

Review

# Application Prospect of Anaerobic Reduction Pathways in *Acidithiobacillus ferrooxidans* for Mine Tailings Disposal: A Review

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**Abstract:** The accumulation of mine tailings on Earth, generated from the extraction, processing, and utilization of mineral resources, is a serious environmental challenge. The importance of the recovery of valuable elements and rare-earth elements, together with the economic benefits of precious and base metals, is a strong incentive to develop sustainable methods to recover metals from tailings. Currently, researchers are attempting to improve the efficiency of valuable elements and rare-earth elements recovery from tailings using bioleaching, a more sustainable method compared to traditional methods. In this work, we report the research status of the application of *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*) anaerobic reduction in tailings disposal. Recent advances in the anaerobic characteristics of *At. ferrooxidans* recovery process and technical difficulties are further described. We found that current research has made significant progress in anaerobic recovery. This is of great significance for the development of bioleaching technologies and industrial production of heavy metals in tailings. Finally, based on the perspectives and directions of this review, the present study can act as an important reference for the academic participants involved in this promising field.

**Keywords:** *Acidithiobacillus ferrooxidans*; anaerobic; bioleaching; mine tailings



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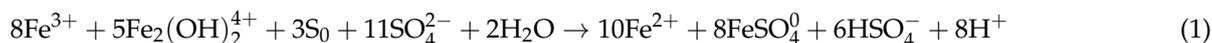
## 1. Introduction

Mineral mining, flotation, and smelting operations are important processes in mineral production, which always generate massive hazardous solid wastes. Mine tailings are the waste or non-economic product generated during mining activities and the processing of minerals [1]. In recent years, the output and stock of mine tailings have increased because of the reduction of metal grade [2]. The improper disposal of the tailings poses geological and environmental issues including tailing dam breakage and the pollution of the ecological system as a consequence of the release of toxic contaminants from the tailings to the surrounding environment [3]. From another point of view, mine tailings may contain significant amounts of valuable metals including indium, magnesium, cobalt, platinum group elements, or rare-earth elements, which are of high economic interest [4]. Therefore, in order to effectively alleviate the potential environmental threat posed by tailings and avoid wasting valuable resources, the recovery of valuable metals from tailings is becoming increasingly important [5].

The traditional metallurgical technology can be divided into pyrometallurgy and hydrometallurgy according to its different characteristics [6]. Pyrometallurgical technology can be considered as a general solution to extracting valuable metal elements from industrial wastes. It is highly efficient for the recovery of metals such as Ti, Zr, Nb, Ta, and Mo [7]. However, due to the heat source needed to maintain high temperatures, pyrometallurgy needs to burn carbon fuel for its heat supply. At the same time, a lot of waste gas

such as fluorine and flue gas will be produced in the production process, polluting the environment [8]. Hydrometallurgy is a metal recovery process involving chemical reactions performed in aqueous or organic solutions. The hydrometallurgical process provides several substantial advantages such as the ability to control the level of impurities, low investment cost (which is highly suitable for small-scale applications), less environmental impact, and high metal recovery potential [9]. However, a large number of chemical agents are used to extract heavy metals in the hydrometallurgy process, but the safety of most agents is not satisfactory. Some studies have shown that chemical solvents used in the hydrometallurgy process contain endocrine disruptors. Biohydrometallurgy (bioleaching technology) is potentially a more sustainable approach where microorganisms are used for metal extraction from their iron and sulfide ores as well as from minerals [10]. Unlike conventional methods, this approach is less energy demanding and eco-friendly, having no discharge of injurious chemicals and gasses into the environment [11]. Jiang, et al. [12] reported that the energy consumption, greenhouse gas emissions, sulfur emission, and toxic byproducts of copper smelting by biohydrometallurgy are only 43.2%, 45.2%, 5.1%, and 5.0% of that of copper smelting by pyrometallurgy. The biohydrometallurgy is not only applied to high-grade ore leaching, but it also plays an important role in tailings leaching. Therefore, biohydrometallurgy of low-grade tailings is beneficial for the efficient utilization of mineral resources.

*Acidithiobacillus ferrooxidans* (*At. ferrooxidans*) is a significant and facultative bacterium that exists widely in acid mine drainage environments and is broadly applied in the bioleaching field [13]. It is known to use a variety of electron donors ( $\text{Fe}^{2+}$ , elemental sulfur  $\text{S}_0$ , reduced inorganic sulfur compounds, hydrogen  $\text{H}_2$ , and formic acid) and either molecular oxygen,  $\text{S}_0$ , or  $\text{Fe}^{3+}$  as an electron acceptor [14,15]. Pronk, et al. [16] and Das, et al. [17] showed that the bacterium grew on the oxidation of  $\text{S}_0$  by  $\text{Fe}^{3+}$  under oxygen-limited conditions. In addition, *At. ferrooxidans* can also obtain energy under anaerobic conditions with  $\text{S}_0$ ,  $\text{H}_2$ , and  $\text{Fe}^{2+}$  as electron donors and  $\text{Fe}^{3+}$  and  $\text{S}_0$  as electron acceptors [15,18]. *At. ferrooxidans* has been shown to anaerobically oxidize  $\text{S}_0$  with  $\text{Fe}^{3+}$  according to the stoichiometry shown below (Equation (1)).



