



## Influence of Seawater Addition on the Bioleaching Process of Chalcopyrite Ore Using *Acidithiobacillus ferrooxidans*

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**Abstract:** In this study, bioleaching experiments on Kastamonu Hanönü copper ore were conducted using the bacterium *Acidithiobacillus ferrooxidans* in the presence of seawater. The characterization of the sample was performed using XRD, XRF, and SEM analysis methods. During the bioleaching experiments, bacteria concentration, pH, copper and iron concentrations were monitored over the 360-hour test period. The results demonstrated that an appropriate proportion of seawater significantly promoted copper recovery, with the solid-to-liquid ratio playing a key role. A maximum copper recovery of 81.43% was achieved in the presence of 30.00% seawater, 7% solid rate compared to only 71.02% in its absence. This study highlights the potential of seawater as an alternative solvent medium, offering both environmental and economic benefits. Moreover, the findings emphasize the applicability of the bioleaching method as an environmentally friendly and efficient process.

**Keywords:** *Acidithiobacillus ferrooxidans*, bioleaching, copper ore, environmental sustainability, recovery, seawater.

## *Acidithiobacillus ferrooxidans* Kullanılarak Gerçekleştirilen Kalkopirit Cevherinin Biyolojik İşlemine Deniz Suyu İlavesinin Etkisi

**Öz:** Bu çalışmada, Kastamonu Hanönü bakır cevheri üzerinde, deniz suyu varlığında *Acidithiobacillus ferrooxidans* bakterisi kullanılarak biyolojik deneyleri gerçekleştirilmiştir. Numunenin karakterizasyonu XRD, XRF ve SEM analiz yöntemleri ile yapılmıştır. Biyolojik deneyleri sırasında, 360 saatlik test süresi boyunca bakteri konsantrasyonu, pH, bakır ve demir konsantrasyonları izlenmiştir. Sonuçlar, uygun bir deniz suyu oranının bakır geri kazanımını önemli ölçüde artırdığını ve katı-sıvı oranının bu süreçte kritik bir rol oynadığını göstermiştir. %30,00 deniz suyu varlığında ve %7 katı oranında, maksimum %81,43 bakır geri kazanımı elde edilirken, deniz suyu olmadan bu oran yalnızca %71,02 olarak gerçekleşmiştir. Bu çalışma, deniz suyunun hem çevresel hem de ekonomik faydalar sunan alternatif bir çözücü ortamı olarak potansiyelini vurgulamaktadır. Ayrıca, biyolojik yönteminin çevre dostu ve verimli bir süreç olarak uygulanabilirliği vurgulanmaktadır.

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**Anahtar kelimeler:** *Acidithiobacillus ferrooxidans*, bakır cevheri, biyolojik, çevresel sürdürülebilirlik, deniz suyu, geri kazanım.

### INTRODUCTION

In recent decades, the steady decline in ore grades and the depletion of easily recoverable metal resources have necessitated the utilization of low-grade reserves to satisfy the growing global demand for copper, emphasizing the importance of sustainable and efficient extraction techniques (Nkuna et al., 2022). Chalcopyrite is a copper-bearing sulfide ore and the most abundant copper mineral found worldwide (Owusu et al., 2014; Granata et al., 2019; Torres et al., 2020). Chalcopyrite has traditionally been processed

using conventional pyrometallurgical methods, which include flotation, smelting, refining (Baba et al., 2012). These methods allow for high metal recovery; however, the emission of SO<sub>2</sub> gas into the atmosphere, especially during the roasting and smelting stages, leads to environmental problems (Lopez Juarez et al., 2006). As a result, there is a growing need to explore more sustainable and environmentally friendly hydrometallurgical alternatives.

Bioleaching is an environmentally friendly hydrometallurgical method suited for processing low-grade ores compared to traditional mineral processing techniques

