NON-FERROUS METAL BIOLEACHING FROM COPPER SLAG WITH A SPENT MEDIUM OF *SPOROSARCINA UREAE*

Plamen Georgiev, Marina Nicolova, Irena Spasova

University of Mining and Geology "St. Ivan Rilski", 1700 Sofia; E-mail: ps_georgiev@mgu.bg
Corresponding author: ps_georgiev@mgu.bg

ABSTRACT. The aim of this study is the indirect bioleaching of non-ferrous metals from pyrometallurgical copper slag containing 0.36% Cu, 1.93% Zn, and 0.09% Co using a spent culture medium from the bacterial strain *Sporosarcina ureae* CCM 981 with a pH of 9.43. Bioleaching was performed using the flask shaking technique at 150 rpm for 72 hours, yielding the best results for the extraction of copper (21.5%), zinc (21.2%), and cobalt (41.3%) at 55 °C and a pulp density of 7.5%. Iron and silicon co-leached, resulting in selectivity values of 0.307 and 0.98, respectively, for non-ferrous metal extraction from the copper slag sample under these experimental conditions.

Key words: bioleaching, non-ferrous metal, copper slag, selectivity

Introduction

The higher demand for base metals and some critical raw materials over the past few decades, driven by sectors such as IT and green transition technologies, has led to annual growth of 2.6% in copper pyrometallurgy and to worldwide annual copper slag generation of 37.7 million tons (Phiri et al., 2022). Apart from copper, copper slags also contain valuable metals, such as cobalt and nickel. Therefore, copper slag is regarded as a raw material whose processing and extraction are tools in the circular economy (Whitworth et al., 2022).

Base metals occur in different phases in copper slag, including sulphides, zero-valence states, oxides, and those absorbed or encapsulated in the crystal lattices of main minerals, such as fayalite, silicates, or magnetite (Deng & Ling, 2007; Prince et al, 2016; Dzinomwa et al., 2023). Therefore, classical methods of mineral processing, such as flotation and pyrometallurgy, are insufficient due to copper recovery of around 50% of the total copper content, the loss of cobalt and nickel in the generated flotation tailings, and higher operational costs for energy and reagents.

Another approach to recover base metals from copper is (bio)hydrometallurgy, which offers the main advantages of operating at ambient temperature and pressure and of generating / recycling lixiviants for the leaching process through microbial growth (Weng et al., 2025; Abraham and Shopf, 2026). A suitable group is the chemolithotrophic bacteria with sulphur-oxidising/ ferrous-oxidising abilities, related to the species *Acidithiobacillus thiooxidans*, *A. ferrooxidans*, and *Leptospirillum ferrooxidans*, which grow at highly acidic pH levels, thereby enhancing efficient copper and other base metal extraction (Mikoda et al., 2018; Watkin et al., 2024), as well as intensive co-leaching of silicon and iron, as the main drawback.

Heterotrophic microorganisms also participate in mineral transformations, especially at pH levels ranging from slightly acidic to alkaline. In that case, several bioleaching mechanisms, including acidolysis, complexolysis, redoxolysis, biosorption, bioaccumulation, and alkalolysis, driven by heterotrophic microorganisms, participate in the bioleaching of base metals (Kumar et al., 2023). Each microbial species with heterotrophic metabolism utilises a narrow range of the mechanisms mentioned above.

This study aims to recover base metals from a copper slag sample containing 0.36% Cu, 1.93% Zn, and 0.09% Co via indirect bioleaching using the spent medium of the bacterial strain *Sporosarcina ureae* CCM 981 at a slightly alkaline pH.

Materials and methods

Pyrometallurgical copper slag

The copper slag sample containing 1.93 % Zn, 0.36 % Cu, and 0.09 % Co as valuable elements and 27.2 % iron and 15.9 % Si (Table 1) was subjected to grinding to particle size – 75 μm to + 25 μm. In that form, the copper slag was used in bioleaching experiments.

