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BIOCHEMICAL FEATURES OF ACIDOPHILIC BACTERIA INVOLVED IN THE BIODEGRADATION PROCESS OF ORGANIC AND INORGANIC COMPOUNDS

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Abstract

Successful biodegradation process of different complexes required attention to biological parameters (such as choice of microorganisms), control of the chemical and physical environment (pH, redox potential, temperature and metallic ions) and consideration of the metal mines condition. In this context, the present paper presents the study regarding the main interactions between acidophilic bacteria and toxic metal ions can occur through active processes, involving the metabolic sequences of living microorganisms or passive processes, independent of cellular metabolism. The acidophilic bacterial cultures used in the degradation experiments of organic compounds were selected on the basis of their capacity to hydrolyse the starch in the presence of different inorganic forms of heavy metal ions The heterotrophic bacterial capacity by producing organic acid was very high, which confirm the adaptation of these populations to higher concentrations of heavy metallic ions. Comparative results bring an improved bacterial growth and sensitivity to specific environmental conditions and bacterial populations although the effect is not maintained after longer times. Our analysis regarding the efficiency of extracellular enzymes from Acidiphilium populations shows continuous agitation conditions (21 days) in the presence of 2-3g/l starch induce an increased extracellular hydrolytic activity (50-60%) when sulphates is present in the environment.

Key words**:** *enzymatic activity, heavy metal ions, acidity, bioremediation technology.*

INTRODUCTION

In the last years, environmental pollution with heavy metallic ions to the aquatic environment in particular, has attracted attention especially in order to the toxic metals leave, deposits to build up in sediments and tailings. The vast majority of heavy metallic ions are not found in the form of water soluble or, if it exists, the respective chemical species are complexed to inorganic or organic ligands which radically affect their toxicity. The main criteria for selection of chemicals in terms of the environmental impact, prevailing in the world are: (1) toxicity, (2) resistance; (3) ability for bioaccumulation; (4) carcinogen and teratogenic effects [7, 16, 23, 34].

The research activities on the microbial ecology of acidic extreme environments provide a basis for the uncontrolled discharge of industrial waste waters into the river systems containing metallic ions induce profound changes of water quality with inhibiting mineralization, accumulating of heavy metals in elevated concentrations in certain the aquatic organisms and are ultimately to humans [6, 24, 27].

Due to the serious changes of environmental factors in the certain areas close to major industrial platforms are found disappearance of local species of the flora and fauna. In other areas there was a change in the structure of ecological communities due to the mainly eutrophication of the water and soil degradation [3, 15, 26].

On the whole, the accumulation of products with inhibitory activity and the antagonism phenomena contribute, together with the quality and quantity changes of the nutrients, to the appearance of changes in the microorganism communities' structure underlying the succession of populations in an ecosystem. In addition, in the pilot centers of coal desulphurization it has been found that the number of acidophilic bacteria that oxidize

minerals decreases rapidly in the presence of flagellates acidophilic species [18, 29, 35].
Therefore, these eukaryotes are able to bring control in the situations mentioned above (for example, mine waste). At the same time, indigenous microorganisms act on the lack of balance produced by the temporary ecological disorders (eg. discharges of wastewater into the soil or natural waters) [4, 8, 11, 19].
New Gram-positive and Gram-negative bacteria were isolated from Poeni oily sludge, using enrichment procedures. The Gram-positive strains belong to *Bacillus*, *Lysinibacillus* and *Rhodococcus* genera. The Gram-negative strains belong to *Shewanella*, *Aeromonas*, *Pseudomonas* and *Klebsiella* genera. Isolated bacterial strains were tolerant to saturated, monoaromatic and polyaromatic hydrocarbons, and also resistant to different antimicrobial agents (i.e., antibiotics, dyes, synthetic surfactants) [31, 32].
However, many bacterial strains have already been identified as oil-degrading bacteria, many of them belonging to other genera as those isolated by Stancu and Grifoll (2011), which could be used for remediation of oily sludge contaminated environments.
Bioremediation is a complex process of treating a contaminated area by using microorganisms able to metabolize certain harmful substances with structural complexity, by reducing them to biodegradable simpler compounds, with a low degree of toxicity [2, 7, 11, 21].
The study conducted for the first time in Romania on the influence of some intercalation graphitic compounds on the morphogenetic processes over *Sequoia sempervineus*. Endel in vitro culture has demonstrated positive effects on their existence, especially for extended periods of time (60 days or 90 days). For two of the three graphitic compounds it has been noticed that they are non-toxic and do not inhibit the normal processes of the growth and the development in the species studied in vitro cultures, probably due to the existence of specific mechanisms involved in the development of these processes [28, 37].
Recent research has revealed that certain groups of acidophilic microorganisms are able to concentrate metals in solution due to their different qualities: reduction/oxidation, absorption and precipitation. Increased resistance of these microorganisms to the Cu^{2+} ion could result from their adaptation to contaminated environments because they were isolated mainly from mining effluents and tailings, containing increased concentrations of Cu^{2+} [10, 12, 38].
In this context, due to the effect of inorganic compounds on the bacterial degradation of organic compounds, our results revealed the efficiency of biodegradation processes of organic compounds using acidophilic heterotrophic populations in the presence of different inorganic substances.

