Acidithiobacillus thiooxidans DSM 26636: An Alternative for the Bioleaching of Metallic Burrs

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Abstract: Metallic wastes from the metal-mechanic industry represent a serious environmental problem. The possible strategies to reduce the metal content of these industrial wastes is their biotreatment by means of sulfur-oxidizing bacteria, such as Acidithiobacillus thiooxidans DSM 26636, which has been reported as an excellent metal-leaching microorganism by its capability to oxide sublimed sulfur and produce sulfuric acid in the presence of metallic burrs, and leach metals. The metallic composition of burrs was determined by ICP-OES before and after its exposure to biological treatment. The bioleaching process was kept for 21 days at 30 °C at an orbital shaking of 150 rev/min by using Erlenmeyer flasks of 125 mL containing 30 mL of Starkey-modified media added with 0.33 g (1% w/v) of sublimed sulfur and 0.33 g (1% w/v) of metal burrs, and 3 mL of inoculum at logarithmic phase. Results showed that A. thiooxidans was able to grow at these conditions with a maximum sulfate production of 11,028 mg/L, sulfuric acid corresponded to 0.16 M, but no statistical difference was observed for days 14 and 21. A reduction in pH was observed from 2.5 to 1.3 units. Metal bioleaching in mg/kg corresponded Fe (4658.5 ± 291), Cr (237 ± 46), Al (185 ± 12), Si (71 ± 10.3), Mo (63 ± 3.6), Mn (46 ± 3.3), V (18 ± 0.94), Mg (22.2 ± 3.7), Ni (15.8 ± 1.5), and Cu (5.7 ± 1.9). Results showed that A. thiooxidans DSM 26636 was able to grow in the presence of metal-containing wastes, and although metal removal was feasible, more studies are needed to enhance metal removal.

Keywords: Acidithiobacillus thiooxidans; metal burrs; leaching; metals; sulfuric acid; sulfur-oxidizing activity

1. Introduction

During the production of metal-containing parts, such as structures or any other products, large amounts of metallic burrs are produced; in most of the cases, these wastes are disposed of in landfills; in fewer cases, they are recycled; however, the high amount of metallic wastes generated has created a serious environmental concern. Metal burrs are produced during milling, turning, and facing and can be discharged outside the machining area through a burrs-conveyor or could be moved directly to the burrs disposal plant. On the other side, if the discharged metal burrs are not processed, a problem of space occurs due to the discharged metal chips occupying a lot of space [1]. Metal burrs could result in environmental pollution derived from their heavy metal content, so the accumulation of these heavy metals leads to major ecological and health problems. As it has been reported, heavy metals can cause malfunctioning of the cellular processes via the displacement of essential metals from their respective sites. Oxidative deterioration of biological macromolecules has been primarily found due to metal binding to DNA and nuclear proteins [2]. Not only its high level of toxicity is alarming, but the metallic resources also represent a viable option before the depletion of natural reserves by its non-renewable nature. It is, therefore, imperative to search for alternatives for the
exploitation of these resources that result in important technological developments and economic value. At the present time, the technologies employed for the treatment of solid wastes contaminated with metals are divided into physical, chemical, and biological methods [3]. Since these biological methods have the advantages of reducing energy costs, they have few industrial requirements and are safer for the environment [4]. Biohydrometallurgy is a promising technology for recovering metals from industrial wastes. In processes that operate at ambient temperature, the most important bacteria are iron- and sulfur-oxidizing Acidithiobacillus ferrooxidans, sulfur-oxidizing Acidithiobacillus thiooxidans and Acidithiobacillus caldus, and iron-oxidizing Leptospirillum spp. (Leptospirillum ferrip hilum and Leptospirillum ferrooxidans) [5,6]. However, other genera, such as Acidianus, Metallosphaera, Sulfobacillus, Sulfolobus, Ferroplasma, are usually present in commercial bioleaching processes [7]. Other organisms, including thermophiles Sulfobacillus thermosulfidooxidans, Bacillus stearothermophilus, and Metallosphaera sedula, and heterotrophic fungi, including Aspergillus niger, Penicillium Simplicissimum, and Cyanobacterium violaceum, have also been used to effectively dissolve various metallic fractions from e-wastes [8,9].

