

Comparative and Synergistic Bioremediation of Chromium and Iron Using *Pseudomonas fluorescens* and *Bacillus subtilis*

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ABSTRACT

This research investigates the biosorption capabilities of two bacterial species, *Bacillus subtilis* and *Pseudomonas fluorescens* against heavy metals. A time-course *in-vitro* experiment was conducted to evaluate their tolerance and biosorption efficiency when exposed to iron (Fe^{3+}) and chromium (Cr^{6+}). Using ICP-MS analysis, *B. subtilis* was found to biosorb approximately 27% Cr^{6+} and 46% Fe^{3+} after 72 hours, while *P. fluorescens* biosorbed about 38% Cr^{6+} and 32% Fe^{3+} . Interestingly, a consortium of both species demonstrated enhanced biosorption compared to individual cultures, indicating a synergistic effect in reducing heavy metal concentrations. Results suggest

that consortia of these microbes can serve as effective bioremediation agents for heavy metal contamination.

Keywords Biosorption, Heavy metals, *Bacillus subtilis*, *Pseudomonas fluorescens*, Consortium.

INTRODUCTION

Environmental pollution continues to be the greatest threats to human health and ecosystems, with heavy metal contamination being a major contributor. Anthropogenic activities including mining, industrialization, urbanization and intensive farming with chemical fertilizers are the key sources of heavy metal release into agro-ecosystems (Ukaogo *et al.* 2020, Patil *et al.* 2024, Wu *et al.* 2022). Heavy metals adversely affect soil fertility along with population of beneficial microbes with contaminations often exceeding geo-baseline or legal limits in contaminated lands (Jayakumar *et al.* 2021). Globally an estimated 5 million hectares of land are contaminated with hazardous metals or metalloids, causing serious ecological and health risks. A variety of physico-chemical and biological remediation solutions have been developed to mitigate this issue. Among these, bioremediation is particularly very popular due to its cost-effectiveness, sustainability and efficiency (Aparicio *et al.* 2022). Bioremediation employs microorganisms and their metabolic by-products to immobilize, transform, or remove heavy metals from

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contaminated soils, thereby able to restore ecological balance (Aurica *et al.* 2021).

Among microbes, there is a large variation exist in resistance to heavy metals, with resistant cultivable microbes ranging from 10% to nearly 100% depending on soil conditions (Abdu *et al.* 2017, Kamal *et al.* 2009). Mechanisms of microbial remediation include a variety of processes such as biosorption, precipitation, oxidation and reduction. The aim of this study was to develop a consortium of *B. subtilis* and *P. fluorescens* and to evaluate their biosorption efficacy for iron (Fe) and chromium (Cr) under *in-vitro* conditions. ICP-MS was used to quantify changes in concentration of heavy metals.

MATERIALS AND METHODS

All chemicals used in this study were of analytical grade. The Gram-negative bacterium *Pseudomonas fluorescens* and the Gram-positive bacterium *Bacillus subtilis* were procured from the National Collection of Industrial Microorganisms (NCIM), Pune, India. Each strain was maintained in nutrient medium. 1 mL of nutrient broth containing specific strain with 1.5×10^8 used in appropriate proportions for experimental assays. Heavy metals were procured from V-consultancy, as Fe in Fe^{3+} form and Chromium as Cr^{6+} .

Spiking and sample preparation of heavy metals

Heavy metals were spiked into conical flasks that contained 100 ml of nutrient broth. A total of 10 flasks

