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An overview of bioreactors and their application in the biosorption of heavy metals

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ABSTRACT

Atmospheric toxicants in the form of heavy metals are major threat to human species on earth. Heavy metal ions are accumulative in nature and non-biodegradable. There minute amount in human body is very dangerous, henceforth there removal is necessary. Many physical and chemical methods like ion- exchange, ultra filtration, reverse osmosis, electrochemical treatment, chemical precipitation, evaporation, membrane technologies are employed for remediation of heavy metals from wastewaters but due to some disadvantages there is a requirement of such bioremediation technology which should be environmental friendly and a natural method. Biosorption is one such method which is of great attractive alternative of conventional methods. Bioremediation technologies are now linked to bioreactors to make removal process more efficient from wastewater. Performance of some bioreactors is found to be very good for the removal of heavy metals through biosorption. So, one objective of this paper is to highlight the efficiency of biosorption involved in bioreactors. Here we discuss different types of bioreactors fixed bed, fluidized bioreactor, stirred tank, airlift bioreactors which are being used for the biosorption process. This article also highlights the significance of standard bioreactors and a possible usage of nanoparticles based bioreactors which will definitely be very useful in near future. Further, green synthesis of nanoparticles is also an interesting technology that will revolutionize the process of biosorption.

***Keywords**: Bioreactors, Biosorption, Heavy metals, Metal nanoparticles, Green synthesis.*

Introduction

Industrial revolution has made humans life easier. However, comfort and ease has its own advantages and disadvantages. One major disadvantage of this revolution is increase in the global pollution levels. Pollutants categorized as industrial pollutants are usually discarded mostly without treatment either in open land or in open water bodies (Ghori *et al.,* 2016). These pollutants in water bodies further leads to imbalance of nature and change in the ecosystem which is always harmful for all living beings. Liquid pollutants even penetrate and enter the underground water thereby causing severe consequences to life. One major component of pollutant is the heavy metals which causes threat to all life forms even in bio-accumulated or in minor amount.

Heavy metals exist in nature and are defined as metals having high atomic weight (Masindi & Muedi, 2018). Heavy metals have highest density than water which is 5g/cm^3 (Banfalvi, 2011). As heavy metals are non-biodegradable in nature so there ions easily bio-accumulate in ecosystem. They enter the food chain and increasing concentration become toxic for human life (Ghori *et al.,* 2016).

Heavy metals are divided into two major groups from biological point of view. The first group enlists heavy metals that are required in micro amounts by organisms for their physiological function such as Copper, Chromium, Cobalt and Zinc. The second group comprises of metals such as Arsenic, Mercury, Cadmium, Nickel, Lead that cause toxic effects to human and environment and are classified as non- essential heavy metals (Banfalvi, 2011).

Toxicity due to heavy metals leads to destruction or minimized functions of Central nervous system and thereby harming liver, kidney, lungs and other vital organs of the body. Exposure of heavy metals for long time leads to Alzheimer's disease, Parkinson's disease, muscular dystrophy etc. Further repeated exposure to toxic heavy metals also leads to cancer (Verma and Dwivedi, 2013).

Methods for removal of heavy metals

Due to the toxic, persistent and non-biodegradable nature of heavy metals it is necessary to remove them from environment and methods advocated uses principles of ion exchange, chemical precipitation, ultrafilteration, membrane filteration, reverse osmosis etc. However, all these methods have certain drawbacks like membrane's fouling, high in cost, require costly reagents, formation of toxic sludge or insufficient removal of metal ions. Because of these drawbacks there is a pronounced requirement of innovative technologies which should be cost-effective and eco-friendly. In recent times, biosorption is used as a method that is economical, environment friendly, fruitful and utilizes innovative approaches for the treatment of toxic heavy metals.

Bioreactors used for biosorption process

For the application of industrial scale various types of bioreactors are used. Use of bioreactors for biosorption in heavy metal removal is very efficient. Bioreactors are defined as a container in which biological reactions are done. These biological reactions incorporate enzymes, microbes, plant and animal cells. Inside the bioreactors there is a perfect environment provided externally in order to obtain high yield of bioprocess and to meet the requirement of biological reaction systems (Wang and Zhong, 2007). There are various factors on which efficiency of bioreactors rely on; concentration of biomass which should always be high to maintain high production, sustained aseptic conditions for pure culture system and in order to have for stable scattering of microorganisms and substrate effective stirring is required in working capacity of bioreactor. The bioreactor is to be operated at uniform temperature for perfect microbial growth and temperature should be designed to achieve correct shear conditions as high and low shear conditions are disadvantageous (Najafpour, 2007). Various types of bioreactors used for biosorption are air lift bioreactors, stirred tank bioreactors, fixed bed bioreactors, fluidized bed bioreactors. These can be employed either in continuous modes or in batches or both.

