International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:12, No:11, 2018

Bioleaching of Metals Contained in Spent Catalysts by *Acidithiobacillus thiooxidans DSM 26636*

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Abstract**—**Spent catalysts are considered as hazardous residues of major concern, mainly due to the simultaneous presence of several metals in elevated concentrations. Although hydrometallurgical, pyrometallurgical and chelating agent methods are available to remove and recover some metals contained in spent catalysts; these procedures generate potentially hazardous wastes and the emission of harmful gases. Thus, biotechnological treatments are currently gaining importance to avoid the negative impacts of chemical technologies. To this end, diverse microorganisms have been used to assess the removal of metals from spent catalysts, comprising bacteria, archaea and fungi, whose resistance and metal uptake capabilities differ depending on the microorganism tested. Acidophilic sulfur oxidizing bacteria have been used to investigate the biotreatment and extraction of valuable metals from spent catalysts, namely *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferroxidans*, as they present the ability to produce leaching agents such as sulfuric acid and sulfur oxidation intermediates. In the present work, the ability of *A. thiooxidans* DSM 26636 for the bioleaching of metals contained in five different spent catalysts was assessed by growing the culture in modified Starkey mineral medium (with elemental sulfur at 1%, w/v), and 1% (w/v) pulp density of each residue for up to 21 days at 30 °C and 150 rpm. Sulfur-oxidizing activity was periodically evaluated by determining sulfate concentration in the supernatants according to the NMX-k-436-1977 method. The production of sulfuric acid was assessed in the supernatants 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. On the other hand, Inductively Coupled Plasma - Optical Emission Spectrometry was used to analyze metal removal from the five different spent catalysts by *A. thiooxidans* DSM 26636. Results obtained show that, as could be expected, sulfuric acid production is directly related to the diminish of pH, and also to highest metal removal efficiencies. It was observed that Al and Fe are recurrently removed from refinery spent catalysts regardless of their origin and previous usage, although these removals may vary from 9.5 ± 2.2 to 439 ± 3.9 mg/kg for Al, and from 7.13 ± 0.31 to 368.4 ± 47.8 mg/kg for Fe, depending on the spent catalyst proven. Besides, bioleaching of metals like Mg, Ni, and Si was also obtained from automotive spent catalysts, which removals were of up to 66 ± 2.2, 6.2±0.07, and 100±2.4, respectively. Hence, the data presented here exhibit the potential of *A. thiooxidans* DSM 26636 for the simultaneous bioleaching of metals contained in spent catalysts from diverse provenance.

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Keywords**—***Acidithiobacillus thiooxidans,* spent catalysts, bioleaching, metals, sulfuric acid, sulfur-oxidizing activity.

I. Introduction

DIVERSE anthropogenic activities have caused serious environmental threats. In this regard, metals are commonly present in liquid and solid residues from industrial processes [1]. It is known that metals are potentially dangerous not only for the environment, but also for human health due to their high toxicity, which causes detriment to living organisms through different mechanisms, as destabilization of cellular membranes, alterations in enzymatic specificities, disruption of cellular functions, and/or genetic material damage [2].

Spent catalysts are residues produced from diverse industrial processes that contain several metals in elevated concentrations, and which disposal produce serious environmental impacts. Hydrometallurgical, pyrometallurgical and chelating agent methods are currently available to remove and recover some metals contained in spent catalysts. However, these procedures transfer the pollution to the residues generated by these processes, because they produce potentially hazardous wastes and the emission of harmful gases [3]. To overcome these circumstances, biotechnological approaches have been assessed using different types of microorganisms: bacteria, archaea, or fungi, for the removal of metals contained therein [4].

Bioleaching technologies are novel approaches that allow metal removal from diverse solid industrial wastes, and present advantages in comparison with traditional processes of metal extraction, as they involve lower costs, lower energy requirements, and are simpler to perform and maintain, among other features [3]. Acidophilic sulfur oxidizing bacteria have been used to investigate the biotreatment and extraction of valuable metals from spent catalysts, namely *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferroxidans*, as they have the ability to produce leaching agents such as sulfuric acid and sulfur oxidation intermediates [5].

