AL-KAUNIYAH: Jurnal Biologi, 19(2), 2026, 271-282
Website: http://journal.uinjkt.ac.id/index.php/kauniyah
P-ISSN: 1978-3736, E-ISSN: 2502-6720

# CHROMIUM BIOREMEDIATION USING A COMBINATION OF TWO INDIGENOUS RHIZOBACTERIA *IN VITRO*

## BIOREMEDIASI KROMIUM MENGGUNAKAN KOMBINASI DUA RHIZOBAKTERI INDIGENUS SECARA *IN VITRO*

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Submitted: 23 December 2025; Revised: 21 September 2025; Accepted: 4 February 2026

**Abstract**

Sukaregang, known for its leathercraft industry, faces chromium pollution from poorly managed tannery waste, affecting soil and water resources. The dynamic soil area influenced by plant roots, known as the rhizosphere, becomes a habitat for various microorganisms with unique metabolic capabilities that have significant potential as bioremediation agents for heavy-metal-contaminated environments. This study aims to evaluate the potential of indigenous bacterial consortia from the chromium-contaminated rhizosphere as bioremediation agents. Five bacterial genera: *Pseudomonas*, *Citrobacter*, *Bacillus*, *Azotobacter*, and *Micrococcus*, were identified as chromium-resistant *in vitro*. Compatibility tests showed that these bacterial combinations exhibited synergies without growth inhibition. Among the five consortia combinations tested (AB, BC, CD, DE, EA), consortium DE (*Azotobacter* and *Micrococcus*), exhibiting the most stable growth dynamics, characterized by a sustained stationary phase, suggesting a superior adaptive response to chromium-induced stress. Under chromium stress, the consortium EA (*Micrococcus* and *Pseudomonas*) achieved the highest chromium removal percentage at 30.78%. This study suggests that indigenous rhizobacterial consortia could be a practical approach to remediate chromium pollution in areas like Sukaregang.

**Keywords:** Bioremediation; Chromium; Consortia; Rhizobacteria

***Abstrak***

*Daerah Sukaregang, Kabupaten Garut, yang dikenal dengan industri kerajinan kulitnya, menghadapi pencemaran polusi kromium akibat limbah penyamakan kulit yang tidak dikelola dengan baik, sehingga memengaruhi sumber daya tanah dan air. Daerah dinamis tanah yang dipengaruhi oleh akar tumbuhan atau dikenal sebagai rhizosfer menjadi habitat bagi beragam mikroorganisme dengan kemampuan metabolik unik yang berpotensi besar sebagai agen bioremediasi lingkungan tercemar logam berat. Penelitian ini bertujuan untuk mengevaluasi potensi konsorsium bakteri indigenous dari rhizosfer tanah tercemar kromium sebagai agen bioremediasi. Lima genus bakteri; Pseudomonas, Citrobacter, Bacillus, Azotobacter, dan Micrococcus, diidentifikasi sebagai resisten terhadap kromium secara in vitro. Uji kompatibilitas menunjukkan bahwa kombinasi bakteri ini menunjukkan sinergi tanpa penghambatan pertumbuhan. Di antara lima kombinasi konsorsium yang diuji (AB, BC, CD, DE, EA), konsorsium DE (Azotobacter dan Micrococcus) menunjukkan pertumbuhan terbaik di bawah cekaman kromium, sementara konsorsium EA mencapai persentase penghilangan kromium tertinggi, yaitu 30,78%. Studi ini menyarankan bahwa konsorsium rhizobakteri dari lingkungan dapat menjadi pendekatan praktis untuk mengatasi polusi kromium di daerah seperti Sukaregang.*

***Kata Kunci**: Bioremediasi; Konsorsium; Kromium; Rhizobakteri*

**Permalink/DOI: http://dx.doi.org/10.15408/kauniyah.v19i2.44529**

## INTRODUCTION

The leathercraft industry plays a vital role in Indonesia's economy, with Sukaregang, Garut Regency, a prominent center known for producing high-quality leather goods that are well-recognized domestically and internationally. The tanning process, a key stage in leather production, involves converting raw animal hides into stable, soft leather using chemical and physical methods (Suparno et al., 2008). Approximately 85–90% of leathercraft industries utilize trivalent chromium compounds ($Cr^{3+}$ or Cr(III)), such as chromium sulfate ($Cr_2(SO_4)_3 \cdot 6H_2O$), in the tanning process (Krishnamoorthy et al., 2013). While this method, known as chrome tanning, is favored for its speed, ease, and cost-effectiveness, it produces substantial wastewater that can lead to severe chromium pollution if not adequately treated (Yoshinaga et al., 2018).

Chromium pollution poses significant environmental threats, especially to natural resources like soil and water, and can have toxic effects on health if accumulated in high amounts (Prasad et al., 2021). Although Cr(III) is considered less toxic due to its limited solubility and mobility, hexavalent chromium ($Cr^{6+}$ Cr(VI)) is highly toxic and poses a greater risk due to its high oxidation potential and ability to penetrate living organisms (Tumolo et al., 2020). Addressing this pollution, bioremediation—using biological processes to neutralize or remove chromium—has emerged as a cost-effective, environmentally friendly solution (Azubuike et al., 2016).

