RESEARCH ABOUT THE USE OF BACTERIAL BIOMASS OF *THIOBACILLUS FERROOXIDANS* IN THE RECOVERING OF HEAVY METALS FROM POLLUTED MEDIUM

Tatiana PASCU[1], T. VISAN[2]

[1]National Institute of Research and Development in Soil Science, Agrochemistry and Environment-ICPA Bucharest, Bd. Marasesti 61, Bucharest 011464, Romania
[2]University POLITEHNICA of Bucharest, Spl. Independentei 313, Bucharest 066004, Romania
E-mail: tpascu@yahoo.com

Abstract: *Pollution has become along the years a serious problem, difficult to solve. Developing of the industry was lead to a rising of wastes quantities, those has been accumulated in air, water and soil, when the raw resources are fewer and fewer. Classical wastes contain a large variety of pollutant but the heavy metals, toxic for human and also for environment represent a important part. In present is wide accepted idea of using biotechnology and microbiological meaning for clearing pollution of certain ecosystems from an-organics compound of heavy metals type. This involves certain phenomenon's, being trans-location, such bioremediation, being biological modification of those metals for forming compounds less toxics or with low mobility. Recovering of heavy metals from solution can be achieved using two big group of technique, which resides by involving of electrochemical reaction. In parallel with electrochemical treatment (electrolyses, electrodeposition, electrodialysis, electrocoagulation) exist physicochemicals based on chemical precipitation, cementation and absorption on ion exchange resins, osmoses and sticking metals to biomass. This last technique can resides by bio-absorption of metallic ions on biological surfaces dead or alive, by intercellular accumulation (living biomass) or by bio-precipitation. For studies regarding evaluation of retaining capabilities by microorganisms, presented in this work, was used two strain of bacteria's Tf DSM583 and Tf BRGM and 3 growing mediums: 9K, 9KM si 9Opt. Was used standard solution of* uSO_4, $ZnSO_4$, $CdSO_4$, $CrSO_4$ *with a concentration of* 10^{-3}*M. Thiobacillus ferrooxidans, an acidophilic, aerobic, chemolithoautotrophic, gram-negative bacterium, shows a high, natural level of resistance to heavy metals. Microbial growth has been estimated by the measurement of bacterial metabolism characterized by ferrous iron oxidation. This bacterial oxidation is characterized by the* $Fe^{2+}/(Fe^{2+} + Fe^{3+})$ *ration.* Fe^{2+} *concentration has been determined by colorimetric dosing of iron on a probe (10µl) by the phenanthroline method. Quantities of metallic ions recovered by bacteria Thiobacillus ferrooxidans was determined by polarographic technique for ions of* $Zn^{(II)}$, $Cd^{(II)}$, $Cu^{(II)}$ *and by colorimetric technique for* $Cr^{(VI)}$.

Key words: *heavy metals, Thiobacillus ferrooxidans, strain, biomass*

INTRODUCTION

In present is wide accepted idea of using biotechnology and microbiological meaning for clearing pollution of certain ecosystems from an-organics compound of heavy metals type. This involves certain phenomenon's, being trans-location, such bioremediation, being biological modification of those metals for forming compounds less toxics or with low mobility.

In condition of industrialized society, often, some toxic compounds for plants and animals pollute the environment. Some of them get lost, being degraded to an inoffensive level, other remain.

As it seems, disappearing of those from environment by dilution to very small concentration, hard to determine by classic methods, do not represent a guaranties of ecologic security. Certain biological process on long terms can determine a concentration for certain toxic substances some times to a value multiplied by hundred or thousands against superior level from environment, by complex mechanism of bioaccumulation.

In those processes beside detoxification function, microorganisms, can action as a extern factor highly important for including of heavy metals in tropic network because intense metabolic activities, also the level of rapport surface/volume tend to unity.

Recovering of heavy metals from solution can be achieved using two big group of technique, which resides by involving of electrochemical reaction. In parallel with electrochemical treatment (electrolyses, electrodeposition, electrodialysis, electrocoagulation) exist physicochemicals based on chemical precipitation, cementation and absorption on ion exchange resins, osmoses and sticking metals to biomass. This last technique can resides by bio-absorption of metallic ions on biological surfaces dead or alive, by intercellular accumulation (living biomass) or by bio-precipitation.

From recent past, bio-absorption is studied in detail for bio absorbents which are not sub-product un unvalued of many industries. Microorganism had demonstrated a high capacity of recovering metallic ions, highly above bio-absorbents researched as usual until present (industrial waste).

