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THE EFFECT OF ZEOLITE ADMINISTRATION IN THE CONSORTIUM OF HEAVY METAL REDUCING BACTERIA Pb ON LIQUID WASTE OF TEXTILE INDUSTRY

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ABSTRACT

Textile industry wastewater treatment must be managed properly so as not to cause environmental pollution that has an impact on human health. Industrial wastewater treatment by utilizing microbial activity with the addition of zeolite with varying concentrations measured using UV-Vis Spectrophotometry. This study aims to find out the effect of variations in zeolite concentration on bacterial consortiums in reducing lead heavy metals (Pb) and find out the characteristics of bacteria reducing heavy metals Pb after added zeolite. The results of the study using UV-Vis Spectrophotometry showed that the concentration of zeolite 3% was able to reduce heavy metals Pb on the 14th day with consecutive levels of 0.473 with an initial lead content of 2,460 ppm. The most significant decrease occurred in the addition of a zeolite concentration of 3%. This is shown in its effectiveness value of 86.772%. From this study, it can be concluded that the administration of a concentration of 3% in a consortium of bacteria can lower the levels of heavy metals Pb. Based on the characteristics of bacteria in biochemical tests, namely the confectionery test (Maltose, Glucose, Sucrose) obtained positive results, negative Lactose test, red /red TSIA test, negative marker test, and positive. Basil-shaped gram-negative bacteria refer to the genus *Desulfobacte*r.

Introduction

The textile industry plays an important role in Indonesia because it can absorb labor, meet clothing needs, and encourage economic growth (Riyardi et al., 2015). In addition to having a positive impact on the state, textile industry activities hurt the environment, namely causing pollution. Based on Law Number 32 of 2009 concerning Environmental Protection and Management, the definition of environmental pollution is the entry or inclusion of living things, substances, energy, and/or other components into the environment by human activities so that they exceed the established environmental quality standards. . Environmental pollution produced by the

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textile industry is in the form of textile waste which contains a lot of heavy metals.

One of the heavy metals contained in textile waste is lead. Lead (Pb) is produced from the dyeing process of chemicals and water as a solvent medium so that it is discharged into the environment when the process is complete. The presence of heavy metals in the aquatic environment that exceeds the threshold can cause health problems for living things around polluted waters. This is related to the nature of heavy metals, which are difficult to decompose so that they are easy to accumulate in the aquatic environment. Heavy metal pollution can be caused by human activities, such as industrial waste that is discharged into the waters without being treated first.

High concentrations of Pb are usually found in wastewater produced from battery manufacturing, electroplating, and the paint or pigment manufacturing industry with concentration ranges of 1-50mgL-1, 8-10 mg-1, and 1-25 mg-1, respectively. This metal is toxic and needs to be removed from the water so as not to harm the environment. The maximum recommended level of Pb in waste according to the Regulation of the Minister of the Environment No. 5 of 2014 is 0.1 mg/L, while according to SNI 013553-2006 the maximum permissible level of Pb is 0.005 mg/L.

Pb poisoning causes major damage to ecosystems and human health such as central nervous system disorders, liver, kidneys, reproductive systems, blood circulation, and death of aquatic biota (Milani, 2016). Heavy metal Pb respiratory, oral, and skin contact (Sembel, 2015). In children, lead poisoning will be higher if there is a lack of calcium, iron, and zinc because it hurts intelligence (IQ) which causes a decrease in their learning achievement, even though lead levels in their blood are not considered toxic (Rahayu, 2018). Therefore, heavy metal Pb in textile industry waste must be handled properly and appropriately so as not to cause harm to the environment and humans.

In line with this, Wage's research (2016) states that the quality of the soil in the Cikijing river environment, Rancaekek District has been contaminated with heavy metals, especially Pb with levels of 9.06 to 16.64 mg/L. Thus, it can be assumed that the heavy metals contained in the Cikijing river can accumulate in soil and plants. Therefore, the government requires a company to build or have a Waste Water Treatment Plant (IPAL).

