The Potential of Mercury-Resistant Bacteria Isolated from Small-Scale Gold Mine Tailings for Accumulation of Mercury

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ABSTRACT

In certain small-gold mining activities in West Lombok, Indonesia, the tailings containing mercury are discharged to agricultural lands, reducing their productivity. One of the efforts to restore the land is by bioremediation of mercury, using mercury-resistant microbes. This study was aimed to isolate the mercury-resistant bacteria from small-scale gold mine tailings containing mercury, and to test their capability in accumulating mercury. Bacterial isolation and identification were conducted from nutrient broth supplemented with 5 ppm $HgCl_2$. The isolated bacteria were tested for mercury accumulation in the nutrient broth containing 10, 20 and 30 ppm Hg for 24 hours, and in small-scale gold mine tailing containing 41.37 ppm Hg for 2 weeks. The results showed that there were four pure isolates of mercury-resistant bacteria which were identified as *Brevundimonas vesicularis, Nitrococcus mobilis, Fusobacterium aquatile* and *Fusobacterium necrogenes*. The highest Hg accumulation from nutrient broth liquid media containing 10, 20 and 30 ppm Hg was observed for *Brevundimonas vesicularis*. The mercury accumulation efficiency of the four bacteria applied to small-scale gold mine tailing containing mercury was in the order of *Fusobacterium aquatile* (76.1%) > *Brevundimonas vesicularis* (75.6%) > *Fusobacterium necrogenes* (74.4%) > *Nitrococcus mobilis* (74.2%). On the basis of the Hg accumulation efficiency of more than 75%, *Fusobacterium aquatile* and *Brevundimonas vesicularis* are prospective for bioremediation of mercury-contaminated soils.

Keywords: mercury-resistant bacteria, small-scale gold mine tailing

INTRODUCTION

Gold mining in Indonesia consists of large-scale gold mining, medium-scale gold mining, and artisanal and small-scale gold mining (ASGM). In almost all ASGMs, the amalgamation process using mercury followed by cyanidation process is the method used to recover gold (Veiga et al., 2006). Amalgamation with mercury is a simple process and requires only a small investment. The mercury recovered from the amalgamation process is always reused in subsequent amalgamation processes. Tailings of the amalgamation and cyanidation processes in the form of sludge that still contain Hg, Au and various other heavy metals, are generally discharged to the nearby agricultural land and water bodies. In every gram of gold produced, there are about 1-3 g Hg released into the air, and partly released into the water, together with mud (Telmer, 2007). The results of a survey conducted by Krisnayanti et al. (2012a) at ASGM locations in Sekotong District of West Lombok showed that the amalgamation process tailing still contains 3002 mg Hg kg⁻¹, on average. This high content of mercury in the waste of gold treatment process led to the increasing content of Hg in the soil contaminated with small-scale gold mining waste. The results of the analysis carried out by soil laboratory of Mataram University showed that the mercury levels in the soils of Sekotong District of West Lombok ranged from 25 mg / kg to 40 mg kg⁻¹, while the mercury content in the seeds of maize and rice grown in the location of tailing disposal was around 0.20

