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Review

Continuous Systems Bioremediation of Wastewaters Loaded with Heavy Metals Using Microorganisms

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Abstract: Heavy metal pollution is a serious concern of the modern era due to its widespread negative effects on human health and to the environment. Conventional technologies applied for the uptake of this category of persistent pollutants are complex, often expensive, and inefficient at low metal concentrations. In the last few years, non-conventional alternatives have been studied in search of better solutions in terms of costs and sustainability. Microbial adsorbents are one of the biomass-based sorbents that have extensively demonstrated excellent heavy metals removal capacity even at low concentrations. However, most of the carried-out research regarding their application in wastewater treatment has been performed in discontinuous systems. The use of microorganisms for the uptake of metal ions in continuous systems could be an important step for the upscale of the remediation processes since it facilitates a faster remediation of higher quantities of wastewaters loaded with heavy metals, in comparison with batch systems removal. Thus, the current research aims to analyze the available studies focusing on the removal of metal ions from wastewaters using microorganisms, in continuous systems, with a focus on obtained performances, optimized experimental conditions, and the sustainability of the bioremoval process. The present work found that microbial-based remediation processes have demonstrated very good performances in continuous systems. Further sustainability analyses are required in order to apply the bioremediation technology in an optimized environmentally friendly way in large-scale facilities.

Keywords: bioremediation; bioreactor studies; heavy metals; microorganisms; wastewater treatment

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1. Introduction

Industrial and technological development has, on the one hand, led to solutions to many problems in the world, and on the other hand, it has generated a significant degree of pollution that affects the environment and human health [1]. Among the pollutants that pose a major risk are heavy metals [2]. These are metals which have a density value surpassing 4 g/cm^3 or five times higher than the one associated with water [3]. Their biodegradability is very low, while their mobility is high, so once released in the environment, they easily reach living organisms [4]. For example, analyses of metal concentrations from the water and aquatic organisms from Snagov Lake from Romania, have registered the highest levels for aluminium and barium [5]. Some metal ions such as Cr(III), Ni(II), Zn(II), Fe(II), and Fe(III) are considered nutrients in small concentrations for humans, animals, or plants [6,7]. Metal toxicity usually causes an inhibition of plant development [8] or adversely affects animal and human health [7].

Heavy metals are persistent pollutants that bioaccumulate in living organisms and are expensive and difficult to remove from wastewaters at low concentrations when using conventional techniques [9]. Successful remediation of heavy metals using active or inactive biomass has been carried out using terrestrial [10] and aquatic plants [11,12], as well as

microorganisms [13,14]. Metal removal from wastewaters through bioremediation can occur through two types of processes. The first one, biosorption, consists of the binding of the pollutant(s) with the inactive biomass through external functional groups of the cell wall. The other type of process is called bioaccumulation and it takes place in two main phases: the biosorption or adsorption stage as previously described and the absorption, through which the heavy metals are incorporated into the internal structures of the living cells [15].

The use of microorganisms (algae, bacteria, fungi) as sorbents for the removal of heavy metals has been studied as a sustainable alternative to the conventional ones. Their ability to remove metals has been mostly studied and successfully demonstrated in batch systems [16]. However, studies performed in continuous systems are in a much smaller number, and metal removal using microorganisms is still not applied at the level of wastewater treatment plants [2,17]. These are important, however, for scaling up microbial remediation processes, as they facilitate faster remediation of larger amounts of wastewater [16,18]. Microorganisms cultivation in continuous systems has been scaled-up for fermentation, for example, by Crater and Lievense [19]. According to these authors, the lab-scale processes should be carried out using conditions that resemble as closely as possible the industrial-level process.

Taking these into consideration, the current paper reviews: (1) the potential of microbial biomass immobilization for heavy metals removal from wastewaters; (2) the results in terms of the performances obtained so far in the scientific literature regarding the removal of heavy metals using microorganisms in continuous systems; (3) the possibility of microbial sorbents regeneration through desorption for its multiple uses in the remediation of metal-loaded waters; and (4) the evaluation of microbial processes from an environmental point of view by applying sustainability assessment methodologies. An overview of these aspects is the key for understanding the current status of research in this domain and to continue the study towards a successful future upscale of microbial remediation of wastewaters containing heavy metals.

2. Parameters in Metal Removal from Wastewaters—Discontinuous versus Continuous Systems

Biosorption and bioaccumulation research were performed in both discontinuous (batch) mode and continuous flow mode. Out of these, discontinuous mode is the most preferred choice of researchers due the fact that it can be operated in small volumes and can easily be carried out with a constant volume [20]. The bioreactor operated in continuous mode is defined as the system with the inlet and outlet fluid flow rate being approximately the same [21]. In this mode of operation, the volume varies and flow regulators, strainers, filter plugs, and other accessories are required, thus making the process more complex than in the discontinuous mode of operation. Furthermore, the continuous flow system for bioremoval of heavy metals requires that the bioreactor vessel to be filled with biosorbents in the form of solid beds/matrices, and these matrices to be fixed, mobile, or to be operated under fluidized conditions [20].

The metal removal from wastewaters using microorganisms is influenced in discontinuous, as well as in continuous, systems by various factors. The optimization of process parameters is of utmost importance for a successful bioremediation of pollutants [22]. Common parameters to both systems are pH, temperature, initial metal concentration, and biomass dosage [15,20]. Metal uptake performance in continuous systems also depends on factors associated with the configuration and the operation mode of the bioreactors (Figure 1) [15,21]. Mass transfer through reactors, phases mixing efficiency, gas bubble size, bed height, pressure drop, bioreactor geometry, diameter of beads, hydraulic retention time, air flow rate, wastewater flow rate, and feeding flow rate are among the most relevant factors related to the configuration and the operation mode of the bioreactor [23,24].

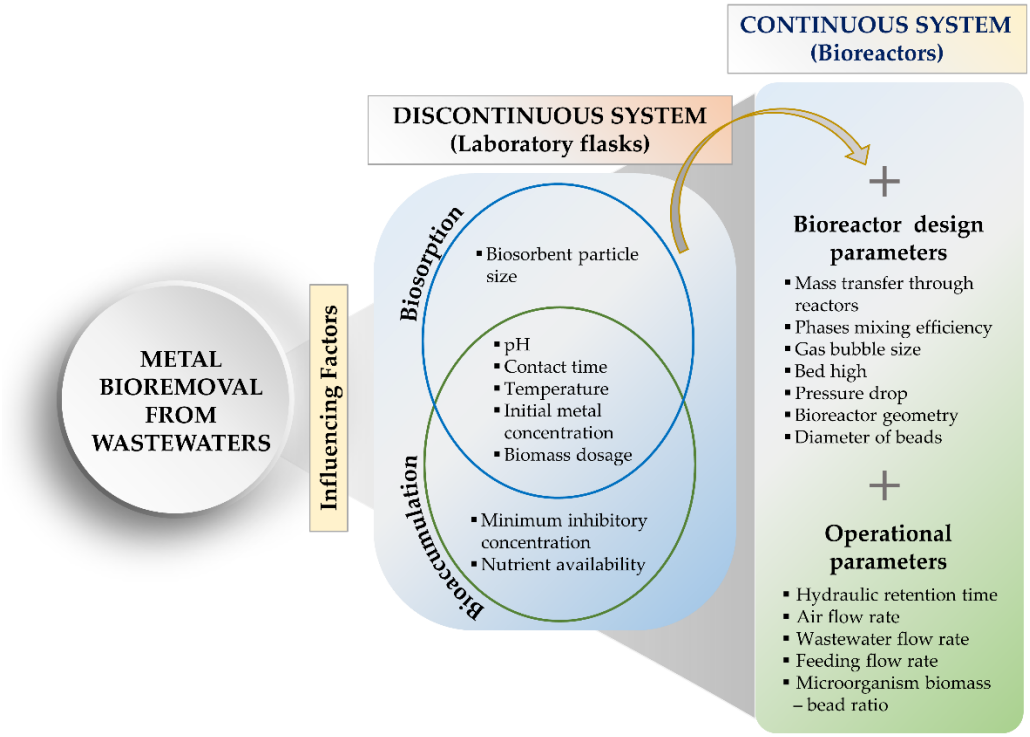

Figure 1. Influencing factors of biosorption and bioaccumulation processes applied in discontinuous and continuous systems.

The pH value determines the surface charge of the sorbent, the availability of metal species, and the interaction between the biomass and the pollutants. Additionally, the pH of the treated effluent influences the growth of the active microbial biomass. For example, the microalgae species *C. pyrenoidosa* cannot develop at extreme pH values 1 and 12 [25]. A pH value of 2 has been demonstrated as optimum for Cr(VI) uptake by microorganisms, but this value affects the microbial cell development [26]. Higher pH values can inhibit the uptake and the release of carbon by microalgae, thus limiting their growth [25].

Temperature is another important factor, since it influences the chemical kinetics. Furthermore, the temperature has a more significant impact on the living microorganisms, thus affecting the bioaccumulation process more in comparison with biosorption [27]. Different temperatures can be used in batch versus column studies. Gopi Kiran et al. [28] applied, for example, for the removal of several metal ions at a temperature of 30 °C in batch mode, whereas in the column system, a temperature value of 25 ± 2 °C was used, the latter being usually specific to wastewater treatment procedures.

Minimum inhibitory concentration (MIC) is another specific parameter that impacts the removal process when the living microbial biomass is used and indicates the minimum concentration of metals which determines the inhibition of the microorganism. The MIC of a strain of *Pseudomonas* sp., for example, generated a value of 6 mM for Cd(II), Pb(II), and Cu(II) and 8 mM in the case of Mn(II) and Zn(II) [29].

