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The Role of Coenzymes on Mercury (Hg^{2+}) Bioremediation by Isolates *Pseudomonas aeruginosa* KHY2 and *Klebsiella pneumonia* KHY3

Liswara Neneng *, Yohanes Edy Gunawan

Biology Education Study Program, University of Palangka Raya, Palangka Raya, Indonesia

ABSTRACT

Mercury pollution is dangerous to health. Previous research was found two potential Gram-negative bacteria for mercury bioremediation, from gold mining in Central Kalimantan, Indonesia. These isolates were identified as *Pseudomonas aeruginosa* KHY2 and *Klebsiella pneumonia* KHY3. Mechanisms of mercury bioremediation had not known yet by these isolates. This study purposed to test the role of coenzymes on mercury bioremediation by these isolate and to determine the coenzymes best level of mercury bioremediation. Experimental design was Completely Randomized Design in a laboratory. Treatment factors were coenzymes obtained from vitamins B1, B6, B12, with 6 levels of treatments, included 1 control. All treatments were done in Luria Broth media that contain 12 ppm of mercury. Mercury was measured by AAS Shimadzu AA-6200. The results showed that coenzymes effect was very significant to improve mercury bioremediation by P. aeruginosa KHY2 and K. pneumonia KHY3. Supplementation of vitamin B12 in culture media, more enhance of mercury bioremediation compared with vitamin B1 and B6. These result above, indicated the mechanism of mercury bioremediation in both isolates, were the enzymatic process.

Keywords: *Coenzymes, mercury bioremediation, Pseudomonas aeruginosa KHY2, Klebsiella pneumonia KHY3*

INTRODUCTION

Mercury is the poisonous substance in nature [1]. This compound is very poisonous for biologic system [1, 2]. The contamination of mercury could be decreased by bioremediation method because this method proved more efficient and economic than physical or chemical ways [3, 4]. Organisms respond to heavy metal stress using different defense systems, such as exclusion, compartmentalization, formation of complexes and synthesis of binding proteins like metallothioneins [5]. Bacteria uses the intracellular mechanism in mercury detoxification process, by reducing the Hg^{2+} to nontoxic Hg^0, by a group of mercury reductase enzyme that incorporated in the mer operon. Hg^0 formed then diffuses out of the cells [6]. Structure of mer operon is varied, consists of genes that encode functional proteins for regulation (*merR*), transportation (*merT*, *merP* and or *merC*, *merF*) and for reduction (*merA*). In species with broad-spectrum resistance, *merB* genes, these gene required resistance to organomercury compounds, such as methyl mercury and phenylmercury, by hydrolysis of the C-Hg bond before reducing Hg^{2+}. Additional genes are located in the downstream of the gene in the operon *merA*. *merB* gene is rarely found in Gram-negative bacteria [7-8].

In many Gram-negative bacteria, bioremediation is enzymatic process. As far as known mechanism of mercury bioremediation by these bacterial isolates can occur due to: 1) reduction of mercury by mercury reductase enzyme works continuously; 2) occurrence of methylation and demethylation process; 3) establishment of hydrogen sulfide (H_2S) under aerobic conditions, can precipitate dissolved Hg^{2+} ions into insoluble HgS [7, 8]. All these processes require the enzymes, that act as biological catalysts.

Previous research was found two potential Gram-negative bacteria of mercury bioremediation, from gold mining in Central Kalimantan, Indonesia. The identification result by Kit Microbact System, the isolate KHY2 was 99,53% similar to *Pseudomonas aeruginosa*, and

*Corresponding author:
Liswara Neneng
Biology Education Study Program, University of Palangka Raya
Jalan Yos Sudarso C-11, Palangkaraya, Indonesia 73112
E-mail: liswara.neneng@yahoo.com

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KHY3 was 80,58% similar to *Klebsiella pneumonia*. These isolates were gram-negative bacteria [9]. Mechanism of mercury bioremediation by these isolates, still not yet known. This study purposed to test the role of coenzymes on mercury bioremediation by these isolates, and to determine the coenzymes best level of mercury bioremediation in these isolates.

