

# Potentials of Biosorption and Bioaccumulation Processes for Heavy Metal Removal

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## Abstract

Biosorption and bioaccumulation can be applied to remedy environments contaminated with heavy metals as complementary methods to currently used physical and chemical methods. The overall goal of this work was to analyze potentials of biosorption and bioaccumulation for removal of heavy metals from the contaminated environment. The most frequently used methods for removal of heavy metals from water and wastewater were presented. Select biosorbents applied in the process of removal of heavy metals from contaminated environment were characterized. Factors that influence biosorption were analyzed. Also, methods for improving the efficiency of removal of heavy metals with biosorbents through biomass modification and immobilization were presented. Types of reactors applied in the processes of biosorption and bioaccumulation were described. It was found that removal of heavy metals from the environment with biotechnological methods should consider a number of physicochemical factors such as temperature, pH, contact time of biomass, and a solution containing metals, concentration and age of biomass, and toxicity when living microorganisms are applied. Improving the efficiency of removal of metals can be performed through physical and chemical modifications and immobilization of biomass. The most frequently applied reactors include: stirred tank reactors, fixed-bed, reactors and fluidized-bed reactors.

**Keywords:** biosorption, bioaccumulation, heavy metal removal

## Introduction

The rapid development of industry and modern technologies has resulted in introducing diverse toxic substances, including heavy metals into the environment. Heavy metals, are used in the following industries: mining, metallurgy, electronics, and electroplating. Many technological processes generate waste containing heavy metals that are toxic for lower and higher organisms.

There are a number of methods that are currently applied to remove ions of heavy metals. The most common

include: precipitation, ion exchange, membrane processes, evaporation, and filtration. The application of these methods is often associated with technological difficulties such as management of generated waste.

The application of biotechnological methods that use biological material such as microorganisms and plants can be an alternative to currently applied chemical and physical methods.

Biological material can bind metals through processes of biosorption and bioaccumulation. In the process of biosorption, ions of metals are adsorbed on the surface of a sorbent. Biosorption is a metabolically passive process that uses dead biomass. Biosorption is the first step of bioaccumulation.

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Table 1. Anthropogenic sources of toxic metals and metalloids, t/year [2].

Element	Fuels, power engineering, metallurgy	Agriculture	Industry	Wastewater treatment
Arsenic	45,952	7,73	7,55	13,635
Cadmium	16,444	3,666	2,45	25,466
Chromium	344,853	92,68	50,61	81,629
Copper	26,953	411	33,74	68,875
Mercury	6,879	5,245	1,145	2,018
Nickel	239,901	60,246	7,44	60,969
Lead	325,884	192	9,3	58,57
Selenium	70,355	6,566	4,25	6,252
Zinc	162,871	824,935	85,015	168,815

However, bioaccumulation can occur only with living organisms through transport of contaminants to the cell and accumulation of metals inside the cell.

The overall goal of this work was to analyze potentials of biosorption and bioaccumulation for removal of heavy metals from the contaminated environment. The most frequently used methods for removal of heavy metals from water and wastewater are presented. Select biosorbents applied in the process of removal of heavy metals from contaminated environments were characterized. Factors that influence biosorption were analyzed. Also, methods for improving the efficiency of removal of heavy metals with biosorbents through biomass modification and immobilization were presented. Types of reactors applied in the processes of biosorption and bioaccumulation were described.

### Heavy Metals in the Environment

Recently, the development of civilization has resulted in excessive flow of heavy metals into the ecosystems. Heavy metals can create undefined mineral and organometallic connections in aqueous and land environments [1].

Table 1 presents the quantities of toxic metals and metalloids present in wastewater produced by various industries.

Wastewaters are produced in many technological processes, mostly from steel mills, plating plants, tanning plants, production of chemical fertilizers and pesticides, fabric dye plants, electroplating plants, motor and power engineering plants, and battery and accumulator production plants. Heavy metals that are introduced into the environment show high mobility. They can be taken up by plants and end up in the digestive systems of animals and humans. Due to toxic effects on particular elements of the environment and bioaccumulation in the food chain, heavy metals pose serious threats to living organisms [2].

#### Heavy Metals in Surface Water

Heavy metals enter surface water with industrial and municipal wastewater. Surface water also can be contami-

nated by leaching of chemical substances from landfills and deposition of atmospheric particular matter. Also, leaching of mineral fertilizers and pesticides from soil can cause water contamination.

Heavy metals in water environment can be present in the following forms:

- ionic form (this is the most toxic form for living organisms)
- ion bound with diverse ligands (complex compounds)
- precipitated molecules.

Most heavy metals undergo bioaccumulation in surface water. Significant quantities of metals (in particular manganese and lead) are accumulated by algae [3].

#### Heavy Metals in Soil

The main sources of contamination of soil with heavy metals include industry and power engineering, atmospheric emissions and landfilling. Metals can be introduced to soil with sewage sludge applied for fertilization, and also with pesticides. Transportation causes contamination of soil along the route systems.

Depending on soil properties, e.g. pH, metals present in soil can be dissolved, transported, and taken up by various organisms. At lower pH – in acidic soils – metals are present in soluble form, and thus are more available to plants.

