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Effectiveness of Indigenous Bacteria of Salt-Washing Wastewater (Bittern) As Plastic Waste Degraders

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ARTICLE INFO

Paper Type:

Research Paper

Keywords:

Degradation, Plastic, Yeast Extract Broth, Bittern

Article History

Received: 08-11-2024
Revised: 16-02-2025
Accepted: 04-03-2025
Available online: 13-04-2025

ABSTRACT

Plastic is a man-made synthetic polymer that has stable and durable characteristics The process of decomposing plastic takes a long time and the use of plastic which is increasing every year results in the accumulation of plastic waste in the environment, causing environmental pollution. Biodegradation is one solution to reduce the amount of plastic with the help of microorganisms or bacteria. This study has an objective to analyze the OD value; TPC; and the percentage value of plastic waste weight loss from each Indigenous bittern bacterial isolate in degrading plastic waste on YEB media + 1% glucose. The research procedure includes sterilization of tools and materials until data analysis. Data on TPC and OD values during the degradation process were analyzed descriptively and the percentage value of plastic dry weight after 7 days of incubation time was analyzed statistically. The highest OD value was 1,613 (BB1), the highest TPC value was 13,93 Log CFU/ml (BB4), and the highest plastic dry weight percentage value was 25%+0^{b} (BB4). This research provides new insights into the potential of Indigenous bittern bacteria as plastic waste biodegradation agents and enriches knowledge in the field of environmental biotechnology. This research can be used as a basis for the waste management industry and government to design more sustainable policies in waste management to reduce dependence on conventional methods that are expensive and less effective.

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Cite this article as: Saputri, W. D., Ardani, A. M., Junaedi, A. S., & Triajie, H. (2025). Effectiveness of Indigenous Bacteria of Salt-Washing Wastewater (Bittern) As Plastic Waste Degraders. *Airlangga Journal of Innovation Management, 6(1), 13-29.* https://doi.org/10.20473/ajim.v6i1.65188

Introduction

According to the World Bank, the world generates 2,01 billion tons of municipal solid waste every year. As much as 33% of this waste is not managed properly and damages the environment. The use of plastic materials to fulfill the needs of daily life cannot be reduced and there is even an increase in their use. This is in line with the times and the increase in population which results in more and more plastic waste in the environment (Ainiyah & Shovitri, 2014). Malihah & Nazairin (2024) revealed that in the last ten years, the amount of plastic waste has continued to increase by 6% until 2021. The total national plastic waste reached 11,6 million tons or about 17% of the total national waste or on average each resident produces 0,11 kilograms of plastic waste every day.

Plastic waste has become a serious threat to humans because it is a pollutant that causes damage to marine ecosystems around the world (Suryono, 2019). Some aquatic biota in marine ecosystems that experience threats from plastic waste contamination include coral reefs, seagrasses, and mangroves. The closure of coral reef ecosystems by plastic waste results in damage to tissue structures or loss of *zooxanthella* in corals which can increase the number of diseases and deaths in corals. Inhibited photosynthetic activities carried out by seagrasses or several types of aquatic plants in producing oxygen gas due to plastic waste cover, and plastic waste caught by mangrove roots will break down into microplastic particles (S. V. Febriyanti et al., 2024). More than 690 species of marine biota have been impacted by plastic debris in the form of both debris and microplastics (Carbery *et al.*, 2018).

Husmayani *et al.* (2024) suggested that most of the waste in the sea comes from land that usually flows from rivers and empties into the ocean during the rainy season. Waste from land alone accounts for around 309,625 million tons, while waste from the sea amounted to 88,374 tons in 2022. The food and beverage sector are the largest contributor to plastic waste. Based on data presented by the Ministry of Environment and Forestry (KLHK) of the Republic of Indonesia in 2022, the largest source of waste production in Indonesia came from households which reached 35,42% (Mustikasari, 2021). This fact is reinforced by data from the National Waste Information System which shows that the largest amount of national waste in 2022 is plastic waste which is ranked second with a proportion of 17,9%, while food waste is ranked first with a proportion of 41,55% (Rafi & Perkasa, 2023). Based on this data, a high amount of plastic waste occurs because the use of disposable plastics as functional materials is still a choice that is often used by the community.

The handling that has been done to overcome the problem of plastic waste in water includes recycling. Recycling can be done by making new products that have a quality almost equivalent to the original product or with a quality below it (Surono, 2018). However, some treatments for plastic waste in water are still not effective in reducing plastic waste because the recycling process will produce new waste that is also difficult to decompose. Recycling plastic into new items is less effective because it can only utilize certain types of plastic so this effort is not very effective in reducing the amount of plastic in the environment (Purwaningrum, 2016). Therefore, waste treatment techniques are needed. One technique that is widely utilized is bioremediation. Bioremediation is a technique to degrade or detoxify organic and inorganic pollutants using biological agents. Bioremediation will utilize biological mechanisms to degrade polluting substances to a harmless level with the working mechanism of degradation and transformation (Melati, 2020).

