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Biodegradation Test of Polluted River Caused By Domestic Wastewater Using Indigenous Bacteria in The Way Tomu Watershed, Ambon City

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Abstract. Nowadays, the rivers are often used by residents around the river as the final disposal of domestic wastewater that causes the rivers to become polluted. Biodegradation is an alternative to wastewater treatment as an effort to solve water pollution which is often carried out using bacteria that have the potential to decomposers in the biodegradation process, especially indigenous bacteria. This study aims to identify and obtain isolates, combinations, and characteristics of the indigenous bacteria isolate that have the potential as a biodegradation agent for polluted rivers caused by domestic wastewater in the Way Tomu watershed, Ambon City. In this study, isolates of indigenous bacteria that were isolated and purified were tested for protease and amylase enzyme activity to select isolates to be used in the biodegradation process testing consisting of parameters pH, COD, and TSS as indicators of the biodegradation. Afterward, the isolates that have the best potential as biodegradation agents will be characterized and biochemical tests will be carried out to identify the general characteristics of these isolates. The results showed that the combination of Genus *Aeromonas*, *Bacillus*, and *Pseudomonas* as isolates of indigenous bacteria that has the best potential as an agent for the biodegradation of polluted rivers caused by domestic wastewater. The benefit of this research is as initial information and consideration for alternative treatment of polluted rivers caused by domestic wastewater using indigenous bacteria isolates as an effort to resolve river pollution, especially the rivers in Ambon City.

Keywords: Biodegradation, Domestic Wastewater, Indigenous Bacteria, Polluted River, Way Tomu Watershed.

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INTRODUCTION

Nowadays, the rivers are often used by residents around the river as a final disposal of wastes that cause the rivers to become polluted. One of those wastes is domestic wastewater which polluted the rivers in the amount of 60%–70% (Suswati and Wibisono, 2013). Domestic wastewater is wastewater originating from activities that produce kitchen wastewater, bathroom wastewater, washing wastewater, sink wastewater, runoff water from septic tanks, and other wastewater (Mubin et al., 2016).

Polluted rivers caused by domestic wastewater also occur in the rivers of Ambon City. One of the polluted rivers in Ambon City is the Way Tomu River, as explained by BPS that in 2016 the quality status of Way Tomu River was heavily polluted. Performance Report by KemenLHK in 2018 also reported that the water quality status of Way Tomu River is still heavily polluted. Alternative treatment of wastewater as an effort to resolve water pollution is often carried out using bacteria that have the potential to decomposers in the biodegradation process. The reduction of organic contaminants in the waste by degrading bacteria can be observed through indicators of the quality of the liquid waste which include a decrease in the levels of BOD, COD, and TSS (Oljira et al., 2018).

Biodegradation is a natural event that can occur because the ecosystem will normally balance the existing ecological cycle. However, waste with high organic material content cannot be decomposed by bacteria in rivers naturally, due to the high volume and level of waste and the inadequate number and types of bacteria in the rivers (Achyani et al., 2018). Therefore, human intervention is needed to help to characterize the types of bacteria that have potential biodegradation agents. The search for bacteria that play a role in the biodegradation process is often best done in the polluted site itself because organisms living in polluted sites will develop resistance to polluted chemicals and can be useful for the biodegradation process. Local biota bacteria from the polluted environment are called

indigenous bacteria (Novianty et al., 2020). Therefore, to find out what bacteria have the potential to degrade polluted rivers caused by domestic wastewater in the Way Tomu watershed, it can be done by isolating indigenous bacteria from the watershed itself.

In this study, researchers used parameters pH, COD (Chemical Oxygen Demand), and TSS (Total Suspended Solid) as an indicator of the biodegradation process. COD is the amount of oxygen (mg O_2) needed to oxidize organic substances in 1 liter of water sample (Ningsih, 2017). TSS is all solid substances or particles suspended in water in the form of biotic and abiotic components (Jiyah et al., 2017). After the biodegradation test, the isolates that had the best degradation potential were characterized morphologically and biochemical tests were carried out to determine the characteristics of bacteria isolates.

Based on this, efforts are needed to identify and obtain isolates, combinations, and characteristics of the indigenous bacteria isolate that have the potential as a biodegradation agent for polluted rivers caused by domestic wastewater. So, the researchers are interested to conduct this study as initial information and consideration for alternative treatment of polluted rivers caused by domestic wastewater using indigenous bacteria isolate as an effort to resolve river pollution, especially the rivers in Ambon City.

METHODS

Time and Location of Study

The study was conducted from August 2021 to April 2022. Sampling was carried out in the Way Tomu Watershed, Ambon City, then the study was conducted at the Microbiology Laboratory of Biology Department, Faculty of Mathematics and Natural Sciences, Pattimura University including bacterial isolation until biodegradation test, while measuring parameters biodegradation including pH, COD, and TSS was conducted at Chemical Physics Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Pattimura University.

Media Preparation

The media used in this study consisted of Nutrient Agar (NA), Skim Milk Agar 1% (SMA), Starch Agar 1% (SA), Nutrient Broth (NB), and Triple Sugar Iron Agar (TSIA). NA is used for isolation, purification, and for streaking the isolates onto new media before inoculum preparation for biodegradation test and also as an ingredient in the preparation of SMA and SA. SMA 1% is used in testing the activity of the protease enzyme, while SA 1% is used in testing the activity of the amylase enzyme. NB is used in the preparation of bacterial inoculum to adjust the OD of the bacterial isolates suspension before being used in the biodegradation test process. TSIA is needed to test the carbohydrate fermentation of isolates.