Indigenous microorganisms, particularly those found in rhizosphere soils contaminated with chromium, are considered effective agents for bioremediation due to their adaptability to polluted environments. Studies have shown that these microorganisms, including rhizobacteria, can reduce Cr(VI) to the less toxic Cr(III), making them potential candidates for in-situ bioremediation (Su et al., 2019). Additionally, bacterial consortia have been found to enhance the bioremediation process compared to single bacterial isolates, as the diverse species within a consortium can synergistically interact to improve resistance to environmental stress and increase the efficiency of chromium removal (Su et al., 2022; Kuanar et al., 2022).

This study employs two genera of indigenous rhizobacteria in each consortium, which is considered the minimal number required for an effective consortium. Using a smaller number of bacteria in the consortium allows for a more precise observation of the dynamics of bacterial interactions under chromium stress. This research aims to investigate the potential of consortia comprising two genera of indigenous rhizobacteria from the chromium-contaminated rhizosphere soils in Sukaregang, Garut, for chromium remediation *in vitro*.

## MATERIALS AND METHODS

### Soil Sampling & Analysis

Soil samples were collected from the rhizosphere of dominant plants growing in chromium-contaminated soil in Garut Kota District, Garut Regency. Sampling plots were determined based on plant dominance in the area, with each plot measuring 1 × 1 m (Ellenberg & Dumbois, 2016). Soil samples were taken from a depth of ±30 cm using a soil corer, then the soil pH and temperature were measured using a soil tester, and the chromium content was analyzed using an Atomic Absorption Spectrophotometer (AAS) at the Environmental Quality Testing Laboratory, Binalab Bandung.

### Bacterial Isolation and Chromium-Resistant Bacteria Screening

Rhizobacteria were isolated from the soil samples using qualitative and quantitative methods. The qualitative screening of bacterial isolates for chromium resistance was conducted by growing the isolates on media supplemented with potassium dichromate ($K_2Cr_2O_7$) at 1,500 mg/kg and 2,000 mg/kg. Initially, bacterial isolates obtained from chromium-contaminated soils were inoculated onto nutrient agar plates containing these chromium concentrations to assess their tolerance and survival ability under heavy metal stress. Growth was monitored visually by observing colony formation and size after incubation, indicating the isolates' resistance to hexavalent chromium. Isolates demonstrating robust growth at these high chromium levels were selected as potential chromium-resistant candidates for further studies. This screening approach aligns with established methods in the literature, where bacterial isolates are exposed to incrementally increasing concentrations of

chromium compounds to identify strains capable of tolerating and potentially reducing toxic chromium species.

The quantitative isolation of rhizobacteria was carried out from the soil samples after serial dilution, following a serial dilution technique, carried out up to a dilution factor of $10^{12}$. Aliquots from the $10^{-7}$ to $10^{-12}$ dilutions were plated on nutrient agar (NA) medium for the isolation of distinct bacterial colonies (Ed-har et al., 2017). For screening chromium-resistant isolates, a quantitative assay was performed by inoculating 1 mL of actively growing bacterial culture (approximately $10^8$ CFU/mL, aged 18–24 hours) into 100 mL of nutrient broth (NB) supplemented with 1,000 mg/kg $K_2Cr_2O_7$. The cultures were incubated at 30 ± 1°C with shaking at 150 rpm, and bacterial growth was monitored using a spectrophotometer by measuring optical density at 600 nm ($OD_{600}$) at 2-hour intervals for a total duration of 48 hours (Sari, 2022).

**Bacterial Identification**

The chromium-resistant isolates were then identified at the genus level based on morphological and microscopic characteristics (Holt et al., 1994), as well as biochemical tests following the method of Cappuccino and Welsh (2020). Subsequently, growth curves of individual isolates were generated to determine the optimal age of the isolates for use in compatibility and bioremoval tests. The growth curves were created by observing changes in optical density (OD) values in NB media without and with 1,000 mg/kg $K_2Cr_2O_7$ over 48 hours.

**Bioremoval Potential Analysis**

The potential of the consortia as chromium bioremediation agents was evaluated through three main tests: 1) compatibility testing; 2) growth curve analysis of the consortia; and 3) chromium bioremoval percentage. Compatibility testing ensures that the bacterial strains can coexist without antagonistic effects, which is crucial for stable and effective consortium performance. Growth curve analysis monitors the microbial growth dynamics, providing insight into the adaptation and proliferation of the consortia under chromium stress. Finally, the chromium bioremoval percentage quantifies the efficiency of the consortia in reducing toxic hexavalent chromium [Cr(VI)] to less toxic forms, reflecting their bioremediation capability. Such a multi-faceted evaluation approach is commonly employed to assess microbial consortia for heavy metal remediation, as it integrates microbial interaction, viability, and functional performance (Ma et al., 2018).

