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Review

Novel biofiltration methods for the treatment of heavy metals from industrial wastewater

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Abstract

Most heavy metals are well-known toxic and carcinogenic agents and when discharged into the wastewater represent a serious threat to the human population and the fauna and flora of the receiving water bodies. In the present review paper, the sources have discussed the industrial source of heavy metals contamination in water, their toxic effects on the fauna and flora and the regulatory threshold limits of these heavy metals. The various parameters of the biofiltration processes, their mechanism for heavy metals removal along with the kinetics of biofilters and its modeling aspects have been discussed. The comparison of various physico-chemical treatment and the advantages of biofiltration over other conventional processes for treatment of heavy metals contaminated wastewater have also been discussed. The applications of genetic engineering in the modification of the microorganisms for increasing the efficiency of the biofiltration process for heavy metals removal have been critically analyzed. The results show that the efficiency of the process can be increased three to six folds with the application of recombinant microbial treatment.

Keywords: Heavy metals; Biofiltration; Biosorption; Modeling; Genetic engineering

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Contents

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1. Introduction

1.1. Heavy metals in industrial wastewater

Heavy metals are elements having atomic weights between 63.5 and 200.6, and a specific gravity greater than 5.0. Living organisms require trace amounts of some heavy metals, including cobalt, copper, iron, manganese, molybdenum, vanadium, strontium, and zinc. Excessive levels of essential metals, however, can be detrimental to the organism. Non-essential heavy metals of particular concern to surface water systems are cadmium, chromium, mercury, lead, arsenic, and antimony. Heavy metals which are relatively abundant in the Earth's crust and frequently used in industrial processes or agriculture are toxic to humans. These can make significant alterations to the biochemical cycles of living things [1].

Most of the point sources of heavy metal pollutants are industrial wastewater from mining, metal processing, tanneries, pharmaceuticals, pesticides, organic chemicals, rubber and plastics, lumber and wood products, etc. [1–5]. The heavy metals are transported by runoff water and contaminate water sources downstream from the industrial site. All living things including microorganisms, plants and animals depend on water for life. Heavy metals can bind to the surface of microorganisms and may even penetrate inside the cell. Inside the microorganism, the heavy metals can be chemically changed as the microorganism uses chemical reactions to digest food.

The objective of this paper is to analyze various physico-chemical and biological methods used for the treatment of heavy metals from industrial wastewater and compare the improvement in the efficiency of the biofiltration processes by emerging methods such as the application of genetic engineering for the treatment of heavy metals.

1.2. Regulatory limits and necessity of treatment

The maximum contaminant level (MCL) values as per EPA and the position in the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), 2005 list of priority chemicals of some of the toxic and heavy metals are summarized in Table 1 [6,7]. The above limits are mandatory for all the water supply systems. But naturally occurring water (both surface and ground water) sometimes contain some of these heavy metals in 100 or 1000 times more in concentration than the prescribed MCL value. All water treatment facilities, therefore, are required to treat the heavy metals contaminated water to meet the regulatory requirements.

1.3. Biological treatment for heavy metals removal

Several physico-chemical methods have been widely used for removal of heavy metals from industrial wastewater, such as ion-exchange, activated charcoal, chemical precipitation, chemical reduction and adsorption, etc. [8–11]. The conventional methods used for the treatment of heavy metals from industrial wastewater present some limitations. There are still some common problems associated with these methods such as these are cost-expensive and can themselves produce other waste problems, which has limited their industrial applications [12,13].

Table 1
Rank of heavy metals as per CERCLA list of priority chemicals 2005 and their regulatory limits

Heavy metals	Rank	Maximum concentration limit (mg/l)
Arsenic (As)	01	0.01
Lead (Pb)	02	0.015
Mercury (Hg)	03	0.002
Cadmium (Cd)	08	0.005
Chromium (Cr (VI))	18	0.01
Zinc (Zn)	74	5.0
Manganese (Mn)	115	0.05
Copper (Cu)	133	1.3
Selenium (Se)	147	0.05
Silver (Ag)	213	0.05
Antimony (Sb)	222	0.006
Iron (Fe)	–	0.3

Among the available treatment processes, nowadays the application of biological processes is gradually getting momentum due to the following reasons:

- Chemicals' requirement for the whole treatment process is reduced.
- Low operating costs.
- Eco-friendly and cost-effective alternative of conventional techniques.
- Efficient at lower levels of contamination.