But so far the government is considered too loose and unclear in enforcing regulations on the importance of Wastewater Treatment Plants (IPAL) for small-scale industries (Indrayani, 2018). So that many industries still do not have WWTPs. Until now, many environmentalists have doubted the effectiveness of the WWTPs owned by large industries, especially small-scale industries, which are more numerous than large industries and are focused on industrial centers, which do not have WWTPs (Indrayani, 2018).

Efforts to reduce heavy metals in waste have been carried out. One of them by Hapsari et al (2018) is using kale in reducing heavy metal Lead in good wastewater. The result was a decrease of 0.077 mg/l, but the decrease was still not able to reduce lead levels according to the applicable regulations.

One of the alternative processes for handling textile industrial waste containing heavy metal Pb is simply to use a biological method utilizing microbial activity. The microbes used were a consortium of metal-reducing bacteria derived from cow dung. Purnamaningsih's research (2017) stated that heavy metal-reducing bacteria from cow dung had optimal activity in reducing sulfate and precipitating heavy metals with a batch culture scale. A batch culture scale is cells grown by giving nutrients in a medium with a certain volume until they grow. This research is different from existing research by using a consortium of bacteria obtained from animal waste and using natural zeolite which is used to help immobilize metal reducing bacteria so that it can increase activity in reducing metals.

The activity of metal-reducing bacteria is expected to be more effective by giving zeolite. This is because a consortium of metal-reducing bacteria with the addition of zeolite

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can form a biofilm to adhere to the surface of the zeolite so that the biofilm and extracellular polymers can grow and adhere to the supporting media (Purnamaningsih et al., 2017). Research has been carried out by Purnamaningsih et al (2017) regarding the effectiveness of zeolites in the efficiency of heavy metal deposition, which is 61.16%.

Environmental pollution is a very serious problem to be solved because it involves the safety, health, and life of living things. Among the many problems of environmental pollution that receive special attention, namely the problem of environmental pollution caused by heavy metals. Pollution causes huge losses because in general, the environmental waste contains toxic substances, one of which is the heavy metal Pb (Lead).

Materials and Methods

The research was conducted for two months at the Bacteriology and Parasitology Laboratory of the Rajawali Health Institute. The research sample was in the form of textile industry wastewater which was suspected to contain heavy metal Pb in the Ujung Berung area, Bandung. The zeolite activation process is carried out by pounding the zeolite to a size of 0.8 – 1 cm. Zeolite was washed with distilled water 3 times and then soaked in distilled water for 24 hours. Zeolite was filtered and then dried in an oven at 70^{o}C for 24 hours (Purnamaningsih et al., 2017).

A sampling of the textile industry is carried out by inserting a water sample into a holding bottle that has been given weight at the bottom. Measurement of water pH using a pH meter. The bottle was tightly closed and then immediately taken to the laboratory.

Measurement of the initial level of Pb was carried out by pipetting a sample of 10 mL and then adding 1 mL of ARS reagent and 0.13 mL of 0.13 NaOH. After that, it was incubated for 30 minutes, then the absorbance was measured at a wavelength of 510 nm to determine the initial levels of Pb in the sample before batch culture treatment.

Batch culture treatment was carried out by preparing 5 sterilized bottles of 500 mL size and then adding zeolite with varying levels, each containing 0%, 1%, 3%, and 7% (Sofith et al., 2020). Then 250 mL of Postgate B medium was added to each bottle. and add 5 grams of dry cow dung. Then added 150 mL of liquid waste sample. After being incubated for 24 hours, the samples were homogenized to avoid contamination, until the 14th day. Furthermore, Pb levels were measured at 0, 1, 3, 7, and 14 days using UV-Vis spectrophotometry.