(Rathna & Nakkeeran, 2020). It involves the use of microorganisms to extract metals from low-grade ores by catalyzing the dissolution of metal sulfides (Akçıl & Çiftçi, 2006; Schippers et al., 2014). Among these microorganisms, *Acidithiobacillus ferrooxidans* is particularly notable for its ability to oxidize ferrous iron and sulfur compounds, thereby facilitating metal recovery (Valdés et al., 2008). Challenges like prolonged bioleaching durations and low efficiency remain to be resolved (Gu et al., 2018; Petersen, 2023). To address these challenges, various studies have been explored, including the use of specific additives to enhance copper recovery. Liang et al. (2010) investigated the effect of activated carbon on chalcopyrite bioleaching by *Acidianus manzaensis* YN25 at 65°C and reported a significant increase in copper yields from 64% to 95% at an optimum concentration of 2 g/L, attributed to galvanic interactions between activated carbon and chalcopyrite. Sulfur speciation analysis indicated no change in the sulfur-containing species but revealed an accelerated formation of them. Liang et al. (2012) found that the addition of sodium chloride during chalcopyrite bioleaching with *Acidianus manzaensis* YN-25 reduced elemental sulfur accumulation on the mineral surface, increasing copper ion concentration from 2.37 g/L to 2.67 g/L. Dong et al. (2013) studied the effects of quartz on chalcopyrite bioleaching by *Acidithiobacillus ferrooxidans* and found that quartz concentration increased copper extraction by approximately 20% at 50 g L<sup>-1</sup> after 32 days. XRD and SEM analyses suggested that quartz contributed to the reduction of jarosite formation on the chalcopyrite surface and enhanced copper extraction by eroding the chalcopyrite particles through mechanical friction. Zhang et al. (2016) investigated the effect of polyethylene glycol (PEG) on chalcopyrite bioleaching with *Acidithiobacillus ferrooxidans* and found that PEG significantly improved leaching efficiency by enhancing bacterial attachment and promoting sulfur oxidation. XRD, SEM, and XPS analyses revealed that PEG reduced the inhibitory elemental sulfur and jarosite on the chalcopyrite surface, accelerating the biooxidation process. Sajjad et al. (2020) studied the use of indigenous iron-oxidizing bacteria in combination with acid-treated bamboo sawdust (BSD) for copper recovery from low-grade ores. The study demonstrated that 2 g/L BSD significantly enhanced metal recovery, achieving up to 92.2% copper within 8 days, while also influencing bacterial community dynamics, iron speciation, and the bioleaching microenvironment.

Among the more than 100 elements identified worldwide, 80% are found in seawater (Atzori et al., 2019). Seawater, an abundant and readily available resource, is becoming an increasingly promising option for metal extraction (Yang et al., 2018). In line with the increasing significance of seawater, its applications have gained

growing importance in recent years. The chemical composition of seawater can influence metal solubility during leaching processes (Akakçe, 2023). Chen et al. (2020) examined bacterial diversity and dynamics during the bioleaching of copper sulfide ore with the addition of seawater, and found that 20% seawater improved copper recovery from 72.49% to 84.70%, while *Acidithiobacillus ferrooxidans* remained the dominant bacterial species throughout the bioleaching process. More recently, Huynh et al. (2024) isolated a novel halophilic-acidophilic iron-oxidizing bacterium (*Alicyclobacillus* sp. SO9) from a tailings-contaminated beach and demonstrated that, under high chloride concentrations and artificial seawater, this bacterium enhanced copper extraction from chalcopyrite, highlighting the potential of halophilic-acidophilic microorganisms in saline bioleaching environments. Gu et al. (2025) investigated chalcopyrite bioleaching with an enriched microbial community in acidified seawater. They reported that copper extraction was significantly enhanced (from 13.1% to 62.1%) due to acidification. The adaptability of the microbial community to a high-salt environment was also improved.

Based on the review of previous literature, reveals that additives such as activated carbon, sodium chloride, quartz, PEG, seawater, and bamboo sawdust have been effective in the bioleaching process of copper sulfide minerals. These studies have shown that these additives improve copper dissolution by reducing elemental sulfur formation, enhancing bacterial activity, or optimizing the redox potential. Despite its advantages, the use of seawater in copper extraction has not been sufficiently explored, particularly regarding its effects on the bioleaching of copper sulfide minerals; therefore, further research is needed to develop more comprehensive and sustainable strategies to enhance bioleaching efficiency. This study aims to evaluate the feasibility and effectiveness of using seawater as a solvent medium in the bioleaching of copper ores from the Kastamonu Hanönü region. Utilizing *Acidithiobacillus ferrooxidans*, the research focuses on key parameters such as bacterial concentration, pH, Cu concentration, and Fe concentration. By exploring the role of seawater, this study seeks to contribute to the development of sustainable and environmentally responsible mining practices.

