Chapter 12

Yeast Biomass: An Alternative for Bioremediation of Heavy Metals

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Abstract

Heavy metal pollution has become one of the most serious environmental problems throughout the world. Among the innovative solutions for treatment of contaminated water and soil, bioremediation that use biological materials like living or dead micro-organisms is a promising, safe and economical technology. One of the most ubiquitous biomass types available for bioremediation of heavy metals is yeast. Yeast cells represent an inexpensive, readily available source of biomass that retains its removal ability for a broad range of heavy metals to varying degrees. Furthermore, yeasts exhibit the ability to adapt to extreme conditions such as temperature, pH and high levels of organic and inorganic contaminants. To understand the different mechanisms of interactions between metals and yeast strains in the environment, this paper will give an overview on the role that yeasts play in the immobilization/mobilization of toxic metals and factors affecting these processes. Biotechnological applications in the bioremediation of heavy metal such as bioaugmentation using degradation abilities of yeasts will also be discussed.

Keywords: bioremediation, heavy metals, yeast, interaction mechanisms, bioaugmentation

1. Introduction

The industrialization has long been accepted as a hallmark of civilization. However, million tons of contaminating compounds such as toxic heavy metals (Cd, Cu, Hg, Pb, Mn, As, Ni, Zn, etc.) are produced and directly or indirectly released into the environment [1]. Unlike organic contaminants, these pollutants are not biodegradable and can be transferred through the food chain via bioaccumulation [2]. Actually, the build-up of dangerous concentrations

of toxic metals in water sources and in grains and vegetables grown in contaminated soils is critically alarming due to the harmful effects of metals on human life and aquatic biota [1]. The actual challenge is to develop innovative and cost-effective solutions to decontaminate polluted environments and to protect the functioning of the ecosystems. Volesky [3] and Domenech [4] shortlisted some available conventional methods for removing the dissolved heavy metals including chemical precipitation, filtration, ion exchange, oxidation or reduction, reverse osmosis, evaporation, membrane technology, and electrochemical treatment. But most of these techniques become ineffective when the concentrations of heavy metals are less than 100 mg/L [5]. Additionally, strong and contaminating reagents are used for desorption, resulting in toxic sludge and secondary environmental pollution [1].

In order to minimize the effects of environmental pollution, the biological methods of metal removal such as bioremediation were considered. Different kinds of organisms isolated from contaminated soil, waste waters, compost and extreme environments are proved useful for bioremediation, from plants to microbes [6, 7]. Their success to survive in such a harsh environment can be attributed to metabolic possibilities allowing biological organisms to explore, detoxify and survive in exotic and complex substrates. The microbial metabolic diversity and versatility are two of the reasons why they are suitable as agents for remediation among many living organisms [8]. These microorganisms have evolved various measures to respond to heavy-metal stress via processes such as transport across the cell membrane, biosorption to cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation-reduction reactions [9–14].

One of the most ubiquitous biomass types available for bioremediation of heavy metals is yeast cells that retain their ability to accumulate a broad range of heavy metals to varying degrees under a wide range of external conditions [15]. Yeast can serve as a suitable model for studying physiological and molecular mechanisms of eukaryotic cell interactions with heavy metals. Studies on bacteria, yeast, fungi and microalgae showed that yeasts are better biosorbent for the removal of heavy-metal ions from wastewater due to their high growth rate and cell wall structure [16]. Indeed, cell wall phosphates and carboxyl groups have been reported to be the major determinant of negative yeast cell surface charge which enhances the ability of yeast cells to bind heavy-metal cations. This is likely due to electrostatic interactions [17]. It was also reported that the various metal-binding groups, viz., amine, imidazole, phosphate, sulfate, sulfhydryl and hydroxyl, are present in the polymers of the cell wall of fungi [18]. In addition, yeasts are larger than bacteria and can physically intertwine the mycelia/pseudomycelia to form floes. The net-structured yeast floc facilitates oxygen diffusion and eliminates the necessity of excessive dissolution of oxygen in water. Therefore using yeasts can give high efficient oxygen supply and reduce energy consumption by reducing the supplied air flow [19].