Data analysis was carried out with the help of computer software. The standard calibration curve obtained will provide information about the value of the regression coefficient and the linear equation needed for the determination of the metal content of Pb. Then the data on the concentration of Pb that has been obtained were analyzed using the Microsoft Excel application and then compiled in the form of a table for measuring the concentration of Pb heavy metal.

Results and Discussion

First, the absorbance measurement of the Pb standard solution was carried out to obtain the wavelength that produced the maximum absorption in determining the Pb content in the solution. The wavelength range used to find the maximum wavelength is 400 nm - 800 nm. In this study, the maximum wavelength obtained is 510 nm.

Next, the absorbance value was measured in the standard solution series. Determination of the standard curve aims to determine the relationship between the concentration of the solution with the absorbance value so that the concentration of the sample can be known. The measurement of the absorbance of the standard solution was carried out on a standard solution whose concentration was known to be 1 ppm; 2 ppm; 3 ppm; 4 ppm and 5 ppm using a UV-Vis spectrophotometer at a wavelength of 510 nm. The data on the results of the determination of absorbance in the standard solution series can be seen in Table 1.

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Table 1. Pb Standard Solution Absorbance Value

Concentration	Absorbance
1 ppm	0,945
2 ppm	1,823
3 ppm	2.674
4 ppm	3,596
5 ppm	4,253

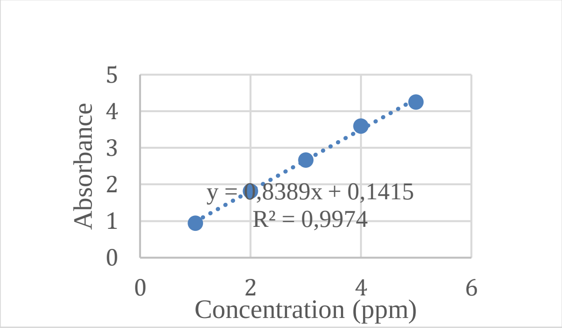

Figure 1. Pb Standard Solution Calibration Curve

From the data above, a standard solution curve was made so that the equation of the linear line used in the search for sample levels whose absorbance has been measured can be determined. The presentation of the standard calibration curve can be seen in Figure 1.

Based on the linear regression analysis in Figure 1, the standard curve regression equation y = 0.8389x + 0.1415 and R^2 = 0.9974. This equation is used to calculate the lead content in the liquid waste sample. Where (y) is the absorbance value and (x) is the lead content in the sample. Measurements of the initial levels of Pb were carried out in the samples before Batch Culture treatment. The results of the measurement of Pb levels in the sample can be seen in Table 2.

Table 2. Initial Level of Heavy Metal Pb Before Batch Culture Treatment

Sample	pH	Absorbance	Average	Level (ppm)
		2,254		
Wastewater	4,02	2,252	2,253	2,460
		2,253		

Measurement of heavy metal Pb in samples was carried out using UV-Vis spectrophotometry. The absorbance obtained was then entered into a linear equation, so that the pure Pb concentration in the sample was 2.460 ppm. The concentration of Pb in industrial wastewater samples exceeded the maximum threshold due to the recommended maximum level of Pb in waste according to the Regulation of the Minister of the Environment No. 5 of 2014 is 0.1 mg/L.

Furthermore, a physical test was performed on the sample. Parameters used in determining the quality of wastewater are physical, chemical, and biological parameters. The physical parameters tested consisted of color, odor, and pH which were tested on day 0, day 1, day 3, day 7, and day 14.

