

Zinc removal from metallurgical dusts with iron- and sulfur-oxidizing bacteria

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ABSTRACT

The generation of currently partially recyclable metallurgical dusts during the steel production represents a challenge for the realization of a circular economy in this industrial sector. Due to a high iron concentration, the reutilization of blast furnace and cast house dusts in internal crude steel processes, is state of the art. However, heavy metal impurities such as zinc, act as an interference due to diverse negative effects. The aim of this study is to utilize *Acidithiobacillus ferrooxidans*, an iron- and sulfur-oxidizing bacteria, to solubilize zinc whilst retaining iron in the dust residue intended for reuse. To achieve this, the biooxidation of elemental sulfur is of crucial relevance. Two different strains of *A. ferrooxidans* (DSM 583 and CCM 4253) were cultivated and their leaching performances were compared to each other using a stirred tank reactor operated in a fed batch set-up, with a working volume of 3 L, at an operation temperature of 30 °C. The dust concentration was gradually increased until a maximum of 125 g/L, after which the reactors were left to run for three additional cycles to ensure replicability of results. For both strains, at a cast house dust concentration of 125 g/L paired with a leaching duration of two weeks, an average zinc removal of 56 % was achieved whereas solely an average of 4 % of iron was leached.

1. Introduction

The yearly global steel production in 2021 was estimated to reach around 1900 million tons, with the outlook of a slight increase in production for 2023 (Mercier et al., 2022). In the blast furnace (BF), dusts such as the cast house dust (CHD) and the BF flue gas dust, are generated. CHD is a by-product of the periodical casting of liquid iron and slag from the BF, originating mainly during the tapping process (Lanzerstorfer, 2017). An average amount of 0.6–5.1 kg CHD produced per ton of hot metal has been reported for European blast furnaces (Remus et al., 2013), characterized by a high iron content and thus opening a discourse to its potential recyclability (Stewart & Barron, 2020). As BF dusts contain high amounts of iron (Fe), and calcium (Ca), partial reuse is achieved by reintroducing the dusts into the sintering process, where currently the recycling rate of said dust in European steelworks approximates to 12.7 kg/t of sinter (Remus et al., 2013). However, a major disruptive element hindering the recyclability of BF dust is zinc (Zn), which depending on the ore source and furnace operation can reach up to 5 wt% (Luo et al., 2022; Wang et al., 2022). Zn is characterized by its low boiling point and undergoes a reduction to its elemental form once charged into the BF. A recondensation of its vapor accelerates the formation of Zn scaffolds on the brickwork in addition to harming

refractories found in the upper stack, ultimately impairing the overall stability of the BF (Binnemans et al., 2020). Unfortunately, landfilling is the conventional solution upon confrontation with metallurgical dusts containing high Zn concentrations, impeding the concepts aligned with a circular economy (Xu et al., 2020).

Current methodologies to extract valuable Zn from BF dusts such as CHD include pyrometallurgical along with hydrometallurgical approaches. An example of an established pyrometallurgical process is represented by the Waelz process, however its high maintenance costs paired with low productivity overshadow its advantages of a reliable Zn output. Meanwhile common hydrometallurgical methods focus on acid leaching using strong acids such as concentrated sulfuric acid (H₂SO₄), achieving high leaching efficiencies at a rapid rate of reaction. Unfortunately, the leaching procedure using concentrated acids, lacks selectivity, as Fe and Zn are equally solubilized (Xue et al., 2022). Furthermore, environmental strategies aim to ameliorate existing linear processes into circular flowsheets, where then difficult regeneration of the used-up H₂SO₄ complicates such undertakings (Binnemans & Jones, 2023). Presently, the production of industrial sulfuric acid is seen as a convenient by-product of the oil and gas industry as harmful sulfur dioxide (SO₂) emissions, derived from combustions reactions, are significantly diminished and through further downstream processing turned

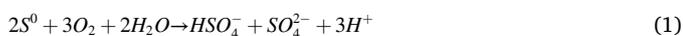
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into H_2SO_4 (King et al., 2013). Yet, considering vital efforts to reduce carbon dioxide (CO_2) emissions will require a drastic restructuring of our global energy system to achieve independency of fossil fuels such as oil and gas in the upcoming future (Intergovernmental Panel on Climate Change (IPCC), (2023)). Taking this into consideration, sulfuric acid ceases to be a simple by-product and as green technologies increase the demand for this vital chemical, alternatives to the energy intensive Frasch process must be found (Maslin et al., 2022).

Bioleaching has been proposed as an alternative, low-cost, and environmentally friendly method utilizing microorganisms or fungi to solubilize metal sulfides as well as oxides (Gu et al., 2018). The mechanisms behind bioleaching lie within the production of inorganic or organic acids, formation of complexing ligands, and oxidation and reduction reactions (Mishra et al., 2005). In a previous study, the bioleaching capacity of a mixed culture of *Acidithiobacillus ferrooxidans* alongside with *Acidithiobacillus thiooxidans* was evaluated using CHD as a substrate (Sasiain et al., 2023). Taking these results into consideration, the focus of the present experimental set-up was to identify the role of *A. ferrooxidans* during the leaching process. The chemolithotrophic *A. ferrooxidans* is a common bacteria used for bioleaching purposes due to its ability to derive energy through the oxidation of reduced sulfides or elemental sulfur (Equation (1) to produce sulfates as well as oxidation of ferrous to ferric iron (Gu et al., 2018; Pakostova & Johnson, 2019). *A. ferrooxidans* is classified as a gram-negative prokaryote, thriving optimally in mineral rich environments at 30 °C and ideally at a pH of 2, however its growth below pH 1 has also been demonstrated (Valdés et al., 2008; Yarzabal et al., 2004).

When elemental sulfur is used as a sulfur source, this process may be referred to as S^0 biooxidation. Sulfate, the final product of S^0 biooxidation, further facilitates the solubilization of metals found in oxides (Equation (2) (Trung et al., 2011). Bioleaching with *A. ferrooxidans* has been investigated previously with the external addition of solely sulfur as an energy source (Chen et al., 2011; He et al., 2019). Nevertheless, the addition of external sulfur contradicts the approaches of a circular economy; thus, to overcome this, attempts have been made to incorporate suitable waste products (Ilyas et al., 2014; Tichy et al., 1994). Apart from technical sulfur, *A. ferrooxidans* has successfully demonstrated biooxidation of biological sulfur derived as a waste product from biogas desulfurization (Kremser et al., 2023), gas purification plant from a paper mill (Seidel et al., 2006), or mining waste deposits (Jiménez-Paredes et al., 2021).