mg kg^{-1} (Krisnayanti et al., 2012b). According to the Indonesian Ministry of Environment, no. 82 year 2001, the mercury threshold level in water and soil is 0.001 ppm. The discharge of tailings of amalgamation process which still contain very high concentrations of mercury in the agricultural lands, can disturb the growth and production of crops as well as harm human health through the consumption of the food produced from heavy metals-contaminated soil (Subowo et al., 2007). The plants that are poisoned by mercury will show the symptoms of chlorosis, brown plant root, root hood damaged, as well as decreased number and size of roots (Patra and Sharma, 2000). In addition to adverse affects to crops, mercury compound in the form of Hg (II) can be bound to the cysteine residues of human proteins so that proteins will lose their activity. In addition to Hg (II), the mercury compound that is most harmful to human health is an organic mercury compound; in particular, methyl mercury and phenyl mercury that are highly reactive and can attack the human nervous system through the bloodstream (Rasmussen et al., 2008). In relation to the above-mentioned matters, efforts are needed to detoxify mercury in the gold-mine tailings prior to the discharge to agricultural land in order not to hamper the plant growth and human health. One of efforts is the bioremediation of mercury using mercury-resistant microbes. Mercury can indeed kill some types of microbes, although there are some bacteria that are able to survive in an environment contaminated with mercury. The bacteria have a mechanism of resistance to the mercury contamination (Nascimento and Chartone-Souza, 2003). Some of the heterotrophic bacteria are resistant to mercury, like *Pseudomonas*, *Psychrobacter spp.* (Pepi et al., 2011), *Nitrosomonas eutropha, Ralstonia eutropha, Sphingomonas* sp. *and Actinobacteria* (Rasmussen et al., 2008). Bacterial resistance to mercury is the first step of the detoxification process. Detoxification of mercury, especially methyl mercury, is generally preceded by demethylation. Methyl mercury is demethylated into Hg^{2+} ion that is then later reduced to Hg^{o} that is volatile and less toxic (Barkay, 1992; Chang et al., 1998; Misra, 2000; Nascimento and Chartone-Souza, 2003). The mercury conversion process involves an enzyme complex comprising organomecurylyase and mercury reductase (Barkay, 1992; Chang et al., 1998; Misra, 2000; Nascimento and Chartone-Souza, 2003). The bacteria that are resistant to mercury are thought to have one or both of these

enzymes allowing the mercury entering through the cytoplasmic membrane into the cell and accumulate. The bacteria that accumulate mercury can be used as biological agents which can be developed to clean up the environment contaminated with mercury or heavy metals. This study was aimed to isolate and identify indigenous bacteria that are able to accumulate mercury from small-scale gold mine tailings in West Lombok.

MATERIALS AND METHODS

Location of bacterial isolation

The study was conducted at microbiology laboratory of Brawijaya University from July to December 2016. The tailings of small-scale gold mining were obtained from the Sekotong District, West Lombok Regency located at 115^{0}46'-116^{0}20'E and 8^{0}25'-8^{0}55 S (Figure 1). The area is located at 400 m above the sea level with average annual rainfall of 1500 mm. Composite samples of gold mine tailings from cyanidation processing facilities were collected from several points representing the tailing disposal areas. The laboratory analysis carried at Soil Science Department of Brawijaya University showed that the tailing samples had the following characteristics: pH 3.9; 0.08% organic C, 0.01% total N, 11.39 ppm available P, and 41.37 ppm total Hg. The value of total Hg exceeded the Indonesian Government threshold level of Hg in water and soil of 0.001 ppm.

Bacterial isolation and identification

Ten grams of tailings were mixed with 90 mL of sterile 0.85% NaCl for 30 minutes. The mixture was then diluted into seven concentrations (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}). From each dilution, 0.5 mL suspension was drawn and poured into a petri dish containing nutrient broth medium that had been supplemented with 5 ppm HgCl$_2$. The culture was then incubated for 48 hours. The bacterial colonies that grew in the medium were then purified to obtain pure isolates. Every isolate grown was then inoculated to the solid nutrient medium using the 16-stroke method and then incubated for 24 hours. Displacements of colony isolates were done three times. The colony parameters under observation were form, surface, edge, and colour of the colonies. Identification of bacteria was based on the system of Bergey's Manual of Determinative Bacteriology. In the

Figure 1. Sampling site of bacteria isolation at Sekotong District of West Lombok

early stages of identification of mercury+resistant bacteria, morphological tests were conducted using gram staining.

Biochemical test of bacterial isolates

The identified bacteria were then subjected to biochemical test that included carbohydrate fermentation, indole formation, H_2S production, motility, oxygen consumption, oxidase fermentation, citrate usage, catalase, and oxidase tests. Carbohydrate fermentation test was conducted using 15% glucose, 0.5% lactose and 0.5% mannitol media that each was dissolved in 100 mL of peptone water-10% NaCl and 1 mL of 0.1% bromthymol blue indicator. The bacterial isolates were inoculated into the fermentation medium and incubated for 24 hours at 37°C. The Indole formation test was performed by inoculating bacteria in a liquid medium of Treptic Soy Broth-10% NaCl and then incubated for 24 hours at 37°C; Kovacs reagent was added afterwards. Positive test result is marked with red colour formation (Cappuccino and Sherman, 2002). Test of production of H_2S and gas was carried out by inoculation of isolates to Triple Sugar Iron Agar -10% NaCl medium and further incubated for 24-48 hours. The positive test of H_2S production is characterized by the formation of black colour on the medium. The positive test of gas formation is characterized by the formation of gas at the bottom of the medium (Cappuccino and Sherman, 2002). Motility of bacteria can be determined from the test of H_2S and gas production. Motility

