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Potential of biological agents (*Pseudomonas sp.*) in plastic waste biodegradation process

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ABSTRACT

Pseudomonas sp. can degrade plastics because it has an inducible operon system that produces certain enzymes (esterase, serine, hydrolase, and lipase) in the process of carbon source metabolism. The purpose of this study was to determine the potential of *Pseudomonas sp.* as a plastic biodegradation agent. This research method is a systematic literature review which is carried out by collecting, understanding, analyzing and concluding as many as 30 national and international journal articles published from 2008 to 2022 from several search engines such as Google Scholar, Ncbi, Sinta, and Plos one with the keywords degrading microorganisms plastic. The steps of this research were made samples to be used, tested samples with bacterial isolation, filtered the bacteria, bacterial bacteria and biodegradation within a specified time period by looking at the % loss of plastic before treatment. The results of the literature review analysis showed that *Pseudomonas sp.* able to degrade plastic around 2-19% by utilizing plastic as a carbon source which will convert addition and condensation polymers into simple molecules such as monomers, dimers and oligomers during the inventory period within 1-3 months. Thus, the microorganisms in the form of *Pseudomonas sp.* can decompose plastic waste naturally and faster than other methods and does not cause adverse effects.

Keywords: biodegradation, plastic, *Pseudomonas sp.*

INTRODUCTION

The demand for and consumption of plastic in Indonesia continues to rise yearly, increasing plastic waste (Fadlilah & Shovitri, 2014). Based on an Indonesian Institute of Sciences (LIPI) survey, there was a 2-fold rise in plastic trash during the Covid-19 epidemic, which was the result of online shopping activities where 96% of the packaging material was made of plastic (Musdary et al., 2021).

Plastics' use in modern life will increase over time because of their lightweight, strength, transparency, anti-corrosion, easy coloration, and strong insulating capabilities (Fadlilah & Shovitri, 2014). Processing of plastic waste is a serious problem considering that plastic materials take a very long time to degrade (Bhardwaj, Gupta, & Tiwari, 2012). At this time, plastic waste is being processed through burning and recycling. However, this method of processing has a negative impact on the environment. Therefore, alternative ways are needed to deal with plastic waste (Novia, 2021).

Another technique for dealing with plastic is biodegradation, which is accomplished with the help of microorganisms (Setyati & Subagiyo, 2012). One type of microorganism that plays a role is bacteria. Certain types of bacteria have the ability to decompose natural polymers (lignin and cellulose) and synthetic polymers (polyethylene and polyurethane) by utilizing them as a carbon source in their growth. *Pseudomonas sp.* is a type of bacteria that can degrade plastic waste (Sriningsih & Shovitri, 2015).

Pseudomonas sp. is a group of gram-negative, rod-shaped bacteria (Rahmadian et al., 2018), which have one or more flagella at the edges, do not produce spores, and are classified as aerobic bacteria, but several species can grow anaerobically, including positive catalase bacteria (oxidatively metabolizing sugar)

(Kurnia et al., 2015). This bacterium does not use hydrolytic enzymes in polymer degradation into monomers. However, it has an inducible operon system to produce specific enzymes in the metabolic process of carbon sources that are generally not used (Sriningsih & Shovitri, 2015).

The decomposition by microorganisms becomes an essential component of nature to prevent contaminants at the waste site. Some anaerobic bacteria use nitrate, sulfate, iron, manganese, and carbon dioxide as their electron acceptors to break down organic chemicals into more minor compounds. Microorganisms cannot transport polymers directly through their outer cell membranes because the polymer molecules are long and insoluble in water. Microbes develop strategies for using carbon and energy sources by secreting extracellular enzymes that depolymerize polymers outside the cell. Depolymerization enzymes aid polymer degradation. During degradation, microbial exoenzymes break down complex polymers to produce short chains or smaller molecules such as oligomers, dimers, and monomers. These molecules are small enough to dissolve in water and can pass through the semi-permeable outer bacterial membrane to be used as carbon and an energy source (Shah & Alshehrei, 2017).

Based on the characteristics of the reactions that occur in synthetic plastics, all the atoms in the monomer that are combined into the polymer is called polymer adhesion *Polymer Adhesion*. Whereas if some monomer atoms are released into small molecules, it is called condensation polymer. Most polymer adhesions are made of monomers that contain double bonds between carbon atoms. Such monomers are called olefins. Condensation polymers are made from monomers with two different groups of atoms that can be joined together, such as an ester or amide bond. Such monomers are called Polyolefins. Such polymers include polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyurethane, and polyethylene terephthalate (Shah & Alshehrei, 2017).

