

# Effect of Cyanide as a Nutrient Source on bacteria, *Bacillus Cereus*, for use in Bioleaching of Gold Tailings

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Mining generates tailings and wastewaters containing extractable gold alongside toxic contaminants such as cyanide (CN<sup>-</sup>), thiocyanates (SCN<sup>-</sup>), and other heavy metals. Conventional cyanidation struggles to process low-grade ores and high-sulphide refractory gold tailings due to cyanide's reactivity with non-gold minerals. This study evaluated the potential of *Bacillus cereus* to utilize CN<sup>-</sup> as a growth substrate compared to a conventional carbon source like glucose. Aerobic batch experiments were conducted under varying conditions of pH, temperature, and initial CN<sup>-</sup> concentrations (1, 3, and 5 mmol/L). Results showed that CN<sup>-</sup> supported *B. cereus* growth, with optimal conditions at pH 7.5–8.5 and 35°C, achieving 95 % cyanide removal within 72 h. While glucose enhanced early metabolic activity, it was depleted within 8 h, emphasizing cyanide's role as a potential substrate. These findings will contribute to optimizing bioleaching processes for sustainable gold extraction and bioremediation of cyanide-contaminated environments thus enhancing the environmental feasibility of mining waste management.

## 1. Introduction

Mining activities have resulted in the accumulation of large deposits of mine tailings that contain extractable gold of significant market value (Pineda et al., 2023). However, much of this gold exists in matrices with other minerals, such as sulphides, sulphates, carbonates, and silicates, where it is strongly adsorbed. This leads to gold encapsulation or passivation, increasing reagent consumption and reducing gold recovery (Bazhko and Yahorava, 2017). Conventional cyanidation methods are inefficient for low-grade ores and high-sulphide refractory gold tailings because cyanide preferentially reacts with other minerals, such as copper and sulphides, instead of gold, further limiting its availability for gold extraction (Soto-Urbe et al., 2023).

In recent years, bioleaching has emerged as a promising eco-friendly alternative for extracting valuable metals from such challenging ores and tailings. Bioleaching, also known as bio-mining, is a biotechnological process that uses microorganisms to recover metals from ores and mineral concentrates. This method is particularly effective for low-grade ores and tailings that are uneconomical to process with conventional techniques (Saldaña et al., 2023). Its advantages include reduced environmental impact and enhanced recovery efficiency, particularly for gold encapsulated in high-sulphide matrices (Mutimutema et al., 2022). However, bioleaching efficiency depends on several factors, including temperature, pH, oxidizing conditions, nutrient availability, surface area, and the presence of toxic ions such as heavy metals (Ubal dini et al., 1997).

The role of environmental conditions and nutrient sources in bioleaching has been extensively studied, particularly for acidophilic and sulphide-oxidizing bacteria. For example, mixed microbial cultures have shown superior performance compared to pure cultures due to their adaptability and synergistic interactions (Chen et al., 2021). This study demonstrates the importance of optimizing parameters such as pH, temperature, and lixivium return. Understanding how these environmental factors coupled with the nutrient sources impact the bacteria in the context of bioleaching is essential for optimizing the process and improving its efficiency and yield (Sadeghi, et al., 2021). Despite this progress, limited research has focused on the bioleaching of high-sulphide refractory gold ores or tailings, particularly under extreme conditions such as high cyanide concentrations.

Cyanide, a common contaminant in mine tailings, is highly toxic to living organisms as it forms stable complexes with transition metals essential for protein function. However, certain bacteria have developed mechanisms to

tolerate and even metabolize cyanide. These mechanisms include a cyanide-insensitive respiratory chain, siderophore-mediated iron acquisition, and cyanide assimilation pathways (Huertas et al., 2006). By utilizing cyanide as a nutrient or electron source, these bacteria can potentially degrade cyanide while sustaining growth, making them ideal for applications in both bioleaching and bioremediation of cyanide-contaminated environments. Local mixed culture consortia, consisting of diverse microbial species native to the mining environment, have shown promise in enhancing the bioleaching process due to their adaptability and potential synergistic interactions (Brune et al., 2012). However, their performance and stability of the process under varying environmental conditions have not been thoroughly investigated.

