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SEMINAR NASIONAL BIOTEKNOLOGI VI

Bioteknologi untuk Indonesia Makmur Sejahtera

Yogyakarta, 2 November 2019

Program Studi Bioteknologi
Universitas Gadjah Mada

PROSIDING

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UNIVERSITAS GADJAH MADA

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Program Studi Bioteknologi, Universitas Gadjah Mada
Yogyakarta, 2 November 2019

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Dr. Boya Nugraha
(Hannover Medical School, Jerman)
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PENGANTAR

Kemakmuran Indonesia yang berbasis pengelolaan sumber daya alam perlu dioptimalkan dengan memperhatikan faktor keberlanjutan. Bioteknologi memiliki peranan penting untuk meningkatkan kualitas sumber daya alam demi mewujudkan kemakmuran dan kesejahteraan rakyat dalam berbagai aspek seperti pertanian, lingkungan, kesehatan, dan industri.

Melalui komunikasi ilmiah Seminar Nasional Bioteknologi VI dengan mengusung tema "Bioteknologi untuk Indonesia Makmur Sejahtera", yang beberapa makalahnya diterbitkan dalam Prosiding ini, diharapkan menjadi salah satu sumbangsih dari dunia akademik untuk mendukung pelaksanaan pembangunan Indonesia menuju Indonesia makmur sejahtera.

Kami mengucapkan terima kasih yang sebesar-besarnya kepada para pemakalah yang telah mempublikasikan makalahnya di Prosiding ini, serta para reviewer yang telah membantu proses review naskah. Semoga Prosiding ini memberikan manfaat yang sebesar-besarnya bagi kesejahteraan masyarakat dan bangsa Indonesia.

Ketua Panitia,

Dr. Abdul Razaq Chasani, M.Si.

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JADWAL ACARA
SEMINAR NASIONAL BIOTEKNOLOGI VI
UNIVERSITAS GADJAH MADA
2019

Waktu	Jenis Kegiatan
07.00 – 08.00	Registrasi
08.00 – 08.30	Pembukaan Acara oleh MC
	Menyanyikan lagu Indonesia Raya dan Hymne UGM
	Sambutan Ketua Pelaksana
	Pembukaan Acara oleh Dekan Sekolah Pascasarjana UGM
08.30 – 08.40	Pentas Seni
08.40 – 09.00	Coffee Break
09.00 – 10.40	**Keynote Speech I** 1. Dr. Widodo (Universitas Gadjah Mada) "Indigenous Lactic Acid Bacteria for The Next Generation of Functional Food"
	Keynote Speech II 2. Dr. Boya Nugraha (Hannover Medical School, Jerman) "Application of Neuroscience in Pathomechanism Study of Chronic Pain"
10.40 – 11.00	**Sesi Poster**
11.00 – 12.30	**Sesi Pararel I**
12.30 – 13.15	Lunch Break
13.15 – 14.05	**Sesi Pararel II**
14.05 – 14.25	Coffee Break
14.25 – 15.30	**Keynote Speech III** 3. Dr. Pascal Montoro (Cirad, Perancis) 4. "A New Avenue in Rubber Tree Biotechnology"
15.30 – 16.00	Pengumuman Presenter Oral dan Poster Terbaik
	Penutupan

Effectiveness of UV Rays on The Ability of Selected Soil Bacteria in Decomposing LDPE Plastic

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Abstract. LDPE plastic is a kind of plastic which is most widely used in the food packaging industry since its characteristics are light and thin but it is strong enough. As we know that plastic cannot be degraded easily. Therefore many studies are currently looking for special potential of microorganisms, especially bacteria, as natural plastic degrading agents. This research was designed to improve the ability of plastic degrading bacteria by inducing it with UV A and UV B rays. This study used some samples taken from three main locations in Jatibarang Landfill, Semarang. There are four methods used in this research. The first screening method was conducted to select the LDPE degrading bacteria and it was followed by the second screening method in order to obtain the culture in solid media. Then, the bacteria was isolated by quadrant streak technique and regrown in other NA plates to obtain the 10 hours bacteria culture. The next one was irradiated with UV A and UV B for 15 minutes then the bacteria goes to the biodegradation rate process. The final result obtained 11 samples from UV A, 5 samples from UV B, and 3 samples from the control samples, that be able to reduce 10% of LDPE sheets' initial weight.

