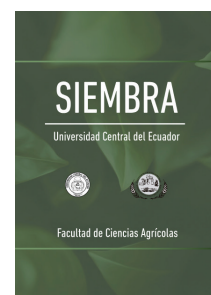


*Bioleaching of heavy metals in mining and metallurgical wastes**Biolixiviación de metales pesados en residuos minero-metalúrgicos*

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**Abstract**

Bioleaching of mining-metallurgical waste to remove heavy metals is a topic of great importance in environmental, public health, and secondary recovery fields. Heavy metals, due to their harmful effects on health, pose a latent risk when dispersed in the environment, primarily in water and soil, at elevated concentrations. In this study, a microbial consortium of acidophilic-mesophilic microorganisms was cultivated from natural biomass resulting from mineral oxidation processes. These microorganisms were then applied in the bioleaching process of mining-metallurgical waste originating from an area with high levels of heavy metal contamination and a producer of mining-metallurgical waste. The process was carried out in two phases: initially in agitated flasks with 50 grams of waste for 15 days, followed by scaling up to 640 grams in 8.3 liters. This scaling-up was performed with agitation at 85 rpm and a controlled temperature between 20 and 33 °C for 30 days, simulating an experimental-scale reactor. Successful copper and iron solubilization from the waste was achieved, with a removal of 63.27% in copper, 16.19% in iron, and 58.82% in arsenic, assessed through concentration measurements before and after bioleaching. The reduction in contaminant concentration underscores the potential of this bioleaching process to mitigate pollution and revalorize metals present in the waste. These findings guide research towards more environmentally friendly methods. The application of bioleaching not only provides environmental benefits but also lays the groundwork for action in favor of public health. Reducing the presence of heavy metals in waste significantly contributes to the protection of ecosystems and the health of communities near mining-metallurgical waste disposal sites.

**Keywords:** bioleaching, waste, heavy metals, pollution, public health.

**Resumen**

La biolixiviación de residuos minero-metalúrgicos para la remoción de metales pesados es un tema de gran relevancia en los campos ambiental, de salud pública y recuperación secundaria. Los metales pesados representan un riesgo latente para la salud cuando se dispersan en el ambiente, especialmente en el agua y el suelo, en concentraciones elevadas. En este

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estudio, se cultivó un conglomerado microbiano de microorganismos acidófilos-mesófilos obtenidos de biomasa natural generada por la oxidación de minerales. Estos microorganismos se aplicaron en el proceso de biolixiviación de residuos minero-metalúrgicos provenientes de una zona con altos niveles de contaminación por metales pesados y productora de residuos minero-metalúrgicos. El proceso se dividió en dos fases: una inicial en matraces agitados con 50 gramos de residuos durante 15 días, seguida de un escalamiento a 640 gramos en 8,3 litros. Este último se llevó a cabo con agitación a 85 rpm y una temperatura controlada entre 20 y 33 °C durante 30 días, simulando así un reactor a escala experimental. Los resultados destacan la solubilización exitosa de cobre y hierro de los residuos, con una remoción del 63.27% en cobre, 16.19% en hierro y 58.82% en arsénico, evaluados mediante mediciones de concentraciones antes y después de la biolixiviación. La reducción en la concentración de contaminantes subraya el potencial de este proceso para mitigar la contaminación y revalorizar los metales presentes en los residuos. Estos hallazgos orientan la investigación hacia métodos más sostenibles para el ambiente. La aplicación de la biolixiviación no solo brinda beneficios ambientales, sino que también establece una base para acciones en favor de la salud pública. La disminución de la presencia de metales pesados en los residuos contribuye significativamente a la protección de los ecosistemas y la salud de las comunidades cercanas a los sitios de disposición de residuos minero-metalúrgicos.

**Palabras clave:** biolixiviación, residuos, metales pesados, contaminación, salud pública.

## 1. Introduction

Areas of economic relevance driven by mining activities can be found in Ecuador, being the province of Azuay, in the canton of Camilo Ponce Enriquez, an example (Escobar-Segovia et al., 2020). The geological zone of Ponce Enriquez contains high amounts of sulfide-type minerals such as pyrite, pyrrhotite, arsenopyrite, chalcopyrite and sphalerite, constituting part of the raw material extracted for the purpose of mineral processing (Salazar & Lozada, 2018). Heavy metals and metalloids typically forming sulfides are Cu, Fe, As, Pb, Cd, Zn among others. These -when released, are hazardous for both the environment and human health (Jiménez-Oyola et al., 2021). Mining activities allow - after mineral processing-, a part of these minerals to remain persistent in the mining-metallurgical waste known as tailings (Peña Carpio & Menéndez-Aguado et al., 2016).

