Chapter 3

Removal of Heavy Metals from Aqueous Solutions by Aerobic and Anaerobic Biomass

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Additional information is available at the end of the chapter

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1. Introduction

Industrialization and urbanization have promoted the generation of great quantities of aqueous effluents that may contain high levels of toxic compounds [1]. Every day, 2,000,000 tons of wastes (from sewers or agricultural and industrial residues) are released into rivers and seas, spreading disease and damage to ecosystems. Achim Steiner, executive chief of the United Nations Program for the Environment stated: "If the world is to thrive, let alone to survive on a planet of 6 billion people heading to over 9 billion by 2050, we need to be collectively smarter about how we manage waste, including wastewaters" [2].

Heavy metals, or potentially toxic elements, constitute a specific group of pollutants that are released into the environment as a result of industrial activities, such as the mining industry. These elements can cause health problems. In México, the mining industry is one of the most important economic activities, with gold, silver, and copper being the precious metals with higher production rates [3].

The metallurgical process of the mining industry involves a series of extraction and purification techniques that result in the disposal of metals into water bodies through acid mine drainage (DAM). Heavy metals can then accumulate at toxic concentrations for a functional ecosystem, which constitutes an economic problem of public health [4].

Controlling and reducing water pollution is a significant concern for our society. Wastewater spills create eutrophication and toxic problems. The wastewater penetrates the soil, contaminates groundwater, and reduces the quality necessary for human consumption [5].

Discharge limits have been established for heavy metals, among many other water pollutants. Most heavy metals are soluble and form aqueous solutions; hence, they cannot be separated by ordinary physical treatments [6].

Contamination of soil and water is the result of numerous industrial activities such as mining, melting, fabrication of jewelry, batteries, and automobiles, and volatile ashes from incineration processes. This type of contamination poses a serious threat for human and animal health since heavy metals remain in the environment for an indefinite time [7].

México has several sites contaminated by heavy metals and other residues from the mining industry. A particular example of pollution is found in the San Pedro River, located in the state of Sonora, México, where silver and copper production has been exploited for decades. The San Pedro River stream originates near Cananea – a mining town known for having the biggest mining districts of the state – and culminates in the state of Arizona, in the United States. Surface water pollution in the San Pedro river was reported in 1997 and 1999 [8]. In 2008, the presence of heavy metals in the river sediments was also evaluated [9]. The river has been contaminated by heavy metals due to its proximity with the metallurgical activity of the state. Metals found in the river are: cadmium, cobalt, chromium, iron, manganese, copper, zinc, nickel, and lead. However, two of the metals with higher concentrations were copper and iron, which exceeded the maximum permissible values established in the Mexican laws for water quality. These laws consider lead, zinc, mercury, silver, nickel, cadmium, aluminum, copper, and arsenic, as water pollutants due to the toxicity they pose for aquatic and terrestrial organisms (NOM-001-ECOL-1996; NOM-002-ECOL-1996; NOM-003-ECOL-1996).

More recently, on August 7, 2014, the Buenavista Copper Mine in Cananea was under the spotlight when approximately 40,000 cubic meters of sulfuric acid were spilled into the Bacanuchi River (also situated in Sonora). This toxic leakage affected an estimate of about 800,000 people [10]. Heavy metals pollution has been reported, but the remediation projects aiming to recover the quality of these sites have been extremely scarce. Thus, it is of great importance for research institutions and industries to evaluate technological alternatives for the removal and stabilization of inorganic contaminants, keeping into consideration the specific environmental conditions of each polluted site [11].

2. Heavy metal removal processes

The removal of heavy metals can be carried out by a number of conventional treatments, such as reverse osmosis, electrodialysis, ultrafiltration, chemical precipitation, and ionic exchange. These methods, however, have the disadvantage of requiring high operation costs. The ionic exchange resins, for example, have been commercially known for their effectiveness as pollutant adsorbents in wastewater treatments, but their high cost hinders their application at industrial levels [1]. Chemical processes, although simple to perform, end up being even more expensive because of the active agent that cannot be recovered for future uses. Besides, the final product is a high concentrated sludge difficult to handle [4].

