Microbiologically Influenced Corrosion of Iron by Nitrate Reducing Bacillus Sp

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Abstract. Iron has played a crucial role in the human ecosystem currently in transportation, manufacturing, and infrastructure. Iron oxide is known as rust, usually the reddish-brown oxide formed by iron and oxygen reactions in moisture from water or air. Microbiologically influenced corrosion (MIC) is a significant problem to the economic damage, especially in industrial sectors and its direct presence with nitrate/iron-reducing bacteria. This paper aims to explore the MIC of iron by nitrate-reducing Bacillus sp. including the redox reaction occurs, microbiologically influenced corrosion, iron/nitrate-reducing and mechanisms of microbial iron/nitrate reduction.

1. Introduction
Iron (Fe) is the most abundant transition metal element in the earth's crust and distribute in natural environments such as soils and sediments as iron minerals. The iron phase contributes to vital roles in geochemistry, particularly the reduction of Fe. The reduction of iron in such small amounts would cause the organic geochemistry and inorganic of the environments. Biocorrosion, so-called microbial corrosion, microbial induced corrosion (MIC) or microbiologically influenced corrosion is one of the different forms of corrosion. It is a form of corrosion by microorganisms depending on the appropriate metal substrate and the environment. The process can lead to pitting or localizing the corrosion in an aqueous environment [1].

MIC can further articulate as material degradation, commonly metal, because of the behavior of microorganisms. It will classify corrosion resulting from microorganisms’ presence and behavior on metal surfaces within biofilms. However, MIC applies to metals and non-metallic surfaces like concrete [2]. The MIC study generates much research and debate among researchers, especially corrosion mechanisms [6-8]. Bacteria are the most significant microorganisms involved in microbiological corrosion. They are common in soil, water, air, food, and skin. Generally, the presence of sulfate-reducing bacteria (SRB), iron-reducing bacteria (IRB) and nitrate-reducing bacteria (NRB) has mentioned as contributing to the corrosion to occurs [9-10].

As one of the most common microbial species, the Bacillus sp is widely available in diverse environmental conditions. It can form biofilms with spores and their metabolites include cyclic peptides, antibiotics, polyglutamic acid, polyaspartic acid and polysaccharide. According to the research, extracellular polymer substances (EPS) formed by Bacillus sp. were the major part of the biofilm formed upon on the metal surface, which affected the corrosion process [3].
2. Redox Reaction

Oxidation and reduction reactions often occur together and are called redox reactions. Generally, oxidation-reduction is a process involving the movement of electrons. Oxidation can define electron loss and reduction as electron gain. Hence, the oxidation increases oxidation state and reduction results in a decrease in oxidation state [4].

The oxidized substance was called the reducing agent, which lost the electron during the redox reaction as shown in Figure 1. Then, the reduced substance was called the oxidizing agent, which gained an electron. Besides, potent oxidizing agents such as oxygen, hydrogen, peroxide and chlorine tend to gain electrons, whereas good reducing agents such as sodium and hydrogen tend to lose electrons [5].

![The substance losing electron is oxidized. The substance gaining electron is reduced.](image)

Figure 1. The mechanism of oxidation-reduction.

3. Microbiologically Influenced Corrosion

There were three types of MIC distinguished by electrogenic microbes such as SRB, IRB and NRB. SRB can use sulfate as a terminal electron acceptor. NRB removed nitrate by denitrification in anaerobic conditions to sustain the metabolism [6]. In methanogenesis, methanogens can oxidize the iron to cause corrosion [7]. Type two was the consequence of the corrosive metabolites formed by fermenting microbes like acid-producing bacteria (APB). Proton reduction can be paired with iron oxidation to affected the MIC at a locally acidic pH. While type three was different from type one and type two. It has known as biodegradation and was not electrochemical.

Nevertheless, some bacteria with various physiological properties are also known to play roles in corrosion processes. It represents IRB, which under anaerobic conditions will convert ferric ion to ferrous ion. However, IRB's role and significance in MIC has been a contentious topic and still debated [12]. Based on the MIC researched, SRB was a concentrate on the role of IRB in biocorrosion. IRB strains were capable of reducing ferric ion to ferrous ion. Fig. 2 shows that iron-reducing bacteria derive benefit from Fe$^{3+}$ reduction by using this ion in their metabolism as a terminal electron acceptor. Iron-reducing can achieve in two ways: using an electron transport chain in anaerobic respiration or using Fe$^{3+}$ as an electron trap during fermentation.

