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Oxodegradable Polyethylene Biodegradation Using Lactobacillus casei

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Abstract

The biodegradation of oxodegradable polyethylene has traditionally been carried out using pure strains of polyethylene-degrading agents. Research on polyethylene biodegradation is using other microbes than pure strains still scarce. This study utilized lactic acid bacteria, specifically Lactobacillus casei, to know the capability of Lactobacillus casei to degrade polyethylene. The results indicate that Lactobacillus casei can degrade polyethylene plastic. Polyethylene plastic biodegrades by 27.92% over 28 days without preheating. The optimal temperature and pH for the process are 26°C and pH 5, respectively. On the other hand, the preheated polyethylene plastic performed at 9.65% biodegradation over 28 days, with an optimum temperature of 28°C and an optimum pH of 5.6..

1. INTRODUCTION

The issue stemming from the indiscriminate disposal of polyethylene plastic into the environment is escalating. The daily use of polyethylene as food containers or plastic bags for packaging goods, food, and beverages results in their disposal into the environment[1]. This is also the case at UIN Sayyid Ali Rahmatullah Tulungagung, that have many students and everyday use polyethylene plastic bags for packaging and other purposes. And the absence of integrated waste management at UIN Sayyid Ali Rahmatullah Tulungagung causes polyethylene is escalating.

Polyethylene plastic, widely used as bags and packaging containers, is a polymer of ethylene (C_2H_4), made from resin derived from petroleum. Single-use plastic bags or packaging containers made of polyethylene are usually discarded after one use and should not be reused[2]. The use of these single-use plastic bags contributes to the accumulation of plastic waste in the environment.

The waste management principle has traditionally followed the 4R principle (Reuse, Reduce, Recycle, Replace). However, when applying the principles of reuse and recycle to plastic waste, there are drawbacks. Although reusing and recycling plastic waste hold promising prospects for business and product improvement, if the recycled products are not utilized, they still contribute to waste accumulation in the environment[3]. The utilization of the "replace" principle, especially in Indonesia, particularly in the UIN Sayyid Ali Rahmatullah Tulungagung area, has not been fully maximized. Therefore, the best solution is to process waste using the "reduce" principle, which aims to reduce the amount of waste. One method to reduce plastic waste is through biodegradation, the decomposition of plastic waste using environmentally safe organisms, as opposed to physical and chemical degradation methods[4].

Currently there is no definitive solution to the plastic waste problem. The increased use of plastic as food and beverage packaging containers, coupled with the lack of awareness among the Indonesian population.

One commonly researched effort to reduce polyethylene plastic waste is through biodegradation using bacteria, fungi, or other organisms. For example, bacteria obtained from waste disposal sites or bacteria from oil drilling have been studied[2]. *Bacillus subtilis* demonstrated a 1.75% reduction in plastic weight over 30 days[4]. Additionally, the use of *Penicillium simplicissimum* YK fungi has been reported to thrive on polyethylene plastic media Bacteria and fungi commonly used are pure strains with inherent polyethylene degradation capabilities[5].

Biodegradation of polyethylene using bacteria from non-pure strains also needs to be explored. This can potentially be used as agents for polyethylene degradation, contributing to new insights in the field of plastic biodegradation. For instance, Lactic Acid Bacteria (LAB), specifically *Lactobacillus bulgaricus* combined with other bacteria, achieving a biodegradation percentage of 19.47% in 20 days[6]. Therefore, further research was conducted using only lactic acid bacteria, specifically *Lactobacillus casei*, without the addition of other bacteria.

2. MATERIALS AND METHODS

The type of research conducted is qualitative descriptive research, focusing on the results of plastic degradation in the form of biodegradation percentages. Additionally, qualitative analysis of temperature and pH during the biodegradation process is performed.

