



# BIODEGRADASI *STYROFOAM* OLEH BAKTERI TANAH DARI TEMPAT PEMBUANGAN AKHIR SARIMUKTI CIPATAT BANDUNG

## *STYROFOAM BIODEGRADATION BY SOIL BACTERIA ISOLATED FROM LANDFILL SITE IN SARIMUKTI CIPATAT BANDUNG*

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### Abstrak

*Styrofoam* digunakan sebagai kemasan makanan atau minuman, dibentuk oleh stiren dan benzena. Migrasi benzena dari bahan kemasan ke makanan dapat menyebabkan berbagai penyakit. Cara untuk mengurangi limbah *styrofoam* adalah dengan mencari bakteri yang dapat mendegradasi *styrofoam* secara alami. Sumber potensial untuk menemukan bakteri tersebut adalah di Tempat Pembuangan Akhir Sarimukti. Metode penelitian ini menggunakan metode eksplorasi yang dianalisis secara deskriptif kualitatif. Tahapan penelitian terdiri atas uji biodegradasi dengan metode *Winogradsky Column*, perhitungan persentase penurunan berat kering *Styrofoam*, analisis fisik dengan *Scanning Electron Microscope* (SEM), dan analisis perubahan gugus fungsi dengan FT-IR. Hasil penelitian ini ditemukan 4 spesies bakteri pendegradasi polistiren yaitu *Pseudomonas aeruginosa*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, dan *Bacillus firmus*. Persentase penurunan berat kering polistiren menunjukkan pada minggu kedelapan mencapai 18,23% dan analisis fisik dengan *Scanning Electron Microscope* (SEM) menunjukkan bahwa proses degradasi oleh bakteri tanah menghasilkan pembentukan pori-pori di permukaan *Styrofoam*. Analisis gugus fungsi menunjukkan bahwa gugus fungsi menjadi lebih sederhana setelah degradasi dengan munculnya gugus fungsi C-O pada bilangan gelombang 1.030,02  $\text{cm}^{-1}$ . Bakteri pendegradasi polistiren dari tempat pembuangan akhir Sarimukti ini dapat direkomendasikan sebagai metode yang ramah lingkungan untuk mengurangi limbah *styrofoam*.

**Kata kunci:** Bakteri; Degradasi; Polistiren

### Abstract

*Styrofoam* is commonly used in food and beverage packaging. *Styrofoam* or polystyrene is made from styrene and benzene. The migration of benzene from packaging materials into foods can cause various diseases. One effort to reduce *styrofoam* waste is possible by searching for bacteria that can degrade *styrofoam* naturally. Potential source of place where the bacteria will be discovered is the landfill site in Sarimukti. This research applied exploratory methods through descriptive qualitative analysis. The stages of the study consisted of biodegradation testing using the *Winogradsky* method, calculation of the percentage of dry weight loss of *Styrofoam*, physical analysis using *Scanning Electron Microscope* (SEM), and analysis of changes in functional groups using FTIR. This study discovered 4 species of polystyrene degrading bacteria, namely *Pseudomonas aeruginosa*, *Bacillus amyloliquefaciens*, *Bacillus cereus* and *Bacillus firmus*. The percentage of dry weight reduction of polystyrene was shown in the eighth week which reached 18.23% and physical analysis by *Scanning Electron Microscope* (SEM) indicated that the process of degradation by soil bacteria resulted in formation of pores on the surface of *styrofoam*. Functional group analysis produced a simpler functional groups after the degradation as marked by the appearance of C-O functional groups at wavenumber of 1,030.02  $\text{cm}^{-1}$ . The use of these polystyrene degrading bacteria from Sarimukti landfill can be recommended as an environmentally friendly method for reducing *styrofoam* waste.