Organisms that use an electron transport chain under anaerobic generated more energy than fermentation and these organisms were usually facultative anaerobes. Recent studies show that IRB can accelerate corrosion by reducing insoluble ferric ion compounds to soluble ferrous ions, removing protective corrosion scales formed on the exposed surfaces and biofilm concentration formation cells. However, IRB could inhibit the corrosion process by removing oxygen from the systems via their aerobic respiration [8].
4. Dissimilatory Iron and Nitrate Reducing Bacteria

The role of IRB and NRB in carbon steel corrosion is controversial whether it inhibits or enhances the corrosion. The group of IRB and NRB strains is known to reduce the capacity of insoluble iron ions to soluble iron ions. Iron reducers gain from the reduction of iron by using this ion as a terminal electron acceptor in their metabolism. It can be achieve in two ways: using an electron transport method in anaerobic respiration or during fermentation by using Fe$^{3+}$ as an extra electron sink. The IRB and NRB group is abundant and can found in enriched sediments, where *Geobacter sp.*, *Shewanella sp.*, and *Bacillus sp.* have been monopolized [9]. Moreover, IRB and NRB were involved in biofilms that create differential microbial metabolism that facilitates corrosion [10]. Hence, it indicates that the IRB and NRB play an essential role in converting organic contaminants and metals.

4.1 Genus Shewanella

Gram-negative, rod-shaped facultative anaerobes, gamma-proteobacteria are of the genus *Shewanella*. *Shewanella oneidensis MR-1* was among the first strains of bacteria recorded to conserve energy by reducing dissimilar iron. The bulk of the *Shewanella* strain, followed by sediments, were mainly isolated from marine environments in using a wide variety of terminal electron acceptors, including oxygen, nitrate, fumarate, sulfur compounds sulfite thiol sulfate and soluble and insoluble transition metals such as iron citrate, iron oxide, hematite, Mn$^{2+}$ oxide and Mn$^{3+}$.

In bioremediation analysis, some *Shewanella* strains can reduce contaminants such as Uranium and Chromium from the dissolved liquid state. Some can develop polyunsaturated fatty acids and, during low-temperature fish storage, some have been known as the primary bacterial pathogens [11]. Moreover, they are environmentally ubiquitous and are well known for their ability, like Fe$^{3+}$ oxides and anodes, to use strong electron acceptors. Their involvement in MIC was primarily due to their reduction properties of Fe$^{3+}$, which can either enhance or inhibit corrosion [12].

4.2 Genus Bacillus

*Bacillus sp.* is gained traction due to its ability to withstand and flourish under harsh industrial conditions. *Bacillus sp.* was a heterogeneous group of Gram-positive, facultative anaerobic and endospore-forming bacteria. Their rod-shaped cell morphology distinguished them, catalase development and soil usually was its primary reservoir. Hence, they also can live in ubiquitous circulation. *Bacillus sp.* could create endospores and help it to survive harsh environmental conditions. Moreover, the certain types of Bacillus sp. were heterotrophic nitrifiers, denitrifiers, nitrogen fixators, iron precipitators, selenium oxidizers, manganese oxidizers and reducers. They can also survive in
different conditions, such as acidophiles, alkaliphiles, psychrophiles, thermophiles, etc. Furthermore, according to researchers’ state, the Bacillus sp. can act as corrosion induction and corrosion inhibition for carbon steel based on certain enzymes' ranges and concentrations [13]. It can enhance the corrosion at any specified range and could inhibit the enzymes' higher concentrations.

Nevertheless, Rai et al. [14] had reported that some bacterial strains could also prevent the corrosion of metals. Indeed, for many metal substrates in both surface and groundwater, corrosion inhibition by spore-forming Gram-positive bacteria has been widely documented and reviewed in the literature. This microbe modifies the metal dissolution rate as they remain viable in the media by reducing oxygen concentration at the metal surface with aerobic respiration.

Hence, biofilms reported having a tremendous influence on the electrochemical process as the metabolic activities [15]. Based on the previous study, Bacillus licheniformis could prevent metal corrosion and it was one of the Bacillus species with Gram-positive and spore-forming bacteria [16]. However, Wan et al. [17] stated that Bacillus cereus also from this kind of Bacillus group can accelerate the pitting corrosion with NRB under anaerobic environment. The study shows that this Bacillus sp. can inhibit or enhance the corrosion depending on their microbial metabolism conditions and electron acceptors.