This study aimed to investigate the ability of *Bacillus cereus* to utilize cyanide as a growth substrate while degrading cyanide and facilitating gold bioleaching from high-sulphide refractory tailings. Key objectives included evaluating the influence of environmental conditions such as pH, temperature, and cyanide concentration on bacterial growth and cyanide degradation. By leveraging cyanide as a nutrient source, this research seeks to optimize bioleaching processes, reduce reliance on conventional carbon sources like glucose, and contribute to sustainable mining waste management and environmental remediation.

## 2. Materials and Methods

Bacteria isolated from gold mine tailings was characterized, operating conditions optimized and was utilized in the degradation of various concentrations of cyanide and glucose as described below.

### 2.1 Sampling and source of microorganism

Samples of the local, adapted cultures were taken as grab samples from the soil at the site of contamination. These were collected from Golden Kopje Mine in Chinhoyi, Zimbabwe and stored in a cooler box at 4 °C.

### 2.2 Growth media

Tryptone Soy Agar (TSA) and Tryptone Soy Broth (TSB) (Oxoid Ltd., Basingstoke, Hants, UK) were used as the primary growth media. TSA was prepared by dissolving 40 g of TSA powder in 1 L of distilled water, while TSB was prepared by dissolving 30 g of TSB powder in 1 L of distilled water. Both media were sterilized by autoclaving at 121 °C and 4 MPa for 15 minutes (Mangwandi et al., 2022). The mineral salt medium (MSM) used in this study was made up of the following constituent as described by Tendenedzai et al., (2021).

### 2.3 Bacterial isolation, cultivation and culture storage

For bacterial isolation, 0.2 g of soil samples stored at 4 °C was inoculated into 100 mL of sterile TSB amended with 1000 ppm NaCN and stabilized with 1 M NaOH to prevent HCN volatilization. The cultures were incubated for 24 h at 35 ± 0.2 °C on a rotary shaker at 120 rpm (Tendenedzai et al., 2021). Following incubation, the cultures were streaked onto agar plates containing 1000 ppm NaCN and incubated at 35 °C for 24 h. Colonies from the agar plates were gently scraped and transferred to fresh TSB amended with NaCN for another 24 h under the same conditions. For long-term storage, 20 mL of sterile glycerol (20%, v/v) was added to 80 mL of bacterial culture. The mixture was homogenized, transferred into 2 mL screw-cap tubes, and stored at -70 °C as pure stock solutions. For experimental runs, frozen cultures were thawed for 10–20 minutes, streaked onto sterile nutrient agar plates, and incubated at 30 ± 0.2 °C for 24 h.

### 2.4 Experimental setup

#### Growth experiments

All chemicals used in this study were sourced from Merck (Sigma-Aldrich) unless otherwise stated. A cyanide stock solution of 1000 ppm (20 mmol/L) NaCN was prepared and diluted to varying concentrations (1, 3, and 5 mmol/L) for experimental use. These cyanide solutions were utilized across all experiments conducted. Growth optimization experiments were performed to assess bacterial survival and performance under different conditions. The bacteria were exposed to varying cyanide concentrations (1, 3, and 5 mmol/L), pH levels (7.5, 8.5, and 9.5 ± 0.2), and temperatures (25 °C, 35 °C, and 45 °C) in an incubator equipped with a rotary shaker. Phosphorus and carbonate buffers were used to adjust and maintain the desired pH levels

#### Batch cyanide utilization experiments

One of the primary objectives of this study was to assess whether cyanide could serve as a carbon/nitrogen source for *Bacillus cereus*. Batch experiments were conducted to evaluate the potential of cyanide as a nutrient source in comparison to conventional nutrients. The starting pH was set to 9.5 (to minimise volatilisation of NaCN), and the temperature was maintained at 35 °C as initial conditions. Reactor setups were prepared in triplicate, with the experimental conditions detailed in the table 1 below.

