



Bioleaching of carbide waste using spent culture of *Acidithiobacillus* bacteria: Effective factor evaluation and ecological risk assessment

Meghdad Pirsaeheb^a, Sara Zadsar^{b,*}, Hooshyar Hossini^a, Seyed Omid Rastegar^c, Hyunjung Kim^d

^a Department of Environmental Health Engineering, Faculty of Health, Kermanshah University of Medical Sciences, Kermanshah, 6714869914, Iran

^b Students Research Committee, Kermanshah University of Medical Sciences, Kermanshah, 6714869914, Iran

^c Department of Chemical Engineering, Faculty of Engineering, University of Kurdistan, Sanadaj, 6617715175, Iran

^d Department of Earth Resources & Environmental Engineering, Hanyang University, 222-Wangsimni-ro, Seongdong-gu, Seoul, 04763, 133791, Republic of Korea

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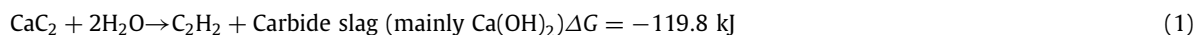
ABSTRACT

This study investigates the bioleaching of Li, Ni, Cu, Zn, and Ba from carbide slag using presecreted metabolites (spent cultures) of *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) and *Acidithiobacillus thiooxidans* (*A. thiooxidans*). The effects of pH (1–3), pulp density (0.1%–2% w/v), and contact time (up to 8 days) in achieving high metal recovery were determined. The highest metal biosolubilize rate was achieved at a pH of 1.5, pulp density of 0.5% w/v, and retention time of 6 days. Results showed that the spent cultures of *A. ferrooxidans* and *A. thiooxidans* recovered 100% of Ni and Li, respectively. In addition, the results of the spent of mixed culture revealed an improved metal recovery compared with individual strain mediums. Furthermore, investigations on the pollution indexes of the leached slag revealed that leaching using spent of mixed culture was more effective than other leaching methods. Investigation of the contamination factor, ecological risk factor, pollution load index, and geo-accumulation index demonstrated that the metabolites of *A. ferrooxidans* and the spent of mixed culture significantly improved the waste quality in terms of reducing heavy metals pollution.

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1. Introduction

Calcium carbide (CaC_2) is synthesized industrially from calcium carbonate (CaCO_3) and coke (C) at elevated temperatures of approximately 3000 °C. Carbide slag is an industrial waste of CaC_2 generated from hydrolysis during the production process of acetylene, which is used for welding and cutting. The hydrolysis is presented as follows (Eq. (1)) (Yang et al., 2014):



The high alkaline carbide slag has several deteriorating effects, such as land and environmental pollution (Lei et al., 2011). Consequently, its management and disposal requires special caution. The chemical components of impurities in the carbide

* Corresponding author.

E-mail address: zadsar.saraa@gmail.com (S. Zadsar).

Table 1

Carbide slag characteristics by ICP and XRF analyses.

ICP	Li	Ni	Zn	Ba	Cu	Ag	Au	Pd	V
Value (mg/L)	0.03	0.128	5.63	0.064	1.78	0.012	0.006	0.006	0.006
XRF	CaO	K ₂ O	Al ₂ O ₃	SiO ₂	Na ₂ O	MgO	MnO	P ₂ O ₅	Organic matter
Value (%)	24	0.288	0.225	0.24	0.183	0.273	0.022	0.029	24

slag mainly include heavy metals, silicon dioxide (SiO₂), iron and aluminum oxides, and organic carbon (Yang et al., 2014). Toxic metals attack living cells and destroy the balance between reactive oxygen species (ROS) production (free radicals) and the subsequent defense by antioxidants (Jaishankar et al., 2014).

Semi-industrial workshops and automobile repair shops produce acetylene from CaC₂, which is frequently used in carbide welding and metal cutting. Hence, it is expected that carbide slag is produced in large quantities daily. However, there are environmental concerns due to the indiscriminate disposal of these slags into the environment without adequate treatment and caution. Waste dumping practices are the only strategy for solid waste management in several low-income countries. A large amount of carbide slag, mainly composed of Ca(OH)₂, is discharged from the polyvinyl chloride (PVC) and chlor-alkali industries every year. However, they are not strongly implemented due to lack of stringent laws and regulations. The best solution, which is rarely discussed, is the recycling or extraction of toxic heavy metals (Weijun and Naibin, 2010).

Conventional technology, including pyrometallurgy and hydrometallurgy, has been used to recover heavy metals. These processes require large amounts of chemicals, have high operating costs, and are complex, involving complicated operations with low recovered amounts (Dewil et al., 2007; Pathak et al., 2009). Thus, it is essential to develop new technologies for extracting metals. Bioleaching is a new method for recovering metals from solid wastes based on the ability of microorganisms to produce inorganic or organic acids, resulting in soluble and extractable metal elements that can be recovered. This method is simpler, cheaper, and more environmentally friendly than conventional technology (Rastegar et al., 2015). According to studies on bioleaching, two species of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* have been extensively investigated for extracting various metals. Their saline characteristics are extremely acidophilic, chemolithoautotrophic, and mesophilic. These bacteria grow by obtaining energy from the oxidation of ferrous iron (Fe²⁺) or elemental sulfur (S⁰), thereby achieving metal solubilization (Marlenne et al., 2020).

