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The acidophilic chemolithotrophic bacteria involved in the desulphurization process of lignite and pit coal from Halanga, Mintia and Petrila mines

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Abstract

The acidophilic chemolithotrophic bacteria used in coal bioleaching processes for the elimination of sulphur are also considered to play a major role in the dissolution of heavy metals. The acidity of the coal mine drainage is caused primarily by the oxidation of the mineral pyrite, which is found in coal, coal overburden, and mine waste piles.

In our study the investigations focused on the chemolithotrophic Acidithiobacillus ferrooxidans, due to its ability to develop in such polluted areas and the potential bioremediation application. Strains isolated in previous studies were used to investigate its sulphur oxidative activity, this being the first attempt of its kind in Romania.

The tolerance of acidophilic chemolithotrophic bacteria to low pH (1-4) is the result of their adaptation, these species being able to maintain their cytoplasm pH near the neutral value. Studying the influence of physical-chemical parameters on the A. ferrooxidans metabolic activity it was observed that the culture oxidative activity was higher at pH values close to the acidity of the origin habitats. The results revealed that the adapting of the Acidithiobacillus ferrooxidans population at higher concentrations of metallic ions determined an increased coal desulphurization activity (74.23-88.46%). High solid/liquid ratio determined the decreases of the bacterial oxidizing activity of the coal, correlated with desulphurization %.

Keywords: *Acidithiobacillus ferrooxidans*, biodesulphurization, coal mining.

Introduction

Acid mine drainage refers to the outflow of acidic water from metal mines or coal mines. In many localities the liquid that drains from coal stocks, coal handling facilities, coal washeries, and even coal waste tips can be highly acidic, and in such cases it is treated as acid rock drainage. AMD is a chemically complex, but biologically simple ecosystem dominated by microbial communities, with a variety of autotrophic (both photo- and chemo-autotrophic), heterotrophic and decomposers (JOHNSON [5], NORRIS & JOHNSON [19])

Coal has been accepted as a major source of energy for centuries. Sulfur emission from coal combustion presents many environmental problems. It is believed that the best method to limit the amount of sulfur oxides emitted into the atmosphere is to reduce the amount of sulfur in coal before combustion. The techniques used include physical, chemical and biological processes. Most of research work on coal desulphurization focused on pyritic sulfur removal, which represent a half of the total sulfur content in coal (PRAYUENYONG [21], MISHRA [18], KOMNITSAS & al.[13]).

Coal biodesulphurization is a microbial processes that involves heterotrophic and chemolithotrophic bacteria, which would remove sulphur from fuels without degrading the fuel value of the product. Successfully desulphurization required attention to biological parameters (bacteria and nutrient supplements), control of the physical environment (pH, redox potential, temperature), as well as consideration of the coal conditioning and its source (ACHARVA & al. [1], KARAVAIKO [8], KLEIN [12])

The economic implications of ecological effects are identified to the extent they can be determined within acceptable limits. A variety of ecological processes are affected and altered by air pollution. Such processes include community succession and retrogression, nutrient biogeochemical cycling, photosynthetic activity, primary and secondary productivity, species diversity and community stability (ACHARYA & al. [1], GLASS [4], NAKAOKA & al. [19]).

The acidophilic chemolithotrophic bacteria used in the processes of sulphur emission from coal by bioleaching are currently considered to play an important part in the dissolving of other elements. The structural and physiological characterization of the acidophilic bacteria from microbial communities may lead to the discovery of new species of bacteria, which could play an important role in bioremediation processes (KINNUMEN & al. [11], LAZAR [15]).

The activity of the acidophilic bacteria at ecosystem level is very complex: (a) in the trophic chains and nets; (b) in limiting the development of some organisms populations; (c) in forming and preserving the soil structure, essential for water, air and nutrients circulation; (d) bioindicators for the "health" of the ecosystems. They have a major contribution to the circuit of biogenic elements in the nature and on the global plane; they prevent the accumulation of residual materials of different environmental contaminants (JOHNSON & al. [6], KOMNITSAS & al. [13]).