High concentrations of metals in soils always leads to disturbance of chemical equilibrium, and in consequence impairs functioning of individual ecosystems. Metals accumulated in soils cause inhibition of growth of soil microorganisms. Basic physiological functions of microorganisms related to decomposition and transformation of organic substances are impaired [3, 4].

#### Heavy Metals in Atmosphere

The presence of heavy metals in atmospheric air is caused mostly by the emission of particular matter from industry, transportation, and power engineering. Contamination of atmosphere is of global character. This is due to the small size of molecules that can float in the air

for long periods of time and reach remote parts of the globe. The scale of emissions of heavy metals to atmosphere depends on economic development of a given country as well as methods preventing atmospheric contamination.

Contamination of air with heavy metals has a negative effect on climate change, results in economic loss (mostly in agriculture and forestry) and also poses a serious threat to human health [5].

### Removal of Heavy Metals from Water and Wastewater

#### Removal of Metals with Physicochemical Methods

Conventional methods for removal of heavy metals from water solutions include physicochemical methods [5]. The most common methods are:

- chemical precipitation
- ion exchange
- membrane processes
- sorption
- infiltration
- coagulation [6].

Heavy metals can be removed with one process or in combination with two or more. Selection of a method depends on many factors such as wastewater types, composition, and forms and concentration of constituents subjected to removal or required removal degree [2].

The most frequently used method for removal of heavy metals from industrial wastewater is precipitation of metals in forms of hydroxides that are later separated from the solution during filtration or decantation. Temperature, pH, and the presence of organic and selected inorganic compounds can have a negative effect on process efficiency. Additionally, managing hydrated sludge increases the costs [2].

Ion exchange is another method used to remove heavy metals. The process involves exchange of ions bound to ion-exchanger for ions present in the surrounding solution. Ion-exchangers can be natural or synthetic. Prior to ion exchange the solutions should be pretreated as contaminants present in water that may disturb the process [6].

Heavy metals also can be removed from wastewater by membrane processes. These processes allow for separation of contaminants. Membranes are crucial for the efficiency of water treatment. A membrane is a thin partition that allows for selective flow of molecules. Membranes can be manufactured from natural and synthetic materials. They should show high hydraulic efficiency, separation properties, and resistance to mechanical, chemical and thermal damage [7].

Removal of heavy metals can also be achieved with sorption on activated carbon or zeolite [8]. Adsorption is performed on activated carbon (present in granular or fine forms). Removal of metals occurs on the surface of activated carbon where they are retained. If the concentration of heavy metals is too high in treated water, adsorption alone is not sufficient. The application of combined coagulation, sedimentation, filtration and adsorption on activated carbon would allow for removal of heavy metals even up to 95-99% [3, 4].

The application of the presented methods is often time-consuming and expensive. Therefore, there is a need for new, cost-efficient, and simpler methods for removal of heavy metals.

#### Removal of Heavy Metals with Biosorption and Bioaccumulation

The awareness about protection of the natural environment has been increasing since the end of the 20<sup>th</sup> century. Due to gradual deterioration of the environment with diverse contaminants there is a need for developing new methods for removal of contaminants from the environment. Biotechnological methods can be alternatives for physicochemical methods as they use natural properties of microorganisms to adsorb and accumulate heavy metals.

All microbial species show the ability to capture and accumulate heavy metals from water solution. Sorption of heavy metals is related to a microbial mechanism that allows for taking up elements required for growth and metabolic processes. Biomass properties to bind and also accumulate toxic metals can be used to develop efficient and affordable technology for treatment of wastewater from mining and electroplating industries.

Processes that utilize biomass for removal of metals are referred to as biosorption and bioaccumulation [9]. Fig. 1

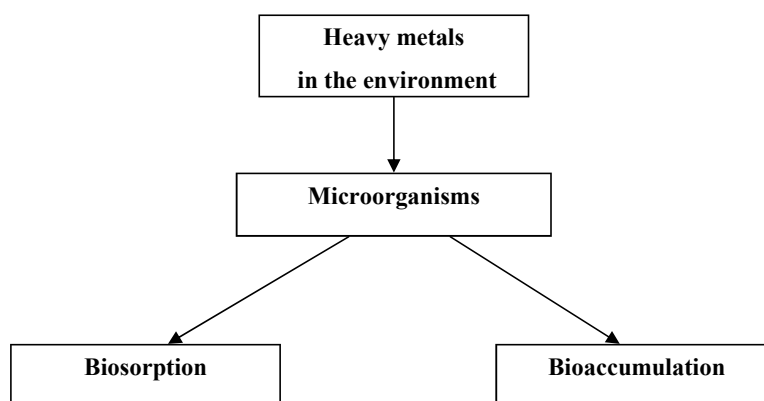


Fig. 1. Biological methods for removal of heavy metals.

presents biological methods for removal of heavy metals by microorganisms.

Both methods differ in terms of a mechanism that allows for binding contaminants. Biosorption allows for binding contaminants mostly on the surface of the microbial cell wall whereas in the case of bioaccumulation these contaminants are transported into microbial cells. Bioaccumulation occurs only when microbial cells are alive because removal of metals requires metabolic activity. Biosorption is a process that uses dead biomass [9].