The identified bacterial isolates were then subjected to compatibility testing through the cross streak method on NA media to ensure no antagonistic interactions between isolates (Sadiq et al., 2023; Azizah et al., 2024). The compatibility of the bacterial consortia was assessed to establish whether they could work synergistically without inhibiting each other's growth. The growth of the consortia in NB media containing 1,000 mg/kg $K_2Cr_2O_7$ was monitored over 10 hours using the same method as for single isolates. Following this, chromium content in the media was measured using a microplate reader according to the method of Hagiri et al. (2024). The percentage of bioremoval was calculated using the formula: bioremoval percentage (%)= H0-H1$\frac{H0-H1}{H0} \times 100$. $H_0$ is the initial chromium concentration and $H_1$ is the final chromium concentration, to determine the effectiveness of the bacterial consortia in remediating chromium.

**Statistical Analysis**

The population data of the consortium and the bioremoval percentage were statistically analyzed using IBM SPSS Statistics 20. Normality and homogeneity tests were conducted, followed by Kruskal-Wallis tests and Dunnett T3 post-hoc analysis for data that did not meet assumptions. Simple linear regression analysis was employed to evaluate the relationship between the population of the consortium and the reduction in chromium concentration by the bacterial consortium.

**RESULTS**

Analysis of soil samples using AAS revealed a chromium concentration of 7.582 mg/kg, indicating high contamination levels at the sampling site, exceeding safe environmental limits (0.500

mg/kg) according to the Badan Standardisasi Nasional (Badan Standardisasi Nasional, 2004). Dominant plant species at the site included *Mikania micrantha*, *Alternanthera sessilis*, and *Amaranthus viridis* (Figure 1). These plants are known for their ability to grow in heavy metal-contaminated environments due to specific mechanisms that tolerate, accumulate, and remediate heavy metals, including chromium.


**Figure 1.** The most dominant plants at the sampling site: (a) Mikania micrantha; (b) Alternanthera sessilis; (c) Amaranthus viridis

The bacterial isolates obtained from the rhizosphere soil samples were characterized based on macroscopic colony morphology. A total of 16 bacterial isolates were observed, each displaying distinct colony characteristics as shown in Figure 2. Colony shapes varied from round to irregular, with some exhibiting filamentous forms. Colony edges ranged from smooth to undulating or irregular, and elevation was mostly flat, though some colonies were raised. Colors included shades of white, cream, and yellow. This morphological diversity provides valuable information for distinguishing between isolates.

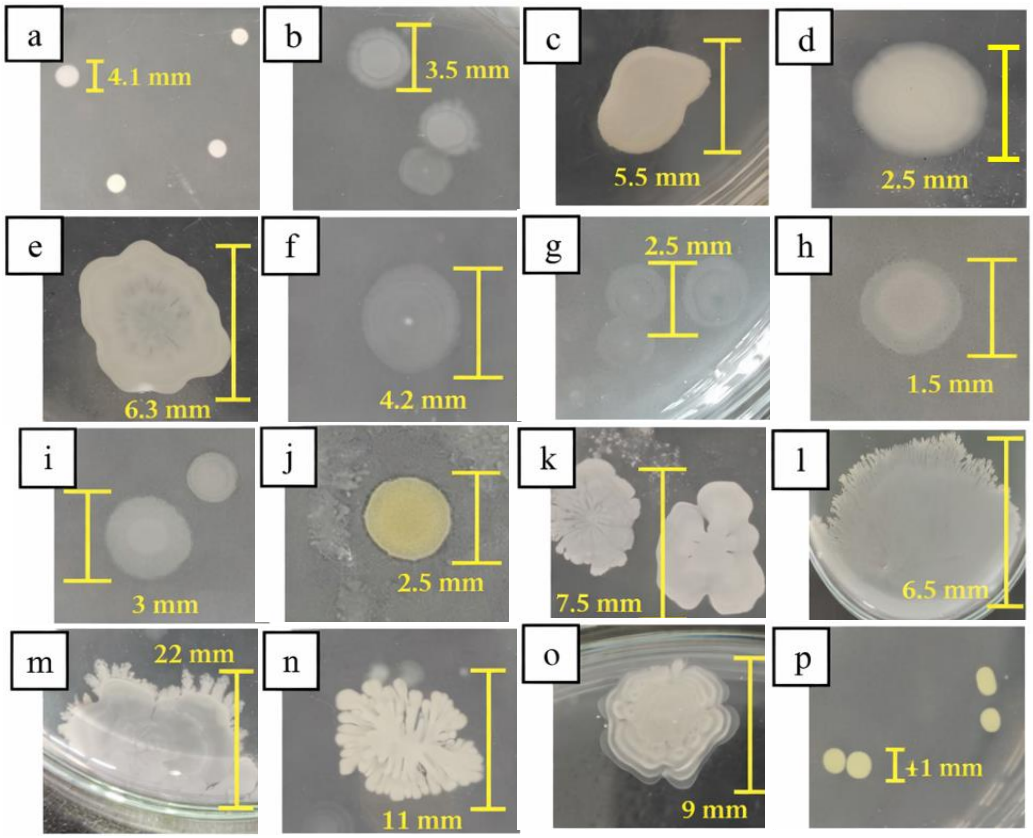



**Figure 2**. Macroscopic colony morphology of bacterial isolates

The qualitative screening revealed that none of the isolates grew at 2,000 mg/kg $K_2Cr_2O_7$, but some showed growth at lower concentrations (1,500 mg/kg and 1,000 mg/kg). Quantitative analysis of bacterial growth in NB with 1,000 mg/kg $K_2Cr_2O_7$ was performed, measuring OD at 600 nm. The

OD values, ranging from 0.034 to 0.097 at 0 hours and increasing to 0.241 to 0.348 after 24 hours, indicated varying levels of bacterial growth and chromium tolerance. The isolates with the highest ΔOD after 48 hours (A5, A14, A12, A4, A16, A13, and A15) were selected for further identification.

