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# Biological Absorption of Pb2+ Metal by *Escherichia coli* Bacteria

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***Abstract***— *The biological absorption of Pb2+ metal by Escherichia coli bacteria has been conducted. This study aims to determine the ability of Escherichia coli bacteria to absorb Pb2+ metal based on variations in Pb2+ exposure concentrations (0, 3, 6, 9, 12, 15 ppm) over different exposure times (days) and to identify the optimal concentration and time required for Escherichia coli in the biological absorption of Pb2+ metal. The maximum wavelength of the standard Pb2+ solution is 520 nm. Based on the test results, Escherichia coli was able to absorb Pb2+ at varying exposure concentrations (3, 6, 9, 12, 15 ppm) with biosorption percentages of 49.118%, 23.985%, 14.500%, 10.150%, and 7.808%, respectively. The optimal concentration and time required for Escherichia coli to absorb Pb2+ occurred on the 3rd day with a Pb2+ exposure concentration of 3 ppm.*

***Keywords***— *Biosorption, Pb2+ metal, Escherichia coli*.

## I. INTRODUCTION

The pulp and paper industry is one of the most significant forest products industries. There are almost no human life activities that do not use this industrial commodity, ranging from life activities in households, offices, industries, learning, trade and so on. Indonesia as a country that still has a very large forest, has the potential to compete in the world industry in the field of pulp and paper industry because of the availability of forests, as the main source of raw materials, which is the main driver for the development of this industry. In addition to having a very large forest and tropical air that encourages plants to develop faster, Indonesia also has alternative sources of raw materials, such as agricultural waste.[1]

The paper industry uses organic and inorganic materials in its production process. Heavy metals Pb and other compounds are listed in dyes used in the pulp and paper industries. This was proven by the author through a preliminary test where heavy metal Pb was found in the liquid waste.[2]

Heavy metals are a natural factor that can be found in the erosion of mining rocks, volcanic, marine and so on. Heavy metals in waters can endanger the life of organisms directly or indirectly to human health.[3] Heavy metals are metals with high toxicity values and can be at risk if they enter the body beyond the threshold. Heavy metals can also be a risk caused by the bioaccumulation process. Bioaccumulation refers to a rise in the concentration of chemical substances within the bodies of living organisms in accordance with the food chain. Heavy metals can have a negative impact on the lives of living things, such as disrupting chemical responses, thus limiting the absorption of essential nutrients.[4]

Pb metal is a non-essential and toxic metal that is not needed by living organisms that can cause accumulation in the tissues of the organism so that it can interfere with the metabolic process.[5] Pb metal has properties that cannot be regulated by aquatic organisms so that Pb metal will continue to accumulate in the organism.[5]

Adsorption is a substance (molecule or ion) on the surface of an adsorbent. The adsorption mechanism is where the molecules present in the adsorbate, are bound to the surface of the adsorbent substance. The adsorption process can be established if the adhesion form between the adsorbate molecule and the adsorbent molecule is greater than the cohesion form of each molecule.[6] Heavy metal binding to biosorbents can be established by the method of exchange of heavy metal ions that have a positive charge in relation to the surface of the cell chamber that has a negative charge in extracellular polymers, such as proteins and polysaccharides as the source of functional groups in the process of binding heavy metal ions.[7]

Biosorption is the ability of biological materials to accumulate heavy metals through metabolic media or psycho-chemical pathways. The biosorption process can occur due to the presence of biological materials or biosorbents and the presence of solutions that contain heavy metals with high affinity so that they are easy to bind to biosorbents. The mechanism of absorption of heavy metals by microorganisms by means of an ion exchange process similar to ion exchange in resins. The ion exchange mechanism can be formulated with the following: A2+ (B-biomass) -> B2+ + (A-biomass).[8] The Gospel of Jesus

Based on the description above, this study aims to determine the ability and optimal conditions of biological absorption of Pb2+ metal by *E. coli* bacteria using variations in metal concentrations and changes in measurement time. UV-Vis spectrophotometers are used to determine the concentration of Pb2+ metals after biological absorption.

## II. METHOD

### *A. Tools and materials*

#### *2.1.1. Tool*

The equipment used in this study is a Visible spectrofometer instrument *(Rayleight Vis* 7220G), *an eppendorf micropipette*, an analytical balance, a glass cup, an ose needle, *an autoclave*,

a spatula, a stirring rod, a spray bottle, tweezers, bunsen, sterile swab cotton, an incubator, *laminar air flow, a hotplate with magnetic stirer* and a weighing bottle.

