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Determination of Biodegradation Rate of Bioplastic with Controlled Environment

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ABSTRACT

The use of synthetic plastics in everyday life has a negative impact but can be reduced in effect, if replaced with biodegradable plastics. Polymer biodegradation can occur because of microbiological infestation of the material. Microorganisms could produce various kinds of enzymes that can react with polymers. This study aims to study the effect of adding organic matter spearhead bacteria on the biodegradation rate in test samples with the help of *Bacillus sp* bacteria and the bacterium *Pseudomonas* sp. In this case, bioplastic samples were characterized using Fourier Transform Infrared (FTIR) spectroscopy and continued with quantitative analysis using gravimetric methods to determine biodegradation rates based on CO_2 weight. The results of the characterization test study showed that the test sample containing cellulose is characterized by the presence of glycosidic -OH, -CH, and C-O functional groups. The determination of the rate of biodegradation gave the value of the rate constant microcrystalline cellulose as a positive control of 0.0489 day^{-1}, Bioplastic A without inoculum addition of 0.0451 day^{-1} while Bioplastic A with the addition of inoculum by 0.0423 day^{-1} . Meanwhile, Bioplastic B without inoculum addition is 0.0254 day^{-1}, and bioplastic B with inoculum addition is 0.0391 day^{-1}.

Keywords: Biodegradation, Biodegradation Rate, Characterization Test, Biodegradable Plastics

1. INTRODUCTION

National waste production shows a trend that continues to increase along with economic growth and an increase in population. One type of waste that is currently of concern is plastic waste. Plastic is a material that is widely used in daily needs. The use of plastic materials is increasingly widespread because they are strong, not brittle, and not easily damaged by weathering. In 2018, world plastic production was 360 million tons. Plastic demand for Asia is 50% of the total production (360 million tons) of plastic materials in the world. In different plastic applications, plastic packaging achieves the highest recycling rate of 39.5% and represents more than 80% of the total recycling amount[1]. This data shows that there is a need for alternative materials and/or substitutes for plastic that are more environmentally friendly[2].

In general, non-biodegradable plastic cannot be degraded in the environment so it accumulates in the environment and creates problems for living creatures and their habitats. Soil conditions that contain too many toxic plastic particles can cause the death of decomposing microorganisms, such as worms, which can cause soil fertility to decrease. As for the impact on humans, the residue produced from burning synthetic plastic in the form of smoke can trigger respiratory problems, cancer, hepatitis and nervous system disorders[3].

Another problem that plastic can cause is the risk of transferring toxic compounds to the ecosystem and disturbing the living creatures in it because the plastic is ingested by them. Plastic crumbs contain PCBs (Polychlorinated biphenyl), aromatic hydrocarbons, organochlorine pesticides, phthalates, and other substances that are added during production, but also become adhering substances from the

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environment. On the other hand, the use of biodegradable bioplastics such as polylactides (PLA) from corn starch, polyhydroxyalkanoates (PHA), and aliphatic polyesters is an alternative.

Seeing these conditions what can be done is through the synthesis of polymers (polymer raw materials or plastic making) which can be degraded by soil microbes [4]. Biodegradable plastics are generally made from starch or starch which can be degraded by bacteria. The bacteria that are known to degrade starch or starch are the genus Pseudomonas sp. and Bacillus sp. Both genera of bacteria can break polymer chains into simpler monomers. Apart from that, compounds resulting from polymer degradation produce carbon dioxide and water as well as other organic compounds, such as organic acids and aldehydes which are safe for the environment.

This research aims to determine the reaction order and constant value of the biodegradation rate and determine the effect of adding organic material decomposing bacteria, namely Bacillus sp. and Pseudomonas sp. on the rate of biodegradation.

2. METHODS

2.1. Materials and Tools

The materials used in this research include soda talc, soda lime, anhydrous calcium chloride ($CaCl_2$), sulfuric acid (H_2SO_4) 1 M, sodium hydroxide (NaOH) 0.1 N, hydrogen chloride (HCl) 0.1 N, sea sand, methyl-orange indicator, silica gel and mature compost containing various vegetable residues, other organic materials that contain minerals, borax, $Na_2B_4O_7$, concentrated H_2SO_4, 0.1 N H_2SO_4 solution, 0.1 N HCl, 40 NaOH solution % distilled water, Conway's mixed indicator (methyl red and bromo cresol green), and 1% phenolphthalein indicator. The test samples were bioplastic and microcrystalline cellulose (MCC) with a particle size of 20 µm as a positive control. The tools used in this research include a series of modified ISO 18455-2 tools, clock bottles, ovens, thermometers, analytical balances, autoclaves, pH meters, Shimadzu FTIR, 100mL Erlenmeyer, statives, spectrophotometers, clamps, volume pipettes, measuring flasks, drop pipettes , watch glass, rubber suction (bulp), spray bottle, petri dish, micro pipette, Erlenmeyer 500mL, measuring cup, beaker, glass stirrer, Kjeldahl flask, complete digestion unit, funnel, heater, complete distillation unit, and burette.

2.2. Research Methods

This research procedure consists of four stages, including test sample preparation, making the degradation medium, characterization of the degradation medium and determining the biodegradation rate. The first stage, test sample preparation. A total of two different types of bioplastics were collected from various sources. The test samples are collected in the form of sheets, then they will be cut into small pieces of each type of bioplastic. The bioplastic pieces will weigh as much as 10 g from each sample. Before being homogenized with compost, the sample will first be analyzed using FTIR. This is done to determine the functional groups of compounds contained in bioplastics. Testing was carried out by cutting biodegradable plastic samples and then adjusting them to the existing spectrum. The FTIR spectrum was recorded using a spectrophotometer at room temperature. The data obtained is in the form of a spectrum image between wave number and transmittance so that the functional groups contained in the bioplastic material can be identified. The next stage is making the degradation media.

