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# ISOLATION AND CHARACTERIZATION OF IRON BACTERIA FROM RUSTED IRON MATERIALS

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## ABSTRACT

Iron corrosion is one of the most complicated and costly problems faced by drinking water utilities. Iron pipes have been used to transport potable water and iron pipe corrosion has been a problem for just as long. The brownish slimy growth of iron bacteria on rusted iron materials is responsible for corrosion. These iron oxidizing bacteria show several advantages, when grown and implemented in various sectors. Some of the applications of iron bacteria include removal of excess of iron from water bodies, extraction of metals from poor ores, reduction of dyes, reduction of coliforms in water bodies and bioleaching. This review focuses on the isolation, identification and characterization of iron oxidising bacteria and discusses some of the applications the same. Organisms are isolated by scrapping the rusted iron materials such as iron pole exposed to water and air for a long time and using these scrappings as inoculum. The sample is enriched in a medium containing different salts and iron ammonium citrate as a major component. After enrichment of about 3days isolation is carried out using Winogradsky's medium. Isolated colonies are then identified and subjected to use in different industrial sectors.

**KEYWORDS:**

## INTRODUCTION

Iron is the most abundant element in planet earth where it is present at a mean concentration of 5%. Iron is usually required in trace amounts. In nature, iron occurs in two oxidation states. The form of iron that predominates depends greatly on prevailing environment, physiochemical parameters, such as pH, oxygen concentration and redox potential. Iron bacteria include one of the first microorganisms to be observed and described and continue to be a subject of a considerable body of fundamental and applied microbiological research. Iron bacteria can be subdivided into four groups (1) acidophilic aerobic iron oxidizers,(2) neutrophilic aerobic iron oxidizers, (3) neutrophilic anaerobic iron oxidizers, (4) anaerobic photosynthesis iron oxidizers. They were originally considered to be bacteria that catalyze the oxidation of ferrous to ferric, often causing the latter to precipitate and accumulate.

Iron bacteria that are also called metal-depositing microorganisms are commonly referred as causing microbiologically influenced corrosion (MIC) in many studies. They are possible to metabolize reduced iron in their aqueous habitat, and then release it in the form of hydrate ferric oxide on or in their mucilaginous secretions. They can cause piping clogged with rusty sludge and unpleasant smell and taste. Besides, iron bacteria are capable of increasing the organic content in bulk water favoring the multiplication of other bacteria although they do not cause health problems in people directly. For example, the opportunities of sulfur bacteria infestation may be improved, and SRB probably grow quickly in the locally anaerobic environments provided by iron bacteria beneath the iron-rich tubercles.

Iron bacteria have the capability of converting ferrous to ferric ions, that afterwards precipitates as ferric hydroxide, and they can obtain energy from the redox process for growth. It is reported that iron bacteria may produce large amounts of iron oxide precipitates during a very short time, and the iron oxidation rate under biotic condition can be ten to hundreds of times higher than that of abiotic condition. The role of iron bacteria is to produce oxygen concentration areas and divide the metal surface into small anodic sites and large surrounding cathodic zone beneath biofilms and deposit layers. In addition, the deposit layers formed by iron bacteria could also create condensed oxygen zones and initiate crevice corrosion in water systems containing corrosion inhibitors. The corrosion rate beneath the rust deposits remains high because of the low penetrability of corrosion inhibitors and declining of local oxygen concentration where the site might turn anodic.

Though they are responsible for corrosion can also be applied to several industrial sectors discussed below. Iron-oxidizing bacteria are chemotrophic bacteria that derive the energy they need to live and multiply by oxidizing dissolved ferrous iron.There is a class of bacteria that feed on Ferrous iron, converting it to a slimy matrix of bacterial bodies and into Ferric iron.The problem typically exists in well water areas where the groundwater aquifers are formed mainly of sandy soils or organic musk soils (very common in Florida) usually with a pH of below 7.0 and in the absence of dissolved oxygen. These waters contain Ferrous iron (Fe+2) which is chemically reduced, 100% water soluble and which serves as the primary raw material for potential slime formation. The dissolved iron may precipitate out of the water due to changes in temperature and pressure, an increase in pH, or through the action of bacteria. The result is a sludge or slime that may reduce system performance.Iron bacteria present in the water react with the Ferrous iron ($Fe^{+2}$) through an oxidation process. This changes the iron form to Ferric iron ($Fe^{+3}$) which is insoluble. The insoluble Ferric iron is surrounded by the filamentous bacteria colonies and creates the sticky iron slime gel. Iron-oxidizing bacteria colonize the transition zone where de-oxygenated water from an anaerobic environment flows into an aerobic environment. Groundwater containing dissolved organic material may be de-oxygenated by microorganisms feeding on that dissolved organic material. Where concentrations of organic material exceed the concentration of dissolved oxygen required for complete oxidation, microbial populations that contain iron-reducing bacteria can reduce insoluble ferric oxide in aquifer soils to soluble ferrous hydroxide and use the oxygen released by that change to oxidize some of the remaining organic material.

