Biosorption of Pb^{2+} Ion by Bacterium *Pseudomonas* sp.

Riki Riki[a)], Rudi Kartika, and Rahmat Gunawan

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, St. Barong Tongkok No.4, Samarinda 75123, Indonesia

[a)] Corresponding author: riqieborneo9@gmail.com

Abstract. The study aimed to determine the ability of *Pseudomonas* sp. bacteria to absorb Pb^{2+} ions based on variations in the concentration of Pb^{2+} ion exposure to the time (day) required for bacteria in the process of biosorption of Pb^{2+} ions. Determination of maximum wavelength using a UV-Vis Spectrophotometer with a standard solution of 1.2 ppm Pb^{2+} ions with a yield of 510 nm. Based on the test results, *Pseudomonas* sp. bacteria were able to absorb Pb^{2+} ions at exposure concentrations (4, 8, 12, 16, and 20) ppm with the percentage (%) of biosorption respectively 36,874; 30,548; 20,236; 13,178; and 10,020. The optimum concentration and time required for *Pseudomonas* sp. bacteria to absorb Pb^{2+} ions occurred on the 14th day with an exposure concentration of 4 ppm with a biosorption efficiency of 36,388% %.

INTRODUCTION

The presence of heavy essence in submarine surroundings is known to beget severe damage to submarine life. Fact that these essence kill microorganisms during natural treatment of wastewater with a consequent detention of the process of water sanctification. Utmost of heavy essence mariners are answerable in water and form waterless results and accordingly cannot be separated by ordinary physical means of separation.

Physico –chemical styles, similar as chemical rush. Chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, ion exchange, and membrane technologies have been extensively used to remove heavy essence ions from artificial wastewater. These processes may be ineffective or precious, especially. The heavy essence ions are in results containing in the order of 1-100 mg dissolved heavy essence ions/L [1,2]. Biological styles similar as biosorption/bioaccumulation for the junking of heavy essence ions may give a seductive volition to physico-chemical styles [3].

Pseudomonas sp. is a type of bacteria that is widely used for biocontrol and utilization in heavy metal processing. *Pseudomonas* sp. is a gram-negative bacteria in the form of a rod (basil) or coccus and has a very high flagella for motility. These bacteria grow well at 4 °C or below 43 °C. This genus of bacteria produces types of enzymes such as proteases, amylase and a lipase [1].

Lead (Pb) is one of the heavy metals that has a high level of toxicity. Pb concentrations that exceed the threshold of 10 µg/dl can pollute the environment. One of the efforts to overcome pollution is with adsorption. When the lead metal content (Pb) is 188 mg/L, it can kill microorganisms in the body of the water [2]. Other symptoms of Pb metal poisoning: nausea, anemia, and abdominal pain [3].

Biosorption of heavy essence by microbial cells has been honored as an implicit volition to being technologies for recovery of heavy essence from artificial waste aqueducts. Utmost studies of biosorption for essence junking have involved the use of their laboratory-grown microorganism or biomass generated by the pharmacology and food processing diligence or wastewater treatment units. Adsorption method is one of the methods used for purification, adsorption method involved the formation of bonds between adsorbens and adsorbates (adsorption substance) [4].

Microorganisms (bacteria) can serve as adsorbents that bind to heavy metals. Based on research [5] capacity of absorption by *Flavobacterium* bacteria and *Micrococcus* sp. bacteria adsorb by 96% and 99% with different concentrations and times. Accordingly, this study aimed to investigate the continuous bisorptive potential of *Pseudomonas* sp. isolated from a contaminated waste treatment plant.

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MATERIAL AND METHOD

Equipments and Material

Equipments

Used in the study is the Visible Spectrophotometer (*Rayleight Vis* 7220G), micropipette eppendorf, analytical balance sheet, glass glass, ose needle, autoclave, spatula, stirrer rod, spray bottle, tweezers, bunsen, sterile cotton swab, incubator, laminar water flow, hotplate with magnetic stirrer and weighing bottle.