Keyword: *biodegradation rate, LDPE plastic, plastic degrading bacteria, UV induction*

Introduction

Plastic is made of petrochemical-based material that synthetically making by using monomer and few chemicals addition to shaping it into long polymer chains (Shimao, 2001). Indonesia is the second-largest producer of plastic waste in waters

after China which reached 187.2 million tons. The Ministry of Environment and Forestry states that from 100 shops as the members of the Indonesian Retail User Association (APRINDO) can produce 10.95 pieces of plastic bag waste that equal to 65.7 hectares of plastic bag in a year (Purwaningrum, 2016). Meanwhile, based on the data from Semarang City Government Office in 2017, the daily waste production in Semarang City was 1.200 tons and it reached up to 438.000 tons annually, in which 38% of it was non-organic wastes.

There are some kinds of plastic, one of them is polyethylene polymer that consist of –CH2-CH2 repeating units as the polymer backbone (Sharma *et al.*, 2015). Polyethylene plastic is categorized in two types, there are LDPE plastics (Low Density Polyethylene) that has MW (molecular weight) up to 4800 and HDPE (High-Density Polyethylene) that has MW 93000 (Sangale, 2012). LDPE is widely used in daily life because it is light and not too thick but it is strong enough. Mostly it is used to wrap food and drink containers. The researcher chooses LDPE plastic as the main material in this research because it has a low MW, so it will be easier to cut than another kind of plastic such as HDPE that has a strong straight bonding structure. Some research has been revealed that plastic with low MW will degrade faster and better than plastic with high MW (Yamada-Onodera *et al.*, 2001; Gu, 2003).

There are some technologies used for degrading plastic material such as photo-oxidative degradation, thermal degradation, ozone-induced degradation, mechanochemical degradation, catalytic degradation, and biodegradation (Singh & Sharma, 2008). The biodegradation method is considering to be the safest way for the plastic degradation process because the outcome is expected to be non-toxic to the environment (Singh & Sharma, 2008; Pathak and Navneet 2017a) and redistributed in carbon, nitrogen, and sulfur cycles. Biodegradation process can be enhanced by using abiotic hydrolysis, physical integration, and photo-oxidation, also using such additives and bio-surfactant will increase the hydrophilicity of the polymer and increasing the biodegradation rate (Singh & Sharma, 2008).

Recently researchers are trying to upgrade the microbial ability by using pretreatment in chemicals or certain conditions in order to maximize the ability of microorganisms degrading the plastic component effectively. Microorganisms are able to adapt in the continuous exposure of recalcitrant material such as synthetic polymer and develop new pathways by modifying their own preexisting genetic components by either mutation(s) in single structural and/or regulatory gene or maybe adding a single silent gene when they encounter the foreign compounds (Talkad *et al.,* 2014). This theory creating an idea to enhance the mutation development by using of UV rays or others induced mutation method in order to encourage the ability of microorganisms to be survival by "forcing" them to change the structure of the cell, whether it is the DNA, protein cell, or the fat cell (Santos *et al.*, 2012).

There is some research that studying the effect of induced mutation by UV rays in bacterial species and finding that the mutation bacteria are able to degrade the material better than without the induced mutation. Talkad *et al*., (2014) using *Pseudomonas putida* that have been induced mutation by using EMS and UVC to degrade pretreated LDPE. Their research finds that induced with UV and EMS in *Pseudomonas putida* are showing better result at biomass reduction. The species of *Deinococcus ficus* that have been exposed to UVC showing that one of the mutants (strain CC-ZG207) is better growth, a slight increase in protease activities, and higher keratinolytic activity after 10 days incubation with a slightly higher in the cell number (Zeng *et al.,* 2011).

The aim of this research is to select the soil bacteria that have a high ability to degrade the LDPE plastic effectively, then it is mutated with the UV (Ultra Violet) rays induction in order to see the effect of UV rays on the effectiveness of bacteria in degrading LDPE plastic.

