From AMD to Pigments: Biological Iron Oxidation and Characterization of Recovered Iron Precipitates

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Abstract

This study investigates biological iron removal and recovery from the real mine water at the Smolnik site (Slovakia). Ferrous iron oxidation was enhanced using native acidophilic microbial consortia, achieving over 99% removal within 30 hours under acidic conditions. A sequential process combining aeration, microbial oxidation, and controlled pH adjustment enabled the formation of iron precipitates such as schwertmannite and ferrihydrite. Elemental and structural analyses confirmed low arsenic content in biologically produced precipitates, meeting regulatory limits for pigment use. This approach highlights a sustainable strategy focusing on contaminant removal with value-added material recovery.

Keywords: Mining influenced water, acidophiles, mineral pigments, circular economy, iron oxidation

Introduction

Iron is a common contaminant in natural and industrial waters, particularly in effluent associated with mining and metallurgical activities, where elevated concentrations can adversely affect aquatic ecosystems and technical infrastructure. At the same time, iron constitutes a valuable secondary resource, as its precipitate forms can be reutilized as mineral pigments in a wide range of industrial applications, including paints, ceramics, plastics, and construction materials.

In nature, iron occurs mainly in two oxidation states, as ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions. The prevailing form is dependent on pH, oxygen concentration, and redox conditions (Stumm and Morgan 1996).

The oxidation of ferrous iron to ferric iron by dissolved oxygen is a complex and multi-step process. It involves the formation of partially oxidized intermediate phases which are metastable and challenging to characterize or predict. Over time, these intermediates undergo transformation into more thermodynamically stable iron oxide minerals, including hematite, magnetite, goethite, and lepidocrocite (Morgan and Lahav 2007).

Mining influenced waters are commonly acidic due to the oxidation of certain sulfide minerals exposed during mining activities, which generates acidity and lowers the pH of the drainage waters (Johnson and Hallberg 2005; Singer and Stumm 1970; Wolkersdorfer 2022).

In acidic environments, the abiotic oxidation of ferrous iron by dissolved oxygen is kinetically limited, as Fe^{2+} remains relatively stable at low pH (Morgan and Lahav 2007). Consequently, spontaneous chemical oxidation proceeds slowly and is insufficient to account for the rapid iron cycling observed in acid mine drainage (AMD) systems. Instead, this process is largely driven by the metabolic activity of acidophilic iron-oxidizing microorganisms, which catalyze the oxidation of ferrous to ferric iron at rates several orders of magnitude higher than abiotic reactions (Johnson *et al.* 2012).

The microbial oxidation of iron plays a central role in both natural and engineered systems, enabling efficient iron removal and influencing the formation of secondary iron minerals. Biohydrometallurgical studies have demonstrated that near-complete ferrous iron oxidation can be achieved at pH values as low as 1.5 without the need for chemical

neutralization, highlighting the advantages of biological processes over conventional chemical treatments (Kaksonen *et al.*, 2014). These processes promote the formation of iron precipitates such as schwertmannite, jarosite, and goethite, which are critical for the removal of iron and associated contaminants from mine waters.

This study focuses on iron removal regarding acid mine drainage from the historical mining site in Smolnik, Slovakia. In the recent years, pH of the mining influenced water from the main drainage shaft Pech, ranges from 3.95 to 4.12 and produces a high volume of mine water (approximately 6.5–10 L/s). Concentration of metals analysed in the water ranges according to the weather conditions and rainfall volumes (Bártová 2025).

The purpose of this study was to optimize Fe^{2+} oxidation processes and characterize iron precipitates formed from real mine water under laboratory conditions. Mine water samples were either inoculated with iron-oxidizing acidophiles (pre-grown cultures of native strains) or left uninoculated, allowing a comparison of products formed by biological and chemical oxidation.

