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Microalgal bioremediation : Current practices and perspectives

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Abstract

During last two decades, extensive attention has been paid on the management of environmental pollution caused by hazardous materials. A number of methods has been developed for removal of such substances like precipitation, evaporation, ion-exchange etc. However these methods have several disadvantages. This review highlights the alternative biological agent abundantly present in nature i.e Microalgae as a potential sink for removal of such toxic substances from the surrounding. Microalgal biomass has also been used to remove heavy metal and in wastewater treatment facilities, the microalgae can be used to reduce the amount of toxic chemicals needed to clean and purify water The microalgae either accumulate or adsorb or metabolise these noxious elements into substantial level.

Key words: Biosorption, heavymetal, microalgae, phyco-remediation, toxicity.

Introduction

The term microalgae refers to the aquatic microscopic plants (organisms with chlorophyll *a* and a thallus not differentiated into root, stem and leaf), and the oxygenic photosynthetic bacteria, that is, the cyanobacteria, formerly known as Cyanophyceae (Tomaselli 2004). Micro-algae are microscopic photosynthetic organisms that are found in both marine and freshwater environments. Their photosynthetic mechanism is similar to land based plants, but due to a simple cellular structure, and submerged in an aqueous environment where they have efficient access to water, CO_2 and other nutrients, they are generally more efficient in converting solar energy into biomass. They are the source of oxygen and the first ring of the food chains in aquatic systems. Many microalgae species may accumulate extracellular polysaccharides, such as a gelatinous mass enclosing their cells, which are called envelopes, sheaths or capsules. Microalgae contain lipids and fatty acids as membrane components, storage products, metabolites and sources of energy. In terms of biomass, microalgae form the world's largest group of primary producers.

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The microalgae which exist in the freshwater environment and the oceans are important in global ecology, extremely efficient, and taxonomically diverse (Brown and Zeiler 1993). These microalgae (phytoplankton) in the oceans live in an environment which comprises more than 70% of the earth's surface and is responsible for at least 32% of global photosynthesis (Whittaker 1975). Through 500 million years of evolution and within this large competitive environment, these microalgae have developed a myriad of polymers which can scavenge the metals of interest, many of which are essential nutrients utilized at low concentrations in microalgal metabolism. In fact, these polymeric materials in the particulate fraction of the world's oceans and lakes regulate the distribution of heavy metal ions in natural waters. The propensity for sequestration of heavy metals by cell walls of these unique and diverse microbes makes them an ideal source of the complex multifunctional polymers which can be used to sequester many different metals through adsorption or ion-exchange processes. This tendency to absorb toxic metals is a problem in microalgal biomass cultures destined for food use. Microalgae are so efficient at scavenging of metals from effluent water, from contaminants in nutrients, or from atmospheric deposition into open ponds, that the biomass produced sometimes can contain amounts at the upper limit of metal content for food use (Kajan et al. 1992). In natural environments, organisms living in chronically polluted sites are exposed to low concentrations of heavy metals for long periods. In the other cases, the organism may be abruptly exposed to high levels of metals upon the outfall of a pollutant in coastal waters. The storage of metals by cellular detoxifying mechanism makes them available for assimilation by the biota and bio-magnification along the aquatic food chains. The heavy metals may accumulate in food crops, vegetables and fruit plants, thus entering in food chain. Their presence in the atmosphere, soil and water, even in traces, can cause serious problems to all organisms. Once in the food chain, these heavy metals can profoundly disrupt biological processes and pose a serious threat to human health (Krishnan et al. 2004). Heavy metal contamination of agricultural soils has become a serious issue in crop production and human health in many developed countries of the World. Heavy metals are among the conservative pollutants that are not subjected to bacterial attack or other break down or degradation process and are permanent additions to the environment. Subsequently, their concentrations often exceed the permissible levels normally found in soil, waterways and sediments.