Determination of Sampling Station

Determination of the sampling station in this study using the Purposive Sampling method. The researchers chose the location of the sampling station that is considered close to the pollutant sources, which is surrounded by settlements, offices, and hotels, so the location of the sampling station was divided into 2 stations, namely station 1 was in the middle of the watershed at the bridge of Jalan Kakialy and station 2 was in the downstream part of the watershed at The Losari Bridge, Jalan Pantai Mardika. At each station, sampling was carried out at 2 points, the left and right of the watershed flow.

Sampling

Sampling in this study used the Integrated Sample method. Sampling was conducted on weekdays and at the time that was considered to be the peak of community activity producing domestic wastewater, which is at 7-10 AM. In this study, sampling was conducted from the bridge, by means of a sterilized sample bottle wrapped with a rope and given a stone for the weight, aseptically lowered from the bridge using a rope wrapped around the sample bottle, until the sample bottle sinks and believed has been in the middle of the depth in the river, wait until the sample bottle is full and aseptically pour the sample into another sterile sample bottle as storage. To check the pH quality of the river, immediately analyzed at the sampling location using a pH meter. Next, put the entire sample into the coolbox and then take it to the laboratory.

Isolation and Purification of Indigenous Bacteria

Isolation of indigenous bacteria was conducted up to 10^{-5} serial dilutions. Then, aseptically pipette 0.1 ml from a 10^{-1} dilution test tube and isolate using the spread plate method on NA, do the same thing until 10^{-5} dilutions on another new NA and incubated it all in an incubator at 37°C for 24 hours. After the isolation process was carried out,

next purified the isolates used the quadrant streak method to obtain pure bacterial cultures and incubated all the results in an incubator at 37°C for 24 hours.

Enzyme Activity Test

Before the biodegradation test, the pure isolates obtained were tested for the activity of protease and amylase enzymes to select isolates to be selected for the biodegradation test. The procedure for testing the activity of the enzyme was conducted following Fatichah (2011), which is by dividing the outside of the petri dish into 3-4 parts using a marker. After that, aseptically inoculate the pure isolates obtained by spotting on the surface of SMA 1% for the protease enzyme activity test and SA 1% for the amylase enzyme activity test, in each petri dish 3-4 different bacteria isolates were inoculated according to the sign marker on the petri dish. Then incubate it in an incubator at 37^{o}C for 24 hours. After that, only for the amylase enzyme activity test have to drip the iodine solution first to the overgrown bacteria and then observe the clear zone formed around the bacteria colony for both enzyme activity tests and then measure its diameter using a ruler and calculate the Proteolytic Index and Amylolytic Index with the equation based on Asril and Leksikowati (2019) as follows:

$$\text{Proteolytic Index or Amylolytic Index} = \frac{\text{Clear Zone Diameter (mm)} - \text{Colony Diameter (mm)}}{\text{Colony Diameter (mm)}}$$

Selection of Single and Combination of Isolates for Biodegradation Test

In this study, the single isolates of bacteria selected for the biodegradation test consisted of 3 pure isolates from each sampling station which had enzyme activities, as follows:

1. High Proteolytic Index (Isolate A)
2. High Amylolytic Index (Isolate B)
3. Moderate Proteolytic Index (Isolate C)

This determination was made because according to Amri and Wesen (2017) the solids in domestic waste consisted of 65% protein and 25% carbohydrates. Then from those three single isolates made a combination of isolates, as follows:

1. Combination of Isolates A+B
2. Combination of Isolates B+C
3. Combination of Isolates A+B+C

Inoculum Preparation for Biodegradation Test

The preparation of bacteria inoculum for biodegradation test in this study was carried out following Oljira et al (2018), with the following steps: first, inoculate pure isolates including single isolates and combination of isolates into each Erlenmeyer containing NB separately using an aseptic needle. Afterward, incubate the entire Erlenmeyer in a shaker incubator for 24 hours at 37^{o}C with 150 rpm. Afterward, adjust the results of each inoculum to $OD_{0.5}$ at a wavelength of 660 nm using a spectrophotometer. This was done because, in this study, the number of bacteria cells was not calculated, only based on the same OD value hoping that the growth speed of bacteria would be the same.

Biodegradation Test of Polluted River caused by Domestic Wastewater

The biodegradation test of polluted river caused by domestic wastewater in this study was carried out following Oljira et al (2018), which is using a simple laboratory scale batch culture, with the following stages: first, pour 80 ml of water river samples into each Erlenmeyers used as treatment and pour 100 ml for another Erlenmeyers used as a control and then sterilize it all using an autoclave. Afterward, wait until the Erlenmeyers were cold and pipette 20 ml of bacteria inoculum from a single isolate onto the Erlenmeyer using a micropipette, for the combination of 2 isolates pipetted each isolate 10 ml and for the combination of 3 isolates pipetted each isolate 6.6 ml, except for the Erlenmeyer used as control doesn't need to be inoculated with bacteria. Next, homogenize the Erlenmeyers by shaking gently, then pour 30 ml of each treatment onto a sterile beaker glass to measure the value of the parameters pH, COD, and TSS. COD parameter required 10 ml sample while TSS parameter required 20 ml sample. Measurement of pH, COD, and TSS parameters were carried out on day 0 and day 7. Then incubated all the Erlenmeyers for 7 days in a shaker incubator at 37^{o}C with 150 rpm. Next, compared the results to the river water quality criteria regulated in PP No 82 of 2001.

Measurement of River Water Quality Parameters and Biodegradation Test Parameters

The value of pH parameter was measured used a pH meter. The value of COD parameter was

measured based on SNI 6989.15:2019, and the value of TSS parameter was measured based on SNI 066989.3:2004.