Bioleaching can be implemented in several ways, including one-step, two-step, and spent media, each with its unique advantages. Recently, the spent or presecreted metabolite usage of bacteria has gained attention owing to easy storage of metabolites, fast metal extraction, fast reaction rate, as well as no requirement of oxygen or other nutrients, and negligible bioaccumulation or biosorption of metals (Natarajan and Ting, 2015; Rasoulnia and Mousavi, 2016; Kudpeng et al., 2020). Produced metabolites, such as citric acid and ascorbic acid from fungi as well as ferrous iron and biosulfuric acid from *A. ferrooxidans* and *A. thiooxidans*, respectively, act as oxidizing agents to extract metals from a solid matrix.

With dumping the residues of solid wastes after bioleaching into the soil, heavy metals accumulation from waste disposal can affect its quality and fertility for agricultural purposes. Metals can adversely affect plant growth, interfere with soil microbial activities, or degrade soil quality and fertility (Abdullah et al., 2017). Thus, for the residues of solid wastes after bioleaching, the risk potential, including the contamination factor (CF), ecological risk factor (RF), pollution load index (PLI), and geo-accumulation index (*I*_{geo}), must be evaluated, which is the key to manage soils and achieve sustainable development goals. No studies in the literature have dealt with bioleaching using the spent culture of carbide slag. Therefore, the present work aims to recover toxic metals from carbide slag using spent culture of *A. ferrooxidans* and *A. thiooxidans* and their mixed culture. After finding the best conditions for toxic metal extraction, the carbide slag residue was evaluated based on the ecological risk assessment.

2. Material and methods

2.1. Carbide slag

Samples of original carbide slag were obtained from the Kermanshah industrial workshop, Iran. After preparing carbide slag, approximately 500 g of the slag was first washed with distilled water and dewatered at 60 °C for 72 h and then homogenized by milling and sieving with a No. 50 mesh (particle size of 300 μm). The results of the analysis of metal concentration are summarized in Table 1. The obtained results revealed that the primary metals were Zn (5.63 ppm), Cu (1.78 ppm), and Ni (0.128 ppm), others at a lower concentration were Ba (0.064 ppm), Li (0.03 ppm), and Ag (0.012 ppm), and those less than 0.006 ppm were Au, Pd, and V. XRF results revealed that amount of calcium oxide and Loss on ignition (LOI) were approximately 75% and 24% (wt%), respectively.

2.2. Initial analysis of waste

Heavy metals in the carbide slag were separated using wet extraction of hydrochloric acid and nitric acid. Approximately 1 g of waste was digested with 40 mL of HNO₃:HCl solution (1:3 by volume) in a 250-mL beaker at 80 °C for 44 h. Afterward, the digested waste was filtered using a fiberglass filter (0.2-μm pore size). Inductively coupled plasma (ICP) (Perkin Elmer, Optima 7300, US) was then applied to determine the concentration of the extracted metals.

Table 2
Ecological risk assessment.

Ecological risk factor	Description	Pollution load index	Description	Contamination factor	Description	I_{geo}	Description
RF < 40	Low	<1	Perfection	<0.1	Very slight contamination	$I_{geo} \leq 0$	Unpolluted (class 0)
40–80	Moderate			0.1–0.25	Slight contamination	$0 < I_{geo} \leq 1$	Unpolluted/Moderately (class 1)
80–160	Considerable			0.26–0.5	Moderate contamination	$1 < I_{geo} \leq 2$	Moderately (class 2)
160–320	High	1	Baseline levels of pollutants	0.51–0.75	Severe contamination	$2 < I_{geo} \leq 3$	Moderately/Heavily (class3)
≥ 320	Very high	>1	Deterioration of site quality	0.76–1	Very severe contamination	$3 < I_{geo} \leq 4$	Heavily/Extremely (class 4)
				1.1–2	Slight pollution	$4 < I_{geo} \leq 5$	Extremely (class 5)
				2.1–4	Moderate pollution	$5 < I_{geo}$	Strongly Extremely (class 6)
				4.1–8	Severe pollution		
				8.1–16	Very severe pollution		
				16<	Excessive pollution.		