2.3. Making Degrading Media

The compost to be used is added with distilled water (if necessary) until it has a water content of 65%, then half the mass of the compost used is added to sea sand. Sea sand plays an important role as a regulator of humidity and microorganisms.

2.4. Characterization of Degrading Media

2.4.1. Total Bacteria Count

Total Plate Count (TPC) was carried out to determine the number of bacteria contained in the biodegradation media. The degradation medium was weighed 10 g first and then dissolved using distilled water with a ratio of 1:9 before the dilution process was carried out. Next, dilution is carried out starting from the first dilution to the eighth dilution. 1 mL of the sample

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was pipetted and poured into a petri dish, which was then poured into Nutrient Agar (NA) medium and then homogenized. Incubation was carried out for 224 hours at 36 ^{o}C. This treatment also applies to blanks and is carried out in duplicate. Then it ends with calculating the number of colonies of microorganisms that grow.

2.4.2. Calculation of Total Fungi

The degradation medium was weighed 10 g first and then dissolved using distilled water with a ratio of 1:9 before the dilution process was carried out. Next, dilution is carried out starting from the first dilution to the eighth dilution. 1 mL of the sample was pipetted and poured into a petri dish, which was then poured into Potato Dextrose Agar (PDA) medium and then homogenized. Incubation was carried out for 224 hours at 37 ^{o}C. This treatment also applies to blanks and is carried out in duplicate. Then it ends with calculating the number of colonies of microorganisms that grow.

2.4.3. pH measurement

pH measurements using a pH meter which is standardized first using pH 4 and 7 buffers, then measurements are carried out by dipping the pH meter electrode into the sample. pH measurements were carried out by dissolving 1 g of soil sample in 5 mL of water and homogenizing it for 30 minutes using a shaker.

2.4.4. Determination of Phosphate Content

Phosphate content was measured by weighing 0.5 g of sample into a digest tube and then adding 5 mL of nitric acid and 0.5 mL of perchloric acid and leaving it overnight. Then the next day it was heated at 100 ^{o}C for 1 hour 30 minutes. Then the temperature is increased to 130 ^{o}C for 1 hour, the temperature is increased again to 150 ^{o}C for 2 hours 30 minutes (until the yellow steam runs out, if there is still yellow steam then the heating time is increased again), after the yellow steam runs out the temperature is increased to 170 ^{o}C for 1 hour, then the temperature is increased again to 200 ^{o}C for 1 hour (until white steam forms). Digestion is complete with the formation of a white precipitate or a clear solution remaining of approximately 0.5 mL. The extract was cooled then diluted with deionized water to 50 mL, then shaken until homogeneous and left overnight.

Pipet 1 mL of sample extract and standard series 0-200 ppm PO_4 into a chemical tube. Add 9 mL of ion-free water and then homogenize. Pipette 1 mL each of the sample aqueous extract and the standard series of phosphate into a reaction tube. Add 10 mL of dye reagent P. Shake using a vortex until homogeneous and leave for 30 minutes. The phosphate in solution was measured using a spectrophotometer at a wavelength of 693 nm.

2.4.5. Determination of Nitrogen Content Kjeldahl Method

Standardization of HCl 0.1 N Borax was carried out by weighing 0.2 grams dissolved in 25 mL of distilled water. The solution mixture was homogenized then 3 drops of methyl red were added, then titrated using HCl until the end point was pink.

Standardization H_2SO_4 0.1 N was carried out by weighing 0.2 grams $Na_2B_4O_7$, then dissolved using 25 mL of distilled water. The mixture was homogenized, and 3 drops of methyl red were added, which was then titrated until it turned pink.

The sample was carefully weighed at 0.5 gram and placed in a Kjeldahl flask. The sample was added with 25 mL H_2SO4 is concentrated. The sample is digested for ± 2 hours at a temperature of ± 350 ^{o}C until the color of the solution becomes clear. The sample solution was cooled then diluted with distilled water then transferred quantitatively into a 100 mL volumetric flask and measured with distilled water then homogenized. The sample solution was pipetted 10 mL into a Kjeldahl analyzer distillation flask, then 1% PP was added. The sample is collected into 50 mL of 1% H_3BO_3 in a 500 mL Erlenmeyer flask containing several drops of Conway indicator, the tip of the cooler must be immersed in the collecting solution. The sample was added with 40% NaOH solution until the solution turned red. Addition of the 40% NaOH solution must be done quickly. The sample solution is distilled until all the nitrogen is distilled (approximately 100 mL of distillate). Remove and remove the Erlenmeyer flask then rinse the cooling tip with distilled water. Titrate

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the distillate contained in the reservoir solution with 0.1 N H_2SO_4 solution until the end point of the titration is reached. Carry out the determination of H_2SO_4 (blank).

2.4.6. Determination of C-Organic Content

C-organic measurements were carried out by weighing 0.05 g of sample in a test tube. Then add 5 mL of 2 N Potassium dichromate ($K_2Cr_2O_7$) solution while shaking, then add 7 mL of concentrated sulfuric acid, shake again, and let sit for 30 minutes. Dilute with ion-free water, it becomes 10 ml when the solution has cooled. Measure the absorbance of the clear solution using a spectrophotometer after 3-4 hours of incubation at room temperature with wavelength 561 nm. Standard organic carbon solutions were prepared at concentrations of 0;25;50;75; 100; 250 ppm and treated the same as in the example.

2.4.7. Determination of Biodegradation Rates

In biodegradation testing of bioplastics using ISO 14855-2 which has been carried out to determine the biodegradation of various types of bioplastics by duplo measurements under controlled conditions on a laboratory scale. The degradation media used is compost inoculum tested with bioplastic materials. The inoculum used is divided into two types, namely compost with added bacterial isolates and compost without added bacterial isolates. The degradation rate is periodically measured by determining the mass of carbon dioxide in an absorption column filled with soda lime and soda talc.