Upon completion of the bioleaching process, Zn found in the leachate, can be recovered via precipitation, solvent extraction, ion exchange, or (bio)electrochemical systems (Jha et al., 2001; Palimaka et al., 2018; Spiess et al., 2023). Zn is a relevant metal in the galvanizing industry in addition to being utilized in metal alloys to ameliorate their chemical and physical properties (Xue et al., 2022). The remaining Fe-enriched solid leaching residue can then ideally be recycled at the sinter plant of an integrated steel-plant, fulfilling the criteria of a circular flowsheet.

This study aims to assess the potential of the bacterium *A. ferrooxidans* to selectively bring Zn into solution whilst retaining Fe in the solid, using high concentrations of CHD. Bacteria possess the ability to adapt to their surroundings and different strains of *A. ferrooxidans* have demonstrated divergent S and Fe oxidation capacities (Suzuki et al., 1990; Wakao et al., 1991). Thereby the comparison of two strains of *A. ferrooxidans* from two different institutes was attempted in an endeavor to further elucidate the effect of previous adaptation steps on the leaching ability of these strains. There is a fundamental lack of studies evaluating the potential of bioleaching beyond the scope of batch tests, using pulp densities above 10 % w/v (Tezyapar Kara et al., 2023).

Therefore, the focus in this study lies especially on higher pulp densities to support assessment for plausible industrial applications. The experimental set-up included a stirred tank reactor operated in fed batch mode using industrially relevant concentrations of CHD, where a progressive increase of CHD induced bacterial adaptation to high mineral concentrations. This study provides first insights into long-term bioleaching experiments associated with industrial steelmaking waste products. At the final concentration of 125 g/L CHD, the experiment was run for eight weeks to ensure replicability of the results, as a stable methodology is a prerequisite prior to up-scaling.

2. Materials and methods

2.1. Materials and chemicals

The CHD was obtained from an Austrian steelmaking company. A sample was dried previously in a drying cabinet at 105 °C until a constant mass solid content was reached. Then the difference between the weight of an original undried sample and the dried sample was calculated to determine a dry mass of 99.9 %. Furthermore, in order to determine the initial metal concentrations, CHD was solubilized using a wet digestion procedure and then analyzed via ICP-MS (Thermo Scientific iCAP RQ, Waltham, MA, USA) or ICP-OES (SPECTRO Analytical Instruments GmbH, Kleve, Germany) according to the method described by Sasiain et al. (Sasiain et al., 2023).

The results of the analysis are listed in Section 3.1.

2.2. Mineralogical characterization

To further characterize the sample material, X-ray diffraction analysis (XRD) was performed with the original CHD along with the solid leaching residue. For this, solid samples were withdrawn after the completion of each leaching cycle, dried, and stored until analysis. Samples were manually grinded to improve signal quality. The analysis was conducted using the X'Pert PRO MPD diffractometer (Malvern Panalytical Ltd, Malvern, United Kingdom) and interpreted using Panalytical HighScore Plus 3.0.5 software. Further technical details are published previously by Sasiain et al. (Sasiain et al., 2023).

2.3. Chemical leaching using sulfuric acid

Chemical leaching of CHD was conducted in 250 mL Erlenmeyer flasks containing 100 mL of diluted 0.5 M H_2SO_4 and either 5, 7.5, 10, or 12.5 g of CHD. The flasks were shaken in a digital orbital shaker (Witeg Labortechnik GmbH, Wertheim, Germany) at 160 rpm and incubated at 30 °C and 50 °C for one week with samples being withdrawn at day 1, 3 and 7 for ICP-MS analysis and pH measurements. All experiments were carried out in triplicates.

2.4. Bacterial strain and growth conditions

Two different bacterial strains, *Acidithiobacillus ferrooxidans* DSM 583 and *Acidithiobacillus ferrooxidans* CCM 4253, were cultivated for the bioleaching experiments. Both strains were precultivated at 30 °C and 100 rpm in a basal salt medium according to a previous study (Wakeman et al., 2008). This medium consists of 0.15 g/L $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$, 0.45 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.05 g/L KCl, 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g/L KH_2PO_4 , and 0.014 g/L $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ prepared with deionized water and autoclaved at 121 °C for 20 min. The bacterial cultures were supplemented with 10 g/L technical elemental sulfur (finely ground with a purity of $\geq 99.5\%$), which was separately autoclaved (Atlas, 2010; Tilman Gehrke et al., 1998). The medium remained pH unadjusted. Bacteria were cultured at 30 °C in 250 mL Erlenmeyer flasks containing 90 mL of selected medium and 10 mL of inoculum with shaking at 100 rpm until a pH around 2 was reached.

2.5. Semi-continuous mode bioleaching setup

The bioleaching experiments were conducted in two double jacketed stirred tank reactors (Schmizo AG, Oftringen, Switzerland) with a working volume of 3 L at 30 °C. Using an automated control (Awite Bioenergy GmbH, Langenbach, Germany) the temperature, pH, and redox were regularly monitored. The pH and redox electrodes were purchased from Mettler Toledo (Vienna, Austria). The reactors were heated with a water bath (LAUDA, Lauda-Königshofen, Germany) and the content was stirred with a motor-stirrer (Heidolph Instruments GmbH, Schwabach, Germany) at 160 rpm. To maintain an adequate aeration, an air pump (Scheego GmbH, Offenbach, Germany) was set to 75 L/h.

2.6. Semi-continuous mode bioleaching methodology

For each bacterial strain, a reactor was setup with inoculum of the *A. ferrooxidans* DSM 583 strain and inoculum of the *A. ferrooxidans* CCM 4253 strain. The reactors were filled up with the basalt salt medium to reach a working volume of 3 L and the previously described parameters were applied. An initial amount of 30 g of elemental sulfur was added to reach a concentration of 10 g/L. The reactors were operated for three weeks, where each week, two-thirds of the spent medium was exchanged whilst one-third of the content was kept as inoculum. After completion of these three initial weeks and reaching a pH of around 1.0, the CHD was added to reach a concentration of 50 g/L. From now on, the bioleaching was performed for two weeks, and two-thirds (2 L) of the spent medium was exchanged by fresh medium on a biweekly basis, again allowing one-third (1 L) of the solution to act as an inoculum, to ensure a semi-continuous process (Kremser et al., 2022; Osorio et al., 2013). To compensate evaporation losses, the reactors were regularly refilled with deionized water to ensure a working volume of 3 L. These procedures were repeated for a concentration of 75, 100 and 125 g/L of CHD, resulting in a gradual increase in the CHD and adaptation of the bacterial cultures over a total period of eight weeks. The vitality of the microorganisms was confirmed via light microscopy at the end of each leaching cycle.