were prepared. One flask served as the zero-hour control containing only 500 μ L of metal solution. Three flasks were spiked with 500 μ L of metal solution and inoculated with 1 ml of 24 hr old *B. subtilis* culture. Other three flasks were spiked with 500 μ L of metal solution and inoculated with 1 mL of 24 hr old *P. fluorescens* culture and the remaining three were spiked with same concentration of metal and inoculated with a mixed consortium of *B. subtilis* and *P. fluorescens*. All flasks were incubated at 37 °C for 24, 48 and 72 hours respectively. Each experiment was conducted in triplicate to minimize variability. At each time interval (24, 48 and 72 hours), 10 ml nitric acid was added to the flasks for digestion of organic matter. The control flask, containing only metal solution was similarly treated with nitric acid and placed on a hot plate for approximately 2 hours. After digestion, the volume of each sample was adjusted to 100 ml with distilled water. Samples were filtered using syringe filters and transferred into 15 ml centrifuge tubes. Heavy metal concentrations (Fe and Cr) were quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) following standard protocols (Meermann and Nischwitz 2018). All recorded data were statistically analysed using ANOVA and Duncan's multiple range test to validate the results.

RESULTS

The final values representing biosorption efficiency of tested microbes were calculated after subtracting the control values (zero-hour) which was also estimated using ICP-MS. The biosorption potential of *B. subtilis*

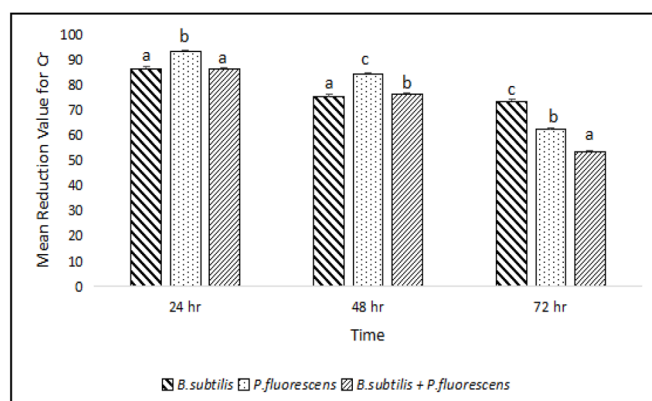


Fig. 1. Cr reduction efficacy of pure and mixed culture of bacteria over time intervals.

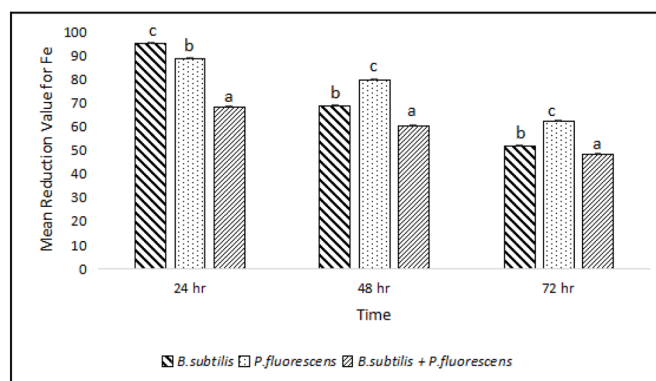


Fig. 2. Fe reduction efficacy of pure and mixed culture of bacteria over time intervals.

for Cr showed a mean value of 72.23 after 72 hours while in the case of *P. fluorescens* it was exhibited 62.23 (Fig. 1). The mixed culture demonstrated enhanced efficiency (Fig. 1) with a mean value of 53.40, indicating greater Cr reduction compared to individual strains.

For Fe, represented in Fig. 1, *P. fluorescens* showed a mean value of 62.21, while it was 51.85 when treated with *B. subtilis* after 72 hours. The mixed culture was more effective, with a mean value of 48.41. Fig. 1 & 2 illustrates the comparative biosorption efficiencies of pure and mixed cultures for both metals.

Statistical analysis using SPSS confirmed that differences among treatments were significant at the 0.05 level ($p < 0.05$). Mixed cultures consistently demonstrated higher biosorption efficiency than pure cultures, highlighting synergistic interactions between the two bacterial strains.