Observations revealed that the bacterial isolates predominantly exhibited a rod-shaped morphology, except for one isolate, which was coccus-shaped. Gram staining displayed a mix of Gram-positive and Gram-negative bacteria, with two isolates showing visible endospores. The biochemical tests summarized in Table 1 indicated diverse metabolic activities among the isolates, reflecting their varied biochemical profiles. Identification of bacterial isolates was achieved through morphological and biochemical assessments, matching the characteristics to Bergey's Manual of Determinative Bacteriology (9th Edition). The isolates were identified as follows: *Pseudomonas* (A4), *Citrobacter* (A5), *Bacillus* (A12 and A14), *Azotobacter* (A13 and A15), and *Micrococcus* (A16).

**Table 1.** Observation results of biochemical activity tests of bacterial isolates

| Isolate code | Microscopic characteristics of cells | | | Biochemical activity test | | | | | | | | | | | | | | Genus identified |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Hydrolysis | | | | IMVIC | | | | Carbohydrate fermentation | | | Catalase | $H_2S$ | Motility | |
| | Shape | Gram staining | Endospore staining | Starch | Lipid | Gelatin | Casein | Indole | MR | VP | Citrate | Dextrose | Lactose | Sukrose | | | | |
| **A4** | Bacillus | - | - | - | - | - | + | - | + | - | + | - | + | + | + | - | + | *Pseudomonas* |
| **A5** | Bacillus | - | - | + | - | - | + | - | + | - | - | - | + | + | + | - | + | *Citrobacter* |
| **A12** | Bacillus | + | + | - | + | - | + | - | + | - | + | + | + | + | + | - | + | *Bacillus* |
| **A13** | Bacillus | - | - | + | - | - | - | - | + | - | + | + | + | + | + | - | + | *Azotobacter* |
| **A14** | Bacillus | + | + | - | + | - | + | - | + | - | + | + | + | + | + | - | + | *Bacillus* |
| **A15** | Bacillus | - | - | + | - | - | - | - | + | - | + | + | + | + | + | - | + | *Azotobacter* |
| **A16** | Coccus | + | - | - | - | - | + | - | - | - | + | + | - | - | + | - | - | *Micrococcus* |

Figure 3 shows the growth curves of five bacterial isolates (*Pseudomonas*, *Citrobacter*, *Bacillus*, *Azotobacter*, and *Micrococcus*) in NB media without chromium, revealing significant differences in growth rates and dynamics. *Pseudomonas* and *Citrobacter* exhibited very short lag phases and reached peak OD more quickly. *Bacillus* and *Micrococcus* showed similar growth patterns, and *Azotobacter* displayed slower growth without achieving a stationary phase within the observation period. The addition of $K_2Cr_2O_7$ at a concentration of 1,000 mg/kg accelerated bacterial cell death and reduced bacterial populations in each growth phase. Overall, bacterial growth entered the stationary and death phases more quickly, indicating that chromium accelerates the effects of stress and cell death on bacteria.

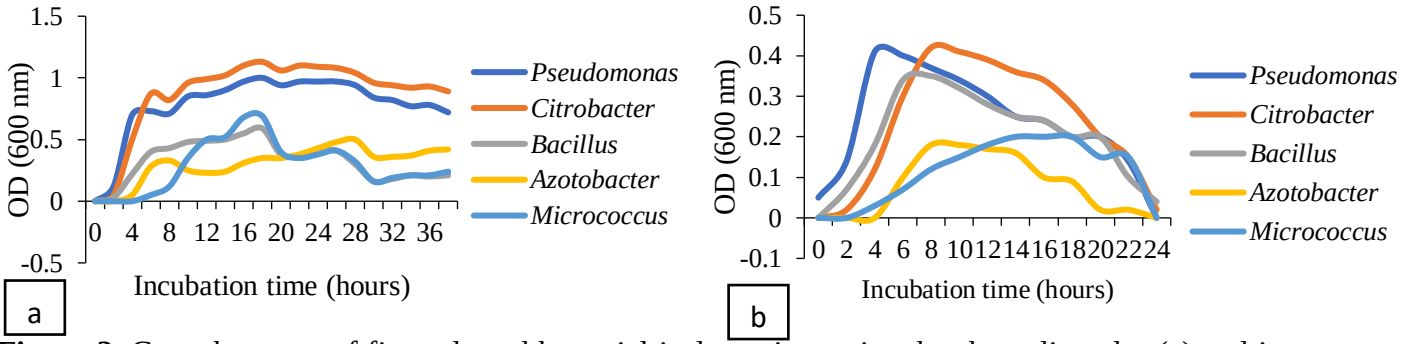



**Figure 3**. Growth curves of five selected bacterial isolates: in nutrient broth media only (a) and in nutrient broth media with $K_2Cr_2O_7$ 1,000 mg/kg (b)