The acidophilic bacteria present in the habitats mentioned above can be heterotrophic or chemolithotrophic being involved in the metals solubilization, the coal desulphurization, the metals bioaccumulation processes. They signal the appearance of some negative modifications of the ecosystems, which result from the activity of some polluting or other perturbing factors, before they affect more evolved organisms (LAZAR [15], NAKAOKA & al. [19], RAHESH [22]).

The growth and activity of the acidophilic chemolithotrophic bacteria in the acidic biotopes depend greatly on the ecological conditions of the environment. Through their activity, the acidophilic bacteria determine changes of the environment pH and of the oxide-reduction potential and they can elaborate different useful substances, which have complex oxidizing or reductive properties. The acidity influences in a different way the life and activity of the different types of microorganisms present in low pH media (, JOHNSON [8], KARAVAIKO & al [10]).

In specialty literature was shown that the best results of coal desulphurization have been obtained with mixed cultures of *Acidithiobacillus thiooxidans* and *A. ferrooxidans*. The role of acidophilic chemolithotrophic bacteria in oxidation of insoluble iron sulfide and other metal sulfides to soluble ions is well established. Pyrite solubilization has been most studied in the presence of the gram-negative chemolithotrophic *A. ferrooxidans*, which utilizes either ferrous iron or reduced inorganic sulfur compounds as a sole energy source. The rate of pyrite oxidation depends on the following: reactive surface area of the pyrite, the oxygen concentration and pH of the water, the forms of pyrite, and the presence of iron-oxidizing bacteria like *A. ferrooxidans*. (KARAVAIKO & al. [10], LUNDGREN & al [16], ROHWERDER & al [24]).

After isolating more strains from the acid effluents of the sulphidic mining area (Tulcea county, Romania), identified according to the morphological and physiological characteristics as being part of *Thiobacillus* genus (CISMASIU [2]), the study focused on their sulphur oxidative activity.

Materials and Methods

In our experiments there were used six samples of pit coal (from Lupeni, Paroşeni, Lonea, Mintia, Vulcan, Petrila), one sample of lignite (from Turceni) taken in 2006 year and two samples of lignite (from Turceni and Halânga) taken in 2007 year.

Selective liquid culture medium 9K was used in order to obtain the strains and populations of *Acidithiobacillus ferrooxidans* from these samples (mineral medium with a pH of 2.5). The energetic substratum was represented by the ferrous sulphate in the concentration of 43.22g/l, which corresponds to 8.6g/lFe^{2+}. The 9K solid medium (KARAVAIKO [9]) was used for strains isolation.

The isolated colonies obtained on agar medium were reddish-brown and were cultivated in 9K liquid medium under continuous agitation conditions, at a temperature of 28^0C for 12 days. The growth was determined by the amount of oxidized Fe^{2+}. The concentration of oxidized Fe^{2+} was determined volumetrically through titration with a solution of $2x10^{-2}$N $K_2Cr_2O_7$ in the presence of sulphur diphenylamine as an indicator.

In order to obtain populations of acidophilic chemolithotrophic bacteria, isolated colonies on agarized selective culture media were used, following the dynamics of the physiological activity in specific inorganic media. Isolated colonies obtained were of brownish-red colour. Using this technique, 8 populations of *Acidithiobacillus ferrooxidans* were isolated.

In the experiments of coal desulphurization by *Acidithiobacillus ferrooxidans* cultures, pit coal from Lupeni mine and lignite taken from two mines: Turceni and Halânga mines were used. In these experiments a solid/liquid ratio of 5-10g/100ml in Leathen medium (KARAVAIKO [9]) was used.

The experiments of testing the coal desulfurization were performed in Erlenmeyer glasses (750ml) with 90ml specific medium and 10ml inoculums aged 7 days. The bacterial cultures were incubated at 28^0C, on the rotational shaker, for 28 days at 150rpm.

In the experiments of coal desulphurization there were used *Acidithiobacillus ferrooxidans* bacterial cultures (8 populations) selected on their resistance to higher concentrations of iron (18g/l Fe^{2+}), Cu^{2+} and Zn^{2+} (5000ppm) (CISMASIU [2]).

