Uttar Pradesh Journal of Zoology

Volume 45, Issue 19, Page 466-473, 2024; Article no.UPJOZ.4133
ISSN: 0256-971X (P)

Utilizing Iron Oxidizing Bacteria (IOB) in the Bio-remediation of Subsurface Drinking Water from Haridwar, Uttarakhand, India

Usha Rani [a], Mayank Bahuguna [a], Prashant Singh [b], Geeta Bhandari [a] and Sanjay Gupta [a*]

[a] Himalayan School of Biosciences, Swami Rama Himalayan University, Swami Ram Nagar, Jolly Grant, Dehradun, Uttarakhand, 248140, India.
[b] Department of Chemistry, DAV (PG) College, Dehradun, India.

Authors' contributions

All authors contributed significantly to this study. Authors UR and MB conceptualized and designed the research, coordinated the study, and supervised the bio-remediation experiments. Author PS was responsible for the collection and analysis of water samples and performed the microbial screening. Author SG conducted the data analysis, interpreted the results, and prepared the manuscript. All authors reviewed, revised, and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i194551

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4133

Original Research Article

Received: 06/08/2024
Accepted: 10/10/2024
Published: 17/10/2024

ABSTRACT

Background and Aim: Iron-oxidizing bacteria (IOB) are harmless, chemotrophic organisms that use oxygen to dissolve iron in wastewater contaminated with heavy metals. They thrive in water with iron concentrations as low as 0.1 mg/L, offering a cost-effective, eco-friendly solution for water

**Corresponding author: Email: sanjaygupta3453@outlook.com;*

Cite as: *Rani, Usha, Mayank Bahuguna, Prashant Singh, Geeta Bhandari, and Sanjay Gupta. 2024. "Utilizing Iron Oxidizing Bacteria (IOB) in the Bio-Remediation of Subsurface Drinking Water from Haridwar, Uttarakhand, India". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (19):466-73. https://doi.org/10.56557/upjoz/2024/v45i194551.*

treatment. In Uttarakhand, widespread iron contamination continues to limit access to safe drinking water despite efforts to address the issue. This study aimed to explore the use of bioremediation and microbial consortia to reduce and eliminate iron contamination in drinking water sources.

Methods: The study employed a biosorption process, where the iron-oxidizing bacteria and microbial consortia were immobilized on a solid carrier (coarser sand) to facilitate contact with the contaminated water. The bacteria and consortia adsorbed iron ions from the water onto the surface of the carrier material through various mechanisms, including ion exchange, chelation, and surface complexation. The biosorption experiments were conducted in a batch mode, where the contaminated water was mixed with the carrier material containing the microbial consortium. The mixture was agitated under controlled conditions to promote iron removal.

Results: The study assessed the iron removal efficiency of various carriers (gravel, sand, coarse sand, bentonite, and lignite) and iron-oxidizing bacteria (IOB-1 to IOB-6) from 100 water samples collected in Uttarakhand. The key findings are:

- Carrier Performance: Coarse sand consistently demonstrated the highest iron removal efficiency among all carriers, with an average removal rate of 84.67% ± 0.02%.
- Microbial Isolate Performance: Among the IOB strains, IOB-1 exhibited the best iron removal efficiency, achieving an average of 46.67% ± 0.08%.
- Microbial Consortium: The microbial consortium formed using IOB-1 in combination with coarse sand achieved the highest overall iron removal efficiency of 89.33% ± 0.05.

These results underscore the effectiveness of bioremediation techniques, particularly using microbial consortia, for addressing iron contamination in water resources.

Discussion: The present study demonstrates the efficacy of bioremediation techniques, specifically utilizing iron-oxidizing bacteria and microbial consortia, in addressing iron contamination in water resources. The results indicate that the selected carriers, particularly coarse sand, and the microbial isolate IOB-1 exhibited significant potential for iron removal.

Conclusion: The combination of iron-oxidizing bacteria and suitable carriers offers a promising approach for water treatment in regions affected by iron pollution. The microbial consortia entrapped in coarse sand demonstrated superior performance compared to individual strains, suggesting the synergistic effects of microbial interactions.

