Cercetări Agronomice în Moldova
Vol. XLIII , No. 2 (142) / 2010

BIO-SOLUBILISATION CAPACITY OF *BACILLUS MEGATERIUM* STRAIN OF SOME MICRONUTRIENTS FROM THE POLLUTED SOIL

Ioana Adriana ŞTEFĂNESCU[*1], Raluca MOCANU[2], M. DUNCIANU[2]

[1] *Bacău University, Bacău*
[2] *"Alexandru Ioan Cuza" University", Iaşi*

Received December 21, 2009

ABSTRACT - The goal of this study was to test the bio-solubilisation capacity of *Bacillus megaterium* strain, isolated previously by the author in a polluted medium. The literature showed that *Bacillus megaterium* had bio accumulative properties of some heavy metals (Pb, As, Cd) or of bio solubilisation of phosphate, silicates, etc. We have tested the bio solubilisation capacity of insoluble compounds or partial soluble compounds of Fe, Mn and Zn, which have high importance for plant growth. These elements were added to the growth medium of microorganisms at different proportions, which are required by plant nutrients. In this case study, microbial metal mobilization from polluted soil by *Bacillus megaterium* has resulted in manganese and iron mobilization of 60-80%, whereas zinc was mobilized by 20%.

Key words: *Bacillus megaterium,* phosphogypsum, bio-solubilisation of heavy metals

REZUMAT – Capacitatea de biosolubilizare a tulpinii *Bacillus megaterium* a unor micronutrienți din solul poluat. Scopul acestui studiu a fost testarea capacității de biosolubilizare a tulpinii *Bacillus megaterium,* izolată de către autor într-un mediu poluat. Literatura de specialitate a arătat că *Bacillus megaterium* are capacități bioacumulative ale unor metale grele (Pb, As, Cd), precum şi de biosolubilizare a fosfatului, silicaților etc. S-a testat capacitatea de biosolubilizare a compuşilor insolubili sau a compuşilor parțial solubili ai Fe, Mn şi Zn, care au importanță deosebită pentru creşterea plantelor. Aceste elemente au fost adăugate în mediul de creştere a microorganismelor în diferite proporții, necesare elementelor nutritive ale plantelor. În acest studiu de caz, mobilizarea metalelor din solurile poluate a dus la o mobilizare de 60-80% a magneziului şi fierului, în timp ce mobilizarea zincului a fost de 20%.

Cuvinte cheie: *Bacillus megaterium,* fosfogips, biosolubilizarea metalelor grele

[*] E-mail: ioana.stefanescu@ub.ro

INTRODUCTION

In soil, microbes are involved in metal mobilization from minerals, in metal precipitation and deposition. These microbiological principles and processes can be adapted to treat solid wastes. Biological processes may contribute at a large extent to future technologies, including also soil unpollution and waste treatment. The biological techniques are highly accepted by people, suggesting a “natural” way of solving environmental problems. However, these “green” technologies have to be seen in juxtaposition to chemical and physical waste treatment techniques. In relation to inorganic compounds (minerals, metals) present in polluted soil, microorganisms can play an important role. The mobilization of metals from minerals, the reduction and oxidation of metals and the metal precipitation and deposition are microbiological principles and these processes have the potential to be adapted for technical waste treatment applications (Brandl, Faramarzi, 2006).

Microorganisms are able to mobilize metals by the formation of organic or inorganic acids (e.g., citric acid, sulphuric acid), by oxidation and reduction reactions and by the excretion of complexion agents (e.g., cyanide). Proton-promoted and ligand-promoted mineral solubilisation may occur simultaneously in the presence of ligands under acidic conditions. The major metabolic reactions (metal transformations) may occur when microbes are in contact with solid metal-containing particles or metals in solution (Brandl, 2001; Ledin and Pedersen, 1996). The mineralytic effects of bacteria and fungi on minerals are based mainly on three principles, namely acidolysis, complexolysis and redoxolysis (Bosecker, 1997; Brandl, 2001).