Table 1. *Chemical content of the copper slag sample used in this study*

Chemical element, %	Value
Fe	27.2
Si	15.9
Ca	6.9
Al	2.78
Cu	0.36
Zn	1.93
Co	0.09
Mn	0.8
P	0.08
S	0.91
pH (H_2O)	7.95

Characterisation of the copper slag

The mineralogical composition of copper slag, which was carried out by X-ray Diffraction analyser EMPYREAN using Cu-Kα radiation and operated at 40 kV and 30 Ma, revealed the presence of compounds with the phase structures of protomangano-ferroanthophyllite ($H_2Fe_{4.48}Mg_{1.08}Mn_{1.44}O_{24}Si_8$), fayalite ($Fe_2SiO_4$) and an unidentified type of pyroxene ($Ca_{0.949}Fe_1Na_{0.051}O_6Si_2$) with content of 56.8 %, 33.2 %, and 10.0 %, respectively. The pH of the copper slag was determined

in distilled water after 1 hour of agitation with an overhead mixer at 150 rpm.

The chemical leaching of non-ferrous metals was studied over a pH range from slightly acidic to alkaline by adding sulphuric acid or ammonia solution to the prepared pulp, with agitation at 300 rpm and 25 °C. The acid/ base addition stopped when the pulp's pH reached the targeted value (6.0, 5.0, or 9.0, 10.0) and remained constant for 6 hours. The pulp sample was collected and centrifuged, and by AAS-ICP methods, the concentration of the elements in the transparent solution was determined.

Bacterial culture and maintenance

Sporosarcina ureae CCM 981 (hereinafter referred to as *S. ureae*) was obtained from the Czech Collection of Microorganisms at the Department of Experimental Biology at Masaryk University. The strain was maintained in a medium suitable for *Sporosarcina* spp. and stored at 4 °C as needed. The medium was inoculated with 1 mL bacterial suspension/ 100 ml and incubated for 7 days at 35 °C under continuous shaking (150 rpm). The duration was the only variable; all other parameters were constant across experiments.

Indirect biological leaching of copper slag

The indirect technique was carried out in two steps. The first step was initiated by inoculating the nutrient medium with the following content: peptone (20 g/ L), K_2HPO_4 (0.75 g/ L), $MgSO_4.7H_2O$ (0.3 g/ L), urea (20.0 g/ L), yeast extract (2.0 g/ L), with 1 mL of bacterial suspension. The flasks were incubated for 7 days at 35 °C under continuous shaking at 150 rpm. Water loss due to evaporation was compensated for by adding distilled water. After the 7th day, the bacterial biomass was separated from the spent medium by centrifugation at 10,000 rpm for 15 minutes. The second step of indirect bioleaching of non-ferrous metals from copper slag was carried out using a transparent, microbial-free spent medium produced under continuous shaking at 150 rpm for 3 days.

Alkalinity, the concentration of NH_4^+ and TOC of the obtained spent medium of *Sporosarcina ureae* were determined by standard analytical methods (APHA, 1995), such as acidic titration with 0.1 N HCl to a pH of 8.3, salicylate colourimetric method, and standard method 5310D (at a temperature of 200°C in a highly acidic pH and in the presence of strong chemical oxidants such as P_2O_5 and CrO_3), respectively.

The effect of leaching duration (1-4 days), pulp density (7.5, 10.0, or 12.5 %), and temperature (35 °C, 55 °C, or 75 °C) on the efficiency and selectivity of copper, zinc, and cobalt recovery was studied. Monitoring of pH and leaching of non-ferrous metals was carried out daily.

All experiments were performed in triplicate, and the results are reported as mean values.

The efficiency of metals (non-ferrous metals and iron) and silicon extraction from the copper slag sample under relevant experimental conditions (Er) was calculated according to the following formula:

$$Er = \frac{C1 - C2}{C1} \times 100, \% \quad (1)$$

where Er-element recovery, %; C_2-metal concentration in the copper slag residue, mg/ kg; C_1-metal concentration in the raw copper slag, mg/ kg.