MATERIALS AND METHODS

Mercury preparation

A stock solution of 1000 ppm Mercury (Hg) from Merck was dissolved by using an HNO_3.

Coenzymes preparation

Coenzymes derived from the solution of vitamins B1, B6, and B12. Five of coenzymes level concentration were: vitamin B1 (1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, 5000 ppm), vitamin B6 (16 ppm, 32 ppm, 64 ppm, 128 ppm, 256 ppm), vitamins B12 (8 ppm, 16 ppm, 32 ppm, 64 ppm, 128 ppm), and control (without coenzyme). Those level determined by a pre-experiment result. Then, coenzymes were sterilized by filter sterilization.

Bacterial and growth culture preparation

The isolates of *P. aeruginosa* KHY2 and *K. pneumonia* KHY3 were obtained from water samples around gold mining area in Kahayan River, Central Kalimantan. Both isolates grew in Luria Broth (LB) Media. Hg solution then added in LB media until 12 ppm concentration. Coenzymes in right doses then added to each treatment tube. Finally, inoculation of *P. aeruginosa* KHY2 and *K. pneumonia* KHY3 in each tube. Incubation in room temperature for 2 × 24 hours.

Experimental models

Experimental design is Completely Randomized Design. The treatments were: 1) Types of microorganisms for mercury bioremediation, 2) Levels of coenzymes concentration: The addition of vitamin B1 in cultured starting from level 1000 ppm up to 5000 ppm. Vitamin B6 from level 16 ppm up to 256 ppm, and treatment of vitamin B12 from level 8 ppm up to 128 ppm. The effects of coenzymes on an activity of mercury bioremediation process by these isolate, measured by Atomic Absorption Spectrophotometer (AAS) Type Shimadzu AA-6200.

Analysis of mercury bioremediation effectivity

The effectivity of mercury bioremediation was measured by compared concentration of mercury in LB medium before and after inoculated with bacteria. Measurement using AAS.

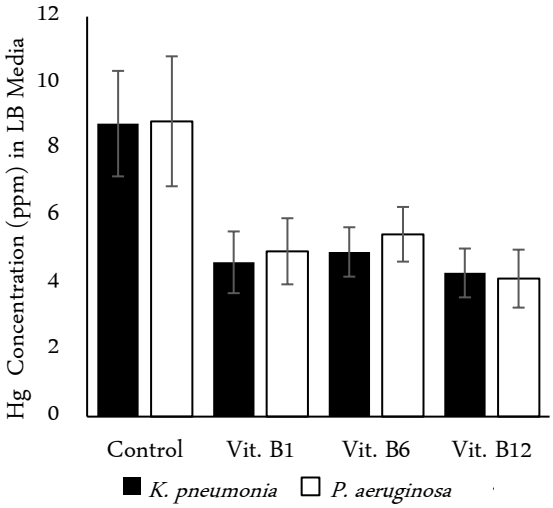

Figure 1. The Effect of Coenzymes on Mercury Bioremediation by *P. aeruginosa* KHY2 and *K. pneumonia* KHY3

Data analysis

Analyses of variance were calculated for each measuring date. All statistical calculations were carried out using the statistical-analysis software *SPSS version 16.0* for *Windows*.

RESULTS AND DISCUSSION

Effect of coenzymes on mercury bioremediation by P. aeruginosa KHY2 and K. pneumonia KHY3

Treatment with coenzymes on mercury bioremediation by isolates *of P. aeruginosa* KHY2 and *K. pneumonia* KHY3 showed decreasing mercury concentration more than 60% in liquid media from initial mercury concentration at 12 ppm, compared to the control. The effectivity of Hg bioremediation in *P. aeruginosa* KHY2 and *K. pneumonia* KHY3 increased by coenzymes (Figure 1).

These finding result supported by ANOVA test, which showed coenzymes (vitamins B1, B6, and B12), had very significant effect on mercury bioremediation in *P. aeruginosa* KHY2 and *K. pneumonia* KHY3.