Acidithiobacillus thiooxidans, an extremely acidophilic, chemolithoautotrophic, Gram-negative, rod-shaped microorganism, is typically related to copper mining operations (bioleaching) and has been well studied for industrial applications. A. thiooxidans grows and survives autotrophically, utilizing elemental sulfur and reducing inorganic sulfur compounds (RISCs) as an energy source, but it cannot use energy or electrons acquired from the oxidation of ferrous iron (Fe (II)) for carbon dioxide fixation as well as other anabolic processes [10]. The metabolite, mainly sulfuric acid, plays a major role in bioleaching. The microbial leaching processes of industrial wastes, like fly ash, sewage sludge, sediment, tannery sludge, electronic scrap, spent battery, spent refinery catalyst, spent petroleum catalyst, spent fluid cracking catalyst, waste electric device, jewelry waste/automobile catalyst, have allowed the recovery metals, such as Al, Zn, Cu, Cd, Mn, Ni, Sn, Co, Li, V, Mo, Au, Ag, Pt [6,11–13]. Based on the previous data, the purpose of the investigation was to show the potential of Acidithiobacillus thiooxidans DSM 26,636 to grow in the presence of metal burrs and produce sulfuric acid as a leaching agent to remove metals from an industrial waste that came from a metal-mechanical industry.

2. Results

2.1. Sulfur-Oxidizing Activity of Acidithiobacillus Thiooxidans in the Presence of Metal Burrs

The present results report the potential use of A. thiooxidans DSM 26,636 to leaching metals contained in metal burrs in the presence of sublimed sulfur, both at 1% (w/v). During sulfur-oxidizing activity, sulfate is produced, and pH reduction due to sulfuric acid production is detected. A. thiooxidans shows the ability to oxidize sublimed sulfur in the presence of metal burrs. During sulfur-oxidizing activity, sublimed sulfur is oxidized by the microorganism to sulfate, whose concentration increases over time, reaching values of 11,028 mg/L at day 21, without statistical difference at days 14 and 21 (Figure 1).

In the presence of metal burrs, the microorganisms reach the highest concentration of 0.15 M (14.7 g/L) of sulfuric acid at days 14 and 21 (Figure 2), and the non-statistical difference is detected between the two days.
Figure 1. Monitoring of sulfates production during microbial treatment of metal burrs plus sublimed sulfur at 1% each by *A. thiooxidans* at 30 °C, 150 rev/min. Statistically significant differences (one-way ANOVA with Tukey’s HSD (*p* < 0.05)) are indicated by different letters. In the presence of metal burrs, the microorganisms reach the highest concentration of 0.15 M (14.7 g/L) of sulfuric acid at days 14 and 21 (Figure 2), and the non-statistical difference is detected between the two days.

Figure 2. Production of sulfuric acid by *A. thiooxidans* during the treatment of metal burrs plus sublimed sulfur at 1% each, incubated at 30 °C, 150 rev/min. Statistically significant differences (one-way ANOVA with Tukey’s HSD (*p* < 0.05)) are indicated by different letters.

Figure 3 shows changes in pH due to sulfuric acid production by *A. thiooxidans* in the presence of metal burrs, and the pH decreases from 2.5 to 1.7 at day 7 and 1.1–1.3 without significant statistical difference between days 14 and 21. In the control system, the presence of metal burrs originates an increase in pH, reaching values of 5.9 to 6.2 without significant statistical difference between days 14 and 21. An increase in sulfate concentration (Figure 1) is accompanied by the increase in sulfuric acid (Figure 2) and the decrease in pH of the culture medium.
2.2. Bioleaching Activity during Metal Burrs Treatment

During sulfur oxidation by *A. thiooxidans*, sulfuric acid is produced and then can be used as a bioleaching agent to remove metals contained in metal burrs, situation that can be attributed to protons that attack metal burrs, enhancing the leaching of metals [14]. The metals leached during biological treatment were evaluated by ICP-OES after 21 days of treatment. Figure 4 shows concentrations of metal removal in mg/kg and removal in percentage, Fe (4658.5; 1.1%); Cr (236.7; 0.7%); Al (184.5; 1.7%); Si (71.3; 0.5%); Mo (63.2; 0.7%); Mn (45.3; 1.0%); Mg (22.2; 1.2%); V (17.9; 0.2%); Ni (15.8; 1.0%); and Cu (5.7; 0.9%).

