

REFERENCES



REFERENCES

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LIST OF PUBLICATIONS



LIST OF PUBLICATIONS

1) Participated in:

- a) 2nd International Conference on Recent Advances in Biotechnology and Nanobiotechnology 2022 (Oral Presentation)
- b) 14th Uttarakhand State Science and Technology Congress 2019-20 (Poster Presentation)
- c) 3rd International Conference on INCD 2018 (Poster Presentation)

2) Paper published:

- a) Rani U, Gupta S, Kumar V. Isolation and characterization of Iron oxidizing bacteria from water samples collected from different regions of Haridwar, Uttarakhand. Zeichen, 2022; Vol. 8; 483-491.
- b) Rani U, Gupta S. Study of Iron removal efficiency of Iron oxidizing bacteria (IOB) using different substrates in sub surface drinking water of Haridwar, Uttarakhand, India. BioGecko, Web of Science. 2023; Vol. 12;647-653.

3) Review article published:

- a) Rani U, Gupta S, Kumar V. An Update on Bioleaching Technology: Iron Bacteria as A Source of Oxidizing Iron Traces from Water Samples. IJPSR, 2021; Vol. 12(2): 744-753.

4) Books chapter published:

- b) Gupta S, Dobhal R, Gupta A, Rani U, Kumar V. Water Quality Assessment and Treatment of Pharmaceutical Industry Wastewater. A Case Study of Pharmacity Selaqui, Dehradun of Uttarakhand State, India. In *Phytobiont and Ecosystem Restitution 2018* (pp. 329 – 377). Springer, Singapore.

CERTIFICATES



2nd INTERNATIONAL CONFERENCE

ON

RECENT ADVANCES IN BIOTECHNOLOGY AND NANOBIOLOGY

(Int-BIONANO-2022)

Amity Institute of Biotechnology, Amity University Madhya Pradesh, Gwalior

CERTIFICATE

This certificate is awarded to Ms. Usha Rani from HHT University, Jolly Grant, Dehradun, Uttarakhand for her presentation of research paper titled Screening of isolates of iron oxidizing bacteria (iob) for removal of iron from fresh water in the 2nd International Conference on Recent Advances in Biotechnology and Nanobiotechnology (Int-BIONANO-2022) during 10-11 February 2022.

Prof.(Dr.) Rajesh Singh Tomar
Convener

Lt. Gen. V. K. Sharma, AVSM (Retd.)
Chairperson



Certificate of Participation



State Council for Science
and Technology, Dehradun

This is to certify that

Usha Rani

has actively participated in

14th Uttarakhand State Science and Technology Congress 2019-2020

27th - 29th February, 2020

presented a research paper (Oral/Poster) entitled

Bioprocess Development for the biological Removal of

Iron from Sub-Surface Drinking water in Uttarakhand.

under the discipline

BioTechnology, Biochemistry & Microbiology.

at Uttarakhand Council for Science and Technology, Vigyan Dham, Dehradun.

Dr Ashutosh Mishra
Organizing Secretary
14th USSTC
Council for Science and Technology
Uttarakhand

Dr Rajendra Dobhal FNASc
Chairman - 14th USSTC
Director General
Council for Science and Technology
Uttarakhand



3rd International Conference
On
“Nutraceuticals and Chronic Diseases-2018”
(INCD-2018)

Certificate of Appreciation

This is to certify that Usha Rani has delivered oral presentation / presented poster / participated at the Third International conference on “Nutraceuticals and Chronic Diseases” (INCD-2018).

Conference accredited from Uttarakhand Medical Council for 10.0 Hours.

Jointly organized by Swami Rama Himalayan University (Dehradun, India), International Society of Nutraceuticals & Chronic Diseases and University of Tennessee Health Science Center (USA), in Dehradun, India, from 14th -16th September, 2018.

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Study of Iron removal efficiency of Iron oxidizing bacteria (IOB) using different substrates in sub surface drinking water of Haridwar, Uttarakhand, India

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Abstract

In Uttarakhand, the availability of clean, safe drinking water is a major challenge. Despite significant expenditures and efforts, the majority of the state's population still lacks access to clean drinking water because of greater levels of iron pollution in both rural and urban regions. The current investigations comprise findings based on the analysis and reduction/elimination of iron from various water resources employing effective bio-remediation approaches and the right microbial consortia. Different agencies' efforts haven't yet shown fruitful results, and all of the imported or market technologies used have disappointed for a variety of reasons, including the use of chemicals, the lack of a local supply of regeneration material, trash disposal, and isolated sites in hilly areas. In the present investigation, iron oxidizing bacteria were screened for reduction of iron content in water samples. The results were found to be effective as the isolated iron oxidizing bacteria were found to have significant iron oxidizing (removal) potential from the water samples collected.

Keywords -Microbial consortia, bio-remediation, contamination, iron content, iron oxidizing bacteria

INTRODUCTION

The term "iron bacteria" includes prokaryotes that, like *Geobacter* spp., catalyze the dissimilatory reduction of Fe⁺ to Fe²⁺. Originally, Bacteria were supposed to catalyze the oxidation of Fe²⁺ to Fe³⁺, 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 Fe²⁺ to Fe³⁺ 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

647

A Journal for New Zealand Herpetology

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*, *Siderocapsa*, *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].