3. RESULTS AND DISCUSSION

3.1. Test sample preparation.

Test sample preparation needs to be carried out to determine the functional group content contained in the sample before testing ISO 14855-2. Figures 1 and 2 show the spectrum of bioplastic A and bioplastic B respectively. The stretching absorption bands at 3264.50 cm^{-1} and 3262.81 cm^{-1} indicate the presence of hydroxyl (OH) groups from cellulose as also reported by several researchers, for example in the absorption area 3355 cm^{-1} and 3388.93 cm^{-1}. Apart from that, the next read absorptions were at wave numbers 2921.82 cm^{-1} and 2915.80 cm^{-1} which were stretching absorptions from the CH group. The CH functional group is the cellulose framework visible at wave numbers 2800-3000 cm^{-1} [5]. This group is located to the right of the OH group.

The absorption of the C=O group indicating the presence of hemicellulose was read at a wave number of 1710.39 cm^{-1}. Unfortunately, the C=O carbonyl group was only read in bioplastic B samples due to the absence of covalent bonds between hemicellulose and lignin. The stretching vibration area of the C=O group appears at absorptions of 1724 cm^{-1}, 1700 cm^{-1} [5], 1730 cm^{-1} [6]. Cellulose consists of glucose units. This form of glucose is not absolute during a cyclic state and glucose can be stable when the form of the glucose chain is open during the Fisher projection. This can be strengthened by the stretching absorption of the aromatic group with C=C symmetry at a wavenumber of 1583.5 cm^{-1}; 1503.65 cm^{-1}; 1467.56 cm^{-1}; and 1506 cm^{-1} [5]. Meanwhile, in the sample this occurred at wave numbers 1636.30 cm^{-1} and 1538.56 cm^{-1}.

CH bending absorption appears at a wave number of 1415.33 cm^{-1} and 1409.11 cm^{-1}. Nomanbhay et. al. [7] reported that the CH group was found at a wave number of 1316 cm^{-1} for cellulose compounds and 1372 cm^{-1} for cellulose and hemicellulose compounds. The wave number 1159.22-1033.85 cm^{-1} is the CO vibration area. The CO group is the structure of lignin which is the constituent of wood. Absorption of this group in the sample occurred at wave numbers 1076.66 cm^{-1} and 1181.79 cm^{-1}.

Cellulose bond absorption area β-glycosidic appears at wave numbers 923.11 cm^{-1} and 930.34 cm^{-1}. The absorption of this bond will appear at wave numbers 950-800 cm^{-1} [6] or 897 cm^{-1} [7]. The -OH, -CH and CO glycosidic functional groups are the main groups of cellulose. This also proves that the sample contains carbon elements which are expected to be contained in the sample as a source of nutrition for microorganisms.

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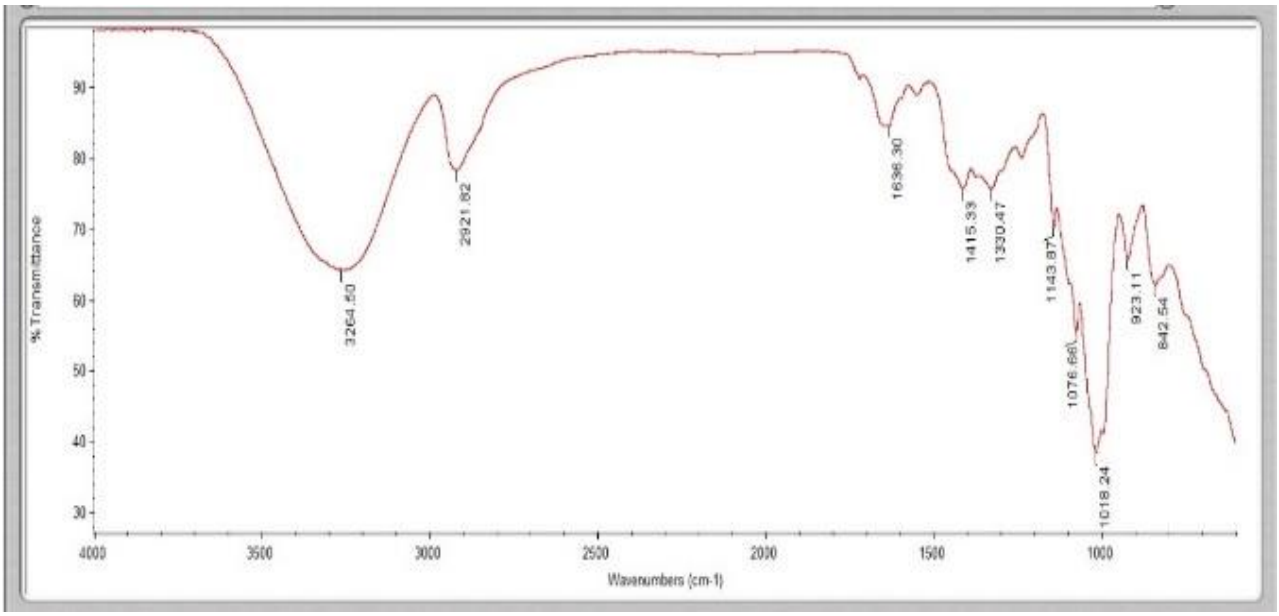

