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Methodology for Analyzing the Capability of Microorganism to Deal with Polystyrene Waste Through Biodegradation

Balqis Ramadhillah[1*], Filson Maratur Sidjabat[2]

[1,2]Environmental Engineering Department, President University, Jawa Barat
***Correspondent author**: balqissr01@gmail.com

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Abstract
The challenge to solve plastic pollution in Indonesia has always been a concern for environmentalists, Moreover, the polystyrene plastic resin has been proved as the major contributor for marine debris in Indonesia coastline. This paper attempts to discuss and examine the analytical methodology to measure the ability of biodegradation by microorganisms in dealing with polystyrene waste using the literature review method and findings from prior studies. The analytical methodologies for measuring the capability of microorganisms in degrading polystyrene is examined. Thus, the most frequent and common analytical methodologies used to measure the biodegradability of polystyrene by microorganisms are the FTIR spectroscopy, SEM, GPC, and TGA. These analytical methodologies along with other complementary methods have proved that specific bacteria, fungi, and worms are capable of degrading polystyrene waste. This perhaps may be one of the less toxic, environmentally friendly solutions for plastic pollution in Indonesia, specifically polystyrene based waste.
Keywords: *microorganism, plastic pollution, polystyrene, biodegradation, analytical methodology*

Abstrak
Tantangan untuk mengatasi pencemaran plastik di Indonesia selalu menjadi perhatian para pemerhati lingkungan, apalagi resin plastik polistirena yang telah terbukti sebagai penyumbang utama sampah laut di garis pantai Indonesia. Makalah ini mencoba untuk membahas dan mempelajari metodologi analisis untuk mengukur kemampuan biodegradasi oleh mikroorganisme dalam menangani limbah polistirena dengan menggunakan metode tinjauan literatur dan penemuan dari penelitian terdahulu. Metodologi analisis yang paling sering dan umum digunakan untuk mengukur kemampuan biodegradasi polistirena oleh mikroorganisme adalah spektroskopi FTIR, SEM, GPC, dan TGA. Metodologi analisis ini bersama dengan metode pelengkap lainnya telah membuktikan bahwa bakteri, jamur, dan cacing tertentu mampu mendegradasi limbah polistirena. Ini bisa menjadi salah satu solusi yang kurang beracun dan ramah lingkungan untuk polusi plastik di Indonesia, terutama sampah berbahan dasar polistirena.
Kata Kunci: *mikroorganisme, polusi plastik, polistirena, biodegradasi, metodologi analisis*

1. Introduction

Plastic pollution has always been a challenging issue that requires immediate action and solution, particularly in Indonesia. The Ministry of Environment and Forestry of Indonesia reported a significant increase in waste generation on national level of about 64 million tons in 2019 [1]. Of equal importance, Indonesia has been infected by the Covid-19 virus since early 2020 which forced the world to experience a pandemic and regional lockdown. Local residents are being quarantined and are restricted to engage in normal activities outside the house and be quarantined. If outdoor activities are truly necessary, personal protective equipment (PPE) must be used. This increases the waste generation of single use plastics, like masks, sanitizer bottles, food and good packaging, as well as takeout plastic bags [2].

The durability properties of plastic make it available in the Earth body for centuries and is accumulated both on the land and aquatic environment. Plastic debris in marine environments is mostly derived from land [3]. A study conducted in Jakarta Bay proved that 59% of marine debris is in the form of plastic [4] The same author also conducted a case study in northern coastal waters of Surabaya and revealed that the most dominant polymer type of plastic debris is polystyrene, which contribute to approximately 58.4 % [5]. The statement is supported by further study which confirmed that the polymers dominating marine debris is polystyrene, contributing to almost half of the total percentage (about 44.6%), followed by polypropylene and polyethylene, which accounted for about 29% and 15% respectively [6].

Polystyrene is commonly recognized with the term Styrofoam. In fact, Styrofoam is a trademark name introduced and popularized by Dow Chemical in 1937 [7]. It is a plastic resin polymer substance with

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Styrene as its co-monomer combined with air to form foam. Despite the fact that styrene itself is known to be toxic for both human body and the environment, polystyrene shows a great performance as one of the safest food packaging as the National Agency of Drug and Food Control (BPOM) have stated that Styrofoam or polystyrene that is used for food packaging contain styrene substance for about 10 to 43 ppm which is far below the standard limit proposed by European Union Directive Migration Limit, which is below 1000 ppm, and below 5000 ppm according to World Health Organization and Food Agriculture Organization [8], [9]. Be that as it may, BPOM do not recommend using Styrofoam for food containing oil or fat and alcohol as it may trigger the release of the toxic styrene monomer. Moreover, the International Agency for Research on Cancer (IARC) categorized styrene as carcinogenic substance class 2B which may activate cancer cells in the human body when exposed to styrene [10].

