IDENTIFICATION OF BACTERIA FROM POST TIN MINING POND AND THEIR ABILITY TO FORM BIOFILMS AT DIFFERENT PH

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Abstract

A number of water quality indicators in the tin post-mined pond of a certain age indicate that the water condition is acidic, low dissolved oxygen content, low cation exchange capacity, and polluted by heavy metals. Restoration of the water quality of post-tin mining pond can use microorganisms as bioremediation agents. Microorganisms live by forming microbial community structures called biofilms. The aims of this study was to identify and find out the optimal pH of biofilm formation biofilm-forming bacteria from post-tin mining pond. The steps of research method was the isolation of bacteria by the spread plate technique, the biofilm formation test by the crystal violet technique, and the identification of bacteria macroscopically, microscopically, and physiologically. The isolation results showed that the highest bacterial density was at station 3 with a total of 8.1x10³ cfu/ml. The results of the visualization of biofilm formation find out the A8 isolate at pH 5 with the most concentrated staining, while the highest Optical Density (OD) value for each pH was 0.11245 (pH 3) for A8 bacteria, 0.1901 (pH 5) for I1 bacteria and 0.1901 (pH 5) for A8 bacteria of 0.08945 (pH 7). There were 14 isolated bacterial belonging to the Genus Branhamella, Bacteroides, Aeromonas, Bacillus and Clostridium 08945 (pH 7). There were 14 isolated bacterial belonging to the Genus Branhamella, Bacteroides, Aeromonas, Bacillus and Clostridium.

Keywords: Biofilms; Bioremediation; Optical density; Pond

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INTRODUCTION

Continuous exploration of mineral resources in Bangka Belitung in the form of tin (Sn) has an impact on environmental damage. One of the consequences of these exploration activities is the formation of excavated soil in the form of a lake which is well-known as Kolong (pond) in Bangka Belitung community (Triswiyana et al., 2019). Based on Suryadin (2011), kolong (pond) is interpreted as stagnant water caused by tin sand excavation carried out by the miners. The formed of pond started from the excavation of tin sand, then was filled with water so that the volume of water increased and filled the pond made by the miners.

After tin mining the pond has a lot of potential water to be utilized, but it has not been carried out optimally, both for primary needs and secondary activities such as for agriculture, fisheries, and animal husbandry (Suryadin, 2011; Prasetiyono, 2015). The lack of utilization is due to the certain age, the water quality shows poor conditions, namely the acid pond water reaches a pH < 4.0 which is part of Acid Mining Water (AAT), low DO levels, low value of cation exchange capacity, and contaminated by heavy metals such as Cr, Cu, and Pb (Kurniawan, 2017; Kurniawan, 2019; Kurniawan & Mustikasari, 2019).

The effort to restore the pond water condition after tin mining with a bioremediation approach is very potential alternative because of the low cost. In an effort to explore potential microbes for bioremediation, understanding the characteristics of microbes is very important, one of them is the ability to form biofilms in water. The community structure of microorganisms known as biofilms is very important in supporting microbial life in aquatic ecosystems. A collection of microbial cells that are irreversibly attached to a surface and covered by a polysaccharide matrix is the definition of a biofilm (Donlan & Costerton, 2002). The biofilm matrix has the potential to be a biological agent that can be used to reduce water pollutants in the form of heavy metals such as Cr (VI), Cu²⁺ and Pb²⁺ (Kurniawan et al., 2018; Kurniawan, 2019).

Several studies have also shown that a number of bacteria that can play a role in forming biofilms are Vibrio alginolyticus, V. natriegens, Bacillus pumilus, Pseudomonas sp., Staphylococcus sp. Esterichia coli, and Alphaproteobacteria denitromonas. These microorganisms have the ability to improve water quality through their metabolism (Merina et al., 2011; Gao et al., 2012; Julistiono et al., 2018).

Biofilms are composed of diverse microorganisms on surfaces which are in unfavorable conditions can help protect these bacteria. Deb et al., (2014) explained the basic steps of biofilm formation,
namely: deposition and film formation, microorganisms attached to the film sheet, bacterial growth and colonization and finally a biofilm is formed. The presence or absence of nutrients in the bacterial growth medium, the growth stage of bacterial cells and the pH of the contact surface are factors that affect the attachment of these biofilm-forming microorganisms (Purbowati, 2016). One of these factors is the pH value (acidity) which is the main factor affecting diversity, community structure, abundance, and composition of microorganisms (Kamika & Momba, 2013).

