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Research Article

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Application of Superior Mercury Resistant Bacteria as a Mercury Remediator on Small Scale Soil using Simple Open and Closed Bioreactor

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ABSTRACT

This study aimed to apply superior mercury resistant bacteria isolate that has been evaluated on a prior study. This experimental study was done in laboratorium for the research preparation, and in green house for application isolate bacteria using simple open and closed bioreactor. Observation was done on day thirty and sixty after incubation. The result shows application of superior mercury resistant bacteria isolate on a small scale with ex-situ using simple open bioreactor could lower mercury level from 200 ppm to 0.08-4.08 ppm or about 97.96-99.96% until day 60 (2 months) after incubation. The best isolate in lowering mercuty is MRB5. Meanwhile, application of superior mercury resistant bacteria isolate on a small scale with ex-situ using simple closed bioreactor could lower mercury level from 200 ppm to 0.18-11.03 ppm or about 94.49-99.91% until day 60 (2 months) after incubation. Isolate MRB5 is the best isolate used in the research.

Keywords: Bioremediation; Gold mining; Mercury resistant bacteria; *Ex-situ*; Bioreactor

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INTRODUCTION

Gold mining near civilization, river, or rice fields quite raise some concerns. However, like two sides of a coin, gold mining is one of the occupations for some people, however, on the other hand, this activity could also lead to serious problems related to many mining done without legal consent (illegal mining). The most concern thing about illegal mining is the use of mercury without supervision, hence there is no definite information about the amount of mercury used in the field. The contamination of mercury could lead to various problems in environment and human well-being. Mercury in the form of Hg^{2+} is very reactive, if accumulated in soil and Hg^{2+} sediment bonded with CH_3 from organic materials could result in methyl mercury thus increasing the toxicity, biomagnification, and becoming lipophilic. Methyl mercury is also carsinogenic, teratogenic, and mutagenic. The second concern about illegal mining is post gold mining, the former land of gold mining is left behind, without field remediation force, so it could decrease the field function, and also decrease aesthetic value of the environment. Field contamination by mercury could be caused by mining activity or industrial activity. Field contamination by mercury is a serious problem that needs to be taken care of soon [1-6]. The remediation effort in former mining soil to anticipate hazard from mercury by using bacteria is known as bioremediation technique. Bacteria isolate that is usually used is mercury resistant bacteria that could reduct Hg^{2+} catalyzed by reductase mercury to form Hg^0 easily vaporized and less toxic, so it is safe for the environment [7,8]. The prior research has found five superior mercury resistant isolate from former gold mining soil. The ability of the five isolates in reducing mercury in liquid medium nutrient broth contained $HgCl_2$ in laboratory scale found that all five superior mercury resistant bacteria isolates could decrease mercury 70.69-85.44% in $HgCl_2$ 150-250 ppm [3].

This study is a second research following the prior research which will apply three superior mercury resistant bacteria isolate to bioremediate *ex-situ*. The aim of this study is to establish the ability of three superior resistant bacteria isolates chosen to be applied as bioremediation agent in mercury-contaminated soil and to establish the best bioreactor type used to apply the three superior mercury resistant bacteria isolates in rescuing soil, marine, and biota inside, along with the citizens around against mercury.

EXPERIMENTAL SECTION

This experimental study applied three superior resistant bacteria isolates (isolate MRB3, MRB5, MRB6) in mercury-contaminated soil sample by using simple open and closed bioreactor in the green house. The observation is taken on day thirty and sixty after incubation. The equipments used in this study is: simple open bioreactor, simple closed bioreactor, sacks for soil sample, plastic sample, petri dish, test tube, micropipet, microtip, aluminium foil, wrap plastic, Erlenmeyer, pincet, ose needle, Bunsen, cotton, kassa, equipment box, plastic container, tissue, beaker, autoclaf. The materials used in this study are as follow: medium nutrient agar (NA), sterile aquadest, mercury ($HgCl_2$), alcohol, and spiritus.

The steps of this study:

- Compose nutrient broth medium modified with $HgCl_2$ 200 ppm.
- Prepare the soil sample to apply bacteria isolate.
- Rejuvenate superior mercury resistant bacteria isolate using medium NA and NB modification.
- Make the bacteria inoculum in modified NB medium.
- Apply bateria isolate *ex-situ* by using simple open and closed bioreactor observed on day 30 and 60 after incubation.
- Analyze mercury decrease in soil sample by using inductively couple plasma (ICP) 9000 Shimadzu.