Consequently, proper disposal of polystyrene waste is very essential to minimize the risk of negative consequences. However, most of the polystyrene waste ends up in landfill without appropriate treatment. The objective of this paper is to discuss how biodegradation could be used to treat polystyrene in landfills. Biodegradation is considered as one of the environmentally-friendly and safe ways to treat plastic waste. In theory, biodegradation is the process of decomposing complex substances into simpler molecules with the help of microorganisms via enzymatic activity [11].

2. Material and Methods

The literature review approach was utilized to complete this paper which involves collecting, analyzing, and correlating the findings of prior studies. This paper only considered literature with the focus on biodegradation of polystyrene. Literature review approach serves as the basic understanding and overview of the chosen topic [12]. In order to stay relevant with current events and concerns, we limit the publication year of the literature to be in the last five years, or to put in other words, the literature that was published from 2017 to 2022. Literature was obtained from online information sources and databases, like Google Scholar, Elsevier, Springer, ResearchGate, and Open Access Journal.

There are a total of eight literature being reviewed in this paper, discussing various microorganisms that have the potential of degrading polystyrene, such as worms or larvae, fungal culture, and bacteria. Analytical methodology used to examine the degradation rate of each microorganism are evaluated. All the literature was going through intense evaluation to extract relevant findings and the key points are summarized in Table 1. Several other literatures were also studied to get a better understanding of the biodegradation concept.

Table 1. Literature on Polystyrene Biodegradation

Authors	Year	Study Location	Microorganism	Analysis Method	References
Peng et al.	2019	China	*Tenebrio obscurus* larvae or dark mealworm and *Tenebrio molitor* larvae or yellow mealworm	THF extraction, GPC, FTIR, TGA	[13]
Yanto et al.	2019	Indonesia	Fungi: *Pestalotiopsis sp., Ceriporia sp., Cymatoderma dendriticum.* Bacteria: *Pseudomonas aeruginosa, Bacillus Subtilis, Serratia marcescens.*	DEV FTIR SEM	[14]
Wang et al.	2020	China	*Acinetobacter* bacterium, *Tribolium castaneum*	TGA, SEM	[15]
Kim et al.	2020	South Korea	*Pseudomonas aeruginosa* isolated from (Larvae of *Zophobas atratus*)	SEM ATR-FTIR TGA	[16]

Authors	Year	Study Location	Microorganism	Analysis Method	References
Peng et al.	2020	China and U.S.	Larvae of *Zophobas atratus*	GPC FTIR ^{1}H NMR TGA	[17]
Yang et al.	2020	China	*Zophobas atratus*	GPC NMR FTIR DTG	[18]
Hidayat et al.	2020	Indonesia	*Pseudomonas aeruginosa, Bacillus amyloliquefaciens, Bacillus cereus,* and *Bacillus firmus*	SEM FTIR	[19]
Chaudhary et al.	2020	India	Fungal culture: *Cephalosporium sp. Mucor sp.*	WRM FTIR SEM DTG GC-MS GPC	[20]

Source: Research data (2022)

3. Results and Discussion

Popular analytical methodologies used to examine biodegradation rate are Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), and Gel Penetration Chromatography (GPC), and Thermogravimetric Analysis (TGA). Explanation of how each methodology assists analysis of biodegradation of polystyrene by microorganism is further described as follows.

Fourier Transform Infrared (FTIR) analysis applies spectral observation of functional group formation as the evidence of chemical changes in the polystyrene after degradation. FTIR usually uses the spectroscopy device Nicolet iS50 spectrometer. The absorbance spectrum of polystyrene demonstrates evidence that a new functional group formed during the biodegradation process. Brandon et al. [21] demonstrated that FTIR spectra of polystyrene incubated with microbiome shows oxygen absorption as indicated by peak appearance corresponding to R-OH stretch in wavelength range of 3000-35000 cm-1 and C-O stretching around 1000-1300 cm-1. In addition, benzene derivatives were also observed, marked by the spectral peak around 700-750 cm-1 associated with C-H bending as shown in **Figure 1**.