On the other hand, yeast biomass is an inexpensive, readily available source of biomass. It can be easily cultivated in cheap growth media, and it can be cheaply available in good quantities from fermentation industries for wastewater remediation [20, 21]. Many industrial waste-biomass types were investigated for their biosorptive potential. These include the yeasts, *Saccharomyces cerevisiae*, from the food and beverage industry and *Candida albicans*, a clinical isolate [14].

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2. Bioremediation

Bioremediation is considered as an alternative processing method to reduce the environmental pollutants into less toxic forms [2]. It is defined as the process by which organic or inorganic wastes are biologically degraded or transformed usually to innocuous materials [22]. Mueller et al. also defined bioremediation as a process where organic wastes are biologically degraded under controlled conditions to an innocuous state or to levels below concentration limits established by regulatory authorities [23].

The major strategies for implementing bioremediation processes include biostimulation and bioaugmentation [22]. Biostimulation is the bioremediation process that can be enhanced by adding an electron acceptor, nutrient or other factors to a contaminated site with the objective of stimulating growth of the microbial population already present there [2]. When microorganisms are imported to a contaminated site to enhance degradation, we have a process known as bioaugmentation. Thus, the microorganisms used in bioremediation may be indigenous to a contaminated area, or they may be isolated from elsewhere. Recent studies show that microorganisms isolated from contaminated sites present high-tolerance adaptation of multiple environmental conditions and have excellent capability of removing significant amounts of metals [2, 24–26].

It was reported that yeasts and fungi are able to grow in matrices that have high concentrations of metal compounds compared to other microorganisms. In addition to their resistance, Ksheminska et al. [27] reported that yeast strains are capable of removing significant quantities of these pollutants. Other studies with yeasts showed also that, upon metal exposure, the main goal of the yeast cell is to protect and detoxify the environment by rendering the metal ions unavailable to promote cytotoxic effects. Furthermore, in comparison with bacteria which only use the active metabolizing capabilities, yeasts have the property of being used whether they are alive (metabolically active) or dead (metabolically inactive/passive) to remove these contaminants [24, 28, 29].

3. Yeast bioremediation mechanisms

Information about metal detoxification mechanisms in yeasts is considerably less available when compared to prokaryotes. The most studied yeasts mainly belong to the ascomycetous group, such as *S. cerevisiae*, *Schizosaccharomyces pombe* and *Candida* sp. [30]. The studies showed that yeasts evolved several different detoxifying mechanisms by which they can mobilize, immobilize or transform metals. The immobilization mechanisms include (i) biosorption, interaction of metals with the cell membrane via different processes such as ion exchange, complexation, crystallization, adsorption and precipitation; (ii) biotransformation, toxic metals are reduced to less toxic forms; and (iii) bioaccumulation, intracellular uptake of metal ions by living microorganisms [2]. The mobilization mechanisms involve mainly bioleaching through production and excretion of some acids which interact with metal ions to produce insoluble complex. Thus, in general the immobilization and mobilization are the two main techniques used for the bioremediation of metals by yeast and fungi (**Figure 1**) [31–33].

