reported to have efficiency of 5.47% and 70.67% according to gravimetry and FTIR analysis, respectively. Afterwards, SEM analysis was conducted to confirm whether there were morphological changes in LLDPE plastic sample surface.

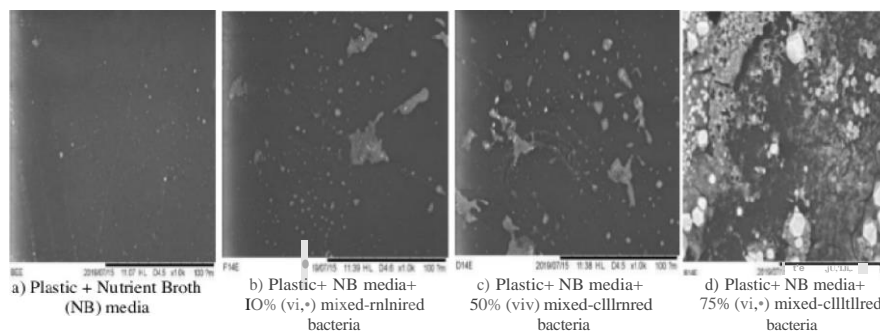


Figure 7. Morphological Changes of LLDPE Sample Surface at 30°C, pH 7

Figure 7 visually depicted morphological changes on LLDPE plastic surface, from smooth, non-porous to damage with fine pores on the surface. Incomplete biodegradation process seemed to occur by mixed-cultured bacteria which were suspected to utilize the plastic as carbon or nutrient source for their growth. The loss of 5.47% plastic weight during 30 days of incubation showed that decomposition or degradation of LLDPE plastic occurred very slowly because morphological changes only occurred on the plastic surface. Physically, the LLDPE sample had not completely broken down on Day-90. Slow biodegradation process may be due to linear low-density polyethylene (LLDPE) is a synthetic polymer consisted of molecules with linear polyethylene with monomer ethylene long chain (C₂H₄) with 0.90-0.94 g/cm³ density [5], [7]. Other than that, plastic is a substance or substrate foreign to biological system, thus produced resistance due to unsuitability of substrate with available enzyme [9], [10], [14] Biofilm layer consists of microorganism, 80-95% water, inorganic particles, and 85-98% extracellular

polymer substance that came from organic materials. The formation of microorganism cells on polymer surface as biofilm is known as biofouling [8], [12].

4. Conclusion

Mixed-cultured *Pseudomonas aeruginosa* and *Brevibacterium sp.* in controlled environment on all variations of temperature and pH can degrade LLDPE sample, although the process was very slow. The highest degradation based on removal efficiency from lost weight and efficiency of functional group intensity reduction were each 5.47% and 70.67% and occurred when 75% of mixed-cultured bacteria was added as degradator, and environmental condition was set to 30 °C and pH 7. Degradation did not increase until Day-90. This may be due to reduced nutrients and inappropriate culture batch condition. Therefore, further studies should be conducted with the addition of co-substrate in continuous culture to ensure nutrient availability until the end of observation period.

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