Morphological Characterization and Biochemical Test

The morphological characterization of bacteria included morphological observations, gram staining, and spore staining. The biochemical tests included catalase, oxidase, and carbohydrate fermentation tests.

Data analysis

Descriptive Data Analysis

Descriptive data analysis was carried out by calculated the percentage of efficiency of increasing or decreasing pH value and efficiency of decreasing COD and TSS value in biodegradation test, with the efficiency formula following Turista (2017), as follows:

$$\text{Increase Efficiency} = \frac{n_2 - n_1}{n_2} \text{ x } 100$$

$$\text{Decreasing Efficiency} = \frac{n_1 - n_2}{n_1} \text{ x } 100$$

n_1 = Parameter's value day 0

n_2 = Parameter's value day 7

ANOVA Category One-Way

ANOVA analysis was performed using the SPSS program to determine the significant difference in the percentage of efficiency increase or decrease of biodegradation test parameters in each treatment, to see which single isolate or combination of isolates had the best potential for degradation of the polluted river caused by domestic wastewater. If the results of the analysis show a significant difference, then proceed with Duncan's test with a 95% confidence level to determine the value of the difference significantly.

RESULTS AND DISCUSSION

Isolation and Purification of Indigenous Bacteria

Total isolates of indigenous bacteria that were isolated and purified in this study were 22 isolates, consisting of 13 isolates from station 1 and 9 isolates from station 2.

Enzyme Activity Test

Activity Test of Protease Enzyme

Based on Table 1 it can be seen that from all 13 isolates of indigenous bacteria that were isolated from station 1 only 3 isolates of indigenous bacteria had the activity of protease enzyme, while at station 2 only 2 isolates had the activity of protease enzyme out of 9 isolates that were isolated. Bacteria isolates selected for biodegradation test were isolates that had high and medium proteolytic index value, namely, isolate S1.6 and S1.9 represent station 1 also S2.5 and S2.9 represent station 2.

In this study, testing the activity of protease enzyme was carried out using SMA 1%. According to Ramadhan et al (2021), the milk contained in SMA 1% contain casein, which is a milk protein consist of phosphoproteins and then binds with calcium and forms calcium caseinate. These molecules not dissolve in water. The color of this suspension is white and can be observed directly when suspended in solid culture media. Extracellular protease enzymes of bacteria will hydrolyze casein into soluble peptides and amino acids. The loss of casein particles in SMA 1% was indicated by the formation of a clear zone around the bacterial colonies which was an indicator that the isolates were able to breakdown casein in SMA 1%.

Table 1. Proteolytic Index Value of Indigenous Bacteria Isolates from Polluted River caused by Domestic Wastewater in the Way Tomu Watershed, Ambon City

Sampling Station	Isolate Code	Clear Zone Diameter (mm)	Colony Diameter (mm)	Proteolytic Index (mm)
Station 1 (Kakialy Bridge)	S1.6	16	8	1
	S1.9	19	10	0.9
	S1.10	13	8	0.6
Station 2 (Losari Bridge)	S2.5	13	6	1.1
	S2.9	54	43	0.2

Activity Test of Amylase Enzyme

Based on Table 2 it can be seen that from 13 isolates of indigenous bacteria that were isolated at station 1, 7 isolates had the activity of amylase enzyme, while from 9 isolates at station 2, 4 isolates had the activity of amylase enzyme. The

bacteria isolates selected for the biodegradation test were isolates that had high amylolytic index values, namely, isolate S1.4 representing station 1 and isolate S2 representing station 2.

In this study, testing the activity of the amylase enzyme was carried out using SA 1%. According to Susilawati et al (2015), bacteria isolates that produce extracellular amylase can be seen from the formation of a clear zone around the bacteria colonies. To clarify the presence of a clear zone, the solid starch medium that has been overgrown with bacteria is dripped with a solution of iodine. The area outside the clear zone will be blue-purple after being given the solution because the iodine solution will react with the starch that is not hydrolyzed. The clear zone is not stained because the starch in that zone has been hydrolyzed into simple compounds such as disaccharides or monosaccharides.

Table 2. Amylolytic Index Value of Indigenous Bacteria Isolates from Polluted River caused by Domestic Wastewater in the Way Tomu Watershed, Ambon City

Sampling Station	Isolate Code	Clear Zone Diameter (mm)	Colony Diameter (mm)	Amylolytic Index (mm)
Station 1 (Kakialy Bridge)	S1.1	7	2	2.5
	S1.2	26	10	1.6
	S1.3	6	5	0.2
	S1.4	19	5	2.8
	S1.5	30	20	0.5
	S1.6	13	7	0.8
	S1.10	11	6	0.8
Station 2 (Losari Bridge)	S2.4	10	3	2.3
	S2.5	13	10	0.3
	S2.6	15	10	0.5
	S2.9	53	46,6	0.1

Selection of Single Isolates and Combination of Isolates for Biodegradation Test

Based on the results of calculated the proteolytic index value in Table 1 and amylolytic index value in Table 2, the selection of single isolates and combinations of isolates for the biodegradation test in this study can be seen in Table 3.