$4H_2O + 2Fe_2O_3 \rightarrow 4Fe(OH)_2 + O_2$

(water) + (Iron[III] oxide) → (Iron[II] hydroxide) + (oxygen)

When the de-oxygenated water reaches a source of oxygen, iron-oxidizing bacteria use that oxygen to convert the soluble ferrous iron back into an insoluble reddish precipitate of ferric iron.

$4Fe(OH)_2 + O_2 \rightarrow 4H_2O + 2Fe_2O_3$

(Iron[II] hydroxide) + (oxygen) → (water) + (Iron[III] oxide)

Since the latter reaction is the normal equilibrium in our oxygen atmosphere while the first requires biological coupling with a simultaneous oxidation of carbon, organic material dissolved in water is often the underlying cause of iron-metabolizing bacteria populations. Groundwater may be naturally de-oxygenated by decaying vegetation in swamps; and useful mineral deposits of bog iron ore have formed where that groundwater has historically emerged to be exposed to atmospheric oxygen. Anthropogenic sources like landfill leachate, septic drain fields, or leakage of light petroleum fuels like gasoline are other possible sources of organic materials allowing soil microbes to de-oxygenate groundwater.

**Scope:** Activities of iron bacteria are very important in turn over of organic and inorganic matter on earth. Neutrophilic bacteria associated with ferric oxide precipitation have been known for a long time. Bacterial iron oxidation might promote coupling between iron oxidation and reduction by producing amorphous or poorly crystalline ferric oxides which are readily available for ferric reducing bacteria. Iron bacteria can be used in bioleaching and coliform reduction.

**Extraction of metals from poor ores:** In microbial leaching (bioleaching), metals can be extracted from large quantities of low grade ores. Although recovery of metals (e.g. copper) from the drainage water of mines has been known for centuries, the involvement of microbes in this process was recognized about 40 years ago. The bacteria which are naturally associated with the rocks can lead to bioleaching by one of the following ways.

1. Direct action of bacteria on the ore to extract metal.
2. Bacteria produce certain substances such as sulfuric acid and ferric iron which extract the metal (indirect action).

In practice, both the methods may work together for efficient recovery of metals.

**Organisms for bioleaching:** The most commonly used microorganisms for bioleaching are *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. *Thiobacillus ferrooxidans* is a rod-shaped, motile, non-spore forming, Gram-negative bacterium. It derives energy for growth from the oxidation of iron or sulfur. This bacterium is capable of oxidising ferrous iron ($Fe^{2+}$) to ferric form ($Fe^{3+}$), and converting sulfur (soluble or insoluble sulfides, thiosulfate, elemental sulfur) to sulfate (SO2-4). *Thiobacillus thiooxidans* is comparable with *T. ferrooxidans*, and grows mostly on sulfur compounds.

Several studies indicate that the two bacteria *T. ferrooxidans* and *T. thiooxidans*, when put together, work synergistically and improve the extraction of metals from the ores. Besides the above two bacteria, there are other microorganisms involved in the process of bioleaching. A selected few of them are briefly described below.

**Mechanism of bioleaching:** The mechanism of bioleaching is rather complex and not well understood. The chemical transformation of metals by microorganisms may occur by direct or indirect bioleaching.

**Direct bioleaching:** In this process, there is a direct enzymatic attack on the minerals (which are susceptible to oxidation) by the microorganisms. For instance, certain bacteria (e.g., *T.*

*ferrooxidans*) can transfer electrons (coupled with ATP production) from iron or sulfur to oxygen. That is these organisms can obtain energy from the oxidation of Fe2+ to $Fe^{3+}$ or from the oxidation of sulfur and reduced sulfur compounds to sulfate as illustrated below.

$4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O$

$2S° + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$

$2FeS_2 + 7O_2 + 2H_2O$

As is evident from the third reaction given above, iron is extracted in the soluble form the iron ore pyrite ($FeS_2$).

**Indirect bioleaching:** In this indirect method, the bacteria produce strong oxidizing agents such as ferric iron and sulfuric acid on oxidation of soluble iron or soluble sulfur respectively. Ferric iron or sulfuric acid, being powerful oxidizing agents react with metals and extract them. For indirect bioleaching, acidic environment is absolutely essential in order to keep ferric iron and other metals in solution. It is possible to continuously maintain acidic environment by the oxidation of iron, sulfur, metal sulfides or by dissolution of carbonate ions.