of bacteria is characterized by bacteria distribution in the former inoculation areas, which means that bacteria were mobile (motile). The test of oxygen demand was carried by inoculating the isolates to a mixture of agar, thioglycollate and NaCl, which was then incubated for 48 hour at 37°C (Cappuccino and Sherman, 2002). The fermentation ability was observed by means of inoculation of bacteria in 10% NaCl oxidase, 1% glucose at 37°C for 24 hours. The positive result is marked by a change in the medium to yellow. The test on the use of citric was conducted by inoculating the isolate in 5 mL of medium Simmons citrate-10% NaCl Agar and Bromthymol blue indicator then incubated at 37°C for 24 hours (Cappuccino and Sherman, 2002). The positive result is characterized by the growth of bacteria and the change of colour of the growing medium from green to blue. For catalase test, a drop of 3% H_2O_2 was placed on top of a sterile glass slide and then the bacterial isolate was aseptically streaked. The positive results are marked by the emergence of gas or air bubbles around the scratches (Harley and Prescott, 2005). For oxidase test, the bacterial isolates were applied to the surface oxidase paper. The positive test result is obtained if the purple colour is formed on the oxidase paper after 5 seconds (Harley and Prescott, 2005).

Preparation of growth curve for starter

Aseptically, a single ose of 24-hours isolate (rejuvenated isolate on solid nutrient agar medium) was inoculated onto 10 mL nutrient broth

(NB) liquid medium in a test tube, and then incubated for 24 hours on a rotary shaker (100 rpm). Afterwards, 5 mL was extracted from the 24-hour culture, and inoculated into 45 mL NB liquid medium with a concentration of 10 ppm $HgCl_2$ in a beaker glass, and incubated for 24 hours on a rotary shaker (100 rpm). One milliliter of culture was then dissolved in 9 mL sterile distilled water and the value of its Optical Density was measured with a spectrophotometer (Boeco S-22, Germany) at a wavelength (λ) 600 nm. The Optical Density measurement was performed for 24 hours at 1-hour interval. The obtained Optical Density data were then used to prepare the growth curve with x axis as time (t) and y-axis as Optical Density. Once the growth phase is known, the determination of starter can be determined, i.e. in the middle of the exponential phase.

Hg accumulation capability test on nutrient broth liquid and tailing media

5 mL of bacterial starter (10^3 cfu mL^{-1}) culture was incorporated into a 250 mL beaker glass containing 50 mL NB+$HgCl_2$ liquid medium. The treated concentrations of $HgCl_2$ used were 10 ppm (5 ppm NB + 0.5 ppm $HgCl_2$), 20 ppm (5 ppm NB + 1.0 ppm $HgCl_2$), and 30 ppm (5 ppm NB + 1.5 ppm $HgCl_2$). After incubation on a rotary shaker (100 rpm) for 24 hours (Badjoeri, 2008), the culture was then centrifuged 4000 rpm for 20 minutes to separate the bacterial cell with the filtrate. The filtrate was placed into a test tube and two drops of HNO_3 were added, and then placed in an autoclave for 30 minutes. Hg accumulation measurement was performed at 24 hours. For Hg accumulation capability test on small-scale gold mine tailing, each bacterial isolate as much as 0.1 mL (10^3 cfu mL^{-1}) was applied to small-scale gold mine tailing and incubated for 4 weeks. Hg accumulation measurements were made 1 week and 4 weeks after inoculation. All Hg measurements were performed using F732-S Cold Atomic absorption Mercury Vapor Analyzer (Shanghai Huaguang Instrument Company). The data obtained were subjected to analysis of variance at 5% significance level.

