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(RESEARCH ARTICLE)

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Potential of IAAT-3 Bacterial Isolate from Coal Mine Acid Mine Drainage Treatment Pond for Iron (Fe) Removal

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Abstract

The IAAT-3 bacterial isolate, recovered from a coal mine acid mine drainage (AMD) treatment pond, was evaluated for its bioremediation potential in reducing iron (Fe) concentrations. Experiments conducted from January to May 2025 examined the isolate's growth kinetics in Nutrient Broth supplemented with 25 ppm Fe and its Fe removal efficiency. A completely randomized design with three replicates and a negative control (Fe-free medium) was employed. The isolate exhibited significantly enhanced growth in the Fe-amended medium, attaining an optical density at 600 nm (OD_{600}) of 1.503 markedly exceeding the control indicating effective Fe tolerance and potential utilization as a micronutrient. Following 30 days of incubation, atomic absorption spectroscopy revealed a 94.79% reduction in Fe concentration. Phenotypic characterization confirmed that IAAT-3 is a Gram-positive, rod-shaped (bacillary) bacterium capable of forming central endospores. These results demonstrate the robust potential of IAAT-3 as an environmentally friendly, low-cost bioremediation agent for passive treatment of iron-contaminated acid mine drainage in coal mining-impacted environments.

Keywords: Bioremediation; Metal-tolerant bacteria; Acid mine drainage (AMD); Iron removal; Fe tolerance

1. Introduction

Coal serves as a critical energy resource and industrial fuel, contributing 26.9% to the global primary energy consumption mix and ranking as the second largest component after petroleum (31%) [1]. In 2021 worldwide coal production reached 8.173 billion tonnes, reflecting a 6.0% increase compared to the previous year [2]. The coal mining industry predominantly employs open-pit mining techniques [3]. Upon completion of mining operations, these activities typically leave behind open pits and generate acid mine drainage (AMD) [4].

Acid mine drainage (AMD) represents a significant byproduct of mining operations. Its formation arises primarily from the oxidative weathering of pyrite (FeS_2) or other sulfide-bearing minerals upon exposure to water (H_2O) and atmospheric oxygen (O_2) [5]. AMD is typified by markedly low pH levels and high concentrations of dissolved metals in solution [6], with iron (Fe) frequently constituting the dominant contaminant [7]. Associated heavy metals and metalloids commonly detected in AMD include aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), iron (Fe), lead (Pb), nickel (Ni), sodium (Na), and zinc (Zn) [8].

If not appropriately managed, acid mine drainage (AMD) exerts profound adverse effects on the environment, including contamination of aquatic ecosystems and degradation of adjacent terrestrial and riparian habitats in mining-impacted areas [9]. AMD treatment approaches are generally divided into two principal categories: active methods, which utilize chemical reagents such as slaked lime (calcium hydroxide) for neutralization and metal precipitation, and passive

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methods, which exploit microbial-mediated processes for natural attenuation and contaminant removal [10]. The mining industry largely favors active treatment systems owing to their rapid response and operational control; however, these approaches are associated with significant limitations, notably the production of large volumes of metal-laden sludge that accumulates in treatment basins, coupled with elevated operational and maintenance costs [11].

In contrast, bacterial bioremediation offers several distinct advantages over conventional chemical treatments, including high environmental compatibility, negligible or absent generation of secondary waste, rapid microbial metabolic rates, and significantly lower operational and maintenance costs. These characteristics position bacterial bioremediation as a highly promising and sustainable strategy for reducing dissolved metal concentrations in acid mine drainage (AMD)-affected waters. The IAAT-3 bacterial isolate, originally obtained from previous investigations, is a metal-tolerant strain capable of sustained growth and metabolic activity in the presence of iron (Fe) concentrations up to 10 ppm.

2. Method

This study employed an experimental approach, beginning with the recultivation (revival) of the IAAT-3 bacterial isolate. The isolate was grown in Nutrient Broth (NB) supplemented with 10 ppm iron (Fe) to confirm its previously demonstrated tolerance to this metal ion.

The ability of the IAAT-3 isolate to reduce iron (Fe) concentration was evaluated by culturing the bacterium in Nutrient Broth (NB) supplemented with 25 ppm Fe. The experiment was conducted in triplicate and included a negative control consisting of NB medium without Fe supplementation. Bacterial growth was monitored over a 30-day incubation period by measuring optical density at 600 nm (OD_{600}), which served as an indicator of biomass accumulation and metabolic activity. Following the incubation period, the reduction of Fe concentration in the culture medium was quantitatively analyzed using Atomic Absorption Spectroscopy (AAS).