Upon completion of the eight weeks, three more rounds at the highest concentration of 125 g/L CHD were conducted.

2.7. Sample withdrawal and chemical analysis

For each 2-week period, a total of three samples were taken. Sample withdrawal occurred right upon the exchanging of the reactor content, then at day 7 and day 14. The samples were filtered using a syringe filter (Chromafil, Macherey-Nagel GmbH, Düren, Germany) with a 0.2 µm pore size. The leachate samples were then analyzed via ICP-MS (Thermo Scientific iCAP RQ, Waltham, MA, USA) or ICP-OES (SPECTRO Analytical Instruments GmbH, Kleve, Germany) according to the method described by Sasiain et al. (Sasiain et al., 2023) to determine the concentration of Fe, Zn, Al, and Cr. Ionic chromatography was used to determine sulfate concentration of samples.

2.8. Calculation of bioleaching efficiency

In order to determine the bioleaching efficiency, the ratio of the metal concentration in the leachate to the metal content of the untreated CHD was calculated by applying following formula:

$$\text{Bioleaching efficiency}[\%] = \frac{(M_C \times V_R) - (M_P \times V_L)}{M_i \times C_A} \times 100$$

The progressive increase of CHD had to be taken into consideration during these calculations where the assumption was made that during the exchange of the spent medium with fresh medium, the solid was distributed evenly in the solution. In the equation above, M_C [g/L] represents the measured metal concentration in the leachate sample, V_R

[L] indicates the total working volume of the reactor (3 L), M_P [g/L] is the metal concentration found in the leachate from the previous stage of CHD concentration, V_L [L] stands for the remaining volume in the reactor (1 L), M_i [mg/kg] indicates the metal concentration found in the untreated CHD, and C_A [g] is the amount of CHD added to the reactor. Fig. 1.

3. Results and discussion

3.1. Results of CHD analysis

The results of the ICP-MS analysis of the original CHD are displayed in Table 1. Fe is the dominating metal in this dust whereas only minimal amounts of Cr and Al were detected. The results of the XRD analysis are illustrated in Fig. 2. Fe_2O_3 and Fe_3O_4 were found to be the dominating phases. Zn occurred in the phases ZnO , ZnFe_2O_4 , as well as ZnAl_2O_4 .

3.2. Monitoring of pH

Fig. 3 illustrates the comparison between the two bacterial strains and their pH development. Both strains demonstrate the capacity to lower the pH from around 2.0 (value at the addition of CHD) to a pH minimum of 1.0, indicating a potential to oxidize elemental sulfur to sulfuric acid.

3.3. Chemical leaching of Zn and Fe

The results of the chemical leaching using diluted H_2SO_4 is depicted in the Fig. 4 below. The majority of Zn is leached within the first day (up to 85 %), and a concentration and temperature dependency is observed. At a lower CHD concentration and higher temperature, more Zn is solubilized within the first 24 h. On the other hand, the rate of Fe leaching is slower, yet above 7 % within the first day of leaching at either temperature, for all concentrations tested. As the leaching process continues, more Fe is released from the matrix into the solution, obtaining a maximum leaching efficiency of 35 % at 50 g/L at a temperature of 50 °C. These results are comparable to data found in literature where a selective Zn extraction of around 60 % is reached within the first 10 min of leaching at 30 °C, using 0.5 M sulfuric acid, and at a liquid to solid ratio of 20 (Kukurugya et al., 2015; Makkonen et al., 2016). Overall, the results of these preliminary experiments demonstrate the feasibility to selectively solubilize Zn prior to Fe, using a diluted sulfuric acid solution

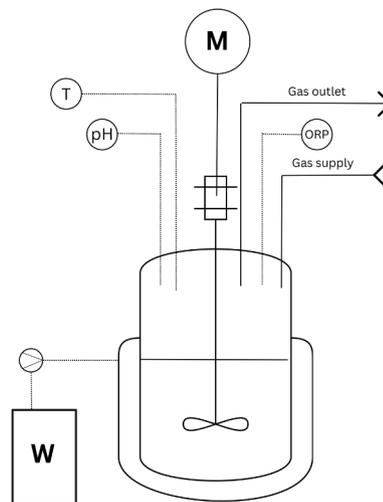


Fig. 1. Schematic illustration of a stirred tank reactor. M represents the motor-stirrer, W indicates the water bath, T equates to the temperature electrode, pH is the pH electrode and ORP stands for the redox electrode measuring the oxidation-reduction potential.

Table 1
Selected metal concentration in CHD determined by ICP-MS analysis.

Metal	Mass percentage [%]
Fe total	63.750
Zn	2.630
Cr	0.008
Al	0.110
V	0.005
Ni	0.003
Cu	0.004
Co	0.001
Cd	<0.00005

at moderate temperatures.

3.4. Leaching efficiencies of selected metals

The leaching efficiencies achieved by both bacterial strains were calculated for Zn, Fe, Cr, and Al and are illustrated in Fig. 5a–d (DSM 583) and Fig. 6a–d (CCM 4253), respectively. For each element an increase in leaching efficiency was observed, however the leaching rate differed. Both strains demonstrated a similar leaching efficiency, with no significant deviations in rate and maximum leaching efficiencies. The DSM 583 strain leached 64 % of Zn at CHD concentrations of 75 and 125 g/L after 14 days, whereas the CCM 4253 strain achieved a maximum of 61 %. Al was leached at around 60 % by both strains, here the leaching occurred mainly within the first week, indicated by a leveling off the

