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Bioleaching of metals from machining process wastes

Biolixiviación de metales a partir de residuos de procesos de mecanizado

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Abstract
The present study evaluates the ability of *Acidithiobacillus thiooxidans* DSM 26636 to grow and leach metals from industrial wastes (IW) containing different metal compositions, coded as IW-steel and IW-scrap. For this purpose, biolixiviation was assessed during 21 days at 30°C and 150 rpm, with 1% (w/v) of IW pulp density. Microbial growth was evaluated periodically by measuring sulfate production; pH and sulfuric acid concentration were also monitored, and metal bioleaching was evaluated by ICP-OES. Results showed that *A. thiooxidans* sulfur-oxidizing activity was higher in the presence of IW-steel. Specifically, sulfates and sulfuric acid concentrations in IW-steel corresponded to 21,522 ± 1,059 mg/L and 0.0988 ± 0.0030 M at day 21, respectively, and a pH diminution from 3.0 to 1.46 ± 0.10 was detected. In the case of IW-scrap, sulfate production only increased to up to 4,392 ± 335 mg/L on day 21, and no pH reduction was detected. Besides, metal removal was different in IW-steel and IW-scrap. The results obtained reinforce the fact that *A. thiooxidans* DSM 26636 presents potential to be employed for the bio-treatment of high-metal-content industrial residues, and that its biolixiviation capability strongly depends on the metal load present in them.
Keywords: *Acidithiobacillus thiooxidans*, bioleaching of industrial wastes, direct bioleaching, metals, sulfur-oxidizing activity.

Resumen
Se estudió la habilidad de *Acidithiobacillus thiooxidans* DSM 26636 para crecer y lixiviar metales de residuos industriales (IW) con diferentes composiciones, codificados como IW-steel e IW-scrap. Para ello, fue evaluada la biolixiviación durante 21 días a 30°C y 150 rpm, con 1% (p/v) de residuo. Se determinó periódicamente el crecimiento microbiano midiendo la producción de sulfatos; el pH y la concentración de ácido sulfúrico también fueron monitoreados, y la biolixiviación de metales fue evaluada por ICP-OES. Los resultados mostraron que la actividad sulfooxidante de *A. thiooxidans* fue mayor en la presencia de IW-steel. Específicamente, las concentraciones de sulfatos y ácido sulfúrico con IW-steel fueron de 21,522 ± 1,059 mg/L y 0.0988 ± 0.0030 M en el día 21, respectivamente, y se detectó una disminución de pH de 3.0 a 1.46 ± 0.10. Para IW-scrap, la producción de sulfatos incrementó a 4,392 ± 335 mg/L en el día 21, y no se detectó una disminución en el pH. Además, la remoción de metales fue diferente. Los resultados obtenidos refuerzan que *A. thiooxidans* DSM 26636 presenta el potencial para emplearse en el biotratamiento de residuos industriales con alto contenido metálico, y que su capacidad biolixiviante depende fuertemente de la carga metálica presente.
Palabras clave: *Acidithiobacillus thiooxidans*, biolixiviación de residuos industriales, biolixiviación directa, metales, actividad sulfooxidante.

1 Introduction

Metals play an important role in modern societies and, historically, they have been linked to industrial development and the improvement of living standards. Environmental pollution by heavy metals has been dramatically accelerated during the last few decades. Heavy metals exert a wide variety of adverse effects on human health, as some metals have extremely long biological half-lives, which enhance their accumulation. In 2016, the global steel production was over 1,360 million tons (World Steel Association, 2017), generating around 170 to 250 million tons of steel slags as a major by-product. Consequently,

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the recycling of slag has received special attention. Although metal recovery from mineral deposits is a key economical activity that is on the search of novel strategies (Calla-Choque and Nava-Alonso, 2020) and metal recovery from wastes is becoming a key process in the context of a circular economy, metal recovery from steel slags has been largely confined to bench-scale studies (Gomes *et al*., 2018).

Among other wastes, electronic scraps, metal finishing, petroleum spent catalysts, batteries, fly ashes, etc., are some of the major industrial wastes. These solid wastes mostly contain metals such as Au, Ag, Ni, Mo, Co, Cu, Zn, and Cr, causing serious environmental concerns, as they represent an important source of toxic metals (Jadhav and Hocheng, 2012). Various pyrometallurgical and hydrometallurgical methods are used for the extraction or recovery of heavy metals from industrial wastes (Rocchetti *et al*., 2018; Pinna, *et al*., 2019; Borda and Torres, 2021). However, despite their high extraction efficiencies, the large-scale applications of these processes have shown important limitations, since they require huge investments for leaching reagents and their operations. Additionally, these processes create secondary pollution (Cui and Zhang, 2008).