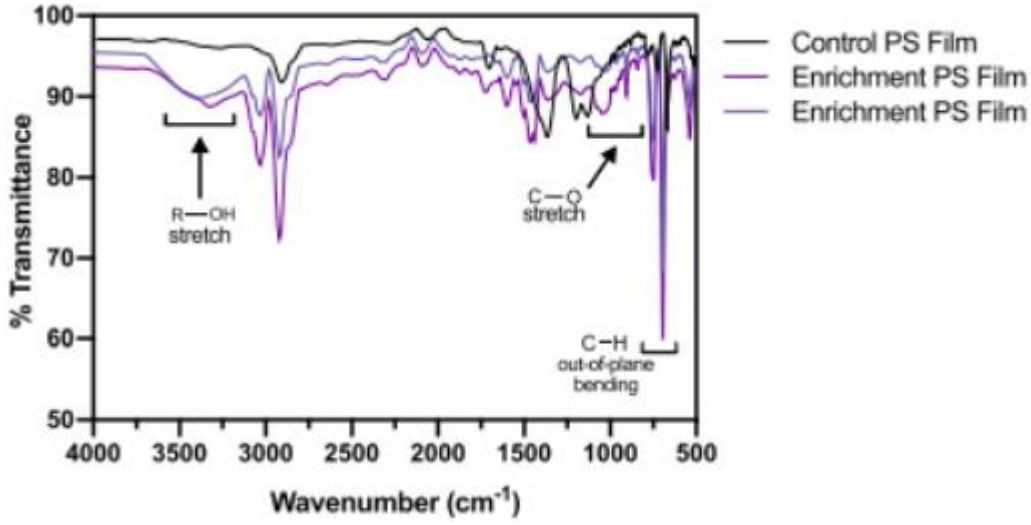

Figure 1. FTIR spectra of polystyrene incubated with microbiome.
Source: Brandon et al. (2021)

Peng et al. [13] support the idea and confirmed that degradation of polystyrene by Tenebrio obscurus (dark mealworm) and Tenebrio molitor (yellow mealworm) which demonstrate a decrease in polystyrene

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concentration in frass samples indicated by lower benzene ring observed around 625-970 cm-1 on the FTIR spectra. The occurrence of oxidation and depolymerization processes in both mealworms are confirmed by the appearance of peaks associated with the formation (−C−O−) around 1075-1150 cm-1, (−C=C− stretch) on 1600 cm-1, (−C=O stretch) around 1700 cm-1 signify the new simpler carbonyl group formation [13]. Similar result was also found in polystyrene biodegradation with isolated fungi Pestalotiopsis sp. [14], Pseudomonas aeruginosa bacteria [14], [16], larvae of Zophobas atratus [17], [18], and bacteria Bacillus sp. [19].

Scanning Electron Microscopy (SEM) observed the subtle physical damages on the surface of polystyrene as the result of biofilm presence which trigger penetration of microorganisms resulting in initiation of degradation and cavities formation using electron microscope. This method is also used to confirm the proliferation of polystyrene by microorganisms [16]. Hidayat et al. discover holes and pores on the surface after several weeks incubation with degrading bacteria *Bacillus sp*. [19]. Incubation of polystyrene with bacteria Acinetobacter sp. for 30 days detects formation of biofilm and cavities in the SEM imagery shown in **Figure 2** [15].