RESULTS AND DISCUSSION

Isolates of mercury resistant bacteria

The result of isolation of mercury resistant bacteria showed a total population of 4.8×10^8. In the purification stage, there were seven selected isolates, but at the stage of gram staining there were only four pure isolates that grew on media containing 5 ppm Hg. The medium used for the isolation of bacteria from gold mine tailings was nutrient agar that has been supplemented with 5 ppm $HgCl_2$. According to Canstein et al. (2002) the isolation of bacterium isolate that can grow on synthetic media with a minimum of 5 ppm $HgCl_2$ is a highly mercury-resistant bacterium isolate. In addition to the gram staining, the four isolates had different characteristics (colour and form colony shape) (Table 1). The results of biochemical tests of mercury resistant isolates are presented in Table 2.

On the basis of the morphological characteristics and biochemical tests, as well as referring to Bergey's Manual of Determinative Bacteriology, isolate A was identified as *Brevundimonas vesicularis.* Another name of *Brevundimonas vesicularis* is *Pseudomonas vesicularis* (Segers et al., 1994). This bacterium is a gram-negative, rod-shaped, and aerobic bacterium. The diameter of bacterial colony was 2.05 mm. The bacterium that can grow at temperatures of 26°C is motile. The colour of colony of this bacterium is generally orange. *Brevundimonas vesicularis* is capable of hydrolyzing esculin, and also able to assimilate D-glucose, maltose, L-ranose and acetate. *Brevundimonas vesicularis* is included in the protein Oma subclass bacteria that are effective in bio-sorption (Hu et al., 1996). Isolate B was identified as *Nitrococcus mobilis*. This bacterium is a gram-negative, cocci shaped, 2.03 mm diameter colony, colony colour was beige, and grew at a temperature of 25-30°C at pH of 7.8-8. This group of bacteria is chemoautotrophic because it uses inorganic nitrogen compounds in its life cycle (Madigan et al., 2009). *Nitrococcus mobilis* is a nitrifier that can oxidize nitrite to nitrate (HNO_2 + H_2O à HNO_3) (Bartosch et al., 1999). Isolates C and D were identified as *Fusobacterium aquatile* and *Fusobacterium necrogenes*, respectively. *Fusobacterium* is a genus of anaerobic, gram-negative bacterium with rod shape. *Fusobacterium aquatile* can ferment several types of sugar and produces catalase enzymes (Komala et al., 2012). *Fusobacterium aquatile* and *Fusobacterium necrogenes* are able to produce energy from oxidation of inorganic chemicals. The habitat of the bacteria involves soil, waste, aquatic environments, and natural environments that contains sulfur, iron or manganese, such as acid mine water and sulfur hot springs (Madigan and Martinko, 2006).

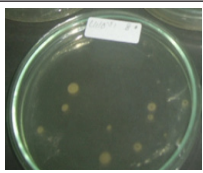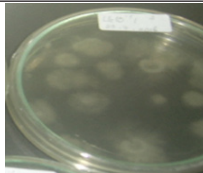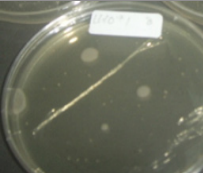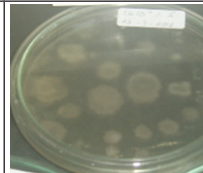**Table 1.** Morphological characteristics of four isolates of mercury resistant bacteria
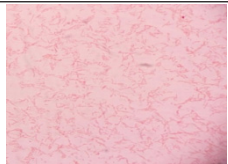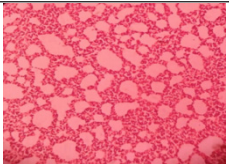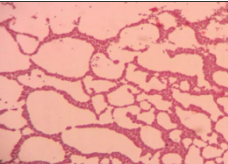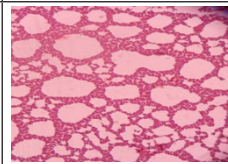
Parameter	Isolates			
	S-1 (A)	S-2 (B)	S-3(C)	S-4 (D)
Colony	Orange	Cream	Cream	Yellow
Colour				
Colony shape	round	round	round	round
Colony diameter (mm)	2.05	2. 03	0. 74	0.67
Colony edge	not flat	not flat	flat	flat
Elevation	convex	convex	convex	convex
Consistency	dry	dry	dry	wet
Gram reaction	negative	negative	negative	negative
Cell shape	rod	cocci	rod	rod