Because of high iron solubility at a low pH, iron mobilization due to ferric iron reduction is more significant at a low pH than at a neutral pH. Since the late 1990s, it has been shown that many species of extremely acidophilic bacteria (e.g., *At. ferrooxidans*) can catalyze the reductive dissolution of ferric iron minerals under anaerobic or microaerophilic conditions [19,20]. Under anaerobic conditions, *At. ferrooxidans* can alternatively catalyze the oxidation of sulfur or reduced inorganic sulfur compounds (RISC) using ferric iron as an electron acceptor [21]. More recently, anaerobic reductive dissolution using a pure culture of *At. ferrooxidans* maintained at 30 °C and pH 1.8 has been found to extract nickel, cobalt, and copper from limonitic ores [22], asbolane [23], and copper-containing laterite [24]. The use of the *At. ferrooxidans* has been proposed to recover nickel by anaerobe reductive dissolution (AnRD) of low-grade nickel laterite ores containing 7% of the total iron at 5% pulp density to extract target metals (e.g., extraction of 70% nickel and 50% cobalt within 30 days) [20]. Yang, et al. [25] demonstrated that jarosites could be anaerobically bio-dissolved by *At. ferrooxidans* using hydrogen as an electron donor. After bio-dissolution by *At. ferrooxidans* for 22 d, ferrous ion concentrations reached 10.07 mM (biologically produced jarosites), 7.68 mM (potassium jarosite), and 1.45 mM (lead jarosite). Compared with other methods of tailing leaching, the bioleaching of tailings via anaerobic reduction has great advantages for industrial applications, including lower costs, environmental friendliness, and a simple control and management process, among others [26]. Therefore, further investigation of the bioleaching field via anaerobic reduction is of great importance.

This review provides an overview of the application of *At. ferrooxidans* anaerobic reduction in tailings disposal and provides a general understanding of the current status of the anaerobic characteristics of *At. ferrooxidans*, the recovery process, and technical difficulties.

The future research perspectives and directions for the recovery of heavy metals from tailings by biological leaching are also discussed, which will provide a valuable reference for the full exploitation and utilization of secondary resources and the industrial development of the recovery of heavy metals from tailings using biological leaching technologies.

## 2. Anaerobic Characteristics and Metabolic Pathways of *At. ferrooxidans*

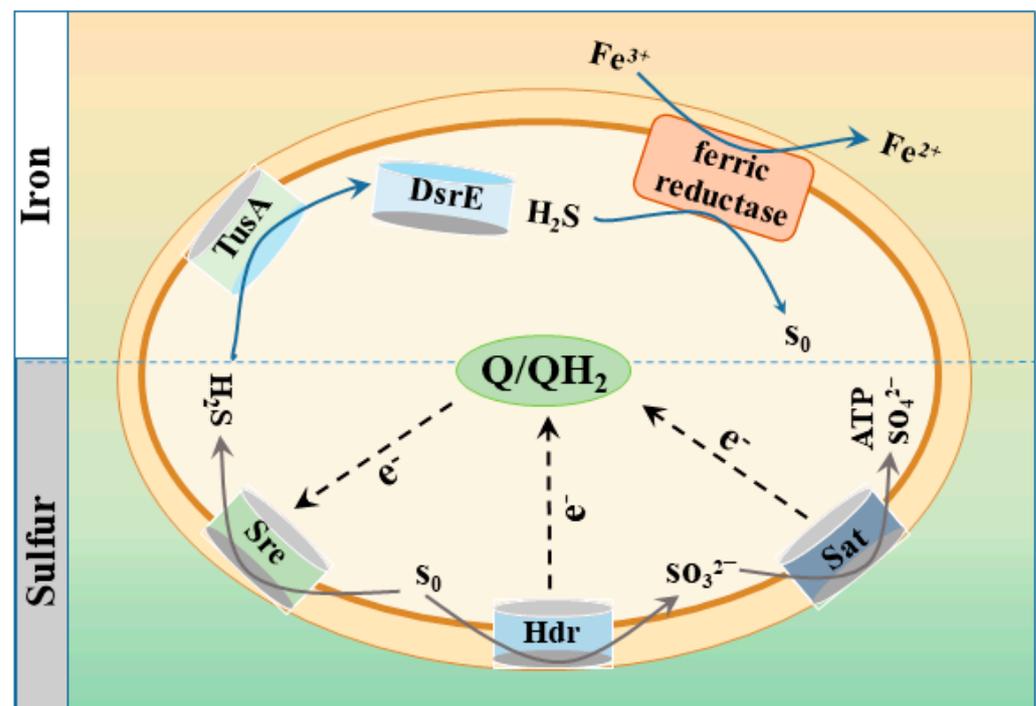
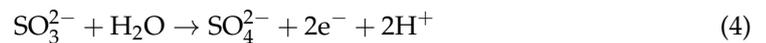
*At. ferrooxidans* (originally named *Thiobacillus ferrooxidans* (*T. ferrooxidans*)) belongs to the phylum Proteobacteria and class *Acidithiobacillia*, and was first isolated by Colmer and Hinkle in 1947 from acid mine drainage [27,28]. With optimum growth at pH 2.0 and 30 °C, this Gram-negative bacterium can grow in a wide range of temperatures (4–37 °C) under acidic conditions (pH 1.0–4.5), and some isolates can thrive below pH 1.0 [13,29]. As a facultatively anaerobic chemoautotrophic bacterium, *At. ferrooxidans* uses iron and sulfur as respiratory sources and was found to grow not only on H<sub>2</sub>/O<sub>2</sub> under aerobic conditions but also on H<sub>2</sub>/Fe<sup>3+</sup>, H<sub>2</sub>/S<sub>0</sub>, and S<sub>0</sub>/Fe<sup>3+</sup> under anaerobic conditions [14]. *At. ferrooxidans* uses Fe<sup>3+</sup>; S<sub>0</sub>; H<sub>2</sub> as an electron donor and Fe<sup>3+</sup>; S<sub>0</sub> as an electron acceptor during anaerobic respiration, and Fe<sup>3+</sup> can be used by it as a terminal electron acceptor. The use of Fe<sup>3+</sup> or S<sub>0</sub> as electron acceptors and H<sub>2</sub> or S<sub>0</sub> as electron donors during anaerobic respiration is the main energy source of *At. ferrooxidans*. In addition, hydrogen, formic acid, uranium, and electrical current have been characterized as additional energy sources for growth and respiration in *At. ferrooxidans* [14,15,30]. These characteristics allow *At. ferrooxidans* to be widely distributed in natural environments where iron and reduced inorganic sulfur compounds are abundant, such as coal deposits, metal sulfide ores, sea water, and acid mine drainage [31].