Biodegradation is the chemical degradation of a material such as polymers carried out by utilizing the work of microorganisms such as bacteria, fungi, and algae (Hasanah & Shovitri, 2015). Plants can also degrade organic pollutants. Plant species reported to have the ability to degrade are Elodea canadensis and Pueraria thunbergiana (Melati, 2020). Some studies show that fungi such as *Trichoderma viride* and

Aspergillus nomius can degrade plastic waste (Abidin *et al.,* 2023). However, although algae, fungi, or plants are capable of degradation, it is not as effective when compared to the degradation carried out by bacteria. Fungi, algae, and aquatic plants need weeks or even months to carry out degradation activities effectively while bacteria can carry out degradation activities effectively in just a few days. Bacteria can produce specific enzymes that can break down complex chemical compounds into simpler and less harmful forms faster than fungi and algae. Bacteria also have fast reproduction rate and can adapt to extreme environmental conditions (Anuar *et al.,* 2014). One type of bacteria that can be utilized for organic compound degradation activities is indigenous bacteria.

Indigenous bacteria are bacteria that can live freely in nature and are usually native to the area. Some previous studies mentioned that *Indigenous* bacteria are quite effective as bioremediation agents in terms of waste decomposition (Rochman *et al.,* 2016). Many *indigenous* bacterial isolates have been reported to be able to degrade plastics. Indigenous plastic degrading bacteria are plastic polymer degrading bacteria that originate from the habitat of origin such as soil or landfills (Mafruchati, Ismail, et al., 2023). Some bacteria capable of degrading plastics include *Pseudomonas* sp., *Staphylococcus* sp., *Streptomyces* sp., and *Bacillus* sp., and *Mycobacterium* sp. (Mafruchati et al., 2022).

Junaedi *et al.* (2024) in their research revealed that Indigenous bittern bacteria have the potential to carry out cellulolytic activity on substrates in the form of cow dung waste and straw. The ability possessed by these indigenous bacteria allows them to degrade plastic components which are also composed of organic matter. This is emphasized by Fatwa *et al.* (2021) who reported that indigenous bacteria are fiber-degrading bacteria that are quite resistant to polluting materials such as heavy metals, oil spills, and also microplastics. In the metabolic process, bacteria will utilize these contaminants as a source of carbon and nutrients for their life using the help of different enzymes in each bacterium. By only utilizing unused salt wash wastewater can be utilized to take bacterial isolates that can be used as an alternative to degrading plastic waste in a short time, namely 7 days of incubation period using only an orbital shaker at 50 rpm agitation.

This study was conducted to determine the ability and effectiveness of indigenous bittern bacteria as one of the plastic waste degradation agents. The results showed that BB4 had the highest ability to degrade plastic waste and was effective as a plastic waste degrader. This research provides technological innovation through microbial-based plastic waste management by formulating Indigenous bittern bacteria that can degrade plastic waste types. Further research needs to be carried out on several treatment variations as a comparison including the length of degradation time, pH variation, rotation speed on the orbital shaker, as well as the type of growth medium, and additional nutrients needed by bacteria in carrying out plastic waste degradation activities. Therefore, it is necessary to conduct research related to the ability and effectiveness of indigenous bacteria in degrading plastic waste in the waters.

Literature Review

Plastic Waste

Plastics are known as synthetic polymers developed through the polymerization of monomers obtained from petrochemicals and combined with other chemicals (Pilapitiya & Ratnayake, 2024; Torres-Agullo *et al.,* 2021). Monomers are repeating units of long-chain polymers composed of carbon, hydrogen, and oxygen joined by covalent bonds. Ethylene and propylene are common examples of lightweight monomers that are widely used in plastic production (Pilapitiya & Ratnayake, 2024; Hassan *et al.,* 2022).

Plastic is a macromolecule formed through the polymerization process of monomer units that are

combined to form long-chain polymers, causing plastics to be difficult to decompose and require decades of biodegradation time (Asmi *et al.,* 2022). Plastic has properties that are more resistant to microbial attack in a short time. Plastic is an organic material made from synthetic polymer compounds consisting of carbon, hydrogen, silicon, oxygen, chloride, and nitrogen and has a more economical, flexible, and lightweight value (Purwaningrum, 2016). Plastic is a polymer product consisting of a variety of synthetic or semisynthetic organic and inorganic compounds (Saminathan *et al.,* 2014).

Plastic is a man-made synthetic polymer that has stable and long-lasting characteristics that make plastic widely used for human interests and needs such as packaging food products, cosmetics, detergents, chemicals, and pharmaceuticals. Every year, the amount of plastic production in various countries is more than 260 million tons. The accumulated plastic waste can cause environmental changes such as the emergence of unpleasant odors due to the difficulty of degrading plastic waste (Sriningsih & Shovitri, 2015). Plastic is a hydrocarbon derivative product such as *Polyvinyl Chloride* (PVC), *Polyethylene* (PE), *Polypropylene* (PP), *Polystyrene* (PS), and *Polyethylene Terephthalate* (PET) that has contributed significantly to environmental problems. Large quantities of plastics accumulate in various environments and are naturally difficult to degrade (Lwanga *et al.,* 2018).

Dangers of Plastic Waste

Every year, 14 million tons of plastic are dumped into the ocean accounts for about 80% of the marine debris found in various aquatic environments. The main contributors to marine plastic debris come from land-based sources. These sources include stormwater and urban runoff, wastewater overflows, littering, inadequate waste disposal and management practices, industrial activities, tire abrasion, development activities, and illegal dumping. Plastic pollution in the marine environment has a huge impact on marine organisms and ecosystems, resulting in many adverse impacts such as asphyxiation, entanglement, injury, and infection. Plastic debris cannot decompose, so it persists for long periods and causes significant disruption to marine ecosystems (Zaini *et al.,* 2024).