Materials and Methods

Soil Sampling Process

The soil sampling location was in Jatibarang Landfill, Semarang, Central Java. The samples were collected from three

different sites (figure 1). Each site consists of three spots (figure 2, figure 3, and figure 4), in which the soil temperature and pH were measured and recorded. Soil samples were taken from a depth of 5-10 cm and collected in ziplock plastics.

Figure 1. Location mapping in Jatibarang Landfill

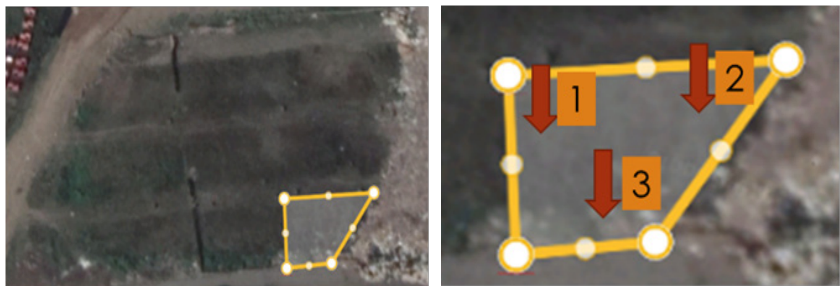


Figure 2. Sampling spot in location A

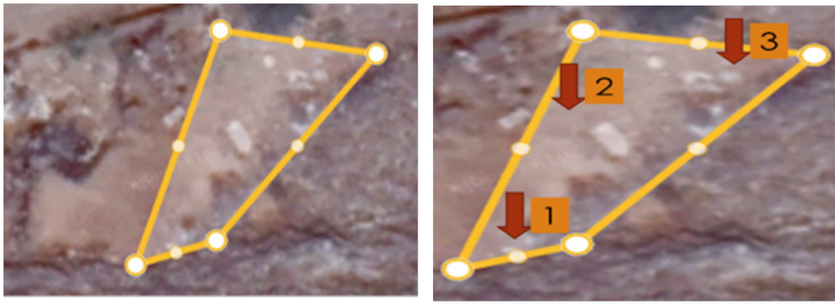


Figure 3. Sampling spot in location B

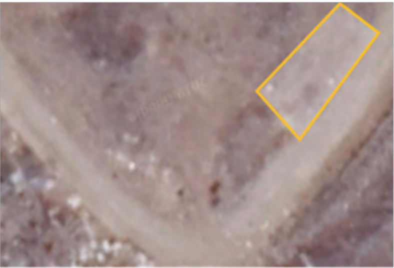


Figure 4. Sampling spot in location C

Making the LDPE (Low Density Polyethylene) Powder

This method was conducted by modified the method proposed by (Deepika *et al.*, (2016), with modifications. LDPE sheets were cut into 2 x 2 cm in size for about 3.5 gram and immersed in 20 ml of xylene in beaker glass. It was boiled for 15 – 20 minutes in 115^{0}C until the LDPE plastic dissolved. The LDPE solution poured to the mortar and crushed while it was still warm with a pestle. Then the LDPE powder was sprayed with 96% of ethanol to remove the residual xylene and let it be evaporated so that the ethanol can be removed from the powder. Then, pour the powder into the porcelain plate and put in the oven at 60^{0}C for 24 hours. After that, took the porcelain out of the oven and sieved it to obtain the same size powder.

Isolation and Screening Plastic Degrader Soil Bacteria

Five grams of soil sample was dissolved in Erlenmeyer flask with 50 ml of sterile distilled water. The sample was incubated overnight under shaking conditions (130 rpm) at the room temperature. The composition for mineral salt broth media were consist of 1000 ml distilled water (K_2HPO_4, 1.73g; KH_2PO_4, 0.68g; $MgSO_4$-7H_2O, 0.1g; $FeSO_4$-7H_2O, 0.03g; NH_4NO_3, 1g; $CaCl_2$-2H_2O, 0.02g). It was conducted based on the composition proposed by (Sekar *et al.*, 2011), with some modifications by getting rid of sodium chloride and glucose, also 0.1% (wt v-1) of LDPE powder and 0.1% (v v-1) of Tween 80 that was added to this media.