MATERIAL AND METHODS

Has been used two types of strains of *Thiobacillus ferrooxidans : Thiobacillus ferrooxidans DSM583* (*Tf DSM583*), which come from German Collection of Microorganisms and Cell Culture and *Thiobacillus ferrooxidans BRGM* (*Tf BRGM*), which been isolated by Bureau de Recherche Geologique et Miniere, France.

For growing of those two strains of the bacteria *Thiobacillus ferrooxidans* was used three growing media: **9K** (0.4g/L $MgSO_4{\cdot}7H_2O$, $(NH_4)_2SO_4$, K_2HPO_4, 33.3 g/L $FeSO_4{\cdot}7H_2O$), **9KM** (0.5g/L $MgSO_4 \cdot 7H_2O$, 3g/L $(NH_4)_2SO_4$, 0.5g/L KH_2PO_4, 0.1g/L KCl, 0.01g/L $CaNO_3$, 0.4g/L $CuSO_4{\cdot}$ $5H_2O$, 44.2 g/L $FeSO_4 \cdot 7H_2O$) and **9KOpt** (0.1g/L $MgSO_4 \cdot 7H_2O$, 10g/L $(NH_4)_2SO_4$, K_2HPO_4, 0.001g/L $CaSO_4$, $CuSO_4{\cdot}$ $5H_2O$, $ZnSO_4$, H_3BO_3, 100 g/L $FeSO_4 \cdot 7H_2O$).

Thiobacillus ferrooxidans, an acidophilic, aerobic, chemolithoautotrophic, gram-negative bacterium, shows a high, natural level of resistance to heavy metals.

Precultures was been realized in 100 ml-flasks which contain 20 ml of sterile 9K medium. Sterilized solution of salt and of water pH 1.4 has been done with autoclave (20 min at 120^0 C) in mean time iron solution has been sterilized by 0.2 µm filters. Steering has been done with a plane rotating at 100 rpm and aeration by diffusion of starting from surfaces of liquid. Cultures have been carried out in Erlenmeyer glasses of 1 L which contain 500ml of un-sterile medium. Agitation and aeration has been carried out by rotating agitation.

Microbial growth has been estimated by the measurement of bacterial metabolism characterized by ferrous iron oxidation. This bacterial oxidation is characterized by the Fe^{2+}/ (Fe^{2+} + Fe^{3+}) ration. Fe^{2+} concentration has been determined by colorimetric dosing of iron on a probe (10µl) by the phenanthroline method.

Recovering of concentrated biomass has been done by centrifugation, after the percentage of Fe^{2+}/ (Fe^{2+} + Fe^{3+}) has reach 10%.

Protein concentration was determined by Lowry method. The bacterial dry weight was calculated by multiplication of protein concentration by factor of 1.67.

RESULTS AND DISCUSSIONS

Studies regarding capacity of recovering ions of $Cu^{(II)}$, $Zn^{(II)}$, $Cd^{(II)}$, Cr^{VI} by those two strains of *Thiobacillus ferrooxidans* has been carried out using standard solution of $CuSO_4$, $ZnSO_4$, $CdSO_4$, $CrSO_4$ with a concentration of 10^{-3}M. For all solution was used H_2SO_4 pH 1.4

Quantities of metallic ions recovering by bacteria *Thiobacillus ferrooxidans* was obtained by polargraphic technique, for ions of $Zn^{(II)}$, $Cd^{(II)}$, $Cu^{(II)}$ and colorimetric technique for $Cr^{(VI)}$.

Influence of the growing medium over recovery capacity of metallic ions by *Tf DSM 583* si *Tf BRGM* are shown in fig. 1 and fig. 2.

Was noted that in cases of ions of $Zn^{(II)}$, $Cd^{(II)}$, $Cu^{(II)}$ and $Cr^{(VI)}$ capacity of recovering by strain of *Tf BRGM* was clear superior against *Tf DSM583* for medium 9K and 9KM, with exception of $Cr^{(VI)}$ for medium 9KM. Same observation can by made for recovery capacity ions of $Zn^{(II)}$ by strain *Tf BRGM* developed in medium 9KOpt is inferior or equal with a strain of *Tf DSM583* developed in same medium.

