

Toxic Metal Removal by Biosorption

Jyotsna Lal

Associate Professor

Department of Chemistry

Christ Church P.G College, Kanpur U.P. 208001

Corresponding author's email id: jyotsna_lal@yahoo.com

DOI: <http://doi.org/10.5281/zenodo.2638778>

Abstract

The goal of all water treatment process is to remove existing contaminants in the water, or reduce the concentration of such contaminants so the water becomes fit for its desired end-use. One such use is returning water that has been used back into the natural environment without adverse ecological impact. The processes involved in treating water for drinking purpose may be solids separation using physical processes such as settling and filtration, and chemical processes such as disinfection and coagulation.

Biological processes are employed in the treatment of wastewater and these processes may include, for example, aerated lagoons, activated sludge or slow sand filters.

Recovery and removal of toxic metal ions is a major problem. Biosorption offers cheap alternative for developing countries like India. This paper studies the various microorganisms and the mechanism of removal.

Keywords: *Biosorption, Toxic metal, Ions, Contaminants, Microbial Biomass*

INTRODUCTION

It has long been known that many biological materials will accumulate

metals This phenomenon tends to exacerbate the environmental impact of various metals that are released in

the environment because they become concentrated in the flora and \or fauna and ultimately get into the food chain . However , the same phenomena potentially can be used in a controlled environment to remove dissolved metal from processing streams either for recovery of valuable resources or to remove pollutants . The interaction between microbes and metals has been studied by scientists of different disciplines. Agricultural scientists are primarily interested in the microbicidal action of metals , bio inorganic chemists basically look at the metal coordinating geometry and their environment in metallo proteins and biological functions Scientists in life sciences study the toxicological effects , its bio accumulation and bio magnification while the environmental scientists and engineers try to utilize this property of bio accumulation for monitoring metal pollution as well as removal \ recovery of metals from natural and waste waters .

There is an increasing interest in investigating such biological adsorbents for variety of applications , as biologically derived material can be produced at low cost , yet have adsorption capacities near those of

commercial ion-exchange resins . Adsorption of the metal ions on to materials of biological origin is now regarded as one of the most promising technologies for treating wastes economically. In this paper the author wishes to direct attention towards the application of micro organisms for metal reclamation and recovery It has been observed that many microbial species exhibit metal binding properties the adsorption capacities of microbial biomass are comebUnparable to or can exceed those of inorganic adsorbents . The use of bio adsorbents .for removal and recovery of dissolved metals has been demonstrated on a laboratory scale or with small pilot plants 1,2 Biosorption has emerged as a viable alternative to conventional metal scavenging techniques . To make this technology economically viable, however the dynamics of this reaction must be enhanced significantly Some mechanisms have been proposed by reseachers for metal accumulation by micro organisms

BIO SORPTION BY MICROBIAL BIOMASS

Use of yeast cells *Saccharomyces* sp for starch fermentation in ethanol

production is well known. Research in use of mixed culture of *Saccharomyces diastaticus* and *Zymomonas mobilis* in ethanol production has been reported [3]. Biosorption, is the process in which live or dead microbes are used for removal of metals from their dilute solutions. In recent years investigation of various types of micro organisms for metal adsorption has been reported. Heavy metals, which can be toxic to living organisms are present in many industrial aqueous waste streams. These metals may originate from a variety of sources including the nuclear power defence and fuel reprocessing industries; surface finishing processes in the aerospace industry; and silk – screening processes in the computer industry. In addition, ground waters at many sites are contaminated with these metals ions making their removal and concentration a necessary step in the restoration of these areas.

Large scale industrial growth has caused serious concern regarding the susceptibility of ground water due to heavy metals, waste materials near the factories are subjected to reaction with percolating rain water and reaches the ground water level [4]. There are a

number of toxic trace elements found in natural and waste waters, some of these are essential at low levels of concentrations serving as nutrients for animals and plants but toxic at higher levels. [5] Zn essential in many metallo-enzymes toxic to plants at high levels, Cu essential trace element, non to animals but toxic to plants and algae while As, Hg, Pb, Cd are highly toxic even at low concentrations. These metals attack the active sites of enzymes, inhibiting essential enzyme activity. Heavy metals act as effective enzymes inhibitors. Zn (II) in some metallo - enzymes is substituted by Cd (II).