3. Discussion

Metallic content of metal burrs shows that these wastes are mainly composed of Fe > Cr > Si > Al > Mo > V > Mn > Mg > Ni > Cu > Sb > Li > Zn. Zhai and Yuan [15] analyzed metal burrs provided...
provided by the Briggs and Stratton Corporation by the ICP-MS method, showing that they were mainly composed of iron (67.30%) and aluminum (32.70%). In the present study, lower concentrations for iron and aluminum were found to be 41% and 1.1%, respectively. The metal composition depends on the kind and origin of the industrial wastes; although the metal content is too diverse, some of them like Cr, Cd, Pb, Zn, Cu, Ni, As, Hg are toxic and carcinogenic and cause various diseases and disorders in human, animals, and aquatic life, so their removal is desirable prior to their disposal [16].

In the case of metal burrs, recycling can enhance the economic profit, reducing the environmental impact of the manufacturing industry; since iron is the most commonly used metal, its recycling requires firstly separating the ferrous and non-ferrous metal burrs. Currently, there is a lack of effective and economical techniques in separating these ferrous and non-ferrous metal burrs to fulfill the needs of the manufacturing industry. Current manufacturing companies either put these wastes in landfills or pay a third party to drag them away [15]. On the other hand, the growing demand for metals as solid urban wastes, such as fly ash, electronic scrap, and spent catalysts, may be considered as “secondary ores” for metal recovery, so most studies are focused on bioleaching of these kinds of wastes. Due to the increasing pressure for industries to adopt environmentally, friendly, and sustainable processes, bioleaching could be considered as a clean and green technology for metal extraction [17].

In the presence of sulfur and RICSs, sulfur-oxidizing microorganisms, such as *A. thiooxidans*, produce sulfuric acid, which is considered a bioleaching agent. Sulfur can exist in various oxidation states, which results in a variety of RISCs, including tetrathionate, thiosulfate, sulfite, sulfide, and elemental sulfur. A variety of enzymes and proteins are involved in the oxidation of RISCs. The sulfur-metabolic enzymes in *Acidithiobacillus* spp. can be categorized as elemental sulfur oxidation enzymes, enzymes in thiosulfate oxidation, sulfide oxidation enzymes, and sulfite oxidation enzymes. These enzymes work cooperatively to oxidize the RISCs to sulfate [18].

Previous studies with *A. thiooxidans* DSM 26,636 have shown its capability to remove sulfur contained in spent catalyst at different pulp densities, within 8.25 to 100% (w/v) corresponding to different sulfur concentrations within 0.7 to 8.63%; under these conditions, the maximum sulfate production is 281 and 262 mg/L of solid waste at 8.25 and 16.5% (w/v) of pulp density, and the concentrations higher than 33% totally inhibit the sulfur-oxidizing activity [19]. *A. thiooxidans* DSM 26,636 has demonstrated its ability to oxidize sulfur when it is added when treating wastes from a coal combustion plant (slag and ash) [20], mine tailing (MT) [11], and spent catalyst from petrochemical industries and catalytic converter [12], all of them in the presence of 1% (w/v) of solid waste. Results show a maximal sulfate production of 28,544 mg/L and 1700 mg/L at day 14 from ashes and slags, respectively [20]. In the presence of mine tailing MT1 and MT2, the sulfate production is 14,871 and 13,575 mg/L, respectively, during 21 days, and no differences are observed for both the residues [11].

