

KINETIC MODEL OF BIOLEACHING OF WASTE FROM THE NICKEL INDUSTRY WITH ACIDITHIOBACILLUS THIOOXIDANS

ABSTRACT

The improper disposal of nickel mining waste represents a major environmental concern, especially in countries like Cuba with significant nickel and cobalt reserves. This study investigates the bioleaching potential of the sulfur-oxidizing bacterium *Acidithiobacillus thiooxidans* applied to Cuban nickel industry waste. Experimental procedures included growth media preparation, physical-chemical and mineralogical characterization of mining residue, and bioleaching trials at varying temperatures (27°C, 32°C, and 37°C) and sulfur concentrations (0.5%, 1%, and 2% as pyrite). Bacterial growth and leaching efficiency were monitored through pH control and cell counts. The best metal recovery was obtained using 1.1% sulfur, with extraction efficiencies of 59.46% for Ni, 78.13% for Co, 24.80% for Fe, and 81.26% for Mg. Kinetic analysis using the Michaelis-Menten model yielded constants of $K = 0.589^\circ\text{C}$ and $V_{\text{max}} = 0.73 \text{ cells/mL}\cdot\text{day}$. These results highlight the adaptability and effectiveness of *A. thiooxidans* in the biotechnological treatment of mining waste, offering a sustainable solution for mitigating environmental impact.

Keywords: bioleaching, *Acidithiobacillus thiooxidans*, nickel waste, kinetic modeling, Michaelis-Menten, environmental remediation, heavy metals recovery

1. INTRODUCTION

The Initial research on the bioleaching of nickel minerals was conducted using heterotrophic microorganisms such as fungi (e.g., *Aspergillus niger* and *Penicillium*). However, their use led to reduced efficiency in metal recovery due to reabsorption by the biomass and spore production. These spores dispersed easily through the air, contaminating plants, animals, and humans (Decheng J. et al., 2019; Han W. et al., 2021).

To overcome these disadvantages, recent studies have explored the use of autotrophic microorganisms in the bioleaching of nickel ores (laterites), particularly sulfur-oxidizing bacteria like *A. thiooxidans*, which pose no health risks. Research by Mohammad J. et al. (2019) and Rory A. et al. (2021) demonstrated that thermoacidophilic microorganisms adapted well to the pulp and enabled high recovery rates of copper and cobalt.

A. thiooxidans, characterized by Waksman and Joffe (1921), belongs to a family of autotrophic bacteria that obtain energy from inorganic substances and carbon from atmospheric CO_2 . It is believed to be among the earliest life forms on Earth, potentially contributing to the foundation of organic life. This genus comprises several Gram-negative, rod-shaped bacteria, known for their

sulfur chemolithotrophic metabolism. Electron donors include compounds like H_2S , S° , and S_2O_3 (Gholamreza K. et al., 2022; Meicen L. et al., 2023). MDPI reviews and pure-culture studies between 2020–2023 demonstrate that *A. thiooxidans*, alone or in consortia with *A. ferrooxidans*, can recover >80 % of Ni and Co from PCBs under optimized pH and aeration regimes.

Studies focused on bioleaching of spent Ni-based catalysts using *A. thiooxidans* show extraction efficiencies between 90–95 %. For example, Tayar et al. (2020) reported up to 94.4 % Ni recovery from Ni/ Al_2O_3 catalysts using *A. thiooxidans* in a two-step leaching process, highlighting its potential for industrial residue valorization.

Frontier studies apply shrinking-core and Michaelis–Menten models to describe Ni dissolution rates. Yi et al. (2021) describe thiosulfate-mediated pathways, noting ferric diffusion as rate-limiting. Such models facilitate scale-up from bench to pilot reactors.

Beyond single cultures, combining *A. thiooxidans* with thermoacidophiles like *Acidithiobacillus caldus* improves leaching of metallurgical slags and steelmaking dust. Novel consortia recover Ni along with Mo and V, with >60 % extraction in lab columns. Studies applying spent-media bioleaching address metal-ion stress in Li-ion waste. They show that controlling metal toxicity via periodic medium renewal significantly enhances Ni and Co recovery in acidophiles.