Figure 1. FTIR Spectrum of Bioplastics A

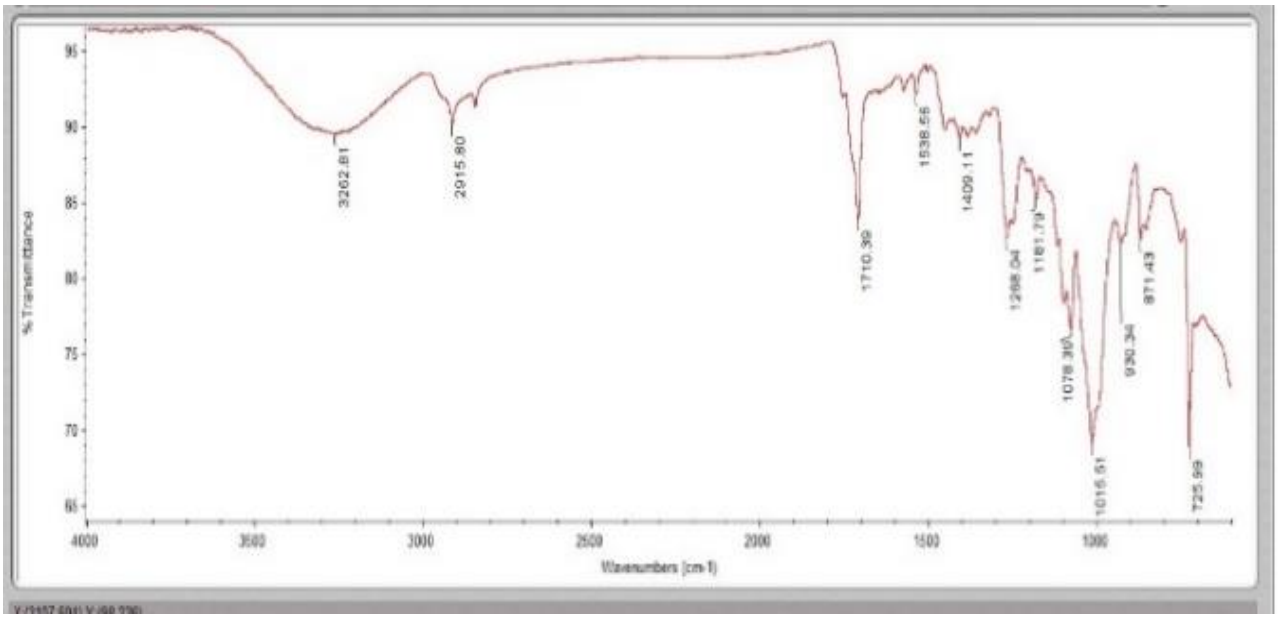

Figure 2. FTIR Spectrum of Bioplastic B

3.2. Results of total bacterial and total fungal populations

Calculation of the total number of bacteria and fungi in the degradation media was carried out using the Total Plate Count (TPC) method. In Table 1 is the total number of bacteria and total fungi before treatment and after treatment. The results shown in Table 1 show that the total number of microbes on blank and microcrystalline cellulose did not have a significant difference in population numbers, namely around 2.30 x 10^8 - 2.2 x 10^8 cfu/g and 2.86 x 10^8 - 2.9 x 10^8 cfu/g while the fungal population ranged between 1.86 x 10^5 - 2.8 x 10^5

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propagules/g and 1.70 x 10^5 - 3.2 x 10^5 propagules/g. The values of these two treatments tend to be stable because the blank and microcrystalline cellulose do not add microbes which can cause major changes in the number of microorganism populations.

In bioplastic degradation media A with the addition of Bacillus sp. and Pseudomonas sp. The bacterial population obtained was 2.40 x 10^9 cfu/g to 3.6 x 10^9 cfu/g and the fungal population was 2.90 x 10^5 propagules/g to 4.1 x 10^5 propagules/g. This value is higher than bioplastic degradation media A without the addition of Bacillus sp bacteria. and Pseudomonas sp where the total bacteria obtained were 3.56 x 10^8 cfu/g to 4.9 x 10^8 cfu/g and the total fungi were 1.93 x 10^5 propagules/g to 3.9 x 10^5 propagules/g.

In bioplastic degradation media B with the addition of Bacillus sp. and Pseudomonas sp. The bacterial population obtained was 2.25 x 10^9 cfu/g to 4.18 x 10^9 cfu/g and the fungal population was 2.62 x 10^5 propagules/g to 4.42 x 10^5 propagules/g. This value is higher than bioplastic degradation media B without the addition of Bacillus sp bacteria. and Pseudomonas sp where the total bacteria obtained was 3.75 x 10^8 cfu/g to 3.15 x 10^8 cfu/g and the total fungus was 2.56 x 10^5 propagules/g to 3.75 x 10^5 propagules/g.

Table 1 shows that the microbial population before treatment and after treatment experienced an increase in the number of microbes in the bioplastic A and bioplastic B samples that were added with Bacillus sp. bacteria. and Pseudomonas sp. This proves that the microbes used can reproduce well in the degradation media. According to Madigan [8], bacterial growth is influenced by several environmental factors such as: temperature, pH, humidity, water activity and bacterial nutrition (food composition).

Cappuccino [9] added that the nutrients needed by microorganisms for growth include carbon, nitrogen, non-metal elements such as sulphur and phosphorus, metal elements such as Ca, Zn, Na, K, Cu, Mn, Mg and Fe, vitamins, water, and energy, so that decreasing the carbon content in compost has an influence on increasing the number of bacteria as occurs in the data in Table 1.

Table 1. Results of Microorganism Populations in Degrading Media

Treatment	Initial population of microorganisms		Final microorganism population	
	Total Bacteria (CFU/g)	Total Fungi (Propagules/g)	Total Fungi (Propagules/g)	Total Bacteria (CFU/g)
Blank (Compost mix)	2.30 x 10^8	1.86 x 10^5	2.8 x 10^5	2.2 x 10^8
Compost mixture + Bioplastic A + Inoculum	2.40 x 10^9	2.90 x 10^5	4.1 x 10^5	3.6 x 10^9
Compost + Bioplastic A mixture	3.56 x 10^8	1.93 x 10^5	3.9 x 10^5	4.9 x 10^8
Compost + Microcrystaline mixture	2.86 x 10^8	1.70 x 10^5	3.2 x 10^5	2.9 x 10^8
Compost mixture + Bioplastic B + Inoculum	2.25 x 10^9	2.62 x 10^5	4.42 x 10^5	4.18 x 10^9
Compost mixture + Bioplastic B	3.75 x 10^8	2.56 x 10^5	3.75 x 10^5	3.15 x 10^8

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Water functions as a source of oxygen for cell organic matter in respiration. In addition, water functions as a solvent and transport tool in metabolism. Bacillus sp. acts as a reducer (decomposer) whose function is to break down organic materials and the remains of dead living bodies into chemical elements. The enzymes it produces include lipase, amylase, and protease. Meanwhile, Pseudomonas sp. has an inducible operon system that can produce certain enzymes in the metabolic process of carbon sources that are not normally used. Therefore, the bacteria Pseudomonas sp. has an important role in the biodegradation process of various polymers, including xenobiotic compounds and pesticides.