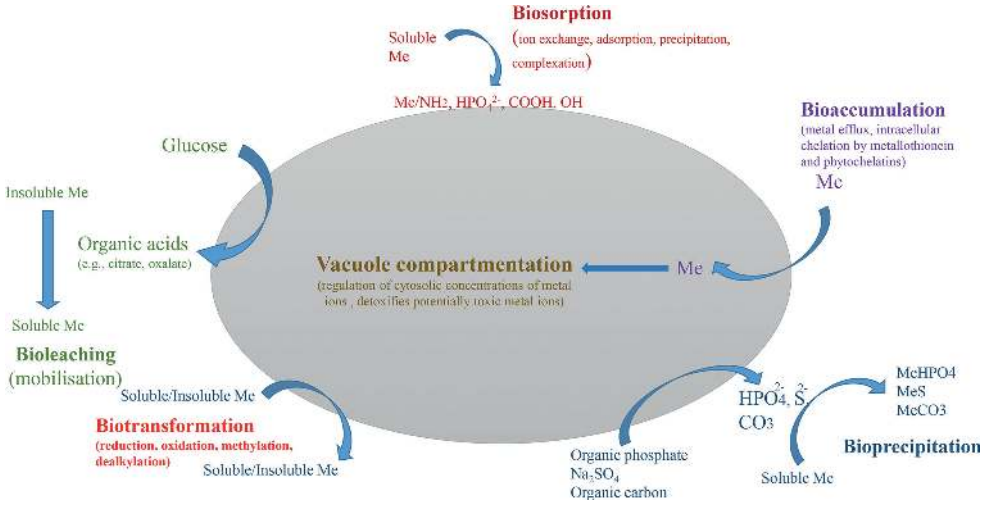

Figure 1. Interactions of metal and fungi cells (adapted from Refs. [31–33]).

3.1. Biosorption

Biosorption is a nondirected physico-chemical interaction that may occur between metal and cellular compounds of biological species [34]. It consists of the ability of biological materials to bind and concentrate heavy metals through metabolically mediated or physico-chemical pathways [35].

Among the promising types of biosorbent studied, yeast and fungal biomass seems to be good sorption materials with a sufficiently high metal-binding capacity and selectivity for heavy metals [36].

3.1.1. Yeast cell wall properties

The first stage of metal ion binding in microorganism cells does not depend on their metabolism and consists in ion chemisorption into cell wall components. Thus, the biosorption efficiency of heavy metals by microbial biomass is mainly connected with the structure of the microorganism cell wall and consequently with cell surface properties in which structure determines the interaction nature between micro-organism and metal cation [36–38].

Yeast cell walls are negatively charged, and the ability of yeast cells to bind heavy-metal cations is likely due to electrostatic interactions [39]. Indeed, heavy metals can be biosorbed by microbes at binding sites present in cellular structure without the involvement of energy. Among the various reactive compounds associated with cell walls, the extracellular polymeric substances such as exopolysaccharide (EPS) are well known to have a considerable effect on acid–base properties and a great ability to complex heavy metals [40]. The structure and the distribution of homopolysaccharides (mannans and glucans), single saccharides and acid components, which are good binding agents, also dictate the cell wall's biosorption capacity [41].

3.1.2. Mechanisms of biosorption

It is likely that various mechanisms can be involved in biosorption and can operate simultaneously to various degrees. Interaction of metal with yeast cell wall involves a complex mechanism that includes several processes such as ion exchange, complexation, adsorption and precipitation [2, 42]. Many evidences have proved that ion exchange mechanism exists in biosorption system [38, 43, 44]. However, it was suggested by many researchers that ion exchange is neither the sole nor the main mechanism for metal biosorption [45]. Ion exchange is the replacement of an ion in a solid phase in contact with a solution by another ion. More specifically, it is the replacement of an absorbed, readily exchangeable ion by another [46]. Rapid release of 70% of cellular K^+, followed by a slower release of approximately 60% of cellular Mg^{2+}, but little loss of Ca^{2+}, was observed in Cu^{2+} removal by *S. cerevisiae* [47], indicating the existence of an ion exchange mechanism. Chen and Wang [48] also reported that *S. cerevisiae* acts as a biosorbent for the removal of Zn (II) and Cd (II) through the ion exchange mechanism. According to Vasudevan et al. [49], the release of Ca^{2+}, Mg^{2+} or H^+ was also observed in the biosorption process of heavy-metal ions (Sr^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Ti^+) by living, non-metabolizing cells of *S. cerevisiae*, which also confirmed the existence of ion exchange. Although it is a simple concept, in reality, ion exchange can be a mechanistically highly complex process depending on the system [50, 51].

Metal precipitation is also involved in biosorption. The term precipitation in most cases refers to the formation of insoluble inorganic metal precipitates [52]. This may be more easily understood when metals are bound to extracellular polymeric substances excreted by eukaryotic microorganisms such as yeast and fungi. The precipitates may be formed and remain in contact with or inside the microbial cells or may be independent from the solid phase of the microbial cell. Some researches proved that purified biomolecule products from isolated cells such as glucan, mannan, and chitin accumulate greater quantities of cations than the intact cells and can form metal precipitates [53].