Table 2. Biochemical test on four mercury-resistant isolates

No	Biochemical test	Isolates			
		S-1 (A)	S-2 (B)	S- 3(C)	S-4 (D)
1	Spore	-	-	-	-
2	Oxidase	+	-	-	-
3	Motility	+	-	+	+
4	Nitrate	+	+	+	+
5	Lysine	-	-	-	-
6	Ornithine	-	-	-	-
7	H_2S	-	-	-	-
8	Glucose	-	+	-	-
9	Mannitol	+	-	-	+
10	Xylose	-	-	-	-
11	Indole	-	-	+	-
12	Urease	-	-	+	-
13	Citric	-	-	-	-
14	Gelatin	+	-	-	-
15	Malonate	-	-	-	-
16	Inositol	-	-	-	-
17	Rhamnosa	-	-	-	-
18	Sorbitol	-	-	-	
19	Sucrose	-	+	+	+
20	Lactose	-	-	-	-
21	Arabinose	-	-	+	-
22	Adonitol	-	-	-	-
23	Raffinosa	-	-	-	-
24	Salicin	-	-	+	-
25	Arginine	-	-	-	-
26	Catalase	+	+	-	-
27	Coagulase	-	-	-	-
28	Beta hemolysis	-	-	-	-
29	Starch hydrolysis	+	+	+	+
30	Casein hydrolysis	-	-	-	-

Growth of bacteria

The growth of four isolates of mercury-resistant bacteria (*Brevundimonas vesicularis, Nitrococcus mobilis, Fusobacterium necrogenes* and *Fusobacterium aquatile*) for 58 hours of incubation showed a uniform pattern (Figure 2). The stationary phase that was the best time for the production of bacterial cell numbers of *Brevundimonas vesicularis* and Nitrococcus *mobilis*, was at 36 hours, while that of *Fusobacterium necrogenes* and *Fusobacterium aquatile* was at 42 hours (Figure 2). The stationary phase difference presented in Figure 2 was probably due to the resistance mechanism of each of the different bacterium. The bacterial growth is the addition of cells and the ability of bacteria to breed that can be illustrated with a growth curve (Cooper, 1991). The growth curve depicts the pattern of bacterial growth that can be divided into four phases: lag, exponential, stationary, and death (Harley and Prescott, 2005). The growth curve corresponds to the bacterial growth curve, which is generally in the form of a sigmoid curve (Harley and Prescott,

2005). The starter lifetime can be determined from the exponential mid-phase. According to Hogg (2005), at the exponential phase, microorganism cells are in stable state, new cells are formed at a constant rate and microorganism cells divide optimally during the doubling time so that it is usually reached in the middle of an exponential phase.