3.3. Acidity Degree Value (pH)

The acidity level or pH value of compost is one of the influencing factors during the composting process. Before starting the process of measuring the rate of biodegradation of the degrading media that will be used, the pH value must first be measured. Then, after the biodegradation rate measurement process is complete, the pH value is measured again to determine the final pH value after the degrading medium undergoes the decomposition process. The results of pH measurements can be seen in Table 2. Initial pH measurements were carried out to ensure that the initial conditions of the degradation media in each sample had reached the required optimum pH.

The results of measuring the pH of the substrate containing the bioplastic A sample along with the inoculum and the substrate containing the bioplastic B sample along with the inoculum showed a decrease in pH, namely from pH 7.17-6.88 for the substrate containing the bioplastic A sample along with the inoculum, pH 7.17-6.97 for substrates containing bioplastic B samples along with inoculum. This can happen because in the metabolic process of microbes they tend to produce acid. The decrease in pH is caused by the formation of organic acids such as acetic acid, hydrogen, and carbon dioxide in the acidogenesis and acetogenesis phases [10]. This is different with the substrate containing samples without the addition of inoculum, microcrystalline cellulose, and blanks which have a pH value in the pH range of 6.5 to 7.5, which is the optimal atmosphere. This condition is expected because bacteria will be more active in neutral conditions and fungi in acidic conditions.

The increase and decrease in pH values in Table 6 is not influenced by water content but is influenced by the presence of nitrogen and anaerobic conditions. This is because certain microorganisms will convert organic waste into organic acid. The next process is that other microorganisms will eat the organic acid, causing the pH level to rise again, approaching neutral. In general, the condition of mature compost has an atmosphere close to neutral or pH 7. However, it should be remembered that in this experiment, fungal growth needs to be avoided so as not to interfere with the results of the level of bioplastic disintegration [11].

Table 2. Compost pH Measurement Results Before and After Treatment

Treatment	Initial pH	Final pH
Blank (Compost mix)	7.17	7.19
Compost mixture + Bioplastic A + Inoculum	7.17	6.88
Compost + Bioplastic A mixture	7.17	7.13
Compost + Microcrystaline mixture	7.17	7.20
Compost mixture + Bioplastic B + Inoculum	7.17	6.97
Compost mixture + Bioplastic B	7.17	7.24

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3.4. C/N Ratio and C/P Ratio

The C/N ratio value is an important factor in composting which is needed by microorganisms as a source of nutrition for the formation of their body cells. The characteristics of compost that has undergone good weathering are that it has a different color from the color of the material from which it is made, has no odor, low water content and has room temperature. Compost maturity based on SNI No. 19 – 7030 – 2004It is characterized by a blackish color, earthy texture, and earthy smell. The temperature of the mature compost is the same as the temperature of the water in the soil, namely no more than 30 ^{o}C. The C/N ratio which continues to decrease is related to the activity of decomposer microbes which liberate CO_2 so that the C element tends to decrease while N remains the same.

Based on Table 3, it shows changes in the C/N ratio during the biodegradation process. At the beginning of the biodegradation process, the C/N ratio value for the compost to be used was 38.92. The C/N ratio value in each treatment decreased during the biodegradation process, because of the decomposition of organic carbon compounds and changes in nitrogen compounds contained in the compost material. A decrease in the C/N ratio value occurred after the biodegradation process took place, this decrease was different for each treatment. In the blank there was a decrease in the C/N ratio value from 38.92 to 9.74, in microcrystalline cellulose the C/N ratio value from 38.92 to 28.03. In samples of bioplastic A and bioplastic B without additional bacterial treatment, the C/N ratio value was 38.92 to 23.27 for bioplastic A and 38.92 to 21.74 for bioplastic B. The more effective the decomposition process, the greater and faster the reduction. C/N ratio. The final C/N ratio in ideal compost is close to the soil C/N ratio, namely 12 or < 20. The value of microcrystalline cellulose and samples without additional bacterial treatment was ≥ 20. This can happen because the compost material is not mixed homogeneously or because the number and types of microorganisms naturally present are smaller so that the effectiveness of reducing the C/N ratio is also less than optimal.

In bioplastic A and bioplastic B with the addition of Bacillus sp. bacteria. and Pseudomonas sp. had a change in the C/N ratio value from 38.92 to 19.95 for bioplastic A and 38.92 to 18.74 for bioplastic B. This C/N ratio value is considered good because it is close to the soil C/N ratio value. During the composting process, CO_2 will evaporate so that carbon will also be reduced. In aerobic composting approximately two thirds of the carbon elements evaporate into CO_2 and the remaining one third reacts with nitrogen in living cells. It can be said that the addition of bacterial inoculum can influence a decrease in the C/N ratio because the more bacteria contained in the compost, the more microbes will carry out the decomposition process. In fact, as organic C levels decrease (reduced nutrients) the nitrogen levels will increase (metabolic results from increased microbial activity). However, this does not rule out the possibility of other uncontrollable factors occurring.

The decrease in the C/N ratio value in Table 3 is due to a decrease in the amount of carbon used as an energy source for microbes to break down or decompose organic material. In the biodegradation process, organic material changes into CO_2 + H_2O + nutrients + humus + energy. During the biodegradation process CO_2 evaporates and causes a decrease in carbon and an increase in nitrogen levels so that the C/N ratio value decreases. If C/N is too high, microbes will lack N for protein synthesis so that decomposition is slow. Basically, the smaller the C/N value, the easier and faster composting. On the other hand, a C/N ratio that is too high will result in a slow decomposition process due to a lack of carbon elements.

