Lead Biosorption (Pb) And Cadmium (Cd) By Flavobacterium Sp Bacteria

Apri Susanto, Rudi Kartika, Soerja Koesnarpadi

Abstract: This research studies the ability of Pb and Cd biosorption by Flavobacterium sp bacteria and the characteristics of bacteria after being exposed to the heavy metal. Heavy metals Pb and Cd with concentrations (2, 4, 6, 8 and 10) ppm were exposed to bacteria as much as 0.4 mL and measured reduction in metal content every 3 days for 30 days. Analysis of the decrease in Pb and Cd levels uses an Atomic Absorption Spectrophotometer (AAS) while the bacterial characteristics test after exposure to heavy metals uses Scanning Electron Microscopy (SEM). This research shows that the Flavobacterium sp bacteria is able to absorb heavy metals Pb and Cd, this can be seen from the decrease in metal content in the growing media that gets smaller with increasing exposure time until all metals are absorbed 100%. The most maximum biosorption process occurred in Pb 2 ppm metal solution on day 18 achieved metal reduction percent of 96.08%, while the highest absorption of Cd metal occurred at a concentration of 10 ppm on day 24 achieved a reduction percent of 77.21 %. SEM photo showed that the Flavobacterium sp bacteria experienced increased levels of heavy metals Pb and Cd after being exposed to the metals for 30 days with an increase of < 1.5% in Pb metals and < 0.5% in Cd metals.

Index Terms: Biosorpsi ,Flavobacterium,Timbal (Pb),Cadmium (Cd)

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1. INTRODUCTION

Environmental pollution, especially in water areas, has become a national to international problem. The impact of environmental pollution that occurs is related to toxicology, i.e. the understanding of chemicals that affect and interfere with living organisms. Proven in recent times, toxicology regarding heavy metals such as Mercury, Lead, Copper, and Cadmium has become a hot topic of discussion in the world. Thus, industrial waste is the main source of metal pollution for aquatic organisms. It has been quoted that heavy metals are the main pollutant in the environment. Heavy metal is a harmful pollutant for fish, because it is not removed from the aquatic system by natural methods, such as organic pollutants, and enriched with mineral organic substances. Metal contaminants are mixed in the water system through the process of smelting, waste, sewage, and washing of garbage which causes severe damage to the water system [1]. Efforts to control wastewater pollution (effluent) are generally carried out by preventing and overcoming pollution of wastewater through the use of technology that considers the type and nature of wastewater as well as the standard of wastewater content.

The technology of choice is expected to be able to change the

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- *Apri Susanto is currently as Student of Magister Program of Chemistry Department, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia, and previously worked as Laboratory Analyst at PT. Pupuk Kalimantan Timur, Bontang, Indonesia*
  E-mail : apris@pupukkaltim.com
- *Rudi Kartika is currently as Lecturing of Chemistry Department, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia.*
  E-mail: rudi_biokimia@yahoo.com
- *Sorja Koesnarpadi is currently as Lecturing at Departement of chimistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia.*
  E-mail: soerja.koes @gmail.com

maximum level of wastewater (effluent standard) so that it can meet the limit of the level of wastewater (stream standard) that can be used optimally in order to maintain waste discharged into the environment. Wastewater treatment is generally carried out by adding chemicals in the form of coagulants, which we know that the price is increased, therefore to reduce the risk, the addition of coagulants is replaced with those from microorganisms, the development of technological planning for maximum level of wastewater (effluent standard) and limits of wastewater level (stream-standard) which introduces bioremediation technology using microorganisms has received considerable attention. Some examples that have been made are the use of indigenous isolates in the control of wastewater bodies contaminated with Lead and Cadmium metals by using several bacteria including Micrococcus sp, Corynebacterium sp, Phenylobacterium sp, Enhydrobacter sp, Morrococcus sp, Flavobacterium sp. Based on the background description above, to absorb Lead and Cadmium in wastewater. Previously, bioremediation of Pb metal was carried out by using a mixture of bacteria. Among Pseudomonas pseudomallei and Pseudomonas aeruginosa bacteria which become one of the researchers' references to develop the reduction of Pb waste by using other bacteria [2]. There are many mechanisms involved in biosorption, some are not fully understood. Mechanism biosorption can be classified based on the dependence on metabolism cell which is called metabolism dependent or according to the location where the metal removed from the solution found which is called dependent Non-metabolic / independent metabolism of extracellular accumulation/precipitation, absorption of cell surface/precipitation and Intracellular accumulation [3, 4]. During metabolism in bacteria, metal absorption is carried out by physicochemical interactions between metals and functional groups on microbial cell surfaces. This is based on physical adsorption, exchange ion, and chemical absorption, which are not dependent on cell metabolism [5]. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins, and lipids have abundant metal bond groups such as carboxyl, sulfate, phosphate and amino groups [5,6]. The mechanism of detoxification of these bacteria can be categorized into intracellular absorption by exports keeping toxic ions out of cells by changing the membrane transportation system involved in initial cellular accumulation, reducing permeability, extracellular absorption by binding of