Generally, bioleaching is a microbiological process described as being "the dissolution of metals from their mineral source by certain naturally occurring microorganisms” or "the use of microorganisms to transform elements so that the elements can be extracted from a material when water is filtered through it" (Brandl, 2001). Additionally, the term "biooxidation" is also used (Brandl, 2001). Usually, "bioleaching" refers to the conversion of solid metal values into their water soluble forms using microorganisms. In the case of manganese, manganese sulphide is microbial oxidized to manganese sulphate and metal values are transferred to the aqueous phase.

The microorganism of *Bacillus* genus such as *Bacillus megaterium* is used as biological agent and grown in the presence of heavy metals. These microorganisms have good percentage mobilized elements such as cadmium, copper, zinc or manganese.

MATERIALS AND METHODS

The *Bacillus megaterium* strain was isolated from a soil polluted by phosphogypsum. Phosphogypsum is a solid waste produced in the technology of a wet-process phosphoric acid production. The basic materials for the production of phosphoric acid are phosphoric rocks. The composition of phosphate rock includes metals such iron, copper, manganese, zinc, cadmium, cobalt, etc. All these metals can be found at a smaller percentage in phosphogypsum. For this reason, the bio-solubilization capacity of *Bacillus megaterium* for manganese, zinc and iron will be analysed.

Bacillus megaterium strain was isolated from polluted soil near phosphogypsum dump, at a distance of 30 m.

Composition of mineral salt liquid medium. Mineral salt liquid medium was made by adding distilled water per litter; sucrose - 10g, K_2HPO_4 – 2.5g, KH_2PO_4 – 2.5g, $(NH_4)_2HPO_4$ - 1g, $MgSO_4$ – 0.2g, $FeSO_4$ – 0.01g, $MnSO_4$ – 0.007g. The average of pH was adjusted to 7.0 by adding 1N NaOH and later it was sterilized to 121ºC, 1.2 atm. for 15 minutes.

Screening of bacterial isolates for bio-solubilisation capacity of monitoring metals. Late-exponential-phase starter (1 ml, OD_{600}=2.2848) was used to inoculate 100 ml of liquid medium in 500 ml conical flasks, and supplied with: variant 1 - 10 ppm of Mn / 85 ppm of Zn / 9 ppm of Fe, for the variant 2 - 30 ppm of Mn / 255 ppm of Zn / 27 ppm of Fe, and for the variant 3 - 50 ppm of Mn / 425 ppm of Zinc / 45 ppm of Fe. Cells were grown to the late-exponential phase in a Shaking Orbital Incubator GFL 3033 at 30°C and 190 rpm. The bacterial cells were sampled at 6000 rpm and 4º_C using a centrifuge (Rotofix 32 A, Germany) and then were washed three times with sterilized 0.9% NaCl solution to remove soluble remaining components of media that might interact with metal binding. (Philippe *et al.*, 1999). We prepared eight Erlenmeyer glasses, one for every test, so the bio-solubilisation capacity can be observed at 6h, 18 h, 22 h, 26 h, 30 h, 42 h and 54 h periods. The supernatants resulted by centrifugation were mineralized with HNO_3 conc. and the quantity of monitored metals was determined by AAS.

Statistical analysis. The obtained results in experiments were expressed in terms of average and standard error (S.E.). Data were statistically defined by SPSS 15.0 software.

Probability (*p*-value) (less than 0.05 and 0.001) was considered significant and highly significant, respectively.

RESULTS AND DISCUSSION

The quantitative determination of Mn, Zn and Fe was done with Atomic Absorption Spectrometer (Flame) - Perkin Elmer 3300. For calibration curve of Mn, Zn and Fe, the standard solution of 100ppm (Merck) was used. All solutions for the calibration curve were diluted with HNO_3 1% (Merck). From Mn and Zn were preparation 0.2, 0.4, 0.6, 0.8 and 1 ppm concentration standards, and from Fe 1.00, 2.00, 3.00, 4.00 and 5.00 ppm concentration standards.