Another prominent application of *A. thiooxidans* is in the bioleaching of mining waste, where the residue is pre-treated with stoichiometric sulfur. Lingkai W. (2023) and Guanglin C. (2023) studied the kinetics and transformation of waste containing Ni, Zn, Mn, Fe, and Cu, establishing Michaelis-Menten constants for bacterial growth.

Recent work (2025) by Li et al. optimized spent-medium bioleaching under high pulp density (up to 40 g/L) with *A. thiooxidans*, achieving ~40 g/L sulfate and $\Delta\text{pH} \sim 1.87$. This study emphasized the need for sulfur dosage and inoculum tuning to sustain bacterial activity under stress

Cuba ranks sixth globally in nickel and cobalt reserves, generating approximately 70 million tons of mining waste annually. This untreated waste poses a severe environmental threat, impacting soil, flora, and fauna (Rodriguez Y. et al., 2001). This study aims to evaluate the feasibility of using *A. thiooxidans* for the bioleaching of Cuban nickel industry waste to mitigate environmental damage.

2. MATERIALS AND METHODS

This study involves examining the microorganism, its growth medium, nickel mining residue, and pyrite concentrate, which serves as a sulfur source.

2.1 PREPARATION OF THE GROWTH MEDIUM

According to Samir P. et al. (2020) and Guanglin C. et al. (2023), A. thiooxidans requires specific conditions for optimal growth. One of the most effective media is the 0K medium (iron-free), which contains essential nutrients and the necessary acidity. Table 1 lists its chemical composition. The reagents are dissolved in one liter of distilled water acidified with H₂SO₄ to pH 2.5, sterilized at atmospheric pressure, and supplemented with 0.5% to 2% sulfur.

Table 1 Chemical Composition of 0K growth medium

Chemical Compounds	Concentración (g/L)
(NH ₄) ₂ SO ₄	3,0
KCl	0,1
K ₂ HPO ₃	0,5
MgSO ₄ ·7H ₂ O	0,5
Ca (NO ₃) ₂	0,01

2.2 MINING WASTE CHARACTERIZATION

The success of the bioleaching process largely depends on the characteristics of the waste material. Therefore, physical, chemical, and mineralogical analyses are conducted as a baseline. Samples are collected from 69 wells based on the geological prospecting method outlined by Rodriguez Y. et al. (2001).

2.3 PHYSICAL, CHEMICAL AND MINERALOGICAL CHARACTERIZATION

The Samples are subjected to particle size analysis per ISO 4701:2019 for iron ores, covering particles from 36 µm to 55 mm. The mass retained on each screen is measured to generate a granulometric curve. To determine the elemental distribution before and after bioleaching, chemical characterization is performed using an ICP-AES Spectroflame model.

Mineralogical analysis involves X-ray diffraction to identify fine mineral components not easily detectable by other methods. Diffractograms are obtained using the powder method, with a 0.050° angular pitch and 3-second measurement intervals. Mineralogical characterization, are used and roentgenographic analyses are carried out to identify more precisely the nature of the mineral components of the deposit, especially those that, due to their small grain size, are not easily identifiable with other techniques. Diffractograms are performed by the powder method and recorded according to the point-to-point measurement variant; with angular pitch of 0,05⁰, at a measurement time of 3 seconds at each angular position.

2.4 MICROORGANISM GROWTH METHOD

An inoculum is prepared from an active culture in the exponential phase (10⁸ cells/mL). Twelve mL are added to 90 mL of 0K medium adjusted to pH 2.5 in 300 mL Erlenmeyer flasks. Tests are conducted at room temperature (27°C), and at 32°C and 37°C using an orbital shaker and thermostatic control, with 1% pre-sterilized elemental sulfur. Samples are shaken at 2.5 s⁻¹ (150

rpm) and monitored for pH and cell count until exponential growth is observed. Figure 1 lists the equipment used.