certain ion-minerals. Extracellular detoxification of toxic cations or anions by enzymatic conversion from a more toxic form to a less toxic form [7].In determining the characteristics of bacteria before and after the metal biosorpsi process is carried out using SEM (Scanning Electron Microscopy) instrument. SEM instrument consists of two main components, console electronics and electron columns. The electronic console provides control buttons and switches that allow adjustments of instruments such as filament current, acceleration voltage, focus, magnification, brightness and contrast. The FEI Quanta 200 is a sophisticated electron microscope that uses a system computer in conjunction with an electronic console so there is no need to have a large console that holds control buttons, CRT, and image capture devices. All major controls are accessed through a computer system using mouse and keyboard. Users only need to be familiar with GUI or software that controls instruments rather than controlling knobs and switches normally found on old-style electron microscope scanning. Images produced by SEM are usually seen on a CRT located on an electronic console but, instead of with FEI, the image can be seen on a computer monitor. The picture captured can be saved in digital format or printed directly [8].

2   MATERIAL AND METHOD

2.1 Manufacture of Metal Solution
The 100 ppm mother solution of Pb and Cd metals was diluted again by taking (2 mL; 4 mL; 6 mL; 8 mL; and 10 mL) diluted to 10 mL so that it obtained standard Pb solution with a concentration of 2 ppm; 4 ppm; 6 ppm; 8 ppm; and 10 ppm.

2.2 Manufacture of Bacteria Media
The bacterial culture used in this study was Flavobacterium sp. The nutrient broth (NB) powder was weighed as much as 8 grams, dissolved in 1 L of demin water. The solution is stirred using magnetic stirer while heating until all the NB powder was completely dissolved and the color of the solution turns to be clear. The media is sterilized in an autoclave at temperature of 121°C for 20 minutes.

2.3 Bacterial Rejuvenation
Bacterial rejuvenation is carried out aiming to get active bacteria, because previously the bacteria were in the refrigerator with inactive bacterial conditions. 100 mL of NB solution is poured into 250 mL erlenmeyer flask with 2 flasks, then 1 ose of Flavobacterium sp bacteria was added to each of the erlenmeyer flasks. The mixture was stirred slowly, then incubated at temperature of 37°C and the number of bacteria was measured after 24 hours.

2.4 Bacteria Count (Total Plate Count / TPC Method)
The bacterial solution was pipetted as much as 0.4 mL dissolved in 1000 mL of demin water in a measuring flask, then stirred slowly. The solution was poured as much as 1 mL into 20 mL of PCA medium in a petri dish by spread technique, then flattened and incubated at temperature of 37°C for 24 hours. The number of colonies growing on PCA medium was observed and counted using haemocytometer colony counter with Colony Forming Unit or colony forming unit (CFU/mL).

2.5 Biosorption of Metals by Bacteria
Metal solution with varying concentrations (2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm), added with 0.4 mL of bacteria. Metal analysis was carried out every 72 hours (3 days) for 30 days using an Atomic Absorption Spectrophotometer (AAS). During the biosorption process, environmental conditions are maintained including pH 6-8, temperature of 22-25 °C.

2.6 Determination of Characteristics of Bacteria Using SEM (Scanning Electron Microscopy) and EDS (Energy dispersive system)
The sample is dried with a vacuum system to be free of water, after that it is placed in the sample container (speciment holder), is coated (coating) with gold (Au) by using ion sputter. The sample is put in specimen chamber for observation before shooting. The photo shoot is carried out with a certain magnification. The microstructure of resulting SEM image is analyzed. The EDS results show the percentage of metal content in the sample