Linearity statistic test. After tracing curve linearity, method sensibility and determination of LOD and LOQ were verified.

From Mn – pant b – 8.5682, s_{xo} – 0.00151 and linearity (1-Sb/b)*100 = 99.85. Limit of detection from Mn was calculated LOD ((3*SD)/b) – 0.00376008 ppm, and the limit of quantification was calculated LOQ ((10*SD)/b) – 0.01253 ppm.

From Zn pant b – 5.9795, s_{xo} – 0.00205 and linearity (1-Sb/b)*100 =

98.52. Limit of detection from Mn was calculated LOD ((3*SD)/b) – 0.00407381 ppm, and the limit of quantification was calculated LOQ ((10*SD)/b) – 0.01357 ppm.

From Fe pant b – 15.011, s_{xo} – 0.0104 and linearity (1-Sb/b)*100 = 98.96. Limit of detection from Mn was calculated LOD ((3*SD)/b) – 0.002068237 ppm, and the limit of quantification was calculated LOQ ((10*SD)/b) – 0.00689 ppm.

AAS Analysis. The repeatability of all samples was three, and the numbers in the *Table 1* represent the average.

Table 1- Supernatant metal concentration

Sample	Age (h)	Zn LOQ=0.01357	Mn LOQ=0.01253	Fe LOQ=0.00689
Control	6	0.049338	1.430743	0.827381
	18	0.035839	0.098536	0.077317
	22	0.030746	0.092262	0.069159
	26	0.025867	0.087363	0.067941
	30	0.031338	0.034126	0.065214
	42	0.033883	0.089367	0.320488
Variant 1	6	0.043747	0.162649	0.459654
	18	0.027003	0.985196	0.419914
	22	0.021433	1.156560	0.403837
	26	0.027579	1.413606	0.278984
	30	0.030579	1.105151	0.292806
	42	0.021433	2.184744	0.260455
Variant 2	6	0.054003	0.205490	0.025547
	18	0.038477	0.244058	0.571894
	22	0.027003	0.282627	0.487639
	26	0.021433	0.316877	0.431720
	30	0.018166	0.299604	0.585981
	42	0.010765 <LOQ	0.280036	0.684940
Variant 3	6	0.107684	0.205490	0.005467 <LOQ
	18	0.270033	0.393684	0.076932
	22	0.254074	0.402559	0.126487
	26	0.207315	0.371150	0.395194
	30	0.158673	0.312609	0.296648
	42	0.172632	0.231195	0.007630
Detection frequency DF%		**96.55**	**100**	**96.55**

Control (C) = 100 ml mineral liquid medium + 1 ml inoculums;
Variant 1 (V_1) = 100 ml mineral liquid medium + 1 ml inoculum + 10 ppm of Mn / 85 ppm of Zn / 9 ppm of Fe;
Variant 2 (V_2) = 100ml mineral liquid medium + 1 ml inoculum + 30 ppm of Mn / 255 ppm of Zn / 27 ppm of Fe;
Variant 3 (V_3) = 100 ml mineral liquid medium + 1 ml inoculum + 50 ppm of Mn / 425 ppm of Zinc / 45 ppm of Fe.