Methods

Bacterial strains and incubation conditions

For the purposes of the experiment, native microbial consortia were cultivated in mine water from the main discharge shaft (Pech), supplemented with 9K salts (1.6 mmol/L $MgSO_4 \cdot 7H_2O$, 0.76 mmol/L $(NH_4)_2SO_4$, 0.23 mmol/L K_2HPO_4, and 120 mmol/L $FeSO_4 \cdot 7H_2O$). Cultivation was carried out in magnetically stirred, aerated baffled reactors (0.5 L working volume) at 25 °C and 240 rpm.

Mine water parameters

pH/ ORP analysis

A combined glass electrode (InLab Micro, Mettler Toledo) was used for pH measurement, and oxidation–reduction potential (ORP) was monitored using a Pt–Ag/AgCl redox electrode (InLab Redox Micro, Mettler Toledo). Measurements were performed with a Seven2Go S2 meter (Mettler Toledo).

Iron speciation

Ferric iron was determined with a UV-spectrophotometric method at 300 nm (Basaran and Tuovinen 1986). Ferrous iron concentrations were determined by a modified o-phenantroline spectrophotometric method, insensitive to Fe3+ interference (Herrera *et al.* 1989).

Chemical analyses

Elemental analysis of the water was done using AAS (Varian AA240Z, AA240FS) and ICP-MS 7700 (Agilent). Sulfate concentration was analyzed by ion chromatography (Dionex ICS 5000).

The microstructure and morphology

Iron powders were investigated by MIRA 3 FE-SEM scanning electron microscope (TESCAN GROUP, a.s., Czech Republic) with an EDX detector (Oxford Instruments, Abingdon, UK).

XRPD patterns were obtained using a D8 Advance diffractometer (Bruker) operating with Cu Kα radiation in the Bragg–Brentano configuration.

Elemental composition was determined using a handheld X-ray fluorescence (XRF) analyzer (X-MET8000 Expert Geo, Hitachi High-Tech).

Color measurements

Color was measured using a Nix™ Spectro 2 sensor (D50, 2°), with results expressed in the CIELab* color space (Robertson 1977).

Results and discussion

Mine water from the Smolnik site was analyzed and treated in order to remove dissolved iron and recover precipitates usable as mineral pigments in form of powders. Air-dried precipitates were then characterized using X-ray diffraction (XRD), X-ray fluorescence (XRF), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and NIX™ color determination instruments. Liquid samples and resulting mine water after Fe-precipitates removal were analyzed using atomic absorption spectroscopy (AAS).

Initial composition of the mine water is given in Tab. 1. During the experiment, concentrations of Fe, As, Al, Mg, Mn, Cu, Co and SO_4^{2-} were followed, as these elements

Table 1 Parameter sand composition of the Smolnik mine water (2026).

pH	ORP	Conductivity	Temperature	Flow rate
	mV	mS/cm	°C	L/s
3.95	286	2.53	11.7	n.a.

Shaft Pech mg/L	SO_4^{2-}	Fe	Al	Mn	Zn	Mg	Ca
2026	2871	238	40.7	12.9	50.3	275	111

Shaft Pech mg/L	Cu	Co	Pb	As	Cd	Cr	Ni
2026	1750	360	47.1	39.4	5.8	0.6	90

either form a pigment structure (Fe, Al, S) or they are considered an unwanted impurity, often exhibiting toxic characteristics (As) or they are considered a valuable component of the water (Cu, Co, Mg, Mn) where leaving them in the water might lead to possibilities of their subsequent recovery from already iron-free solutions.

Iron precipitate (pigment) formation in mine water was carried out as a sequential three-stage process: (i) initial aeration of MIW (30 h) to lower pH and partially oxidize and precipitate Fe, removing phases that can sorb arsenic; (ii) bacterial inoculation to accelerate ferrous iron oxidation; and (iii) pH adjustment to promote precipitation of poorly crystalline phases such as ferrihydrite or schwertmannite. Between stages, Fe precipitates were removed by filtration using a 0.22 µm PES filter.