On the other hand, biotechnological processes can be an option to remove metals from liquid or solid sources (Abba *et al*., 2020; Salazar-Pinto *et al*., 2021). Specifically, bioleaching approaches could represent an alternative to remove metals from high metal content wastes, offering advantages that include low cost, easy operation, low energy requirements, a high degree of metal dissolution, and the generation of non-hazardous by-products. Bioleaching processes encompass direct and indirect reactions of certain microorganisms, including oxidation, reduction, chelation, adsorption, and dissolution processes, which can dissolve some of the insoluble substances (heavy metals, sulfur, and other metals) from solids (Li *et al*., 2012). The most predominant mesophiles used in the bioleaching of metals from wastes belong to the genera *Acidithiobacillus* and *Leptospirillum*, which are capable of oxidizing Fe (*Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans, Leptospirillum ferriphilum*), and the sulfur oxidizers (*Acidithiobacillus thiooxidans, Acidithiobacillus caldus*). These microorganisms are autotrophic in nature, using inorganic carbon (CO_2) as their carbon source, being also strict chemolithotrophs, deriving their energy for growth and other metabolic functions from the oxidation of ferrous iron, sulfur compounds, or metal sulfides, and some species also derive their energy from the oxidation of hydrogen sulfide (Liang *et al*., 2010; Ilyas and Lee, 2014).

Particularly, *A. thiooxidans* strain DSM 26636, which was previously isolated from a high sulfur content environment (Rojas-Avelizapa *et al*., 2013), can grow and use elemental sulfur, producing sulfuric acid as a byproduct of its metabolism. It has been demonstrated that this bacterium is able to leach Al, Fe, Ni and V contained in a hydrotreating spent catalyst (Medina-Arriaga, 2017), and V, Fe, Mg, Al, Si, and Ni from slag wastes. Also, *Acidithiobacillus thiooxidans* DSM 26636 is capable of bioleaching Al, Ni, Sn, Mg, Zn, and Si contained in fly ashes from a carbon combustion plant (Rojas-Avelizapa *et al*., 2018). Thus, the present research assessed the ability of *A. thiooxidans* DSM 26636 to grow and bioleach metals contained in solid industrial wastes at 1% (w/v) of pulp density plus elemental sulfur at 1% (w/v), demonstrating its potential use to reduce the metallic load in high-metal-content industrial wastes, which could be substantially beneficial to reduce the risks and adverse environmental impacts produced by the management of this kind of residues.

2 Materials and methods

2.1 *Microorganism and growth conditions*

The sulfur-oxidizing bacterium used throughout this study was *Acidithiobacillus thiooxidans* DSM 26636, which was isolated from a Mexican soil with high sulfur content (Rojas-Avelizapa *et al*., 2013). The culture medium used for experimentation was the modified Starkey medium, composed of (g/L): KH_2PO_4, 3; $(NH_4)_2SO_4$, 0.2; $MgSO_4.7H_2O$, 0.5; $CaCl_2.2H_2O$, 0.3; $FeSO_4.7H_2O$, 0.1; and, 30 ppb of molybdenum as $Na_2MoO_4.2H_2O$ (Takakuwa *et al*., 1977). Elemental sulfur and industrial wastes were added at a concentration of 1% (w/v) each (Rojas-Avelizapa *et al*., 2018). The pH of the medium was adjusted to 3 with sulfuric acid at the beginning of the experimentation, and growth conditions were 30 °C and 150 rpm (Rojas-Avelizapa *et al*., 2013).

2.2 *Industrial wastes*

Two industrial wastes (IW) were used: IW-steel and IW-scrap. Both residues were donated by two industries located in Mexico City and were crushed to reduce their particle size to < 1000 μm prior to their storage at room temperature until usage. Metal content was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), following the protocol described below. Results from the metal content determination are shown in Table 1.

2.3 Bioleaching studies

The inoculum was prepared in 250-mL flasks containing 50 mL of modified Starkey medium at pH 3, with elemental sulfur at 1% (w/v), and incubated at 30 ºC and 150 rpm during 4 days. Afterward, the experimental sets were prepared as follows: 125-mL Erlenmeyer flasks containing 30 mL of modified Starkey medium were supplemented with the corresponding IW at 1% (w/v) and were inoculated with 3 mL (10%) of the inoculum, previously set at $2x10^8$ CFU/mL. Flasks were incubated at the same conditions (30 °C and 150 rpm) for up to 21 days. Controls without inoculum were also included to determine abiotic sulfur oxidation. Every 7 days, treatments and controls were taken and the total content of each flask (30 mL) was collected and centrifuged at 6000 rpm for 7 min, to eliminate the elemental sulfur, the IW, and the biomass (Hipólito-Juárez, 2018). Supernatants were filtered using a syringe driven filter unit of a membrane, type MF-Millipore (mixed cellulose esters) of 0.22 μm (Catalogue No. R3HN06272) and collected in 50-mL glass tubes to be stored at 4 °C until use. Microbial growth was evaluated through sulfate production, which was determined according to the Mexican standard method. Sulfate and sulfuric acid productions were determined as previously described (Gomez-Ramirez *et al*., 2018; Rivas-Castillo *et al*., 2018), performing each determination in triplicate. The sulfuric acid production in the supernatant was also evaluated by pH measurement, using a digital potentiometer (ORION, Model 310) and by titration with a 5 M sodium hydroxide solution, using bromothymol blue as the acid-base indicator (Cerruti *et al*., 1998; Gómez-Ramírez *et al*., 2014). Finally, metal bioleaching was determined by ICP-OES.