Table 3. Selection of Single Isolates and Combination of Isolates for Biodegradation Test

Sampling Station	Designation	Isolate Code	Explanatory
Station1 (Kakialy Bridge)	Isolate A	S1.6	High Proteolytic Index
	Isolate B	S1.4	High Amylolytic Index
	Isolat C	S1.9	Moderate Proteolytic Index
	Combination of Isolates A+B	S1.6 + S1.4	
	Combination of Isolates B+C	S1.4 + S1.9	
	Combination of Isolates A+B+C	S1.6+S1.4+S1.9	
Station 2 (Losari Bridge)	Isolate A	S2.5	High Proteolytic Index
	Isolate B	S2.4	High Amylolytic Index
	Isolate C	S2.9	Moderate Proteolytic Index
	Combination of Isolates A+B	S2.5 + S2.4	
	Combination of Isolates B+C	S2.4 + S2.9	
	Combination of Isolates A+B+C	S2.5 +S2.4 +S2.9	

Measurement of Biodegradation Test Parameter

pH Parameter

Based on the results in Figure 1 and Figure 2, the pH average during 7 days of incubation in control and all treatments from station 1 and station 2 is neutral and still within the regulated maximum concentration limit as stipulated in PP No 82 of 2001. pH average of control at station 1 and station 2 has decreased, this result was different from all treatments at station 1 and station 2 which has increased. According to Ratna et al (2017), at the beginning of the process, pH will always fall which is caused by a certain

number of microorganisms that will convert organic waste into organic acids in the next process, other types of microorganisms will consume organic acids and will cause the pH to rise again.

The pH decrease due to the acidification process (acid formation). After the acidification process is complete, the next step is to enter the methanogenesis stage, which is the conversion of acid to methane. The acid formed in the acidification stage will be used by bacteria as a substrate in the formation of methane and CO_2 gas. The decrease of pH in the control indicated that the control was in the acidification stage, while the treatment of single isolates or a combination of isolates that experienced an increase in pH was in the methanogenesis stage.