In the presence of spent catalyst from the petrochemical industry (HDS2R, ULSD/2010, and CAT_US) and catalytic converters (SAC-M1 and SAC-M2), the sulfate production in mg/L is 2627, 16,824, 21,430, 28,674, and 15,951 respectively, at the end of 21 days [12]. Thus, in the presence of solid wastes-containing metals, different factors and parameters can affect the sulfur-oxidizing activity of microorganisms, so the bioleaching process would be optimized by the manipulation of the follows parameters: (a) pulp density/solid–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 to the microorganism, (q) microbial requirements, (r) metal tolerance, (s) microorganisms adapted to waste, (t) waste’s physiochemical properties, and (u) type of released metal, all of them could affect the sulfur-oxidizing activity [21–24]. Besides, it has been mentioned that during bioleaching, aeration with compressed air has a positive influence on sulfur-oxidizing activity, and an increase in the sulfate concentration improves the bioleaching process by the bacteria [24]. So, the manipulation of the experimental conditions plays an important role in order to reach higher leaching efficiency by the
microbial way [23]. In the case of *A. thiooxidans* DSM 26,636, it has the ability to produce sulfuric acid within 0.04 M to 0.32 in the presence of different solid wastes as slags, ashes, mine tailing, spent catalyst of petrochemical and automobile industries, all of them at 1% when elemental sulfur is added at 1%; a lower concentration of sulfuric acid is correlated with a lower sulfur-oxidizing activity [11,12,20]. Few studies have focused on metal bioleaching from metallic burrs. Yamanea et al. [25] investigated the influence of bacterial adaptation on copper bioleaching from printed circuit boards. Results showed that in the case of a bacterial adaptation, the final pulp density reached after 3 months was of 28.5 g/L (2.85% w/v); at this pulp density, the extraction of copper was 64% for printers and 52% for computers, but at pulp densities below 25 g/L, the extraction of copper was above 85% for both. The previous report shows that during sulfur removal from spent catalyst containing 7.2% sulfur at 16.5% (w/v) of pulp density, the pH value decreases during the log phase from 1.95 to 1.4, which indicates that bacteria can oxidize elemental sulfur to sulfates [19]. During bio-treatment of slags, ashes, mine tailing, spent catalyst of petrochemical and automobile industries, a pH reduction is detected with values within 3.0 to 0.53 units due to sulfuric acid production [11,12,20]. In the presence of metal burrs and elemental sulfur at 1% (w/v) each, *A. thiooxidans* can oxidize the elemental sulfur using it as a sulfur energy source, then protons are produced, maintaining the pH for metal leaching, sulfates are detected at 11,028 mg/L, sulfuric acid at 0.15 M (14.7 g/L), and pH changes are within 2.5 to 1.3 (treatment) and 3.0 to 6.2 (control) after 21 days of treatment. During bioleaching of metals contained on metal burrs and due to higher iron concentration detected, the most probable mechanism implied could be the next: in the presence of elemental sulfur, *A. thiooxidans* oxidizes the sulfur, producing sulfates and protons (Equation (1)), then the sulfuric acid produced causes metal solubilization, leaching Fe mainly and other metals in a low quantity (Equation (2)) [14,26]. The previous supposition is due to the observation of brown precipitates, possibly due to the presence of ferrous sulfate (data not shown).

\[
\begin{align*}
2S^0 + 3O_2 + 2H_2O & \rightarrow 2SO_4^{2-} + 4H^+ \quad \text{(Microbial)} (1) \\
H_2SO_4 + Me^*O & \rightarrow H_2O + Me^*SO_4 \quad \text{(Chemical)} (2)
\end{align*}
\]