In general, the anaerobic metabolic pathway of *At. ferrooxidans* is mainly the electron transfer for sulfur and iron (Figure 1). Thereinto, an important functional coupling of S<sub>0</sub> oxidation to Fe<sup>3+</sup> reduction occurs via an indirect mechanism involving the reduction of Fe<sup>3+</sup> by H<sub>2</sub>S generated by the disproportionation of S<sub>0</sub> under anaerobic conditions (Equation (2)) (Figure 1) [15]. With anaerobic sulfur, studies have demonstrated the components of the iron oxidation system: a 92-kD outer membrane protein, a copper protein (ferritin), many a- and c-type cytochromes and Fe<sup>2+</sup> oxidase, among others [32,33]. The Fe<sup>2+</sup> oxidase is present in the periplasmic space, it is part of the chain of constituent electron transfer pathways starting from ferrous ions and is an electron transfer body, located in front of ferricyanin and cytochromes [34]. The electrons generated by Fe<sup>2+</sup> oxidation in *At. ferrooxidans* is first transferred to the periplasmic cyanocyanin Rus through the outer membrane mosaic of cytochrome protein Cyc2, and then most of the electrons are transferred along the cis-potential gradient pathway via cytochrome protein Cyc1 and cytochrome oxidase aa3, to the final electron acceptor O<sub>2</sub>, and combined with protons to produce H<sub>2</sub>O, which provides energy for growth [31].

In *At. ferrooxidans*, two mechanisms have been shown to exist for the oxidation of elemental sulfur: first, the ultimate electron acceptor for sulfur oxidation is oxygen; second, when grown anaerobically, *At. ferrooxidans* can utilize three enzymes: Fe<sup>2+</sup> oxidase, sulfite: -Fe<sup>3+</sup> oxidoreductase, and hydrogen sulfide-Fe<sup>3+</sup> oxidoreductase, which together oxidize elemental sulfur to sulfate [25].

When sulfur is used as a substrate, sulfite is oxidized to sulfate in two ways: directly by sulfite:cytochrome c oxidoreductase and the electrons released in this pathway can enter coenzyme Q; or indirectly by ammonium persulphate (APS) reductase, adenosine diphosphate (ADP) sulfatase and adenosine microlase, producing sulfate and adenosine triphosphate (ATP) [15]. In addition, when both iron and sulfur are used as energy substrates, sulfite can be oxidized to sulfate by ferrous sulfite oxidoreductase and produce Fe<sup>2+</sup>, which is reoxidized to Fe<sup>2+</sup> by the action of the iron oxidase system, and the electrons are finally passed to O<sub>2</sub> [25]. The oxidation of S<sub>0</sub> to SO<sub>3</sub><sup>2-</sup> yields four electrons and six protons (assuming that oxygen is derived from water, which is necessarily the case in an anaerobic culture (Equation (3))); and the oxidation of SO<sub>3</sub><sup>2-</sup> to SO<sub>4</sub><sup>2-</sup> produces two electrons and

two protons (Equation (4)). The reduction of  $S_0$  to  $H_2S$  consumes two electrons and two protons (Equation (5)) (Figure 1).



**Figure 1.** Metabolic pathways of electron transfer for iron and sulfur (Hdr: isodisulfide reductase; Sat: ATP sulfurylase; Sre: sulfur reductase; TusA, DsrE: sulfur transporter; Q/QH<sub>2</sub>: quinol pool).

