

Geochemical Pathways and Kinetic Controls of Fe(II) Oxidation and Secondary Mineral Formation in Acid Sulfate Waters from Siderite Deposit (Slovakia)

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Abstract

This study examines indigenous iron-oxidizing bacteria in the initial stage of acid mine drainage treatment, focusing on selective iron removal for subsequent base metal recovery. Slightly acidic mine water (pH 5.6; net acidity \approx 160 mmol/L) from siderite deposit in the Spiš–Gemer Ore Mountains - Slovakia was used. Under aerobic conditions, abiotic Fe²⁺ oxidation was followed by accelerated oxidation driven by indigenous bacteria, resulting in complete oxidation of Fe²⁺ to Fe³⁺. Subsequent Fe³⁺ hydrolysis and precipitation, coupled with pH decrease $<$ 2.6, enabled selective iron removal while retaining Ni, Co, Mn, and Mg in solution for potential recovery in the following steps.

Keywords: Fe-oxidizing bacteria, iron oxidation, metals recovery, acid mine drainage, mining influenced water

Introduction

The mining industry plays a major role in global economic development. However, it is also associated with environmental challenges, most notably the formation of acid mine drainage (AMD) (Yang *et al.* 2020). Although the chemical composition of AMD differs across sites, it is typically characterized by high concentrations of sulfuric acid, metals, and metalloids such as Fe, Mn, Al, and As, which represent a valuable secondary source of mineral resources (Wang *et al.* 2019; Kelly 1999; Singovszka *et al.* 2020). The pH of AMD is usually defined as pH below 5.6 (Nordstrom 2011) and/or between pH 2 and 6 (Nordstrom *et al.* 2000, 2015; Rakotonimaro *et al.* 2017). The more acidic the effluents, the higher the concentrations of metals, particularly Cu, Zn, Ni, Co, and REEs (Plumlee 1999). In general, AMD can be treated using active and passive technologies (García *et al.* 2001). Active treatment involves the addition of alkaline reagents to increase pH and promote metal precipitation (Moodley *et al.* 2018). Passive

treatment systems utilise natural biological and geochemical processes, including microbial-mediated Fe and Mn oxidation, sulfate reduction, alkalinity generation, and reactions with alkaline materials such as limestone or steel slag (Skousen *et al.* 2017). The biological oxidation of ferrous sulfate by acidophilic iron and sulfur-oxidising bacteria has been broadly studied (Jensen and Webb 1995; Nemati *et al.* 1998). After oxidation of Fe²⁺ to Fe³⁺, which subsequently precipitates at substantially lower pH values compared to ferrous iron, enabling selective iron removal (Sandström and Mattsson 2001). While passive systems are generally more sustainable and cost-effective than active treatments, their application is often constrained by larger spatial requirements, longer hydraulic retention times, and reduced efficiency under fluctuating or high contaminant loads (Johnson and Hallberg 2005). Slovakia has a long-standing, historically relevant mining tradition, which has played a key role in the economic development of several regions. In



the 1990s, dozens of ore mines were closed in Slovakia due to a state reduction program in the ore mining sector, most of which are located just in the Spiš-Gemer Ore Mountains (Bajtoš 2025). Smolník and Nižná Slaná sites are two well-known mining influenced sites in the Spiš-Gemer Ore Mountains. Mining influenced water utilised in this paper is coming from flooded mine from area Spiš-Gemer Ore Mountains and represents a model mining influenced water sample. The water is highly supersaturated with respect to ferric hydroxide. In this paper, only the initial stage of bacterial oxidation of ferrous iron will be discussed. Biologically mediated Fe^{2+} oxidation under acidic conditions enables the selective precipitation of Fe-jarosite while retaining divalent metals (Mn^{2+} , Ni^{2+} , Co^{2+} , Mg^{2+}) in solution for potential recovery and minimizing sludge production.