Bed height represents the height of the biomass used in the column and influences the microbial-based bioremediation process as well, having an effect on the breakthrough time and the saturation time. A higher bed height can determine reaching the breakthrough time faster. Additionally, a higher bed height determines a higher sorption performance in the column system [30].

The optimization of the contact time determines the amount of time which is required in a batch system to achieve the highest metal removal values by the microbial sorbent, thus having a high influence [31,32]. Saturation time is the parameter used in continuous systems to characterize the time required for the sorbent to reach its maximum uptake capacity of metals. In both cases, usually the metal uptake process follows an upward

trend, after which it reaches a plateau. In the case of the biosorption process, this is called equilibrium [29]. The saturation time is directly influenced by the bed height, a higher value of the latter determining a higher saturation point [18].

Flow rate is one of the most important parameter characteristics only for the continuous systems and represents the volume of effluent passing through the column in a certain amount of time to be treated. This influences the rate of reaching the saturation point and breakthrough time. A recirculation system must be implemented in the column in order to keep constant the metal uptake rate [33]. In the continuous system, a high metal concentration associated with a high flow rate determines a low saturation point and breakthrough time [33,34].

According to Atmakidis and Kenig [35], pressure drop, retention time, and mass and heat transfer coefficients are parameters that need to be estimated with high accuracy in the upscaling of fixed-bed bioreactor. In immobilized cell systems, the heavy metals removal is affected by gas-liquid-solid mass transfer fluxes, the hydrodynamic regime, and the metabolic activity of the microorganism. Thus, the gas-liquid and liquid-solid mass transfer coefficients, the effective oxygen diffusivity, and oxygen consumption rates are essential parameters to fully understand the removal of heavy metals by microorganisms in fixed-bed bioreactors [36,37]. In columns, for example, due to the immobilization of microbial cells on the surface of the fixed layer, there are two types of mass transfer: one refers to the transfer of nutrients from the liquid phase to the cells and the other to the transfer of oxygen from the gas phase to the cells [36,38]. The amount of dissolved oxygen reaching the cell influences the activity of the microorganism and not the amount of dissolved oxygen in the bulk liquid medium. Therefore, when living microorganisms are used for heavy metals bioremoval, an adequately aeration of the fluid is necessary. The oxygen transfer rate from bulk liquid medium to microbial cells depends mainly on four parameters: the contact area between gas and liquid, the driving force (for example, the difference in oxygen concentration between the two phases), the fluid dynamics, and the composition of the wastewater [38].

Other parameters related to the immobilization of the microbial biomass, such as the diameter of beads formed with different polymers (alginate, chitosan, agar, etc.) and the ratio between the microorganism biomass and the bead, are also important to be considered and optimized for obtaining the highest possible removal performance. These influencing factors concerning the immobilization of microorganisms are applicable to both discontinuous and continuous systems.

3. Microorganisms Immobilization for Heavy Metals Removal

The microbial biomass immobilized on different types of materials could be an ideal combination to solve the problems encountered in the removal of metal ions by free-suspended biomass [39]. The immobilization of microorganisms can be very advantageous because it ensures a higher mechanical strength, reduces the biomass deterioration, controls the biosorbent size, and offers a stable contact surface [40]. It also facilitates the recovery of the microbial cells [40] and in some cases, due to the properties of the carrier, the removal performance is increased [18].

The immobilization of microorganisms can be achieved by several methods such as encapsulation, adsorption, aggregation, cross-linking, covalent bonding, and entrapment in a matrix (Figure 2) [26]. According to Emami Moghaddam et al. [41], the attachment and entrapment are the most-used immobilization techniques. The microorganism cells can be naturally immobilized due to their ability to attach to the surface of natural or synthetic materials and grow on them (passive immobilization), or by active-assisted immobilization using covalent bonding, cross-linking, encapsulation, and entrapment in a matrix. In natural attachment are involved both physical (electrostatic and hydrophobic interactions) and chemical (covalent bond formation) interactions between matrix and biomass cells, the electrostatic interactions being the most common involved in the initial stage of immobilization [41]. By adsorption, the microbial cells are attached on the surface of

the matrix through electrostatic interactions and Van der Waals forces [42], while in covalent binding, the attachment is done by the formation of covalent bonds between the cells and matrix in the presence of a binding agent [41,42]. For the entrapment of the microbial cells, a three-dimensional sol-gel matrix is used (mostly silica-based materials) [43].

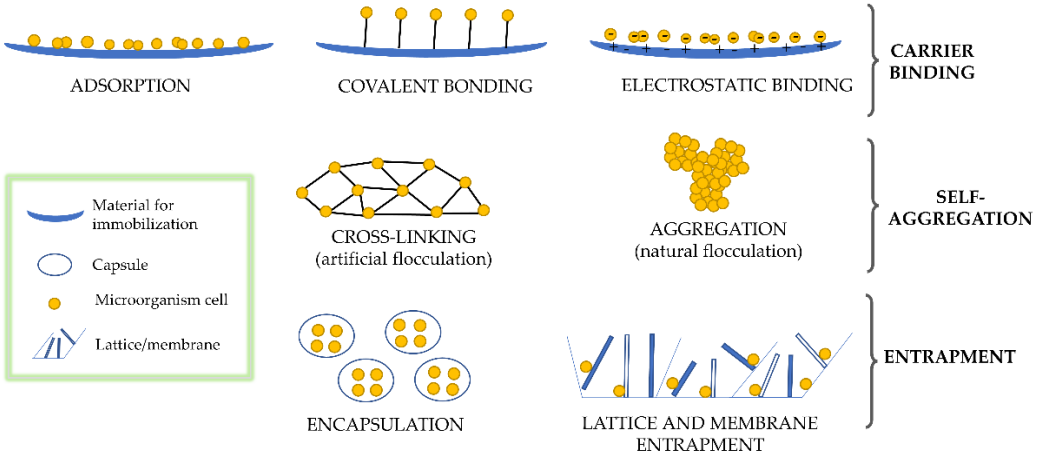

Figure 2. Methods of microorganisms' cells immobilization.

To achieve the highest possible performance in the removal of metal ions, it is important to select a suitable matrix, fulfilling a number of characteristics such as being lightweight, having a porous structure that facilitates the immobilization of cells, to be inert, nonbiodegradable in the test condition, nontoxic and a noninhibitor for microbial cells, and to allow the mass transfer. Furthermore, the matrix should have a high chemical, mechanical, and biological stability, to provide an irregular, rough surface for colonization, and last but not least, to be environmentally friendly and cheap [41].

For the removal of heavy metals from wastewater in a continuous system, the immobilization of microorganisms is often applied using different types of organic and inorganic materials [18,44]. They can be applied directly or can be subjected to certain treatments. The organic materials used for cell immobilization due to their higher absorptivity and the presence of amino, hydroxyl, carboxyl, and other functional groups can also interact with heavy metal ions. Among the most used organic materials are alginate, agar, carrageenan, polyacrylamide, and polyvinyl alcohol. From the category of inorganic materials, the most frequently used are clay, activated charcoal, and zeolite. In bioremediation of wastewaters, these materials are selected due to their resistance to microbial degradation, having a suitable thermostability, and low cost [41].

Kőnig-Péter et al. [45] determined an adsorption capacity of Pb(II) and Cd(II) ions in the case of chitosan beads with *Spirulina* that was lower than in the case of beads made only of chitosan, but in the case of Cu(II), a higher value was observed. Much of the ongoing studies report the use of microorganisms in alginate balls [46–49]. They can be applied directly or they can be subjected to certain treatments. *Spirulina platensis* thus immobilized in alginate and treated with distilled water demonstrated a 10% higher Cu(II) adsorption capacity [45]. The application of alginate balls also has the disadvantage of reducing the adsorption efficiency, thus needing to use a larger amount of biosorbent [50]. Regeneration of the biosorbent bed in the column for reuse is performed using acids or distilled water. However, the results show that acid washes can reduce the adsorption capacity by 50% [45]. Some gels made from alginate or carrageenan, however, degrade easily or can dissolve by exposure to other solutions. An alternative to these metabolites is polysulfone as it is not toxic to microorganisms and has chemical stability, heat resistance, and high durability [51].

In recent years, researchers have started to combine the microbial cells with nanomaterials with the purpose to improve the microbial bioremediation of wastewaters loaded with heavy metals [39]. Nanomaterials are innovative materials, which have important characteristics that make them a good substrate. Various types of nanomaterials such as iron oxide

magnetic nanoparticles [52], nanosilica [53], chitosan-coated magnetic nanoparticles [40], and synthesized titania [54] have already been tested successfully.

For a better efficient exploitation of the immobilized microorganisms in the bioremediation of the contaminated liquid effluents, the disadvantages of the applied immobilization methods must also be taken into account. For example, the main disadvantage of the adsorption method is that the adsorbed microbial cells can be easily detached due to the relative weakness of the adsorptive binding forces, which leads to a low efficiency in their use during the bioremediation of wastewaters [41,42]. In case of some immobilization methods such as entrapment and cross-linking, the processes are irreversible, which means that regeneration and reuse of the biomass is not possible or more difficult to perform. Another aspect that should be considered in terms of quality and quantity used, as well as the potential environmental impact, are acids or bases used for biosorbent treatment in order to enhance removal performance, but also to maintain reduced levels of impact and ensure sustainability. On the other hand, immobilization means an increased consumption of materials and can be expensive [18].

During bioremediation, the immobilized microbial cells, and sometimes together with the support, can remove the heavy metal ions from contaminated wastewater by one or various mechanisms, as described in Figure 3. The mechanisms involved in metal ions removal depend on various characteristics related to the condition of the cells (viable or dead), the metal ion type, the microorganism species, and the type of support (organic or inorganic), but are influenced by the conditions in which the bioremediation process takes place [41,42,55–57].

