MICROBIOLOGICAL PROCESSES OF PLATINUM METAL LEACHING (PLATINUM, PALLADIUM AND RHODIUM) FROM FERROUS AND NON – FERROUS INDUSTRIAL WASTE UNDER THE ACTION OF *Acidithiobacillus ferrooxidans*

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Abstract. The potential enzyme evaluation of the acidophilic, chemolitotrophic and heterotrophic bacteria specific to the mining biotope is of high interest in the solubilization, recovery and / or removal of platinum metals from industrial waste car tires. The obtained data show that the formation of extracellular polymeric substances plays an important role in the attachment of the chemolithotrophic bacteria on the surface of minerals, as well as sulphur. In this sense, a direct interaction between the bacterial cells and the solid surface is mandatory for an efficient mobilization of platinum metals from waste car tires. These occur at two levels: (a) physical sorption due to electrostatic forces with low pH in the leaching media; (b) chemical sorption is carried out only in the type of chemical bonds between the bacterial cell disulphide bond and minerals. The optimization of the technological process such as solubilization and the recovery of platinum metal ions contained in ferrous and non-ferrous industrial waste was materialized through a series of laboratory experiments based mainly on the affinity of bacterial cells for certain organic and / or inorganic surfaces. It is based on the results of the physical-chemical characterization of the liquid samples taken. This study deals with the optimization of the solubilization process of metal ions contained in such samples.

Keywords: biohydrometallurgy, platinum metals, acidophilic chemolithotrophic bacteria, Romania.

Rezumat. Procedee microbiologice de solubilizare a metalelor platinice (platina, paladiu şi rodiu) din deşeuri industriale feroase şi neferoase sub acţiunea lui *Acidithiobacillus ferrooxidans.* Evaluarea potențialului enzimatic al bacteriilor acidofile, chemolitotrofe și heterotrofe, specifice biotopului minier este de mare interes în solubilizarea, recuperarea și/sau îndepărtarea metalelor platinice din deșeuri industriale auto uzate. Datele obținute au evidențiat faptul că formarea substanțelor polimerice extracelulare joacă un rol important în atașarea bacteriilor chemolitotrofe pe suprafața mineralelor, precum și de sulf. În acest sens, interacțiunea directă între celula bacteriană și suprafața solidă este obligatorie pentru o mobilizare eficientă a metalelor platinice din deșeuri auto uzate. Acestea au loc la două niveluri: (a) sorbția fizică din cauza forțelor electrostatice datorită pH-ului scăzut din mediile de lești ere; (b) sorbția chimică în care se realizează numai legături chimice de tip legături bisulfide între celula bacteriană și minerale. Activitatea de optimizare a procesului tehnologic de solubilizare și recuperare a ionilor metalici platimici conținuți în deșeuri industriale feroase și neferoase s-a concretizat printr-o serie de experimente de laborator care se bazează în principal pe afinitatea unor celule bacteriene pentru anumite suprafețe de natură organică și/sau anorganică. Pe baza rezultatelor caracterizării fizico-chmice a probelor lichide prelevate s-a considerat important studiul de față privind optimizarea procesului de solubilizare a ionilor metalici conținuți în acestea.

Cuvinte cheie: biohidrometalurgie, metale platinice, bacterii chemolitotrofe acidofle, România.

INTRODUCTION

The activity and selectivity of acidophilic, chemolithotrophic and heterotrophic bacteria in the processes of solubilization and accumulation / recovery of metallic ions from self-used industrial waste are determined by their metabolic activity in accordance with the physiological conditions of the leaching environment. Acidophilic chemolithotrophic bacteria of the *Acidithiobacillus* genus have the ability to intervene in the solubilization process of sulfides through the regeneration of ferric sulfate and to catalyze the oxidation – reduction reactions of sulphur compounds in the presence of oxygen. Following these reactions, the sulfide ion is oxidized and the metal ion is eliminated in solutions (CISMAȘIU et al., 2015; STANCU, 2019).

