OVERVIEW OF USING LIVING AND NON-LIVING MICROORGANISMS FOR THE REMOVAL OF HEAVY METALS FROM WASTEWATERS

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Abstract. *Heavy metal pollution is a very serious threat to the natural environment and human health due to the fact that it cannot be remediated through chemical or biological processes. Although these persistent pollutants are harmful even in small concentrations, the problem becomes critical due to the significant issue posed by the bioaccumulation of these pollutants in various living organisms. Furthermore, the industries that generate large quantities of wastewaters containing heavy metals are in an increasing development. Sources of large fluxes of wastewaters containing heavy metals include electrical and electronic waste recycling, mining, electroplating and batteries, pesticides, pigments, dyes and textiles production. Hence, viable, environmentally-friendly, low-cost and efficient solutions are required in order to address this problem. Biomass-based removal methods represent a promising alternative to the conventional ones. The capacity of many biological materials for heavy metals removal from aqueous systems has been analyzed so far owing to the very good preliminary results obtained in related research studies. Microorganisms are very potent in this regard since their cells are highly adaptable to toxic environments. They have chemical structures with affinity to metals and the capacity to transform very toxic metal species into less harmful ones. The main processes that can be applied in wastewater treatment through the use of microbial organisms are biosorption for inactivated forms, bioaccumulation, biotransformation and biomineralization, respectively, for living forms. For these processes to be used successfully, different parameters such as pH, temperature, metal concentration and dosage of microbial biomass have been studied. The current paper gives an overview of the obtained lab-scale results of heavy metals removal from wastewater using both living and non-living microorganisms. The non-conventional methods based on the use of microorganisms (algae, bacteria and fungi), as well as the conventional techniques typically used for heavy metals removal are described and discussed.*

Keywords: *biosorption, bioaccumulation, lab-scale application, heavy metals, microorganisms*

INTRODUCTION

Water has an essential role in the metabolism of living organisms, representing a major component in their fluids, and the majority of biochemical reactions take place in its presence, thus becoming the support of the entire system of life on the planet and an indispensable element for all ecosystems (SINGH, 2017). According to the European Environmental Agency (2018) "*38% of European surface waters have a good status, 46% do not have a good status, and 16% have an uncertain status in terms of water quality*". A relatively small category of persistent pollutants is responsible for these results, the heavy metals being included in this category. The metals with significant environmental impact are mercury, lead, nickel and cadmium. These four heavy metals are included in the list of priority persistent pollutants for which 100 aquatic environments in the European Union do not have a good level in terms of chemical composition (EEA, 2018).

The heavy metals are released in surface water body both by various natural activities, such as volcanic eruptions, but also by various anthropogenic activities (e.g. chemical industry, energy sector, production and processing of metals, waste and wastewater management, etc.). According to European Pollutant Release and Transfer Register (E-PRTR, 2020), in 2017, in European surface waters were released 70.1 tons of As, 13.9 tons of Cd, 834 tons of Cr, 913 tons of Cu, 3.76 tons of Hg, 261 tons of Ni, 86.4 tons of Pb and 1899 tons of Zn, and, for example, 58.6% of Cd, 59.9% of Pb, 10.1% of Cr, 24.7% of Hg and 49.4% of Zn released in waters were from urban wastewater treatment plants. Also, the heavy metals are elements that cannot be metabolized by living organisms and their release in the aqueous environment leads to their bioaccumulation along the food chain, causing negative effects on the environment and human health (DIACONU ET AL., 2020; GAVRILESCU, 2004). The toxic effect of heavy metals depends on their concentration, oxidation form, environmental factors and the type of organism or environment in which the contamination has occurred (DIACONU ET AL., 2020).

In this context, this paper provides an overview of the effects of heavy metals on the environment and human health, as well as the main methods that can be applied for the removal of heavy metals from wastewater, especially by the biological processes which use microorganisms as biosorbents.

EFFECTS OF HEAVY METALS ON HUMAN HEALTH AND THE ENVIRONMENT

Certain metals such as zinc, iron, manganese and copper are important elements in the development of organisms due to the role that they play in enzymatic processes and the maintenance of protein structures. However, the industrial development has determined a large release of heavy metals that have bioacccumulated to concentration levels that overcome the healthy limits (ASLAM ET AL., 2020). Furthermore, some heavy metals such as lead, cadmium and mercury are toxic even in very small concentrations (ROȘCA ET AL., 2015).

