Studii şi Cercetări Ştiinţifice 2015, **16** (1), pp. 093 – 097
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

BIOACCUMULATION OF HEAVY METALS BY *BACILLUS MEGATERIUM* FROM PHOSPHOGYPSUM WASTE

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Received: March, 02, 2015
Accepted: March, 31, 2015

Abstract: The aim of present study was to characterize the bioaccumulation capacity of heavy metals by *Bacillus megaterium from phosphogypsum waste.* The *Bacillus megaterium* strain (BM_{30}) was isolated from soil near the phosphogypsum (PG) dump. For the bioaccumulation quantification produced by BM_{30} strain were used three experimental treatments respectively with 2, 6 and 10 $g \cdot L^{-1}$ PG. Cellular biomass samples were collected punctually at ages corresponding to the three stages of the development cycle of the microorganism: exponential phase, stationary phase and decline phase and the heavy metals concentrations were measured by atomic absorption spectroscopy. The bioaccumulation yields in cell biomass, relative to the total amount of analyte introduced in the reaction medium were between 20 - 80 %, the lowest value was recorded by Cu and highest by Mn. The study results indicated that the isolated strain near the dump PG, BM_{30}, bioaccumulate heavy metals monitored in cell biomass in the order Cu > Fe > Zn = Mn.

Keywords: *Bacillus megaterium, bioaccumulation process, bioremediation polluted soils, heavy metals, phosphogypsum*

INTRODUCTION

The soils pollution with phosphogypsum (PG) has been a problem for the fertilizer industry. Presently, about 120000 tons of PG were generated as by-product from phosphoric acid in a fertilizer factory located in Bacau, Romania. PG wastes are stored in a stack occupying a surface of approximately 16 ha, less than 2 km away from the city.

The content of heavy metals in PG waste from Bacau ($mg \cdot kg^{-1}$) [1] is: 0.5 Cd; 2.5 Co; 0.7 Cr; 45 Cu; 7 Mn; 4 Ni; 212 Zn and 1199 Fe. The heavy metals, at high concentrations, exhibit toxic effects on normal biological development for all organisms which come into contact with such polluted environments [2, 3].

The removal of these metals from polluted environments or the reduction of their toxic potential can be realized by the bacteria, and therefore the *Bacillus megaterium*. *Bacillus megaterium* play an important role in the biogeochemical cycle of metals and processes involved in bioremediation [4 - 6]. Bacteria, using their own metabolic processes, actively participate in the mobilization of heavy metals by production of organic and inorganic acids, oxidation or reduction reactions or excretion of complexing agents.

Bacillus megaterium is mentioned in the literature as producing nitric acid [7], in a secondary metabolic pathway in which glycine is converted to hydrogen cyanide after the oxidative decarboxylation reaction catalyzed by the glycine decarboxylase [8].

Bacillus megaterium contribute to the immobilization (bioaccumulation) of metal by sorption in organelles or precipitation as organic or inorganic compounds, such as oxalates, sulfites or phosphates [9]. The literature reports a growth sporulation of *Bacillus megaterium* in the presence of heavy metals in their growth medium, an increase in the amount of extracellular proteins that this bacterium produces and a most significant cell biomass accumulation [10, 11].

Stimulating the sporulation process of *Bacillus megaterium*, noted for the presence in the growth medium of Fe, Cu, Mn, Zn, and As [12], lead to positive effects on cell multiplication and implicitly biomass accumulation, thereby increasing the bioremediation of contaminated site.

This study aims to quantify the heavy metals bioaccumulation capacity from the waste PG by *Bacillus megaterium*.

MATERIALS AND METHODS

Microbial strain

The *Bacillus megaterium* strain, abbreviated as BM_{30} next in the text, was isolated from a soil collected from a PG dump near Bacau region, Romania, accordingly to the Standard Methods specifications [13, 14] and the results were interpreted using Bergey's Manual of Systematic Bacteriology [13].

