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The Effect of Microorganisms on Soil Remediation

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***Abstract*- some heavy metals are important and essential trace elements, at high concentrations, such as those found in many environments today, most can be toxic to microbes. Microbes have adapted to tolerate the presence of metals or can even use them to grow. Thus, a number of interactions between microbes and metals have important environmental and health implications. Microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediating metal-contaminated environments. The goal of this work is to study biosorption processes of heavy metal in polluted soil by the microorganisms.**

Keywords--component; Heavy Metal, Biosorption, Biogeochemical Cycling

I. INTRODUCTION

Rapid industrialization and urbanization have resulted in elevated levels of toxic heavy metals entering the biosphere; this is evident in soil and sediment depth profiles. The total amount and types of waste have increased due to rapid industrial development in recent years. The generation of domestic and hazardous waste is increasing at an unprecedented rate and parallels production rates [5] **.** For treating heavy metal contaminated soils, bioremediation is the most efficient and least costly method [12]. One of the most promising new fields of the remediation of soils contaminated by heavy metals is the development of the processes of bacterial transformation of metals from the bound into mobile forms that are accessible to extraction. Natural remediation of soils contaminated by heavy metals proceeds very slowly [6]. So far, we have only a limited understanding of the many complex interactions between metals and the soil environment [5]. Microbial interactions with metals may have several implications for the environment. Microbes may play a large role in the biogeochemical cycling of toxic heavy metals also in cleaning up or remediating metal-contaminated environments [2]. Strong biosorbent behaviour of certain micro-organisms towards metallic ions is a function of the chemical make"up of the microbial cells. This type of biosorbent consists of dead and metabolically inactive cells [7]. Microorganisms have complex structures and diverse cell products, this result the complexity of adsorption processes and diversity of mechanisms, and make the biosorption of heavy metals quite different from conventional physical–chemical adsorption. The studies regarding adsorption of heavy metals by microorganisms have been conducted in the areas of strain screening adsorption efficiency adsorption mechanisms and models effect factors, adsorbent immobilization, etc. the purpose of this study is to investigate the effect of bacteria on soil remediation [3].

II. SEVERAL RESEARCHES:

A. Biosorption of Heavy Metals

- Biosorbent material:

Strong biosorbent behaviour of certain micro-organisms towards metallic ions is a function of the chemical make"up of the microbial cells. This type of biosorbent consists of dead and metabolically inactive cells. Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals. Some laboratories have used easily available biomass whereas others have isolated specific strains of microorganisms and some have also processed the existing raw biomass to a certain degree to improve their biosorption properties! Recent biosorption experiments have focused attention on waste materials, which are by-products or the waste materials from large-scale industrial operations. For example the waste mycelia available from fermentation processes, olive mill solid residues, activated sludge from sewage treatment plants [1] biosolids aquatic macrophytes used dewatered waste activated sludge from a sewage treatment plant for the biosorption of zinc from aqueous solutions. The adsorption capacity was determined to be 0.564 mM/g of biosolids. The use of biosolids for zinc adsorption was favourable compared to the bioadsorption rate of 0.299 mM/g by the seaweed Durvillea potatorum. Keskinkan et al. 2003 studied the adsorption characteristics of copper, zinc and lead on submerged aquatic plant Myriophyllum spicatum. The adsorption capacities were 46.69 mg/g for lead, 15.59 mg/g for zinc and 10.37 mg/g for copper. The information gives a

