

Aquatic Bioremediation of Metals

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Abstract: Heavy metals have been used by humans for thousands of years. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries, though emissions have declined in most developed countries over the last 100 years. Excessive levels of heavy metals can be damaging to the organism. Some of them are dangerous to health or environment (e.g. mercury, cadmium, lead, chromium), some may cause corrosion (e.g. zinc, lead), some are harmful in other ways (e.g. arsenic may pollute catalysts). Treating the polluted environment with these bioremediators is the most efficient and least costly method. As far as health effects of metals on aquatic flora and fauna is concerned, there are two categories of metals; one, which are nutritionally important but exceeding concentrations tend to be toxic and other category of metals have no role in the physiology of body and are toxic even at low levels. Cu, Zn, Fe, Ni, Co, Se, Mo and Cr are the metals belonging to the first category of metals having biological roles, while Ag, Al, Cd, Pb, Hg, As, Sr, and U belong to the second category. There are other toxic metals too but these are the major elements which have aroused lot of public sentiments and have been extensively studied by the researchers. Maintaining the optimal levels of the elements mentioned by pumping and treating is not only expensive but also less effective. Therefore, bioremediation is a viable option but the toxicity of contaminants to microorganisms and plants to be used for such purpose have to be considered. Another factor to be considered while using biological agents for cleanup is the bioavailability of the metals. Water pH affects the speciation and bioavailability to a considerable level, for example, at pH 7, 6811M of cobalt is predicted to exist in the free ionic form while only 4.1 iiM of nickel remains in this form. Hence, using a buffering system to maintain pH or using lime to increase pH or alum to reduce pH is advisable.

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Introduction

Our environment, and especially the aquatic environment, has been under focus within the past decades because of the large amount of chemical substances released into it. Thousands of synthetic chemical compounds are currently registered for use in industry and agriculture, and thousands of tons of these are produced annually. In addition, several tons of more unintended byproducts accompany these synthetic compounds. Regardless of the source or original intended use, substantial amounts of these chemicals end up in the aquatic environment due to physico-chemical, hydrologic and atmospheric processes. Organic micropollutants such as metals and pesticides will only cause detrimental effects to organisms if they are taken up by the organism and can reach a target site where they can do harm (Escher & Hermens, 2004; Schwarzenbach *et al.*, 2006). The processes of uptake, biotransformation, and elimination, also termed bioaccumulation or toxicokinetics, modify the concentration of organic chemicals in organisms, and kinetic rate constant models of these processes quantify and yield the time

course of internal concentrations (Landrum *et al.*, 1992; Mackay & Fraser, 2000; Hendriks *et al.*, 2001). Bioaccumulation and biotransformation are key factors modifying toxicity (McCarty & Mackay, 1993; Escher & Hermens, 2002; Meador *et al.*, 2008) and bioaccumulation itself is one of the assessment end points in risk assessment of chemicals (van Leeuwen *et al.*, 2007). Bioaccumulation based on total radioactivity measurements of ¹⁴C-labeled compounds varies greatly among species (Rubach *et al.*, 2010), and compounds (Ashauer *et al.*, 2010), but the causes remain partially unresolved because the contribution of biotransformation cannot be quantified with these methods.

Biotransformation in freshwater arthropods has been shown to greatly modify internal concentrations of organic chemicals for various biological species and chemical compounds (Akkanen & Kukkonen, 2003; Nuutinen, *et al.*, 2003; Ikenaka *et al.*, 2006; Richter & Nagel, 2007). Differentiation between parent and metabolites increased accuracy of bioaccumulation parameters compared to total ¹⁴C measurements. Biotransformation dominated toxicokinetics and

strongly affected internal concentrations of parent compounds and metabolites. Many metabolites reached higher internal concentrations than their parents, characterized by large metabolite enrichment factors. (Roman *et al.*, 2012).

Microbial Bioremediation of Metals

Living system requires special transport and handling mechanisms to keep them from toxic metals (Rehman *et al.*, 2008). The toxicity occurs in humans due to environmental pollution via soil or water contamination or due to occupational exposure. Some of these metals are useful to us in low concentrations but are highly toxic in higher concentrations (Ge *et al.*, 2009).

Bioremediation processes are very attractive in comparison with physicochemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and sorption for heavy metal removal techniques because they can have lower cost and higher efficiency at low metal concentrations (Bogdanova *et al.*, 1992; Gadd and White, 1993).