Figure 1. Equipment used: (1) Bioreactors, (2) reactor, (3) Stirrer screen, (4) Orbital screen with thermostat, (5) Analytical balance, (6) Optical microscope. Source: authors

2.5 WASTE BIOLEACHING METHOD

10% solid pulp samples are placed in three agitated batch tanks (TK1 to TK3) at 27°C, 32°C, and 37°C, respectively. *A. thiooxidans* inoculum and pyrite (0.93 g, 1.87 g, and 3.77 g, corresponding to 0.5%, 1%, and 2% sulfur) are added. The tanks operate discontinuously until exponential growth is reached, maintaining a constant pH of ~1.8. pH and cell counts are monitored daily and every 30 days, respectively. To determine microbial growth kinetics, the Michaelis-Menten model is applied. The kinetic constant (K) and maximum growth rate (V_{max}) are derived from the slope and intercept of the exponential growth curve. (Eq.1).

$$\frac{1}{V} = \frac{K}{V_{max}T} + \frac{1}{V_{max}} \quad (1)$$

3. ANALYSIS AND DISCUSSION OF RESULTS

3.1 PHYSICAL, CHEMICAL AND MINERALOGICAL CHARACTERIZATION RESULTS

Grinding and classification result in a fine black powder with 100% particle size < 0.074 mm, within the optimal range (< 0.2 mm) for bioleaching with *A. thiooxidans*. Table 2 presents the chemical composition of the glue samples. The waste exhibits low concentrations of Ni, Co, and

Mg, but high Fe content, which supports bioleaching. Notably, rare earth minerals (*TR*) are present at approximately 3%.

Table 2. Chemical composition of waste

Elements	Fe	Ni	Mg	Co	TR
(%)	45	0,35	2,82	0,081	<3

Figure 2 shows the waste diffractogram, indicating magnetite (M), chromite (C), and forsterite-fayalite (F), with minor antigorite and quartz (Q). According to Samir P. et al. (2020), magnetite and chromite favor bioleaching, while the others remain inert.