Mercury and a few other persistent pollutants that prevail through their spread and impact, limit the achievement of good surface water quality by about 81%. The main sources that prevent the desired quality from being achieved are atmospheric deposits and discharges from treatment plants. Mercury is the main metal limiting the evolution of water remediation efforts in Europe. Urban wastewater treatment plants also generate contamination with mercury, cadmium, lead and nickel. The main countries having a problem of mercury pollution in surface waters are Belgium, Germany, Sweden, Slovenia and Austria (EEA, 2018).

Chromium is present under different oxidation states (from -2 to +6), Cr(0), Cr(III) and Cr(VI) being the only stable forms found in the environmental components. Cr(VI) is highly soluble in aqueous solutions and is one of the most polluting and harmful form to human health, being a carcinogenic and mutagenic (HLIHOR ET AL., 2011, 2013). Cr(III) in low concentrations is needed in the metabolic processes of glucose, stabilization of the tertiary structure of proteins and conformation of DNA and RNA, but Cr(VI) is toxic, even at very low concentrations and has high mobility (DIACONU ET AL., 2020; PAVEL ET AL., 2010). Workers in industries that apply chromium compounds such as stainless steel welding, chromium production, chromium plating and the chromium pigment industry are frequently diagnosed with nasal mucosal irritations, runny nose and bleeding, ulcers and holes in the nasal septum (PAVEL ET AL., 2010).

A source of lead pollution is also represented by electrical and electronic waste. High levels have been detected because of this, including in aquatic environments (ZHANG ET AL., 2016). In China, many children are often exposed, with large amounts of lead being found in blood tests. The level of development in terms of cognitive abilities and other serious conditions are among the negative effects on children's health. Lead is released into the environment by battery and paint industries, and according to USEPA, it has a limit of 15 µg / L in drinking water (PUGAZHENDHI ET AL., 2018). For example, mercury, lead and cadmium do not have a beneficial role in metabolism, they are not metals with important functions in living organisms (RZYMSKI ET AL., 2014). Cadmium is also included in the category of carcinogens. This metal is a cofactor in oxidation reactions, amplifying tissue degradation and the overall negative effect of oxidation in living organisms (DIACONU ET AL., 2020). It causes health problems such as respiratory failure, hypertension, gastrointestinal disorders and osteoporosis (DIRBAZ AND ROOSTA, 2018).

The negative effects of heavy metals on the endocrine system are also known (GAVRILESCU ET AL., 2015). Problems with the kidneys and endocrine system are encountered as an effect of contamination with high concentrations of lead and cadmium (ASLAM ET AL., 2020). Nickel causes skin and respiratory diseases (THYSSEN ET AL., 2013), gastrointestinal problems, lung and kidney problems (NOORMOHAMADI ET AL., 2019).

Average levels of nickel bioaccumulation of approximately 0.99 mg/kg were measured in chestnut leaves (MUREȘAN ET AL., 2019). Low concentrations of mercury, lead, cadmium, chromium and arsenic greatly affect plant development by inhibiting root growth and degradation of plasma membranes and can thus reduce food reserves by significantly decreasing crops (POPOOLA ET AL., 2020).

WASTEWATER TREATMENT METHODS FOR HEAVY METALS REMOVAL

Wastewater treatment is "the complex process whose purpose is to remove and neutralize the dissolved, suspended or colloidal harmful substances present in industrial or urban wastewater before being discharged into aquifers or natural bodies of water such as river, lake, sea, ocean etc." (BARTOLOMEU ET AL., 2018; ENGLANDE ET AL., 2015). After treatment, the quality of wastewaters is improved and can even be reused in certain fields or technological processes. Depending on its source, the composition of wastewater varies significantly, the main pollutants present in all urban and industrial wastewaters in different concentrations are: suspended solids, biodegradable organic matter and nutrients (e.g. nitrogen and phosphorus compounds), non-biodegradable organic matter (e.g. pesticides and detergents), metals (e.g. cadmium, mercury, nickel and lead), dissolved inorganic solids and pathogens. Thus, the toxicity of wastewater depends on pollutants concentrations, as well as their nature (BARTOLOMEU ET AL., 2018). Due to the complex composition of wastewater, in the treatment plants, a combination of physical, chemical and biological processes are used through which the concentrations of pollutants in treated waters are reduced to the allowed legislative limits (BARTOLOMEU ET AL., 2018; PESCOD, 1992). In order of increasing the purity of wastewater, in a wastewater treatment plant, different levels of treatment are applied, known as preliminary, primary, secondary and tertiary or advanced wastewater treatments (PESCOD, 1992). A possible flow chart of a wastewater treatment process containing heavy metals is shown in Figure 1.