The study of how acidophilic chemolithotrophic bacteria interact with the substrate of organic and / or inorganic origin has a strong influence on mining technologies. Also, in commercial leaching operations, bacterial consortia are used because biogeochemical processes take place in the leaching environments (JOHNSON, 1998; CISMAȘIU, 2010; CÎRSTEA & ȘTEFĂNESCU, 2018a, b).

The research in the field of microbial ecology of extreme acid environments has intensified in recent years in the field of basic and applied research. Data from the literature reflect the fact that the role of chemolithotrophic bacteria, as well as heterotrophic ones, is and continues to be of great practical interest for the processes of solubilization of metals or their absorption from liquid effluents (CÎRSTEA & ȘTEFĂNESCU, 2017; CHEMYSH et al., 2019). Studies carried out in the field of biohydrometallurgy have shown that the solubilization of ferrous and non-ferrous industrial waste under the action of *Acidithiobacillus ferrooxidans* is dependent on the nature of the metal ion, as well as on the physico-chemical conditions in which the bacterial culture acts. (KARAVAIKO, 1988; GARG et al., 2020; BRÎNZA et al., 2021).

MATERIAL AND METHOD

In the composition of ferrous and non-ferrous industrial waste, most often, sulphur can be identified in two forms: inorganic in the form of pyrite or marcasite and organic in the form of organic sulphur complexes (coal), as well as other metal sulfides with soluble ions. In the bacterial solubilization experiments of platinum metals from powder (A) and plates (B / C) of non-ferrous industrial waste, acidophilic bacterial cultures represented by 2 strains of *A. ferrooxidans* were used, marked with T_2 isolated from Valea Şesei and with T_4 isolated from Baia, grown on a selective 9K medium (pH = 2.5) (CISMAŞIU et al., 2015).

Experiments of controlled bio-oxidation of platinum metals from non-ferrous industrial waste (platinum metal powder marked A and platinum metal plates marked B / C) in the presence of *Acidithiobacillus ferrooxidans* were performed in 500ml bottles containing 200ml of Leathen medium (KARAVAIK & GROUDEV, 1985) having ferrous sulfate, 80ml of bacterial inoculum (T_2 / T_4) and catalyst (powder or plates of non-ferrous industrial waste) as the only source of growth and energy. In the experiments to test the action of acidophilic chemolithotrophic bacteria of *Acidithiobacillus ferrooxidans* type on non-ferrous industrial waste of platinum metals, the analysed bacterial cultures were incubated, for 4 months, under different experimental conditions: (1) acidity - pH 3.5; (2) temperature - 300C; (3) conditions of continuous stirring (150 rpm).

Experiments of controlled bio-oxidation of platinum metals from carbonless platinum metal powder under the action of Acidithiobacillus ferrooxidans were performed in 500ml bottles containing 200ml of Leathen medium (KARAVAIKO, 1998) having ferrous sulfate, 80ml of the bacterial inoculum (T_2 / T_4) and catalyst (carbonless platinum metal powder) as the only source of growth and energy. In the experiments for selecting the solubilization action of acidophilic acid-oxidizing chemolithotrophic bacteria of *Acidithiobacillus ferrooxidans* type on the aforementioned catalyst, the analysed bacterial cultures were incubated, for 4 months between 3.08.2018 - 7.12.2018, under different experimental conditions: (1) acidity - pH 3.5; (2) temperature - 30^0C; (3) conditions of continuous stirring (150 rpm). In the experiments of bacterial solubilization of palladium and platinum from platinum metal powder without carbon acidophilic bacterial cultures were used, represented by 2 strains of Acidithiobacillus ferrooxidans, marked with T2 isolated from Valea Şesei and T4 isolated from Baia, grown on selective medium 9K (pH = 2.5).