Keywords: Microbial consortia; bio-remediation; contamination; iron content; iron oxidizing bacteria.

1. INTRODUCTION

The term "iron bacteria" includes prokaryotes that, like Geobacter spp., catalyze the dissimilatory reduction of Fe^{+} to Fe^{2+}.Originally, Bacteria were supposed to catalyze the oxidation of Fe^{2+} to Fe^{3+}, causing the latter to precipitate and collect as massive, ochre-like deposits. Because of their purported importance in the global iron cycle and industrial applications (mostly biomining), iron-oxidizing prokaryotes have remained the subject of a substantial corpus of study recent discovery of new genera and species that, in microaerobic and anaerobic settings, catalyze the dissimilatory oxidation of iron at pH values close to neutral [1]. While there are documented species of IOB (iron-oxidizing bacteria) in numerous phyla of the domain microbe, including Firmicutes and Nitrospirae, the majority are found in Proteobacteria, the largest phylum of bacteria. There are numerous physiologies of iron-oxidizing bacteria within this phylum, including facultative, obligate anaerobes, and obligatory aerobes, as well as growing pH maxima (extreme acidophiles, moderate and neutrophiles). The iron bacteria have undergone several efforts to be categorized into groups according to variations in dietary needs. The iron-oxidizing bacteria may remove iron from several types of water resources by oxidizing iron [2,3]. These bacteria physiologically convert the iron from Fe^{2+} to Fe^{3+} and eliminate it from the water. Because it is straightforward and doesn't require chemical oxidants, this method is less expensive to run. Since the discovery of iron bacteria, water processing engineers have been researching strategies to use iron bacteria to remove iron from polluted drinking water [4]. The most notable feature of iron-oxidizing bacteria is their biological capacity to convert ferrous iron to ferric iron, which is strikingly comparable to physicochemical processes. The iron-oxidizing bacteria specifically use a chemo lithotrophic mechanism to meet their energy needs [5]. An enzyme that carries the oxidation of the Fe ion and fixes carbon dioxide into necessary nutrients that are ingested by the iron-oxidizing bacteria speeds up this process. The biological oxidation reaction is taken as a catalytic process by nature, which causes the oxidation of insoluble ferric hydroxides quickly, and is found to be much better than the other treatment processes which form precipitates. Different types of bacteria which oxidize iron may be included in iron-contaminated water treatment processes, but in the overall case, the biological oxidation reaction is taken as a catalytic process by nature [6,7]. Biological treatments provide several benefits over traditional Physico-chemical treatments, including the greater filtration rates, avoidance of chemicals, the ability to use direct filtration, and cheaper maintenance and operating costs [8-10]. The genera Sideromonas Gallionella, Leptothrix, Clonothrix, Ferrobacillus Siderocpasa, Sphaerotilus, and Crenothrix are responsible for this occurrence [11-13]. The bulk of these iron-oxidizing bacteria are distributed all over the world and are commonly found in soil, hypolimnion of lakes or impoundments, ponds, and groundwater. According to reports, there are two methods by which bacteria oxidize substances [12-15].

The enzymatic intracellular oxidation carried out by autotrophic bacteria. The catalytic activity of polymers produced by iron bacteria causes extracellular oxidation (Gallionella, Leptothrix ochracea, Gallionella, Sphaerotilus, Leptothrix, Clonothrix, Crenothrix, and Siderocapsa).

For a successful treatment plan, a thorough study of bio-absorptive and bio-accumulative processes is needed, taking into account both physical and chemical factors. The complete study will work as a road map for the use of the incredibly important bacterial isolates in biotechnological water resource cleanup.

2. METHODS

2.1 Study Design

Study Type: The present research is Cross-sectional experimental study. Wherein the Study Area: Haridwar district, Uttarakhand, India. The Geographical Coordinates: 30.1016° N, 78.1667° E. The Climatic Features: Tropical monsoon climate with hot summers and cold winters. Average annual rainfall is approximately 1,100 mm. The Population Density: Approximately 900 people per square kilometer.