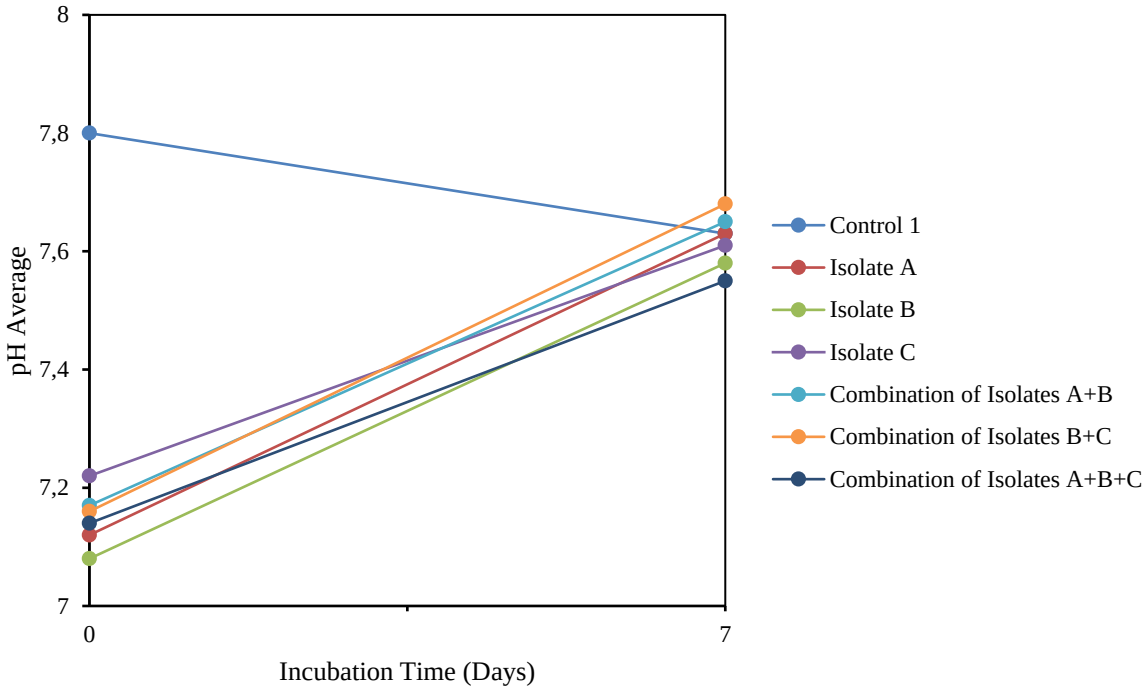

Figure 1. pH Average of Station 1 During The 7 Days of Incubation

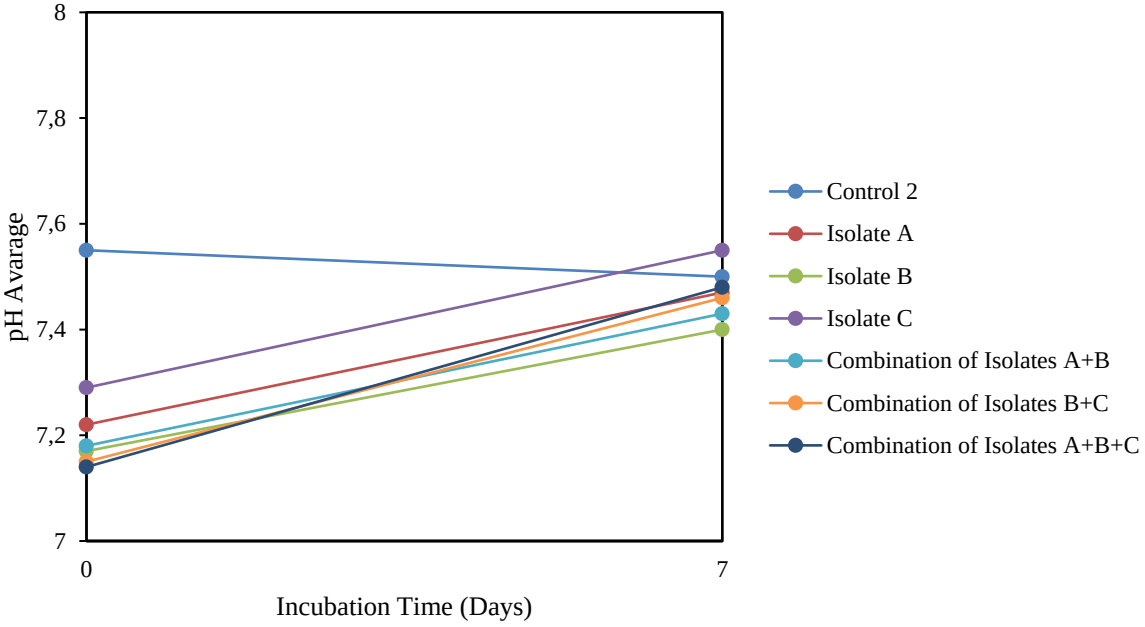

Figure 2. pH Average of Station 2 During The 7 Days of Incubation

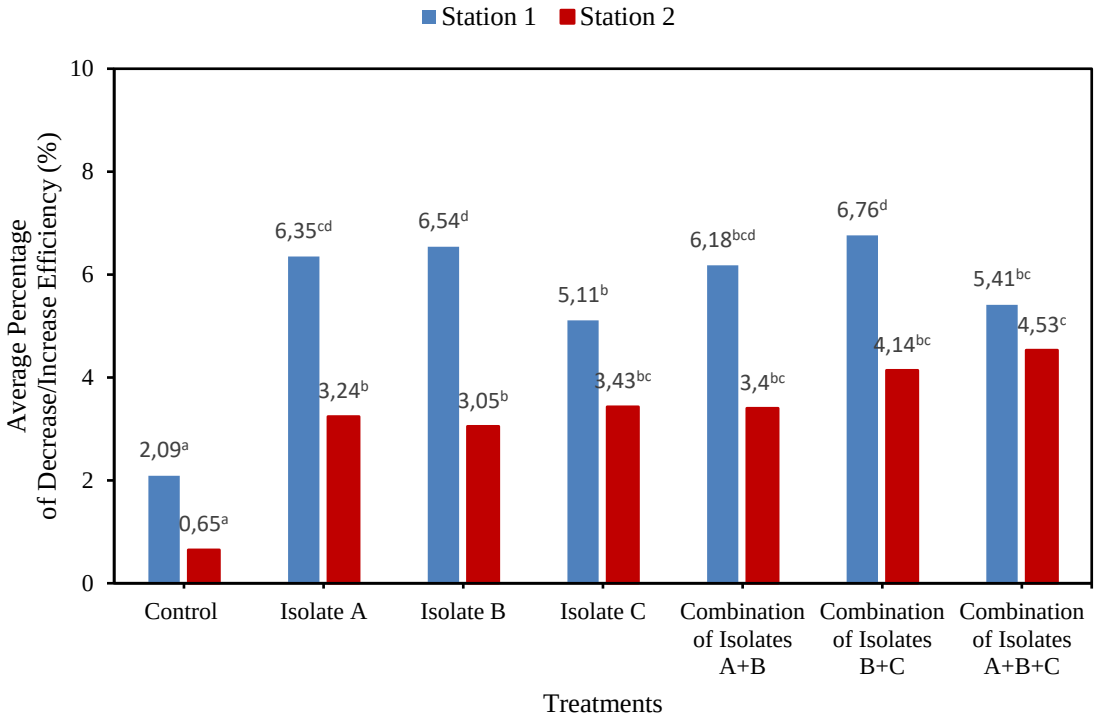

Figure 3. Comparison of pH Average Percentage of Decrease or Increase Efficiency at Station 1 and Station 2. Different Letters Following Values Show Significant Differences ($\alpha < 0.05$).

Based on Figure 3, it can be seen that the best treatment for the biodegradation test for pH parameter is at station 1 treatment because station 1 has a higher average percentage of pH reduction efficiency than station 2. The treatment at station 1 which had the highest average percentage of pH reduction efficiency is the combination of isolates B+C with a value of 6.76%, but after Duncan's test was performed, it is seen that the potential for the combination of isolates B+C was not significantly different with Isolate B which has a decreasing efficiency value of 6.54%. This means that the treatment of a single isolate, which is Isolate B alone, has the potential as a biodegradation agent for the best pH parameter.