Previous studies have reported the promising capability of *Acidithiobacillus thiooxidans* for the bioleaching of metals like As, Zn, Pb, and Cu contained in mine tailings [6]. Specifically, *A. thiooxidans* strain DSM 26636, which was isolated from a Mexican soil with high sulfur content, is able to leach Al, Ni, V, Fe, Mg, Si, and Ni contained in slags from coal combustion wastes [7]. Moreover, this strain successfully lixiviated Al, Fe, Ni and V contained in a hydrotreating spent

catalyst [8].

In the present work, the ability of *A. thiooxidans* DSM 26636 for the bioleaching of metals contained in five different spent catalysts was assessed, observing that Al and Fe are recurrently removed from refinery spent catalysts regardless of their origin and previous usage. Besides, bioleaching of metals like Mg, Ni, and Si was also obtained from automotive spent catalysts. Hence, the data presented here exhibit the potential of strain DSM 26636 for the bioleaching of metals contained in spent catalysts from different provenance.

II. Materials and Methods

A. Microorganism and Culture Media

The sulfur-oxidizing bacteria used throughout this study was *Acidithiobacillus thiooxidans* AZCT-M1256, currently called DSM 26636, which was isolated from a Mexican soil with high sulfur content [9]. The culture medium used was a 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; 30 ppb of molybdenum as $Na_2MoO_4 \cdot 2H_2O$. Also, elemental sulfur was added at concentration of 1% (w/v). The medium was adjusted to pH 3 with sulfuric acid [10].

B. Spent Catalyst

Spent catalysts, coded as HDS2R, ULSD/2010, and CAT US were provided by Mexican Petroleum Institute, spent automotive catalyst (SAC) coded as SCA-M1 and SCA-M2 were provided by a company located in Mexico City. All catalysts were crushed to reduced particle size by using a mortar. Metal characterization was evaluated by inductively coupled plasma optical emission spectrometry (ICP-OES). Table I shows the metal composition of these spent catalysts.

TABLE I
Metal Composition of Spent Catalysts

Metals (mg/kg)	HDS2R	ULSD/2010	CAT_US	SCA-M1	SCA-M2
Ag	nd [a]	nd	nd	118.0	44.0
Al	152892	1935.0	47142.9	107760.5	112507.2
As	nd	nd	nd	114.6	nd
Ba	nd	nd	nd	8210.3	nd
Co	13220	nd	nd	nd	nd
Fe	86.2	880.2	44205.4	3138.2	3173.2
Li	307	308.7	309.3	309.4	302.9
Mg	780	6185.6	1743.9	37488.8	44097.5
Mn	nd	nd	579.1	183.4	nd
Mo	65578	56.2	nd	nd	nd
Ni	21	nd	nd	4200.5	6207.9
Si	nd	nd	nd	31861.8	48870.4
Zn	nd	nd	nd	177.8	nd
T	73	119.5	10870.3	1779.1	2267.4
Au	nd	25.1	nd	12.7	23.8
Ir	nd	12.3	nd	96.4	147.7
Os	1,7	4.3	283.8	nd	nd
Pd	nd	nd	nd	1931.8	2751.3
Pt	nd	15.1	nd	661.5	196.5
Rh	513.4	nd	nd	603.8	163.5
Ru	231.3	138.0	159.1	1862.9	1377.0