As (III) attacks -SH groups of an enzyme i.e inactivation of pyruvate dehydrogenase by complexation with As (III) preventing the generation of ATP in the citric acid cycle. As (III) ion causes arsenicosis, widely prevalent in regions of rural West Bengal. Pb inhibits several key enzymes involved in process of heme synthesis in humans. Hg came into the limelight with the incidence of the Minamata disease.

Many microbial species exhibit metal binding properties. Most research in this area has been associated with

heavy metals , such as Pb, Cd, Cu , Cr ,and radio nuclide accumulation. Intra cellular accumulation of many metals has been observed in bacteria , fungi and algae . It has been shown in [Table 1] by several researchers 7 that for some types of microbial biomass , most of the metal adsorption occurs in the cell or membrane wall , this included microbial biomass from fungi such as Ganoderma lucidum which adsorbs Cu 17 , bacteria such

as Micrococcus luteus for adsorption of Sr 18 and yeast Saccharomyces cerevisiae for U , Zn , Mg , Co 19 Bioaccumulation of Ni, Cu by non pathogenic yeast Candica sp 23 Copper was accumulated by Pseudomonas sp 34 Artificial ponds and streams contain filamentatous algae and cyanobacteria can greatly reduce levels Pb ,Cu , Zn and Mn from waste waters

Table 1 Micro Organisms

WHICH CAN BIND IN THEIR BIOMASS METALS		
MICRO ORGANISM	METAL	SITE OF BINDING
Rhizopus arrhizus	U , [180 mg more than biomass]	Cell wall [Chitin]
Pencillum sp	U ,[80- 120 \ kg biomass]	Cell wall
Bacillus subtilus	Fe ,Mn ,Cu , Zn , Mg , Ni Pb	Cell wall [Peptidoglyan]
Mucor flavus	Fe (OH) ₂	Chitosan
Bacillus lichencforms	Cu, Fe III , Ni ,Mn , Au .	Cell wall [Teichoic acids]
Denitrifying bacteria	U [140 mg \ g biomass]	
Pseudomonas aeruginosa	U	Li poly saccharides
Sacchromyces cerevisiae	U, Zn , Mg	Cell wall

Cyano bacteria	Ni	
Entero bacter cloaca	U, Cr (IV) - Cr (III) -	
Ganoderm lucidum	Cu	Cell wall
Micro coccus luteus	Sr	Peptidoglyan content [Glutamic acid]
Ascophyllum nodosum	Co	
Arthro bacter ,Klebsilla	Ag 30 % of biomass	
Consortia of bacteria , Staphylococcus aureous P .maltophilia		
Citro bacter sp	Cd	Envelope bound Phosphatase at cell surface
Aspergillus niger	Cd	

The live cyano bacteria *Anabena cylindrica* , *Anabena flos-aquae* and *Nostoc* sp accumulates Ni 11 *Arthrobacter* has high Ni uptake 12 *Chlorella vulgaris* has affinity for Au ,Ag Hg 9, 33 *Oscillatoria perornata* var *attenuate* and *Scenedesmus quadricauda* var *longispuia* are able to accumulate Fe Zn , Cu and also affected by high concentrations of heavy metals In Table 2 the toxicity and uptake of heavy metals on Algae are given 6 Blue green algae 35 *Westiellopsis prolifica* has significantly reduced high levels of Na , K , Ca , Cl , SO₄²⁻ , PO₄³⁻ from Paper mill

waste waters . *Thiobacillus* species are involved in oxidation of sulphide minerals *Aspergillus niger* and *Trichoderma harzianum* oxidises sulphides of Cu , Pb , Zn , Co , Mg . *Bacillus* sp and *Vibrio* sp reduce Ferromanganese nodules 7 *Escheria coli* can be used to accumulate Ni 10 *Bacterium* such as *Enterobacter cloacae* will reduce chromate to trivalent chromium which can then be removed using other bioadsorption technology. 8

Table 2 Toxicity and Algae

UPTAKE OF HEAVY METALS TO ALGAE		
	METAL ION	MAXIMUM [ug\ml] CONCENTRATIONS THAT FAVOURED GROWTH
Oscillatoria perornata var attenuate	Fe (III),	27.56
	Zn (II),	0.833
	Cu (II)	0.0207
Scenedesmus quadricauda var longispuia	Fe (III),	20.67
	Zn (II),	0.595
	Cu (II)	0.0188
ALGAE	METAL ION	MAXIMUM UPTAKE ug\ml
Oscillatoria perornata var attenuate	Fe (III),	07.506
	Zn (II),	0.1086
	Cu (II)	0.0111
Scenedesmus quadricauda var longispuia	Fe (III),	06.449
	Zn (II),	0.1086
	Cu (II)	0.0076