The biofilters are the latest and the most promising development in biological processes for the treatment of heavy metals contaminated industrial wastewater.

2. Biofilters

2.1. Biofiltration methods

Microorganisms fixed to a porous medium are used in the biofiltration process to break down pollutants present in the wastewater stream. The microorganisms grow in a biofilm on the surface of the medium or are suspended in the water phase surrounding the medium particles. The filter bed medium consists of relatively inert substances which ensure large surface attachment areas and additional nutrient supply. The overall effectiveness of a biofilter is largely governed by the properties and characteristics of the support medium, which include porosity, degree of compaction, water retention capabilities and the ability to host microbial populations. Critical biofilter operational and performance parameters include the microbial inoculation, medium pH, temperature, the medium moisture, and nutrient content [14].

In a biofiltration system, the biodegradable pollutants are removed due to biological degradation rather than physical straining as in the case in normal filter [15]. With the progress of filtration process, microorganisms (aerobic, anaerobic, facultative, bacteria, fungi, algae, and protozoa) are gradually developed on the surface of the filter media and form a biological film or slim layer known as biofilm. The crucial point for the successful operation of biofilter is to control and maintain a healthy biomass on the surface of the filter.

Since, the performance of the biofilter largely depends on the microbial activities, a constant source of substrates (organic substance and nutrients) is required for its consistent and effective operation, though some chemoautotrophic bacteria may also use inorganic chemicals as energy source. The removal efficiency of biofilters is controlled by some parameters like pH, temperature, O_2 content, initial concentration of toxic pollutants, etc. The removal efficiency may be improved by chemical modification of the filter media or genetic modification of microorganisms.

2.2. *Mechanism of biofiltration*

The underlying mechanisms, which allow biofilters to work and which must be controlled to ensure success, are complex. The biofilter contains a porous medium whose surface is covered with water and microorganisms. The contaminant may form complexes with organic compounds in the water and may be adsorbed by the support medium. Ultimately, biotransformation converts the contaminant to biomass, metabolic by-products or carbon dioxide and water. The biodegradation is carried out by a complex ecosystem of degraders, competitors and predators that are at least partially organized into a biofilm [16]. There are three main biological processes that can occur in a biofilter:

- Attachment of microorganisms.
- Growth of microorganisms.
- Decay and detachment of microorganisms.

The mechanisms by which microorganisms can attach and colonize on the surface of the filter media of a biofilter are transportation of microorganisms, initial adhesion, firm attachment and colonization [17]. The transportation of microorganisms to the surface of the filter media is further controlled by four main processes [15]:

- Diffusion (Brownian motion),
- convection,
- sedimentation due to gravity, and
- active mobility of the microorganisms.

As soon as the microorganisms reach the surface, initial adhesion occurs which may be reversible or irreversible depending upon the total interaction energy, which is the sum of van der Waal's force and electrostatic force. The processes of film attachment and colonization of microorganisms depend on influent characteristics (such as organic type and concentration) and surface properties of the filter media. The following parameters are taken into consideration to estimate the attachment of microorganisms on the surface of the filter media:

- The steric effect,
- hydrophobicity of the microorganisms,
- contact angle, and
- electrophoretic mobility values.

The factors that influence the rate of substrate utilization within a biofilm are substrate mass transport to the biofilm, diffusion of the substrate into the biofilm, and utilization kinetics within the biofilm. Adsorption and biodegradation performs simultaneously in biofilters to remove biodegradable and water soluble hazardous organic molecules, which results in simultaneous adsorption and biodegradation [18,19]. Though the mechanism of heavy metals removal by biofilter differs from that of the organic chemicals removal, but all the above conditions regarding growth of biomass in the biofilm are the same. Only exception in this case is that there is no biodegradation in case of heavy metals removal. The mechanism of heavy metals removal from contaminated water in biofilter is as follows.