Techniques presently in existence for removal of heavy metals from contaminated waters include: reverse osmosis, electro dialysis, ultra filtration, ion-exchange, chemical precipitation, phytoremediation etc. However, all these methods have disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that required careful disposal (Ahalya et al. 2003). With increasing environmental awareness and legal constraints being imposed on discharge of effluents, a need for cost effective alternative technologies are essential. In this endeavour, microbial biomass has emerged as an option for developing economic and eco-friendly waste water treatment process. In addition to providing nutrition, microalgae can be explored for a variety of other uses such as fertilizer and pollution control. Certain species of algae can be land applied for use as an organic fertilizer, either in its raw or semi-decomposed form (Riesing 2006).

Biosorption and bioaccumulation processes involve metal ions binding by either non-living (biosorption) or living (bioaccumulation) biomass in addition to environmental factors influencing such remediation as mentioned in fig. 1.

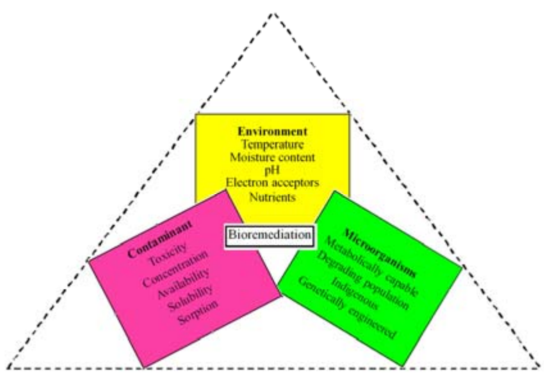

Figure 1. Main factors of influence in bioremediation process (Bitton 2005)

These techniques can be used in either bioremoval or biobinding methods. The first one finds an application in wastewater treatment whereas the second technique involves the production of biological mineral feed supplements.In this context microalgae have several useful characteristics which enable them to be used in a variety of ways. The high lipid, carbohydrate, and protein contents of many microalgal species have driven research in a wide spectrum of uses.

These vary from food products to biofuels to use for phycoremediation. The exponential growth of microalgae under ideal nutrient loads has lead to the idea of microalgae as a phycoremediation tool (Olguin 2003), considering that the nutrients that microalgae needs are often a waste product, such as nitrogen and phosphorous. Microalgae, when grown using photosynthesis, also need carbon dioxide, which is often a waste stream from factories. Microalgal biomass can also be used as a biosorpent to clean contaminated waste streams (Abdelwahab et al. 2006) Absorption of metal ions from aqueous system by microalgae has spent much attention for wastewater treatment, which may reduce the metal ion concentration significantly. The basic procedure of bioabsorption for meal recovery involves several steps like selection of biomass (microalgae), pre-treatment, immobilization etc (Fig 2). The Biosorption process utilizes the ability of biological materials to accumulate heavy metals from wastewater by either metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux 1992). Application of biosorbents / biomass from various microbial sources, moss, aquatic plants and leaf-based adsorbents was reported by various investigators (Chang et al. 1997; Niu et al. 1993; King et al. 2007) with the aim of finding more efficient and cost-effective metal-removal biosorbent. Among them, microalgae have proved to possess high metal binding capacities (Schiewer et al. 2000) due to the presence of polysaccharides, proteins or lipid on the surface of their cell walls containing some functional groups such as amino, hydroxyl, carboxyl and sulphate, which can act as binding sites for metals (Yu et al. 1999). Of the many types of biosorbents recently investigated for their ability to sequester heavy metals, microalgal biomass has proven to be highly effective as well as reliable and predictable in the removal of heavy metals from aqueous solutions (Volesky and Holan 1995; Schiewer and Volesky 2000).

On the other hand the bioaccumulation process refers to the accumulation of substances, such as pesticides, or other organic chemicals in an organism and is defined as the transfer of organic or inorganic pollutants into the interior of living cells (Barron 1995). In this process, also nutrients can be removed from treated effluents (nitrates, phosphates, sulfates, organic and inorganic carbon compounds). For instance, artificial ponds containing photosynthetic microorganisms can be used to treat mining leachates with the use of controlled eutrophication (Ehrlich 1986). Bioaccumulation occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost. Thus, the longer the biological half-life of the substance the greater the risk of chronic poisoning, even if environmental levels of the toxin are not very high (Bryan et al. 1979).

