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Highly Effective In-vitro Mercurial Reduction by Indigenous Bacteria as Single Strains Compared to the Consortium Culture

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Abstract: Mercury has become one of the major pollutants that degrade human health and significantly affect the environment worldwide. Hence, developing cost-effective and highly productive procedures to remove mercury is crucial. Bioremediation of heavy metals, including mercury, has been well studied, yet the diversity of resistant bacteria offers unlimited possibilities to discover beneficial microbes for pollutant abatement. The current study demonstrated the capacity of five indigenous bacterial species isolated from mercury-polluted sites to reduce $HgCl_2$ to 30 ppm in solution when inoculated in pure and mixed cultures over a 48-hour incubation period. The microbes reduced mercury in the solution by as much as 89-97%, while the consortium culture reduced 98%. The percentage of mercury removal in inoculated liquids, either by single or consortium culture, was 3 to 4-fold higher than in the non-inoculated liquids, thus confirming that the addition of Hg^{2+} reducing bacteria was much better than the control. The difference between reduced mercury and the measured volatilized fraction indicates that multiple processes contribute to mercury transformation in the system. Future studies should investigate the genetic determinants of mercury resistance and evaluate these bacteria in field-scale bioremediation systems. This research contributes to our understanding of microbial mercury transformation and suggests potential directions for developing sustainable strategies to mitigate mercury pollution in contaminated environments.

Keywords: bioaugmentation; bioremediation; clean technology; heavy metal pollution; Hg-reducing bacteria.

1. Introduction

Despite its toxicity [1], mercury is a very common raw material or catalysused in various industries, including chemical production, fluorescent lamps, thermometers, pesticides, dental amalgams, and gold amalgamation. Mercury tends to accumulate in the ecosystem and magnify in the tissues of organisms through the food chain [2,3]. Due to the extensive use of mercury, especially in industrial processes worldwide, contamination by this heavy metal has become significant environmental problem [4,5].

Currently, the primary source of mercury contamination is gold mining practices, accounting for up to 38% of total pollution recorded by theSEPA [6]. In Indonesia, for example,

artisanal gold processing using mercury contributed at least 380 tonnes of mercury releases to the environment [7]. Traditional and small-scale businesses discharge wastes and by-products directly into the surrounding environment without proper treatment. Many studies, e.g., by Arifin *et al*. [8], Basri *et al*. [9], and Mallongi *et al*. [10], reported that the mercury contamination in water bodies, soils, and biota such as paddy rice has reached an alarming level, which reminds us of the Minamata tragedy in the 1950s [11].

The standard approaches for treating metal-polluted sites, mainly by physical means, such as excavation, are costly [12]. Besides, the displacement of contaminated matrices will potentially spread the pollutant to undisturbed areas. For these reasons, biological-based remediation methods have been investigated as affordable, practical, and environmentally friendly alternatives [13]. Such methods utilize a variety of organisms, mainly bacteria, to absorb, degrade, or transform metal ions into less or non-toxic compounds [14,15]. Recently, the search for suitable bacterial strains has been a thriving effort globally [16,17].

Bacterial strains have been reported to reduce heavy metals [18,19]. Moreover, the benefits of using bacterial consortia have been reviewed, with studies showing that bacterial strains work more effectively as a group [20–23]. In terms of mercury-resistant bacteria, many studies have been conducted to isolate indigenous bacteria from mercury-contaminated sites [24,25]. The advantage of indigenous bacteria is that they are well-adapted to contaminated sites, enabling faster bioremediation[26].

In a previous study, we isolated and characterized 13 Gram-negative bacterial species resistant to mercury at concentrations up to 100 ppm from a mercury-contaminated site in Sukabumi, Indonesia [27]. The discovery of resistant bacteria at mercury-polluted habitats suggested the potential of the strains as bioremediation agents for the cleanup of such sites [28]. These microbes have potential as practical, cost-efficient bioremediation agents for mercury reduction, thus applicable in developing countries such as Indonesia.

The most comprehensive report on the detoxification of mercury by bacteria is the conversion of inorganic mercury into metallic mercury [25]. Microorganisms' capacity to convert ionic mercury to the volatile metallic form may be associated with their resistance gene to mercury [29]. Studies on the reduction and volatilization of mercury by various bacterial genera have already been reported, such as Bacillus [30,31], Vibrio [32,33], and Pseudomonas [34,35].

Despite the notorious risks posed by mercury, there remains a critical gap between the scale of contamination, particularly in artisanal and small-scale gold mining (ASGM) regions, and the availability of affordable, field-applicable remediation technologies. Conventional approaches such as soil excavation and physicochemical treatments are either expensive or environmentally disruptive, making them unfavorable for many resource-limited scenarios. The use of indigenous mercury-resistant bacteria represents a promising, low-cost biotechnological strategy that can be applied in situ with minimal resources.