2.4 Metal digestion and ICP-OES analyses

Liquid samples (1 mL) of treatments and controls were placed in cylindrical silicon carbide vials, and 6 mL of concentrated HNO_3 and 2 mL of concentrated HCl were added. Samples were digested in a microwave reaction system (Multiwave PRO, Anton Paar), using an HF100 rotor. Digestion conditions were as follows: 600 W for six vessels, 40 bar, 210 - 240 °C, with pRate of 0.3 bar/sec, ramp 15 min, hold 15 min and cooling at 55 °C. Subsequently, 20 mL of deionized water was added to each cylindrical vial, and the supernatant was collected and set to 50 mL using also deionized water. Analyses of Ag, Al, As, Ba, Be, Cd, Co, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sb, Se, Si, Sn, Sr, Tl, V, and Zn were performed by ICP-OES (Varian Model 710-Es) at their appropriate wavelengths (nm), as follows: Ag (328.068), Al (396.152), As (188.980), Ba (455.403), Be (313.042), Cd (214.439), Co (238.892), Cr (267.716), Cu (327.395), Fe (238.204), Li (670.783), Mg (279.553), Mn (257.610), Mo (202.032), Ni (231.604), Pb (220.353), Sb (206.834), Se (196.026), Si (251.611), Sn (189.925), Sr (407.771), Tl (190.794), V (292.401), Zn (213.857). Metals leached were calculated based on a calibration curve of 0.25 to 6 ppm using commercial standards (High Purity, Cat. # ICP-200.7-6). Distilled water was used as a blank, and metal quantification was processed by the software ICP expert II version 1.1. The effectiveness of the biological treatment was determined by comparing the metal content at the beginning and the end of the experimentation. To determine the metals leached by the biological treatments, the amounts of the metals leached in the abiotic system were subtracted from the values obtained of the leached metals from the bio-treated samples (Fierros-Romero *et al*., 2016).

2.5 Statistical analyses

Basic statistical parameters and analyses of variance (one-way ANOVA) were performed using the commercial statistical software OriginPro 9.0. Differences in P values ≤ 0.05 were considered as statistically significant, and lower-case letters in each case represent groups of statistically different data.

3 Results and discussion

3.1 Growth of Acidithiobacillus thiooxidans DSM 26636 in the presence of the industrial wastes (IW)

Table 1 reports the metal composition of both IW used in the present study. Sulfates, sulfuric acid concentrations, and pH changes during the

Table 1. Metal content of Industrial Wastes (IW).

Metals (mg/Kg)	IW	
	Scrap	**Steel**
Al	484,590.0 ± 2864.0	388.0 ± 32.0
Cr	354.0 ± 37.4	412.4 ± 12.1
Cu	1986.0 ± 129.0	6534.0 ± 95.0
Fe	38,654.3 ± 3163.0	416,238.0 ± 2688.0
Li	295.1 ± 10.2	297.0 ± 6.1
Mg	5,125.0 ± 515.4	849.1 ± 17.1
Mn	1,428.2 ± 760.0	10,748.3 ± 610.0
Ni	171.4 ± 21.0	409.3 ± 72.0
Si	4,649.1 ± 554.0	1,838.0 ± 300.0
Zn	447.0 ± 172.4	11,831.0 ± 142.3

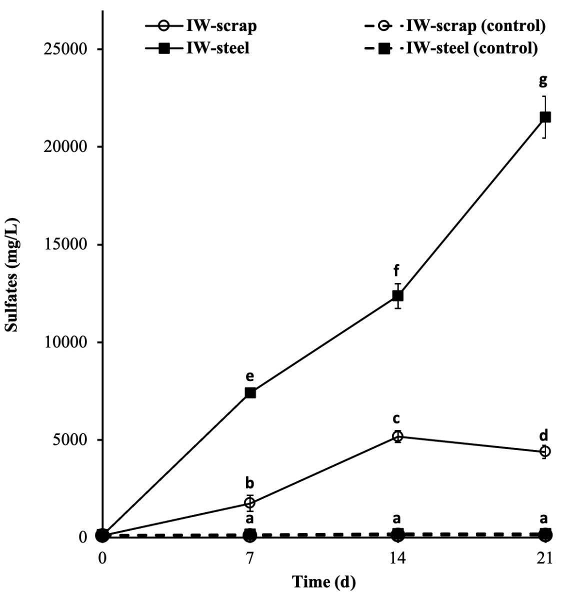

Fig. 1. Monitoring of sulfate production during A. thiooxidans treatment of IW-scrap and IW-steel wastes at 1% (w/v), plus elemental sulfur at 1% (w/v), at 30 °C and 150 rpm. Data are presented as averages ± standard deviations, and lower-case letters represent groups of data that show statistically significant differences ($P \leq 0.05$).