Sources of materials used for removal of metals with biological methods include spent biomass and organisms naturally present in the environment. This makes biological methods affordable for a wide range of applications.

### Biosorption

Removal of heavy metals with living microorganisms poses many difficulties. The main problem is that heavy metals have a negative effect on cells of living organisms. However, advanced investigations showed that dead cells are also able to bind ions of metals through various physicochemical mechanisms. This new discovery attracted a lot of attention to biosorption and biosorbents of different origin. Numerous investigations in this area contributed to dissemination of knowledge about biosorption. This resulted in an increase in the number of new potential applications.

It is known that this process not only depends on type and chemical composition of biomass but also on external physicochemical factors. Also, the mechanisms behind the process of biosorption have been recognized and described [10].

Many research facilities worldwide are doing research in order to get a better understanding of biosorption. Also, the potential of different biological materials present in nature is being analyzed in order to improve the properties of these

materials for removal of heavy metals, to learn about binding mechanisms, and to develop the most efficient biosorbents that can be used for removal of contaminants including heavy metals [10].

Biosorption is a physicochemical process that binds ions of metals (that are mostly present in the form of cations) by cell membranes, i.e. by compounds with negative charge that are present in cell membranes. Understanding of biosorption mechanisms that allow for removal of heavy metals is essential for optimization of the process. There are many different mechanisms that occur during sorption. Due to the complexity of biological materials it is possible that different mechanisms can occur at the same time at different rates [11].

Biosorption covers the following mechanisms [3, 4]:

- Ion exchange – it is a reversible chemical reaction of exchanging mobile ions for other ions of the same charge occurring on solids that contain relevant functional groups.
- Complexation – ions of heavy metals are bound to functional groups present in cell membranes.
- Physical adsorption – is triggered by an intermolecular interaction – van der Waals forces. In the case of physisorption no chemical binding occurs.

The biggest advantage of biosorption is that heavy metals are bound by cells that no longer show metabolic activity. This allows for removal of contaminant by dead organisms, and thus the process is simpler and less expensive because supporting living biomass requires an additional supply of nutrients and energy [3, 4].

Cell membranes play an important role in the process of sorption. All ions of metals before entering a cell membrane and cytoplasm go through a cell wall. The structure of a cell wall comprises various polysaccharides and proteins, and thus there are many active spots for binding ions of metals. A cell wall can be treated as a complex ion exchanger sim-

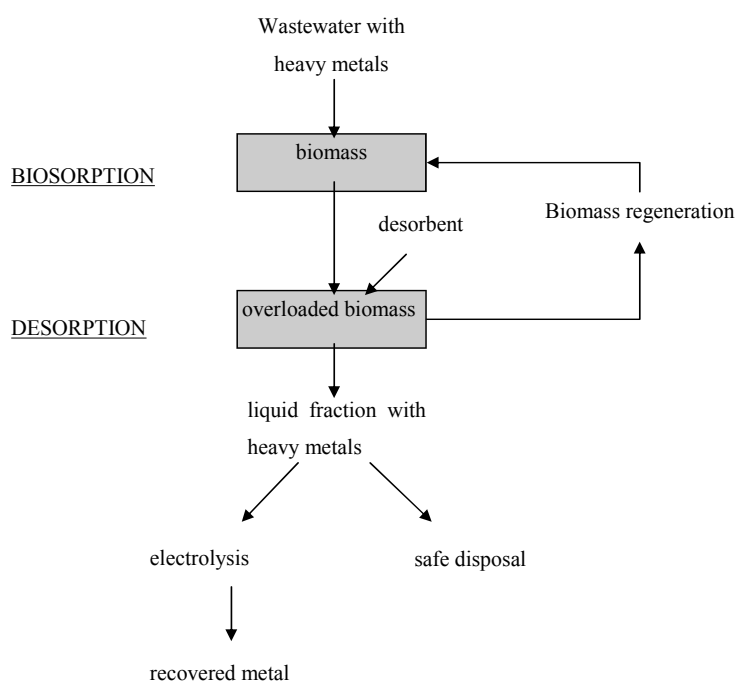


Fig. 2. Removal and recovery of heavy metals from wastewater by microbial biomass [3, 4].

ilar to commercially available resins. Diverse compositions of microbial cell walls and intercellular variations have a significant impact on different quantities of adsorbed ions of metals. The surface of microbial cells is composed of macromolecules with a high number of charged functional groups such as [12]:

- carboxylic
- hydroxyl
- phosphate
- amino.

Cell surface is often negatively charged mainly due to lack of carboxylic and phosphate acid residues. This allows for passive binding of cations on the cell surface [11].

Positively charged ions of metals present in the solution are attracted to the cell and adsorbed on a negatively charged surface of the cell. The entire process is passive and occurs without metabolic functions of the cell.

The complete cycle of wastewater treatment with biosorption is presented in Fig. 2. First, wastewater containing heavy metals is mixed with biomass. This results in adsorption of metal ions on the surface of microorganisms (biosorption). Then, regeneration of biomass (desorption) is performed, and finally metals can be recovered from the remaining liquid fraction.