Following the investigation of the biosorption potential, an examination of comparisons was con-

ducted using SPSS. The relative effectiveness of bacterial strains at various time periods has been calculated. It was found that mixed cultures were more effective in the biosorption of heavy metals. The mean difference turned out to be significant at the 0.05 level ($p < 0.05$). Heavy metals are more bio-sorbed by mixed cultures than by pure cultures. The findings concerning the percent reduction of heavy metals demonstrate how effective the inoculants are. Thus, bacterial inoculants showed synergism. The percentage reduction relative to both Fe and Cr, two heavy elements, is depicted in Tables 1 and 2. *Bacillus subtilis* showed 4.93% reduction of Fe in 24 hrs, 31.26% in 48 hrs and 48.15% in 72 hrs whereas *Pseudomonas fluorescens* showed 11.59% in 24 hrs, 20.39% in 48 hrs and 37.79% in 72 hrs for Fe. A mixed culture has significant potential for iron reduction. Mixed culture indicated a reduction of 31.64% in 24 hrs, 39.54% in 48 hrs, and 51.59% in 72 hrs.

In case of Cr (Table 1), *B. subtilis* reduced 13.86% at 24 hrs, 24.83% at 48 hrs and 26.77% at 72 hrs percent reduction. *P. fluorescens* exhibited 6.84%, 15.79%, and 37.77% of reduction respectively. The

Table 1. Percentage reduction of Fe by pure culture and mixed culture at different time intervals.

| Time (Hours) | <i>B. subtilis</i> (% reduction) | <i>P. fluorescens</i> (% reduction) | Mixed culture (% reduction) |
|--------------|----------------------------------|-------------------------------------|-----------------------------|
| 24 | 4.93% | 11.59% | 31.64% |
| 48 | 31.26% | 20.39% | 39.54% |
| 72 | 48.16% | 37.79% | 51.59% |

Table 2. Percentage reduction of Cr by pure culture and mixed culture at different time intervals.

| Time (Hours) | <i>B. subtilis</i> (% reduction) | <i>P. fluorescens</i> (% reduction) | Mixed culture (% reduction) |
|--------------|----------------------------------|-------------------------------------|-----------------------------|
| 24 | 13.86% | 6.84% | 13.81% |
| 48 | 24.83% | 15.79% | 23.60% |
| 72 | 26.77% | 37.77% | 46.60% |

mixed culture showed 13.81% at 24 hr, 23.60% at 48 hr and 46.60% at 72 hr. Overall, *P. fluorescens* was more effective in Cr reduction while *B. subtilis* maintained relatively stable biosorption over time. The mixed culture displayed synergistic potential for Fe reduction particularly at longer incubation periods (72 hrs).

DISCUSSION

This study evaluated the heavy metal tolerance and biosorption capacity of *B. subtilis* and *P. fluorescens*. The findings indicate that *B. subtilis* was particularly effective in Fe reduction (48.15% at 72 hr) while *P. fluorescens* demonstrated superior Cr reduction (37.77% at 72 hrs). The mixed culture showed enhanced biosorption of Fe over time (24-72 hrs), suggesting possible cooperative interaction between these strains. In nature, microbes act as scavengers, and able to transform heavy metals into less toxic forms or immobilizing them in stable structures (Sharma *et al.* 2022). Previous studies have reported that *Bacillus* sp. can biosorb multiple heavy metals such as Cd, Cu, Cr, Ni, and Pb (Chen *et al.* 2022). *B. cereus* has also been shown to remediate mercury from synthetic effluents (Sinha *et al.* 2012). Similarly, *Pseudomonas* strains have demonstrated tolerance to elevated concentrations of Cr (VI). For instance, *Pseudomonas* sp. Cr13 was capable of tolerating up to 250 mg/L (Gong *et al.* 2020), whereas *Pseudomonas* sp. NChrA20 tolerated only 10.4 mg/L (Viti *et al.* 2003). Given the global concern regarding Cr (VI) pollution (Ashraf *et al.* 2017), the biosorption potential of these strains is remarkable. The mixed culture approach (as observed in this study) may provide greater efficiency in bioremediation of heavy metals compared to pure cultures particularly for iron. The results of this study establish a foundation for the application of *B. subtilis* and *P. fluorescens* in the bioremediation of hazardous heavy metal from contaminated environments.

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