Plastic is a synthetic polymer that is difficult to decompose in nature and takes about hundreds of years to decompose plastic completely in nature. The process of plastic decomposition takes a long time and the use of plastic which is increasing every year has resulted in the accumulation of plastic waste in the environment, harming the environment (Nasution, 2015). Plastic that does not decompose causes various problems, including environmental pollution, soil degradation and negative impacts on marine life. Poorly managed plastic waste tends to end up in rivers, seas, and wastelands, causing environmental pollution that harms ecosystems and living things (A. R. Febriyanti et al., 2022). Plastics that enter the waters can degrade into microplastics that are very small and widely distributed in the environment (Kamsiati *et al.,* 2017). These microplastics can enter the food chain and eventually reach humans through seafood consumption.

Biodegradation

The largest group of microorganisms, bacteria are found primarily in water, soil, and the surrounding environment (Das *et al.,* 2024). Various bacteria are known to have the ability to break down contaminants (Mafruchati et al., 2024). Currently, several areas of studies have examined the use of microbes to degrade plastic waste. The application of undiluted bacterial cultures has been the main focus of most research on the breakdown of plastic waste through microbial intermediates in the laboratory. Another purpose of using a single strain in plastic waste degradation research is to facilitate the examination of metabolic pathways and evaluation of the impact of various environmental factors on MP degradation

(Wardhana & Ratnasari, 2022). In addition, changes occurring within the MP as well as the entire process of MP elimination by active bacteria can be closely monitored (Das *et al.,* 2024).

Biodegradation is the process of breaking down complex compounds into simpler compounds such as water and carbon dioxide. This decomposition process uses the activity of microorganisms so that changes in molecular integrity occur. Biodegradation is the process of microorganisms being able to decompose natural polymers (such as lignin, cellulose) and synthetic polymers (such as polyethylene, polystyrene) and microorganisms have different degradation abilities, so they vary from one microorganism to another (Fadlilah & Shovitri, 2014; Kaseem *et al.,* 2012)

The ease or difficulty in degrading plastic is influenced by various factors, such as the type of plastic, the size and molecular structure of the plastic, environmental conditions, and the type and ability of microorganisms involved in the biodegradation process. In addition, environmental factors such as temperature, humidity, and nutrient availability can also affect the ability of microorganisms to decompose plastics (Abidin *et al.,* 2023).

Indigenous Bacteria

Indigenous bacteria are microorganisms that naturally occur in a certain environment and have adapted to the physical, chemical, and biological conditions in that habitat. Indigenous bacteria are bacteria that currently provide many benefits in various fields of human life. Indigenous bacteria can live freely in nature and are usually native to the area. One of the roles of indigenous bacteria is to break down pollutants in an environment (Tanasupawat *et al.,* 2016). Some previous studies have mentioned that indigenous bacteria are quite effective as bioremediation agents in terms of waste decomposition (Rochman *et al.,* 2016). This is *because indigenous* bacteria are fiber-degrading bacteria that are quite resistant to polluting materials such as heavy metals, oil spills, and also microplastics (Fatwa *et al.,* 2021).

Fachrul & Rinanti (2018) mentioned that indigenous bacteria have significant potential to degrade plastic waste, mainly due to their ability to produce enzymes that can break down plastic polymers. Some indigenous bacteria found in various ecosystems, such as soil, water, or organic waste, have shown the ability to degrade plastics, although the degradation process is slow and depends on environmental conditions and the type of plastic being degraded. Khastini *et al.* (2022) suggested *that indigenous* bacteria found in plastic-polluted places have often adapted by developing mechanisms to use plastic as a carbon or energy source. This type of bacteria can utilize plastic by breaking it down into smaller molecules that can be taken up and metabolized by cells. Indigenous bacteria often form biofilms on plastic surfaces. This biofilm helps in the plastic degradation process as it provides a stable microenvironment and supports enzymatic activity.

Indigenous Bacteria of Salt Wash Wastewater (Bittern)

Bittern is a residual solution rich in salt, mainly magnesium chloride produced after the evaporation of seawater for the production of table salt. This salt wash wastewater or bittern is a very extreme habitat for many microorganisms due to the high salt content and the presence of other chemical compounds (Nuzula *et al.,* 2021). Megawati *et al*. (2021) suggested that *indigenous* bacteria in bittern usually have diverse metabolic capabilities, including the ability to decompose complex organic compounds or even chemical compounds that may be toxic to many other microorganisms. Indigenous bittern bacteria have a unique ability to degrade plastic waste, mainly because they originate from environments with extreme conditions, such as high salinity and the presence of certain chemical compounds. These extreme conditions cause the bacteria to develop specialized adaptation mechanisms that allow them to survive and break down difficult-to-degrade materials, including plastics.

Methodology

Time and Place

The reseach was conducted from September to October 2024. The research included sampling to the degradation process of plastic waste. Plastic sampling was carried out in the waters of the East Harbor to Karang Kiring, Kamal, Bangkalan at 3 stations and each station was replicated 3 times. The plastic samples used were black plastic bags. The plastic waste degradation process was carried out at the Marine Biology Laboratory and Basic Laboratory of Trunojoyo University Madura.

Sterilization of Tools and Materials

The tools used in plastic waste biodegradation activities include tools made of glass and plastic. Tools made of glass include Erlenmeyers, measuring pipettes, and culture bottles, test tubes, petri dishes, beakers while tools made of plastic are TIP. Sterilization of tools is done by means of steam heat sterilization using an autoclave with a temperature of 121ºC for 15 minutes. Tools that will be sterilized, first washed using clean water and dried using tissue. Sterilization of materials in the form of media is carried out to kill bacteria in the media using the same autoclave as sterilization on tools.