The materials used in this research include *Lactobacillus casei*, soil, polyethylene plastic, distilled water (aquades), 70% and 90% alcohol. The equipment used consists of a shaker, autoclave (Biobase BKQ-Z301), laminar air flow (LAF) FlowFast V 12 P, petri dishes, test tubes, micropipettes, micropipette tips, colony counter, microscope, spatula, Bunsen burner, microscope slides, cover slips, pipettes, test tube racks, oven, incubator Hettcube 200, cotton, measuring glass, beaker glass, erlenmeyer flask, inoculation needle, aluminum foil, tissue paper, analytical balance, and sample bottles.

The research is carried out in two stages[6]. The first stage involves sterilizing the necessary equipment and materials using an autoclave at a temperature of 121°C and a pressure of 1 atm for 15 minutes. Before sterilization, items such as glass bottles, pipettes, blue tips, tweezers, spatulas, petri dishes, test tubes, and measuring glasses are cleaned with 90% alcohol and wrapped in aluminum foil. Soil is prepared by filtering and weighing 100g, then placing it in glass bottles. Test plastic, sourced from polyethylene and oxodegradable plastic bags, is cut and weighed before being placed in the glass bottle with the soil. The bottle is sealed with aluminum foil and sterilized using an autoclave at 121°C and 1 atm pressure for 15 minutes. 20 ml *Lactobacillus casei* bacteria (20×10^9 CFU/ml) is added to the bottle. Initial temperature and pH are measured, and data are collected every

7 days for 28 days, observing final plastic weight, temperature, and pH. Biodegradation percentage is calculated.

The second stage involves preparing equipment by sterilizing them using an autoclave at 121°C and 1 atm pressure for 15 minutes. Similar to the first stage, items are cleaned with 90% alcohol and wrapped in aluminum foil. Soil is prepared, and plastic is cut and heated for 12 hours at 100°C. The remaining steps are similar to the first stage, including placing the plastic in a glass bottle with soil, sealing with aluminum foil, autoclaving, adding *Lactobacillus casei* bacteria, measuring initial temperature and pH, and collecting data every 7 days for 28 days. Biodegradation percentage is calculated using the formula:

Biodegradation Percentage= $\left(\frac{Wi-Wf}{Wi}\right) x\ 100\%$ where *Wi* is the initial dry weight before degradation (in grams) and *Wf* is the final dry weight after degradation (in grams).

3. RESULTS

This study focused on how Robusta green coffee extract could modulate immune cells, specifically through macrophage phagocytosis and lymphocyte proliferation. Robusta green coffee was extracted using a water solvent and infusion method. To remove the water content, Robusta green coffee extract was dried using freeze-drying until it became a powdered extract. This drying method aimed to prevent damage to the active compounds contained in the extract and extended its utility without altering the active compounds within.

Effect of Robusta Green Coffee Extract on Macrophages Phagocytosis Activity

Macrophages were cells about 9-12 µm in size with a relatively large nucleus and often had many branches or pseudopodia (Figure 1a). To determine the immunomodulatory effect of Robusta green coffee extract on macrophage activity, a phagocytosis test was conducted in vitro. The parameters used were Active Phagocytic Cells (APC), which referred to the percentage of macrophage cells (PP) that phagocytized latex particles out of 100 macrophage cells, and phagocytic capacity (PC), which referred to the number of latex particles phagocytosed [14]. Phagocytic activity was indicated by latex particles contained in phagosomes in the macrophage cytoplasm (Figure 1b).

The results of this research are divided into two parts: firstly, the biodegradation of oxodegradable polyethylene without heating the plastic, and secondly, after inoculating *Lactobacillus casei*, the weight of polyethylene decreases, as shown in Table 1,Figure 1 and Figure 2.