### 3. Anaerobic Bioprocesses in Mine Tailings Leaching and Recovery of Valuable Elements

#### 3.1. Anaerobic Biobleaching of Mine Tailings

Biobleaching is the process of converting insoluble metal compounds into soluble compounds through the interaction between microorganisms and metals [35]. As a highly active autotrophic microorganism, *At. ferrooxidans* plays an important role in mineral processing by utilizing its unique metabolic pathways and biocatalytic properties, and is considered to be the main strain in biohydrometallurgy [36]. At present, *At. ferrooxidans* has been applied in leaching Cu, Zn, Ni, Pb, etc. from mine tailings since discovered [37,38].

*At. ferrooxidans* has the capability to reduce  $Fe^{3+}$  under anaerobic conditions by using  $H_2$  and/or  $S_0$  as electron donors [14], thus indicating the potential application of *At. ferrooxidans* in biobleaching for recovering valuable metals [39]. Pronk, de Bruyn [34] reported that *At. ferrooxidans* grows anaerobically using  $Fe^{3+}$  as the electron acceptor and suggested that this could be the major biological process during the oxidation of metal ores in leaching operations. In 1983, Goodman, et al. [40] predicted anaerobic leaching in a pyritic waste rock dump, where they discovered high cell numbers at a 3-m depth (oxygen < 0.1 vol%,  $CO_2$  > 2 vol%) and the highest copper concentrations in the

dump. In their laboratory-scale experiments, 63% zinc was recovered from a zinc–iron sulfide under a CO<sub>2</sub>-rich, O<sub>2</sub>-free atmosphere in a ferric-rich solution at pH 2.5. Das and Mishra [41] used *At. ferrooxidans* under anaerobic conditions to dissolve copper and iron from sulfide ores such as pyrite, pyrrhotite, and chalcopyrite. Donati, et al. [42] confirmed the anaerobic leaching of covellite in the presence of ferric-iron-reducing *At. Ferrooxidans*. Sulfur formed during the chemical attack of covellite by ferric iron serves as an electron donor for iron reduction by *At. Ferrooxidans*, which is followed by an increase in copper leaching from covellite. The metabolic capacity of *At. ferrooxidans* can effectively decompose high concentrations of sulfides, making it highly efficient. Additionally, anaerobic reductive dissolution using a pure culture of *At. ferrooxidans* maintained at 30 °C and pH 1.8 has been found to extract nickel, cobalt, and copper from limonitic ores [22], asbolane [23], and copper-containing laterite [24]. The use of *At. ferrooxidans* has been proposed to recover nickel by anaerobe reductive dissolution (AnRD) of low-grade nickel laterite ores containing 7% total iron at 5% pulp density to extract target metals (e.g., extraction of 70% nickel and 50% cobalt within 30 days) [20]. Furthermore, *At. ferrooxidans* can simultaneously degrade other toxic and harmful substances such as hydrogen and nitrite, making it more widely applicable [43]. With the decline of sulfidic ores and increasing demand for valuable metals, novel technologies focusing on the dissolution of reduced ores under anaerobic conditions becomes more and more attractive [44].

In fact, there are many acidophilic bacteria and archaea that are able to reduce ferric iron under anaerobic conditions [45], and many of these (e.g., *At. ferrooxidans*) are also well known for their ability to oxidize ferrous iron and are already used in (oxidative) bioleaching operations (see above). The majority of acidophilic bacteria are known to reduce ferric iron under anaerobic conditions coupled to the oxidation of either inorganic (e.g., sulfur, hydrogen) or low-weight organic compounds [17,34,44]. Please see Table 1.

**Table 1.** Acidophilic bacteria that are able to grow by ferric iron reduction (anaerobic respiration) (adapted from Schippers, Hedrich [44]).

Species	Atmosphere Required for Ferric Iron Reduction	Electron Donor
<i>At. ferrooxidans</i>	Anaerobic	Reduced S <sub>0</sub> , H <sub>2</sub>
<i>Acidithiobacillus. ferrivorans</i>	Anaerobic	Reduced S <sub>0</sub>
<i>Acidiferrobacter thiooxydans</i>	Anaerobic <sup>a</sup>	Reduced S <sub>0</sub>
<i>Acidimicrobium ferrooxidans</i>	Anaerobic	Reduced S <sub>0</sub> , organic
<i>Acidicaldus organivorans</i>	Anaerobic	Organic
<i>Ferrimicrobium acidiphilum</i>	Anaerobic	Organic
<i>Ferrithrix thermotolerans</i>	Anaerobic	Organic
<i>Sulfobacillus acidophilus</i>	Anaerobic <sup>a</sup>	Reduced S <sub>0</sub> , organic
<i>Sulfobacillus. thermosulfidooxidans</i>	Anaerobic <sup>a</sup>	Reduced S <sub>0</sub> , organic
<i>Sulfobacillus. benefaciens</i>	Anaerobic	Organic

<sup>a</sup> This is also capable of ferric iron reduction under microaerobic conditions.