Sampling

Plastic sampling was carried out in the waters of the East Harbor area to the Karang Kiring area, Kamal Bangkalan. The plastic samples that will be tested are black plastic bags. The sampling point consists of 3 places with each point being repeated 3 times. Plastic samples are characterized based on type, size, color, quantity, and condition. Plastic that will be degraded in YEB + 1% glucose media is the least common or least plastic found in a 1 × 1 m transect.

Preparation of Nutrient Agar Slant Media

The preparation of NA slant media was carried out by weighing NA media as much as 1.68 grams and then dissolved in 90 ml of distilled water. The solution was heated with a hot plate until boiling. 6 ml of NA media was pipetted into a test tube. NA media was sterilized using an autoclave at 121°C for 20 minutes. NA media was tilted with a tilt angle of 15°. The solidified NA media was stored in a refrigerator for 24 hours.

Rejuvenation and Propagation of Bacterial Isolates

Rejuvenation and multiplication of bacterial isolates is done by taking 1 ose of bacterial isolate aseptically from the stock culture. Growing bacteria by streak plate on NA media. Incubate at 37ºC for 24 hours. Take 1 ose of a single colony of bacteria that grows on nutrient agar media on a petri dish. Growing bacteria by scraping (streak plate) on slanted NA media. Incubate the stock culture at 37ºC for 24 hours. Storing the stock culture in the refrigerator.

Preparation of Bacterial Inoculum

Making bacterial inoculum is done by taking 1 ose of bacterial isolate aseptically. Homogenize 1 ose of bacterial isolate into a test tube containing 10 ml of sterile distilled water. Incubate the bacterial inoculum in an incubator at 37ºC for 24 hours.

Preparation of Liquid Media (Yeast Extract Broth + 1% Glucose)

YEB media was weighed as much as 30 grams. YEB media was dissolved with 3000 ml of distilled water. YEB media was put into 30 culture bottles with each filled with 100 ml. Made a 1% glucose solution

and then mixed it into the YEB media. Sterilize culture bottles containing liquid media (YEB + 1% glucose) using an autoclave at 121ºC for 15 minutes.

Plastic Biodegradation Activity

The bacterial inoculum that has been incubated for 24 hours is pipetted 1 ml. Inoculate 1 ml of bacterial inoculum into 100 ml of liquid medium. Incubate the bacterial culture for 24 hours at 37ºC. Samples of 1 × 1 cm black plastic that had been sterilized in 70% alcohol for 15 minutes were dried in an oven at 37ºC for 24 hours. The plastic was weighed as the initial weight before the biodegradation process. Put the plastic sample into a culture bottle containing liquid media. Incubate for 7 days on an orbital shaker with 50 rpm agitation.

Optical Density (OD) Measurement of Bacterial Culture During Degradation Activity

OD measurement was performed by pipetting 3 ml of bacterial culture in liquid media into a sterile test tube. Inserting 3 ml of bacterial culture into the liquid to be inserted into the cuvette. Measuring the OD value using uv-vis spectrophotometer with a wavelength of 600 nm.

Calculation Of Total Plate Count (Tpc) Value During Degradation Activity

Prepare a series of dilutions containing 9 ml into sterile petri dishes. Day 0 dilution series 10^{-4} sequentially until day 7 10^{-11}. NA media as much as ± 10 ml was poured into a petri dish that already contained bacterial cultures from the dilution series. The mouth of the Petri dish was closed using plastic wrap. Code the bacterial culture samples using label paper. Incubate the incubation for 24 hours. Count the colonies formed and record them in the research notebook.

Calculation of Percentage Dry Weight Loss of Plastics

Test plastics from culture bottles that had been incubated for 7 days were cleaned by soaking the test plastics using 70% alcohol for 15 minutes and then dried using an oven at 37ºC for 24 hours. The plastic was then weighed to determine the final dry weight. Calculating the percentage of dry weight loss of the test plastic using the following formula:

$$\text{Weight Loss} = \frac{W_1 - W_2}{W_1} \times 100\%$$

Description:
W1: Initial dry weight of plastic before degradation activity (g)
W2: Final dry weight of plastic after degradation activity (g)

Research Design

a) b) c)

Figure 1 Documentations

Note: a) test plastics, b) culture media, c) incubation period

Source: Author (2024)

This study used a complete randomized design (CRD) using 1 type of independent variable, namely variations in the type of indigenous bittern bacteria inoculated on liquid culture media (YEB + 1% glucose) as much as 100 ml. The types of indigenous bittern bacteria used were BB1 to BB9. The controlled variable used was *Bacillus Megaterium* (BPS4). This study used 10 treatment samples that were repeated 3 times. The dependent variables observed were OD, TPC, and percentage of dry weight of the test plastic.

Data Analysis

OD and TPC values during the 7-day degradation process were analyzed descriptively while the percentage value of dry weight loss of the plastic after 7 days of incubation time was analyzed statistically.