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Table 3. Carbon (C), Nitrogen (N), C/N Ratio and C/P Ratio Measurement Results

Treatment	%C Initial	%C Final	%N Initial	%N End	Initial C/N	Final C/N	C/P
Blank (Compost mix)	39.84	1.3435	1.0234	0.1379	38.92	9.7425	33.77
Compost mixture + Bioplastic A + Inoculum	-	3.6333	-	0.1821	-	19.9522	-
Compost + Bioplastic A mixture	-	4.7116	-	0.2024	-	23.2786	-
Compost + Microcrystaline mixture	-	5.8957	-	0.2103	-	28.0347	-
Compost mixture + Bioplastic B + Inoculum	-	3.1667	-	0.1689	-	18.7489	-
Compost mixture + Bioplastic B	-	4.2134	-	0.1938	-	21,740	-

The element phosphate (P) is the second essential element after N which plays a very important role. An increase in total P_2O_5 levels indicates a decrease in compost C levels due to decomposition into simple compounds and this is a good thing in the composting process. Total P_2O_5 levels increase as the C/N ratio decreases. The increase in total P_2O_5 levels is thought to occur due to the dissolution of phosphate in organic acids produced by the activity of the microorganism Bacillus sp. which converts glucose into lactic acid so that the environment becomes acidic. The more microorganisms will make the compost mature quickly so that the microorganisms can suck up the phosphorus in the mature compost.

Apart from functioning as a source of nutrients in compost, phosphate is also needed by Pseudomonas sp. and Bacillus sp. because both bacteria are phosphate solubilizing microorganisms which require the presence of phosphate in the form available in the media for their growth. These microorganisms excrete several low molecular weight organic acids such as oxalate, succinate, tartrate, citrate, lactate, α-ketoglutarate, acetate, formic, propionate, glycolate, glutamate, glyoxylate, malate, fumarate. The increase in organic acids is followed by a decrease in pH. This is what causes the difference between compost that has added bacterial isolates and compost that has not added bacterial isolates.

3.5. Results of Determination of Biodegradation Rates

ISO Standards14855-2 in a controlled environment is used to determine biodegradation on a laboratory scale under aerobic conditions. The biodegradation process is characterized by the production of CO2 by microorganisms as an indicator that the material used is capable of being degraded. CO2 which reacts with sodium hydroxide and calcium hydroxide contained in the absorbent (soda lime and soda talc) through weighing is calculated quantitatively. The series of ISO 14855-2 modification tools can be seen in Figure 3.

Figure 3. ISO 14855-2 Biodegradation Tool Suite

Based on the series of tools in Figure 3, the container used is a Scott Duran bottle with a rubber cap as a connecting hose, which functions as a place for air to exit and enter (aeration), as well as a place for CO2 gas produced by bacteria to flow. The connecting hose must not be closed or blocked so that oxygen can easily

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enter, and the composting process can occur aerobically. Aerobic microorganisms need air to survive (especially bacteria and fungi) so that the decomposition process occurs [12]. During the process, distilled water is added which functions to maintain humidity and temperature, and periodic stirring is carried out to provide an even supply of air and moisture. This is done to avoid poor mixing will cause the compost maturity level to be uneven, if this happens it will result in the decomposition process being not optimal. Carbon dioxide reacts quantitatively with the sodium hydroxide and calcium hydroxide contained in the absorber. The chemical reaction is explained as follows:

$CO_2 + 2\ NaOH \rightarrow Na_2CO_3 + H_2O$
$CO_2 + Ca(OH)_2 \rightarrow CaCO_3 + H_2O$

Determination of the reaction order and reaction rate constant in bioplastic samples is determined by the relationship between reaction time and CO_2 conversion obtained using graph and substitution methods. The graphic method is obtained by plotting the relationship between concentration [A] and time, while the substitution method is obtained using a first order equation formula. The CO_2 results obtained in the sample are results that have been corrected by the blank so that the results are the original CO_2 results produced in the sample.

First order reactions provide an illustration of the change in [A] and the reaction rate which increases as time changes. The first order graphic method is determined by plotting ln[A] versus time which can be seen in Figure 4 and 5.