Selectivity coefficient (S) of the non-ferrous leaching from the copper slag sample at the relevant experimental conditions was calculated according to the following formula:

$$\boldsymbol{S} = \frac{\sum(CCu + CZn + CCo)}{(\mathrm{Cse})} \quad (2)$$

where S – selectivity coefficient; $\sum$ (C_{Cu} +C_{Zn} + C_{Co}) - sum of copper, zinc, and cobalt concentrations in the pregnant leach solution, mg/L; C_{SE} - concentration of structural element (iron or silicon) in the pregnant leach solution, mg/ L.

Results and discussion

Non-ferrous metal chemical leaching from copper slag

The studied copper slag sample had a slightly alkaline pH of 7.95 (Table 1). The chemical leaching of non-ferrous metals at slightly acidic/slightly alkaline pH was chosen due to the higher content of silicate minerals in the raw material and the significant effect of certain microbial groups on the microenvironmental pH in the presence of suitable substrates for their growth (Rezza et al., 2001).

The chemical leaching of non-ferrous metals from the copper slag sample with ammonia was studied at a slightly alkaline pH (8-11). Biological processes, such as the bio-oxidation of organic acids and the ammonification of nitrogen-enriched organic compounds, carried out by heterotrophic microorganisms, consume free hydrogen ions, thereby shifting the pulp's pH toward alkalinity. It enhances the degradation of silicate minerals and the recovery of valuable elements from copper slag by liberating their cations and forming soluble complexes with ammonia ions (Rezza et al., 2001). Under such conditions, the amount of non-ferrous metals recovered by chemical leaching with ammonium ions ranges from 2.5 % for cobalt to 0.5 % for copper, which is insignificant compared to the results of chemical leaching at a slightly acidic pH (4-6) (Figure 1).

Biological processes of sulphide oxidation and oxidation of suitable organic compounds (monosaccharides/ alcohol), carried out by chemolithotrophic and heterotrophic bacteria, respectively, produce sulphuric acid or weak organic acids. Those compounds are sources of free hydrogen ions, turning the pH from slightly alkaline to acidic. Non-ferrous metal leaching from the copper slag sample under these conditions was simulated using sulphuric acid leaching. In that case, the minimum amount of sulphuric acid was needed to drop the native pH of the raw copper slag sample to a slightly acidic pH of 5.0 (Figure 1).

This results in the substantial leaching of non-ferrous metals, which varies between 19.8 % recovery for zinc and 14.2 % of copper, as well as substantial co-leaching of silicon and iron; 13.2% and 19.3 %, respectively. The higher content of protomangano-ferroanthophyllite in the copper slag sample indicated that weathering occurred during storage of the slag on the dump for more than sixty years. Weathering involves the sequential alteration of the initially deposited copper slag mass from larger grains with lower surface area to smaller grains with higher surface area, and changes in their chemical content due to leaching, co-precipitation, and adsorption. Therefore, non-ferrous metal leaching strongly depended on the leaching of iron and silicon, the main structural elements in the raw sample and in the minerals that contain them.

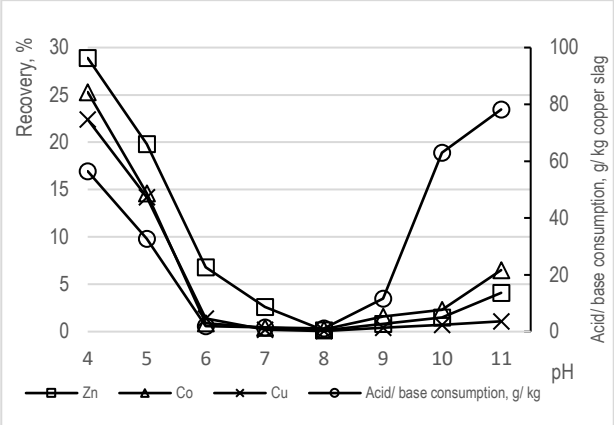


Figure 1. Acid/ base consumption of the copper slag sample and its effect on the non-ferrous metal recovery in dependence on the target pH