Regarding the study of raising the efficiency of the coal biodepyritization process, the experiments were accompanied by chemical controls and biological controls (the P_9 population - with a low resistance to ferrous sulphate). At the end of the experimental period it was determined the diminished weight of the different coal under the action of *Acidithiobacillus ferrooxidans* cultures, which solubilized pyrite into soluble sulphate.

The tests have been realized on the chemistry laboratory of the Research and Development National Institute for Metals and Radioactive Resources, by gravimetric determination in the $BaSO_4$ form (CISMASIU & al. [3])

Results and discussion

The coal and metal ore mines, and the neighboring areas, are known as extreme habitats (KOZLOV & ZVEREVA [14]). In Europe many mine galleries are now considered as geothermal heat exchangers, with the potential of being used as a renewable source of energy (KINNUNEN & al. [11] , RODRIGUEZ & DIAZ [23]). Besides the high temperatures, the mining areas present usually high concentrations of heavy metals and very low pH (JOHNSON & HALLBERG [7]). Their metabolic activity is influenced by the acidity and temperature levels (NORRIS & JOHNSON [20], SRIVASTAVA & al. [25]).

The strains and populations of acidophilic chemolithotrophic bacteria were analyzed having in view the inoculation into specific medium and the following features were considered: morphology of colonies, rate of growth and potential of growth into nutrient medium containing different concentration of substratum. On the basis of these phenotypical features and of the development and the production of pigments on specific media, the isolates were characterized that belong to *Acidithiobacillus ferrooxidans*. The most representative structures and colonies had been included in photographic database (figs 1-2).

The research regarding the tolerance at high concentration of metallic ions of the *Acidithiobacillus ferrooxidans* populations, confirms the data in the specialty literature about the increased capacities of the bacterial population to adapt to the extreme medium conditions (acid pH and high concentrations of metallic ions).

The *Acidithiobacilllus ferrooxidans* cultures used in the experiments of sulphur biooxidizing from the three tested coals were selected on the basis of their capacity to oxidize the sulphur in the presence of higher concentrations of metallic ions. In a view to raising the efficiency of the bacterial desulphurization processes from two coals (lignite/pit coal) the P_9 population was used as a reference strain, due to its higher sensitivity at big concentrations of ferrous sulphate (16-20g/l Fe^{2+}) in a culture medium (fig.3).

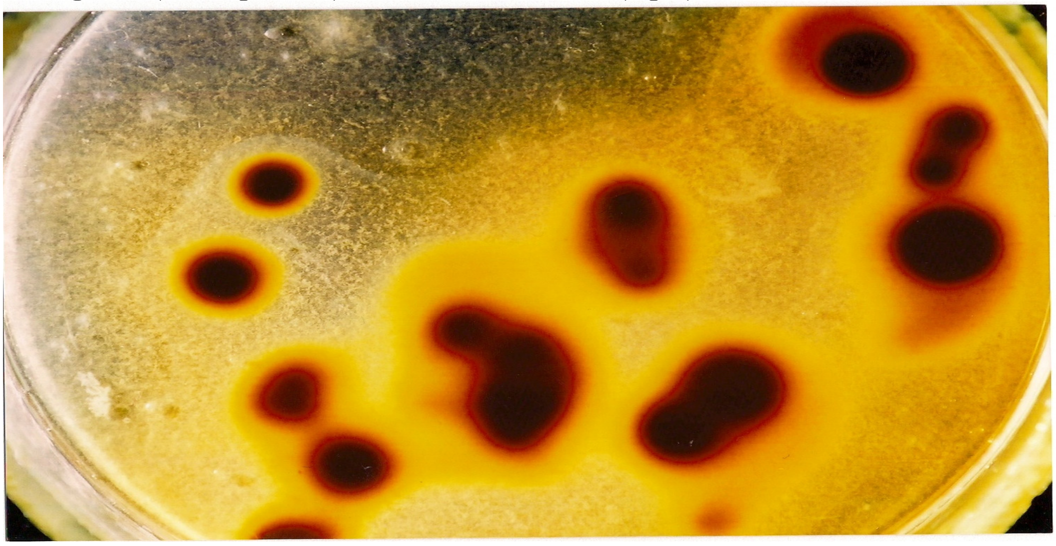

Figure 1. *Acidithiobacillus ferrooxidans* colonies isolated from acidic water samples taken from Baia sulphidic dump (Tulcea county)