MATERIALS AND METHODS

Starting from the identification of the environmental pollution source, the pollutants characterization and the degree of the contamination that cause extreme and hostile conditions that influence the zonal microbiota, due to changes in physic-chemical characteristics of the affected area, the presence of the physiological groups of bacteria with biotechnological potential is microbiologically analysed [1, 5, 33, 36].
The research regarding the microbiological analysis of samples from processing plant revealed a big variety of acidophilic bacteria from different physiological groups. Acidophilic bacteria, like as *Acidithiobacillus ferrooxidans* and *Acidiphilium* sp., isolated from metal mines in Romania [14, 19, 39].
In order to obtain isolates of the *Acidiphilium* genus were used selective liquid culture medium GYE (organic medium with a pH of 3.0), in which the energetic substratum was represented by the starch in the optimum concentration of 3g/l.
In the biodegradation experiments of organic substrate by the cultures of *Acidiphilium* sp. were followed the growth of acidophilic heterotrophic bacteria and extracellular enzymatic activity by Wohlgemuth method in six experimental variants with two types of toxic metallic ions sub forms of chloride, such as: (1) GYE medium + 3g/l starch + 0.1% $NiCl_2$ + 0.1% $MgCl_2$; (2) GYE medium + 3g/l starch + 0.1% $NiCl_2$ + 0.1% $MgCl_2$ + 0.1%

$CaCl_2$; (3) GYE medium + 3g/l starch + 0.1% $NiCl_2$ + 0.1% $MgCl_2$ + 0.1% NaCl; (4) GYE medium + 3g/l starch + 0.1% $ZnCl_2$ + 0.1% $MgCl_2$; (5) GYE medium + 3g/l starch + 0.1% $ZnCl_2$ + 0.1% $MgCl_2$ + 0.1% $CaCl_2$; (6) GYE medium + 3g/l starch + 0.1% $ZnCl_2$ + 0.1% $MgCl_2$ + 0.1% NaCl.

RESULTS AND DISCUSSIONS

The study on enzymatic hydrolytic activity of acidophilic bacteria, such as heterotrophic cultures belonging to the *Acidiphilium* genus, present in extreme acidic environments has strong influences on the mining technologies.
In this context, these biodegradation experiments of organic compounds using acidophilic heterotrophic populations mentioned above improved the mineralization mechanisms of complex inorganic forms from polluted industrial environments subject to organic substances contamination.
The test of microbial populations for their capacity to degradation complex organic forms in the presence of different metallic ions composition was made by comparative experiments of the study that metallic ions compounds present in industrial effluents have on the extracellular hydrolytic activity of acidophilic isolates belonging to the *Acidiphilium* genus. Growth experiments were carried out at different species type, heavy metal ion type and contact times between cell and these ions. The comparative studies regarding the effects of toxic metallic ion concentrations on the acidophilic heterotrophic bacteria revealed a increase of growth in GYE medium with 0.1% $NiCl_2$, respectively 0.1% $MgCl_2$ and 0,1% $CaCl_2$, at a concentration value of the organic substratum is established at 3g/l starch.
The biodegradation experiments have revealed the high capacity of the bacterial isolates belonging to the *Acidiphilium* genus to reduce the organic compounds in the presence of Ni^{2+} in the experimental variant V_2 and in the presence of Zn^{2+} in the experimental variant V_6. They have degraded the starch as nutritive substratum in percentages between 35-65% depending on the type of metal ion in solution, pH and bacterial cultures used (fig. 1-4).