In this context, the present study addresses current environmental management challenges by evaluating the mercury-reducing efficiency of five native bacterial strains isolated from a heavily contaminated ASGM site [27] in both single and consortium cultures in a laboratory-scale experiment. The performance of the cultures was measured by the reduction in mercury concentration over 48 hours of observation in an open-flow bioreactor system. Our findings provide foundational evidence for practical bioaugmentation solutions that can support site-level remediation planning, reduce ecological and human exposure risks, and complement national mercury reduction efforts under the Minamata Convention.

2. Materials and Methods

2.1. Molecular identification of mercury-resistant bacteria.

Five isolates were selected from the 27 mercury-resistant bacteria previously reported in Amandita *et al*. [27]. The selection was primarily based on their strong tolerance to mercury observed during preliminary resistance screening, in which these isolates exhibited the highest growth at elevated $HgCl_2$ concentrations compared with other isolates. To identify the selected bacteria, genomic DNA was extracted, and 16S rRNA (~1300–1500 bp) gene analysis was conducted by Macro-gen® (South Korea) using the universal primers of 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The 16S rRNA gene is widely used for bacterial identification because it contains highly conserved regions that allow amplification with universal primers, as well as variable regions that enable differentiation among bacterial taxa and phylogenetic analysis [36]. The raw chromatogram files were visually inspected to verify base-calling accuracy and detect ambiguous peaks. Low-quality regions at the ends of the sequences were trimmed before assembling the forward and reverse reads into contigs using CodonCode Aligner (CodonCode Corporation, USA). This step ensures only high-confidence sequences are used for subsequent similarity searches and phylogenetic analysis. All successful contigs were compared with other sequences in the Ezbiocloud database (https://www.ezbiocloud.net/) to find the most similar strains and then named accordingly. The alignment of contigs and reference sequences from closely related bacterial species was conducted to construct a phylogenetic tree using the Neighbor-Joining algorithm with 1,000 bootstrap replicates in MEGA X[37].

2.2. Evaluation of MIC.

To evaluate the minimum inhibitory concentration (MIC) of mercury to the selected strains, 1 mL of fresh bacterial cultures with turbidity of 0.5 McFarland was grown on Mueller-Hinton agar incorporated with 0 ppm, 30 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm, and 1000 ppm of $HgCl_2$ under aerobic conditions at 37°C. MIC was considered the lowest concentration of $HgCl_2$ supplemented in the media agar in which no bacterial growth was observed after 24 h incubation [38]. Bacterial growth intensity was evaluated using a semi-quantitative scoring system based on visible colony development on agar plates. The symbols (+ to ++++) represent increasing levels of colony growth relative to the control plate without mercury, while (–) indicates the absence of visible growth. All MIC experiments were conducted in triplicate.

2.3. Experimental design.

The ability of single and consortium cultures of mercury-resistant bacteria to degrade mercury in vitro was analyzed in an open-flow bioreactor system (Figure 1), which consisted of a reduction bottle (R) containing inoculated culture medium and a volatilization bottle (V) containing an oxidizing trapping solution designed to capture volatilized mercury released during bacterial activity. Five pure strains (Mer07, Mer18, Mer20, Mer23, Mer27) were prepared in Luria Bertani broth with 50 ppm $HgCl_2$ and kept at a temperature of 37°C for 24 h. Prior to inoculation, all cultures were adjusted to a 0.5 McFarland turbidity, corresponding to the early-to-mid logarithmic growth phase for these bacterial species. Cultures were used immediately after reaching this phase to ensure consistent metabolic activity across strains, recognizing that mercury reduction is strongly influenced by physiological state [39]. From

each culture, 10 mL was inoculated into 150 mL of Luria Bertani broth with 35 ppm $HgCl_2$ and then labeled as Mer07, Mer18, Mer20, Mer23, and Mer27. For the consortium culture, five bacterial solutions with equal volumes and a 0.5 McFarland turbidity were mixed, and then 10 mL of the homogenized solution was inoculated into the same media as a single culture.

For each of the experimental units, the bioreactor consisted of two Duran bottles: 1) an R bottle filled with 150 mL inoculated media and supplemented with 35 ppm of $HgCl_2$, 2) a V bottle filled with 150 mL mixture of $KMnO_4$ (0.9 g in 15 mL deionized water), 1.5 mL HNO_3, and 127.5 mL H_2SO_4 to bind the volatilized mercury from the R bottle. The mixture of $KMnO_4$, HNO_3, and H_2SO_4 solution was used as an oxidizing trapping system, in which $KMnO_4$ acts as a strong oxidizing agent that converts gaseous Hg^0 into soluble Hg^{2+}, while HNO_3 provides the acidic environment, and H_2SO_4 stabilizes the oxidized mercury and prevents its re-volatilization. This trapping approach is commonly employed in mercury volatilization studies because it enables efficient oxidation and quantitative recovery of gaseous mercury species, allowing accurate measurement of mercury released during microbial reduction processes [40,41].