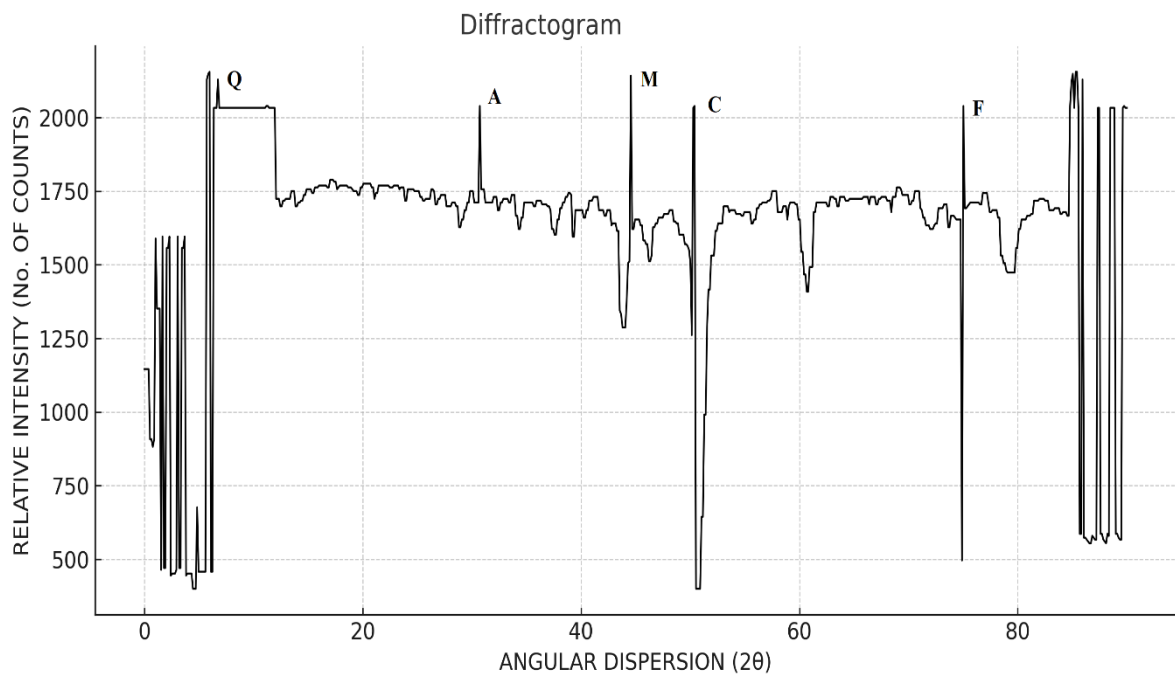


Figure 2. Diffractogram of waste. Source: authors

3.2 BACTERIAL GROWTH RESULTS

Figure 3 illustrates bacterial growth curves at 27°C, 32°C, and 37°C, simulating local seasonal temperatures. Exponential growth begins between days 6 and 8, with faster progression at 27°C and 32°C, aligning with regional climate data (Barcia S. et al., 2022).

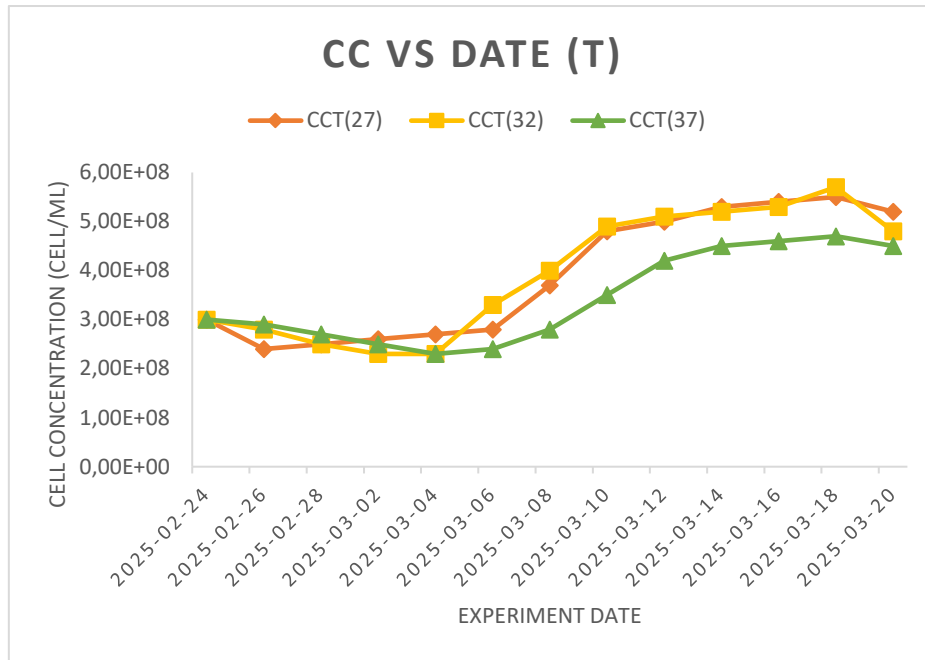


Figure 3. Growth curve at different temperatures Source: authors

3.3 WASTE BIOLEACHING PROCESS RESULTS

The Bacterial growth is evidenced by increased cell counts and decreased pH. Figure 4 shows pH variation during the tests. TK1 and TK2 exhibit a consistent pH decline, falling sharply after day 10. TK3 maintains higher pH levels initially, followed by a similar drop. This indicates a 10-day adaptation period to tank agitation conditions. The steady pH decline confirms that cell rupture due to impeller motion was not significant.