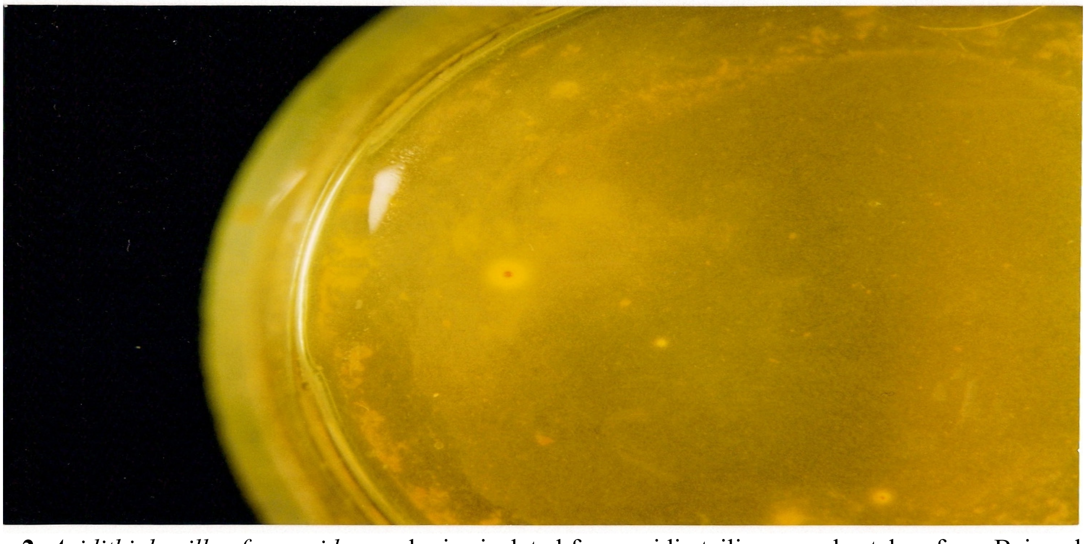

Figure 2. *Acidithiobacillus ferrooxidans* colonies isolated from acidic tailing samples taken from Baia sulphidic dump (Tulcea county)

The sulphur oxidizing from the pit coal for different solid/liquid ratios (1-5g/100ml) in the presence of *Acidithiobacillus ferrooxidans* cultures in stirring conditions are presented in figures 3-6.

The desulphurization experiments of the pit coal (Petrila and Mintia mine) with cultures of *A. ferrooxidans* for different solid/liquid ratios showed the fact that at a solid density of 4g/100ml and 5g/100ml a higher efficiency of sulphur biooxidizing from coal was obtained, getting to percentages of 55-75%.

The experiments of coal desulphurization with cultures of *A. ferrooxidans* for the tested solid/liquid ratios revealed a significant difference between the efficiency of sulphur biooxidizing in the two coals, shown by the weight loss of mass. Thus, it was noticed that *A. ferrooxidans* cultures oxidized the sulphur in higher percentages from lignite (58.72-88.46%), compared to pit coal (60.19-74.23%), a fact correlated with the presence of the highest quantities of mass loss.

The comparative analyses regarding the sulphur oxidizing efficiency from lignite (Halânga mine) at solid/liquid ratios between 1-5g/100ml using the *A. ferrooxidans* populations showed that the highest percentages of coal desulphurization were obtained in the presence of the P_7 population (60-75%).

Raising the solid/liquid ratio from 1g/ to 3g/100ml determined the increase of the bacterial oxidizing efficiency of the coal, which was evidenced through the desulphurization of lower percentages, which get to 80.45% for the lignite and 70.45% for pit coal.

