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Application of *Indigenous* Bacterial Formulation of Salt-Washing Wastewater (*Bittern*) in Degrading Plastic Waste

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ABSTRACT

The accumulation of plastic waste can lead to environmental changes. One solution to reduce plastic waste is through a biodegradation process involving microorganisms or bacteria. One solution is biodegradation by using microorganisms or bacteria to break down plastic. Biodegradation is a solution to reduce plastic numbers with the help of microorganisms or bacteria. This study aims to measure the *Optical Density* (OD), *Total Plate Count* (TPC), and degradation effectiveness of indigenous bacteria from salt wash wastewater (*bittern*) in degrading plastic on *Nutrient Broth* (NB) media with 1% glucose. The research process included sterilization of tools and materials, sampling, media preparation, rejuvenation of bacterial cultures, inoculation, OD measurement, TPC calculation, and percentage of plastic weight loss. Data on OD and TPC were analyzed quantitatively with visual aids. The plastic degradation percentage was measured after 7 days of incubation. The percentage of plastic degradation was measured after 7 days of incubation. The results showed that the highest OD value was bacteria BB8 which amounted to 1.614. TPC for plastic degradation was highest in BB9 which amounted to 13.37 Log CFU/g. BB4 showed the highest percentage of plastic dry weight reduction 36.4% after 7 days. These findings highlight the potential of salt-washing wastewater bacteria for plastic waste degradation.

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Introduction

Plastic is the most common waste because it is difficult to decompose and return to the environment. Syauqiah *et al*. (2021) explained that plastic is a hydrocarbon polymer hard to decompose. Based on data from Jenna Jambeck (2018), a waste researcher from the University of Georgia, Indonesia is ranked second in the world for plastic waste, which reaches 187.2 million tons, and China, which reaches 262.9 million tons. Alamsyah & Fadli (2023) reported that one of the countries in the Asian continent that contributes the largest amount of waste as well as contributing to plastic waste contamination in the environment is Indonesia. Director General of Waste Management, Waste, and Hazardous and Toxic Substances (B3) of the Ministry of Environment and Forestry, Tuti Hendrawati Mintarsih reported that the total amount of Indonesian waste in 2019 will reach 68 million tons with the amount of plastic waste expected to reach 9.52 million tons or around 14 percent of the total waste.

Octavianda *et al*. (2016) reported that the increase in plastic use that occurs every year is in line with the increase in plastic waste that could harm the environment. Pollution of the aquatic environment by synthetic polymers such as plastic waste is recognized as a major problem. This is emphasized by several institutions such as the European Union (EU), which has banned various disposable plastic products such as straws, coffee stirrers, and plastic cutlery since 2021 (Mafruchati et al., 2022). Several non-governmental organizations are active in the field of aquatic environmentalists who also strongly oppose the use of plastics, one of which is the ECOTON Conservation Foundation. ECOTON emphasizes that plastics, especially those that are difficult to decompose, contribute greatly to the pollution of water, soil, and aquatic ecosystems. In addition, plastics absorb pollutants produced by chemicals in the environment and seawater, which indirectly introduce harmful substances into organisms through the food chain. As a result, contaminated food could harm human health. The extensive use of plastics in food processing and packaging results in a significant volume of plastic waste. However, the current government in Indonesia has no definite law enforcement to address this issue (Riesti *et al*., 2023)

However, this fact is still not matched by the results of a significant reduction in the amount of plastic waste in the aquatic environment. This is emphasized by the statement of Sriningsih & Shovitri (2016) which states that plastics have persistent properties. In addition, Suryono. (2019) added that plastics also contain non-plastic substances called additives, including colorants, antioxidants, ultraviolet light absorbers, and anti-adhesives. This can also be caused by differences in the components of each constituent type of plastic in circulation, including *Low-Density Polyethylene* (LDPE), *High-Density Polyethylene* (HDPE), and *Polystyrene* (PS) (Hartono & Rachmat, 2022). One type of plastic that is often used in everyday life is polyethylene plastic. Polyethylene is a plastic polymer composed of several ethylene monomers (García-Astrain & Avérous, 2018).

The persistent properties found in plastics are caused by the presence of macromolecular compounds that are processed through the polymerization stage to form long-chain polymers with the main constituents being carbon and hydrogen atoms making plastics difficult to break down (Sari *et al*., 2023). Other additives contained in plastics such as bisphenol A and phytates that enter the waters can cause significant damage to aquatic ecosystems (Mohan *et al*., 2014). Plastic can absorb toxic chemicals such as POPs (Persistent Organic Pollutants) and PBTs (Persistent, Bioaccumulative, and Toxic Substances) so that it has the potential to cause a large impact in the waters that cause aquatic biota to experience disturbances and even high concentrations can cause death (Mafruchati et al., 2024).

This persistent nature of plastics must be reduced in the environment due to their difficulty in degrading without harming the environment. Plastics that are not properly recycled end up as litter and can

pollute aquatic ecosystems. Therefore, alternative efforts are needed to reduce plastic waste more efficiently. One of the efforts that can be made is using microorganisms as biodegradation agents for plastic waste. Restrepo-Flórez *et al*. (2014) defined biodegradation as the process of degradation or breakdown of natural polymers such as lignin, cellulose, and synthetic polymers such as polyethylene and polystyrene carried out by microorganisms. Bacterial isolates as plastic degrading agents derived from the waste of aquatic industrial activities such as bittern (salt wash wastewater) have not yet been carried out. The use of indigenous bacterial isolates of plastic degraders is still derived from samples of aquatic environments (Mafruchati, 2023).

Nadia *et al*. (2015) stated that bittern is a concentrated liquid resulting from the activity of salt production activities with a relatively abundant amount containing various compounds such as magnesium sulfate (MgSO4), sodium chloride (NaCl), magnesium chloride (MgCl2), potassium chloride (KCl), calcium chloride (CaCl2). Budiharjo *et al*. (2017) added that bittern also contains microorganisms that are resistant to high salt levels, one of which is halophilic bacteria. Halophilic bacteria can produce hydrolytic enzymes in the form of proteases that catalyze the hydrolysis reaction of proteins into oligopeptides and their amino acids (MAFRUCHATI et al., n.d.).