The non-biodegradable water soluble heavy metals are either oxidized or reduced by the microorganisms and produce less soluble species. The less soluble form of these metals which are formed due to microbial reactions are adsorbed or precipitated/co-precipitated on the surface of the adsorbent and the extra cellular protein of the microorganisms in the biolayer [20]. The methylation of metals is also another important route for bioremediation of heavy metals in water [21]. Though the microbial action on metal ion transformation is still a matter of research, it is assumed that there are two paths. In one path oxidation or reduction of heavy metal ions takes place by extra cellular enzymes where the metal ions do not enter into the bacterial cell. In the other path the metal ions are transported into the microbial cells by trans-membrane proteins and are converted to other less soluble forms by metabolic actions of enzymes in the cells followed by subsequent excretion from the cells, yet both the paths are plasmid mediated [20]. Whether the microbial action on a metal ion is performed by only one path or by both the paths is a matter of research.

2.3. *Mathematical modeling of biofilters*

Biofilter modeling started in the early 1980s and was based on earlier work on submerged biofilm models. The models assumed basic mass balance principles, simple reaction kinetics, and a plug flow stream. More recently, fundamentally different but potentially promising type of models, use quantitative structure activity relationships and seek to predict the performance of biofilters from data describing the removal of a few known pollutants.

The difficulty in modeling a biofilter lies in the complexity of the fundamental processes. Biofiltration involves many physical, chemical, and microbiological phenomena. In order to simulate biofilter effectiveness with varying operating conditions, a model must include these various phenomena. Further, a number of unknowns or difficulties exist in the definition of equations for a biofilter model [22].

There are only a few models reported in the literature that can predict the performance of a biofilter. Most of these models are based on the assumption of stationary and uniform flow.

A biological filter may be described as a three-phase packed column.

The solid phase consists of two components:

- Carrier particles (packing) and
- biofilm (biomass) consisting of water, microorganisms and saccharidic polymers.

The gaseous phase also consists of two components:

- Air (for aerobic reactions) and
- products of the reaction (carbon dioxide, and/or gaseous nitrogen).

Generally two main parts can be distinguished in the model of a packed biofilter:

- A reactor model (hydrodynamic aspect) and
- a biofilm model with substrate uptake, product formation and biomass development.

In most biofilter models, a diffusion phenomenon is taken into account and is described by Fick's law. In biological filtration the biofilms are very irregular and filamentous which accords poorly with the diffusion phenomena hypothesis. Furthermore, experimental studies on aerobic and anaerobic biofilms show that the substrate removal reaction occurs only at the edge of the biofilm in a very thin part of it, the active thickness is estimated at about 10 μm where as, the biofilm may reach 500 μm [23]. Thus, the reaction may be considered as a surface reaction.

Rittman and McCartey [24] first introduced a steady-state biofilm model in 1980. It was assumed that minimum bulk substrate concentration ($S_{\min}$) is required to maintain the steady-state biofilm in the filter. The model describes the fundamental biological processes but does not take into account the biofilm growth with time. Chang and Rittman in 1987 [25] developed a model for the kinetics of biofilm on activated carbon (BFAC) incorporating film mass transfer, biodegradation, and adsorption of a substrate, as well as biofilm growth. All the fundamental biological processes have been included in this model. However, the non-steady state condition due to backwashing, change in the filter bed porosity and hence the filter depth have not been considered in this model.