Factors like pH, temperature, stirring, nutrient requirement which effect biosorption process carried out in reactors are managed and improved by feeding lines which supply nutrients, stirrers/baffles used for stirring, adding acid or base solutions for maintaining pH and cooling jackets for temperature.

Types of Bioreactors

Fixed bed bioreactors

Fixed or packed bed bioreactors are most commonly used bioreactors. Benefit in using fixed bed bioreactors are excellent rate of reaction and operation simplicity (Wang and Zhong, 2007). Other advantage of using fixed bed bioreactor is that biosorption process can be carried when current is flowing in opposite direction i.e. countercurrent flow.

It is created with the two columns, biological material fixed onto a bed and a vessel having the bed with-in. Process of biosorption is carried out on one column while regeneration of biosorbent is carried out on other column by washing with appropriate chemical reagent. During the process of biosorption heavy metals present in wastewater pass through column and biosorbent absorbs the sorbent. Biological material biosorbs the sorbent till the saturation capacity is reached. The material used in fixed bed reactor should have high surface area, high structural reliability and chemical constancy, high porosity (Wang and Zhong, 2007). This method has a handling disadvantage. Sometimes fixed bed gets block due to the solid impurities present in water. So to avoid column blocking clear liquid is required (Ilamathi *et al*., 2014).

Fluidized bed bioreactor

Studies have proven that it is found that fluidized bed avoids the channeling problem and permit the treatment of muddy or turbid water. Fluidized bed reactors are most commonly used bioreactors because of superior rate of heat transfer and mass between the molecules and side walls of the column and the fluid and the molecules (Ilamathi *et al.*, 2014).

Quality of fluidization depends on the molecule size and size distribution, density of molecule, features of molecule surface. In fluidized bed reactor fluidization means situation of suspended molecules (Ilamathi *et al*, 2014). This reactor has 3 phases solid, liquid and gas. The biological material is solid phase on solid molecules which absorbs metal ions.

In fluidized bed bioreactor through bed of biosorbent (solid molecules) liquid or gases are moved up with certain velocity. The force of gravity causes the molecule to fall molecule will remain arise (Wang and Zhong, 2007; Ilamathi *et al*., 2014). Fluidization is accomplished by the merged upward and downward movement of molecules (Wang and Zhong, 2007). There are some advantages of fluidized bioreactor over fixed bed bioreactor like; The system is consistent so it is simple to watch and control the operating factors like pH, dissolved oxygen concentration and temperature. Fine mixing is attained in such a way that no gradients occur beyond the reactor. In between even during operations there is a possibility of displacement of mobile or active fragments and easy sampling of molecules. Because of great specified surface area of small molecules and free motion of molecules high mass transfer and heat transfer rates are anticipated between the liquid and molecules (Wang and Zhong, 2007). There are some problems during operations. First one it is difficult to forecast about the patterns of fluidization and back mixing. Secondly, fluidized beds has contract range of operating situations at comparatively high bed extension and low stability levels and in this situation it is very problematic to sustain non fluctuating operations (Wang and Zhong, 2007).

Airlift Bioreactors

Airlift bioreactors are unique variant of fluidized bed bioreactors. Main advantage of airlift bioreactor above fluidized bed bioreactor is good fluidization characteristics (Zhu, 2007).

Airlift bioreactors have a draft tube internal or an external loop. Due to the draft tube airlift bioreactor has several advantages:

a. By directing bubbles in one direction it prevents bubble merging;
b. Provide more beneficial situations for cell growth;
c. Spread the shear strain evenly through out the reactor;
d. Intensify the mass and heat transfer rates, by increasing the cyclical movements of fluid in reactor (Wang and Zhong, 2007).

a. Air lift bioreactors with external loop are considered as better bioreactor because they have benefit of having good turbulence near jacket and hence good heat transfer ability. Heating or cooling jacket is present on the walls of air lift bioreactor and external loop air lift bioreactors produce less foam than internal loop airlift bioreactors (Wang and Zhong, 2007).

Air lift bioreactor has some pitfalls such as due to bubble merging there is a decrease of interfacial area in viscous liquids. Another one is back mixing occurs in continuous liquid phase (Wang and Zhong, 2007).

Stirred Tank Bioreactors

Stirred tank bioreactors are one of the most traditional bioreactors. The chief element of stirred tank bioreactor is impeller. Impeller executes various tasks such as;

Aeration

Transfer of heat and mass

Proper mixing to achieve homogenization (Wang and Zhong, 2007).