Studies have corroborated that, of the total material subjected to processing, which reaches enormous figures worldwide, a percentage higher than 95 % ends up turning into tailings (Falagán et al., 2017; Schoenberger, 2016). The processing of sulfide ores represents a risk to water resources. The problem of sulfide oxidation reduces water quality with sulfates, iron (III), heavy metals and metalloids (Lindsay et al., 2015). The waste deriving from the process is not adequately managed. This has led to the contamination of surface water bodies by the direct discharge of mining-metallurgical waste. Rivers nearby the area contain high amounts of heavy metals in both water and sediments (Carling et al., 2013; Reyes et al., 2016).

Metals such as Cu, Fe, Zn present in the tailings have a high mobility at low pH. The process of sulfide oxidation by microorganisms generating acid drainage is widely spread. This phenomenon enables the capacity of mining-metallurgical wastes to generate acid drainage, and to facilitate the mobility of heavy metals such as copper, iron and arsenic (Tabelin et al., 2020). It has been used in copper recoveries in controlled-media low-grade ores, using strains of unicellular organisms. Bioleaching, by being responsible for precursor chemical reactions bioleaching (Latorre et al., 2016; Zhang et al., 2020), is not only used as a secondary method to increase the recovery of metals from tailings. Also, its use has been proposed as a low-cost, more environmentally friendly alternative for environmental remediation since it focuses on removing heavy metals (Park et al., 2014).

The presence of Heavy metals and metalloids represents a serious problem, since water resources are the basis of any activity. Food security and global public health are compromised by anthropogenic activities. Heavy metals and metalloids affect in different ways, ranging from damage to vital organs to development of various types of cancer (Reyes et al., 2016).

The oxidation of sulfur and iron  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$ , is the effect of microorganisms responsible for bioleaching (Hoffman, 2020). Unicellular species used for bioleaching include the genus *Thiobacillus*, which gained recognition in biohydrometallurgy being industrially used for the recovery of metals from mining and metallurgical waste (Gao et al., 2021).

## 2. Materials and Methods

### 2.1. Mining and metallurgical waste samples

In the location of the study ores with high amounts of sulfides are mined, and part of these minerals are present in the mining-metallurgical tailings (Mäkinen *et al.*, 2020). The tailings sample was donated by Compañía Minera Gaocas S.A. Ore beneficiation plant, located in the Zhumiral sector, Camilo Ponce Enriquez, Azuay, Ecuador. This beneficiation plant carries out processes of concentration by flotation and hydrometallurgy by cyanidation. A representative sample was taken for the analysis directly from the pipe discharging to the tailings dam.

### 2.2. Mining and metallurgical waste characterization

Table 1 shows the tests carried out at the Instituto de Investigaciones Geológico Energéticas del Ecuador (IIGE) and the Facultad de Ingeniería en Minas Petróleos y Ambiental (FIGEMPA).

**Table 1.** Physical, chemical and mineralogical characterization

Test	Standard
Granulometry	ASTM D422-C136
ICP-OES	ASTM E2941-14
XRF	ASTM E1605-04
XRD	Análisis Retvield
Atomic absorption	NCH - ISO 17025

### 2.3. Culture media and microbial enrichment

Two liquid culture media were used for bacterial culture, 9k medium (Temple & Colmer, 1951): 3 g l<sup>-1</sup>; KCl, 0.1 g l<sup>-1</sup>; 0.5 g l<sup>-1</sup>; 0.5 g l<sup>-1</sup>; 0.1 g l<sup>-1</sup>, 44.2 g l<sup>-1</sup>. Modified 9k medium (TK) was also used, the latter having 10 g l<sup>-1</sup> thiosulfate instead of ferrous sulfate. Microbial cultures were carried out in 250 ml Erlenmeyer flasks at a temperature of 30 °C with magnetic stirring at 120 RPM.

