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Feasibility Study of Mine Tailing's Treatment by *Acidithiobacillus thiooxidans* DSM 26636

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***Abstract*—**Among the diverse types of pollutants produced by anthropogenic activities, metals represent a serious threat, due to their accumulation in ecosystems and their elevated toxicity. The mine tailings of abandoned mines contain high levels of metals such as arsenic (As), zinc (Zn), copper (Cu), and lead (Pb), which do not suffer any degradation process, they are accumulated in environment. Abandoned mine tailings potentially could contaminate rivers and aquifers representing a risk for human health due to their high metal content. In an attempt to remove the metals and thereby mitigate the environmental pollution, an environmentally friendly and economical method of bioremediation has been introduced. Bioleaching has been actively studied over the last several years, and it is one of the bioremediation solutions used to treat heavy metals contained in sewage sludge, sediment and contaminated soil. *Acidithiobacillus thiooxidans*, an extremely acidophilic, chemolithoautotrophic, gram-negative, rod shaped microorganism, which is typically related to Cu mining operations (bioleaching), has been well studied for industrial applications. The sulfuric acid produced plays a major role in bioleaching. Specifically, *Acidithiobacillus thiooxidans* strain DSM 26636 has been able to leach Al, Ni, V, Fe, Mg, Si, and Ni contained in slags from coal combustion wastes. The present study reports the ability of *A. thiooxidans* DSM 26636 for the bioleaching of metals contained in two different mine tailing samples (MT1 and MT2). It was observed that Al, Fe, and Mn were removed in 36.3±1.7, 191.2±1.6, and 4.5±0.2 mg/kg for MT1, and in 74.5±0.3, 208.3±0.5, and 20.9±0.1 for MT2. Besides, < 1.5 mg/kg of Au and Ru were also bioleached from MT1; in MT2, bioleaching of Zn was observed at 55.7±1.3 mg/kg, besides removal of < 1.5 mg/kg was observed for As, Ir, Li, and 0.6 for Os in this residue. These results show the potential of strain DSM 26636 for the bioleaching of metals that came from different mine tailings.

Keywords*—A. thiooxidans*, bioleaching, metals, mine tailings.

I. Introduction

METAL pollution is a serious environmental threat worldwide, as metals are broadly involved in a variety of industrial processes, and as a consequence, they are commonly present in solid and liquid residues produced by these anthropogenic activities [1].

Mining-metallurgy sector in Mexico corresponds to the 4% of the Gross Domestic Product, as this country is the first investment destiny for mining exploitation in Latin America, and the forth destiny globally [2]. Mining activities generate elevated amounts of solid residues, known as mine tailings, which are commonly stored in environmentally open piles. These mine tailing deposits are major concern, since they contain the simultaneous presence of numerous metals in elevated concentrations [3].

Sulfur oxidizing bacteria like *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferroxidans* possess the ability to produce sulfuric acid as a metabolic byproduct [4], which confers these microorganisms with the potential to be used in bioleaching processes.

Previous reports have shown the capability of *Acidithiobacillus thiooxidans* for the bioleaching of metals like As, Zn, Pb, and Cu contained in mine tailings [5]. Particularly, *A. thiooxidans* strain DSM 26636, which was isolated from a high sulfur content site in Mexico, was able to bioleach Al, Fe, Ni and V contained in a hydrotreating spent catalyst [6], and Al, Ni, V, Fe, Mg, Si, and Ni were also removed from slags contained in coal combustion wastes [7].

Thus, the present study reports the capability of *A. thiooxidans* DSM 26636 for the bioleaching of metals contained in two different mine tailing samples, establishing the potential that this microorganism possesses for mine tailing biotreatment.