Characterization of the IAAT-3 bacterial isolate included macroscopic observation of colony morphology, such as shape, margin, elevation, and color. In addition, Gram staining was performed to determine cell wall characteristics, and microscopic examination was conducted to assess cellular morphology, including cell shape and arrangement.

3. Result and Discussion

The growth of the IAAT-3 bacterial isolate in Nutrient Broth (NB) supplemented with 25 ppm Fe was significantly higher than that observed in the control treatment (NB without Fe supplementation), as indicated by a greater increase in optical density at 600 nm (OD_{600}). This enhanced growth suggests a positive adaptive response of the isolate to the presence of iron (Fe), indicating that Fe may function as a beneficial micronutrient or activate metabolic pathways that promote bacterial proliferation under these conditions. The growth curves of isolate IAAT-3 under both the 25 ppm Fe-supplemented condition and the Fe-free control are shown in Figure 1.

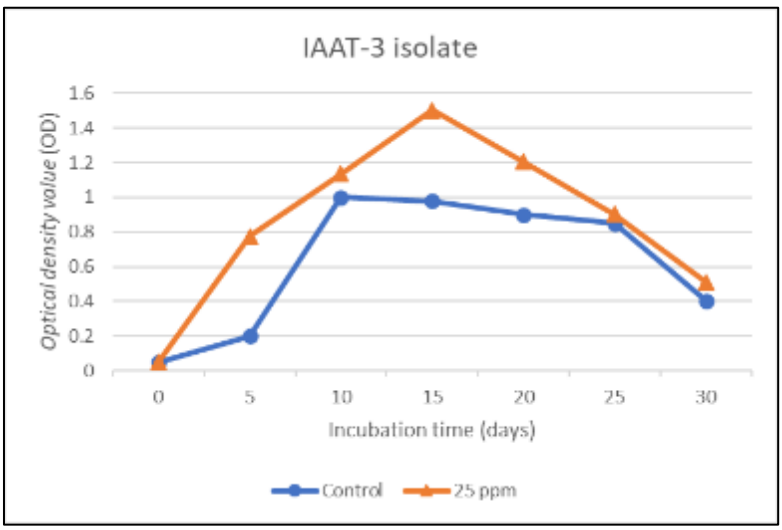

Figure 1 Growth curve of the Fe-tolerant IAAT-3 isolate at 25 ppm Fe

As shown in Figure 1, the IAAT-3 isolate exhibited significantly higher growth in Nutrient Broth supplemented with 25 ppm Fe than in the unsupplemented control, as reflected by higher optical density values at 600 nm (OD_{600}). This enhanced growth indicates that iron at low concentrations functions as an essential micronutrient, supporting key biological processes such as enzymatic activity, respiratory pathways, and overall cellular metabolism. The observed decline in growth at day 30 of incubation is likely attributable to the depletion of essential nutrients in the culture medium, causing the IAAT-3 isolate to enter the stationary phase, during which cell proliferation decreases due to nutrient limitation and the accumulation of metabolic byproducts. These findings are consistent with previous studies reporting enhanced bacterial growth in metal-supplemented media.

Bacterial isolates were able to proliferate in Nutrient Broth supplemented with graded concentrations of $HgCl_2$, as indicated by progressive increases in optical density (OD) values [12]. The highest OD value for isolate IB1 was observed on day 2 at a concentration of 100 ppm, whereas the lowest OD value occurred on day 7 at 200 ppm, demonstrating concentration-dependent growth dynamics and adaptive responses to metal-induced stress [13]. The capacity of bacteria to sustain growth under elevated metal concentrations reflects the activation of physiological adaptation mechanisms, including siderophore production for metal chelation and acquisition, the operation of active efflux pump systems for intracellular detoxification, and the sequestration of metal ions by cellular proteins or exopolysaccharides [14].

Quantitative analysis of iron (Fe) reduction was conducted using Atomic Absorption Spectroscopy (AAS). The IAAT-3 bacterial isolate exhibited substantial removal of Fe from the culture medium after 30 days of incubation. The extent of Fe reduction achieved by isolate IAAT-3 is shown in Figure 2.