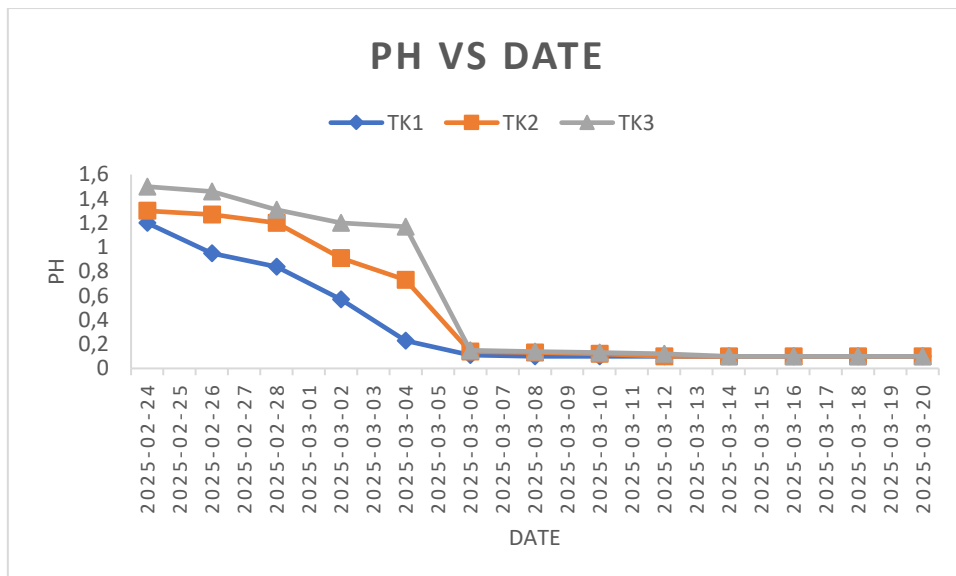


Figure 4. Behavior of pH vs. Date tests with Sulphur. Source: authors

3.4 KINETIC MODEL

Table 3 presents results for six samples from each tank, with sulfur concentration and temperature directly influencing bioleaching efficiency (Effic%). The best Ni, Co, and Fe extraction

efficiencies occurred with 1.1% pyrite. Temperature had minimal effect on Ni and Co, with Co reaching 78.13% recovery in test 6. Fe recovery peaked at 26.40% in test 4. For Mg, optimal recovery occurred at 1% sulfur.

Table 3. Efficiency of the bioleaching process

# Sample	S (%)	T(°C)	Effic%			
			Ni	Co	Fe	Mg
TK1.A	1	27	53,21	61,34	18,72	80,51
TK1.B	1,1	27	58,68	73,31	24,18	78,53
TK2.A	1	29	56,70	65,76	24,68	79,04
TK2.B	1,1	29	59,46	72,29	26,40	76,16
TK3.A	1	32	54,01	68,38	22,99	81,26
TK3.B	1,1	32	58,44	78,13	24,80	78,40

Each experiment was replicated twice. Simple regression analysis was performed using STATGRAPHICS 16.3 software, after confirming the normal distribution of the data. This resulted in four equations to estimate the leaching efficiency for each element, along with the corresponding coefficients of determination (R^2) and standard deviations (SD). Analysis of the influence of each variable on the models indicates that the bioleaching efficiency is more strongly dependent on the percentage of sulfur added than on the temperature of the medium, which is favorable for potential future scaling of the research. (Eq.2-5)

$$\%Ni = 12,358 + 42,20 \cdot S + 0,0028 \cdot T, R^2 = 0,78, SD = 2,61 \quad (2)$$

$$\%Co = -64,314 + 94,166 \cdot S + 1,203 \cdot T, R^2 = 0,954, SD = 5,96 \quad (3)$$

$$\%Fe = -19,778 + 29,967 \cdot S + 0,407 \cdot T, R^2 = 0,68, SD = 2,64 \quad (4)$$

$$\%Mg = 102,606 - 25,333 \cdot S + 0,116 \cdot T, R^2 = 0,64, SD = 1,79 \quad (5)$$

Finally, Michaelis-Menten constants under the tested conditions were $K = 0.589$ °C and $V_{max} = 0.73$ cells/mL-day (Table 4).