COD Parameter

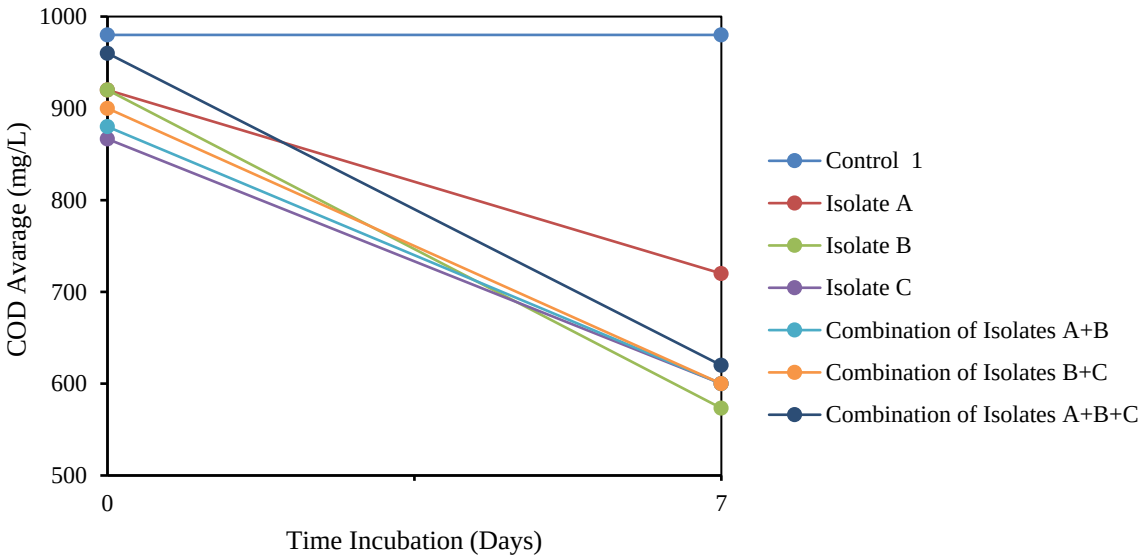

Figure 4. COD Average of Station 1 During The 7 Days of Incubation

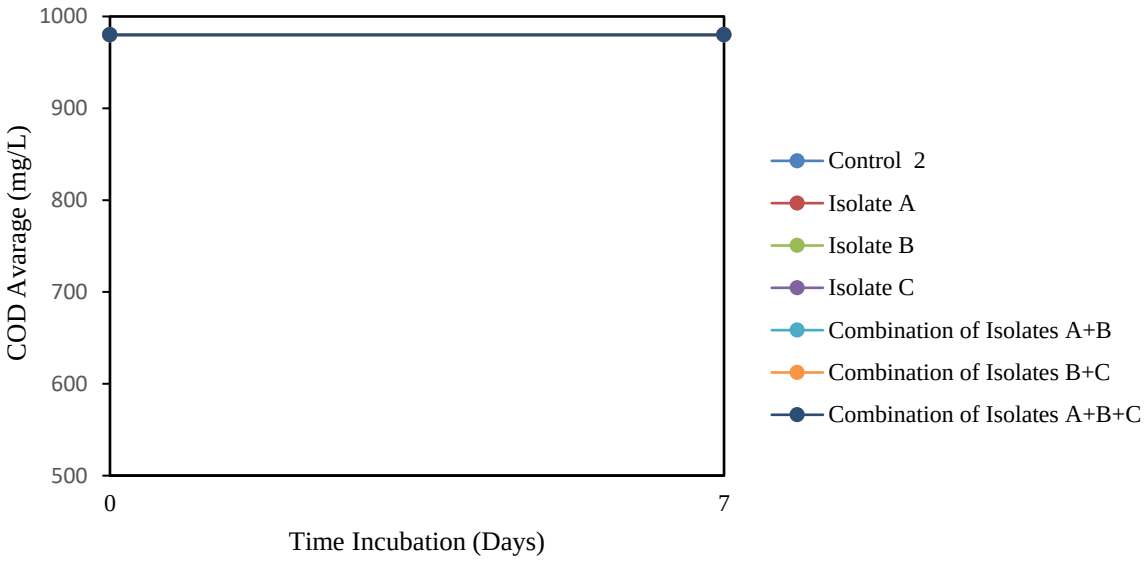

Figure 5. COD Average of Station 2 During The 7 Days of Incubation

Based on the results in Figure 4 and Figure 5, the average value of the COD parameter during 7 days of incubation in control and all treatments of station 1 and station 2 greatly exceeded the maximum concentration limit as stipulated in PP No 82 of 2001. The average value of COD parameter during 7 days of incubation in control station 1 and station 2 did not change. This means that without the addition of bacteria inoculants, polluted river caused by domestic wastewater cannot be degraded in the COD parameter. All treatments of station 2 isolates also did not change. This is because the bacteria isolates at station 2 carried out anaerobic respiration. These results were different from all treatments of station 1 isolates which experienced a decrease in COD value, meaning that the bacteria isolates at station 1 carried out aerobic respiration. The decrease in COD was due to the occurrence of bacteria activity during biodegradation test. This can happen because the organic material contained in wastewater serves as a substrate for microbial metabolism, thereby causing a decrease in COD concentration (Oljira et al, 2018).