Principles: Hg in the soil is extracted with wet vaporization using concentrated nitrat acid. Metal concentration is measured by ICP spectrophotometry. Calculation: the ICP extract is measured with standard each logam as the comparison.

RESULTS AND DISCUSSION

From the application of three superior mercury resistant bacteria isolates (isolate MRB3, MRB5, MRB6) in mercury-contaminated soil sample using simple open and closed bioreactor and the result showed as follow:

Application of superior mercury resistant bacteria isolate *ex-situ* using simple closed bioreactor

The three superior mercury resistant bacteria isolates applied in mercury-contaminated soil using simple open bioreactor, analyzed on day thirty after incubation is shown on Figure 1.

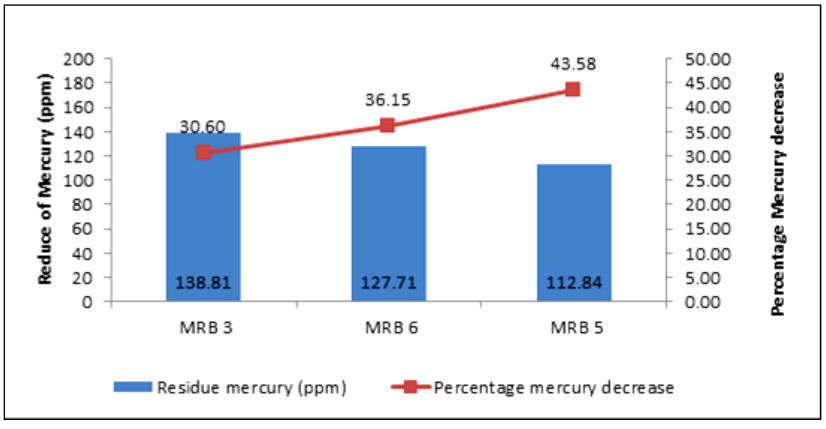

Figure 1: Residu mercury and percentage mercury decrease on day thirty after incubation using superior mercury resistant bacteria isolate *ex-situ* with simple open bioreactor

Figure 1 showed on day thirty after incubation superior mercury resistant bacteria isolate on simple open bioreactor, there is a decrease of mercury in the soil. This trend occurs from 200 ppm to 112.84-138.81 ppm. This decrease

showed that superior mercury resistant bacteria isolate work well in degrading mercury. Mercury decrease on day thirty after incubation is around 30.60-43.58%. This showed a good improvement since there is certain difficulty faced in the field to apply bacteria isolate which is very complex. One of the contributing factor affecting the successful rate of remediation process is temperature, weather, and the isolate itself. On day sixty (two months) after incubation, the decrease of mercury showed a very good result, as seen in Figure 2.

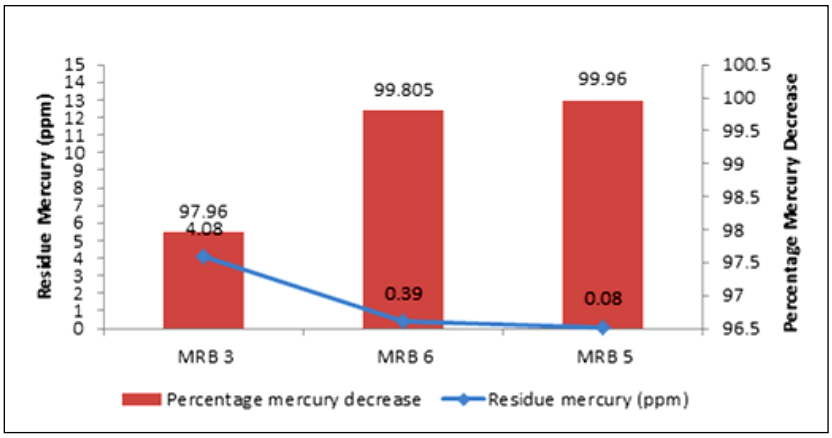

Figure 2: Residu mercury and percentage mercury decrease on day sixty (two months) after incubation using superior mercury resistant bacteria isolate *ex-situ* with simple open bioreactor

Figure two showed that the residu mercury in soil sample is around 0.08-4.08 ppm. This marked that the mercury remediation process is taking place well. This also means percentage mercury decrease more than ninety percent, which is approximately 97.96-99.96%. Mercury contaminated soil remediation using superior mercury resistant bacteria isolate with simple open bioreactor, on observation day thirty after incubation and day sixty (two months) after incubation showed that reside mercury and percentage mercury decreased is best achieved by isolate MRB 5, followed by MRB 6 and MRB 3.