**Keywords:** Bacteria; Degradation; Polystyrene

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## INTRODUCTION

Styrofoam is widely used for various purposes, for example as a protective electronic material even for packaging foods or beverages. This waste does not only harm the environment but also bring a negative impact on human health. The environmental impact of the small component of polystyrene, which is found on the surface and throughout the water column, is likely to be digested by marine organisms and is a threat to ecosystems in the ocean. If ingested by organisms, it can endanger the quality of marine catches. Other impacts can pose a risk to public health from eating fish and shellfish that have ingested polystyrene fragments (Brink, Schweitzer, Watkins, & Gionfra s gionfra, 2017). Other health impact as mentioned by the International Agency for Research on Cancer (IARC) in 2014 which determined that styrene is a carcinogen. This is based on the results of research on styrene metabolites (i.e. styrene oxide) as epoxides that are very chemically reactive with ability to bind to DNA and trigger carcinogenesis. Differences between individuals in metabolism may lead to different effects of these carcinogens, as well as possible differences in susceptibility to carcinogenicity between species and individuals (National Toxicology Program, 2014).

The method of styrofoam waste treatment which is only limited to waste disposal will put burden on nature regarding waste decomposition (Fitidarini & Damanhuri, 2011). Therefore, we need other methods that are environmentally friendly to reduce styrofoam waste. One method is possibly applied through biodegradation process that utilizes microorganisms that are able to deteriorate synthetic polymers in the environment. Bacteria are the most dominant group of microorganisms in the soil and cover half of the microbial biomass in the soil. Polymer biodegradation process can be carried out using *Rhodococcus pyridinivorans* (Kundu, Hazra, & Chaudhari, 2015), *Pseudomonas putida* and *Pseudomonas aeruginosa* (Hilliard & Jacob, 2013), *Enterobacter* sp., *Citrobacter sedlakii*, *Alcaligenes* sp. and *Breyundimonas diminuta* with a degradation rate of 12.4% for 30 days (Mohan, Sekhar, Bhaskar, & Nampoothiri, 2016), *Staphylococcus aureus*

and *Streptococcus pyogenes* (Asmita, Shubhamsingh, Tejashree, Road, & Road, 2015).

Sarimukti Landfill is managed by the West Java Solid Waste Management Center (P3JB) of West Java Province, which serves the Bandung Metropolitan Region in the western region including Bandung City, Cimahi City, Bandung Regency and West Bandung Regency. The Sarimukti Landfill (TPA) is the only Waste Recycling Site to date, where all waste generation in the waste service area of West Bandung Regency reached  $140 \text{ m}^3 \text{ day}^{-1}$  disposed of at the Sarimukti Landfill, while its capacity is  $\pm 900 \text{ ton day}^{-1}$  (Kusumaningrum, 2018).

Many problems are caused by the existence of plastic waste in the environment, making plastic waste a major focus in the field of research, thus raising concern on plastic biodegradation. Biodegradation can be carried out by microorganisms such as bacteria, fungi, yeast and algae. Biodegradation of organic materials can occur both aerobically or anaerobically. The process of biodegradation occurs through several stages, namely biodegradation, biofragmentation, assimilation, and mineralization (Guzman, Gnutek, & Janik, 2011).

One type of bacteria that has been investigated for its ability to degrade polystyrene is *Pseudomonas* that generally does not have hydrolytic enzymes that are important in degrading polymers, but have an inducible operon system that is capable of producing certain enzymes in the process of metabolizing carbon sources that are not normally used. The type of enzymes produced by *Pseudomonas* spp. which play a role in biodegradation are serine hydrolase, esterase, and lipase. *P. aeruginosa* could change polystyrene into acetyl CoA which enters the TCA cycle. The result of the degradation of styrene into acetyl CoA was not able to break the benzene compound, hence polystyrene degradation by *P. aeruginosa* still left toxic compounds and not environmentally friendly (Nugraha, 2018).

The control samples showed a smooth surface, while degradation of polystyrene with *Pseudomonas* and *Bacillus* treatments showed the formation of holes or pores on the surface.

Percentage of reduction in dry weight after incubation treatment was obtained by a decrease of 23.7% in *Bacillus* sp. and less than 10% in *Pseudomonas* sp. (Mohan et al., 2016). The process of biodegradation can be observed in the presence of bacterial biofilms in polystyrene. Characterization in the calculation of weight loss percentage and physical analysis using Scanning Electron Microscope (SEM) was indicated by the occurrence of degradation process by soil bacteria. and analysis of changes in functional groups with FTIR also can help prove the existence of the degradation process.