biological treatment of IW were analyzed, to indirectly assess growth by the sulfur-oxidizing activity of the bacterium. Figure 1 shows the ability of *A. thiooxidans* to oxidize elemental sulfur in the presence of IW-scrap and IW-steel. Sulfate concentrations increased over time in the presence of the residues, reaching values of 4,392 ± 335 mg/L and 21,522 ± ,059 mg/L on day 21, for IW-scrap and IW-steel, respectively. In the case of both bio-treated samples, significant statistical differences in sulfate concentrations were observed during all the bio-treatment period, in contrast to the control systems, where no significant statistical differences in sulfate concentrations were detected (Figure 1). The sulfuric acid production was higher in the presence of IW-steel, being of 0.0988 ± 0.0030 M at day 21, compared to the one observed in IW-scrap, which was of 0.0085 ± 0.0001 M (Table 2). In the control systems (without the microorganism), acid concentrations of 0.0047 ± 0.0001 M and 0.0010 ± 0.0002 M were detected in the presence of IW-scrap and IW-steel, respectively, which can be attributed to the acid used to adjust the pH of the modified Starkey medium at the beginning of the experimentation (data not shown). Regarding pH changes, pH decreased to 1.46 ± 0.10 in the sample with IW-steel after 21 days of growth, in contrast to the samples with IW-scrap, where pH was observed in 3.82 ± 0.08 and 4.09 ± 0.07 on days 14 and 21, respectively.

Previous studies with *A. thiooxidans* DSM 26636 showed its ability to grow autotrophically using elemental sulfur as the energy source, at concentrations of up to 9 % (w/v) in a pH range of 3 to 7 (Rojas-Avelizapa *et al.*, 2013; Ilyas and Lee, 2014), and with a sulfur removal rate of 0.185 mg S $g^{-1}h^{-1}$ in the presence of a pulp density of 16.5% (w/v) of industrial waste (Gómez-Ramírez *et al.*, 2014). When the sulfur-oxidation process takes place in the microorganism, like in other sulfur-oxidizing bacteria, sulfuric acid is produced and it works as a bioleaching agent to remove metals contained in high-metal-content residues. The increment in sulfate production is commonly interpreted as an indicator of bioleaching efficiency, which is likewise correlated to sulfuric acid production and, hence, pH reduction (Gómez-Ramírez *et al.*, 2018).

The pH reduction in the sample containing IW-steel is in agreement with the higher 2.8-fold sulfuric acid production, in contrast to the conditions observed during the biolixiviation of metals contained in IW-scrap, where sulfate production (Figure 1) and sulfuric acid production were less evident, in addition to the increase in pH, instead of pH diminishing (Table 2). In the presence of metal-containing solid wastes, different factors and parameters can affect the sulfur-oxidizing activity of microorganisms, so the bio-treatment processes could be optimized by the manipulation of the following: (a) pulp density/solid-

Table 2. Sulfuric acid production and pH changes during the biolixiviation process.

Time	**IW-Scrap**		**IW-Steel**	
(days)	**H_2SO_4 [M]**	**pH**	**H_2SO_4 [M]**	**pH**
7	1, 0, 00.0036 ± 0.0005a*	1, 0, 03.66 ± 0.15 a	0.0213 ± 0.0014 a	1, 0, 03.34 ± 0.08 a
14	1, 0, 00.0084 ± 0.0013 b	1, 0, 03.82 ± 0.08 b	1, 0, 00.0454 ± 0.0009 b	1, 0, 01.76 ± 0.04 b
21	1, 0, 00.0085 ± 0.0001 b	4.09 ± 0.07 c	1, 0, 00.0988 ± 0.0030 c	1, 0, 01.46 ± 0.10 c

* Lowercase letters indicate groups of data in each column that were significantly different by ANOVA ($P \leq 0.05$).