Material

The materials used in the study are solids $Pb(NO_3)_2$, Alizarin Red S (ARS) 0.01 M, phosphate buffer solution, KH_2PO_4 0.1 M, NaOH 0.1 M, aquadestt, *Pseudomonas* sp., Nutrient Broth liquid media (NB), Nutrien Agar solid media (NA).

Methods

Standard Solution Pb^{2+}

In the manufacture of standard solution Pb^{2+} the first thing that is done is the manufacture of a 1000 ppm Pb^{2+} solution after which the solution is diluted to 100 ppm and re-diluted to 10 ppm. Then, the manufacture of a standard Pb^{2+} curve by way of 10 ppm Pb2+ is taken as much as 2 mL, 4 mL, 6 mL, 8 mL, and 10 mL and inserted into each of the measuring pumpkins 50 mL and added aquades to the tera mark.

Sterilization Glass

Glass tools used such as Erlenmayer squash, test tubes and petri dishes are washed thoroughly, dried and wrapped using paper and plastic. The glass device is then sterilized in an autoclave for 1 hour at a temperature of 121 ºC.

Preparation of Nutrient Broth

A total of 6 250 mL Erlenmeyer squashes are each added 1 gram *of nutrient broth,* dissolved with aquadest of 200 mL, boiled using a hot plate at a temperature of 60 ºC and homogenized with magnetic stirrer. Medium is sterilized with autoclave at a temperature of 121 ºC for 15 minutes. The medium is put in an incubator at a temperature of 30 ºC for 24 hours.

Regeneration of Bacteria

The first step of determining the starter of pseudomonas sp bacteria i.e. 10 mL sterilized NB is inserted into 50 mL Erlenmeyer. Pseudomonas sp. Injected into the medium as much as 1 ose, the inoculation process is carried out in laminar airflow. The medium is stored at a temperature of 37ºC for 24 hours before use.

Then, the second step is the determination of OD by means of a standard solution of Pb (II) with a concentration of 1.2 ppm as much as 10 mL, then added 1 mL Alizarin Red S 0.01 M in each solution. Then added 0.15 mL NaOH 0.1 M and 1 mL buffer pH 7. The standard solution that has been mixed with Alizarin Red S is left for ±30 minutes. Measured absorbance uses a Vis Spectrophotometer at wavelengths of 400-550 nm [6].

Determination of Maximum Wavelength

The standard solution Pb^{2+} with a concentration of 1.2 ppm as much as 10 mL, then added 1 mL Alizarin Red S 0.01 M in each solution. Then added 0.15 mL NaOH 0.1 M and 1 mL buffer pH 7. The standard solution that has

been mixed with Alizarin Red S is left for ±30 minutes. Measured absorbance using a Vis Spectrophotometer at wavelengths of 400-550 nm [6].

Determination of Optimum pH of Pb-ARS Complex

A standard solution of Pb^{2+} with a concentration of 1.2 ppm of 10 mL, then added 1 mL Alizarin Red S 0.01 M to each solution, added 0.15 mL NaOH 0.1 M and 1 mL buffer pH (4, 7, and 12). The standard solution that has been mixed with Alizarin Red S is left for ±30 minutes. Measured its absorbance using a Vis Spectrophotometer at maximum wavelength [7].

Pb^{2+} Standard Curve

A standard solution of Pb^{2+} 10 ppm is incorporated into a 50 ml with variations in concentration (0.4; 0.8; 1,2; 1.6 and 2.0) ppm. Each solution with a variation in concentration is taken as much as 10 mL, transferred into Erlenmeyer 50 mL and added Alizarin Red S 0.01 M as much as 1 mL and NaOH as much as 0.15 mL. Then, added 1 mL of buffer in accordance with the optimum pH obtained. Measured its absorbance using a Vis Spectrophotometer at maximum wavelength [7].

Biosorption of Ions Pb^{2+} By Pseudomonas sp.