The purpose of this study was to characterize morphological changes of polystyrene after degradation. The expected results are bacteria from landfill site in Sarimukti could degrade polystyrene, therefore the use of these bacteria will necessarily be a recommended method to reduce Styrofoam waste.

## MATERIALS AND METHODS

The soil samples used in this study were collected from landfill site in Sarimukti, Bandung, while styrofoam samples were obtained from styrofoam seller in the community. This study applied exploratory methods through descriptive qualitative analysis.

### Biodegradation Test

Styrofoam samples were cut into a diameter of 2 cm. Bottles were filled with soil sample and Mineral Salt Medium (1:1). This medium contained 12.63 grams MSM Broth Base (M1864) in 1,000 mL distilled water. Approximately one vial of Growth Supplement I was added into MSM (FD28) and one vial of Growth Supplement II was put into MSM (FD288). Styrofoam was bottled then incubated at room temperature for 1–8 weeks (Sriningsih & Shovitri, 2015).

### Calculation of Percentage of Dry Weight Loss

This stage was conducted weekly. The pieces of Styrofoam were put into a tube, added with 13 mL of sterile distilled water, then homogenized using vortex. Styrofoam pieces were separated to be weighed (Ainiyah & Shovitri, 2014). Styrofoam pieces were

sterilized, dried at 80 °C for 24 hours, and further measured to obtain its dry weight loss (Sriningsih & Shovitri, 2015).

### Analysis of Scanning Electron Microscopy (SEM)

Styrofoam samples were sprayed with 70% alcohol, mounted on the SEM specimen holder using a double-sided adhesive carbon tape with a cross-section that was directed vertically upward or to the objective lens. The sample chamber is at vacuum up to  $10^{-6}$  torr. SEM was operated following the standard operating parameters. Topographic images were observed (Sujatno, Salam, Dimiyati, & Bandriyana, 2015).

### Analysis of functional group changes by FT-IR

Styrofoam samples (1 mg) were put into mortars and KBr was added (20 mg). Samples were crushed and placed at the mold and pressed using a mechanical pressure device to form tablets, then placed at the sample site in an infrared spectroscopy for analysis (Moreira et al., 2018).

## RESULTS

### Biodegradation by Bacteria

The results of degradation was indicated by the presence of biofilm on the surface of styrofoam. Biofilm produced from these results were then identified using Vitek 2 Compact Biomerieux and identified 4 species of bacteria, i.e. *Pseudomonas aeruginosa*, *Bacillus amyloliquefaciens*, *Bacillus cereus* and *Bacillus firmus*.

### Calculation of Percentage of Dry Weight Loss

The dry weight loss was already found since the first week of observation. The graph of the percentage of styrofoam dry weight loss (Figure 1) shows an increase from the first week to the fourth week, while there was a slight decrease in the fifth week, but it increased again from the sixth week until the eight week that reached 18.23%.

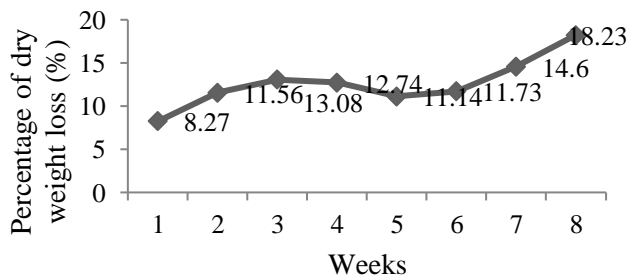
### Analysis of Scanning Electron Microscopy (SEM)

Morphological changes can indirectly be seen through Scanning Electron Microscope