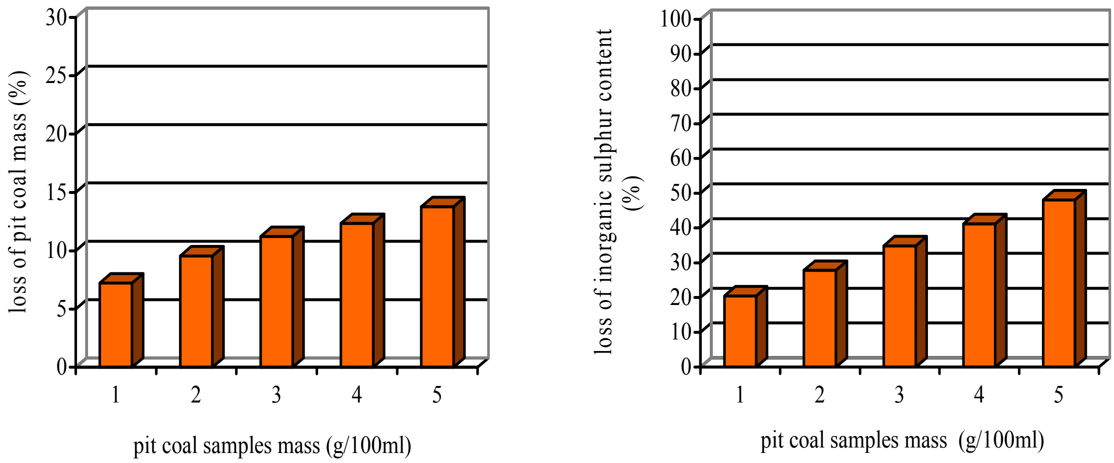

Figure 3. Variation of mass loss and inorganic sulphur content of pit coal from Petrila, in the presence of *Thiobacillus ferrooxidans* P_9 population, with the low tolerance to ferrous sulphate.

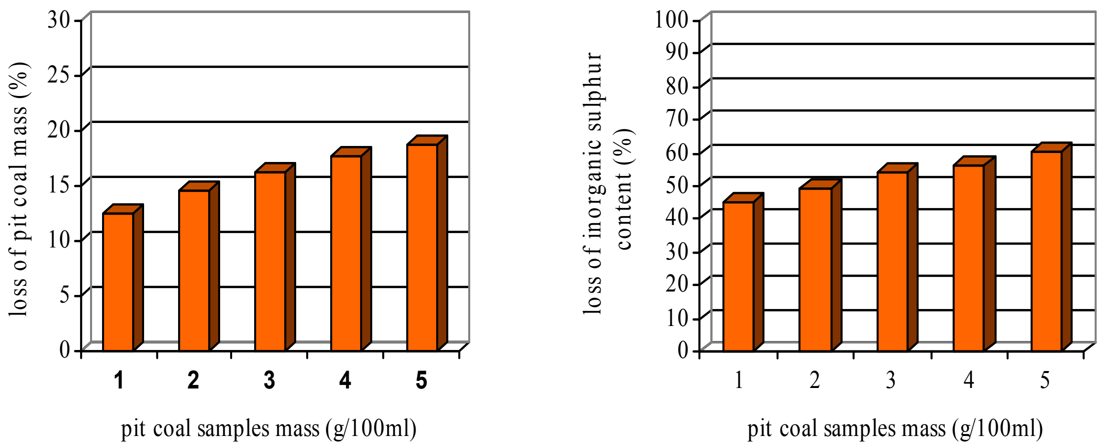

Figure 4. Variation of mass loss and of inorganic sulphur content of pit coal from Petrila, in the presence of *Thiobacillus ferrooxidans* P_7 population, with the high tolerance to ferrous sulphate.