comparison of heavy metal uptakes of various macrophytes. Pagnanelli, et al2002 have carried out a preliminary study on the 'Useof olive mill residues as heavy metal sorbent material The results revealed that copper was maximally adsorbed in the range of 5.0 to 13.5 mg/g under different operating conditions. The simultaneous biosorption capacity of copper, cadmium and zinc on dried activated sludge [1] were 0.32 mmol/g for metal system such as Cu-Cd; 0.29 mmol/g for Cu-Zn and 0.32 mmol/g for Cd-Zn. The results showed that the biomass had a net preference for copper followed by cadmium and zinc. Another inexpensive source of biomass where it is available in copious quantities is in oceans as seaWeeds, representing many different types of marine macro-algae. However most of the contributions studying the uptake of toxic metals by live marine and to a lesser extent freshwater algae focused on the toxicological aspects, metal accumulation, and pollution indicators by live, metabolically active biomass. Focus on the technological aspects of metal removal by algal biomass has been rare. Although abundant natural materials of cellulosic nature have been suggested as biosorbents, very less work has been actually done in that respect. The mechanism of biosorption is complex, mainly ion exchange, chelation, adsorption by physical forces, entrapment in inter and intrafibrilliar capillaries and spaces of the structural polysaccharide network as a result of the concentration gradient and diffusion through cell walls and membranes. There are several chemical groups that would attract and sequester the metals in biomass: acetamido groups of chitin, structural polysaccharides of fungi, amino and phosphate groups in nucleic acids, amido, amino, sulphhydryl and carboxyl groups in proteins, hydroxyls in polysaccharide and mainly carboxyls and sulphates in polysaccharides of marine algae that belong to the divisions Phaeophyta, Rhodophyta and Chlorophyta. However, it does not necessarily mean that the presence of some functional group guarantees biosorption, perhaps due to steric, conformational or other barriers.

- Choice of metal for biosorption process:

The appropriate selection of metals for biosorption studies is dependent on the angle of interest and the impact of different metals, on the basis of which they would be divided into four major categories: (i) toxic heavy metals (ii) strategic metals (iii) precious metals and (iv) radio nuclides. In terms of environmental threats, it is mainly categories (i) and (iv) that are of interest for removal from the environment and/or from point source effluent discharges. Apart from toxicological criteria, the interest in specific metals may also be based on how representative their behaviour may be in terms of eventual generalization of results of studying their biosorbent uptake. The toxicity and interesting solution chemistry of elements such as chromium, arsenic and selenium make them interesting to study. Strategic and precious metals though not environmentally threatening are important from their recovery point of view.

- Biosorption Mechanisms:

The complex structure of microorganisms implies that there are many ways for them etal to be taken up by the microbial cell. The biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

1. Metabolism dependent
2. Non -metabolism dependent.

According to the location where the metal removed from solution is found, biosorption can be classified as:

1. Extra cellular accumulation/ precipitation
2. Cell surface sorption! precipitation
3. Intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal. During non-metabolism dependent biosorption, metal uptake is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible. In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface [4]. Further, it may be dependent on the cell's' metabolism if, in the presence of toxic metals, the microorganism produces compounds that favour the precipitation process. Precipitation may not be dependent on the cells' metabolism, if it occurs after a chemical interaction between the metal and cell surface.

B. Remobilization of Toxic Heavy Metals

Clays and bacterial walls and envelopes usually have a net negative charge over a wide pH range. This anionic character is due to their complex chemical structures producing a variety of sites that attract and interact with metallic aquo-ions. One mechanism contribitting to the retention of metals on these soil constituents is coulombic attraction between the charged site and the ion [11]. Attractive forces and affinity between the metal and site can be relatively weak and nonspecific, resulting in easily exchangeable cations, or they can be strong and specific resulting in less easily remobilized cations. A second major mechanism involved in cation retention on surfaces is precipitation of, for example, hydroxy or carbonate species on clays [11] or on carboxyl or phosphoryl groups of bacterial surfaces

- Metal-binding experiments.