*Me = Fe, Cr, Al, Si, Mo, Mn, V, Ni, Cu

The metal selective recovery from bio-leachates could be done by biosorption, bioelectrochemical, and bioprecipitation processes; these strategies require low energy input and are being integrated into novel hydro-, bio-, and hybrid-metallurgical system [27]. During the sulfuric acid quantification by titration, sodium hydroxide 0.5 M is used for leaching, causing changes in pH, and the appearance of a brown-dark precipitate is detected, but the content of metals is not quantified. Bioleaching uses microorganisms (bacteria or fungi) to mobilize and leach metals from solid materials based on three principles: (i) the transformation of organic or inorganic acids (protons); (ii) oxidation and reduction reactions; (iii) the excretion of complexing agents. Bioleaching is an emerging technology that shows considerable potential for adding value to the industry for delivering attractive environmental and social benefits to all the associated industries [28]. *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* are the main microorganisms involved in the process of metal compounds dissolution. These bacteria can grow by obtaining energy from the oxidation of ferrous iron (Fe$^{2+}$) or elemental sulfur (S$^0$) and thereby carrying metal solubilization. Such metal bioleaching techniques have been used in biomining on an industrial scale. This metal-extraction property of microorganisms has been exploited for metal machining [29]. The *Acidithiobacillus* group possesses genes for most of the elements known to human society. These genes determine transport systems of nutritive substances, including K, P, and Fe, which are necessary to intercellular balance between needs and toxicity, as well as for detoxification or elimination of toxic elements, such as Hg, Pb, As, Cr, Cd, and Ag [30]. Nareshkumar et al. [31] carried out bioleaching of heavy metals from contaminated soil by *A. thiooxidans* varying sulfur/soil ratio from 0.03 to 0.33 (1–10 g/L of sulfur). It was observed that the bioleaching efficiency was assessed based on a decrease in pH, oxidation-reduction potential, sulfate production, and solubilization of heavy
metals from the soil. It was shown that solubilization of Cr, Zn, Co, Pb, Cd from the contaminated soil was in the range of 11–99%. Previous study with *A. thiooxidans* DSM 26,636 has shown its ability to produce sulfuric acid during bio-treatment of slags and ashes at 1% (w/v) and elemental sulfur; at the end of time incubation, V > Fe > Mg > Al > Si > Ni leach from slags, and Al > Ni > Sn > Mg > Zn = Si for ashes. The higher leached metal concentration is detected for ashes, and these differences could be attributed to the properties of the raw material, as it has been reported previously [20]. During metal bioleaching from mine tailings by *A. thiooxidans*, a metal leaching in mg/kg is observed for Al (36.3±1.7), Fe (191.2 ± 1.6), and Mn (4.5 ± 0.2) for MT1 and Al (74.5 ± 0.3), Fe (208.3 ± 0.5), and Mn (20.9 ± 0.1) for MT2. Besides, 1.3 ± 0.4 Au is bioleached from MT1 and 0.9 ± 0.6 of Ir, 0.8 ± 0.6 of Os, and 55.7 ± 1.3 of Zn from MT2 [11]. From different spent catalysts, *A. thiooxidans* shows the ability to leach the following metals in mg/kg, Fe (38.5 ± 0.6) > Al (9.5 ± 2.2) from the catalyst coded as ULSD/2010; Fe (368.4 ± 47.8) > Ti (310.6 ± 16.9) > Al (110.7 ± 22.4) from catalyst CATUS; Al (439 ± 3.9) > Si (100 ± 2.4) > Mg (66 ± 2.2) > Fe (21 ± 2.2) > Zn (10.2 ± 0.21) > Ni (5.4 ± 0.14) from catalyst SAC-M1, and Al (237 ± 3.6) > Si (48 ± 1.1) > Mg (12 ± 3.3) > Fe (7.13 ± 0.3) > Ni (6.2 ± 0.07) from catalyst SAC-M2. For the catalyst coded as HDS2R, no metal removal is observed, this fact can be correlated with both lower sulfur-oxidizing activity and sulfuric acid production [12]. Ferreira et al. [32] evaluated the recovery of heavy metals by *A. thiooxidans* FG-01 from a spent catalyst from diesel hydrodesulfurization (HDS), without chemical, thermal, or physical pretreatment by two-stage (indirect) bioprocess at 50 g/L of pulp density; the best recovery results observed were 26% Al, 26% V, and 39% Mo, and it was not possible to recover Co, Cu, and Ni (<5%) under any of the tested conditions. Some studies have focused on microbial leaching of solder waste for metal recovery by *Aspergillus niger* and *A. ferrooxidans* separately. The culture supernatant of *A. niger* shows better metal removal than *A. ferrooxidans*. Metals are removed faster from Sn–Cu–Ag solder than Sn–Cu and Sn–Pb solder by *A. niger* culture supernatant. Considering the toxicity of metals toward microbial cells, a two-step bioleaching process is found to be effective. In the bioleached solution from Sn–Cu–Ag, the addition of NaOH and NaCl precipitates Sn and Ag, respectively. The H2S gas at pH 8.1 selectively precipitates Pb from the Sn–Pb bioleached solution [33]. Many developed countries send their e-wastes to several countries, such as Malaysia, Thailand, Nigeria, Ghana, Indonesia; however, once these countries stop doing this, the recycling of e-waste will be a challenge for several well-developed countries. In fact, e-waste management is not well regulated, particularly in many developing countries [13]. The correct disposal and treatment of hazardous solid wastes containing metals will avoid problems of soil, air, and water pollution with the consequent alteration of the ecosystem and damage to animals, plants, and humans. Then, it is urgent to develop eco-friendly technologies for the recycling of metal burrs; the recycling of these metal burrs can enhance the economic profit and reduce the environmental impact of the manufacturing industry. The use of *A. thiooxidans* could represent a low-cost process for the recycling of these materials.