There are a number of bio materials that can be used to remove metal from waste water, such as molds, yeasts, bacteria, and seaweeds (Vieira and Volesky, 2000; Waisberg *et al.*, 2003). The ability of microbial stains to grow in the presence of heavy metals would be helpful in the waste water treatment where microorganisms are directly involved in the decomposition of organic matter in biological processes for waste water treatment (Prasenjit and Sumathi, 2005; Munoz *et al.*, 2006), because often the inhibitory effect of heavy metals is a common phenomenon that occurs in the biological treatment of waste water and sewage (Filali *et al.*, 2000). Mechanisms of metal resistance in microbes include precipitation of metals as phosphates, carbonates and/or sulfides; volatilization via methylation or ethylation; physical exclusion of electronegative components in membranes and extra cellular polymeric substances (EPS); energy-dependent metal efflux systems; and intra cellular sequestration with low molecular weight, cysteine-rich proteins (Gadd, 1990; Silver, 1996).

There are some yeast like *Rhodotorula mucilaginosa* which is efficient in lead bioadsorption (Chatterjee *et al.*, 2011). Hexavalent chromium (Cr (VI)) and trivalent chromium (Cr (III)) are the most prevalent species of chromium in the natural environment (Chung *et al.*, 2006).

These identified heavy metal resistant bacteria could be useful for the bioremediation of heavy metal contaminated sewage and waste water. Biotechnological approaches are recommended for extraction of metal forms can be grown in ponds where effluents (rich in heavy metals) are discharged. The microbes will extract the heavy metals and sequester

them inside their cell membranes (Sabyasachi *et al.*, 2012).

Organic compounds are detoxified or removed by the microbes by converting them into harmless water, carbon dioxide and other volatile gases but metals are just transformed by the microbes to less soluble or bioavailable form (Lovley and Coates, 1997). This generally is made possible by converting inorganic forms into organic forms by redox conversions and coupling in respiratory pathways (Lovley and Coates, 1997; Tebo *et al.* 1997). Microbes have capabilities to immobilize metals by bioaccumulation and biosorption. The process of active uptake of metals by bacteria is termed as bioaccumulation while passive uptake is called biosorption (Unz and Shuttleworth, 1996).

Bioaccumulation is an interaction between the microorganism and the metal ion in relation to metabolic pathways. Metal ions required for biological functions are actively taken up by the microbes and converted into organic forms. Accumulation of radio nucleotides through the pathways of their stable isotopes or of chemical homologous elements can be considered as bioaccumulation. One such example is of accumulation of cesium by potassium channels (Avery, 1995).

Biosorption is simply a physicochemical process of accumulating metal species by sorption, surface complexation, ion exchange and entrapment (Gadd, 2004; Le Cloirec and Andre A, 2005). The biosorption qualities of *Saccharomyces cerevisiae* have a special mention. This is a by-product of fermentation and brewery industry and is hence quite cost effective to treat water bodies dissolving certain metals (Unz and Shuttleworth, 1996; Lovley and Coates, 1997). In fact, the dehydrated yeast *Candida utilis* demonstrated improved chromium sorption (Simmons *et al.*, 1995). *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Escherichia coli* have also been attributed with biosorption properties of metals like chromium, copper, nickel and cobalt (Churchill and Churchill, 1995).

Peptidoglycan carboxylic groups of the Gram-positive bacteria phosphate groups in gram-negative bacteria (Beveridge and Doyle, 1989; Schultze-Lam *et al.*, 1996; McLean, *et al.*, 2002), chitin in fungal cell walls, chitosan and other chitin derivatives (Simmons *et al.*, 1995) have been attributed with metal adsorptive properties. Fungal phenolic polymers and melanins possess many potential metal binding sites with oxygen-containing groups such as carboxyl, phenolic and alcoholic hydroxyl, carbonyl and methoxyl groups (Gadd and White, 1993). Due to its cost effectiveness and easy availability of raw material, this technology is being widely appreciated and accepted. This method not only remediates effluents and water in the water bodies but recovery of soluble metals is also possible (Gavrilescu, 2004). This technology is being used as

immobilized living biomass mainly in the form of bacterial biofilms on inert supports in a variety of bioreactor configurations such as rotating biological contactors, fixed bed reactors, trickle filters, fluidized beds and air-lift bioreactors (Macaskie and Dean, 1989; Gadd and White, 1993; Schiewer and Volesky, 2000; Gadd, 2001).

Not only microorganisms but also other biosorbent substrates like tamarind shell, rice husk, cottonseed hull, corncobs, almond and peanut hulls have been shown to remove heavy metal ions (Johnson *et al.* 2002; Shen and Duvnjak, 2005; Kumar and Bandyopadhyay, 2006; Rao Popuri *et al.*, 2007).