### 3.2. Recovery of Valuable Metal Elements

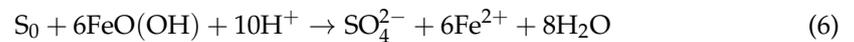
Tailings was defined as valuable metal stocks in the technosphere by Johansson, et al. [46], indicating that reprocessing might also be categorized into an innovative reclamation technology. There are various types of mine tailings discharged, including iron, gold, copper, manganese, lead, zinc, vanadium, rare-earth, and platinum tailings [47]. Therefore, there has been significant interest in studying and developing technically feasible and environmentally acceptable technologies for metal recovery from different types of mine tailings. These technologies include, but are not limited to, the following representative technologies: solvent extraction, electrolysis, chemical processing, and bioleaching [48]. Chemical processing is limited by tailings complexity and extractants [49]. Solvent extraction is generally more costly due to involving expensive extractants [48]. Electrochemical repair techniques are expensive and require complex operations [49]. However, bioleaching is considered as a convincing technology for the recovery of valuable metal elements from mine tailings due to the low

cost and simple infrastructure, with broad development prospects [50,51]. Moreover, under anaerobic conditions, *At. ferrooxidans* can also effectively recover Ni, Co, Mn, and rare-earths from mine tailings [20,39].

### 3.2.1. Nickel and Cobalt Recovery via Anaerobic Bioleaching

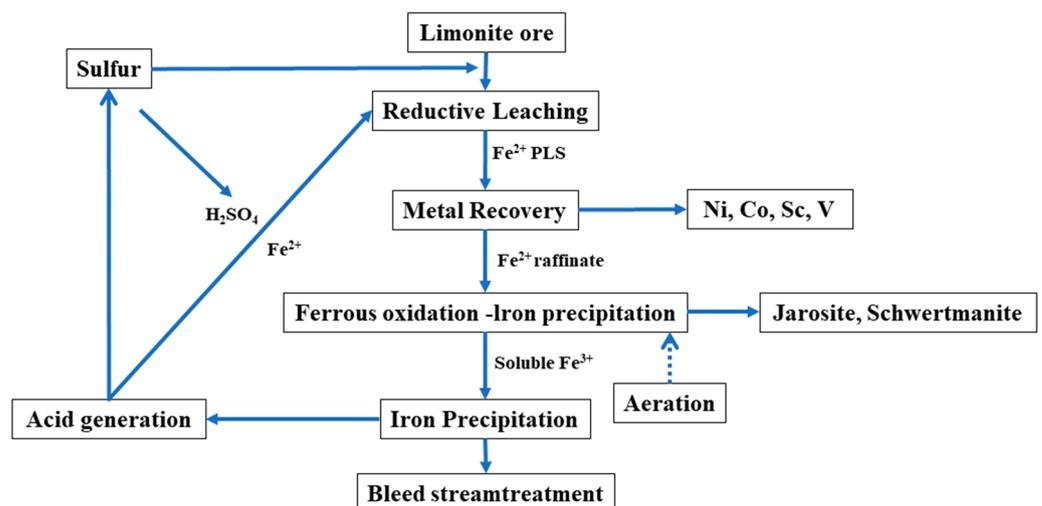
*At. ferrooxidans* grown under anaerobic conditions has been shown to recover nickel and cobalt from tailings. Specifically, laterite tailings were treated using *At. Ferrooxidans*, which has shown its potential in recovering nickel and cobalt from tailings [52].

Reductive acid leaching of limonitic (goethite in particular), resulting in nickel release, has been demonstrated in mildly acidic conditions (pH range 1.5–2) at atmospheric pressure and ambient temperature (25–30 °C) using *At. ferrooxidans* [53]. The leaching process is based on the reduction of ferric iron contained in the goethite host mineral, where the electrons are derived from elemental sulfur. In addition, a novel recently developed bioprocess allows the leaching of nickel and cobalt under acidic conditions and at an ambient temperature (30 °C) by iron-reducing bacteria (autotrophic or heterotrophic) that are able to dissolve the goethite structure [22]. The process utilizes a low-cost electron donor (sulfur), whereby electrons are shuffled from sulfur to ferric iron according to Equation (6).



The iron-reducing bacteria involved in this process belong to species *At. ferrooxidans* and reduce ferric iron under anaerobic conditions coupled to the oxidation of elemental sulfur. The reductive dissolution process catalyzed by *At. ferrooxidans* at low pH and ambient temperatures achieved a 58% extraction of nickel and 56% extraction of cobalt from the laterite tailings in only 7 days [52].

Nickel can also be leached from limonite ore in an anaerobic bioreactor with *At. ferrooxidans*. Within the course of 30 days this bioleaching yielded an over 75% nickel extraction from limonite ore [54]. Du Plessis, Slabbert et al. [55] have proposed a conceptual flow sheet for targeting tropical limonitic laterites and called it a Ferredox process. The process components are designed to (a) facilitate simplified and low-intensity processing, (b) be amenable to modularization, (c) reduce technical process implementation risk, and (d) reduce capital costs for tropical limonite projects (Figure 2).