## MATERIAL AND METHOD

**Characteristics of ore sample and seawater pretreatment:** The copper sulfide ore sample used in this study were obtained from a copper Mine, Hanönü, Kastamonu, Türkiye. The sample was ground to a particle size of less than 75 µm and then dried prior to being used in the experimental tests. The sample's chemical composition and mineralogical identification were analyzed using X-ray fluorescence spectrometry (XRF, Minipal 4 Panalytical), an

X-ray diffraction instrument (XRD, Panalytical Empyrean), and an SEM device (FEI Quanta 650 field-emission) respectively, at Çukurova University. The SEM micrographs were captured at 10000 magnification. The chemical composition analysis revealed that the Cu grade was 1.45%, with the main components of the copper ore being 31% SiO<sub>2</sub>, 16% Al<sub>2</sub>O<sub>3</sub>, 1.38% TiO<sub>2</sub>, 2.84% S, 4.46% K<sub>2</sub>O, 2.05% Ca, 20.96% Fe, and 0.18% Zn. The mineralogical analysis of the ore sample indicated that quartz, chalcopyrite, muscovite, clinocllore and pyrite, were the mineral phases, as shown in Figure 1.

In Figure 2, the presence of minerals such as quartz, muscovite, and clinocllore, consistent with SEM observations and XRD analysis results, indicates that silicate phases are dominant and influence the surface texture. EDX analysis of the identified thirteen elements, with the distribution as follows: 41.07% O, 18.76% C, 13.01% Si, 9.38% Al, 0.32% Ca, 1.19% S, 0.23% Cu, 0.65% Mg, 2.66% K, 0.39% Ti, 4.64% Fe, 6.44% Au and 1.25% Na. The detection of gold in EDX is due to the plating process. The inclusions observed in the SEM image can be explained by the separation of different phases, such as pyrite or quartz, within the microstructure as identified by XRD.

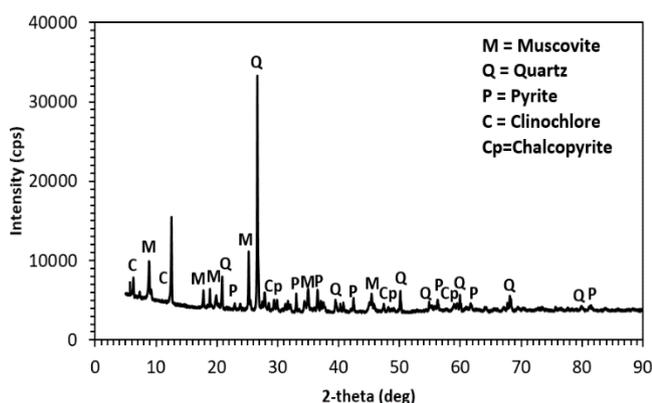


Figure 1. XRD pattern of the copper sulfide ore sample.

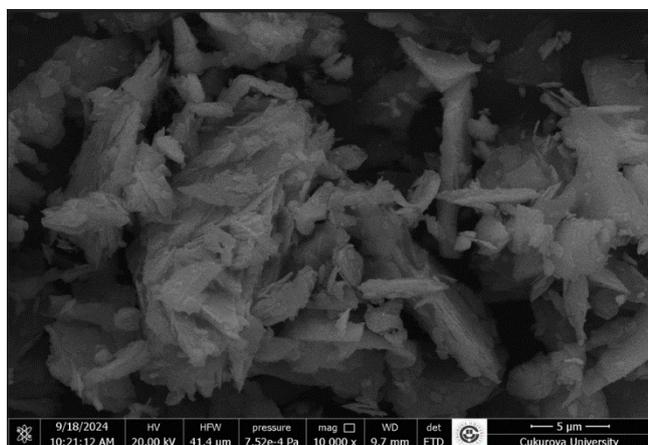


Figure 2. SEM analysis images of the copper sulfide ore sample.

Seawater used in this study was sourced from Erdemli Province, Mersin. To prevent the potential influence of unidentified bacteria in the seawater on the bioleaching

process, the seawater was subjected to high-temperature treatment prior to the copper ore bioleaching experiments. This preprocessing step involved heating the seawater in an autoclave at 121 °C for 15 minutes. After cooling, the treated seawater was used in the bioleaching experiments. The composition of the seawater is provided in Table 1.

Table 1: Composition of seawater used in the study.