2.3. Spent culture preparation

Pure cultures of *A. ferrooxidans* (PTCC No. 1646) and *A. thiooxidans* (PTCC 1717) were prepared at the Iranian Research Organization for Science and Technology, Tehran, Iran. The culture medium of 9K composition used in growing *A. ferrooxidans* was contained in 500 mg/L of K_2HPO_4 , 500 mg/L of $MgSO_4 \cdot 7H_2O$, 3000 mg/L of $(NH_4)_2SO_4$, 100 mg/L of KCl, 10 mg/L of $Ca(NO_3)_2$, and 44,680 mg/L of $FeSO_4 \cdot 7H_2O$ (Chen et al., 2009). The culture medium of TK for *A. thiooxidans* contained in 250 mg/L of K_2HPO_4 , 250 mg/L of $MgSO_4 \cdot 7H_2O$, 2000 mg/L of $(NH_4)_2SO_4$, 100 mg/L of KCl, and 10,000 mg/L of S (Rastegar et al., 2016). The pH of the mediums was set to 2 at the beginning using 1-M H_2SO_4 and 1-M NaOH. Before inoculation, *Acidithiobacillus* species were grown over a log phase period of 8 days. To prepare the spent culture as a leaching agent, 10% v/v of the pure strains was inoculated into a 250-mL Erlenmeyer flask containing specific growth media and then transferred to a shaker incubator set at 33 °C and 130 rpm. The spent culture after 8 days was used for the bioleaching experiment. During the bacterial growth, the pH and oxidation–reduction potential (ORP) were simultaneously analyzed.

2.4. Leaching experiments

In the bioleaching experiment, four methods were adopted, including the spent culture of pure *A. ferrooxidans* and pure *A. thiooxidans*, mixed spent cultures of *A. ferrooxidans* and *A. thiooxidans*, and chemical leaching with 4-M inorganic acids of H_2SO_4 , HCl, and HNO_3 at 70 °C for 24 h and 130 rpm. In each method, the effect of different parameters, including pH, pulp density, and time, was investigated.

2.5. Analyses

To identify the changes in carbide functional groups before and after bioleaching, Fourier-transform infrared (FTIR) spectroscopy (GX ATR-FTIR, Spectrum, US) was performed. The surface morphology of the samples was analyzed using a scanning electron microscope (SEM; XL30, Philips, Netherlands). The pH and ORP were determined by a pH meter (Hanna, US) and a portable ORP meter (ORPTestr10, Eutech, Singapore), respectively. X-ray fluorescence (XRF) spectra was analyzed by X' Pert (Philips, Netherlands).

2.6. Assessment of ecological risk

The CF, PLI, and I_{geo} were evaluated to estimate the level of pollution, whereas ecological risk index (RI) and risk factors (RFs) were evaluated to estimate the ecological risk. The number of I_{geo} can be calculated according to Eq. (2), (Feng et al., 2019b):

$$I_{geo} = \log_2 \frac{\text{heavy metal concentration (mg/kg)}}{1.5 \times \text{reference sample}} \quad (2)$$

The reference sample used was obtained from Bradl reference for heavy metals (Bradl, 2005). To localize the results, Iranian agriculture soil references (targets) were investigated. The I_{geo} for the reference soils is listed in Table 2.

The RI and PLI are useful tools in assessing environmental pollution because they provide a more comprehensive evaluation of the level of pollutants from a cumulative pattern from existing metals. However, using the index individually can lead to incorrect assessment. The PLI is obtained using Eq. (3) (Kumar and Thakur, 2017).

$$PLI = \sqrt[n]{CF_1 + CF_2 + \dots + CF_n}, \quad (3)$$

where n represents the number of heavy metals, and CF can be determined from Eq. (4) (El-Amier et al., 2017).

$$CF = \frac{\text{metal concentration in the sample}}{\text{target or background value}}. \quad (4)$$

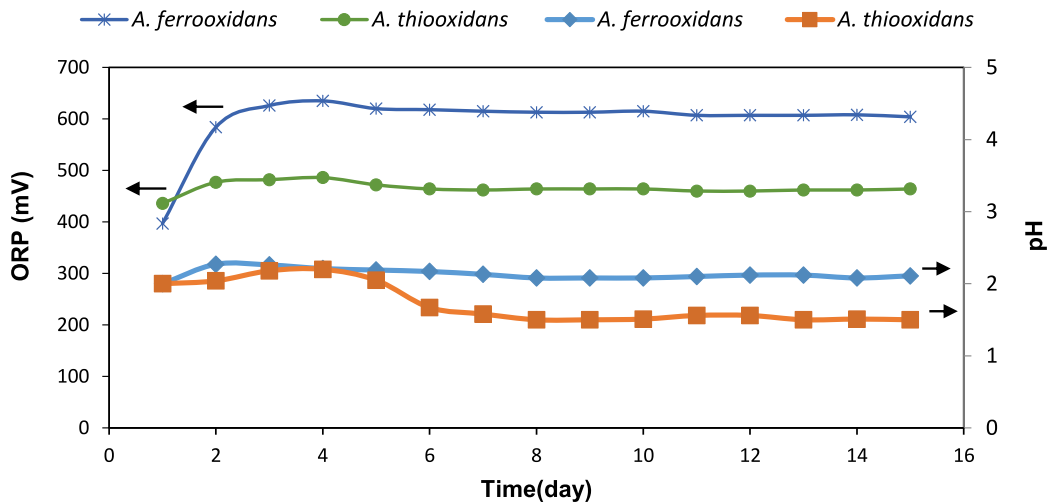


Fig. 1. pH and oxidation–reduction potential (ORP) variation during the growth of the acidic bacterium *A. ferrooxidans* and *A. thiooxidans* [carbide slag dose: 0.2% w/v, initial pH: 2, inoculum percentage: 10% v/v, and temperature: 33 °C].