### 2.4. Leaching tests at laboratory level

Microbial leaching experiments were carried out using Erlenmeyer flasks of 1 liter capacity, placed on magnetic stirring plates at a speed of 120 revolutions per minute and at a temperature of 30 °C, using a pulp density of 8 %. Each flask was filled with 700 ml of 9K Culture Medium previously dissolved at 7 %. In addition, leaching tests were carried out without the addition of microbial inoculum, in order to compare the oxidation rate of the chemical reactions with those taking place in the presence of a biological catalyst.

### 2.5. Larger scale bioleaching tests

With a Temperature oscillating between 20 and 33 °C, acidity  $\square$  1.5 was obtained. The RPM generated was 85 RPM. In a volume of 8.3 liters, 640 g of metallurgical mining residues were tested. As summary scheme in Figure 1 shows.

## 3. Results

The tests performed on the residue sample prior to bioleaching process lead to the following results. Figure 2 shows the microbial staining, Table 2 shows the results of the waste characterization by ICP-OES, XRD and XRF assays. The ICP-OES metal concentrations are described in units mm kg<sup>-1</sup>, its equivalent is ppm. The XRF (X-ray fluorescence) assay results of characterization prior to the bioleaching process present the chemical

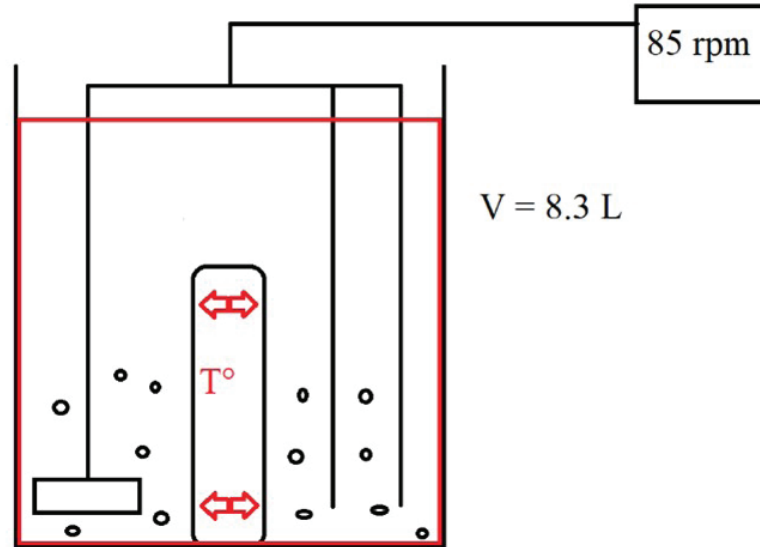


Figure 1. Schematic of the operation of a controlled environment for scaling of the process.

elements constituting the mineral residues. For the identification of the minerals present (major elements) in the residues, the results of X-ray diffraction (XRD) were used.