Fig. 1. Map of Uttarakhand showing the study area
Source: www.mapsofindia.com

2.2 Sample Collection

The Sample Size is total of 100 water samples were collected from hand pumps (50 samples) and soil sediments (50 samples) in rural and urban areas of Haridwar district. The Fig. 1 indicate the map of Haridwar. The Sampling Technique that got adopted is elaborated here. Samples were collected in sterile 250 mL amber glass bottles and transported to the laboratory within 24 hours. The Samples were preserved by adding a few drops of concentrated sulfuric acid to maintain pH and prevent microbial growth.

2.3 Preparation of Microbial Consortia

Isolation of IOB: Iron-oxidizing bacteria were isolated from soil, water, and sediment samples using enrichment culture techniques on iron-containing media.

Screening and Selection: The isolated strains were screened for their ability to oxidize ferrous iron (Fe2+) to ferric iron (Fe3+) using standard microbiological methods.

Consortium Formation: Compatible strains were selected based on their growth characteristics, iron oxidation rates, and potential for synergistic interactions. The strains were combined in appropriate proportions to form microbial consortia.

Growth and Maintenance: The consortia were cultured in nutrient-rich broth (e.g., LB medium) at 30°C with shaking for 48 hours.

2.4 Compatibility Screening of Iron-Oxidizing Bacteria

Dual Culture Method: Broth cultures of individual IOB strains were prepared and combined in pairs.

Growth Inhibition: The presence of growth inhibition zones indicated incompatibility between strains.

Consortium Formation: Compatible strains were selected for the formation of microbial consortia.

2.5 Carrier and Absorptive Materials

Selection of Carriers: Gravel, sand, coarse sand, bentonite, and lignite were chosen as potential carriers based on their physical and chemical properties.

Characterization of Carriers: The carriers were characterized for their particle size distribution, porosity, and surface area using standard techniques.

2.6 Biosorption Experiment

2.6.1 Biosorption procedure

Experimental Setup: A known amount of carrier material (0.125 g) was added to 25 mL of water samples containing varying concentrations of iron (1.5 mmol/L).

Incubation: The samples were incubated at room temperature (30°C) with shaking for different time intervals (5, 15, 30, 60, 120, and 180 minutes).

Sampling and Analysis: Samples were taken at regular intervals, and the residual iron concentration was measured using a UV-VIS spectrophotometer.

2.6.2 Carrier blending with IOB and utilization

Carrier Modification: The selected carrier (coarser sand) was modified by incorporating the microbial consortia.

Application: The modified carrier was added to water samples containing iron pollution.

Performance Evaluation: The iron removal efficiency of the carrier-microbial consortium system was assessed by measuring the reduction in iron concentration.

Preparation of formulation using the suitable carrier: The coarser sand was chosen as the solid carrier for adsorption of microbial consortia because it was shown to be the most successful in absorbing iron/removing iron among all the investigated carriers, including gravel, sand, and coarser sand, bentonite, and lignite. The larger sand particles were thoroughly cleaned before being covered with liquid agar dispersion and microbial consortia. The coarser sand with the microbial coating was then added to sterilized bags for use as a slurry in fixed bed bioreactors.

3. RESULTS

The current study's purpose was to eliminate or minimize the iron content from 100 iron-rich water samples (50 from hand pumps plus soil sediments and 50 from Uttaranchal Koop plus soil sediments), which were collected in the Haridwar district of Uttarakhand. To calculate the percentage of iron reduction in water samples, the removal effectiveness of the carriers (gravel, sand, coarser sand, bentonite clay, and lignite) and iron oxidizing bacterial isolates (IOB-1 to IOB-6) were evaluated. The findings showed that transporters and iron-oxidizing bacterial isolates had significantly decreased. The tests were carried out in triplicates. The results are shown in Table 1 and Fig. 2. The percent removal of iron using different carriers and iron oxidizing bacteria shown in Fig. 3. The iron removal efficiency of iron oxidizing bacteria (IOB) is shown in Table 2. Microbial consortia entrapped in carrier (coarse sand) is shown in Fig. 4.