There were five consortium combinations observed in this study; each consortium combination was composed of two genera of bacterial isolates from five selected bacterial genera, as shown in Table 2. The five consortium combinations were tested for compatibility to determine the synergism between members of the bacterial consortium. This study tested five consortium combinations to

ensure they can grow together without inhibiting each other. The compatibility test results showed that all bacterial consortium combinations exhibited synergy, as indicated by the absence of inhibition zones at the intersection lines (Figure 4). This finding suggests that all bacterial consortium combinations can work synergistically in the culture medium.

**Table 2.** Bacterial consortium combination for treatment

| Consortium combination name | Consortium members |
|---|---|
| AB | *Pseudomonas, Citrobacter* |
| BC | *Citrobacter, Bacillus* |
| CD | *Bacillus, Azotobacter* |
| DE | *Azotobacter, Micrococcus* |
| EA | *Micrococcus, Pseudomonas* |

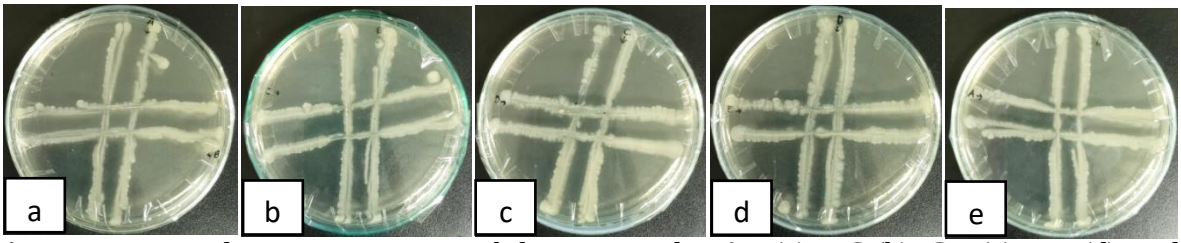
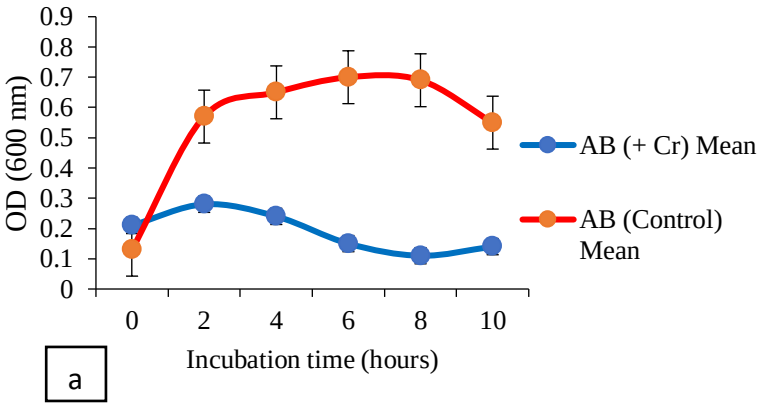
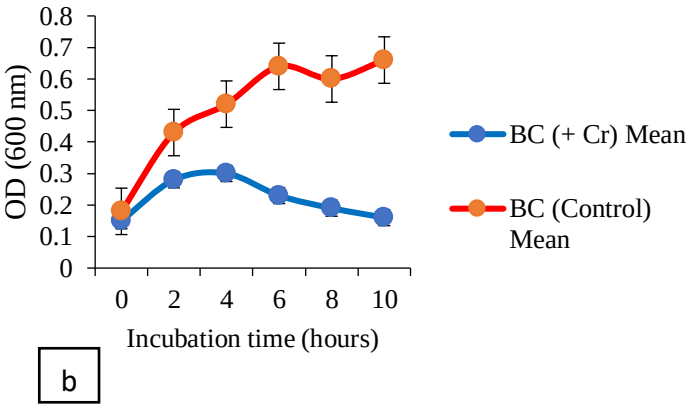



**Figure 4**. Bacterial consortium compatibility test results: AB (a); BC (b); CD (c); DE (d); and EA (e)

The growth curves showed that all consortia exhibited an extended lag phase and a slower exponential growth rate in the presence of chromium than in the control without chromium (Figure 5). The DE consortium (*Azotobacter* and *Micrococcus*) displayed the most consistent growth pattern, maintaining a stable stationary phase, indicating a more effective resistance to chromium stress. Figure 6 shows that the EA consortium (*Micrococcus* and *Pseudomonas*) demonstrated the highest bioremoval efficiency with a 30.78% reduction in chromium concentration after 10 hours, followed by CD (13.60%), AB (10.47%), BC (7.44%), and DE (6.71%). These results indicate that the EA consortium has the most effective ability to remove chromium from the media, possibly because the combination of bacteria in the consortium has a more efficient adaptation and detoxification mechanism for chromium.