Application of superior mercury resistant bacteria isolate *ex-situ* using simple closed bioreactor

Application of superior mercury resistant bacteria in mercury-contaminated soil, in single culture and consorsium with simple closed bioreactor, after analyzed on day thirty showed the result as seen in Figure 3.

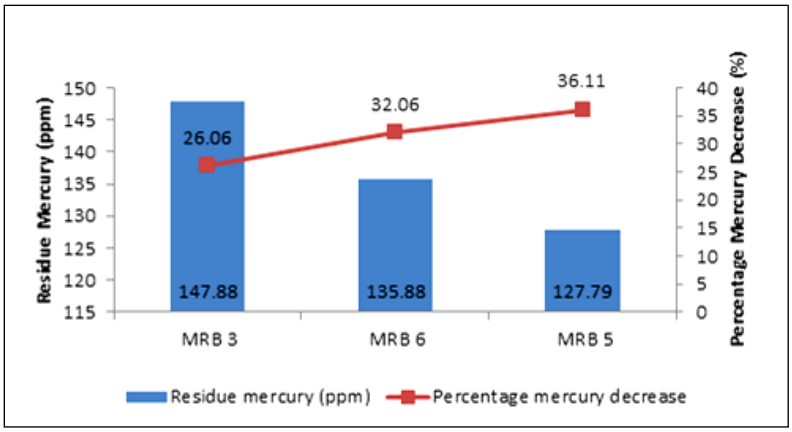

Figure 3: Residu mercury and percentage mercury decrease on day thirty after incubation using superior mercury resistant bacteria isolate *ex-situ* with simple closed bioreactor

In Figure 3, the application of superior mercury resistant bacteria isolate *ex-situ* with simple closed bioreactor on day thirty after incubation showed there is a decrease of mercury in the soil. This means that the bacteria isolate has worked well. The decrease of mercury from 200 ppm to 127.79-147.88 ppm. This decrease showed that superior

mercury resistant bacteria worked well in degrading mercury in the soil. The decrease of mercury in the soil analyzed on day thirty after incubation reached 26.06-36.11%. This improvement showed that superior mercury resistant bacteria could also work well in remediating mercury in closed bioreactor. The decrease of mercury on day sixty, (two months) after incubation with closed bioreactor showed a very good result as seen in Figure 4.

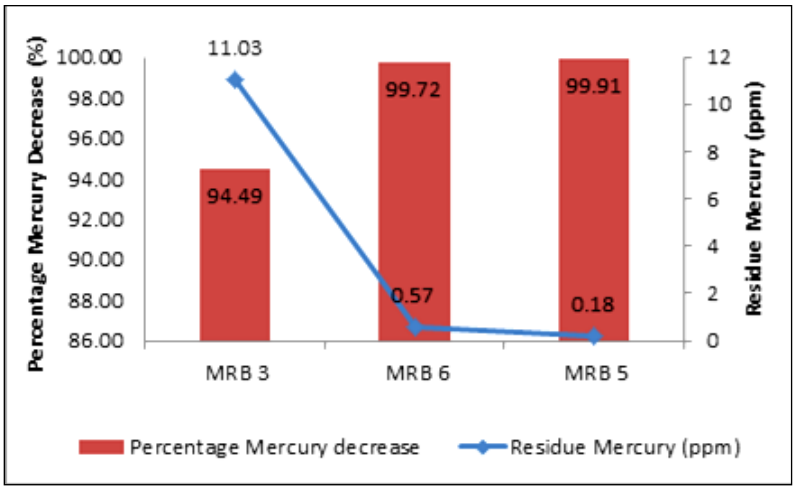

Figure 4: Residu mercury and percentage mercury decrease on day sixty (two months) after incubation using superior mercury resistant bacteria isolate ex-*situ* with simple closed bioreactor