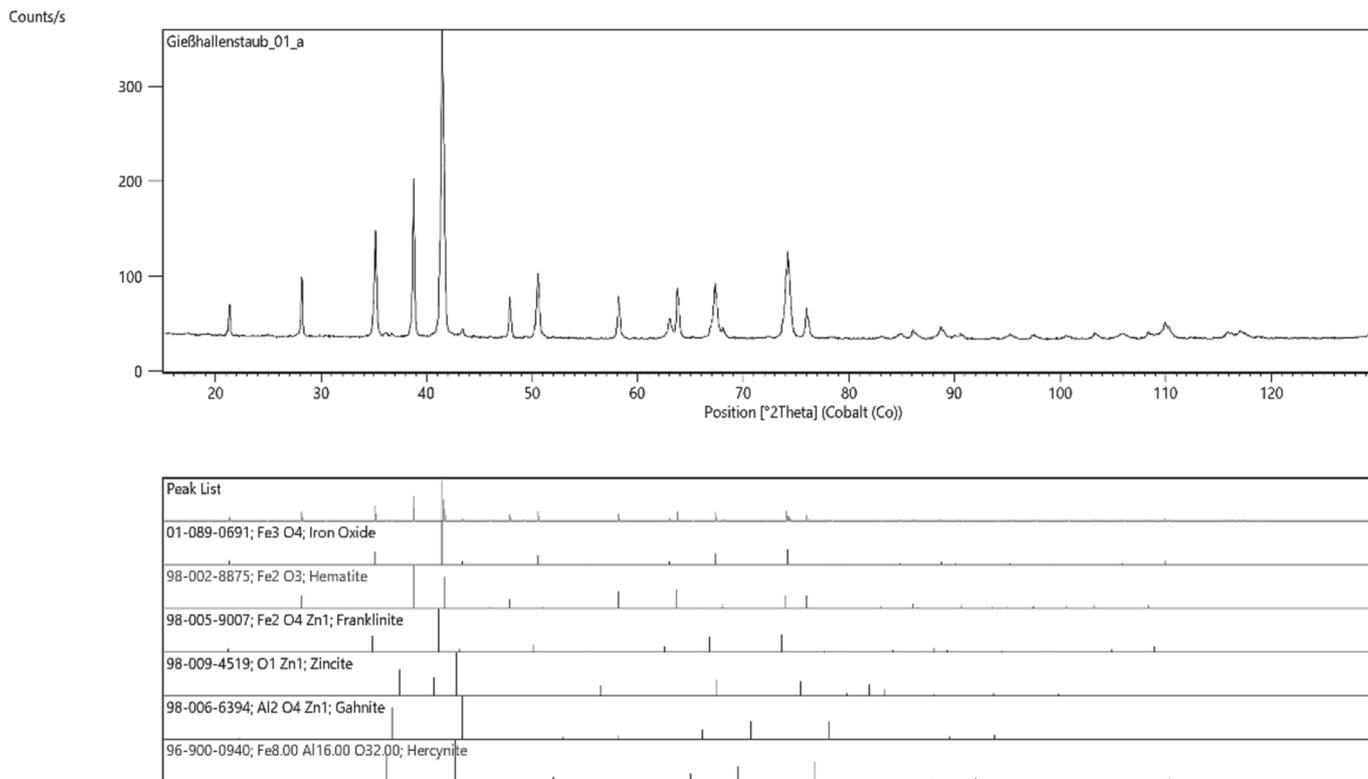


Fig. 2. XRD analysis for original CHD sample.

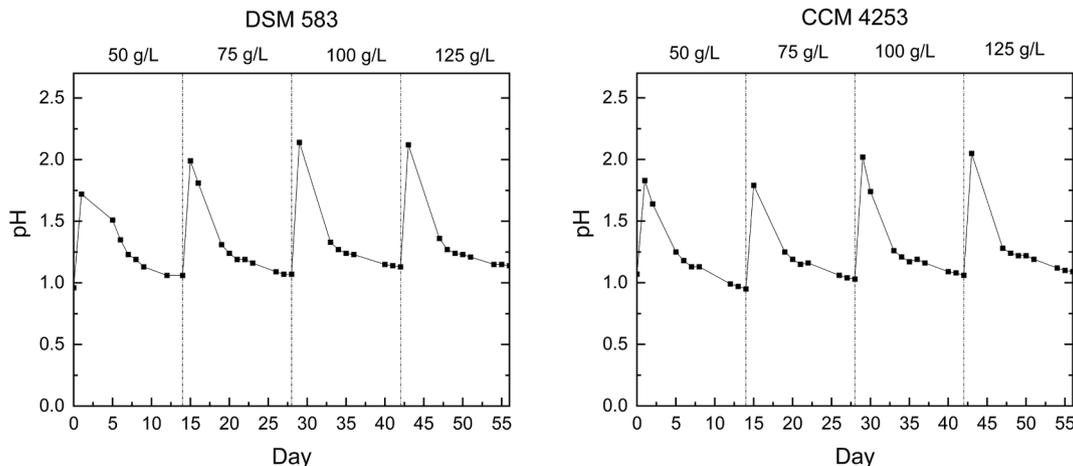


Fig. 3. Comparison of the two *A. ferrooxidans* bacterial strains DSM 583 and CCM 4253 in terms of their pH course during the first four runs. The dashed lines represent an increase in CHD concentration along with a two-third exchange of the reactor content.

