academicJournals

Vol. 13(17), pp. 1756-1771, 23 April, 2014
DOI: 10.5897/AJB2013.13311
Article Number: 5139C8044266
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Review

Review of challenges in the escalation of metal-biosorbing processes for wastewater treatment: Applied and commercialized technologies

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Received 24 September, 2013; Accepted 14 April, 2014

Despite the eminent consequences, wastewaters containing toxic heavy metals are still discharged in the environment without prior treatment, certainly because of the high cost associated with the effective management of these effluents. Difficulties emanated from the fact that most of the technologies available for the remediation of the pollution require high capital and operational cost, which cannot be justified for the treatment of diluted effluents. Biosorption technique for its relatively competitive cost has been immensely investigated in laboratories for remediation of heavy metals polluted effluents. This paper reviews the major progresses achieved in the field of biosorption technology since it was first introduced; enhancement of the performance of suitable biosorbents through regeneration and immobilization techniques are some of the approaches that have contributed to improve metal removal processes. Despite some few progresses, efforts devoted in the development of biosorption technology have not been translated into successful implementation everywhere, hence, the persistence of problems related to pollution of water sources by toxic heavy metals in most part of the world. Challenges hindering the commercialization of biosorption technology are clearly discussed and critical aspects related to the characteristics of waste effluents and potential of biosorbents are highlighted. Suggestions are made for consideration of hybrid technologies, carefully designed and informed by the complexity of waste effluents.

Key words: Mine waste, toxic heavy metals, biosorption technology, immobilization, desorption, hybridization of techniques, escalation of processes.

INTRODUCTION

The dispersion in the environment of toxic heavy metals mostly from hydrometallurgical plants has led to deterioration of the quality of water sources (Figure 1) all over the world. The presence of toxic heavy metals in ground and surface waters represent a serious threat to human health, especially in developing countries where communities in rural and poor urban areas rely on untreated water for their basic needs.

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Conventional methods or physic-chemical methods such as ion exchange, activated carbon, and electrodialysis used for removal of metals from solutions have been effective in some applications. However metal pollution of environmental waters could not be effectively remediated with such techniques because of the relatively low level of metals in such solutions. Hence, researches carried out over the years have mainly focused on ecological and cheap approaches to resolve the problem. A host of biosorbents have so far been tested for their potential in metal uptake with various successes (Volesky and Holan, 1995; Zhou et al., 1998; Matsunaga et al., 1999; Murphy et al., 2007; Vijayaraghavan and Yun, 2008). The extensive and desperate search for adequate biosorbents has led researchers to investigate the potential of microorganisms. Microorganisms have a large surface area, quick growth and can be obtained at relatively low-cost from their biotope or as industrial wastes (Park et al., 2010). Their cell surface contains identified active groups mainly responsible for passive/chemical uptake of metal from their environment. They can also sequester metal into the cytoplasm by active mechanism involving specific proteins (Fosso-Kankeu et al., 2011). Bacteria, fungi, algae and seaweeds are among the microorganisms often used in biosorption processes; although exhibiting different affinities for metals, microbial biosorbents have shown better adsorption capacities than other sorbents in most cases (Vieira and Volesky, 2000). However, most of experiments conducted at bench scale have never been escalated to industrial level, for various reasons. First of all, the use of living cells has mainly suffer the drawback of cell inhibition and cost of media from production; on the other hand, some dead biomasses collected from the environment have lower adsorption capacities (Wang and Chen, 2006; Volesky, 2001) and pretreatment required to obtain suitable biosorbents with high adsorption capacity will enhance the process cost. The quest for better microbial sorbent over the years has motivated consideration of new technical approaches which are intended for the improvement of the adsorption potential of microbial sorbents and increase of their life span. For example the idea of immobilization of cells was suggested by many researchers for minimization of cells degradation and facilitation of solid/liquid separation (Vijayaraghavan and Yun, 2008; Wang and Chen, 2009); while, the regeneration and reuse of cells has been considered in biosorption processes for the reduction of the cost of biomass production. Despite significant improvement/optimization of biosorption processes at bench scale, numerous attempts to escalate the process at industrial level have been unsuccessful.

This review extensively covers current challenges in the application of biosorption technology at large scale, and then substantiates the different scientific approaches which have been contemplated for improvement of biosorption processes. Reasons for failure in the escalation of biosorption processes are discussed and remedies suggested based on successful cases of commercialization of the technology.

THE POTENTIAL OF MICROORGANISMS FOR METAL UPTAKE

Prior to its uptake, metal ions firstly come into contact with the cell membrane of microorganism. It is reported (Kefala et al., 1999) that in the first five to fourty minutes of exposure, the passive or physic-chemical mechanism is mainly responsible of the removal of metal ions from solution. This mechanism occurs through interaction of metal ions with the functional groups present on the cell surface. Although extracellular polysaccharides of all bacteria are involved in metal binding (McLean et al., 1992), the main components responsible for metal-binding capacity of the cell wall in Gram-positive bacteria are anionic functional groups present in the peptidoglycan, teichoic acids and teichuronic acids, while in Gram-negative bacteria, peptidoglycan, phospholipids and lipopolysaccharides play the major role (Vijayaraghavan and Yun, 2008).

The cell walls of fungi and algae are different in chemical composition from prokaryotic cell walls. The cell walls of fungi are dominated by polysaccharide completed with proteins, lipids, polyphosphates and inorganic ions. Algal cell walls are similar to the one of fungi in structure and contain xylans, pectin, mannans, alginic acids or fucinic acid.

The functional groups which are directly responsible for the binding of metals at the surface of all the microorganisms have been listed by Talaro and Talaro (2002) and include: hydroxyl, carboxyl, amino, ester, sulfhydryl, carbonyl terminal end, carbonyl internal and phosphate. These groups mainly influence cell surface affinity for ligands as illustrated in the Table 1.

Metal binding to cell wall occurs through two basic mechanisms: stoichemistry interaction between the metal and the reactive chemical groups (phosphate, carboxyl, amine, phosphodiester etc) in the cell wall, and inorganic deposition of metals (Gupta et al., 2000). The passive adsorption of metal species by various functional groups on microbial cell wall includes non-metabolic mechanisms such as ion exchange, complexation, chelation, coordination, microprecipitation and reduction (Volesky, 1990a, b; Liu et al., 2002).