Both bottles were covered with silicon lids that were customized to hold two glass straws in each of the bottles: the first straw in the first bottle was connected to an electrical pump to push filtered air into the inoculated media with a constant flow of 150 L per hour; the second straw in the first bottle was connected to the first straw in the second bottle with silicon pipe to let the mercury gas to be transported and bind into the acid liquid in the second bottle; the second straw in the second bottle was connected to an air vacuum machine to suck the gas out of the system and suspended the gas into NaOH to avoid further spread. All bioreactors were placed in incubators with a temperature of 37°C during the observation period. All experiments were conducted in triplicate with uninoculated media as a control.

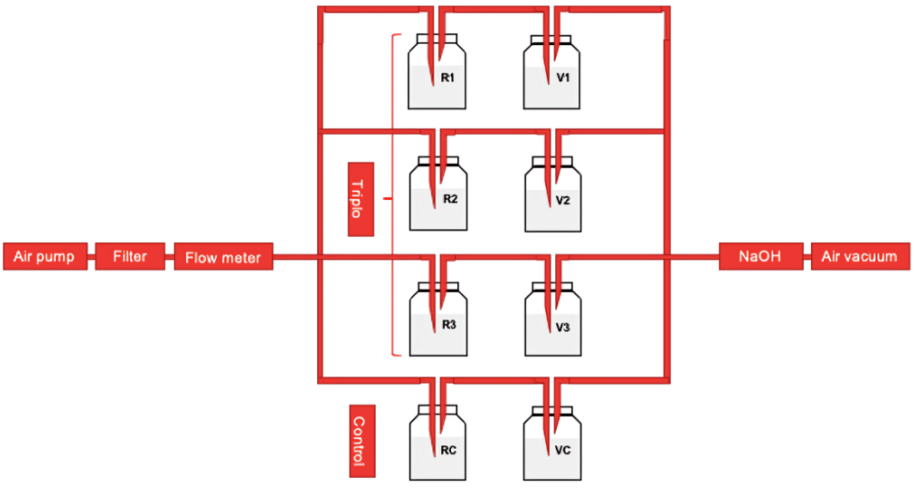

Figure 1. Experimental setup for in-vitro mercury reduction and volatilization testing using mercury-resistant bacterial strains. The system is divided into reduction bottles (R) and volatilization bottles (V). Filtered air is introduced into the R bottles through a controlled air pump to maintain fixed aeration and ensure gas flow into the V bottles, and the remaining gas is sucked by the air vacuum and passed into a NaOH solution. A control system (C) without bacterial inoculation was operated in parallel.

2.4. Bacterial growth and total mercury measurement.

As much as 5 mL of liquid from each bottle was taken to measure bacterial growth and total mercury concentration. These measurements were carried out at 0 h, 6 h, 24 h, 30 h, and 48 h after the bioreactor started running. Bacterial growth in each reduction bottle (R) with and

without $HgCl_2$ supplementation (C) was evaluated as optical density (OD) using a spectrophotometer (Shimadzu, Japan) at 600 nm and as colony count on Plate Count Agar.

The concentrations of mercury remaining in the inoculated liquid in reduction bottles (R) and the acid liquid in volatilization bottles (V) were measured using a Mercury Analyzer (NIC, Japan). The measurement was performed immediately at 0 h, 6 h, 24 h, 30 h, and 48 h after inoculation once the liquid was removed from the bioreactor bottles. The per cent reduction was calculated as the proportion between the reduced concentration in R at 48 h and the initial concentration in R. Meanwhile, the per cent volatilization was calculated as the proportion between the total mercury measured in V at 48 h and the reduced concentration in R at 48 h.

Because components of the growth medium and bacterial metabolites may influence mercury detection, uninoculated media (LB + $HgCl_2$) were analyzed as matrix blanks at each time point. All mercury concentrations reported for inoculated samples were corrected by subtracting the corresponding blank values. Prior to sample analysis, the Mercury Analyzer (NIC, Japan) was calibrated using standard mercury solutions prepared from certified reference material. The limit of detection (LOD) of the instrument was approximately 0.01 ppm, and recovery tests using spiked mercury standards yielded rates within 95–105%, indicating satisfactory analytical performance.

2.5. Statistical analysis.

All treatments were performed in triplicate (n = 3). Mercury-reduction efficiencies and growth measurements are reported as mean ± standard deviation. Differences among bacterial strains were assessed using one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test, with significance determined at $p < 0.05$. Prior to conducting ANOVA, the data were evaluated for normality and homogeneity of variance to ensure that the assumptions of the test were satisfied. Statistical analysis and graphical production were conducted using JMP software (SAS, USA).