[a] nd = not detected

C. Growth of Acidithiobacillus thiooxidans DSM 26636 in the Presence of Spent Catalysts

The inoculum was prepared in 250 mL flasks containing 50 mL of modified Starkey medium at pH 3, elemental sulfur at 1% was added, and incubated at 30 ºC and 150 rpm during 3 days. Afterwards, the inoculum was used to study the ability of *A. thiooxidans* DSM 26636 to grow in presence of spent catalysts at 1 % (w/v) pulp density plus elemental sulfur at 1 %. Five different experimental sets were prepared as follows: 125 mL Erlenmeyer flasks containing 30 mL of modified Starkey medium were inoculated with 3 mL (10%) of the inoculum (2×10^8 CFU/mL) and supplemented with the corresponding spent catalyst. Flasks were incubated at 30 °C and 150 rpm, during 21 days. Controls without inoculum were also included. Every seven days, flasks of each experimental set were removed and each flask content was collected in 50 ml Falcon tubes and centrifuged at 6000 rpm during 7 min. The supernatants were collected in 50 mL glass tubes with screw caps and stored at 4 ºC. Microbial growth was evaluated through sulfate turbidimetry determination according to a Mexican standard method [11]. Each determination was performed in triplicate. The production of sulfuric acid in the supernatants was also evaluated by pH measurement, using a digital potentiometer (ORION, Model 310), and by titration using a 5 M sodium hydroxide solution with bromothymol blue as an acid-base indicator [12].

D. Bioleaching Activity

Liquid samples (1 mL) coming from day 21 of each treatment, including those samples from abiotic controls, were placed in cylindrical silicon carbide vials, and 6 mL of concentrated HNO_3 and 2 mL of concentrated HCl were added. Then, 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 ºC, with pRate of 0.3 bar/sec, ramp 15 min, hold 15 min, and cooling at 55 ºC. Afterwards, 20 mL of deionized water were added to the cylindrical vials, and the supernatant was collected and set to 50 mL using also deionized water. Assessments of Ag, Al, As, Ba, Be, Cd, Co, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sb, Se, Si, Sn, Sr, Tl, V, Zn, Au, Ir, Os, Pd, Pt, Rh, Ru, Ti and W, were performed at their respective wavelengths (nm): 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), Au (242.794), Ir (224.268), Os (225.585), Pd (340.458), Pt (214.424), Rh (343.488), Ru (267.876), Ti (336.122), W (207.912) by ICP-OES. Metal leaching was calculated based on a calibration curve of 0.25 to 6 ppm using High Purity commercial standards: Cat. #ICP-200.7-6; Cat. #ICP-MS-68A-C and Cat. #ICP-MS-68A-B. The effectiveness of the biotreatment was determined by the difference in the metal concentrations at the beginning and at the end of the treatment.

III. Results and Discussion

A. Ability of Acidithiobacillus thiooxidans DSM 26636 to Grow in the Presence of Spent Catalysts

For this study, a sulfur-oxidizing bacterium was used, which has previously shown the ability to grow autotrophically using elemental sulfur in concentrations between 1% and 9% [9], with a sulfur removal rate of 0.185 mg S g^{-1} h^{-1}, in the presence of 16.5% (w/v) pulp density of an industrial waste [13]. Thus, this study evaluated the ability of *A. thiooxidans* to grow in the presence of five different spent catalysts with elemental sulfur at 1% (w/v) during 21 days. Fig. 1 shows sulfate production of *A. thiooxidans* during sulfur oxidation in presence of spent catalysts coded as HDS2R, ULSD/2010 and CAT_US that came from a petrochemical industry, and SAC-M1 and SAC-M2 that came from catalytic converters. Under these conditions, the higher and the lower sulfur oxidizing activities of *A. thiooxidans* were detected in the automotive catalyst coded as SAC-M1, and petrochemical catalyst coded as HDS2R respectively, with a maximal sulfate production of 28.674 mg L^{-1} and 2.627 mg L^{-1} at the end of the biological treatment. The lower sulfur oxidizing activity in catalyst HDS2R could be attributed to the toxicity of metals like Co, which was absent in the other four spent catalysts (Table I). In presence of the spent catalysts HDS2R, ULSD/2010 and CAT_US, sulfate productions was similar after 7 and 14 days. Nevertheless, at day 21, sulfate production changed to 16.824 mg L^{-1} and 21.430 mg L^{-1} to ULSD/2010 and CAT_US respectively. In the case of SAC-M2, sulfur oxidizing activity increased to up to 15.951 mg L^{-1} at day 21. The low sulfur oxidizing activity of *A. thiooxidans* in presence of the spent catalyst coded as SAC-M2 at time 7 and 14 days could be possibly attributed to differences in its metal content, which is slightly higher for some toxic metals, as Al, Ni, Si and Pd, in comparison to the composition of SAC-M1 catalyst, although this premise would need further demonstration (Table I).