II. Materials and Methods

A. Bacterial Strain and Growth Conditions

A. thiooxidans strain, firstly coded as AZCT-M125-5, was isolated from the Natural Park Los Azufres, located in Ciudad Hidalgo, Michoacán de Ocampo, Mexico [8]. Afterwards, this strain was deposited at the Deutsche Sammlung von Mikroorgamismen und Zellkuturen GmbH (DSMZ) collection and is currently identified as *A. thiooxidans* DSM 26636 [7]. Growth conditions were set at 30 °C and 150 rpm, and modified Starkey culture medium was used for culture growth, which composition is of (g/L): KH_2PO_4, 3; $(NH_4)_2SO_4$, 0.2; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2.2H_2O$, 0.3; $FeSO_4.7H2O$, 0.1; 30 ppb of molybdenum. Elemental sulfur was added to the medium at a concentration of 1% (w/v), and pH was adjusted to pH 3 with sulfuric acid [9].

B. Mine Tailing Samples

The mine tailing (MT) samples, coded as MT1 and MT2,

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were obtained from a company located in San Luis Potosí, Mexico. The residues were obtained as a fine powder, with a particle size smaller than 1000 µm and were used without any additional treatment. Before experimentation, mine tailings metal content was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), following the methodology described below, and such metal composition is presented in Table I.

TABLE I
METAL COMPOSITION IN MINE TAILINGS

	mg / kg	
Metal	MT1	MT2
Al	23250.8 ± 2610.8	24736.3 ± 2258.2
As	202.8 ± 6.9	127.2 ± 4.6
Au	14.3 ± 0.6	12.8 ± 0.4
Ba	836.6 ± 253.6	827.8 ± 66.1
Cd	nd-	55.7 ± 18.3
Cu	893.1 ± 136.8	1080.3 ± 572.0
Fe	62631.4 ± 672.2	56333.9 ± 1915.7
Ir	221.8 ± 25.7	197.3 ± 35.2
Li	329.8 ± 3.0	329.0 ± 5.2
Mg	6307.8 ± 519.3	6346.7 ± 214.9
Mn	2029.9 ± 335.0	1916.1 ± 141.8
Os	174.3 ± 27.3	177.3 ± 12.9
Pb	8678.7 ± 103.3	6199.3 ± 330.0
Pd	4.3 ± 1.9	3.8 ± 0.4
Pt	19.4 ± 6.2	25.4 ± 4.0
Ru	40.0 ± 4.6	40.2 ± 7.8
Si	37296.4 ± 342.0	35459 ± 679.3
Zn	15538.3 ± 1638.7	22763.6 ± 1506.6

nd: not detected

C. Evaluation of Sulfur-Oxidizing Activity

Acidithiobacillus thiooxidans DSM 26636 was grown in 125-mL flasks containing 30 mL of modified Starkey mineral medium (with elemental sulfur at 1 %, w/v) and 1 % (w/v) of each mine tailing, for up to 21 days, at 30 °C and 150 rpm. At days 7, 14, and 21, sulfur oxidation was evaluated by determining sulfate ($SO4^{2-}$) concentration in the supernatant according to the NMX-k-436-1977 method. The production of sulfuric acid was assessed in the supernatant as well, by a titration procedure using NaOH 0.5 M with bromothymol blue as acid-base indicator, and by measuring pH using a digital potentiometer (Thermo Scientific, Orion) [7], [8].

D. Bioleaching Tests

An inoculum was grown in 125-mL flasks containing 30 mL of modified Starkey mineral medium (with elemental sulfur at 1 %, w/v). After four days of growth, 125-mL flasks containing 30 mL of medium were inoculated with $2x10^8$ colony forming units (CFU). After 21 days of growth, samples were filtered to separate the mine tailings from the cell culture, and solid mine tailing samples were dried at room temperature for 48 h. Afterwards, metal residual concentrations in these samples were determined as described below. Sets were prepared in triplicate, and three controls without inoculum were also prepared in order to assess abiotic removal of metals.