Component	Concentration (g/L)
Cl <sup>-</sup>	2.39
Na <sup>+</sup>	1.22
Mg <sup>2+</sup>	0.53
Ca <sup>2+</sup>	0.36
K <sup>+</sup>	0.06
SO <sub>4</sub> <sup>2-</sup>	0.49

**Bioleaching experiments:** *Acidithiobacillus ferrooxidans* (DSM 583) was procured from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). The growth 9 K medium included the following components: (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> at 0.4 g/L, KH<sub>2</sub>PO<sub>4</sub> at 0.2 g/L, MgSO<sub>4</sub>·7H<sub>2</sub>O at 0.4 g/L, KCl at 0.1 g/L and FeSO<sub>4</sub>·7H<sub>2</sub>O at 55.6 g/L based on Akçıl and Çiftçi (2003) and Demirci and Bayat (2017). The bacteria were inoculated into flasks containing 9K medium. The optimal growth conditions for this bacterium were determined as a temperature of 30 °C, an initial pH of 2 (adjusted with dilute H<sub>2</sub>SO<sub>4</sub>), and agitation at 120 rpm during laboratory-scale culture experiments. Consequently, the bacterial concentration reached 7.6×10<sup>7</sup> cells/mL at the start of the bioleaching experiments. The bioleaching experiment was conducted under various experimental conditions outlined in Table 2.

Table 2: Experimental conditions for bioleaching experiment.

Experiment no	Seawater (%/mL)	Solid ratio (%)
SA-0	0.00/0.00	5
SA-1	10.00/15.00	5
SA-2	20.00/30.00	5
SA-3	30.00/45.00	5
SA-4	40.00/60.00	5
SB-0	0.00/0.00	7
SB-1	10.00/15.00	7
SB-2	20.00/30.00	7
SB-3	30.00/45.00	7
SB-4	40.00/60.00	7

The tests were divided into two groups, SA and SB, each with varying seawater concentrations and solid-to-liquid ratios. For the SA series (SA-0 to SA-4), the solid ratio was kept constant at 5%. Seawater concentrations were adjusted progressively, starting from 0.00% (0.00 mL) in SA-0 and increasing to 40.00% (60.00 mL) in SA-4. In these experiments, the medium consisted of a 0 k solution with decreasing volumes from 130.00 mL in SA-0 to 70.00 mL in SA-4, supplemented with 20.00 mL of bacteria. Similarly, the SB series (SB-0 to SB-4) had a higher solid ratio of 7%, with seawater concentrations following the same progression, from 0.00% (0.00 mL) in SB-0 to 40.00% (60.00 mL) in SB-4. The 0 k solution volume in the medium was decreased accordingly, starting at 130.00 mL for SB-0 and reducing to 70.00 mL for SB-4, while the bacteria volume remained consistent at 20.00 mL in all experiments. In all experiments, the volume of 0 k medium was adjusted

based on the amount of seawater added to maintain total liquid volume constant (150.00 mL). This design facilitated a systematic analysis of the influence of seawater and solid-to-liquid ratios on bioleaching efficiency. Since chalcopyrite samples are rich in Fe, which can provide sufficient energy for bacterial growth, the culture medium herein referred to as 0 K medium (i.e., 9 K medium without  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) containing  $(\text{NH}_4)_2\text{SO}_4$  at 0.4 g/L,  $\text{KH}_2\text{PO}_4$  at 0.2 g/L,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  at 0.4 g/L, KCl at 0.1 g/L was used in the bioleaching experiments in this study (Chen et al., 2020). The experiments were conducted under identical initial conditions in 250 mL flasks. The pH of the leach solution was adjusted to 2.00 by adding dilute  $\text{H}_2\text{SO}_4$ . Subsequently, the flasks were incubated at a temperature of 30 °C and agitated at 120 rpm. Parameters such as pH,  $\text{Cu}^{2+}$  concentration, and bacterial concentration were monitored throughout the experiments.

In this experimental study, the concentrations of  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES). The concentrations of copper and iron in the leach solution were analyzed using a PerkinElmer PinAAcle 900H Atomic Absorption Spectrometer (AAS). After microbial growth processes were completed, bacterial counting was performed on bacterial solutions using a Nikon YS-100 model biological microscope with a Petroff-Hausser counting chamber. The pH value was measured with a pH meter (WTW S-720). The residue ores were analyzed using X-ray diffraction instrument (Panalytical Empyrean) after bioleaching experiments. All experiments were carried out in triplicate. The results are presented as mean values with standard deviations (SD). Error bars in the figures represent the standard deviations of triplicate measurements ( $n=3$ ).