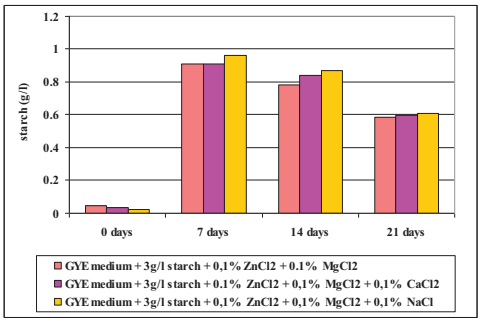

Fig.1. The growth of the P_4 population in GYE medium with 0.1% $NiCl_2$ in the presence of different cations (Mg^{2+}, Ca^{2+} and Na^{+}) sub form of chloride at 3g/l starch

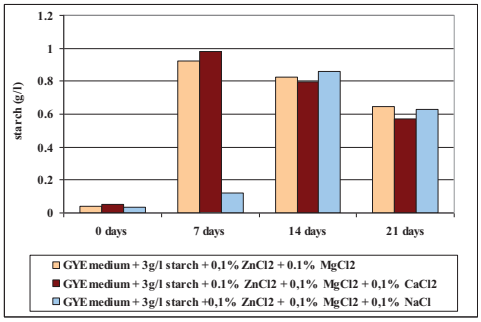

Fig.2. The growth of the P_7 population in GYE medium with 0.1% $NiCl_2$ in the presence of different cations (Mg^{2+}, Ca^{2+} and Na^{+}) sub form of chloride at 3g/l starch

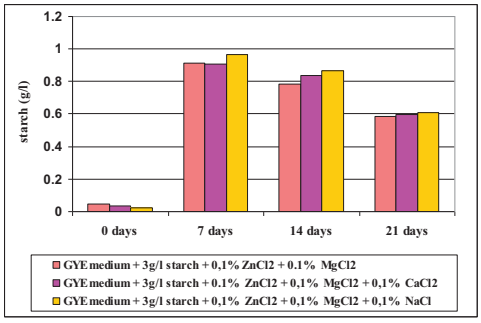

Fig.3. The growth of the P_4 population in GYE medium with 0.1% $ZnCl_2$ in the presence of different cations (Mg^{2+}, Ca^{2+} and Na^{+}) sub form of chloride at 3g/l starch

Appreciable percentage of the starch degradation in the experimental variants demonstrated the efficiency of the microbiological process of the environments remediation contaminated with toxic metal ions.

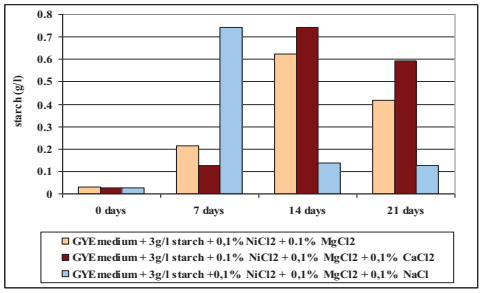

Fig.4. The growth of the P_7 population in GYE medium with 0.1% $ZnCl_2$ in the presence of different cations (Mg^{2+}, Ca^{2+} and Na^{+}) sub form of chloride at 3g/l starch

Also, depending on the variation of their habitat essential parameters (temperature, pH, Eh, concentration of O_2 and CO_2, type of metal ions) are produced enzymatic activity changes of the *Acidiphilium* population (fig. 5-8).

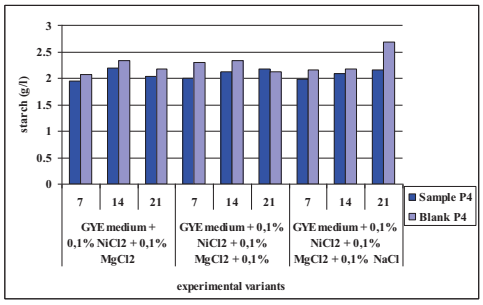

Fig.5. The extracellular degradation activity of the P_4 population in GYE medium with 0.1% $NiCl_2$, in the presence of different cations (Mg^{2+}, Ca^{2+} and Na^{+})

A slight influence of sodium sulphates on the extracellular organic degradation activity was obtained under the action of the acidophilic bacterial population with slower tolerance at higher concentrations of toxic metal ions. High percentages degradation of organic compounds were obtained using populations of acidophilic heterotrophic bacteria, which confirms the adaptation of the extracellular hydrolytic activity to the concentration of toxic metallic ions used. The biodegradation experiments of the organic substrate with cultures of *Acidiphilium* sp. at different salts composition showed the fact that at 0,1% $ZnCl_2$ in the presence of Mg^{2+} and Na^{+} it was obtained a higher efficiency of extracellular hydrolytic activity from organic compounds, getting to percentages of 40-75% (fig. 6-8).