E. Digestion of Samples and Metal Analysis

Dry mine tailings (20 mg samples) were subjected to metal analysis using ICP-OES (Model 710-ES, Varian, Palo Alto, CA, USA) after acid digestion. Briefly, the samples were placed in cylindrical vials of silicon carbide, and acids were added (HCl 6 mL; HNO_3 2 mL). Then, the samples were digested in a Microwave Reactions System Multiwave PRO (Anton Paar, Ashland, VA, USA) using HF100 rotor. Digestion conditions were: power 800 W, 40 Bar, temperature 210 °C, with pRate of 0.3 bar seg-1, ramp 15 min, hold 30 min and cooling during the time needed to lower the temperature to 55 °C. Afterwards, 20 mL of deionized water was added to the cylindrical vials and each supernatant was collected in a volumetric flask, where volume was set to 50 mL with deionized water. Analyses for the diverse metal concentrations were performed at the following wavelengths (nm): Ag (328.068), Al (396.152), As (188.980), Au (242.794) Ba (455.403), Be (313.042), Cd (214.439), Co (238.892), Cr (267.716), Cu (327.395), Fe (238.204), Ir (224.268), Li (670.783), Mg (279.553), Mn (257.610), Mo (202.032), Ni (231.604), Os (225.585), Pb (220.353), Pd (340.458), Pt (214.424), Rh (343.488), Ru (267.876), Sb (206.834), Se (196.026), Si (251.611), Sn (189.925), Sr (407.771), Tl (190.794), V (292.401), and Zn (213.857). Metal residual concentrations were calculated based on a calibration curve of 0.25 - 6 ppm using two commercial standards (Cat. # ICP-200.7-6 and Cat. # ICP-MS-68A-C; High-Purity Standards, Charleston, SC, USA).

F. Statistical Analysis

Basic statistical parameters and analyses of variance (ANOVA) were performed using the commercial statistical software OriginPro 9.0. Differences with P values of ≤ 0.05 were considered statistically significant.

III. RESULTS

A. Evaluation of A. Thiooxidans Sulfur-Oxidizing Activity in the Presence of Mine Tailings

It has been previously reported that *A. thiooxidans* DSM 26636 is able to oxidize elemental sulfur to sulfuric acid in concentrations between 1 to 9% of the former, and in a pH range between 3 to 7 [8]. Thus, in order to evaluate the sulfur oxidizing activity of the strain in the presence of each mine tailing at 1 % (w/v) pulp density, *A. thiooxidans* DSM 2663 was grown in the presence of MT1 and MT2 residues at an initial pH of 3, 30°C, and 150 rpm for up to 21 days. As can be observed in Fig. 1, the microorganism was able to produce sulfates for up to 14870.9±3473.2 and 13574.5±786.2 mg/L in the presence of MT1 and MT2, respectively, not showing a significant production difference between the two different residues. Also, sulfuric acid concentrations and pH values in mine tailing samples were verified as indicators to support sulfur oxidizing activity; finding out that sulfuric acid concentration increased from day 7 and beyond in both tailing residues, from 0.02 M at the beginning of experimentation, to 0.20 M, and 0.22 M in day 7 and day 14 for MT1, and to 0.19

M, and 0.24 M for these same days in the case of MT2, until they reached concentrations of 0.32 and 0.25 M for MT1 and MT2 after 21 days of growth, respectively, and that pH descended consecutively from 3.0 in MT1 to 1; 0.87, and 0.64 at days 7, 14, and 21, respectively. In the case of MT2, initial pH was 3.0, diminishing to 1.02 and 0.89 in days 7 and 14, and finally reaching 0.63 after 21 days of growth (data not shown). In control system, pH remained unchanged throughout the treatment with final pH of 3.03 and 3.05 to MT1 and MT2 respectively.