Methods

Sampling and Chemical analyses

Samples of mine water were collected from the main shaft that receives the majority of water draining the flooded mine area. The water sample intended for the experiment was processed immediately upon transport to the laboratory to minimize physicochemical and microbiological alterations. The mine water was poured into four sterile 500 mL magnetically stirred reactors equipped with the pH and oxidation–reduction potential (ORP) probes. Air supplied to the reactors was sterilised by filtration through 0.2 μm membrane filters. All experiments were conducted at 30 °C, corresponding to the in-situ temperature of the mine water at the time of sampling. Regarding iron monitoring, the experiments were performed in quadruplicate. In two reactors, pH probes were inserted, and in the other two, ORP electrodes were used. Concentration of metals in mine water was determined using atomic absorption spectrometer VARIAN AA240FS. Sulfate, magnesium, calcium and potassium concentrations were analysed by ion chromatography Dionex ICS 5000. Ferric iron was determined by a UV-spectrophotometric method at 300 nm (Basaran and Tuovinen 1986). Ferrous iron concentrations were determined using

a modified o-phenanthroline spectrophotometric method insensitive to Fe^{3+} interference (Herrera *et al.* 1989). Physical-chemical parameters, such as pH and ORP were measured using probes Radiometer Analytical/HACH Lange for pH and Mettler Toledo InLab Redox-L type for ORP.

Gas analysis

The rates of chemical (abiotic) and bacterial ferrous iron oxidation, as well as bacterial growth, were monitored by online measurements of gas-phase oxygen consumption and carbon dioxide production/consumption. Oxygen and carbon dioxide concentrations in the inlet and outlet air were measured using a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer (Sable Systems, USA), respectively. The analyzers were calibrated using high-purity nitrogen (99.99% N_2) for zero point and ambient outdoor air for span calibration of O_2 (20.95%) and CO_2 (0.042%) concentrations. Air of the same quality was used to aerate the reactors. The data acquisition was performed by a software application developed in LabVIEW® (National Instruments).

Results and discussion

Characterisation of water

The real acid mine water originating from a flooded siderite ore deposit in the Spiš-Gemer Ore Mountain was used as a model system for the biological iron-oxidation step. The slightly acidic pH of the mine water (pH values ≈ 5.7) was influenced by the siderite buffering system (Wolkersdorfer 2008). The mine water effluent transported substantial quantities of potentially toxic metals and metalloids into the river system. Elevated iron concentrations (1261.5 mg/L) caused intense reddish-brown discoloration of the water (Zeman *et al.* 2022). The retention of these metals in soluble form enables their potential recovery from the treated mine water. Characterisation of the AMD sample is summarised in Table 1.

For the potential recovery of divalent metals (such as Mn, Ni, Co and/or Mg), it is necessary to first remove the major element Fe from the solution (Sandström and Mattsson 2001). This can be achieved



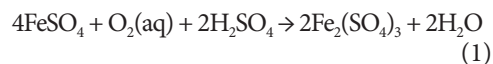
Table 1 Characterisation of the initial sample of acid mine drainage.

Parameter	Concentration	Parameter	Concentration
Al	0.785 mg/L	Net acidity	160 – 190 mmol/L
As	15.4 mg/L	Ni	2.88 mg/L
Ca ²⁺	565.4 mg/L	Sb	0.0082 mg/L
Cd	<0.1 mg/L	Pb	0.035 mg/L
Co	0.370 mg/L	SO ₄ ²⁻	30,162 mg/L
Fe	1261.5 mg/L	Zn	0.87 mg/L
K ⁺	90.87 mg/L	TDS	53 g/L
Mg ²⁺	5460 mg/L	t	30.9 °C
Mn	97.5 mg/L	pH	5.7
Na ⁺	69.85 mg/L	Eh	182 mV

through the biological oxidation of Fe²⁺ to Fe³⁺ by iron-oxidizing bacteria, followed by the precipitation of Fe³⁺ as Fe-hydroxysulfates under low-pH conditions. This process operates without the addition of neutralizing agents, allowing divalent metal cations (Mn²⁺, Ni²⁺, Co²⁺ and Mg²⁺) to remain in solution (Johnson and Hallberg 2005).

Upon discharge from the anoxic underground environment into the surface, mine water commonly undergoes rapid physico-chemical transformations driven by the degassing of dissolved CO₂, oxidation of Fe²⁺ and Mn²⁺, and hydrolysis of Fe³⁺ and Mn⁴⁺ as the system equilibrates with atmospheric conditions (Geroni *et al.* 2012; Cravotta *et al.* 2013). The chemical oxidation rate of Fe²⁺ by molecular oxygen is strongly pH-dependent (Singer and Stumm 1970; Lowson 1982). The initial rapid abiotic oxidation of Fe²⁺ in aerated water progressively slows down as pH decreases, particularly below pH 3.5. Consequently, a substantial amount of ferrous iron remained in solution. The onset of exponential growth of indigenous iron-oxidising bacteria was observed up to 72 h after the mine water equilibrated with atmospheric conditions (Fig. 1). The rate of Fe²⁺ oxidation increased proportionally with the exponential growth of bacterial biomass. Subsequently, the oxidation-reduction potential (Eh) rose in parallel with a further decrease in solution pH (Fig. 3). The exponential increase in O₂ consumption rate (Fig. 1a) accompanied by CO₂ consumption, indicates the activity