Table 1: Reagents employed for batch growth experiments in broth and cyanide utilisation in MSM

Reagent	R control	R1	R2	R3	R4
Broth (for growth)/MSM (for utilisation experiments)	150 mL	150 mL	150 mL	150 mL	150 mL
NaCN	-	5 mmol/L	3 mmol/L	3 mmol/L	1 mmol/L
Glucose	-	-	-	5mmol/L	-
Bacteria	1 mL	1 mL	1 mL	1 mL	1 mL

## 2.5 Analytical methods

The cyanide concentration in the samples was measured using an Ion Chromatography (IC) 940 Professional IC Vario ion chromatograph (Metrohm, Herisau, Switzerland) equipped with a Metrosep C 10-250/4.0 separation column (Metrohm, Switzerland). Bacterial growth was monitored spectrophotometrically by measuring optical density at 600 nm (OD600) using a UV-Vis spectrophotometer. The pH and oxidation-reduction potential (ORP) of the samples were measured using a calibrated pH and conductivity meter. The identification of bacterial species was performed via 16S rRNA sequencing, outsourced to Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa).

## 3. Results

### 3.1 Results on optimum pH and growth

Reactors were set up at 35°C with 150mls of growth medium and 1ml of bacteria aliquot in an incubator.

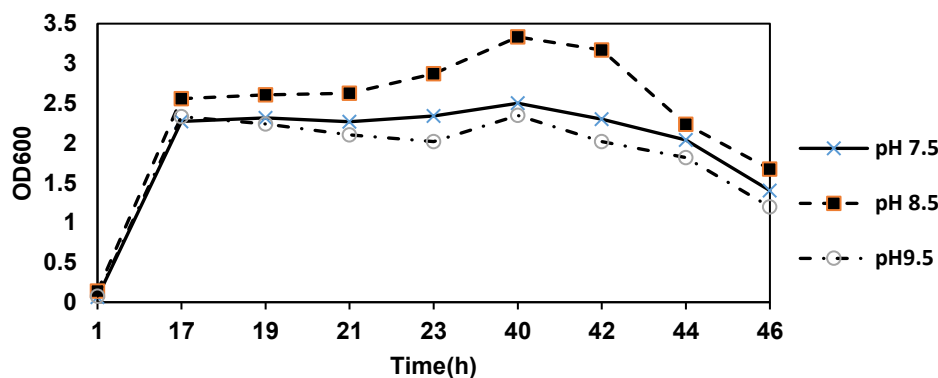


Figure 1: OD600 of reactors at various pH values

At pH 8.5, the growth rate was the highest, with OD600 reaching approximately 3.5 at around 40 h, which was significantly higher than in the other pH conditions. However, after peaking, there was a sharp decline in OD600 after 40 h, indicating that the cells likely entered a stationary or death phase. These results suggest that while pH 8.5 supports the most rapid and highest growth initially, it may not sustain bacterial viability over a prolonged period. At pH 9.5, *Bacillus cereus* showed slower as reaction proceeds compared to pH 7.5 and 8.5. The peak OD600 at pH 9.5 was lower (~2.3) and occurred around 40 h. At 7.5 there was more stable growth over a longer duration, indicating its suitability for sustained bacterial activity, it was also found to be the optimum pH by Rodrigo et al., 2021.

Conversely, pH 9.5 is less ideal for *Bacillus cereus* growth, as it limits the efficiency and biomass yield, likely due to stress factors associated with higher alkalinity (Clavel et al. 2004). In applications such as bioleaching or industrial processes, pH 8.5 may be optimal for short-term bacterial activity, while pH 7.5 would be more suitable for longer-term operations requiring sustained bacterial growth. pH 9.5 should generally be avoided, as it inhibits bacterial growth and reduces biomass yield. The ability for *B. Cereus* to grow at alkaline pH is significant for this study as the alkaline conditions do not support the generation of HCN, toxic gas (Ruiz-Bermejo et al., 2021). Overall, pH 8.5 facilitated the fastest and highest bacterial growth, suggesting that *Bacillus cereus* can tolerate slightly alkaline conditions and thrive more efficiently at this pH in the short term.

### 3.2 Results on optimum temperature

Three sets of reactors were subjected to different temperatures, 25 °C, 35 °C and 45 °C.