## RESULTS AND DISCUSSION

The changes in parameters (bacteria concentration, pH, copper and iron extractions) during the bioleaching of copper sulfide ore with seawater are shown in Fig. 3(a)–(h), respectively. The maximum copper recoveries for samples SA-0 to SA-4 were 70.19%, 72.44%, 75.19%, 77.84%, and 62.12%, respectively, while iron recoveries were 40.19%, 43.12%, 45.67%, 52.15%, and 34.86% (Figures 3(a) and 3(c)). For samples SB-0 to SB-4, the maximum copper recoveries were 71.02%, 73.68%, 78.86%, 81.43%, and 65.67%, while iron recoveries were 44.23%, 47.62%, 54.78%, 57.58%, and 41.19%, respectively (Figures 3(b) and 3(d)). Under the presence of seawater at a 5% solid ratio, a maximum copper recovery of 77.84% was achieved, compared to 70.19% in sample SA-0 without seawater. Similarly, at a 7% solid ratio with seawater, the maximum copper recovery reached 81.43%, while it was 71.02% in sample SB-0 without seawater. These results suggest that the addition of seawater at an appropriate ratio can enhance the

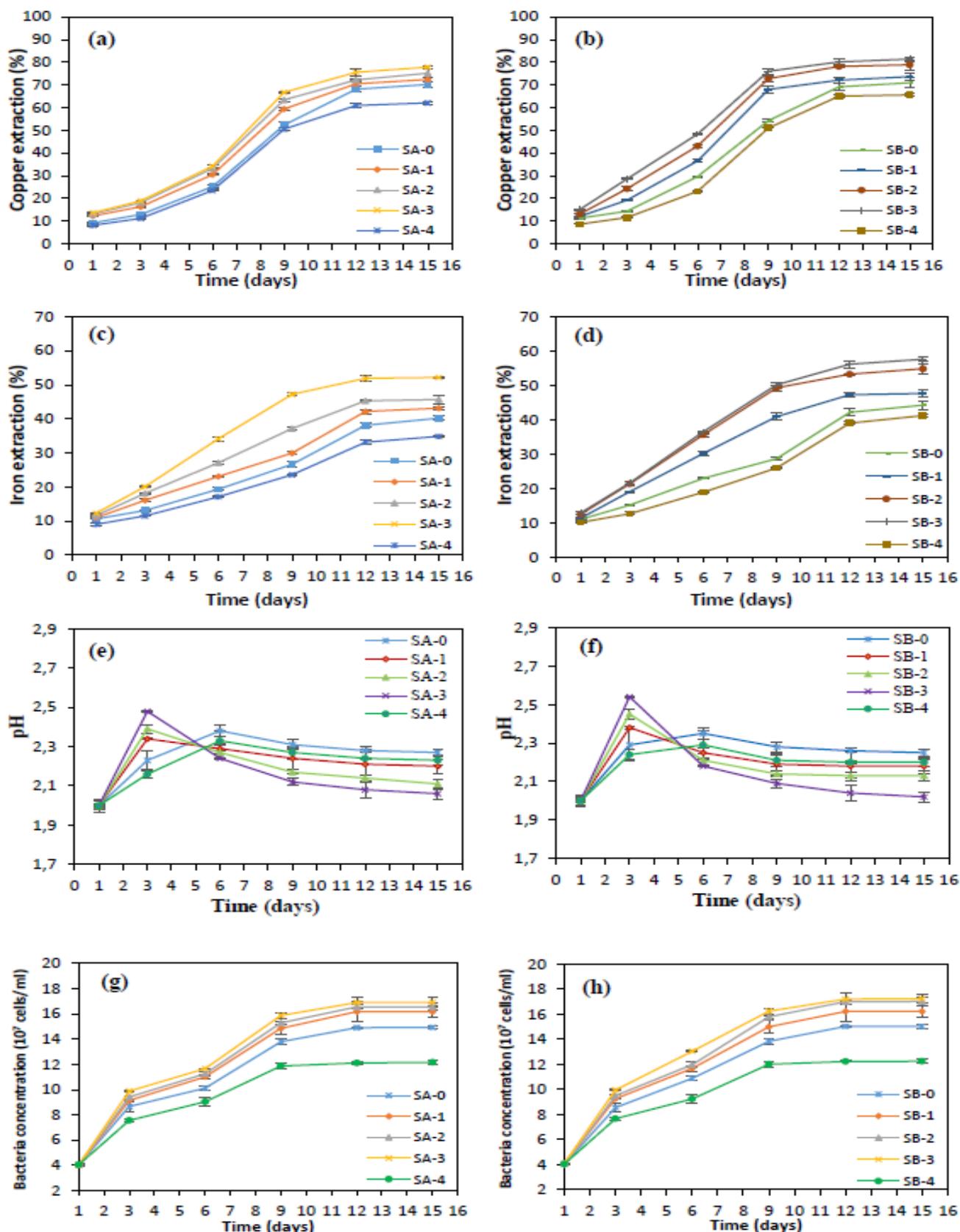
efficiency of bioleaching. A study on polymetallic sulfidic mining residues demonstrated that a gradual increase in solid content up to 20% (w/w) allowed microbial adaptation and enabled efficient metal solubilization. However, at a solid concentration of 30%, biological activity was significantly reduced (Joulian et al., 2023). This highlights the importance of optimizing solid concentrations for effective bioleaching processes.