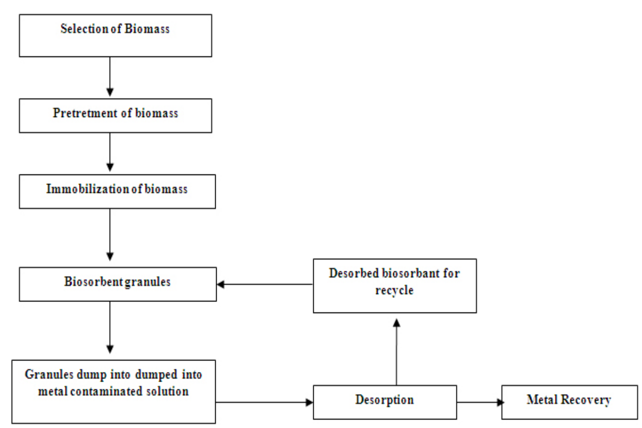

Figure -2 Schematic representation of biosorption procedure

Bioremediation of Heavy metals

The presence of heavy metals in the environment is a major concern because of their toxicity to flora and fauna. Moreover, recovery of heavy metals from industrial waste streams is becoming increasingly important as society realises the necessity for recycling and conservation of essential metals. Industrial wastes, geo-chemical structure and mining of metals create a potential source of heavy metal pollution in the aquatic environment (Gumgum et al. 1994). Heavy metals are major pollutants in marine, ground, industrial and even treated wastewaters. Rapid urbanization, industrialization, fertilizer and pesticide use has resulted in heavy metal pollution of land and water resources. The increasing load of heavy metals has caused imbalance in aquatic ecosystems and the biota growing under such habitats accumulate high amounts of heavy metals (Cu, Zn, Cd, Cr and Ni etc.) which in turn, are being assimilated and transferred within food chains by the process of magnification (Pergent et al. 1999). The toxic metals can be broadly divided into two groups. The first group consists of metals that are essential as nutritional requirements at trace amount for many organisms but are toxic when present in greater amounts. This group includes As, Cr, Co, Cu, Ni, Se, Va and Zn. The second group includes Pb, Hg, Cd, Ur, Ag and Be, all of them are highly poisonous and are not known to have any nutritional value (Inthorn 2001). Heavy metals are not biodegradable and tend to accumulate in living organisms. To curtail heavy metal pollution problems, many processes have been developed for the treatment of metal containing waste waters. Heavy metals showed to affect a wide range of microalgal cellular activities including cell viability and membrane structure and properties**.** Many chemical contaminants, including organochlorine compounds, herbicides, domestic and municipal wastes, petroleum products and heavy metals are now recognized to have adverse affects on ocean environments, even when released at low levels (Haynes and Johnson 2000). The need for economical, effective and safe methods for removing heavy metals from waste waters has resulted in the search for unconventional materials that may be useful in reducing the levels of heavy metals in the environment. In this light, biological materials have emerged as an ecofriendly and economic option. Biosorption, which uses the ability of biological materials to remove and accumulate heavy metals from aqueous solutions, has received considerable attention in recent years because of few advantages compared to traditional methods. Biosorption uses cheaper materials such as naturally abundant microalgae or by products of fermentation industries as biosorbents (Davis 2000). The ability of microalgae to absorb metals has been recognized for many years.(Table 1a and Table 1b). Algae possess the ability to take up toxic heavy metals from the environment, resulting in higher concentrations than those in the surrounding water (Megharaja et al. 2003). Microalgae, related eukaryotic photosynthetic organisms, and some fungi have preferentially developed the production of peptides capable to bind heavy metals. These molecules, as organometallic complexes, are further partitioned inside vacuoles to facilitate appropriate control of the cytoplasmic concentration of heavy metal ions, thus preventing or neutralizing their potential toxic effect (Cobbett and Goldsbrough 2002). In contrast to this mechanism used by eukaryotes, prokaryotic cells employ ATP consuming efflux of heavy metals or enzymatic change of speciation to achieve detoxification**.** Microalgae are superior in remediation processes as a wide range of toxic and other wastes can be treated with algae and they are non pathogenic. The risk of accidental release of pollutants into the atmosphere causing health safety and environmental problems are avoided when microalgae are employed for remediation. Microalgae utilizes the wastes as nutritional sources and enzymatically degrade the pollutants. The xenobiotics and heavy metals are known to be detoxified/ transformed/or volatilized by microalgal metabolism. They have the ability to take up various kinds of nutrients like nitrogen and phosphorus (Ayse et al. 2005).