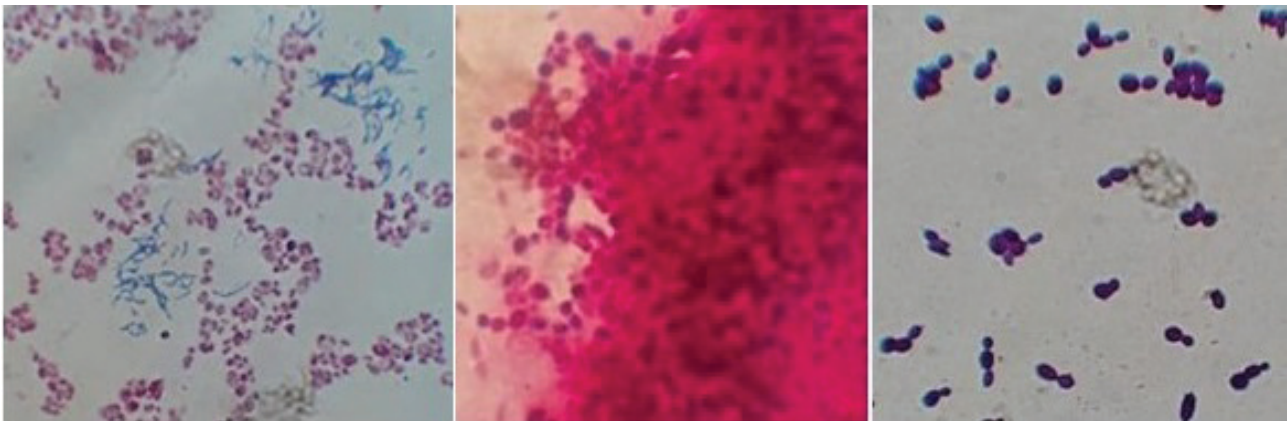


Figure 2. Gram staining of the consortium of acidophilic microorganisms. Magnification 100x.

Table 2. Concentration of elements of interest by ICP-OES, XRD and XRF.

ICP-OES		DRX		FRX	
Element	Concentration mm kg <sup>-1</sup>	Compound	Weight %	Element	Weight %
Cu	536,71	MgO	2,025	Quartz	70,3
Fe	64.708,09	Al <sub>2</sub> O <sub>3</sub>	5,613	Nonrite	12,9
Zn	996,00	SiO <sub>2</sub>	74,556	Albite-Anortite	11,0
As	888,50	SO <sub>3</sub>	0,733	Amphibole	3,9
Cd	27,31	K <sub>2</sub> O	0,150	Calcite	1,9
Pb	27,94	CaO	2,107		
		TiO <sub>2</sub>	0,542		
		Mn <sub>2</sub> O <sub>3</sub>	0,086		
		Fe <sub>2</sub> O <sub>3</sub>	9,967		
		PPC**	3,741		

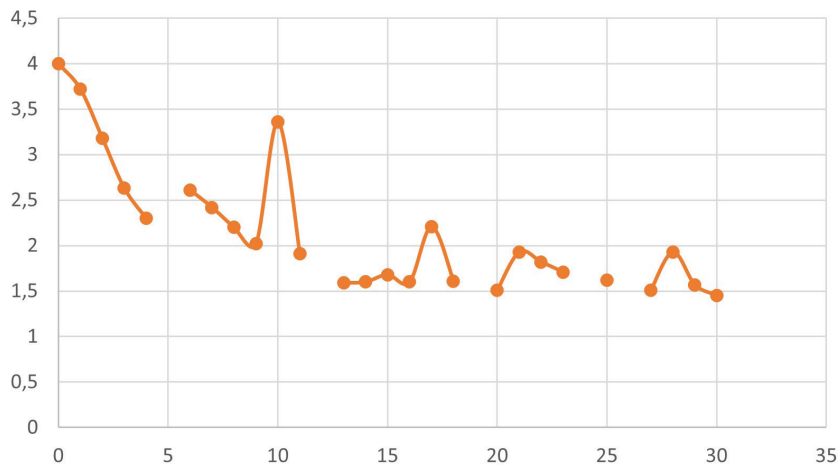


Once the data were obtained, the first laboratory tests in flasks were carried out as previously specified. The results showed that the bioleaching process of mining-metallurgical waste inoculated with microorganisms capable of biooxidation presented changes in the solution. In the flask without microbial inoculum, pH and TDS values did not change significantly throughout the experiment (Table 3). Both copper and iron remained undetectable in the solution.

**Table 3.** Bioleaching results in flask without microbial inoculum.

Date	pH	TDS ppm	Fe Ppm	Cu ppm
09/1/2023	6,94	1040	-0,178	-0,85
12/1/2023	7,3	1280	-	-
16/1/2023	7,55	1390	-	-
19/1/2023	7,55	1440	-	-
23/1/2023	7,73	1490	-0,166	-0,87

As part of the bioleaching process, the evolution of pH over the days of the experiment is shown (Figure 3), as well as the changes in dissolved copper (Figure 4).