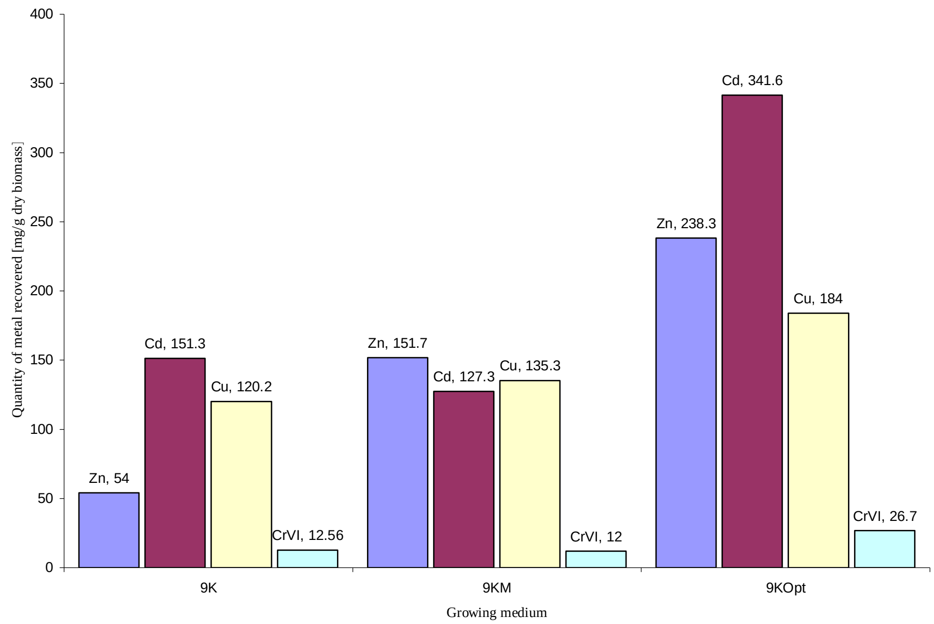

Figure 1. Variation of metallic quantities recovered by bacterial biomass of *TfDSM583* depending on the medium

Influence of the variation of concentration of $FeSO_4$ in medium 9K against recovering capacities of those two strains used in this work - *Tf DSM583* and *Tf BRGM* - was studied for three growing media, which have been: medium 9K20 which contain 20g/L $FeSO_4$ instead of 33.3g/L $FeSO_4$ contained in initial medium 9K, medium 9K40 which contain 40g/L $FeSO_4$ and medium 9K50 which contain 50 g/L $FeSO_4$.

In case of variation quantities of $FeSO_4$ in all cases, recovery capacity of strain *Tf BRGM* is superior recovery capacity of strain *Tf DSM583* for all four metals taken into account with exception of $Cr^{(VI)}$ which represent a better recovery capacity of strain *Tf DSM583* in case of medium 9K40.

Influences of the ions of $Ca^{(II)}$, $Cu^{(II)}$ or $Zn^{(II)}$, present in medium 9K against recovering capacities of strain *Tf DSM583* has been studies in case of adding in medium 9K of a quantities of 0.4 g/L $CuSO_4$, 10^{-3}g/L $CaSO_4$ or 10^{-3}g/L $ZnSO_4$.

When adding to medium 9K a quantities of $CuSO_4$, $CaSO_4$ or $ZnSO_4$, recovery capacity of heavy metals by strain *Tf BRGM* is net superior or equal with strain *Tf DSM583*. Also, exist exceptions: in case of recovering ion of $Zn^{(II)}$ capacity of recovering is bigger for strain *Tf DSM583* developed in medium 9KCu than strain *Tf BRGM* similar obtained and in case of recovering ion of $Cr^{(VI)}$ for medium 9KZn.

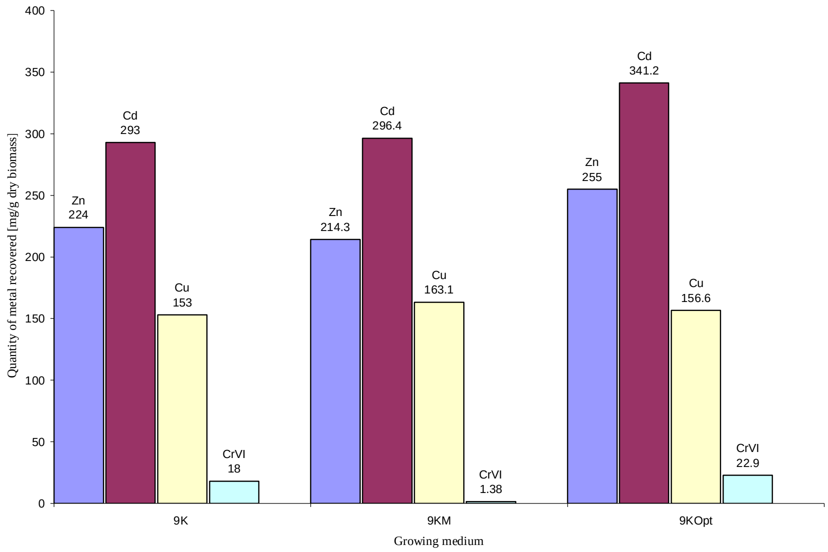

Figure 2. Variation of metallic quantities recovered by bacterial biomass of *Tf BRGM* depending on the medium