Zooglea ramigera , common in natural water have been used to accumulate Ni , Fe , Cr from waste waters 19 Micro organisms involved in bio reduction and bio oxidation of minerals 7 are given in Table 3 & 4 Citrobactor sp immobilised in poly acrylamide gel scavenged large quantities of Cd 16

Most research in this area has been associated with heavy metals such as

of Cd Cu , Pb , Cr, but a broad range of micro organisms has been shown to effectively remove metals like Sr from a typical waste water containing radioactive pollutants As seen in Table 5 the distribution coefficients for Sr adsorbtion (quantity of Sr adsorbed per unit mass of dry biosorbent divided by equilibrium concentration of the metal within the bulk solution) 14

Table 3 Micro-Organisms Involved in Bio- Reduction

ORGANISMS	ORE
Vibrio sp	Ferro manganese nodules
Clostridium sp	Goethite , Haematite
Entero bactor sp	Goethite , Haematite, Cr(IV) to Cr (III)
Bacillus sp	Ferro manganese nodules
Pseudomonas sp	“ “
Chromatiphilia sp	Chromium ore
Sulphate reducers	Sulphates of Fe(II),Ni(II) ,Cu(II) ,Co(II)
Bacteria and Fungi	SeO , SeO

Table 4 Micro- Organisms Involved In Bio-Oxidation

ORGANISM	ORE
1 Thio bacillus ferro oxidans 2- 40 C pH 1.4 –4.8	Metal sulphides, Uranite Rock phosphate,
2 T . thiopains	Sulphide minerals
3 T .cupriums	--
4 T .acidophilus	Pyrite Chalcopyrites
5 Sulfolobus acido caldarium Optimum Temp 80 - 85 C	Sulphide minerals
6 S .brierleyi Optimum Temp 70 C	Sulphide minerals
7 Sulphobacillus thermo sulfido oxidans 20 –60 C	Sulphide minerals
8 Archae bacteria [un identified]	Mn (II) minerals
9 Thio microspira crunogen	Sulphur oxidizing
10 Arthro bacter Optimum Temp 88 C	Mn (II)
11 Hypho microbium sp	Mn (II)
12 Gallionella	Fe 2+ -----> FeO OH
13 Aspergillus niger	Sulphides of Cu Pb , Zn ,Co , Mg
14 Trichoderma harzianum	--
15 Leptothrix discophora	Mn (II)

Table: 5 Adsorption of Strontium By Various Micro- Organisms

Micro organism	Distribution		Coefficient	
	2 Days	7 Days	2 Days	7 Days
Rhizopus arrhizus	1,470		85,533	
Micro coccus lutes	24,943		12,010	
Anabaena	5,554		10,304	
Streptomyces viridochromogenes	4,147		3,949	
Penicillium chrysogenum	7,181		3,625	
Escherichia coli	967		1,961	
Chlorella pyrenoidosa	734		1,915	
Zooglaa ramisera	493		1,858	
Chlamydomonas reinhardtii	1,639		1,785	
Candida sp	764		1,139	

IMMOBILIZED

ORGANISMS

The immobilization of micro organisms can be defined as any technique which limits the free migration of cells There are two types of immobilization

(i) attachement , micro organisms and other cellular materials have a natural inclination to adhere to surfaces and in this way become immobilised

MICRO

(ii) entrapment where micro organisms are caught in the interstices of fibrous \porous material or physically restrained within solid or porous matrix such as a stabilized gel or a membrane To concentrate large quantities of bio mass , the microbial biomass is entrapped into gel matrix like carragenan , alginate , modified bone gel in the form of beads.

