THE RECOVERY OF NOBLE METALS WITH MICROORGANISMS AND INVERTEBRATES: BASIC AND APPLIED IMPORTANCE

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Abstract. The development of processes based on the activity of micro-organisms is very important to increase the efficiency of processes for the recovery of noble metals from industrial wastes by two beneficial effects: (1) *the bioremediation of polluted environment with inorganic waste*, such as heavy metals that are toxic to most forms of life, including humans; (2) *recovering the amounts of metal ions* in order to reuse them for the economic purpose. The evolution of technology, the deepening of the knowledge of the microbial diversity and the action of the microorganisms on the different natural substrates allowed the realization of some economic technological processes. Among them, the use of microbiological methods for the recovery of noble metals from industrial wastes present a particular importance due to the depletion of rich ore stocks, as well as the accumulation of waste poor in metals due to their processing by hydrometallurgical methods. The biodegradative action of microorganisms is the result of the alternative intervention by heterotrophic and chemolithotrophic bacteria in a positive sense in the economy of human society.

Keywords: microorganisms, noble metals, bacterial oxidation, enzymatic reduction, Romania.

Rezumat. Recuperarea metalelor nobile cu ajutorul microorganismelor și nevertebratelor: importanța fundamentală și aplicativă. Dezvoltarea proceselor tehnologice bazate pe activitatea microorganismelor este foarte importantă pentru creșterea eficienței proceselor de recuperare a metalelor nobile din deșeuri industriale cu două efecte benefice: (1) *bioremedierea mediului poluat cu substanţe anorganice reziduale*, cum sunt metalele grele care sunt toxice pentru majoritatea formelor de viață, inclusiv omul; (2) *recuperarea unor cantităţi de ioni metalici* în vederea refolosirii acestora în scop economic. Evoluţia tehnologiei, aprofundarea cunoașterii diversității microbiene şi acţiunii microorganismelor asupra diferitelor substraturi naturale a permis realizarea unor procedee tehnologice economice. Dintre acestea utilizarea metodelor microbiologice pentru recuperarea metalelor nobile din deșeuri industriale prezintă o importanță deosebită datorită epuizării stocurilor de minereuri bogate, cât şi acumulării de deşeuri sărace în metale datorită prelucrării acestora prin metode hidrometalurgice. Acţiunea biodegradativă a microorganismelor reprezintă rezultatul intervenţiei alternative a bacteriilor heterotrofe şi chemolitotrofe în sens pozitiv în economia societăţii umane.

Cuvinte cheie: microorganisme, metale nobile, reducere enzimatică, oxidare bacteriană, România.

THE IMPORTANCE OF TECHNOLOGICAL PROCESSES DEVELOPMENT BASED ON THE ECOLOGY OF MICROORGANISMS AND INEVERTEBRATES

The development of technological processes based on the activity of microorganisms is very important to increase the efficiency of processes for the recovery of noble metals from of waste industrial two beneficial effects: (1) the bioremediation of polluted environment with inorganic waste, such as heavy metals that are toxic to most forms of life, including man; (2) recovery of quantities of metal ions in order to reuse them for economic purposes (LAZĂR, 2001). The optimizing activity of the bioreduction process of metallic ions contained in mining effluents was materialized through a series of laboratory experiments that are based mainly on the affinity of microbial cells for certain surfaces (ZARNEA, 1994).

The most popular methods of immobilizing microbes are the following: (a) inclusion of the inert materials (exp. Agar-agar), (b) adsorption on a solid, inert, (c) immobilizing the links of affinity between the molecules and biological (d) immobilization of cells by covalent or coordinative bonds with materials other than inert ones. Through their activity, microorganisms cause changes in the pH of the environment and in the redox potential, and they can develop various useful substances with completing properties during their metabolism. In this context, microorganisms have a predominant role in the solubilization and recovery of noble metals from industrial waste. It is already well known that some bacteria are able to solubilize inorganic sulphur from ferrous and non-ferrous industrial waste (CISMAȘIU, 2004).

The study of the aquatic microbiota includes the investigation of the presence and quantitative distribution of microorganisms present in water and the water sediment. Also, a particular interest is the interaction between the microorganisms' components and their relations with other bodies, such as invertebrates and plants. The knowledge of these interactions leads to the establishment of the role that microorganisms play in the flow of matter and energy in the aquatic ecosystem.