Erlenmeyer 250 mL squash contains a medium nutrient broth of 6 pieces added 0, 10, 20, 30, 40 and 50 mL standard solution Pb^{2+} 100 ppm, added nutrient broth and homogenized. Each medium is calculated with a 1 mL starter of *Pseudomonas* sp.. Medium of *Pseudomonas* sp. is put into an incubator at a temperature of 37 ºC

Sample

Nutrient broth solution that has been exposed by *Pseudomonas* sp. with variations in concentration (0, 4, 8, 12, 16 and 20 ppm. Each solution of concentration variation is taken 10 mL and then filtered using 0.45µ membrane millipore filter paper and then added Alizarin Red S 0.01 M as much as 1 mL and NaOH 0.1 M as much as 0.15 mL. Added 1 mL buffer solution that corresponds to the optimum pH obtained. Measured its absorbance using a Vis Spectrophotometer at maximum wavelength. Sample testing is done every 48 hours [7].

Data Analysis

The analysis was performed on each sample with the number of measurements taken 1 time. The relationship between the average analytical response obtained with the theoretical concentration of analytes in the preparate can be calculated by equation:

$$y = ax + b \quad (1)$$

Information:
a = intersep
b = slope
x = sample concentration (mg/L)
y = absorbance

RESULTS AND DISCUSSIONS

Determination of Maximum Wavelength and Optimum pH of Pb-ARS Complex

Determination of the maximum wavelength of solution Pb^{2+} is done by measuring the absorbance value of the standard solution Pb^{2+} 1.2 ppm which has been complexed then measured using Vis Spectrophotometer and obtained a length of 510 nm. Graph of maximum wavelength determination results can be seen in Fig. 1.

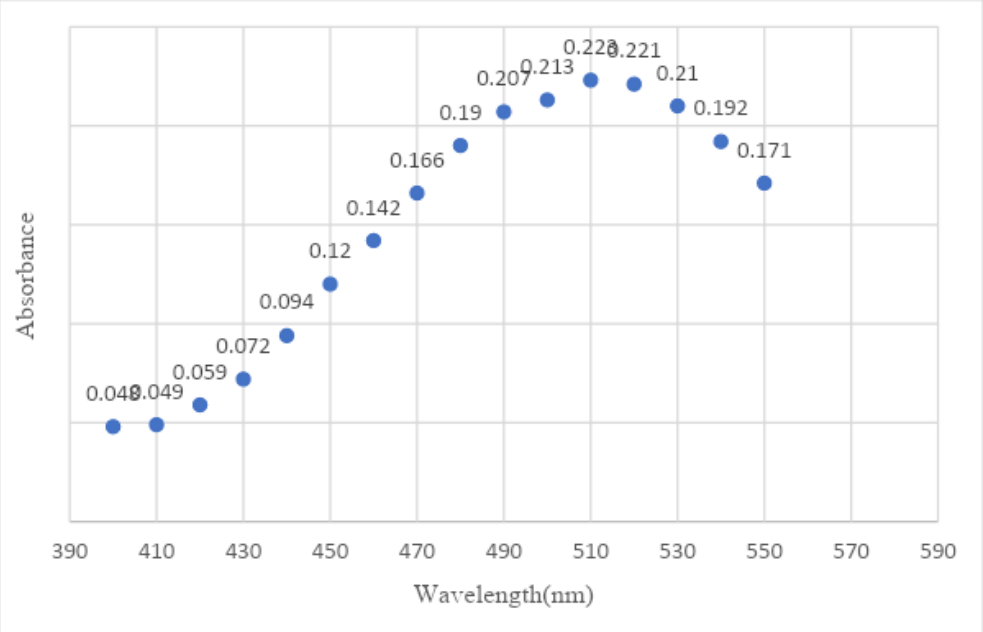

FIGURE 1. Determination of Maximum Wavelength

Determination of optimum pH

To ensure that pH 7 is the optimum pH, the variation in pH at the maximum wavelength that can be seen in Fig. 2 below states that the optimum pH obtained is pH 7 with an absorbance value of 0.223 nm [7].