Different PLI values references are tabulated in Table 1 (Ihedioha et al., 2017). In addition, the value of RFs reflects the degree of contamination, which can be calculated as follows (Eq. (5)) (Wang et al., 2018):

$$RF = Tr \times CF, \quad (5)$$

where Tr represents the toxic response factor of metals (for example; $Tr = 1$ for Zn and $Tr = 5$ for Ni).

3. Results and discussion

3.1. Growth characterization

As shown in Fig. 1, the ORP and pH as growth characterization of the medium had an upward trend for the first 4 days and then decreased, whereas at the end of the 8th day, when the bacteria entered a fixed phase, they remained constant until the 15th day. The pH varied from 2 to 1.5 for *A. thiooxidans*, whereas a small variation in pH was observed for *A. ferrooxidans* for more than 15 days.

An initial increase in the culture pH can be attributed to the initial composition of the carbide slag, which neutralized the medium protons, as shown in the following reaction (Eq. (6)) (Vilcáez et al., 2009):



The ORP variation increased from 397 to 604 mV for *A. ferrooxidans*, indicating a good activity of iron-bacteria. The ORP value revealed the Fe^{3+}/Fe^{2+} ratio in the culture medium according to the Nernst equation (Eq. (7)), and an increase in the ORP is ascribed to the subsequent oxidation reaction of ferrous to ferric by the bacteria (Eq. (8)) (Gu et al., 2018):

$$E = E^0 + \frac{RT}{F} \ln \left[\frac{Fe^{3+}}{Fe^{2+}} \right] \quad (7)$$

and



where E^0 represents the standard reduction potential, R and T represent the universal gas constant and absolute temperature, respectively, and F is the Faraday constant. The transformation of ferrous to ferric (Eq. (8)) is due to the iron-oxidizing bacteria and it results in a high level of ORP. The ORP and pH have a direct relationship, in other words, ORP is sensitive to pH change; a decrease in pH is accompanied by increasing ORP, as shown in the data presented in Fig. 1.

3.2. Effect of initial pH

The effect of the initial pH range of 1–3 on Ni and Li on the second day using 10% v/v inoculum was observed, and the results are presented in Fig. 2. It was observed that the maximum Ni recovery was more than 40% by spent culture of *A. thiooxidans*, whereas it was approximately 80% at pH 1.5 for spent culture of *A. ferrooxidans*. Furthermore, maximum Li

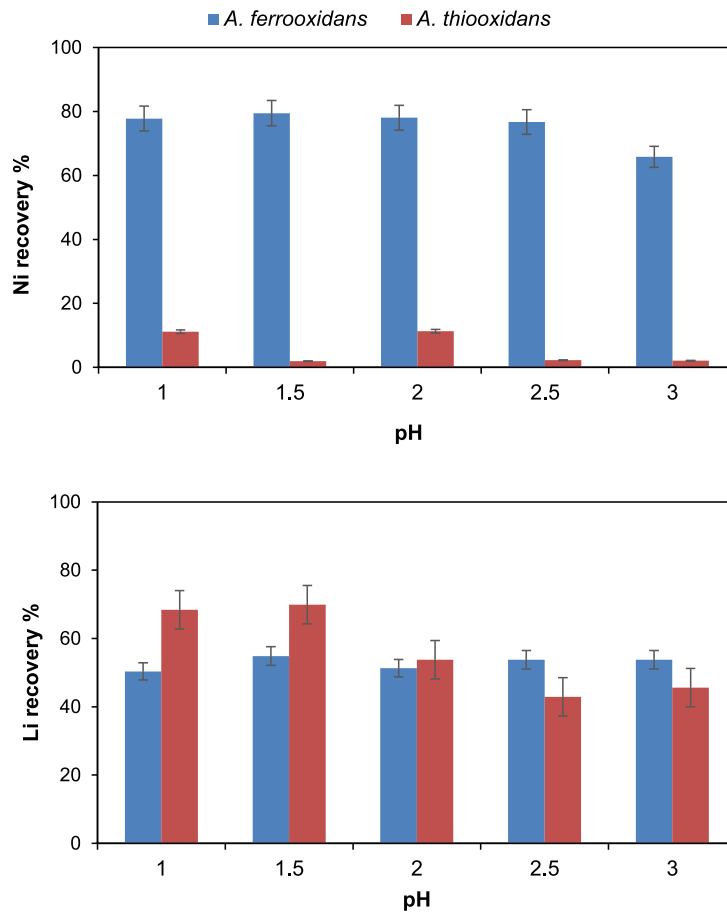
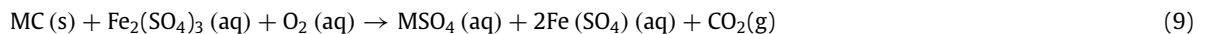