The application of indigenous bacteria from salt wash wastewater (*bittern*) has also been reported by Efendy *et al*. (2023) and Syawallita *et al*. (2024) as a probiotic fish starter in Banyuajuh Village, Bangkalan and Lumbangaol *et al*. (2024) in Durbuk Village, Pamekasan which can remodel organic matter in aquaculture activities. Junaedi *et al*. (2024) also reported the application of Indigenous bacteria of salt-wash wastewater as a starter on the substrate of cow dung and corn cobs of Madura variates to produce biogas. Therefore, it is necessary to carry out the latest research innovations related to the application of Indigenous bacteria of salt-wash wastewater (*bittern*) in degrading plastic waste that pollutes aquatic ecosystems.

Previous studies have examined various bioremediation methods using indigenous bacteria derived from their natural environment. There are still few previous studies that examine how salt-wash wastewater can be utilized for bioremediation. Only a few previous studies explored the potential of Indigenous bacteria derived from salt-wash wastewater in degrading plastic waste. Therefore, this research aims to fill the gap by developing indigenous bacteria application formulations as an innovative and sustainable solution in plastic waste management.

This study aims to determine the *Optical Density* (OD) value as an indicator of microorganism growth on NB media, the *Total Plate Count* (TPC) value to determine the number of microorganisms that grow on NB media, and the percentage value of test plastic degradation activity as a parameter of microorganism effectiveness in decomposing test plastics. The purpose of this research is to develop and apply indigenous bacterial formulations obtained from salt wash wastewater to support the downstream of biotechnology-based products, especially in increasing the efficiency and effectiveness of plastic waste degradation as a sustainable solution to environmental problems. This research is expected to produce indigenous bacterial formulations that are effective in degrading plastic waste, as well as support the downstream of environmentally friendly biotechnology products for sustainable waste management solutions.

Literature Review

Definition of plastic waste

Hastarina *et al*. (2019) stated that plastic waste is one of the largest wastes and is difficult to decompose. Plastic itself is an alkene polymer compound with a large molecular shape. The term plastic in

the chemical sense includes synthetic or semi-synthetic polymerization products. Plastic molecules are formed from organic condensation or addition of polymers and consist of other substances to improve their performance or economic value. The durable nature of plastic and not easily degraded by natural processes, plastic waste is one type of waste that has great potential to pollute the environment, both on land and at sea (Pradnyana et al., 2021).

Indigenous bacteria

Yasid (2014) explains that *indigenous* bacteria are natural microbes isolated from waste of the same type as the type of waste to be treated. Natural consortia already exist in their natural habitat, namely liquid waste, be it carbohydrate degrading bacteria, fat degrading bacteria or protein degrading bacteria. Bacteria that interact with each other in the form of consortia and those isolated from *indigenous* waste can accelerate the degradation process of pollutants. The consortium is a combination of pure cultures referred to as mixed inoculum.

Biodegradation of plastic waste

Biodegradation is the process of decomposing organic substances by microorganisms, especially aerobic bacteria, into simpler substances such as carbon dioxide, water, and ammonia. Hydrocarbon compounds cannot be completely degraded if only carried out by one type of microbe but must be carried out by several types of microbes that have synergistic properties in the form of a consortium. The decomposition process utilizes the activity of microorganisms so that changes in molecular integrity occur. Each microorganism has different characteristics, so the degradation process that occurs will be different or vary between one microorganism and another (Mosconi et al., 2020).

Bacterial nutrients and growth media

Microorganisms utilize nutrients in the media in the form of small molecules that are assembled to form the components of their cells. Different microorganisms require different nutrient materials. Therefore, culture media vary in form and composition, depending on the type of species being cultured. With culture media, isolation of microorganisms into pure cultures can be carried out and the composition of culture media can also be made according to the needs of bacteria. Because different bacteria require different nutritional needs, various kinds of growth media have been developed for use in microbiological diagnostics (Mafruchati, 2020).

Measurement of bacterial growth

Optical Density (OD)

Optical Density (OD) is a measure of the turbidity or darkness of a solution caused by particles or microorganisms in it. OD is often used to measure the growth of bacteria or other microorganisms in liquid culture. Bacterial population density is obtained based on the observation of turbidity and measurement of the optical density OD of bacteria with a spectrophotometer. Measurements made with a spectrophotometer are made by measuring the amount of light absorbed or blocked by a solution at a certain wavelength. The higher the concentration of microorganisms in the culture, the more light will be absorbed or inhibited resulting in a higher OD value (Mu et al., 2015).

Total Plate Count (TPC)

Total Plate Count (TPC) is a common method used to measure bacterial growth by counting the number of live bacterial colonies formed on solid culture media. TPC provides a clear picture of the number

of active bacterial cells in a sample and is often used in microbiological quality testing (Nawangsih *et al*., 2024).

Methodology

Time and place

This research was conducted in September-October 2024 including bacterial rejuvenation and plastic sampling with the same type of plastic bag, namely a thick black crackle bag, and measuring water quality including temperature, pH, and DO (*Dissolved oxygen*) in the East Harbor area to the Karang Kiring area, Kamal Bangkalan, then continued by analyzing samples and data at the Fisheries Resource Management Laboratory, Environmental Laboratory, Marine Biology Laboratory, and Integrated Laboratory of Trunojoyo University Madura. The research flow is presented in (**Figure 3.1**) as follows:

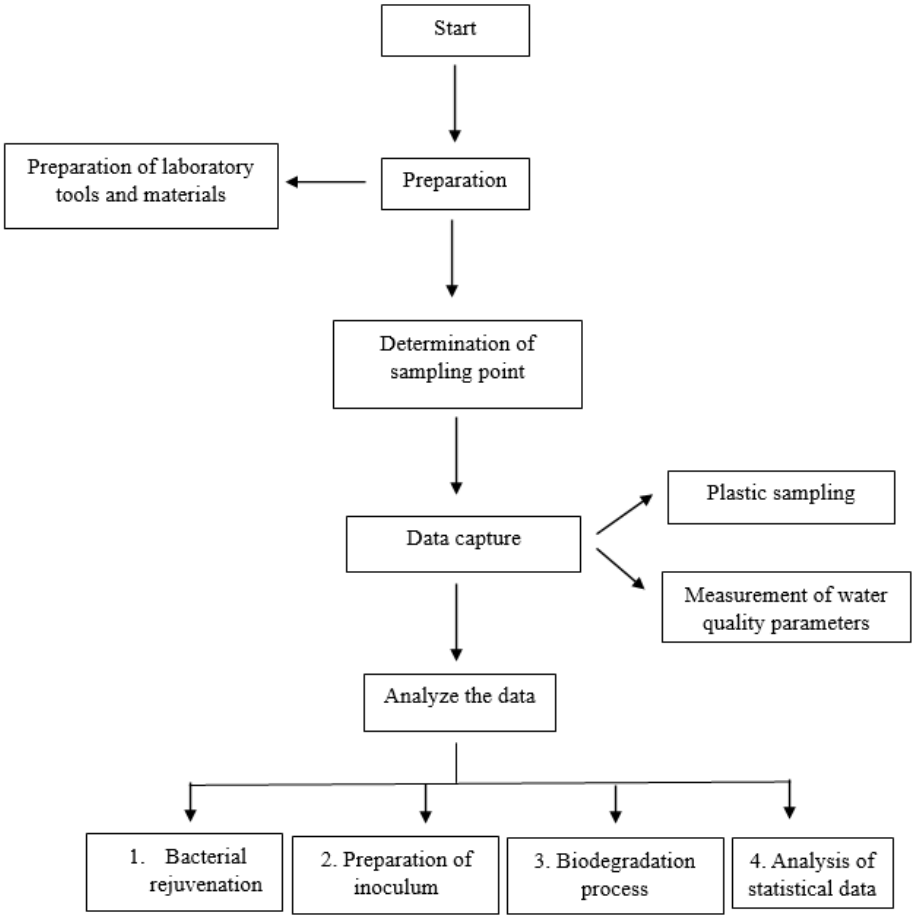

Figure 3.1 Research Flow

Source: Author (2024)

Sterilization of tools and materials

Tool sterilization

Sterilization is the process of removing or killing microorganisms (protozoa, fungi, bacteria, mycoplasma, viruses) in objects or equipment to keep equipment in the laboratory sterile and prevent contamination. Tools that will be sterilized, washed first using running water, and dried with a tissue. Sterilization consists of dry sterilization and wet sterilization. Glass tools are carried out dry sterilization

by covering the mouth of the bottle with cotton then coating it with aluminum foil wrapped in plastic wrap and then put in an oven at 180˚C for 20 minutes. Tools made of glass include Erlenmeyers, beakers, culture bottles, and piper volumes. Tools made from plastic are wet sterilized using an autoclave at 121˚ for 15 minutes with a pressure of 1 atm. Tools made of plastic are TIP micropipettes. Tools and materials are presented in (**Table 3.1**) as follows:

Table 3.1 Tools and materials used during the plastic degradation process

No.	Tools	Materials
1.	Ruler 30 cm	Plastic sample
2.	Analytical scales	*Indigenous* bacteria of salt wash wastewater (*bittern*)
3.	Oven	*Bacillus subtilis*
4.	Scissors	*Nutrient Broth* (NB)
5.	Culture bottle	*Nutrient Agar* (NA)
6.	Incubator	Glucose 1%
7.	GPS	Alcohol 70%
8.	DO meter	Cotton
9.	pH meter	Tissue
10.	Autoclave	Distilled water
11.	Ose needle	Spirtus
12.	Bunsen	Label paper
13.	Micropipette	Plastic wrap
14.	Blue Tip	Alumunium foil
15.	Spektrofotometer	Gloves
16.	Orbital Shaker	Mask
17.	Pipette volume	Matches
18.	Refrigerator	
19.	Erlenmeyer	
20.	Tweezers	
21.	Test tube	
22.	Test tube rack	
23.	spray bottle	
24.	Magnetic Stirer	
25.	Pipette pump	
26.	Petri dish	
27.	Spatula	

Data Source: Primary Data (Integrated Laboratory, 2024)

Material sterilization

Sterilization for media and liquid materials uses wet heat sterilization using an autoclave at 121°C and 1 atm pressure for 15-20 minutes. In addition to the sterilization process above, there are aseptic techniques to maintain contamination of unwanted microorganisms by using a Bunsen flame to sterilize the tip of the ose needle by burning the tip of the ose needle until it glows. The work table is chemically sterilized by spraying 70% alcohol.

Sampling

Plastic sampling was conducted at 3 stations with 3 repetitions using transects with a size of 1 x 1 m in the East Harbor area to the Karang Kiring area, Kamal Bangkalan. Plastic samples were characterized based on type, size, color, quantity, and condition at the time of sampling. Plastic that will be degraded in Nutrient Broth + 1% glucose media is the rarest or least plastic found in a 1 x 1 m transect. Each station has different conditions including at station 1 the location is close to residential areas so many canoes are in the

area, and the substrate conditions in the area are sandy mud. The condition of station 2 is not much different from the condition at station 1, but the canoes at station 2 tend to be few, and the substrate at station 2 is a little rocky so the plastic waste found is small. The condition of station 3 is not much different from the conditions of stations 1 and 2, at this station no canoes were found but there was a water drain, the substrate at station 3 was rocky.

Preparation of Inclined Nutrient Agar (NA)

Weighing 1.68 grams of Nutrient Agar using an analytical balance. Dissolving Nutrient Agar in 90 ml of distilled water. Heating the solution with a hotplate at 380 ° C until boiling, while using a magnetic stirrer to accelerate the homogenization process. Pipetting 6 ml of Nutrient Agar medium into 10 test tubes. Sterilize Nutrient Agar media using an autoclave at 121 ° C for 20 minutes. Tilting the Nutrient Agar media with a tilt angle of 15 °. Placing Nutrient Agar media that has solidified into a plastic bag. Storing Nutrient Agar media in the refrigerator for 24 hours.

Bacterial rejuvenation

Take each Indigenous bacterial isolate of salt-wash wastewater (bittern) using a sterile ose. Growing bacteria by scratching them on a slanted Nutrient Agar medium. Incubating the stock culture at 37°C for 1 x 24 hours. Storing the stock culture in the refrigerator.

Preparation of bacterial inoculum

Take each indigenous bacterial isolate of salt wash wastewater (bittern) in stock culture using a sterile ose needle. Inoculate the ose needle into a test tube containing 10 ml of sterile distilled water. Incubate the bacterial inoculum for 1 x 24 hours at 37°C.

Preparation of Liquid Media (Nutrient Broth + 1% Glucose)

Weigh 39 grams of Nutrient Broth media using an analytical balance. Dissolved Nutrient Broth media in 3 liters of distilled water. Pouring Nutrient Broth media into 30 culture bottles, with each bottle filled with 100 ml of Nutrient Broth. Sterilize the Nutrient Broth media with an autoclave at 121°C for 20 minutes. Weighing 30 grams of glucose using an analytical balance. Dissolved glucose in 30 ml of distilled water (1% glucose solution concentration). Mixing 1% glucose solution into Nutrient Broth media. Sterilize the liquid media (Nutrient Broth + 1% glucose) using an autoclave at 121°C for 20 minutes.