In the preliminary stage of wastewater treatment, solids and other large materials are being removed by retention on gratings, sieves, commutators, disintegrators (PESCOD, 1992; BARTOLOMEU ET AL., 2018); in the primary stage fats and oils are removed by flotation, and suspended solids and larger solids (sand, gravel) by decantation (PESCOD, 1992). After these two stages, the wastewater is treated by biological processes which are mainly applied to remove the organic content (secondary stage). The processes take place in biological reactors and are based on the biochemical degradation that naturally occurs in water bodies, such as rivers and lakes. Secondary treatment is performed using activated sludge (suspended biomass) in bioreactors or in lagoons (BARTOLOMEU ET AL., 2018). Sometimes, after the secondary stage, it is necessary to apply a tertiary treatment, such as disinfection for microorganisms removal, or other methods to remove the suspended solids, the excess of nutrients and specific toxic compounds (e.g. heavy metals, persistent organic pollutants) (PESCOD, 1992).

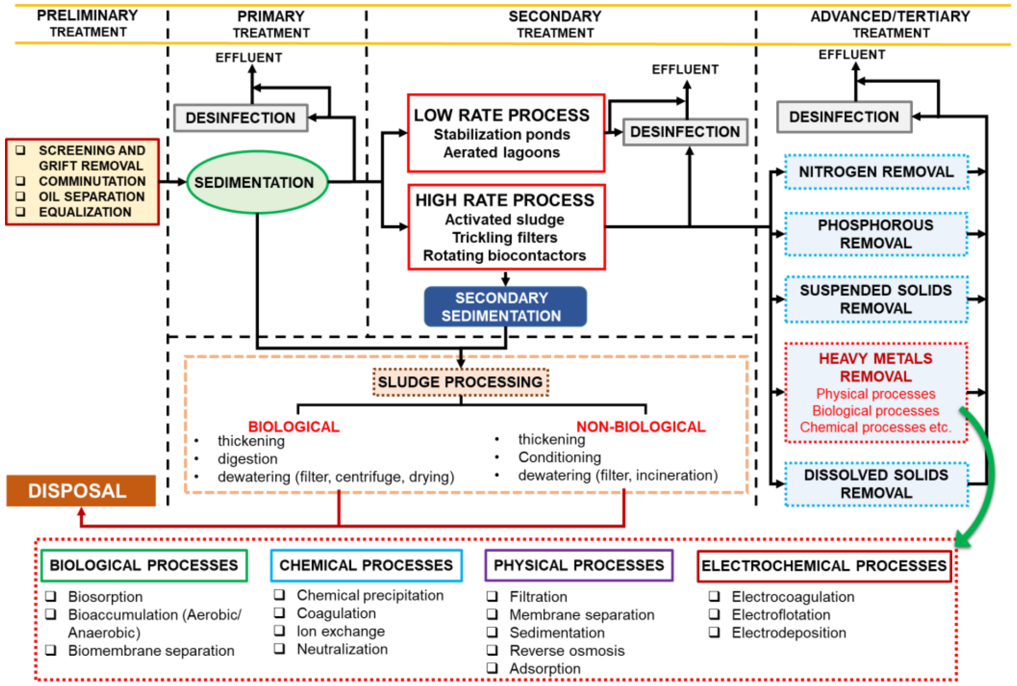

Figure 1. Possible flow chart of a wastewater treatment process and the conventional and non-conventional processes for heavy metals removal (adapted by MODESTRA ET AL., 2017; PESCOD, 1992)

According to the European Commission Directive no. 271 from 1991 on urban wastewater treatment, at EU level it is mandatory that the wastewater is collected and that at least the secondary treatment is applied, plus when the wastewater is discharged in sensitive areas, the tertiary treatment is also required (BARTOLOMEU ET AL., 2018). In brief, the physical, chemical and biological processes are combined for an efficient wastewater treatment and their selection generally depends on the characteristics of the wastewater components, the pollutant concentrations and the economic feasibility of methods (BARTOLOMEU ET AL., 2018).

