Artikel Biodegradable Plastics

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Research Paper

Biodegradable Plastics: Biodegradation Percentage and Potential Microplastic Contamination in Seawater

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Abstract

Increasing plastic production, which causes the problem of plastic garbage polluting the oceans, has increased the use of biodegradable plastics to address the problem. However, it is still debatable how much microplastic contamination it can cause. So, this study aims to determine the proportion of biodegradable plastics in the marine environment, identify the microplastics it produces, and analyze the relationship between the two. Seawater sampling is located in the Bangka Strait. The research was conducted in the Genetics and Biotechnology Laboratory, Department of Biology, Sriwijaya University. The biodegradable plastic test material used was made from a mixture of polyhydroxyalkanoates (PHA) and starch. Biodegradation test method using standard ASTM D6691-17 with respirometry system design. The stage of microplastic identification is carried out through filtration with a 4.75 mm-size filter; density separation using $ZnCl_2$ solution and Whatman No. 1 filter paper; as well as visual observation of microplastics under a microscope. last Pearson Correlation analysis with bootstrap to see the relationship of the percentage of biodegradation with microplastics. The results obtained in this study were the percentage of biodegradable plastic (26.5$\pm$1.4%) and positive control kraft paper (33.2$\pm$4.2%) for 70 days, which produced 9 microplastic particles from biodegradable plastic with fragment and film types. Correlation analysis concluded that there was no relationship between the percentage of biodegradation and the microplastics produced.

Keywords

Biodegradable Plastic, Biodegradation Percentage, Microplastic

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1. INTRODUCTION

Plastic production in the world in the past 5 years has continued to increase. In 2017, world plastic production was at 348 million tons and in 2021, despite experiencing stagnation during the COVID-19 pandemic, world plastic production data reached 390.7 million tons. In 2021, there will also be a significant increase in bio-based plastic production at 5.9 million tons compared to 2020 with a total production of 3.9 million tons (PlasticsEurope, 2018; PlasticsEurope, 2022).

The increase in the world plastic production has made people aware of the impact of plastic waste on the environment. Limiting the use of plastic, banning the use of plastic, and increasing plastic waste processing, are various efforts that have been made to solve the problem of plastic waste. But with the comparison between higher plastic production and, the low understanding of society about wise waste management, these efforts have not been enough to have an impact on a wider scale (Shen et al., 2020).

Poorly managed plastic waste from land can end up in the marine environment due to polluted river flows (Lebreton and Andrady, 2019). In 2016 it was estimated that 19 to 23 million metric tons, or 11% of the total plastic waste generated globally, entered aquatic ecosystems. Given the trend of government commitments, this figure will reach 53 million metric tons per year by 2030 (Borrelle et al., 2020).

The current development of biodegradable plastics in the 21st century is important for the global environment, especially in an effort to support environmentally sustainable development (Shen et al., 2020). Biodegradable polymers have been touted as a solution to the plastic waste problem, but the controlled and uncontrolled environmental degradation processes of these materials are still not fully understood. This will be a problem when people do not understand how to manage the end-of-life of these polymers and dispose of them in the environment. The blending of biodegradable polymers to increase their functionality raises other concerns about how they impact waste management and the environment (Narancic et al., 2018).

There is a strong suspicion that biodegradable plastics will also generate microplastics. The reason is that during the degradation process, changes in the physical characteristics of the polymer cause the polymer to easily fragment and become biodegradable microplastics (Liao and Chen, 2021). Basically, biodegradable plastics were created to easily facilitate the degradation process from long-chain carbon into small debris to H_2O, CO_2, and biomass. However, it remains unclear whether biodegradable plastics can be used to solve the world's plastic waste problem (Shen et al., 2020).

The capability of biodegradable plastics to biodegrade naturally in seawater without causing microplastic contamination is still a matter of debate among researchers therefore, this study aims to determine how much biodegradable plastics can degrade in seawater and identify the impact of microplastic contamination from biodegradation, so that the relationship between them can be determined.

2. EXPERIMENTAL SECTION

2.1 Materials

The seawater used was collected at the surface in the Bangka Strait using sterilized 20-liter jerry cans. Kraft paper is used as a positive control, and biodegradable plastic bags are a commercially available under the Avani-eco brand with a starch and polyhydroxyalkanoates (PHA) base material. Chemicals used such as 70% alcohol, distilled water, Merck HCl solution (0.50 N), Smart-Lab 30% H_2O_2, Merck NaOH (0.25 N), Merck NH_4Cl, Merck $KH_2(PO_4)$, Smart-Lab PP indicator, silica gel, Merck Whatman No.1 filter paper, and Pudak Scientific $ZnCl_2$, were obtained from Microbiology Laboratory, Department of Biology, Sriwijaya University. Laboratory instruments such as aerator hose (3/16 inch), analytical balance, autoclave, beaker, compressor, Erlenmeyer, glass bottle (100 mL), measuring cup, respirometer bottle, volumetric flask, vacuum pump, and Shacker-Incubator were obtained from the Genetics and Biotechnology Laboratory, Department of Biology, Sriwijaya University.