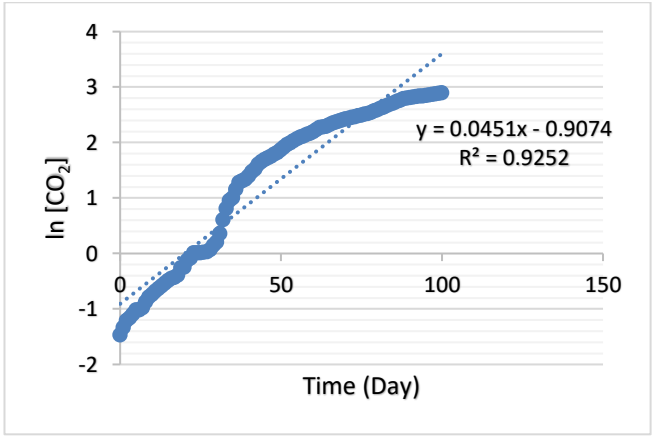

Figure 4. Bioplastic A without inoculum

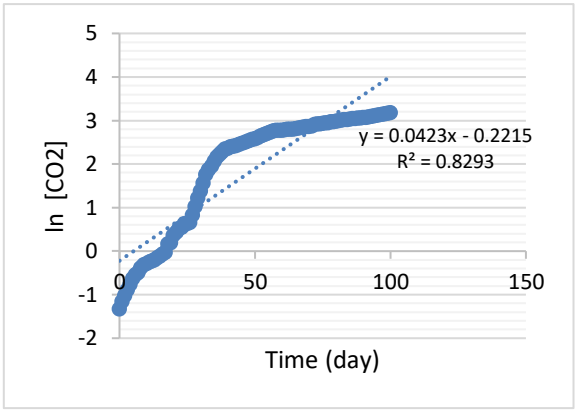

Figure 5. Bioplastic A with inoculum

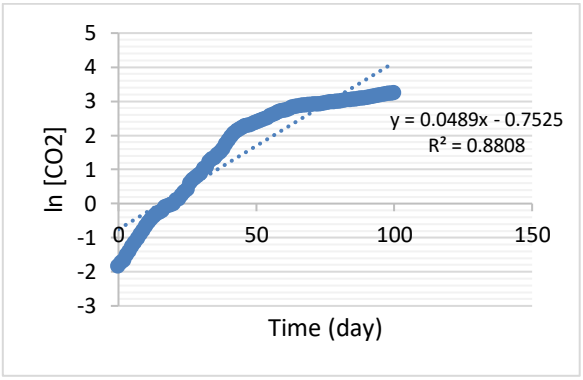

Figure 6. Microcrystalline, curve of ln[A] versus time

Based on Figure 4, bioplastic A without the addition of inoculum obtained an R value of 0.9252. Based on Figure 5, it shows that the rate constant value obtained is 0.0451 day-1 and in Figure 6 for Bioplastic A with the addition of inoculum, the value of R = 0.8293 is obtained. Based on Figure 6, it shows that the rate constant value obtained is 0.0423 day^{-1}. The reaction rate constant (k) is a value that states the reaction rate constant. The greater the k value, the greater the reaction rate, the k value is influenced by the reaction order. The kinetic equation that was obtained using the substitution method was then validated by looking at the linear regression value. The greater the linear regression value or closer to one, the kinetic equation is suitable for the data obtained.

Based on the rate constant values from Figure 5 and Figure 6, the rate constant value in Figure 6 has a smaller value. This matter can be caused by the bacteria Bacillus sp. and Pseudomonas sp. added to the sample cannot work optimally due to several factors, for example due to a lack of oxygen. This can cause

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aerobic microorganisms to die, thereby reducing the population of microorganisms. If microorganisms are reduced, the decomposition process will decrease so that they are unable to decompose protein.

In Figure 6 microcrystalline cellulose used as a positive control obtained an R value of 0.8808 and produced a rate constant value of 0.0489 day^{-1}. Microcrystalline cellulose has a rate constant value that is greater when compared to the two test samples. Even though microcrystalline cellulose is not added to microcrystalline cellulose, this can happen because microcrystalline cellulose is composed of cellulose which is easily degraded by microbes. Cellulase $(C_6H_{10}O_5)_n$ is an enzyme complex that works together to hydrolyze cellulose into glucose. There are at least 3 enzymes involved in the complete hydrolysis of cellulose into glucose, namely exoglucanase (breaks cellobiose units from the non-reducing end of the cellulose chain), endoglucanase (breaks internal bonds of cellulose), and cellobiose (specifically breaks glucose units from the non-reducing end of cell - oligosaccharides.

The first stage, the endoglucanase enzyme attacks the amorphous area of cellulose randomly and forms non-reducing ends which facilitate the work of endoglucanase. Next, the exoglucanase enzyme hydrolyzes the crystalline area of cellulose by liberating two glucose units. System the works for these two enzymes produces smaller saccharide units which are then hydrolyzed by -glucosidase to produce glucose. This is supported by research using ISO 14855-2 [13], cellulose powder is used as a reference material. Preincubation procedure is also the same for all tests. These results indicate that cellulose powder is biodegraded by almost the same process. This means that cellulose powder can decompose at least 60% after 40 days. It is characterized by the presence of a Cartesian graph which states the relationship between the x and y axes where the x axis represents time (days) and the y axis represents the CO_2 produced β.

In Figure 7 Bioplastic B without the addition of inoculum obtained an R value = 0.8344 with a rate constant value obtained of 0.0254 day^{-1}. Meanwhile, Bioplastic B with the addition of inoculum obtained an R value = 0.9047 with a rate constant value of 0.0391 day^{-1}. The rate constant value in the bioplastic test sample B is smaller when compared to Microcrystalline cellulose and the bioplastic test sample A. The difference in results can be caused by several factors including the influence of microorganisms (bacteria), fungi, and enzyme activity, in addition to the hydrophobic nature of the addictive material, production process, polymer structure, morphology and molecular weight of the plastic, other external factors including environmental conditions (temperature, sunlight intensity and humidity).

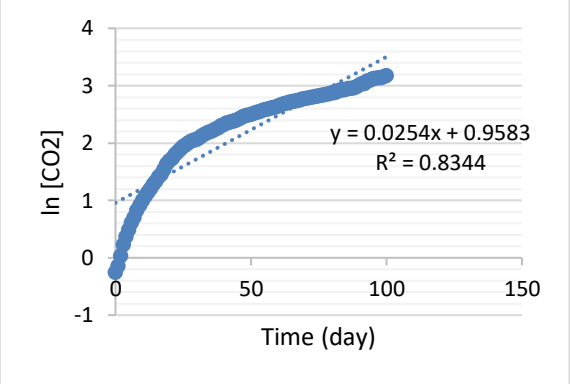
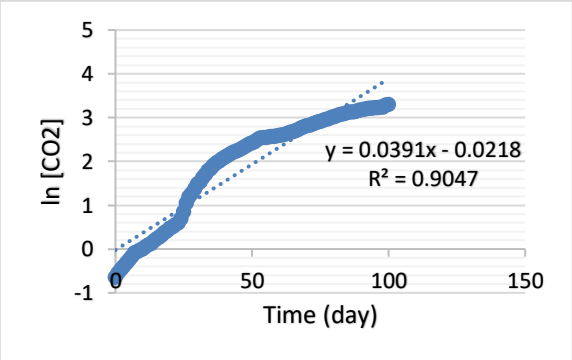


Figure 7. Bioplastic B without inoculum



Picture 8. Bioplastic B with inoculum

The response of bioplastics to bacteria can be strengthened by paying attention to the characteristics of the media, including water content and pH, as well as proving the presence of Pseudomonas sp. bacteria. in the media used [14]. Pseudomonas sp. was chosen because of the ability of Pseudomonas sp. in degrading biopolymers which are the building blocks of bioplastics. Sand has the lowest water content compared to agricultural soil and compost. Meanwhile, at neutral pH (7-8), the ability of bacteria to degrade tends to increase. If the pH tends to be acidic, it will reduce the rate of degradation of the bioplastic itself,

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because bacteria will find it difficult to live in such conditions.