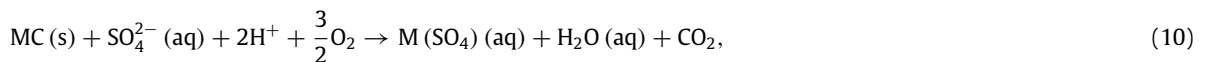
Fig. 2. Recovery percentages of Ni and Li corresponding to spent culture of *A. ferrooxidans* and spent culture of *A. thiooxidans* at different initial pH values [pulp density 0.2% w/v and time: 8 days].

recoveries were 54.8% and 70% by spent culture of *A. ferrooxidans* and spent culture of *A. thiooxidans*, respectively, in pH 1.5, which was considered the optimum pH for the next experiments. According to the nature of heavy metals, such as V, Ni, and Cu, many of them frequently undergo dissolution at a fast rate when in contact with an acidic medium (Mishra et al., 2007). Hence, metal recovery at low pH values is expected to increase.

The mechanism for bioleaching of metals of different valences using spent culture of *A. ferrooxidans*, which is based on Fe^{3+} in the form ($\text{Fe}_2(\text{SO}_4)_3$), and spent culture of *A. thiooxidans*, which is based on SO_4^{2-} , is based on the following reactions (Eqs. (9) and (10)) (Pirsaeheb et al., 2021):



and

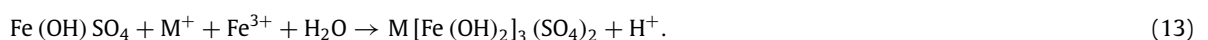


where M represents Li and Ni.

On the other hand, there is a potential risk of iron precipitation with increasing pH, which can affect the coprecipitation of other metals in the extracted solution because of jarosite formation, as represented by following reactions (Eqs. (11)–(13)) (Akbari and Ahmadi, 2019; Nazari et al., 2014):



and



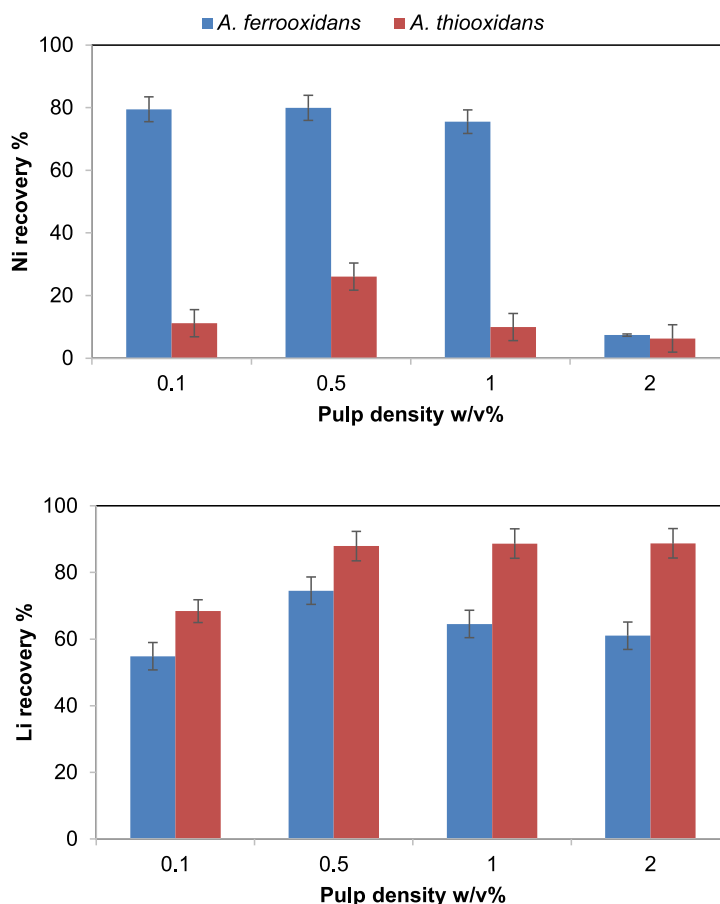


Fig. 3. Recovery percentages of Ni and Li corresponding to spent culture of *A. ferrooxidans* and spent culture of *A. thiooxidans* at different pulp densities [pH: 1.5 and time: 8 days].

In addition, pH can increase because of the reduction of proton-consuming reactions, for example, the acidic dissolution of metals, such as Cu, Zn, and Ni, regeneration reaction of ferrous, or consumption by gangue minerals (Akbari and Ahmadi, 2019). Therefore, the recovery rate of metals is reduced in the presence of low concentrations of protons.