Substantial amounts of metals were bound to the bacterial cells walls of B. subtilis and E. *coli* and the smectite

clay. Kaolinite has a lower cation-exchange capacity than smectite or the bacterial walls and retained much smaller amounts of metals. The quantities of metals retained by the walls and smectite in these experiments were significantly higher than those in the experiments by reserchers Since the walls, envelopes, and the clays used in the present experiments were from the same batches as those for the study of Walker et al. and since the only technical difference was the use of high-purity water versus deionized distilled water the importance of subtle environmental changes in these types of experiments is aptly illustrated. In our present study, electron-dense amorphous Cr precipitates and elemental Ag deposits were seen on BW after 48 h of incubation in UPW at pHs 7 and 3. Silver deposition on some EE also was observed after remobilization treatments at various pHs but was observed less frequently on envelopes than on walls. Hydroxy-bridged precipitates of Cu also are thought to occur [11] but, if present, were not large enough to be resolved by electron microscopy on the walls.

- Remobilization of metals.

In all cases of remobilization, whether it is a single-component system or a composite, mechanistic view must incorporate the exchange of site associated cations, the dissolution of chemically complexed

Metallo-aggregates, and, possibly, the breakdown of the particle fabric itself (e.g., the peptidoglycan of bacterial walls). Our experiments revealed a variety of remobilization eresponses that were frequently metal, desorption agent, cell-wall, or composite dependent. Each constituent has a range of unique properties which contribute to the multiplicity of responses. We recognize that each experiment will have a multitude of interpretations and associated complexities and offer only those explanations which seem most compelling.

The cases, the mobilities of organic material-clay-associated metals were similar to those of metals associated with individual components; i.e., the percentage of remobilized metal from composites was between those determined for the individual components under the same conditions. In these cases, the behavior of the metal in composites could be predicted by the metal mobility data obtained from the single-component systems. However, this was not always the case. In 22% of the cases, metal remobilization approached that of one of the components (usually the wall or envelope) and was much different from that in the other. For example, Ag remobilization on treatment with 500 ,Um EDTA from EE+SK and EE+SS mixtures was like that from EE (5%) and six to eight times lower than that from SS or SK alone (41 and 30%, respectively). These results suggest that EE were responsible for much of the Ag binding in the composite and hence dictated Ag remobilization; in addition, the affinity of clays for Ag is low, that its chemical complex is unstable, and that most Ag binds to the organo-components. Knowing this information, we could have predicted these mobilities, too. Conversely, at pH 3, Cu remobilization from EE+SS (51%) is like that from EE (52%) and greater than that from SS (21%). On an individual basis, SS binds more Cu than EE does, but SS does not have much influence on the outcome of Cu mobility in the EE+SS composite. Again, remobilization was dictated by the organic component, but this was not as predictable as was the previous example. Other results from our studies have shown that the remobilization of organic material-clay-associated metals was, in 23% of experiments, totally unique and quite unpredictable compared with data from single organic material or clay systems. In some cases, metals associated with the wall-clay mixtures were easier to remobilize than metals associated with either component. For example, at 160-ppm Ca, 1.5 to 10 times more Ag was mobilized from EE+SK than from EE or SK. Also, at pH 3, Ag was remobilized to a greater extent from EE+SK and EE+SS (1.2 to 3.2 times more) than from EE, SS, or SK alone. Alternatively, Cu mobility was reduced by 25% in BW+SS and EE+SS on treatment with 500, M EDTA. Why do we see unpredictability in the remobilization of metals associated with some composites? In some cases, the sum of the two components (clay plus wall) must profoundly alter their individual physicochemistries. Certainly, the production of the composites alters the total surface charge and blocks reactive sites. It also is possible with certain wall-clay combinations that previously unexposed sites become available and that their binding affinities will be metallo-ion specific. New interfacial sites must be produced at the adhesion zones between cell walls and clay particles. Charge and hydrophobic interactions at these zones will determine accessibility of not only metalloions but also remobilization agents. It is quite possible that small ion exchangers, such as H+ or Ca2+, have readier access to immobilized metals in these zones, whereas larger, negatively charged agents such as EDTA or FA are excluded.

- Metal remobilization by lysozyme.

