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Microbial inoculum production for bioleaching of critical metals from tailings

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Abstract. In this paper, research is presented regarding the development and testing of strategies to identify the optimal solutions for the production of microbial inoculum for the bio-recovery of critical metals from tailings. The versatility and diversity of biotechnologies available offer the opportunity for an economically viable solution for treating ores with complex mineralogy and those not suitable for processing by traditional methods. Bioleaching is a real economic alternative for the treatment of mine tailings, which allows, depending on the chosen strategy, two possible outcomes: (i) a leachate enriched in target metals or (ii) a tailings enriched in target metals by leaching interfering components. Continued population growth, ever-increasing levels of economic activity and technological innovation lead to a rapid increase in resource consumption, making the sustainable use of resources an imperative issue for humanity. Therefore, in recent years, new ideas regarding the use of materials and circularization have emerged. On the one hand, traditional mining activity produced tailings that were deposited in tailings ponds, containing metals as by-products of the extraction processes. On the other hand, as we aim to expand human presence in space, we need to find viable approaches to achieve independence from terrestrial resources and space biomining becomes of great interest.

Keywords: critical metals, bio-recovery, microbial consortium, biomining

1. Introduction

The bio-recovery of critical metals from tailings has become a significant area of research due to the increasing demand for metals and the need for more sustainable and environmentally friendly extraction methods. Bioleaching is an important process of critical metal recovery from tailings (Pollmann K., 2018; Xiufang Gao, 2021; Butu A., 2023). Microbial inoculum, consisting of microorganisms capable of extracting metals from tailings, is a crucial component of the bio-recovery process (Homayoun Fathollahzadeh, 2018; Reynier N., 2021; Rito B, 2022). The optimal production of microbial inoculum is essential for efficient and cost-effective metal recovery. This article outlines the development and testing of strategies for identifying the optimal solutions for the production of microbial inoculum for bio-recovery.

2. Production of microbial inoculum for bioleaching of critical metals

The first step in developing a strategy for optimal microbial inoculum production is the identification of microorganisms that are capable of extracting metals from tailings. This can be achieved through screening and selecting microbes based on their metal-extracting abilities. Various screening methods, such as culturing on metal-rich media and determining metal uptake, can be used to evaluate the metal-extracting abilities of microorganisms. A variety of metal-extracting microorganisms can be obtained from environmental samples, such as soil and water, or from previously isolated cultures.

Once the optimal microorganisms have been identified, the next step is to optimize the culture conditions to enhance their metal-extracting abilities. This includes adjusting factors such as temperature, pH, and nutrient levels, to ensure that the microorganisms are able to grow and extract metals effectively. The optimization of growth conditions can also be achieved by manipulating the composition of the culture medium to provide the microorganisms with the necessary nutrients for optimal growth and metal extraction.

After optimizing the culture conditions, the next step is to scale-up the production of microbial inoculum. This involves increasing the size of the culture from a laboratory scale to a larger scale that is suitable for practical applications. A variety of techniques can be used to scale-up the production of microbial inoculum, such as batch, fed-batch, and continuous culture methods.

Once the microbial inoculum has been produced, it must be tested and evaluated to determine its efficacy for metal extraction from tailings. This can be achieved through laboratory-scale tests, such as batch experiments or

column tests, or through pilot-scale tests, where the microbial inoculum is tested on a larger scale. The results of these tests can then be used to determine the optimal conditions for metal extraction and to evaluate the effectiveness of the microbial inoculum.

3. Strategies for microbial inoculum production

The production of microbial inoculum for the bio-recovery of critical metals from tailings is a crucial aspect of the overall process. The development and testing of strategies for identifying the optimal solutions for the production of microbial inoculum can lead to efficient and cost-effective metal recovery.

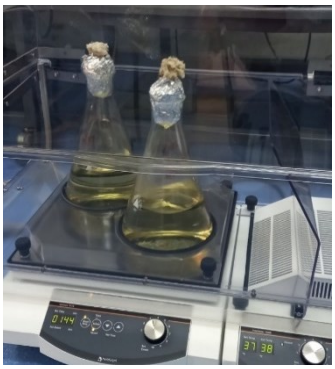

Figure 1. Individual cultivation of autochthonous bacterial isolates on synthetic media (S1)

The experiments were carried out with the aim of optimizing the production of microbial inoculum with application in the biorecovery of critical metals.