of indigenous autotrophic iron-oxidizing bacteria naturally present in mine water (Johnson and Hallberg 2005). Isolation of bacterial strains and methanogenic analysis of biodiversity in the mine water before and after treatment are currently underway. The bacteria completely oxidized ferrous iron to ferric iron within the next 80 h. The maximum iron oxidation rate reached ca. 30 mg L⁻¹ h⁻¹ (Fig. 1B). Due to Fe³⁺ hydrolysis the pH decreased to ≈ 2.6. At this low pH, Fe³⁺ precipitation was less pronounced because hydrolysis reactions were limited (Johnson and Hallberg 2005). Part of the oxidized iron precipitated as secondary Fe-minerals, while approximately half of the Fe³⁺ remained in solution as a sulfate complex (Fig. 2A, B). Variations in pH, redox potential (Eh), O₂ consumption and CO₂ production/consumption values were recorded online. The rate of iron oxidation was continuously monitored using online gas analysis, based on oxygen consumption (Fig. 1), and calculated according to the stoichiometry of Eq. 1.



The Fe²⁺ oxidation rate derived from O₂ and CO₂ consumption rates (Fig. 1), was used to calculate the decline in Fe²⁺ concentration by integrating $r_{\text{Fe}^{2+}}$ over time. The Fe²⁺ concentration decline calculated from gas analysis showed good agreement with the data obtained from chemical determination of ferrous iron in the liquid phase (Fig. 2A).

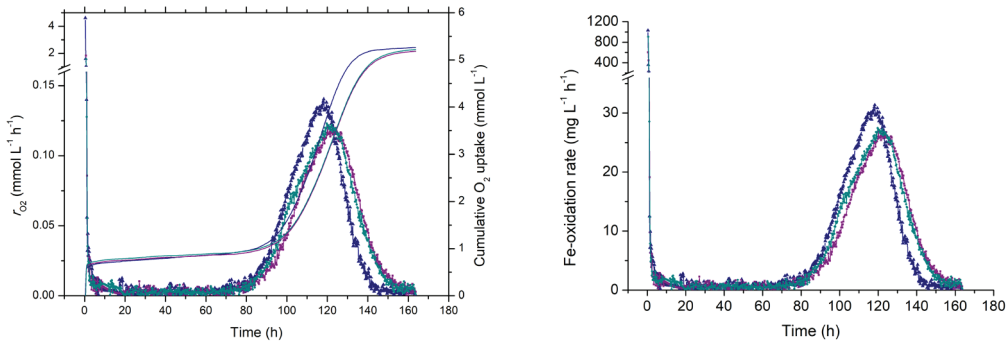


Figure 1 Oxidation of ferrous iron in the mine water under aerobic conditions. A) O₂ consumption rate and cumulative O₂ consumption related to Fe²⁺ oxidation. Chemical iron oxidation was followed by increased O₂ consumption due to exponential growth of indigenous Fe-oxidizing bacteria. B) Fe²⁺ oxidation rate derived from gas analysis based on O₂ and CO₂ consumption.