The gel is generally formed into useful biocatalyst beads by adding the cells as a suspension to the aqueous solution of the gelling material. This material is formed into droplets either by forcing it drop wise through a nozzle or orifice or by dispersing into a non interacting liquid medium. Immobilization or entrapment of micro organisms is done for various applications like ethanol fermentation, production of antibiotics and therapeutics. Micro organism *Cephalosporium acremonium* was immobilized in poly acrylamide gel, used for the production of Cephalosporin β lactam antibiotic. 27 *Citrobactor* sp immobilised in poly

acrylamide gel scavenged large quantities of Cd 16 L.K Jang et al dispensed green algae *Microcystis* in sodium alginate to form a biopolymer gel for the recovery of Cu^{2+} and Co^{2+} ions. 25 Researchers 14, 15 29 have discussed the use of various types of bioadsorbent materials plant tissue and microbial mass for immobilization in gel matrix for removal of dissolved metals A unique columnar system has been proposed for the use of crosslinked bone gel beads Significant quantities of heavy metal ions will adsorb on bone gel beads in a Fluidized - bed contactor. The loaded beads can easily be removed and regenerated 36

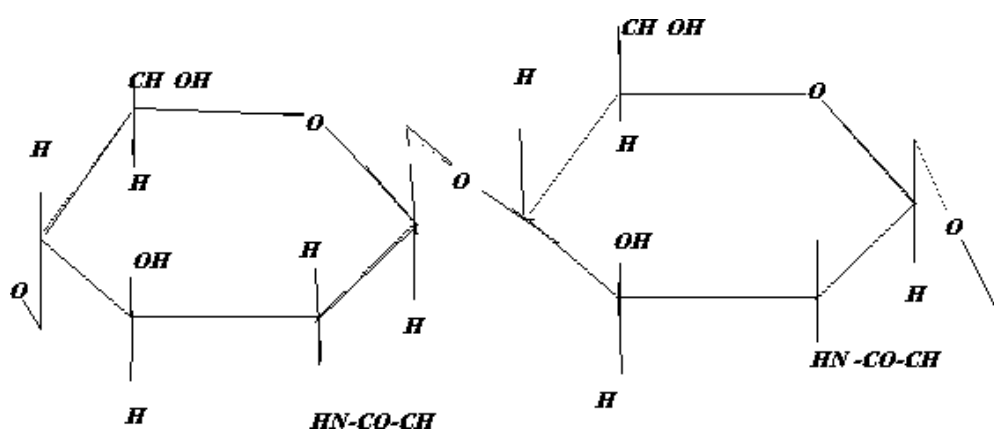


Figure: 1 Structure of CHITIN Molecule

MECHANISM OF BIO ACCUMULATION

Mechanisms by which micro organisms remove metals from solution are

- i. extracellular accumulation \ precipitation (ii) cell – surface sorption \ complexation
- ii. (iii) Intracellular accumulation ; process (ii) can occur whether the micro organism is living or dead (i) may be facilitated by micro bial viability (iii) process requires micro bial activity

Both active and passive mechanisms are employed by living cells to accumulate dissolved metals. Since, high concentration of heavy metals tend to adversely affect physiological processes, living cells that adsorb such materials must use [Table 2] mechanisms that ensure that only a limited amount of the metal actually reaches in the interior of the cell where it is most toxic. Many cellular mechanisms result in depositing the metals in cell wall or membrane. *Citrobacter* sp in presence of a suitable phosphate donor is able to immobilise large quantities of Cd, Cu, Pb, U by forming insoluble metal

phosphates on the cell surface [16]. Sulphate reducing bacteria may produce H₂S gas which will react with metal ions to form an insoluble precipitate. [14]. Phytochelatins, a class of peptides found in plants are apparently effective in metal binding. These peptides are in the range of 5-17 amino acids in length with a carboxyl terminal glycine that apparently binds metals by thiolate coordination. The Phytochelatins are generated in the living plant tissue that is exposed to metal ions thus providing a means of enhancing the metal adsorption capacity of the biomass [14].

Living cells are affected by metal toxicity and they require the addition of necessary nutrients, maintenance of specific operating conditions. On the other hand nonliving or inactive microbial biomass does not have these requirements and in many cases can be a very effective bioadsorbent for dissolved metals. The cell walls and internal materials within biomass offer abundant sites for metal complexation or micro precipitation. Biological components have amounts of polysaccharides, proteins and lipids that are replete with metal binding functional groups

including carboxylate , hydroxy , sulfate , phosphate and amino groups 14

It has been shown [Table 1] by several researchers that for some types of microbial biomass ,most of the metal adsorption occurs in the cell or membrane wall , this included microbial biomass from fungi such as *Ganoderma lucidum* which adsorbs Cu 17 , bacteria such as *Micrococcus luteus* for adsorption of Sr 18 and yeast *Saccharomyces cerevisiae* for U , Zn , Mg , Co .