Studies on biofilms and the factors that affect their growth in post-tin mining waters has not been carried out and published. This study is an important source of information for elaborating on the presence of potential microorganisms that can help accelerate the process of improving water quality and its growth factors. So the objectives of this study were to determine the abundance of bacteria, to determine the effect of different pH and to identify bacteria that have the potential to form biofilms from different plant substrates under tin mining.

MATERIALS AND METHODS

This research was conducted from November 2020 to April 2021, in post-tin mining pond Rebo Village, Sungailiat District, Bangka Regency, Bangka Belitung Islands (Figure 1).

Figure 1. Map of research location and 3 sampling points in post mining pond, Rebo Village

The research tools used include sample bottle, centrifuge, erlenmeyer, autoclave, stirring rod, beaker glass, measuring cup, GPS (Global Positioning
System), incubator, inoculum needle, measuring flask, Laminar Air Flow Cabinet (LAFC), glass preparation, microplate reader, microscope, microwave, micropipette and tip size 1000 L, 100 L, 2-20 L, 2-200 L, orbital shaker, petri dish, pH meter, tweezers, dropper pipette, test tube with rack, thermometer, analytical balance, spatula and vortex. The main object in this research project was aquatic plants from tin pond in Rebo Village, Sungailiat District, Bangka Regency, Bangka Belitung Islands. The ingredients used include agar, distilled water, alcohol, glycerol, physiological salt, crystal violet, and NB (Nutrient Broth) media.

**Measurement of physical and chemical properties of water**

Measurement of physical and chemical properties was carried out as supporting data in the study and to determine water quality in Kolong Timah, Rebo Village, Sungailiat District, Bangka Regency, Bangka Belitung Islands. The water sample was measured for temperature and pH.

**Sampling**

Samples were taken from post-tin mining water in the form of aquatic plants as much as the size of sterile plastic. Previously, prepared sterile plastic as a sample container. Samples were taken using tweezers by clamping and shaking in order to dry. Then the sample was put in sterile plastic and labeled.

**Dilution of Bacterial Isolation Samples Using Pour Plate Technique**

The dilution process was carried out from pond water plants to a dilution of 10-6. Aquatic plants that had been taken from the location were placed in sterile petri dishes containing sterile under water. Samples of aquatic plants were rubbed and 1 ml of each sample was taken, put into an Erlenmeyer flask containing 9 ml of sterile bottom water, homogenized and obtained a dilution of 10-1. Then from the 10-1 dilution, 1 ml of water was taken using a micropipette, which was put into 9 ml of sterile distilled water and obtained a 10-2 dilution. Dilution to obtain a dilution of 10-3, 10-4 and 10-5 (Azizah et al., 2017). The results of the dilution in the test tube were isolated into NA media using the pour plate method and repeated 3 times.

**Biofilm Forming Test**

The activity of forming bacterial biofilms from bacterial colonies used a purple crystal technique using a microplate (Julistiono et al., 2018) with modifications. Bacterial isolates were grown in liquid media (Nutrient Broth) with pH 3, 5 and 7 for 48 hours at 37°C. Each colony was taken with a sterile loop needle and inoculated
into two parallel wells from a microplate containing 200 l of NB under water and incubated for 7 days at room temperature with a shaker. After the incubation period, the wells were rinsed with physiological saline and fixed with 2 L 99.99% ethanol for 10 min. The attached bacterial material was then stained by adding 2 L of crystal violet (2%) for 20 minutes. The plates were gently rinsed with tap water and the biomass was installed as required using a microplate reader at 450 nm.

**Bacterial Biochemical Staining and Test**

Bacterial cell characterization was carried out by observing the morphology and arrangement of cells and Gram staining. Bacterial colonies were taken with a loop and smeared on the surface of a slide that had been moistened with sterile water and spread evenly before being heated on a bunsen. Afterwards, it was dripped with purple crystal solution and allowed to stand for one minute, then washed with distilled water and dried. Then, it was dripped with iodine solution and left for 2 minutes, washed with distilled water and dried. Then it was dripped with 95% ethanol solution for 30 seconds, washed with distilled water and dried. Afterwards, it was dripped with a solution of safranin and allowed to stand for 30 seconds, then washed with distilled water and dried (Nurhidayati et al., 2015). Bacterial isolates capable of forming biofilms were subjected to other tests for bacterial identification purposes such as biochemical tests. The biochemical tests carried out included motility test, catalase test, oxidase test, OF test, indole test, citrate test and MR-VP test (Widodo & Kusharyati, 2015).