3. Results and Discussion

3.1. Molecular identification and phylogenetic analysis.

Based on a search of the most similar nucleotide sequences through the Ezbiocloud database (https://www.ezbiocloud.net/), the five strains investigated here are identified as *Exiguobacterium acetylicum* (Mer07), *Enterobacter huaxiensis* (Mer18), *Acinetobacter junii* (Mer20), *Bacillus cereus* (Mer23), and *Klebsiella huaxiensis* (Mer27). The similarity of the inquiry sequences with the top-hit sequences is more than 90%, except for Mer18 and Mer23. The contigs Mer23 and Mer27 failed to assemble; identification of these two strains was based on one of the two primers used (Table 1). As a result, the species-level assignments for these two isolates carry inherent uncertainty, which is common when read quality or overlap is insufficient. This taxonomic uncertainty should not be a limiting factor in interpreting the mercury reduction data, since the experimental outcomes reflect physiological performance regardless of precise species identity. Future work involving whole-genome sequencing or multi-locus phylogenetics would help to confirm their taxonomic position and clarify any functional traits associated with mercury resistance.

Table 1. Species identification of investigated bacterial isolates based on DNA sequencing.

Isolate ID	Identification Result		
	Species Name	**Similarity**	**NCBI Accession No.**
Mer07	*Exiguobacterium acetylicum*	99.44	SAMN38441102, SAMN38441103
Mer18	*Enterobacter huaxiensis*	76.24	SAMN38441104, SAMN38441105
Mer20	*Acinetobacter junii*	94.37	SAMN38441106, SAMN38441107
Mer23	*Bacillus cereus*	68.00	SAMN38441108, SAMN38441109
Mer27	*Klebsiella huaxiensis*	95.59	SAMN38441110, SAMN38441111

To infer the relationship between the investigated strains and their closely related species based on the molecular trait, the phylogenetic tree in Figure 2 shows that all strains belong to the specified genera, supporting their preliminary taxonomic assignments based on 16S rRNA gene sequence similarity. However, this phylogenetic tree implies that Klebsiella is not a monophyletic group.

From an ecological perspective, the use of indigenous strains for bioaugmentation offers advantages in compatibility and environmental safety. Because all isolates were recovered from mercury-impacted soils, they are already adapted to the local microbial community and are less likely to disturb native ecological functions than non-native species. However, the introduction of concentrated cultures may still influence microbial nutrient competition or functional community composition. These potential unintended impacts highlight the importance of monitoring changes in microbial diversity during field implementation and conducting site-specific risk assessments. Overall, the ecological compatibility of these native strains makes them promising candidates for bioremediation.

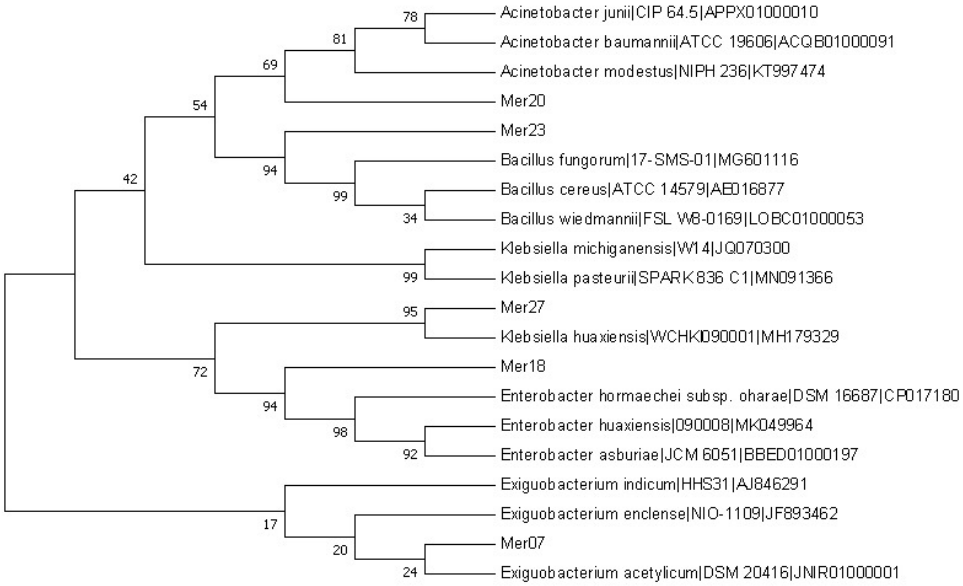

Figure 2. The maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences shows the position of strains and other related genera. Bootstrap values (%) are based on 1000 replicates.

3.2. Mercurial-resistant test.

The resistance of five bacterial strains studied here was evaluated by inoculating them on agar media augmented with $HgCl_2$. In a previous study [27], we tested the resistance of 27 bacterial isolates to 200 ppm, and based on the results, we selected the five most resistant. In the current study, we tested the resistance up to 1000 ppm, and found that all five strains could grow on the media with $HgCl_2$ up to 500 ppm except Mer27. However, the growth intensity of the other four strains decreased as the $HgCl_2$ concentration increased, and at 1000 ppm, growth was completely inhibited (Table 2).

Table 2. The resistance level of investigated bacteria to $HgCl_2$, as implied by the growth intensity compared to the media with 0 ppm $HgCl_2$.