2.2 Methods

2.2.1 Biodegradation Test of Biodegradable Plastics

This biodegradation test is based on American Standard Testing and Material No. D6691-17: Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum (ASTM, 2017), using a modified (Alvarez-Zeferino et al., 2015) respirometry system design (Figure 1), including an aerator, NaOH sorbent, respirometry reactor and silica gel. Natural seawater that has been obtained is filtered with Whatman No. 1 paper, then taken to add NH_4Cl as much as 0.5 g/L and $KH_2(PO_4)$ 0.1 g/L for every 1 liter as a seawater inoculum.

67 mg of biodegradable plastic and craft paper samples were put in a respirometry bottle containing 250 mL of

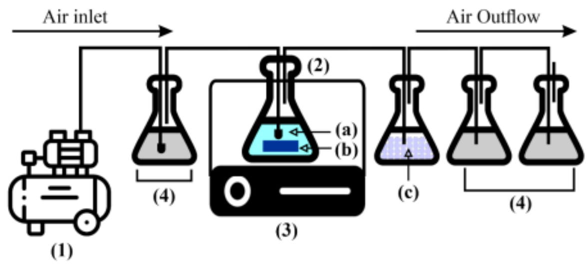

Figure 1. Respirometry System Design: (a) Seawater Inoculum, (b) Plastic Sample, (c) Silica Gel, 1) Aerator, 2) Respirometry Bottle, 3) Shaker Incubator, 4) NaOH Sorbent (Alvarez-Zeferino et al., 2015)

seawater inoculum with a blank containing seawater inoculum only. The respirometry bottle was placed in a shaker incubator at 30±1°C and a rotation of 100±5 rpm for 10-70 days was repeated 3 times. NaOH (0.25 N) sorbent was used to capture CO_2 from the aerator and respirometry reactor. Every week was replaced and the CO_2 level was measured by titration using HCl (0.5 N) solution with phenolphthalein indicator.

2.2.2 Calculate The Percentage of Biodegradation

The calculation of the percentage of biodegradation was done using the percentage of mineralization (Equation 1) by subtracting the CO_2 values of the sample (C_{Test}) and blank (C_{Blank}) and then dividing by the amount of polymer-C added (C_{Total}) or the weight of the sample used.

$$\text{Mineralization (\%)} = \frac{C_{Test}\text{-}C_{Blank}}{C_{Total}} \times 100 \quad (1)$$

2.2.3 Microplastic Identification

After biodegradation testing, seawater samples from the respirometry reactor bottle were filtered into a 250 mL beaker using a 4.75 mm sieve to separate macroplastics. The method Wang et al. (2021) was used to destroy the organic material by adding 5 mL of 30% H_2O_2 per 200 mL into the sample filtrate, after which the sample was allowed to stand overnight and again separated between the filtrate and precipitate using Whatman No. 1 filter paper.

The next process is separation to separate microplastics based on density by using the Konechnaya et al. (2020) method with a slight modification. The filter paper is moved into a petri cup, then added to a $ZnCl_2$ solution of 1.7 g cm^3 made by dissolving 72.07 g of $ZnCl_2$ in 46.84 mL of distilled water. The $ZnCl_2$ solution was added until it filled 3/4 of the Petri dish, then settled for 10 minutes, and the filter paper was gently moved 3 times in 2 minutes. Microplastics will float above because they have a lower density. To collect the microplastics, the petri dish is moved into another wider container and then added back to the $ZnCl_2$ solution until it overflows from the petri dish. The overflow that is collected is then filtered using Whatman No. 1 filter paper. The filter paper is then transferred into a container and dried at room temperature.

The last step of filter paper is observed using a Microscope to identify the number and type based on the visualization. The last step of filter paper is observed using a microscope to see the visualization. Visible microplastics are counted in number and identified by type (fragments, films, pellets, granules, filaments, and foams) based on the description of Viršek et al. (2016). For each identified microplastic, it will be counted as a particle whose number will be averaged.

2.2.4 Correlation Analysis

To see if there is a relationship between the percentage of biodegradation of biodegradable plastics and the resulting microplastics, a Pearson correlation analysis was performed. Pearson correlation is done to see if there is a relationship between the percentage of biodegradation of biodegradable plastics and the microplastics produced. To anticipate that the data is not distributed normally, bootstrapping is done by re-sampling 5000 times.