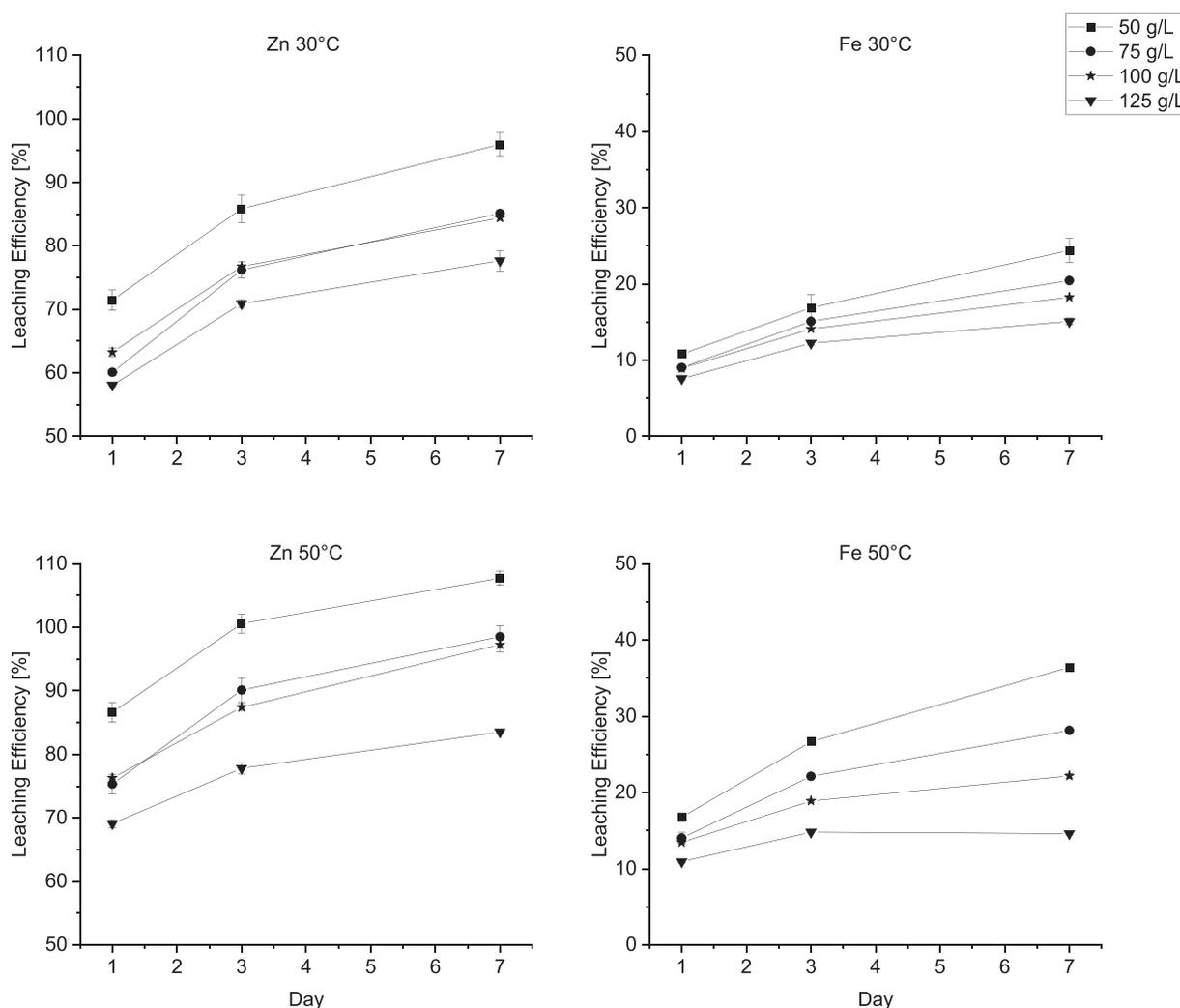


Fig. 4. Chemical leaching results using 0.5 M H_2SO_4 over a duration of 7 days. Results are depicted as the mean of three triplicates.

leaching curve in Fig. 5d and Fig. 6d. Cr was leached minimally, peaking at 13 % at a CHD concentration of 125 g/L after 14 days using the DSM 583 strain.

Previous studies have investigated the leaching potential of *A. ferrooxidans* on steel plant waste products. Bayat et al. demonstrated adapted *A. ferrooxidans* strains to achieve higher leaching efficiencies compared to non-adapted strains, yet at a solid concentration of 1 % w/v, around 35 % of Zn and Fe was solubilized (Bayat et al., 2009). In a column leaching set-up, *A. ferrooxidans* was applied to leach Cr and Al from 100 g of basic oxygen furnace (BOF) steel slag, attaining metal removal rates close to 100 % (Gomes et al., 2018). Nevertheless, the applied pulp densities concerning metallurgical slag in bioleaching experiments tend to range around a maximum of 10 % (Potysz et al., 2018), whereas the bioleaching of CHD, as described in this study, showed the possibility to use higher concentrations.

3.5. Comparison of Zn and Fe leaching

As this investigation aimed to focus on solubilizing Zn whilst keeping Fe in the CHD matrix, a direct comparison of the leaching efficiencies of both elements is illustrated in Fig. 7 (DSM 583) and Fig. 8 (CCM 4253) for the respective strains. As stated previously, Zn peaked at a leaching efficiency of 64 % using the DSM 583 strain at a CHD concentration of 125 g/L after 14 days. Simultaneously only 6 % of Fe was found to be solubilized, implying a high Fe retention in the dust matrix. The leaching rate of both metals did not level off, hence a longer leaching duration

would most likely result in a higher leaching efficiency. Furthermore, the leaching efficiency as well as the leaching rate did not seem to be dependent on the CHD concentration, indicated by a lack of difference between the leaching efficiencies. This suggests that the full leaching capacity of the bacteria was not yet taken advantage of, where higher dust concentrations could potentially further increase the Zn solubilizing whilst maintaining Fe retention. In Fig. 9 the mean leaching efficiency after 14 days is depicted of 4 runs using 125 g/L of CHD. For Fe and Zn, the results remained constant, indicating a stable process where bacterial activity is not significantly deteriorating after a total of 14 weeks running time in semi-continuous mode.

Thermodynamic as well as kinetic aspects may offer an explanation to the differences in leaching rates found for Zn and Fe. From a theoretical perspective, Kukurugya et al. determined the likelihood of the solubilization of ZnO, ZnFe_2O_4 , Fe_2O_3 , and Fe_3O_4 in a 1 M sulfuric acid solution applying concepts of the Gibbs free energy change. The authors found ZnO and ZnFe_2O_4 to undergo a faster rate of reaction compared to Fe, however ZnO requires a lower activation energy compared ZnFe_2O_4 , thus passing into solution significantly prior to ZnFe_2O_4 (Kukurugya et al., 2015). The XRD analysis revealed Zn to be bound as ZnO as well as ZnFe_2O_4 , which could offer an explanation to why Zn leaching efficiency did not surpass 64 %. The XRD analysis of the solid residue demonstrated a loss of ZnO, however traces of ZnFe_2O_4 were still detected (Fig. 10).