Inoculant	$HgCl_2$ concentration (ppm)						
	0	**30**	**50**	**100**	**200**	**500**	**1000**
Mer07	++++	++++	++++	+++	+++	+	-
Mer18	++++	++++	++++	++++	+++	++	-
Mer20	++++	++++	++++	++++	++++	+++	-
Mer23	++++	++++	++++	++++	+++	++	-
Mer27	++++	++++	++++	+++	+++	-	-

-: no growth at all; +: 25% colony growth; ++: 50% colony growth; +++: 75% colony growth; ++++: 100% colony growth

Five strains of bacteria used in this experiment were proven to be resistant to high concentrations of mercury. These strains were molecularly identified by 16S rRNA gene analysis; however, further investigation is warranted to confirm the presence of mercury-resistant genes. The mer operons, which are the genes responsible for mercury resistance in bacteria [42,43], have been reported to be found in *Enterobacter sp.* [44], *Acinetobacter sp.* [45], *Bacillus sp.* [46], and *Klebsiella sp.* [47]. No studies have reported on mercury resistance or mercury degradation by the strains studied here, except *Bacillus cereus* [30,48]. However, there is a possibility of misidentification of Mer23 as *Bacillus cereus* since the level of similarity was low (68%).

In other studies [49,50], it was found that the microbes' capacity to degrade pollutants was related to higher MIC values. However, in the present study, the opposite was observed with the isolate Mer27 (*Klebsiella huaxiensis*), which showed the lowest tolerance to Hg but the second-highest mercury reduction. At the mercury concentration employed in the study reported here, 30 ppm on average, all the strains showed inarguably high resistance levels.

3.3. Bacterial growth assessment.

Figure 3 shows that the growth of single culture and consortium culture, measured as optical density, is congruent with the colony count, in which Mer18, Mer27, and Consortium have relatively higher growth than Mer07, Mer20, and Mer23. The latter slows down in the middle of the observation period, indicating that these three strains reached their maximum growth rate under the given conditions.

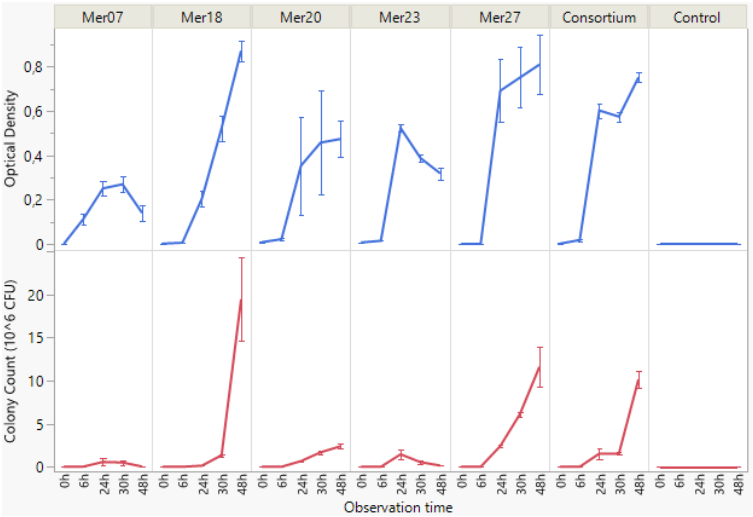

Figure 3. The bacterial growth of five single strains and the consortium was measured as optical density at 600 nm wavelength and colony count (10^6 CFU mL^{-1}) in the bioreactor. Values represent mean ± standard deviation (n = 3).

Furthermore, the highest optical density and CFU can be observed in Mer18 (*Enterobacter huaxiensis*), proving that this strain has the highest adaptation to mercury contamination and thus can reproduce the highest population compared to other strains. Overall, the viable cell counts of the strains and the consortium cultures studied here were higher than 10^6 CFU mL^{-1}. Several studies reported that for successful biodegradation, the cell number inoculated was 10^6 CFU g^{-1} at a minimum [51,52]. The relationship between the growth phase and mercury degradation shows that most mercury concentration was reduced during the logarithmic phase of the growth, which was during the first 24 hours of observation. It should be noted that as mercury is reduced, the medium becomes less toxic to the bacteria; thus, bacterial growth is expected to increase as the mercury concentration decreases. Mer18 and Mer27 provide an excellent example of such a theory since a substantial increase in cell number was recorded between 24 h and 48 h after more than 80% of the mercury had been reduced.

3.4. Mercury reduction and volatilization.

Despite careful, uniform procedures during experiment preparation, the initial total mercury concentration was measured differently, ranging from 21 ppm to 35 ppm. This variability is likely due to a combination of factors, including (i) small pipetting deviations during dilution of concentrated $HgCl_2$ stock solutions, (ii) temporary heterogeneity of Hg^{2+} distribution before full equilibration in the broth medium, and (iii) normal analytical variation associated with the mercury analyzer. Nevertheless, these differences do not affect comparative interpretations because mercury reduction was calculated relative to the initial concentration measured in each bioreactor.

