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Styrofoam Biodegradation By Soil Bacteria Isolated From Landfill Site In Hospital: Literature Review

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ABSTRACT

Introduction: 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 hospital. **Material and Methods:** 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. **Results:** 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. **Conclusion:** 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 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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1. 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(Ricardo Barra & STAP, 2018).

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(Cavalier et al., 2023).

The method of styrofoam waste treatment which is only limited to waste disposal will put burden on nature regarding waste decomposition(Wahyuni et al., 2024). 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 o microorganisms in the soil and cover half of the microbial biomass in the soil. Polymer biodegradation process can be carried out using *Rhodococcus*(Cappelletti et al., 2020), *Pseudomonas putida* and *Pseudomonas aeruginosa, Enterobacter* sp., *Citrobacter sedlakii*, *Alcaligenes* sp. and *Breyundimonas diminuta* with a degradation rate of 12.4% for 30 days, *Staphylococcus aureus and Streptococcus pyogenes* (Bidoia et al., 2021).

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,

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fungi, yeast and algae. Biodegradation of organic materials can occur both aerobically or anaerobically. The process of biodegradation occurs through several stages, namely biodeterioration, biofragmentation, assimilation, and mineralization (Putcha & Kitagawa, 2024).

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(Maisyaroh et al., 2024).

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.(Chaudhary & Vijayakumar, 2020).

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 hospital could degrade polystyrene, therefore the use of these bacteria will necessarily be a recommended method to reduce Styrofoam waste (Ural, 2021).

2. Research Method

Research Design, Setting, and Sample

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The soil samples used in this study were collected from landfill site in hospital, while styrofoam samples were obtained from styrofoam seller in the community. This study applied exploratory methods through descriptive qualitative analysis. Units resources and recovery in existing conditions in order to treat waste water by PICO Analysis.

Measurement and Data Collection

The research began with a field survey, literature study, hypothesis determination, data collection for the evaluation process in the form of primary data and secondary data, recapitulation or data collection, evaluation of each operational unit and process unit, calculation and planning of the effectiveness of the Styrofoam. Other additional data collected included photos of all processing units in the of hospital.

Data Analytics

To discuss the evaluation carried out in this study, it was carried out by comparing data from hospital. The following are the results of the evaluation of the Styrofoam of hospital.

a) **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 (Erlambang et al., 2019).

b) **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. Styrofoam pieces were sterilized, dried at 80·C for 24 hours, and further measured to obtain its dry weight loss (Ainiyah & Setiawan, 2024).

c) **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 verticall upward or to the objective lens. The sample chamber is at vacuum up to 10–6

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torr. SEM was operated following the standard operating parameters. Topographic images were observed (Niko & Basuki, 2022).

d) Analysis of functional group changes by FTIR

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 (Sintim et al., 2021).

3. Results And Discussions

1) 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. 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(Duc et al., 2022), 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(Lim & Thian, 2022).

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 CO2 and H2O(Devi et al., 2023).

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2) Calculation of Percentage of Dry Weigh 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%.

Table 1.
FT-IR spectrum of polystyrene by week (Bidoia et al., 2021)

Weeks	Wavenumber (cm-1) Function	Functional group
control	698	C-H
	2,924	C-H and CH2
	3,446.91	OH
2	696.33	CH
	1,030.02	C-O
	2,924.18	C-H and CH2
	3,446.91	OH
4	698.25	CH
	1,030.02	C-O
	2,924.18	C-H and CH2
	3,446.91	OH
6	698.25	CH
	1,030.02	C-O
	2,962.76	C-H and CH2
	3,427.62	OH

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8	698.25 1,031.95 2,924.18 3,416.05	CH C-O C-H and CH2 OH

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 (Kim et al., 2021).

The wavenumber of 698 cm-1 in the control styrofoam has C-H functional groups, the wavenumber of 2,924 cm-1 has C-H and CH2, and the wavenumber of 3,446.91 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 (Meng et al., 2021). The FT-IR analysis in the 2^{nd} week showed a new functional group, namely the C-O functional group which was detected at a wavenumber of 1,030.02 cm-1. This

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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(Ziad et al., 2022).

3) **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. (Bidoia et al., 2021)

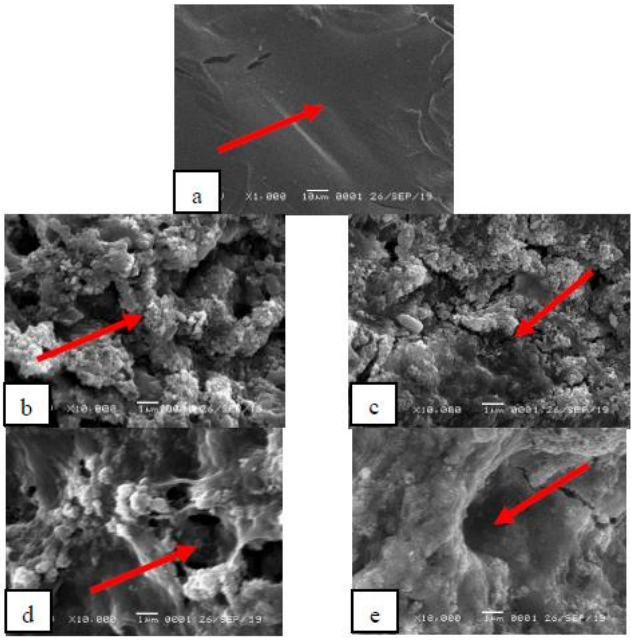

Figure 2. SEM images of degradation process. Before degradation (a), 1 week of degradation (b), 3 weeks of degradation (c), 7 weeks of degradation (d). (Bidoia et al., 2021)

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 (Zhang et al., 2022).

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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 (Zhang et al., 2022).

(Wang et al., 2024) 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.

4. 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.

5. Compliance With Ethical Standards

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Disclosure of conflict of interest

There is no potential for any stakeholder to have a conflict of interest in this research.

Statement of informed consent

In our capacity as writers, every action we perform constitutes a joint agreement or consent.

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