Several yeast species such as *S. cerevisiae*, *Pichia anomala, Candida tropicalis, C. albicans*, and *Cunninghamella elegans* emerged as a promising sorbents against heavy metals [54]. **Table 1** summarizes some of the important results of metal biosorption using these yeast biomasses.

3.1.3. Factors affecting yeast biosorption capacity

It is noted that the same yeasts species have different biosorption capacities for the same metal ion. It was shown that different yeasts species present different cell surface properties and cell wall compositions, which brings about a differentiation in biosorption ability, affinity and interaction specificity. Moreover, biosorption depends on many factors that are related to the biomass or to environmental conditions. Indeed, Nguyen et al. [68] studied the polysaccharide composition of the cell walls of several yeast species, such as *Debaryomyces hansenii*, *Zygosaccharomyces bailii* and *S. cerevisiae,* and results indicated that the cell wall composition varied over the species and strains. Growth, nutrition and age of the biomasscan also influence the variability of cell size, cell wall composition and extracellular product formation [69]. It was found that the cell wall composition and polysaccharide content could vary by more than 50% with the nature of the carbon source, nitrogen limitation, temperature and aeration

Biomass type	Metal studied	Initial concentration of metal studied (mg/L)	Biosorption capacity (mg/g) or % removal	Biomass concentration (g/L)	References
S. cerevisiae	Hg	25–200	76.20	2	[55]
S. cerevisiae	Pb		67–82%		[56]
	Cd		73–79%		[56]
S. cerevisiae	Cd	169	5.96		[57]
Schizosaccharomyces pombe	Ni	400	33.8		[58]
S. cerevisiae	Cr(III)	200	35.00		[59]
S. cerevisiae	Cr(VI)	150	120	0.5	[60]
S. cerevisiae	Pb		270.30	10	[61]
S. cerevisiae	Zn		23.40	1	[62]
S. cerevisiae	Hg		64.20	1	[62]
S. cerevisiae	Cd		15.60		[63]
S. cerevisiae	Pb		17.50		[63]
S. cerevisiae	Pd		40.60		[64]
S. cerevisiae	Cr(VI)		32.60	10	[61]
S. cerevisiae	Ni		46.30	10	[61]
S. cerevisiae sp.	Zn		100%	0.4	[65]
S. cerevisiae	Cd		95%	0.4	[65]
C. pelliculosa	Cu	100	95.04%	13.39	[66]
C. utilis	Cd	50	81.46	3	[67]
C. tropicalis	Cr	100	29.10	2	[24]
W. anomalus	Cr	100	28.14	2	[24]
C. fabianii	Cr	100	18.90	2	[24]

Table 1. Some data on the biosorptive capacities of yeasts for different metal ions reported in literatures.

and pH and with the mode of cell cultivation [70]. Temperature is considered as one of the important factors in the biosorption process. It was reported that adsorption reactions are mostly exothermic and the extent of adsorption augment with decreasing temperature [32]. Thus, a better biosorption capacity for Ni and Pb by *S. cerevisiae* was observed at a low temperature (25°C) and found to diminish as the temperature was increased to 40°C [71].

Metal biosorption was also frequently shown to be strongly pH dependent. Sulaymon et al. [35] indicated that pH of solution affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions. Some studies showed that yeast cells of *S. cerevisiae* are able to remove heavy-metals between pH 5.0 and

9.0, being pH close to 5–6 as the optimal for Cu^{2+}, $Cd^{2+,}$ Pb^{2+}, Ni^{2+}, Zn^{2+} and Cr^{3+} biosorption by yeast cells [47, 72]. However, other studies demonstrated that biosorption of metals like Cu, Cd, Ni, Co and Zn is often reduced at low pH values [73, 74]. Generally, the heavy metal uptake for most of the biomass types declines significantly when pH of the metal solutions is decreased from pH 6.0 to 2.5 [72]. The cell surface hydrophobicity may also affect biosorption capacity, facilitating hydrophobic bonds. Edyta [75] has reported that a lower relative hydrophobicity and a higher negative surface charge can be related to better availability of polar/charged groups such as carboxyls and mannosylphosphates on yeast cell surface.