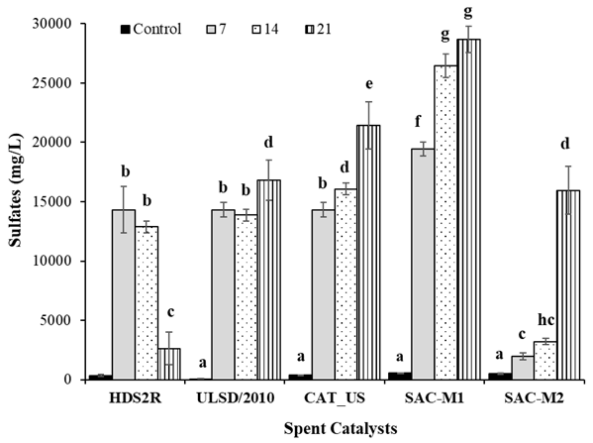

Fig. 1 Monitoring of sulfate production during microbial treatment of spent catalysts at 1% (w/v) pulp density by *A. thiooxidans* at 30 °C, 150 rpm, during 21 days. Statistically significant differences (one-way ANOVA) with Tukey's HSD ($P < 0.05$) are indicated by different lower case letters

Sulfuric acid is produced during sulfur oxidation, and it was detected by titration with a sodium hydroxide solution 5 M, using bromothymol blue as an acid-base indicator [12]. It was observed that in the presence of the spent catalysts coded as ULSD/2010, CAT_US and SAC-M1, sulfuric acid production was similar after 21 days of incubation, being of 0.262 M, 0.282 M and 0.263 M, respectively. However, in the presence of SAC-M2 and HDS2R, lower sulfuric acid concentrations were detected during all the incubation period (Table II).

TABLE II
Sulfuric Acid (M) Production during the Biological Treatment of Spent Catalysts

Spent catalysts	0d	7d	14d	21d
HDS2R	0.008	0.021	0.018	0.021
ULSD/2010	0.011	0.219	0.256	0.262
CAT_US	0.003	0.251	0.255	0.282
SAC-M1	0.003	0.193	0.250	0.263
SAC-M2	0.003	0.018	0.024	0.153

Regarding the pH, this parameter was set to 3.0 for all systems at the beginning of experimentation. In those treatments where *A. thiooxidans* was inoculated, sulfuric acid production caused a pH reduction within 0.53 – 0.73 at the end of the treatments, except for the catalyst coded as HDS2R, where not pH changes were detected (Table III), probably due to this spent catalyst toxicity induced by the presence of toxic metals like Co, or other metals of lowest toxicity, as Mo, or even more, the specific metal combination that is present in this catalyst. During bioleaching processes, metal oxides react with the acid and are solubilized as metal sulfates [14]. The pH decreases due to the bacterial activity and its acid production, which is involved in the bioleaching process. A similar behavior in the pH profile was reported by other bioleaching studies with spent refinery catalyst using sulfur and iron oxidizing bacteria [15]. The decrease in the pH of the medium is indicative of bacterial activity in the bioleaching system, since an increase or decrease in the pH is dependent on the consumption of acid by the material and/or by the bacteria, and the production of acid, resulting from biochemical sulfide oxidation reactions [16].