Strandberg et al studied *Saccharomyces cerevisiae* for U 20 . suggested the role of phosphomannans carboxylic groups of cell wall protein of yeasts in metal binding Yeast cells 23 have been shown to accumulate heavy metals such as Cu , Ni via two distinct process .There is an initial rapid accumulation step that is metabolism and temperature independent ,involves cation binding to negatively charged sites on the cell wall .The outer mannan protein layer of the yeast cell wall as well as the inner glucan -chitin layer are important for heavy metal accumulation Second process is metabolism dependent much slower can accumulate large quantities of cations . Researchers

have shown that nine species of genus *Penicillium* had no significant difference in their ability to accumulate metal ions , this accumulation property is associated with a microbial species rather than genus . 21 Chitin , an amino polysaccharide with a molecular structure shown in Figure 1 has been postulated as the cell wall component responsible for metal coordination Tsezos and Volesky have reported results of a systematic study carried out to delineate the mechanism of biosorption of uranium by *Rhizopus arrhizus* 22. Based on electron microscopy ,X-ray energy dispersion analysis ,and infrared spectroscopic studies , they proposed three processes responsible for metal scavenging :

- i. The first process is complex formation between dissolved uranium ionic species and chitin present in the cell wall of *Rhizopus arrhizus* . Uranium coordinates to the amino nitrogen of the chitin crystallates and is retained within the cell wall of the mycelium.
- ii. The second process is adsorption of additional uranium by chitin network close to those complexes by chitin nitrogen.

- iii. The third process is hydrolysis of uranium chitin complex formed by the first process and the precipitation of the hydrolysis product on the cell wall upon which the chitin nitrogen relieved will re-engage further uranium until accumulation of the hydrolysis product inhibits the cycle.

Free radicals are highly unstable species which are trapped in the very stable cell wall matrix, this free radical has been attributed to chitin nitrogen. Muraleedharan et al. 17 have concluded that the free radical present consistently in the biosorbents is not taking part in the metal uptake.

However, the cell wall matrix which has encompassed and trapped this free radical opens up upon metal uptake, indicating its role in metal uptake. The exposed cell wall matrix thus freely interacts with the metal, resulting in high removal rates, the free radical can be used to probe for further identification of the stable cell wall components responsible for metal sorption.

Tsezos observed that at pH 4, uranium uptake by *Rhizopus arrhizus* was inhibited by competing cations, while at pH 2 no effect was evident. J.M. Tobin et al. 24 studied the effects of cation competition on the uptake of a range of different metals on the fungus *Rhizopus arrhizus*. They have reported the displacement of Ag^+ ions by UO_2^{+2} , indicating that the sites of uptake of these metals are the same. In studies where Ag^+ was in excess, the results support the presence of multiplicity of binding sites in the biomass. In the $\text{UO}_2^{+2} - \text{Ag}^+$ system, it appears that at less than saturation concentrations UO_2^{+2} ions bind preferentially to certain sites, leaving others partially unoccupied and available for Ag^+ binding even at low $[\text{Ag}^+] \setminus [\text{UO}_2^{+2}]$ ratios. As this ratio increases, Ag^+ ions may increasingly displace the UO_2^{+2} ions from the latter type sites. Conversely, at higher UO_2^{+2} concentrations UO_2^{+2} ions occupy all the sites and Ag^+ is not absorbed.