*2.1.2 Material*

The material used in the study is Pb(NO solids)$_3$)$_2$solution *Alizarin Red S* (ARS) 0.01 M, phosphate buffer solution, KH solution$_{2PO4}$ 0,1 M, larutan NaOH 0,1 M, NaCl, Tripton, *yeast extract*, aquades and bacteria *E. coli*.

### B. Research Procedure

*1) Determination of OD Value*

A starter medium of E. coli *bacteria* as much as 1 ml is taken from the starter solution and put into a cuvette. Absorbance values were measured in the range of 610 nm on the vis spectrophotometer (measurements were made at time ranges (0 hours, 1 hour, 6 hours and 24 hours).[9]

*2) Determination of Maximum Wavelength*

A standard Pb2+ solution with a concentration of 1.5 ppm of 10 mL, then 1 mL *of Aliizarin Red S* 0.01 M was added to each solution, 0.15 mL of NaOH 0.1 M and 1 mL of pH buffer solution 7. A standard solution that has been mixed with *Aliizarin Red S* is left for ± 30 minutes. Its absorbance was measured using a vis spectrophotometer at a wavelength of 400-600 nm.[10 The Gospel of Jesus Christ]

*3) Creation of Pb2+ Standard Curves*

A standard Pb2+ solution of 10 ppm is put into a 50 ml measuring flask with variations in concentrations (0.5; 1.0; 1.5; 2.0 and 2.5) ppm. Each solution with the concentration variation was taken as much as 10 mL, transferred to Erlenmayer 50 mL and added 1 mL *of Alizarin Red S* 0.01 M and NaOH of 0.15 mL. Then, 1 mL of buffer solution is added according to the optimum pH obtained. The absorbance was measured using a visible spectrophotometer at the maximum wavelength.[10 The Gospel of Jesus Christ]

*4) Biosorption of Metal Pb2+ by E. coli Bacteria*

Erlenmeyer pumpkin 250 mL contains 6 farmed Luria medium added 0 mL each; 7.5 mL; 15 mL; 22.5 mL; 30 mL; and 37.5 mL of standard Pb2+ 100 ppm solution, added Luria farmed and homogenized. Each medium was inoculated with a starter of 1 mL of E. coli bacteria. Medium of E. coli bacteria is introduced into the incubator at a temperature of 37 °C

### C. Data Analysis

The data analysis technique was carried out by making a standard curve between the concentration (ppm) on the x-axis and the absorbance on the y-axis using a series of standard solutions Pb(II) with concentrations of (0.5; ,1.0; 1.5; 1.5; 2.0; and 2.5) ppm so that the equation was obtained:

$$y=ax+b$$

Information:
a = Slope
b = Intercept
y = Absorbansi
x = Concentration (ppm)

The level of bisorption is determined by the difference between the exposure concentration and the measured concentration (residue) and expressed as a percentage by the equation:

$$\%\ Biosorpsi = \frac{Xpemaparan - Ysisa}{Xpemaparan}\ x\ 100\%$$

Information:
% Biosorption = Biosorption rate
Xexposure = Exposure Concentration
Ysisa = Residual Concentration

## III. Results And Discussion

### A. Determination of OD Value

Determination *of Optical Density* (OD), measured the absorbance of the *starter* medium using a visible spectrophotometer at a wavelength of 610 nm. The absorbance value reflects the level of turbidity present in the medium during the growth of E. coli bacteria. The higher the absorbance value obtained, the higher the turbidity level of the growth medium, which indicates that more bacteria are growing in the medium.[12] The *optical density* values obtained from the bacterial *starter* media are shown in table 3.1

TABLE 3.1. Optical Density *Values*

| **Treatment** | **Nilai *Optical Density* (OD)** |
|---|---|
| Before incubation | 0,381 |
| After incubation | 0,942 |

Based on the above data, the absorbance value obtained in the starter media after incubation is greater than before incubation, this shows a turbidity variability which indicates that the turbidity of the media after incubation is greater than the media before incubation. It can be concluded that *E. coli bacteria* in the starter media have successfully grown.