liquid ratio, (b) sulfur concentration, (c) microorganisms used, (d) culture media, (e) conditions of preliminary cultivations, (f) carbon dioxide, (g) population density, (h) osmotic pressure, (i) redox potential, (j) temperature, (k) water activity, (l) pH, (m) bioleaching period, (n) particle size, (o) shaking speed, (p) bioavailability of metal for the microorganism, (q) microbial requirements, (r) metal tolerance, (s) microorganisms adapted to waste, (t) physiochemical properties of the residues, and (u) type of released metals (Gómez-Ramírez and Tenorio-Sanchez, 2019; Gómez-Ramírez *et al* ., 2020). The differences in the results observed during the biological treatment processes of the IW could be related to the metal composition of each industrial waste, and the diverse cellular responses produced in the microorganism under the two stress conditions caused by the metallic charge of these residues. It has been shown that elevated concentrations of metals have a clear negative impact on bacterial growth, and some general toxic effects include the blockage of biologically important functional groups, enzyme denaturation, and C and N mineralization. However, microbial cells have a remarkable capability to counteract cellular damage, and a minimum threshold concentration of a toxic metal must reach a cellular target, as the cell wall, before any biological response takes place (Aljerf and AlMasri, 2018). Thus, the presence of elevated concentrations of metals in wastes could limit cell viability (Wu *et al*., 2018). Also, the simultaneous presence of a mixture of metals in high concentrations can reduce the usage of higher pulp densities of metal-containing wastes, such as spent catalysts (Rivas-Castillo *et al*., 2019; Rivas-Castillo and Rojas-Avelizapa, 2020). The aluminum (Al) content in IW-scrap, in addition to the presence of the other metals in this residue, may have inhibited sulfate production, and thus growth, as Al concentration is 1249-fold higher in IW-scrap than in IW-steel. Although few reports are available concerning *A. thiooxidans* metal toxicity, Chen *et al*. (2004) carried out a dose-response study based on divalent Cd, Cu, and Zn cations, unraveling a differential metal toxicity in the microorganism, which was Cu > Cd > Zn. Regarding Al toxicity, it has been stated that Al ions can bind to diverse cellular components, altering lipid-protein interactions, modifying the cellular transport activity, and blocking surface potential. Also, once inside the cell, Al ions can alter the metabolism by binding to enzymes or enzyme substrates (Garciduenas-Pina and Cervantes, 1996; de Souza Oliveira *et al*., 2009). However, it has been observed before that Al toxic effects may decrease in previously adapted bacterial cultures to Al-containing residues or increasing concentrations of this metal (Buvignier *et al*., 2019).

3.2 Metal removal from the industrial wastes (IW)

The metals leached during the biolixiviation processes were detected by ICP-OES at the end of the experimentation period (21 days), and the results are shown in Figure 2. In total, 5 metals were lixiviated from IW-scrap, whose lixiviation in ppm/percentage were as follows: Al (1568.3/ 0.32) > Mg (20.3/0.4) > Fe (8.8/0.02) > Mn (6.3/0.44) > Zn (3.5/0.78). On the other hand, 7 metals were removed from IW-steel, with a decreasing order of (ppm/percentage): Fe (4661.9/1.12) > Mn (131.1/1.23) > Mg (34.8/4.10) > Zn (16.6/0.14) = Cu (15.5/0.24) > Cr (4.85/1.18) = Ni (4.46/1.09). It is interesting to note that Fe, Mn, Mg y Zn were lixiviated from both IW, and Al was only removed from IW-scrap, where the Al concentration was 1249 times lower in this residue compared to the one determined in IW-steel. Also, the 4 metals that were removed from both wastes (Fe, Mn, Mg y Zn), were removed 529.8 -fold, 20.9-fold, 1.7-fold, and 4.7-fold higher in IW-steel. In the abiotic controls, metal bioleaching in (ppm) was: Al

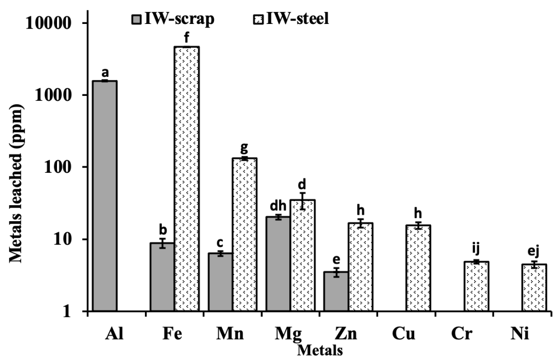

Fig. 2. Bioleaching of metals (in ppm) from IW-scrap and IW-steel wastes at 1 % (w/v) pulp density plus 1 % (w/v) elemental sulfur by A. thiooxidans after 21 days of treatment, at 30 °C and 150 rpm. Data are presented as averages ± standard deviations, and lower-case letters represent groups of data that show statistically significant differences ($P \leq 0.05$).