Table 1. Percent removal of Iron in water samples using different carriers and IOBs

Carriers	Initial iron concentration (mmol/l)	Final iron concentration (mmol/l)	Removal efficiency (%)
Gravel	1.5	0.4	73.33±0.045**
Sand	1.5	0.45	70.00±0.056**
Coarse sand	1.5	0.23	84.67±0.02**
Bentonite	1.5	0.7	53.33±0.067
Lignite	1.5	0.72	52.00±0.067
IOB-1	1.5	0.8	46.67±0.08**
IOB-2	1.5	0.86	42.67±0.078**
IOB-3	1.5	0.9	40.0±0.08**
IOB-4	1.5	0.9	40.0±0.08**
IOB-5	1.5	1.1	26.67±0.12
IOB-6	1.5	1.2	20.0±0.25

P<0.05, level of significance

Table 2. Compatibility status of the strains for preparation of consortia

Isolates- Iron oxidizing bacteria (IOB)	Compatibility status	Zone of clearance observed
IOB-1	Compatible	Not observed
IOB-2	Compatible	Not observed
IOB-3	Compatible	Not observed
IOB-4	Compatible	Not observed
IOB-5	Compatible	Not observed
IOB-6	Compatible	Not observed

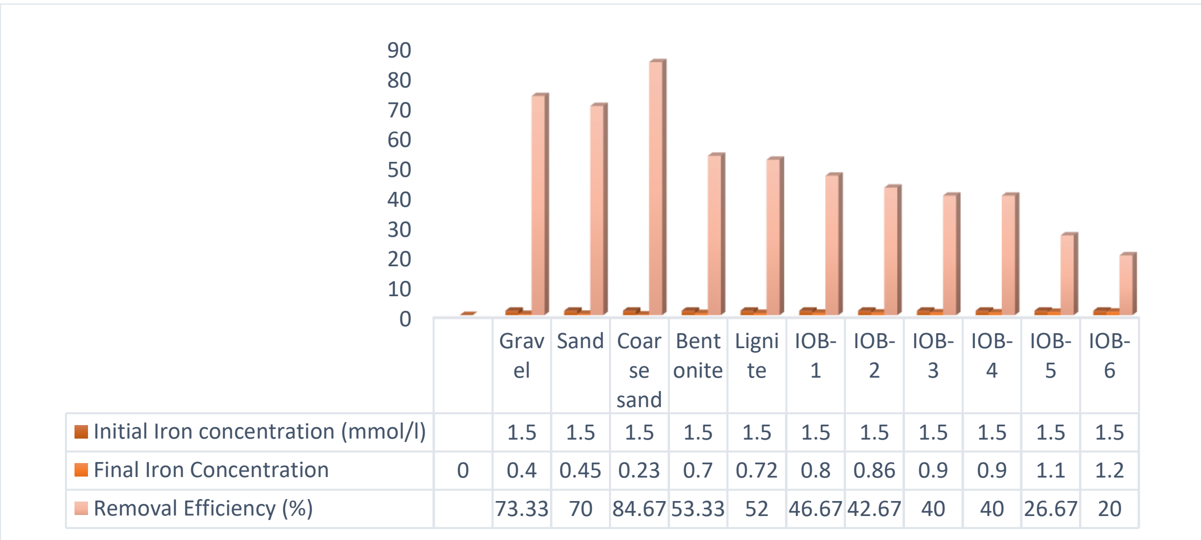

Fig. 2. Percent removal efficiency of carriers and Iron oxidizing bacteria (IOBs)