The concept of dimensionless empty bed contact time (EBCT) which allows comparison of results among different surrogate parameters such as available organic carbon (AOC) and biodegradable dissolve organic carbon (BDOC) was developed by Zhang and Huck [26]. Hozalski and Bouwer in 2001 [27] developed a numerical model called BIOFILT, to simulate the non-steady-state behavior of biologically active filters used for drinking water treatment. The model is capable of simulating the substrate (biodegradable organic matter) and biomass (both attached and suspended) profiles in a biofilter as a function of time. The model also has the capability to simulate the effects of a sudden loss in attached biomass due to filter backwash on substrate removal efficiency. All the models described above are successful in modeling the fundamental biological processes of biofilter. Till now, only a little work has been done in the field of modeling of biofilters for the removal of heavy metals from wastewater.

3. Treatment methods

3.1. Microorganisms used for heavy metal removal from industrial wastewater

The application of microorganisms for the remediation of heavy metals in water is a recent field of research

Table 2
Microorganisms having heavy metals removal capabilities

Microorganisms	Removable toxic and heavy metals	References
Bacterial species		
Rhodospirilium species	Cd, Hg, Pb, Ni	Chatterjee [28]
Gallionella feruginea	As, Mn, Fe	Katsoyiannis and Zouboulis [29]
Leptothrix species	As, Mn, Fe	Katsoyiannis and Zouboulis [29]
Pseudomonous species	Cr, As	Valls et al. [30]
Desulfovibrio species	Cu, Zn, Ni, Fe, As	Jong and Pany [31]
Thiomonous species	As, Fe	Casiot et al. [32]
Escherichia coli	Hg, Ni	Deng et al. [33]
Thauera selenatis	Zn, Cd, Co, Cu, Ni, Pb, Cr, Hg	Mergray et al. [34]
Alcaligenes faecalis	As	Phillips and Taylor [35]
Fungal species		
P. Chrysogenum	Zn, Cu, Ni, As	Loukidou et al. [36]
Aspergillus niger	Ni, Cu, Pb, Cr	Dursun et al. [37]
Coriolus hersutus	Cd	Miyata et al. [38]
Trametes versicolor	Cr, Co	Blanquez et al. [39]
Mucor rouxi	Pb, Cd, Zn, Ni	Yan and Viraraghavan [40]
Algal species		
Brown algae	Cd, Cu, Zn, Pb, Cr, Hg	Davis et al. [41]
Green algae	Cu, Hg, Fe, Zn, Pb, Cd	Haritonidis and Malea [42]
Scenedesmus genus	Cu, Ni, Cd, Cr, Cu	Pena-Castro et al. [43]