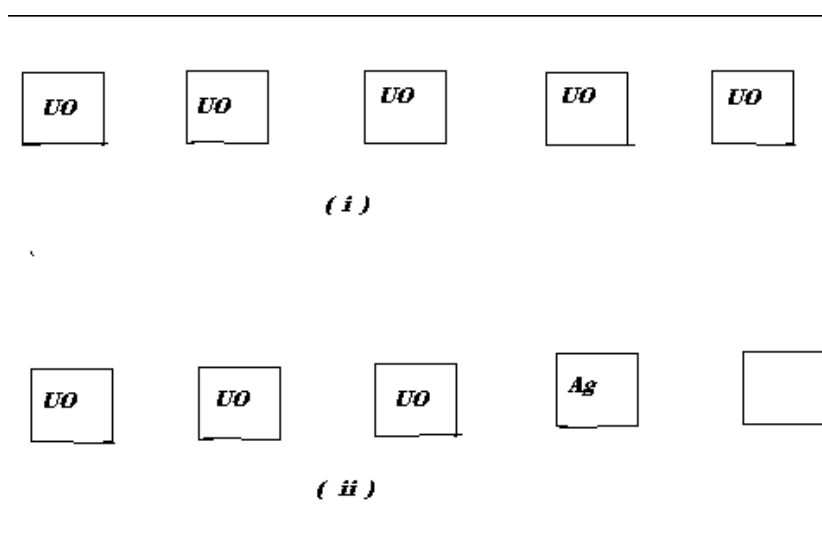
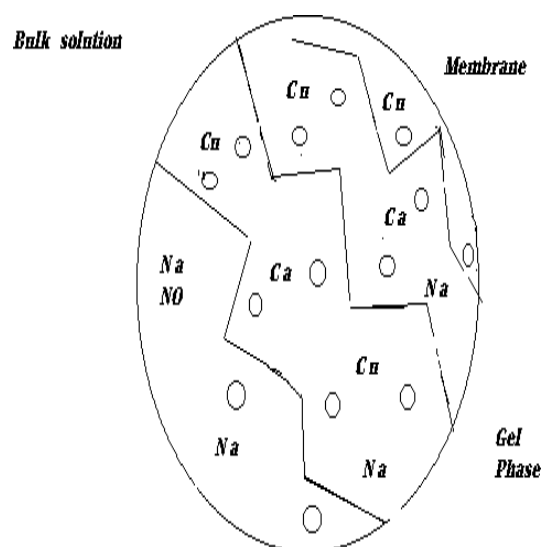


Figure 2 A Diagrammatic model of the multiplicity of uptake sites on *Rhizopus arrhizus*. [Suggested by J.M. Tobin and coworkers]

L.K Jang and coworkers dispensed green algae *Microcystis* in sodium alginate to form a biopolymer gel for the recovery of Cu^{2+} and Co^{2+} ions. The algin contained 4.356 mmol/g uronate residues or repeating monomer units. According to the egg box model of gelatin mechanism, each cavity formed in the alginate gel to trap a divalent metal ion has to involve two carboxyl groups from participating uronate residues.

Bone gelatin based gel beads are able to accumulate large quantities of Cu and Sr and thus enhance capacity of immobilized biosorbent [31].



DISCUSSION OF GEOCHEMICAL DATA

To provide an anaerobic state to support the development of anaerobic microorganisms, all oxygen and other electron acceptors (such as nitrate and

sulfate) have to be consumed. This condition can be achieved by providing substrates such as lactic acid to the saturated zone. HRC is a source of lactic acid. Anaerobic microorganisms metabolize lactic acid to carbon dioxide and water depleting electron acceptors. Subsequently, the oxidation reduction potential (ORP) shifts from a positive to a negative value. Fermentative microorganisms and the HRC-derived lactic acid, in conjunction, form pyruvic acid and acetic acid. This decomposition process provides the hydrogen required for reductive dechlorination (replacing the chlorine in the COCs with hydrogen).

Naturally occurring microorganisms capable of reductive dechlorination then use the hydrogen to progressively remove chlorine atoms from chlorinated hydrocarbon contaminants (i.e., in a simplistic sense, to convert PCE to TCE to DCE to VC to ethane or ethene) (Wiedemeier et al., 1996). Pertinent groundwater geochemical data collected from MW-3 and MW-10 since May 1, 2000 (immediately prior to the HRC injection event). Analysis of perched groundwater samples in and around the source area indicates that the saturated zone is now in an anaerobic condition, which has been promoted by the injection of HRC. ORP

measurements range from -307 millivolts (mV) in source well MW-3 to -39 mV in MW-10, and ORP has reduced significantly in MW-3 and MW-10 since the application of HRC. An ORP of less than 50 mV is required for effective reductive dechlorination, with values of less than -100 mV being optimal (Wiedemeier et al., 1996). In general, the ORP will reduce only when the electron acceptors (nitrates and sulfates, for example) are consumed. Furthermore, the presence of low quantities of methane in monitoring wells in and around the source area indicates that the aquifer is slightly methanogenic (indicative of anaerobic conditions). Elevated levels of HRC-derived metabolic acids (lactic, propionic, pyruvic, butyric, and acetic acids) in MW-3 and MW-10 indicate that these acids have been effectively released into the shallow aquifer and are being utilized to generate hydrogen within the aquifer to stimulate reductive dechlorination. Permanent gases ethane and ethene have been detected in source well MW-3. The presence of ethane and ethene indicates that the reductive dechlorination pathway has proceeded through DCE and VC to the end-product ethene and/or ethane. Geochemical sampling results indicate conclusively that the HRC injection has

been successful in stimulating the reductive dechlorination process.