Table 4. Michaelis-Menten constants

Growth curve	Growth Velocity (cel/mL- d)	Inverse of the Temperature (1/T)	Inverse of the Velocity (1/V)
27°C	$5,5 \cdot 10^7$	0,03703	$0,18 \cdot 10^7$
32°C	$3,6 \cdot 10^7$	0,02941	$0,28 \cdot 10^7$
37°C	$2,4 \cdot 10^7$	0,02702	$0,40 \cdot 10^7$

4. FINAL THOUGHTS

The Michaelis-Menten model effectively describes microorganism growth in nickel industry waste. Although higher temperatures reduce microbial growth rates, they do not impede bioleaching. Using *A. thiooxidans*, extraction efficiencies of 59.46% Ni, 78.13% Co, 24.80% Fe, and 81.26% Mg were achieved with 1.1% sulfur (as pyrite) at room temperature. The bacterium

adapts well under appropriate temperature and acidity, suggesting its potential application for treating Cuban nickel industry waste.

5. REFERENCES

1. Decheng Jin, Lanlan Liu, Guanyu Zheng, Jianru Liang, Lixiang Zhou, A rapid method to quantify the biomass of viable *Acidithiobacillus ferrooxidans* in iron-based bioleaching matrix of sewage sludge, *Biochemical Engineering Journal*, Volume 152, 2019,107360, ISSN 1369-703X, <https://doi.org/10.1016/j.bej.2019.107360>.
2. FANG, D., CHENG, X., ZHENG, Y., et al. Bioleaching of spent lithium-ion batteries using the spent-medium approach under metal toxicity. *Bioresource Technology*, 2022, vol. 363, p. 127950. <https://doi.org/10.1016/j.biortech.2022.127950>.
3. Gholamreza Khodadadmahmoudi, Hadi Abdollahi, Amirhossein Mohammadzadeh, Roozbeh Saneie, Mirsaleh Mirmohammadi, Ali Rezaei, Golnaz Jozanikohan, Hojat Naderi, Green extraction of nickel and valuable metals from pyrrhotite samples with different crystallographic structures through acidophilic bioleaching. *Journal of Environmental Management*, Volume 317, 2022, 115394, ISSN 0301-4797, <https://doi.org/10.1016/j.jenvman.2022.115394>.
4. Guanglin Chen, Hongjie Shi, Huili Ding, Xu Zhang, Tingyue Gu, Minglong Zhu, Wensong Tan, Multi-scale analysis of nickel ion tolerance mechanism for thermophilic *Sulfobacillus thermosulfidooxidans* in bioleaching. *Journal of Hazardous Materials*, Volume 443, Part B, 2023,130245, ISSN 0304-3894, <https://doi.org/10.1016/j.jhazmat.2022.130245>.
5. Han Wang, Liyuan Feng, Rogerio Manica, Qingxia Liu, Selective depression of millerite (β -NiS) by polysaccharides in alkaline solutions in Cu-Ni sulphides flotation separation, *Minerals Engineering*, Volume 172, 2021,107139, ISSN 0892-6875, <https://doi.org/10.1016/j.mineng.2021.107139>.
6. LIN, C., HUANG, W., JIANG, H., et al. Bioleaching of metals from waste printed circuit boards using *Acidithiobacillus* species. *Minerals*, 2023, vol. 13, no. 6, p. 828. <https://doi.org/10.3390/min13060828>.
7. Lingkai Wen, Gongchu Shi, Yingqin Sun, Yanchao Cui, Shihao Zhang, Xiaohui Chen, Jian Cheng, Jia Wang, Baoping Xin, Rapid and efficient extraction of Zn from wasted Zn-rich paint residue by indirect bioleaching and successive production of high-purity ZnCO₃/ZnO by precipitation, *Journal of Environmental Management*, Volume 342, 2023, 118294, ISSN 0301-4797, <https://doi.org/10.1016/j.jenvman.2023.118294>.
8. LI, N., ZHAO, J., WANG, H., et al. Spent-medium bioleaching of lithium-ion battery black mass using *Acidithiobacillus thiooxidans* under high pulp densities. *Scientific Reports*, 2025, vol. 15, p. 16517. <https://doi.org/10.1038/s41598-025-00660-9>
9. Meicen Liu, Jameson Hunter, Jian Shi, Yi Zheng, Bioleaching to produce clean loblolly pine for thermochemical conversion, *Journal Fuel*, Volume 344, 2023,128148, ISSN 0016-2361, <https://doi.org/10.1016/j.fuel.2023.128148>.
10. Mohammad Javad Kazemi, Mohammad Kargar, Jamileh Nowroozi, Abbas Akhavan Sepahi, Abbas Doosti, Zahra Manafi, The wide distribution of an extremely thermoacidophilic microorganism in the copper mine at ambient temperature and under acidic condition and its significance in bioleaching of a chalcopyrite concentrate, *Revista Argentina de Microbiología*, Volume 51, Issue 1, 2019, Pages 56-65, ISSN 0325-7541, <https://doi.org/10.1016/j.ram.2017.09.004>.
11. Rodríguez Yanicet, Blázquez María Luisa, Ballester Antonio, González Felisa, Muñoz Jesús. (2001). La biolixiviación al comienzo del siglo XXI. *Revista de Metalurgia*. 37. <https://doi.org/10.3989/revmetalm.2001.v37.i5.528>.
12. Rory A. Cameron, Rolando Lastra, Yves Thibault, Lucie Morin, W. Douglas Gould, Stirred-tank bioleaching of nickel and cobalt from pyrrhotite-rich tailings from Sudbury, Ontario, *Hydrometallurgy*, Volume 204, 2021, 105592, ISSN 0304-386X, <https://doi.org/10.1016/j.hydromet.2021.105592>.
13. Samir Prioto Tayar, Maria Pilar Yeste, Martín Ramírez, Gema Cabrera, Denise Bevilaqua, Jose Manuel Gatica, Hilario Vidal, Miguel Ángel Cauqui, Domingo Cantero, Nickel recycling through bioleaching of a Ni/Al₂O₃ commercial catalyst, *Hydrometallurgy*, Volume 195, 2020, 105350, ISSN 0304-386X, <https://doi.org/10.1016/j.hydromet.2020.105350>.