As demonstrated in the chemical leaching experiments (Fig. 4), leaching of Zn using 0.5 M H_2SO_4 occurred mainly within the first 24 h

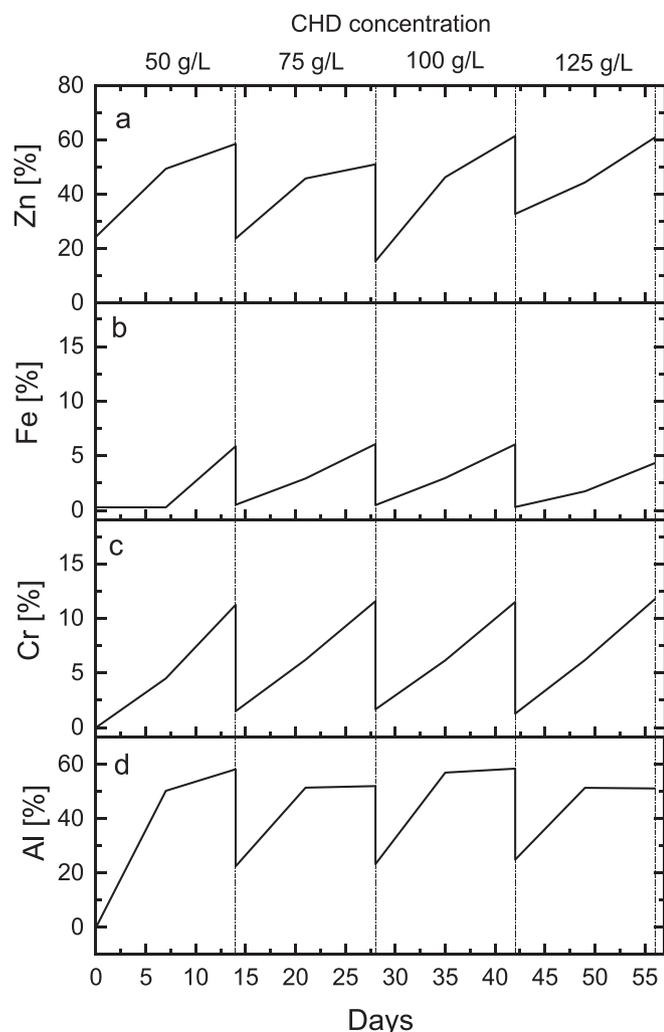


Fig. 5. Leaching efficiency achieved by *A. ferrooxidans* DSM 583 for the metals Zn, Fe, Cr, and Al for the first 4 runs. The dashed lines represent an increase in CHD concentration along with an exchange of the reactor content.

whereas the leaching duration during the bioleaching experiments required 14 days. Using conventional hydrometallurgical methods, Zn was observed to pass into solution significantly quicker than Fe using various concentrations of diluted sulfuric acid. Different parameters such as the solid to liquid ratio, temperature, and acid concentration mainly affected the rate of Fe leaching rather than Zn solubilization, yet relatively high leaching efficiencies of 25–40 % were found for Fe (Cruells et al., 1992; Oustadakis et al., 2010). This suggests that the leaching rate of Zn is not limited by the chemical dissolution of ZnO, but rather by biological processes such as the oxidation rate of sulfur. However, no excessive acid was added to the cultivating medium nor was required to maintain bacterial metabolic activity, thus the production of acid relied solely on biological processes. The determination of SO_4^{2-} concentration at the end of the last 14-day cycle resulted in 17.9 g/L for the CCM 4253 and 19.3 g/L SO_4^{2-} for the DSM 583 strain. These concentrations are high, in comparison, previous batch-scaled investigations have determined the increase in SO_4^{2-} concentration induced by sulfur oxidation by *A. ferrooxidans* in solely medium to reach around 14 g/L SO_4^{2-} at the end of a 14-day period (Konishi et al., 1994) or 13 g/L SO_4^{2-} using a mixed culture including *A. ferrooxidans* at 28 °C after 10 days (Plumb et al., 2008). Meanwhile, Lin et al. evaluated the bioleaching potential of a mixed consortium of sulfur-oxidizing bacteria in a stirred tank reactor to leach heavy metals from industrial waste sludge and achieved a production of around 12 g/L SO_4^{2-} in 14 days (Lin et al.,

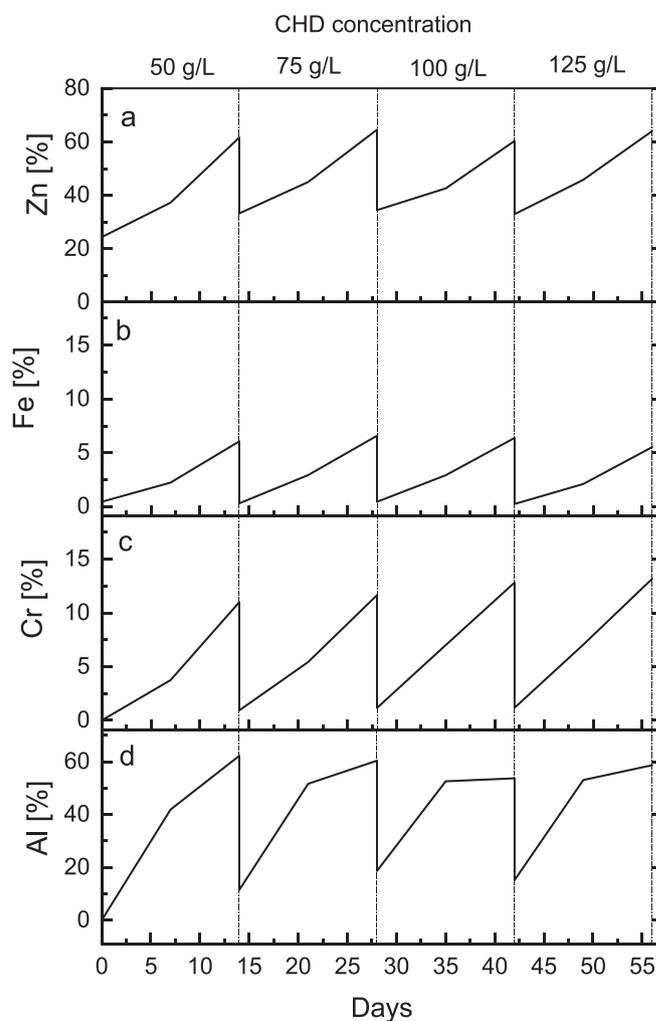


Fig. 6. Leaching efficiency achieved by *A. ferrooxidans* CCM 4253 for the metals Zn, Fe, Cr, and Al for the first 4 runs. The dashed lines represent an increase in CHD concentration along with an exchange of the reactor content.

2010).

Fig. 10 illustrates the mineralogical analysis of the solid material residue upon completion of the last leaching run using the *A. ferrooxidans* DSM 583 strain. Even though ZnO is solubilized, elemental sulfur is still found in the residue, indicating an excess in sulfur addition, which did not undergo bacterial metabolization. This suggests a necessary reduction of sulfur for planned upcoming investigations, as elemental sulfur should be completely removed, in order to successfully recycle the leaching residue in an integrated steel plant. Sulfur impacts the brittleness, weldability, and corrosion resistance negatively and is therefore actively removed during the steel-making process (Schrama et al., 2017). As a newly formed compound, jarosite was observed in the solid residue (Fig. 10), this being also reported by Abdollahi et al. (Abdollahi et al., 2022). Due to the addition of CHD at each cycle, the short-lived rise in pH might offer an explanation to the formation of this compound.