Bioremediation of certain metals by microbes can be affected by mobilization (Gadd, 2004). Microorganisms can affect dissolution of metals by leaching, chelation while they are metabolized by action of siderophores. Once leached or chelated, the metals become unavailable for biological functions in water. Processes such as methylation of certain metals can also volatilize them facilitating the removal from water. In general, mobilization is affected by various methods like chemoorganotrophic leaching, autotrophic leaching, siderophores, biomethylation and redox transformations.

Chemoorganotrophic Leaching (Heterotrophic)

Microorganisms maintain their charge balance through H⁺ efflux through H⁺-ATPase pumps so as to neutralize metabolic carbon dioxide. This proton efflux causes the microenvironment surrounding the biofilm to get acidic and lead to metal release from the soil (Gadd, 2004). There is a dynamic equilibrium between the metals adsorbed in the soil and metal ions dissolved in water. Acidification of water shifts the equilibrium and causes the release of ions into water from soil. Microbes also release some organic acids that are formed during metabolic processes which supply water with protons and metal-complexing anions (Burgstaller and Schinner, 1993; Gadd, 1999). Citrate and oxalate ions have the ability to form complexes with a wide variety of elements (Gadd, 2001).

Metal citrate ions are very stable and hard to break thereby removing many metals from water (Francis, *et al.*, 1992). Oxalate forms stable complexes with Al, Li, Mn and Fe (Strasser, *et al.*, 1994). Acid producing fungi is a better approach for bioremediation as they can tolerate a wider fluctuation in pH than bacteria (Burgstaller and Schirmer, 1993). Some of the acid producing fungi are *Yarrowia lipolytica* (citric), *Mucor* spp. (fumaric and gluconic), *Rhizopus* spp. (lactic, fumaric and gluconic), *Aspergillus niger* (citric, oxalic, gluconic), *Aspergillus* spp. (citric, tartaric, malic, α -ketoglutaric, itaconic, aconitic), *Penicillium* spp. (citric, tartaric, malic, α -ketoglutaric, gluconic) and *Sclerotium rolfsii*

(malic) (Burgstaller and Schirmer, 1993).

Autotrophic Leaching

Some bacteria oxidize ferrous ions or reduce sulphur so as to obtain energy. Such a chemical change results in solubilization of metals as the end product is usually Fe(III) or H₂SO₄ which are soluble (Rawlings, 1997; Schippers and Sand, 1999). Such bacteria are called chemoautotrophic, chemolithotrophic and acidophilic bacteria which fix carbon by obtaining energy from such chemical reactions (Gadd, 2001). Organic acids act as carbon substrates which are oxidized completely to CO₂ or to some other organic intermediates. The ATPs are produced through electron transport chain with sulphur as terminal electron acceptor which is reduced to sulphide (Hansen, 1993; Peck Jr, 1993). The sulphide so generated forms metal sulphide and gets precipitated. Sulphur reducing bacteria are essentially anaerobic (White, *et al.*, 1998). Some of the bacterial species used in bioremediation are: sulphur oxidizing *Thiobacillus thiooxidans*, Iron and sulphur oxidizing *T. ferrooxidans*, Iron oxidizing *Leptospirillum ferrooxidans* (Ewart and Hughes, 1991; Bosecker, 2006). Autotrophic reduction of sulphuric acid causes metal to solubilize from sewage and sludge (White *et al.*, 1998). Both sulphate and iron reducing bacteria have been used to treat the mine waste water in artificial wetlands (Harnmack and Edenborn, 1992).

Siderophores

Siderophores (from the Greek: "iron carriers") are defined as relatively low molecular weight, ferric ion specific chelating agents released by bacteria and fungi growing under low iron stress (Neilands, 1995). Bacteria producing siderophores have been used to treat metal contaminated sandy soils.

Alcaligenes eutrophus is used to solubilize metal from contaminated soils which can be removed by biosorption techniques as mentioned earlier. This method has been used effectively for reduction of Cd, Zn and Pb from contaminated soils (Diels, *et al.*, 1999).

Biomethylation

Microbes can methylate metals so as to yield volatile derivatives such as dimethylselenide and trimethylarsine (Brady, *et al.*, 1996; Dungan and Frankenberger, 1999; Gadd, 1993). Selenium methylation has been widely discussed and the probable mechanism is by transfer of methyl group to selenium by S-adenosyl methionine system (Gadd, 1993). Methylation of Hg, As, Se, Sn, Te and Pb by bacteria has been discussed by Gadd, (2004).