TABLE III
pH Changes during Biological Treatment of Spent Catalysts

Spent catalysts	0d	Control 21 d	7d	14d	21d
HDS2R	3.0	2.94	2.85	2.94	3.01
ULSD/2010	3.0	4.67	0.98	0.86	0.6
CAT_US	3.0	2.93	0.94	0.82	0.58
SAC-M1	3.0	4.91	0.63	0.49	0.53
SAC-M2	3.0	4.23	1.69	1.57	0.73

B. Metal Removal from Spent Catalysts

During *A. thiooxidans* growth, sulfuric acid is produced, and it can be used as a bioleaching agent of the metals contained in spent catalysts. This mechanism is called acidolysis, in which the oxygen atoms covering the surface of a metal compound are protonated. The protons and the oxygen combine with water and the metal is therefore detached from

the surface [17]. As can be observed in Table IV, sulfuric acid leached the metals Fe > Al from the catalyst coded as ULSD/2010; Fe > Ti > Al were leached from catalyst CAT-US; Al > Si > Mg > Fe > Zn > Ni were leached from catalysts SAC-M1, and finally, Al > Si > Mg > Fe > Ni were leached from catalyst SAC-M2 (Table IV). For the catalyst coded as HDS2R, no metal removal was observed at the end of the biological treatment, and this fact can be correlated with both lower sulfur oxidizing activity and sulfuric acid production (2627 mg L^{-1} and 0.021 M). Thus, there were observed different affinities for the bioleaching of metals contained in the five different spent catalysts. It has been reported that diverse parameters such as pH, pulp density, size particle and spent catalysts metal content have important effects on metal bioleaching efficiencies.

Heavy metals are able to exert harmful effects on microorganisms due to their strong coordinating capabilities [17]. The toxicity generated by the heavy metals in culture media may slow down or inhibit the growth of the microorganisms, and their activity as well. During sulfur oxidation, it has also been suggested that intermediate metabolites could affect metal extraction processes, being also suggested that thiosulfate and sulfite can help to mobilize metals from the solid material [15].

TABLE IV
BIOLEACHING OF METALS FROM SPENT CATALYSTS AFTER 21 DAYS OF TREATMENT

Metals leached (mg/kg)	ULSD/2010	CAT_US	SAC-M1	SAC-M2
Al	9.5 ± 2.2	110.7 ± 22.4	439 ± 3.9	237 ± 3.6
Fe	38.5 ± 0.6	368.4 ± 47.8	21 ± 2.2	7.13 ± 0.3
Ti	0	310.6 ± 16.9	0	0
Mg	0	0	66 ± 2.2	12 ± 3.3
Ni	0	0	5.4 ± 0.14	6.2 ± 0.07
Si	0	0	100 ± 2.4	48 ± 1.1
Zn	0	0	10.2 ± 0.21	0

Previous reports of *A. thiooxidans* DSM 26636 showed its ability to leach metals from slags and ashes at 1 % (w/v) pulp density, where metal removal was V > Fe > Mg > Al > Si > Ni, and Al > Ni > Sn > Mg > Zn > Si, respectively, observing the highest bioleaching efficiency in the case of ashes wastes [7]. The bioleaching process of spent catalysts can be considered as a heterogeneous solid–fluid reaction, which can be summarized in four sequential steps: (a) the diffusion of the attacking species from the bulk solution to the reactant; (b) the diffusion of the reactants through the solid matrix; (c) chemical reaction; and (d) the transfer of the resultant species to the bulk solution [17]. The metals present in a spent catalyst are therefore likely to exist in different chemical fractions, which will eventually affect their mobility and bioavailability [18]. Thus, the efficiency of the bioleaching process will also be largely dependent on the speciation of metals contained therein.

IV. CONCLUSION

This study evaluated the metal bioleaching due to sulfuric acid production by *A. thiooxidans* DSM 26636 in the presence of five different spent catalysts. The metal bioleaching affinity from spent catalysts was observed different. Although more studies will be needed to improve the metal bioleaching capability of *A. thiooxidans* DSM 26636, the data presented here exhibit the potential use of this strain for the bioleaching of metals contained in spent catalysts from diverse provenance.

ACKNOWLEDGMENT

This research received financial support by Secretary of Science, Technology and Innovation of Mexico City, Project SECITI/063/2016 and the Instituto Politécnico Nacional.

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