As shown in Figure 3(e), the pH initially increased and then decreased. The maximum pH values of the samples from SA-0 to SA-4 were 2.38, 2.34, 2.39, 2.48, and 2.33, respectively. For the SB-0 to SB-4 samples, the maximum pH values were 2.35, 2.38, 2.45, 2.54, and 2.29, respectively (Figure 3(f)). The increase in pH during the early stage of the bioleaching of low-grade copper sulfide ore may be attributed to the consumption of  $\text{H}^+$  ions, as oxides present in the bioleaching system could react with  $\text{H}^+$  (Chen et al., 2020). Compared with the sample SA-0, which contained no seawater, the samples SA-3 and SB-3 reached their peak pH values more rapidly, indicating that the addition of an appropriate amount of seawater accelerated the rise in pH. Meanwhile, lower pH values were observed in samples SA-3 and SB-3, in which higher  $\text{Cu}^{2+}$  concentrations were produced (Figure 3(e)–(f)). According to a previous study, a decrease in the initial pH enhanced chalcopyrite dissolution (Vilcáez et al., 2009). Thus, the observed pH reduction in this study could have contributed to the enhanced copper extraction from low-grade copper sulfide ores.

As shown in Figure 3(g), the maximum bacterial concentrations for samples SA-0 to SA-4 were  $14.92 \times 10^7$  cells/mL,  $16.18 \times 10^7$  cells/mL,  $16.56 \times 10^7$  cells/mL,  $16.91 \times 10^7$  cells/mL, and  $12.15 \times 10^7$  cells/mL, respectively. For samples SB-0 to SB-4, the maximum bacterial concentrations were  $15.02 \times 10^7$  cells/mL,  $16.21 \times 10^7$  cells/mL,  $17.01 \times 10^7$  cells/mL,  $17.23 \times 10^7$  cells/mL, and  $12.24 \times 10^7$  cells/mL, respectively (Figure 3(h)). Under relatively high seawater conditions, the SB-3 sample reached the highest bacterial concentration of  $17.23 \times 10^7$  cells/mL, which was  $2.21 \times 10^7$  cells/mL higher than that of SB-0 (Figure 3(h)). These findings suggest that, under the given experimental conditions, the addition of seawater at the specified proportion can be concluded to have had a significantly positive effect on bacterial concentration in this study. The reproducibility of these results was confirmed by the triplicate experiments, and the variations are supported by error bars representing the standard deviations, indicating the reliability of the data.

Figure 4 shows the XRD patterns of residual copper sulfide ores after bioleaching experiments at solid concentrations of (a) 5% and (b) 7%. The observed decrease in the intensity of pyrite ( $\text{FeS}_2$ ) and chalcopyrite ( $\text{CuFeS}_2$ ) peaks is consistent with the copper and iron extraction

presented in Figure 3. This suggests effective dissolution of these minerals during the bioleaching process.



**Figure 3.** Effect of seawater on the bioleaching of copper sulfide ore samples. (a–b) Copper extraction at 5% and 7% solid concentrations. (c–d) Iron extraction at 5% and 7% solid concentrations. (e–f) pH variation at 5% and 7% solid concentrations. (g–h) Bacterial concentration at 5% and 7% solid concentrations. Error bars represent the standard deviations of triplicate experiments (n = 3).