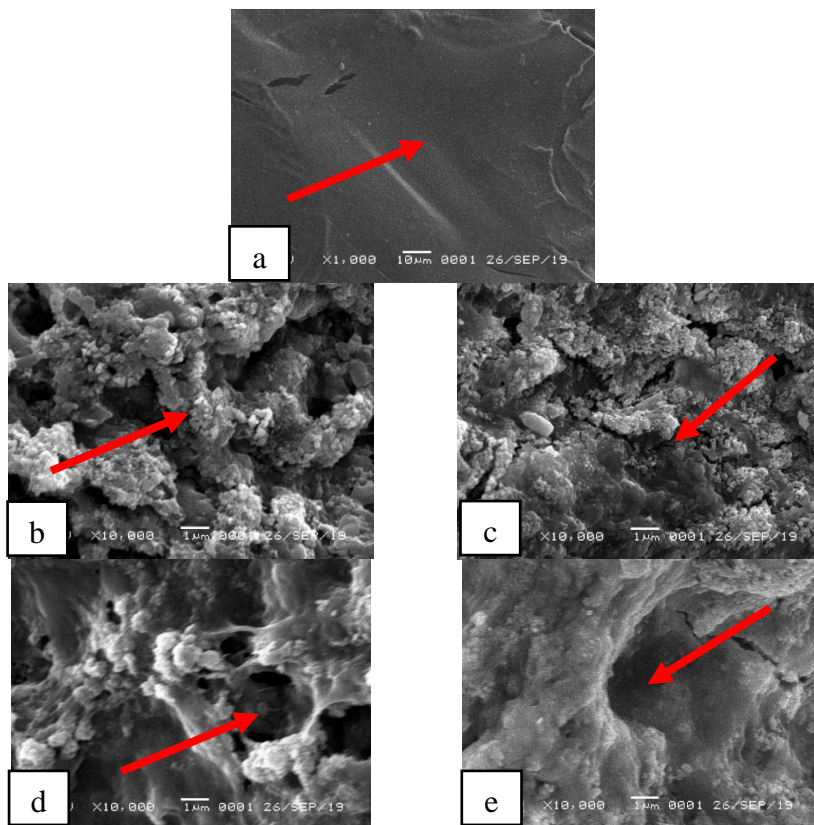
(SEM), resulted in changes in styrofoam surface. Before incubation, styrofoam had smooth and flat surfaces, whereas holes and pores were observed on the surface of styrofoam after incubation. The longer the incubation period, the bigger and wider the hole or pore formed on the surface. The overall degradation process on the styrofoam surface is shown in Figure 2.

**Analysis of functional group changes by using FT-IR**

FT-IR analysis showed a change in functional groups starting from the second week after incubation. Changes in this functional group are characterized by the appearance of new wave peaks at certain wavenumber regions in the FT-IR chart, indicating a redox reaction carried out by the bacteria during the incubation period. Styrofoam control depicted several peaks in the spectrum (Figure 3). The results of this analysis are displayed in Table 1 to facilitate the analysis of changes in functional groups in the Styrofoam.



**Figure 1.** Percentage of Dry Weight Loss



**Figure 2.** SEM images of degradation process. Before degradation (a), 1 week of degradation (b), 3 weeks of degradation (c), 5 weeks of degradation (d), 7 weeks of degradation (e)