3.1.4. Industrial application

Biosorption process has not only been used in laboratory scale (batch and column) but also in pilot plant-scale studies. Tigini et al. [76] tested this process using *C. elegans* biomass in 200 L of pilot plant installation for dye removal. Obtained results showed that biosorption was an effective process for the removal of different pollutants from spent baths and wastewaters [76].

The apparent exploitation potential of biosorption is often cited in the literature, and the commercial applications of biosorption are currently available on the market. The biological materials can be commercialized as powdered biosorbents, e.g. BIO-FIX® (sphagnum, peat moss, algae, yeast, bacteria and aquatic flora immobilized in polysulfone), MetaGeneR and RAHCO Bio-Beads [77]. All the preparations are suitable for the treatment of wastewaters from metallurgical industry or mining operations. The future possible applications may concern using biosorption in the separation and purification of high value molecules, e.g. high-value proteins, steroids, pharmaceuticals or antibodies [77].

3.2. Bioaccumulation

Bioaccumulation is defined as the uptake of toxicant by living cells and their transport into the cell [78]. It is a growth-dependent process mediated only by living biomass [79]. The mechanism of intracellular uptake is more complex than biosorption itself and is not fully understood yet. Generally, the process is regarded as a two-step process [80]. The first step called passive biosorption proceeds rapidly within several minutes by any one or a combination of the following metal-binding mechanisms: coordination, complexation, ion exchange or physical adsorption (e.g. electrostatic). The metal ions are adsorbed to the surface of cells by interactions between metal-functional groups displayed on the surface of cells. The second step which slowly takes place is that metal ions penetrate the cell membrane and enter into the cells. Raspor et al. [81] pointed out that after initial rapid biosorption on cell walls, active transport mechanisms into the cells take place and metal ions penetrate the cell membrane and enter into the cells. Metal ions transported across the cell membrane, are transformed to other species or precipitated within the cell by active cells, including transportation.

Metal accumulation strategies for essential and non-essential metal ions may be different. Literature in model yeasts indicates that upon non-essential metal exposure, cytoplasmic detoxification is the major strategy [30]. This cytoplasmic detoxification can be achieved by

metal transport to the outside of the cell or to less sensitive cellular compartments, making the metal ions unavailable to promote cytotoxic effects [82]. Thus, microorganisms developed different mechanisms including cell membrane metal efflux [83], intracellular chelation by metallothionein proteins and glutathione-derived-peptides called phytochelatins [84, 85] as well as metal compartmentalization in vacuoles [3], but the exact mechanism of intracellular accumulation is not elucidated.

In general, metals enter yeast cells by dedicated transporters, which are often target of negative regulation when the specific metal is present in excess intracellularly. In the past decades, a large number of references on metal ion transport in the baker's yeast *S. cerevisiae* were published [86, 87]. Baker's yeast was found to possess two or more substrate-specific transport systems to accumulate any single metal ion, and a large number of yeast genes that function in metal ion transport or its regulation were confirmed [87]. After entering into the cell, the metal ions react with thiol groups present in Cys residues such thiolated peptides include the glutathione (GSH), phytochelatins (PC), and the metallothioneins (MT) [82]. The resulting metal-thiolated peptide complexes may be used as a substrate for metal(loid) extrusion to the outside of the cell, or for accumulation in cellular compartments such as the vacuole. However, thiolated peptides can be produced to chelate metals, reducing their reactivity and availability to the cells [30].

The most described yeast MT is the Cup1 of *S. cerevisiae*, which is mainly associated with Cu detoxification [88]. Several authors attributed Cd, in addition to Cu, detoxification to chelation by this MT [88, 89]. In *S. cerevisiae*, GSH synthesis was also described. In comparison with a wild-type strain of *S. cerevisiae*, the mutant strain displayed a high sensitivity to arsenite, selenite and cadmium which confirms the role of GSH on detoxification of these metals.