Results and Discussion

Optical Density (OD) Value

OD value measurements were taken from day 0 to day 7 on YEB + 1% glucose media. OD values were measured using a UV-VI spectrophotometer with a wavelength of 600 nm. The results of the OD value can be seen in Figure 2 as follows:

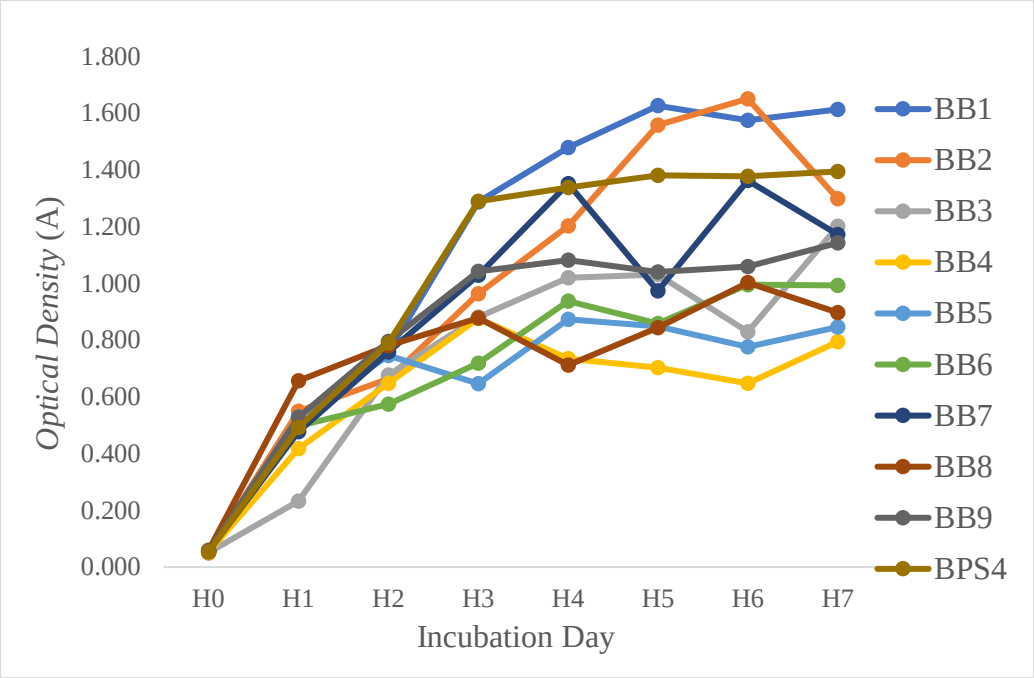

Figure 2 Graph of Optical Density (OD)

Note: BB1 up to BB9: Bittern Bacteria, BPS4: *Bacillus megaterium* (control)

Source: Author (2024)

Figure 2 shows the OD value of 9 indigenous bacterial isolates of salt wash wastewater (bittern), namely BB1 to BB9, and as a control, namely BPS4. The graph shows that most of the bacterial isolates showed an increase in OD value. The highest OD value on day 0 occurred in BB8 which amounted to 0,059, while the lowest OD value in BB2 amounted to 0,050 and the control was 0,053. The highest on day 1 occurred in BB8 which amounted to 0,657, while the lowest in BB3 was 0,233 and the control was 0,492. The highest on day 2 occurred in BB9 which amounted to 0,796, while the lowest in BB6 amounted to 0,575 and the control was 0,790. The highest on day 3 occurred in BB1 which amounted to 1,288, while the lowest in BB5 amounted to 0,646 and the control was 1,290. The highest on day 4 occurred in BB1 which amounted to 1,479, while the lowest in BB8 amounted to 0,712 and the control was 1,338. The highest on day 5 occurred in BB1 which amounted to 1,626, while the lowest in BB4 amounted to 0,703 and the control was 1,381. The highest on day 6 occurred in BB2 which amounted to 1,651, while the lowest in BB4 amounted to 0,648 and the control was 1,377. The highest on day 7 occurred in BB1 which amounted to 1,613, while the lowest in BB4 amounted to 0,794 and the control was 1,396.

Based on Figure 2 the OD value at H0 shows the lag phase (adaptation). Respati *et al.* (2017) mentioned that the lag phase in bacterial growth is the initial period of bacteria adjusting to the new environment or medium. In this phase, although metabolic activity occurs, there is no significant increase in cell number. The duration of the lag phase is strongly influenced by factors such as the type of bacteria, culture age, and medium conditions. Setiawati *et al.* (2014) emphasized that the lag phase is the initial stage of adaptation before exponential growth occurs. Environmental conditions, such as pH, temperature, and nutrient availability greatly affect the length of this phase. This adjustment is needed so that bacteria can activate enzymes and metabolic pathways suitable for their new medium.

The OD values at H1 to H3 indicate that the indigenous bittern bacterial are in the logarithmic (exponential) phase. This phase is characterized by OD values or bacterial density that experience rapid

growth. The rapid growth of bacteria at the beginning of the incubation period can be caused by nutritional factors available in the media tend to be abundant. This logarithmic phase describes the cell dividing itself which is characterized by a constant rate, constant metabolic activity, and a state of balanced growth (Anggraeni & Triajie, 2021).

Optical Density (OD) values at H4 to H7 show that indigenous bittern bacterial are in the stationary phase. This can be seen from the graph which tends to show a stable line means that there is no significant increase or decrease in OD value. Novanti & Zulaika (2018) stated that the stationary phase in bacterial growth is the stage when cell division begins to balance with the rate of cell death. This usually occurs due to nutrient limitation, accumulation of toxic waste products, or changes in environmental conditions that no longer support optimal growth. In this phase, metabolic activity slows down, and the total number of living cells in the population remains stable. This stationary phase is also when bacteria may produce secondary metabolites such as antibiotics or enzymes.