Table 1. Results of biodegradation of oxodegradable polyethylene without heating the plastic

Day	Percentage of biodegradation (%)	Temperature (°C)	pH
0	0	33	5
7	5.38	25	6
14	13.97	25.3	5
21	22.21	26	6
28	27.92	23	5

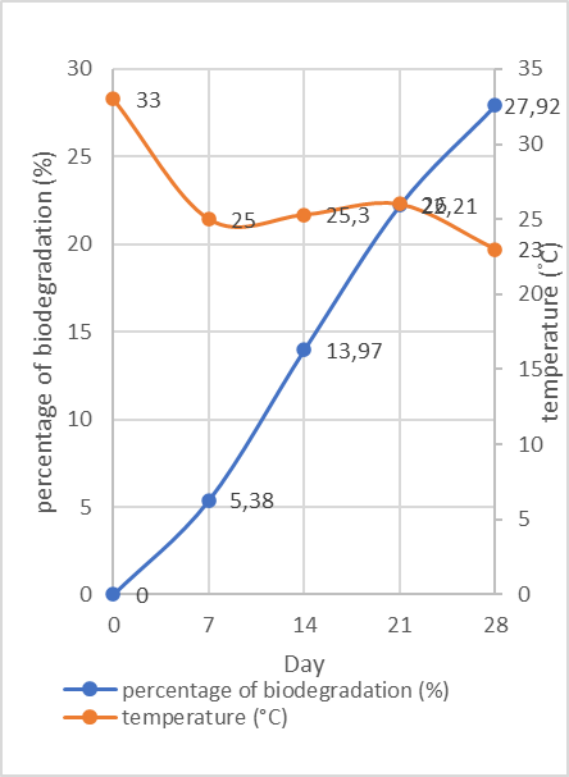

Figure 1. Graph showing the relationship between the percentage of biodegradation of oxodegradable polyethylene without heating the plastic and temperature

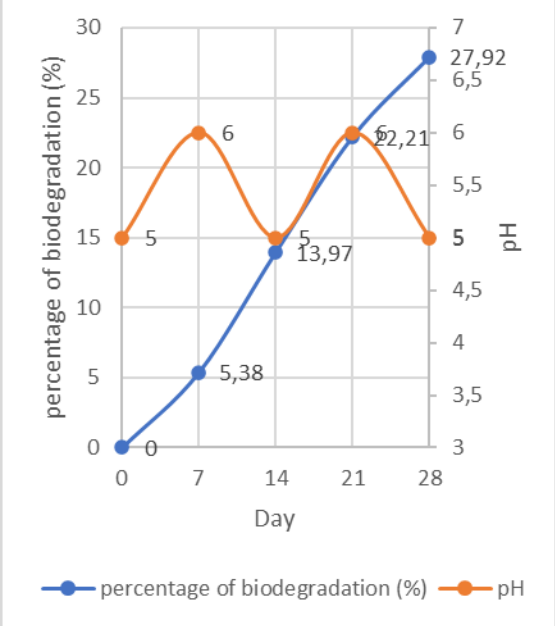

Figure 2. Graph showing the relationship between the percentage of biodegradation of oxodegradable polyethylene without heating the plastic and pH

The data from Table 1 above reveals that the percentage of biodegradation increases with the incubation period or the duration of the biodegradation process from day 7 to day 28. This increase is also evident in Figure 1 and Figure 2, where the curves consistently show an upward trend. It indicates that by adding *Lactobacillus casei* inoculum, a degradation process occurs on the test plastic, as seen from the increasing degradation percentage. This implies that the longer the duration of the biodegradation process, the more plastic will be decomposed.

In Figure 1, it is observed that the initial temperature of the media after the addition of Lactobacillus casei inoculum, which was 33°C, decreases to 23°C by the end of day 28. On day 28, the highest result for the percentage of plastic biodegradation is obtained, indicating an optimum temperature of 23°C for this biodegradation process. Not only temperature but also the pH of the media plays a crucial role in the degradation process.

At the beginning of the addition of *Lactobacillus casei* inoculum, the pH of the media is 5. Over the 28-day biodegradation process, as seen in Figure 2, the pH fluctuates between pH 5 and pH 6. At the end of biodegradation on day 28, the pH of the media is around pH 5. Hence, the optimum pH in this biodegradation process is pH 5. A pH of 5 indicates an acidic media condition, aligning with the fact that *Lactobacillus casei* bacteria can thrive in acidic conditions. This strongly suggests that *Lactobacillus casei* bacteria are indeed involved in the biodegradation process.