3.3. Pulp density

Fig. 3 illustrates the effect of pulp density on the bioleaching of Li and Ni from 0.1% to 2% (w/v) for 2 days using 10% v/v inoculum at pH 1.5. High pulp density decreases the leaching efficiency of Li and Ni. This behavior has been investigated in the literature (Ahmadi et al., 2010; Pradhan et al., 2010; Asghari and Mousavi, 2014). The negative effect of increasing pulp density is attributed to various factors, including an increase in toxicity, which inhibits metabolic activities of microorganisms and limits the mass transfer of the leaching agent and the adsorption of metal ions in the solution to the solid matrix in the medium, thereby decreasing metal solubility in the bioleaching medium (Gu et al., 2018).

On the other hand, a high density of wastes can retain metal ions in the solid matrix, thereby decreasing the solubility of metals. In the presence of a constant oxidizer metabolite, which was considered for different pulp densities, it was observed that a large proportion of high density pulps remains unchanged.

As shown in Fig. 3, the optimum pulp density was 0.5% (w/v). Under this dosage, Ni and Li were extracted at 80% and 74.5% using spent culture of *A. ferrooxidans*, respectively, whereas spent culture of *A. thiooxidans* leached 26% of Ni and 87.9% of Li.

3.4. Effect of time

Fig. 4 (a, b) represents the recovery percentages of Ni and Li at different contact times under pH 1.5 and pulp density of 0.5 wt%. According to Fig. 4a, 100% of Ni and 75% of Li were recovered using spent culture of *A. ferrooxidans* after 4 days. Maximum recoveries of Li and Ni were 100% after 6 days and 26% after 2 days, respectively, using spent

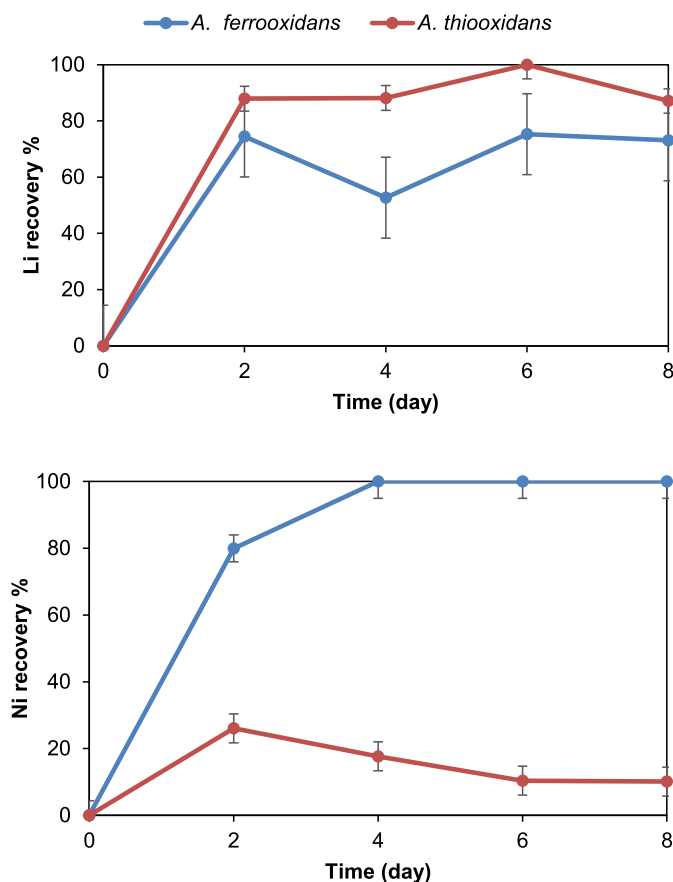


Fig. 4. Recovery percentage rates of Ni and Li corresponding to spent culture of *A. ferrooxidans* and spent culture of *A. thiooxidans* at different contact times [inoculum percentage: 10% v/v, pH: 1.5, and pulp density: 0.5%].

culture of *A. thiooxidans* (Fig. 4b). For better recovery of these metals from a porous matrix, the bacteria are required to secrete extracellular polymeric substances (EPS) directly into the solid phase. It was revealed that the additional chemical derivatives were produced through the “EPS-mediated contact” mechanism, which is a porous matrix promoted by the EPS-mediated contact mechanism (Feng et al., 2019a).