3. RESULTS AND DISCUSSION

3.1 Biodegradation of Plastic

During the 70-day biodegradation test, changes in the seawater inoculum and biodegradable plastic sample on the 7th day, or the first week, as shown in Figure 2, there was a change in the seawater inoculum, which became more cloudy than previously clear. The turbidity is caused by the dissolution of biodegradable plastic containing starch in seawater inoculum. This has been observed by Mohan et al. (2017) in their research dissolving plastic biofilms of starch in solution, demonstrating the ability of biodegradable packaging to dissolve and cause turbidity in the media solution.

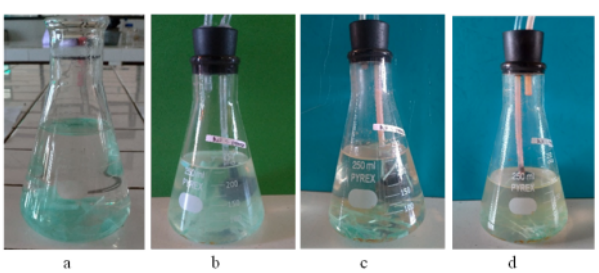

Figure 2. The Changes of Biodegradable Plastic Sample (a) Day-0, (b) Day-7, (c) Day-30, and (d) Day-70

After 30 days, the color of the biodegradable plastic sample changed from green to reddish. This change is a sign that the plastic surface of the sample has been covered by a biofilm, followed by an increase in the growth of bacterial production. Lott et al. (2020) stated that the color change of most samples (PHA and LDPE) during the test period is related to the formation of biofilms and mineral deposits that form. In the formation of biofilms on polymers, bacterial production occurs, which reaches its peak in the second week to the day of the growth phase, or on days 15 to 22 (Dussud et al., 2018). Entering the final period of testing on day 70, the biodegradable plastic sample that was previously floating has completely sunk to the bottom of the respirometry bottle. According to Shruti and Kutralam-Muniasamy (2019), the plastic sphere or biofilm layer from bacterial and fungal colonization that is formed will cause the density of microplastics to increase and the plastic particles to sink.

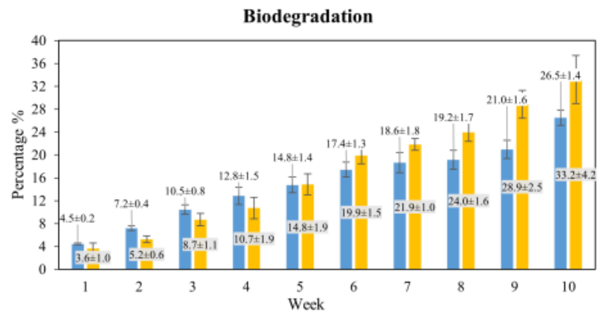

Figure 3. Graph of Increasing Biodegradation Percentage of each Sample

3.2 Percentage of Biodegradation

The CO_2 value measured every week of the biodegradation process will be calculated by equation 1 to be the biodegradation percentage value. Measuring the value of biodegradation based on the change of carbon (polymer) to CO_2 is the best method compared to the weight loss method. This is because there will be an error in the measurement results of weight loss due to the interference of other factors that are not related to the biodegradation process, like hydrolysis, photodegradation, etc. According to Kunioka et al. (2009), there are many ways to measure the percentage of biodegradation. The easiest way that can be used is to measure the weight of the plastic over a certain period after environmental testing. Because the decomposed material has become small particles and is difficult to collect, the accuracy of this method is reduced for biodegradation percentages above 70%. It is important to understand that with all current test methods, the value that counts is the rate of mineralization, not biodegradation; this is done for practical reasons, and the right method to measure biomass production has not been found, so the level of mineralization can be referred to as the level of biodegradation (Pischedda et al., 2019).

During the test period, the biodegradation percentage chart (Figure 3) for both plastic and control samples contin-