**Data analysis**

Data were analyzed descriptively and inferentially. Species data, biophile absorbance values were displayed in tabular or graphic form. Different tests were carried out to determine the best pH for biofilm formation.

**RESULTS AND DISCUSSION**

**Water Parameter Measurement**

The difference in the presence of aquatic plants in the post-mining area is the reason for determining the sampling location. The sampling location points consisted of three points, namely Point 1, Point 2 and Point 3. The three research points were chosen as research locations because they had different dominant plant species that were used as sampling media and bacterial isolates. At each point in the research location, the temperature and pH of the water as well as the types of aquatic plants were measured (Table 1 and Figure 2).
Table 1. Results of measuring physical, chemical and biological properties

| Location | Temperature (°C) | pH     | Plant type               |
|----------|------------------|--------|--------------------------|
| 1 point  | 30               | 7.24   | Kumpai (Huperzia sp.)    |
| Point 2  | 32               | 6.79   | Lotus (Nymphaea sp.)     |
| 3 point  | 30               | 6.97   | Water reeds (Typa angustifolia) |

Figure 2. Types of aquatic plants, namely (a) Kumpai (Huperzia sp.) at point 1, (b) Lotus (Nymphaea sp.) at point 2, and (c) Water reeds (Typa angustifolia) at point 3 (Personal documentation)

The parameters measured in this study were physical (water temperature), chemical (water pH) and biological parameters (plant species). The results of the measurement of water temperature were Point 2 had the highest temperature of 32°C, then Point 1 and Point 3 had the same temperature, which was 30°C. This water temperature measurement was carried out directly during the day. Point 2 has a higher temperature because there are fewer plants at that point than other points so that direct sunlight penetrates the body of water. It is in accordance with Boyd (2015) which states that the temperature of a body of water is influenced by season, latitude, altitude above sea level (attitude), time of day, air circulation, cloud cover and flow and depth of water body.

The acidity (pH) of the water measured the tin of post-tin mining area in this study was in the neutral pH range of 6.79 – 7.24 (Table 1). Whereas the main characteristic of the post-mining site is low or acidic pH condition. Acidic pH conditions involve oxidation processes and complex chemical reactions to produce H+, sulfate (SO42-), Mn3+ and other ions. More and more of these ions were formed in an environment causing acidity to increase and pH to be low (Nurofiq et al., 2016). In addition, Sari et al., (2017) explained that there was a change in the soil layer due to mining activities, namely the underground layer to the ground surface. Rocks that contain heavy metals can have the opportunity to produce acid mine drainage if they are in direct contact with water/air, causing a low pH. The change in the acidity value of the water became neutral due to the age of the post-tin mining water of more than 15 years.
**Bacterial Density**

Bacteria were isolated from plants at each point of the study site. The calculation of bacterial density using the calculation of the Total Plate Number (ALT) in units of cfu/ml of bacteria grown on the media. The highest population density in this study was found in alang - alang air plant (*Typa angustifolia*) which was $8.1 \times 10^3$ cfu/ml (Point 3) (Figure 3).

![Figure 3. Average number of bacterial density](image)

The results of the calculation of the average number of bacterial density there are differences at points 1, 2 and 3 respectively, namely $1.6 \times 10^3$ cfu/ml; $1.9 \times 10^3$ cfu/ml, and $8.1 \times 10^3$ cfu/ml (Figure 3). Point 1 and Point 2 have an average bacterial density which is less than Point 3, which is $1.6 \times 10^3$ cfu/ml and $1.9 \times 10^3$ cfu/ml. Since at Points 1 and 2 there are fewer plants. Plants directly or indirectly have an impact on the waters that affect the growth of bacteria. Mudatsir (2007) states that microbial growth in water is influenced by several factors, including abiotic factors (conductivity of water temperature, turbidity, current, light, salinity, Biochemical Oxygen Demand, dissolved oxygen levels or Chemical Oxygen Demand and pH),

In addition, Point 1 and Point 2 with less plant density will have an impact on limited food for microbes. Microbes that successfully adapt to their environment will more quickly get nutrients for their food sources. Microbes that can interact well with their environment were microbes that get nutrients for food sources more quickly. Some microbes in water have different growth patterns, namely the ability to divide cells faster than others. The results of the metabolism of microbes in the water can inhibit the growth of its competitors (Mudatsir, 2007).
**Biofilm-forming Bacteria**

The activity of biofilm-forming bacteria from bacterial colonies was observed qualitatively using the crystal violet technique on a microplate. Microplate containing bacterial isolates after washing will produce a thick color if it is a biofilm-forming bacteria. The result of visualization of bacterial isolate staining shows a crystal violet color as shown in Figure 4.