3.5. Comparing different leaching methods

The bioleaching efficiency of the spent culture of pure bacteria at the determined optimum condition was compared with that of the spent of mixed culture of bacteria and that of the chemical leaching of 4 M of strong inorganic acids, such as H_2SO_4 , HNO_3 , and HCl . In addition, the extractions of other metals, such as Zn, Ba, Li, and Ni, have been evaluated. Fig. 5 shows the results of metal recovery efficiency after 6 days. The results revealed that the spent of mixed culture method has better efficiency than other bioleaching methods and chemical leaching. In particular, for Ni, 100% recovery was obtained for the spent of mixed culture, whereas it was 92.4% for spent culture of *A. ferrooxidans*, 10.36% for spent culture of *A. thiooxidans*, and 7.16% for chemical leaching. The dissolution rates in descending order of both biological and chemical leaching are shown as follows:

Spent culture of *A. ferrooxidans* bioleaching: Ni > Li > Zn > Cu > Ba

Spent culture of *A. thiooxidans* bioleaching: Li > Zn > Ba > Ni > Cu

spent of mixed culture bioleaching: Ni > Li > Zn > Ba > Cu

Chemical leaching: Li > Zn > Ba > Ni > Cu

3.6. SEM, XRD, and FTIR analyses

Fig. 1S shows particle morphology before and after bioleaching. Fig. 1a reveals the smooth microstructure of the carbide slag in contrast to the rough and increased length of holes evident on the carbide surface after bioleaching by mixed culture spent medium according to Fig. 1b. The particle-size distribution in the raw material ranged mainly from 4 to

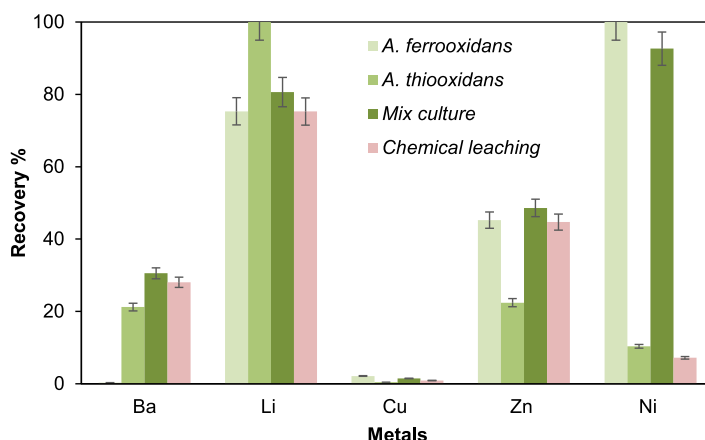


Fig. 5. Comparison of spent culture of *A. ferrooxidans*, spent culture of *A. thiooxidans*, spent of mix culture and chemical leaching processes after 6 days.

Table 3

Ecotoxicity results.

Carbide slag	CF		RF		PLI	I_{geo}	
	Zn	Ni	Zn	Ni		Zn	Ni
Raw material	75.6	4.06	75.6	20.31	5.48	5.92	2.25
spent culture of <i>A. ferrooxidans</i>	41.4	0	41.4	0	2.76	4.78	0
spent culture of <i>A. thiooxidans</i>	58.6	3.64	58.6	18.21	4.95	5.28	1.27
spent of mixed culture	38.8	0.29	38.8	1.45	1.92	4.69	−2.32

44 nm and 10 to 70 nm in the bioleached material (Figs. 1c and 1d). This result demonstrated that a large part of metal-containing particles on the carbide slag surface has been dissolved by the presecreted metabolites. In combination with the SEM image, it can be deduced that the surface of the carbide slag was smooth prior to the bioextraction. FTIR spectra of the carbide slag are shown in Figs. 1e and 1f. The spectra revealed that CaC_2 has four peaks on the broad band (Somnua et al., 2011). Regarding the spectra shown in Fig. 1e, the distinct peaks identified at 651, 730, 974, 1011, 1426, 1643, 3430, 3649, and 3843 cm^{-1} are associated with calcium carbonate, bicarbonate, $\text{Ca}_5(\text{SiO}_4)_2(\text{OH})_2$, SiO_2 , bicarbonate, OH, $\text{Ca}(\text{OH})_2$, and $\text{Ca}(\text{OH})_2$, respectively. Fig. 1f shows the FTIR spectrum of carbide slag after bioleaching using the mixed culture spent medium. It is obvious that the spent culture agent has reacted with the functional groups, as there are changes in the four regions appearing at 600–670, 1137, 1622, and 3400–3540 cm^{-1} .