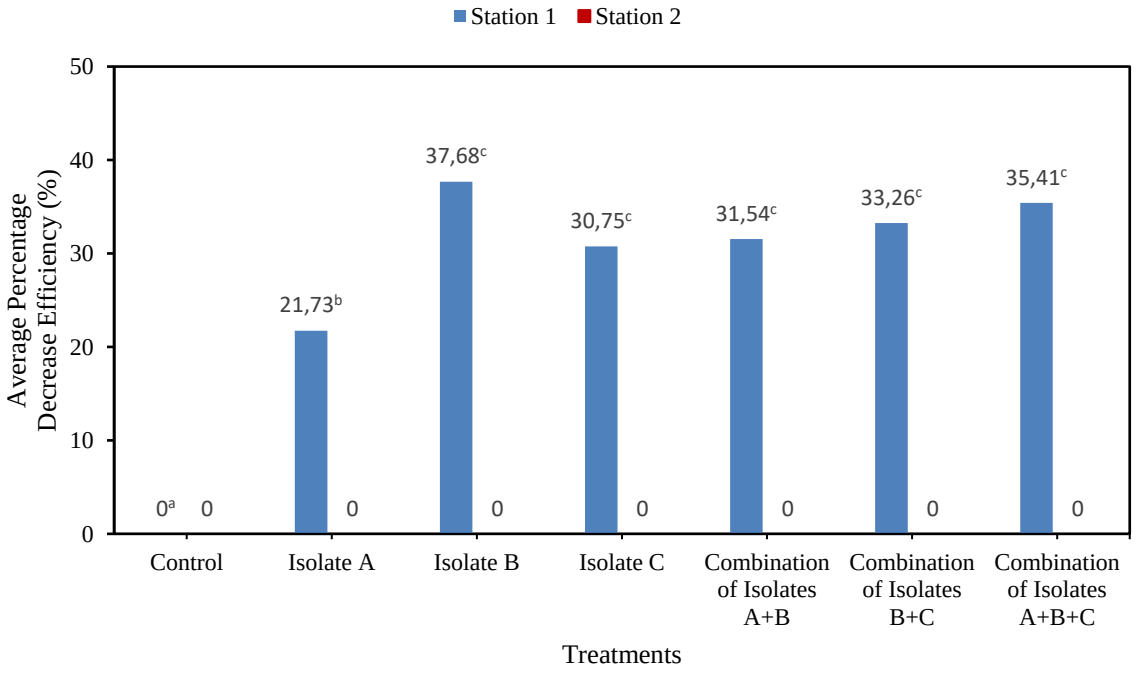

Figure 6. Comparison of COD Average Percentage of Decrease Efficiency at Station 1 and Station 2. Different Letters Following Values Show Significant Differences ($\alpha < 0.05$).

Based on Figure 6, it can be seen that the best biodegradation test treatment for COD parameters is at station 1, because the treatment at station 2 did not change, the average value of the COD reduction efficiency was 0%. The treatment at station 1 which had the highest average COD reduction efficiency is Isolate B with a value of 37.68%, but after Duncan's test was performed, it is seen that Isolate B is not significantly different with combination of isolates A+B+C, combination of isolates B+C, combination of isolates A+B, and isolate C. This means that the treatment of single isolates of Isolate B or Isolate C alone has the potential as a biodegradation agent for the best COD parameter.

TSS Parameter

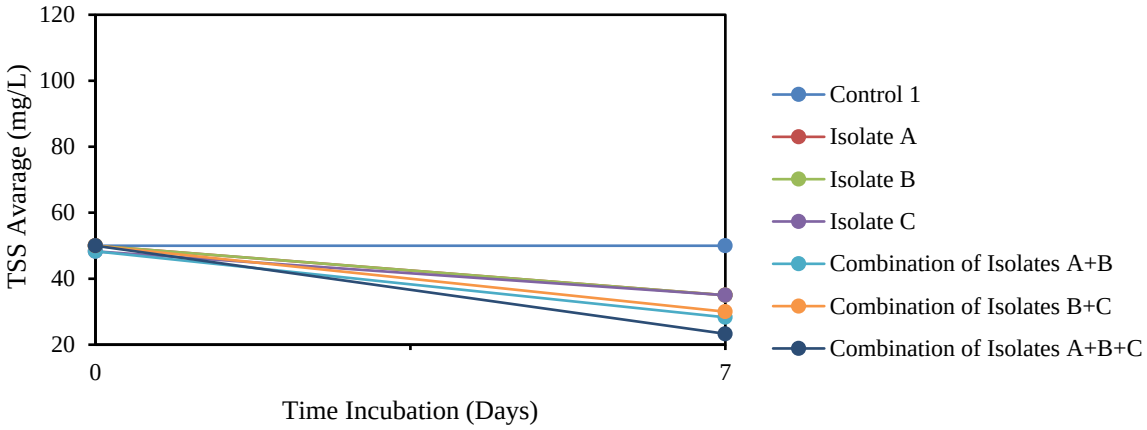

Figure 7. TSS Average of Station 1 During The 7 Days of Incubation

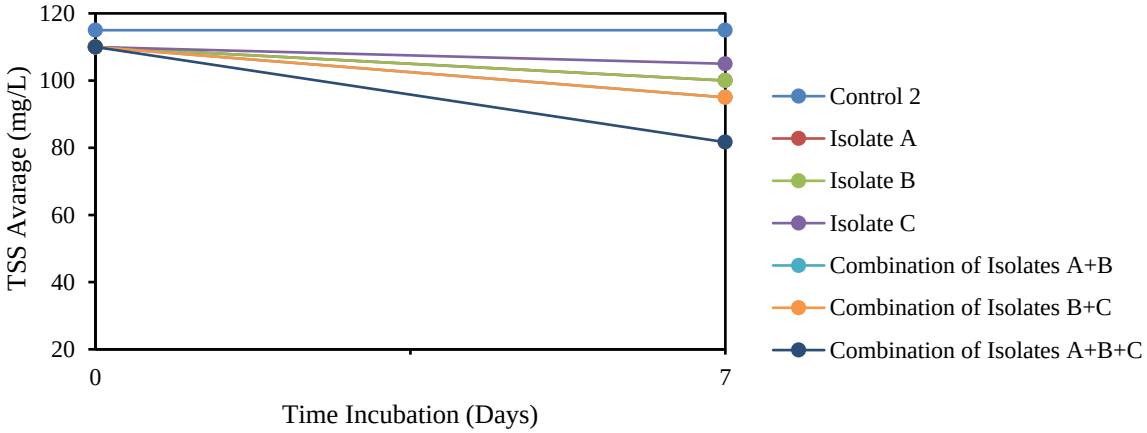

Figure 8. TSS Average of Station 2 During The 7 Days of Incubation

Based on the results obtained in Figure 7 and Figure 8, the average value of the TSS parameter during 7 days of incubation in the control and treatments at station 1 was still within the maximum concentration limit while at station 2 greatly exceeded the maximum concentration limit as stipulated in PP No 82 of 2001. The average value of the TSS parameter during 7 days of incubation in control Station 1 and Station 2 did not change. This means that without the addition of bacteria inoculants, polluted river caused by domestic wastewater cannot be degraded in the TSS parameter. These results were different from all treatments at Station 1 and Station 2 which experienced a decrease in TSS. The decrease in TSS is due to the activity of degrading organic compounds by bacteria (Waluyo, 2017). The decrease in TSS levels can occur in waste because, in the growth phase, the microbes grow rapidly, and when the microbes need nutrients in the form of waste, it will directly have an impact on the decrease in TSS values (Sari et al., 2017).