### 4.3 Bacillus Licheniformis

*Bacillus licheniformis* was widespread in diverse nature and mainly found in soil as spores. Hence, most of these strains can thrive at higher temperatures, the ranged temperature from 30-50°C had also reasonable for vegetative growth. Primarily, *B. licheniformis* was a facultative anaerobic which can undergo on nitrate. Moreover, *the B. licheniformis* shaped can appear as curved or straight rod-shaped and gram positive motile bacterium. The classification of taxonomic *B. licheniformis* as shown in Table 1.

| Phylum       | Firmicutes          |
|--------------|---------------------|
| Class        | Bacilli             |
| Order        | Bacillales          |
| Family       | Bacillacea          |
| Genus        | Bacillus            |

Recent studies show that *B. licheniformis* can act as an inhibitor of corrosion due most of these strains had been secreted their protective biofilm against corrosion [16]. Based on the research by Xu et al. [18] this strain also investigated on carbon steel surface in terms of the nitrate-reducing bacterium. The result was found that *B. licheniformis* against C1018 of carbon steel would act as a corrosive nitrate reducer under anaerobic conditions [18].

### 4.4 Bacillus cereus

*Bacillus cereus* was a gram-positive, rod-shaped bacterium of the aero-anaerobic respiratory type, widely distributed in several biotopes. It belongs to the genus *Bacillus*. As the primary genus of the gram-positive and widespread *Bacillus sp.*, *B. cereus* was an industrial contaminant common in soil, air, marine ecosystems and a public health threat [19]. Moreover, *B. cereus* was common in nature and quickly grew in nature. A few studies show that MIC was concerned in *B. cereus* due its EPS can oxidize on different surfaces like carbon steel, stainless steels, floors and concrete blocks [20]. Throughout this study, *B. cereus* can inhibit corrosion due to its ability to form with inorganic ions. Thus, it can regulate the formation of calcium carbonate in biofilm. However, Rajasekar et al. [21] indicated that *B. cereus* converted ferric and manganese into iron oxides and speed up enhancing pitting assault on the surface.
Besides, Wan et al. [17] show that *B. cereus* could enhance the corrosion when the presence of nitrate in an anaerobic environment. Thus, the mechanism and structure of *B. cereus* were very complicated in the corrosion process.

5. Molecular Mechanisms of Microbial Iron and Nitrate Reduction
Since the early 1900s, microorganisms’ functions on the MIC had been identified and its mechanisms are essential to investigate the MIC. In this review, several mechanisms occur in this study: extracellular electron transfer, electron shuttling pathway, and chelation pathway.

5.1 Extracellular Electron Transfer
This mechanism influenced certain microorganisms to exchange intracellular electrons in the cell membrane with an extracellular electron donor or acceptor such as natural metal compounds and artificial electrodes. [22]. The recent study shows the mechanism of microorganisms such as *Geobacter* and *Shewanella oneidensis* of redox-active proteins in outer membrane c-type cytochromes (OMC’s) had played a significant role in EET. It was responsible for electrons transfer to iron oxides.

The mechanism of EET has been shown in figure 3. Direct contact of extracellular reductases to the electron acceptor was used by *Geobacter* and *Shewanella* species. At soluble shuttle, it secretes soluble proteins to bring electrons from the cell to the electron acceptor. Thus, *Shewanella* secretes their electron shuttle while *Geobacter* secretes and shuttles electrons using a soluble cytochrome. Finally, the production of protein at cellular extensions to carry electrons from the cell to the electron acceptor.

![Figure 3](image_url)

**Figure 3**: The general mechanisms of Extracellular electron transfer.

5.2 Electron Shuttling Pathway
Electroactive bacteria can indirectly transfer their electrons through an electron shuttle to iron (III) oxide. The electron shuttle serves as an agent for the delivery of electrons to the surface of iron oxide. Organic compounds such as humic acids, quinones or other molecules with a quinone-like structure are the possible exogenous electron shuttles in the atmosphere [23]. Like endogenous electron shuttles, including flavin, menaquinone, melanin and organic sulfur (thiol) compounds, some bacteria such as *Shewanella sp* would also generate quinone.

5.3 Chelation Pathway
Via the chelation route, electroactive bacteria can transfer electrons. Iron is solubilized by iron chelators known as siderophores in the chelation pathway and becomes usable to the cell. Siderophores are synthesized from cells and bind to Fe, which holds iron in solution in this low solubility medium. Siderophore specific receptors recognize the soluble iron-siderophore complex and bind it to it on the
cell's surface. The iron is then released from the complex reduced and taken up into the cell as Fe2+. A study shows that cultures of S.oneidensis and S.putrefaciens are electrochemically detected under anaerobic conditions with soluble organic iron(II).

6. Conclusions
Iron has played a vital role in the human ecosystem currently in transportation, manufacturing and infrastructure. Iron oxide is known as rust, usually reddish-brown oxide formed by iron and oxygen reactions in moisture from water or air. Microbiologically influenced corrosion (MIC) has been noticed as a major problem to the economic damage, especially in industrial sectors and its direct presence with nitrate/iron-reducing bacteria.

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