3.7. Ecological risk assessment

The ecological risk of carbide residue before treatment was investigated. The value of I_{geo} in the heavy metal before and after bioleaching of the carbide slag with different leaching methods is listed in Table 3. Based on I_{geo} values, Zn at its present concentration was extremely toxic pollutant (class VI), whereas Ni exhibited moderate to heavy pollution (class III). After the bioleaching corresponding to agriculture reference, it was discovered that bioleaching using spent culture of *A. ferrooxidans* and spent of mixed culture could reduce the toxicity of Ni pollution from class III ($I_{geo} = 2.25$) to I ($I_{geo} \leq 0$, unpolluted). In addition, comparatively, there was a decrease in I_{geo} value in Zn from class VI ($I_{geo} = 5.92$) to class V ($I_{geo} \leq 5$) concentrations in relation to the calculated value in the raw material using spent culture of *A. ferrooxidans* and spent of mixed culture. Table 3 also presents the evaluation of the overall pollution of all analyzed metals based on their PLI values. A remarkable reduction was observed in the index values when spent culture of *A. ferrooxidans* and spent of mixed culture were used for bioleaching. The PLI value reductions were from 5.48 for raw carbide slag to 1.9 in spent of mixed culture bioleaching and 2.76 in spent culture of *A. ferrooxidans*. Although high metal recovery efficiency was achieved in each of the proposed methods, the analysis of PLI values reveals better performance in the mixed culture leaching. Next, the biological RF of each metal was determined, with $\text{RF} < 40$ indicating a low potential risk of Ni in all methods. However, for Zn, among the leaching methods, only the spent of mixed culture could reduce the level of toxicity of pollution from moderate ($\text{RF} = 75.6$) to low ($\text{RF} = 38.8 < 40$). Based on the CF index values from Tables 1 and 3, the toxicity level for Ni is regarded as severely polluting. The spent culture of *A. ferrooxidans* method reduces the CF index to 0 (very slight contamination), and the spent of mixed culture reduces the CF index to 0.29 (slight contamination). These results show that spent culture of *A. ferrooxidans* and the spent of mixed culture reduced CF, RF, PLI, and I_{geo} considerably compared to other bioleaching methods.

Our previous study Pirsaeheb et al. (2021) was based on one-step bioleaching also the ecological risk assessment was determined and the results are different from the present study which is based on spent culture medium. This difference is due to different leaching efficiency in the two methods then the ecotoxicity results are different. In both methods, a decrease in PLI has been observed and in both of them, the use of mixed culture has caused a further decrease in PLI. In one step bioleaching method, in all cases, the amount of PLI has reached below 1 (without contamination) and it has been better than using the spent culture bioleaching method, where none of the cases have been able to reduce the amount of PLI below 1. Based on RF, in the one-step bioleaching, Zn has reached low contamination, but in the spent culture bioleaching method, Zn has been able to reach low contamination only by using the spent mix culture case. Based on CF in the one-step bioleaching method both Ni and Zn have reached very slight contamination, but in the spent culture bioleaching method, Zn does not change the contamination level after bioleaching but Ni was changed to very slight contamination using spent culture of *A. ferrooxidans* and to slight contamination using spent of mixed culture. Generally comparing all of the ecotoxicity results including PLI, RF, and CF shows that the one-step bioleaching method is much more effective than the spent culture method to reduce pollutants both Ni and Zn.

3.8. Practical applications and future perspectives

Bioleaching of metals from carbide slag with high efficiency using spent culture of *Acidithiobacillus* bacteria is obtained. It is a green technology from environmental perspective. The application of the spent culture method on the industrial scale can be more applicable than other bioleaching methods, where microorganisms are in contact with waste directly. The toxicity of waste makes it impossible to keep them for an extended period in direct bioleaching. It is also possible to use this method in a formal industrial bioreactor, such as a stirred-tank bioreactor. In addition, using this method (mainly spent culture on mixed culture of *A. ferrooxidans* and *A. thiooxidans*), the recovery of extracted metal with high purity can be obtained, making the application of this method on the industrial scale is more feasible. Furthermore, this method could reduce critical environmental risk factors, such as I_{geo} , PLI, RF, and CF, indicating that the residue of carbide slag can be dumped in the soil without any concerns about the environment degradation.

From a future perspective, this method can be used for treating other solid wastes, such as waste from electrical and electronic equipment, fly ash, lithium-ion batteries. Among other bioleaching methods, this method seems to be a safe and acceptable technique on a large scale; however, gaps in the research still prevail and need to be eliminated. The major problem using this method is the preparation of the spent culture media. Thus, there is a need to concentrate on the development of new and better strains, process optimization, and the scaling up of the current process. Thus, this research field should focus more on interdisciplinary subjects, such as microbiology, metallurgy, engineering, and biotechnology.

4. Conclusions

In this research, we studied the use of spent culture of *A. ferrooxidans* and *A. thiooxidans* for bioleaching of carbide slag. The results revealed that under a pH of 1.5, pulp density of approximately 0.5%, and a contact time of 6 days, the amount of recovery was 75.27% and 100% for Li and 100% and 10.3% for Ni using spent culture of *A. ferrooxidans* and spent culture of *A. thiooxidans*, respectively. Based on different risk indexes and parameters, the ecological risk assessment studies revealed that there was a significant reduction of waste under different leaching processes, and among them, the spent cultures of *A. ferrooxidans* and spent of mixed culture proved to be the best. Considering the ecological toxicity results, the spent culture of *A. ferrooxidans* and spent of mixed culture bioleaching can be adopted as an effective and environment friendly strategy for reducing heavy metals.

CRediT authorship contribution statement

Meghdad Pirsaeheb: Validation, Writing, Editing. **Sara Zadsar:** Methodology, Sampling, Experiments. **Hooshyar Hossini:** Validation, Writing, Editing, Software. **Seyed Omid Rastegar:** Review, Editing. **Hyunjung Kim:** 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.

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Ethics statement

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.eti.2022.102801>.

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