4. Materials and Methods

The methodology used to determine the sulfur-oxidizing activity and metal removal by *Acidithiobacillus thiooxidans* in the presence of metal burrs plus sublimed sulfur, both at 1% (w/v) of pulp density, was based on the previous research of our group investigation [11,12,20].

4.1. Bacterium Strains and Growth Condition

*Acidithiobacillus thiooxidans* DSM 26,636, previously coded as AZCT-M125-6, is a sulfur-oxidizing bacteria, isolated from Tourist Camp at National Park “The Sulfur”, located in Michoacán State in Mexico [34]. The modified Starkey medium was used for inoculum preparation and for bioleaching experiments of metals, and it was prepared by adding the following salts in (g/L): KH2PO4 (3), (NH4)2SO4 (0.2), MgSO4·7H2O (0.5), CaCl2·2H2O (0.3), FeSO4·7H2O (0.1), 30 ppb of molybdenum as Na2MoO4·2H2O. The pH of the medium was adjusted to 3 with concentrated H2SO4, and sublimed sulfur USP (Fermont CAS 7770-34-9) was added at a concentration of 1% (w/v) [11,12,20,35]. The culture medium was autoclaved at 10 psi for 20 min to avoid the agglomeration of sublimed sulfur.
4.2. Industrial Waste

Metallic burrs were obtained from an industry located in Mexico City, there were cut with scissors and then crushed in a crushing machine to reduce particle size within 10–15 mm long and 1–2 mm wide, and they were stored at room temperature until use. Metallic content was determined by ICP-OES \([11,12]\). For digestion, \(50 \pm 0.6\) mg of burrs were used, and the digestions were held for 45 min. Analysis of metals and digestion conditions are mentioned later. The metal composition of burrs is shown in Table 1.

Table 1. Metallic content of burrs determined by ICP Optical Emission Spectrometers.

| Element | Content in mg/kg |
|---------|------------------|
| Fe      | 410,159.5 ± 21,163.2 |
| Cr      | 39,242.3 ± 2428.2 |
| Si      | 13,819.0 ± 5460.8 |
| Al      | 11,126.9 ± 13,030.5 |
| Mo      | 9664.9 ± 573.4 |
| V       | 7453.1 ± 440.6 |
| Mn      | 4737.9 ± 248.2 |
| Mg      | 1894.0 ± 1287.6 |
| Ni      | 1611.4 ± 229.1 |
| Cu      | 829.9 ± 122.7 |
| Sb      | 450.0 ± 47.4 |
| Li      | 298.3 ± 7.0 |
| Zn      | 176.2 ± 76.3 |