As discussed above, microorganisms have physiological characteristics suitable for the removal of metal from solutions; however the performance of microbial sorbents may vary depending on the strain used and the metal in solution. This is mainly due to the fact that microorganisms may have different active groups on their cell wall and the extracellular polymeric substances (EPS) produced or excreted by some of them are often not identical (Wang and Chen, 2006). This may explain why some microbial sorbents outperform others; Genre of *Bacillus*

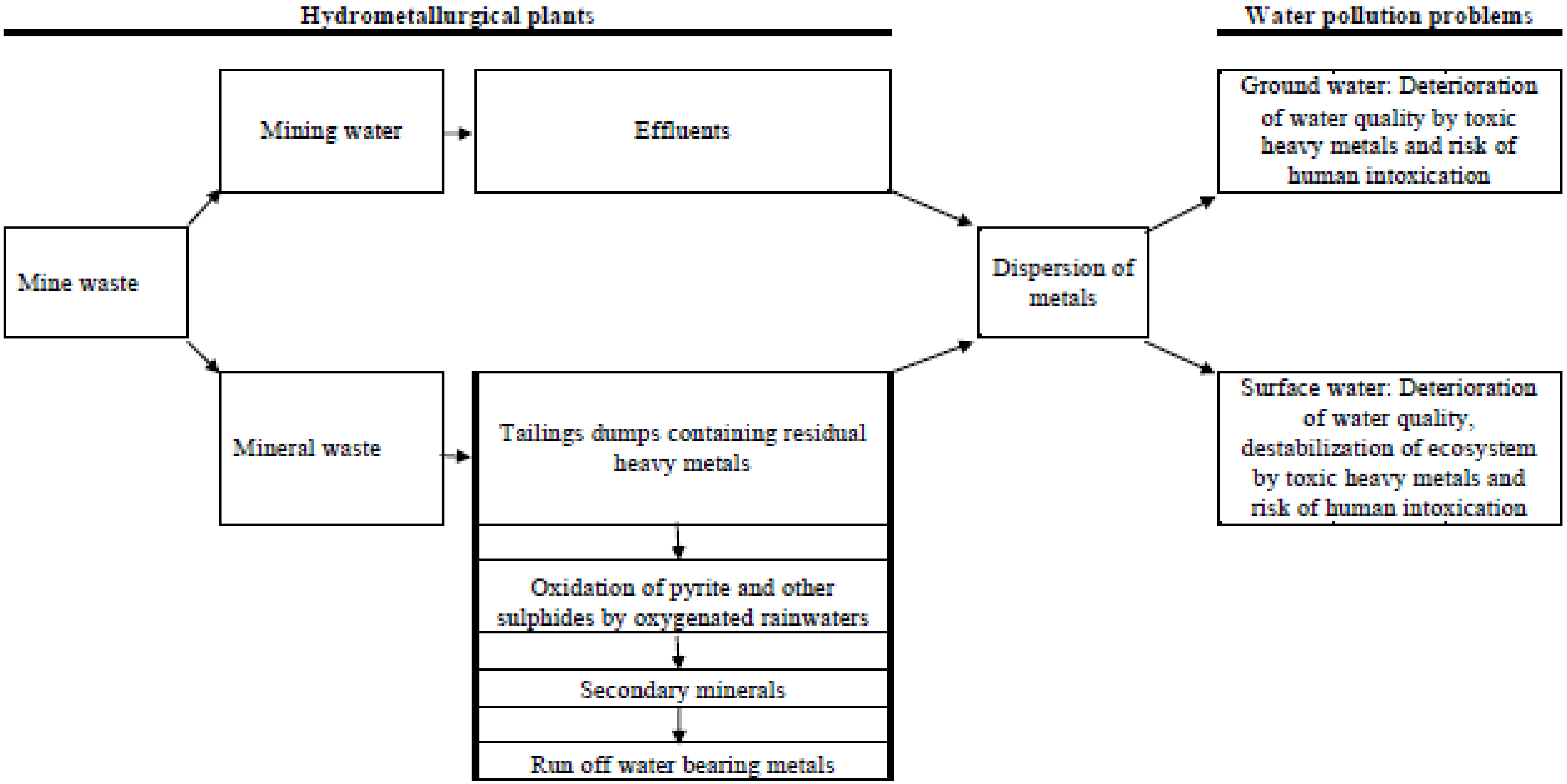

Figure 1. Dispersion of toxic heavy metals from mine wastes

Table 1. The ligands present in biological system and three classes of metals (Remacle, 1990).

Ligand class	Ligand	Metal class
Ligands preferred to Class A	F^-, O^{2-}, OH^-, H2O, CO_3^{2-}, SO_4^-, $ROSO_3^-$, NO_3^-, HPO_4^{2-}, ROH, $RCOO^-$, C=O, ROR	Class A: Li, Be, Na, Mg, K, Ca, Sc, Rb, Sr, Y, Cs, Ba, La, Fr, Ra, Ac, Al, Lanthanides, Actinides
Other important ligands	Cl^-, Br^-, N_3^-, NO_2^-, SO_3^{2-}, NH_3, N_2, RNH_2, R_2NH, R_3N, =N-, -CO-N-, R, O_2, O_2^-, O_2^{2-}	Borderline ions: Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Cd, In, Sn, Sb, As
Ligands preferred to Class B	H^-, I^-, R^-, CN^-, CO, S^{2-}, RS^-, R_2S, R_3AS	Class B: Rh, Pd, Ag, Lr, Pt, Au, Hg, Tl, Pb, Bi

and *Pseudomonas* among bacteria, *Aspergillus*, *Rhizopus* and *Penicillium* among fungi have been identified as good biosorbents (Bai and Abraham, 2003; Tan and Cheng, 2003; Park et al., 2005; Binupriya et al., 2006; Tunali et al., 2006; Uslu and Tanyol, 2006; Vijayaraghavan and Yun, 2008). As far as selectivity of metal is concerned, the physio-chemical nature of metal and active groups on cell surface makes biosorption selective. The affinity or the tendency of microorganisms to preferably bind a given metal is influenced by factors such as the ionic radius and the electronegativity (Mattuschka and Straube, 1993; Brady and Tobin, 1995; Chong and Volesky, 1995).