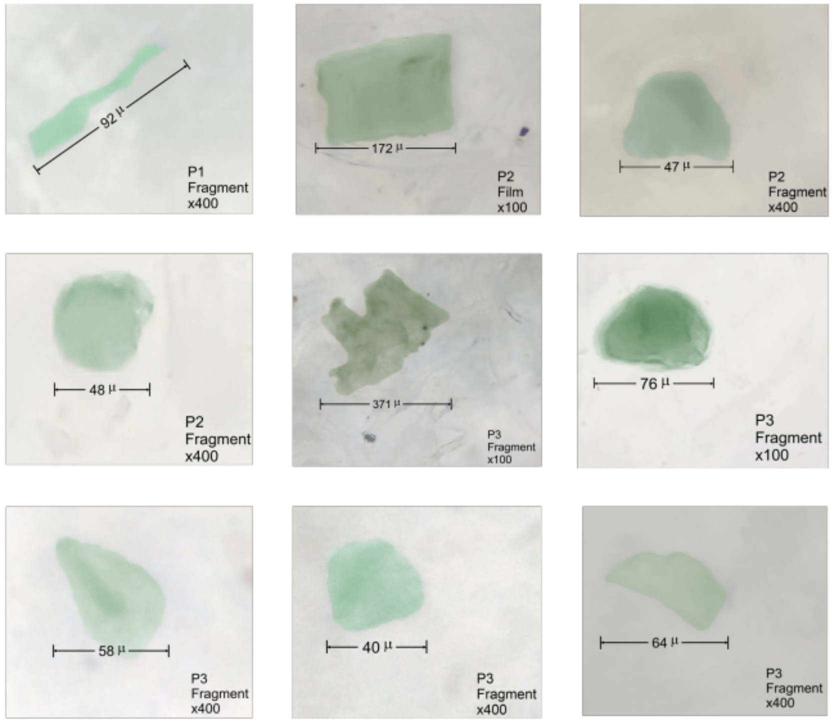

Figure 4. Photos of Microplastic Identification Results

ued to increase every week. The final result of biodegradation, namely the 70th day for biodegradable plastic samples, was 26.5±1.4%, and the positive control of kraft paper was 33.2±4.2%. The biodegradation value is still relatively high when compared to other studies conducted by Greene (2018), who used the same method as ASTM D6691 and a type of plastic made from PHA but without a mixture of biodegradation rates of 37% and 47% for 180 days. According to Dilkes-Hoffman et al. (2019), PHA plastic bottles take 1.5-3.5 years to completely degrade, with an average biodegradation rate of 0.04-0.09 $mg \cdot day^{-1} \cdot cm^{-2}$ (p=0.05) in marine environments.

Based on this research and other studies that have been done, we found that there is an effect of agitation on the rate of biodegradation. This is proven when compared with Alvarez-Zeferino et al. (2015) study, which tested Ecovio® compostable plastic using a water bath without agitation in the same method as ASTM 6691. The results obtained were 10% biodegradation within 48 days, a lower value compared to the value obtained from this study, which used a Shacker Incubator biodegradation percentage of 26.5% in 70 days. Agitation is used to describe the sublittoral zone of the sea. When agitation is not carried out, there will be anaerobic activity in the sediment in parallel, resulting in a lack of oxygen because most of the organic carbon in the sample turns into methane. The result is that the CO_2 measurement value for aerobic biodegradation will be disrupted (Briassoulis et al., 2020), but this is still only an initial assumption that is not completely strong. To ensure this factor, further testing is needed with the same method and type of plastic to ensure a deeper understanding.

3.3 Microplastic Identification

To ensure that no other microplastic contamination occurs during the identification process, all equipment is washed using aquades that have been filtered as many as three times. The results obtained from all repeated biodegradable plastic samples identified nine microplastic particles with an average of 3±1.6 particles (Figure 4), and the types of microplastics produced are in the form of fragments and films. The results of this research show that in the process of degradation in the marine environment, biodegradable plastics can cause microplastic contamination, as well as in Shruti and Kutralam-Muniasamy (2019) research that initial evidence of major degradation of polyhydroxybutyrate (PHB) polymers in marine environments would rapidly lead to the formation of macro (<5 mm), micro (100-1600 μm) and nano(<50 μm) plastic.

Table 1. The Results of Correlation Analysis with Bootstrap 5000 Times

Correlations				Biodegradation	Microplastic
Biodegradation	Pearson Correlation			1	-0.042
	Sig. (2-tailed)				0.973
	N			3	3
	Bootstrap[c]	Bias		0[d]	-0.217[d]
		Std. Error		0[d]	0.827[d]
		95% Confidence Interval	Lower	1[d]	-1.000[d]
			Upper	1[d]	1.000[d]

[c]Unless otherwise noted, bootstrap results are based on 5000 bootstrap samples
[d]Based on 4450 samples

3.4 Correlation Analysis

Correlation analysis by bootstrapping 5000 times sampling on the biodegradation percentage variable and the amount of microplastic produced is shown in Table 1. The results of the analysis show that the level of confidence in the upper (1.000[d]) and lower (-1.000[d]) values is not in the same pole, so there is a possibility of a zero number, or it can be said that there is no relationship between these variables. According to Hayati et al. (2021), data will be said to have a significant effect if the upper and lower poles do not differ.

4. CONCLUSIONS

The conclusion that can be drawn from this research is that biodegradable plastic made from a mixture (PHA + Starch) produces a percentage of biodegradation of 26.5±1.4% and a positive control of kraft paper of 33.2±4.2% within 70 days. The biodegradation of biodegradable plastic produced nine microplastic particles, which based on correlation analysis, do not have a significant relationship between the percentage of biodegradation and the microplastics produced.

5. ACKNOWLEDGEMENT

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