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

For a successful treatment plan, a thorough study of bio-adsorptive 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.

MATERIALS AND METHODS

Characterization and formulation of soil media/ carriers for the treatment of raw water:

a) Gravel

Rock fragments that are more or less rounded in a coarser-grained aggregate than sand (i.e., more than 2 mm [0.08 inch] in diameter). Heavy metallic ore deposits like cassiterite (a important source of tin) or native metals like gold can be found accumulating in gravel beds in some places. Gravel is a frequent building material. The various sizes of gravel pieces include pebbles (4-64 mm), cobbles (64-256 mm), and boulders (larger than 256 mm). Gravel is rounded due to abrasion when being transported by streams or milled by the sea. Gravel deposits form in stream channels and on beaches when the water is too fast for sand to settle. Because of changeable environmental variables, gravel formations are frequently more limited and diverse in thickness, coarseness and structure than clay or sand deposits. On a sandy beach, there may be a persistent buildup of gravel or pebble beds around the inner zone of the breaking waves. Beaches with cobbles and pebbles (shingle beaches) frequently start at the tips of stony cliffs

b) Sand

Sand is a granular substance made up of tiny pieces of rock and minerals. The content of sand varies, but the grain size is what distinguishes it. Sand is coarser than silt and has smaller granules than gravel. A soil type or textural class that contains more than 85% of its bulk in sand-sized particles is referred to as sand. The composition of sand depends upon the local rock sources and conditions, although silica (SiO₂), usually in the form of quartz, is much common component in non-tropical coastal settings and inland continental settings. Fine sand has particles that range in size from 0.075 to 0.425 mm, whereas medium-sized sand has particles that range in size from 0.425 to 2 mm.

c) Coarse sand

The particle size of coarse sand ranges from 2 mm to 4.75 mm. It may be used as a carrier since it is significantly coarser than the fine sand.

d) Bentonite

The main component of bentonite, an absorbent swelling clay, is montmorillonite. It often develops as a result of volcanic ash weathering in saltwater, when the volcanic glass included in the ash turns into clay particles. Bentonite is a useful adsorbent since they increase the clay's overall surface area to a very high level.

e) Lignite

Lignite is a soft, dark, flammable sedimentary rock created from naturally compacted peat and is sometimes referred to as brown coal. Lignite is mined all over the world and is almost solely utilized as a fuel for the production of steam-electric power.

Utilization of carrier for absorption of Iron in water samples

Biosorption technologies are recognized as efficient and cost-effective strategies for removing various heavy metals from aqueous systems due to their numerous advantages (complete recovery of retained heavy metals from depleted bio-sorbents, inexpensive removal of heavy metals, minimum sludge, high efficiency and ease of operation etc.). Several biomasses, including algae, fungus, yeast, peat, and other agricultural wastes, have been explored as bio sorbents for heavy metal removal in a variety of experimental conditions [16-18]. To determine the ideal conditions, the absorptive abilities of gravel, sand, coarse sand, bentonite, and lignite were tested for the heavy metals biosorption processes concerning a) temperature, b) bio sorbent dose, c) heavy metals concentration, d) contact time, and e) initial solution pH. The experimental data were simulated using pseudo-first order, pseudo-second order, intra-particle diffusion kinetic models, Dubinin-Radushkevich, Langmuir, and Freundlich isotherm models to the major aspects of the biosorption mechanism. Additionally computed were the biosorption process' thermodynamic characteristics.

Biosorption experiments

The iron ion biosorption experiments were performed in batches by mixing the prescribed amount of bio sorbents with 25 mL of aqueous solution with a known concentration of heavy metal ions in 150 mL conical flasks. Depending on objectives of the experiment, flasks were periodically swirled for the proper duration. At room temperature (30 °C), iron solutions with beginning concentrations of 1.5 mmol/L with pH levels between 4.5 and 5.5 were used to research the impact of the pH of the starting solution. This pH range was chosen to prevent any secondary reactions from significantly affecting biosorption and to maintain the metal in the solution as free M^{2+} ions. The 25 mL of an aqueous solution containing 1.5 mmol/L iron metal was used in the kinetic investigations. solution was combined with the same amount of each carrier, including sand, coarse sand, gravels, bentonite, and lignite (0.125 g), at various time intervals ranging from 5 to 180 minutes. Investigations were done to understand the concentration of iron metal affected the effectiveness of biosorption. Filtering separated the two phases after biosorption. The iron content of the filtrates was then spectrophotometrically measured (using a UV-VIS spectrophotometer) using calibration graphs that had been previously created.

Data evaluation

The following parameters were generated from experimental findings and used to assess the biosorption process of iron on the relevant carrier material:

Percentage drop in iron content

Each case's standard deviation was less than or equal to 1.0 percent, and all of the data are the means of three duplicate measurements.