The polarizing power of a cation increases as the ionic radius decreases, implying that lower ionic radius may lead to reduce biosorption; on the other hand, the electronegativity which represents the ability of an atom to attract electron for the formation of ionic bond, increases with the affinity of microbial biomass for a metal (Allen and Brown, 1995; Brady and Tobin, 1995; Gabr et al., 2008). Mattushka and Straube (1993) observed that biosorption was selective when attempting to remove metals using *Streptomyces* waste biomass. The equilibrium constant of the Langmuir isotherm was reported (Gabr et al., 2008; Baysal et al., 2009) to positively correlate to the strength of the binding sites on the biomass surface and has been used to illustrate the sorption affinity of microbial biosorbents for metal ions. Gabr et al. (2008) established a positive correlation between an increase of the equilibrium constant with the sorption affinity of *P. aeruginosa* ASU 6a for lead, while on a similar basis Fosso-Kankeu et al. (2011) showed selective binding of heavy metal ions (Ni^{2+} and Co^{2+}) on Bacillaceae bacterium in the presence of light metal ions (Ca^{2+} and Mg^{2+}). Sorption of metal on EPS produced by some microorganisms was also found to be selective; Greater selectivity of copper over cobalt was observed by Jang et al. (1995) when performing competitive uptake on Na-alginate extracted from *M. pyrifera* or *L. hyperborea*. This selectivity was attributed to the guluronic content, as also confirmed by the works of Figueira et al. (1997) and, Haug and Smidsrod (1965; 1967).

Intracellular uptake of metals or bioaccumulation is used by living cells for the uptake of metal from the environment and differs in biosorption mechanism. It can occur through two uptake systems; a fast, unspecific and constitutively expressed system which is driven by the chemiosmotic gradient across the cytoplasmic membrane of bacteria; the second system is much more slower, specific and is a metabolic process that requires hydrolysis of adenosine triphosphate (ATP) as source of energy. Metals Sequestered from the environment interact and bind to specific proteins or chelatins and are transported into the vacuoles and other intracellular sites. A number of protein families are involved in the transport of metals into the cytoplasm of microorganisms; these proteins are specific and are not found in all bacteria. However, they can be engineered in foreign strains, especially to improve their tolerance for toxic heavy metals. The *cnr* operon which is originally present in *Cupriavidus metallidurans* and mediates resistance to nickel and cobalt was recently expressed by Fosso-Kankeu et al. (2012) in foreign Gram-positive strain namely Bacillaceae bacterium to improve their capacity to uptake nickel from solution. This innovative approach of expressing the Resistance-Nodulation-Cell division in Gram-positive strain was inconclusively suspected to be the result of a mutation.

Bioaccumulation is a slower process as compared to biosorption (Malik, 2004; Vijayaraghavan and Yun, 2008). In growing/living cells, metal uptake is a biphasic process, starting with the rapid phase of biosorption, followed by a slower phase of bioaccumulation. There is a conflicting opinion about which of the living or dead cells are better biosorbents for remediation processes. Some researchers (Vijayaraghavan and Yun, 2008) argued that biosorption has a higher degree of uptake, while other findings (Matsunaga et al., 1999; Perez-Rama et al., 2002; Malik, 2004) suggest that intracellular accumulation of metals such Cd by growing culture of marine microalgae accounts for most of the uptake.

MICROBIAL SORBENTS AS LIMITING FACTORS IN BIOSORPTION PROCESSES

The increasing volume of polluted effluents discharged in the environment by industries requires appropriate measures to effectively address the problem. The limitations of the conventional techniques with regards to the treatment of large volume of diluted effluents could not be over emphasized; better technologies are too costly to be used for daily running of bioremediation plants. In addition, chemical treatment often results in the production of toxic sludge and waste by-products. All these disadvantages have led to exploration of alternative method namely biological treatment for removal of toxic metal from effluents. This method presents a number of advantages such as low cost (biosorbents are abundant materials easily produced or collected as waste biomass from industries), high adsorption capacity of biosorbents, possible regeneration of biosorbents and recovery of metals and no sludge produced during the process. This has positioned biosorption as one of the most attractive Techniques for remediation of polluted effluents. However, several attempts to implement laboratories' concepts at large scale processes did not meet the predicted expectations for reasons still speculated and under investigation. Few of those reasons will be discussed in this review followed by palliative measures undertaken for substantial improvement of the process.

It has been observed that the use of living cells requires extra cost for their production, making the process relatively costly. On the other hand, living cells are susceptible to harsh environmental conditions such as extreme pH and temperatures as well as relatively high concentration of metals; therefore the use of living biomasses for metal uptake can be affected by the inhibition of cells (Eccles, 1995). During investigation, Tangaromsuk et al. (2002) observed growth inhibition of *Sphingomonas paucimobilis* biomass exposed to 25 to 200 mg/L of cadmium. Fosso-Kankeu et al. (2012) reported reduction of bacteria surface area following inhibition of living cells by relatively high concentration of nickel; this affected the adsorption capacity and the prediction of microbial sorbent behaviour.

Studying the bioaccumulation of copper (II) and nickel (II) by the non-adapted and adapted growing *Candida* sp., Donmez and Aksu (2001) made and important finding; the adsorption capacity of both cells decreased with increase in initial concentration of metals; while the copper's uptake capacity of adapted *Candida* sp. (36.9 mg/g dry weight) was greater than the one of non-adapted *Candida* sp. (23.1 mg/g dry weight) at relatively higher initial concentrations of copper.

Although adequate for prediction of adsorption performance at the bench scale, the use of suspended biomasses is unsuitable for practical application, because of: 1) low mechanical strength: harsh conditions are likely to degrade microbial sorbents and therefore affect the stability and chemical structure of active groups on cell surface; 2) easy cell loss: free cells are difficult to control and are likely to contaminate the treated water, this may come up with solid-liquid separation problems; 3) ineffective recovery of metals: It will be difficult to recover all the metals adsorbed at the surface of the biomass, since loaded Biomasses are dispersed in the solution; and 4) Inability to regenerate/reuse biosorbents: as the biosorbents are degraded under extreme conditions, their adsorption capacity will be considerably reduced.

One of the predicted advantages of biosorption was the lower cost of the process mainly due to the possibility of using waste biomass from the environment. However it has been noticed that some of the waste biomasses are often supplied wet and there is a need of drying the raw biomasses to prevent its degradation during storage (Volesky, 2007). Furthermore, some of the waste biomasses such as *Saccharomyces cerevisiae* from fermentation industries, have low adsorption capacity because of the transformation undergone in the industries and are therefore considered as mediocre biosorbents in comparison to other biomasses (Volesky, 1994; Bakkaloglu et al., 1998; Wang and Chen, 2006).

DEVELOPMENTS IN BIOSORPTION TECHNOLOGY

After various attempts of biosorption experiments using hundreds of biosorbents for adsorption of metals under different Physic-chemical conditions, it has emerged that optimum conditions observed at bench scale are not sufficient to achieve the objectives specified for the treatment of wastewater in the bioremediation plants. To address some of the limitations elucidated above, researchers have explored methods to stabilize biosorbents, minimise their production costs and increase the chance of recovery of valuable metals.

Immobilization

For the reasons already discussed, free cells are not adequate for application of biosorption processes; it is therefore important to attach microbial sorbent to suitable matrix prior to use in conventional unit systems, such as packed/fluidized bed reactors and continuous stirred tank reactors; therefore ensuring optimum uptake capacity and reuse over several cycles.