CONCLUSION

The preceding discussion shows that many microbial species exhibit metal binding properties. The major potential use for immobilized cells is in biomedical area for production of antibiotics and therapeutics. Waste water treatment is one of the significant application for immobilized cells. To evaluate the suitability of a biosorbent for field application in the treatment of metal rich waste waters, it is necessary to determine the maximum sorption capacity, kinetics of sorption, recovery of the metal and physical state of the biosorbent.

However, not all of them may be suitable, it is better to select the microbial mass which can fulfil the following criteria: (i) the bio sorbent should be produced at low cost and be reusable (ii) uptake and release of the metal should be efficient and rapid (iii) regeneration of the biosorbent should be possible.

An understanding of the mechanisms by which organisms accumulate metals is important to the development of microbial processes for the concentration, removal and recovery of the metal

from aqueous solutions. A knowledge of the chemical and physiological reactions during metal uptake might enable the specification and control of process parameters to increase the rate of metal accumulation.

REFERENCES

- I. Shumate .S.E; Strandberg GW [1985] —Comprehensive Biotechnology| ed Robinson CW ;Howell J A Pergmon Press
- II. J .A .Brierly; G .M. Goyak [1986] Fundamentals and Applied io hydrometallurgy eds R W Lawrence, R M R Bronien ,H G Ebner Elsevier New York
- III. Amutha . R ; Gunasekaran .P [2000] Improved Ethanol production by mixed culture of *Saccaromyces diasticus* and *Zymomonas mobilis* from liquified casava starch . Ind . J . Microbiology, 40, 103- 107
- IV. Sawhney .A.L et .al [2000] Degradation of ground water quality due to heavy metals in industrial areas of India -A review, Ind . J. Environ. Protection 20 : 174

- V. BIS [1996] Indian Standard : Drinking Water Specification [IS 10500:1991] Bureau of Indian Standard ,New Delhi ,8
- VI. Sharad Mittal .; R.M.S .Sengar ; B.D .Kaushik [1992] Uptake and Toxicity of Heavy metals to Algae Ind . J. Microbiology 32, 51-55
- VII. Pawan Sharma ; Ajit Verma [1991] Microbial reclamation of metals from ores and industrial waste waters . Ind. J. Microbiology 31 1-26
- VIII. K. Komori; A .Rivas; K . Toda ; H . Ohtak [1990] Biological removal of toxic Cr using Enterobacter cloaco strain Biotech & Bioengg , 35, 951 – 54
- IX. Aksu .Z ; Kutsal .T [1990] A comparative study for biosorption characteristics of heavy metal ions with Chlorella. vulgaris Environ Tech nol 11, 979-987
- X. Ranul Krihna swamy; David B Wilson [2000] Construction and characterization of an Escheria coli genetically engineered for Ni accumulation Appl .Environ Microb 66 , 5383 - 5386
- XI. Corder S.L; Reeves .M [1994] Biosorption of Ni in complex aqueous streams by Cyano bacteria Appl. Biochem. Biotechnol 45\46 , 847 - 859
- XII. Veglio . F ; Beol/ F; Gasbarro. A [1998] Biosorption of toxic heavy metal using free Arthrobacter sp Process Biochem 32, 99-105
- XIII. L .E .Macaskie and A.C.R .Dean [1989] Advances in Biotechnological processes Vol 12 eds A . Mizrani , AR Liss New York
- XIV. Charles .D .Scott and James N. Petersen [1992] Immobilized biosobents for dissolved metals Separation and Purification Technology eds Norman Li, Joseph M Calo, Marcel Dekker Inc New York. Basel .Hong Kong
- XV. Charles .D .Scott [1987] Immobilised cells A review of recent literature Enzyme .Micro. Techno . , 9 , 66-73