The results of the biodegradation rate analysis show that the use of Pseudomonas sp. and Bacillus sp. and selecting the right media has a significant effect on the constant rate of biodegradation of bioplastic A and bioplastic B. Based on the output, it shows the results of the correlation test or the relationship between the two data or the relationship between the pre-test variables and the post-test variables. From the output data, it is known that the correlation coefficient (Correlation) value is 0.764 with a significance value (Sig.) of 0.000. Because the Sig value. 0.000 < probability 0.05, then it can be said that there is a relationship between the pretest variables and the posttest variables.

The decision-making guideline in the paired sample test based on the significance value (Sig.) of the SPSS output results is if the Sig. (2-tailed) < 0.05, then H_0 is rejected, and Ha is accepted. Conversely, rejected. (2-tailed) > 0.05 then H_0 is accepted, and Ha is rejected accepted, it is known that the Sig. (2-tailed) is 0.000 < 0.05, then H_0 is rejected, and Ha is accepted. So, it can be concluded that there is an average difference between no addition of bacteria and the addition of bacteria to the test sample.

Based on the output in the attachment, it shows the results of the correlation test or the relationship between the two data or the relationship between the pre-test variables and the post-test variables. From the output data, it is known that the correlation coefficient (Correlation) value is 0.810 with a significance value (Sig.) of 0.000. Because the Sig value. 0.000 < probability 0.05, then it can be said that there is a relationship between the pretest variables and the posttest variables. The decision-making guideline in the paired sample test based on the significance value (Sig.) of the SPSS output results is if the Sig. (2-tailed) < 0.05, then H_0 is rejected, and Ha is accepted. Conversely, rejected, (2-tailed) > 0.05 then H_0 is accepted, and Ha is rejected. It is known that the Sig. (2-tailed) is 0.000 < 0.05, then H_0 is rejected, and Ha is accepted. So, it can be concluded that there is an average difference between no addition of bacteria and the addition of bacteria to the test sample.

4. CONCLUSION

The degradation kinetics of the biodegradation rate follows first order with a rate constant value of Microcrystalline cellulose as a positive control of 0.0489 day^{-1}. This rate constant value is greater than the test sample. Where the rate constant for Bioplastic A without inoculum is 0.0451 day^{-1} while Bioplastic A with inoculum is 0.0423 day^{-1}. Followed by bioplastic B without inoculum at 0.0254 day^{-1} and bioplastic B with inoculum at 0.0391 day^{-1}. Addition of Bacillus sp. and Pseudomonas sp. As bacteria that decompose organic matter, they have a significantly different effect on the reaction rate, indicated by a significant value or $p < 0.05$. Where the use of Bacillus sp. is considered better and more efficient.

REFERENCES

[1] Plastics Europe. 2015. Plastics - the facts 2014/2015: An Analysis of European Plastics Production, Demand and Waste Data. 1–34. doi: 10.1016/j.marpolbul.2013.01.015.

[2] DiGregorio and Barry E. 2009. Biobased Performance of Bioplastics: Mirel. Chemistry and Biology. 16(1): 1–2.

[3] Singh RV. 2015. Polyhydroxybutyrate (PHB): Biodegradable, Bioplastics Produced by Microorganisms. International Journal of Pharmaceutical Research. 7:17–23.

[4] Darni Y and Utami H. 2010. Study of the Manufacturing and Characteristics of the Mechanical Properties and Hydrophobicity of Bioplastics from Sorghum Starch. Journal of Chemical and Environmental Engineering. 7(4):88–93.

[5] Kinney, TJ, CA Masiello, B. Dugan, WC Hockaday, MR Dean, K. Zygourakis and RT Barnes.2012. Hydrologic properties of biochars are produced at different temperatures. Biomass and Bioenergy.30: 1-10.

[6] Kumar, A., YS Negi, V. Choudhary & NKBhardwaj.2014. Characterization of Cellulose Nanocrystals Produced by Acid-Hydrolysis from Sugarcane Bagasse as Agro-Waste .Journal of Materials Physics and Chemistry.2 (1): 1-8.

[7] Nomanbhay, SM, R. Hussain & K. Palanisamy.2013. Microwave-Assisted

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e-ISSN: 2776-4508; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

alkaline pretreatment and microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber for enhanced fermentable sugar yield. Journal of Sustainable Bioenergy Systems.3: 7-17.

[8] Madigan, MT, JM Martinko, DA Stahl, DP Clark. 2011. Brock biology of microorganisms, 13th ed.

[9] Cappuccino, JG and Sherman, N., 2014. Microbiology Laboratory Manual, Edition 8. Jakarta, EGC.

[10] Dian APR, Ganjar S, Sri S. 2017. The Effect of Water Content on the Organic Waste Composting Process Using the Takakura Method. Engineering Journal: Vol. 06.

[11] Yuwono D. 2006. Compost. Jakarta (ID): Self-Help Spreade

[12] Jumar, Saputra RA. 2018. Organic Agriculture Technology. Malang (ID): Media Intelligence.

[13] Funabashi M, Ninomiya F, Kunioka M. 2009. Biodegradability Evaluation of Polymers by ISO 14855-2. International Journal of Molecular Sciences. 10:3635-3654.doi:10.3390/ijms10083635.

[14] Marcelinus C, Ismojo, Sidik Marsudi. 2021. Biodegradation Kinetic Study of Cassava & Tannia Starch-Based Bioplastics as Green Material in Various Media. doi: 10.20884/1.jm.2022.17.1.5591