In conventional stirred tank bioreactor axial and radial flow are two types of impellers that are used. Besides impellers type other structural parameters dominant in functioning of stirred tank bioreactor are the size of impeller, baffles, baffles breadth, types of sprinkler and its position, ratio of diameter of tank to the liquid height (Wang and Zhong, 2007; Cronin and Nienow, 1989; Nienow, 1990)

In case of large bioreactors various impellers are used for better mixing and transfer of mass (Wang and Zhong, 2007; Whitton, 1988). Traditonal impellers cannot be directly used for cells of plants and animals which are sensitive to shear. Physical cell damage can be caused by two reasons firstly by strain of shear produced due to shaking and secondly by damage of air bubble caused by sparging of gas (Wang and Zhong, 2007).

These two problems can be solved by constructing new types of impellers, by using accurate protective agents and proper device for oxygenation (Lehmann *et al*., 1988, Papoutsakis, 1991; Nienow *et al.,* 1996).

Stirred tank bioreactors are mostly used in biopharmaceutical industries as they are good in maintaining standardize conditions, better capacity for industrial use, good performance and easy to control (Marks, 2003; Wang and Zhong, 2007).

It is clear that capacity of removal of metal species mainly depends on the type of bioreactors and biosorbent used and also on the operating conditions of bioreactors.

Table 1 shows efficiency of different biosorbent in removing various metal ions by using different bioreactors.

From above table it is clear that biosorption can be efficiently associated with different types of bioreactors for heavy metal removal in wastewater. It is also shown in above table that efficiency of removal of heavy metals through bioreactors is highest achieved in airlift bioreactor 94.3% and than in packed bed bioreactor which is 90%.

Usage of Nanoparticles in Bioreactors

Nanoparticles are defined as nanoscopic dimensions materials in the range of 1-100nm (Pal *et al*., 2019). Nanoparticles shows different chemical nature and can be metallic or metallic oxides, silicates, polymers, organics or carbons and can be produced in different shapes like cylinders, spheres, tubes, sheets etc (Ibrahim, 2015; Yang *et al*., 2015; Pal *et al*., 2019). Different types of nanoparticles synthesized are gold, silver, copper, platinum etc. Mostly synthesized nanoparticle is silver by green synthesis than gold other metal are synthesized in low numbers.

Nanoparticles green synthesis is an eco-friendly method with good stability, properties and dimensions. There will also be no need of high temperature, pH and pressure during synthesis. Green synthesis is defined as the use of biological materials like bacteria, fungi, plants for the synthesis of nanoparticles. The nanoparticles produced are chemical free and environment friendly (Pal *et al*., 2019).

Plant as bioreactors in metal nanoparticles synthesis

Plants having properties of reductive capacity and high metal ion hyper-accumulating power are used for extraction of metals from land. As it is known that plants can reduce metal ions from their surface as well as from various tissues. These metals after harvesting, by method of sintering and smelting can be recovered from plants (Makarov *et al*., 2014). Best example of this is accumulation of silver nanoparticles by using silver nitrate as substrate by *Medicago sativa* and *Brassica juncea* plants (Harris and Bali, 2008; Makarov *et al*., 2014).

In vitro methods like plant extracts are mostly used for metallic nanoparticle synthesis because this have many advantages like 1. Good control over size and shape of nanoparticles and also easy purification of metallic nanoparticles is possible, 2. This invitro process occurs much faster than the synthesis of nanoparticles in whole plant (Ghosh *et al*., 2012; Harris and Bali, 2018).

Besides mentioned above plants extract other plants used are *Nyctanthis arbortristis, Cassia fistula, Avena sativa, Medicago sativa*. Terpenoids, sugars, alkaloids, polyphenols, phenolic acids, proteins, flavones, flavonoids are the various metabolites which