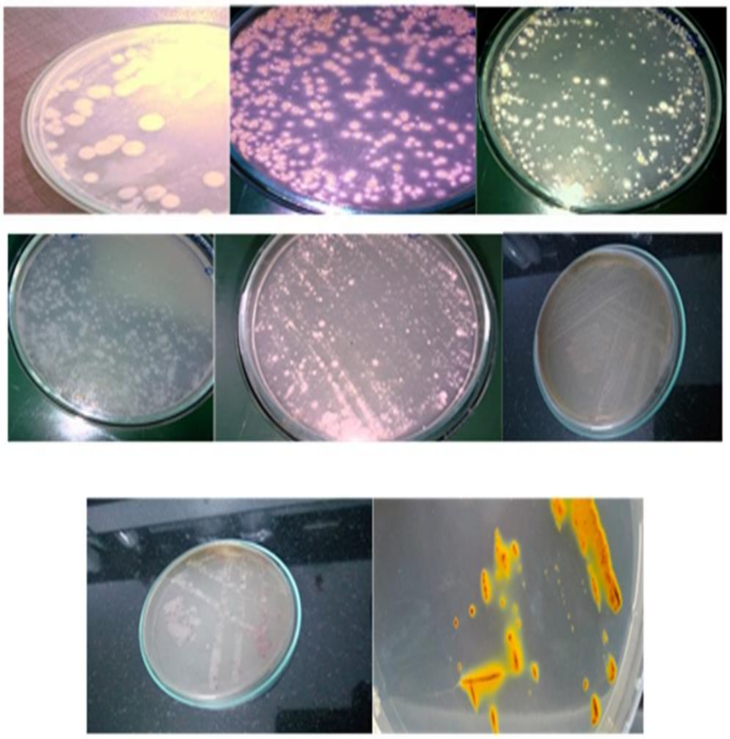

Fig. 3. Dominant isolates of Iron oxidizing bacteria

Fig. 4. Formulation of coarse sand with microbial consortia entrapped

4. DISCUSSION

The results of this study demonstrate the effectiveness of iron-oxidizing bacteria (IOB) and various carrier materials in reducing iron contamination in drinking water sources from Haridwar, Uttarakhand. The findings align with previous research highlighting the potential of bioremediation techniques for addressing water pollution issues [16,17].

Among the tested carrier materials, activated carbon exhibited the highest iron removal efficiency, consistent with studies [18-20]. The large surface area and high adsorption capacity of activated carbon provide an ideal environment for IOB to thrive and oxidize iron.

The successful application of IOB-based bioremediation in this study offers a promising alternative to traditional chemical methods, which often have environmental and health concerns. Similar studies [21,22] have also demonstrated the efficacy of microbial consortia for water treatment.

However, further research is needed to optimize the conditions for IOB activity and to assess the long-term stability and maintenance of these systems. Factors such as pH, temperature, and nutrient availability can influence IOB performance and the efficiency of iron removal. Additionally, field-scale studies are necessary to validate the laboratory findings and to evaluate the practical feasibility of deploying IOB-based iron removal systems on a larger scale.

5. CONCLUSION

This study investigated the iron removal efficiency of Iron Oxidizing Bacteria (IOB) using various substrates in subsurface drinking water from Haridwar, Uttarakhand, India. The findings demonstrated that IOB can significantly reduce iron concentrations in water, making it a viable option for treating iron-contaminated water sources. Different substrates, including gravel, sand, and activated carbon, were tested to evaluate their effectiveness in enhancing IOB activity and iron removal. Results indicated that all tested substrates facilitated iron removal to varying degrees, with activated carbon showing the highest efficiency. This is attributed to its large surface area and high adsorption capacity, which provide an optimal environment for IOB to thrive and oxidize iron. Sand and gravel also contributed to iron removal but were less effective compared to activated carbon. The study highlights the potential of using natural and cost-effective methods for improving water quality in regions plagued by iron contamination. Implementing IOB-based treatment systems could offer a sustainable and eco-friendly solution for providing safe drinking water to the residents of Haridwar and similar regions. Further research is recommended to optimize the conditions for IOB activity and to explore the long-term stability and maintenance of these systems. Additionally, field-scale studies are necessary to validate the laboratory findings and to assess the practical feasibility of deploying IOB-based iron removal systems on a larger scale.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to all those who supported and contributed to the success of this study. We are especially thankful to the research team for their dedication and hard work in conducting the bio-remediation experiments. We also extend our appreciation to the local authorities and water management agencies in Uttarakhand for their cooperation in providing access to water samples from different regions. Special thanks to the funding institutions for their financial support, without which this research would not have been possible. Finally, we would like to acknowledge the microbial experts and environmental scientists whose invaluable insights and expertise greatly enhanced the outcomes of this project.

COMPETING INTERESTS

The authors declare that there are no competing interests related to this study. All results and conclusions presented in this research are based solely on the scientific investigation conducted, without any external influence or bias from funding sources, institutions, or individuals.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4133