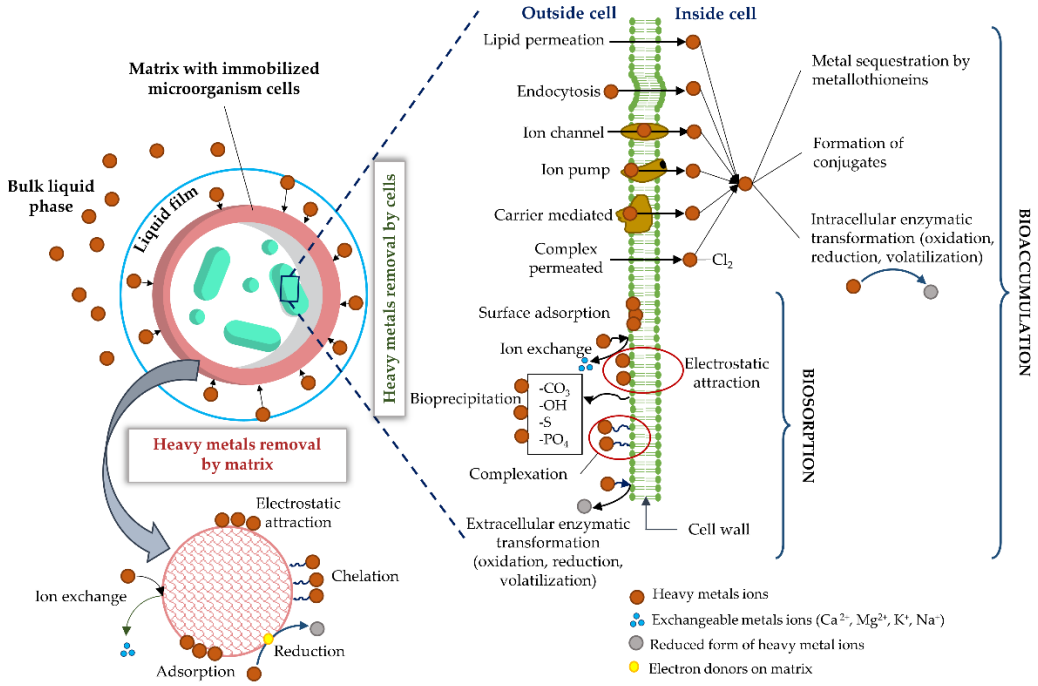

Figure 3. Mechanisms involved in heavy metals removal by a matrix with immobilized microbial cells.

The heavy metals removal by inactive cells is called biosorption and it is considered that, within the physical adsorption, ion exchange, bioprecipitation, electrostatic attraction, complexation or/and extracellular transformation (reduction/oxidation) mechanisms, the ions are retained on the cell surface [4,58]. By complexation between metal ions and the functional groups found in the cell wall components, complexes are formed which may be neutral, positively or negatively charged, mononuclear or polynuclear, and may have different coordination numbers. The ion exchange mechanism involves the exchange of heavy metal ions with counterions present on the biosorbent surface. Depending on the type of ions that can be exchanged, during the biosorption process, the exchange can be

cationic or anionic. For example, carboxylic groups are involved in the cation exchange, while amino/imidazole groups participate in the anionic exchange [59]. The functional groups found in cell wall components can also form precipitates with heavy metal ions in solution. Usually, through this mechanism, insoluble inorganic precipitates are formed, but organic metallic precipitates formed by extracellular polymeric substances secreted by microbial cells can also be obtained [59–61]. For some heavy metal ions, such as Cr(VI), the removal of these consist of extracellular reduction to another form (e.g., Cr(III)). The reduction is achieved as a result of the interaction of Cr(VI) ions with the carboxyl functional group, chromate reductases, and/or metabolites secreted by cells [59]. Immobilized inactive microbial cells have been used as biosorbents in various studies for metal ions removal. For example, Meringer et al. [62] used *Pseudomonas putida* trapped in agar beads for the removal of Cu(II) and Zn(II) and Contreras-Cortés et al. [63] used *Aspergillus australensis* inactive cells immobilized on textile made of 100% polyester for Cu(II). In other studies, the performances of *Saccharomyces cerevisiae* cells immobilized on magnetic chitosan beads to remove Sr(II), Co(II), and Cs(I) [64] or *Aspergillus sydowii* immobilized on γ-Fe_2O_3 magnetic chitosan to remove Cd(II) [65] were assessed. Inactive *Chlorella vulgaris* immobilized in Ca-alginate beads was used by Ahmad et al. [46] for the removal of Fe(II), Mn(II), and Zn(II) ions, and according to the results of this study, the predominant mechanisms involved in the removal of these ions are ion exchange and chemisorption.

The removal of heavy metals from contaminated aqueous solutions has been evaluated in numerous studies and the performance of immobilized viable microbial cells has been assessed. In this case, the bioremediation process is called bioaccumulation and is a process mediated by the cell metabolism [56]. The bioaccumulation process through microorganisms consists of two main stages: one taking place at the cell wall level and another intracellular phase during which the incorporation of the metal ions takes place through metabolic pathways [4]. So, in order to thrive under the stress induced by metal ions, the cells develop protection mechanisms through which heavy metals from aqueous solutions are removed. These mechanisms involve the transport across the membrane of metal ions via lipid permeation, endocytosis, using metal efflux pumps followed by intracellular metal sequestration by metallothionein, the formation of conjugates, and intracellular enzymatic transformation of metal ions (e.g., reduction of Cr(IV) to Cr(III)) [66]. The heavy metals are removed by living microbial cells through extracellular mechanisms such as physical adsorption, ion exchange, bioprecipitation, electrostatic attraction, complexation, or/and extracellular transformation (reduction/oxidation), which are the representative mechanisms of the biosorption process [56,58,66]. According to Huang et al. [67], ion-exchange, complexation, physical entrapment, precipitation, and intracellular accumulation are involved in the removal of Cd(II) ions by living cells of *B. cereus* RC-1 immobilized on three types of biochar derived from rice straw, chicken manure, and sewage sludge. Biochar is known as an innovative material with environmental applications and conductive properties, which also has valuable perspectives in the circular economy context [68]. For example, at 50 mg/L Cd(II) in solution, *B. cereus* RC-1 immobilized on biochar derived from rice straw removed 39.5% of Cd(II) by ion-exchange, 36.3% by complexation, 8.8% by precipitation, 8.2% by intracellular accumulation, and 7.2% by physical entrapment. For the biochar derived from sewage sludge, the representative mechanism for Cd(II) removal was complexation (56.9%), followed by ion-exchange (20.1%), intracellular accumulation (8.3%), physical entrapment (8%), and precipitation (6.7%).

Most often, the materials used for the immobilization of microbial cells can also remove the heavy metals from contaminated liquid effluents through various mechanisms. One study mentions that sodium alginate is able to remove the metal ions through chelation, electrostatic interaction, ion exchange, reduction, and photocatalytic reduction mechanisms [69]. The various types of zeolites used to immobilize cells also have the ability to retain metal ions, the predominant one being the cation exchange mechanism due to the abundance of Na(I), K(I), Ca(II), and Mg(II) exchangeable cations in the zeolite structure [70]. Adsorption and precipitation of heavy metal ions are two other mechanisms

by which zeolites remove metal ions [71]. According to Hong et al. [71], the LTA and FAU zeolites remove the Pb(II) and Cu(II) ions predominantly by cation exchange and the Ni(II) ions by adsorption in the pores. The study performed by Zhao et al. [72] highlights that the biochars derived from different types of feedstock remove the heavy metals by complexation and cation exchange, thus indicating that the performance of microbial cells immobilized on biochar is also due to the support ability to retain heavy metals, not only of the microorganism uptake capacity. Thus, in Table 1 are presented the performances of various microorganism species immobilized on various types of materials for heavy metals removal in batch systems.

Table 1. Microbial cells immobilization on various supports and their performances for heavy metals removal in batch systems.

Support	Immobilized Microorganism Specie	Metal Ion	Experimental Conditions	Performance	Ref.
Iron oxide magnetic nanoparticles	*Bacillus licheniformis*	Pb(II)	pH = 6, Ci = 200 mg/L, D = 0.7 g/L, t = 12 h, T = 30 °C	98% 113.84 mg/g	[52]
Synthesized titania	*Saccharomyces cerevisiae*	Cr(VI)	pH = 1, Ci = 100 mg/L, t = 82.5 min, T = 30 °C	99.92% 162.07 mg/g	[54]
Nanosilica	*Aspergillus ustus*, *Fusarium verticillioides*, *Pencillium funiculosum*	Cr(III)	pH = 7, Ci = 0.1 mol/L, D = 15 g/L, t = 30 min, T = 25 ± 1 °C	128.26, 138.66, and 97.06 mg/g	[53]
		Cr(VI)	pH = 2, Ci = 0.1 mol/L, D = 15 g/L, t = 30 min, T = 25 ± 1°C	336.24, 332.77 and 197.58 mg/g	
Silicon dioxide nano-powder	*Aspergillus ustus*	Cd(II)	pH = 7, Ci = 0.01 mol/L, D = 1.5 g/L, t = 30 min, T = 25 ± 1 °C	112.41 mg/g	[73]
Chitosan-coated magnetic nanoparticles	*Saccharomyces cerevisiae*	Cu(II)	pH = 4.5, Ci = 40–500 mg/L, D = 1.5 g/L, t = 2 h, T = 28 °C	96.8%, 144.9 mg/g	[40]
Zeolite NaY	*Escherichia coli*	Fe(III)	Ci = 6–99 mg/L, pH = 2.7–3.5, t = 10 days, T = 37 °C	100%	[74]
		Ni(II)	pH = 5.7–6.2, Ci = 11–117 mg/L, t = 10 days, T = 37 °C	82.5–85.5%	
Alginate	*Arthrospira platensis* (SAG257.80)	Pb(II)	pH = 4, Ci = 100 mg/L, D = 20 g/L, t = 24 h, T = 27 ± 1 °C	65.91 mg/g	[48]
Silica gel			pH = 5.5, Ci = 100 mg/L, D = 20 g/L, t = 24 h, T = 27 ± 1 °C	2.68 mg/g	
Agarose			pH = 5, Ci = 100 mg/L, D = 20 g/L, t = 24 h, T = 27 ± 1 °C	31.53 mg/g	
Ca-alginate	*Scenedesmus quadricauda*	Cu(II)	pH = 5.0, Ci = 600 mg/L, t = 120 min, T = 25 °C	75.6 mg/g	[75]
		Zn(II)		55.2 mg/g	
		Ni(II)		30.4 mg/g	
Biochar derived from rice straw	*Bacillus cereus* RC-1	Cd(II)	pH = 7, Ci = 180 mg/L, D = 0.2 g/L, t = 24 h, T = 28 ± 2 °C	158.77 mg/g	[67]
Biochar derived from chicken manure				110.14 mg/g	
Biochar derived from sewage sludge				127.71 mg/g	