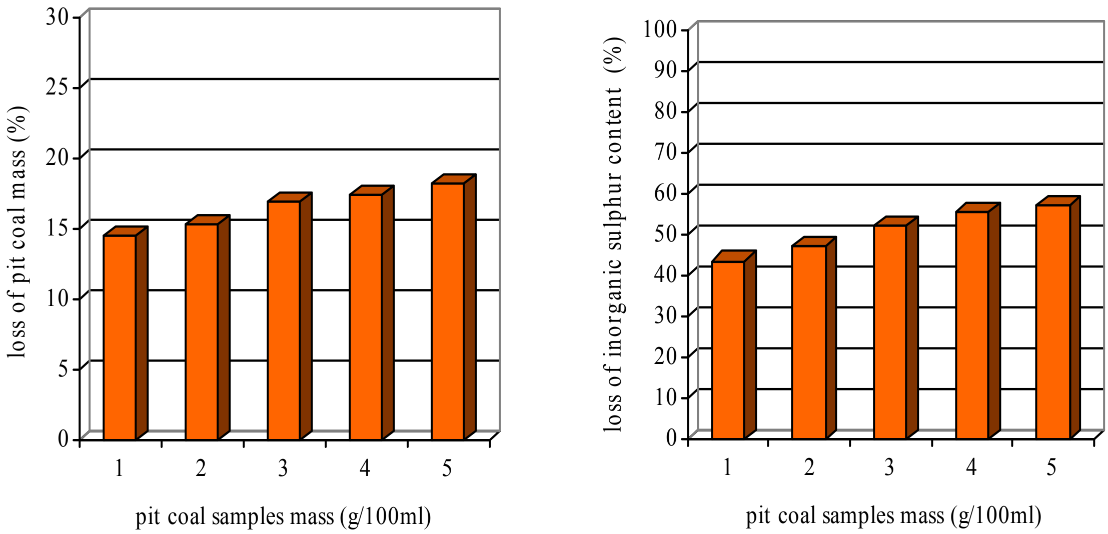

Figure 5. Variation of mass loss and inorganic sulphur content of pit coal from Mintia, in the presence of *Thiobacillus ferrooxidans* P_9 population, with the low tolerance to ferrous sulphate.

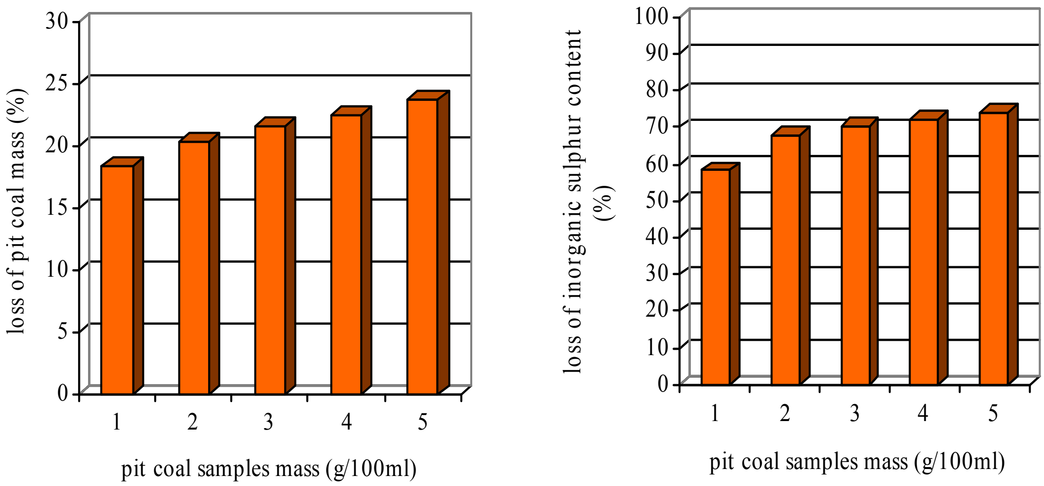

Figure 6. Variation of mass loss and of inorganic sulphur content of pit coal from Mintia, in the presence of *Thiobacillus ferrooxidans* P_7 population, with the high tolerance to ferrous sulphate