Also, invertebrates have an important role in the food chain of industrially polluted ecosystems in the Oltenia Plain, dominated by protozoa, rotifers, copepods, cladocera, oligochaetes, gastropods, bivalves, amphipods, odonata, chironomides. The population of gastropods is an important component of the biological production of the lake ecosystems eutrophic and identified a total of 37 species, including *Viviparus acerosus, Radix balthica*, *Physella (Costatella) acute Lymnaea stagnalis*, *Planorbarius coneus* are characteristic to the floodplain in the Oltenia. Ichthyofauna characterizes and particularizes the functionality of such pre-existing ecosystems to the river dam (CIOBOIU, 2011; 2014; CIOBOIU et al., 2017; 2019).

The persistence and multiplication of acidophilic microorganisms and invertebrates depends on their genetic structure, which is crucial for their adaptation to the respective environment and which rigorously limits the potential of phenotypic adaptation (FIRĂ & NĂSTĂSESCU, 1977; LAZĂR, 2001; CISMAŞIU, 2007). As such, the survival, growth and multiplication of a microorganism in a given environment involves mechanisms of evolutionary adaptation as well as phenotypic adaptation: (1) evolutionary adaptation - is an adaptation to long variations based on genotype modification and better stem selection adapted to the conditions of the respective environment; (2) the phenotypic adaptation (physiological) - is the result of the body's response to temporary changes in population (ZARNEA, 1994; ZARNEA & DUMITRU, 1994).

THE EVOLUTION OF TECHNOLOGY, THE DEEPENING OF THE MECHANISMS STUDY OF MICROORGANISMS AND INVERTEBRATES ACTION ON DIFFERENT NATURAL SUBSTRATES

Mining activities causes serious problems of pollution of the aquatic and terrestrial environment. As a result of the mining activities carried out in various perimeters in the country, the extraction and processing of different types of ores have resulted and continue to result in significant amounts of water and solid waste, which have accumulated in the environment on large areas. Also, these residual materials contain increased concentrations of metal ions with higher economic value. The sulphur waste deposited in landfills, with high concentrations of metal ions, have the ability to generate acidic and toxic waters. The diversity of the acidophilic microbiota resistant to heavy metals may be a potential source of microorganisms, underlying biotechnological applications for the recovery of heavy metals contained in mining effluents (RAKESH, 1990; LAZAR, 1992; RAWLINGS, 1999). The biological desulphurization processes was established as a laboratory technique by using bacterial cultures with a high capacity to oxidize pyrite, thus implicitly removing sulphur from industrial non-peat waste (Fig. 1).