Characteristics of the spent medium of *Sporosarcina ureae*

S. ureae is a heterotrophic and mesophilic bacterium which gains energy using oxygen as a final acceptor of electrons. It is typical in soil and manure, biotopes enriched with nitrogen-containing organic compounds (Coelho et al., 2022). The *S. ureae* strain used in this study was chosen for its ability to produce the enzyme urease, converting urea into ammonia and carbon dioxide at a higher rate according to the following reaction:

$$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2 \quad (3)$$

Urease is an inducible type of enzyme whose synthesis and regulation are strongly dependent on the urea content in the nutrient medium. Its synthesis results in faster microbial growth, a more negative surface charge, and higher biomineralisation ability (Ma et al., 2020).

Ammonia is a compound that dissolves easily in water and transforms into an ammonia ion:

$$NH_3 + H_2O \rightarrow NH_4^+ + OH^- \quad (4)$$

Because of its low toxicity and cost, ease of recovery, and higher selectivity, ammonium has been recognised as an effective leaching agent in hydrometallurgy. Therefore, it is highly used in the recovery of copper from a wide range of raw materials such as ore and concentrate (Radmehr et al., 2014), fly ash (Chiang et al., 2013; Ramanathan and Peng, 2016), slag (Wilcher and Bosecker, 2003; Chiang et al., 2013), sulphide tailings (Yin et al., 2018), and sewage sludge (Wang et al., 2024).

The spent medium obtained after a week of cultivation of *S. ureae* on the nutrient media, is characterised by a slightly alkaline pH, higher alkalinity, and ammonia ions and low-molecular-weight organic compounds, mainly organic anions, with contents exceeding 1.0 g/L (Table 2). Therefore, the chemical composition of the spent medium is more complex than that of the ammonia-based solution used for chemical leaching of copper slag at alkaline pH. Their effect on base metal recovery from raw materials is well recognised (Chiang et al., 2013). *S. ureae* has been widely studied recently because of its role in the microbial-induced carbonate precipitation process (MICP).

Table 2. *Spent medium of Sporosarcina ureae obtained after a week of cultivation*

Index	Value
pH	9.43
Alkalinity, g/ L	11.7
NH_4^+, g/ L	1.05
Total organic carbon, g/ L	1.73

This process could be applied to the practice for bioremediation of ecosystems situated around the abandoned mine sites and affected by acidic effluents and heavy metal ions, such as Cu, Cd, Cr(III), or Zn (Kang et al., 2016; Duarte-Nass et al., 2020; Huang et al., 2022), as well as to enhance the recovery of some critical metals, as lithium, due to the removal of alkaline earth metals, such as calcium and magnesium (Rehmanji et al., 2025). The concentration of carbonate ions produced, as well as the architecture and surface charge of the microbial biofilm, determined the efficiency of heavy metal ion removal via carbonate precipitation and reduced the biotope's ecotoxicity towards typical soil inhabitants (Yee and Fein, 2001; Ma et al., 2020).

Indirect bioleaching of copper slag with the spent medium of *Sporosarcina ureae*

Effect of the duration of the leaching process

The indirect leaching of copper slag at 7.5 % pulp density using the spent medium of *S. ureae* was a promising technique for the recovery of the easily leachable fractions of non-ferrous metal. The experiment revealed that the optimal duration of the process was three days, with recovered amounts of zinc, copper, and cobalt reaching 19.9%, 15.1%, and 39.6%, respectively. The higher cobalt extraction was due to the relatively low content of the critical element and its tendency to accumulate in micro-zones between copper slag particles, thereby enhancing its recovery through desorption (Deng and Ling, 2007).