Figure 2 shows that the growth phase of indigenous bittern bacteria only reaches the stationary phase and does not reach the death phase. Mahjani & Putri (2020) suggested that the death phase in bacterial growth occurs after the stationary phase when the number of dead bacteria begins to exceed the number of living bacteria. Bacteria that experience this death phase show a sharp decrease in biomass concentration. This is due to several factors such as running out of nutrients needed for growth or due to the accumulation of toxins in the environment that are not degraded, thus inhibiting the metabolic process of bacteria. This decline may continue until almost the entire bacterial population dies, depending on the environmental conditions present.

Bacterial growth can be determined by measuring the difference between the OD value before incubation and the OD value after incubation. The OD value shows the amount of light absorbed by cells is directly proportional to the number of cells, where nutrients and growth media as well as environmental factors such as temperature and pH can be one of the inhibiting factors in the process of bacterial growth. The number of bacterial cells is measured by knowing the level of turbidity in bacterial culture media caused by bacterial density, where the more turbid the bacterial culture media, the more the number of cells (Munfaati *et al.*, 2015).

Total Plate Count (TPC) Value

Calculation of TPC values was carried out from day 0 to day 7 on YEB media with the addition of 1% glucose. Calculation of TPC values with the pour plate method using NA media. The results of the TPC value calculation can be seen in Figure 3 as follows:

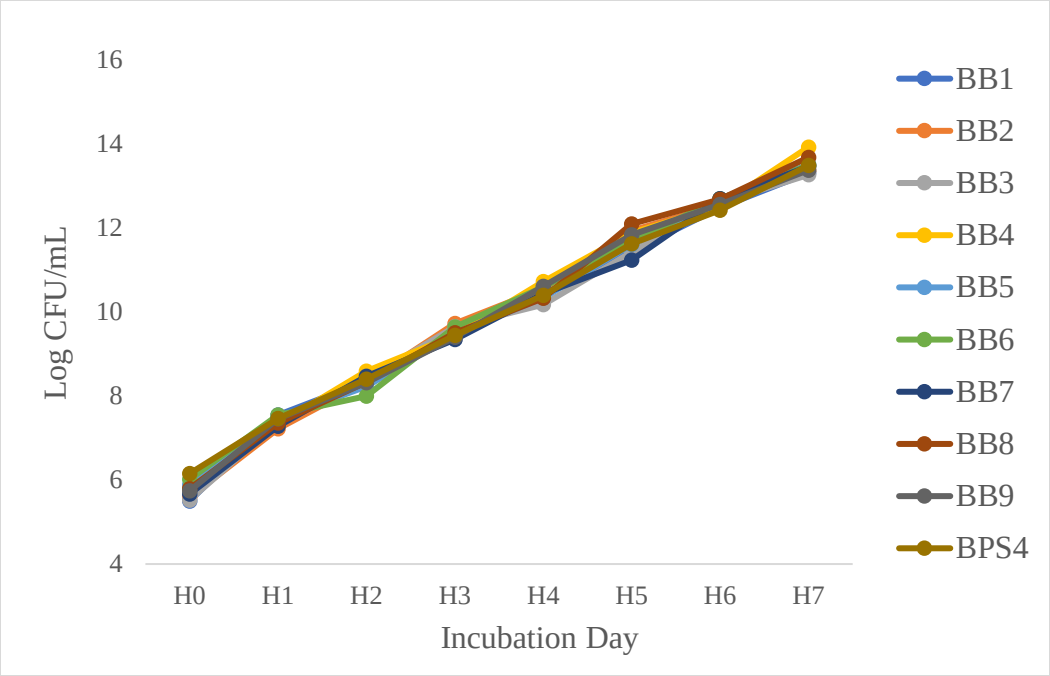

Figure 3 Graph of Total Plate Count (TPC)

Note: BB1 up to BB9: Bittern Bacteria, BPS4: *Bacillus Megaterium* (control)

Source: Author (2024)

The highest TPC value on day 0 occurred in BB6 was 6,02, while the lowest TPC value in BB1 was 5,50, and the control was 6,15. The highest on day 1 occurred in BB6 was 7,55, while the lowest in BB2 was 7,22, and the control was 7,47. The highest on day 2 occurred in BB4 was 8,59, while the lowest in BB6 was 8,00, and the control was 8,40. The highest on day 3 occurred in BB2 was 9,72, while the lowest in BB7 was 9,34, and the control was 9,45. The highest on the 4th day occurred in BB4, was 10,72, while the lowest in BB3 was 10,18, and the control was 10,40. The highest on day 5 occurred in BB8 was 12,10, while the lowest in BB7 was 11,24, and the control was 11,62. The highest on day 6 occurred in BB6 and BB7, which was 12,70, while the lowest in BB1 was 12,48, and the control was 12,43. The highest on day 7 occurred in BB4 which was 13,93, while the lowest in BB3 was 13,27, and the control was 13,49.

Calculation of TPC was carried out by Aida & Manalu (2023) on bacterial isolates degrading palm oil liquid waste. The results showed that the highest TPC value was shown by sample with an average value of 8,5 × 107 CFU/ml or 7,93 Log CFU/ml, while the lowest value was shown by sample 1 with an average of 3,5 × 107 CFU/ml or 7,54 Log CFU/ml. The difference in the number of bacterial colonies that grow depends on the stage of decomposition. Variations in the number of bacterial colonies can also be caused by several parameters such as nutrients, minerals, temperature, oxygen levels, and acidity (pH). Khastini *et al.* (2022) reported that the ability of bacteria to carry out degradation activities on hydrocarbon, xenobiotic, and radioactive molecules or compounds must be supported by optimal growth and cell division capabilities so that the test bacteria can remain active and grow even though the culture medium contains molecules or compounds that are not desired for their metabolic activities.