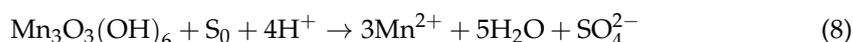


**Figure 2.** Modified scheme of the Ferredox process for reductive dissolution of limonitic laterite ore under anaerobic conditions and metal recovery [55].

### 3.2.2. Manganese Recovery via Anaerobic Bioleaching

The application of bioleach processes to mine tailings for the recovery of manganese has been investigated by a number of research groups [56]. For example, Sharma and

Eisele reported that the recovery of manganese from reagent-grade manganese dioxide and high-grade manganese ore has been studied for over 180 days in an anaerobic environment at room temperature and pH around 5 [57]. Therefore, reductive dissolution using *At. ferrooxidans* has been found effective for the recovery of manganese from the oxyhydroxide mineral asbolane present in a low-grade limonitic nickel laterite ore [23]. Marrero, Coto et al. explored the manganese solubilization from the laterite overburden by bioleaching under anaerobic conditions at pH 0.8 over seven days followed by pH 1.8 [20]. These results indicated that the manganese recovery from laterite overburden by *At. ferrooxidans* reached 86% in 28 days. The reduction of manganese in the asbolane was also enhanced under the present conditions and occurs either indirectly via the ferrous iron produced from goethite (Equation (7)), in the case for cultures of *At. ferrooxidans*, or in the presence of manganese-reducing bacteria similar to ferric iron reduction (Equation (8)) [58,59].



Moreover, it has been recognized that the reduction of the Mn oxides and their subsequent dissolution are necessary to release Cu, Ni, and Co entrained within the oxide matrix. This can be achieved by either the addition of an organic reductant such as glucose or starch in conjunction with bacterial activity [60,61] or by the addition of an inorganic reductant, typically  $\text{Fe}^{2+}$  ions [62].

### 3.2.3. Rare-Earths Recovery via Anaerobic Bioleaching

There is considerable potential for applying reductive bioleaching to the recovery of rare-earths from mine tailings. For example, the recovery of rare-earth elements from red mud has been demonstrated by a two-stage aerobic and anaerobic bioleaching process. Data showed that 86.3% Ce, 93.7% Gd, 90.2% Y, and 74.9% Sc were leached from the red mud after 10 days under anaerobic conditions [39]. Nancucheo, Johnson et al. reported preliminary results of the anaerobic reductive bioleaching of lateritic ore using *Acidithiobacillus* species, and only ~9% of the cerium and 5% of the lanthanum were extracted [63]. Experiments carried out with a sample in which rare-earth-element-containing minerals were occluded by anaerobic reductive bioleaching showed a much greater extraction of the rare-earth following a reductive pre-treatment [64]. Barnett, Palumbo-Roe et al. compared three approaches for the bioleaching of rare-earth elements from bauxite [65]. The presence of *At. ferrooxidans* showed a clear improvement in rare-earth leaching compared to the controls (e.g., 50.2% recovery Nd compared to 35.7%). The total rare-earth was mobilized from gibbsite samples by *At. ferrooxidans* with efficiencies of 67.6% [66].

In addition, anaerobic bacteria have been applied for the mobilization of rare-earths from tailings such as phosphogypsum. Almost 80% of yttrium was mobilized from phosphogypsum in a fixed-bed reactor by *Desulfovibrio desulfuricans* [67].

## 4. Main Technical Difficulties

*At. ferrooxidans* has been demonstrated to recover valuable metal elements from mine tailings under anaerobic conditions in the last few decades [44,68,69]. Although the recovery of valuable metal elements from mine tailings using *At. ferrooxidans* looks promising, there are still some technical problems in practical engineering applications, such as using hydrogen as an electron donor, the anaerobic leaching environment, and the microbial concentration. Therefore, the understanding of the main technical difficulties under the defined bioleaching conditions is essential for improving the efficiency of bioleaching processes.

### 4.1. Supply of Hydrogen as Electron Donor

Hydrogen is a widespread substrate and metabolite in both oxic and anoxic environments and can be utilized as an electron donor in *At. ferrooxidans*. The oxidation of

hydrogen (Equation (9)) is catalyzed by the enzyme hydrogenase, which can also catalyze the reverse reaction of hydrogen production [70,71].



In an acidic environment, hydrogen can be formed through many processes, including acid dissolution of metals and some minerals, so hydrogen is a potential widely available electron donor for acidophiles [70]. Ohmura, Sasaki, Matsumoto and Saiki [14] were not able to grow *At. ferrooxidans* on hydrogen under anaerobic conditions. However, Hedrich and Johnson have revealed that anaerobic respiration with H<sub>2</sub> as an electron donor is the main energy source for the autotrophic growth of *At. ferrooxidans* [70]. *At. ferrooxidans* could utilize hydrogen as an electron donor and grow with ferric iron under anaerobic conditions as an electron acceptor [72]. Kucera, Lochman [73] reported that a multi-omics analysis was applied on *At. ferrooxidans* growing on hydrogen, and a respiratory model was proposed. Under anaerobic conditions, reduced ubiquinol transfers electrons to outer membrane cytochrome *c* (ferrireductase) via the cytochrome *bc*<sub>1</sub> complex and a cascade of electron transporters (cytochrome *c*<sub>4</sub>, cytochrome *c*<sub>552</sub>, rusticyanin, and high-potential iron–sulfur protein), resulting in proton efflux and the reduction of ferric iron. Compared with other inorganic growth substrates, the use of hydrogen as an electron donor has several practical advantages, which can avoid ferric precipitation, medium acidification, and the possibility of producing more biomass [70]. At the same time, using hydrogen as an electron donor to produce water is more environmentally friendly than using elemental sulfur to produce sulfuric acid. Anaerobic oxidation by *At. ferrooxidans* contributes to the global H<sub>2</sub> cycle and may promote microbial productivity in oligotrophic environments [73]. Considering the expected technological development and the reduction of hydrogen cost, hydrogen is more competitive than elemental sulfur [25].