There are a number of techniques used for immobilization (Table 2) of microbial biosorbent and they vary according to the physical mechanism exploited; these techniques include: entrapment within a porous matrix, cross-linking induced by chemical agents; encapsulation which is similar to entrapment, but the microbial sorbents are free-floating within a capsule-like membrane walls; and attachment or adsorption on inert carriers. The latter involves weak forces such as van der Waals forces, ionic and hydrophobic interactions and hydrogen bonds which are responsible for the formation of bonds (Flickinger and Drew, 1999; Guisan, 2006; Kumar, 2009).

The varied chemistry and the nature of biomass as well as the polymeric matrices required that sufficient care is taken during the choice of the immobilization matrix to carry out biosorption processes at industrial scale. Immobilization techniques have been used for enhancement of microbial sorbents for removal of different metal ions from synthetic solutions or real environmental wastewaters. A number of polymeric matrices including poly (vinyl formal), polysulfone, polyurethane, alginate, polyacrylamide, k-carrageenan and polyethylenimine (PEI) were used in the laboratory to immobilize Rhizopus oryzae in an attempt to improve biosorption of copper (Al-Hakawati and Banks, 2000). Developed porous polysulfone beads containing immobilized nonliving biomasses were used by the US Bureau of Mines, Salt Lake City Research Center for extraction of metal contaminants from wastewaters (Jeffers et al., 1991; Jeffers and Corwin, 1993; Beolchini et al., 2003). One of the main challenges often encountered following immobilization of microbial sorbents is the mass transfer resistance which slow the attainment of equilibrium; a delay in the attainment of equilibrium was observed by Vijayaraghavan et al. (2007) during the uptake of dye using *C. glutamicum* immobilized within a polysulfone matrix. Several works have reported improvement of the adsorption capacity of biosorbents after immobilization on matrices (Table 3), however the cost of immobilization together with other disadvantages (Table 2) have to be carefully considered

Table 2. Available techniques for the immobilization of biomass.

Technique	Examples of matrices/reagent	Advantage	Disadvantage	Reference
Entrapment	Polysulfone, alginate, polyurethane, polyacrylamide, etc	Known cheap	Mass transfer resistance	Gorecka and Jastrzebska, 2011; Park et al., 2010; Volesky, 2001; Gilson and Thomas, 1995; Jeffers et al., 1993; Trujillo et al., 1991
Cross-linking	Nitroacetic acid, epoxides, ethylene glycol diglycidiyl ether (EGDE), glutaraldehyde, divinylsulfone, formaldehyde, etc	Increased strength	Loss of activity, not universal	Gorecka and Jastrzebska, 2011; Park et al., 2010; Volesky, 2001; Leusch et al., 1995
Encapsulation	Alginate, chitosan, maltodextrin, cellulose, etc. Often coated with chitosan, polyvinyl acetate (PVA), gelatin, etc	Prevent biosorbents leakage, higher catalyst densities	Mass transfer resistance, fragile capsules	Gorecka and Jastrzebska, 2011; Park et al., 2010; Volesky, 2001; Chang, 1992; Chang, 1995
Adsorption	Active charcoal, ceramic, glass bead, sand, carbon nanotubes (CNTs), etc	Higher biomass loading, simple and cheap technique	Possible leakage of biosorbents, unstable binding	Gorecka and Jastrzebska, 2011; Park et al., 2010

and addressed for successful application of biosorption at industrial level. Polysulfone beads were identified by few researchers (Jeffers et al., 1991; Veglio et al., 1998; 1999) as suitable immobilization matrix because of their lower cost and mechanical strength. Recently, some researchers attempted to use carbon nanotubes (CNTs) as immobilizing support (Liu et al., 2009). CNTs are divided into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs), which are considered as good candidates adsorbers because of their hollow and layered nanosized structures (Chen et al., 2009; Liu et al., 2009; Ren et al., 2011).

Regeneration and reuse of biosorbents

Among the available and realistic ways of minimising the cost in the application of biosorption process and still achieving the maximum removal of toxic metals from wastewater, regeneration and reuse of biosorbents are of utmost importance as they provide the opportunity not only to cut down the cost of biosorbents, but also to recover valuable and scarce metals. It is however equally important to select good quality eluent, which is less aggressive to the biomass, effective and cheap. Different categories of reagents susceptible to serve as eluting agents are available on the market and include mineral acids (HCl, H_2SO_4, HNO_3, H_3PO_4, etc), alkalis (NaOH, NH_4OH, etc.), organic solvents (ethanol, methanol, acetone, etc) and others (EDTA, Na_2CO_3, $CaCl_2$, $KHCO_3$, KSCN, etc.). These reagents all have the potential to unbind the metal from the biosorbents, but the mechanism of desorption may vary; three basic mechanisms of desorption have been identified by some authors (Kapoor et al., 1999; Lezcano et al., 2011): Formation of insoluble compound with eluent can lead to precipitation of immobilized metal (example of eluent: H_2SO_4); Competition among ions for the binding sites on loaded biomass may lead to the displacement of the immobilized ion by the ion in solution, it is an ion exchange mechanism (example of eluents: mineral acids, alkalis, etc.); Sharing of electron between immobilized metal and eluent often results in complexation (example of eluents: EDTA, Na_2CO_3, etc.).

For desorption of cation, mineral acids are often preferred as they can quickly wash off the metals from the loaded biomass and produce metal rich effluent suitable for economic exploitation (Volesky, 2007; Lezcano et al., 2011). The main problem with mineral acids is the aggressiveness toward the biomass which can diminish the capa-city of the biosorbent in the next cycle. Desorbing heavy metals from loaded biomass of *Sargassum* seaweed, Davis et al. (2000) observed that was-hing with solutions of HCl rendered the biomass more fragile. Although large amount of immobi-lized metals can be easily desorbed with large volume of eluent, rapid elution, using the smallest quantity of eluent possible is desirable, in order to achieve high concentration of metals in minimum volume of effluent. Hence the need for the researcher to optimize the elution process through a trial experiment that will enable the adequate solid/liquid ratio (solid representing the loaded sorbent and the eluent being the liquid). Desorbing loaded biomass of *Sagassum* seaweed with calcium

Table 3. Performances of some polymers as immobilizing matrices of biosorbents for metal uptake.