The role of the vacuole in the detoxification of metal ions was also investigated and a large number of researches signaled that fungal vacuole plays an important role in molecular degradation, storage of metabolites and regulation of cytosolic concentrations of metal ions and detoxifies potentially toxic metal ions. The results showed that vacuole-deficient strain displayed much higher sensitivity and decreased large uptake of As, Zn, Mn, Co and Ni [90, 91]. Avery and Tobin [92] also confirmed that Sr^{2+} accumulated mainly stays in the vacuole of the living yeast cell of *S. cerevisiae*.

The active mode of metal accumulation by living cells is dependent on structural properties, physiological and genetic adaptation, environmental modification of metal specification, availability and toxicity [93]. The process also depends of several factors (which are almost identical to the factors influencing the cultivation of an organism): the composition of the growth medium, pH, temperature, the presence of other pollutants or other inhibitors, surfactants, etc. [94]. Metabolic activities such as respiration, nutrient uptake, and metabolite release will alter the microenvironment around the cells which, in turn, may affect mechanisms involved in bioaccumulation (adsorption, ion exchange, complexation and precipitation) [69].

In yeasts, heavy metals can be accumulated by bioaccumulation process more than biosorption. But the biosorption process seems to be more feasible for large scale application compared to the bioaccumulation process, because microbes will require addition of nutrients for their active uptake of heavy metals [27]. Thus, the biosorptive capacity of yeast was studied extensively in comparison to bioaccumulation.

3.3. Bioreduction

The detoxification of metal ions can also be realized by oxidation or reduction. When reduction of a metal to a lower redox state occurs, mobility and toxicity can be reduced, thus offering potential bioremediation applications [2].

Information available for metal detoxification, such as reduction mechanisms in yeasts, only considers neutrophilic yeasts and there is considerably less information available when compared to prokaryotes. Indeed, for eukaryotic microbial cells and primarily yeasts, the data on the metal-reducing systems are more ambiguous. It is generally unknown what system enzymatic or non-enzymatic and intracellular or extra-cellular plays a leading role in the chromate detoxification process [9].

Tamás and Wysocki [82] proved that one mechanism of detoxification of As(V) was the reduction of As(V) to As(III), a process catalysed by arsenate reductase enzymes. The removal of toxic hexavalent chromium from aqueous solution by biosorption by different biomass types was as well extensively reported [9]. Cr(VI) can be reduced as a powerful oxidative agent to Cr(III) by cellular-reducing systems that can include enzymatic and non-enzymatic pathways.

The intracellular reduction of Cr(VI) to Cr(III) is known to be the main detoxification mechanism of chromium. In aerobic condition, microbial reduction of Cr^{6+} is catalysed by soluble enzymes (chromate reductase) [95]. Many yeasts like *Cyberlindnera fabianii*, *P. anomala*, *Rhodotorula pilimanae* D-76, and *Pichia guilliermondii* ATCC 201911 were known for their enzymatic reduction ability of Cr^{6+} to Cr^{3+} [27, 28, 96, 97].

Cr(VI) removal may also be associated with its simultaneous reduction to Cr(III). It was reported that Cr(VI) can be reduced to Cr(III) through a redox reaction unrelated to any enzyme activity [98, 99]. Thus inactivated biomass, e.g. *S. cerevisiae*, *C. tropicalis*, *P. anomala* and *Penicillium chrysogenum*, removes Cr(VI) from aqueous solutions by reduction to Cr(III) when contacted with the biomass [96, 100]. Glutathione and cysteine can be considered as the most powerful non-enzymatic chromate reductants for microbial cells and ascorbate for higher organisms [2, 101]. Therefore, the removal of Cr(VI) from aqueous solution by dead cells may involve two mechanisms: (i) direct reduction, in the aqueous phase, Cr(VI) is directly reduced to Cr(III) by contact with the electron-donor groups of the biomass which has lower reduction potential values than that of Cr(VI) (+1.3 V), and (ii) indirect reduction, which includes three steps—(a) adsorption of Cr(VI) anionic species to the biomass surface containing the positively charged groups, (b) reduction of Cr (VI) to Cr(III) by adjacent electron-donor groups and (c) release of Cr (III) ions in the aqueous phase due to the electronic repulsion between the Cr (III) ions and positively charged groups, or adsorption of Cr(III) with adjacent groups.