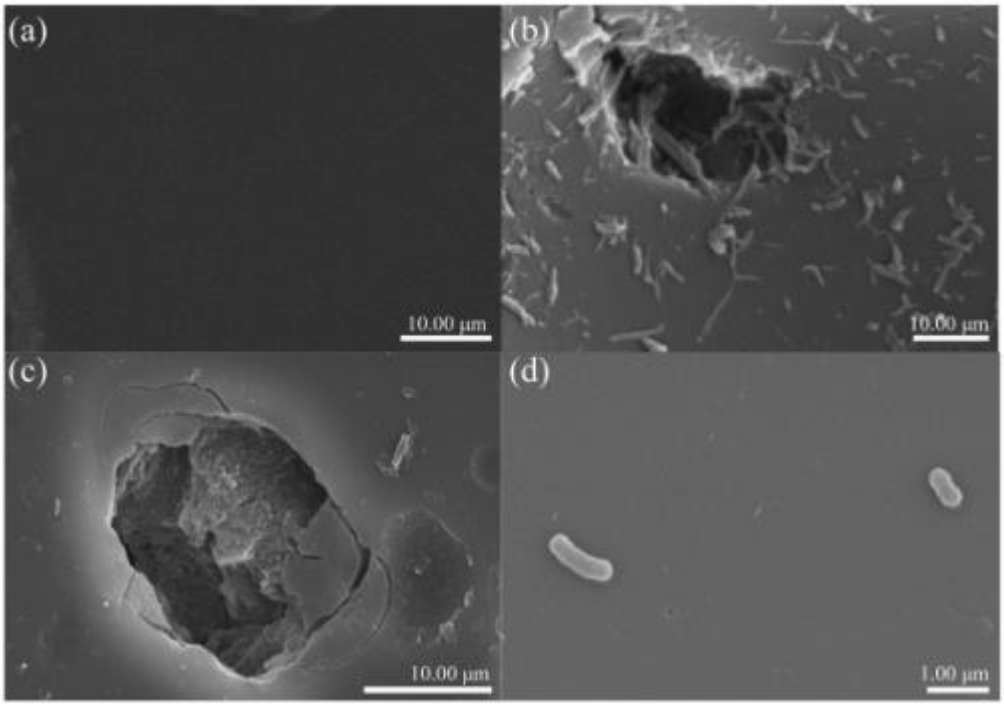

Figure 2. Polystyrene degradation
Source: Wang et al. (2020)

Figure 2a represents the polystyrene control (without incubation) as comparative reference, **Figure 2b** illustrates the *Acinetobacter sp*. degraded film, **Figure 2c** illustrates cavities after incubation with *Acinetobacter sp*., and **Figure 2d** illustrates the *Acinetobacter sp*. itself. **Figure 3** illustrates the optical images of polystyrene surface with respect to incubation time of degradation by Superworm or *Zophobas atratus* [18].

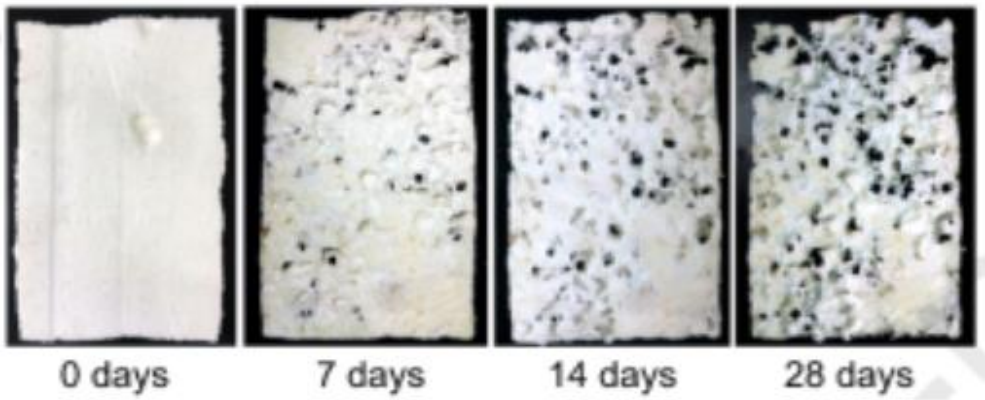

Figure 3. Polystyrene degradation by Superworm.
Source: Yang et al. (2020)

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Gel Penetration Chromatography (GPC) analysis provides information about change in molecular weight of the polystyrene. It measures the average molecular weight in terms of number (Mn), average molecular weight in terms of weight itself (Mw), and molecular weight distribution (MWD) as key factors of polymer modification, depolymerization, and degradation [13], [17]. Common trend in plastic biodegradation is broad depolymerization which include the decrease in both Mn and Mw as well as a shift to lower MWD as evidence of enzymatic depolymerization of microorganism [17].

Thermogravimetric Analysis (TGA) analysis estimates the thermal changes of polystyrene biodegradation into frass. The process observed decomposition of complex polystyrene under continuously increasing temperature, usually from 40 ºC to 800 ºC, under high purity nitrogen flow for protection. Thereafter, samples were cooled down to 500 ºC and heated again to 800 ºC in air ambience [13], [15].

Other analysis methodologies include Derivative Thermogravimetry (DTG), Tetrahydrofuran (THF) extraction, which has the objectives to determine residual polystyrene in frass, the microorganism excrement [13]. Gel Permeation Chromatography (GPC), which is important for analyzing changes of molecular weight of the polystyrene [13]. Degradation Efficiency Value (DEV), which is used to determine the degradation efficiency by finding the percentage of sample weight compared to control weight and is given by the following expression:

$$DE = \frac{W0-W1}{W1} \times 100\%$$

Where, W0 represents the control weight and W1 represents the sample weight [14].