The formation of soluble copper-ammonium complexes at alkaline pH enhanced the base metal recovery:

$$Cu^{2+} + 4(NH_4)^+ \rightarrow [Cu(NH_3)_4]^{2+} + 4H^+ \quad (5)$$

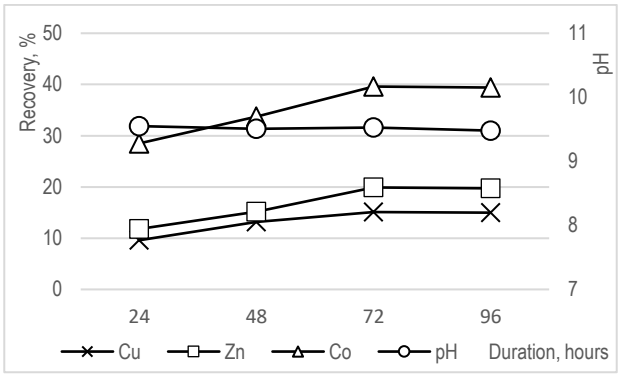


Figure 2. Effect of duration of experiment on the indirect bioleaching of non-ferrous metals from copper slag with spent medium of *S. ureae* at 7.5 % pulp density and at 35 °C

However, the higher base metal content and the fact that approximately 25% of that content remained as a phase-leachable fraction only after an oxidising process significantly

limited the recovery of copper (Georgiev et al., 2025a). Due to sorption, the concentration of base metals in the pregnant solution slightly declined after the third day of leaching.

The main processes involved in base metal leaching were acidolysis, desorption of the metals from the mineral surface, complexolysis of released cations with suitable ligands (organic anions and ammonium cations) in the spent medium, and alteration of the mineral surface due to enhanced co-leaching (Whitworth et al., 2022).

The indirect bioleaching of non-ferrous metals is carried out in two phases, each with its own recovery efficiency (Figure 2). The first phase began at the start of leaching and lasted for the first 24 hours. During that period, approximately 70% of the total leached amounts of copper, zinc, and cobalt were leached efficiently under these conditions. It relates to the amounts of base metals that desorb easily from mineral surfaces into the pregnant solution. However, the recovered amounts of non-ferrous metals for the first 24 hours were more than 10 times higher than the amounts recovered by chemical leaching at alkaline pH. This demonstrated the positive effect of dissolved organic compounds, particularly organic acids and their respective anions in the spent medium, on enhancing the extraction of non-ferrous metals by facilitating their desorption from the mineral surface and promoting the formation of soluble complexes.

The second phase of indirect leaching lasted from the second day until the end of the experiment, with the extraction of non-ferrous metals increasing by an additional 20–30%. It relates to the amounts of base metals leached due to iron and silicon co-leaching and weathering of the minerals` structure. Therefore, the copper slag decreased in weight by 2.7% at the end of the experiment.

The efficiency of microbial leaching of non-ferrous metals from copper slag depends directly on the type and concentration of microbial metabolites produced, as well as on each strain's adaptation to environmental conditions. For example, acidophilic chemoautotrophic bacteria not only oxidise and dissolve the sulphides of non-ferrous metals but also produce sulphuric acid, which, through acidolysis, dissolves their oxide minerals in the mineral feedstock. For this reason, as well as due to this group of bacteria's adaptations to highly acidic pH and high concentrations of non-ferrous metals in the pregnant solution, the efficiency of non-ferrous metal recovery usually exceeds 65% (Georgiev et al., 2017). This is why this group of microorganisms has been used for decades in the copper recovery from low-grade ores. A major drawback of this approach is the low selectivity of sulphuric acid as a leaching agent, leading to high concentrations of iron and, in some cases, silicon and calcium in the leachate.

Fungi, particularly strains of *Aspergillus niger*, are the most promising among heterotrophic microorganisms. The reason for this is that they are natural producers of citric acid, a natural organic compound with the strongest acidic properties and excellent complex-forming ability. For example, indirect biological leaching using spent culture medium of *Aspergillus niger* occurred at a slightly acidic pH (4.4), with non-ferrous metal recovery ranging from 22.5 to 44.4% (Georgiev et al., 2025a).