Figure 4 showed the residu mercury in soil sample is approximately 0.18-11.03 ppm, while the decrease percentage of mercury is also more than ninety percent, approximately 94.49-99.91%. This showed that the mercury remediating process has done well. The same goes to the use of that superior mercury resistant bacteria using simple open bioreactor, the use of that superior mercury resistant bacteria with simple closed bioreactor to remediate mercury-contaminated soil on day thirty after incubation and day sixty (two months) after incubation showed there is residu mercury and the best percentage mercury decrease achieved by isolate MRB6 and MRB3. Overall, the remediation of mercury-contaminated soil with that superior mercury resistant bacteria MRB3, MRB5, and MRB6 with simple open and closed bioreactor observed on the last day, day sixty (two months) after incubation showed that the use of open bioreactor give a better result in degrading mercury in sample soil compared with simple closed bioreactor, as also seen in Figure 5.

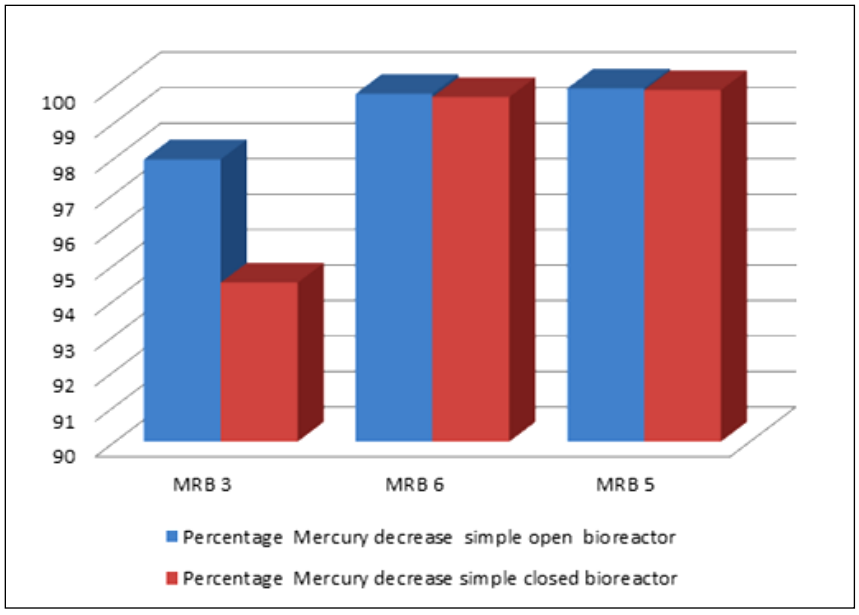

Figure 5: The comparison of superior mercury resistant bacteria using simple open and closed bioreactor on the mercury decrease percentage on day sixty (two months) after incubation

This shows that the isolates used are proven superior than the laboratorium scale before, and when applied in the field with contaminated soil also showed a good work. The mercury decrease percentage is approximately >90%. Figure 5 also showed that the application of superior mercury resistant bacteria on day sixty (two months) after incubation using simple open and closed bioreactor showed the mercury decrease percentage greater found on isolate MRB5 followed by MRB6 and MRB3. This might due to the degradation done by bacteria isolate, environmental factor, like photolysis, temperature, and humidity. The isolate capability in decreasing mercury concentration is due to the appropriate adaptation genetically and physiologically [4]. Mercury resistant bacteria isolate has gene mer operon [1,7]. Structure mer operon is different in various types of bacteria. Generally, mer operon structure consists of gene metalloregulator (merR), gene transport (merT, merP, merC A) and gene mercury reductase (merA) and organomercury liase (merB) so it is very potential as remediation agent in mercury contaminated soil [2,5]. Some other remediation techniques that have been applied are as follow: stabilization/solidification, immobilization, vitrivication, thermal desorption, nanotechnology, soil washing, electro-remediation, phytostabilization, phytoextraction and phytovolatilization [6].

CONCLUSION

All three superior mercury resistant bacteria could be used as bioremediation agent in mercury contaminated soil with capability more than 90% in decreasing mercury in the soil.
Application superior mercury resistant bacteria from pilot project *ex-situ* with simple open bioreactor give the result better than simple closed bioreactor.

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