Biosorbent	State of biosorbent	Biosorption technique	Matrix	Best matrix	Immobilization techniques	Maximum adsorption capacity first cycle	References
Aspergillus niger	Dead cells, powder form	Column	Polysulfone	Polysulfone	Adsorption	Cd(3.6 mg/g), Cu(2.89 mg/g), Pb(10.05 mg/g), Ni(1.08 mg/g)	Kapoor and Viraraghavan (1998)
Sargassum duplicatum	Dead cells, powder form	Column	Silica gel	Silica gel	Adsorption	Cu(280.1 µmol/g), Cd(130.5 µmol/g), Pb(113.7 µmol/g)	Suharso et al. (2010)
Ulva lactuca	Dead cells, powder form	Column	Agar	Agar	Adsorption	Cu(0.85 mmol/g), Zn(0.35 mmol/g), Cd(0.41 mmol/g), Pb(1.55 mmol/g)	Areco et al. (2012)
Phormidium laminosum	Dead cells, powder form	Batch	Polysulfone, epoxy resin	Polysulfone	Adsorption	Cu(19.83 mg/g), Fe(17.83 mg/g), Ni(16.1 mg/g), Zn(18.05 mg/g)	Blanco et al. (1999)
Pseudomonas fluorescens, *Microbacterium oxydans* and *Cupriavidus* sp	Living cells	Batch	Hydroxyapatite	Hydroxyapatite	Adsorption	Zn(0.433 mmol/g), Cd(0.09 mmol/g)	Piccirillo et al. (2013)
Phaseolus vulgaris L.	Plant, powder form	Column	Silica gel	Silica gel	Entrapment	Ni(0.0017 mol/g)	Akar et al. (2009)
Bacillus strain CR-7	Dead cells, powder form	Batch	Sodium alginate, gelatin, polyvinyl alcohol	Sodium alginate (2%)		Cu(19 mg/g)	Xu et al. (2011)
Cupriavidus, *Sphingobacterium*, *Alcaligenes*	Living cells	Column	Alginate, pectate and a synthetic cross-linked polymer	Synthetic cross-linked polymer	Entrapment	Zn(1.7 mM/g), Cd(0.9 mM/g)	Pires et al. (2011)
Mentha arvensis	Living cells	Batch	Sodium alginate (2%)	Sodium alginate (2%)	Entrapment	Cu(104.48 mg/g), Zn(107.75 mg/g)	Hanif et al. (2009)
Phanerochaete chrysosporium	Living cells	Batch	Iron oxide magnetic nanoparticles (MNPs) and Ca–alginate	Iron oxide magnetic nanoparticles (MNPs) and Ca–alginate	Entrapment	Pb(185.25 mg/g)	Xu et al. (2013)
Rhizopus nigricans	Dead cells, powder form	Batch	Calcium alginate, polyvinyl alcohol (PVA), polyacrylamide, polyisoprene, and polysulfone	Polysulfone	Entrapment	Cr(VI) 119.2 mg/g	Bai and Abraham (2003)

Table 3. Contd.

Trichoderma viride	Dead cells, powder form	Column	Ca-alginate	Ca-alginate	Adsorption	Cr(6.9 mg/g), Ni(6.9 mg/g), Zn(4.95 mg/g)	Kumar et al. (2011)
Chlorella sp. and *Chlamydomonas* sp.	Living cells	Batch	Sodium alginate	Sodium alginate	Entrapment	Cu(33.4 mg/g), Zn(28.5 mg/g)	Maznah et al. (2012)
Sargassum baccularia	Dead cells, powder form	Batch	Polyvinyl alcohol	Polyvinyl alcohol	Adsorption	Cu(5 mg/g)	Hashim et al. (2000)

salt, Davis et al. (2000) observed a decrease from 95% to less than 50% elution efficiency after increase of solid/liquid ratio.

Desorption has been performed on suspended as well as immobilized cells. It is quite easy to elute metal from suspended cells than from immobilized cells (Atkinson et al., 1998); however suspended cells are more susceptible to degradation and therefore reduction of adsorption capacity. Using 1% (w/v) $CaCl_2$/HCl-solution at pH 3 to desorb suspended-loaded biomass of *Sargassum*, Volesky et al. (2003) achieved a desorption efficiency around 95% for a maximum of seven cycles, but the biomass loss was 21.6%. In a separate study, Bai and Abraham (2003) investigated chromium (VI) adsorption-desorption on immobilized fungal biomass; using the best matrix (polysulfone), they observed that the biomass beads could be regenerated and reused in more than 25 cycles and the regeneration efficiency was 75 to 78%.

The physiological state of microbial sorbent (dead or leaving cells) and the affinity of the biosorbent for the metal are parameters that can affect the effectiveness of regeneration and reuse of biosorbent (Table 4).

Although total desorption of sorbates attached to biosorbent through passive (metabolic independent) mechanism are easily achieved, it is not the case when the sorbates are inside the cell (bioaccumulation in living cells). Total recovery of intracellular bound metals is possible only if the cells are destroyed using techniques such as incineration or dissolution into strong acids or alkalis (Park et al., 2010).

It is known that ineffective desorption of biosorbent (that may result from high affinity of sorbate or weakness of eluent) diminishes the adsorption capacity in the next cycle as residual ions on biosorbent's surface occupy the binding sites and will compete with ions in solution. On the other hand, if the eluent is too strong, it will result in low displacement of desorbing ions during the next cycle. The need to evaluate the performance of different eluents at various dose prior to selection, should therefore be emphasized.

Hybridization of techniques

Improvement of biosorption processes through immobilization and/or regeneration of biosorbents has not yet contributed to the success expected. In regards of the outcomes of the number of attempts by researchers exploiting these avenues to substantiate the benefits of the unequivocal potential of biosorption, it could be said that alternative measures suggested by previous authors (Tsezos, 2001; Malik, 2004; Wang and Chen, 2006; 2009) and consisting of the use of hybrid technologies (intrabiotechnological or intertechnological) involving biosorption have to be seriously considered and thoroughly investigated. The heterogenic nature of environmental waste waters which are contaminated not only with toxic heavy metal ions, but also with considerable amount of light metal ions, organic matters and therefore higher total dissolved solids. All these can significantly interfere with the performance of the biosorbents and biosorption system; Light metal ions will compete with the ions of interest for the binding sites while the organic matters and other solids in solution will contribute to rapid clogging of the column or saturation of biosorbent surface (Vijayaraghavan and Yun, 2008). It is therefore clear that any venture in the treatment of environmental waste waters relying on the unique application of biosorption will hardly succeed in achieving the effective cleanup of metal pollutants.