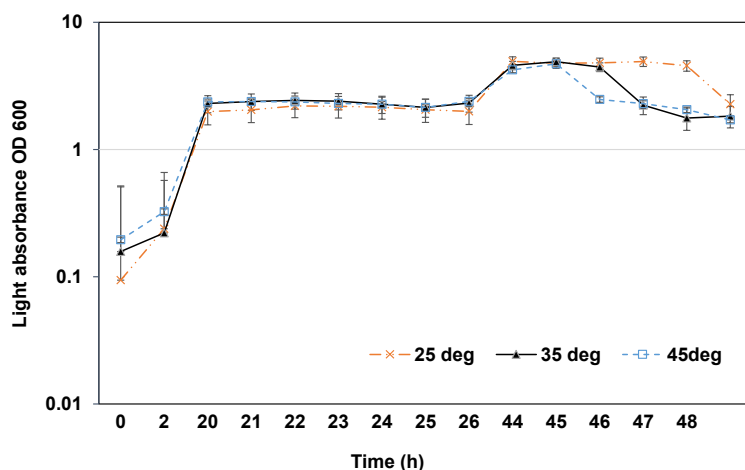


Figure 2: OD600 at different temperatures

At 25 °C growth is slower compared to the other temperatures and achieves the highest at the end of the experiment of 48 hours. At the temperature of 35 °C the fastest growth rate and highest final OD600 was reached, suggesting it is the optimal temperature for this bacterial strain. While growth is observed at 45 °C, it is slower compared to 35 °C, and the final OD600 is slightly lower. This indicates that 45°C may be near the upper limit of the bacteria's tolerance as supported by Dubey (2011) whose study found the range between 20-40 °C and optimum growth at temperature of 30 °C.

### 3.3 pH and ORP Changes

The pH and ORP changes were measured for the different setups.

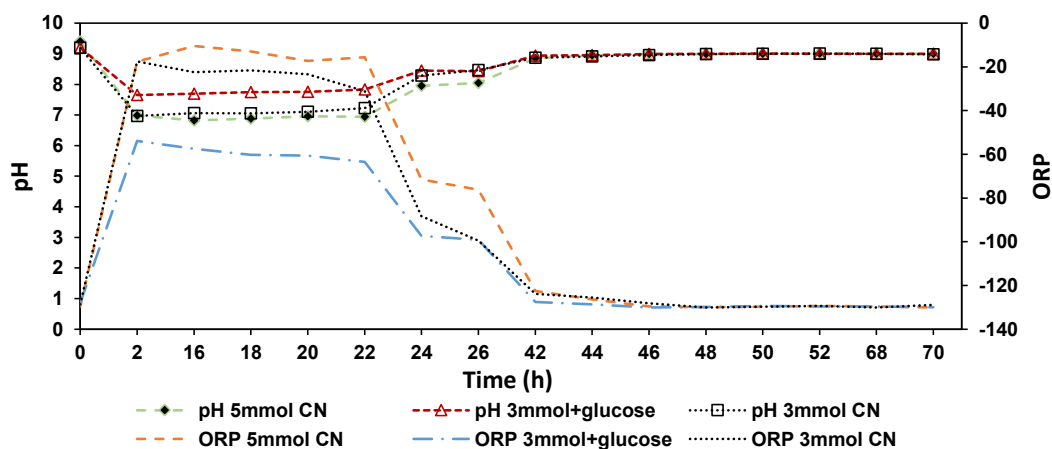


Figure 3: pH versus ORP in 72 hours

The pH values across the different reactors started out at basic then shifted to neutral (ranging from 9.5 to 7). The main pathways of cyanide detoxification are conversion to thiocyanate (Lachowicz et al., 2024). Additionally, hydrolysis of cyanide produces HCN a weak acid, organic acids or other acidic intermediates or release of CO<sub>2</sub> forming carbonic acid (Lachowicz et al., 2024). This was followed by a modest increase or stabilization, which can typically be attributed to microbial or chemical processes occurring in the reactors. In microbial systems, pH changes are often linked to the metabolism of the microorganisms, such as the conversion of cyanide and intermediate products to less toxic products like ammonia, which can increase the pH in the system (Akcil & Mudder, 2003, Quiroz et al., 2023). The ORP values in the reactors were generally negative, fluctuating between -20 mV and -120 mV. Initially, there was some increase in the ORP values among the reactors, but over time, they stabilized at less negative values. Negative ORP values are indicative of reducing conditions, which typically suggest that oxygen or other strong electron acceptors are limited or absent. This is common in microbial processes that involve the reduction of compounds, such as cyanide degradation, where bacteria utilize alternative electron acceptors (Dash et al., 2009). This is likely associated with the consumption of cyanide and the release of by-products like ammonia, which also contribute to the lowering of the ORP (Akcil & Mudder,