The lowest concentration of remaining mercury at 6 h was found in the solution inoculated with Mer27 (8.1 ppm), with the highest initial concentration (35 ppm). At 48 h, the lowest total mercury concentration was recovered from the Consortium-inoculated solution (0.3 ppm), from the initial concentration of 27 ppm. Moreover, the Mer27-inoculated solution has the highest final concentration (3.5 ppm), even though its initial concentration was the lowest. Interestingly, the total mercury concentration in the uninoculated solution (control) was slightly decreased from 22 ppm to 17 ppm (Figure 4).

The mercury reduction and volatilization have been investigated in several studies using different bacteria, i.e., *Klebsiella pneumoniae* was reported to be able to clean $HgCl_2$ up to 99.8% with volatilized mercury reached at least 48% [53]; *Lysinibacillus fusiformis* was evaluated for reducing mercury almost 97% with a portion of volatilized mercury up to 63% [54]; and *Pseudoxanthomonas sp.* was able to reduce mercury as much as 100% with 60% being volatilized [55]. However, none of these studies evaluated the strains used here, four of which have never been previously described for mercury detoxification. The demonstration that *Exiguobacterium acetylicum*, *Enterobacter huaxiensis*, *Acinetobacter junii*, and *Klebsiella huaxiensis* can achieve high reduction efficiencies, therefore, represents a meaningful expansion of the microbial taxa known to participate in mercury biotransformation. The use of these strains for field applications may have beneficial potential because indigenous strains often exhibit superior environmental adaptability and competitive survival in contaminated matrices.

The mercury trapped in the acid solution in the volatilization bottles was measured to add up over time, but the increase was much lower than the mercury reduction. The highest mercury concentration detected in the acid solution from the bioreactor with Mer27 inoculation

was up to 14 ppm at the end of the observation period. At the same time, the mercury reduced by this strain was measured at up to 30 ppm, indicating a 16 ppm difference between the reduced mercury and the volatilized concentration.

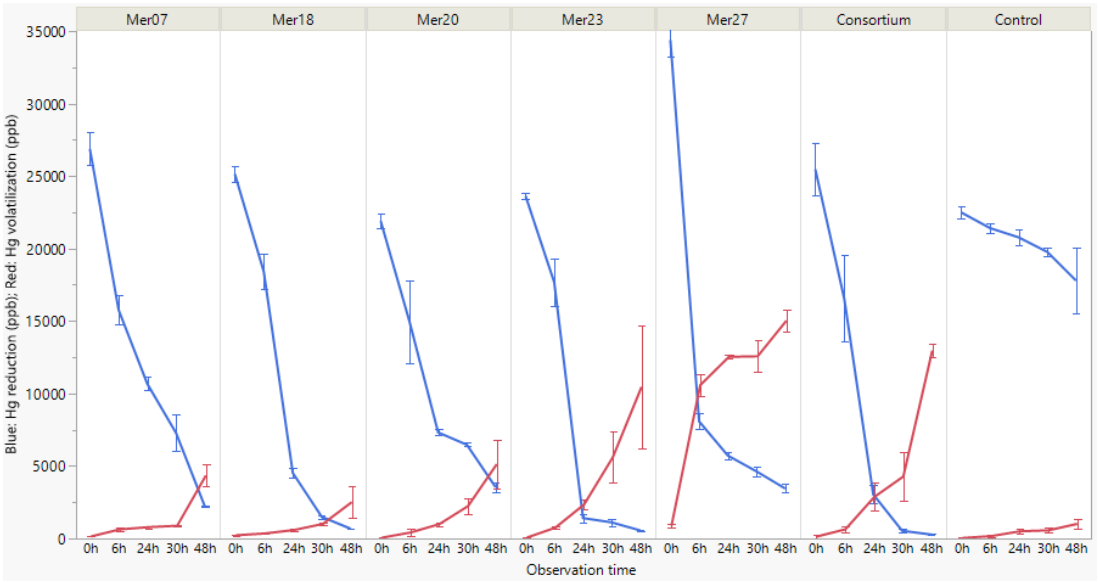

Figure 4. The decrease of total mercury concentration as bacterial-induced reduction (blue line) and the increase of total mercury concentration (red line) show the level of volatilized mercury. Values represent mean ± standard deviation (n = 3).

Furthermore, the comparison between the percentage reduction and the percentage volatilization of mercury by each culture is depicted in Figure 5. The highest mercury reduction was observed in the consortium bioreactor system, reaching 98.90%, followed by Mer23 (97.63%) and Mer18 (97.31%). The lowest reduction was 83.82% by Mer20, 4-fold higher than the reduction detected in the control (20.90%). Conversely, the lowest volatilized mercury occurred in Mer18 (10.28%), lower than the control (21.58%). The statistical analysis of mean variances using Student's t-test shows that the percentage reduction in the control is significantly smaller than that in the inoculated bioreactors. However, the volatilization is proven to be the same as the others.