Proton Nuclear Magnetic Resonance (1H NMR) is used by a study conducted by Wang et al. [15] which is used as an analytical tool for monitoring degradation and demonstrating there might be new hydrogen-containing groups in polystyrene biodegradation process. NMR spectrometer analysis is also employed by Yang et al. to characterize the chemical composition of frass and polystyrene. The fundamental concept of NMR is to assign specific resonance signals to detect the presence of carbonyl group molecules both in the polystyrene and the frass by observing their spectrum [18].

Differential Thermogravimetric (DTG) is employed by Yang et al. [18] and Chaudhary et al. [20] to determine the weight reduction or weight loss by analysis of thermal stability of the polystyrene samples before and after incubation period. Chaudhary et al. revealed that all the polystyrene samples were completely decomposed after 8-weeks incubation with fungal culture in a temperature range of 450 ºC to 600 ºC [20]. Meanwhile Yang et al. shows significant weight loss up to 98% at temperature range of 360ºC to 480ºC after being degraded by superworms [18]. **Figure 4** demonstrates the DTG curve for polystyrene degradation by fungal culture, *Cephalosporium sp*. and *Mucor sp*.

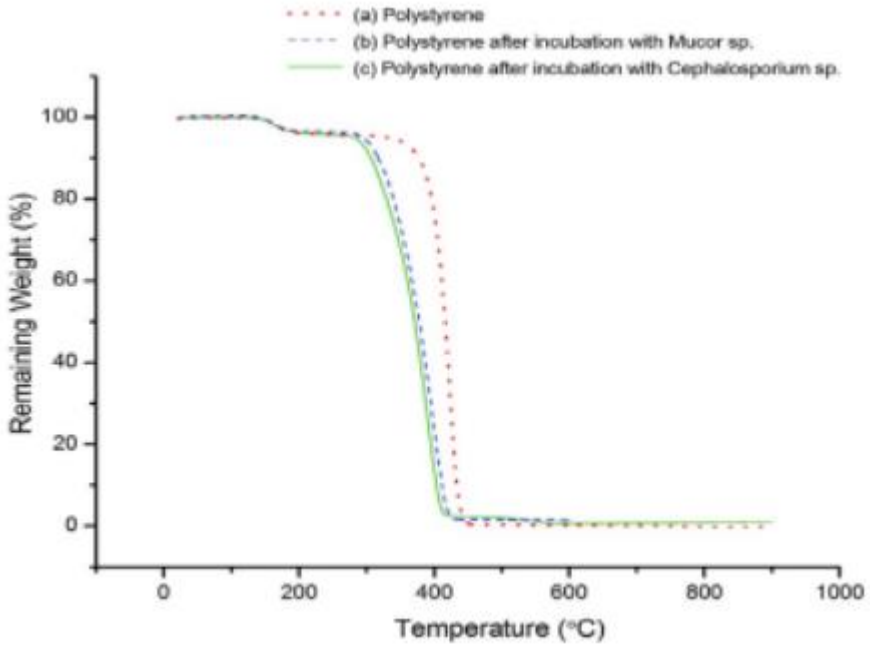

Figure 4. DTG curve for polystyrene degradation by fungal culture.
Source: Chaudhary et al. (2020)

Where, Mi represents the initial weight of polystyrene before degradation and Mf represents the weight after incubation period. However, this equation does not correspond to thermal stability like DTG analysis.

Gas Chromatography-Mass Spectrometer (GC-MS) has the objective of identifying the presence of alkanes, alkenes, acids, alcohols, antioxidants, and aromatic hydrocarbon compounds in the by-product of degradation process. Chaudhary et al. used this analysis method for biodegradation using pure fungi culture, *Cephalosporium sp*. and *Mucor sp*. The list of compounds identified after degradation is represented in **Table 2** and **Table 3** for each fungus respectively [20].

Table 2. Compounds identified after polystyrene degradation with *Cephalosporium sp*.