Plastic biodegradation activity

Pipette 1 ml of bacterial inoculum that has been incubated for 1 x 24 hours. Inoculate 1 ml of bacterial inoculum into 100 ml of Nutrient Broth + 1% glucose media. Incubated the bacterial culture for 1 x 24 hours at 37oC in an incubator. Cutting a 1 x 1 cm piece of black plastic. Soak the 1 x 1 cm black plastic pieces in 70% alcohol solution for 15 minutes. Drying the 1 x 1 cm black plastic pieces for 1 x 24 hours at 37oC in an oven. Weighing the 1 x 1 cm black plastic using analytical scales to determine the initial weight before the degradation process. Put the 1 x 1 cm black plastic sample into a culture bottle containing liquid media (Nutrient Broth + 1% glucose). Place the culture bottle on an orbital shaker with 50 rpm agitation.

Optical Density (OD) measurement of bacterial culture during degradation activity

Pipette 3 ml of bacterial culture in liquid media (Nutrient Broth + 1% glucose) into a sterile test tube. Put 3 ml of bacterial culture in liquid media (Nutrient Broth + 1% glucose) into the cuvette. Measure the Optical Density value using a uv-vis spectrophotometer with a wavelength of 600 nm.

Calculation of Total Plate Count (TPC) Value During Degradation Activity

Prepare a dilution series containing 9 ml of distilled water solution from dilution series 10^{-1}-10^{-4} (day 0), 10^{-1}-10^{-5} (day 1), 10^{-1}-10^{-6} (day 2), 10^{-1}-10^{-7} (day 3), 10^{-1}-10^{-8} (day 4), 10^{-1}-10^{-9} (day 5), 10^{-1}-10^{-10} (day 6), and 10^{-1}-10^{-11} (day 7) into sterile Petri dishes. Pouring Nutrient Agar media as much as ± 10 ml into

Petri dishes. Homogenize the bacterial culture samples in Nutrient Agar media by moving the Petri dish to form a Figure 8. Close the mouth of the Petri dish using plastic wrap. Code the bacterial culture samples using label paper. Incubate the bacterial culture samples for 1 to 7 x 24 hours at 37oC in an incubator. Counting the colonies formed and recording them in the research logbook.

Measurement of pH Value of Bacterial Culture During Degradation Activity

Prepare a digital media pH meter. Measuring the pH value of each culture bottle. Recording the pH value in the research logbook. Calculation of Percentage Dry Weight Loss of Plastics. Taking the test plastic from the culture bottle. Cleaning the test plastic separated from the biofilm using distilled water. Soaking the test plastic with 70% alcohol for 15 minutes. Drying the test plastic for 1 x 24 hours at 37ºC in an oven. Weighing the test plastic using analytical balance after the degradation activity. Calculated 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
W2: Final dry weight of plastic after degradation activity

Research design

This study used a completely randomized design (CRD) using one type of independent variable, namely variations in the type of *indigenous* bacteria of salt wash wastewater (*bittern*) inoculated in liquid culture media (*Nutrient Broth* + 1% glucose) as much as 100 ml. The types of *indigenous* bacteria of salt wash wastewater (*bittern*) used are BB1, BB2, BB3, BB4, BB5, BB6, BB7, BB8, BB9. The controlled variable used was the type of *Bacillus subtilis* bacteria with test plastic in the form of black plastic measuring 1 x 1 cm, which was incubated for 1 to 7 x 24 hours at 37ºC using an *orbital shaker* with a speed of 50 rpm. This study used 10 treatment samples which were repeated three times. The dependent variables observed were *Optical Density* (OD), *Total Plate Count* (TPC), pH, and dry weight percentage of the test plastic. This study refers to Sari *et al*. (2020) so that the same treatment is carried out before and after the degradation activity. Before the degradation activity, the test plastic is rinsed with running water and then cut with a size of 1 x 1 cm after being soaked with 70% alcohol for 15 minutes. After soaking in alcohol, the test plastic oven at 37ºC for 1 x 24 hours. The treatment applied after the degradation activity is almost the same as the treatment before the degradation activity. The test plastic was taken from the culture bottle containing NB media then the plastic was cleaned with distilled water from the biofilm attached to the plastic. After that, the plastic was soaked in 70% alcohol for 15 minutes. After the soaking process was complete, the test plastic was oven for 1 x 24 hours then weighed the plastic as the final weight of the plastic.

Data Analysis

Biodegradation of plastic waste using a completely randomized design (RAL). Data related to the characteristics of plastic waste found in the waters of Kamal, Bangkalan Regency will be analyzed descriptively qualitatively by displaying data on the characterization results of plastic samples found in 1 x 1 m transects. Optical Density (OD), Total Plate Count (TPC), pH, and percentage of plastic dry weight of each indigenous bacterial isolate of salt wash wastewater (bittern) before and after plastic waste degradation activity on NB (Nutrient Broth) media with the addition of 1% glucose will be analyzed descriptively by displaying data on the percentage of plastic degradation activity by indigenous bacteria of salt-washing wastewater (*bittern*).

Results and Discussion

Optical Density Value of Each Indigenous Bacterial Isolate of Salt Wash Wastewater (Bittern) Before and After Plastic Waste Degradation Activity on NB (Nutrient Broth) Media with the Addition of 1% Glucose

Optical Density (OD) measurements were carried out on day 0 (before degradation) to day 7 (after degradation). *Optical Density* (OD) measurement using uv-vis spectrophotometer. *Optical Density* (OD) measurement results are presented in (**Figure 4.1**) as follows:

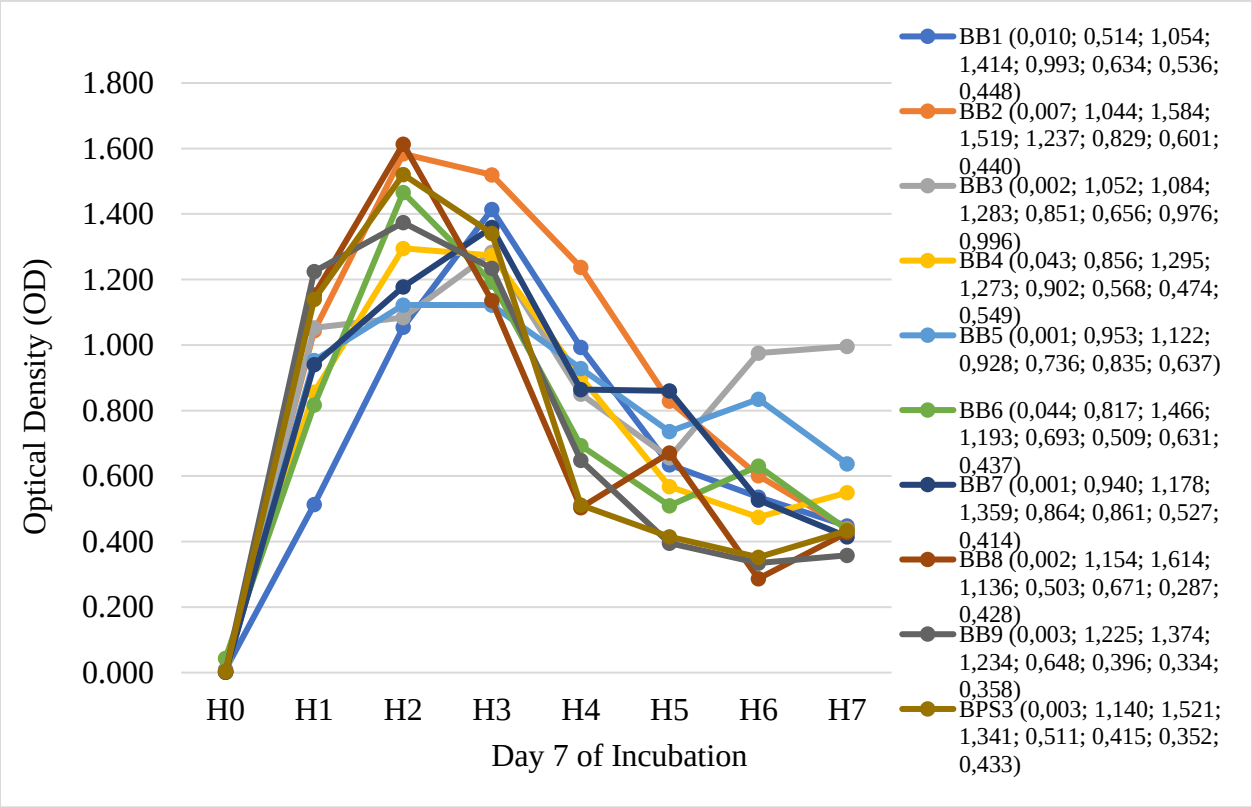

Figure 4.1 Optical Density (OD) Measurement Chart
Note: BB1 up to BB9: Bittern Bacteria One up to Bittern Bacteria Nine
Source: Arranged by author (2024)

The results of Optical Density (OD) measurements using a spectrophotometer with a wavelength of 600 nm will show differences in absorbance values in each growth medium observed. Based on (Figure 4.1) there is a calculation of Optical Density (OD) on each growth medium consisting of indigenous bacterial inoculum of salt washer wastewater (bittern) including BB1, BB2, BB3, BB4, BB5, BB6, BB7, BB8, BB9 and control, namely BPS3. Measurement of Optical Density (OD) value on day 0 was carried out before the degradation activity process by adding bacterial inoculum into the growth medium (Nutrient Broth + 1% glucose) as much as 1 ml with a volume of 100 ml in the culture bottle.

Calculation of the highest Optical Density (OD) value on day 0 was found in BB6, which obtained a result of 0.044, and the lowest Optical Density (OD) value was found in BB5 and BB7, which obtained a result of 0.001 while the Optical Density (OD) value of the control was 0.003. The calculation of the highest Optical Density (OD) value on day 1 was found in BB9, which obtained a result of 1.225, and the lowest Optical Density (OD) value was found in BB1, which obtained a result of 0.514. The control Optical Density (OD) value obtained a result of 1.140. Calculation of the highest Optical Density (OD) value on day 2 was found in BB2, which obtained a result of 1.584. The lowest Optical Density (OD) value was

BB5, which obtained a result of 1.122. The Optical Density (OD) value of the control obtained a result of 1.521. Calculation of the highest Optical Density (OD) value on the 3rd day was found in BB2, which obtained a result of 1.519 and the lowest Optical Density (OD) value was BB5, which obtained a result of 1.122

The Optical Density (OD) value of the control obtained a result of 1.521. Calculation of the highest Optical Density (OD) value on the 4th day was found in BB2, which obtained a result of 1.237 and the lowest Optical Density (OD) value was BB8, which obtained a result of 0.503 Optical Density (OD) value of the control obtained a result of 0.511. Calculation of the highest Optical Density (OD) value on day 5 was found in BB2, which obtained a result of 0.829 and the lowest Optical Density (OD) value was BB9, which obtained a result of 0.396 while the Optical Density (OD) value of the control obtained a result of 0.415. Calculation of the highest Optical Density (OD) value on the 6th day was found in BB3, which obtained a result of 0.976 and the lowest Optical Density (OD) value was BB8, which obtained a result of 0.287 while the Optical Density (OD) value of the control obtained a result of 0.352. The highest Optical Density (OD) value calculation on the 7th day was found in BB3, which obtained a result of 0.829 and the lowest Optical Density (OD) value was BB9, which obtained a result of 0.358, while the Optical Density (OD) value of the control obtained a result of 0.433.

Uthami & Irdawati (2024) explained that Optical Density (OD) measurements provide information about cell density in microbial cultures that allow quantitative monitoring of microbial population growth. Optical Density (OD) testing is based on the principle that light passing through a solution will experience resistance proportional to the number of cells or particles in it. The denser the solution, the higher the resistance. The OD measurement scale ranges from 0 to 2. An Optical Density (OD) value of 0 generally indicates an empty or clean solution or no light resistance, while an Optical Density (OD) value of 2 indicates a solution that is very dense with cells or particles (Urbanek et al., 2018). The growth of bacteria from the Optical Density (OD) test itself can generally be illustrated through a growth curve, which includes four main phases, namely the lag phase, exponential phase, stationary phase, and death phase. This curve represents the entire growth cycle of bacteria, starting from the initial adaptation period (lag phase), then through a period of rapid growth (exponential phase), reaching a phase where growth reaches its peak (stationary phase), and finally entering a phase of decreased growth (death phase) (Gambarini et al., 2022)

Total plate count values obtained from each Indigenous bacterial Isolate of salt-wash wastewater (bittern) before and after plastic waste degradation activity on NB (Nutrient Broth) media with the addition of 1% glucose