2003). In such systems, the degradation of toxic compounds like cyanide often creates anaerobic or facultative anaerobic conditions, which are characterized by negative ORP values (Wang et al., 2022). The bacteria, likely facultative anaerobes, would be able to thrive under these conditions and continue degrading cyanide even in the absence of oxygen (Bottone, 2010). The shift towards these reducing conditions, marked by the negative ORP and stable pH, suggests that the system achieved a steady state, likely after the majority of cyanide had been converted to less toxic compounds, supporting the hypothesis that the bacteria were effectively metabolizing cyanide under these conditions. This aligns with previous studies on cyanide degradation, where anaerobic or facultative anaerobic conditions support the microbial breakdown of cyanide and other toxic compounds (Akcil & Mudder, 2003; Dash et al., 2009; Bottone, 2010).

### 3.4 Substrate Degradation

Cyanide and glucose were used as potential substrates and the degradation was noted as shown in Fig 4.

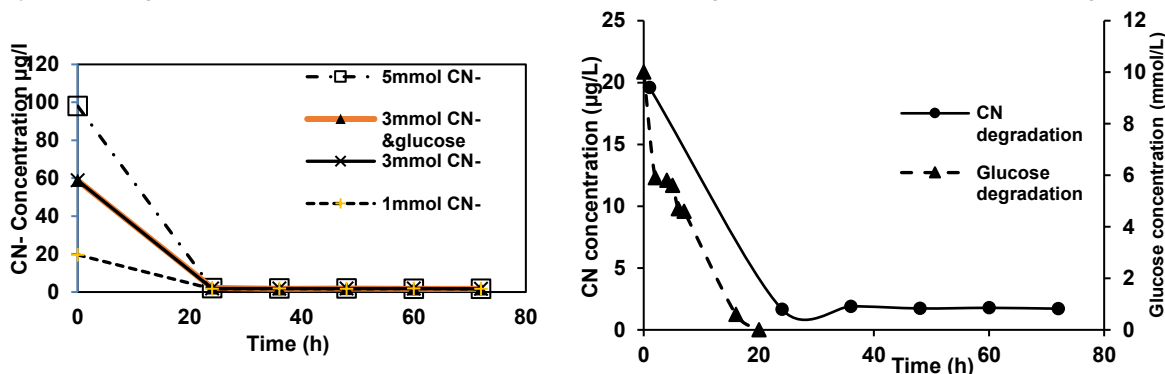


Figure 4: a) Cyanide degradation in 72 hours b) Cyanide vs glucose depletion

Cyanide concentrations decrease with a rate of between 91–98% over time in all experimental setups. The depletion of cyanide appears consistent across 24 h to 72h period, regardless of the initial concentration. The degradation of cyanide results in intermediate products such as thiocyanates and other nitrogen compounds (Ebbs, 2004; Lachowicz, 2024). In the condition with glucose shows a faster rate of glucose depletion as a substrate compared to cyanide as it was all consumed in the first 20 hours. This suggests that glucose enhances bacterial activity, potentially providing an additional energy source that accelerates cyanide degradation (Dash et al., 2009; Akcil & Mudder, 2003). The rapid decline in cyanide concentration suggests effective bacterial degradation across all conditions. The bacteria can metabolize cyanide, likely using it as a nitrogen source. The near-complete depletion of cyanide after 24h indicates efficient cyanide-utilizing bacterial activity. This highlights that cyanide degradation might be enhanced by supplementing with a readily metabolised substrate (Warda et al., 2016). The bacteria *Bacillus cereus* can tolerate cyanide concentrations used in these experiments, as evidenced by successful degradation as confirmed by Alvarado-López et al., 2023. This is relevant for bioleaching applications where free cyanide can be found within the gold sulphide tailings.