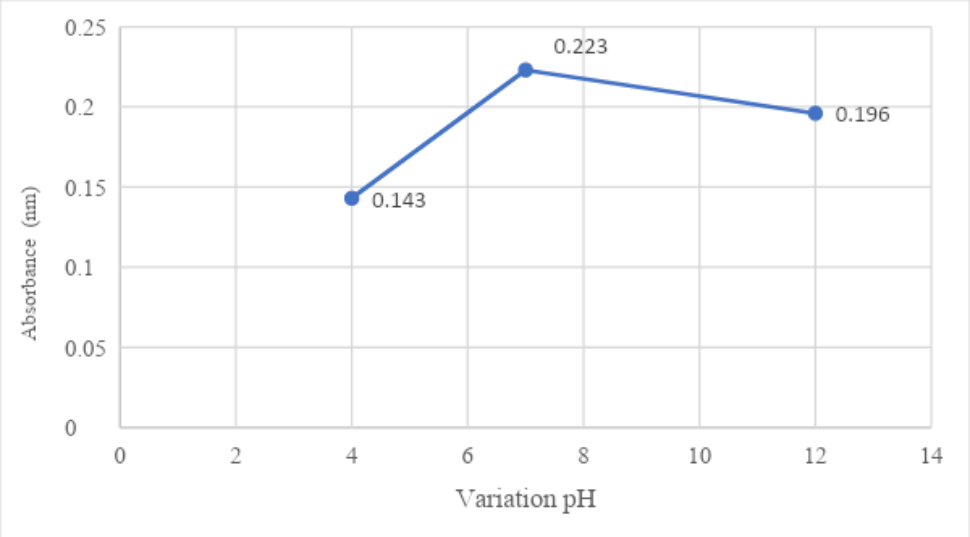

FIGURE 2. Determining the Optimum pH

Pb^{2+} Standard Curve

The manufacture of standard curves Pb^{2+} used variations in the concentration of standard solutions Pb^{2+} among others 0; 0.4; 0.8; 1.2; 1.6 and 2.0 ppm at max wavelengths of 510 nm. The result of the linearity test obtained the regression equation that is y = 0.2004x + 0.0128 with y stating the absorbance value and the value x stating the concentration value of Pb^{2+} indicated in Fig. 3.

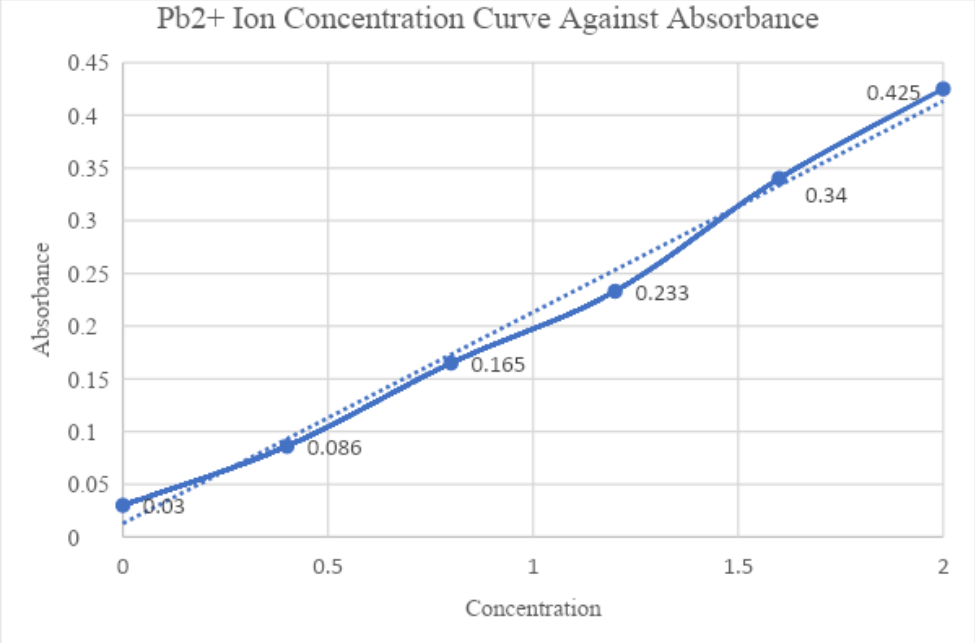

FIGURE 3. Pb2+ Concentration Curve Against Absorbance

Regeneration of *Pseudomonas* sp.