3. FINDING AND DISCUSSION

3.1 Biosorption of Lead by Flavobacterium sp bacteria
Based on the results of the analysis on the periodic days that have been set, it can be seen that the five variations in concentration used decrease based on the increase in time variation used. This means that the biosorption of Pb metal by Flavobacterium sp walks perfectly on day 21, ie the concentration of Pb has been completely absorbed by bacteria. The results of the analysis of the metal remaining on the growing media of Flavobacterium sp bacteria as in the image below:
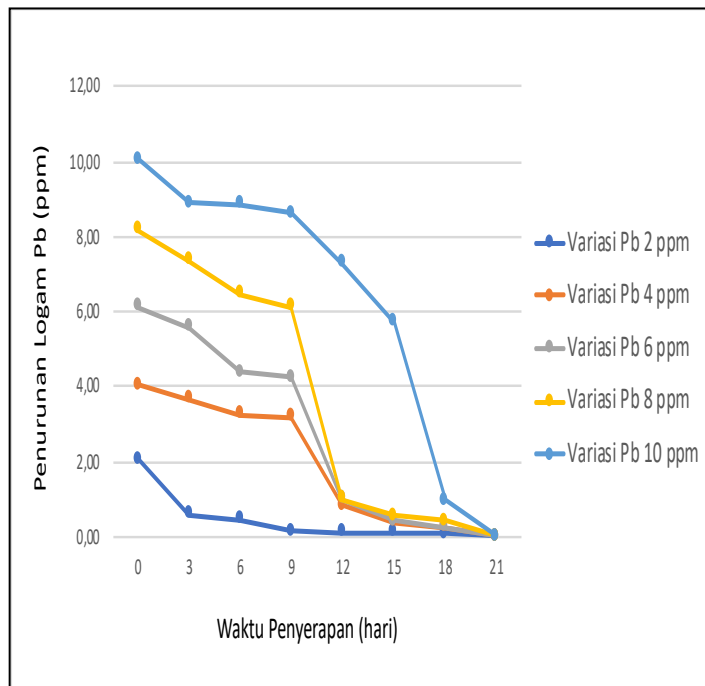

Figure 1. *Graph of decreased Pb metal concentration in growing media of Flavobacterium sp bacteria*

This condition also shows that Flavobacterium sp bacteria can survive even if exposed to Pb metal means that in the body of the bacteria, there was an antibody that can bind to the Pb metal. This also shows that the bacteria has antibody that

contain the cysteine amino acid

3.2 Biosorption of Cadmium by Flavobacterium sp bacteria

The results of the analysis of the remaining Cd metal in bacterial growing media are shown in Figure 2, showing that there is a perfect absorption of Flavobacterium sp bacteria absorb as a whole on day 30 with a concentration of remaining Cd metal 0 ppm (completely absorbed by bacteria). But at an initial concentration of 10 ppm Cd, exposure of Flavobacterium sp bacteria after day 15, there is very drastic decline. This shows that Flavobacterium sp bacteria is easy to absorb Cd metal and also shows that Flavobacterium sp containing anti body which contains sufficient cysteine amino acids to absorb metal.
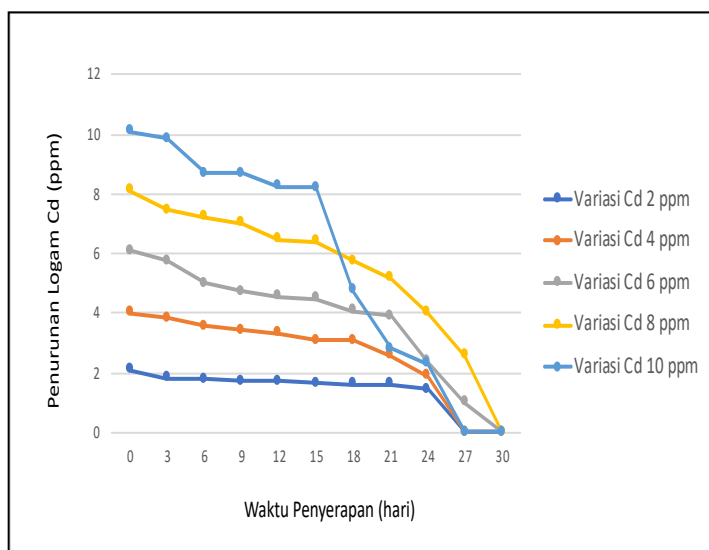

Figure 2 *Concentration of Cd metal decrease in the growing media of Flavobacterium sp bacteria*

3.3 Characteristic of Flavobacterium sp before exposure to Pb and Cd

The surface morphology characteristics and the percentage of metal content in Flavobackterium sp before and after exposure to heavy metals were analyzed by SEM (Scanning Electron Microscopy) and EDS (Energy Dispersive System) methods. Characteristics of Flavobacterium sp bacteria and before