The discrepancy between the amount of reduced mercury and the volatilized fraction observed in this study has also been noted in similar bioreactor experiments and may be attributed to several mechanisms [35,51]. First, mercury species are known to adsorb strongly to glassware, silicone tubing, and reactor surfaces, particularly when Hg^{2+} is partially reduced or when biofilm-like deposits form on internal walls [56,57]. Second, the efficiency of Hg^{o} trapping in the $KMnO_4$ acid solution may be imperfect, especially in open-flow systems where airflow dynamics can reduce the contact time required for complete oxidation and capture. Third, a portion of mercury may remain associated with bacterial biomass through intracellular reduction, binding to thiol-containing proteins, or biosorption to cell walls [29,52]. Finally, the formation of insoluble secondary mercury compounds (e.g., Hg_2Cl_2) may also occur, leading to particulate-bound mercury not quantified in the volatilization trap. Collectively, these mechanisms help explain why volatilized mercury did not fully account for the total observed reduction and highlight the complexity of microbial mercury transformations in bioreactor systems.

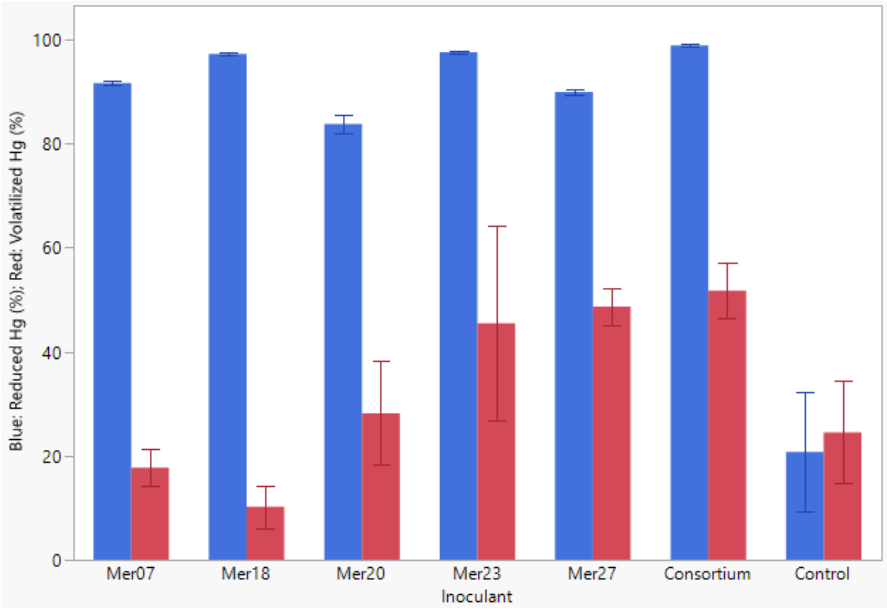

Figure 5. Comparison between the percentage of mercury reduction (blue bars) and volatilization (red bars) at the end of observation (48 h). Different capital letters at the top of each bar indicate the groups of mean variances as calculated with Student's test ($p<0.05$). Values represent mean ± standard deviation (n = 3).

The variation in mercury reduction efficiency observed among the five isolates may reflect differences in their underlying genetic and cellular detoxification systems. In well-characterized mercury-resistant bacteria, Hg^{2+} reduction is primarily mediated by the mer operon, in which the enzyme MerA (mercuric reductase) catalyzes the intracellular conversion of Hg^{2+} to volatile Hg^{0} [42,43]. Some strains also harbor MerB (organomercurial lyase), enabling cleavage of organomercury compounds before reduction [29,42,43]. Efficient detoxification further depends on periplasmic and membrane-associated transport proteins such as MerP and MerT, which facilitate rapid uptake and delivery of Hg^{2+} to the cytosolic reductase, as well as the regulator MerR, which modulates operon expression in response to mercury exposure [42,43]. The higher reduction capacities observed in strains such as Enterobacter huaxiensis (Mer18) and Mer23 may therefore indicate the presence of more active or complete mer operon components, whereas lower-performing strains may rely more heavily on slower processes such as passive biosorption, thiol-binding, or extracellular sequestration [46,52]. While the present study did not evaluate genetic determinants, the observed phenotypic patterns align with the diversity of cellular strategies described in the literature and warrant future genomic or transcriptomic investigation to confirm the distribution and functionality of mer-system genes in these isolates.

The consortium culture showed higher mercury reduction than individual strains, but statistical analysis indicated that this difference was not significant ($p > 0.05$). Previous studies [58,59] reported that microbial consortia were more effective than a single straiat degrading contaminants, possibly due to higher bacterial cell density, which increases bacterial resistance to the contaminant. Meanwhile, a study [60] showed that a single strain performed better in reducing contamination than the consortium. The lower efficiency of the microbial consortium might be due to the nutrient availability and competition between strains. This antagonistic phenomenon occurs when organisms in a shared ecosystem eliminate one another to survive underimiting conditions [61].