RESULTS AND DISCUSSION

A. ***Bacterial solubilization experiments of platinum metals from non-ferrous industrial waste under the action of Acidithiobacillus ferrooxidans, iron- and sulphur-oxidizing bacteria.***

The microbiological processes of biosolubilization of platinum metals from non-ferrous industrial waste is complemented by mechanisms of metal complexation with chemical groups on the outer membrane of Gram-negative bacteria, as well as "nuclear" processes that bite through the bound metal progressive bit deposits. larger metal that are not mutually exclusive (Table 1).

Table 1. Solubilization of platinum and palladium from non-ferrous industrial waste under the action of bacterial strains of *Acidithiobacillus ferrooxidans* marked with the index T_2 and T_4.

Nr. crt.	Sample Id	Pt 265.945 (mg/L)	Pd 340.45 (mg/L)
1.	Ultrapure water	0.000	0.000
2.	Calib Std 1 - 1 mg/L	0.500	0.500
3.	Reagent blank	-.0.001	0.001
4.	AT2_supernatant	0.110	0.012
5.	CT4_supernatant	0.094	0.018
6.	AT2_biomasa	0.007	0.001
7.	CT4_biomasa	0.019	0.001
8.	CT4_supernatant d=0	0.641	0.293
9.	AT2_supernatant d=0	0.700	0.192

The controlled bio-oxidationbio oxidation reaction under the action of *Acidithiobacillus ferrooxidans d*epends on the physiological conditions of the cells, the chemical state of the metal in the impact with the bacterial cell, the metabolic products secreted by the cells, and all these are influenced by the ecological conditions of the leaching environment (Table 1).

The solubilization of pyrite (and for desulphurization) has been extensively studied in Gram-negative chemolithotrophic bacteria *Acidithiobacillus (A.) ferrooxidans*, which use either metal ions or the reduction of inorganic sulphur compounds as the only source of growth and energy (MONROY-FERNANDEZ et al., 1995; BRÎNZA et al., 2021). To isolate strains of sulphur and iron-oxidizing bacteria type *A. ferrooxidans*, 9K agarized selective medium was used (KAVAIKO & GROUDEV, 1988; KLEIN, 1998; CHEMYSH et al., 2019). Isolated colonies are distinguished by the reddish-red colour (CISMAȘIU et al., 2015).

The ability of acidophilic chemolithotrophic bacteria to adapt to extreme environmental conditions (low pH and high concentrations of metal ions) is very useful for their use in solubilizing metals from ferrous and non-ferrous industrial waste. In our study, the investigations focused on the bacterial leaching processes of metals from industrial wastes of platinum metals in powder form and plates under the action of acidophilic chemolithotrophic bacteria of the genus Acidithiobacillus due to their potential application in bioremediation of metal ion contaminated media (CIOBOIU & CISMAȘIU, 2018a, b; GARG et al., 2020; MISHRA et al., 2020).

B. The selection and testing of sulphur- and iron-oxidizing bacteria acidophilic chemolitotrofe performing the process of solubilization of the palladium and platinum group platiniei free of carbon powder.

In the process of biosolubilization of platinum metals an important role belongs to acidophilic chemolithotrophic bacteria type *Acidithiobacillus ferrooxidans* which is based on the elimination in the environment of inorganic acids, as well as some metabolism products, which together form water-soluble compounds. The best results of the controlled bio-oxidation of platinum and palladium metals under the action of Acidithiobacillus ferrooxidans were obtained by using the T2 strain of Acidithiobacillus ferrooxidans. highlighted by the increase in the level and yield of desulphurization of platinum metal powder without carbon (Fig. 1).