Heterotrophic bacteria play a role in the leaching of non-ferrous metals at neutral pH. For example, the indirect leaching of non-ferrous metals from copper slag using a production solution formed by the growth of the silicate bacterium *Bacillus megatherium* in a nitrogen-free medium but with nitrogen-fixing ability ranged from 11.8 to 16.4% (Georgiev et al., 2025b).

Effect of pulp density

The recovery of non-ferrous metals from raw material above a certain pulp density drops significantly, regardless of the leaching technique used. Factors, such as higher concentration of toxic metals in solutions, which resulted in the increased toxicity to microorganisms used in bioleaching; accumulation of ions in the pregnant leach solution, which hinders their further mass transfer from the solid sample; inadequate pulp mixing and insufficient oxygen transfer from the atmosphere into solution; increased shear stress on the microbial cell wall, which increases at higher values of agitation rate, either individually or in combination, have a negative effect on the recovery of non-ferrous metals when a direct leaching technique is applied (Gu et al., 2018).

The effect of pulp density during the indirect bioleaching of a copper slag sample using the spent medium from *S. ureae* showed that the extraction of copper, zinc, and cobalt decreases significantly when the pulp density is increased from 7.5% to 12.5% (Figure 3). For example, the extraction of copper and cobalt decreased from 15.1% to 9.9% and from 39.6% to 26.1%, respectively. However, the mass balance analysis showed that the concentrations of non-ferrous metals extracted remained nearly constant across different pulp densities. This revealed that the main factor limiting the improved non-ferrous metal recovery under the applied experimental conditions is the initial lixiviant concentration in the spent medium of *S. ureae*.

This process drawback can be overcome by applying two distinct approaches. One option is to increase the initial concentrations of lixiviants (such as ammonium and organic anions) by adding suitable reagents to the freshly obtained spent medium. Another option is to apply a multi-stage indirect bioleaching process, in which recovery of non-ferrous metals from the raw material is carried out in a series of reactors, with different batches of spent medium solution used in each reactor. However, the better recovery of non-ferrous metals will undoubtedly come at higher capital and operating costs.

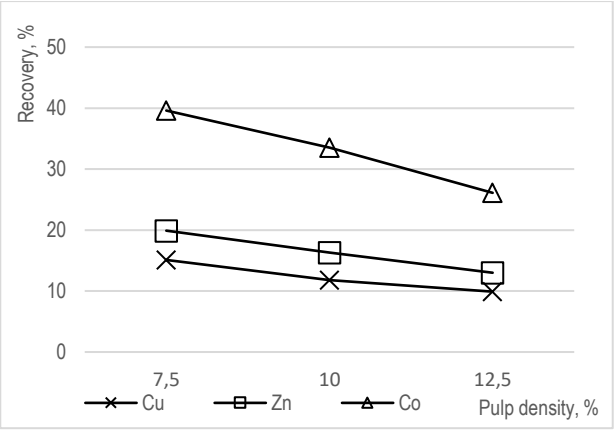


Figure 3. Effect of pulp density on the indirect bioleaching of non-ferrous metals from copper slag with spent medium of *S. ureae* for 72 hours and at 35 °C

Effect of the temperature

The main advantage of the indirect method of raw material leaching is that it is a microbial cell-free approach that can be applied at temperatures significantly above the optimal growth temperature of the relevant microbial strain. Therefore,

increasing the leaching temperature from 35 °C to 55 °C resulted in a slight increase by 1.3-1.7 % in the recovery of zinc and cobalt (Figure 4). The higher temperature had a more pronounced positive effect on copper leaching, with recovery increasing by 6.4%. However, a further increase in temperature during indirect leaching to 75 °C resulted in a significant drop in the recovery of non-ferrous metals (reaching 13.7% for zinc and 27.4% for cobalt) due to partial chemical oxidation, iron precipitation, and secondary sorption of already leached base metals.