The screening of soil bacteria was divided into two steps. On the first step, a dilution series was done from 10^{-1} to 10^{-4} dilution. Then, 1 ml of sample was taken and added to Erlenmeyer flask with 25 ml of sterile mineral salt (MS) broth medium. The sample was incubated at 37^0C for 1 week and every 24 hours the flask was monitored and shaken (120 rpm) for 1 hour. For the second step, mineral salt agar (MSA) media was used with the same composition as mineral salt broth, about 7 g/900 ml of "Swallow" agar, 0.1% (v v-1) of Tween 80, and 0.1% (wt v-1) of LDPE plastic. This step used two different forms of LDPE plastic, powder and sheets. First, LDPE powder was prepared and added before the mineral salt agar medium. For LDPE sheets, five sheets (1x1 cm) were prepared in each petri dish. Next, 15 ml of MSA was poured into the petri dish. After the agar hardened, 20 μl of MS broth medium from the first screening was spread in the agar media using L rod. The plates were incubated at 37^0C for 4-5 days and observed every 24 hours. The bacterial colony from the MSA was inoculated to NA media by quadrant streak method and one-time replication. Then, all the plates were incubated for 24 hours at 37^0C.

Induction of Bacterial Mutation using UV Rays

This stage was carried out by UV A and UV B ray radiation, using UVA (Sankyo Denki F10T8BLB lamp, 10W, Japan, main emission line 352 nm) and UVB (Philips Special PL-S 9W/01/2P lamp, Philips, Poland, main emission line 311 nm). Bacterial culture was incubated for 10 hours. Three replicates were used in this stage, for the UV A radiation, the UV B radiation, and the control samples. The plates were irradiated inside the laminar flow cabinet for 15 minutes with a 20 – 30 cm gap between the lamp and the plates. After that, the bacteria cultures were incubated at 37^0C for 36 hours.

Effectiveness Test of LDPE Degradation Rate

This stage aims to test the rate of decomposition of LDPE plastic by bacteria that have been treated with UV rays in the previous stage. The bacteria cultures were inoculated in 120 ml bottles contained Mineral Salt Broth media with sterilized 2 x 2 cm LDPE sheets. Then,

the bacteria were incubated at 37^{0}C for 1 week and every 24 hours it was monitored and shaken (150 rpm) for 1 hour.

Data Processing of LDPE Plastic's Degradation Rate

This stage aims to determine the effectiveness level of bacteria that were treated with UV A and UV B compared with the control bacteria. This method was according to the method proposed by (Singh *et al.,* 2016), with modifications. The LDPE plastic sheet was taken from the bottle by using tweezers aseptically. The plastic was dipped several times into distilled water and 70% alcohol, then dried with a clean tissue. It was put into a petri dish and heated using an oven at 45^{0}C for 1 hour. After that, the plastic was put into a desiccator to lower the temperature and re-weighed using analytical scales. The results were recorded and calculated using the formula of the percentage of mass loss as follows:

$$\%\ \text{mass loss} = \frac{\text{initial mass-final mass}}{\text{initial mass}}\ \text{x}100\%$$

Morphological Cell Bacteria Analysis

The morphological cell bacteria analysis was using gram coloring method to disguise the bacterial categories, whether it is Gram positive or Gram negative bacteria. Then observed it using light microscope to see the bacteria color, cell shape, and colony shape. Put one drop of sterile distilled water into microscope slides. After that, the bacterial culture that grew in NA medium were taken by ose needle and put into the distilled water drop. In the next step, bacterial cultures were killed by fixation. The dried part of bacteria on top of the microscope slides is called a smear. After the smear was dried, 1-2 drops of primary stain (violet crystal stain) were applied on top of the smear, allowed it to stay for 1 minute and rinsed the slide gently with water. Next, air-dried the slides and applied 1-2 drops of Lugol solution then allowed it to stay for 1 minute and rinsed the slide gently with water. To get rid of the stained, a few drops of alcohol 95% were applied for just 10-20 seconds or until the stained was diminished. Applied a few drops of Safranin solution and allowed it to stay for just 10-20 seconds and rinsed the slides

with water gently then air-dried the slides. Finally, the slides were able to be viewed under a microscope using 10x100 magnified which helped to observe the morphology of bacteria.