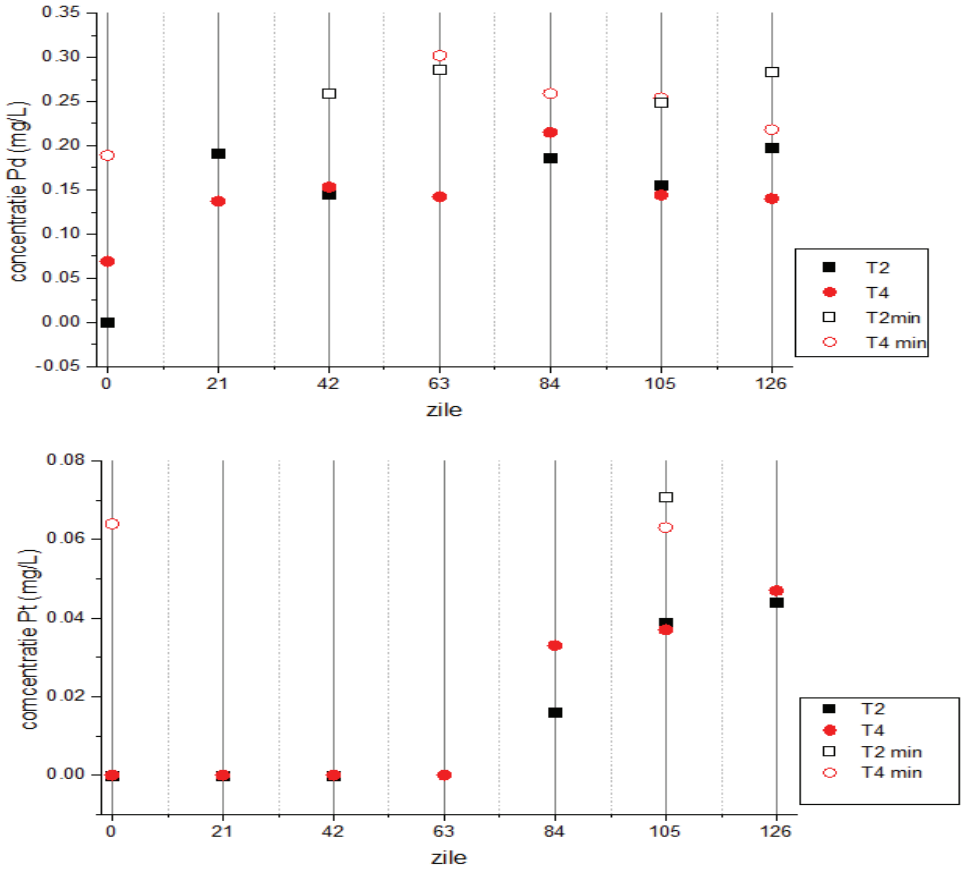

Figure 1. Selection of strains of acidophilic chemolithotrophic bacteria of *Acidithiobacillus ferrooxidans* (marked with index T_2 and T_4) performing in processes of controlled bio-oxidation of platinum and palladium.

In the experiments of bacterial solubilization of palladium, platinum and rhodium from platinum metal powder with carbon and without carbon an acidophilic bacterial culture was used, represented by the strain of *Acidithiobacillus ferrooxidans*, marked with the T_2 index isolated from the Sesei Valley, cultivated on a selective medium 9K (pH = 2.5) according to the data presented above.

The experiments of controlled bio-oxidation of platinum metals from platinum metal powder under the action of Acidithiobacillus ferrooxidans were performed in liquid medium Leathen (KARAVAIKO, 1988) having as the only source of growth and energy ferrous sulphate, 20% bacterial inoculum represented by strain T_2 and 3g catalyst (carbonless platinum metal powder).

In this context, the bioleaching of metal-poor ore waste involves the use of the ability to generate acid waters of bacteria belonging to the genus *Acidithiobacillus* for the solubilization of metals from mining waste with low metal content that cannot be extracted using physico-chemical techniques used in traditional mining, increasing the efficiency of extracting metal scraps from the electrolysis solution (coating of sheet metal objects) and from waste using bacteria of the *Acidithiobacillus* genus resulting from the processing of jewelry before disposal.

The obtained data showed that the microbiological processes of bacterial solubilization represent an economical alternative for biohydrometallurgy because the selectivity of acidophilic chemolithotrophic bacteria of the *Acidithiobacillus* genus is determined by their physiological properties, as well as a number of physical and chemical factors such as pH, concentration. Platinum metals in the composition of the inorganic substrate are represented by carbon-free powder (Table 2).