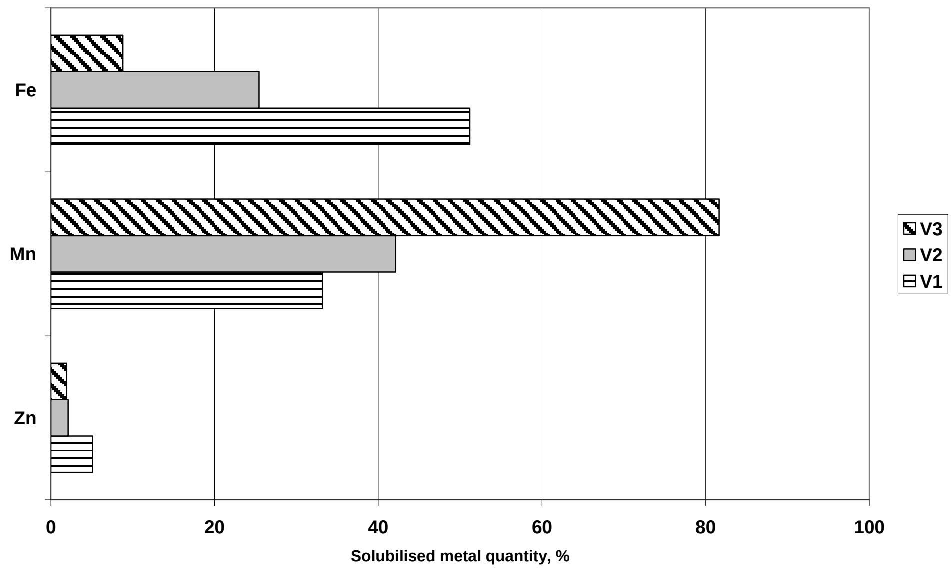

Figure 1 - Solubilised metal quantity by *Bacillus megaterium*

In *Table 1*, we may notice that the frequency detection is very high, explaining that the *Bacillus megaterium* strain solubilised a sufficient amount of the metals of each variant, which can be detected.

In addition, it aims at the bio-solubilisation gradient for each metal in relation to total metal added (the supplement of metal of each variant and composition of mineral liquid medium). This bio-solubilisation gradient is the ratio between total bio-solubilised quantity and the quantity of metals added in mineral liquid medium (*Figure 1*).

The lowest percentage of soluble metal is set for zinc in all the three variants. This suggests that zinc has come into metabolic cycle of *Bacillus megaterium,* having a cofactor role. (Wenze, 2008). As percentage, the order for bio-solubilisation of traced metals is Mn>Fe>Zn.

Based upon statistic analyse, a correlation in reverse ratio between Mn-Zn (r^2 = -0.835; p= 0.039) and Mn-Fe (r^2 = -0.822; p=0.045) is set.

CONCLUSIONS

This study shows that the isolated *Bacillus megaterium* strain from a polluted medium determines a good bio-solubilisation of metals taken from phosphogypsum waste. There are notable bio-solubilisations for manganese (80%) and iron (60%).

Following *self-relations* of traced characteristics – age, Zn, Mn, Fe quantities – important correlations between microorganism age and bio-solubilised Fe and Mn quantity can be noticed, as well as between zinc, manganese and iron. The important negative correlations set on analyzed metals (reverse ratio) suggest that a metal can potentate the metabolic chain, which determines bio-solubilisation for other metal.

The traced strain has a bio-solubilisation/ bioaccumulation potential for predominant metals taken from the sample waste. As a result, this strain is important for retrieval processes of polluted site with heavy metals.

REFERENCES

Bosecker K., 1997 - *Bioleaching: metal solubilization by microorganisms*, FEMS Microbiol. Rev., vol. 20, p. 591-604

Brandl H., Faramarzi M. A., 2006 - *Microbe-metal-interactions for the biotechnological treatment of metal-containing solid waste*, China Particuology, Vol. 4, No. 2, p.93-97

Brandl H., 2001 - Microbial *leaching of metals*, Biotechnology, vol. 10, p. 191-224

Ledin M., Pedersen K. , 1996 - *The environmental impact of mine wastes - Roles of microorganisms and their significance in treatment of mine wastes*, Earth Sci. Rev., vol. 41, p. 67-108

Philippe C., Van der Lelie D., Borremans B., Provoost A., De Lorenzo V., Brown N.L., Lloyd J.R., Hobman J.L., Csoeregi E., Johansson G., Mattiasson B., 1999 - *Whole cell- and protein-based biosensors for the detection of bioavailable heavy metals in environmental samples*, Analytica Chimica Acta vol. 387, p. 235–244

Wenze W.W., 2008 - *Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils,* Plant Soil