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• *Apri Susanto is currently as Student of Magister Program of Chemistry Department, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia, and previously worked as Laboratory Analyst at PT. Pupuk Kalimantan Timur, Bontang, Indonesia*
  E-mail : apris@pupukkaltim.com
• *Rudi Kartika is currently as Lecturing of Chemistry Department, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia.*
  E-mail: rudi_biokimia@yahoo.com
• *Sorja Koesnarpadi is currently as Lecturing at Departement of chimistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia.*
  E-mail: soerja.koes @gmail.com

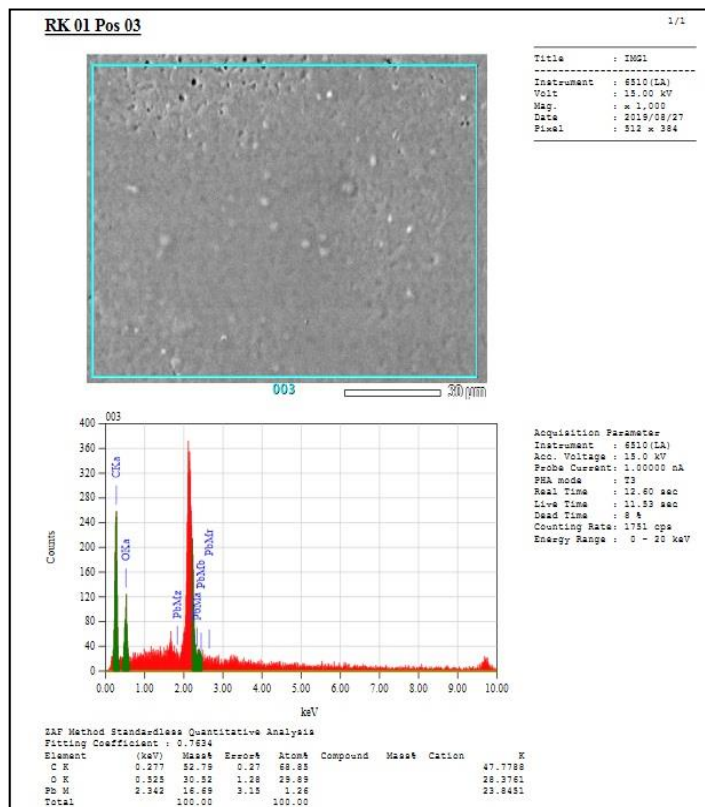exposure to heavy metals, it is shown in the following figure:

Figure 3 *The results of SEM and EDS analysis of Flavobacterium sp before being exposed to metals*

In Figure 3, it shows that hasil SEM bakteri Flavobacterium sp which shows that there is no Cd metal content inside Flavobacterium sp bacteria but there is a metal content of Pb in the bacterium of 1.26% which is indicated from the EDS results. This shows that in Flavobacterium sp bacteria before being exposed, Pb metal has Pb content which is useful for its life in the growing media.

3.4 Characteristic of Flavobacterium sp after being exposed to Pb and Cd metals

Surface morphology characteristics and the percentage of metal content in Flavobackterium sp bacteria after being exposed to Cd heavy metal as shown in Figure 5. Figure 4 shows the analysis result of SEM and EDS of Flavobacterium sp bacteria which grows in media being exposed to Pb metal. That result shows that Flavobacterium sp bacteria contains Pb metal of 1.39%, there is an increase in Pb metal content compared to before exposure to Pb metal, which is 1.26%. The increase in Pb metal content is due to the biosorption process of Flavobacterium sp in absorbing Pb heavy metal until the Pb content is completely gone on day 21. The ability of Flavobacterium sp bacteria is that it can survive until the Pb content increases 0.13% due to the presence of cysteine amino acid antibody in its body to maintain its life. Figure 5 shows the results of SEM and EDS analysis of Flavobacterium sp bacteria after exposure to Cd metal. Increased levels of Cd in Flavobacterium sp bacteria are relatively small at 0.12% where the growth of bacteria before exposure to Cd metal does not indicate the presence of these metal contents. The slight increase of Cd metal content in Flavobacterium sp does