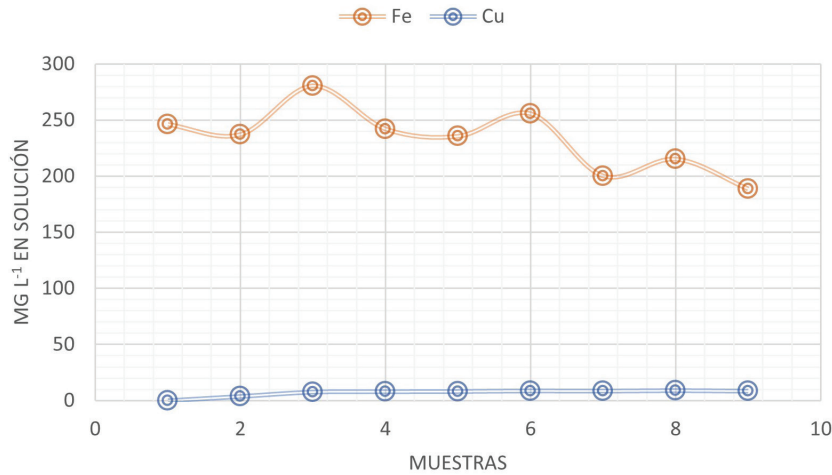


**Figure 3.** Evolution of pH for 30 days of testing.



**Figure 4.** Changes in dissolved copper over the test time at laboratory.

Liquid samples taken periodically on Mondays and Thursdays were subjected to atomic absorption spectrometry generating solubilized Cu and Fe concentrations (Figure 5).



**Figure 5.** Solubilized copper and iron concentrations.

At the end of the bioleaching process, tests were carried out in order to show what changes occurred during the process. Based on these tests carried out by the IIGE it was possible to perform calculation procedures for the evaluation of arsenic removal. From this point on, the test tables were for the sample subjected to bioleaching. The concentrations of metals of interest are shown in Table 4.

**Table 4.** Final concentration of target elements in the analysis.

Element	Concentration mg kg <sup>-1</sup>
<b>Cu</b>	197.12
<b>Fe</b>	54232.18
<b>Zn</b>	48.54
<b>As</b>	365,85
<b>Cd</b>	17,96
<b>Pb</b>	21,25

The changes in mineralogy by XRD and the major elements of the XRF assay are shown in Table 5.

**Table 5.** XRD and XRF test results.

Element	Weight %	Compound	Weight %
<b>Quartz</b>	55,0	<b>Na<sub>2</sub>O</b>	0,239
<b>Nontrite</b>	8,1	<b>MgO</b>	1,741
<b>Albite-Anortite</b>	1,2	<b>Al<sub>2</sub>O<sub>3</sub></b>	4,437
<b>Gypsum</b>	7,5	<b>SiO<sub>2</sub></b>	56,626
<b>Elemental S</b>	28,2	<b>SO<sub>3</sub></b>	0,991
		<b>K<sub>2</sub>O</b>	0,109
		<b>CaO</b>	2,059
		<b>TiO<sub>2</sub></b>	0,312
		<b>Mn<sub>2</sub>O<sub>3</sub></b>	0,055
		<b>Fe<sub>2</sub>O<sub>3</sub></b>	6,756
		<b>PPC**</b>	24,700

With the relevant data on the concentrations of heavy metals present in the waste, both before and after the microbial attack developed in the experimental bioreactor, the percentages of absence of metals were established by comparing the initial values with the final ones. The arsenic removal efficiency in the present work was calculated by means of equation [1].

$$\% \text{ Rec As} = \frac{[As]_i - [As]}{[As]} \cdot 100\% \rightarrow \% \text{ Rem As} = \frac{([888.50]_i - [365.85])}{[888.5]} \cdot 100\% \rightarrow \% \text{ Rem As} = 58.82\% \quad [1]$$

where:

- $[As]_i$  = Initial arsenic concentration in  $\text{mg kg}^{-1}$ .
- $[As]$  = Arsenic concentration after treatment in  $\text{mg kg}^{-1}$ .

The calculations performed are a straightforward way to establish the direct percentage relationship between the amounts of metals. Graphically this is visible in Figure 6, giving the percentage count of element removal.