The results of the second study involve the use of oxodegradable polyethylene plastic that was subjected to heat treatment before inoculating with *Lactobacillus casei*. After the heating process, *Lactobacillus casei* inoculation

was performed, and the biodegradation results are presented in
Table 2, Figure 3 and Figure 4.

Table 2. Results of biodegradation of oxodegradable polyethylene with plastic heating

Day	Percentage of biodegradation (%)	Temperature (˚C)	pH
0	0	28	7
7	7.28	27.3	6
14	7.5	30	7
21	8.11	28	5.6
28	9.65	28	5.6

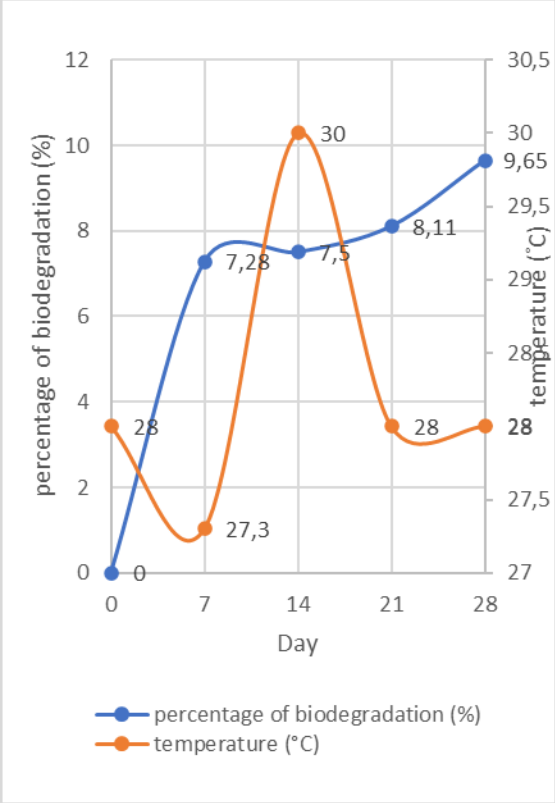

Figure 3. Graph showing the relationship between the percentage of biodegradation of preheated oxodegradable polyethylene and temperature

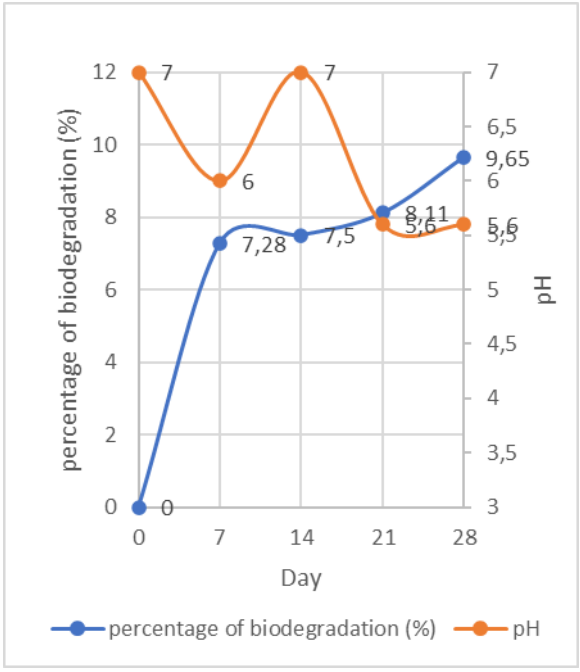

Figure 4. Graph showing the relationship between the percentage of biodegradation of preheated oxodegradable polyethylene and pH

The data from Table 2 above shows that the percentage of biodegradation increases with the incubation period or the duration of the biodegradation process from day 7 to day 28. This increase is also evident in Figure 3 and Figure 4, where the curves consistently show an upward trend. It indicates that by adding *Lactobacillus casei* inoculum, a degradation process occurs on the test plastic, as seen from the increasing degradation percentage. This implies that the longer the duration of the biodegradation process, the more plastic will be decomposed.