Visualization results of bacterial isolate biofilm staining test (Figure 4) was carried out with two repetitions at different pH media on microplate. The pH values were pH 3 and pH 5 and at pH 7. The staining results showed that the most concentrated isolate was isolate A8 at pH 5 (red circle), while the least concentrated isolate was isolate C1 at pH 3 (yellow circle).

Bacteria that can form biofilms and cannot form biofilms can be seen in Figure 5. Adhesion to the bottom of the plate becomes the basis for determining whether or not a bacterial biofilm was formed. In Figure 5 on the left, it was suspected that the bacteria can form biofilms which were marked with arrows, namely the presence of bacterial deposits on the bottom of the plate. The bacterial precipitate will bind to the crystal violet used in the washing process as of the bottom of the plate will look a more concentrated color. On the other hand, Figure 5 on the right represents bacteria that are thought to be unable to form biofilms or if present in small quantities. Seen from the color of crystal violet which was not concentrated and the absence of bacterial deposits.
To be able to ascertain the ability of bacteria to form biofilms not only through visualization, but measurement using a microplate reader is needed to ensure quantitatively biofilm-forming bacteria. The measurements were carried out with a wavelength of 450 nm presented in the form of Optical Dencity (OD) values. Of the three pH treatments (3, 5 and 7) the highest average yield was on bacteria with isolate code I1. The highest Optical Dencity (OD) value for each pH was 0.11245 (pH 3) for bacterial isolate A8 (Fig. 6), I1 bacterial isolate was 0.1901 (pH 5) (Fig. 6) and A8 was 0.08945 (pH 7) (Fig. 6).

![Figure 6. Absorbance values for biofilm-forming bacterial isolates at pH 3, 5 and 7](image)

It was quantitatively carried out by measuring the microplate on a microplate reader using a wavelength of 450 nm. The bacterial isolates of the test were varied with several pHs, namely pH 3, pH 5 and pH 7. The results showed that the bacterial isolates at pH 7 could grow at pH 5 and pH 3. It was suspected that before reaching pH 7 in this old age of post-tin mining, bacteria had already existed when the pond was
still young and lower. Therefore, it can be stated that these bacteria acted as pioneer organisms in the post-tin mine pond. It is same as Purbowati (2016) statement that biofilm-forming microbes have the advantage that microbial cells are able to survive in limited food conditions. Besides that, Watrick & Kolter (2000) suggested that cells in the form of biofilms have a better ability to survive in unfavorable conditions.

Based on the graph, the highest absorbance values for biofilm formation at pH 3 were isolate A8, pH 5 at isolate I1 and pH 7 at isolate A8 (Figure 6). Therefore it means that the pH can affect the growth of bacteria. There are two ways pH affects bacterial growth, namely through the function of the enzymatic system in bacterial cells and the formation of energy in the cell. Then on the graph it can also be seen that the absorbance value of biofilm-forming bacteria is evenly distributed, namely at pH 7. The changing pH directly affects the structure of enzymes and other proteins in cells, because intracellular physiological activity is always in a close neutral condition. Therefore it is needed to be adjustments by bacterial cells if the environmental condition outside the cell is too acidic or too alkaline. In addition, bacteria required longer growth time at low pH compared to close neutral pH. This is because the formation of energy in cells is also influenced by an acidic environment. pH with conditions that are too acidic or too alkaline will slow down the formation of ATP, while at neutral pH conditions the formation of ATP will run faster (Rieger et al., 2021).

Based on the identification results (Table 2, 3 and 4) of the five genera obtained mostly have gram negative compared to gram positive with a ratio of 10: 4. It affected the formation of biofilms because gram-positive and negative bacteria used different signaling molecules (autoinducers) in quorum sensing.

![Figure 7. Bacterial quorum sensing system on gram negative and positive (Kurniawan & Astriani, 2020)](image-url)
The signal molecules (autoinducers) used by gram-negative bacteria were generally acyl homoserine lactones (AHLs-AI-1). As for the group of gram-positive bacteria, namely autoinducer peptides (AIPs). In addition, in interspecies communication involving both groups of bacteria, namely signaling molecules from furanone such as furanosyl borate diester with the characteristics of a combination of AHLs and AIPs called autoinducer-2 (AI-2). Identification of bacteria in this study obtained 5 genera of bacteria with 3 phyla namely Bacteroides, Firmicutes and Proteobacteria.In an aquatic ecosystem, microbial communities have strong or weak interactions between certain bacterial phyla as shown in Figure 7 (Kurniawan & Astriani, 2020).