The dilution series in the process of calculating the TPC value is intended to grow bacterial colonies on limited growth media, making it impossible to count large numbers of bacteria. Dilution aims to reduce the density of bacteria to be grown in liquid (distilled water), where the dilution series used is adjusted to the estimated number of bacteria present in the sample (Puspitasari *et al.*, 2012). The calculation of the TPC

value is intended to show the number of microbes by counting bacterial colonies grown on NA media. Living microbial cells if grown on an agar medium will multiply and form colonies that can be seen directly without using a microscope (Yunita *et al.*, 2015).

Weight Loss Percentage Value of Plastic Waste

The dry weight loss percentage data of the test plastics can be seen in Figure 4 as follows:

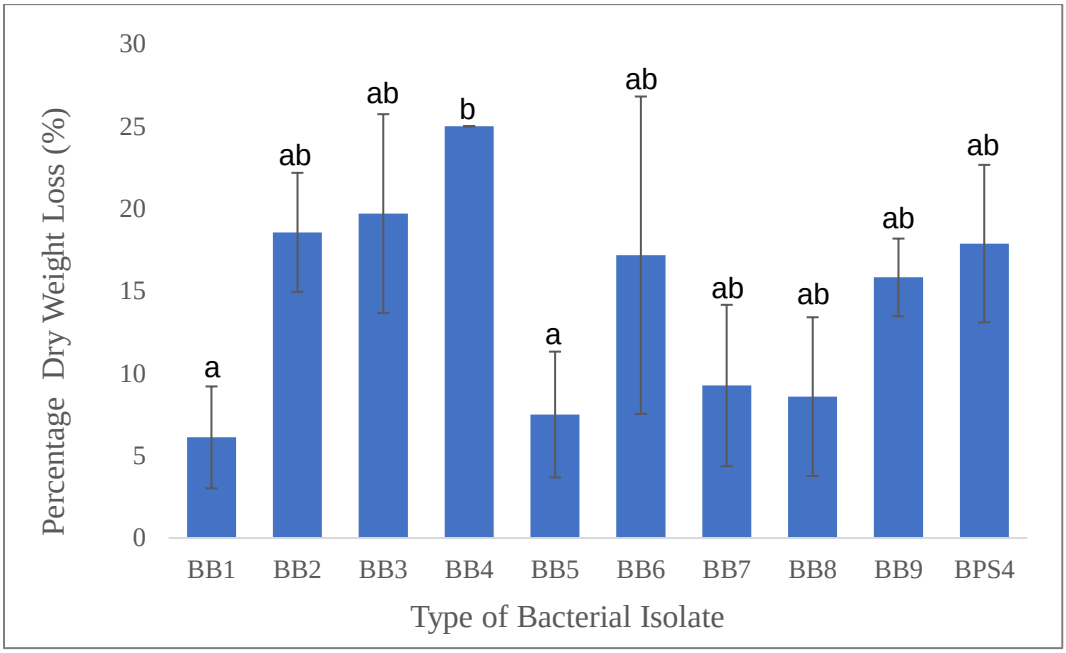

Figure 4 Diagram of percentage of plastic dry weight loss

Note: BB1 up to BB9: Bittern Bacteria, BPS4: *Bacillus megaterium* (control)

Source: Author (2024)

Based on the calculations carried out on the percentage value of plastic weight loss, the results show that the percentage value of plastic dry weight loss in BB1 is 6,1%±3,09^{a} with the difference in each repetition in order of 0,0001 g; 0 g; and 0,0001 g. The value in BB2 is 18,6%±3,61^{ab} with the difference in each repetition in order of 0,0001 g; 0,0002 g; and 0,0003 g. The value in BB3 is 19,7%±6,09^{ab} with a difference in each repetition of 0,0002 g; 0,0001 g; and 0,0003 g, respectively. The value in BB4 is 25%±0^{b} with the difference in each repetition in order of 0,0003 g; 0,0002 g; and 0,0002 g. The value in BB5 is 7,5%±3,82^{a} with the difference in each repetition in order of 0,0001 g; 0 g; and 0,0001 g. The value in BB6 is 17,2%±9,63^{ab} with the difference in each repetition in order of 0 g; 0,0002 g; and 0,0003 g. The value in BB7 is 9,3%±4,90^{ab} with the difference in each repetition in order of 0,0002 g; 0 g; and 0,0001 g. The value in BB8 is 8,6%±4,82^{ab} with a difference in each repetition of 0,0001 g; 0,0002 g; and 0 g, respectively. The value in BB9 is 15,8%±2,36^{ab} with the difference in each repetition in order of 0,0001 g; 0,0002 g; and 0,0002 g. The value in BPS4 is 17,9%±4,78^{ab} with the difference in each repetition in order of 0,0002 g; 0,0003 g; and 0,0001 g.

The results of the one-way ANOVA test conducted showed that the data obtained were normally distributed, with homogeneous data variations. Thus, a post hoc test shows that different types of bacteria affect the degradation activity of plastic waste in the test culture medium. It was shown by BB4 with the highest degradation activity value of 25%+0^{b} (Figure 4). This is consistent with the TPC value shown by BB4 on the 7th day of incubation time which showed the highest TPC value (13,93 Log CFU/mL) among

other indigenous bacterial species of bittern wastewater and when compared to the TPC value shown by the control (BPS4). However, this was different from the OD value shown by BB4 on the 7th day of incubation time which showed the lowest OD value (0,794) among other types of indigenous bacteria of salt-washing wastewater (bittern) and when compared to the OD value shown by the control (BPS4).