Measurement of *Total Plate Count* (TPC) values was carried out from day 0 (before degradation) to day 7 (after degradation). Measurement of *Total Plate Count* (TPC) using the *pour plate* method with an incubation time of 1 x 24 hours using an incubator with *Nutrient Agar* (NA) media. The results of the *Total Plate Count* (TPC) measurement are presented in (**Figure 4.2**) as follows:

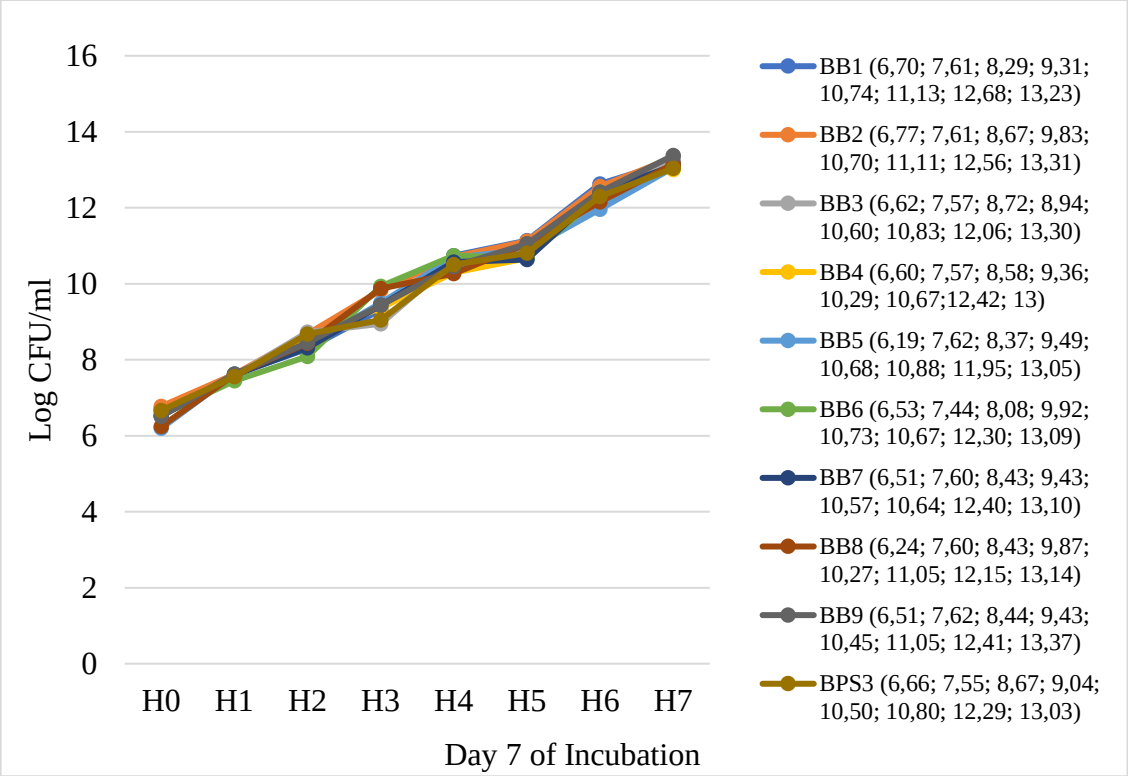

Figure 4.2 *Total Plate Count* (TPC)
Note: BB1 up to BB9: Bittern Bacteria One up to Bittern Bacteria Nine
Source: Arranged by author (2024)

The results of measuring the Total Plate Count (TPC) value with the pour plate method with an incubation time of 1 x 24 hours using an incubator with Nutrient Agar (NA) media. Based on (Figure 4.2) there is a calculation of the Total Plate Count (TPC) on each growth medium consisting of indigenous bacterial inoculum of salt washing wastewater (bittern) including BB1, BB2, BB3, BB4, BB5, BB6, BB7, BB8, BB9 and control, namely BPS3. The graph shows that the Total Plate Count (TPC) value fluctuates greatly. The Total Plate Count (TPC) value is calculated from each dilution factor and then used as Log. Calculation of Total Plate Count (TPC) values by preparing a dilution series consisting of 10^{-1}-10^{-4} (day 0), 10^{-1}-10^{-5} (day 1), 10^{-1}-10^{-6} (day 2), 10^{-1}-10^{-7} (day 3), 10^{-1}-10^{-8} (day 4), 10^{-1}-10^{-9} (day 5), 10^{-1}-10^{-10} (day 6), and 10^{-1}-10^{-11} (day 7). Measurement of Total Plate Count (TPC) value on day 0 was carried out before the degradation activity process by adding bacterial inoculum into the growth medium (Nutrient Broth + 1% glucose) as much as 1 ml with a volume of 100 ml in the culture bottle.

On day 0, the highest TPC value was found in BB2 (6.77 Log CFU/g) and the lowest in BB5 (6.19 Log CFU/g), while the control was 6.66 Log CFU/g. On day 1, the highest TPC value was in BB9 (7.62 Log CFU/g) and the lowest was in BB6 (7.44 Log CFU/g), with a control of 7.55 Log CFU/g. On day 2, the highest value was in BB3 (9.92 Log CFU/g) and the lowest in BB6 (8.08 Log CFU/g), while the control was 8 Log CFU/g. On day 3, the highest value was found in BB6 (9.92 Log CFU/g) and the lowest in BB1 (9.31 Log CFU/g), with a control of 9.04 Log CFU/g. On day 4, BB1 had the highest TPC value (10.74 Log CFU/g) and BB8 the lowest (10.27 Log CFU/g), while the control was 10.50 Log CFU/g. On day 5, the highest value was recorded in BB1 (11.13 Log CFU/g) and the lowest in BB7 (10.64 Log CFU/g), with a control of 10.80 Log CFU/g. On day 6, the highest value was recorded in BB1 (12.62 Log CFU/g) and the lowest in BB5 (11.95 Log CFU/g), while the control was 12.29 Log CFU/g. On day 7, BB9 had the highest TPC value (13.37 Log CFU/g) and BB4 the lowest (13 Log CFU/g), with the control at 13.03 Log CFU/g.

Nawangsih *et al*. (2024) explained that the *Total Plate Count* (TPC) method has the disadvantage that there is a limit to counting bacteria so the counting range is narrow, namely 25-250 CFU bacteria. Another weakness of this method is that the number of bacteria in CFU recorded is sometimes not the same between technicians. The reason was that the recorded bacterial data is raw data. Moreover, the difference can be influenced by different technician experiences, colony morphology, and colony density (Siroli et al., 2017).