Figure 8. Schematic of interaction between phyla pond post-tin mining with different ages. A strong–very strong correlation is indicated by a thick line, while the dotted line indicates a weaker correlation (Kurniawan & Astriani, 2020)

The phyla found at each point in this study were Proteobacteria at Point 1, Bacteroidetes and Proteobacteria at Point 2 and Bacteroidetes, Proteobacteria and Firmicutes at Point 3. Based on Figure 8, interactions between phyla in this study occurred at Points 2 and 3 with a strong interaction between Bacteroides and Firmicutes and weak interactions between Firmicutes and Proteobacteria. Kurniawan & Astriani (2020) also stated that interactions that arose from quorum sensing microbes, especially bacteria, can have a positive impact and mutually support benefits for other individuals in the form of mutualism, commensalism and
synergism interactions. These interactions can also be negative which cancel each other out (predation), competition or amensalism (repression).

**Identification of bacterial isolates**

Bacterial isolates growing at the three research points were identified manually using Cowan and Steel’s Manual for the Identification of Medical Bacteria Third Edition and Bergey’s Manual of Determinative Bacteriology Ninth Edition. Identification was done by observing the macroscopic, microscopic and physiological characteristics of each bacterial isolate (Tables 2, 3 and 4). The results of bacterial identification from Point 1 (3 isolates) showed that the three bacteria were *Branhanella* sp. (Table 2). It is also explained in Table 2 that in the *Branhamella* genus there were differences in macroscopic characteristics with the same microscopic and physiological characteristics.

| Characteristics          | Isolate code |
|--------------------------|--------------|
|                         | C1           | C2           | C3           |
| Macroscopic              |              |              |              |
| Size                     | Large        | Small        | Large        |
| Color                    | White        | White bone   | White        |
| Optical characteristics   | Opaque       | Opaque       | Opaque       |
| Form                     | Irregular    | Irregular    | Irregular    |
| Elevation                | Flat         | Raised       | Flat         |
| Surface                  | Rough        | Wrinkled     | Rough        |
| Margin                   | Undulate     | Lobate       | Lobate       |
| Microscopic              |              |              |              |
| gram                     | -            | -            | -            |
| Cell shape               | Coccus       | Coccus       | Coccus       |
| Physiological            |              |              |              |
| Motility                 | -            | -            | -            |
| Catalase                 | +            | +            | +            |
| Oxidase                  | +            | +            | +            |
| OF                       | -            | -            | -            |
| Indole                   | -            | -            | -            |
| Citrate                  | -            | -            | -            |
| MR                       | -            | -            | -            |
| VP                       | -            | -            | -            |
| Genus                    | *Branhamella*|              |              |

The results of bacterial isolation at Point 2 obtained 3 bacterial isolates with different characteristics. Then the identification of the three isolates obtained results in the form of Genus *Bacteroides* and *Aeromonas* (Table 3).
Table 3. Results of identification of bacterial isolates at point 2

| Characteristics          | Isolate code |   |   |
|--------------------------|--------------|---|---|
|                          | I1           | I2 | I3 |
| **Macroscopic**          |              |   |   |
| - Size                   | Small        | Moderate | Small |
| - Color                  | White bone   | white-yellow | White |
| - Optical characteristics| Opaque       | Opaque | transparent |
| - Form                   | circular     | Circular | circular |
| - Elevation              | Flat         | Convex | Raised |
| - Surface                | Smooth shiny | Smooth shiny | Smooth shiny |
| - Margin                 | Entire       | Entire | Entire |
| **Microscopic**          |              |   |   |
| - gram                   | -            | -  | -  |
| - Cell shape             | Coccus       | Coccus | Coccus |
| **Physiological**        |              |   |   |
| - Motility               | -            | +  | +  |
| - Catalase               | -            | +  | -  |
| - Oxidase                | -            | +  | +  |
| - OF                     | F            | F  | -  |
| - Indole                 | -            | -  | -  |
| - Citrate                | +            | +  | +  |
| - MR                     | -            | -  | -  |
| - VP                     | -            | -  | -  |
| **Genus**                |              |   |   |
|                          | Bacteroides  | Aeromonas | Aeromonas |

Furthermore, at Point 3, more isolates were obtained, namely 8 bacterial isolates. The identification of the eight bacterial isolates showed that there was a genus *Aeromonas* (2), *Bacillus* (2), *Bacteroides* (2) and *Clostridium* (2) (Table 4).