Bioremediation of Petroleum Contaminants

Petroleum and its components drive the present civilization and are the major energy sources. But, where there is use there is a chance for abuse too. Hence, being the prime source of energy, petroleum is also a major environmental pollutant. Since 1992, there

have been 21 major oil spills causing huge economic and immeasurable non-economic losses (Cedre, 2012. <http://www.endgame.org/oilspills.htm>). Petroleum contamination is quite harmful for the higher organisms (Lyons, et al., 1999; Janjua et al, 2006; Cheong et al., 2011) but it is fortunate that microorganisms can thrive on it and assimilate (Atlas, 1995; de Oliveira et al., 2012). Soon after major oil spill incident is reported, the efforts are concentrated at physical removal of oil but they rarely achieve complete clean up. As per Office of Technology Assessment (OTA; USA), such mechanical methods are efficient at removing no more than 10-15 per cent of oil after a major spill. In such cases, bioremediation has a major role to play in neutralizing the harmful effects of oil in the open environment. The basic principle is to use organisms that can use petroleum as carbon source and hence, break them down to harmless end products.

Like any other technology that uses biological agents, success of bioremediation of petroleum contamination also depends on establishing and maintaining conditions that favour proliferation of petroleum scavenging microorganisms. Bioaugmentation and Biostimulation are the two main approaches followed in this regard. Bioaugmentation refers to inoculating the affected area with degrading microorganisms while biostimulation would require favouring growth of such microorganism through addition of nutrients or by providing other growth-limiting substrates (e.g. oxygen, surf washing etc.). As petroleum is hydrophobic in nature, its bioavailability becomes a major constraint in the process of bioremediation. Use of biosurfactants is a common approach to increase the bioavailability. Requirements of a successful bioremediation process of petroleum contamination are as follows:

The very first requirement is the availability of microorganisms that can utilize oil as a metabolic substrate. Finding and transplanting such an organism to the site of contamination would be the first approach. Jones et al. (1983) reported for the first time biodegraded petroleum byproducts in marine sediments (Das and Chandran, 2010). Enzymatic degradation of petroleum can be achieved by bacteria, algae or fungi. Different organisms have varied degradation capabilities and act on different substrates. As petroleum is an assortment of different components, it is advisable to use a cocktail of organisms to effect remediation. Bacteria are the most efficient of all organisms that can degrade hydrocarbons (Rahman et al., 2003; Brooijmans 2009. Floodgate, (1984) mentioned 25 genera of hydrocarbon degrading bacteria" and 25 genera of hydrocarbon degrading fungi which were isolated from marine environment.

Some of the bacteria recognized as hydrocarbon

degrading are *Arthrobacter*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, *Rhodococcus*, *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*, *Bacillus sp.*, *Alcaligenes sp.*, *Acinetobacter lwoffii*, *Flavobacterium sp.*, *Micrococcus roseus*, and *Corynebacterium sp.* (Jones et al., 1983; Adebuseye et al., 2007). Some fungal genera utilized for this purpose are *A-inorphoteca*, *Neosartorya*, *Talaromyces*, *Graphium*, *Candida lipolytica*, *Yarrowia*, *Pichia*, *Aspergillus*, *Cephalosporium*, *Rhodotorula mucilaginosa*, *Geotrichum sp*, *Trichosporon mucoides* and *Penicillium* (Boguslawska-Was and Dabrowski, 2001; Chaillan et al., 2004; Singh, 2006). Alter the potential scavengers have been identified, the conditions for their survival and proliferation have to be ascertained.

Among the physical factors temperature is most important one determining the survival of microorganisms and composition of the hydrocarbons (Das and Chandran, 2010). At higher temperature some fraction may get evaporated and the oil would tend to spread while in low temperature the slick would be more viscous and retention of otherwise volatile fractions thereby delaying the bioremediation process. For freshwater bioremediation process 20-30°C is the ideal temperature while for marine 15-20°C is recommended. For high molecular weight polycyclic hydrocarbons, which are otherwise difficult to degrade, higher temperatures may be required (Bartha and Bossert, 1984; Cooney, 1984). As temperature has effect on enzymatic turnover rate "Q¹⁰" hence, higher temperature would favour bioremediation. It was reported that the rate of hydrocarbon remediation was maximum in the range of 30-40°C in general and above this, the membrane toxicity effect of hydrocarbons was found to inhibit the survival of microorganisms (Bartha and Bossert, 1984). As there is a close relationship between temperature and oil bioremediation, it is easy to understand why an oil leak disaster would be dangerous in polar regions.

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