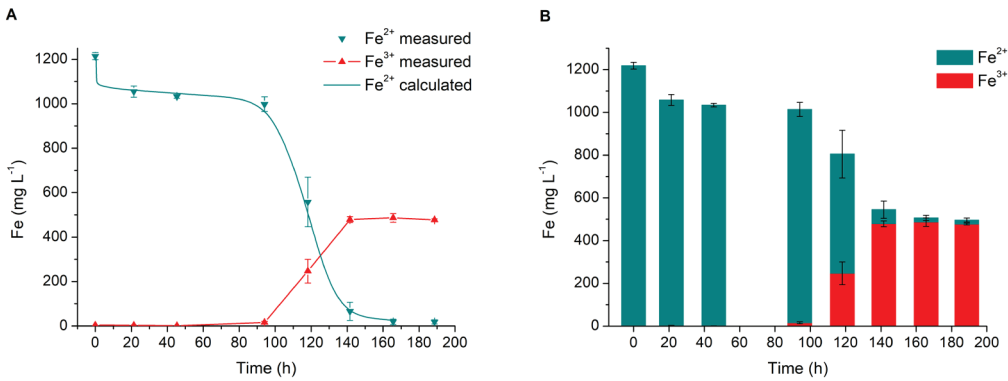


Figure 2 Iron speciation in mine water during oxidation. A) Concentrations of Fe²⁺ and soluble Fe³⁺ determined chemically in the liquid phase and the Fe²⁺ concentration decline calculated by integrating of $r_{Fe^{2+}}$ over time. B) cumulative amounts of dissolved iron species (Fe²⁺, Fe³⁺).

After approx. 180 h of experiments, almost all ferrous iron was oxidised into ferric form.

In acidic aqueous solutions, biological Fe²⁺ oxidation exceeds the rate of chemical oxidation by six orders of magnitude, as chemical oxidation is inhibited by low pH (<3.5) (Kupka 2002; Singer and Stumm 1970). Water in contact with air can exhibit biological activity due to the natural presence of bacteria in the acid-mine-water environment (Johnson and Hallberg 2005). The process also enables partial control of pH and the targeted addition of monovalent cations, both of which are critical in governing the solubility and precipitation behavior of iron and sulfate species under acidic conditions. Such control is essential for

optimizing mineral formation pathways and achieving predictable iron speciation during treatment. During Fe oxidation, the Eh of the mine water increases from approximately 182 mV at the beginning to 832 mV (Fig. 3).

Alkali and alkaline earth cations were monitored during the experiment. Magnesium and calcium remained stable, while minor potassium fluctuations occurred in pH and ORP probed reactors, likely from KCl release. Biologically mediated Fe²⁺ oxidation under acidic conditions enables the selective precipitation of iron as secondary minerals without the need for neutralizing agents. This allows divalent metal cations, such as Mn²⁺, Ni²⁺, Co²⁺, and Mg²⁺, to remain in solution without notable changes after

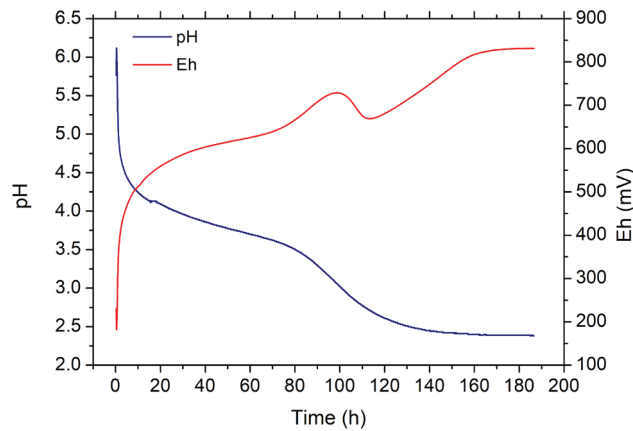


Figure 3 pH and Eh profiles during iron oxidation in the mine water.

Fe-oxidation, which enables their potential recovery in subsequent treatment steps. The concentration of sulfates remained constant during the experiment.

Conclusions

The results demonstrate that indigenous iron-oxidizing bacteria present in AMD can effectively catalyse the oxidation of ferrous iron under acidic conditions. Following an initial phase of rapid abiotic oxidation, biological activity significantly enhanced the overall oxidation rate, leading to complete conversion of Fe^{2+} to Fe^{3+} within approximately 80 hours. The course of Fe^{2+} concentration calculated from online gas analysis agrees with the values of Fe^{2+} concentration measured by offline chemical analysis (Fig. 2a). The study confirms that chemical iron oxidation is strongly influenced by pH, with lower pH values limiting abiotic iron oxidation rate. As a result, a substantial fraction of Fe^{3+} remained in solution under highly acidic conditions ($\text{pH} \approx 2.6$). Nevertheless, this process enables selective iron transformation without the addition of neutralizing agents, allowing divalent metals such as Mn, Ni, Co, and Mg to remain dissolved and available for subsequent recovery. These findings support the applicability of biologically driven processes as an initial step in AMD treatment, particularly in systems aiming to recover metals rather than achieve complete neutralization. The research is ongoing and

focuses on optimizing conditions for Fe^{3+} precipitation as oxyhydroxides or hydroxy-sulfates to improve treatment efficiency and resource utilization.

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