Bioaccumulation studies reveal the accumulation of the contaminant in the organism via uptake of food or water containing the contaminant. In marine environments, the sedimentation of microalgae during algal blooms has been associated with substantial (20–75%) reductions in the level of suspended heavy metals, as well as heavy metal deposition (Luoma et al. 1998). Absorption of metal ions from aqueous system by microalgae has spent much attention for wastewater treatment, which may reduce the metal ion concentration significantly. The idea of the application of microalgae in bioaccumulation of heavy metal ions was proposed for the first time by Oswald and Gootas in the year 1957 , but this topic has gained attention only recently (Oswald 1988; Doshi et al. 2007).

Table 1(a): Microalgae used for bioremediation of heavy metals.

Microalgae	Authors Name	Metal studied
Tetraselmis chuii	Ayşe et al. 2005	Cu
Spirulina (Arthrospira) platensis	Arunakumara et al. 2008	Pb
Oscillatoria sp., Spirogyra sp.	Diwan 2007	Ni(II)
Spirogyra sp., Nostoc commune	Mane et al. 2011	Se
Anabaena variabilis, Aulosira sp., Nostoc muscorum, Oscillatoria sp. and *Westiellopsis sp.*	Parameswari et al. 2010	Cr(VI), Ni (II)
Spirogyra hyalina	Nirmal Kumar and Cini 2012	Cd, Hg, Pb, As and Co
Dunaliella and *Chlorella*	Muhaemin 2004	Pb
Scenedesmus bijuga, Oscillatoria quadripunctulata	Ajayan et al. 2011	Cu, Co, Pb, Zn
Scenedesmus acutus Chlorella vulgaris	Travieso et al. 1999	Cd, Zn and Cr
Scenedesmus sp., Chlorococcum sp., Chlorella vulgaris var. vulgaris and *Fischerella sp., Lyngbya spiralis, Tolypothirx tenuis, Stigonema sp., Phormidium molle, Lyngbya heironymusii, Gloeocapsa sp, Oscillatoria jasorvensis, Nostoc sp. Scenedesmus acutus, Scenedesmus acutus*	Inthorn et al. 2001	Cd, Pb and Hg
Padina sp.	Kaewsarn 2002	Cu
Scenedesmus obliquus, Chlorella pyrenoidosa and *Closterium lunula*	Yan and Pan 2002	Cu
Spirulina platensis	Gannikar 2002	Cu, Hg and Pb
Chlorella minutissima	Singh et al. 2011	Cr(VI)
Chlorella pyrenoidosa	Yao et al. 2011	Zn, Cu, As, Pb, Cd, Cr, Ni, Hg

Table 1(b): Microalgae involve in metal removal/recovery from waste waters

Metal	Organism
Cd(II)	*Chlorella vulgaris, Chlorella salina, Scenedesmus obliquus, Chlamydomonas reinhardtii, Asterionella Formosa, Fragilaria crotonensis, Thalasphaere elongate*
Pb(II)	*Chlorella vulgaris, Euglena sp.*
Zn(II)	*Chlorella vulgaris, Chlorella regularis, Chlorella salina, Chlorella homosphaera, Euglena sp.*
Au(I)	*Chlorella vulgaris*
U(II)	*Chlorella vulgaris, Chlorella sp., Scenedesmus obliquus, Scenedesmus sp., Chlamydomonas sp., Dunaliella tertiolecta, Ankiistroesmus Sp., Selenastrum sp.*
Cu(I)	*Chlorella regularis, Euglena sp., Cricosphaere elongate*
Ni(I)	*Chlorella regularis, Thalassiosira rotula*
Co(II)	*Chlorella regularis, Chlorella salina*
Mn(II)	*Chlorella regularis, Chlorella salina, Euglena sp.*
Mo(I)	*Chlorella regularis, Scenedesmus sp., Chlamydomonas reinhardtii*
Tc(II)	*Chlorella emersonii, Scenedesmus obliquus, Chlamydomonas reinhardtii*
Zn(II)	*Chlorella emersonii, Scenedesmus obliquus, Chlamydomonas reinhardtii*
Hg(II)	*Chlorella sp.*
Al(III)	*Euglena sp.*