In this article, the materials used are included in the thermoplastic category. Thermoplastics are polyethylene polymers consisting of long chains of ethylene monomers that result from reactions from ethylene gas and are assisted by catalysts at specific pressures and temperatures (Khoironi et al., 2021). There are two types of polyethylene: LDPE (Low-Density Poly Ethylene) and HDPE (High-Density Poly Ethylene) (Sukarman et al., 2021). The ability to break down polymer adhesion and condensation into olefin and polyolefin monomers are what makes *Pseudomonas sp.* selected as one of the biological agents to degrade plastic waste. Thus, this study aims to determine the potential of *Pseudomonas* bacteria in degrading plastic waste.

METHOD

This study uses the type of systematic literature review research by identifying and interpreting all the findings on the research topic to answer research questions (research questions) so that it does not only mean reading literature but rather towards an in-depth evaluation of previous studies (Muktiono & Soediantono, 2022). This Systematic Literature Review was synthesized using the narrative method by grouping data that is appropriate to answer the objectives of this research article to then be collected, analyzed, and made a summary of the research article (Wahyudin & Rahayu, 2020). The main object of this review is to provide facts about the effectiveness of the biological agents of the bacterium *Pseudomonas sp.*, which is implemented in the process of degradation of plastic waste.

Data collection was carried out using search engines in the form of Google Scholar, NCBI, Sinta, and Plos One using several keywords related to the topic of discussion and then selected with the journal publication year of at least the last 15 years. Then based on data collection, it can be seen if the materials used in the research findings are in the form of plastic bags of LDPE (Low-density Polyethylene), HDPE (High-Density Polyethylene), and Oxium types. The test was carried out by first isolating the bacteria, then purifying and selecting the bacteria used, then identifying the bacteria, and then testing the effectiveness of the bacteria *Pseudomonas sp.* in degrading plastics.

Data analysis in the form of how effective the bacteria *Pseudomonas sp.* is in degrading plastics using descriptive analysis by looking at the percentage of plastic weight loss before and after each treatment.

RESULTS AND DISCUSSION

The literature study results show that *Pseudomonas sp.* can degrade plastic waste. Several previous studies have proven the potential of *Pseudomonas sp.* as a degrader of plastic waste.

Sriningsih & Shovitri (2015) showed the results of isolates of *Pseudomonas sp.* can degrade plastic after three months incubation period. If seen from the average percentage of dry weight loss, *Pseudomonas sp.* can degrade black plastic by 2.7%, degrade white plastic by 3.3%, and degrade transparent plastic by 4.5% (Table 1).

Table 1. Percentage of plastic degradation by *Pseudomonas sp.*

Treatment	Dry Weight Loss (%)		
	White	Black	Transparent
Pseudomonas	3.3 [a]	2.7 [a]	4.5 [a]
Control	-0.4[b]	-0.4 [b]	0.4 [a]

Note: The same letters and numbers in one column indicate no significant difference to the Tukey One-Way ANOVA test with a truth value of 0.05.
(Source: Sriningsih & Shovitri, 2015)

According to Sari et al. (2020), the results of *Pseudomonas sp.* can degrade plastic within 10, 20, and up to 30 days. If seen from the average dry weight loss percentage at room temperature ±25°C, *Pseudomonas sp.* can degrade oxium plastic or environmentally friendly plastic sequentially by 2.43%, 5.71%, and 9.86%. Whereas in LDPE (Low-Density Polyethylene) plastic, the bacteria *Pseudomonas sp*. can degrade sequentially by 1.13%, 2%, and 1.17% (Table 2).

Table 2. Percentage of Plastic Degradation by *Pseudomonas sp.*

Treatment	Dry Weight Loss (%)		
	Degradation (days)	Oxium	LDPE
Pseudomonas sp.	10	2,43%	1.13%
	20	5,71%	2%
	30	9,86%	1.71%

Source: (Sari et al., 2020)

The study Riandi et al., (2017) used soil samples from 3 landfills and two mangrove forests which were added to pieces of HDPE (High-Density Polyethylene) and LDPE (Low-Density Polyethylene) plastic bags, then tested in the laboratory. The identification results showed that isolate number 24 belonged to the type of *Pseudomonas aeruginosa* bacteria. From the average percentage of dry weight loss, the Pseudomonas biological agent can degrade LDPE plastic bags by 18.75% during the ± 45-day incubation period (Table 3).