Typical laboratory procedure for biosorption is performed in the following phases [3, 4]:

- I. In the first phase a solution sample of volume  $V$  containing heavy metals at concentration  $c_i$  is prepared.
- II. In the second phase biomass  $M$  (g) is added to the investigated sample.
- III. In the third phase the solution with biomass is intensely mixed for about 16 h to obtain the equilibrium.
- IV. In the next phase biomass is separated from the solution (centrifugation, filtration).
- V. Then the solution treated with biosorption is tested for metal ions ( $c_f$ ).

Biosorption is expressed in mg metal per g biomass and calculated from the following formula [4]:

$$q = \frac{V(c_i - c_f)}{M} \quad (1)$$

...where:

- $q$  – quantity of adsorbed metals [mg/g]  
 $V$  – volume of the solution sample [ml]  
 $c_i$  – initial concentration of metal ions in the solution [mg/l]  
 $c_f$  – final concentration of metal ions in the solution [mg/l]  
 $M$  – biomass mass [g]

### Bioaccumulation

Living organisms absorb and retain different kinds of elements from the surrounding environment. Bioaccumulation is a process that allows for binding toxic metals or organic substances inside a cell structure. Biological activity of biomass is essential in the process of bioaccumulation. Cells adsorb contaminants through meta-

bolic functions and thus they have to be alive. In order to achieve desired results the process should be conducted under constant control [11].

During bioaccumulation the entire cell absorbs ions of metals. Metals get inside the cells of living organisms through the same routes that nutrients are supplied. Unicellular organisms absorb heavy metals with elements required for living, such as calcium or magnesium. Plants take up nutrients through the root system from water or soil, whereas animals take up nutrients through the digestive or respiratory system.

Bioaccumulation can be divided into two stages. In the first stage ions of metals are bound on the cell surface. This part of the process is metabolically passive and is identical to the biosorption mechanism. Then ions of metals are transported into the cell. The second part of this process is possible only when the cells are metabolically active. If suitable conditions for the growth of organisms are maintained in the second stage, then the quantity of biomass increases. This allows for binding bigger quantities of metal ions in comparison to biosorption [9].

Metals can be accumulated in microbial biomass through precipitation. However, it is important that precipitated metals were bound to the cell. For example, *Citrobacter* produces acidic enzyme, i.e. phosphatase. In the presence of this enzyme heavy metals are precipitated in the cell wall in forms of hardly soluble acidic phosphate. *Achromobacter denitrificans* contributes to acidification of the environment through denitrification, and thus also enhance precipitation of metals [3, 4].

Polysaccharides such as cellulose, chitin, and alginates that are constituents of cell walls of fungi and algae also participate in capturing metals [12].

### Comparison of Biosorption and Bioaccumulation

Biosorption is a metabolically passive process that occurs without engaging any metabolic functions. Therefore, biomass used in the process of biosorption is not alive. Bioaccumulation occurs in the presence of living cells and requires higher inputs of nutrients and energy. Additionally, there is a need to prevent from toxic effect of heavy metals on cells which may impair the process. In view of this, minimal concentration of heavy metals that inhibits the growth of microorganisms should be determined prior to bioaccumulation. Taking into account all these aspects, bioaccumulation as a method of heavy metals removal is more complex and expensive. Table 2 presents a detailed comparison between these two methods.

### Adsorption Isotherms

During biosorption, equilibrium between metal ions adsorbed on the surface of a biosorbent ( $q_{eq}$ ) and metal ions not adsorbed that are present in the solution ( $c_{eq}$ ) is reached. Passive binding of metals on the surface of cells proceeds usually at a fast rate. The predominating part of equilibrium



Table 2. Comparison of biosorption and bioaccumulation [13].

Characteristics	Biosorption	Bioaccumulation
Cost	Usually low. Biomass can be obtained from industrial waste. Cost covers mostly transportation and production of biosorbent.	Usually high. The process occurs in the presence of living cells that have to be supported.
pH	pH of the solution strongly affects sorption capacity of heavy metals. However, the process can occur in a wide range of pH.	Significant change in pH can heavily affect living cells.
Selectivity	Poor. However can be increased by modifications/biomass transformation.	Better than in the case of biosorption.
Rate of removal	Most mechanisms occur at a fast rate.	Slower rate than in the case of biosorption. Intercellular accumulation takes a long time.
Regeneration and reuse	Biosorbents can be regenerated and reused in many cycles.	Due to intercellular accumulation reuse is rather limited.
Recovery of metals	With an adequate eluent the recovery of heavy metals is possible.	If it is even possible, biomass cannot be used for other purposes.
Energy demand	Usually low.	Energy is required for cell growth.

occurs during the first couple of minutes. Complete equilibrium is reached after several hours. In the case of desorption, which is a reverse process to biosorption reaching the equilibrium requires similar time.

The equilibrium in the process of biosorption can be described with the Langmuir or Freundlich isotherms that are frequently used in wastewater and water treatment [4].