In literature, *A. thiooxidans* is described as the more effective sulfur-oxidizing species, compared to *A. ferrooxidans*. In batch experiments, it was reported that *A. thiooxidans* reaches a concentration of 75 g/L SO_4^{2-} after 14 days, when supplied with 20 g/L of sulfur (Cheng et al., 1999). However, also Yang and Yang observed a strain dependency previously (Yang & Yang, 2018). Based on these results along with the conclusions published by Sasiain et al. (Sasiain et al., 2023), further investigations are intended, evaluating the bioleaching potential of *A. thiooxidans* on CHD. Additionally, methodological optimization is required to ensure

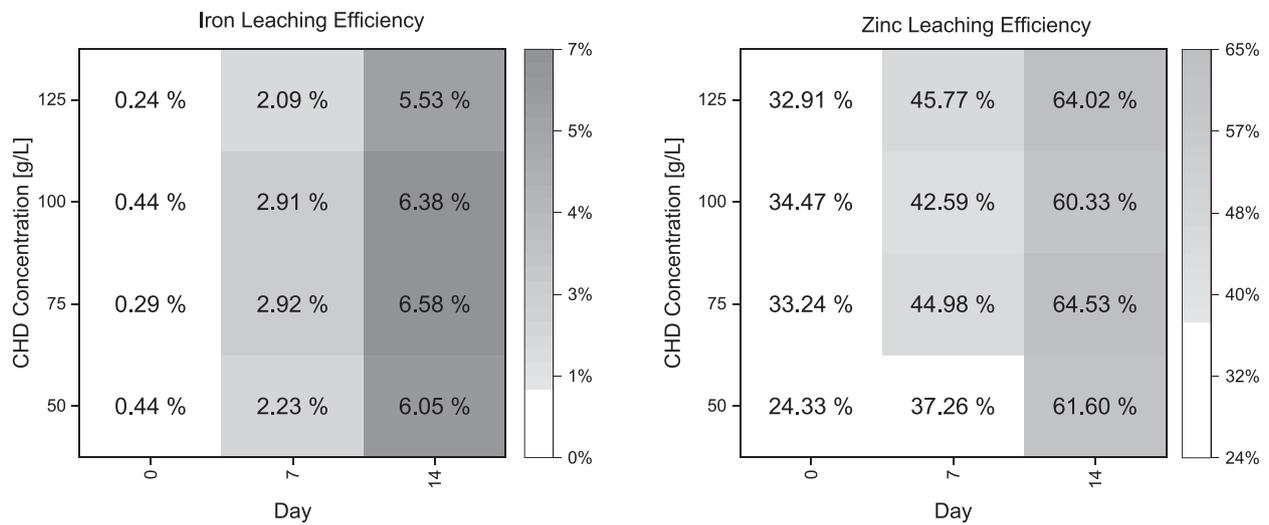


Fig. 7. Leaching efficiency achieved by *A. ferrooxidans* DSM 583 for Zn and Fe illustrated in heatmaps for the first 4 runs.

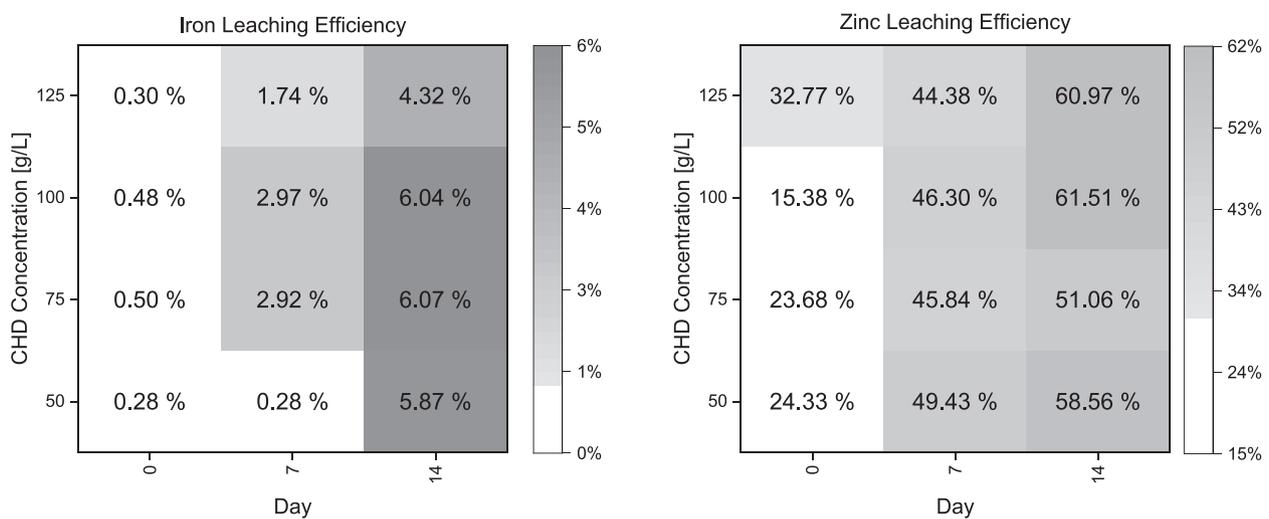


Fig. 8. Leaching efficiency achieved by *A. ferrooxidans* CCM 4253 for Zn and Fe illustrated in heatmaps for the first 4 runs.