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Table 3. Textile Industry Liquid Waste Physical Test

Days	Concentration(%)	Physical Parameter		
		Color	**turbid**	**Stink**
0	0	yellow	turbid	not stinging
	1	yellow	turbid	not stinging
	3	yellow	turbid	not stinging
	7	yellow	turbid	not stinging
1	0	yellow	turbid	not stinging
	1	yellow	turbid	not stinging
	3	yellow	turbid	not stinging
	7	yellow	turbid	not stinging
3	0	brown	clear	stink
	1	brown	clear	stink
	3	brown	clear	stink
	7	brown	clear	stink
7	0	brown	clear	stink
	1	brown	clear	stink
	3	brown	clear	stink
	7	brown	clear	stink
14	0	brown	turbid	stink
	1	brown	turbid	stink
	3	brown	turbid	stink
	7	brown	turbid	stink

The results of the physical test on the sample can be seen that the color characteristics of the waste are the longer the incubation time, the darker the color obtained, and the pungent smell. According to (Punjungsari, 2017) a darker color change indicates the activity of lead (Pb) heavy metal-reducing bacteria in the Batch Culture Scale.

Measurements of the pH of each sample were carried out on days 0, 1, 3, 7, and 14. pH measurements were carried out using a pH meter. The results of pH measurements can be seen in Table 4.

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Table 4. pH Measurement on Sample

Zeolite Concentration (%)	**pH sample**				
	Day				
	0	1	3	7	14
0%	4,05	4,03	5,05	6,10	6,35
1%	4,0	4,08	5,01	6,25	6,37
3%	4,05	4,09	5,05	6,04	7,13
7%	4,05	4,05	5,04	6,25	6,35

Based on Table 3, the increase in pH in the sample was due to the activity of a consortium of Metal Reducing Bacteria that grew in an anaerobic environment causing the pH to increase. The results of the pH measurement are then made into a curve.

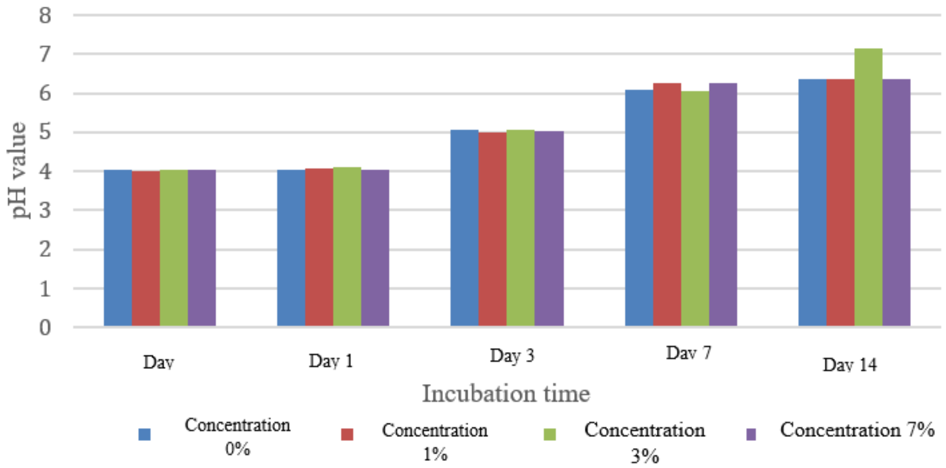

Figure 2. pH Measurement Curve on Sample

One of the important factors in the growth of bacteria is the pH value. Bacteria need an optimum pH to grow optimally. The Postgate B medium was first checked for pH, the result was pH 4. On day 0 after the medium was added zeolite, cow dung, and textile industry wastewater samples obtained pH 4. On the 3rd day all pH concentrations became 5. 7 all pH concentrations became 6. On the 14th day at a concentration of 0%, 1%, 7% pH 6, while at a concentration of 3% the pH increased to 7. The increase in pH could be caused by the activity of a consortium of Metal Reducing Bacteria growing in an anaerobic environment (Márquez-Reyes et al., 2013).

When the pH value becomes too high or low, the basic structure of the enzyme can change. The effect of pH on bacterial growth is related to enzyme activity. This enzyme is needed by bacteria to catalyze reactions related to bacterial growth (Amalina et al., 2005). In this study, the optimum pH for bacterial growth in reducing heavy metals was pH 7.