Table 1. *Cont.*

Support	Immobilized Microorganism Specie	Metal Ion	Experimental Conditions	Performance	Ref.
Sodium alginate	Sulfate-reducing bacteria	Fe(III)	pH = 7, Ci = 10 mg/L and 50 mg/L, t =120 h, T = 30 °C	85–95%	[28]
		Zn(II)		85–95%	
		Cd(II)		85–95%	
		Pb(II)		85–95%	
		Ni(II)		75–95%	
γ-Fe_2O_3 magnetic chitosan	*Aspergillus sydowii*	Cd(II)	Ci = 50 mg/L, D = 0,76 g/L, t = 24 h, T = 28 °C	56.40 mg/g	[65]
Agar beads	*Pseudomonas putida*	Cu(II)	pH = 4.3, Ci = 2 to 60 mg/L, D = 30 g/L, t = 4 h (for Cu(II)) and 6 h (for Zn(II)), T = 24 °C	0.255 mg/g	[62]
		Zn(II)		0.170 mg/g	
Kaolin	*Escherichia coli*	Cr(VI)	pH = 5.0, Ci = 10–200 mg/L, D = 6.66 g/L, t = 24 h, T = 25 °C	91 mg/g	[76]
		Zn(II)		78 mg/g	
	Staphylococcus epidermidis	Cr(VI)		56 mg/g	
		Zn(II)		49 mg/g	
Alginate	*Aspergillus niger*	Th(IV)	pH = 6.0, Ci = 80–200 mg/L, D = 0.04 g/L, t = 480 min, T = 40 °C	303.95 mg/g	[47]
Ca-alginate	*Chlorella vulgaris*	Fe(II)	Ci = 30–300 mg/L; pH = 6.0, D = 0.4 g/L, t = 300 min, T = 25 °C	129.83 mg/g	[46]
		Mn(II)		115.90 mg/g	
		Zn (II)		105.29 mg/g	
Loofa sponge	*Chlorella sorokiniana*	Cr(III)	pH = 4.0, Ci = 10–300 mg/L, D = 0.4 g/L, t = 20 min, T = 25 ± 2 °C	69.26 mg/g	[77]
Magnetic chitosan beads	*Saccharomyces cerevisiae*	Sr(II)	pH = 6, Ci = 5–300 mg/L, D = 2 g/L, T = 30 °C	36.97 mg/g	[64]
		Co(II)		30.92 mg/g	
		Cs(I)		16.67 mg/g	
Magnetic Fe_3O_4 $^-$ phthalate nanoparticles	*Staphylococcus aureus*	Pb(II)	pH = 5, Ci = 0.03–0.5 mmol/L, D = 1.5 g/L, t = 20 min, T = 25 °C	100%, 280.75 mg/g	[78]
		Ni(II)		97.5%, 57.81 mg/g	
		Cu(II)		89.2%, 50.52 mg/g	
Bio-carrier Beads (polysulfone matrix)	*Bacillus drentensis* LMG 21831T	Pb(II)	Ci = 0.01–100 mg/L, D = 40 g/L, t = 24 h, T = 20 °C	0.3332 mg/g	[79]
		Cu(II)		0.5598 mg/g	
Ca-alginate	*Bacillus cereus* $M^1{}_{16}$	Ni(II)	pH = 6.0, Ci = 25–1000 mg/L, D = 3.8 mg/L, t = 200 min	125 mg/g	[49]
Textile made of 100% polyester	*Aspergillus australensis*	Cu(II)	pH = 5.5, Ci = 20 mg/L, t = 24 h, T = 35 °C	34.46%, 2.46 mg/g	[63]

Ci—initial concentration of metal ions in solution, D = biosorbent dosage, t = contact time, T = temperature.

4. Performance of Continuous Removal of Heavy Metals Using Microorganisms

Analysis of heavy metals removal by microbial biomass in continuous systems is an important step towards the successful scale-up of the remediation process. Heavy metals removal from wastewaters using microorganisms has been so far carried out in various types of columns: packed bed [45,80,81], fixed bed [51,82,83], fluidized bed [80], and airlift columns [16]. Fixed bed column systems are, for example, commonly used in separation and purification processes since they can most effectively use the difference in concentration

which ensures the movement in the system [81]. Its advantages in comparison with the use of free cells is the reduction of possibly clogged biomass, and an easier separation and reuse of the microbial biomass [82]. The advantages of packed bed columns are represented by good generated yields, good operability, the residence times, and the capacity to remove metals in low concentrations from large effluent quantities [83]. Additionally, this type of continuous system can be easily scaled-up [82].

Optimized conditions and the associated results of the microbial metal removal process in continuous systems based on available scientific studies are summarized in Table 2 (microalgae), Table 3 (bacteria), and Table 4 (fungi).

Table 2. Removal of heavy metals in a continuous system using microalgal biomass.

Metal	Microalgae Species	Bioreactor Type	Optimal Conditions	Performance	Ref.
Cr(VI)	*Scenedesmus quadricauda* biochar	Fixed-bed column (100 mm height and 6.6 mm internal diameter)	Initial metal concentration (mg/L) = 5; pH= 2; Temperature (°C) = 22; Biosorbent dose (g) = 0,2; Flow rate (mL/min) = 2; Saturation time (min) = 810;	57.58% 13.10 mg/g	[84]
	Spirulina platensis (calcium alginate beads)	Packed-bed column (35 cm height and 2 cm internal diameter)	Initial metal concentration (mg/L) = 100; pH = 1.5; Temperature (°C) = 30; Biosorbent dose (g) = 140 (9.5 g of *S. platensis*); Flow rate (L/h) = 3.5;	99% -	[50]
Pb(II)	*Oscillatoria princeps* (92%), *Spirogyra aequinoctialis* (3%), *Oscillatoria subbrevis* (2%), *Oscillatoria formosa* (1%), and other species (1%)	Fluidized bed system (1 m height and 7.5 cm inner diameter)	Initial metal concentration (mg/L) = 50; pH = 4; Temperature (°C) = 20; Biosorbent dose (g) = 1; Flow rate (L/h) = 100; Bed height (cm) = 2.5; Particle size (mm) = 0.6–1;	- 44.5 mg/g	[80]
	Spirulina platensis (alginate beads, chitosan, respectively)	Packed-bed column (30 cm height and 2 cm inner diameter)	Initial metal concentration (mg/L) = 100; pH = 5–6; Temperature (°C) = 25; Biosorbent dose (g/L) = 1; Flow rate (mL/min) = 2; Beads size (mm) = 2;	- 621.6 mg/g, 124.32 mg/g, respectively	[45]
Cd(II)	*Oscillatoria princeps* (92%), *Spirogyra aequinoctialis* (3%), *Oscillatoria subbrevis* (2%), *Oscillatoria formosa* (1%), and other species (1%)	Fluidized bed system (1 m height and 7.5 cm inner diameter)	Initial metal concentration (mg/L) = 50; pH = 4; Temperature (°C) = 20; Biosorbent dose (g) = 1; Flow rate (L/h) = 100; Bed height (cm) = 2,5; Beads size (mm) = 0.6–1;	- 39.5 mg/g	[80]
	Spirulina platensis (alginate beads, chitosan, respectively)	Packed-bed column (30 cm height and 2 cm inner diameter)	Initial metal concentration (mg/L) = 100; pH = 5–6; Temperature (°C) = 25; Biosorbent dose (g/L) = 1; Flow rate (mL/min) = 2; Beads size (mm) = 2;	- 213.58 mg/g, 89.93 mg/g, respectively	[45]
As(II)	*Oscillatoria princeps* (92%), *Spirogyra aequinoctialis* (3%), *Oscillatoria subbrevis* (2%), *Oscillatoria formosa* (1%), and other species (1%)	Fluidized bed system (1 m height and 7.5 cm inner diameter)	Initial metal concentration (mg/L) = 50; pH = 4; Temperature (°C) = 20; Biosorbent dose (g) = 1; Flow rate (L/h) = 100; Bed height (cm) = 2,5; Beads size (mm) = 0.6–1;	- 35 mg/g	[80]
Cu(II)	*Oscillatoria princeps* (92%), *Spirogyra aequinoctialis* (3%), *Oscillatoria subbrevis* (2%), *Oscillatoria formosa* (1%), and other species (1%)	Fluidized bed system (1 m height and 7.5 cm inner diameter)	Initial metal concentration (mg/L) = 50; pH = 5; Temperature (°C) = 20; Biosorbent dose (g) = 1; Flow rate (L/h) = 100; Bed height (cm) = 2,5; Beads size (mm) = 0.6–1;	- 41 mg/g	[80]
	Spirulina platensis (alginate beads, chitosan, respectively)	Packed-bed column (30 cm height and 2 cm inner diameter)	Initial metal concentration (mg/L) = 100; pH = 5–6; Temperature (°C) = 25; Biosorbent dose (g/L) = 1; Flow rate (mL/min) = 2; Beads size (mm) = 2;	- 196.99 mg/g, 63.54 mg/g, respectively	[45]

Table 3. Removal of heavy metals in a continuous system using bacterial biomass.