Table 4. Results of identification of bacterial isolates at point 3

| Characteristics          | Isolate code |   |   |   |   |   |   |   |   |
|--------------------------|--------------|---|---|---|---|---|---|---|---|
|                          | A1           | A2 | A3 | A4 | A5 | A6 | A7 | A8 |   |
| **Macroscopic**          |              |   |   |   |   |   |   |   |   |
| - Size                   | Moderate     | Moderate | Moderate | Moderate | Moderate | Moderate | Moderate | Moderate | Moderate |
| - Color                  | Yellow-orange | Light | white-brown | White | Yellow | White | White | White | White |
| - Optical characteristics| Opaque       | Opaque | Opaque | Opaque | Opaque | Opaque | Opaque | Translucent |   |
| - Form                   | circular     | circular | circular | circular | circular | circular | circular | Filamentous | Irregular |
| - Elevation              | Flat         | Raised | Flat | Raised | Raised | Raised | Smooth shiny | Smooth shiny | Umbonate |
| - Surface                | Smooth shiny | Smooth shiny | Smooth shiny | Smooth shiny | Smooth shiny | Smooth shiny | Smooth shiny | Smooth shiny | Lobate |
| - Margin                 | Entire       | Entire | Entire | Entire | Entire | Entire | Entire | Entire |   |
| **Microscopic**          |              |   |   |   |   |   |   |   |   |
| - gram                   | -            | -  | +  | -  | -  | +  | +  | +  | +  |
| - Cell shape             | Coccus       | Coccobacilli | Basil | Coccus | Coccus | Coccus | Coccus | Coccus | Coccobacilli |
| **Physiological**        |              |   |   |   |   |   |   |   |   |
| - Motility               | +            | +  | +  | -  | -  | +  | +  | +  | +  |
| - Catalase               | +            | +  | +  | -  | +  | +  | -  | +  | -  |
| - Oxidase                | +            | +  | +  | -  | -  | -  | +  | -  | -  |
| - OF                     | F            | F  | F  | -  | -  | -  | F  | -  | -  |
| - Indole                 | -            | -  | -  | -  | -  | -  | -  | -  | -  |
| - Citrate                | +            | -  | +  | -  | -  | -  | -  | -  | -  |
| - MR                     | +            | -  | -  | -  | -  | -  | -  | -  | -  |
| - VP                     | -            | -  | -  | -  | -  | -  | -  | -  | -  |
| **Genus**                |              |   |   |   |   |   |   |   |   |
|                          | Aeromonas    | Aeromonas | Bacillus | Bacteroides | Bacteroides | Clostridium. | Bacillus | Clostridium |   |
The results of the identification in Table 2, Table 3 and Table 4 obtained the results of point 1 with 3 bacterial isolates of the genus *Branhamella* (Table 2), Point 2 obtained 3 bacterial isolates in the form of Genus *Bacteroides* and *Aeromonas* (Table 3) and Point 3 are Genus *Aeromonas* (2), *Bacillus* (2), *Bacteroides* (2) and *Clostridium* (2) (Table 4). The bacteria of this genus were found because they were bacteria that were tolerant to metal polluted environments. Yulistia *et al.*, (2015) explained that the isolates of coal ash waste obtained were 3 genera of bacteria, including *Aeromonas* and *Branhamella* after the identification and calculation of the bacterial diversity index were carried out. It means the five genera of bacteria in this study were *Branhamella, Bacteroides, Aeromonas, Bacillus* and *Clostridium* have the ability to survive in a polluted environment which in this study comes from tin mining and the content of the post-tin mining pond can be used as a nutrient for growth.

**CONCLUSION**

The highest bacterial abundance found in this study was at Point 3 (8.1x10^3 cfu/ml) with aquatic plant substrates in the form of water reed (*Typa angustifolia*), the optimum pH of biofilm-forming bacteria from pond tin mining was pH 7 because it had a thick color on visualization. microplate and has a more even absorbance value. Bacterial isolates that have the potential to form biofilms from different plant substrates in post-tin-mining waters were identified as Genus *Branhamella, Bacteroides, Aeromonas, Bacillus* and *Clostridium*. The genus *Clostridium* showed that the best biofilm-forming potential with the highest (biofilm parameters) was at pH 7.

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