The Langmuir isotherm is the adsorption isotherm proposed by Irving Langmuir in 1916. It assumes that adsorbed molecules (i.e. an adsorbate) can form a monolayer at the surface of adsorbents (i.e. cells). Langmuir assumed that the surface of an adsorbent is characterized with specified adsorption places (i.e. adsorption centers). Each adsorption place is able to adsorb only one molecule. The Langmuir equation has the following form [4]:

$$q_{eq} = \frac{q_{max} b c_{eq}}{1 + b c_{eq}} \quad (2)$$

...where:

- $q_{eq}$  – amount of adsorbed ions in the equilibrium
- $c_{eq}$  – residual concentration of ions in the solution
- $q_{max}$  – maximum amount of adsorbed ions
- $b$  – ratio of adsorption and desorption rate.

Changing pH of the solution during biosorption allows for changing  $q_{max}$ , which then becomes the function of solution pH. In this case the equation has the following form [4]:

$$q_{eq} = \frac{q_{max}(pH) b c_{eq}}{1 + b c_{eq} n} \quad (3)$$

The  $q_{max}(pH)$  in this equation is the maximum amount of adsorbed ions in the solution of a given pH. The Freundlich equation is used in the process of sorption on heterogenic surface, i.e. energy heterogenic surface. Typical property of heterogenic surface is that the areas where adsorption occurs differ in terms of adsorption energy.

In consequence, adsorption places with the highest adsorption energy are covered first, and then the places with lower energy are covered. The Freundlich equation has the following form [4]:

$$q_{eq} = K \cdot c_{eq}^n \quad (4)$$

...where:

- $q_{eq}$  – the amount of adsorbed ions in the equilibrium,
- $c_{eq}$  – equilibrium concentration of ions in the solution,
- $K, n$  – constants.

## Characteristics of Select Biosorbents

### Bacteria

Bacteria are unicellular organisms that belong to the prokaryote group. Size of bacteria is usually small. The diameter is usually about 1-50  $\mu\text{m}$ , although bigger organisms such as *Thiomargarita namibiensis* with length of about 750  $\mu\text{m}$  also exist. Size of a cell is a very important characteristic of an organism because the size affects the rate of metabolic processes.

Cell walls of all bacteria are not identical. In fact, the composition of the cell wall is the primary determinant for classification of bacteria into two main types [3, 4]. Differences in the structure and composition of gram-positive (G+) and gram-negative (G-) bacteria result in diverse properties of both types of bacteria. Gram-positive bacteria (G+) show higher resistance to alkaline reaction of the environment and elevated temperatures. However, they are more susceptible to the effects of antibiotics and detergents [3, 4].

Cell walls play an important role in binding ions of metals. It was proved that functional amino groups present in peptidoglycan and teichoic acids of gram-positive bacteria and also in peptidoglycan, phospholipids, and polysaccharides of negative-gram bacteria are the main constituents

responsible for anionic character and binding properties of cell walls [13].

Bacteria that originate from areas significantly contaminated with heavy metals are resistant to higher concentrations of metals in comparison to bacteria occurring in uncontaminated areas [14].

### Fungi and Yeasts

Fungi are a widely diversified group of organisms that belong to eukaryote. This group includes unicellular as well as multicellular organisms. Fungi can reproduce both asexually via vegetative and sexually. Fungi are heterotrophs and together with bacteria take part in decomposition of organic matter. These organisms can be classified according to size into macroscopic and microscopic. The latter are of greater importance in biotechnology. Microscopic fungi are divided into yeasts (unicellular) and hyphae that are built of hypha, i.e. a cylindrical thread-like cell surrounded by the cell wall. Reproduction of hyphae results in the formation of a dense structure, i.e. thallus, which is referred to as mycelium. Cytoplasm comprises cellular organelles such as nuclei, mitochondria, ribosomes, and Golgi apparatus [4, 15].

Fungi are heterotrophs and they need organic compounds as carbon and energy sources. A rigid cell wall of fungi prevents them from transporting larger molecules like carbohydrates. Therefore, larger molecules are dissolved by enzymes produced by fungi, and then can be further transported. These compounds pass through cytoplasmic membrane via active transport [15].

Yeasts belong to microorganisms that, due to their properties, are known for thousands of years. Even long before the common era these microorganisms were used to produce beer, wine, and bread, and also used in Asia to produce milk beverages such as kefir. Since the 19<sup>th</sup> century knowledge about these organisms has been growing significantly, which has resulted in a number of applications. *Saccharomyces* is the most commonly used yeast in biotechnology. *Saccharomyces cerevisiae* was the first model in biochemical investigations and now it attracts wide attention from many disciplines such as biotechnology, biochemistry, genetics, or molecular biology [16].

Yeasts are an example of unicellular fungi. In terms of size and shape there is a wide diversity among yeasts which is mostly due to yeast type, physiological state, and environmental conditions. Extreme conditions, e.g. nutrient deficiency and excessive acidity, can contribute to significant changes in yeast cells. Usually, the width and the length of yeast cells are 1-6  $\mu\text{m}$  and 1-8  $\mu\text{m}$ , respectively. The shape of yeast cells is mostly spherical and oval [16].

Yeasts belong to aerobic organisms although they can also grow in anaerobic conditions, resulting in significant alteration in their metabolism. Krebs cycle reactions are inhibited and the process of glycolysis is intensified in order to supply the cells with required metabolic energy. In anaerobic conditions yeasts grow much slower, but only then the production of ethanol occurs that is one of the most common practical applications of yeasts [16].