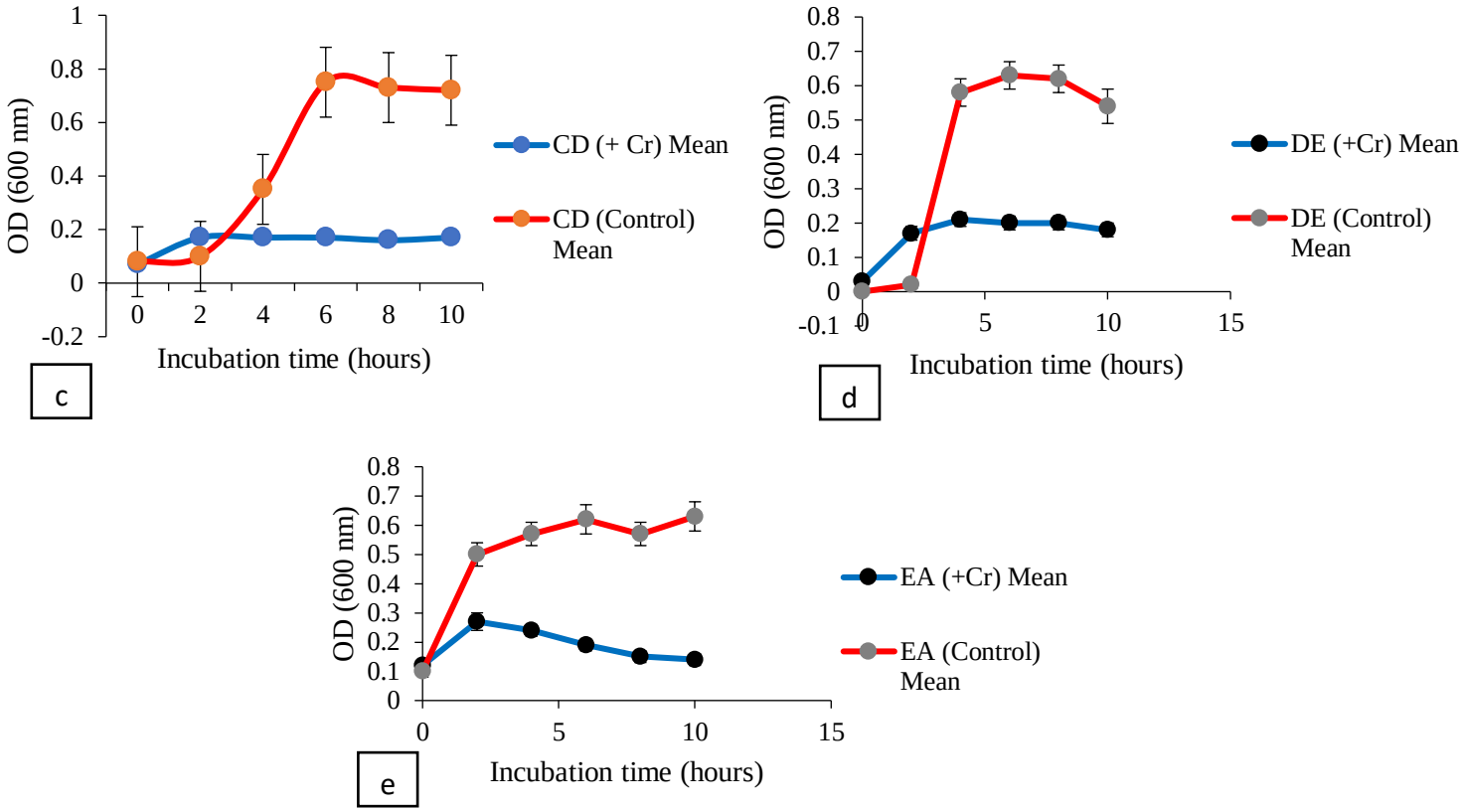



**Figure 5**. Growth curves of five combinations of bacterial consortium on nutrient broth media with $K_2Cr_2o_7$ 1,000 mg/kg: AB (a); BC (b); CD (c); DE (d); and EA (e)