Analyzing the results obtained in the previous research phases, five strategies were developed to test the optimal solution for the production of microbial inoculum for the biorecovery of metals from tailings. These are the following:

S1 - individual cultivation of autochthonous bacterial isolates on synthetic media

S2 - mixed cultivation of autochthonous bacterial isolates on synthetic media

S3 - individual cultivation of autochthonous bacterial isolates on synthetic media with the addition of sterile (100:4, mL/g)

S4 - mixed cultivation of autochthonous bacterial isolates on synthetic media with added sterile (100:4, mL/g)

S5 - individual cultivation of autochthonous bacterial isolates on synthetic media followed by mixed cultivation on synthetic media

Two types of tailings were used, namely one with an alkaline pH (Tl), collected from Teliuc, and one with an acidic pH (Ct), collected from Certej. The sterile ones were used as such, without additional treatments (e.g. grinding, autoclaving, etc.). The experiments were carried out at room temperature and 37º C in Erlenmeyer flasks of 1500 mL, on the rotary shaker at 150 rpm.

The solutions tested for the production of the microbial inoculum were evaluated according to the quality of the inoculum obtained, taking into account the cfu/mL and the time required to reach the logarithmic growth phase. Thus, a scale from 5 to 0 was established, where 5 represents the best results and 0 the worst.

Table 1. Inoculum quality score obtained by different strategies

Tailings saple_Strategy	Scor
Tl_S1	4
Tl_S2	2
Tl_S3	2
Tl_S4	1
Tl_S5	1
Ct_S1	3
Ct_S2	2
Ct_S3	4
Ct_S4	2
Ct_S5	5

From the previous table (Table 1.) it can be seen that the tested strategies behaved differently for the two types of tailings. The S5 strategy of individual cultivation of native bacterial isolates on synthetic media followed by mixed cultivation on synthetic media with added tailings performed best for acid pH tailings (Ct) and performed worst for alkaline pH tailings (Tl). One of the potential factors considered responsible for this behavior is the very low granulometry of the sterile material (close to powder appearance) which acts as a barrier for bacterial cells to access oxygen. Reducing shaking, specifically spotting the experiment using occasional shaking (manual shaking for about one minute, 3-4 times a day) significantly improved the results (score 3), but without equaling the score of strategy S1. So, in the case of Tl waste, the best solution for obtaining the microbial inoculum is strategy S1, namely, the individual cultivation of autochthonous bacterial isolates on synthetic media and their simultaneous inoculation in the lysimeter. The other strategies led to poor and very poor results (score 2 and 1, respectively) for Tl tailings. In the cases of adding tailings to the synthetic culture medium (strategies S3 and S4), the factor that negatively influences inoculum development is the same as in the case of strategy S5, particle size. Another factor leading to a low-scoring inoculum when isolates (S2, S4, S5) are cultured simultaneously is the difference in development time of the isolates in the consortium. For tailings with acid pH, another strategy with very good results is S3 - individual cultivation of autochthonous bacterial isolates on synthetic media with addition of tailings. If there is a time constraint this strategy can be successfully applied to obtain an inoculum of satisfactory quality.

As was also concluded in the previous stage, this time too it was observed that although cultivation at 37ºC leads to a reduction in the duration of obtaining the inoculum, this

solution is not justified from an economic point of view, energy expenses can be reduced through a planning efficient (e.g. advancing the moment of starting the cultivation of the pre-inoculum, respectively the inoculum).

Three experiments for testing in biosolubilization experiments of the inoculum obtained by the most effective strategies taken according to the type of waste in the work were carried out .

In the first experiment, two waste samples were used, one with acid pH (A1) and one with alkaline pH (A2), such a composition is shown in table 2. They were inoculated with the inoculum obtained by strategy S5, respectively with the inoculum obtained by strategy S1. and their simultaneous inoculation in the lysimeter) and water was added up to 50 VWC. No additional water was added after the initiation of the experiment. The experiment was stopped when the moisture in the tailings reached 20 VWC. Liquid collection was difficult due to low humidity. The collected liquid was analyzed for metal content determination (Table 3).

Table 2. Composition of tailings (experiment 1)

		AL	Cu	Fe	Mg	Mn	Na	P	S	Si	Zn
A1	%	1.96	0.013	2.86	2.92	0.53	0.11	0.05	0.49	20.76	0.012
A2	%	4.2	0.007	9.47	0.152	0.0106	0.073	0.017	1.42	31.51	0.017

Table 3. Composition of collected liquids (experiment 1)

		AL	Cu	Fe	Mg	Mn	Na	P	S	Si	Zn
L1	g/L	<0.00002	<0.00002	<0.00002	0.4	<0.00002	0.007	<0.00002	0.41	0	<0.00002
L2	g/L	1.2	0.03	1.3	0.41	0.19	0.012	0.17	0.8	0.08	0.68

From the results obtained by determining the composition of the liquids, it was concluded that it is necessary during the biosolubilization to supplement with water in the lysimeter so as to keep the humidity in the waste at over 30 VWC.