### Algae

The term "algae" refers to a wide and diverse group of organisms. Algae belong to eukaryotic organisms. They are autotrophs, i.e. self-feeding organisms that are able to synthesize organic compounds from simple inorganic compounds. This ability is due to chloroplasts – additional organelle – that are present in the cell. Chloroplasts are green elements of cells that contain green pigments, i.e. chlorophyll that can absorb solar energy required for photosynthesis. Algae can occur in a form of single cellular organisms as well as from large thallus, i.e. large aggregations of single cells. Most algae belong to microorganisms, but macroscopic species referred to as sea weeds also occur in nature. It is important to differentiate algae from cyanobacteria that are also aerobic phototrophs and are classified as bacteria [16, 17].

In favorable conditions algae can reproduce massively in a relatively short period of time. This sudden increase in algae growth in water reservoirs is referred to as water blooming, and is mostly caused by the increase in concentrations of biogenic elements. This phenomenon often lasts for a relatively short period of time, but this is not always the case. In some water reservoirs water blooming can occur more often and lasts longer. Excessive growing and dying away can cause oxygen deficiency, leading to sudden death of organisms, including fish [18]. Algal biomass from eutrophicated reservoirs can be used to produce energy carriers as alternative source of energy [19]. What is more, algal biomass can be applied as a sorbent for removal of heavy metals. Eutrophication occurs during plant vegetation, and thus in order to provide a continuous supply of biomass used as a sorbent, during winter algae can be grown in closed systems.

Algae occur in the environment where water is present, even in trace quantities. Algae in the natural environment perform many important functions. Algae are a food source for many organisms. They participate in natural attenuation of water. They are the main producer of oxygen in water. Industrial utilization of algae mostly includes obtaining many products such as alginate, carrageenan, fertilizer, or food for humans. Recently, due to development of biological methods for removal of heavy metals, algae can also be applied as biosorbents [3, 16].

Algae belong to organisms that can achieve significant increases in biomass growth at minimal environmental requirements [20]. Algae culture can be run in near vicinity of industrial plants that allows for assimilation of  $\text{CO}_2$  and production of carbohydrates, proteins, and lipids in algae cells [21].

Among all algae there are three groups that are of significant importance for humans. They include phaeophyceae (brown algae) (*Phaeophyta*), red algae (*Rhodophyta*), and green algae (*Chlorophyta*).

The process of biosorption by algae is mostly due to the properties of a cell wall that participates in electrostatic attraction of metal ions as well as in the formation of complexes with metals.

Algae show wide diversity in terms of structure and chemical composition of the cell wall. The cell wall in algae is more complex than in bacteria or fungi. It consists mostly of a matrix of cellulosic filaments, but usually comprises different kinds of polysaccharides such as pectin (i.e. a mixture of carbohydrates), xylans, mannans, and alginic acid [22].

The cell wall of some algae can be additionally reinforced with calcium carbonate. Other algae can comprise a polymer called chitin in their cell wall. Brittleworts from the group of chrysophytes are surrounded by a cell wall built of silica, proteins, and polysaccharides. Silica constitutes about 50% of dry mass of the entire cell, and thus the cell wall is very resistant. When brittleworts die and their biological material undergoes decomposition, the inner structure can remain intact for a long period of time. Among algae there are organisms that do not have a cell wall at all (i.e. euglenoids). However, these organisms do not show good properties for removal of metal ions. Lack of a cell wall significantly reduces sorption capacity, therefore such algae are not used in the process of biosorption [22].

Algal cell walls are permeable for substances with small molecular weight such as water, ions, gases and other nutrients. Bigger molecules do not go through the cell wall of these organisms. Algae have pores with width of 3-5 nm that allow for the passage of only small molecules. The cell wall of brown algae contains such substances like cellulose, alginic acid and fucoidan (a sulphate polysaccharide). Alginic acid is an abundant polysaccharide in brown algae and contains carboxylic functional groups (-COOH) that participate in binding metals. It usually constitutes about 10-40% of dry matter of brown algae. Kelps also contain cellulose but their biosorption properties are mostly due to galactanes (glucose polymers that include agar and carrageenan). The cell wall of green algae consists mainly of cellulose and proteins bound with polysaccharides to form glycoproteins. Cell walls can have a fibrillary structure as in the case of fungi. Green algae contain chlorophyll *a* and *b* and carotenoids, and they store carbohydrates in the form of starch [23, 24].

### Chitosan and Chitin

Nowadays, synthetic substances are primarily used for a great number of applications. However, in the case of chitin and chitosan it is different. These natural substances are biodegradable and non toxic. Wastes from chitosan production do not pose any threat to the natural environment because they can be decomposed by microorganisms into simpler compounds [25].

Chitosan is formed by deacetylation (i.e. removal of acetylic group from a molecule) of chitin. Chitin is a white hard substance of little flexibility. In terms of structure it is similar to cellulose (the difference is that chitin contains the acetamide group instead of hydroxyl group). Chitin shows poor solubility in water and in most solvents. It is one of the most abundant natural polysaccharides in the world. It can be found in shells of crustaceans, molluscs, insects, and cell walls of fungi. Availability of chitin allows for production

of chitosan for commercial scale [25, 26]. Chitosan can be prepared in various forms depending on applications. It can be available in a form of powder, solution, gel, granules, or capsules [23].