### B. Determination of Maximum Wavelength

The determination of the maximum wavelength in Pb2+ metal is carried out by measuring the absorbance value of the standard Pb2+ solution of 1.5 ppm that has been complexed, then measured using a spectrophotometer and obtaining a maximum wavelength of 520 nm. A graph of the maximum wavelength determination results can be seen in figure 3.1

Based on the data above, the maximum wavelength in the Pb2+ metal obtained is 520 nm. This is because at a wavelength of 520 nm the Pb2+ Complex solution has the maximum absorption.

### C. Creation of Pb2+ Calibration Curves

In making the standard curve of Pb2+ ions, it is done by making variations in the concentration of the standard solution of Pb2+ ions that have been determined with the absorbance value at the maximum wavelength obtained. The graph of the results of the standard measurement of the Pb2+ ion curve obtained can be seen in figure 3.2

In Figure 3.2, the result is that the absorbent value is getting higher along with the increase in the concentration of Pb2+ ions which means that there is a positive relationship between the absorbance value and the concentration in the form of the equation y = 0.2176x – 0.005, where y expresses the absorbance value and x is the concentration (ppm) and the regression coefficient (R2) value is 0.995. The value of the determination coefficient (R) which is close to 1 states that the calibration curve results obtained have good linearity and have Strong

correlation between absorbance value and concentration (ppm).[13 The Gospel of Jesus Christ]