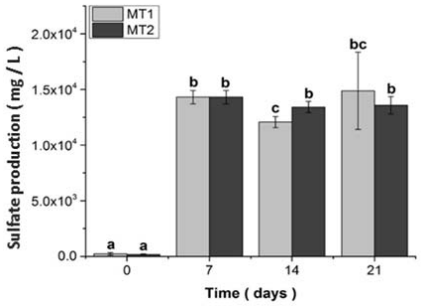

Fig. 1 Sulfate production of *A. thiooxidans* DSM 26636 in the presence of mine tailings at 30 °C and 150 rpm after 21 days of incubation

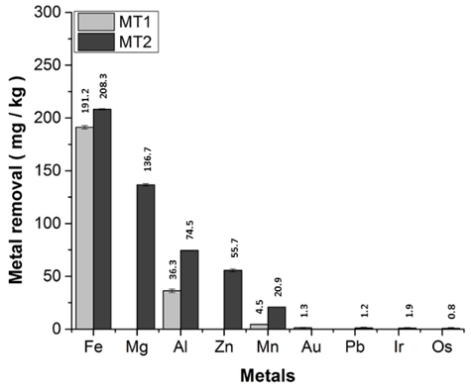

Fig. 2 Metal bioleaching by *A. thiooxidans* DSM 26636 in the presence of mine tailings at 30 °C and 150 rpm after 21 days of incubation

B. Metal Removal from Mine Tailings

Previous results have shown that *A. thiooxidans* DSM 26636 is able to lixiviate Al, Fe, Ni, and V contained in a high metal content hydrotreating spent catalyst [6]. Therefore, to assess this strain capability to remove the metals from the two different mine tailing samples, strain DSM 26636 was grown in the presence of MT1 and MT2 for 21 days, and then metal bioleaching was determined for the two high metal content residues. Abiotic lixiviation of each metal was also assessed and subtracted from each sample before reporting the biolixiviation results presented in Fig. 2, where it can be observed that Al, Fe, and Mn were removed from both mine tailings, in 36.3±1.7, 191.2±1.6, and 4.5±0.2 mg/kg for MT1, and in 74.5±0.3, 208.3±0.5, and 20.9±0.1 for MT2, respectively. Besides, 1.3±0.4 Au was biolixiviated from MT1, and bioleaching conditions in MT2 also favored the removal of 0.9±0.6 Ir, 0.8±0.6 Os, and 55.7±1.3 Zn.

IV. Discussion

An increment in sulfate production can be interpreted as an indicator of bioleaching efficiency, which is likewise correlated with sulfuric acid production and hence pH reduction [10]. The results obtained are in accordance with these previous observations, as sulfate production increased since day 7 and beyond in the presence of both mine tailing samples, and sulfuric acid concentration increased, descending the pH values.

The metal primarily bioleached from both mine tailings was Fe, which is interesting to note since it has been broadly established that *A. thiooxidans* close relative, *A. ferrooxidans* can oxidize this metal to catalyze the dissolution of minerals [11]. However, these results suggest the capability of *A. thiooxidans* DSM 26636 for Fe bioleaching contained in mine tailings.

Although the nature of the two residues may be similar as they represent solid residues coming from mining activities that show similar metal composition (Table I), and not differences were detected in sulfate production nor pH diminishing between both residues, there was observed highest metal bioleaching in the case of MT2, since besides Fe, Al and Mn were removed in a higher extent, and even more, metals like Mg and Zn were also removed in this residue but not in MT1.

In this regard, there has been stated that metal toxicity varies depending on each metal intrinsic properties, and that elements like As and Pb tend to be more toxic than other metals [12]. Also, synergistic, additive or even antagonistic actions have been reported when more than one two or more metals are simultaneously present in a system [13]-[15]. Thus, residue MT1 presents highest As and Pb concentrations, which may have caused a more elevated stress for the microorganism, producing a diminishing in the bioleaching efficiency in the case of this residue. Hence, the results presented in the current report show that *A. thiooxidans* DSM 26636 could be potentially used for the bioleaching of metals contained in mine tailings and in high Fe content residues, while further experimentation will be necessary to optimize this metal removal capabilities.

V. Conclusion

The bioleaching of metals from mine tailings by *Acidithiobacillus thiooxidans* DSM 26636 is feasible, since the microorganisms was able to remove Al, Fe and Mn in both samples. The bioleaching of other metals like Mg, Zn, Au, Pb, Ir and Os was also possible at different extents.

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