Furthermore, the measurement of heavy metal Pb with Batch Culture treatment was carried out. The research process was carried out with the addition of zeolite concentrations of 0%, 1%, 3%, and 7% with the textile industry wastewater samples of 150 mL each. Each postage medium used was 250 mL with an initial pH of 4 (acidic). Measurements in this study using UV-Vis Spectrophotometry. The decrease in Pb heavy metal levels can

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occur after the medium with the Batch Culture Scale treatment is incubated on days 0, 1, 3, 7, and 14. The final results of heavy metal Pb levels can be seen in Table 5.

Table 5. The final concentration of heavy metal Lead (Pb

Zeolit Concentration(%)	The final content of heavy metal Lead (ppm)				
	Day				
	0	1	3	7	14
0%	1,667	1,245	1,169	1,157	0,973
1%	1,536	0,957	0,813	0,710	0,411
3%	1,519	0,878	0,702	0,568	0,336
7%	1,486	0,834	0,776	0,649	0,345

The data in Table 5 shows that the lead (Pb) heavy metal-reducing bacteria added to the zeolite can reduce the lead content contained in the textile industry wastewater samples. From the results of the final heavy metal content of Lead in the sample, a graph is made which can be seen in Figure 3.

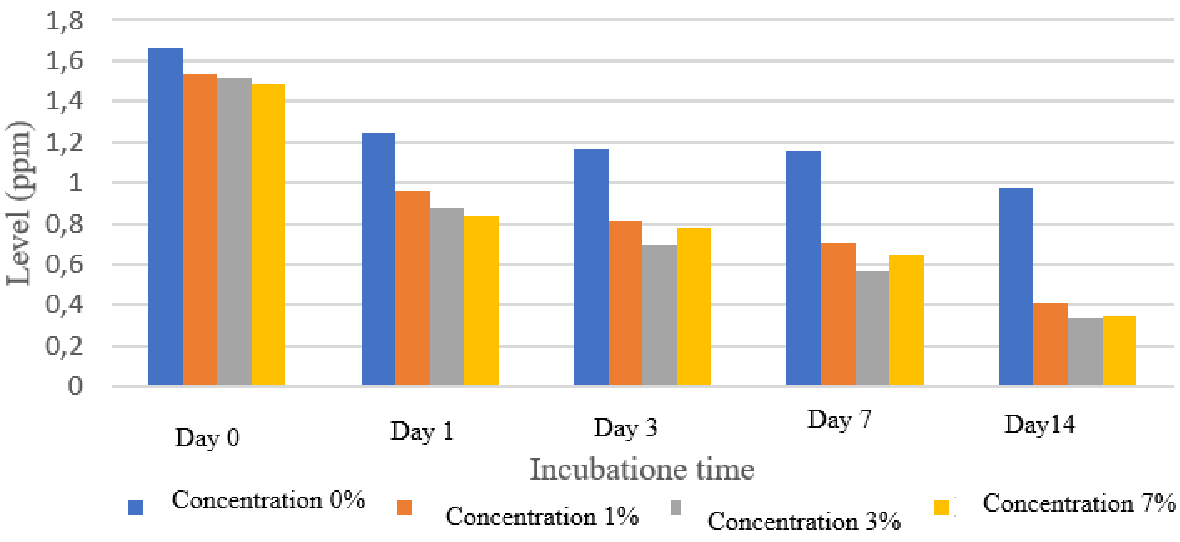

Figure 3. Graph of Final Level of Heavy Metal Pb in the sample

Based on the graph above, it can be seen that the sample with a concentration of 3% experienced the highest decrease. This is because the bacteria tested have a negatively charged cell surface while heavy metals are positively charged ions so bonds can occur between the bacterial cell surface and heavy metal ions (Junovia, 2015). The decrease in heavy metal content can be seen in Table 6.