14. Sardiñas, BS., Hernández González, D., & Otero Martín, M. (2022). The equivalent effective temperature in Cuba. *Investigaciones Geográficas*, (78), 47-66. <https://doi.org/10.14198/INGEO.21447>
15. TAYAR, S.P., YESTE, M.P., RAMÍREZ, M., et al. Nickel recycling through bioleaching of a Ni/Al₂O₃ commercial catalyst. *Hydrometallurgy*, 2020, vol. 195, p. 105350. <https://doi.org/10.1016/j.hydromet.2020.105350>.
16. VARDANYAN, A., MELIKYAN, H., KARAPETYAN, H., et al. Bioleaching of steelmaking dusts by thermophilic consortia containing *Acidithiobacillus thiooxidans*. *Processes*, 2023, vol. 11, no. 9, p. 2447. <https://doi.org/10.3390/pr11092447>.
17. Waksman Selman A. y Joffe J. S. Microorganisms concerned in the oxidation of sulfur in the soil, New Jersey Agricultural Experiment Stations. Department of Soil Chemistry and Bacteriology, 1921.-33: Vol. II.
18. WANG, Y., LIU, J., CAI, Y., et al. Advances in bioleaching of heavy metals from solid waste: a review of microbial resources and mechanisms. *Science of the Total Environment*, 2025, vol. 907, p. 167672. <https://doi.org/10.1016/j.scitotenv.2023.167672>.
19. YI, H., WANG, Z., LUO, G., et al. Insights into thiosulfate and polysulfide pathways in *Acidithiobacillus*-mediated metal sulfide dissolution. *Environmental Science and Pollution Research*, 2021, vol. 28, p. 44822–44835. <https://doi.org/10.1007/s11356-021-14655-x>.