Optical Density (OD)

Optical Density (OD) aims to determine the value of turbidity intensity of a sample based on its absorbant value. The absorbance values in the bacterial starter media before and after incupation can be shown in Table 1.

TABLE 1. Optical density

Treatment	Time (Hours)			
	1	12	24	25
Before incubation (nm)	1,669			
After Incubation (nm)	1,703	1,729	1,751	1,749

Based on Table 1, the absorbance obtained after the starter media is incupted is greater than before incessed, this shows a difference in turbidity which means the turbidity of the media after inclation. This shows that *Pseudomonas* sp. in the starter medium successfully grow.

Biosorption of Ions Pb^{2+} By *Pseudomonas* sp.

The results obtained from the biosorption of Pb^{2+} ions by *Pseudomonas* sp. are shown in Fig. 4.

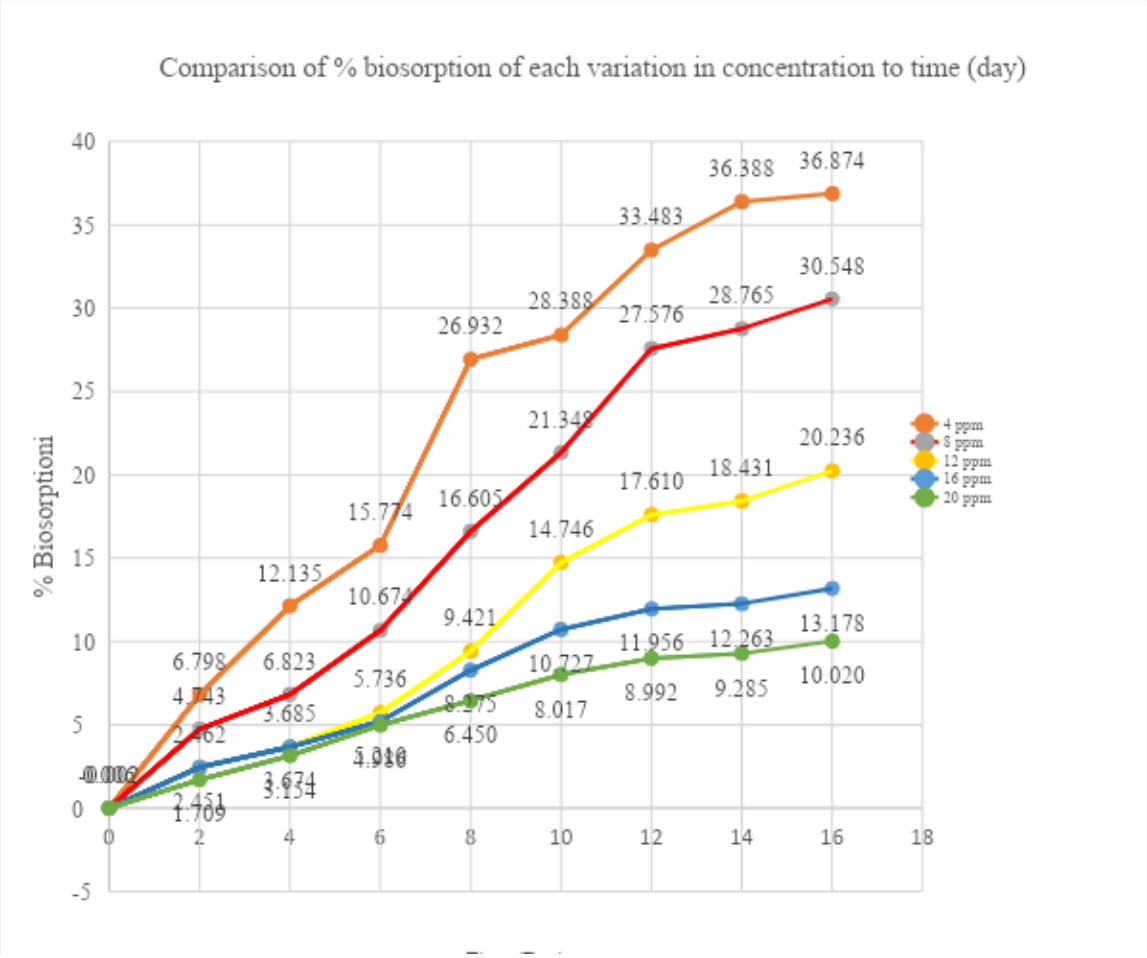

FIGURE 4. Percentage Ratio of Pb2+ Ion Biosorption by Pseudomonas sp on Variations in Concentration Over Time

Time, concentration and pH are factors that can affect the biosorption process. In Fig. 4 the media presented by Pb^{2+} ions amounted to 4, 8, 12, 16 and 20 ppm there was absorption from day 0 to day 16 where *Pseudomonas* sp. were able to absorb Pb^{2+} ions in a row which is 36.874%, 30.548%, 20.236%, 13.178% and 10.020%. In this study, it showed that the higher the concentration of the solution, the decreased the percentage of biosorption of Pb^{2+} ions. It is suspected that the precipitation of metal ions Pb^{2+} with pH biomass *(Pseudomonas* sp.) so that the absorption of the concentration of metal ions in the media is not too large [8]. The pH value of the media grew bacteria on the 0th day by 5.61 while on the 16th day the pH value of the media grew consecutively which is 6.15; 6,27; 7,58; 8.26 and 8.29. The existence of this deposition process can lead to a decrease in biosorption efficiency [2]. The pH value of the media grew bacteria on the 0^{th} day by 5.61 while on the 16^{th} day the pH value of the media grew consecutively which is 6.15; 6,27; 7,58; 8.26 and 8,29. The pH value is above 