Table 3
Removal of heavy metals using microorganisms

Reference	Microbes, its type and energy source	Percentage removal of heavy metals	Reactor type with specifications and operating conditions	Remarks
Katsoyiannis and Zouboulis [29]	*Gallionella ferrigunea* (aerobic and chemoautotrophic), energy source Fe(II) → Fe(III) + energy; *Leptothrix ochracea* (aerobic and heterotrophic), energy source = organic substrate	97.0% removal of Fe and Mn; 80.0% removal of As	Bench scale reactor, plexiglas made, adsorbent = polystyrene bead of diameter 3–4 mm, residence time = 7.3 min, bed volume 3.6 l, total bed porosity 0.37, temperature = 10–15 °C for *Galleonella ferrigunea* and 20–25 °C for *Leptothrix ochracea*, pH 4.5–7.0, D.O. = 3.7 mg/l, feed rate = 111–560 ml/min, $\mathrm{As_o} = 150\ \mu\mathrm{g/l}$, $\mathrm{Fe_o} = 2.8\ \mathrm{mg/l}$, $\mathrm{Mn_o} = 0.6\ \mu\mathrm{g/l}$	Biolayer was formed by indigenous bacteria for 3 months, flow of water through the reactor, suitable for removal up to ppb level to meet the statutory standards
Hope et al. [46]	*Leptothrix discophor SP-6* (aerobic and heterotrophic), energy source = organic substrate	>97.3% removal of Mn	Bench scale and pilot scale, glass made, adsorbent = sand of 0.4 mm grain diameter, residence time = 1.74 h, volume for bench scale = 5 l, pilot scale = 20 l, temperature = 25–26 °C, pH 7.6–7.0, feed rate = 48 ml/min, $\mathrm{Mn_o} = 3000\ \mu\mathrm{g/l}$	Biolayer was developed by inoculated medium, relatively non-problematic, scale up to the large-scale biofilter, suitable for removal up to ppb level to meet the statutory standards
Jong and Pany [31]	Mixed population of sulfate reducing bacteria (SRB) (anaerobic and heterotrophic), energy source = Na lactate anaerobic condition tri sodium citrate prevents metal precipitation	97.5% removal of Cu, Zn and Ni, >77.5% removal of As, 82% removal of Fe, no removal of Mg and Al	Bench scale reactor, PVC made, adsorbent = coarse pool filters sand of diameter >2 mm, residence time = 14 h, empty working volume = 4.78 ± 0.01 l, final pore volume = 2110–2400 ml, porosity = 0.43–0.47, temperature = 25 °C, pH 4.5–7.0, organic substrate and sulfate loading rate = 7.43 and 3.71 kg per day per $\mathrm{m^3}$, respectively, influent feed rate = 2.61 ml/min	Sulfate reduction was >82%, biolayer was developed by inoculated medium, suitable for removal upto ppb level to meet the statutory standards, $\mathrm{Cu_o} = 5\ \mathrm{mg/l}$, $\mathrm{Ni_o} = 10\ \mathrm{mg/l}$, $\mathrm{Zn_o} = 10\ \mathrm{mg/l}$, $\mathrm{Al_o} = 20\ \mathrm{mg/l}$, $\mathrm{As_o} = 50\ \mathrm{mg/l}$

D.O., dissolved oxygen; $\mathrm{As_o}$, $\mathrm{Fe_o}$, $\mathrm{Mn_o}$, $\mathrm{Cu_o}$, $\mathrm{Ni_o}$, $\mathrm{Zn_o}$, $\mathrm{Al_o}$ are the initial concentration of the respective metals.

inenvironmental engineering. Some microorganisms have been identified to possess heavy metals removal capability from contaminated water. The dominant pollutant degraders in biofilters are bacteria and fungi. These simple organisms are capable of utilizing the substrate rapidly. Their small size produces a high surface-to-volume ratio ideal for rapid pollutant uptake. Bacteria are generally smaller and more active than fungi, and they will dominate in biofilter with a high water content treating easily degraded substrate at near neutral pH. Fungi will become more important if the biofilter is drier or more acidic, and they can degrade some complex substances which are beyond the metabolic abilities of bacteria. Table 2 represents some microbial species implicated in heavy metal removal. Almost all the reports are based on laboratory experiments.

3.2. Application of biofilter for metal remediation in water

Biofiltration was first introduced in 1893 in UK as a trickling filter [44]. So far, it has widely been used for the treatment of contaminated water bearing biodegradable organic compounds. But its application for the remediation of non-biodegradable heavy metals from contaminated water is a very recent development. This technology is not yet well established, though the reports of some of the studies are discussed below to establish the high possibility of biofilters application in the treatment of heavy metals from contaminated water.

3.3. Removal of heavy metals by biofilters using microorganisms

In 2002, Erik et al. on a study of iron removal through sand filters of three different fresh water plants, two biotic and one abiotic, in the same area of Denmark, have compared the rate of iron precipitation in biotic and abiotic conditions [45]. As per this report biotic iron precipitation is 60 times faster than abiotic precipitation and biotic sludge is 7–9 times denser than abiotic one.