Heavy metals sources are not renewable, and the natural reserves are being consumed. Therefore, it is imperative that those elements considered dangerous to the environment or those of technological importance and economic value are withdrawn and recovered at their point of origin through appropriate treatments.

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A brief description of the before mentioned processes is presented next:

Reverse osmosis: a process where heavy metals are separated through a semipermeable membrane by using a pressure higher than the osmotic pressure, which is caused by the dissolved solids in wastewaters. The high pressures required for this process are the main reason for the high operating costs of reverse osmosis.

Electrodialysis: in this process, metallic ions are separated by selective semipermeable membranes. An electric current is applied between two electrodes located at each side of the membranes, which produces a migration of cations and anions toward their respective electrodes. The migration of ions results in the formation of metal salts that precipitate out of solution. However, a major disadvantage of electrodialysis is membrane clogging, caused mainly by the formation of metal hydroxides.

Ultrafiltration: this process involves the use of porous membranes and high pressures for the separation of metal ions. Sludge generation is the main disadvantage in this treatment.

Ionic exchange: metallic ions in diluted solutions are exchanged with the ions located in the active sites of synthetic resins by electrostatic forces. Sludge generation and the high costs of exchange resins are the main disadvantages.

Chemical precipitation: precipitation of metallic ions is achieved by the addition of coagulants such as calcium salts, iron, and other organic polymers. The inconvenience of this method is the excessive amounts of sludge (it might include toxic compounds) produced during the precipitation.

Phytoremediation: it involves the use of certain plants as removing or stabilizing agents in contaminated soils, sediments, and water. The time required for effective stabilization of heavy metals is large and can be a constraint in this process; furthermore, plant regeneration is even more complex.

All of the disadvantages previously mentioned, such as incomplete removal, high energy consumption, excessive residual sludge, and formation of other toxic residues requiring careful disposal protocols justify the need for a cost-effective treatment for the removal of heavy metals from wastewater [12].

New technologies are currently being developed, taking into consideration the processing costs and direct scaling up and implementation [13]. The search for effective removal technologies has directed attention toward biosorption, an ecological alternative that uses different biological materials for binding and concentrating metal ions.

Biosorption: This process is based on the capacity of biological materials to concentrate heavy metals by either metabolic or physical–chemical pathways.

Developments in the field of environmental biotechnology have allowed the identification of several species of algae, bacteria, fungi, and yeast as effective metal biosorbents [14]. The main advantages of biosorption over conventional treatments include: lower costs, high removal yields, minimum residual sludge formation, and potential biosorbent regeneration and metal recovery [15].

The biosorption process involves a solid phase – the biosorbent, or biomass – and a liquid phase – the solvent (commonly water). The liquid phase contains the sorbate, i.e., the species to be sorbed (metallic ions). During biosorption, the sorbate is attracted and bound to the biosorbent through a variety of mechanisms. This "binding" process continues until a state of equilibrium is achieved between the amount of sorbate present and the available active sites of the biosorbent [16].

The two mechanisms by which biosorption can take place are [13]:

- Bioaccumulation: based in the intracellular transport of metallic ions by living biomass.
- Bioadsorption: based on the adsorption of metallic ions on the cell surface. This process can occur by ionic exchange, precipitation, complexation, or electrostatic attraction. Figure 1 shows a basic experimental approach that can be used to determine the biosorption capacity, q, a measure of the metal uptake by biomass.

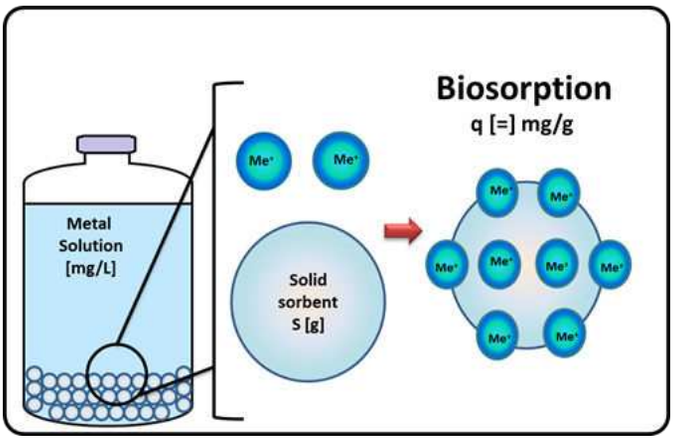

Figure 1. General experimental setup for biosorption of heavy metals.