(59), Mg (4.0), Zn (3.5), Fe (2), and Mg (0.3) for IW-scrap, and Fe (50), Mg (8), Mn (7), Cu (2), Zn (2), Cr (1) and Ni (0.5) for IW-steel. It is important to consider that the differences in the metal removals from solid industrial wastes are influenced by the presence of the metals in particular fractions, such as exchangeable fraction, reducible fraction, oxidizable fraction, and residual fraction; these can influence the metal-leaching susceptibility by diverse processes, including biological treatments (Gomez-Ramirez and Tenorio-Sanchez, 2019). Overall, the differences in the sulfuric acid productions observed in the presence of IW-scrap and IW-steel during the biolixiviation processes, and the additional biological effects caused by the distinct metallic load of the two residues, may have originated lower bioleaching of metals (others than Al) from IW-scrap, compared to the general metal removal obtained from IW-steel. Regarding IW-scrap metal content, it has been previously sustained that the presence of Al in high concentrations does not avoid the bioleaching processes (Veloso *et al.*, 2012) and that previous bacterial adaptation to high-metal-content residues, in addition to promoting the diminishing of the cellular toxic effects, as described above, has also enhanced leaching rates in materials with a high metallic load (Jang and Valix, 2017). In the present study, there were not used previous acclimated cultures to the industrial residues, which could be a major perspective for future assessments.

Additionally, it has been suggested that bioleaching processes in spent refinery catalysts ($Co/Mo/Al_2O_3$ type) present better efficiencies when using *A. thiooxidans* instead of *A. ferroxidans*, because the former may operate at a higher pH than the latter (Gholami *et al.*, 2011), and previous studies of our research group demonstrated that *A. thiooxidans* DSM 26636 has the ability to leach metals from slags and ashes produced in a coal processing plant in the presence of 1% (w/v) of pulp density; the metals that were mainly leached in that previous study were, in mg/Kg: V (48); Fe (29); Mg (25); Al (15); Si (10) and Ni (7.4), while for ashes, in mg/Kg: Al (249); Ni (90); Sn (40); Mg (40); Zn (30) and Si (30) (Gómez-Ramírez *et al.*, 2014). From these past assessments and from the present study, it can be stated that the ability of *A. thiooxidans*, like of the other microorganisms used in bio-treatment processes, may be affected by the metallic load of the material assessed and also by the intrinsic metal stress response mechanisms of the microorganism (Rivas-Castillo *et al.*, 2018; Gómez-Ramirez *et al.*, 2019; Rivas-Castillo and Rojas-Avelizapa, 2020). Even so, *A. thiooxidans* DSM 26636 presents the potential to be used for the bioleaching of metals contained in high-metal-content industrial wastes.

Conclusions

The sulfur-oxidizing activity of *Acidithiobacillus thiooxidans* has attracted extensive attention for metal leaching purposes from different metal-containing solid wastes. In this study, *A. thiooxidans* DSM 26636 was able to oxidize sublimed sulfur and produce sulfuric acid in the presence of two different industrial wastes: IW-scrap and IW-steel, and metal leaching was achieved in the presence of both residues. Thus, DSM 26636 bioleaching capability could be used as an eco-friendly approach for the treatment of this kind of solid wastes, reducing their metal content and diminishing environmental impacts. The results obtained reinforce the knowledge about the potential of this strain for the bio-treatment of high-metal-content residues, especially industrial wastes.

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References

Abba, Z. A., Yahaya, S., Ahmad S. A., Ramírez-Moreno, N. and Yusuf I. (2020). Bioremediation of heavy metals by melanised and non-melanised feathers and heavy metal resistant feather-degrading bacteria. *Revista Mexicana de Ingeniería Química 19*, 243-252. https://doi.org/10.24275/rmiq/Bio1551

Aljerf, L. and AlMasri, N. (2018). A gateway to metal resistance: Bacterial response to heavy metal toxicity in the Biological Environment. *Annals of Advances in Chemistry 2,* 32-44. https://doi.org/10.29328/journal.aac.1001012

Borda, J. and Torres, R. (2021). Comparative study of selective zinc leaching from EAFD using carboxylic agents. *Revista Mexicana de Ingeniería Química 20*, 389-398. https://doi.org/10.24275/rmiq/IA2022

Buvignier, A., Peyre-Lavigne, M., Robin, O., Bounouba, M., Patapy, C., Bertron, A. and Paul, E. (2019). Influence of dissolved-aluminum concentration on sulfur-oxidizing bacterial activity in the biodeterioration of concrete. *Applied Environmental Microbiology 85*, e00302-19. https://doi.org/10.1128/AEM.00302-19

Calla-Choque, D. and Nava-Alonso, F. (2020). Thiourea determination for the precious metals leaching process by iodate titration. *Revista Mexicana de Ingeniería Química 19,* 275-284. https://doi.org/10.24275/rmiq/IA539

Cerruti, C., Curutchet, G. and Donati, E. (1998). Biodissolution of spent nickel-cadmium batteries using *Thiobacillus ferrooxidans. Journal of Biotechnology 62*, 209-219. https://doi.org/10.1016/S0168-1656(98)00065-0

Chen, B. Y., Liu, H. L., Chen, Y. W. and Cheng, Y. C. (2004). Dose-response assessment of metal toxicity upon indigenous *Thiobacillus thiooxidans* BC1. *Process Biochemistry 39*, 735-745. https://doi.org/10.1016/S0032-9592(03)00180-8