4.3. Bioleaching of Metal by Acidithiobacillus thiooxidans DSM 26,636

To obtain the inoculum of Acidithiobacillus thiooxidans DSM 26,636 in log phase, 250 mL Erlenmeyer flasks were prepared containing 50 mL of modified Starkey medium at pH 3 plus sublimed sulfur USP (Fermont CAS 7704-34-9) at 1% (0.5 g) and incubated at 30 °C, 150 rev/min during 4 days. The inoculum obtained was adjusted at \(2 \times 10^8\) CFU/mL by count in the Newbauer Chamber (Blau Brand, Germany) and used for the study of Acidithiobacillus thiooxidans DSM 26,636 in the leach metals in the presence of metal burrs plus sublimed sulfur at 1% \((w/v)\) each. Experimental sets consisted of Erlenmeyer flasks (Fischer Scientific, Madrid) of 125 mL containing 30 mL of modified Starkey medium added with 0.33 g (1% \(w/v\)) of metal burrs plus (0.33 g) (1% \(w/v\)) of sublimed sulfur and inoculated with 3.0 mL (10%) of the inoculum. The inoculum obtained was used to study the ability of Acidithiobacillus thiooxidans DSM 26,636 to grow and leach metals in the presence of metal burrs plus sublimed sulfur at 1% \((w/v)\) each. Eighteen Erlenmeyer flasks were incubated for 21 days, 30 °C temperature, and orbital shaking of 150 rev/min. As controls, nine Erlenmeyer flasks contained 33 mL of modified Starkey medium, plus 0.33 g (1% \(w/v\)) of metal burrs and 0.33 g (1% \(w/v\)) of sublimed sulfur were not inoculated and included to determine abiotic sulfur-oxidizing activity. Six inoculated flasks and three controls were kept out every seven days; the total content of the flasks was centrifuged at 7000 rev/min for 8 min, and the supernatants free of cells were filtered by using syringe-driven filter unit of membrane-type MF-Millipore (Mixed cellulose esters, Merck KGaA, Darmstadt, Germany) of 0.22 \(\mu\)m (Catalogue number R3HN06272) and collected in glass tubes and stored at 4 °C until use. During A. thiooxidans growth, the sublimed sulfur was transformed into sulfuric acid through oxidation-reduction reactions. The amount of sulfate ion in the supernatants was determined according to the NMX-K-436-1977 method by triplicate \([36]\). The concentration of sulfuric acid was evaluated by titration with NaOH 0.5 M with bromothymol sulfone phthalein at 0.25% dissolved in 20% ethyl alcohol as pH indicator as well as pH measurement in supernatants \([20,37]\).
4.4. Digestion and Analyses of Metals

At the end of treatment, liquid samples (1 mL) were put in cylindrical silicon carbide vials, plus 8 mL of a mixture of concentrated HNO$_3$:HCl in a 3:1 ratio. Samples were placed in an HF100 rotor, and the digestion was carried out by using the Multiwave PRO of Anton Paar. The conditions of sample digestion were for six vessels: ramp and hold 15 min, respectively, and cooling at 55 °C. The analysis 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 was done by using an inductively-coupled plasma optical emission spectrometry ICP-OES, Varian Model 710-OES (Agilent, USA), and a calibration curve of 0.25 to 6 ppm of the commercial standards High Purity (Cat. # ICP-200.7–6) was used [20,21]. The metals analyzed and wavelengths (nm) used were as follows: Li (670.783), Ba (455.403), Sr (407.771), Al (396.068), Cu (327.395), Be (313.042), V (292.401), Mg (279.553), Cr (267.716), Mn (257.610), Si (251.611), Co (238.892), Fe (238.204), Cd (214.439), Zn (213.857), Mo (206.834), Se (196.026), Tl (190.794), Sn (189.925), and As (188.980). Metal removal from the metallic burrs was calculated by the difference in concentration (mg/kg) between day 0 and day 21 and subtracting the abiotic loss of metals in control systems. Removal is reported in percentage, and it was calculated by the following formula.

\[
\text{Removal percentage} (\%) = \left( \frac{\text{Metal content in control system at day 21 in mg/kg} \times 100}{\text{Metal removal at day 21 in mg/kg}} \right)
\]

5. Conclusions

The sulfur oxidation is an essential physiological feature of *Acidithiobacillus thiooxidans* and has attracted extensive attention for metal leaching from different solid wastes-containing metals. *A. thiooxidans* DSM 26,636 oxidizes sublimed sulfur and produces sulfuric acid in the presence of metal burrs from machining processes; this ability can be used as an eco-friendly method to treat these kinds of solid wastes, reducing their metal content, avoiding environmental problems. The metals leached could be recovered and reused for another application.

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