Utilization of carrier along with microbes for absorption of iron in water samples

The goal of the study was to quantify the effectiveness of iron removal by employing carriers and microbial consortiums of iron-oxidizing bacteria (IOBs). The isolates of iron-oxidizing bacteria were evaluated for compatibility behavior to create consortia, and they were then further bonded with the best and most appropriate carrier.

Compatibility screening of iron oxidizing bacteria

A total of 06 isolates of iron-oxidizing bacteria (IOB-1 to IOB-6) were subjected to a compatibility test. Each strain's broth culture was made separately and stored for 48 hours for incubation. The broth cultures of each strain were centrifuged at 5000 rpm after 48 hours had passed. Using the good diffusion technique on

nutrient agar plates, the supernatant of each strain was compared to each strain [19]. The zone of clearance discovered by the supernatants of other bacterial strains against the strains was considered to be incompatible.

Preparation of microbial consortia

The isolates of Iron oxidizing bacteria were injected in a well-defined sterile nutritional medium (liquid broth) at a spore count of 10^8 CFU/ml, an orbital shaker with a pH range of 4.5 to 5.5 and a temperature range of 35 to 37°C for 48 hours. The spore count of the broth culture containing the consortia was examined.

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.

RESULTS AND DISCUSSION

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**. The percent removal of iron using different carriers and iron oxidizing bacteria. The iron removal efficiency of iron oxidizing bacteria (IOB) is shown in **Table 2 and Figure 3**. Microbial consortia entrapped in carrier (coarse sand) is shown in **Figure 4**.

CONCLUSION

The present study suggests that, iron oxidizing bacteria can be isolated and explored from iron deposit sources of water/sediments can be utilized as a novel criterion for removal of iron deposits in water. Also, the carriers such as coarse sand can be utilized as a significant carrier for adsorption of iron content. The study will lead as a basis to remove the iron content from water using iron oxidizing bacteria and carrier in combination.

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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**

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IOB-5	1.5	1.1	26.67±0.12
IOB-6	1.5	1.2	20.0±0.25

Table 1: Percent removal of Iron in water samples using different carriers and IOBs
*P<0.05, level of significance

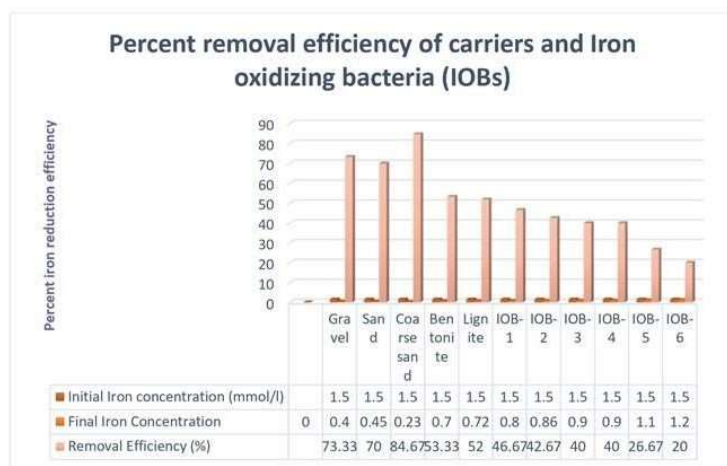


Figure 1: Percent removal efficiency of carriers and Iron oxidizing bacteria (IOBs)

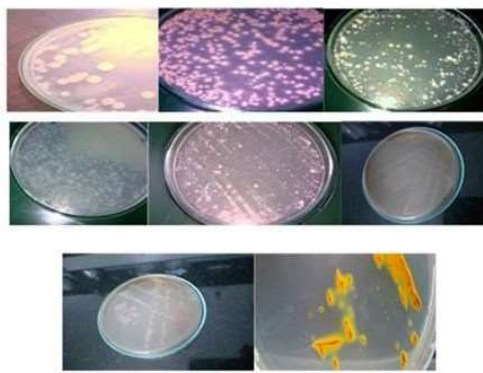
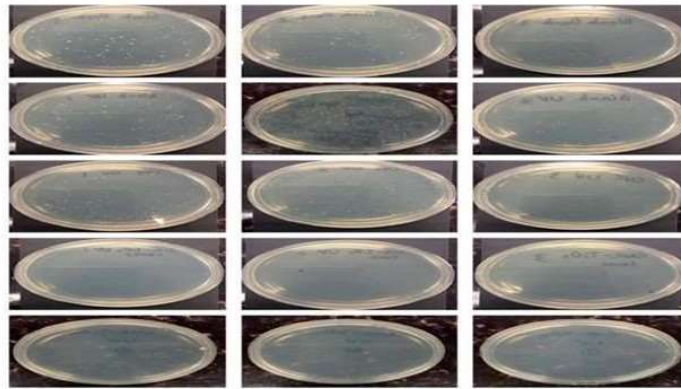


Figure 2: Dominant isolates of Iron oxidizing bacteria (IOB)

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

Figure 3: Compatibility screening amongst the IOB strains



Figure 4: Formulation of coarse sand with microbial consortia entrapped