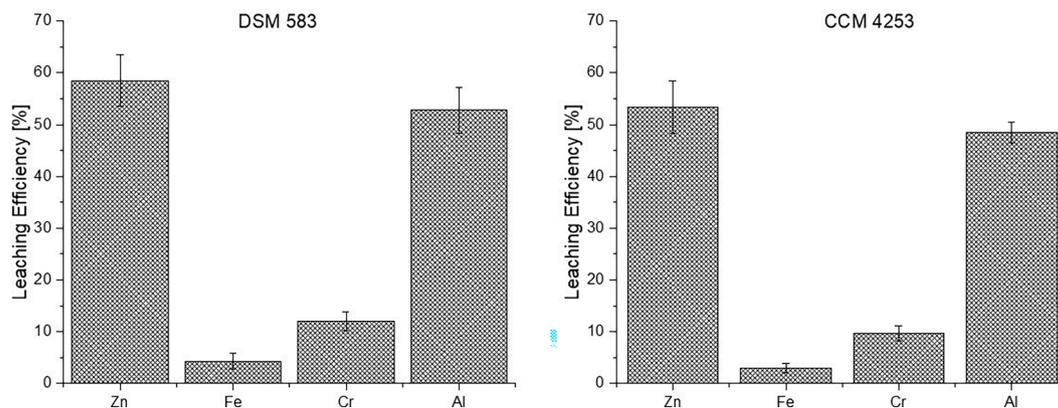


Fig. 9. Leaching efficiencies for both strains after 14 days depicted as the mean of four replicates at a CHD concentration of 125 g/L. The error bars indicate the standard deviation of the results.

competitiveness in this field, especially regarding the long leaching duration in comparison to hydrometallurgical approaches. A plausible approach to increase the biological activity of the microorganisms might be achieved by increasing the amount of available oxygen and by

supplementation of appropriate sulfur concentrations.

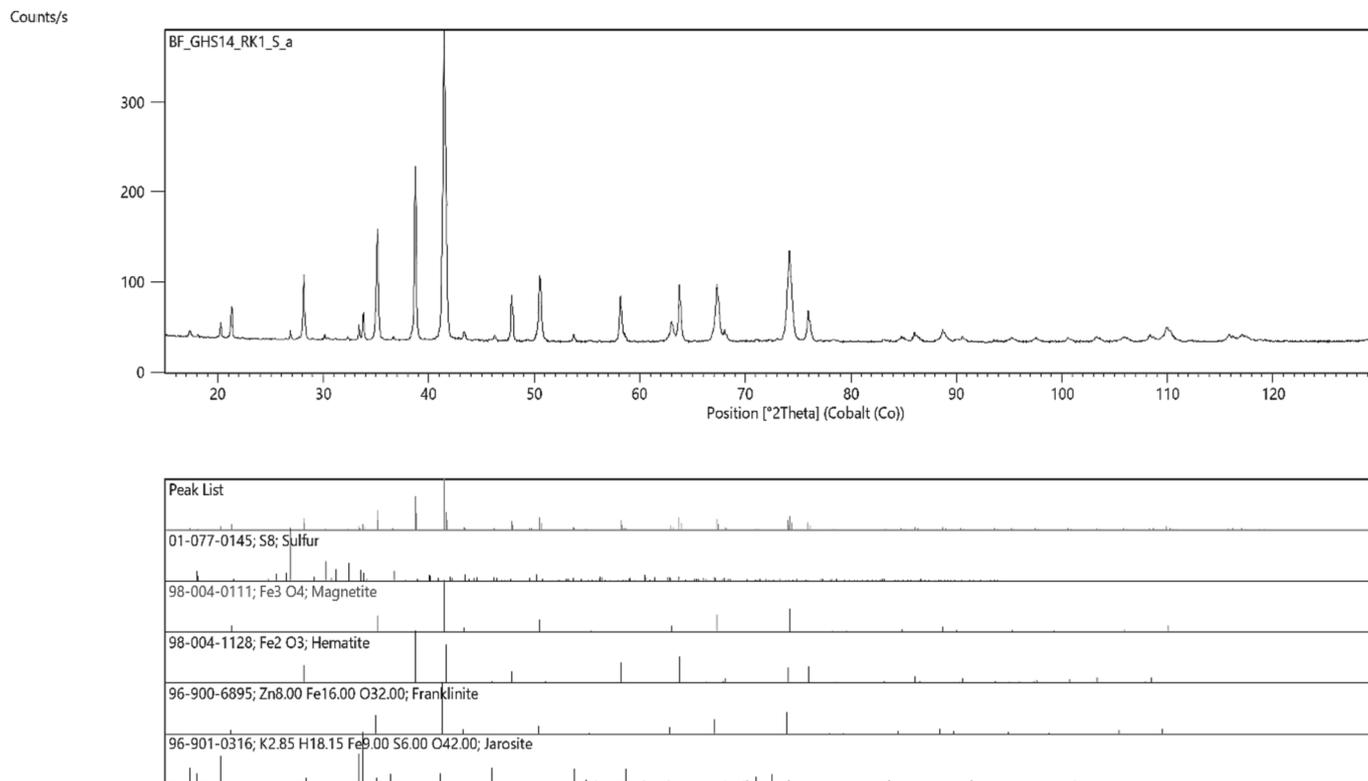


Fig. 10. XRD analysis of leaching residue taken from the last performed cycle at a CHD concentration of 125 g/L and a leaching duration of 14 days using the *A. ferrooxidans* DSM 583 strain.

4. Conclusion

The aim of this study was to assess the applicability of utilizing *A. ferrooxidans* in a bioleaching process to selectively solubilize the metal Zn whilst retaining Fe in the CHD matrix. Two different strains of *A. ferrooxidans* were selected to further analyze plausible discrepancies in leaching efficiencies, however both strains demonstrated nearly identical leaching activity. In summary, a mean leaching efficiency of 55.3 ± 5.1 % for the CCM 4253 strain and 58.5 ± 4.9 % for the DSM 583 strain for Zn was achieved, after four runs using a CHD concentration of 125 g/L, within a leaching duration of 14 days using a mild operation temperature of 30 °C. Simultaneously, during these experiments, an average of 3.0 ± 0.9 % for the CCM 4253 strain and 4.3 ± 1.5 % for the DSM 583 strain of Fe underwent leaching, indicating a suitable selectivity between the two elements. The stability of this leaching set-up was demonstrated by the low standard deviation between four replicates performed at CHD concentration of 125 g/L. The mineralogical analysis demonstrated Zn to be found as ZnO as well as $ZnFe_2O_4$, where the presence of the latter limited the leaching efficiency of Zn.

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CRedit authorship contribution statement

Rebeka Frueholz: Conceptualization, Methodology, Investigation,

Visualization, Writing – original draft. **Amaia Sasiain Conde:** Writing – review & editing. **Clemens Habermaier:** Investigation. **Sabine Spiess:** Writing – review & editing. **Ludwig Birklbauer:** Writing – review & editing. **Martin Wallner:** Mineralogical Analysis. **Marianne Haberbauer:** Project administration, Supervision, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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