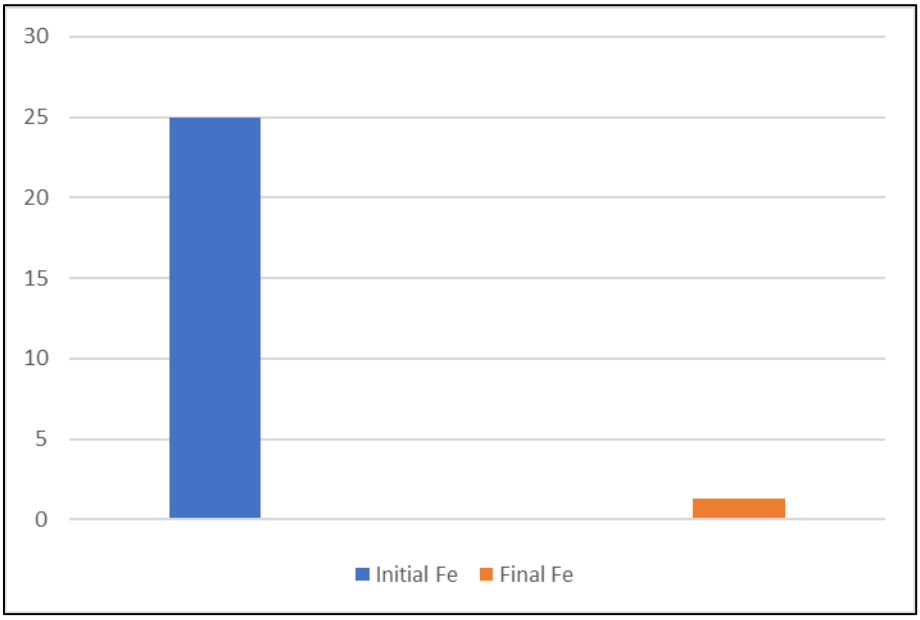

Figure 2 Percentage decrease in Fe concentration by isolate IAAT-3

Figure 2 shows that the IAAT-3 bacterial isolate achieved a substantial reduction in iron (Fe) concentration, with a removal efficiency of 94.79% after 30 days of incubation. This high level of Fe removal suggests that IAAT-3 possesses effective mechanisms for iron sequestration and/or transformation, thereby enabling significant bioreduction of dissolved iron in the culture medium.

Iron is not inherently toxic to microorganisms; at appropriate concentrations, it functions as an essential micronutrient involved in numerous cellular metabolic processes, including respiration, DNA synthesis, and enzymatic activity, particularly as a cofactor in heme-containing proteins and iron–sulfur (Fe–S) clusters. Certain bacteria, especially those isolated from metal-rich environments such as acid mine drainage, exhibit specialized metabolic capabilities, including iron oxidation by acidophilic iron-oxidizing bacteria or iron reduction through dissimilatory Fe(III) reduction pathways. These processes contribute to iron immobilization and detoxification in contaminated environments. The present findings are consistent with previous studies on metal-resistant bacterial isolates. For instance, five resistant isolates (B3Cd, B5Cr, B7Pb, B6Pb, and B3Pb) were reported to form biofilms and reduce heavy metal concentrations by 38.67–

61.19%, highlighting the role of biofilm-mediated mechanisms in enhancing metal tolerance and removal efficiency [15].

Macroscopic characterization of isolate IAAT-3 revealed colonies with irregular shape, white pigmentation, lobate margins, and raised elevation. Microscopic examination showed rod-shaped (bacillary) cells, while Gram staining confirmed Gram-positive cell wall characteristics. Additional physiological tests indicated that the isolate is catalase-negative, suggesting anaerobic or facultative anaerobic metabolism, and exhibits positive motility. A summary of the phenotypic characteristics of bacterial isolate IAAT-3 is presented in Table 1.

Table 1 Phenotypic and physiological characterization of the Fe-tolerant bacterial isolate IAAT-3

Isolate characteristic	**IAAT-3 isolate**
Macroscopic	
Colony shape	Irregular
Colony color	White
Colony margin	Lobate
Colony elevation	Raised
Gram	Positive
Microscopic	
Cell shape	Bacillus
Endospore test	Positive
Catalase test	Negative
Motility test	Motile

Table 1 summarizes the macroscopic colony characteristics of isolate IAAT-3, including irregular colony shape, white pigmentation, lobate margins, and raised elevation. Microscopic examination revealed rod-shaped (bacillary) cells, and Gram staining confirmed that the isolate is Gram-positive. These morphological and staining characteristics are consistent with previous reports describing metal-tolerant or metal-oxidizing bacteria isolated from contaminated environments.