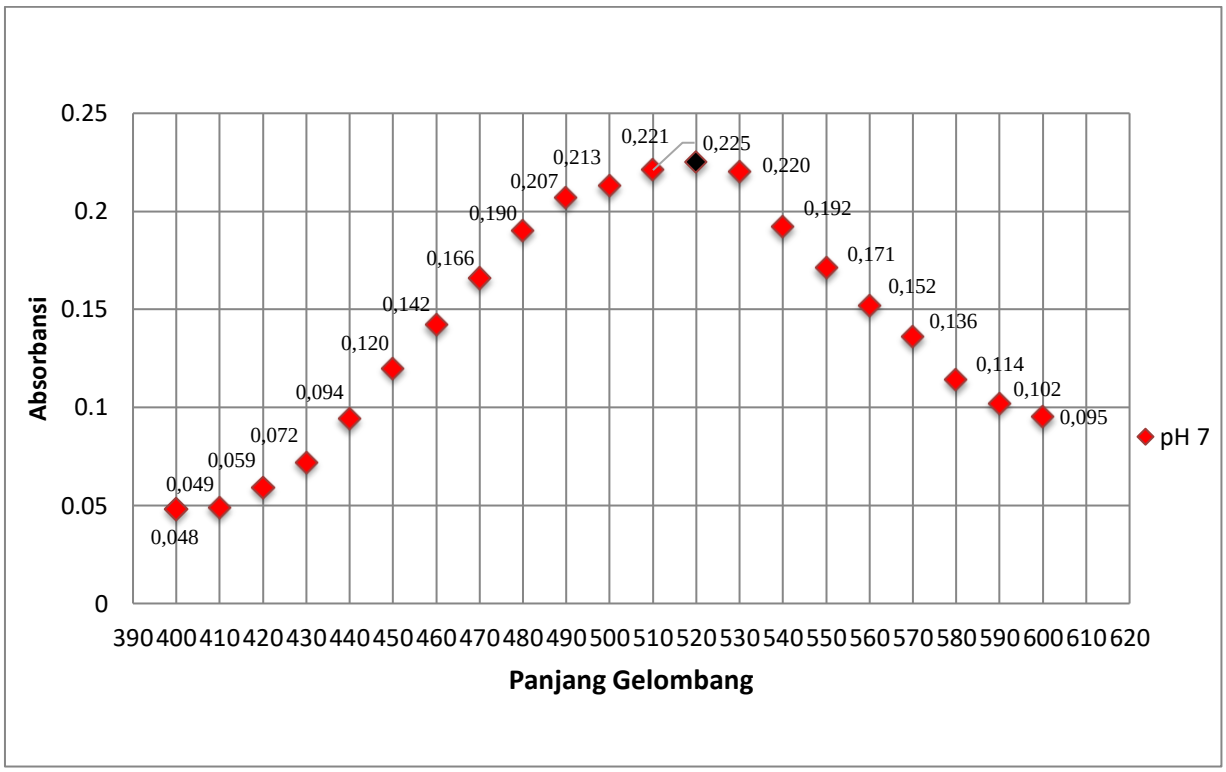


Figure 3.1. Determination of Maximum Wavelength

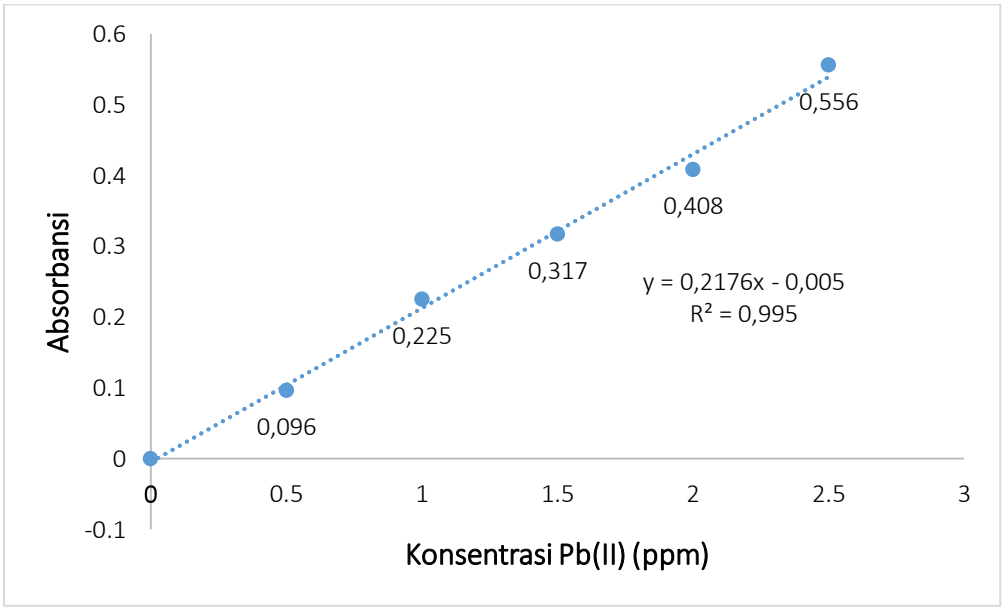


Figure 3.2. Pb2+ Ion Concentration Curve to Absorbance

*D. Biosorption of Metal Pb2+ by E. coli Bacteria*

In this study, *E. Coli* bacteria were used as biosorbents in the biosorption process of Pb2+ ions. Biosorption has the principle of binding metal ions that occur in the structure of microbial cells (cell walls). Metal ion binding can be done in several ways, namely: active cation transfer systems and binding on surfaces and other mechanisms. This binding mechanism is related to the physical and chemical properties and anionic properties of the cell wall, resulting in Pb2+ ions (cations) being bonded by the cell wall by adhesion.[14 The Gospel of Jesus Christ]

In figure 3.3, it can be seen that Pb2+ ions exposed to *the media of E. coli* bacteria with variations in Pb2+ ion

concentrations (3; 6; 9; 12; and 15) ppm, show a decrease in concentration which indicates that *E. coli bacteria* are able to absorb Pb2+ ions. In Figure 3.3, it can also be seen that the biosorption power of *E. coli* bacteria increases with increasing exposure time. This is directly proportional to the [statement,11] that the longer the exposure time, the longer the interaction time between metal ions and biosorbents, so that the active groups bound to metal ions will be more numerous and will increase the amount of metal absorbed.