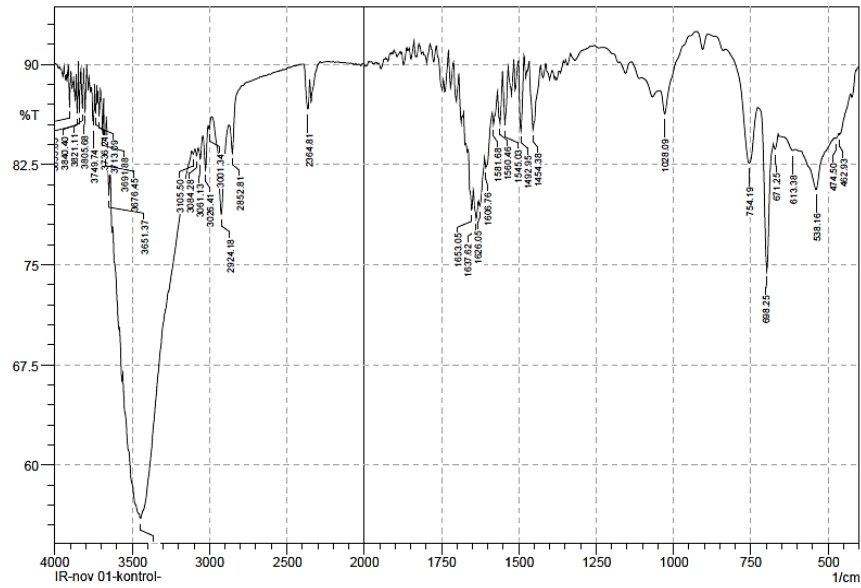


Figure 3. FT-IR analysis results for control treatment

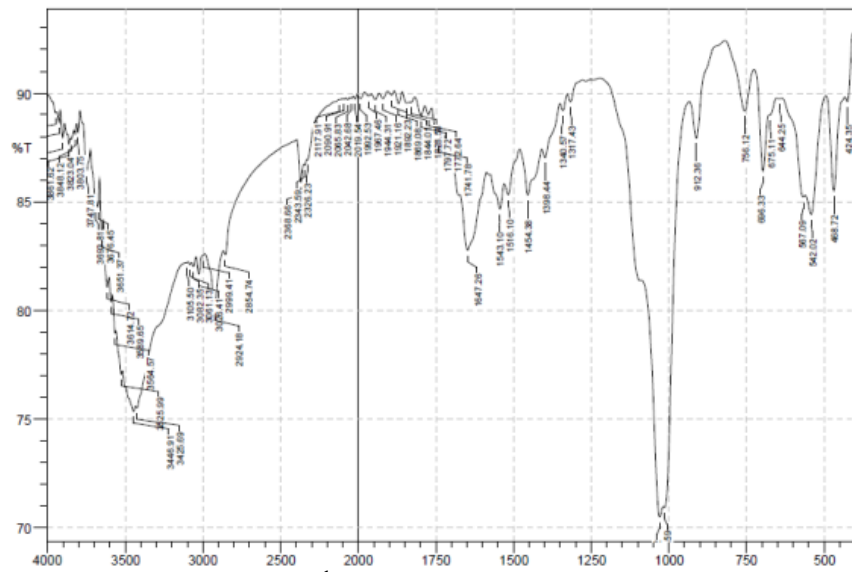


Figure 4. FT-IR analysis results in the 2<sup>nd</sup> week

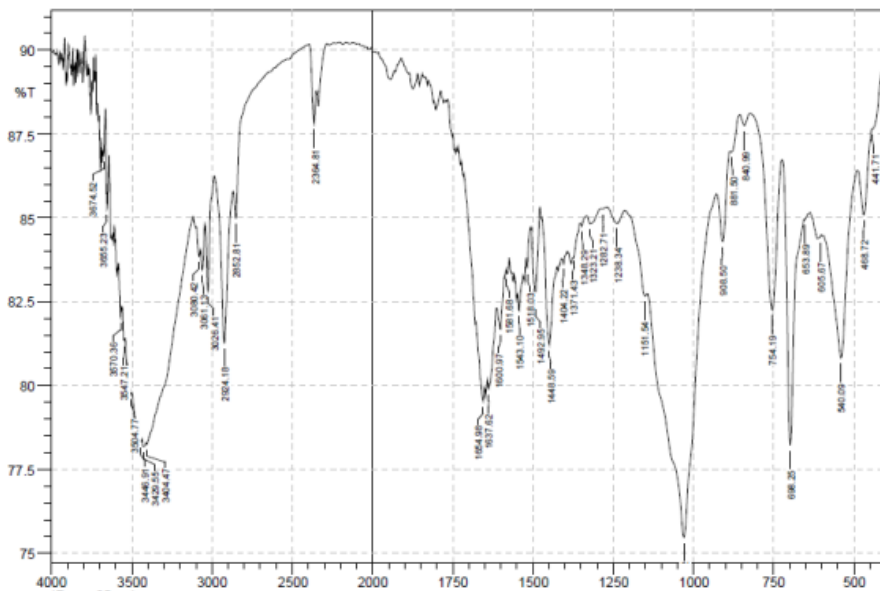


Figure 5. FT-IR analysis results in the 4<sup>th</sup> week

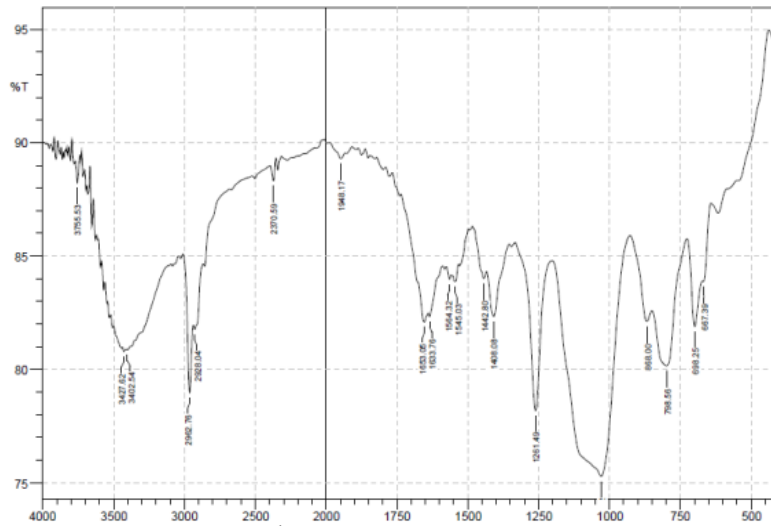


Figure 6. FT-IR analysis results in the 6<sup>th</sup> week

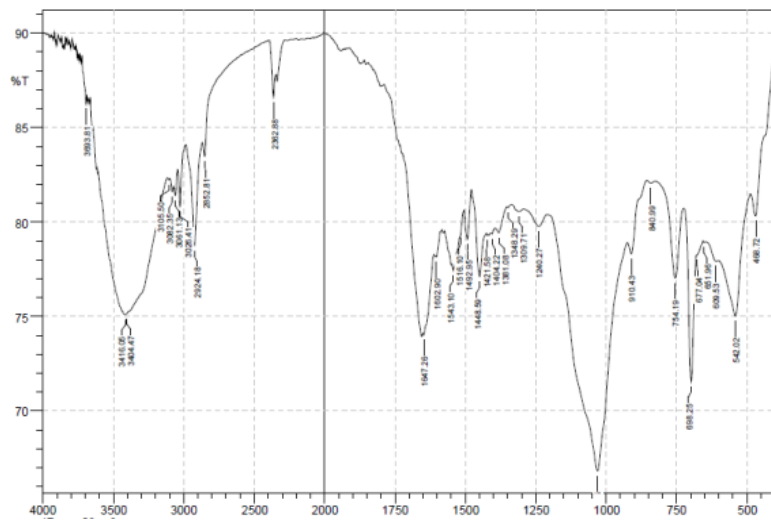


Figure 7. FT-IR analysis results in the 8<sup>th</sup> week

Table 1. FT-IR spectrum of polystyrene by week

Weeks	Wavenumber (cm <sup>-1</sup> )	Functional group
control	698	C-H
	2,924	C-H and CH <sub>2</sub>
	3,446.91	O-H
2	696.33	CH
	1,030.02	C-O
	2,924.18	C-H and CH <sub>2</sub>
4	3,446.91	OH
	698.25	CH
	1,030.02	C-O
6	2,924.18	C-H and CH <sub>2</sub>
	3,446.91	OH
	698.25	CH
8	1,030.02	C-O
	2,962.76	C-H and CH <sub>2</sub>
	3,427.62	OH
	698.25	CH
	1,031.95	C-O
	2,924.18	C-H and CH <sub>2</sub>
	3,416.05	OH



## DISCUSSION

The Winogradsky column method was applied in this study for biodegradation testing. This method can show the ecological picture of soil bacteria in an ecosystem as well as the stratification of electron donors for each layer. The Winogradsky column method used bottles filled with soil from the Landfill site and Mineral Salt Medium (1:1). Soil is a source of bacteria that can degrade polymer since bacteria from Landfill site mostly have adapted to their environment with abundant organic and inorganic waste, including Styrofoam. MSM is used as a nutrient medium for growing bacteria, but in the absence of carbon source bacteria are expected to obtain carbon source from polystyrene.

The biodegradation process will be more effective by using types of microorganisms that have different degradation characteristics. Some microorganisms play an important role in the breakdown of polymers, while some other utilize monomer. According to (Villaverde, Rubio-Bellido, Merchán, & Morillo, 2017), a bacterial consortium can perform more than one task in a population. Biodegradation of synthetic polymers requires mineralization carried out by various enzymes and synthetic pathways are usually not present in a single strain and carried out by a consortium. Research on the microbial consortium has been widely developed in the process of degradation of synthetic polymers, such as mixed cultures from *Lysinibacillus xylanilyticus* and *Aspergillus niger* isolated from Tehran landfill soil, has been used for the degradation of synthetic polymers in the Low Density Polyethylene (LDPE) group.