Metal	Bacteria Species	Bioreactor Type	Optimal Conditions	Performance	Ref.
Total Cr	*Arthrobacter viscosus*	Acrylic column (25 cm height and 3.2 cm inner diameter)	Initial metal concentration (mg/L) = 26; pH = 2; Room temperature; Flow rate (mL/min) = 10; Exhaustion time (min) = 350; Biofilm amount (g/L) = 5.75;	100% 20.37 mg/g	[85]
	Microbial consortia immobilized beads (*Bacillus subtilis*, *Acinetobacter junii*, *Escherichia coli*)	Continuous flow reactor (30 cm height and 2.5 cm inner diameter)	Initial metal concentration (mg/L) = 10; pH = 7; Temperature (°C) = 37; Flow rate (mL/min) = 0.5; Saturation time (min) = 120; Bed height (cm) = 18; Particle size (mm) = 1–2; 5% bacterial consortium in a bead;	51 ± 4.23% 224 ± 8.16 mg/g	[86]
	Alginate beads loaded with *Acinetobacter junii*, *Escherichia coli* and *Bacillus subtilis*	Column (30 cm height and 1.5 cm inner diameter)	Initial metal concentration (mg/L) = 300; pH = 3; Temperature (°C) = 30; Flow rate (mL/min) = 3; Saturation time (min) = 105 Bed height (cm) = 20; Particle size (mm) = 1; 5% (*w*/*v*) bacterial consortium;	23.94% 657 mg/g	[30]
Total Cr	*Arthrobacter viscosus*	Acrylic column (25 cm height and 3.2 cm inner diameter)	Initial metal concentration (mg/L) = 26; pH = 2; Room temperature; Flow rate (mL/min) = 10; Exhaustion time (min) = 350; Biofilm amount (g/L) = 5.75;	42.4% 20.37 mg/g	[85]
Pb(II)	Free, immobilized (respectively) *Aeromonas hydrophila*	Fixed-bed column (30 cm height and 2 cm inner diameter)	Initial metal concentration (mg/L) = 103.6; pH = 5; Temperature (°C) = 30; Flow rate = 2 mL/min; Saturation time (h) = 68.29; Bed height (cm) = 19; Particle size (mm) = 0.1;	85.38% 163.9, 138.88 mg/g (respectively)	[51]
Cd(II)	Alive *Bacillus cereus* (fixed with activated carbon from coconut husk)	Fixed-bed column (3.1 cm inner diameter)	Initial metal concentration (mg/L) = 15.2; pH = -; Room temperature; Flow rate (mL/min) = 7; Saturation time (h) = 40 Bed height (cm) = 21.5; Particle size (mm): -;	>80% -	[87]

Table 4. Removal of heavy metals in a continuous system using fungal biomass.

Metal	Fungi Species	Bioreactor Type	Optimal Conditions	Performance	Ref.
Cr(VI)	*Aspergillus niger* (alginate beads)	Column (4 cm inner diameter)	Initial metal concentration (mg/L) = 100; pH = 1.5; Temperature (°C) = -; Flow rate (mL/min) = 5; Saturation time (h) = 17; Bed height (cm) = 40; Beads size (mm): 3.2 mm ± 0.1 mm; 5% (*w*/*v*) biomass/bead.	- -	[88]
	Trichoderma viride (sodium alginate beads)	Column (2.5 cm inner diameter)	Initial metal concentration (mg/L) = 50; pH = 2.5; Temperature (°C) = -; Flow rate (mL/min) = 5; Equilibrium time (h) = 4.6; Saturation time (min) = 667.8; Bed height (cm) = 20; Particle size (mm) = 4; 5% (*w*/*v*) fungal biomass/bead;	- 6.88 ± 0.03 mg/g	[81]
	Rhizopus arrhizus	Packed-bed column (10 cm height and 2.5 cm inner diameter)	Initial metal concentration (mg/L) = 199; pH = 1.3; Temperature (°C) = -; Flow rate (mL/min) = 0.8; Residence time (min) = 20; Bed height (cm) = 45; Beads size (mm) = 2.429;	49.89% 52.11 mg/g	[89]

Table 4. *Cont.*

Metal	Fungi Species	Bioreactor Type	Optimal Conditions	Performance	Ref.
Cr(III)	*Aspergillus niger*	Airlift bioreactor (3 L volume)	Initial metal concentration (mg/L) =1000–1300; pH = 5.1; Temperature (°C) = 30; Ventilation (*v*/*v*) = 4; Contact time (h) = 32;	96% 208.70 mg/g	[90]
Pb(II)	*Aspergillus caespitosus* (immobilized glutaraldehyde cross-linked calcium alginate beads—AGCCAB beads)	Packed-bed column (35 cm length and 1.5 cm inner diameter)	Initial metal concentration (mg/L) = 600; pH = 5.5 ± 0.5; Temperature (°C) = -; Flow rate (mL/min) = 2.5;	- 670 ± 2.5 mg/g	[91]
	Saccharomyces cerevisiae	Double-draft airlift column (27.9 cm height and 7.6 cm inner diameter)	Initial metal concentration (mg/L) = 120; pH = 5; Temperature (°C) = 22; Biosorbent dose (g/L) = 3; Airflow (L/min) = 3; Mixing rate (rpm) = 200;	78% 72.5 mg/g	[16]
	Aspergillus niger and *Aspergillus terreus*	Fixed-bed column (30 cm height and 2 cm inner diameter)	Initial metal concentration (mg/L) = 250 mM; pH = -; Temperature (°C) = -; Flow rate (mL/min) = 3; Support height (cm) = 6;	18–22%, 14–20%, respectively -	[92]
Cd(II)	*Phanerochaete chrysosporium* (immobilized by growing onto polyurethane foam material)	Packed-bed column (33 cm height and 10 cm inner diameter)	Initial metal concentration (mg/L) = 11; pH = 5.3; Temperature (°C) = -; Flow rate (mL/min) = 125; 10% Breakthrough time (h) = 1.3; Bed height (cm) = 32.5;	42.2% -	[82]
Cu(II)	*Saccharomyces cerevisiae*	Double-draft airlift column (27.9 cm height and 7.6 cm inner diameter)	Initial metal concentration (mg/L) = 50; pH = 5; Temperature (°C) = 22; Biosorbent dose (g/L) = 3; Airflow (L/min) = 3; Mixing rate (rpm) = 200;	42% 29.9 mg/gc	[16]

In continuous systems, the pH of the effluent or metal solution, as well as the flow rate and the amount of adsorbent, are the most important influencing factors [84]. The concentration of the immobilization material can also impact the results of the sorption process. Raising the calcium alginate concentration of *Spirulina platensis* encompassed in calcium alginate beads decreased the sorption of Cr(VI) ions [50]. *Spirulina platensis* isolated in calcium alginate beads was used in a packed-bed column for Cr(VI) removal and generated a 99% removal performance [50]. The *Arthrobacter viscosus* bacteria species, as an active immobilized biomass, was used in the form of a biofilm on a tubular, star-shaped polyethylene support (17 mm external diameter and 10 mm height). The removal process of an effluent with a 25 mg/L Cr(VI) concentration consisted first of the reduction of Cr(VI) ions to Cr(III), the complete reduction taking place after 92 h. Then, the total chromium retention capacity was analyzed. Thus, under these mentioned conditions, an uptake capacity of 20.37 mg total Cr/g biomass was obtained [85].

The biochar form of the microalgae biomass, namely *Scenedesmus quadricauda*, has been applied for the removal of Cr(VI) in a fixed-bed column. Three types of biochar synthesized at three different temperatures (300, 500, 700 °C, respectively) were analyzed in batch mode initially for their capacity to adsorb the metal ions. The best performance was observed in the case of the biochar obtained at 500 °C and the results were even better than the ones generated by the raw biomass. Therefore, the 500 °C microalgal biochar was also used in the continuous system study.

In terms of differences in metal removal capacity between discontinuous and continuous systems, although an initial Cr(VI) concentration three times higher was used in the column study by applying *Acinetobacter junii*, *Escherichia coli*, and *Bacillus subtilis* incorporated in alginate beads, an approximately ten times higher sorption capacity was registered than in the batch mode [30]. Furthermore, the use of *Saccharomyces cerevisiae* for Cu(II)

and Pb(II) biosorption in experimental conditions, which consisted of pH values of 5–5.5 and a biosorbent dose of 1.5 g/L in both batch and column systems, indicated a slightly higher removal capacity for Pb(II) continuous removal than in a discontinuous system for all initial metal concentrations (10–60 mg/L). In the case of Cu(II), removal capacity was higher in batch mode for initial metal concentrations in the range 10–30 mg/L [16].