The biosorption process can be carried out in a bioreactor, where the wastewater flows through a bed of microorganisms which bind the heavy metals. Bioreactors are useful tools where high volumes of wastewaters may be treated continuously, transferring the contaminated "portion" to a considerable smaller volume. However, certain problems can arise during the operation of bioreactors, such as biomass washout, liquid–solid separation difficulties, and pressure drops. These problems originate due to the fact that microbial biomass generally consists of small particles with low density and poor mechanical strength [17]. Immobilization of biomass in a suitable matrix (or material supports) can overcome washout problems by inducing cellular growth in the form of a stable biofilm constituted by microbial cells and extracellular polymeric substances.

3. Heavy metals removal by aerobic biomass

Nowadays, the use of microorganisms for environmental remediation and recovery purposes has grown as a research field. It is believed that the most fitted microorganisms for removal

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treatments are the ones isolated from the same environment where they were naturally selected; however, genetic manipulation techniques can be used to enhance the capacity of different microorganisms [18].

Bioremediation utilizes the catalytic abilities of living organisms to degrade and transform pollutants from aquatic and terrestrial ecosystems. This alternative can be potentially applied to mitigate environmental contamination. Bioremediation has focused on the exploitation of genetic diversity and metabolic versatility, characteristic traits that make bacteria suitable for the transformation of pollutants into harmless products, or less toxic compounds, that can be reintegrated in the natural biochemical cycles. On the other hand, there are other microorganisms such as fungi or plants that have been isolated and used in removal processes like phytoremediation [19].

Microorganisms are naturally exposed to heavy metals in essential or toxic quantities, and the amount of heavy metals in certain sites can be so high that microorganism growth is not possible. Metal toxicity forces microorganisms to develop various strategies to defend themselves against high concentrations of heavy metals [20].

There are several experimental protocols important to effectively examine metal biosorption by aerobic biomass. These protocols are described below.

3.1. Isolation / Inoculation

Isolation is used to identify microorganisms able to grow in polluted environments. Wastewater samples are generally collected from damaged sites, and yeast or bacteria (biomass) cells are grown by inoculating them into a nutrient-rich environment. Inoculation is usually done in cell-culture dishes by the streaking method using selective enriched nutritive media for each microorganism. Commonly, 10 mL of wastewater sample is inoculated in a specific culture medium at 37°C for 24 h.

3.2. Batch biosorption and kinetics of heavy metals

The biosorption batch tests with aerobic biomass are carried out in experimental vessels, such as Erlenmeyer flasks. Wastewater samples are mixed with a known amount of biomass. Flasks are placed in an incubator at specific conditions and tests are carried out in duplicate, using two flasks for every sampling time. For aerobic microorganisms such as yeast, the conditions are usually set as follows: pH 3–4, 37°C, and 100 rpm. Samples are taken at regular intervals until equilibrium is achieved. Every sample is then centrifuged to separate biomass from the solution. Concentration of metals is usually determined by atomic absorption spectrometry.

Biosorption efficiency (E) is calculated as follows:

$$E = \left(\frac{Co - Cf}{Co} \right) \times 100 \quad (1)$$

where:

C_o, C_f are the initial and final metal concentration (mg/L).

The biosorption capacity of the biomass at any given time is calculated as follows:

$$q_{eq} = \frac{m_o - m_{eq}}{V_{ads}} \tag{2}$$

where

m_o is the initial mass (mg), equal to the initial concentration (mg/L) times initial volume;

m_{eq} is the mass at equilibrium (mg), equal to the concentration (mg/L) times volume at equilibrium;

v_{ads} is the volume of biomass used (L).