not affect the ability of Cd metal biosorption so that it can eliminate the level of Cd in the growing media until it is completely gone until day 30.
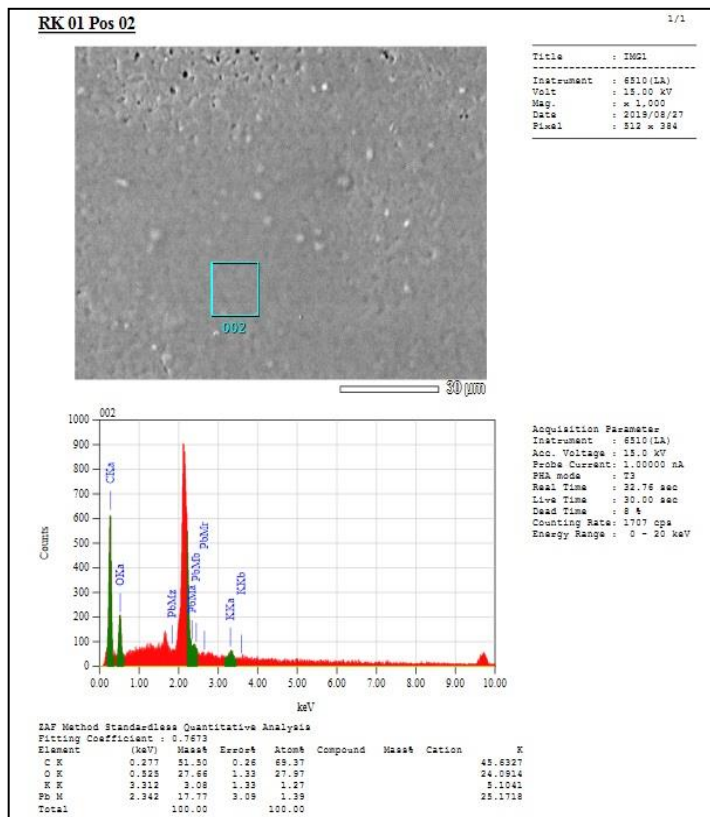

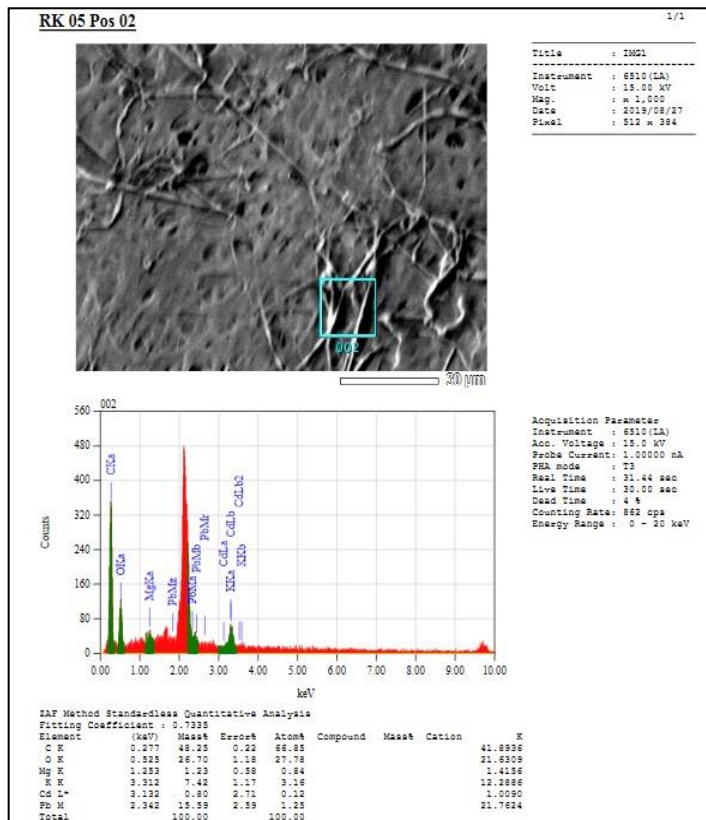*Figure 4 Analysis result of SEM and EDS of Flavobacterium sp bacteria after exposure to Pb metal in the growing media*

Figure 5 The analysis result of SEM and EDS of Flavobacterium sp bacteria after exposure to Cd metal in the growing media

4. CONCLUSION

Flavobacterium sp bacteria is able to absorb Pb and Cd metals up to a concentration of exposure of 10 ppm. The maximum Pb biosorption on Flavobacterium sp is 96% with a concentration of 2 ppm on day 18. The maximum biosorption of Cd in Flavobacterium sp is 77% with a concentration of 10 ppm on day 24. SEM photo shows that Flavobacterium sp has increased levels of Pb and Cd heavy metals after being exposed to these metals for 30 days with an increase of < 1.5% in Pb metals and < 0.5% in Cd metals.

5. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

6. ACKNOWLEDGMENT

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