An approach based on the simultaneous or consecutive application of different techniques bearing in mind the affinity principle, could help address the above challenges in an integrated and effective manner. A number of techniques that can be combined with biosorption (Figure 2) have been suggested and include bioprocesses such as bioreduction and bioprecipitation with other processes namely electrochemical processes, chemical precipitation, flotation, membrane technology and more (Tsezos, 2001; Wang and Chen, 2006). Successful removal of inorganic and organic pollutants from environmental effluent was achieved by Diels et al. (2001) using a combination of metal biosorbing and bioprecipitating bacteria in moving bed sand filters (effective bed height, 2 m) biofilm. Another hybrid approach

Table 4. Examples of Eluants used for the recovery of metals and regeneration of biomasses.

Biomass	Eluants used	Best eluant(s)	Maximum sorption capacity (cycle 1)	Maximum % desorption (cycle 1)	Maximum Adsorption capacity (last cycle)	Maximum loss of biomass, total number of cycle	Reference
Cyanobacterial mats	HCl, $CaCl_2$, SDS, HNO_3, NaOH	0.1 mM HCl	Pb(1 mM/g), Cu(0.35 mM/g), Cd(0.3 mM/g)	Pb(93.4%), Cu(92.7%), Cd(84.6%)	Pb(1 mM/g), Cu(0.35 mM/g), Cd(0.3 mM/g)	11%, 6	Kumar and Gaur (2011)
Streptoverticillium cinnamoneum	HCl, HNO_3, EDTA, H_2SO_4, Na-citrate, $NaHCO_3$, Na_2CO_3, KCl	0.1 M HCl, 0.1 M HNO_3, 0.1 M EDTA	Pb(57.7 mg/g), Zn(21.3 mg.g)	Pb(10%), Zn(90%)	Pb(43 mg/g), Zn(14 mg/g)	24.3% (Na2CO3), 3	Puranik and Paknikar (1997)
Phormidium laminosum	0.1 M HCl	0.1 M HCl	Cu(950 µg), Fe(900 µg), Ni(750 µg), Zn(900 µg)	Cu(90%)), Fe(85%), Ni(95%), Zn(120%)	Cu(900 µg), Fe(950 µg), Ni(1000 µg), Zn(1000 µg)	(immobilized biomass), 10	Blanco et al. (1999)
Enterobacter sp. J1	0.1 M HCl	0.1 M HCl	Pb(50 mg/g), Cu(32.5 mg/g), Cd(46.2 mg/g)	Pb(90%), Cu(90%), Cd(100%)	Pb(75%), Cu(79%), Cd(90%)	Na, 4	Lu et al. (2006)
Pithophora oedogonia	HCl(0.1 M), HNO_3(0.1 M), NaOH(0.1 M), H_2SO_4(0.1 M), $CaCl_2$(0.1 M), Na_2CO_3(0.1 M), EDTA(0.1 M)	HCl(0.1 M), EDTA(0.1 M)	Cu(23 mg/g), Pb(52 mg/g)	Cu(92.3%), Pb(96.2%)	Cu(12 mg/g), Pb(35 mg/g)	15%, 5	Singh et al. (2008)
Rhizopus nigricans	0.01N of acids, salts, alkalies, deionized distilled water and buffers	0.01 N NaOH, 0.01 N Na_2CO_3, 0.01 N $NaHCO_3$	Cr(VI)44.34 mg/g	Cr(VI)90 to 95%	Cr(VI)15 mg/g	Immobilized biomass-polysulfone), 25	Bai and Abraham (2003)
Sargassum	Hydrochloric, nitric, oxalic and diglycolic acids; calcium nitrate and chloride, EDTA-disodium	0.3 M of HCl	La(0.23 mmol/g), Eu(0.24 mmol/g), Yb(0.24 mmol/g)	La(90%), Eu(100%), Yb(100%)	La(0.25 mmol/g), Eu(0.39 mmol/g), Yb(0.23 mmol/g)	30%, 4	Diniz and Volesky (2006)

reported was conducted in two steps involving the bioleaching of toxic metals (Cu, Ni, Mn) by acidophilic sulphur-oxidizing bacteria in the first step, and precipitation (80 to 98%) of the leached metals in the next step by the activity of sulphate-reducing bacteria in an anae-robic bioreactor

Table 4. Contd.

Saccharomyces cerevisiae	0.1 M of HCl, HNO3, H_2SO_4, CH_3COOH and EDTA	H_2SO_4 pH1	Cr(III) 3.75 mg/g	Cr(III) 52%	Cr(III) 2 mg/g	17.50%, 3	Ferraz et al. (2004)
Spirogyra neglecta	0.1 M HCl	0.1 M HCl	Cu(30.17 mg/g), Pb(49.11 mg/g)	Cu(88.8 %), Pb(97.5%)	Cu(21.24 mg/g), Pb(38.33 mg/g)	Na, 10	Singh et al. (2012)
Streptomyces zinciresistens	0.1 M HCl	0.1 M HCl	Zn(160 mg/g), Cd(65 mg/g)	Zn(87.33%), Cd(98.11%)	na	Na, 1	Lin et al. (2012)
Scenedesmus obliquus CNW-N	0.1 M HCl, 0.1 M NaOH and 0.1 M $CaCl_2$	0.05 M $CaCl_2$	Cd(68.6 mg/g)	Cd(80%)	Cd(60 mg/g)	Na, 5	Chen et al. (2012)
Macrocystis pyrifera	HNO_3, EDTA, $Ca(NO_3)_2$	0.1 M HNO_3, 0.1 M EDTA	Zn(0.91 mmo/g), Cd(0.89 mmol/g)	Zn(99%), Cd(100%)	na	Na, 1	Cazon et al. (2012)
Microcystis	Distilled water, 0.5 M, 1 M, 2 M, 4 M, 6 M and 8 M of HCl	8 M HCl	Sb(III) 85%	Sb(III) 69%	Sb(III) 60%	Na, 5	Wu et al. (2012)

(White et al., 1998; Malik, 2004).

ESCALATION OF BIOSORPTION PROCESSES

Some of the reasons hindering successful implementation of biosorption processes at large scale has been discussed above. Innovative approaches gearing toward transformation of biosorption processes as realistic, reliable, sustainable, accessible and competitive industrial techniques for the treatment of heavy metal polluted waste wasters are still to be invented by researchers.

Efforts applied in the field of biosorption over four decades have not been satisfactorily translated into industrial applications (Tsezos, 2001; Volesky, 2007). Host of innovations patented since the early 80's have certainly contributed in their own right to the development of biosorption. From the understanding of the basic mechanisms of metal uptake by microbial sorbents, to the identification of suitable microbial sorbents, immobilization techniques, regeneration of biosorbents and biosorption process design, a lot has certainly being done to develop biosorption processes; however, there is still a long way to go as far as the implementation of an industrially competitive technique is concerned. Couple of biosorption processes have been tested at pilot scale and few have reached the stage of commercialization.