Conventional processes applied for the removal of heavy metals from wastewater are chemical precipitation, ion exchange, chemical oxidation, electrodialysis, ultrafiltration, adsorption, reverse osmosis and others. These processes have the advantage to be easily applied and to remove a large amount of heavy metals, but they have high operating costs and are energy consuming (KAUR AND ROY, 2020). For example, ion exchange has high removal performances for an extended range of metals species and concentrations, but the adsorbent used requires regeneration or disposal. Oxidation is considered a quick process for the removal of toxic contaminants but has high costs and during the process, by-products are generated (SEZGIN, 2018). Chemical precipitation is the most widely used method for heavy metals removal due to its easy application, but it requires a high amount of chemicals and additional remediation methods for sludge treatment. Metals such as Cu(II), Zn(II), Cd(II) and Mn(II) easily form hydroxide precipitates and can thus be removed from solutions (ZHAO ET AL., 2016).

Adsorption is also a method intensively applied in wastewater treatment "*to purify, decolorize, detoxify, deodorize, separate and to remove and to recover the harmful pollutants from wastewater*" (CRINI ET AL., 2019). This process is a cost-effective method, has a simple design, it is easy to operate and a wide variety of sorbents are available (CRINI ET AL., 2019; RASALINGAM ET AL., 2014). In wastewater treatment plants, the activated carbon is the most used adsorbent, due to its high performance in removing contaminants such as "*dyes, phenolic and aromatic derivatives, metals, radionuclides, rare earth elements, pesticides, pharmaceuticals and drugs*" (CRINI ET AL., 2019). To remove heavy metal ions from wastewater, other materials can be used as sorbents such as activated alumina, polymeric resins and zeolites, but also biological materials, such as agriculture wastes and microorganisms, which have been shown in lab-scale studies to have high performance in removal of pollutants (CRINI ET AL., 2019; FIL ET AL., 2018). Thus, Figure 2 shows the main categories of adsorbents that are able to remove the heavy metal ions from aqueous media.

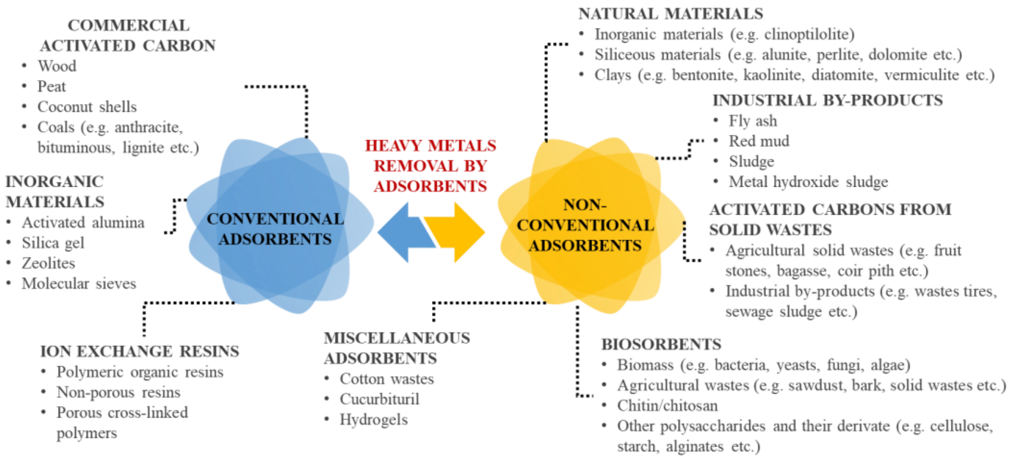

Figure 2. Conventional and non-conventional adsorbents used for heavy metals removal (adapted by CRINI ET AL., 2019)

BIOREMEDIATION OF WASTEWATERS USING MICROORGANISMS

Unconventional processes used in the removal of heavy metals from wastewaters are represented by the methods that use microorganisms, agricultural waste, phytoremediation and those that apply nanomaterials (KAUR ET AL., 2020). The main processes that are involved in the bioremediation of heavy metals are biosorption and bioaccumulation. Biosorption is a type of adsorption process which uses biological materials and implies the binding of the metal ions on the surface of the biosorbent or the living biomass through different chemical groups. The bioaccumulation process consists of two sub-processes: the adsorption of the pollutant on the surface of the used biomass and the absorption of the pollutant inside the living organism where it is digested through metabolic pathways (ROSCA ET AL., 2015). Other processes involved in the fulfillment of the adsorption process are ion exchange, precipitation and surface complexation (GUPTA ET AL., 2015).