When using the anaerobic reduction technology of *At. ferrooxidans*, it is necessary to provide sufficient hydrogen as an electron donor to support the metabolic electronic pathway of *At. ferrooxidans*. However, the solubility of H<sub>2</sub> in the liquid phase is low, and the concentration of hydrogen is difficult to control accurately. The flammable and explosive characteristics of H<sub>2</sub> not only have potential safety hazards, but also limit its supply of hydrogen through a high-pressure headspace. If the hydrogen supply is not sufficient, it will lead to the imbalance of the system, and the metabolic activity of *At. ferrooxidans* will be inhibited, which will affect the reduction reaction and the treatment effect of tailings. Therefore, how to safely and reliably improve the H<sub>2</sub> accessibility of *At. ferrooxidans* in an anaerobic environment is a key problem to be solved in this process [74].

#### 4.2. Leaching Anaerobic Environment

*At. ferrooxidans* can grow on metal minerals with H<sub>2</sub> as an electron donor under anaerobic conditions. Its efficient leaching of valuable metals under anaerobic conditions has been widely confirmed. Pronk, et al. [34] reported that *At. ferrooxidans* grew anaerobically with trivalent iron ions as electron acceptors, and according to Brock and Gustafson [74], this may be the main biological process of metal ore oxidation during leaching [42]. Li, et al. used *At. ferrooxidans* and pyrite to carry out the bacterial leaching of valuable metals in the manganese module, and found that under anaerobic conditions, microorganisms can use MnO<sub>2</sub> as the final electron acceptor in the respiratory chain of their metabolism, rather than oxygen [68]. Therefore, the reduction activity of pyrite under anaerobic conditions is stronger than that under aerobic conditions, and the leaching efficiency is higher. Under anaerobic conditions, when Mn<sup>4+</sup> is reduced to Mn<sup>2+</sup>, the Mn<sup>2+</sup> nodule matrix is decomposed, and the valuable metals in the matrix are easily leached [68]. Yang, et al. used *At. ferrooxidans* to realize the biological dissolution of jarosites by anaerobic reduction, which further expanded the field of bioleaching [25]. Under anaerobic conditions, *At. ferrooxidans* can use iron ions as electron acceptors on copper sulfide to leach copper, and the leaching rate (68%) is higher than that under aerobic conditions with a similar medium composition. At the same time, *At. ferrooxidans* can also grow on sulfide ores containing sulfur and zinc

under anaerobic conditions. Only in the presence of *At. Ferrooxidans* is the dissolution of copper blue by iron ions under anaerobic conditions significant [42].

However, the reaction needs to be carried out in the leaching solution, and the environment needs to be completely anaerobic to ensure the leaching efficiency of *At. ferrooxidans*. In general, the leaching of impurities in tailings leads to environmental changes that are difficult to grasp, and the leaching of tailings is affected by factors such as the soil, water source, and compaction of the tailings disposal site, and the leachate in the tailings will participate in chemical processes in the environment and may release various gases [75], making it difficult to ensure that the leachate is completely in an anaerobic state, thus affecting the redox conditions of the environment and causing the metabolism of *At. ferrooxidans* to be seriously affected [76]. At the same time, the anaerobic environment itself has a higher process difficulty, and it is necessary to establish the corresponding treatment device design, operation methods, detection control, and other aspects of effective control and adjustment. Therefore, how to ensure a stable anaerobic environment has become a difficult part of the application of this technology.

#### 4.3. Microorganism Concentration

Different growth conditions of *At. ferrooxidans* resulted in different cell concentrations. Das, Mishra et al. demonstrated anaerobic growth on elemental sulfur using dissimilar iron reduction by *At. ferrooxidans* [17]. The total cell count increased from  $3.8 \times 10^5$  cells/mL to  $2.78 \times 10^6$  cells/mL after about 128 h. Data showed that the ferric ion reducing activity (FIR) of the anaerobic cells was double that of the aerobic cells. Furthermore, a series of aerobic/anaerobic cultivation experiments were then carried out with the *At. ferrooxidans* with medium containing  $\text{Fe}^{3+}$  as the electron acceptor and  $\text{H}_2$  as the electron donor. Under aerobic conditions, Ohmura, Sasaki et al. found that the cell density increased from  $2.56 \times 10^7$  to  $2.29 \times 10^8$  cells/mL after 98 h of incubation [14]. Under anaerobic conditions, the cell density of *At. ferrooxidans* increased a minimum of >15-fold, increasing from  $2.18 \times 10^8$  to  $4.95 \times 10^8$  cells/mL. In a comparison of *At. ferrooxidans* growth in oxic and anoxic conditions, both cultures reached similar levels of cell density [77].