Microorganisms play a role in a biological degradation of polymer. Bacteria will break down the polymer into monomer to be further used in the metabolism to produce energy sources. Biodegradation can occur due to cooperation between bacteria that can degrade polystyrene, i.e. bacteria that produce specific enzymes that is able to break down polystyrene into simpler styrene monomers.

Bacteria isolated and identified in this study consisted of 4 species, namely *Pseudomonas aeruginosa*, *Bacillus amyloliquefaciens*, *Bacillus cereus* and *Bacillus firmus*. Asmita et al. (2015) revealed

that polystyrene can be degraded by bacteria like *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Aspergillus niger*, which exist in different types of soils. The methods used in the present set of experiments are easy to perform, cost effective, and environmentally friendly. Bacteria successfully identified from the Bengkala Landfill in Buleleng belonged to 10 bacterial genera, namely: *Acetobacter*, *Paracoccus*, *Bacillus*, *Agrobacterium*, *Zooglea*, *Lactobacillus*, *Alcaligenes*, *Acinetobacter*, *Microbacterium* and *Carnobacterium* (Ristiati, Suryanti, & Indrawan, 2018).

Biodegradation is the process by which organic material is broken down by microorganisms such as bacteria and fungi. During the polymer biodegradation process, exoenzymes from microorganisms break down complex polymers into smaller molecules, such as oligomers, dimers and monomers that are small enough to pass through semipermeable bacterial membranes and then are used as a source of carbon and energy and release products such as CO<sub>2</sub> and H<sub>2</sub>O (Pramila & Ramesh, 2015).

The simplest method for measuring the biodegradation of a polymer is to determine the weight loss and degradability of the polymer. Weight loss is determined by calculating the weight difference in sample pieces after the incubation period. The calculation of dry weight loss percentage of styrofoam resulted in a percentage of 8.27% in the first week after the incubation process. This weight loss of styrofoam was caused by soil bacteria that used polystyrene as a carbon source for their metabolism. This study used mineral salt medium which does not contain carbon source, thus providing bacterial environment and forcing bacteria to use polystyrene as a carbon source for their metabolism. The changes in styrofoam dry weight loss percentage as displayed in the chart might due to changes in bacterial metabolic patterns, sensitivity characteristics, and antagonistic characteristics between bacterial species in the same environment.

*Bacillus* bacteria have the potential to degrade plastics, because they are able to grow in Winogradsky columns for 4 months of

incubation period. Degradability percentage of *Bacillus* isolates for degrading white and black plastics were 1.9% and 2.3%, respectively (Fadlilah & Shovitri, 2014). Inoculum of microorganisms in the final disposal site was found to be able to degrade plastic with monthly average percentage of dry weight loss for clear white and black plastic of 1% and 1.87%, respectively. Microorganism inoculums in the landfill site were isolated and biochemically characterized to discover 13 isolates of waste soil bacteria that were able to degrade plastic (Ainiyah & Shovitri, 2014).

The morphology of polyethylene after biodegradation in compost inoculated with bacteria was examined and observed using SEM. Smooth surfaces of polyethylene was eroded as a result of the presence of biodegradation. Many bacterial cells are attached to the surface of polyethylene and can be seen through SEM (Gyung Yoon, Jeong Jeon, & Nam Kim, 2012).

The direct and indirect effects of the bacterial degradation process on styrofoam can be seen after an incubation process for 1 week. Morphological changes possibly observed directly were changes in color, texture and formation of biofilms on the surface of the Styrofoam. Before incubation, the styrofoam surface had a clean white color with a smooth and flat texture, but the surface color of the styrofoam turned brown with a rough and slimy texture after 1 week incubation. The change in color, texture and formation of mucus is expected due to the growth of Styrofoam degrading bacteria on the Styrofoam surface.

Mohan et al. (2016) in their research using High Impact Polystyrene (HIPS) found that samples without microbial treatment had smooth surfaces, while organisms were found to be around the surface of samples treated with microbes besides the formation of holes that indicated degradation by microbes.