Real effluents have also been used for the study of heavy metals removal in continuous systems by microbial biomass. A study conducted in an airlift bioreactor for the removal of Cr(III) ions from a real tanning effluent by the fungi species *Aspergillus niger*, demonstrated a chromium removal performance of 208.70 mg/g (88%) in optimized conditions. The chemical characterization of the treated effluent had a Cr(III) concentration of 1000–1300 mg/L, pH 3–3.5, a total Kjeldahl nitrogen value of 160–245 mg/L, and total organic carbon value of 27,000–41,000 mg/L. A removal of Cr(VI) ions was also performed with the fungal species *Trichoderma viride* [81] and *Rhizopus arrhizus* [89] in the form of sodium alginate beads in a packed-bed column. The influence of other ions, including metals in effluent treatment on the removal of Cd(II) by the heat-inactivated fungal species *Aspergillus ustus* and its immobilization in silicon dioxide nanopowder, was also analyzed [73]. Regarding the optimized experimental conditions, a pH of 7, a biosorbent dose of 50 mg, a contact time of 30 min, and 25 °C were applied. The remarks showed a minimum degree of interference from sodium ions and potassium with a concentration of 113.76 mg/g; the observed cadmium concentration in this case being 122.87 mg/g. The maximum degree of interference was identified in the case of Cu(II) ions, a Cd(II) concentration of 85.86 mg/g being obtained. In comparison with these results, cadmium sorption capacity without the interference of ions was 124.66 mg/g [73].

Consortiums of various microbial species have also been applied for metal removal in continuous systems. The consortiums can actually be more effective, since in the environment, such conformations are usually found [93]. For example, six bacterial species and two fungal species were immobilized in alginate beads, pre-treated with 0.1 M of HCl, and altogether grouped in cellulose tissue. This combination successfully removed all chromium from the treated industrial effluent in the discontinuous system and 98% in the continuous system. Optimum parameters applied in the continuous system were a pH value of 6 and a flow rate of 5 mL/min. In the case of lead ions, the consortium generated a removal efficiency of 55% in both batch and continuous modes [94].

5. Sorption-Desorption of Heavy Metals Removal Using Microorganisms

Sorption-desorption studies carried out in continuous systems are important for the future upscale of wastewaters bioremediation by microbial biomass. The desorption process consists of the application of an eluent (namely a base or acid), which facilitates the unbinding and recovery of the metal ions from the surface of the microbial biomass [95]. In the case of the bioaccumulation process though, the desorption cannot be performed for metal recovery. As in the case of the biosorption, the pH is the most important factor influencing the efficiency of the desorption process [96]. The sorption-desorption mechanism is illustrated in Figure 4.

The application of the desorption process in one or more cycles ensures the extension of the sorbent's life cycle, as well as the recovery of the removed metal for its reuse in the industry. Using eluents for the unbinding of the metal ions from the cell wall surface is thus important for ensuring the sustainability of the microbial remediation process. After applying the feasible number of sorption-desorption cycles, the biomass residue with ideally no metal at all in its structure can be used, depending on its chemical composition, in compost for plant growth and development, or will be sent to deposits/landfills depending on the quality and quantity of the metal left in the microbial biomass. Metals that can be used in compost are iron, copper, cobalt, zinc, nickel, and molibdenum [97].

Sorption-desorption cycles have also been carried out at a laboratory level in continuous systems. The *Scenedesmus quadricauda* microalgae species, immobilized in a polyethylene column, was applied for the removal of Cd and Pb. Three sorption-desorption cycles

were successfully performed using 0.5 M of HNO_3. After these three cycles, the obtained desorption performances were 96% and 100% for Cd and Pb, respectively. However, sorption performance was quite low, at 9% and 12% for Cd and Pb, respectively [9]. Another study used *Trichoderma viride* immobilized in sodium alginate beads in a fixed-bed bioreactor. Five sorption-desorption cycles were applied for Ni, Zn, and Cr removal, respectively, from wastewaters and recovery which was carried out using HCl as an eluent. After these five cycles, the desorption performance was 75%, 53%, and 40.1%, respectively [81]. Furthermore, *Phanerochaete chrysosporium* immobilized on polyurethane in a fixed-bed bioreactor was applied in two sorption-desorption cycles for the bioremediation of Cu and Cd. The desorption performance using 0.1 M of HCl as an eluent was 65 and 75%, respectively [82].

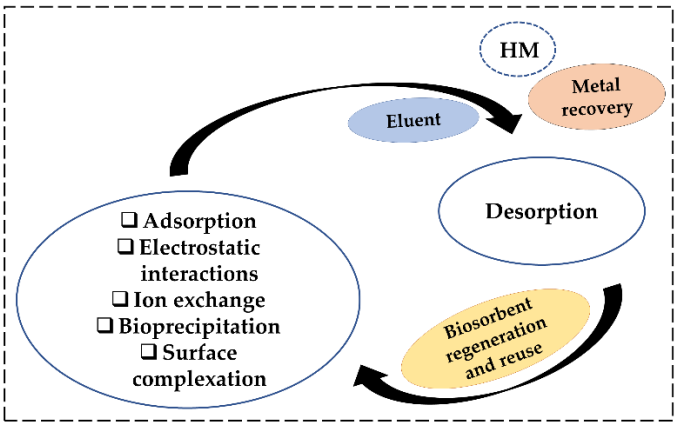

Figure 4. Sorption-desorption mechanism for heavy metals (HM) removal from wastewaters using microbial biosorbents.

6. Life Cycle Analysis (LCA) of Metal Removal from Wastewaters Using Microorganisms in Continuous Systems

Life-Cycle Sustainability Assessment (LCSA) provides a framework for the sustainability of processes and products from the perspective of all three dimensions of sustainability: environment, economic, and social [98]. The impact of a product or process on the environment component is analyzed by Life-Cycle Assessment methodology (LCA or E-LCA), considering environmental as well as human health effects. Furthermore, the economic dimension is attributed to the Life-Cycle Costing (LCC) methodology, which assesses all the costs associated with the analyzed product or process system. Social Life-Cycle Assessment (S-LCA), on the other hand, analyzes the impact on the social dimension including stakeholders, employees, communities, etc., and it is less developed than LCA and LCC [99]. The methodologies part of the LCSA framework that have been mostly applied so far in research studies are thus Life-Cycle Analysis (LCA) and Life-Cycle Costs (LCC). A schematic representation of the three methodologies comprised by the LCSA framework is included in Figure 5.

Life-Cycle Assessment (LCA) methodology is a very well-established methodology defined by the standards ISO 14040 and ISO 14044 and includes four main phases of application: goal and scope definition, inventory analysis, impact assessment, and interpretation. The starting point of the LCA analysis is the establishment of the system boundaries. This can be used in several forms: cradle to gate, gate to gate, gate to grave, and cradle to grave (Figure 6). Out of the four potential options, the cradle to grave type of system is the one that includes the input and output emissions and flows from the raw materials to waste disposal [100]. Taking into consideration the lack of environmental impact data regarding the metal removal from wastewaters using microorganisms in column systems, studies approaching any of the possible system boundaries options can bring added value at this point.

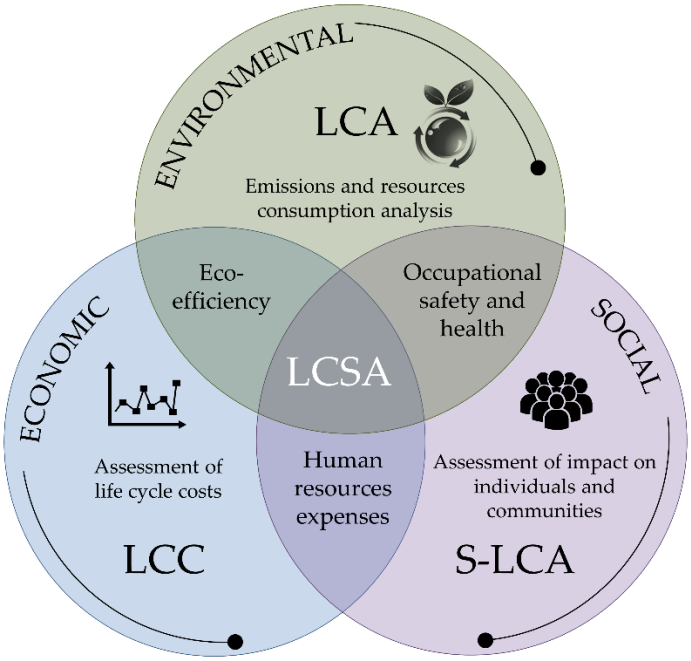

Figure 5. Life-cycle sustainability assessment framework considering the three pillars of sustainability.