The dominant role of some algal polysaccharides (carrageenan) in accumulation of metals was proved in microalgae and is considered to be correlated with the ability of the polysaccharides to form gels (Pengfu et al. 2001). It was also shown that the green algae appeared to be more tolerant to metals such as zinc, lead and copper than blue green algae and diatoms in general. Chojnacka et al. 2005 studied heavy metal biosorption equilibrium of *Spirulina* in the absence of metabolic processes. Rangsayatorn et al. 2004 reported that its metal adsorption rate was rapid. And the bioremediation potential of *Spirulina platensis* was also assessed by Chen and Pan 2005.

Bioremediation of Oil

An oil spill is a release of a liquid petroleum hydrocarbon into the environment due to human activity, and is a form of pollution. It includes release of crude oil from tankers, offshore platforms, drilling rigs and wells, spills of refined petroleum products like gasoline, diesel and their by-products. It also includes heavier fuels used by large ships like bunker fuel, spill of any oily refuse or waste oil. Oil spill is a big environmental concern. Microalgal growth on the spills are to be monitored so as to check whether Microalgae has the potential to clean up the spill or not.. Marine oil pollution has been receiving increasing attention since the middle of the 19th century with the intensification of tanker operations and oil use, marine tanker collisions, pollutant release from coastal refineries (Wake 2005) and continuous operative discharges from ships (Carpenter and MacGill 2001). Annually, 48% of the oil pollution in the oceans is due to fuels and 29% to crude oil. Microalgae are potential biofuel feedstocks that can provide solutions to the twin challenges of energy security and environmental pollution. They have great potential for the removal of excess nitrogen and phosphorus from wastewater including the farm runoff. They can capture carbon dioxide from coal fired power plants thereby reducing greenhouse gas and also producing algal biomass, which can be converted into biofuel. Walker et al. (Walker et al. 1975) performed experiments with the microalgae which was found to degrade petroleum hydrocarbons found in crude and motor oils. Interestingly, in the crude oil, 38-60% of the saturated aliphatic hydrocarbons and 12-41% of the aromatic compounds were degraded, whereas in the motor oil, 10-23% of the saturated aliphatic hydrocarbons and 10-26% of the aromatic compounds were degraded. This suggested that the microalga were capable of degrading different oils to varying levels alongwith other microorganisms (Table.2). Praepilas and Pakawadee (2011) investigated the potential of microalgae (*Scenedesmus quadricauda* and *Chlorella* sp.) for utilizing industrial wastewater as a cheap nutrient for their growth and oil accumulation. The cultures gave the highest lipid content at 18.58 % and 42.86% in cases of *S. quadricauda* and *S. obliquus*. In addition, under salt stress (1.0 M NaCl), *S. obliquus* demonstrated the highest lipid content at 50% which was much more than the case of no NaCl adding. However, the concentration of NaCl does not affect on lipid accumulation in case of *S. quadricauda.* Many microalgae can accumulate lipids due to excess photosynthate and some species can accumulate amount of lipids under heterotrophy or environment stress, such as nutrient deficiency or salt stress (Takagi et al. 2006).