Hence, the concentration of *At. ferrooxidans* is an important factor affecting leaching. The higher the microorganism concentration, the higher the leaching efficiency. Therefore, the concentration of *At. ferrooxidans* is also one of the reasons for the efficient treatment of tailings. The bacterial concentration improved the probability of bacterial contact with the tailings, thus increasing the dissolution efficiency [78]. For instance, the extracellular polymeric substances (EPS) composition and content of *At. ferrooxidans* have obvious influence on the interaction between bacteria and minerals, with a high concentration of EPS being able to increase the adhesion ability of bacteria to minerals [79]. However, the cell wall of *At. ferrooxidans* is thicker, the growth rate is slower, and the biological accumulation is less, thus, the microorganism concentration is often a technical difficulty for general tailings treatment. Moreover, the leaching of impurities in tailings will change environmental parameters, such as the change in the anaerobic environment, temperature, pH, the change in the electron donor [49], as well as the production of toxic and harmful substances [80], competition and interference of other microorganisms, etc., which can also affect the population and metabolic activity of *At. ferrooxidans*. For example, Marrero, Coto [20] reported that under anaerobic conditions, due to the low amount of ferric iron in the solution at pH 1.8, there was an almost absence of cell growth (from  $4 \times 10^9$  to  $5 \times 10^9$  cell/mL). Therefore, the strict control of *At. ferrooxidans* in the active metabolic period and dormant period requires a certain physical environment, surface volume, and impurity concentration constraints. If these constraints are not sufficient or destroyed, the microbial flora will also be too sparse or it will be too difficult to survive. Hence, measures must be taken to control environmental parameters and optimize culture conditions to increase the concentration and activity of microorganisms, thereby improving the treatment effect. Yang, Chen [78] found that with the addition of  $\text{Fe}^{3+}$ , the rate and efficiency of jarosite bio-dissolution can be significantly accelerated and improved. Dong, Chong [81] explored

the enhanced effect of biochar on the bioleaching of stone coal tailings by *At. ferrooxidans*. On the one hand, the unique porous structure of biochar created a microenvironment for free microorganisms for inhabitation, while storing abundant nutrients. On the other hand, biochar can also act as an excellent electronic medium to promote electron transfer, improving the oxidation ability of *At. ferrooxidans* on  $\text{Fe}^{2+}$ . Furthermore, the presence of biochar may effectively inhibit the formation of jarosite precipitation on tailings in bioleaching, thereby improving the dissolution of tailings and the release of metal elements.

## 5. Perspectives and Directions for Future Research

Although significant progress has been made on *At. ferrooxidans* anaerobic reduction, there are a number of theoretical and practical issues that remain, which need to be explored and clarified. It can be hoped that a notable breakthrough will be achieved in the following aspects.

- (1) Since *At. ferrooxidans* and other chemolithoautotrophic bacteria produce cellular components through the fixation of  $\text{CO}_2$ , whether  $\text{CO}_2$  could be added to the cultivation under anaerobic conditions to improve cell growth, produce EPS, and increase cell numbers is in question. In addition, EPS plays an important and active role in bioleaching. Therefore, under anaerobic conditions, how to induce *At. ferrooxidans* to produce more EPS to promote leaching will be important research in the future. At the same time, the EPS produced by *At. ferrooxidans* leaching different tailings at different periods may be different, and the research on this aspect needs to be supplemented, so as to make the study of leaching mechanisms more precise.
- (2) The mechanism and growth kinetic model of *At. ferrooxidans* leaching different tailings are different, and further research can be conducted based on the existing research in the future.
- (3) The specific function of genes and proteins involved in anaerobic reduction should be studied systematically and concertedly. Although several genes and proteins have been identified and characterized in anaerobic reduction pathways [33,82], their dynamic changes and roles in sole and double substrate anaerobic reduction have not been revealed [f]. The more functional genes of *At. ferrooxidans* could be predicted through the whole genome information of different *At. ferrooxidans* [83]. We can combine protein isolation and identification technology to research the anaerobic reduction system and the energy metabolism mechanism of *At. ferrooxidans* and further modify and improve the anaerobic reduction system model of *At. ferrooxidans*.

## 6. Conclusions

Mine tailings disposal still has many challenges to overcome in the coming years, but one more clearly observes the importance of the role that anaerobic reduction pathways in *At. ferrooxidans* have developed in mine tailings disposal. Therefore, it is necessary to know more about the anaerobic characteristics of *At. ferrooxidans*, the recovery process, and technical difficulties. In order to improve the effect of *At. ferrooxidans* in the anaerobic reduction process, more technical difficulties need to be overcome, including involving hydrogen as an electron donor, looking at the anaerobic leaching environment, and microbial concentration. Several research activities are being developed, increasing the recovering yield obtained from mine tailings and reducing environmental pollution.

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