Microorganisms can be applied through biosorption and bioaccumulation processes. They are very strong in this respect due to the fact that their cells are extremely adaptable to toxic environments. They have chemical structures with affinity for metals and the ability to turn highly toxic metal species into less harmful ones. Their use is advantageous as they reduce costs and energy consumption. On the other hand, the disadvantage of this technology is that it can generate toxicity problems depending on the living organisms used and how they work (KAUR ET AL., 2020).

The use of microorganisms is a method already widely applied in the secondary treatment stage. Biofilm reactors composed of microalgae and bacteria can be used in treatment plants for various inorganic as well as organic compounds. Microalgae absorb nitrogen and phosphorus which they use in their metabolic processes and produce oxygen which is used by bacteria to degrade organic pollutants, thus forming symbioses that can reduce the amount of pollutants such as acetate, from a concentration of 323 mg/L to 39 mg/L (BOELEE ET AL., 2014). For heavy metals removal, this method has only been applied in laboratory studies, the implementation at industrial level or in municipal treatment plants has not yet been fulfilled.

The biosorption process of heavy metals can be achieved with inactive and active microorganisms. A comparative description of the use of living and dead microorganisms for the removal of heavy metals is included in Table 1.

Table 1

Comparison between the application of living and dead microorganisms for heavy metal removal

Living microorganisms	***Dead microorganisms***
The active biomass is used in two main processes (biosorption + bioaccumulation)	The inactive biomass is used in a single passive process (biosorption process only)
Their application is achieved through a slow process and low absorption of metals (living cells are sensitive to large amounts of toxic metals)	Their application is achieved through a rapid process, and the adsorption of metals is in large quantities
Nutrients that support the production of metabolic energy are constantly needed to grow and maintain the microbial culture	No nutrients are needed to grow the microbial culture, and storage is very easy to maintain
The possibility of regeneration and reuse is limited (metals are integrated into intracellular structures)	It is possible to regenerate and reuse the biomass through sorption-desorption processes of heavy metals
They have a higher selectivity because the active metabolic processes can adapt to the metallic pollutant	They have low selectivity, but can be improved by chemical treatment

The bioremediation process using microorganisms depends on many types of factors which characterize the biosorbent, the biosorbate and the environmental conditions applied (Figure 3). For example, microalgae, bacteria and fungi have various functional groups on the surface of the cell wall to which both anions and metal cations adhere very easily (ROȘCA ET AL., 2018).

Many species of bacteria have been used in heavy metal biosorption processes. Among them, isolated species from areas contaminated with heavy metals, such as soils, mining areas, polluting waters, have been frequently used, as they have a high tolerance to metallic pollutants. Species of the genus *Pseudomonas* were tested by immobilization in 3% sodium alginate for wastewater contaminated with As(V) (BANERJEE ET AL., 2016). Removal of Zn(II) using a *Streptomyces* strain demonstrated a higher removal capacity through bioaccumulation (4.4 mmol/g) than by applying the biosorption process (0.75 mmol/g) (SEDLAKOVA-KADUKOVA ET AL., 2019). *Bacillus* species are among the most extensively tested for the removal of heavy metals in lab-scale studies and some examples are presented in Table 2. *Bacillus cereus* bacterium isolated from polluted soils due to galvanization generated a removal efficiency of 82% for an initial Cd(II) concentration of 200 mg/L (ARIVALAGAN ET AL., 2014).

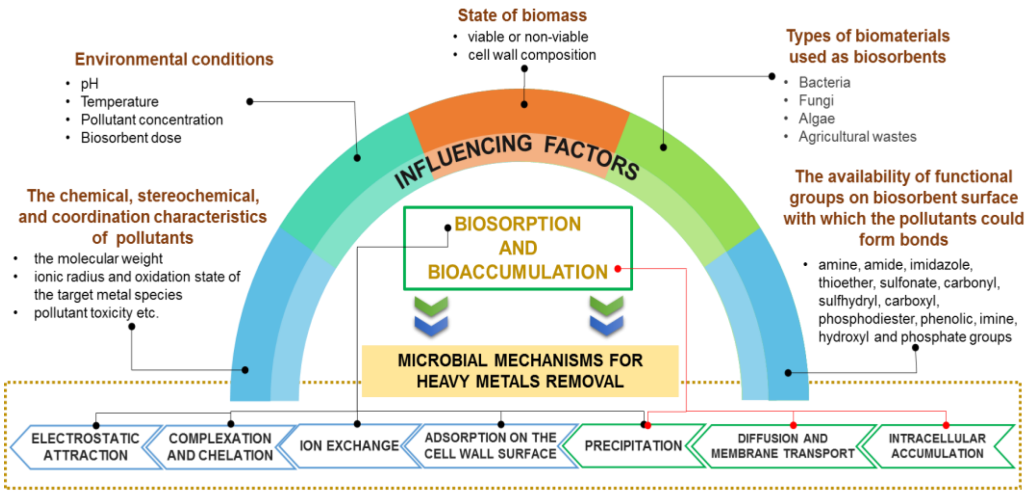

Figure 3. Biosorption and bioaccumulation influencing factors and microbial mechanisms involved in heavy metals removal from aqueous solutions