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Tabel 6 Reduction of Lead heavy metal content

Zeolite Concentration (%)	Reduction of Lead heavy metal content (ppm)				
	Days				
	0	1	3	7	14
0%	0,793	1,215	1,291	1,303	1,487
1%	0,924	1,503	1,647	1,750	2,049
3%	0,941	1,582	1,758	1,892	2,124
7%	0,974	1,626	1,684	1,811	2,115

The data in Table 6 shows that the highest decrease in Pb heavy metal levels was at a concentration of 3% on the 14th day, which was 2.124 ppm. The final reduction curve for heavy metal Pb can be seen in Figure 4.

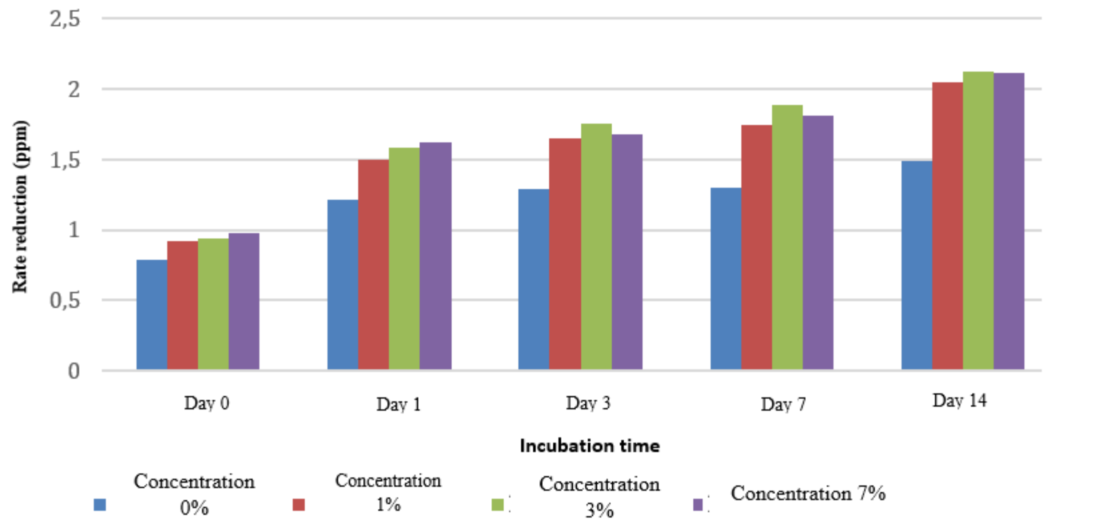

Figure 4. Pb Level Reduction Curve

Based on the curve above, it can be seen that the sample with a concentration of 3% experienced the highest decrease in levels of 2.124 ppm. The effectiveness of reducing the concentration in the sample can be seen in Table 7.

Table 7 Treatment effectiveness of batch culture

Konsentrasi Zeolit (%)	Efektivitas perlakuan (%)				
	Hari ke-				
	0	1	3	7	14
0%	40,366	49,390	52,479	52,967	60,447
1%	37,561	61,097	66,951	71,138	83,293
3%	20,122	64,308	71,463	76,910	86,772
7%	39,593	66,098	68,455	73,618	85,976

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Batch Culture Scale treatment was performed, and postage B medium was measured using a UV-Vis Spectrophotometer at a wavelength of 510 nm periodically on days 0; 1; 3; 7, and 14. The initial level of Pb in the liquid waste sample was 2.460 ppm. Based on table 4.5 shows all treatments experienced a decrease in Pb concentration. In 41 treatments, 0% decreased from day 0 of 1.667 ppm to day 14 to 0.973 ppm. The decrease in concentration was 0.694 ppm. The effectiveness of the reduction in the 0% treatment was 60.447%. In the 1% treatment, it decreased from day 0 of 1.536 ppm to day 14 to 0.411 ppm with a decrease of 1.125 ppm. The effectiveness of the reduction in the 1% treatment was 83.293%. The highest decrease was in the sample with the addition of 3% zeolite concentration because it was seen in its effectiveness, which was 86.772%. Day by day, the level of Pb in the sample decreased. The decrease in Pb concentration in the media was due to the interaction between the sulfide produced in the sulfate reduction process with Pb^{2+} metal to form insoluble metal sulfide, resulting in a decrease in Pb concentration. Biomass that has been loaded with heavy metal Lead (Pb) will form floc and settle by gravity, resulting in the accumulation of heavy metals in biological floc or activated sludge (Amalina et al., 2015).