The experiment was repeated with two new samples of acid tailings (A3 and A4). For these, complete determinations of the content of elements were made, and the data obtained are presented in table 4. Each of the two tailings samples was introduced into a lysimeter, they were inoculated with the microbial consortium obtained according to the S5 strategy (individual cultivation of autochthonous bacterial isolates on synthetic media followed by mixed cultivation on synthetic media with added sterile) and supplemented with water up to 50 VWC. During the experiment when the humidity recorded values of 25 - 35 VWC it was corrected by adding water up to 45 - 50 VWC.

Table 4. Content of tailings samples used in experiment 2

		AL	Cu	Fe	Mg	Mn	Mo	Na	P
A3	mg/Kg s.u.	9049.48	101.95	86342.81	29487.71	4536.20	0.88	301.74	560.05
A4	mg/Kg s.u.	1723.77	72.95	34822.80	294.00	46.36	0.80	283.36	247.72
		Zn	Ti	V	Ag	Hg	Be	As	Sn
A3	mg/Kg s.u.	118.69	268.60	11.91	0.13	0.09	0.15	119.30	0.72
A4	mg/Kg s.u.	314.20	5.40	6.07	0.72	1.20	<0.1	362.52	0.21
		Te	Se	Sr	Li	B	Cr	K	Ca
A3	mg/Kg s.u.	<0.14	0.41	86.32	2.82	2.43	8.60	2161.92	77814.89
A4	mg/Kg s.u.	7.15	3.20	26.53	0.46	1.35	5.14	2365.42	18806.79
		Ni	Pb	Co	Cd	Sb	Ba	Tl	Bi
A3	mg/Kg s.u.	6.55	70.53	4.16	0.20	0.34	3260.89	<0.05	<0.08
A4	mg/Kg s.u.	2.87	1576.15	0.58	0.51	22.04	76.76	<0.05	<0.08

Table 5. Composition of liquids from the first collection (experiment 2)

		Cu	Mg	Mn	Mo	Zn
A3	mg/L	35.2937	387.8000	142.7250	0.0109	508.9000
A4	mg/L	57.6374	463.7000	321.8000	0.0095	1,046.7000

Table 6. Composition of liquids from the final harvest (experiment 2)

		Cu	Mg	Mn	Mo	Zn
A3	mg/L	0.0260	369.2000	0.0120	0.0780	<0.002
A4	mg/L	80.8000	543.4500	310.5500	0.0820	604.5000

Two liquid collections were made from each lysimeter at equal time intervals (6 weeks) and the concentrations for the main metals in these liquid samples were analyzed (Tables 5 and 6). Under the conditions of experiment 2, the microbial consortium remained active and, as can be seen from tables 5 and 4, optimal conditions for biosolubilization were maintained.

For the third experiment, two samples of alkaline tailings (A5 and A6) were used. For these, the concentrations of the main metals were determined, and the data obtained are presented in table 6. Each tailings was introduced into a lysimeter, they were inoculated with the microbial consortium obtained according to strategy S1 (individual cultivation of autochthonous bacterial isolates on synthetic media and their simultaneous inoculation in the lysimeter) and were supplemented with water up to 50 VWC. Humidity was monitored throughout the experiment and corrected to 45 - 50 VWC by adding water.

Table 7. Concentrations of the main metals in tailings samples used in experiment 3

		AL	Cu	Mg	Mn	Mo	Zn	Ti	V	Ag
A5	mg/Kg s.u.	826.85	8.08	2,942.72	595.41	0.52	153.85	644.92	17.76	0.05
A6	mg/Kg s.u.	217.09	5.44	24.85	14.33	0.81	1,267.56	12.31	12.55	0.51

Table 8. Composition of liquids from the final harvest (experiment 3)

		Cu	Mg	Mn	Mo	Zn
L5	mg/L	0.0190	290.3000	163.7250	<0.005	612.9000
L6	mg/L	4.3230	36.0300	37.5000	0.0280	615.2500

This experiment showed that even in the case of tailings samples with alkaline pH, the addition of water provides the necessary conditions for biosolubilization with the microbial consortium obtained by the optimal strategy (S1).

Once the optimal solution for the production of microbial inoculum has been achieved, the inoculum can be implemented in the bio-mining process. The inoculum can be added directly to the tailings, or it can be used to inoculate a separate culture, which can then be added to the tailings. The effectiveness of the inoculum can be monitored over time to ensure that the bio-mining process is achieving the desired results.

4. Conclusion

The development and testing of strategies to identify optimal solutions for the production of microbial inoculum for the bio-recovery of critical metals from tailings involves several steps. The results demonstrate that the choice of the microbial inoculum production strategy for bio-recovery of critical metals should be correlated with tailings characteristics.

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