The advantage of chitosan and chitin is that they can be modified chemically and physically in order to change their properties. This allows for expanding the range of potential applications [23].

Due to a great number of valuable properties chitosan and chitin can be widely applied in many industries. They can be used in cosmetics, medicine, biotechnology, and in wastewater treatment [25].

### Biosorbents Produced from Waste Materials

In recent years, biosorbents from waste materials have attracted more and more attention. A biosorbent produced from peanuts husks shows higher sorption efficiency of Cr(III) ions in comparison to activated carbon. Biosorbents from waste materials show higher separation capacity toward heavy metal ions [27].

### The Impact of Environmental Factors on Biosorption

The efficiency of biosorption of heavy metals depends on many environmental factors including pH, temperature, contact time, concentration, biomass age, and presence of other ions in a solution.

#### pH

In some respects biosorption is similar to ion exchange, and therefore pH of a solution has a significant impact on removal of heavy metals. The number of available binding spots on the surface of cells depends on pH. At low pH the available binding spots in a cell bind to hydrogen cations present in a solution. This leads to limitation of the number of available spots and fewer metal cations can be adsorbed. However, with the increase in pH the number of active spots with negative charge that attract cations also increases [3].

#### Temperature

The stability of metal ions in a solution and the stability of metal-cell complexes depend on temperature. However, temperature in the range of 20 to 35°C does not significantly affect biosorption. Higher temperature allows for improving sorption capacity of biomass, but it can result in damaging sorption material [9].

#### Contact Time

Biosorption is also affected by contact time between biomass and the solution containing metals. Biosorption proceeds fast and most metals are adsorbed at the very beginning of the process. Equilibrium is reached during the first couple of minutes starting from the moment of exposure of biomass to the solution [9].



### Concentration and Age of Biomass

At high concentration of biomass the degree of metal removal from the solution is also high. However, at high concentration of biomass the quantity of bound metals to the quantity of dry matter is rather low. Higher concentration of biomass leads to the formation of bigger cell aggregates that may disturb the equilibrium in the reactor.

The age of biomass can influence the properties of cell walls that are crucial for adsorption of heavy metals. According to numerous observations the relationship between the age of biomass and adsorption of heavy metals is not fully understood. Depending on organisms used for biosorption, it can be stated that older cultures can show better ability for removal of metals or the other way round [9].

### Presence of Other Ions in Solution

Wastewaters are contaminated with various contaminants, including different kinds of metals at the same time, which has an effect on biosorption dynamics. The presence of other substances dissolved in a solution can inhibit biosorption of metals. This is due to competitiveness between ions of metals that are removed and other ions for binding spots on the surface of cells [9].

### Improvement of Efficiency of Metal Removal by Sorbents

Improvement of the efficiency of metal removal by biosorbents can be performed through modification and immobilization of biomass.

#### Modification of Biosorbents

A cell wall is an element that plays a predominating role in the process of biosorption. Therefore, any modifications of the cell wall can significantly influence the efficiency of binding of metal ions. This led to new methods incorporated into existing technologies based on biosorption. The application of such methods results in modifications of cells. These methods include physical and chemical methods.

Physical treatment includes heating/boiling, freezing/defrosting, drying, and lyophilization (freeze drying) [22]. *Chlorella* sp. cells exposed to heating showed significantly higher ability to remove Mn (5,100 mg/g) than living cells. Biomass of *Pseudomonas aeruginosa* exposed to high temperature showed superior ability to bind zinc. This was probably due to destruction of a cell membrane resulting in an increased number of additional spots for binding zinc inside the cell [10].

Biomass can also be subjected to treatments with different chemical substances such as alkali or acids. Ross and Townsely [28] observed more efficient sorption of Cu by *Penicillium spinulosum* biomass that was treated with a detergent. Similar results were obtained by Gadd et al. [29] for *Saccharomyces cerevisiae* and *Rhizopus arrhizus*. Treatment of cyanobacteria (*Phormidium laminosum*) with

alkaline substances enhanced the degree of metal removal by 5-34%. However, treatment with acidic substances resulted in a decrease in adsorption of metals. This was probably caused by higher competitiveness between protons and dissolved metals [10].

All these treatments aim at improving the efficiency of biosorption. It has to be pointed out that in many cases after physical and chemical treatment the ability of biomass to bind metals may be impaired or remain the same.

### Immobilization of Biomass

Currently, the practical application of biosorption on a bigger scale is still limited by many factors, mostly by high costs of separating biomass from processing water and poor mechanical strength of biomass. Biosorption in a laboratory scale where cells are freely suspended allows for better understanding of the process. However, prior to applications of biosorption for bigger scales the cells should be subjected to immobilization. The process of biosorption in reactors requires suitable flow rates that result in a pressure increase. Under these conditions, freely suspended cells can undergo disintegration. Due to these problems the utilization of microorganisms in subsequent cycles of sorption and desorption is not feasible. In order to overcome these obstacles cells can be immobilized in a way that does not impair the ability to adsorb ions of metals [10].