Percentage value of plastic waste weight loss after plastic waste degradation activity in NB (Nutrient Broth) media with the addition of 1% glucose

The percentage of dry weight loss of plastic was done by calculating the dry weight of plastic after it was degraded by indigenous bacteria of salt-washing wastewater (bittern) consisting of BB1, BB2, BB3, BB4, BB5, BB6, BB7, BB8, BB9 and control, namely BPS3 during the incubation time of 7 x 24 hours using an analytical balance. The percentage of dry weight loss of plastics was calculated using the formula and the data on the percentage of dry weight loss of the test plastics are presented in (**Table 4.1**) as follows:

Table 4.1 Dry Weight Loss Percentage of Test Plastics

No.	types of bacteria	Plastic Weight Loss Difference			Percentage Weight Loss of Plastic
		1	2	3	
1.	BB1	0	0,0003	0	12,5%
2	BB2	0,0004	0	0	16%
3.	BB3	0	0,0001	0,0001	7,4%
4.	BB4	0,0002	0,0004	0,0002	36,4%
5.	BB5	0	0	0	0%
6.	BB6	0,0001	0,0002	0,0001	13,8%
7.	BB7	0	0,0001	0	3,8%
8.	BB8	0,0003	0,0002	0,0001	25%
9.	BB9	0,0004	0	0	15,3%
10.	BPS3	0	0	0,0001	4,3%

Source: Arranged by author (2024)

Based on the results of the percentage of dry weight loss of plastic can be seen in (**Table 4.1**) which shows that there are several indigenous bacteria of salt wash wastewater (*bittern*) capable of degrading the test plastic during the incubation time of 7 x 24 hours. The initial process of biodegradation of test plastics is the attachment of microorganisms or the formation of biofilms on plastics to obtain carbon sources. The test plastic in BB1 repetition 1 has a weight difference of 0, repetition 2 has a weight difference of 0.0003 repetition 3 has a weight difference of 0 and the total percentage of plastic weight loss is 12.5%. The test plastic on BB2 repetition 1 has a weight difference of 0.0004, repetition 2 has a weight difference of 0, and repetition 3 has a weight difference of 0 and the total percentage of plastic weight loss is 16%. The test plastic in BB3 repetition 1 has a weight difference of 0, repetition 2 has a weight difference of 0.0001, and repetition 3 has a weight difference of 0.0001 and the total percentage of plastic weight loss is 7.4%. The test plastic in BB4 repetition 1 has a weight difference of 0.0002, repetition 2 has a weight difference of 0.0004 repetition 3 has a weight difference of 0.0002 and the total percentage of plastic weight loss is 36.4%.

The test plastic in BB5 repetition 1 has a weight difference of 0, repetition 2 has a weight difference of 0. Repetition 3 has a weight difference of 0 and the total percentage of plastic weight loss is 0%. The test plastic on BB6 repetition 1 had a weight difference of 0.0001. Then, repetition 2 had a weight difference of 0.0002. Moreover, repetition 3 had a weight difference of 0.0001 and the total percentage of plastic weight loss was 13.8%. The test plastic in BB7 repetition 1 has a weight difference of 0, repetition 2 has a weight

difference of 0.0001 repetition 3 has a weight difference of 0 and the total percentage of plastic weight loss is 3.8%. The test plastic on BB8 repetition 1 has a weight difference of 0.0003 repetition 2 has a weight difference of 0.0002 and repetition 3 has a weight difference of 0.0001 and the total percentage of plastic weight loss is 25%.

The test plastic in BB9 repetition 1 has a weight difference of 0.0004 repetition 2 has a weight difference of 0. Repetition 3 has a weight difference of 0 and the total percentage of plastic weight loss is 15,3%. BPS3 repetition 1 had a weight difference of 0, repetition 2 had a weight difference of 0, and repetition 3 had a weight difference of 0.0001 and the total percentage of plastic weight loss was 4.3%. The highest percentage of test plastic degradation activity was found in BB4 with a percentage value of 36.4%. The lowest percentage of test plastic degradation activity was found in BB7 with a percentage value of 3.8%. This data was analyzed using descriptive quantitative by displaying Excel data along with displaying the Duncan test. The bar chart of the percentage of degradation effectiveness of the test plastics is presented in (**Figure 4.4**) as follows:

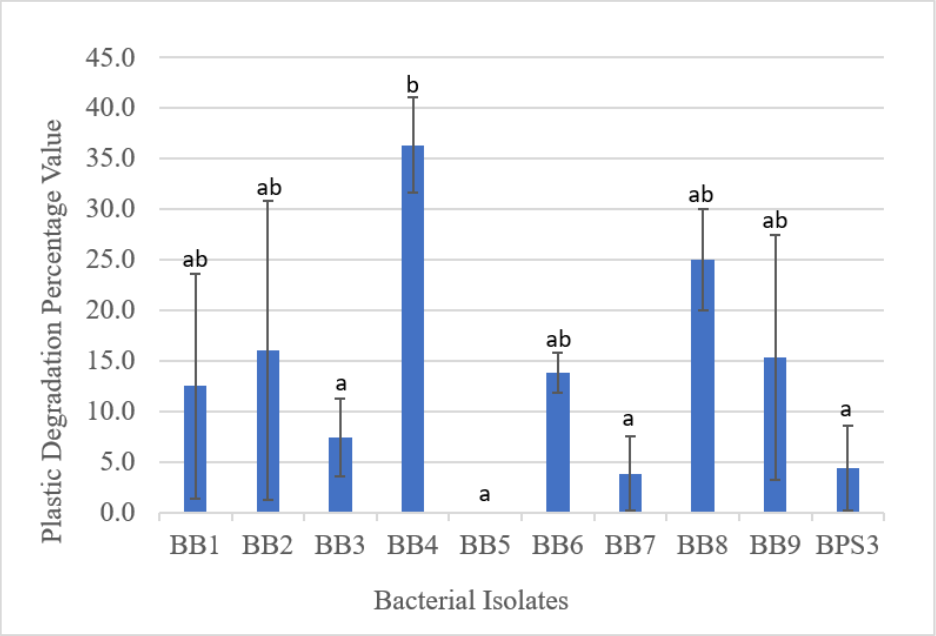

Figure 4.4 Diagram of Percentage of Plastic Dry Weight Loss
Note: BB1 up to BB9: Bittern Bacteria One up to Bittern Bacteria Nine
Source: Arranged by author (2024)