The microbe used in this study was *Gallionella ferruginea*. The morphology of the iron precipitates has been investigated by using light, X-ray, scanning electron and transmission electron microscopy. The physico-chemical conditions governing precipitation and precipitated iron sludge has also been investigated. This report was very much interesting to

Table 4
Recombinant bacterial bioassay for heavy metals removal

Heavy metal	Promoter (origin)	Reporter	Microorganism	Time of induction	Concentration of metals	Reference
Aluminium	fliC (*E. coli*)	luxAB (*V. harveyi*)	*E. coli*	20 min	40–400 μM	Guzzo et al. [48]
Antimonite	arsRD’	lacZ	*E. coli*	17 h	100 μM	Ramanathan et al. [49]
Arsenic	arsB (*S. aureus*)	luxAB (*V. harveyi*)	*E. coli, S. aureus*	1–2 h		Corbisier et al. [50]
Cadmium	cadA (*S. aureus*)	luxAB (*V. harveyi*)	*E. coli, S. aureus*	1–2 h	1–100 μM	Corbisier et al. [50]
Chromate	chr (*A. eutrophus*)	lux	*A. eutrophus*	1–2 h	1–50 nM	Peitzsch et al. [51]
Copper		luxABCDE (*V. fisheri*)	*E. coli*		1 μM–1 mM	Holmes et al. [52]
Mercury	mer (*S. aureus*)	blaZ (*S. aureus*)			5 μM	Chu et al. [53]
	mer	lux (*V. fisheri*)	*E. coli*	30 min	10 nM–4 μM	Tescione and Belfort [54]
Zinc	smtA (*Synechococcus* PCC7942)	luxCDABE (*V. fisheri*)	*Synechococcus PCC7942*	4 h	0.5–4 μM	Erbe et al. [55]

the bioengineers for the development of a new technique to remove heavy metals from contaminated water and some new reports came out in successive years which have been analyzed in Table 3. From the above table it is evident that biofilters can remove selective metals to a considerable amount. It is also noted that in some cases organic carbon is not required as an energy source for the microbes and indigenous microbes may be used to develop a biolayer. The relatively non-problematic scale up to the large-scale biofilter in case of Mn removal suggests that it would be possible to seed a new manganese biofilter with re-circulating, laboratory grown batch cultures of *Leptothrix discophora SP-6*. The percentage removal is also high in many cases although for arsenic it is only 80%. But within lower range of pollutants this 80% observed removal may also be effectively utilized as polishing stage of water treatment. Moreover, chemical modification of adsorbents can improve the filter efficiency and microbes are susceptible to engineering improvements of their capabilities [47]. The recent attempts of some researchers to improve the heavy metal removal efficiency of some microbes are described below.

4. Emerging biofiltration methods

4.1. Improvement in the biofilters efficiency by genetic modification of the microorganisms

Bioremediation is the transformation or degradation of contaminants into non-hazardous or less hazardous chemicals. Bacteria are generally used for bioremediation, but fungi, algae and plants have also been used. There are three classifications of bioremediation:

Table 5
Improvement of heavy metals removal efficiency by genetic modification of the microorganisms

Reference	Microorganism used	Improvement in performance of heavy metal considered	Plasmids used and protein expressed	Remarks
Valls et al. [30]	*Pseudomonas putida* KT2442	Three folds w.r.t bacteria for Cd	pTn-MTβ1 and pCNB1; IgaAβ-MT protein	No study on the effect of other metals on removal efficiency was performed
Deng et al. [33]	*Escherichia coli*	Six folds w.r.t bacteria for Ni	pSUN1 and pGMPT3; GSM-MT	Pb^{2+}, Cd^{2+} do not have significant effect on removal efficiency; Mg^{2+}, Hg^{2+} have adverse effect on removal efficiency
Kostal et al. [57]	*Escherichia coli*	Five folds w.r.t. bacteria for arsenate and 60 folds w.r.t bacteria for arsenite	pMal-c2x and pETR; ELP153AR (ArsR incorporated with elastin like polypeptide)	The high affinity of ArsR allowed 100% removal of 50 ppb of arsenite from contaminated water; no significant improvement in Cd^{2+} or Zn^{2+} accumulation
Mondaca et al. [58]	*Pseudomonas putida* KT2441	20 folds w.r.t. bacteria for chromate	*Pseudomonas putida* KT-6	The resistant level observed in the transconjugant was 10 mmol/l chromate while the control was resistant only to 0.5 mmol/l

w.r.t., with respect to.