LARGE SCALE APPLICATION

More than ever, there is a crucial need for eco-friendly techniques capable to operate with natural resources using the minimum of power as possible. The enormous potential of biosorption technique for business opportunity (Volesky (2001) estimated the immediate and existing market for new biosorbents materials in North America to around US$30 million) is the major catalyst for the application and commer-cialization of biosorption-based products, however most of the attempts so far are premature, judging by the low percentage of successes achieved (Tsezos, 2001; Volesky, 2007; Wang and Chen, 2009). As from the early 1990's, the first proprietary biosorption processes or biosorbents were commercialized, and since then a number of attempts have been made to bring more to the market (Table 5). Several companies mainly from North America have developed immobilized biosorbents deriving from

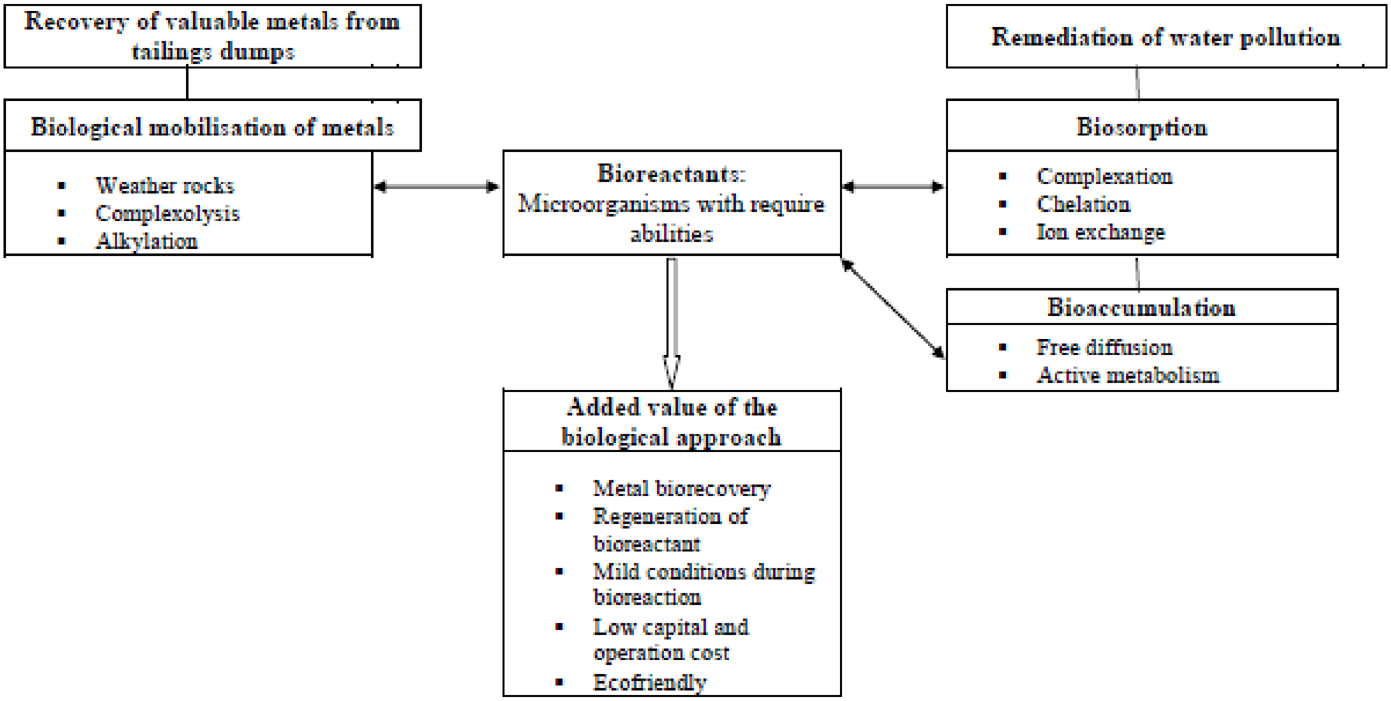

Figure 2. Illustration of hybridization of bioleaching and biosorption for prevention of metal dispersion

different natural raw materials; mainly from algae, yeast and bacteria biomasses. These biosorbents were proven to perform under harsh conditions such as extreme physic-chemical levels (pH, conductivity, temperature, etc.) and adsorbed mainly toxic heavy metals.

These biosorbents often have characteristics allowing great flexibility in engineering process design and applications. There is a number of metal-removal systems designed for industrial application of biosorption processes; these include fixed bed upflow and downflow reactors, fluidised beds, rotating biological contactors, trickle filters and air-lift reactors. Downflow systems are associated with some operational drawbacks, but the fact that they depend on gravitational forces to transfer the water body through the bed, provide a major advantage of lower power consumption over all the other systems requiring pumps or motors for water transfer (Atkinson et al., 1998; Volesky and Naja, 2005). On the basis of several parameters, Volesky and Naja (2005) found that fixed-bed continuous flow columns are the sorption system arrangement suitable for effective biosorption of heavy metals. According to Vijayaraghavan and Yun (2008), the design and type of process to be employed (batch/continuous) is entirely dictated by the choice of biomass and its method of immobilization.

The technological principles for industrial application of biosorption as elucidated above are similar for ion exchange; one could therefore expect that biosorption products will easily outperformed ion exchange products on the market, at least for the treatment of large volume of dilute effluents. However reports show that the ion exchange market is as well established as before and worse, the biosorption technology has been unable to sufficiently penetrate markets such as clean-up operation applications so far non-accessible to ion exchange process (Volesky, 2007; Wang and Chen, 2009). The limitations of industrial application of biosorption are well known and have been reported by several researchers (Tsezos, 2001; Wang and Chen, 2009):

i) Lack of reliable supply of waste microbial biomass suitable for specific application; fermentation industry was reluctant or unable to secure a steady supply of waste microbial biomass as the inexpensive raw material that would be used for production of the new biosorbents.

ii) The cost for producing the required biomass for the sole purpose of transforming this biomass into biosorbents was shown to be too expensive.

iii) The process has become more complex when considering distribution of immobilized biomass, regeneration, recycling and reuse of biosorbents.

iv) Challenges in technology development, researchers require the support of business partners for innovative process venture.

The knowledge and know-how acquired in the process of laboratories' investigations must be complemented with pilot studies and when needed, business expertise for successful commercialization of biosorption products.

Viability of industrial application

Enough care and investigations must be exercised to minimize the risk of failure and maximize the chances

Table 5. Examples of commercialised biosorption techniques.