Iron oxidation

Ferrous iron oxidation is limited at pH below 4 (Jones *et al.* 2014; Morgan and Lahav 2007). As the pH of Smolník MIW fluctuates around this value and decreases below 3.5 after aeration, acidophilic microbial consortia were introduced to enhance oxidation. Despite genotypic differences, many acidophilic iron oxidizers share similar physiological traits (Kupka *et al.* 2023). Therefore, native microbial species were preferred over pure collection species, which can be costly and their purity needs to be confirmed over a longer period of time to ensure that we are still working with the declared species. Native consortia isolated from Smolník MIW were pre-cultivated and subsequently used for iron oxidation.

A 2 L of Smolník MIW were aerated for 30 h and subsequently filtered to collect the initial iron phase (A). The filtrate was transferred to a 5 L flask, and 50 mL of actively growing microbial culture (exponential phase; ≈2.0×10^6 cells/mL) was added. Iron introduced with the growth medium was not considered. Iron oxidation was monitored for 29 h, with a substantial decrease in Fe^{2+} concentration observed within the first 21 h. After 29 h, Fe^{2+} levels dropped below 2 mg/L, corresponding to a removal efficiency exceeding 99%. The solution was then filtered again to collect the resulting iron precipitates (B) for further analysis.

After filtration, the water was divided into separate flasks for the final pH adjustment. Starting from pH 2.6, the pH was increased to (i) 3.2 (optimal for schwertmannite formation), (ii) 4.1 (natural Smolník MIW pH), (iii) 5.5 (favorable for Al precipitation), and (iv) 6.2 (rapid ferrihydrite formation) using 20% NaOH. After each adjustment, precipitates were removed by filtration, and the water was either subjected to further treatment or stabilized with concentrated HNO_3 for AAS analysis (Tab. 2). Note that a pH level of 5.5 was achieved using the remaining water after filtering water that had already been adjusted to a pH of 3.2, as a high iron concentration was still present in the sample.

As table 2 shows, majority of Fe was effectively removed already after the bacterial oxidation, without any further pH adjustments. Applying pH levels above 4.1 the Fe concentration complied with the Regulation of the Government of the Slovak

Table 2 Final elemental composition of mine water after the pH adjustment; n.d.: below detection limit.

	Name	Fe	Al	Mn	Mg	Cu	As	Co
		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
–	**Initial MIW**	238.8	40.7	12.88	275.3	1750	39.4	359.9
A	**30 hours aeration**	196.5	40.9	13.12	277	1740	15.7	369.4
B	**Bacterial oxidation**	15.68	42.2	13.22	273.1	1780	3.3	368.3
C	**pH 3.2**	8.39	38.8	13.18	246.3	1750	2.3	404.2
D	**pH 4.1**	0.21	32.7	13.03	236	1610	1.9	394.1
E	**pH 5.5**	0.05	n.d.	11.7	216.3	210	1.8	400.2
F	**pH 6.2**	0.05	n.d.	10.99	210.9	60	1.8	334.6

Republic (2010), which sets a limit of 2 mg/L. Aluminum was effectively removed from the solution at pH 5.5, as it was expected. Co, Cu, Mg and Mn did not co-precipitate from the water at pH below 4.1 and such water can be subject for their later recovery. The majority of arsenic was removed during the first aeration step, and the remaining concentration already complied with the Regulation of the Government of the Slovak Republic (2010), which sets a limit of 0.02 mg/L.

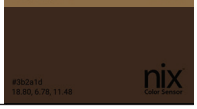
Pigments obtained from the first two steps (i, ii) of iron precipitation were analyzed using SEM-EDS and a handheld X-ray fluorescence (XRF) analyzer to confirm their elemental composition and to ensure that the resulting products did not contain high concentrations of arsenic or other potentially toxic elements. XRF analysis showed that Product A (30-hour aeration) contained a high arsenic concentration of above 350 mg/kg. In contrast, Product B (bacterial oxidation) exhibited minor arsenic levels of less than 30 mg/kg. This value complies with the European Commission criteria, which state that pigments must not be based on Cd, Pb, Cr (VI), Hg, As, Se, Sb, or Co, and that impurities from these elements must not exceed 100 mg/kg in the final product formulation (European Commission 2025). Based on these criteria, Sample B can be considered suitable for use as a pigment.