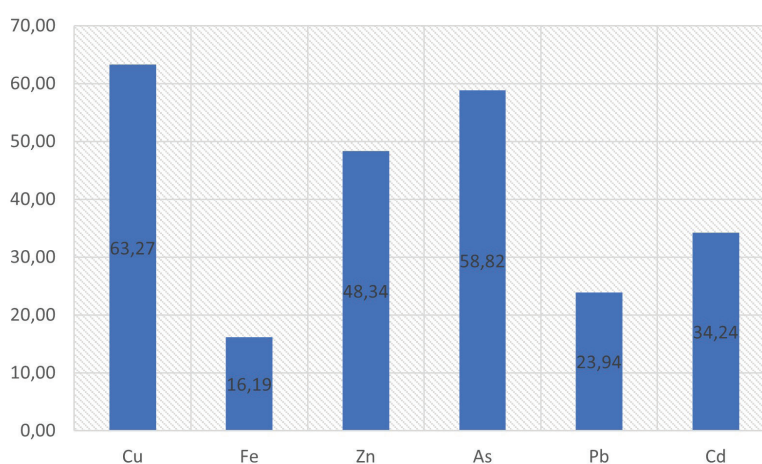


Figure 6. Metal removal rates.

## 4. Discussion

Normally the residues from the process of separating economic minerals and waste rock are considered hazardous, as over time the remaining sulfide minerals oxidize, thus generating leachates enriched in potentially contaminating metals (Lindsay *et al.*, 2015; U.S. Environmental Protection Agency, 1994). The elimination of sulfur in mining waste by microbial attack implies the possibility of completely inhibiting the generation of acid drainage; and, therefore, stopping the migration of heavy metals to soil and bodies of water. The present research replicated natural processes in a controlled environment with the objective of chemically stabilizing a hazardous waste, by increasing the favorable conditions for the action of microorganisms in order to enhance the removal of heavy metals.

Metals can become soluble under acidic conditions and remain in a liquid phase within a controlled environment. This process is evidenced by atomic absorptions. It is possible to verify the absence of metals by repeating tests for analysis of compounds and elements.

The significant removal of 63.27 % of the concentration of copper, 16.19 % of iron and arsenic is an achievement that should be further studied, because they are highly toxic and polluting heavy metals. The method requires further tests in order to determine the new compounds that formed in the liquid phase of the leachate. It seems reasonable to accept that such a finding may represent the way to neutralize the mining-metallurgical waste. Similarly, an interesting aspect is the analysis of the recovery of metals with economic value. This could be recovered by assigning a value to the waste (Fomchenko & Muravyov, 2020). Thus, revalorization and environmental remediation create the picture suitable for sustainability.

The experiment still requires experimental processes that will provide additional data, which will allow to determine the efficiency of all processes given in bioleaching. However, the external parameters controllable with experimental technology had a positive impact. By working on the improvement of microorganisms characteristics such as their metabolism, resistance to the amount of dissolved metals, growth rate and generational duplication, it will be possible to obtain improvements in the results, given that the ramification of research in this field offer many opportunities; and, capitalizing on the fact that the microorganisms come from a direct isolation from nature.

## 5. Conclusions

Leachate-generating microorganisms with hazardous metals and contaminants represent problems for the management of mining and metallurgical wastes, and even operating mines. But, with a different approach, they can be beneficial in a controlled environment, as long as their vital functions are identified on wastes with usable elements for them. Given the characteristic of releasing heavy metals from minerals, and in parallel the generation of acidity with ferric oxidation produces a medium that increases the amount of metals released to a controlled liquid medium.

The passivation of wastes is an issue that should be urgently treated, as the generation of mining-metallurgical wastes is constant, and it moves a large part of the economy of a developing country. This study demonstrates that exist options to generate methodologies which can be implemented within mind the objective of a sustainable industry.

Heavy metals and metalloids constitute a point of interest within public health and should be disseminated. Diseases associated with heavy metal intoxication in populations that consume water, and other water-dependent elements must be treated in time once the problem is known.

## Contributor Roles

- Santiago Martín Salinas García: conceptualization, investigation, methodology, resources.
- Juan David Rosero Mosquera: investigation, methodology, resources, writing - original draft.
- Félix Daniel Andueza Leal: methodology, writing - original draft.
- Diana Karina Fabara Salazar: conceptualization, writing - original draft, writing - review & editing.
- Danny Santiago Burbano Morillo: writing - review & editing.
- Sonia Elizabeth Chamorro Armas: investigation.
- Darío Damián Lozada Fiallos: conceptualization, supervision, writing - original draft, writing - review & editing

## Ethical Issues

Ethics approval Not applicable.

## Conflict of interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

## Additional information:

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