Unlike all other agents used in our study, lysozyme was capable of hydrolyzing the bacterial walls into their soluble constituents. This enzyme breaks select P-1,4 bonds along the glycan strands of peptidoglycan to liberate N-acetylglucosaminyl-N-acetylmuramyl dimers, which may or may not be bonded by transpeptide linkages to adjacent dimers; all of these degradation products, including those containing secondary polymers [10] are soluble. The cross-linkage in our B. subtilis walls was 35% (9). Enzymatic action makes the mode of metal remobilization of lysozyme quite different from those of the other agents. Only the biopolymeric fraction is hydrolyzed, and the metal is not ripped from its chemical linkage; instead, the biofabric is solubilized with the metal attached. Yet, even though there can be no enzymatic attack on the clay fraction, lysozyme-clay interaction is quite possible since, like many proteins, the enzyme is electropositive at neutral pH (pl = 11; 27). It could compete for metal sites on clays, and a proportion could be adsorbed from solution. This proportion would reduce the enzymatic activity of the fluid phase. Although heat-denatured lysozyme was used as an inactivated enzyme control, it retains its electropositive character and should have the same exchange attributes on the clays as the native form. It was of interest that the denatured enzyme did remobilize some metal, and this should reflect the exchange

capacities of both the native and denatured forms. The increased remobilization capacity of the active enzyme was due to its wall degradation power. At 80 to 160 ppm, lysozyme degraded the walls from the Cu-wall composite and remobilized 100% of the Cu. Cu was also mobilized completely (100%) from BW+SK but only 46% from BW+SS. The large difference between SK and SS composites with Cu is striking. From the single-clay experiments, it seems certain that this difference cannot be attributed solely to their individual sportive powers. Instead, it would appear that smectite has somehow inhibited lysozyme activity, allowing a proportion of the walls to remain as discrete structures together with their metal and clay associates. This is not surprising, because smectite has the capability of absorbing small proteins into the interlayers between its platelets [8]. Once absorption is complete, the plates clamp down and trap the enzyme, thereby removing it from reaction. Compared with Cu, 66 and 93% less Ag and Cr were remobilized from BW by lysozyme, suggesting that these cations are inhibitory. Certainly it is not unusual for heavy metals to inhibit wall-degrading enzymes, because Fe3+ retards autolysins associated with BW, and various metals have been shown to inhibit lysozyme activity. Our own enzyme inhibition studies showed that the degradation of BW by lysozyme was reduced by Cr, Ag, and smectite, but not by Cu or kaolinite. Published evidence of heavy metal inhibition of lysozyme is conflicting; some investigators argue for reversible inhibition by Cu, while others suggest that it is irreversible inhibition, the inhibitory concentrations of metals vary among the reports.

C. Hg(II) adsorption by Bacillus mucilaginosus

- Hg concentrations-absorbances relationship equation

The experimental results showed a good linear relationship between Hg concentrations (0–10 ppb) and the absorbances as follow:

$$Y = 0.15X-0.0872, \text{ with } R^2 = 0.9996$$

Here, Y was measured Hg concentration, and X was absorbance.

- Adsorption of Hg at different concentrations by the culture of B. *mucilaginosus*

Adsorption ratio A, the removal efficiency of Hg from the solutions, was calculated as

$$A = (C0–C)/C0 * 100\% \quad \text{and}$$

Adsorption capacities q, the amount of adsorbed Hg(mg) by 1 l bacterial culture, was determined as