## 4. Conclusion

The finding of this study is that *Bacillus cereus* can thrive in cyanide conditions of 5mmol/L and the fluctuations in ORP show a strong likelihood of successful degradation of cyanide by *B. Cereus* within the first 24 hours. It is important to analyse the residual metabolic by-products after cyanide depletion (e.g., ammonia, thiocyanate) to fully ascertain the level of degradation. Anaerobic conditions can also be explored to assess the performance of *B. Cereus* under such conditions.

## References

- Akcil, A. (2003). Destruction of cyanide in gold mill effluents: Biological versus chemical treatments. *Biotechnology Advances*, 21(6), 501–511. DOI: 10.1016/S0734-9750(03)00091-4.
- Akcil, A., & Mudder, T. (2003). Microbial destruction of cyanide wastes in gold mining: Process review. *Biotechnology Letters*, 25(6), 445–450. DOI: 10.1023/A: 1022850705647.
- Alvarado-López, M. J., Garrido-Hoyos, S. E., Raynal-Gutiérrez, M. E., El-Kassis, E. G., Luque-Almagro, V. M., & Rosano-Ortega, G. (2023). Cyanide Biodegradation by a Native Bacterial Consortium and Its Potential for Goldmine Tailing Biotreatment. *Water*, 15(8), 1595. <https://doi.org/10.3390/w15081595>.
- Bazhko, V, and Yahorava, V (2017), "Evaluation of ozonation technology for gold recovery and cyanide management during processing of a double refractory gold ore" *Journal of the Southern African Institute of Mining and Metallurgy*, 117(8), 749-756.
- Bottone, E. (2010). *Bacillus cereus*, a volatile human pathogen. *Clin. Microbiol. Rev.* , 23, 382–398.