Immobilization or fixing (from Latin *immobilis* – immovable) can be defined as a group of methods that allow for total or partial limitation of movement of particular atoms, molecules, and substances of biological material on solid base or inside specific structures. Immobilization improves mechanical strength and resistance of biomass, allowing for expanding potentials of biomass for applications in the reactors with continuous flow. Dead as well as living cell microorganisms can be immobilized [22].

Kuhn and Pfister [30] removed Cd, Zn, Mn, Pb, Cu, and Sr by *Zoogloea ramigera* cells immobilized with alginate with the degree of removal above 95%. Alginate beads showed themselves sorption properties but not sufficient as in case of binding metals with immobilized cells. Other investigations show that cells of *Chlorella salina* immobilized with calcium alginate allowed for removal of higher quantities of cobalt, zinc and manganese than by freely suspended cells [10].

### Reactors

#### Stirred Tank Reactors (STRs)

This type of reactor is equipped with a stirrer that allows for maintaining biomass in a state of suspension. STR can work in continuous or batch modes. Solid phase can be separated from liquid phase by sedimentation due to gravity force in the same reactor or by filtration in a separate process unit. Biosorption run in stirred tank reactors, is less complex than in fluidized-bed or fixed-bed reactors but the operational costs are higher [12].

### Fixed-Bed Reactors (FXRs)

Similarly to ion exchange and adsorption, biosorption can be performed on fixed beds. During biosorption the solution contaminated with metals is passed through a column. When the maximal sorption capacity of a column is reached, regeneration of a fixed bed is conducted. In order to maintain continuous mode of work there is a need for two columns. Biosorption is performed in the first column, whereas regeneration of a biosorbent is performed in the second column. A biosorbent is recovered by rinsing the spent biomass with a chemical substance [12].

### Fluidized-Bed Reactors (FBRs)

In contrast to fixed-bed reactors, fluidized-bed reactors are movable. In the process of biosorption the bed is maintained in the state of suspension by water flowing into a column in a counter-flow system. With the increase in water flow the bed undergoes minor expansion at the beginning. When the flow rate still increases the bed converts into a so-called fluidized state. In the fluidized state the particles are in continuous movement and constantly migrate in the entire volume of the column [12].

In this type of reactor a biosorbent is placed inside a column and a solution with metals is supplied in the counter-flow system. The advantage of fluidized-bed reactors over fixed-bed reactors is that they do not clog.

### Regeneration of Biomass

Removal of metals from a solution is conducted until all active spots on the surface of cells are filled. When adsorption of ions is completed, then the biosorbent becomes a waste material and needs to be utilized. There are two methods for utilization of spent biosorbents: incineration and landfilling. Also, spent biosorbents can be subject to desorption. Incineration of biosorbents only allows for reducing the volume of generated waste. Desorption solves the problem of generated waste entirely. The additional benefit of this method is regeneration of biomass for further reuse and recovery of metals [3, 4].

In contrast to bioaccumulation, biosorption is a process that is not affected by metabolism and as such it is reversible in most cases. Desorption is a reverse process to sorption and allows for releasing adsorbed molecules, atoms, and ions. In order to perform desorption properly it is necessary to select a suitable substance, i.e. an eluent. Selection of an eluent depends primarily on a biosorbent and mechanisms responsible for biosorption. Additionally, an eluent should [13]:

- not be toxic to biomass
- be efficient
- show high capacity
- be affordable
- be environmental friendly

Desorption of metals is frequently performed with mineral acids (e.g. sulfuric acid, hydrochloric acid, nitric acid),

organic acids (e.g. citric acid, acetic acid, lactic acid), or complex compounds such as EDTA and NTA [19]. Binding metal ions with microbial cells is influenced by pH. By pH modifications it is feasible to reverse adsorption of such ions as Cu(II), Cr(II), Ni(II), Zn(II), Cd(II), and Co(II). These ions bound by *Chlorella vulgaris* at pH=5.0 were released by decreasing pH to 2.0 [10]. Prior to desorption, concentrations of applied compounds and contact time of an eluent and biomass should be determined in order to prevent from any damage to biosorbents.

### Conclusions

With reference to the presented analysis of biosorption and bioaccumulation potentials, the following conclusions can be formulated:

1. Heavy metals belong to the group of contaminants that are the most difficult to remove. Biosorption and bioaccumulation can be applied to remedy environments contaminated with heavy metals as complementary methods to currently used physical and chemical methods.
2. Removal of heavy metals from the environment with biotechnological methods should consider a number of physicochemical factors such as temperature, pH, contact time of biomass, and a solution containing metals, concentration, and age of biomass, and toxicity when living microorganisms are applied.
3. Improving the efficiency of removal of metals can be performed through physical and chemical modifications and immobilization of biomass.
4. The most frequently applied reactors include: stirred tank reactors, fixed-bed reactors, and fluidized-bed reactors.
5. The biggest advantage of applying biosorbents is that they can be subject to regeneration. Other advantages of biosorption and bioaccumulation applications are:
  - low cost due to biological origin of materials
  - removal substantial quantities of metals
  - recovery of metals.

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