3.3. Continuous biosorption studies

Continuous studies are carried out in bioreactors. Bioreactors consist commonly of acrylic or glass columns with lateral sampling points. Perhaps, the simplest configuration is the Upflow Aerobic Reactor packed with material supports and biomass recirculation. An example of material support is clinoptilolite, a zeolite with a particle size of 4.76 mm, a pore diameter of $3.22E^{-03}$μ m and a Si/Al ratio of 4.53. Aerobic conditions are met by supplying air from the bottom of the column through peristaltic pumps.

3.4. Biosorption tests in aerobic reactors

Once reactors are inoculated with the selected aerobic biomass, mineral medium is used for biomass acclimation at pH levels optimum for growth. Mineral medium is only used during startup as a source of nutrients for biomass growth and immobilization. In the case of yeasts, pH is generally 3–4, and the medium consists of the following compounds: (g/L): ammonium phosphate 1, glucose 5, sodium chloride 5, magnesium sulfate 0.2, and phosphate potassium 1 [21].

Figure 2 shows a schematic diagram of two Upflow Aerobic Reactors connected in series that were used to remove heavy metals by Hernández-Mata et al., 2014 [22]. In this scheme, the first reactor (R1) was inoculated with biomass and the effluent was recirculated until the biomass reached a concentration of 1 g/L. When the desired biomass concentration was achieved, the biosorption stage was initiated with mining effluents. After the biosorption stage, a desorption (purification) step was carried out to remove the metallic ions adsorbed by the biomass. Biomass concentration was measured once again until the concentration reached 1 g/L. The effluent of R1 was then fed to R2 (containing the same biomass produced in R1) and biosorption was examined in both reactors. Samples were taken at regular intervals at the inlet and outlet points until column saturation was evident [22].

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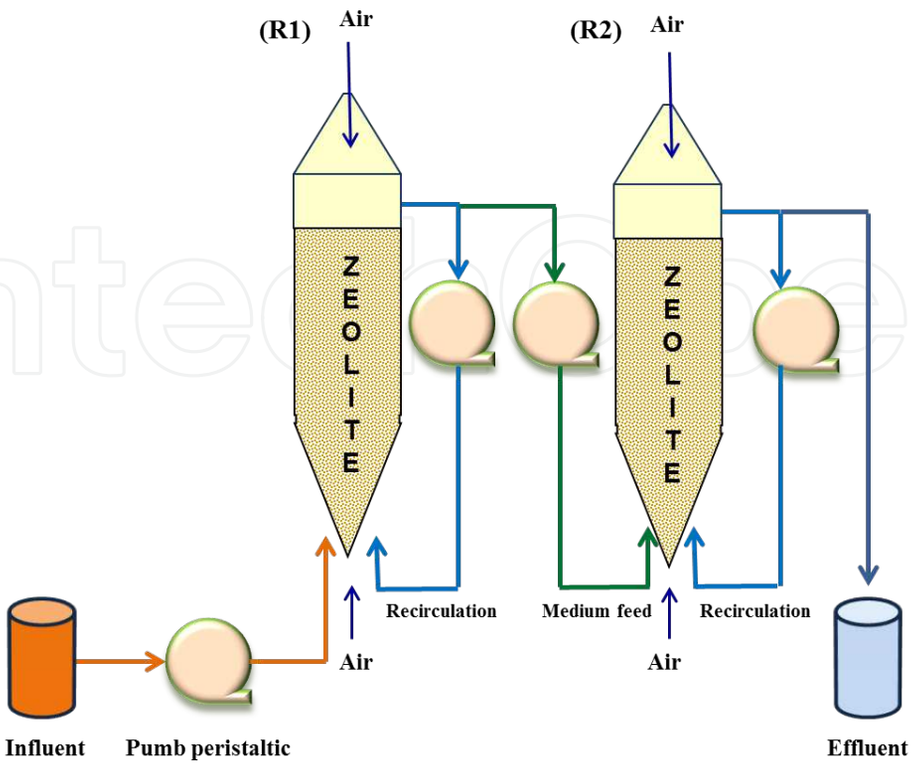

Figure 2. Schematic diagram of two upflow aerobic reactors packed with zeolite.