Media and culture growth conditions

Cells were cultured in mineral liquid medium (MLM = sucrose, 10 $g \cdot L^{-1}$; K_2HPO_4, 2.5 $g \cdot L^{-1}$; KH_2PO_4, 2.5 $g \cdot L^{-1}$; $(NH_4)_2HPO_4$, 1 $g \cdot L^{-1}$; $MgSO_4 \cdot 7H_2O$, 0.2 $g \cdot L^{-1}$;

$FeSO4 \cdot 7H_2O$, 0.001 $g \cdot L^{-1}$; $MnSO_4 \cdot 7H_2O$ 0.007 $g \cdot L^{-1}$ dissolved in water). MLM was prepared and autoclaved at 121 ºC, 1.2 bar, for 15 minutes [14]. Final *p*H was around 7.4-7.6. The inoculum was prepared by placing a single colony into 100 mL MLM for 24 h at 30 ºC and 119 rpm in a rotary shaker incubator. The optical density of inoculum was OD600 = 2.2735.

Metal bioaccumulation quantification

For the bioaccumulation quantification produced by BM_{30} strain, the cells were inoculated in three experimental treatments respectively with 2.6 and 10 $g \cdot L^{-1}$ PG, and incubated on a rotary shaker incubator (GPL 3033) at 30 ºC and 190 rpm. The bioaccumulation of heavy metals was observed during the life cycle at 18, 24 and 30 hours. The cells were grown up at the established time, harvested by centrifugation at 9.000 rpm for 30 min at 4 ºC. For bioaccumulation quantification, the metal concentration was determined from the biomass cell. The experiment was carried out in triplicate for each concentration of PG added and every established time. The heavy metals concentrations were measured by atomic absorbtion spectroscopy (Perkin Elmer 3300).

RESULTS AND DISCUTIONS

Cellular biomass samples were collected punctually at ages corresponding to the three stages of the development cycle of the microorganism: exponential phase, stationary phase and decline phase. As established experimentally complete life cycle for BM_{30} strain is 42 hours. In relation to literature, specifying duration of *Bacillus megaterium* life cycle of 36 - 42 h [12], and a maximum of exponential phase of up to 24 h [15], we say with certainty a good adaptation of the BM_{30} strain at PG, meaning the maximum exponential phase for this strain is up to 18 hours. In Figure 1 is shown dynamic distribution of Cu, Fe, Zn and Mn in cellular biomass for BM_{30} strain.
From Figure 1 we can see that in the case of Cu and Fe, the results show a good correlation with the metabolized amounts of Cu from each microbial growth phase, exchange between the aqueous and the cell phase being performed during the stationary phase (18 - 24 hours).
Identification of the point of inflection presents industrial importance because it establishes the time at which the analyte can be removed by treating the aqueous phase or cell biomass processing.
Moreover, opting for bioremediation techniques of polluted soil with PG by phytoremediation, this inflection point can establish the relationships between the time of application biofertilizer (*Bacillus megaterium*), the time of the cultivation and harvesting of plants in order to obtain maximum results.