Several studies have reported bacterial isolates from heavy metal–polluted sites that were predominantly Gram-negative and exhibited coccoid or bacillary cell morphologies [16]. In addition, iron-oxidizing bacteria have been characterized as Gram-negative, chemoautotrophic, acidophilic, and aerobic organisms, highlighting the diversity of Gram reactions and metabolic strategies among metal-metabolizing taxa [17]. Notably, Fe-oxidizing bacteria are frequently rod-shaped (bacillary), which is consistent with the cellular morphology observed in isolate IAAT-3 [18].

Based on the observed phenotypic traits particularly the Gram-positive reaction, rod-shaped cells, irregular and raised colony morphology, and the reported endospore-forming capability (as documented in related characterizations) isolate IAAT-3 is provisionally assigned to the genus *Bacillus* spp. in accordance with standard microbiological identification keys [19]. Nevertheless, definitive species-level identification requires further molecular analyses.

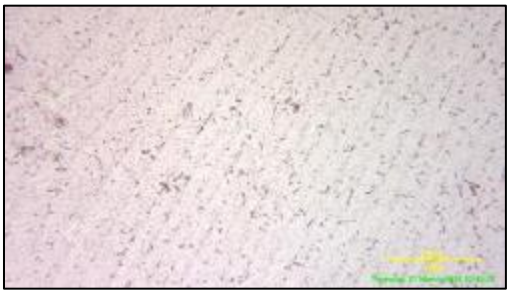

Figure 3 Gram staining results of the Fe-tolerant bacterial isolate IAAT-3

Gram staining results revealed that the bacterial isolate IAAT-3 consists of rod-shaped (bacillary) cells exhibiting a Gram-positive reaction, with the additional observation of central endospore formation, as clearly illustrated in Figure 3.

Endospore staining is conventionally performed selectively on Gram-positive rod-shaped isolates to detect the presence of these highly resistant, dormant structures, which serve as survival mechanisms under adverse environmental conditions. The procedure employed in this study followed the standard Schaeffer-Fulton method, which utilizes heat to facilitate the penetration of the primary stain (malachite green) into the endospore core, followed by counterstaining with safranin to differentiate vegetative cells (pink/red) from endospores (green) [20].

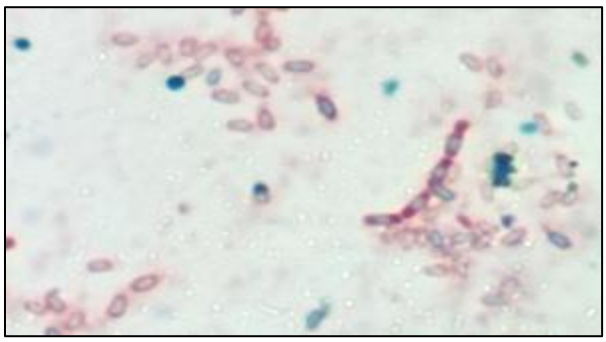

Figure 4 Endospore staining results of the Fe-tolerant bacterial isolate IAAT-3, demonstrating the presence of central endospores

The ability of isolate IAAT-3 to form endospores reflects a high level of adaptation to harsh, metal-contaminated environments such as acid mine drainage (AMD), which is characterized by low pH and elevated metal concentrations. Endospores are metabolically dormant structures that function as a key survival and defense mechanism under extreme environmental stress. Their formation is generally induced by adverse conditions, including exposure to heavy metals, extreme temperatures, desiccation, nutrient limitation, or oxidative stress [21].

4. Conclusion

Based on the results of this study, the following conclusions can be drawn: The bacterial isolate IAAT-3 demonstrates tolerance to iron (Fe), achieving maximum growth on day 15 of incubation with an optical density at 600 nm (OD_{600}) of 1.503. The isolate was capable of substantially reducing Fe concentration, with a removal efficiency of 94.79% after 30 days. Phenotypic characterization indicated that IAAT-3 consists of rod-shaped (bacillary), Gram-positive cells and is capable of forming central endospores.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing interests in relation to this study.

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