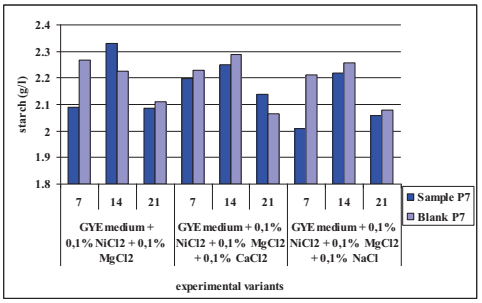

Fig.6. The extracellular degradation activity of the P_7 population in GYE medium with 0.1% $NiCl_2$, the presence of different cations (Mg^{2+}, Ca^{2+} and Na^{+})

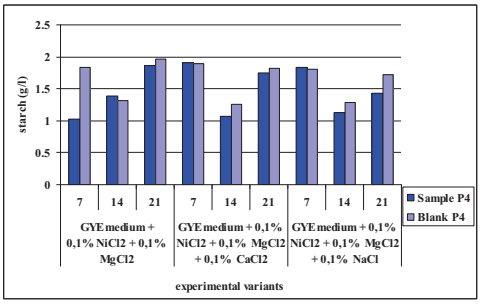

Fig.7. The extracellular degradation activity of the P_4 population in GYE medium with 0.1% $ZnCl_2$ in the presence of different cations (Mg^{2+}, Ca^{2+} and Na^{+})

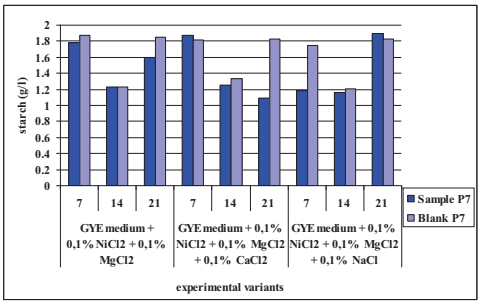

Fig.8. The extracellular degradation activity of the P_7 population in GYE medium with 0.1% $ZnCl_2$, the presence of different cations (Mg^{2+}, Ca^{2+} and Na^{+})

Also, studies of the influence of the metal ions on the metabolic activity of acidophilic heterotrophic bacteria has proved the effectiveness of using starch as an energy underlayer in the stimulation and intensification of bioaccumulation or biosorbtion of heavy metal ions.

The use of *Acidiphilium* populations in the biodegradation experiments of organic compounds has shown a higher correlation

between the growth and the extracellular hydrolytic activity of these bacteria (fig. 1-8).
To understanding the extracellular activity of acidophilic heterotrophic bacteria constitute a landmark in establishing the high influence of chemical conditions on the biodegradation process of organic complexes in the presence of inorganic salts by their action on the heavy metals sorption in the polluted environments with these ions [13, 17, 25, 30].

CONCLUSIONS

The comparative studies regarding the influence of the metallic ions solution on the growth and the starch degradation activity is developed with a maximum intensity up to 7 days of incubation periods at the same toxic metal ion concentrations.
The biodegradation experiments of organic complexes revealed a selective sensitivity to actions in the presence of chloride compounds in environments with different toxic metal ions (Zn^{2+} and Ni^{2+}), which reflects the correlation between specific culture conditions and corresponding bacterial populations.
An advantage of the biodegradation process of organic and inorganic compounds under the action of *Acidiphilium* populations is that the optimum pH and temperature value coincides with their optimum growth values. The experiments of organic compounds degradation under the action of acidophilic heterotrophic bacteria belonging to the *Acidiphilium* genus have revealed the possibility of using starch as a nutritive substratum in processes of ecological restoration of the habitats contaminated with heavy metal ions.

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