In Figure 3, it is observed that the initial temperature of the media after the addition of *Lactobacillus casei* inoculum, which was 28°C, decreases to 27.30°C on day 7, increases to 30°C on day 14, and then decreases again to 28°C by the end of day 28. On day 28, the highest result for the percentage of plastic biodegradation is obtained, indicating an optimum temperature of 28°C for this biodegradation process where the plastic is preheated. Not only temperature but also the

pH of the media plays a crucial role in the degradation process.

In contrast to the pH in the biodegradation process of non-preheated plastic, in the process of biodegradation with preheated plastic, at the beginning of the addition of *Lactobacillus casei* inoculum, the pH of the media is 7. Over the 28-day biodegradation process, as seen in Figure 4, the pH fluctuates. On day 7, the pH of the media is at pH 7, on day 14, the pH decreases to pH 6, and from day 21 to day 28, the pH decreases further to pH 5.6. On day 28, the percentage of biodegradation achieves the highest result, which is 9.65%. Therefore, the optimum pH for the biodegradation process of preheated plastic is at pH 5.6, indicating an acidic media condition suitable for the pH of *Lactobacillus casei* bacteria. The biodegradation results for preheated plastic show a lower percentage compared to the biodegradation of non-preheated plastic.

4. DISCUSSION

Methods of degrading polyethylene plastic using bacteria, fungi and other organisms have been extensively researched.. However, there has been limited research on the degradation process using bacteria not typically employed in the field of plastic biodegradation. Filayani conducted biodegradation using lactic acid bacteria, specifically *Lactobacillus bulgaricus*. The study not only utilized lactic acid bacteria alone but also combined it with other bacteria, resulting in a plastic weight reduction with a biodegradation percentage of 19.47% within 20 days[6].

This research focuses solely on the use of single lactic acid bacteria, meaning it does not involve the simultaneous use of other bacteria in the biodegradation process. The objective is to understand and explore whether lactic acid bacteria, particularly *Lactobacillus casei*, can indeed reduce the weight of oxodegradable polyethylene plastic. *Lactobacillus casei* is commonly known as a probiotic bacterium that plays a role in maintaining intestinal microflora, producing lactic acid through lactose fermentation[7]. Therefore, *Lactobacillus casei* has not been previously known for its role in the plastic biodegradation process.

The results of this research demonstrate that *Lactobacillus casei* can indeed degrade oxodegradable polyethylene plastic, evidenced by the reduction in plastic weight during the biodegradation process (Table 1 and Table 2). The decrease in oxodegradable polyethylene plastic weight occurs because Lactobacillus casei utilizes polyethylene as a carbon source for nutritional needs and ATP formation[8].

Plastic is an organic polymer molecule consisting of repeated units of organic monomers bound together. These monomers are made up of carbon, hydrogen, and other atoms connected by covalent bonds. Plastic degradation is divided into two different categories: abiotic degradation and biotic degradation. Abiotic degradation breaks down plastic through light, heat, chemicals, or mechanical pressure. This pressure causes polymer chain degradation through oxidation, dechlorination, chain scission, cross-linking, ablation, and fragmentation[9]. Biotic degradation refers to plastic degradation caused by an organism. The degradation process can occur through biochemical processes or simply by organisms biting, chewing, or digesting plastic. It has been found that fungi[10], bacteria[11], and insects can degrade plastic. Two bacterial strains found in the intestines of worms are capable of degrading polyethylene. The final step of degradation is the mineralization of polymer fragments into water and carbon dioxide[12].

5. CONCLUSION

The research on the biodegradation of oxodegradable polyethylene using *Lactobacillus casei* bacteria can be concluded that *Lactobacillus casei* is capable of degrading oxodegradable polyethylene plastic, as evidenced by the reduction in plastic weight. The biodegradation process of oxodegradable

polyethylene plastic without preheating resulted in a biodegradation percentage of 27.92% over 28 days. Meanwhile, in the biodegradation process of preheated oxodegradable polyethylene plastic, the achieved biodegradation percentage was 9.65% over the 28-day degradation process.

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