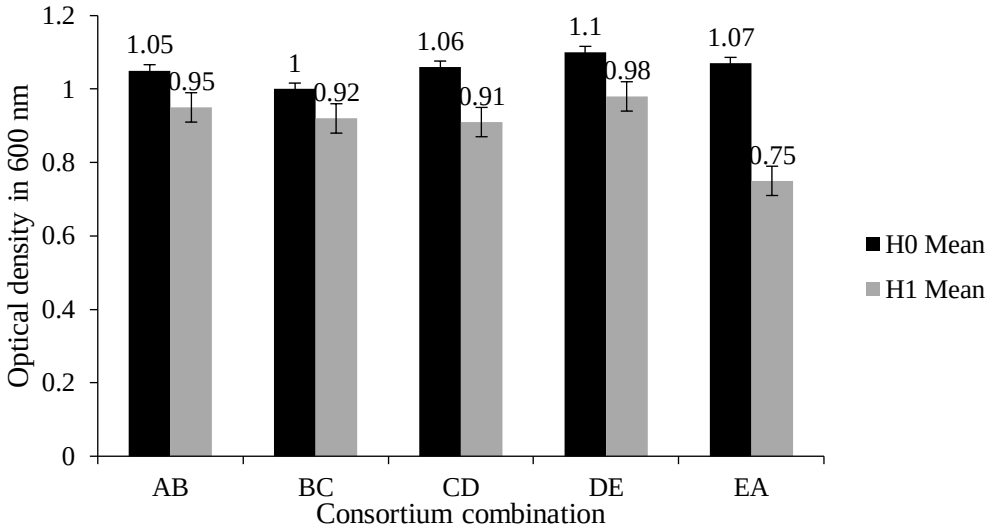



**Figure 6**. Graph of bioremoval ability of bacterial consortium on chromium after 10 hours (H0= initial concentration of chromium, H1= final concentration of chromium)

The bacterial population and chromium reduction data from five consortium combinations (AB, BC, CD, DE, EA) were analyzed statistically. Both datasets were non-normal and non-homogeneous, leading to the use of the Kruskal-Wallis test, which showed significant differences inbacterial populations and chromium reduction ($P < 0.05$). Dunnett's T3 post-hoc test revealed that consortia AB (*Pseudomonas* and *Citrobacter*) and BC (*Citrobacter* and *Bacillus*) had significantly higher bacterial populations, while the EA consortium (*Micrococcus* and *Pseudomonas*) showed the most effective chromium reduction. However, due to the short time of bioremediation, simple linear regression found no significant relationship between bacterial population and chromium reduction in any consortium, with $R^2$ values close to zero, indicating a weak or nonexistent linear correlation.

## DISCUSSION

The high chromium concentration at the sampling site underscores significant contamination that surpasses safety thresholds. *Mikania micrantha, Alternanthera sessilis,* and *Amaranthus viridis* suggest these species possess mechanisms to tolerate and remediate heavy metal contamination. *Mikania micrantha* is recognized for its potential in heavy metal removal (Leung et al., 2019), while *Alternanthera sessilis* has been identified as a hyperaccumulator of heavy metals. *Amaranthus viridis* has also shown chromium accumulation, particularly in its leaves (Ramanlal et al., 2020). The tolerance and accumulation abilities of these plants are linked to their structural adaptations, such as high pectin cell walls, developed vacuoles, and efficient antioxidant systems. Furthermore, the role of rhizosphere microorganisms in enhancing phytoremediation and bioaccumulation through collaborative mechanisms with plants underscores the importance of this symbiotic relationship in effective soil remediation.

The microscopic and macroscopic observations revealed that the bacterial isolates were primarily rod-shaped, with one coccus-shaped isolate. Gram staining results showed a mix of Gram-positive and Gram-negative bacteria, highlighting the diversity in cell wall structure. The presence of endospores in two isolates suggests adaptation to harsh conditions, such as chromium contamination, aligning with findings from Prabhakaran et al. (2016), who noted that Gram-positive bacteria like *Bacillus* can form highly resistant endospores. The biochemical test results demonstrate diverse metabolic capabilities among the isolates. These variations are consistent with the findings of Talaiekhozani et al. (2015), who emphasized the importance of biochemical tests in identifying bacterial genera. The distinct biochemical profiles of the isolates further support the identification of *Pseudomonas, Citrobacter, Bacillus, Azotobacter,* and *Micrococcus,* reflecting their ability to adapt and utilize various metabolic pathways. These results are in line with some studies, which state that these genera of bacteria are bacteria that can bioremediate heavy metals (Meliani & Bensoltane, 2016; Wang et al., 2018; Pandit et al., 2023; Kurniawan et al., 2019).

The differences in growth patterns among bacterial isolates in chromium-free media can be attributed to each bacterium's ability to adapt to the growth medium. *Pseudomonas* and *Citrobacter* showed short lag phases and rapid growth, likely due to efficient adaptation to the medium and quick utilization of available nutrients. In contrast, *Bacillus* and *Micrococcus* exhibited longer lag phases and slower growth, possibly due to longer adaptation times. *Azotobacter* demonstrated diauxic growth, characterized by a slow growth phase followed by secondary growth, which may reflect efficient use of alternative nutrients (George et al., 1985). The addition of chromium accelerated the transition to the stationary phase and cell death in all bacterial isolates, indicating significant toxic effects of chromium. High chromium concentrations inhibit growth by damaging cell membranes and disrupting cellular processes (Kothari et al., 2011). Research indicates that chromium concentrations above 100 mg/L can significantly inhibit bacterial growth, with varying toxic effects depending on bacterial species and chromium concentration (Karuppiah & Rajaram, 2011). However, bacteria have developed mechanisms to cope with chromium stress through biotransformation, biosorption, and bioaccumulation (Munawaroh et al., 2017).