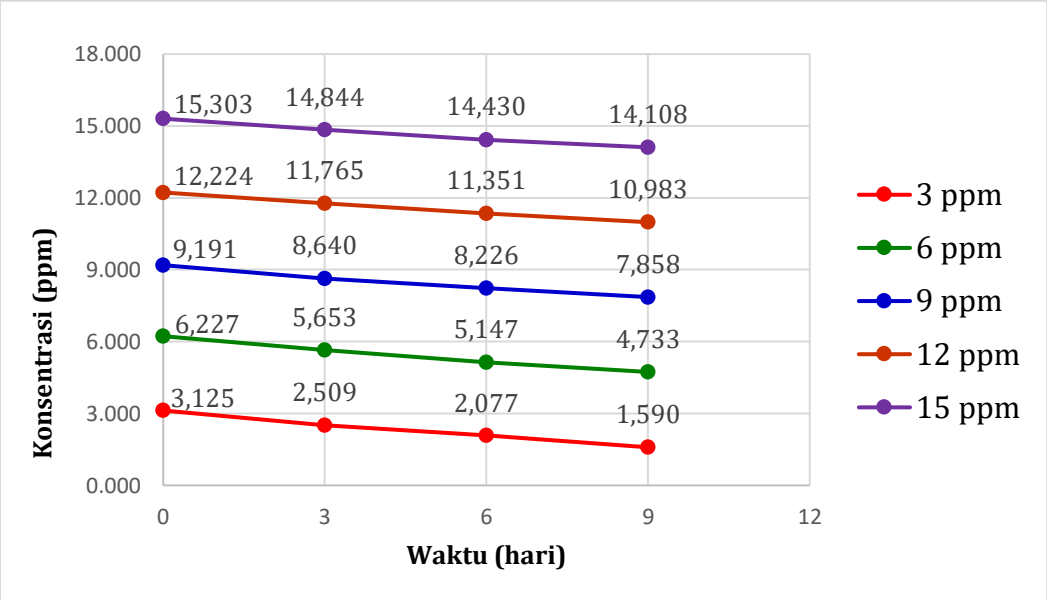


Figure 3.3. Decrease in Pb2+ ion concentration at each concentration variation over time (days)

TABLE 3.2. Percent Biosorption of Pb2+ Ions by *E. coli Bacteria*

| Day | Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 15 |
| 0 | 0 | 3,125 | 6,227 | 9,191 | 12,224 | 15,303 |
| 3 | 0 | 2,509 | 5,653 | 8,640 | 11,765 | 14,844 |
| 6 | 0 | 2,077 | 5,147 | 8,226 | 11,351 | 14,430 |
| 9 | 0 | 1,590 | 4,733 | 7,858 | 10,983 | 14,108 |

In table 3.2, it can be seen that the biosorption power of *E. coli* bacteria increases with increasing exposure time. This is directly proportional to the statement of Adriansyah (2018), that the longer the exposure time, the longer the interaction time between metal ions and biosorbents, so that the active groups bound to metal ions will be more and will increase the amount of metal absorbed.[11 The Gospel of Jesus Christ]

Table 3.2 shows that as the concentration of Pb2+ ions increases, the biosorptive power of bacteria *E. coli* Decreased. This is due to the fact that the high initial concentration of the metal affects the mass transfer of ions in the absorption efficiency. The absorption capacity of metal ions by biosorbents will increase as the concentration of the metal increases. However, the high initial concentration causes the efficiency of the metal biosorption percentage to be reduced because the biosorbent capacity in the biosorption process has reached an equilibrium and saturation point. Meanwhile, a small initial concentration of metals is able to improve the performance of the interaction between metal ions and the functional groups that bind the metal so that absorption is optimal.[15]

*E. Optimum Concentration and Time of Biosorption of Pb2+ Metals by E. coli Bacteria*

Figure 4.4 is a graph of the percentage biosorption ratio of each variation in the concentration of Pb2+ ions to time (days). In table 4.3, *the E. coli* bacterial media exposed to Pb2+ ions with concentration variations of 3 ppm, 6 ppm, 9 ppm, 12 ppm and 15 ppm occurred absorption from day 0 to day 9 where *E. coli* bacteria were able to absorb Pb2+ ions by 49.118%, 23.985%, 14.500%, 10.150% and 7.808% respectively. Based on the data obtained from this study, it shows that the higher the concentration of the solution, the lower the percentage of biosorption of Pb2+ ions.

According to (Komari, 2021), the factor that greatly affects adsorbs is time, where to reach the equilibrium point (maximum adsorbtion) of Pb2+ ions, so that the absorption of Pb2+ ions is time-sensitive. At a certain time frame, there will be an equilibrium between biosorbents (*E. coli*) and adsorbate (Pb2+ ions), where the time needed to reach this equilibrium state is called the optimal time for the absorption of Pb2+ ions.