Table 2. The bacterial solubilization of rhodium under the action of the T2 strain of *Acidithiobacillus ferrooxidans* from platinum metal powder without carbon.

Nr.crt. falcone	**The analysis of the platinum metals (platinum, palladium and rhodium)**		
	Pt (ppm)	Pd (ppm)	Rh(ppm)
Nr. 1. The biological control (Mb) from the untreated powder at time 0 (initially) on 22.02.2019.	0	0	0.011
Nr. 2. Chemical control (Mc) from untreated powder at time 0 (initial) on 22.02.2019.	0	0	0.015
Nr. 3. Chemical control (Mc) from untreated powder at the final time of experimentation on 28.06.2019.	0	0	0
Nr. 4. The biological control (Mb) from the untreated powder at the final time of experimentation on 28.06.2019.	0	0	0.006

The solubilization of pyrite (and for desulphurization) has been extensively studied in Gram-negative chemolithotrophic bacteria *Acidithiobacillus (A.) ferrooxidans*, which use either metal ions or the reduction of inorganic sulphur compounds as the only source of growth and energy (KARAVAIKO, 1988; LAZĂR & DUMITRU, 1998; KOMNITSAS et al., 2001; VIDYALAKSHMI et al., 2009). In order to isolate the strains of sulphur and iron-oxidizing bacteria type *A. ferrooxidans*, the 9K agarized selective medium was used (KAVAIKO & GROUDEV, 1985; VOICU et al., 2009). Isolated colonies are distinguished by the reddish-red color (LAZAR et al., 2004; CISMAŞIU et al., 2015).

The development of such biotechnological processes, based mainly on the activity of acidophilic bacteria demonstrates the efficiency of bioremediation of the environment polluted with solid waste and toxic substances (CIOBOIU & CISMAŞIU, 2016; CÎRSTEA & ŞTEFĂNESCU, 2018a, b; STANCU, 2019). The unique properties of extremophilic microorganisms make them useful for a number of biotechnological applications in a variety of industrial fields and in environmental protection (ZARNEA & DUMITRU, 1994; CISMAŞIU et al., 2016).

In the experiments of desulphurization of coal concentrates, under the action of the populations of Acidithiobacillus ferrooxidans at the tested solid / liquid ratios, a significant difference was observed between the bio-oxidation yields of inorganic sulphur from the two coal concentrates. Thus, it was found that bacterial cultures of Acidithiobacillus ferrooxidans biooxidized inorganic sulphur in higher percentages of lignite concentrate compared to coal, which correlated with the presence of higher amounts of inorganic sulphur in coal (BOOMSTA et al., 1999; BOSECKER, 1999; BRANDL, 2001; BRÎNZA et al., 2021).

CONCLUSIONS

The accumulation of metabolic products with stimulating activity contributes together with the chemical changes of the inorganic substrate (platinum metal powder without carbon) to increase the efficiency of microbiological processes for solubilizing platinum metals from non-ferrous industrial waste under the action of Acidithiobacillus ferrooxidans in a leaching environment.

The paper represents a contribution to the research and development of technological processes based on the activity of acidophilic chemolithotrophic bacteria with efficient applications in bioremediation of environments polluted with residual inorganic substances as well as for the recovery of quantities of metal ions. The paper represents a contribution to the research and development of technological processes based on the activity of acidophilic chemolithotrophic bacteria with efficient applications in bioremediation of environments polluted with residual inorganic substances as well as for the recovery of quantities of metal ions.

The methods of bacterial mobilization of platinum metals are the following: (1) inclusion in inert materials of organic and inorganic nature; (2) absorption on a solid material; (3) mobilization by affinity bonds between biological molecules; (4) mobilization of platinum metals and cells by covalent or coordinative bonds with materials other than inert ones. Also, the data obtained from controlled oxidation experiments of inorganic sulphur reflect the fact that the presence of acidophilic chemolithotrophic bacteria of the genus *Acidithiobacillus ferrooxidans* is evidenced by the accumulation of biomass and not by metabolic products.

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