Results and Discussion

1st Screening

This 1st screening process was aim to find bacteria that able to degrade LDPE plastic. This has be done by using visual observation of the culture growth and after observed the sample shook for 1 hour at a speed of 120 rpm. The quantitative data was getting from checking the level of turbidity and the level of dirtiness by using spectrophotometry at the end of incubation time.

These are the samples documentation for daily sample observations and using a code as the sample name. The 1st capital alphabet is describe the sample location at A, B, or C location when sampling; the number in the middle describe the sample obtained from spot number 1, 2, or 3; the lowercase alphabet describe the sample obtained from the 1st or 2nd dilution test; the sign (-) describe sample is the repeated treatment in 1st screening process.

Table 1. Observation result at B2b and B2b- samples

1st Day / 24 hours		2nd Days / 36 hours	
B2b sample	B2b- sample	B2b sample	B2b- sample
5th Days / 110 hours		**6th Days / 158 hours**	
B2b sample	B2b- sample	B2b sample	B2b- sample

From the table 1, it was obtained visually that the longer incubation time, the level of media turbidity will more decrease and the level of dirtiness will more increase. The presence of these impurities was indicated as the presence of microorganism cells that clump with one another. There are a lot of hypothesis and theory behind of the cell clumping and for this occasion the closest theory is about the formation of biofilms on the LDPE surfaces. Biofilms is a mechanisms which allow the bacterial cell to adapt in the environment by producing some proteins such as collagen, fibrin, fibronectin, and laminin as a coating material that enhance the ability of cell-to-cell adhesion in biofilm. The mechanism shown as a matrix of extracellular polymeric substance (EPS) and form a three-dimensional structure that depends on the environment conditions. The inside of this biofilm can be a single species or mixed population of microorganism (Pathak and Navneet 2017b). The LDPE powder as a substrate attracting the bacteria to make a biofilm and it was probably visually observed as a biofilm.

On the table 1, it can be seen that bacteria was able to grow only by utilizing carbon sources from LDPE plastics for their growth energy. Bacteria was using the LDPE plastic powder as a sole carbon source by cutting them into a small molecules by using extracellular enzyme and further absorbed by microbial cells and proceeds by intracellular enzyme (Gu, 2003). The spectrophotometry test was also done in this stage to reinforce the visual observation result in turbidity and dirtiness from the sample.

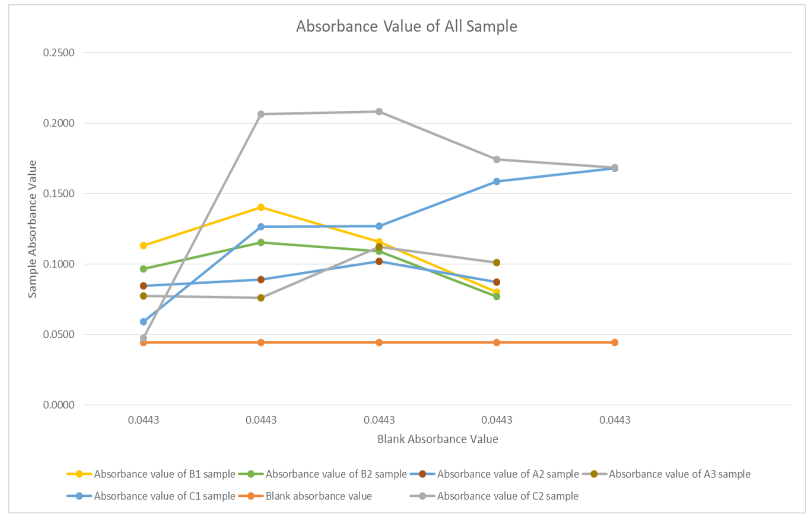


Figure 5. Absorbance value result in the end of incubation time

From this graph (figure 5), it was found that all samples contained microorganisms that grew in them because the absorbance value of the sample is higher than the blank of 0.0443 which only contained growth media. Thus it can be said from the screening stage 1 has been found that bacteria have the potential to degrade LDPE. It can be seen from the graph that a high absorbance value means that there is a significant growth of the bacteria and the forming of biofilms.