All samples were characterized using XRPD. The diffraction patterns indicated poorly crystalline iron phases consistent with schwertmannite or ferrihydrite-like materials, clearly in samples A and B and less distinctly in sample C. Samples E and F exhibited predominantly amorphous characteristics, while sample D showed intermediate properties. EDS analysis revealed a transition from Fe-rich oxyhydroxysulfate phases (schwertmannite-like) at low pH to Al-rich hydroxide phases at higher pH, confirming a sequential precipitation mechanism controlled by microbial oxidation and pH adjustment.

The color of iron precipitates is a key parameter for their potential use as pigments because it directly determines their aesthetic and commercial value. Variations in color are closely linked to mineral phase, crystallinity, particle size, and chemical composition, all of which influence optical properties such as light absorption and scattering (Cornell and Schwertmann 2003). In addition, impurities and substitutions (e.g., Al, sulfate, or trace metals) can modify hue and stability, affecting pigment performance (Bigham *et al.* 1990; Schwertmann and Cornell 2000). To quantitatively evaluate these properties, it is convenient to express color values in the CIE L*a*b* color space system, which provides a standardized, device-independent framework based on lightness (L*) and chromatic coordinates (a*, b*). This system correlates well with human visual perception and enables precise comparison between samples making it particularly suitable for pigment characterization and quality control (Mclaren 1976; Robertson 1977). To determine color, Nix™ Spectro 2 Color Sensor was used.

The synthesized iron precipitates (Tab. 3) exhibited a wide range of color coordinates in the CIE L*a*b* space (L* = 14.81–48.80, a* =

Table 3 Final elemental composition of mine water after the pH adjustment; n.d.: below detection limit.

		CIE L*a*b*	Nix Color
A	30 hours aeration	CIELAB: 32.84, 16.42, 34.09	
B	Bacterial oxidation	CIELAB: 46.63, 18.09, 47.59	
C	pH 3.2	CIELAB: 34.16, 14.59, 26.14	
D	pH 4.1	CIELAB: 14.81, 5.94, 10.97	
E	pH 5.5	CIELAB: 48.80, 8.82, 26.42	
F	pH 6.2	CIELAB: 18.80, 6.78, 11.48	

5.94–18.09, b* = 10.97–47.59), corresponding to dark brown, brown, and yellow- to orange-brown (ochre-like) hues typical of iron-based pigments. These color differences can be related to the identified mineral phases and compositions, where Fe-rich oxyhydroxides (ferrihydrite- to schwertmannite-like) tend to produce darker brown to orange-brown hues, while Al-rich hydroxide/hydroxysulfate phases are associated with lighter, more yellowish tones (Cornell and Schwertmann 2003; Schwertmann and Cornell 2000). In addition, sulfate incorporation and the presence of trace elements can influence color intensity and saturation, particularly in poorly crystalline Fe phases such as schwertmannite (Bigham *et al.* 1990).

Conclusions

Despite extensive research on AMD treatment, most studies address iron removal, microbial oxidation, or mineral formation separately. The integration of biological iron oxidation with controlled precipitation for simultaneous contaminant removal and resource recovery remains insufficiently explored, particularly in real AMD systems. In addition, the performance of native microbial consortia under field-relevant conditions has received limited attention.

This study demonstrates that coupling microbial Fe^{2+} oxidation with staged pH adjustment enables efficient iron removal from authentic mine water while producing iron-rich precipitates suitable for use as mineral pigments. The use of native acidophilic microorganisms proved effective under acidic conditions without the need for extensive chemical inputs.

Beyond iron removal, the process preserves dissolved metals such as Cu, Co, Mn, and Mg in solution, allowing their potential downstream recovery. Overall, this approach represents a sustainable and economically attractive strategy that links wastewater treatment with the production of value-added materials.

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