The percentage value of plastic degradation activity is also displayed in the bar chart presented in (**Figure 4.5**) as follows:

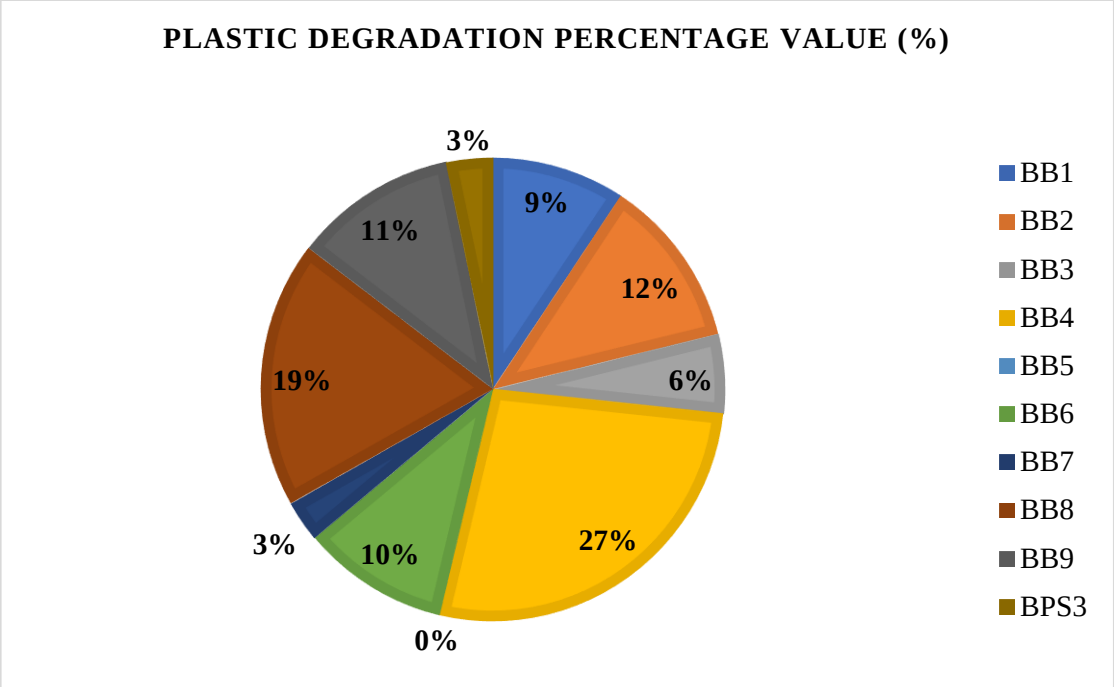

Figure 4.5 Plastic Degradation Percentage Pie Chart
Note: BB1 up to BB9: Bittern Bacteria One up to Bittern Bacteria Nine
Source: Arranged by author (2024)

Mardalisa *et al*. (2021) explained that the difference in the ability of bacterial isolates to degrade plastic polymers is influenced by certain factors such as environmental conditions and the type of test plastic. The ability of biodegradation by bacteria is influenced by several factors such as the type of substrate, pH, temperature, and humidity adjusted to the type of microorganism used. The substrate consists of components. On the other hand, the size of the constituent compounds affected the degradation. Degradation will take place quickly when the substrate size is smaller and the constituent compounds are simpler. The mechanism of plastic biodegradation starts from the attachment of microbes to the polymer, and then surface colonization occurs. Enzyme-based plastic hydrolysis occurs when the enzyme attaches to the polymer substrate followed by hydrolytic cleavage. Polymer degradation products such as oligomers, dimers, and monomers have a much lower molecular weight and are eventually converted into CO2 and H2O through mineralization. Under aerobic conditions, oxygen is used as an electron acceptor by bacteria followed by the synthesis of smaller organic compounds, thus, CO2 and water are produced as end products. Under anaerobic conditions, the polymer would be destroyed in the absence of oxygen by microorganisms (Pieters & Maes, 2019).

Conclusion

1. The *optical density* values of *Indigenous* bacteria in degrading polyethylene plastic waste in *Nutrient Broth* (NB) + 1% Glucose media by BB1, BB2, BB3, BB4, BB5, BB6, BB7, BB8, BB9, and BPS3 at the 7th day incubation time are 0.448; 0.440; 0.996; 0.549; 0.637; 0.437; 0.414; 0.428; 0.358; and 0.433
2. The *total plate count* (TPC) value of *indigenous* bacteria in degrading polyethylene plastic waste in *Nutrient Broth* (NB) + 1% Glucose media by BB1, BB2, BB3, BB4, BB5, BB6, BB7, BB8, BB9, and BPS3 at the 7th day incubation time was 13.23 Log CFU/ml; 13.31 Log CFU/ml; 13.30 Log CFU/ml; 13.00 Log CFU/ml; 13.05 Log CFU/ml; 13.09 Log CFU/ml; 13.10 Log CFU/ml; 13.14 Log CFU/ml; 13.37 Log CFU/ml; and 13.03 Log CFU/ml.
3. The dry weight percentage values of polyethylene plastic after degradation during 7 days incubation in *Nutrient Broth* (NB) + 1% Glucose media by BB1, BB2, BB3, BB4, BB5, BB6, BB7, BB8, BB9, and

BPS4 at the 7th day incubation time are 12.5%; 16.0%; 7.4%; 36.4%; 0.00%; 13.8%; 3.8%; 25.0%; 11.5%; and 4.3%.

This study provides important implications in plastic waste management, by utilizing indigenous bacteria found in salt wash wastewater as biodegradation agents. The results of this study can open new opportunities in the development of environmentally friendly biotechnology technology to overcome the increasing problem of plastic waste. Future research can explore the potential of other indigenous bacteria found in various types of liquid waste, as well as examine the specific mechanisms used by bacteria in deconstructing plastic. Further research also needs to be done to increase the efficiency of the plastic degradation process by optimizing environmental conditions such as temperature, pH, and oxygen levels. In addition, large-scale tests and field applications need to be conducted to assess the effectiveness and sustainability of using bacteria in commercial plastic waste management.

Author's Contribution

All authors have contributed to the completion of this paper. The contributions of each author include, those who has provided excellent guidance and provided critical revisions to the article. All authors discussed the research results and contributed to the final draft.

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Declaration of Competing Interest

The study is independent internal grant research that does not have a tendency or interest in the various parties involved. The data obtained is pure data used for science and knowledge.

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