2nd Screening

This screening stage 2 began with the growth of bacteria which was able to degrade LDPE plastic in a solid Mineral Salt Agar media so that later the growing bacteria which could be isolated and obtain a pure culture. This stage used 2 treatments for carbon sources in the form of LDPE plastic sheets and also LDPE powder. These serves to see the effectiveness of bacteria to grow and find the best method to be able to isolate pure culture easily. These are the result that obtained from the second screening using Mineral Salt Agar media with the addition of LDPE powder.

Table 2. 2nd screening observation in mineral salt agar media with LDPE powder

4th Days / 96 hours		6th Days / 144 hours	
A2c** sample	B2a- sample	A2c** sample	B2a- sample

Table 3. 2nd Screening observation in mineral salt agar media with LDPE sheets

2nd Days / 48 hours		4th Days / 96 hours	
A2c** sample	B2a- sample	A2c** sample	B2a- sample

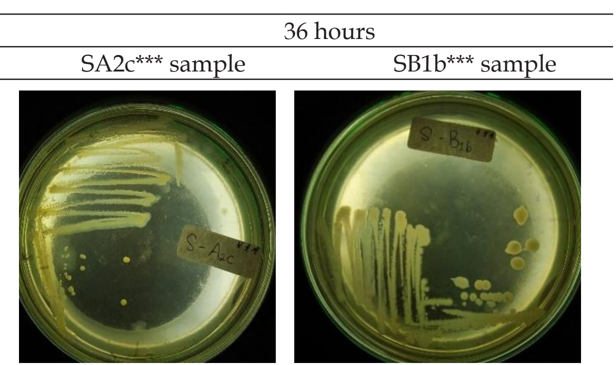
**sample was the result of the current sample repeat screening stage 2.

On table 3, it can be seen that there are bacterial colonies which grew attached to the edges of the 5 LDPE sheets inside the petri dish, making it easier to isolate LDPE plastic degrading bacteria than in the plates with LDPE powder (table 2) because the growth was spreading in all parts of the petri dish. Some bacteria grew not attached either to the LDPE plastic sheet or LDPE powder. It can happen because other carbon sources accidentally contained in the media used such as agar as a growth media, which is one of the polysaccharides that have carbon chains in their constituent structures. Then the bio-surfactant Tween 80 is a modulator of hydrophobic interaction between the organism and the polymer (Gilan *et al.*, 2004; Talkad *et al.*, 2014), so it may modulate the biodegradation process, tween 80 which is polysorbate 80 or polyethylene sorbitol ester assuming

20 units of ethylene oxide, 1 sorbitol, and 1 oleic acid. With this explanation it can be seen that there are 2 sources of carbon chains namely sorbitol with the chemical formula $C_6H_{14}O_6$ (PubChem), then oleic acid with the chemical formula $C_{18}H_{34}O_2$ (PubChem). Because of not having media comparison without using tween 80, the researcher assumes that the survived bacteria were able to degrade the ethylene that comes from tween 80 and also comes from LDPE powder and plastic sheets. The next step was isolated the culture to separate it from other species in the plate and the results obtained in the isolation method are as follows:

Table 4. Observation result of bacterial isolation

36 hours	
SA2c*** sample	SB1b*** sample



Code S = sample obtained from the culture that uses LDPE sheets (strips)
*** sample was a result of sample repetition from the sample with ** at screening stage 2