In this study, the final levels of Pb in samples that meet the criteria for water quality standards in the Minister of the Environment Regulation no. 5 of 2014 which is 0.1 mg/L. Than, Gram staining was performed for the microscopic test of bacteria. Coloring gram aims to see the type of gram positive or negative bacteria. gram bacteria positive has a thick peptidoglycan which will bind to the main dye crystal violet so that the bacteria will appear purple. While the bacteria gram-negative does not have peptidoglycan so it will bind to the comparison dye namely safranin so that the bacteria will appear red. Once done Gram staining was observed under a microscope with 100x magnification. Bacteria which have been stained in preparation A have the form of bacilli or stems that are separated from each other. Whereas in preparation B it has a round shape piled up. Furthermore, biochemical tests were carried out to determine the bacterial species.

Biochemical test is a treatment performed to identify and determine a pure culture isolated by its physiological characteristics (Rahayu & Gumilar, 2017). Biochemical tests carried out in this study including sugar test, TSIA, MR-VP, Indole, Simmon Citrate and Urease. A confectionery test is used to ferment each sugar form acids. The candy medium was separated into five different tubes and the medium used is each sugar with a concentration of 1% in peptone then added phenol red indicator. On the test glucose, maltose and sucrose obtained a positive result which was marked by a change in the media to yellow color, meaning that the bacteria can ferment sugar.

The TSIA test contains three kinds of sugar namely glucose, lactose and sucrose which aims to determine the ability of a bacterium to ferment sugar to produce acid or gas (Touelle, 2014). In this test it was found red color results on the slant and butt because the bacteria are alkaline. The results obtained in the Methyl Red (MR) test were positive, which means, These bacteria produce mixed acids from the glucose fermentation process contained in methyl red. Meanwhile, in the VP test, negative results were obtained indicating that there was no change in the color of the medium to red after addition of 5% a-naphthol and 40% KOH.

The indole test aims to identify the ability of bacteria produce tryptophanase enzymes so that bacteria are able to oxidize acidstryptophan to form indole. The presence of indole can be detected by the addition of kovacs reagent (Rahmadian et al, 2018). In this study obtained negative indole test results, which means that these bacteria do not form indole from tryptophan as a carbon source.

Simmon citrate test aims to detect the ability of a organisms to utilize citrate as the sole source of carbon and energy. The citrate medium contains the indicator brom thymol

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blue which, if bacteria using citrate as a carbon source, then the media will turn into blue (Touelle, 2014). But the results obtained are negative, the media is not turns blue, meaning that these bacteria do not use citrate as the only carbon source.

From the biochemical tests that have been carried out based on the properties of the bacteria, characteristics and identification tend to refer to bacteria by genus Desulfobacter. Desulfobacter bacteria are bacteria that have ability to oxidize acetate to CO2, does not form spores on growth and able to grow using alcohol, fatty acids high molecular weight and benzoate.

Conclusions

The highest decrease occurred in the addition of a 3% concentration of zeolite with decreased effectiveness of 86.772%. Based on the characteristics of the bacteria in the biochemical test, namely the carbohydrate test (maltose, glucose, and sucrose) the results were positive, the lactose test was negative, the TSI test was red/red, the indole test was negative, the MR test was positive, and the Simmon Citrate test was negative. Bacilli-shaped gram-negative bacteria refer to the genus *Desulfobacter.*

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