The presence of the four identified bacteria (*Brevundimonas vesicularis, Nitrococcus mobilis, Fusobacterium aquatile* and *Fusobacterium necrogenes*) in the environment containing mercury is because the bacteria have the genes that are resistant to mercury (Mer Operon). Highly mercury-resistant bacteria have *mer* operon that encodes flavoenzime, a mercury reductase that can reduce Hg^{2+} ion to the less toxic Hg^0 (Vetriani et al., 2004). The mercury resistant gene is generally composed of a metal regulator gene (MerR), a mercury transport gene (MerT, MerP, MerC), a reductase mercury gene (MerA) and an organo mercurylyase (MerB) (Brown et al., 2002; Nofiani and Gusrizal, 2004). The results of a study reported by Lima de Silva et al. (2012)
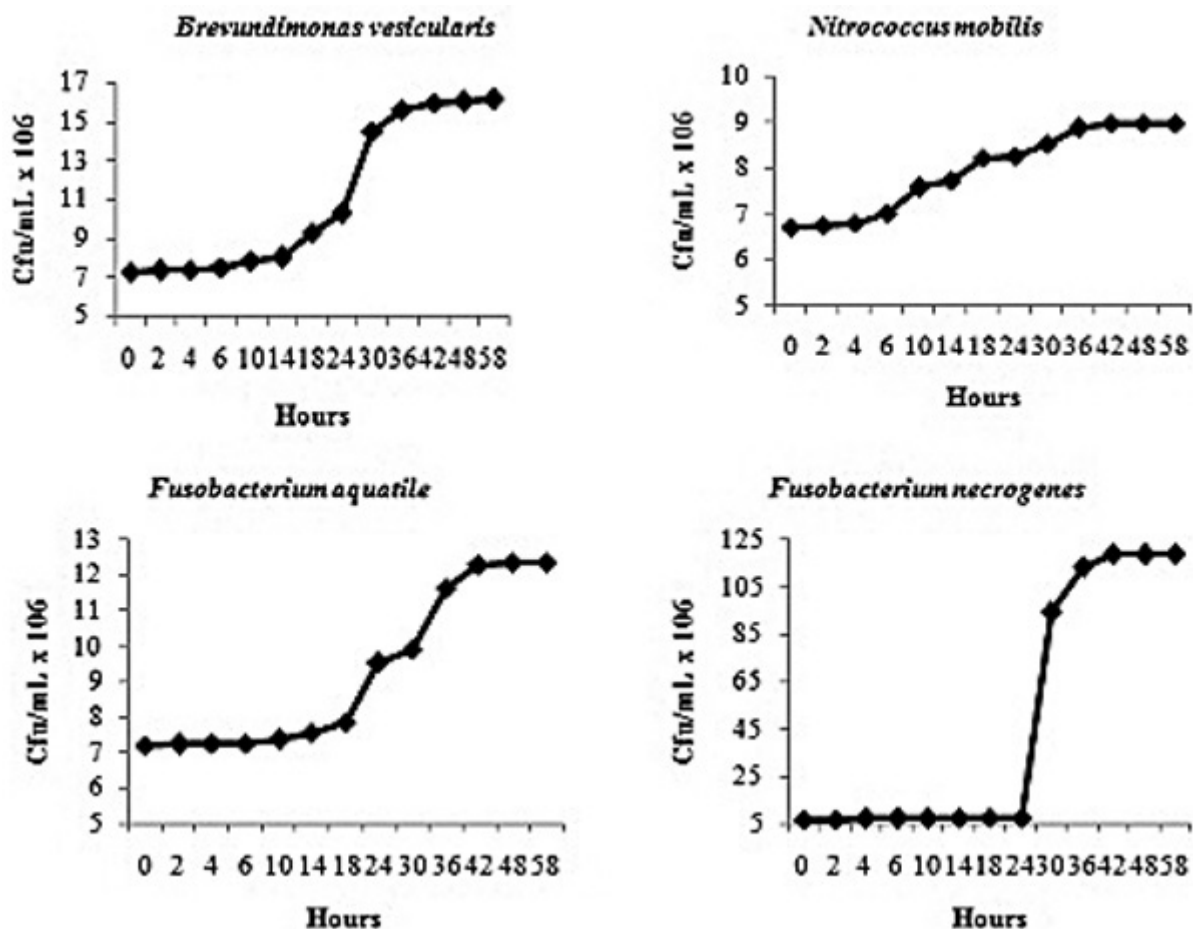

Figure 2. Growth of four isolates of mercury-resistant bacteria

showed that *Brevundimonas vesicularis* was one of strains found in the media containing mercury ($HgCl_2$). Irawati et al. (2012) reported that *Brevundimonas sp.* accumulated Hg^{2+} up to 1.09 and 2.7 mg g^{-1} dry weight of cells and removed 64.38 and 57.10% Hg^{2+} from the medium containing 50 and 100 ppm $HgCl_2$, respectively.

Mercury accumulation from nutrient broth liquid media

After incubation for 24 hours, the highest Hg accumulation was observed for bacteria A (*Brevundimonas vesicularis*), i.e. 1.68, 2.08, and 2.17 ppm Hg at 10, 20, and 30 ppm Hg treatments, respectively (Figure 1). Meanwhile, bacteria D (*Fusobacterium necrogenes*) performed the lowest Hg accumulation at all treatments (Figure 3). In general, bacteria A (*Brevundimonas vesicularis*) accumulated Hg higher than other bacteria at all treatments of Hg concentrations, except for *Nitrococcus mobilis* and treatment Hg concentration treatment of 30 ppm Hg. However, the difference of Hg accumulation by *Brevundimonas vesicularis* and *Nitrococcus mobilis* at the treatment of 30 ppm Hg was not significant. The decrease of Hg concentration in nutrient broth media is because the organic and inorganic materials in the medium are able to bind or react with different types of metals. Some organic compounds, such as polysaccharides, humic or protein can react with metal ions by redox or binding reactions that form metal-organic complexes, but these bindings are influenced by environmental factors (Buffle et al.,