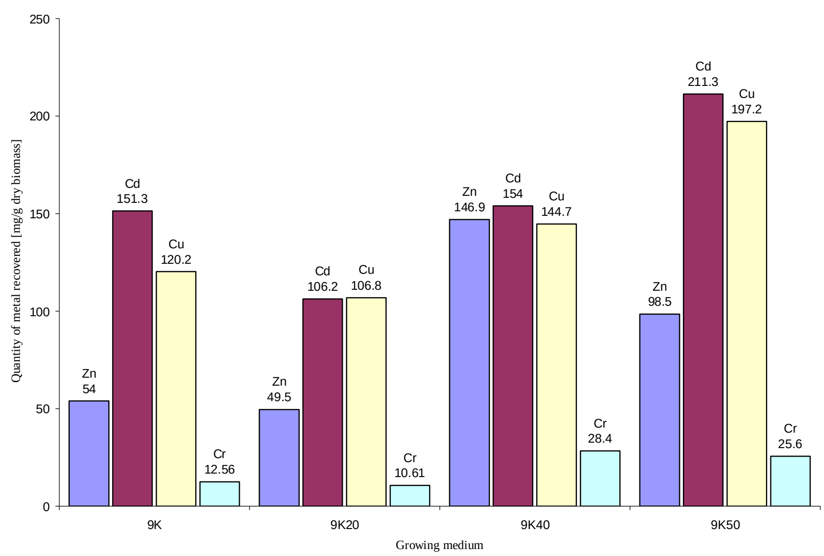

Figure 3. Variation of metallic quantities recovered by bacterial biomass of *TfDSM583* depending on the quantities of $FeSO_4$ from medium 9K

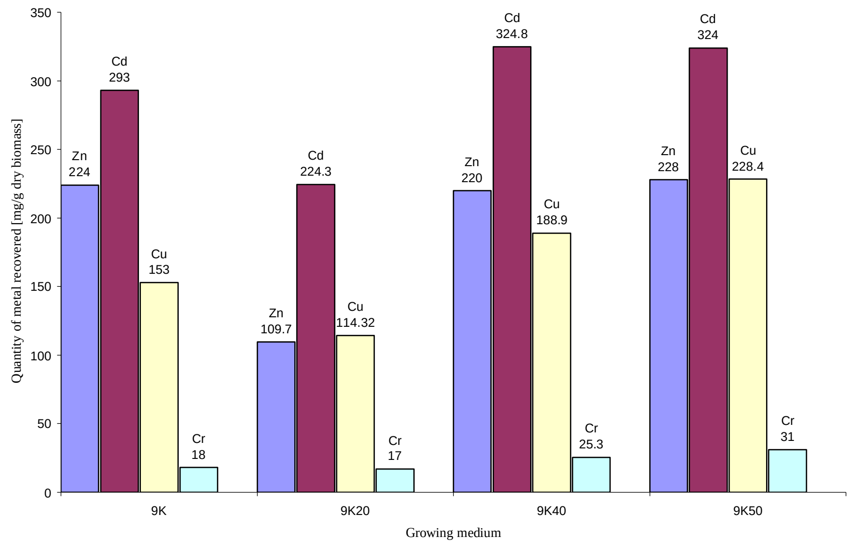

Figure 4. Variation of metallic quantities recovered by bacterial biomass of *TfBRGM* depending on the quantities of $FeSO_4$ from medium 9K