Commercialized biosorbent	Family of biosorbent	State of cell free/immobilized	Company	Affinity	Application equipment	Reference
AlgaSORB^{TM}	*C. Vulgaris*	Immobilized on silica	Biorecovery Systems	Metallic cations and metallic oxoanions	Two columns operating in serie or in parallel	Chojnacka, 2010; Park et al., 2010; Wang and Chen, 2009
B.V. SORBEX	*S. natans, A. nodosum, Halimeda opuntia, Palmyra pamata, Chondrus crispus and C. Vulgaris*	Powder or granules	BV SORBEX Inc	Specific to toxic heavy metals	Fixed bed system, fluid bed system and completely mixed tanks	Park et al., 2010; Wang and Chen, 2009
AMT-BIOCLAIM^{TM}	Bacillus treated with caustic soda	Immobilized in extruded beads-polyethyleneimine and glutaraldehyde		Suitable for accumulation of gold, cadmium and zinc from cyanide solutions	Fixed bed canisters or fluid-bed reactor systems	Chojnacka, 2010; Park et al., 2010; Wang and Chen, 2009
BIO-FIX ^{R}	Sphagnum, peat moss, algae, yeast, bacteria and aquatic flora	Immobilized in polysulfone	U.S. Bureau of Mines (Golden Colorado)	Selective for toxic heavy metals		Chojnacka, 2010; Park et al., 2010; Wang and Chen, 2009
MetaGeneR				Remove heavy metals from electroplating and mining waste streams		Chojnacka, 2010; Park et al., 2010; Wang and Chen, 2009
RAHCO Bio-Beads	Variety of sources including peat moss	Immobilized within an organic polymer		Remove heavy metals from electroplating and mining waste streams		Chojnacka, 2010; Park et al., 2010; Wang and Chen, 2009

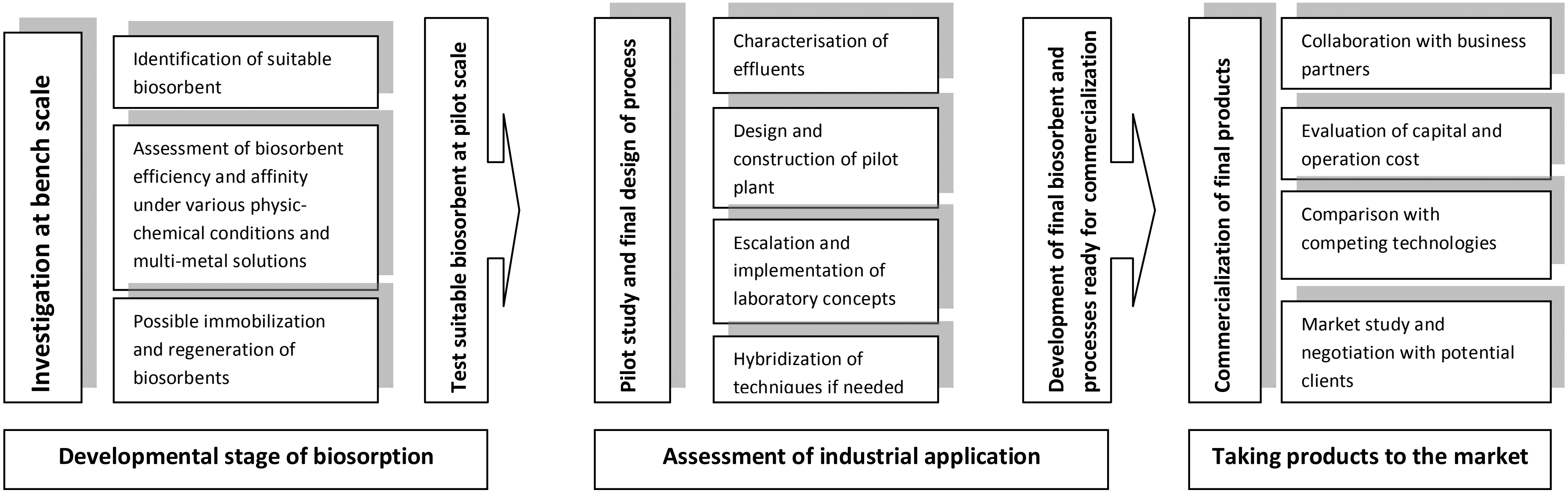

Figure 3. Schematic representation of the pathway of biosorption development.

for successful commercialization of biosorption products. In the process of escalating biosorption process, technical aspects related to the possible application of laboratories' concepts to the treatment of real industrial effluents have to be carefully investigated by constructing pilot plants (Figure 3). Points to be considered during such investigation were extensively discussed by other researchers (Atkinson, 1998; Volesky, 2007; Vijayaraghavan and Yun, 2008; Wang and Chen, 2009) and have to do with the informed selection of biosorbent based on the characteristic of the effluents including the nature of pollutants, the cost and the availability of biomass.

A very important point that requires collaboration with a business partner is the knowledge of the overall commercial potential of the biosorption technology. Strategic approach in positioning biosorption products on the market must be taken, prioritizing the focus on the market share of biosorption technology and the advantages of this new technology over the existing ones. Practically, information on the possibility for biosorption technology to penetrate the market can be acquired by conducting studies based on:

i) Comparison of the cost of the new biosorbent-based technology with the cost of conventional technology for the treatment of a given solution.
ii) Determination of the approximate figure of the market size requiring ecofriendly treatment of metal polluted effluents.

It is important to mention here that, the main struggle with biosorption is undoubtedly at the technological level. There is still a need to develop a finish product (that may include hybrid biological technologies) capable to effectively remedy heavy metal pollution of effluents at competitive cost. To a large extend we have not reached that stage and most of the attempts of commercialization are premature.

CONCLUSION

It is very important for all those who envisage escalating the biosorption processes for comercialization, not to consider applying biosorption as a "unique solution for decontamination of all pollutants in the effluent". One must bear in mind the potential of the biosorbents as well as their limitations. Environmental effluents have a complex physic-chemical characteristic often containing a multitude of pollutants likely to inhibit the action of the biosorbents. The capacity of biosorbents to remove metals is unequivocal, capable and excellent biosorbents have been identified and developed for metal removal and only require to be used under suitable conditions. Characterization of effluents and involvement of hybrid technologies if needed for the pretreatment of effluents must be considered upfront. There is a growing demand for ecofriendly and cheap technologies, hence a huge portion of the market which cannot be covered by conventional technologies; the bottom line is cheap, reliable, effective and an industrial processes (possibly a hybrid biological system involving biosorption) for treatment of toxic metals polluted effluents developed on the basis of effluents characteristics and tested accordingly in pilot plants prior to consideration for commercialization.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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