Bioremediation is effective cost and nature-friendly biotechnology that is supported by microbial enzymes. The main process of bioremediation depends to microorganisms enzymatically attack the pollutants and convert them into innocuous products [10]. Basic of biochemical resistance to inorganic mercury compounds such as HgCl2 looks same on several different species.

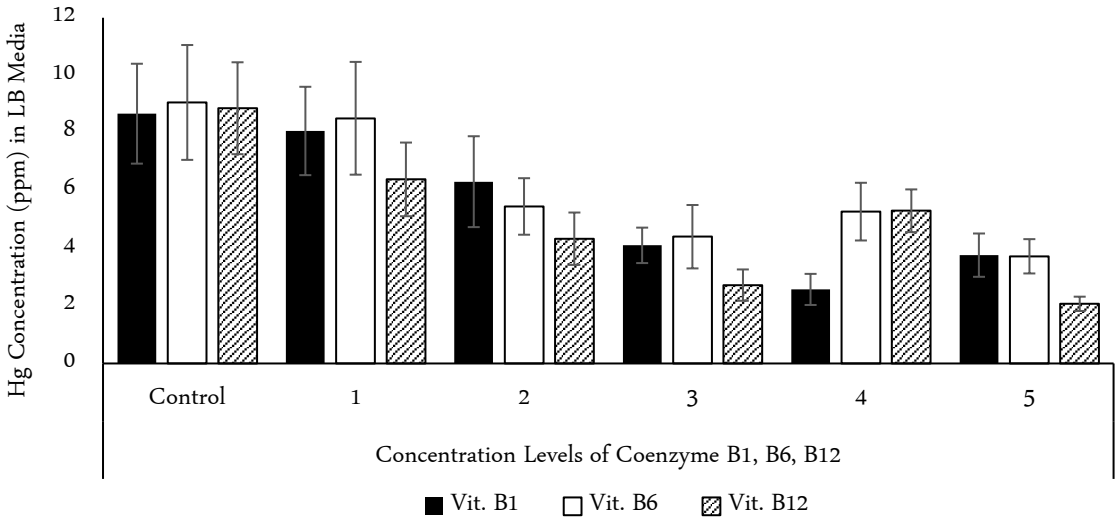

Figure 2. The Effect of coenzymes on mercury bioremediation by *P. aeruginosa* KHY2

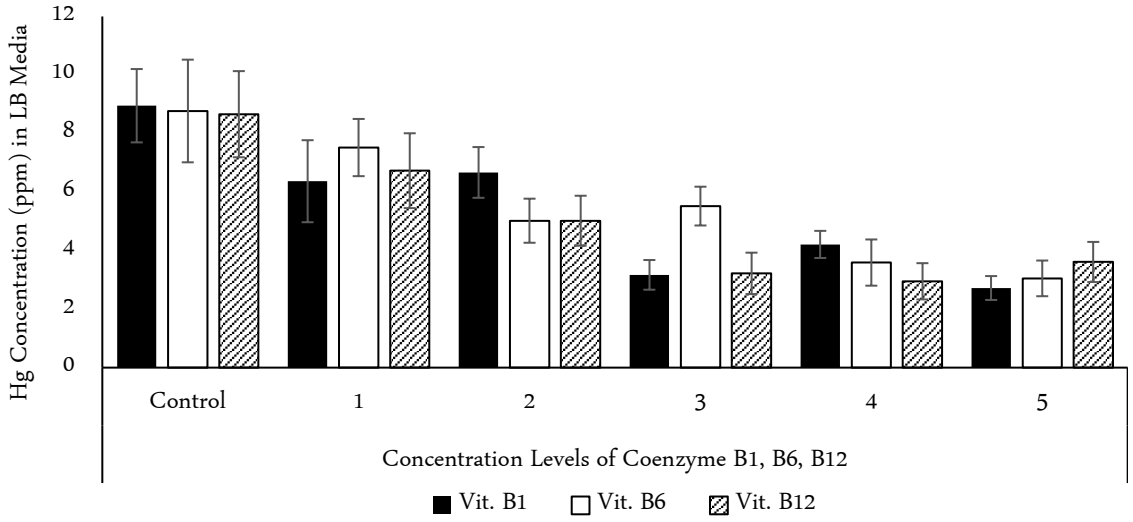

Figure 3. The Effect of Coenzymes on Mercury Bioremediation by *K. pneumonia* KHY3

The mechanism includes reduction of Hg^{2+} to Hg^0 volatile by mercury reductase enzyme. The enzyme is a flavoprotein, which catalyzes the NADPH-dependent reduction of Hg^{2+} into Hg^0 [7].