Cui, J. and Zhang, L. (2008). Metallurgical recovery of metals from electronic waste: A review. *Journal of Hazardous Materials 158*, 228-256.https://doi.org/10.1016/j.jhazmat.2008.02.001

de Souza Oliveira, R. P., Rivas Torres, B., Zilli, M., de Araújo Viana Marques, D., Basso, L. C. and Converti, A. (2009). Use of sugar cane vinasse to mitigate aluminum toxicity to *Saccharomyces cerevisiae*. *Archives of Environmental Contamination and Toxicology 57*, 488-494. https://doi.org/10.1007/s00244-009-9287-x

Fierros-Romero, G., Rivas-Castillo, A. M., Gómez-Ramírez, M., Pless, R. and Rojas-Avelizapa N.G. (2016). Expression analysis of Ni- and V-associated resistance genes in a *Bacillus megaterium* strain isolated from a mining site. *Current Microbiology 73*, 165-171. https://doi.org/10.1007/s00284-016-1044-6

Garciduenas-Pina, R. and Cervantes, C. (1996). Microbial interactions with aluminium. *Biometals 9*, 311-316. https://doi.org/10.1007/BF00817932

Gholami, R. M., Borghei, S. M. and Mousavi, S. M. (2011). Bacterial leaching of a spent Mo-Co-Ni refinery catalyst using *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. *Hydrometallurgy 106*, 26-31. https://doi.org/10.1016/j.hydromet.2010.11.011

Gomes, H. I., Funari, V., Mayes, W. M., Rogerson, M. and Prior T. J. (2018). Recovery of Al, Cr and V from steel slag by bioleaching: Batch and column experiments. *Journal of Environmental Management 222*, 30-36. https://doi.org/10.1016/j.jenvman.2018.05.056

Gómez-Ramírez, M., Moreno-Villanueva, F. and Rojas-Avelizapa Norma G. (2020). *Acidithiobacillus thiooxidans* DSM 26636: An alternative for the bioleaching of metallic burrs. Catalysts *10*, 1230. https://doi.org/10.3390/catal10111230

Gómez-Ramírez, M., Rivas-Castillo, A. M., Rodríguez-Pozos, I., Avalos-Zuniga, R. A. and Rojas-Avelizapa, N. G. (2018). Feasibility study of mine tailing's treatment by *Acidithiobacillus thiooxidans* DSM26636. *International Journal of Biotechnology and Bioengineering 12*, 468-471. https://doi.org/10.5281/zenodo.2363155

Gómez-Ramírez, M., Rojas-Avelizapa, N. G., Hernández-Gama, R., Tenorio-Sánchez, S. A. and López-Villegas, E. O. (2019). Potential use of *Bacillus* genera for metals removal from spent catalysts. *Journal of Environmental Science and Health Part A, Toxic/Hazardous Substances and Environmental Engineering 54*, 701-710. https://doi.org/10.1080/10934529.2019.1585720

Gómez-Ramírez, M. and Tenorio-Sánchez, S. A. (2019). Parameters involved in biotreatment of solid wastes containing metals. In: *Biotechnology for treatment of waste containing metals*, (N. G. Rojas-Avelizapa, ed.), Pp. 43-64. River Publishers, Denmark.

Gómez-Ramirez, M., Zarco-Tovar, K., Aburto, J., García de León, R. and Rojas-Avelizapa, N. G. (2014). Microbial treatment of sulfur-contaminated industrial waste. *Journal of Environmental Science and Health Part A, Toxic/Hazardous Substances and Environmental Engineering 49*, 228-232. https://doi.org/10.1080/10934529.2013.838926

Hipólito-Juárez, I. V. (2018). Tratamiento de metales no ferrosos por *Acidithiobacillus thiooxidans*. Informe Técnico de Residencia Profesional, Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada del IPN, Unidad Querétaro, México.

Ilyas, S. and Lee, J. C. (2014). Biometallurgical recovery of metals from waste electrical and electronic equipment: A review. *ChemBioEng Reviews 1*, 148-169. https://doi.org/10.1002/cben.201400001

Jadhav, U. U. and Hocheng, H. (2012). A review of recovery of metals from industrial waste. *Journal of Achievements in Materials and Manufacturing Engineering 54*, 159-167.