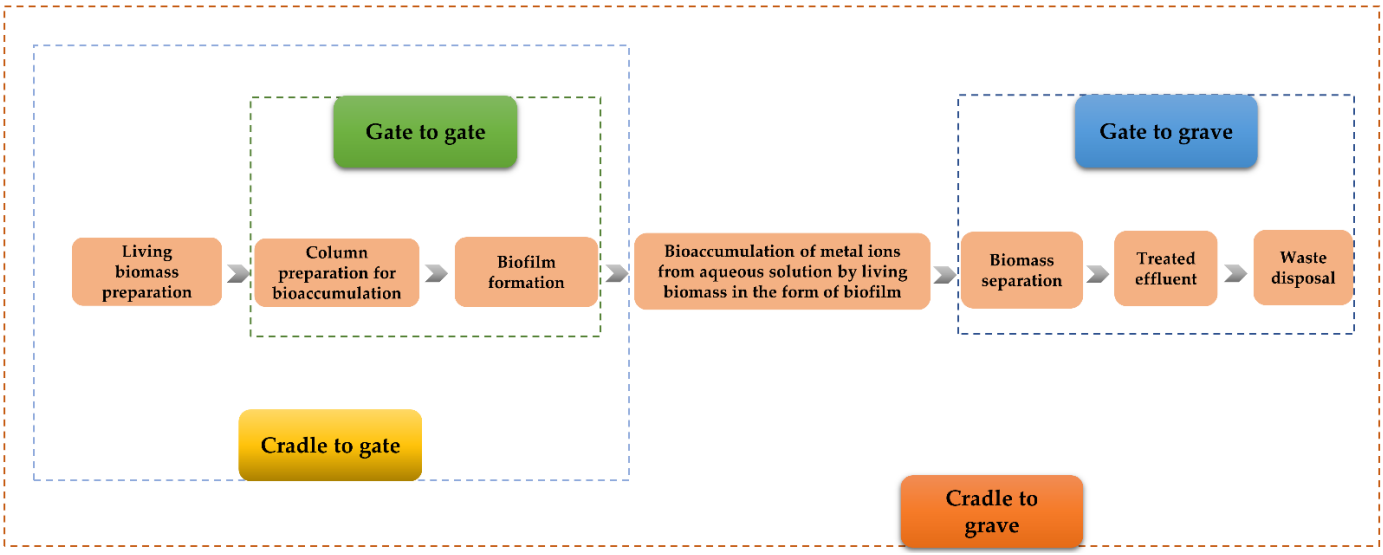

Figure 6. Defining system boundaries for the LCA of metal removal from wastewaters in continuous systems using microorganisms.

The application of LCA methodology enables the conversion of different consumed resources and generated emissions into a quantified environmental impact. These flows are converted into impact categories through characterization factors and models. The Life Cycle Inventory (LCI) includes the inputs and outputs in terms of emissions and materials which are finally quantified into an environmental impact according to the established objective [101]. A schematic overview of the Life-Cycle Inventory applied to the continuous removal of metals from wastewaters using microorganisms is included in Figure 7.

The need to optimize energy efficiency in wastewater treatment has been acknowledged by many authors. The energetic consumption is even higher and thus important to be reduced at a higher scale. LCA studies in this field have also identified energy consumption as usually having the highest contribution to the quantified environmental impact [102,103]. The use of renewable sources of energy can reduce some of the impacts such as CO_2 emis-

sions. However, in the case of biofuels and biogas, there are drawbacks which consist of deforestation, a high waterfootprint, and land occupation for biomass production [104]. The possibility to recover and reuse the used solvents and water ensures a decrease in the final environmental impact [98]. The process flow differs to some extent between batch and column systems and between microbial-based biosorption and bioaccumulation processes. Using microorganisms for metal removal in continuous systems involves complex equipments and materials that can determine a higher environmental impact as well as increased costs. Furthermore, the LCI phase and data analysis require a different approach in the case of the continuous system depending on the type of column. Reusing treated effluents as a source of water is also a common practice inside wastewater treatment plants (WWTPs), with demonstrated reduced environmental impact [104]. Water recovery and reuse can ensure a reduction in both environmental impact and the total costs, whereas the application of sorption-desoption cycles in metal biosorption by microorganisms can extend the life cycle of the used microbial biomass [105]. Further extensive research is therefore needed to reduce the environmental impact of metal uptake from wastewaters in continuous systems, energy consumption being one of the hottest issues to investigate. Thus, to make this possible, investments, economic support, and mutual effort is required from stakeholders, leaders who have the ability to make technological decisions, as well as policymakers [106].

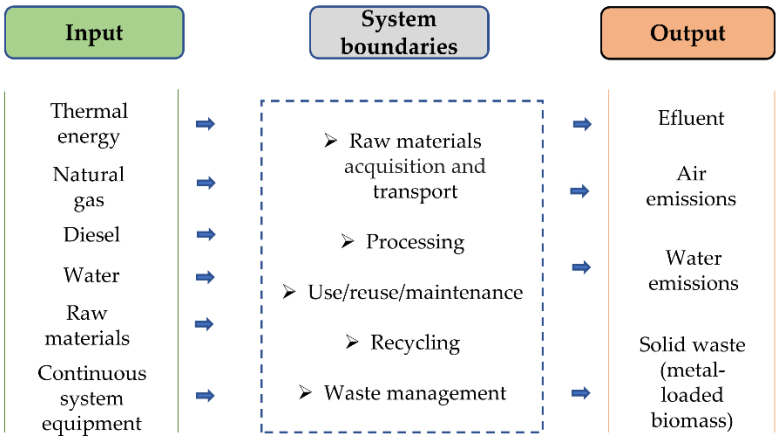

Figure 7. Overview on the Life-Cycle Inventory for the continuous removal of metal ions using microorganisms.

Industrial wastewaters typically contain more than only one type of metal ion. This makes the microbial remediation process in scaled systems more complicated, with lower performances and higher contact time for metal uptake being possible due to the competition of the ions for the available binding sites [31,105]. The higher contact time can entail higher energy consumption and thus a higher environmental impact. The composition of metal-loaded wastewaters and the interaction between the metal ions can differ, depending on the development stage, profile, and regional distribution of the generating industry/industries [107]. Thus, the multiple involved parameters and factors make the environmental impact analysis and ecodesign of the associated processes a complex matter. Most conducted research regarding metals removal from wastewaters using microorganisms has been applied to mono-metal solutions in both batch and column studies. It is therefore important to also develop research studies concerning the performance as well as the environmental impact of microbial-based remediation of wastewaters loaded with multiple metal ions.

It is worthwhile to consider that the use of microalgae in comparison with bacteria and fungi could have a lower environmental impact in wastewater treatment, especially for global warming impact categories, due to the fact that these microorganisms are photosynthetic and thus contribute to climate change mitigation. An LCA study on the impact of

microalgae cultivation for bio-oil extraction and pyrolysis processing revealed that for 1 ton of cultivated microalgal biomass, 220 kg of CO_2 are removed from the atmosphere through photosynthesis. Furthermore, comparing the microalgae cultivation with terrestrial plants cultivation (soybean and canola seed), a negative value was generated in the case of microalgae use (−222 CO_2 equivalent), thus environmental savings were recorded for the impact category of global warming, whereas in the case of the plants use, a positive value was generated (243.3 and 738.5 CO_2 equivalent, respectively). The value of the water use impact category was much higher though for microalgae cultivation [108]. Thus, to succesfully scale-up the microbial-based metal removal processes, LCA is necessary to be performed in order to have a perspective on the environmental impact of the bioremediation and to ensure minimum negative effects on the natural world [109]. A short overview of the research and technology implementation status regarding the application of LCA, S-LCA, and LCC for the analysis of microbial-based heavy metals removal using microorganisms, from laboratory to industrial scale, is shown in Figure 8.

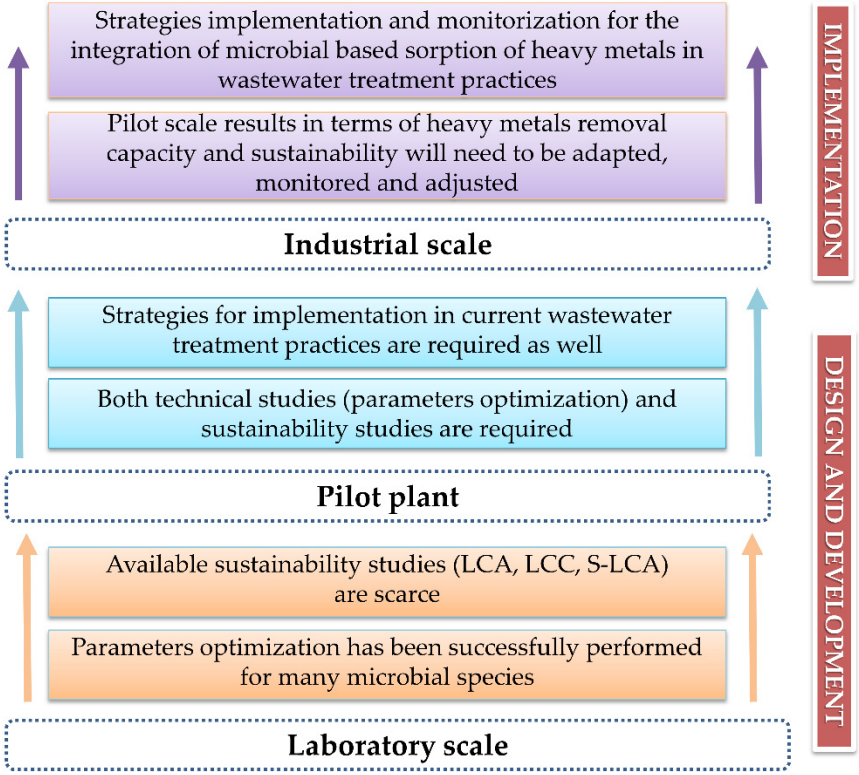

Figure 8. Life-cycle sustainability assessment status research from a lab scale to industrial scale concerning metal removal from wastewaters using microorganisms.

The choice of a specific LCA method is also important, since the results can differ depending on the chosen model [110]. Various methods of environmental impact quantification should be applied in research studies in order to have a significant outlook on the final impact.