-
- XVI. Macaskie , M. ; Dean .A.C.R. , [1986] Cd accumulation by immobilised cells of *Citrobacter* sp Biotech & Bioengg , 28 ,1358 –65
- XVII. Muraleedharan, T.R; C. Venkobacchar [1990] Mechanism of biosorption of Cu II by *Ganoderm luciderm* . Biotech & Bioengg [1989], 35, 320 – 325
- XVIII. B .D. Faison; C. A . Cancel ; S.N. Lewis ; H.I. Adler [1990] Appl . Environ Microbiol 56 ,36-49
- XIX. Norberg . A B ; Pearson . H ; [1984] Accumulation of heavy metals by *Zooglea ramigera* Biotech & Bioengg 26 , 239
- XX. Strand berg G.W. ; Shumate S. E ; J. R . Parrot [1981] Microbial cells as Biosorbents for Heavy metals : Accumulation by *Saccharomyces cerevisiae* ; *Pseudomonas aeruginosa* Appl . Environ Microbiol , 41 ,237 .
- XXI. Niu H; Xu . X .S ;Wang J .H [1993] Removal of Lead by *Penicillium* biomass Biotech & Bioengg 42 ,785-787
- XXII. Tsezos ; Volesky [1981] Mechanism of biosorption of Uranium by *Rhizopus arrhizus* Biotech & Bioengg , 23 , 583
- XXIII. G. Donmez ; Z .Aksu [2001] Bio accumulation of Cu and Ni by the non adapted and adapted growing *Candida* sp Wat Res ,35 , 1425 – 1434
- XXIV. Tobin . J.M ; Cooper D .G ; Neufeld .R .J . [1988] The effects of cation competition on metal adsorption by *Rhizopus arrhizus* Biotech & Bioengg 31, 282 – 286
- XXV. Jang L K ; Lopez S L; Eastman ;Pryfogie .P [1991] Recovery of copper and cobalt by Biopolymer Gels Bio technol & Bioengg ,37 ,266-73
- XXVI. Wulf .C [1984] Biotechnology : A Textbook of Industrial Microbiology [English trans]
- XXVII. Saurabh Gupta; Vinod Bihari ; S. C. Agarwal et. al [2000] studies on free and immobilised cells of *Cephalosporium acremonium* gel for the production of
-

- Cephalosporin [β lactam antibiotic] Ind J . Microbiology 40 , 141 -144
- XXVIII. Macaskie, M ; J. M . Wates ; Dean .A.C.R. , [1987] , Cadmium Accumulation by Citrobacter immobilised on gel and solid supports Biotech & Bioengg 30 , 66
- XXIX. Good man G.T; Roberts T . M; [1971] Plants and Soils as indicators of metals in air . Nature 231 , 287
- XXX. Watson J.S ;Scott C.D ; Fasison B D ; [1990] Emerging Technologies in Hazardous Waste Management ACS Symposium Series no 422 [DW Tedder ; FG Pohland] American Chemical Society Washington
- XXXI. Petersen ,J.N ;Scott C.D ; et al [1990] Accumulation of Cu on to Modified bone gelatin beads Bio technol .Techniqes ,4 ,435 – 440
- XXXII. Yi-Tin Wang ; Evans ,M. Chirwa [2000] Cr (VI) reduction in continuous flow coculture Bio reactor . J . Environ . Engg . 126 , 300-306
- XXXIII. Frencessa Podda ; Paola Zudda et al [2000] Heavy metal co precipitation with Hydro zincite from mine waters caused by photosynthetic micro organisms App . Environ . Micro biology ,11, 5092 -5098
- XXXIV. M . Vasanth; P . Lakshmana perumala samy ; [2000] Copper accumulation by Pseudomonas sp ; IJEP 20 , 280-283
- XXXV. A .K .Dash ; P .C. Misra [1999] Role of Blue green algae Westiellopsis prolifica in reducing pollution load from paper mill waste water IJEP ,19 ,1-5
- XXXVI. J.N. Petersen ; B.H . Davidson ;C.D. Scott ; SL Blankinship [19991] Size changes associated with metal adsorbtion onto Modified bone gelatin beads Biotech & Bioengg 38 , 923-928
- XXXVII. Jyotsna Lal , Remediation of toxic metals: biosorption-a viable

technology V.S. Magar and M.E. Kelley (Eds.), In Situ and On-Site Bioremediation—2003.

XXXVIII. Proceedings of the Seventh International In Situ and On-Site Bioremediation Symposium (Orlando, FL; June 2003). ISBN 1-57477-139-6, published by Battelle Press, Columbus, OH, www.battelle.org/bookstore.