Jang, H. C. and Valix, M. (2017). Overcoming the bacteriostatic effects of heavy metals on *Acidithiobacillus thiooxidans* for direct bioleaching of saprolitic Ni laterite ores. *Hydrometallurgy 168* , 21-25.https://doi.org/10.1016/j.hydromet.2016.08.016

Li, Q., Wang, Q., Li, B., Sun, C., Deng, F., Song, C. and Wang S. (2012). Isolation of *Thiobacillus* spp. and its application in the removal of heavy metals from activated sludge. *African Journal of Biotechnology 11*, 16336-16341. https://doi.org/10.5897/AJB12.607

Liang, G., Mo, Y. and Zhou, Q. (2010). Novel strategies of bioleaching metals from printed circuit boards (PCBs) in mixed cultivation of two acidophiles. *Enzymes and Microbial Technology 47*, 322-326. https://doi.org/10.1016/j.enzmictec.2010.08.002

Medina-Arriaga, M. B. (2017). Optimización de la biolixiviación de Ni y V de un catalizador agotado por *Acidithiobacillus thiooxidans* . Tesis de Maestría en Tecnología Avanzada, Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada del IPN, Unidad Querétaro, México.

Pinna, E. G., Martínez, A. A., Tunez, F. M., Drajlin, D. S. and Rodriguez, M. H. (2019). Acid leaching of $LiCoO_2$ from LIBs: Thermodynamic study and reducing agent effect. *Revista Mexicana de Ingeniería Química 18*, 441-450. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n2/Pinna

Rivas-Castillo, A. M., Gómez-Ramirez, M., Rodríguez-Pozos, I. and Rojas-Avelizapa, N. G. (2018). Bioleaching of metals contained in spent catalysts by *Acidithiobacillus thiooxidans* DSM 26636. *International Journal of Biotechnology and Bioengineering 12*, 430-434. https://doi.org/10.5281/zenodo.2021685

Rivas-Castillo, A. M., Guatemala-Cisneros, M. E, Gómez-Ramírez, M. and Rojas-Avelizapa, N. G. (2019). Metal removal and morphological changes of *B. megaterium* in the presence of a spent catalyst. *Journal of Environmental Science and Health Part A, Toxic/Hazardous Substances and Environmental Engineering 54,* 543-550. https://doi.org/10.1080/10934529.2019.1571307

Rivas-Castillo, A. M. and Rojas-Avelizapa, N. G. (2020). Microbiological approaches for the treatment of spent catalysts. *TIP, Revista Especializada en Ciencias Químico-Biológicas 23*, e20200214. https://doi.org/10.22201/fesz.23958723e.2020.0.214

Rocchetti, L., Amato, A. and Beolchini, F. (2018). Printed circuit board recycling: A patent review.

Journal of Cleaner Production 178, 814-832. https://doi.org/10.1016/j.jclepro.2018.01.076

Rojas-Avelizapa, N. G., Gómez-Ramírez, M., Hernández-Gama, R., Aburto, J. and García de León, R. (2013). Isolation and selection of sulfur-oxidizing bacteria for the treatment of sulfur-containing hazardous wastes. *Chemical and Biochemical Engineering Quarterly 27*, 109-117.

Rojas-Avelizapa, N. G., Hipólito-Juárez, I. V. and Gómez-Ramírez M. (2018). Biological treatment of coal combustion wastes by *Acidithiobacillus thiooxidans* DSM 26636. *Mexican Journal of Biotechnology 3*, 54-67. https://doi.org/10.29267/mxjb.2018.3.3.54

Salazar-Pinto, B. M., Zea-Linares, V., Villanueva-Salas, J. A. and Gonzales-Condori, E. G. (2021). Cd (II) and Pb (II) biosorption in aqueous solutions using agricultural residues of *Phaseolus vulgaris* L.: Optimization, kinetics, isotherms and desorption. *Revista Mexicana de Ingeniería Química 20*, 305-322. https://doi.org/10.24275/rmiq/IA1864

Takakuwa, S., Nishiwaki, T., Hosoda, K., Tominaga, N. and Iwasaki H. (1977). Promoting effect of molybdate on the growth of a sulfur-oxidizing bacterium *Thiobacillus thiooxidans*. *Journal of General and Applied Microbiology 23*, 163-173. https://doi.org/10.2323/jgam.23.163

Veloso, T. C., Sicupira, L. C., Rodrigues, I. C. B., Silva, L. A. M. and Leao, V. A. (2012). The effects of fluoride and aluminum ions on ferrous-iron oxidation and copper sulfide bioleaching with *Sulfobacillus thermosulfidooxidans*. *Biochemical Engineering Journal 62*, 48-55. https://doi.org/10.1016/j.bej.2012.01.003

World Steel Association (2017). World steel in figures. Available at: www.worldsteel.org/en/dam/jcr:0474d208-9108-4927 -ace84 ac5445c5df8/World+Ste el+in+Figures+2017.pdf. Accessed: September 25, 2019.

Wu, W., Liu, X., Zhang, X, Zhu, M. and Tan, W. (2018). Bioleaching of copper from waste printed circuit boards by bacteria-free cultural supernatant of iron-sulfur-oxidizing bacteria. *Bioresources and Bioprocessing 5*, 1-13. https://doi.org/10.1186/s40643-018-0196-6