Table 1. Efficiency of different bioreactors in biosorption of heavy metals

Biorectors Types	Metal species	Biosorbent	Efficiency of Biosorption (in %)	References
Packed Bed bioreactors	*Aspergillus niger*	Cu	86.93%	Mukhopadhyay *et al*., 2008
	Ulva reticulate	Cu, Co, Ni	56.3%, 46%, 46.5%	Vijayaraghavan *et al*., 2005
	Microcystis aeruginosa	Pb, Cd, Hg	80%, 90%, 90%	Chen *et al.*, 2005
	Escherichia coli	Cr	77.7%	Chakraborty *et al.*,2013
	Turbinaria ornate	Cu	63.94%	Vijayaraghavana *et al*.,2006
Fluidized Bed Bioreactor	*Pseudomons aeruginosa*	Cd, Cr	67.17%, 49.25%	Ilamathi *et al*., 2014
	Bacillus thuriengenesis	Ni	62.02%	Nirmala *et al.*, 2012
	Rhizopus arrhizus	Cr	94.23%	Prakashama *et al*., 1999
	Thiobacillus ferroxidans	Cu	84.62%	
	Bacillus subtilis	Cu	58%	
Airlift Bioreactors	*Trichoderma viridae*	Cr	94.3%	Morales *et al*., 2006
	Scenedesmus incrassatulus	Cr	43.5%	Jacome *et al*., 2009
Stirred Tank Bioreactors	*Rhizopus arrhizus*	Cr	70.5%	Prakasham *et al.*, 1999
	Trichoderma viridae	Cr	60%	Morales *et al*., 2006

Table 2. Plant bioreactors proficiently used for synthesis of nanoparticles.

Plant Extracts used	Metal Nanoparticles	Size and Shape	References
Pelargonium graveolens	Gold	20-40 nm decahedral	Shiv Shankar *et al*., 2003.
Cymbopogon flexuosus	Gold	0.05-18 μm nanotriangles and nanospheres	Shiv Shankar *et al*., 2003.
Azadirachta indica	Gold	50-100nm triangles	Shiv Shankar *et al*., 2004.
Juice of Azadirachta indica	Silver	5-25 nm spherical	Shiv Shankar *et al*., 2003.
Leaves of Aloe barbadensis	In2O3	5-50 nm cubic	Maensiri *et al*., 2008
Nicotiana benthamiana	Iron	500 nm	Makarov *et al*., 2014
Nicotiana benthamiana	Gold	50 nm	Makarov *et al*., 2014
Nicotiana benthamiana	Silver	100 nm	Makarov *et al*., 2014
Lawsonia inermis	Gold	21-30 nm anisotrophic	Kasthuri *et al*., 2009
Lawsonia inermis	Silver	21-30 nm spherical	Kasthuri *et al*., 2009
Oscimum basilium	Silver	n.d.	Ahmad *et al*., 2010
Cinnamomum zeylanica	Silver	n.d.	Singh *et al*., 2010.
Pinus thunbergii	Silver	n.d.	Velmurugan *et al*., 2013
Tridax procumbens	Silver		Dhanlakshmi and Rajendran, 2012.
Nerium oleander	Silver	n.d.	Suganya *et al*., 2013
Euphorbia nivulia	Silver	n.d.	Valodkar *et al*., 2012
Cyamopsis tetragonoloba	Silver	n.d.	Pandey *et al*., 2012.
Osimum sanctum	Platinum	n.d.	Soundarrajan *et al*., 2012.
Sesbania drummondii	Gold	n.d.	Makarov *et al*., 2014

n.d.: Not defined

play important role in reduction of metal ions (Makarov *et al*., 2014).

"Green" synthesis of nanoparticles using plants as bioreactors is of more interest and these nanoparticles are employed in various practical applications like;

i. These nanoparticles good antimicrobial activity against *E.coli, Vibrio chlorae* (Dhanlakshmi and Rajendran, 2012).
ii. Exhibit antibacterial activity against gram positive and gram negative bacteria like *Psuedomonas syringae, Xenthomonas oryaze* (Velmurugan *et al*., 2013).
iii. Cytotoxic activity against many tumor cell lines.
iv. Strong larvicidal activity against larvae of malaria vector *Anopheles stephensi* (Suganya *et al*., 2013).
v. Inhibit the survival and growth of HeLa cell lines (Valodkar *et al*., 2012). .
vi. Toxic to A549 cell line of human lung cancer (Valodkar *et al*., 2012).
vii. Contains catalytic activity and used in production of hydrogen fuel elements (Soundarrajan *et al*., 2012).
viii. Act as biosensor to determine ammonia which has applications in agriculture and biomedicines (Pandey *et al*., 2012).
ix. Convert highly toxic aromatic compounds into less toxic as 4-nitrophenol to 2- nitrophenol.

Conclusion

Bioreactors are systems that sustain and enable growth of biological active organisms in the controlled environment for the production of active substances. Other than these utilities they can be used in method of bioremediation particularly for the biosorption of heavy metals. A step forward is the utilization of nano-particles and in particular nano-particles which are produced by the method of green synthesis in bioreactors with the same objective. Studies are being undertaken and the adoption of green nano-particles will revolutionize the process of bioremediation.

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