Bioremediation of Pesticides

A wide variety of organochlorine compounds are introduced into aquatic environments via domestic sewage, pesticide runoff from agricultural lands and industrial effluents. Recent studies have indicated that different microalgal species apparently have different sensitivity to pesticides (Solomon 1996).The response of pesticides vary widely depending upon concentrations used,

Table 2: Degradation of petroleum compounds and fuel components by different groups of microorganisms

Algae	Compound
Selanastrum capricornatum	Benzene, toluene,naphthalene, phenanthrene, pyrene
Cyanobacteria(Blue-green algae) *Microcystis aeruginosa*	Benzene, toluene,naphthalene, phenanthrene, pyrene
Mixed cultures(Yeasts, molds, protozoa, bacteria, activated sludge)	Acrylonitrile
Activated sludge	Dibenzanthraceae
Sewagw slude	Fluoranthene
Acinetobacter calcoaceticus	Petroleum derivatives
Strains of *Pseudomonas putida*	Phenol cresols
Trichosporon pullulans	Paraffins
Aeromonium sp.	Total petroleum hydrocarbons
Mycobacterium sp.	n-Undecane

duration of exposure, and algal species tested. Generally, there was a broad range of sensitivity among species and between divisions of algae tested.

Biodegradation of pesticides is determined by two groups of factors, the first relates to microbial consortium and the optimum condition for their survival and activity while the second relates to the chemical structure of the pesticides. Factors related to microorganisms including the presence and number of appropriate microorganisms, the contact between microorganisms and the substrate (pesticide), p^H, temperature, salinity, nutrients, light quality and intensity, available water, oxygen tension and redox potential, surface binding, presence of alternative carbon substrates and alternative electron acceptors. The second group of factors including chemical structure, molecular weight and functional groups of the applied pesticides, their concentration and toxicity and their solubility in water. Some information on the interactions between pesticides and algae was compiled by Kobayashi and Rittman (Kobayashi and Rittam 1982), showing that not only were microalgae capable of bioaccumulating pesticides, but they were also capable of bio transforming some of these environmental pollutants.(Table 3).

Table 3: Microalgae in bioaccumulation and biotransformation of pesticides.

Microalgae	Bioaccumulation	Biotransformation
Chlamydomonas sp.	Mirex	Lindane, naphthalene, phenol
Chlorella sp.	Toxaphene, methoxychlor	Lindane, chlordimeform
Chlorococcum sp.	Mirex	
Cylindrotheca sp	DDT	
Dunaliella sp.	Mirex	DDT, naphthalene
Euglena gracilis	DDT, parathion	Phenol
Scenedesmus obliquus	DDT, parathion	Naphthalene sulfonic acid
Selenastrum capricornutum	Benzene, toluene, chlorobenzene, 1,2-dichlorobenzene, nitrobenzene naphthalene, 2,6-dinitrotoluene, phenanthrene, di-n-butylphthalate, pyrene	Benzo[a]pyrene

Bioremediation of Toxins

Bioaccumulation of toxins is a separate issue to environmental toxicity, though there are likely to be direct relationships between toxin accumulation and the nature and strength of toxic effects. Bioaccumulation occurs where tissue-based concentrations exceed those available in the environment: the latter may include algal toxins available through drinking, dietary, and/or direct contact routes. Algae comprise the greatest abundance of plant biomass in aquatic environments and are a logical choice for aquatic toxicological studies, yet have been underutilized in this capacity. The lipid content of many algal species provides a point of entry for trophic transfer of lipophilic organic contaminants.

Bioremediation of Radioactive compounds

Uranium is one of the most seriously threatening heavy metals because of its high toxicity and some radioactivity. Excessive amounts of uranium have found their ways into the environment through the activities associated with the nuclear industry. Uranium contamination posses a threat in some surface and ground waters (Laul 1992). There is a need for controlling the heavy metal especially uranium emissions into the environment. Conventional methods for removing heavy metals from industrial effluents (*e.g.* precipitation and sludge separation, chemical oxidation or reduction, ion exchange, reverse osmosis, membrane separation, electrochemical treatment and evaporation) are often ineffective and costly when applied to dilute and very dilute effluents (Aksu 1998). Marine algae are capable of biosorbing radionuclide such as radium, thorium and uranium has been known for a long time.The biosorption of uranium by *Cystoseira indica* , a brown algae biomass has been reported (Edgington et al. 1970).

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