Table 3. Percentage of plastic degradation by *Pseudomonas sp.*

Isolate name	Dry weight without treatment (gram)	Dry weight after treatment (gram)	Mean	Degradation (%)
isolate 24 (repeat 1)	0,0254	0,0179	0,0044	18,75%
K2T3U1 KRG (repeat 2	0,0144	0,0132	0,0044	18,75%

Source : (Riandi et al., 2017)

Degradation is a series of processes of breaking or breaking down complex compounds into simple compounds (Dewi et al., 2018). Several factors influence the success of the decomposition process, including the type of microorganism, temperature, humidity, pH, nutrients, minerals, and the type of polymer being degraded (Purba et al., 2020). Other influencing factors are crystallinity, type of functional group, molecular weight, substituents such as plasticizers and additives (Sari et al., 2020). Based on Table 2, the degradation process in oxium plastic is faster due to cofactors such as cobalt salts (Co) which help the extracellular enzymes in *Pseudomonas sp*. to hydrolyze the substrate. Cobalt Co salt is an ingredient used in the production of Oxium plastic. Cobalt (Co) salt is considered safer than other additions, such as Cadmium, because it increases enzyme activity faster when exposed to organisms. Meanwhile, LDPE plastic deterioration is slower due to the need for more additives that act as cofactors (Sari et al., 2020).

The primary mechanism in biodegradation is the process of breaking polymer complex chains (Octavianda et al., 2016). The interaction between enzymes catalyzing hydrolysis reactions released by bacteria and non-enzyme compounds that degrade polymer structures from the environment or the microorganisms themselves affects biodegradation (Riandi et al., 2017).

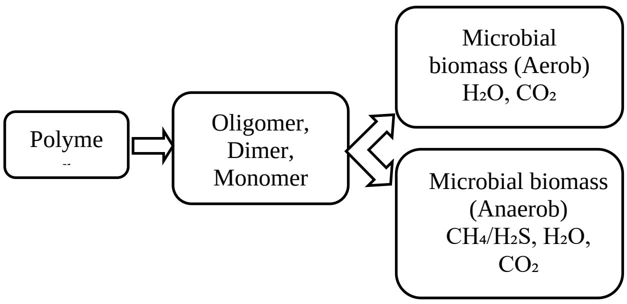

Figure 1. Plastic biodegradation mechanism

Figure 1 depicts the mechanism of plastic biodegradation, which demonstrates that microorganisms cannot bring in polymer molecules via the cell membrane (Shah & Alshehrei, 2017). As a result, bacteria have a specific method: first, to excrete extracellular enzymes that break down polymers outside the cell. The process of breaking down polymer molecules is also assisted by exoenzymes, extracellular and intracellular polymerase enzymes (Octavianda et al., 2016). Polymers will be converted into simple molecules such as monomers, dimers, and oligomers (Rizqah et al., 2019). Because this small molecule dissolves in water, it can pass through the cell membrane of microscopic organisms. Microorganisms will utilize this substance as a source of energy and carbon The end products produced from microorganisms are H_2O, CO_2, CH_4, and biomass through a process called mineralization (Sendjaya et al., 2021).

The result of degradation will be a change in the morphology of plastic waste, which a decrease in plastic mass can prove. This decreased plastic mass is caused by bacteria using plastic as the only carbon source (Riandi et al., 2017). Scanning Electron Microscopy (SEM) forms a microscopic image of the sample. The basic idea of SEM is the same as that of a light microscope, except this microscope focuses on an electron beam than a photon beam (Islami, 2019).

According to Muhonja et al., (2018), biodegradation makes plastic degradation higher because it involves organisms. From the results of research on contamination, decomposing bacteria have the ability to degrade effectively. Based on biodegradation indicators, it shows changes in the FTIR spectrum (functional groups present in the sample) (Dang et al., 2018).

One advantage of the biodegradation of plastic waste by *Pseudomonas sp* is that the degradation process will be aided by microorganisms, which will speed up the process. Microorganisms with large numbers found in the soil will be able to produce extracellular enzyme compounds (Rori et al., 2020) which serves to accelerate the process of plastic degradation in the soil. Moreover, because it uses microorganisms, the environment will remain natural without additional mixed materials used to degrade plastic waste (Sari et al., 2020).

This deficiency of biodegradation can be seen from the many factors that affect the degradation process, such as the type of microorganism, temperature, pH, humidity, amount of minerals, and others. So if one of these factors is unsuitable, it will affect biodegradation. Temperatures and pH levels that are too high or too low may prevent microorganisms from surviving. The type of microorganism also influences the speed and effectiveness of biodegradation. Thus different varieties can produce varied results (Sari et al., 2020).