S. No.	Retention time (min)	Area %	Name of the compound
1.	3.803	29.32	Pyridine
2.	4.276	5.97	1,3,5 Cycloheptatriene
3.	6.951	100	Benzene, Chloro
4.	16.973	5.64	Methane, tris(methylthio)-
5.	21.425	3.35	1H-Indene,2,3-dihydro-1,1,3-trimethyl-3-phenyl
6.	21.99	3.29	Benzene, 1,1'-(1,1,2,2-tetramethyl-1,2-ethanediyl)bis-2,4-Diphenyl-4-methyl-2(E)-pentene
7.	22.329	12.25	2,4-Diphenyl-4-methyl-2(E)-pentene
8.	24.954	2.16	Pentadecanoic acid, 2,6,10,14-tetramethyl-,methyl ester 13-Tetradece-11-yn-ol
9.	25.403	1.59	13-Tetradece-11-yn-ol
10.	25.975	2.4	9-Octadecanoic acid, ethyl ester
11.	26.399	10.56	Octadecanoic acid, ethyl ester

Source: Chaudhary et al. (2020)

Table 3. Compounds identified after polystyrene degradation with *Mucor sp*.

S. No.	Retention time (min)	Area %	Name of the compound
1.	2.154	10.53	n-Hexane
2.	2.634	3.6	Cyclohexane
3.	3.802	31.88	Pyridine
4.	4.276	9.83	1,3,5 Cycloheptatriene
5.	6.95	100	Benzene, chloro
6.	16.973	4.43	Methane, tris(methylthio)-
7.	20.268	4.27	2,4-Diphenyl-4-methyl-1-pentene
8.	20.336	1.97	Benze, (1,1-dimethyldecyl)-
9.	21.492	3.22	Phenol, 2,4-bis(1-methyl-1phenylethyl)
10.	22.408	1.93	1H-Indene,2,3-dihydro-1,1,3-trimethyl-3-phenyl

Source: Chaudhary et al. (2020)

After going through previously mentioned analysis methodologies, biodegradation capability of microorganisms can be concluded. Peng et al. (2018) [13] summarized the consumption rate of T. obscurus or dark mealworm under sole diet of polystyrene and incubation time 31 days is 5.06±0.08 mg/day PS per g larvae or 32.44±0.51 mg/day PS per 100 larvae. Meanwhile, T. molitor or yellow mealworm shows smaller consumption rate of 3.89±0.47 mg/day PS per g larvae or around 24.30±1.34 mg/day PS per 100 larvae [13]. Consumption rate of superworm, *Zophobas atratus*, is observed by Yang et al. (2020) to be the average close to 0.58 mg/day PS per larvae or approximately 58 mg/day PS per 100 larvae, almost double the consumption rate of yellow mealworm [18].

A group of fungi, for instance, *Cymatoderma dentricium* and *Ceriporia sp*. shows the ability of degrading polystyrene up to 15.7% and 19.4% within 30 days respectively. The same study reported that fungi *Pestalotiopsis sp*. demonstrate significant capability of degrading polystyrene for more than half of the total percentage or approximately 74.4% [14]. Polystyrene incubated with fungi *Cephalosporium sp*. and *Mucor sp*. for eight-weeks period demonstrate weight loss around 2.17% and 1.81% [20].

Yanto et al. (2019) [14] observed the ability of bacteria to degrade polystyrene and revealed that Serratia marcescens is able to degrade 38.3% polystyrene. Much greater ability is shown by *Bacillus subtilis* and *Pseudomonas aeruginosa* which contribute to 52.6% and 63.4% polystyrene degraded [14]. Other *Bacillus sp.* bacteria were also observed by Hidayat et al. (2020) which stated that they have the capability to degrade polystyrene as they reduce the dry weight of polystyrene by about 18.23% [19].

4. Conclusion

Studies on the capability of microorganisms to degrade polystyrene have confirmed that there are fungi, larvae or worms, and bacteria that depend on polystyrene waste as carbon and energy source for growth. The fundamental ways for determining a biodegradation of polymer is to measure the loss weight and analyze degradability of the polymer. The most frequent and common analytical methodology used for both measurements are the Fourier Transform Infrared (FTIR) spectroscopy for examining the characterization of degraded molecule, Scanning Electron Microscopy (SEM) for detecting physical changes on the surface of polystyrene, Gel Penetration Chromatography (GPC) for measuring molecular weight of the depolymerized molecule, and Thermogravimetric Analysis (TGA) for observing thermal stability of decomposition process. These analytical methodologies along with other complementary methods have proved that specific bacteria, fungi, and worms are capable of degrading polystyrene waste. This perhaps may be one of the less toxic, environmentally friendly solutions for plastic pollution in Indonesia. Moreover, the fact that depolymerization process of the microorganism is carried out by enzymatic activities suggest the demand for further study about extraction of the enzymes for easier and better biodegradation and plastic recycling alternatives.

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