$$q = V0 * (C0–C)/V \text{ mg/l},$$

Here V0 was the total volume of the reaction solution (100 ml), V was the volume of the culture (5 ml), C was the equilibrium Hg concentration, and (C0-C) was adsorbed Hg concentration. In this case, the bacterial adsorption sites were far more than the amount of Hg complexes. Thus the majority of Hg complexes were adsorbed, while the bacterial adsorption sites were far from being saturated. In contrast, at higher concentrations of Hg, A was lower and q was higher (374 mg/l, maximum). In this case, bacterial adsorption sites were far less than the amount of Hg. Thus the majority of adsorption sites were occupied by Hg complexes, while a large amount of Hg has not been adsorbed. The results showed that as Hg(II) concentration increased q increased; rapidly at lower levels of Hg(II), and it tended to be saturated at higher levels of Hg, which suggested that the adsorption curve was in accordance with the Langmuir model. Control experiment showed that sterile medium (free of bacteria) could not adsorb mercury at various concentrations. pH values of experimental solutions were at around 6, and decreased gradually with the increasing concentrations of mercury.

III. CONCLUSION

Bioremediation of soil contaminated by organic chemical pollutants benefits considerably from the use of soil microorganisms to metabolized the organic chemical compounds. It is natural therefore to expect that there would be naturally occurring consortia of microorganisms-ranging from bacteria and fungi to viruses- available to successfully address synthetic organic chemicals since they would be expected to be well adapted to the specific habitat. The available energy sources and all the other micro environmental factors such as pH, temperature, water content, etc. will produce suites of biomass that have adapted to the micro environmental [9]. The result showed that Biosorption is being demonstrated as a useful alternative to conventional systems for the removal of toxic metals from industrial effluents. The development of the biosorption processes requires further investigation in the direction of modeling, of regeneration of biosorbent material and of testing immobilized raw biomasses with industrial effluents.

REFERENCES

[1] A. Hammaini. Simultaneous uptake of metals by activated sludge. *Minerals Engineering*. 16: 723-729. 2003..

[2] A. Spain1, E Alm. Implications of Microbial Heavy Metal Tolerance in the Environment. *Reviews in Undergraduate Research*. 2: 1-6. 2003.

[3] B. Mo, B Lian. Hg(II) adsorption by *Bacillus mucilaginosus*: mechanism and equilibrium parameters. *World J Microbiol Biotechnol.* Published online, 2 sep 2010.

[4] C. Ercole, F. VegIio, L. Toro, G. Ficara and A. Lepidi, Immobilisation of microbial cells for metal adsorption and desorption. In: Mineral Bioprocessing II. Snowboard. Utah. 1994

[5] C.A Flemming, F.G. Ferris T.J. Beveridge, G.W. Bailey. Remobilization of Toxic Heavy Metals Adsorbed to Bacterial Wall-Clay Composites. *Applied and Environmental Microbiology*. 56(10): 3191-3203. 1990

[6] G.N. Nikovskaya, Z.R. Ul'berg, L.A. Koval. Colloidochemical Processes in the Biotechnology of Heavy Metal Removal from the Soil. *Colloid Journal*. 63(6). 750–754. 2001.

[7] N. Ahalya, T.V. Ramachandra, RD. Kanamadi. Biosorption of Heavy Metals. *Research Journal Of Chemistry And Environment.* 7(4): 71-79. 2003.

[8] O. Talibudeen, Interlamellar adsorption of protein monolayers on pure montmorillonoid clays. *Nature* (London) 166:236. 1950.[9]- R.N. Yong. geoenvironmental engineering contaminated soils, pollutant fate, and mitigation. Boca Raton Washington, D.C. CRC Press. 2001

[9] T. J. Beveridge.. Ultrastructure, chemistry, and function of the bacterial wall. *Int. Rev. Cytol.* 72:229-317. 1981.

[10] W.F. Pickering. Copper retention by soil/sediment components p. 217-253. In J. 0. Nriagu (ed.), Copper in the environment. I. Ecological cycling. John Wiley & Sons, Inc., Toronto. 1979.

[11] X. Xie, J. Fu, H Wang, J. Liu. Heavy metal resistance by two bacteria strains isolated from a copper mine tailing in China. *African Journal of Biotechnology*. 9(26): 4056-4066. 2010