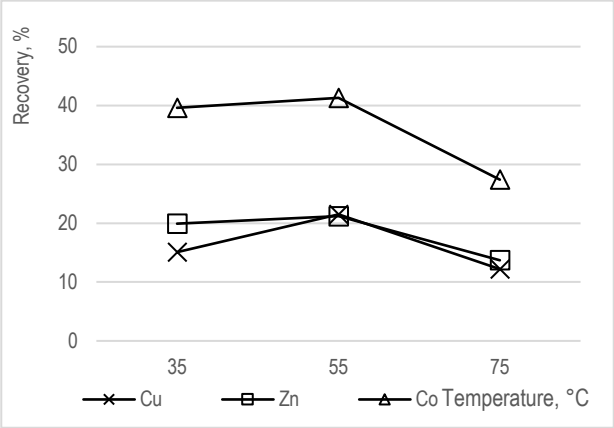


Figure 4. Effect of temperature on the indirect bioleaching of non-ferrous metals from copper slag with spent medium of *S. ureae* for 72 hours and at 7.5 % pulp density

Effect of the temperature on the selectivity of base metals leaching

The effect of temperature on the leaching of base metals from the studied copper slag sample was closely related to the leaching of silicon and iron, the main structural elements of the raw sample, and to changes in their concentrations in the pregnant leach solutions. Interrelations between those processes were characterised by the calculated selectivity coefficient values under relevant experimental conditions. Graphs presented in Figure 5 show that the indirect leaching of non-ferrous metals from copper slag with a spent medium of *S. ureae* was more selective than their alternative leaching with a spent medium generated from fungal cultivation and enriched with organic acids, such as citric and oxalic (Georgiev et al., 2025a).

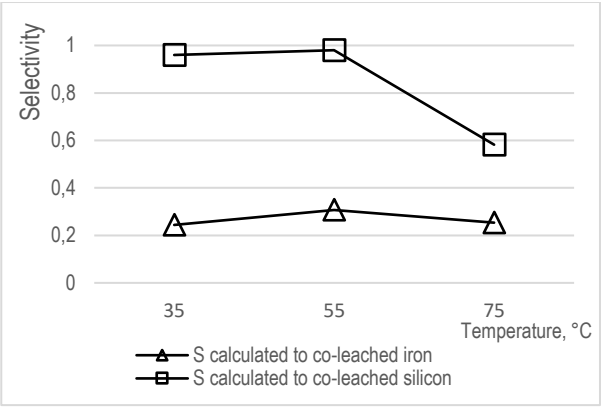


Figure 5. Effect of temperature on the selectivity of non-ferrous metals, indirect bioleaching with the spent medium of *S. ureae* from copper slag at 7.5 % pulp density

Despite the slightly alkaline pH at which the process occurred, a significant concentration of ferrous iron accumulated in the pregnant leach solution. It resulted in an S value of 0.244 at 35 °C. Despite the better leachability of silicon at slightly alkaline pH than at slightly acidic pH, the value of the relevant selectivity coefficient for base metal recovery changed only slightly at temperatures ranging from 35° to 55° C.

Indirect leaching of copper slag at 75 °C showed that higher temperatures negatively affected the recovery of base metals. Therefore, the values of both selectivity coefficients decreased due to pronounced chemical oxidation and hydrolysis of iron, as well as enhanced silicon leaching. Therefore, silicon leaching and calculated selectivity factor were not sensitive indicators of the selectivity of non-ferrous metal leaching from copper slag compared with those of iron co-leaching.

Conclusions

Sporosarcina ureae growth in media enriched in nitrogen organic compounds resulted in a spent medium with slightly alkaline pH and a significant concentration of ammonium ions. Indirect leaching of copper slag with the spent leaching solution obtained for 3 days at 35 °C resulted in recoveries of 15.1%, 19.1 and 39.6% for copper, zinc, and cobalt; the selectivity coefficient calculated with respect to the co-leached iron better characterises the non-ferrous metals recovery than selectivity calculated with respect to the co-leached silicon and the effect of temperature on the entire process. The constant concentration of the lixiviants in the spent medium, as well as the presence of 25% copper in phases leachable only under oxidising conditions, limited the further recovery of base metals.

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