In the comparison between consortium AB (*Pseudomonas*, *Citrobacter*) and the growth of each pure culture, it was observed that the population density in the consortium tended to be lower (OD <0.4) than in the respective pure cultures (OD >0.4). A similar trend was found in consortium BC (*Citrobacter, Bacillus*), where the population growth in the consortium was lower compared to each pure culture. In consortium CD (*Bacillus, Azotobacter*), population growth was lower than that of *Bacillus* in pure culture but closely resembled the growth pattern of *Azotobacter*, with more stable population dynamics observed up to 10 hours of incubation. Consortium DE (*Azotobacter, Micrococcus*) demonstrated relatively consistent population levels and more stable growth throughout the observation period. Meanwhile, in consortium EA (*Micrococcus, Pseudomonas*), the population growth pattern resembled that of the *Micrococcus* pure culture. Based on these observations, consortium DE showed the highest synergistic potential among the five tested combinations. Microbial consortia capable of maintaining stable growth under co-culture conditions are thought to exhibit supportive interactions and hold strong potential for application in

environmental biotechnology, particularly in bioremediation efforts. Growth stability within microbial consortia is a key indicator for the successful application of microorganisms in complex environmental systems such as contaminated soil and water (Nayak et al., 2024).

The synergy observed in the AB consortium, composed of *Pseudomonas* and *Citrobacter*, is consistent with previous studies reporting that mixed bacterial consortia enhance Cr (VI) bioremediation through synergistic microbial interactions (Wang et al., 2021), who stated that the synergy between these bacteria involves interactions in resource utilization or the ability to produce factors that support bacterial survival and growth. The BC consortium (*Citrobacter* with *Bacillus*) also demonstrated synergy, which can be linked to the role of both bacteria as plant growth-promoting rhizobacteria (PGPR). Denaya et al. (2021) reported that *Citrobacter* can synergize with *Bacillus* strains by increasing the solubility of nutrients beneficial to plants. A similar phenomenon was observed in the CD consortium, composed of *Bacillus* and *Azotobacter*, where *Azotobacter*, also a PGPR, works synergistically with *Bacillus* through its role as a biocontrol agent for plants and its contribution to the solubilization of nutrients, particularly nitrogen and phosphorus (Proboningrum et al., 2019). Although the synergy of the DE consortium (*Azotobacter* with *Micrococcus*) and EA consortium (*Micrococcus* with *Pseudomonas*) has not been previously reported, the non-inhibitory growth of both isolates may be due to various cooperative mechanisms, such as complementing each other in resource utilization, producing secondary metabolites beneficial to other bacteria, and controlled competition for resources.

The extended lag phase and inhibited exponential growth observed across the consortia suggest that chromium significantly impacts bacterial growth and metabolism. The stability of the DE consortium may be related to the chromium tolerance of *Azotobacter* and *Micrococcus*, as previous studies reported their ability to survive and reduce chromium under heavy-metal stress conditions (Rahayu, 2017; Meitiniarti et al., 2022). The synergy between these two bacteria may enhance their collective chromium tolerance, consistent with findings by Plestenjak et al. (2022), who reported that microbial communities were better adapted to elevated Cr(VI) concentrations than individual bacterial isolates.

The superior bioremoval performance of the EA consortium suggests that the combination of *Pseudomonas* and *Micrococcus* may possess complementary detoxification mechanisms, enabling more efficient chromium reduction. While previous studies have highlighted the individual roles of *Pseudomonas* and *Micrococcus* in heavy metal bioremediation (Aravindhan et al., 2007; Gong et al., 2020; Arroyo-Herrera et al., 2023), the potential synergy of this consortium in chromium bioremoval warrants further investigation. The findings of this study underscore the importance of selecting bacterial combinations that not only survive in contaminated environments but also work synergistically to enhance bioremediation efficacy.

The significant differences in bacterial populations and chromium reduction across the consortia suggest that specific bacterial pairings, such as *Pseudomonas* with *Citrobacter* and *Micrococcus* with *Pseudomonas*, can enhance both growth and bioremediation efficiency. The lack of a significant linear relationship between bacterial population and chromium reduction, as indicated by the low $R^2$ values, might be due to the high chromium concentration (1,000 mg/kg) in the culture medium, which could induce bacterial stress and reduce their bioremediation capacity. This outcome underscores the potential limitations of *in vitro* studies in replicating natural environmental conditions, where bacteria might demonstrate different bioremediation capabilities.

## CONCLUSION

Five chromium-resistant bacterial genera from rhizosphere soil (*Pseudomonas, Citrobacter, Bacillus, Azotobacter,* and *Micrococcus*) exhibited synergism in consortium combinations, with the DE combination (*Azotobacter* and *Micrococcus*) showing the most stable growth curve and the EA combination (*Micrococcus* and *Pseudomonas*) achieving the highest bioremoval capability, at 30.78%. However, the growth of the consortium did not significantly affect the chromium bioremoval capacity.The successful application of bacterial consortia for chromium bioremediation highlights a promising eco-friendly approach for addressing chromium contamination from tanning industry

waste. Further development of this method, including the optimization of bioreactors with indigenous rhizobacteria, could enhance bioremediation efficiency.

## ACKNOWLEDMENTS

We are grateful to everyone who helped with this research.

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