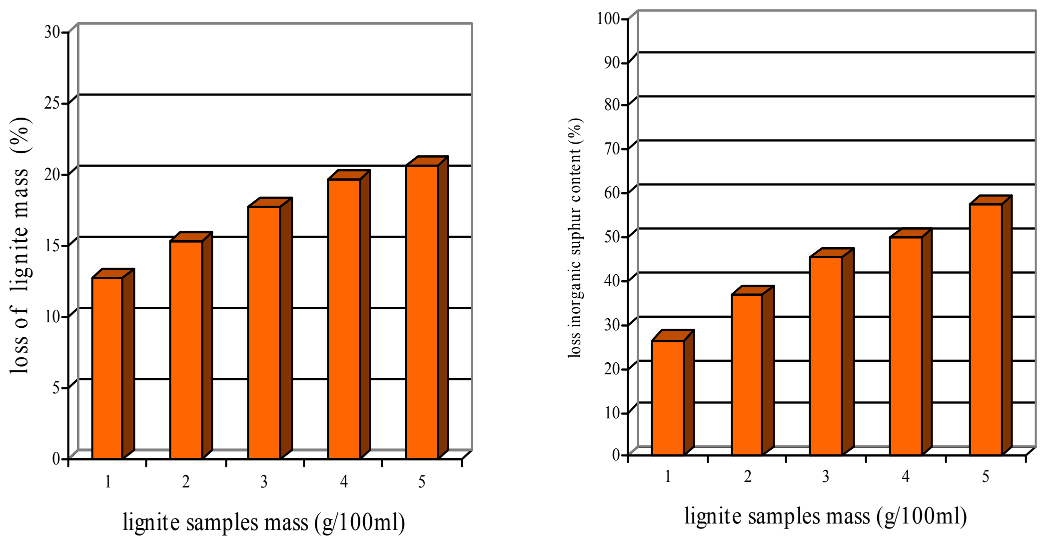

Figure 7. Variation of mass loss and of inorganic sulphur content of lignite from Halânga, in the presence of *Thiobacillus ferrooxidans* P_9 population, with the low tolerance to ferrous sulphate.

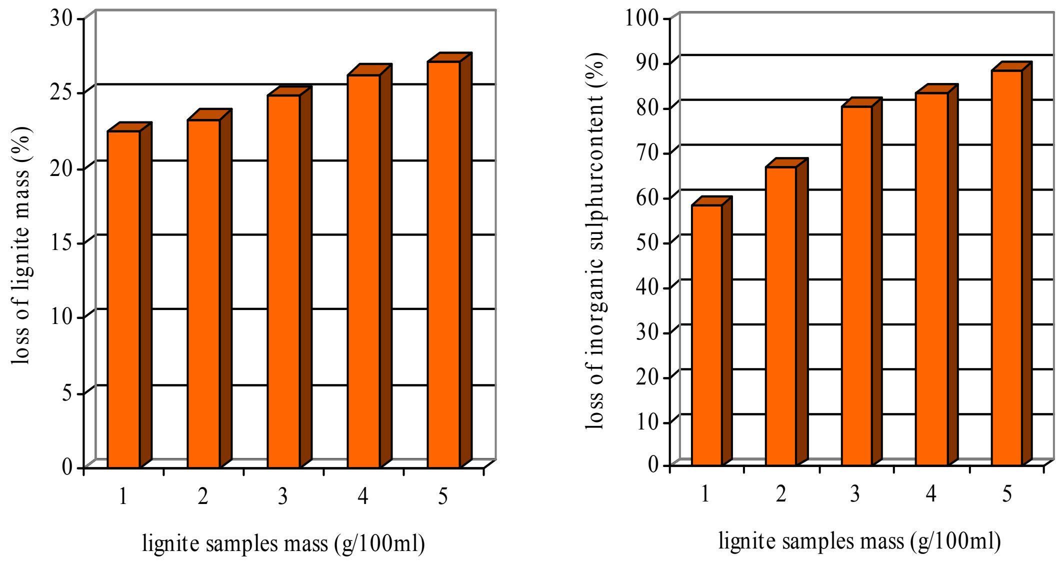

Figure 8. Variation of mass loss and of inorganic sulphur content of lignite from Halânga, in the presence of *Thiobacillus ferrooxidans* P_7 population, with the high tolerance to ferrous sulphate.

The sulphur biooxidizing from lignite (Halânga mine) and pit coal (Mintia mine) for different solid/liquid ratios (5-10g/100ml) in the presence of *Acidithiobacilllus ferrooxidans* cultures in conditions of continuous stirring are presented in figure 9.