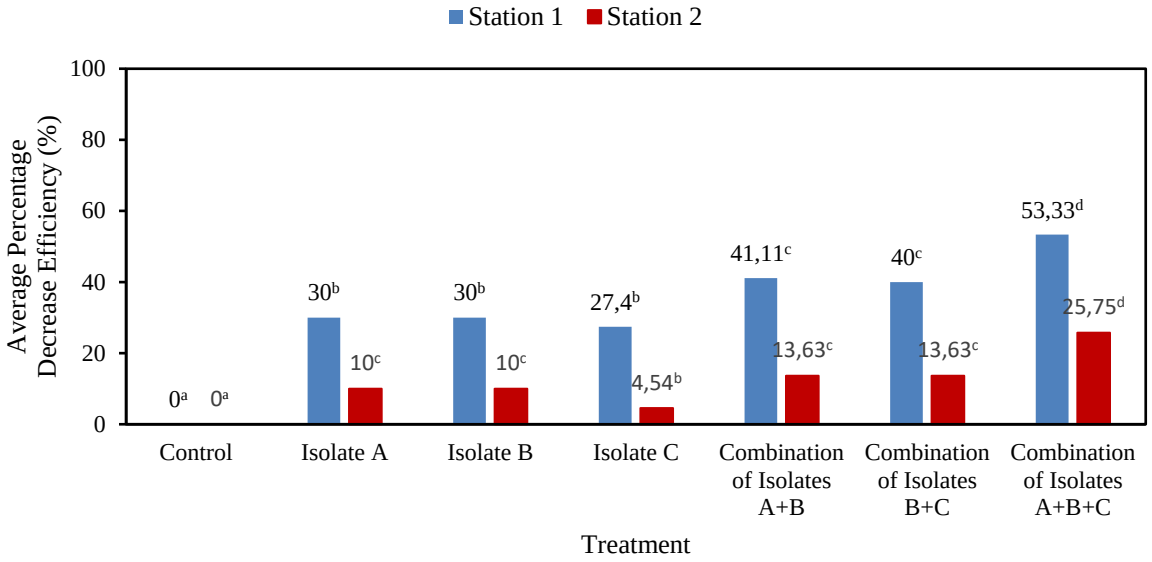

Figure 9. Comparison of TSS Average Percentage of Decrease Efficiency at Station 1 and Station 2. Different Letters Following Values Show Significant Differences ($\alpha < 0.05$).

Based on Figure 9, it can be seen that the best biodegradation test treatment for the TSS parameter is found in the treatment of station 1 because station 1 has a higher average percentage of TSS reduction efficiency than station 2. The treatment at station 1 which had the highest average percentage of TSS parameter reduction efficiency is the combination of isolates A+B+C with a value of 53.33%, and after Duncan's test, it was proven that the combination of isolates A+B+C is the best treatment to degrade polluted river caused by domestic wastewater for TSS parameter.

Morphological Characterization and Biochemical Test

The results of morphological characterization and biochemical tests of the isolates used in the biodegradation test can be seen in Table 4. The results were matched with the bacteria characters in Cowan and Steel's Manual for the Identification of Medical Bacteria and obtained 3 different genera at each Station.

Table 4. Morphological Characterization and Biochemical Tests

Tests	Station 1 Isolate A	Isolate B	Isolate C	Station 2 Isolate A	Isolate B	Isolate C
Colony Shape	Circular	Circular	Circular	Circular	Circular	Circular
Margin	Entire	Undulate	Entire	Entire	Entire	Undulate
Elevation	Convex	Flat	Raised	Raised	Raised	Flat
Color	Bone White Thick Shiny	Milky White Thick	Bone White Moist Shiny	Bone White Moist Shiny	Bone White Moist Shiny	Milky White Thick
Gram Stain	Gram – Basil	Gram + Basil	Gram – Basil	Gram – Basil	Gram – Coccus	Gram + Basil
Endospora Stain	–	+	–	–	–	–
Katalase Test	+	+	+	–	+	+
Oksidase Test	+	–	+	+	–	–
Carbohydrate Fermentation Test	Glucose	Glucose	Can't ferment carbohydrates	Lactose Sucrose	Glucose	Glucose
Suggested Genus	*Aeromonas*	*Bacillus*	*Pseudomonas*	*Cardiobacterium*	*Acinetobacter*	*Corynebacterium*

The benefit of this research is as initial information and consideration for alternative treatment of polluted rivers caused by domestic wastewater using indigenous bacteria isolates as an effort to resolve river pollution, especially the rivers in Ambon City.

CONCLUSION

Based on the study that has been done, to thoroughly carry out the biodegradation test of the polluted river caused by domestic wastewater which includes the best percentage efficiency of pH, COD, and TSS parameters simultaneously, must use a combination of isolates from the Genus *Aeromonas*, *Bacillus*, and *Pseudomonas*. It can also be concluded that the data generated from this study can serve as initial information and consideration for alternative treatment of polluted rivers caused by domestic wastewater using indigenous bacteria as an effort to solve river pollution, especially rivers in Ambon City.

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