- Brune, K., Bayer T. (2012). Engineering microbial consortia to enhance biomining and bioremediation. *Frontiers in Microbiology*. 3. 203. 10.3389/fmicb.2012.00203.
- Cáceda Quiroz, C. J., Fora Quispe, G. d. L., Carpio Mamani, M., Maraza Choque, G. J., & Sacari Sacari, E. J. (2023). Cyanide Bioremediation by *Bacillus subtilis* under Alkaline Conditions. *Water*, 15(20), 3645. <https://doi.org/10.3390/w15203645>.
- Clavel, T.; Carlin, F.; Lairon, D.; Nguyen-The, C.; Schmitt, P. (2004). Survival of *Bacillus cereus* spores and vegetative cells in acid media simulating human stomach. *J. Appl. Microbiol.* , 97, 214–219.
- Dash, R. R., Gaur, A., & Balomajumder, C. (2009). Cyanide in industrial wastewaters and its removal: A review on biotreatment. *Journal of Hazardous Materials*, 163(1), 1–11. DOI: 10.1016/j.jhazmat.2008.06.051.
- Dubey, S.K., Dubey, J., Mehra, S., Tiwari, P.K., & Bishwas, A.J. (2011). Full Length Research Paper Isolation of protease producing novel *Bacillus cereus* and detection of optimal conditions. *African Journal of Biotechnology*, 10, 1160-1164.
- Ebbs S. Biological degradation of cyanide compounds. *Curr Opin Biotechnol*. 2004 Jun;15(3):231-6. doi: 10.1016/j.copbio.2004.03.006. PMID: 15193331.
- Jiang, S., Chen, Y., Chen, S., & Hu, Z. (2023). Removal and Reclamation of Trace Metals from Copper and Gold Mine Tailing Leachates Using an Alkali Suspension Method. *Water (Switzerland)*, 15(10).
- Huertas, M. J., Luque-Almagro, V. M., Martínez-Luque, M., Blasco, R., Moreno-Vivián, C., Castillo, F., & Roldán, M. D. (2006). Cyanide metabolism of *Pseudomonas pseudoalcaligenes* CECT5344: Role of siderophores. *Biochemical Society Transactions*, 34(1), 152–155. <https://doi.org/10.1042/BST0340152>
- Kuyucak N, Akcil A (2013) Cyanide and removal options from effluents in gold mining and metallurgical processes. *Miner Eng* 50:13–29. <https://doi.org/10.1016/j.mineng.2013.05.027>.
- Lachowicz JI, Alexander J, Aaseth JO. Cyanide and Cyanogenic Compounds-Toxicity, Molecular Targets, and Therapeutic Agents. *Biomolecules*. 2024 Nov 7;14(11):1420.
- Mangwandi, T., Tendenedzai, J., Chirwa, E. & Brink, H. (2022). Optimisation of Selenite Reduction using *Enterococcus* spp. under Anaerobic Conditions. *Chemical Engineering Transactions*. 92. 139-144. 10.3303/CET2292024.
- Mutimutema, P., Akdogan, G., and Tadie, M. 2022 Evaluation of pre-treatment methods for gold recovery from refractory calcine tailings. *Journal of the Southern African Institute of Mining and Metallurgy*, vol. 122, no. 10, pp. 561–570. <http://dx.doi.org/10.17159/2411-9717/2070/2022>.
- Pineda, Y. S., Devries, S. L., Steiner, N. C., & Block-Cora, K. A. (2023). Bioleaching of Gold in Mine Tailings by *Alcaligenes faecalis*. *Minerals*, 13(3), 1–13. <https://doi.org/10.3390/min13030410>
- Rodrigo, D., Rosell, C. M., & Martinez, A. (2021). Risk of *Bacillus cereus* in Relation to Rice and Derivatives. *Foods*, 10(2), 302. <https://doi.org/10.3390/foods10020302>
- Ruiz-Bermejo, M., de la Fuente, J. L., Pérez-Fernández, C., & Mateo-Martí, E. (2021). A Comprehensive Review of HCN-Derived Polymers. *Processes*, 9(4), 597. <https://doi.org/10.3390/pr9040597>
- Sadeghi, P., Diaz, A., Vila, M. C., Fiúza, A., & Nunes, O. (2021). Appraisal of the Laboratory-Scale Tests for Bioleaching of Low-Grade Heavy Metal-(oid) s Resources. *Water, Air, and Soil Pollution*, 232(7).
- Saldaña, M, Jeldres, M, Galleguillos Madrid, FM, Gallegos, S, Salazar, I, Robles, P, and Toro, N (2023), "Bioleaching Modeling-A Review" *Materials (Basel)*, 16(10).
- Soto-Uribe, JC, Valenzuela-Garcia, JL, Salazar-Campoy, MM, Parga-Torres, JR, Tiburcio-Munive, G, Encinas-Romero, MA, and Vazquez-Vazquez, VM (2023), "Gold Extraction from a Refractory Sulfide Concentrate by Simultaneous Pressure Leaching/Oxidation" *Minerals*, 13(1), 116.
- Tendenedzai, J. T., Chirwa, E. M. N., & Brink, H. G. (2021). Performance evaluation of selenite (Seo32-) reduction by *enterococcus* spp. *Catalysts*, 11(9). <https://doi.org/10.3390/catal11091024>.
- Ubal dini, S., Veglió, F., Toro, L., & Abbruzzese, C. (1997). Biooxidation of arsenopyrite to improve gold cyanidation: Study of some parameters and comparison with grinding. *International Journal of Mineral Processing*, 52(1), 65–80. [https://doi.org/10.1016/s0301-7516\(97\)00041-0](https://doi.org/10.1016/s0301-7516(97)00041-0)
- Wang, X.; Wu, Y.; Chen, N.; Piao, H.; Sun, D.; Ratnaweera, H.; Maletskyi, Z.; Bi, X. Characterization of Oxidation-Reduction Potential Variations in Biological Wastewater Treatment Processes: A Study from Mechanism to Application. *Processes* 2022, 10, 2607. <https://doi.org/10.3390/pr10122607>
- Warda AK, Siezen RJ, Boekhorst J, Wells-Bennik MH, de Jong A, Kuipers OP, Nierop Groot MN, Abee T. Linking *Bacillus cereus* Genotypes and Carbohydrate Utilization Capacity. *PLoS One*. 2016 Jun 7;11(6):e0156796. doi: 10.1371/journal.pone.0156796. PMID: 27272929; PMCID: PMC4896439.