The desulphurization experiments of the coal with cultures of *Acidithiobacilllus ferrooxidans* showed the fact that for a solid density of 10g/100ml it was obtained a higher efficiency of sulphur biooxidizing from lignite, getting to percentages of 76.40-82.50%

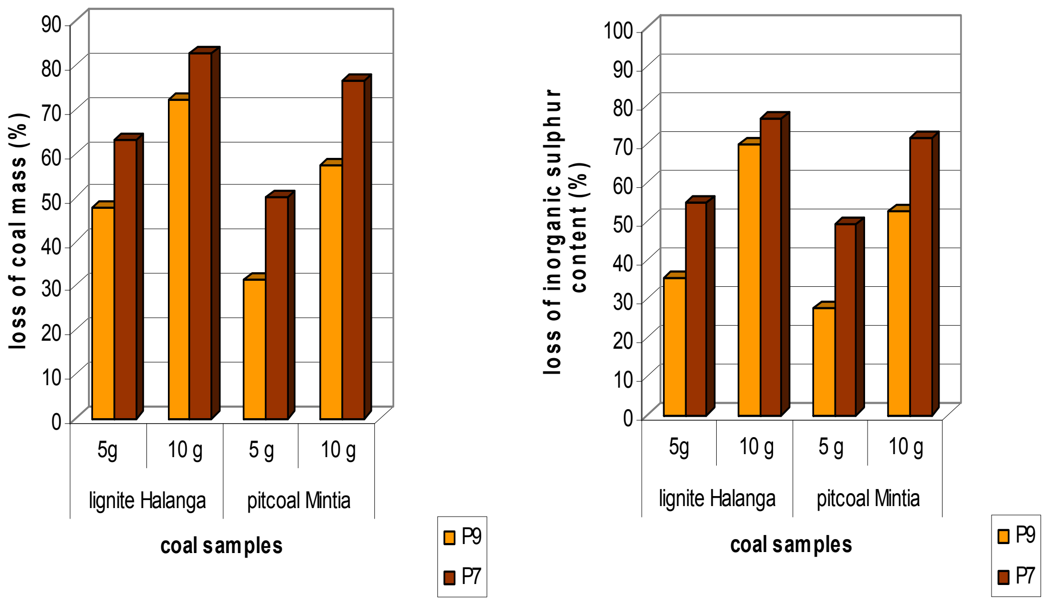

Figure 9. Comparative results regarding the loss of coal mass and inorganic sulphur content by *Acidithiobacillus ferrooxidans* population activity

The comparative analyses regarding the sulphur biooxidizing efficiency from coal at solid/liquid ratios between 5-10g/100ml in the presence of the populations of *A. ferrooxidans* showed that the highest percentages of desulphurization coal were obtained in the presence of the P_7 population which oxidized the sulphur from coal in percentages of 63.10-82.50%, confirming previous studies (CISMASIU & al. [3]).

Previous studies of the *A. ferrooxidans* isolated from different samples have shown that the acidophilic chemolithotrophic bacteria isolated from the coal mine wastewater are more active than the lab cultures probably as a consequence of their adaptations at the high acidic conditions from the mine (CISMASIU [2]).

Conclusions

A diminution of coal mass was obtained in the desulphurization experiments in which acidophilic chemolithotrophic bacteria cultures have been used. The results have showed that the loss of coal mass was more important in the lignite sample comparatively with the mass reduction observed in pit coal sample.

In time, the adapting of the *Acidithiobacillus ferrooxidans* population at higher concentrations of ferrous sulphate (18g/l Fe^{2+}) determined a raised efficiency of coal desulphurization at values between 63.10-88.46%. Also, raising the solid/liquid ratio from 5 g/100ml to 10 g/100 ml determined the increasing of the coal biodesulphurization efficiency, which gets to 57.30-76.41% for the pit coal and 72.21-82.50% for lignite. The comparative results regarding the efficiency of coal desulphurization in the presence of *A ferrooxidans* cultures illustrated that the P_7 population oxidized the highest percentages of sulphur from coal (54.78-63.10%).

Unlike other extremophiles, whose maximum oxidative activity occurs at higher temperatures and bigger depths, *A. ferrooxidans* are able to cope with low pH even at 28^0 – 30^0C, the surface temperature of the acid mine drainage in coal mine area; their potential for bioremediation (eg. retention of heavy metals) seems high and could be further investigated in a local pilot study. Future studies will focus on identifying a narrower range of the optimum physical and chemical parameters where *Acidithiobacillus ferrooxidans* presents its highest enzymatic activity, as well on the implementation of these results in the ecological reconstruction of coal mining area.

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