From the results of degradation, the advantages and disadvantages can be seen that microorganisms will naturally decompose plastic waste by themselves. Then to prove this, we can do the testing in stages. The first stage is exploring or exploring, then looking for information on the abundance of plastic-degrading bacteria at a point in place. After the location is determined, sampling (Sari et al., 2020).

Moreover, the initial isolation stage was completed by taking a soil sample, suspending it in an Erlenmeyer, and allowing it to stand for 10 minutes to produce a precipitate. The suspension is then diluted before implanted in agar media and incubated for 48 hours (Sari et al., 2020).

After the bacterial isolates were obtained, morphological identification was carried out utilizing gram staining using crystal violet, iodine solution, 95% alcoholic alcohol, and safranin. Then it was observed with a microscope with a magnification of 1000 times (Sari et al., 2020).

The plastic material is cut to the desired size before being sterilized and dried in an oven at 37°C for 24 hours. The plastic is then weighed to determine the initial dry weight (Sari et al., 2020).

The biodegradation test began with Mineral Salt Medium (MSM) and was then supplemented with glucose prepared earlier in the study (Riandi et al., 2017). Addition of sterile sand as a plastic planting medium in research (Sriningsih & Shovitri, 2015), or maybe nothing was added like in the research (Sari et al., 2020). Only then are sterile plastic pieces previously weighed and incubated for a predetermined period of 10, 20, or up to 30 days. After completion, they were dried, and the dry weight was re-weighed (Sari et al., 2020).

The effectiveness of the number of *Pseudomonas sp* bacteria used to degrade the amount of plastic waste may depend on the type of plastic being degraded. It is supported by previous studies on the subject. Based on research by Riandi et al. (2017), a suspension of 1 mL of *Pseudomonas sp.* bacteria during an incubation period of 45 days could degrade LDPE plastic with a percentage of 18.75%. Meanwhile, Sriningsih & Shovitri (2015) discovered that 35 mL of *Pseudomonas sp*. isolates degraded black plastic with an average degradation of 2.7%, white plastic with 3.3%, and transparent plastic with 4.5% after a 3-month incubation period.

Based on these studies, there are different types of degradable plastics. The physical and chemical properties of each type of plastic affect the mechanism of biodegradation. Surface conditions (surface, area, hydrophilic and hydrophobic properties), chemical structure, melting point, molecular weight, and crystal structure play an essential role in biodegradation (Tokiwa et al., 2009).

It needs to be realized that the increase in plastic waste continues from year to year, reaching 38 million tonnes/year, with the most significant contributor being a plastic waste (Hendiarti, 2018). In various parts of the world, the use of plastic reaches 100 kg/person each year (Purwaningrum, 2016). It can be detrimental to humans and the environment considering the nature of plastic which is difficult to decompose, reduces soil fertility, pollutes water sources, makes the environment dirty, and becomes a source of disease (Surono, 2013).

With this research, it is hoped to increase public awareness and students in science, especially biology, so that they are wiser in using plastic in their daily lives. In addition, it is also hoped that the public can see the existing natural potential and then make the best use of it for future welfare by creating the latest innovations to minimize plastic waste. One of the efforts that students in biology can make is to study and understand microbiology, enzymology, and biotechnology (Wahyudin & Rahayu, 2020) primarily related to the biodegradation process that uses certain microorganisms, and can then practice the biodegradation process, which is packaged in practical learning in the laboratory (Anwar, 2008).

Apart from using microorganisms to prevent the abundance of plastic waste, other efforts that can be made at this time are recycling plastic waste into useful and valuable products (Warlina, 2019).

CONCLUSION

The conclusion drawn from this literature review is that *Pseudomonas sp*. can degrade plastic waste by producing serine hydrolase, lipase, and esterase enzymes so that they can decompose plastic waste more quickly. It is when compared to other methods, such as burning or recycling, wherein the degradation process, plastic is used by *Pseudomonas sp*. as a carbon source in its metabolic activities. The results of biodegradation by *Pseudomonas sp*. caused changes in the morphology of plastic waste, as evidenced by a decrease in the mass of plastic.

It is hoped that this literature review within the scope of society can become a reference in utilizing existing natural potential without making things worse with the latest innovations in the form of biodegradation to solve the problem of plastic waste, and then it can be applied massively. Then within the scope of science, this research is expected to become a library for researchers to continue to develop existing innovations to become more developed and attract people to care more about the environment both in the present and in the future.

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