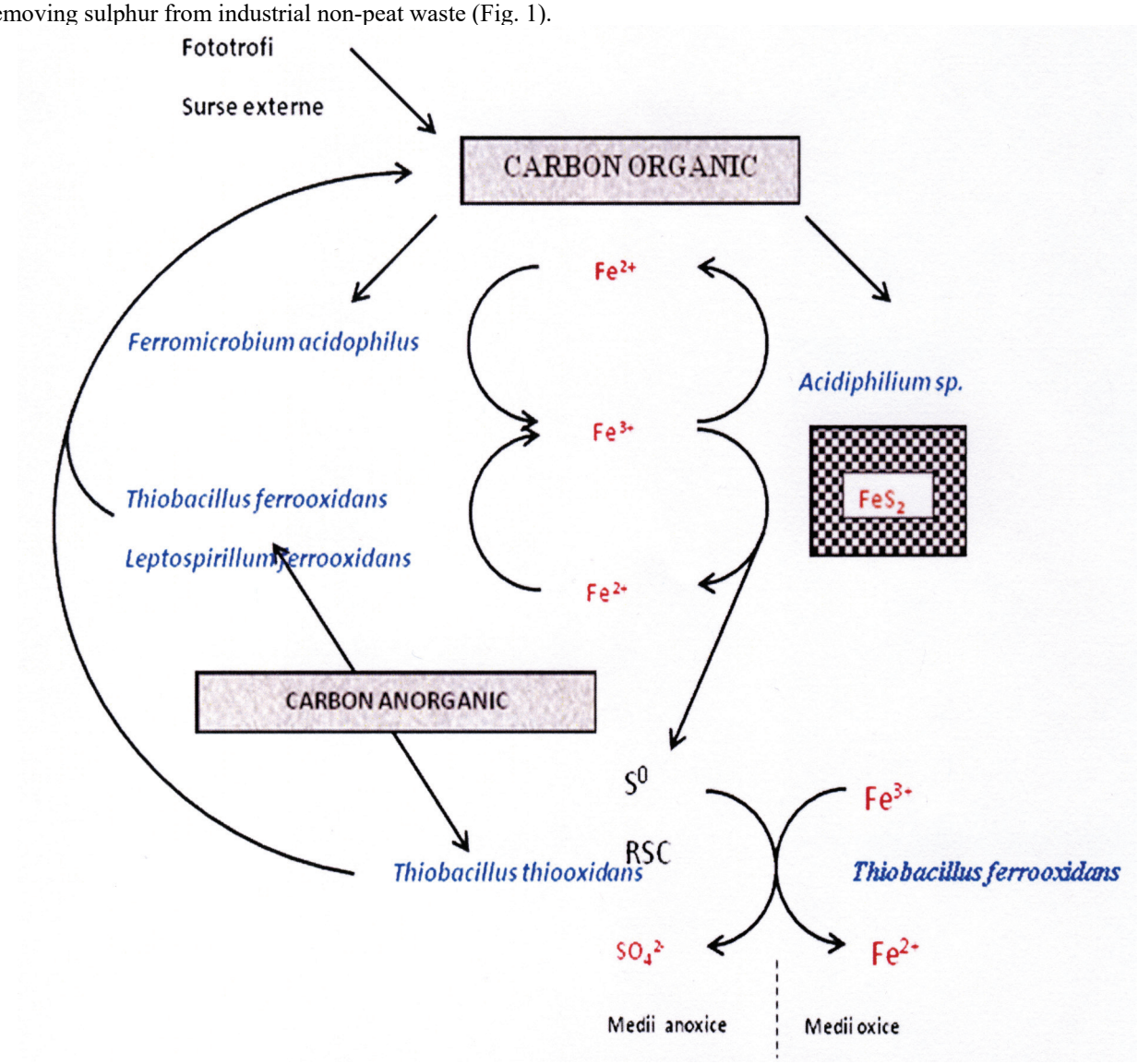

Figure 1. Schematic representation of the carbon circuit and the dissimilatory oxidation-reduction of iron and sulphursulphur in ore leaching media (after JOHNSON, 1999).

Regarding the presence of bivalent heavy metals in sediments and the shell of freshwater snails, it was found that they can accumulate higher levels of Mn^{2+}, Fe^{2+}, Cu^{2}+ and Zn^{2+} than the permissible environmental values (0.001 - 0.01 mg / l), in accordance with Ord. 161/2006. The analyses performed in the lacustrine ecosystems from the Danube Plain in the Oltenia sector illustrate the capacity of the lung snail species such as *Radix balthica* and *Lymnaea stagnalis* to accumulate metal ions of Mn^{2+}, Fe^{2+}, Cu^{2+} and Zn^{2+} type in direct correlation with the chemical peculiarities of the Danube floodplain. Also, studies have shown the adaptation of these species into the environment of the slugs in the presence of divalent metal ions derived from industrial solid waste processing (CIOBOIU & CISMAŞIU, 2016).

Biotechnologies applied for the removal of metal ions from industrial effluents and inorganic sulphur from solid fuels are based on processes of bio oxidation, biosorption and bioaccumulation by acidophilic bacteria through three mechanisms: (a) oxidation of inorganic sulphur from coal under the action of *Acidithiobacillus* bacterial cultures; (b) binding of metal ions to certain sites on the surface of the cell wall (hydroxyl, sulfhydryl, phosphate, amino and carboxyl groups); (c) intracellular accumulation by active or passive transport of metal cations through the cell wall and membrane. Microbiological methods for the recovery of precious metals from industrial wastes are of particular importance due to the stock depletion of minerals, rich and poor waste accumulation in metals due to processing by conventional hydrometallurgical methods (CISMAŞIU, 2004). In this sense, it was found that these invertebrate species are biological indicators of industrially contaminated areas in Oltenia, because they signal the emergence of negative ecological changes in lacustrine ecosystems (CIOBOIU et al., 2018; CIOBOIU & CISMAŞIU, 2018; MARINESCU & MITITELU-IONUŞ, 2019; MITITELU-IONUŞ et al., 2021; RĂDUCA et al., 2021).

Laboratory analyses confirm the increased capacity of invertebrate and microorganism species to adapt to extreme environmental conditions and to accumulate metallic ions in the water and the sediment depending on their concentration (27.22 mg / kg $Zn^{2=}$; 15.6 mg / Kg Ni^{2+}; 11.39 mg / Kg Pb^{2+}; 9.49 mg / Kg Cr^{3+}; 6.75 mg / Kg Cu^{2+}). The conclusion is that gastropod species and microorganisms species are bioaccumulators, biocatalysts and bioindicators of metallic ions in water and soil (CIOBOIU et al., 2017).