The effect of coenzymes treatment on mercury bioremediation by P. aeruginosa KHY2

The supplementation of vitamin B12 in the culture medium of *P. aeruginosa* KHY2, average more enhanced of mercury bioremediation, compared than vitamin B1 and B6. Based on LSD result, known the treatment with coenzymes in different concentration level, had a significantly different effect on mercury bioremediation by *P. aeruginosa* KHY2. Coenzyme best treatment based on the result of LSD is the addition of vitamin B12 in concentration 128 ppm (Figure 2).

Coenzyme is an organic molecule that is a non-protein cofactor of the enzyme, which is required for catalytic function. Coenzymes consist of small organic molecules, which are found in vitamins. The series of vitamin B components comprise of coenzymes [11].

Many microorganisms avoid Hg toxicity by reducing ionic Hg (Hg^{2+}) into volatile Hg^0, a potential application use to remove Hg from the Hg-contaminated water. The reduction of Hg^{2+} to Hg^0 can mediate by a number of microorganisms, including enteric bacteria *Pseudomonas*. The ability of bacteria to reduce Hg2+ is linked to Hg resistance (mer) operon. In the cytoplasm, Hg^{2+} is reduced to Hg^0 by a soluble FAD-containing mercuric reductase [12].

P. aeruginosa NRRL B-30604 has been able to degrade a variety of PCB congeners including a complete

degradation of CB-126 and CB-181. This culture was able to remove over 70% Cd from growing media when supplemented with 100 ppm Cd. [13]. Other strains of *P. aeruginosa*, namely, isolate CH07 and isolate Bro12 from marine sediment successful run more than one month, the bioreactors are able to retain the toxic metal, which resulted recovery of approximately 64% from mercury influent. Mercury removal rate was the highest in are action with 1 ppm Hg^{2+}, despite of the efficiency was relatively good up to 8 ppm Hg^{2+}, in the normal M9 medium [14].

The effect of coenzymes on mercury bioremediation by K. pneumonia KHY3

The result of ANOVA test showed the treatment with coenzymes had very significant effect on mercury bioremediation by *K. pneumonia* KHY3. The treatment by vitamin B1 at level 5000 ppm, showed the highest of decreased mercury concentration in LB media, compared with other treatments in *K. pneumonia* KHY3. Mercury decreased below on this level is resulted by treatment of 64 ppm vitamin B12 (Figure 3).

In general, enzyme activity influenced by the available coenzyme or cofactor. Coenzyme is an organic molecule which is a non-protein cofactor of the enzyme, that required for its catalytic function. Enzyme cofactor although in small numbers in the cells, but essential to some enzymes, and important in cell metabolism. There are several types of enzymes whose activity depends on the coenzymes and cofactors, but there are also several enzymes that can work without coenzymes and cofactors [15].

Based on the results of this research, there are strong indication that the mechanism of mercury bioremediation occurred in both isolated *P. aeruginosa* KHY2 and *K. pneumonia* KHY3 were an enzymatic reaction because it required coenzymes to improve their mercury bioremediation process.

CONCLUSION

From this research, it can be concluded that coenzymes had a significant effect on mercury bioremediation by isolates P. aeruginosa KHY2 and K. pneumonia KHY3.

Coenzyme form vitamin B12 more improved effectivity of mercury bioremediation in *P. aeruginosa* KHY2, while in *K. pneumonia* KHY3 more effective with supplementation of vitamin B1. Coenzyme best treatment based on the result of the LSD in these isolate is the addition of vitamin B12 in concentration 128 ppm.

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