Table 2

Biosorption of priority metal pollutants by bacteria (*Bacillus* species)

Metal	***Bacterial specie***	***Optimal conditions***	***Performances***	***Reference***
Hg(II)	*Bacillus licheniformis*	Initial metal conc. (mg/L) = 50; pH = 7; Temperature (°C) = 30; Biosorbent dose (g/L) = 0.5; Equilibrium time (h) = 1	70%	UPADHYAY et al. (2017)
Pb(II)	*Bacillus licheniformis*	Initial metal conc. (mg/L) = 200; pH = 6; Temperature (°C) = 20-22; Biosorbent dose (g/L) = 0.7; Equilibrium time (h) = 12	113.84 mg/g	WEN et al. (2018)
Cd(II)	*Bacillus cereus*	Initial metal conc. (mg/L) = 200; pH = 6; Temperature (°C)= 35; Biosorbent dose (g/L) = -; Equilibrium time (h) = 20	82%	ARIVALAGAN et al. (2014)
	Bacillus megaterium	Initial metal conc. (mg/L) = 100; pH = 4; Temperature (°C)= 30; Biosorbent dose (g/L) = 3; Equilibrium time (h) = 2	15.39 mg/g	ROȘCA et al. (2018)
Ni(II)	*Bacillus laterosporus*	Initial metal conc. (mg/L) = 10-20; pH = 7; Temperature (°C)= 30; Biosorbent dose (g/L) = 40; Equilibrium time (h) = 2	44.44 mg/g	KULKARNi et al. (2014)

Also, different species of fungi and yeasts have been used to analyze their capacity in heavy metal adsorption processes. *Saccharomyces cerevisiae* has successfully reduced a concentration of 100 mg/L of Cr(VI) (HLIHOR ET AL., 2013). Maximum removal efficiency of Hg(II) using the same specie for a Hg(II) initial concentration of 0.0789 mg/L was 88.9%, value obtained at pH 5.45 (HADIANI ET AL., 2018). *Phanerochaete chrysosporium* was applied to remove Cd(II) and Ni(II) ions in concentrations between 15-35 mg/L and 5-25 mg/L, respectively (NOORMOHAMADI ET AL., 2019).

Microalgae have been successfully used for the removal of a large range of heavy metals concentrations. The scientific literature records results for the use of species such as *Chlorella pyrenoidosa* for the removal of copper ions (MOREIRA ET AL., 2019), *Spirulina platensis* (NITHYA ET AL., 2019) and *Scenedesmus sp.* (PRADHAN ET AL., 2019) for the removal of chromium. The use of *Parachlorella sp.* for the removal of cadmium ions was 1.5 to 3 times more effective than *Scenedesmus sp.*, *Spirulina sp.* and *Nannochloropsis sp.* (DIRBAZ ET AL., 2018). Maximum adsorption capacity was 90.20 mg/g determined at pH 7, 35 °C and 1 g/L biosorbent dosage. Optimum agitation speed was determined to be 150 rpm for an initial metal concentration of 60 mg/L.

CONCLUSIONS

The main persistent inorganic pollutants are heavy metals and aquatic environments are mainly contaminated with mercury, lead, cadmium and nickel. The sources of heavy metal pollution are mainly represented by the production and processing of metals, the chemical industry, the mineral industry, the energy sector and the management of waste and wastewater. The removal of heavy metals from wastewater and contaminated aquatic environments requires effective solutions so that the effect of these substances on human health and the environment can be prevented in the long term. In this respect, non-conventional technologies based on bioremoval techniques are currently in the spotlight for scientific research due to the low costs compared to the application of conventional technologies. The use of microorganisms for the removal of heavy metals has been extensively researched in lab-scale studies in both active and inactive form for process optimization. Further upscaling analyses with technical and economic perspectives are required.

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