7, the biosorben's ability to bind to The Pb^{2+} ion will become smaller [9]. At the base pH the metal ion will spontaneously react with hydroxide ions to form a metal-hydroxide bond, while at the acidic pH there will be competition between metal ions with H+ ions to bind to microbial cell walls [10]. This causes the accumulation of metals in microbial cells at a neutral pH greater than the pH of acids and bases.

To achieve the optimal adsorption state of the metal by the adsorbent, a time span is required. In Fig. 4, it can be known that the concentration of 4 ppm of Pb^{2+} ions there is an increase in the efficiency of good biosorption on days 6 to 8 which indicates that the biosorption process is running well in that time span with biosorption percent of 15.774% to 26.932%. This happens because of a significant and high increase in biosorption power which means that in that time span *Pseudomonas* sp. work optimally in absorbing Pb^{2+} ions [8]. Then, on the 14^{th} to 16^{th} day obtained a percent biosorption value of 36.388% to 36.874 percent and the biosorption process leads to a constant condition and is expected to stop the next day. This can happen because bacteria have reached their maximum capacity in absorbing metals [11]. The active side of the saturated biosorben surface causes absorbed solutes to reach the maximum limit, as a result of the biosorben surface contained in it can no longer absorb adsorbate. So it can be assumed that the optimum absorption of Pb^{2+} ions occurs on the 14^{th} day with an absorption result of 36,388%.

CONCLUSION

Pseudomonas sp. are able to absorb Pb^{2+} ions at exposure concentrations (4, 8, 12, 16, and 20) ppm. The optimum biosorption of Pb^{2+} ions per variation in successive metal concentrations amounted to 36.874%; 30.548%; 20.236%; 13.178%; and 10.020%. The optimum concentration and time required *Pseudomonas* sp. to absorb Pb^{2+} ions occurs on the 14^{th} day with a biosorption percent of 36.388 % varying the concentration of Pb^{2+} 4 ppm ions.

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