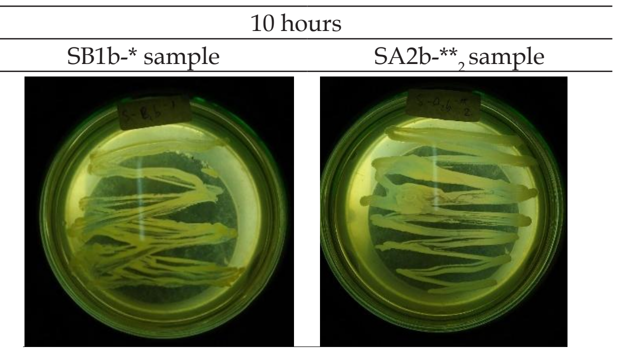
Therefore, the researcher selected the sample for the next stage based on the characteristics of the samples which are still considered to be able to grow and be isolated such as having a surface that was still slimy or wet. The isolation stage is done to obtain pure culture distribution in a small colony by using the quadrant streak method on the petri dish containing NA media (table 4).

Induce Mutations with UV Rays

These are the results of growing bacteria for 10 hours at 37^0C and before they get irradiate:

Table 5. 10 hours of incubation for preparation before UV rays treatment

10 hours	
SB1b-* sample	SA2b-**$_2$ sample



Code S = sample obtained from the culture that uses LDPE sheets (strips)
**sample was a result of sample repetition at screening stage 2
[2] sample was a different culture but is located on the same plate

The purpose of using UV rays is to create microbiological biodiversity and their functions in the ecosystem. Beside it, UV rays can be used as a way to improve the biocatalytic activity of microorganisms for a specific purpose. UV rays radiation can be divided into three groups, there are UV A (320 – 400 nm), UV B (280 – 320 nm), and UV C (10 – 280 nm) (Santos *et al.*, 2012). Santos said that UV rays with shorter wavelengths will be able to kill and decrease the activity of microorganisms, while longer-wavelength will be able to broke the DNA structure and restructure it into a better characteristic such as a capability to survive in the extreme condition. In his journal, Santos said that "the DNA reaction into UV A ray has a very strong responsibility which is shown as the growth of ROS (Reactive Oxygen Species) generation, fat oxidation (TBARS), and carbonylation of protein. UV B rays also give an impact with a medium effect between UV A and UV C, which can improve the ability of bacteria to survive better than before by breaking down the structure of TBARS and DNA so bacteria can change the structure and function of the membrane during the exposure of UV B. The effects from UV C radiation is more related to the extent of damage to DNA and producing

mutagenic organisms as well as their inhibitory effects to the organism activity".

From the above explanation, the researcher decides to use UV A and UV B rays to find their impact on increasing the ability of bacteria to degrading LDPE plastic material. Jagger (1995) at (Santos *et al.,* 2012) said that "the UV irradiation will have more damage effect in Gram-negative bacteria than in Gram-positive bacteria because of the shielding effect from the cell wall". Research from Zeng *et al.,* (2011)found that induced mutation to *Deinococcus ficus* with the UVC exposure energy is 999 mJ/cm2 able to produce 2 mutant strains of *D. ficus* (CC-ZG207 and CC-ZG227) which have opposite result between two of them. The existence of the *lexA-imuB-dnaE2* gene cassette in *D. ficus* serving the possibility of creating superior keratinolytic mutant after the UV induction process. This was working well too in this project, by using UV A and UV B to mutate the bacterial sample before the LDPE biodegradation proceeds.

Effectiveness Test of LDPE Degradation Rate

The result of incubation process for 112 hours for knowing degradation rate of bacteria are as follows:

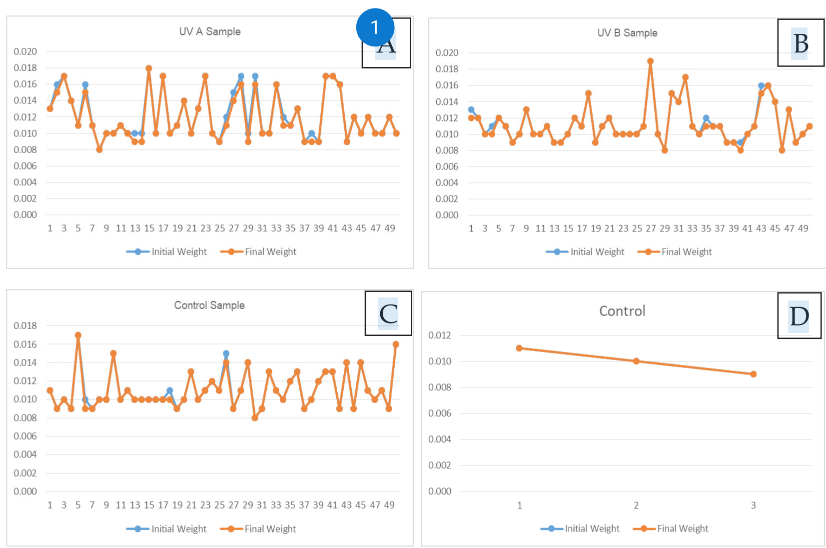


Figure 6. The result of LDPE plastic degradation by using bacteria

Total of 11 LDPE Sheets are reduced in weight at UV A sample, 5 LDPE sheets are reduced in weight at UV B sample, and 3 LDPE sheets are reduced in weight at the Control sample (figure 6). The change in mass of the plastic was 10% (0.001 g) of the initial weight, Based on the results, it can be said that the decomposition rate of LDPE plastics will increase if the culture is irradiated using UV A and UV B. It was compared with the cultures which do not get any UV rays at all.

According to another research at the same topic (Singh *et al.*, 2016), their bacteria show a positive result of biodegradation rate at 10 days (240 hours) of incubation, with a decrease of plastic weight 0.003 gram for the 10- micron thickness of polyethylene and 0.001 gram for the 40-micron thickness of polyethylene. Another research from (Zusfahair *et al.*, 2007) shown a result that in 5 days of incubation, the polyethylene can loss 0.46% of initial weight.

Identification of Bacteria

The following figure is the morphological shape of bacteria culture.

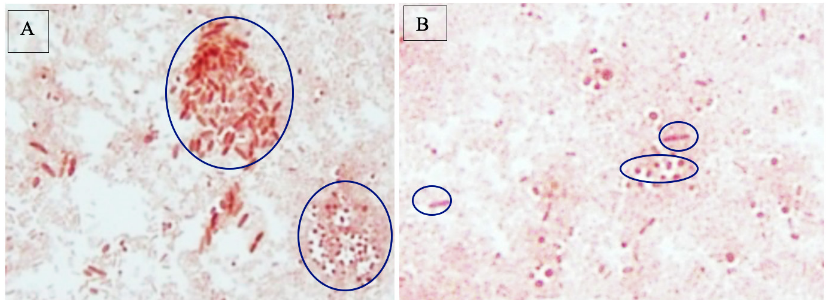


Figure 7. Picture A and B is a SA3c** sample

At this stage, the researcher has not been able to finish the research well for this time, since after observing morphologically with a microscope, it can be known that the bacteria used are still mixed colonies (figure 7). Therefore if the researcher wants to proceed to the identification stage of the bacteria, it needs a long time to isolate the culture to obtain a pure bacterial culture consisting of only one species. These are the documentation we took using a light microscope.

Conclusion

UV A and UV B rays irradiation have an impact on the ability of bacteria in degrading LDPE plastic compared to non-treatment bacteria. Even though the effect of the UV irradiation can be known, the species of the bacteria still unknown, considering the inadequate research time. The result of this research is able to be developed and applied on a large scale, such as for degrading LDPE plastic in the landfills.

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different sites (figure 1). Each site consists of three spots (figure 2,figure 3, and fig...

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Figure 4. Sampling spot in location CMaking the LDPE (Low Density Polyethylene) ...

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The screening of soil bacteria was divided into two steps. Onthe first step, a dilutio...

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x100%initial massMorphological Cell Bacteria AnalysisThe morphological cell bact...

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From the table 1, it was obtained visually that the longerincubation time, the level ...

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Figure 5. Absorbance value result in the end of incubation timeFrom this graph (fig...

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Table

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4th Days / 96 hoursA2c** sampleB2a- sample**sample was the result of the curre...

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