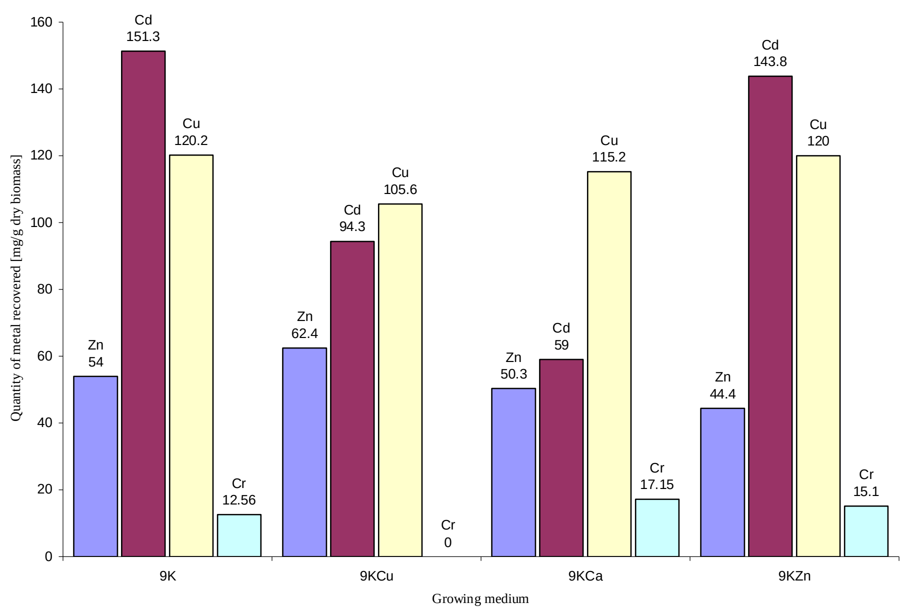

Figure 5. Variation of metallic quantities recovered by bacterial biomass of *TfDSM583* depending on the quantities of $CuSO_4$, $CaSO_4$, $ZnSO_4$ from medium 9K

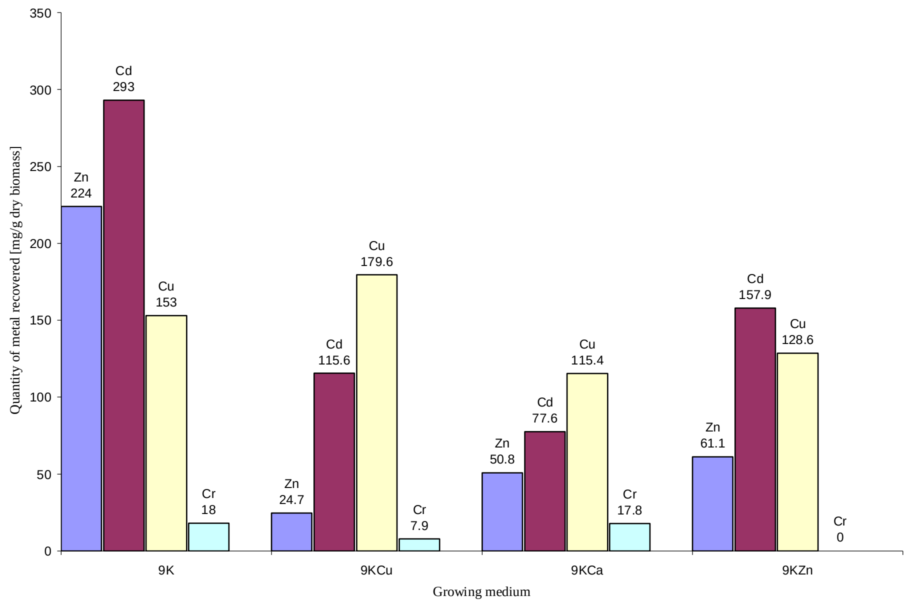

Figure 6. Variation of metallic quantities recovered by bacterial biomass of *TfBRGM* depending on the quantities of $CuSO_4$, $CaSO_4$, $ZnSO_4$ from medium 9K

CONCLUSIONS

- Growing medium used for growing of those two strains of *Thiobacillus ferrooxidans – Tf DSM583 and Tf BRGM*- has influence against metallic ion recovery capacity.
- The strain *Tf BRGM* represent a high capacity for recovering heavy metals ions than strain *Tf DSM583* indifferent of use medium: ex. *Tf BRGM* can recovery twice more Cadmium (293 mg/g dry biomass) than *Tf DSM583* (151.3 mg/g dry biomass) in medium 9K.
- Recovery capacity of heavy metals by those two strains of *Thiobacillus ferrooxidans* is direct proportional with rising of quantities of $FeSO_4$ in growing medium.
- Generally speaking, adding $CaSO_4$ or $ZnSO_4$, components of medium 9KOpt, and $CuSO_4$, medium 9KM component in the medium 9K leads to decrease the recovery capacity of the two strains of *Thiobacillus ferrooxidans* used.

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