The quantification of the environmental and economic impact of continuous systems for metal removal from wastewaters using microorganisms is very important to be analyzed for the short term as well as for the long term. Furthermore, it is important that a thorough sustainability analysis is conducted at an early phase of a process or product development or if the process is not yet implemented in the design phase [111,112]. However, the temporal distribution of the environmental impact resulting from LCSA, even that of LCA, has not been sufficiently explored in general. The lack of development of the temporal component of LCA methodology has been pointed out by many research studies especially in terms of the evolution of the resulted emissions and environmental impact over the

course of time [113,114]. The temporal analysis extrapolation is especially important for the assessment carried out concerning impact categories related to human and ecological toxicity [115]. Furthermore, considering the toxicity of heavy metals, especially the one of certain ions such as Cr(VI), As(V), Cd(II), Hg(II), and Pb(II), as well as the potential fluctuation/variation in the metal concentration of wastewaters, the temporal evolution of the generated emissions should be performed for an accurate and realistic perspective, regarding the associated environmental impact. For example, an important aspect is represented by the management of the residual microbial biomass loaded with heavy metals, especially obtained after the bioaccumulation process, in which case the metal ions cannot be recovered through desorption. Additionally, considering the bioaccumulation of heavy metals in living organisms, the determination of the long-term impact should be carefully considered. According to Shimako et al. [114], the toxicity distribution is not enough for a complete temporal understanding of the environmental impact identified through LCA. Time variability expressed in the LCI step is also important in order to understand the temporal component in the analyzed processes [116,117]. The main temporal aspects in life-cycle assessment methodology are included in Figure 9.

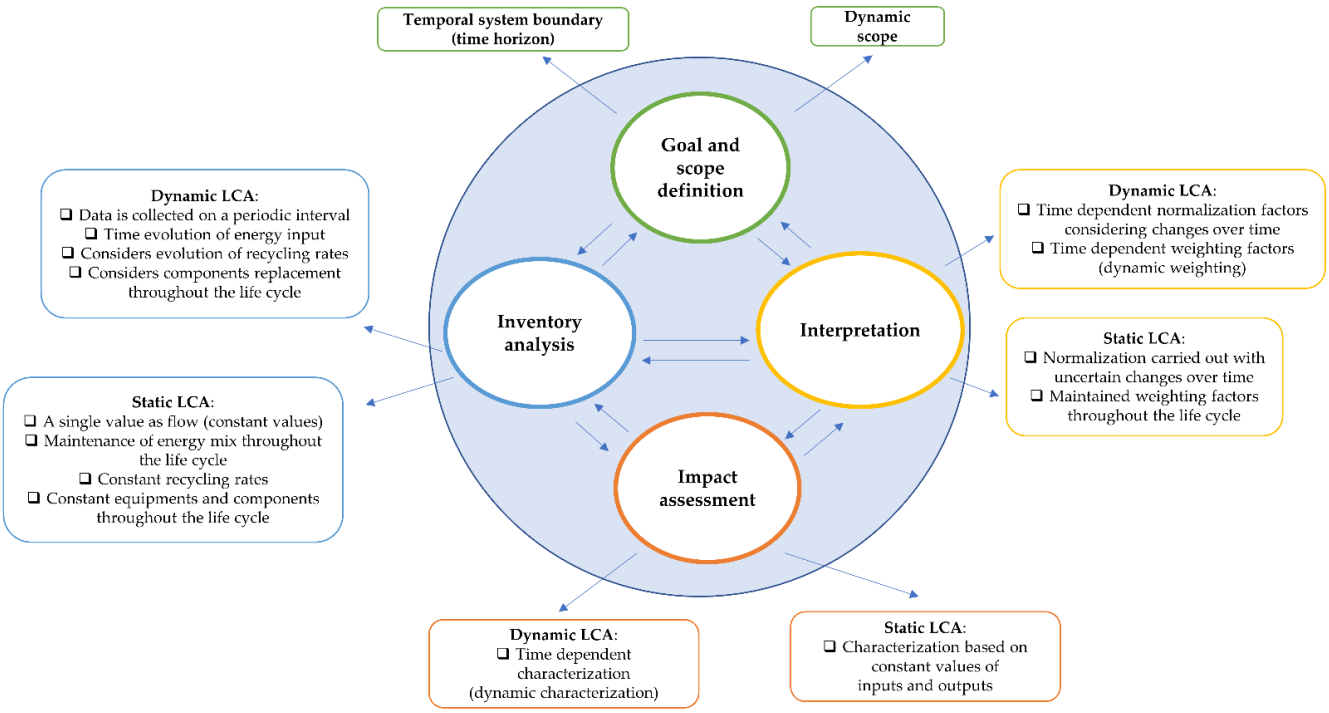

Figure 9. Temporal considerations in LCA.

Dynamic LCA (DLCA) was introduced with the scope of solving the temporal dimension issues of LCA. The principle underlying dynamic of LCA is processing the data concerning emissions collected in the LCI step as a function of time. Furthermore, in dynamic LCA, characterization factors are calculated in order to apply the analysis for any time frame [118]. The Monte-Carlo Analysis can be applied to analyze the uncertainty of several temporal aspects, including the ones of long-term emissions [116]. According to Lueddeckens et al. [116], the outcome of the LCA analysis depends greatly on the time horizon. The time horizon (TH) can be set as finite or infinite and it is an important aspect and also one of the most commonly used for the environmental impact analysis through DLCA. The option usually applied as the recommendation is the choice of infinite time horizons, one of the reasons being the fact that some impact categories use infinite THs. An exception in this sense is the land use impact category for which the time horizon is not detailed [119]. There is also the concept of partial dynamic LCA. Yang et al. [120], applied a partial dynamic LCA as well as a dynamic LCA for the global warming impact assessment

of a crop residue gasification system. The authors performed the dynamic LCA by applying instantaneous radiative forcing based on the results of the partial dynamic LCA. The use of the static, as well as the dynamic approach, is, however, recommended in order to obtain a thorough perspective on the generated environmental impact.

Taking into consideration the lack of data regarding the use of active or inactive microorganisms for metal removal from wastewaters at a pilot or industrial level, data simulation is an approach that can enable obtaining a larger perspective on the associated environmental impact. de Faria et al. [121] applied dynamic LCA in wastewater treatment using Python™ for the simulation of data, which was further processed in Umberto for final LCA results. Although dynamic LCA has not been applied yet in the removal of heavy metals from wastewaters using microorganisms, its use in wastewater treatment plants from Poland [103] and Spain [122] has been studied. The establishment of the limits of the analyzed system enables the identification of the main input and output data based on the main sequential processes that are performed within the wastewater treatment using microorganisms. The chosen system boundaries for the analyzed system describes the temporal scope of the studied environmental impact. The description of the temporal dimension in this way can be limiting, though, and can generate uncertainties [119].

The accuracy of LCA results can be improved not only by developing the temporal analysis of the generated impact, but also through the study of the spatial dimension [123]. Countries differ in terms of economic situation and environmental pollution. In order to be able to adapt the LCSA technology effectively to different geographic zones, these aspects must be taken into consideration. The spatial dimension in LCA is included in a relatively new concept named territorial LCA. There are two types of territorial LCA: one that concentrates on the analysis of one activity only from a certain region and another which evaluates the impact of all associated activities in the selected region [124].

The chemical composition of wastewaters loaded with heavy metals can differ significantly regionally, which makes the introduction of local data very important for obtaining the most realistic perspective on the generated impact. However, related characterization factors cannot be used with most of the available LCA methods [110]. The characterization factors should be used in order to determine the impact based on the pollutants involved and the local geographic area [123]. Characterization models which are developed considering Europe area are IMPACT 2002+, ILCD, EDIP2003, and CML-IA, whereas for North America, LUCAS, BEES, and TRACI are applied. Furthermore, IMPACT world+ was created based on IMPACT 2002+, LUCAS, and EDIP2003 models and includes regionalized characterization factors for most of its impact categories. ReCiPe2016, one of the most commonly applied LCA methods, also includes some country or region-based characterization factors. In terms of inventory databases, Sphera and Ecoinvent include data on different geographical scales [125].

The USEtox model is an established method for the quantification of the environmental impact on human health, being the general recommendation of the European Union in this matter. The spatial dimension can be a problem because of the too general geographic area taken into consideration by this method. USEtox enables the analysis of the impact on both global and country level, comprising 8 continental and 17 subcontinental zones. The available LCA methods are generally superficially considering the impact on a country level, whereas the environmental conditions and data can differ significantly even inside the territory of a state. This necessary regional data are scarce though [123]. Another approach for addressing the spatial dimension for environmental impact quantification is applying LCA methodology, as well as ERA, a method analyzing the ecological effects of single-substances or different combinations of chemicals. This is due to the fact that, in comparison with the LCA methodology, which typically lacks the time and space component, ERA method includes these aspects. Another recommendation is using the LCA for a global assessment and ERA for a local analysis of the impact [126].

The integration of both temporal and spatial perspectives is significant for accurate results. On the other hand, variability due to data availability and quality in LCA of

wastewater treatment is expected. Many factors, such as meteorological, geographic, number of households, businesses, and industries connected to a WWTP and their usual degree of water use, influence the total LCA results. Taking this into consideration, uncertainty analysis is an important step in order to ensure maximum accuracy of LCA results [127].

7. Conclusions

The use of microorganisms for sorption processes of heavy metals represents a promising alternative to the conventional sorbents and other physico-chemical methods. Desorption of heavy metals enables the recovery of the metal ions and multiple uses of the microbial biomass, ensuring in this way a more sustainable remediation process. Heavy metals sorption-desorption processes using microorganisms has demonstrated good results in discontinuous (batch), as well as continuous (column), systems. Furthermore, different immobilization materials, including nanomaterials, can be used for a more stable and efficient remediation process. The current study shows the potential of performing metal removal processes in continuous systems using microorganisms as sorbents as analyzed in various studies in the field. Based on the synthesized scientific information, we conclude that more studies concerning the parameters of optimization, performance analysis in multiple sorption-desorption cycles, and multi-metal removal, as well as sustainability analyses applied for continuous systems and pilot scale, are required in order to finally upgrade the microbial-based metal removal process. An approach focused on the analysis of real effluents is recommended for future research work in continuous systems.

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