3.4. Bioleaching

In the context of bioremediation, immobilization processes such as biosorption, accumulation and precipitation may enable metals to be transformed in situ into insoluble forms. They are particularly applicable to remove metals from mobile phases such as ground waters and leachates. In contrast, the process of metal solubilization provides a way to remove metals from soils and sediments by leaching. Generally bioleaching is a process described as being

"dissolution of metals from their mineral source by certainly and naturally occurring microorganisms". However, there are some slight differences in definition: Usually, "bioleaching" is described as the conversion of solid metal values into their water soluble forms using microorganisms [102].

In the soil environment, metals can be held on inorganic soil constituents through various sorption or ion exchange reactions, complexed with soil organic materials or precipitated as pure or mixed solids [103]. However, in most acidic soils, metals may be speciated into more mobile forms [104]. It was reported that in such locations, fungi are the most predominating and often comprise the largest biomass suggesting its intervention in metal solubilization [33, 104]. Gadd [33] confirmed that microbes interact with metal and mineral in natural and synthetic environments, altering their physical and chemical state. Therefore, the biochemical activity of fungi and other microorganisms can affect metal speciation and mobility in the soil, modifying their biogeochemical cycles. The most important mechanisms of metal and minerals solubilization by fungi are acidolysis and complexolysis. It was revealed that some excreted metabolites with metal-complexing properties, e.g. phenolic compounds, and organic acids may be involved in metals solubilization [105]. Gadd [104] also indicated that low molecular weight organic acids, such as citric and oxalic acid, are the most important chemical fungal and yeast products and they were used for heavy metal solubilization. In fact, previous studies showed that many metal citrates are highly mobile. They also proved that oxalic acid can act as a leaching agent for those metals, which forms soluble oxalate complexes, including Al and Fe [106]. It was shown that organic acids provide both source of protons for solubilization and metal-chelating anion to complex the metal cation [107]. They have the double function: (i) to acidify the substrate, thus enhancing ions solubility, and (ii) to form complexes with solubilized ions, which leads into mobilizing them [104, 105].

Yeast doesn't show a broad area of examples in the field of bioleaching and the available information about the metal extraction from solid substrates using yeasts is limited. Some yeasts associated with bioleaching are *Rhodotorula rubra* and *Rhodotorula mucilaginosa*. Studies of yeast *R. mucilaginosa sp. lm*9 isolated from Kupferschiefer black shale showed that organic acids (malic and oxalic) produced by this strain can effectively mobilize copper from sedimentary rock [108]. Marcinčáková et al. [109] also demonstrated that 0.17% of lithium can be recovered from lepidolite by microbial leaching using the heterotrophic microorganism of *R. rubra*.

The bioleaching process is carried out at the mine site. It is indicated that the bioleaching process can be used to process low-grade ores and arsenic-containing ores that could not be processed effectively by high temperature smelting. Two types of bioleaching processes exist: bioleaching in stirred tank reactors (STR), which is dedicated to high-grade ores due to the relatively high costs of investments, and heap leaching, which is dedicated to low-grade ores. Bench scale columns were also used with ore in Australia in the period from 1964 to 1968 [110].

There are many and various applications of bioleaching. It can be applied to a wide variety of base-metal sulfides, mainly in large operations located in many countries and several interesting projects were conducted in order to develop this technology. The earliest commercial

applications of the process involved in situ leaching of uranium in Canada, heap bioleaching of copper in Toromocho and dump leaching of copper in the United States (bioleach, Chili). Nowadays, the production of copper from low-grade ores is the most important industrial application. Early application of bioleaching to copper mining was centered in its recovery from heap or dump/stockpile [111, 112]. In these operations, copper is extracted from ores containing minerals: secondary copper sulfides such as covellite (CuS), chalcocite (Cu_2S) and bornite (Cu_5FeS_4) and the primary copper sulfide, chalcopyrite ($CuFeS_2$).