THE BIODEGRADATIVE ACTION OF MICROORGANISMS AND INVERTEBRATES TO INCREASE THE EFFICIENCY OF NOBLE METAL RECOVERY PROCESSES

The biodegradative action of microorganisms is the result of the alternative intervention of heterotrophic and chemolithotrophic bacteria in a positive sense in the economy of human society. Studies deriving directly or indirectly from ecological research have shown that both reducing and oxidative processes can be catalysed by microorganisms and have shown that, while these reactions are considered to favour the oxidation of sulphur ores, they may have a potential for bioremediation of industrial waste (LAZAR, 2001; JOHNSON & HALLBERG, 2005). Through their activity, microorganisms cause changes in the pH of the environment, the redox potential, and during their metabolism they can develop various useful substances with complexing properties. In this context, microorganisms have a predominant role in the solubilization and recovery of noble metals from industrial waste (ZARNEA, 1994; SILVER & PHUNG, 2009).

Studies arising directly or indirectly from ecological research showed that both processes - reduction and oxidation - can be catalysed by the acidophilus bacteria and indicated that, while these reactions are considered to disadvantage the oxidation process of sulphur ores, they may have the potential to bioremediate waste produced in active or abandoned mines. Invertebrates have an important role in biogeochemical circuits, in the transfer of matter and energy in ecosystems in natural and industrial environments.

Benthic invertebrates (oligochaetes, chironomids, amphipods, molluscs, bivalves) modify the sedimented material in different ways: (1) the particles they ingest are partially digested, and their mineralization is accelerated by the intestinal transit; (2) their activity facilitates the penetration of oxygen and other e-acceptors in the deep layers, favouring mineralization; (3) interface exchanges are modified by bioturbulence; increasing the redox potential due to oxygenation can reduce the solubilization of phosphorus and its release into water; (4) when migrating to the water mass, many benthic invertebrates carry phosphorus to other compartments.

Fish represent the end of the food chain, but at the same time an important compartment of pelagic phosphorus (40-50% of it is found at this level) (BREZEANU et al., 2011). Indigenous microorganisms compete for substrates, as they contain both organic and inorganic electron donors. Various environmental factors, such as temperature, pH, concentrations of dissolved metals have a great influence on the microorganisms which grow in extreme environmental conditions. In extreme environments, it is easy to establish the characteristics that allow resistant microorganisms to survive in an ecosystem, to colonize it, to multiply and to persist (CISMAŞIU et al., 2015; CÎRSTEA et al., 2015). These in turn influence, sometimes significantly, the physical and chemical properties of the environment through their action in the ecosystem (CISMAŞIU et al., 2015).

Microorganisms and invertebrates present in the industrially contaminated areas of Romania are sources of new species for the recovery of economically valuable metal ions, chlorides and sulphur compounds, in accordance with the values allowed by international standards (***. Ord. 756/1997; ** *. Ord. 161/2006). Research has shown an increase in the efficiency of microbiological processes for the recovery of noble metals from ferrous and non-ferrous industrial waste under the action of acidophilic chemolithotrophic bacteria of the *Acidithiobacillus* genus They also

demonstrated the stimulation of the degradation activity of inorganic substances under the action of microorganisms and invertebrates species in the presence of mineral salts existing in industrially contaminated areas in Romania.

The bacterial biosolubilization remains an efficient biotechnological alternative for the recovery of metals from poor ores and aquatic systems. In the last millennium, a large-scale expansion of ore bioprocess is expected. Different strains, species or genera of acidophilic microorganisms (probably members of microbial consortia) may become the most important oxidizing agents for ores in some of these cases. The reductive dissolution of ferrous iron containing in ores by iron-oxidizing bacteria can be used to remove iron oxide impurities from ferrous industrial wastes (JOHNSON, 1999; CISMAȘIU, 2007).

CONCLUSIONS

Microorganisms and invertebrates present in the industrially contaminated areas of Romania are sources of new species for the recovery of economically valuable metal ions, chlorides and sulphur compounds in accordance with the values allowed by international standards. Also, the results obtained highlighted the ecological characteristics of gastropod species belonging to the Lymnaeidae family that have an important role in the biogeochemical circuit, being indicators of industrially contaminated environments of Oltenia, as well as bioaccumulators of metal ions from natural and anthropogenic ecosystems. Studies arising directly or indirectly from ecological research showed that both processes reducing and the oxidation can be catalysed by microorganisms and indicated that, while these reactions are considered to disadvantage the oxidation process of sulphur ores, they may have a potential for the bioremediation of industrial wastes.

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