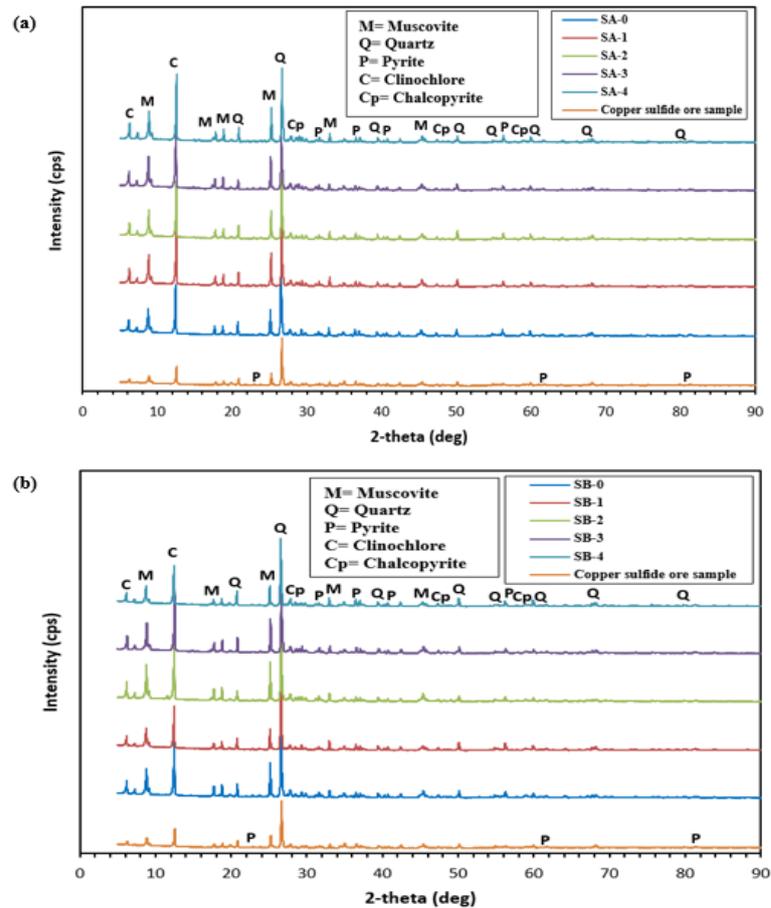


Figure 4. XRD patterns of residual copper sulfide ores after bioleaching experiments at (a) 5% and (b) 7% solid concentrations.

## CONCLUSION

In this study, the bioleaching of chalcopyrite ore from the Kastamonu Hanönü region was investigated using *Acidithiobacillus ferrooxidans* in the presence of seawater. The results demonstrated that seawater significantly enhances copper recovery, with a maximum recovery of 81.43% achieved at a 30% seawater concentration, 7% solid rate compared to 71.02% in the absence of seawater. This highlights the potential of seawater as an alternative solvent medium in bioleaching processes, offering both environmental and economic benefits. The solid-to-liquid ratio was also found to play a critical role in optimizing copper recovery. The findings of this study contribute to the growing body of research on sustainable and environmentally friendly mining practices. By utilizing seawater, which is an abundant and readily available resource, the mining industry can reduce its reliance on freshwater and mitigate the environmental impact associated with traditional extraction methods. Furthermore, the use of *Acidithiobacillus ferrooxidans* in bioleaching processes aligns with the principles of green chemistry, as it minimizes the release of harmful emissions and reduces the need for energy-intensive pyrometallurgical processes. However, several challenges remain to be addressed. The prolonged duration of

bioleaching and the need for optimal conditions, such as pH, temperature, and bacterial concentration, require further investigation. Future studies should focus on optimizing these parameters to enhance the efficiency of the bioleaching process. Additionally, the potential effects of other seawater components, such as magnesium and calcium ions, on the bioleaching process should be explored to better understand their impact on metal recovery. In conclusion, this study underscores the potential of seawater as a viable medium for bioleaching copper ores, offering a sustainable alternative to conventional extraction methods. By continuing to explore and refine bioleaching techniques, the mining industry can move towards more environmentally responsible practices, ensuring the efficient recovery of valuable metals while minimizing ecological impact.

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**Author contribution:** The entire process of the article was carried out by the corresponding author.

**Conflicts of interest:** The author declares that no conflict of interest.

**Ethical approval:** This study was conducted within the scope of a research that did not require ethical approval.

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