As well as detoxification of pollutant metals and copper production, the recovery of precious metals such as gold is also a potential area for exploitation. Industrial-scale bioleaching of refractory gold concentrates was practiced in South Africa, Brazil, Australia, Ghana, Peru, China and Kazakhstan. In this case, the process is used to leach sulfide minerals such as pyrite (FeS_2) and arsenopyrite (FeAsS), which encapsulate microscopic and sub-microscopic gold particles. By dissolving these sulfide minerals, the gold particles are exposed and can be recovered by further treatment [113].

Although yeasts have a high potential for bioleaching, there are no studies on the use of these microorganisms in bioleaching projects. So, it would be interesting to develop this technology using yeasts by testing their capacity on a large scale.

4. Biotechnological approaches: bioaugmentation

Bioaugmentation is one of the promising techniques of bioremediation; it is referring to the process of adding selected microbial strains or mixed cultures to biological waste treatments or contaminated sites in order to enhance effectively the removal of specific pollutants [114]. The rationale for this approach is that indigenous microbial populations may not be able to degrade the wide range of potential substrates present in complex mixtures [115] or when the indigenous pollutant-degrading population is low. Thus, the acceleration of decontamination is the primary advantage for the introduction of microorganisms.

The approach of this technology is taking advantage of microbial consortia designed for specific physico-chemical properties of the bioprocess [116]. This remediation way was shown to be more efficient than using undefined inocula [114]. However, one of the difficulties of bioaugmentation processes is the presence of many uncharacterized organisms that enter in competition with introduced populations. It was reported that some foreign microorganisms (those in inocula) were applied successfully in laboratory, but their efficiency depends on their ability to compete with indigenous microorganisms, predators and various abiotic factors. Thus, successful bioaugmentation treatments depend on the use of inocula consisting of microbial strains or microbial consortia well adapted to the site to be decontaminated [117].

Bioaugmentation was carried out using different microorganisms. The yeast-based bioaugmentation was specifically shown to be an advantageous soil and water clean-up approach for contaminated sites, containing heavy metals and/or organic pollutants [118–120].

Many isolated and laboratory-qualified microorganisms were reviewed, but they are not valid in situ yet. The soil bioaugmentation with *C. fabianii* removed more than 60% of a soil's chromium contamination of 40 $mg.Kg^{-1}$. It has reduced by the way its phytotoxic effects on *Phaseolus vulgaris L.* and promoted its growth under chromium stress [121]. In another work, both alive and dead *C. tropicalis* biomass showed a great ability to significantly reduce the bioavailability of 40 $mg.Kg^{-1}$ of Cr(VI) in soils (up to 58.7 and 72.25% of reduction, respectively) [120]. In that work, clover plants were used as bioindicator where significant increase in seed germination and growth of seedlings was detected in the inoculated soils by *C. tropicalis* cells. In a further work, the bioaugmentation with the specific yeast strain *C. tropicalis SK21*showed a great efficiency for the bioremediation of petroleum-contaminated soil [119] where 96 and 42% of total petroleum hydrocarbons (TPH) were degraded by the strain at the initial diesel oil concentrations of 0.5 and 5% (v/v), respectively. The bioaugmentation of acidic oily sludge-contaminated soil with *Candida digboiensis* showed its great ability to degrade the acidic petroleum hydrocarbons under laboratory and field conditions [118]. From an application perspective, the bioaugmentation using microbial consortia rather than pure cultures is surely more advantageous [122–125]. It provides divers metabolic pathways and robustness required for field applications.

The application of bioaugmentation in different countries around the world was extensively reported using bacteria. Numerous works suggested the use of yeast strains as potential tools for bioaugmentation process [117]. Nevertheless, an impending gap between laboratory trials and on-field studies was detected. Hence, further efforts should be deployed by scientific community for a higher-scale application. A successful industrial application of bioaugmentation requires the application of strategies customized for the specific environmental conditions of the contaminated sites [124]. Thus, many studies were carried out to take advantages of this treatment technology for industrial application, as it represents an economical and environmental friendly remediation way [126].

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