- Biotransformation:
  - ■ The alteration of contaminant molecules into less or non-hazardous molecules.
- Biodegradation:
  - ■ The breakdown of organic substances in smaller organic or inorganic molecules.
- Mineralization:
  - ■ The complete biodegradation of organic materials into inorganic constituents.

These three types of bioremediation can occur either *ex situ* or *in situ*. There are both advantages and disadvantages associated with *ex situ* and *in situ* processes. In the former case, the contaminants are removed and placed in a contained environment, which makes the remediation process faster by allowing easier monitoring and maintaining of conditions and progress. However, the removal of the contaminant from the contaminated site is time consuming, costly and potentially dangerous which are the major disadvantages of the process. In contrast, the *in situ* process does not require the removal of the contaminant from the contaminated site. Instead, either biostimulation or bioaugmentation is applied. The former is the addition of nutrients, oxygen or other electron donors or acceptors to the coordinated site in order to increase the population or activity of naturally occurring microorganisms available for remediation, while the latter is the addition of microorganisms that can biotransform or biodegrade contaminants.

Bioremediation technology involves the use of microorganisms to reduce, eliminate, contain or transform to benign products contaminants present in soils, sediments, water and air. *Staphylococcus*, *Bacillus*, *Alcaligens*, *Escherichia*, *Pseudomonas*, *Citrobacteria*, *Klebsilla* and *Rhodococcus* are the organisms that are commonly used in bioremediation. This process involves biochemical reactions or pathways in an organism that result in activity, growth and reproduction of that organism. Chemical processes involved in microbial metabolism consist of reactants, contaminants, oxygen or other electron acceptors, which convert metabolites to well defined products. A key factor to the remediation of metals is that metals are non-biodegradable, but can be transformed through sorption, methylation and complexation and changes in valence state.

Although using bioremediation is a great idea, quite often the contaminants are also toxic to the active microbes involved in the bioremediation process. This problem can make it very difficult to keep the rate of bioremediation high. A solution to this problem is genetically engineered microbes which are resistant to the extreme conditions of the contaminated site and also have bioremediation properties. Bioremediation is gaining importance in recent times as an alternate technology for removal of elemental pollutants in soil and water, which require effective methods of decontamination. Table 4 represents the summary of the genetic modification work done on various microorganisms for the removal of heavy metals.

The pretreatment of fungal biomass of *P. chrysogenum* with common surfactants (as hexadecyl-trimethyl ammonium bromide and dodecyl amine) and a cationic-polyelectrolyte was found to improve the biosorption efficiency. The reported improvement in biosorption efficiency was 37.8, 33.3 and 56.1% for hexadecyl-trimethyl ammonium bromide, dodecyl amine and polyelectrolyte, respectively [36]. Moreover, this biosorptive process reduces capital costs by 20%, operational cost by 36% and total treatment cost by 28% when compared with conventional processes [56]. The improvement of heavy metals removal efficiency by genetic modification of microbes is described in Table 5.

The microbial cloning in the studies mentioned in Table 5 was plasmid mediated cloning. From the table it is evident that the engineered bacteria achieve more removal efficiency with respect to the natural ones. The engineered microorganisms are more selective. The improvement in removal efficiency for all the cases is noticeable. It is hoped that in combination, these techniques would improve filter efficiency.

5. Conclusions

From the above discussion it can be concluded that there is a high possibility for effective application of biofilters for removal of toxic heavy metals from contaminated water in large scale. The success in microbial cloning technique may improve the removal efficiency and hence the reduction in treatment cost. As it is capable of removing heavy metals up to ppb level and is cheaper, application of this technique for the treatment of wastewater of the industries like chemicals and fertilizers, textiles, pulp and paper, dyes and pigments, pharmaceuticals, etc. will help these units to meet the statutory mandate and to alleviate the threat for survival due to high wastewater treatment cost of these units. In short, the biofilters are having emerging applications for the treatment of heavy metals contaminated wastewater.

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