The results of the FT-IR analysis were produced in the form of a spectrum with several peaks in the absorption area at a specific wavenumber. The peak of the spectrum has a different intensity and band shape, higher intensity indicates more number of functional groups. The shape of the band on the FT-IR spectrum shows the purity and

specifications of the functional groups that vary depending on the type.

The wavenumber of  $698\text{ cm}^{-1}$  in the control styrofoam has C-H functional groups, the wavenumber of  $2,924\text{ cm}^{-1}$  has C-H and  $\text{CH}_2$ , and the wavenumber of  $3,446.91\text{ cm}^{-1}$  has O-H. The functional groups are in line with the literature for functional groups in polystyrene, except for the absence of O-H. The presence of O-H groups in the FT-IR analysis may be caused by several factors, including the trapping of water or alcohol compounds in the styrofoam during sterilization process before the FT-IR analysis was carried out. The FT-IR analysis in week 1 did not show any new functional groups since there was only an increase in intensity and the peak point of wavenumber in O-H functional group. Both of them showed broad band, hence O-H functional group could be observed earlier in the first week. The FT-IR analysis in the 2<sup>nd</sup> week showed a new functional group, namely the C-O functional group which was detected at a wavenumber of  $1,030.02\text{ cm}^{-1}$ . This functional group has strong intensity and narrow band. The C-O functional group was formed due to the activity of bacteria that conducted the process of polystyrene degradation. This further reaction is expected to be an esterification reaction which can produce a functional group in the form of C-O.

The FT-IR analysis in the 4<sup>th</sup> week showed that C-O functional groups were detected at wavenumber of  $1,030.02$ ,  $1,448.50$  and  $1,654.98\text{ cm}^{-1}$  with intensity ranged from moderate to strong. The band belonged to the C-O functional group was observed to be wide and sharp. Strong intensity is related to the number of functional groups present in the reaction. The reaction observed in this (4<sup>th</sup>) week is estimated to be the same esterification reaction that can produce a functional group in the form of C-O which existed in the previous week, only the reaction occurred more often. Result of FT-IR analysis in the 6<sup>th</sup> week showed that C-O functional groups were detected at three wavenumber points with range of intensity from moderate to strong. The band belonged to C-O functional group was found to be narrow and wide. The reaction in this (6<sup>th</sup>) week is estimated to be the same esterification reaction as in the previous week.



FT-IR analysis in the 7th and 8th week showed that C-O functional groups were detected at three wavenumber points and the intensity of these functional groups ranged from moderate to strong in the 8th week. The band belonged to the C-O functional group was indicated to be wide, narrow and sharp. The reaction of this (8<sup>th</sup>) week is expected to be similar to the esterification reaction which can produce a functional group in the form of C-O as in the previous week.

As confirmed by Umamaheswari and Margadan (2013) FTIR spectroscopy showed that stretching in the bonds like C=C, C-H, OH, C-O, and C=O of polymer in styrofoam buried in soil, cow-dung, and sewage can be degraded by fungi by cleaving bonds of styrofoam polymer.

This study found several bacteria that can degrade styrofoam. The bacteria are expected to have adapted to the soil in Sarimukti area. This adaptation is necessary for these bacteria to survive in a nutrient-poor environment and force the bacteria to obtain energy sources from styrofoam inorganic waste.

Based on the results of this study, there are indications of changes that occurred in the styrofoam morphological structure, indicating the degradation process carried out by Styrofoam degrading bacteria from Sarimukti landfills. The results of this study can be used as a reference in further research as an environmentally friendly method for reducing or handling styrofoam waste.

## CONCLUSION

*Styrofoam* degrading bacteria isolated from Sarimukti landfill were identified as *P. aeruginosa*, *B. amyloliquefaciens*, *B. cereus*, and *B. firmus*. Percentage of reduction in dry weight of polystyrene amounted to 18.23% in the eighth week and physical analysis using a *Scanning Electron Microscope* (SEM) showed the formation of holes or pores on the *Styrofoam* surface. The FT-IR analysis indicated the occurrence of decomposition which resulted in a simpler functional group.

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