The same research was conducted by Ardani *et al.* (2024) using test plastics in the form of 1 × 1 cm black plastic waste in NB media, and indigenous bittern bacteria as degradation agents. The results showed that the highest percentage of plastic weight loss was in BB4 36,4%. This result is in accordance with the results of the study which showed that BB4 is the most effective bacteria to degrade plastic waste. Fadlilah & Shovitri (2014) their research used Bacillus bacteria with white and black plastic types in Salt Mineral Medium (MSM) within 4 months of incubation. The results showed that the percentage of Bacillus bacteria degradation on white plastic was 1,9% and on black plastic was 2,3%.

Similar research was also conducted by Sriningsih & Shovitri (2015) who tested the degradation activity of Pseudomonas bacteria on test plastics in Mineral Salt Medium (MSM) for 3 months of incubation. The results of the percentage of dry weight loss on black plastic showed an average value of 2,7%, white plastic of 3,3%, and transparent plastic of 4,5%. Filayani (2020) conducted a polyethylene plastic degradation test using the Winogradsky Column method with the addition of *Lactobacillus bulgaricus* and *Streptococcus thermopilus* bacteria for 20 days of incubation time and obtained the highest percentage value of polyethylene plastic degradation reaching 19,4%. Based on the three previous studies, the best plastic degradation activity was BB4 with a value of 25% showed a higher value compared to the plastic degradation activity value shown by the test bacteria in the three previous studies.

This could be due to the different types of plastic tested. Octavianda *et al.* (2016) stated that the type of plastic is one of the factors that can affect plastic weight loss during the degradation process by bacteria. The chemical structure and physical properties of the test plastic are the main components that must be recognized by bacteria to be broken down from the polymer form into simpler compounds. Factors such as the type of chemical bonds in the polymer, molecular weight, degree of hydrophobicity, presence of certain functional groups, as well as physical properties such as surface texture and porosity of the plastic, play an important role in determining the level of accessibility and effectiveness of enzymes produced by bacteria. Some plastics have a more complex or inert structure, which makes them more difficult to degrade, while other plastics degrade more easily due to a simpler chemical structure or are more susceptible to enzymatic degradation.

The mechanism of plastic biodegradation occurs through two stages: abiotic and biotic. The abiotic mechanism is characterized by the process of carbon oxidation that produces functional groups that cause the hydrocarbon polymer properties to change to hydrophilic (Mafruchati, Othman, et al., 2023). The polymer surface becomes able to absorb water, making it easier for bacteria to carry out degradation activities (Leja & Lewandowicz, 2009). The biotic mechanism or biodegradation occurs when bacteria attached to the plastic surface form a biofilm. Bacteria can break down complex plastic compounds into compounds that can be used as carbon and energy sources (Mohan & Srivastava, 2010).

Environmental factors that affect the degradation process include humidity, temperature, pH, plastic chemical structure, oxygen availability, and nutrient supply (Sangale *et al.,* 2012). Plastic-degrading bacteria tend to grow more to form biofilms or adhere to the surface of the plastic rather than live freely in the liquid media column. Bacteria must be able to form a stable biofilm to attach to the plastic which is a carbon source so that degradation activities can occur optimally (Sriningsih & Shovitri, 2015).

Conclusion

Based on the research conducted, it can be concluded that the OD value of indigenous bittern bacteria in degrading plastic waste in YEB + 1% Glucose media by BB1 to BB9 and BPS4 at the 7th day incubation time is 1,613; 1,299; 1,201; 0,794; 0,846; 0,993; 1,172; 0,897; 1,143; and 1,395, respectively. The TPC value of indigenous bittern bacteria in degrading plastic waste in YEB + 1% Glucose media by BB1 to BB9 and BPS4 at the 7th day incubation time was 13,31; 13,37; 13,27; 13,93; 13,52; 13,49; 13,47; 13,68; 13,38; and 13,49 Log CFU/ml. The percentage values of the dry weight of plastic after degradation during 7 days incubation in YEB + 1% Glucose media by BB1 to BB9 and BPS4 at the 7th day incubation time were 6,1%; 18,6%; 19,7%; 25,0%; 7,5%; 17,2%; 9,3%; 8,6%; 15,8%; and 17,9%. These results show that BB4 has the highest ability to degrade plastic waste.

This research provides new insights into the potential of indigenous bittern bacteria as plastic waste biodegradation agents to enriches knowledge in the field of environmental biotechnology, especially in microbial-based plastic waste management. This research can be used as a basis for the waste management industry and government to design more sustainable policies in plastic waste treatment to reduce dependence on conventional methods that are expensive and less effective. Further research is needed to specifically identify the genus and species of each indigenous bittern bacterial isolate used in this study and investigate environmental factors such as pH, temperature, and bacterial concentration so that the plastic degradation process takes place more quickly and efficiently.

Author's Contribution

All authors have contributed to this article. The first and second authors were responsible for data collection and material preparation. The third and fourth authors revised and provided feedback and an overview of the research.

Acknowledgments

The authors are grateful to LPPM UTM for the internal independent grant in 2024 that was given so that this research could be carried out smoothly.

Declaration of Competing Interest

This study did not have any tendency or interest in the various parties involved. The data obtained is pure data used to develop science and knowledge.

Fundings

The author received research funding from the Institute for Research and Community Service.

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