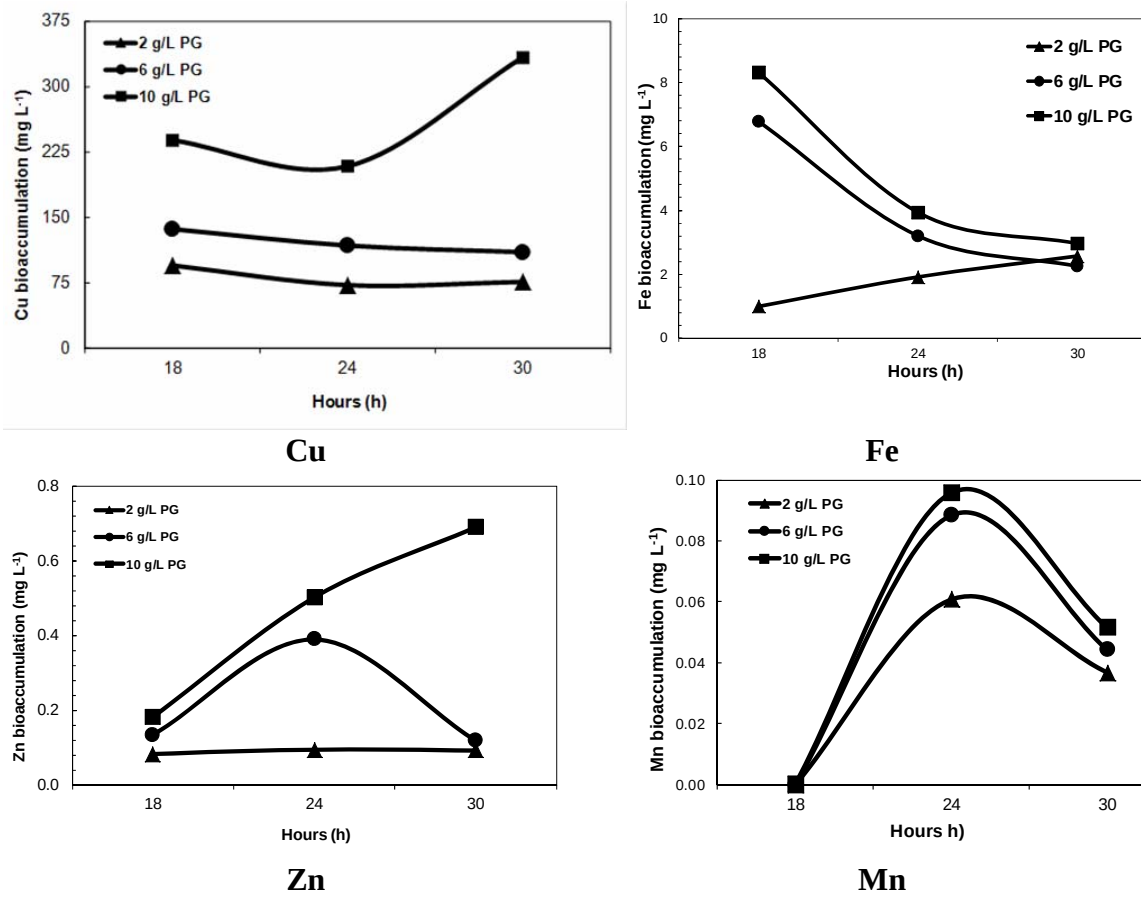

Figure 1. *The dynamic distribution of heavy metals in cell biomass of BM_{30} strain*

The case of Zn shows that the inflection point between Zn import-export is performed at the same age (24 h) at which the Fe and Cu ions are removed from the cell. Similar import system of Zn (bioaccumulation process) in the cell, export systems (process Bioleaching) are made also of protein but, opposed to those who are less specific for import zinc, the exporters are located in the membrane periplasmic and they have a high specificity for each ionic species and implicitly for Zn. This aspect favors the possibility of cells to remove until tolerance limit these amounts of ionic species which becomes toxic for cell viability. Looking at Zn distribution between the two phases, aqueous respectively cell biomass (behavior observed with 6 $g \cdot L^{-1}$ PG concentration), we cannot attribute for BM_{30} strain a predominantly bioaccumulative effect. Similar behavior was observed for Mn. Considering that Mn was found in a high proportion in soluble form, because the contribution made by MLM, it can be said with certainty that the strain isolated in this study possess a high level of resistance and adaptability to PG.

CONCLUSIONS

So far the literature has not reported *Bacillus megaterium* data involvement in bioremediation of polluted soils PG and no studies have been reported on bioaccumulative metals distribution in biomass cell from PG waste during the complete life cycle. Because PG is almost insoluble in water, the presence of heavy metals in the

cell biomass of the reaction system is certainly due to the metabolic activity of the *Bacillus megaterium.* Moreover, bioaccumulation yields in cell biomass, relative to the total amount of analyte introduced in the reaction medium were between 20 - 80 %, the lowest value was recorded by Cu and highest by Mn. The study results also indicated that the isolated strain near the PG dump, BM_{30}, bioaccumulate heavy metals monitored in cell biomass in the order Cu > Fe > Zn = Mn. The trend of Cu and Mn bioaccumulation increases with increasing PG concentration in the environment, and for Fe and Zn trend is decreasing with increasing concentration PG.

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