In figure 4.5, it can be seen that the concentration of 3 ppm of Pb2+ ions resulted in a good increase in biosorption efficiency on day 0 to day 3, which shows that the biosorption process went well at that time vulnerability with a biosorption percentage of 0.000% to 19.706%. This is due to a significant increase in biosorption power so that it can be concluded that in this time frame *E. coli* bacteria work optimally in absorbing Pb2+ ions.[17] Then at the concentration of 15 ppm of Pb2+ ions on the 6th to 9th day, a biosorption percentage value of 5.706% to 7.808% was obtained, the biosorption process that occurred showed a decrease in biosorption power and allowed it to experience a constant condition and was suspected to stop the next day. This is because bacteria have reached their maximum capacity to absorb metals. The active side of the saturated

biosorbent surface causes the absorbed solute to reach the maximum limit, which results in the bisorbent surface or the functional group contained in it can no longer absorb the adsorbate.[11] So it can be concluded that the optimal absorption of Pb2+ ions occurred on the 3rd day with a concentration of 3 ppm with an absorption result of 19.706%.

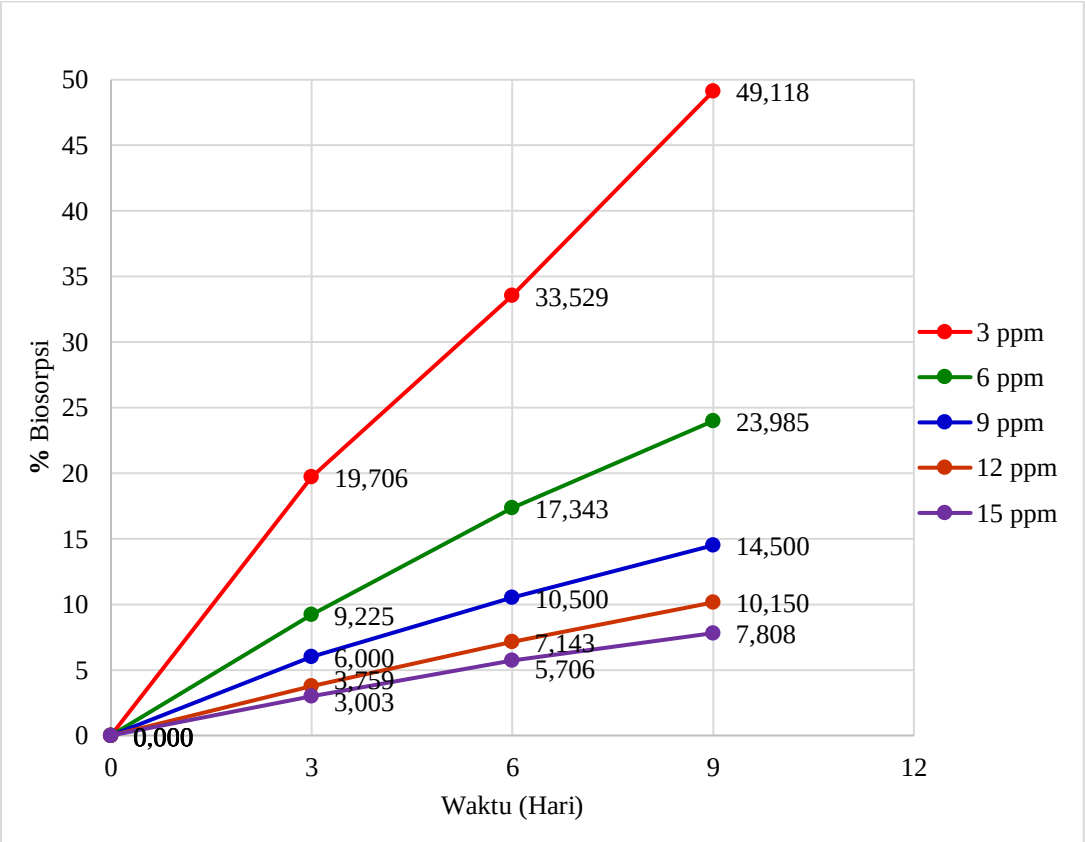


Figure 3.4. Comparison of Percent Biosorption of Pb2+ Ions by E. *coli* Bacteria Per Concentration Variation Over Time (days)

## IV. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that *E. coli* bacteria are able to absorb Pb2+ metal with optimal conditions where the optimal time and concentration required *by E. coli bacteria* to absorb Pb2+ metal occurs on the 3rd day with a biosorption percentage of 19.706% in the variation of Pb2+ metal concentration of 3 ppm.

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