1994). The decrease of mercury concentration in the cultures containing mercury-resistant bacteria occurs also because the bacteria have cytosolic flavoenzyme mercury ion reductase that catalyzes the reduction of Hg^{2+} to Hg^0 with NADPH as the electron donor (Fox and Walsh, 1982; Zeroual et al., 2003). The formed $Hg^0$is eliminated from the cell by passive diffusion under normal physiological conditions, due to the high vapour pressure of mercury metal, resulting in the evaporation of mercury from the medium (Chang et al., 1999). This process is a mercury detoxification in which the mercury metal formed is volatile, so it can penetrate out of the medium (Barkay et al., 2003).

Mercury accumulation from small-scale gold mine tailing

After 1 week incubation, the highest accumulation of Hg (31.34 ppm) was found in the bacteria A treatment (*Brevundimonas vesicularis*), whereas the lowest Hg accumulation (29.62 ppm) was observed in the bacteria D treatment (*Fusobacterium necrogenes*) (Figure 4). This was in agreement with Hg accumulation in nutrient broth liquid media where *Brevundimonas vesicularis* was the best Hg accumulator (Figure 3). After 4 weeks, Hg accumulation by *Brevundimonas vesicularis* was still high, although the value was slightly lower than that at 1 week. While the Hg accumulation by *Fusobacterium aquatile* and *Nitrococcus mobilis* at 4 weeks remained constant, the Hg accumulation by *Fusobacterium aquatile* and *Fusobacterium necrogenes* increased (Figure 3). This was
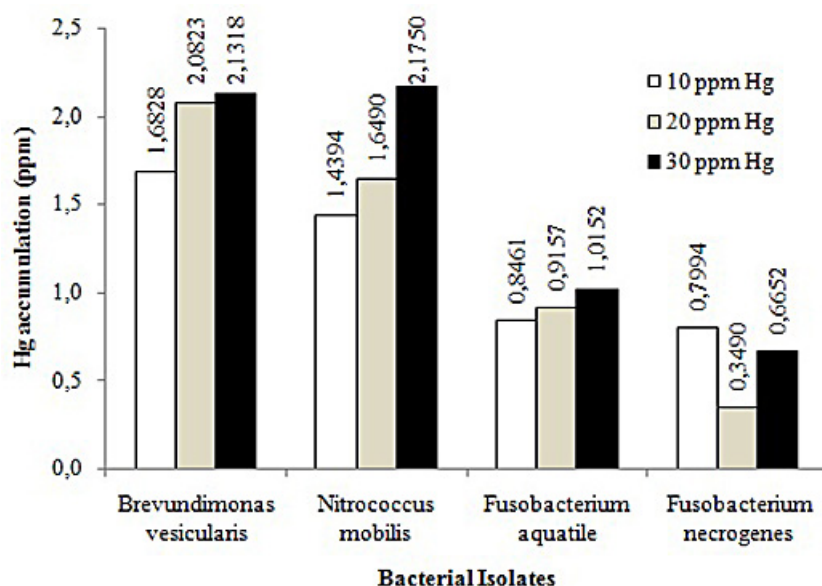

Figure 3. Accumulation of mercury by isolates of mercury resistant bacteria from nutrient broth liquid medium
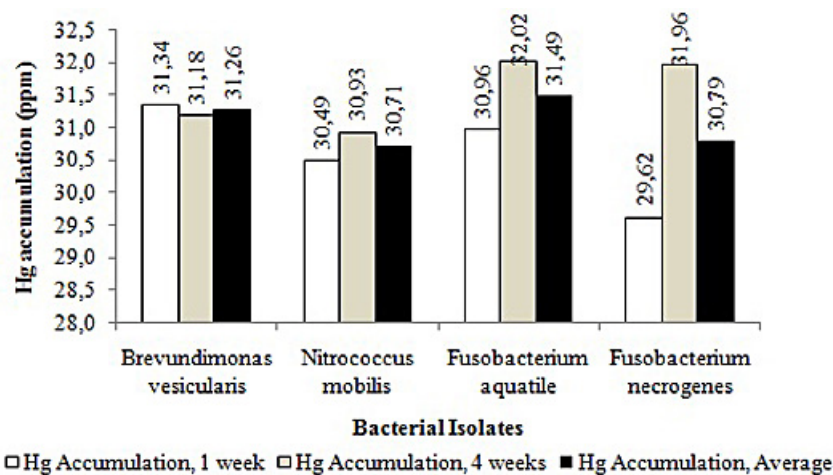
Figure 4. Accumulation of mercury by four isolates of mercury resistant bacteria in small-scale gold mine tailing