**Commercial Process of Bioleaching:** The naturally occurring mineral leaching is very slow. The microbial bioleaching process can be optimized by creating ideal conditions—temperature, pH, and nutrient, $O_2$ and $CO_2$ supply etc. The desired microorganisms with nutrients, acid etc., are pumped into the ore bed. The microorganisms grow and produce more acid. The extracted leach liquor is processed for the metal recovery. The leach liquor can be recycled again and again for further metal extraction.

**Coliform Reduction by Iron Bacteria:** The aquarium water collected and is subjected to most probable number test to estimate the number of fecal coliforms present in 100 ml of the sample by following the method of Gruett (1993). Lauryl tryptose broth (LTB) is prepared in three double strength test tubes by taking 20ml of LTB medium in each and three single strength test tubes by taking 10ml of LTB medium in each tube. Durhams tubes are placed in all the test tubes and medium is sterilized and cooled to room temperature. A loop full culture of iron isolated bacteriais inoculated to each of the test tubes and incubated at 37°C for 24 h. The positive result are indicated by the gas formation in Durhams tubes.

A Brilliant green lactose bile broth (BGLB) is prepared, sterilized and cooled to room temperature. A loop full of culture form the positive result shown in LTB test tubes are transferred to BGLB test tubes and incubated at 37°C for 48 h. The result is noted by the gas formation in the Durhams tube. The same procedure is followed with 100ml of aquarium water treated with 50ml of iron isolated bacterial culture and incubated at 37°C for 48 h. After incubation coliform count is noted.

**Dye Reduction Test:** Dye reduction test performed with the iron isolated bacterial culture by using 0.1g/100ml concentration of various dyes such as violent, orange, green and yellow dye solutions is prepared. The isolated iron bacteria culture are inoculated into individual dye solution and incubated at 37°C for 7 days at an interval of each day the absorbance values for the dye solution is observed on the Spectrophotometer at 620nm.

**Detection and culture of iron bacteria:** The seed bacteria used in the study were isolated from rust deposits collected from the inner surface of iron pipes in water distribution systems during maintenance. They were incubated into the culture medium for enrichment at 30°C, and subsequently purified by picking up several single colonies using a sterile inoculation loop. The culture medium of iron bacteria consists of $(NH_4)2SO_4$ 0.5g, $NaNO_3$ 0.5g, $K_2HPO_4$ 0.5g, $MgSO_4{\cdot}7H_2O$ 0.5g, $CaCl_2{\cdot}6H_2O$ 0.5g, ammonium ferric citrate 10.0g, and distilled water 1000ml and sterilized at 121°C for 20 min. Iron bacteria were enumerated using Plate Counts method with Winogradsky's medium (g/L) 0.5 $K_2HPO_4$, 0.5 $NaNO_3$, 0.2 $CaCl_2$, 0.5 $MgSO_4{\cdot}7H_2O$, 0.5, $NH_4NO_3$ and 6.0 ammonium iron citrate under aerobic chamber.

## DISCUSSION

The iron oxidizing bacteria isolated from rusted materials showed distinct growth characteristics. The isolated organisms from various sources can be used for processes such as in bioleaching, dye reduction, reduce coliform number, extraction metals from poor ores and removal of excess iron from water bodies.

## REFERENCES

1. Li L B, Liu Z J, Zhuang C Y, Zhou J X, Yang K, Han J G. Population diversity of soil bacteria along the Qinghai-Tibet railway by DGGE analysis [J]. Chinese Journal of Ecology, 2008; 5: 013.
2. Emerson D, Moyer C. Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. Applied and Environmental Microbiology, 1997 Dec 1;

63(12): 4784-92.

3. Banfield JF, Welch SA, Zhang H, Ebert TT, Penn RL. Aggregation-based crystal growth and microstructure development in natural iron oxyhydroxide biomineralization products. Science, Aug 4, 2000; 289(5480): 751-4.
4. Yilmaz EI. Metal tolerance and biosorption capacity of Bacillus circulans strain EB1. Research in Microbiology, Jul 1, 2003; 154(6): 409-15.
5. Lone TA, Lone RA. Extraction of cannabinoids from Cannabis sativa L. plant and its potential antimicrobial activity. Universal Journal of Medicine and Dentistry, 2012; 1(4): 51-5.
6. Emerson D, Moyer C. Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. Applied and Environmental Microbiology, Dec 1, 1997; 63(12): 4784-92.
7. Brock TD, Gustafson JO. Ferric iron reduction by sulfur-and iron-oxidizing bacteria. Applied and Environmental Microbiology, Oct 1, 1976; 32(4): 567-71.
8. Emerson D, Fleming EJ, McBeth JM. Iron-oxidizing bacteria: an environmental and genomic perspective. Annual review of microbiology, Oct 13, 2010; 64: 561-83.