The partial conversion of Hg^{2+} to elemental Hg^{o} observed in this study has important environmental implications, especially when considering potential field-scale applications.

Although microbial reduction of ionic mercury lowers its aqueous toxicity and mobility, the volatilized Hg^0 can enter the atmosphere and undergo long-range transport. Such atmospheric cycling is well documented as a major pathway contributing to the global distribution of mercury pollutants [4]. Therefore, while microbial Hg^{2+} reduction is a desirable detoxification mechanism, its implementation in real contaminated sites must incorporate safeguards to prevent uncontrolled volatilization. Field systems should be designed to promote capture or re-oxidation of Hg^0, for example, through soil covers or sorbent amendments. Addressing volatilization risk is essential to ensure that bioremediation strategies do not unintentionally shift mercury contamination from terrestrial to atmospheric compartments.

In practice, the performance of these indigenous strains will be influenced by environmental factors, such as pH, ion presence, and organic matter content,hich can affect mercury speciation and microbial detoxification pathways [62]. Fluctuating pH, for example, can alter Hg^{2+} solubility and cell-surface charge, thereby modifying the efficiency of microbial uptake and reduction [63]. Similarly, the presence of competing ions such as Ca^{2+}, Mg^{2+}, Fe^{3+}, or sulfide can form stable complexes with mercury, potentially reducing its bioavailability or redirecting it into particulate phases [64]. Organic matter may either enhance or inhibit detoxification depending on its composition; for example, humic substances can bind Hg strongly and limit microbial access, whereas low-molecular-weight organics may serve as additional carbon substrates supporting microbial activity [65].

Because the strains in this study are indigenous to mercury-impacted soils, they are likely pre-adapted to such dynamic geochemical conditions. Nonetheless, future field-scale bioremediation systems should consider stabilizing pH within microbially favorable ranges, minimizing excessive ionic competition, and optimizing organic matter inputs to support sustained microbial growth and reductive activity. These considerations will be important for translating laboratory efficiencies into robust mercury-removal performance at the level of a field trial.

4. Conclusions

The long-term aim behind this research is to exploit microorganisms for the in situ bioremediation of mercury. Therefore, bacterial strains were isolated from contaminated sites to explore their capacities as bioremediation agents. The mercury-tolerant bacterial isolates reported here show great potential to be exploited in managing mercury pollution. This study concluded that mercury degradation in vitro was enhanced by bioaugmentation of mercury-resistant bacteria, namely *Exiguobacterium acetylicum*, *Enterobacter huaxiensis*, *Acinetobacter junii*, *Bacillus cereus*, and *Klebsiella huaxiensis*. This study also demonstrated that mercury volatilization occurred in part of mercury degradation. These results suggest that indigenous mercury-resistant bacteria may have potential for future bioaugmentation approaches aimed at mercury remediation. However, additional studies, including investigation of the specific molecular mechanisms underlying mercury detoxification, were not directly addressed in this study; further genomic or molecular analyses will be required to confirm the genetic basis of the observed mercury resistance. Field-scale validation will be required to confirm their effectiveness under environmental conditions and to assess practicality in systems such as in situ soil bioreactors, amended landfarms, or ex situ slurry treatments, where environmental conditions, particularly aeration, moisture, pH, and nutrient availability, can be adjusted to support microbial reduction. To ensure environmental safety, field implementation should include mechanisms to minimize mercury volatilization, such as gas-capture materials

or semi-closed treatment cells. Furthermore, this study provides initial evidence that several indigenous bacterial isolates, which have not been previously reported in the context of mercury reduction, exhibit strong mercury-reducing capabilities under laboratory conditions. Their performance broadens the current spectrum of microbial agents available for Hg bioremediation and underscores the importance of exploring local microbial diversity in contaminated environments.

Author Contributions

Conceptualization, F.Y.A., E., H.A., and Y.T.M.; methodology, F.Y.A. and E.; investigation, F.Y.A. and E.; formal analysis, F.Y.A. and E.; resources, F.Y.A., E., and H.A.; writing—original draft preparation, F.Y.A. and E.; writing—review and editing, F.Y.A., E., H.A., and Y.T.M.; supervision, H.A. and Y.T.M.; project administration, F.Y.A. and E.; funding acquisition, F.Y.A. All authors have read and agreed to the published version of the manuscript.

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Data supporting the findings of this study are available upon reasonable request to the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
Hg	Mercury Element Symbol
$HgCl_2$	Mercury Chloride
Hg^{2+}	Mercuric Cation
USEPA	U.S. Environmental Protection Agency
ASGM	Artisanal and Small-scale Gold Mining
DNA	Deoxyribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
MIC	Minimum Inhibitory Concentration
ppm	Parts Per Million
$KMnO_4$	Potassium Permanganate
HNO_3	Nitric Acid
H_2SO_4	Sulfuric Acid
NaOH	Sodium Hydroxide

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