related to the growth of the bacteria (Figure 2). The stationary phase of *Brevundimonas vesicularis* and *Nitrococcus mobilis,* was at 36 hours, while that of *Fusobacterium necrogenes* and *Fusobacterium aquatile* was at 42 hours. The stationary phase is the best time for the production of bacterial cell numbers.

In comparison with the initial Hg concentration in tailings (41.37 ppm), at 1 week, the highest Hg accumulation efficiency (75.8%) was observed for *Brevundimonas vesicularis* followed by *Fusobacterium aquatile* (74.8%), *Nitrococcus mobilis* (73.7%, and *Fusobacterium necrogenes* (71.6%). At 4 weeks, however, the highest Hg accumulation efficiency (76.6%) was observed for *Fusobacterium aquatile* followed by *Brevundimonas vesicularis* (75.6%), *Fusobacterium necrogenes* (74.4%), and *Nitrococcus mobilis* (74.2%). The accumulation efficiency of Hg in four bacterial isolates tended to increase at 4 weeks incubation, except for *Brevundimonas vesicularis*. The signs of bacterial life indicated that the accumulation process was still ongoing, so the efficiency of accumulation at four weeks was higher than that of one week of incubation, although the difference was not significant. On average, the Hg accumulation capability of the mercury-resistant bacteria isolated from small-scale gold mine tailings was in order of *Fusobacterium aquatile* (76,1%) > *Brevundimonas vesicularis* (75,6%) > *Fusobacterium necrogenes* (74,4%) > *Nitrococcus mobilis* (74,2%). The process of bacterial resistance to mercury ions (Hg^{2+}) via the reaction of the ligand binding and enzymatic reactions that can reduce Hg^{2+} into volatile Hg^0, so Hg^{2+} will not poison the bacterial cells (Nascimento and Chartone-Souza, 2003). The bacterial resistance to mercury can be

through the mechanisms of biosorption and accumulation. The biosorption mechanism is a passive process, so that the metal is not toxic to the bacterial cell. The accumulation mechanism is an active process in which heavy metals are toxic to the bacterial cell (Chojnacka, 2010). According to Iyer et al. (2005), the biosorbtion mechanism is associated with the presence of exopolysaccharide in the bacterial cell wall that serves as chelating heavy metals on the cell surface. Complex molecules on the bacterial cell wall consist of peptidoglycan that is composed of simple molecules such as phosphoryl, carboxyl, and amino acids that have a negative charge. The negative charge will interact with the ions or molecules with a positive charge on the outside environment that shaped the bonding ligand. Metal ions are positively charged, so that they bind to the cell surface electrostatically (Langley and Baveridge, 1999).

CONCLUSION

Four isolates of mercury-resistant bacteria i.e. *Brevundimonas vesicularis, Nitrococcus mobilis, Fusobacterium aquatile*, and *Fusobacterium necrogenes* could be isolated from small-scale gold mine tailings from Sekotong District of West Lombok. The highest Hg accumulation from nutrient broth liquid media was observed for *Brevundimonas vesicularis* at all Hg treatments, while *Fusobacterium necrogenes* performed the lowest Hg accumulation. The highest mercury accumulation from small-scale gold mine tailing containing mercury (average of 1 week and 4 weeks incubation) was by *Fusobacterium aqua-*

tile followed by *Brevundimonas vesicularis, Fusobacterium necrogenes, and Nitrococcus mobilis*. On the basis of the Hg accumulation efficiency of more than 75%, *Fusobacterium aquatile* and *Brevundimonas vesicularis* are potential for bioremediation of mercury-contaminated soils.

Acknowledgements

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