4. Heavy metals removal by living anaerobic biomass

Anaerobic microorganisms perform as part of their metabolism a process known as anaerobic digestion, which has been widely implemented in the treatment and stabilization of effluents with high organic loads. Two of the main bacterial groups that participate in anaerobic digestion are acidogenic microorganisms (responsible for the conversion of organic matter into volatile fatty acids, VFAs) and methanogenic microorganisms (methane producers).

Generally, it is considered that methanogenic bacteria are less resistant to external changes in their growing conditions such as pH, temperature, and/or presence of toxic metals [23]. It was also reported in a previous study that inhibition by heavy metals was less noticeable for acidogenic bacteria [24].

4.1. Biomass treatment (Acidogenic phase)

To achieve acidogenic conditions, biomass can be inoculated in Erlenmeyer flasks for a large period of time (up to 8 weeks), mixing anaerobic sludge and material supports (if desirable). The flasks are kept at 30°C. The feed medium is changed continuously and prepared according to the requirements of the microorganism [25]. The medium pH is kept at acidic levels (3–4) to inhibit the growth of methanogenic organisms, which is favored at neutral pH.

Dextrose is generally used as substrate. This substrate is the source of organic matter that enhances volatile fatty acids (VFAs) formation, mainly: acetic acid, propionic acid, and butyric acid. In order to verify that the anaerobic sludge is carrying out the acidogenic phase of digestion, VFAs formation and concentration can be measured by HPLC (high performance liquid chromatography) taking samples from the flasks at regular intervals. pH can be measured daily and the growth of biomass can be indirectly calculated by determining the volatile suspended solids (VSS), which are obtained according to the gravimetric method [26].

4.2. Toxicity studies

Toxicity tests are carried out prior to any biosorption test with living biomass to obtain inhibitory concentrations. For acidogenic biomass, VFAs formation or substrate consumption are a direct measurement of microbial activity. During a toxicity experiment, a known amount of biomass (or immobilized biomass, if desirable) is put into a series of flasks and mixed with fixed volumes of metallic solutions and a selected substrate. The concentration of heavy metals in the metallic solutions varies according to each experimental setup and metallic ion, but one flask must be selected as a blank. The concentration of the organic substrate is kept constant in all flasks. Solution pH has to be adjusted to acidic levels (3–5) to avoid metal precipitation. Once the biomass and solutions are mixed, the flasks are closed and placed in an incubator at a specific temperature and rpm (for instance, 35°C and 50 rpm). Small liquid samples are taken from each flask at regular intervals to determine substrate or VFAs concentration. Sampling can stop when concentrations in all flasks remain constant for at least two consecutive points.

Once all measurements are done, toxicity is determined in terms of the half-inhibitory concentration, IC_{50}, which is the concentration at which microbial activity is decreased by 50%. Microbial activity is determined by calculating the difference between the initial concentrations and final concentrations in each flask and dividing it by the concentration difference of the blank (Equation 3). IC_{50} is then determined graphically from a plot of "% activity" versus "metallic concentration". The blank is considered to have a 100% microbial activity since no metallic inhibition takes place, but the activity decreases with increasing heavy metal concentration.

$$A(\%) = \frac{C_{0-}C}{C_{B0-}C_{Bt}} \times 100\% \tag{3}$$

where

A(%): microbial activity.

D_0, D_{48}: concentration of substrate or VFAs at times 0, and t, respectively. D_{c0}, D_{c48}: concentration of substrate or VFAs in the blank flask at times 0, and 7, respectively.

4.3. Biosorption isotherms

Biosorption isotherms are plots of biosorption capacity versus metallic concentration at equilibrium. Isotherms can be adjusted to adsorption models to determine other parameters

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useful in the scaling up of biosorption processes, such as maximum biosorption capacity and affinity coefficients. To determine biosorption capacity, batch tests are carried out in a similar fashion to toxicity tests, but the variable of importance is the heavy metals concentration. A known amount of biomass (or immobilized biomass, if desirable) is put into a series of flasks and mixed with fixed volumes of metallic solutions. Metallic ions concentrations are determined by atomic absorption spectrometry. Biosorption equilibrium takes place when concentrations in all flasks remain constant for at least two consecutive points, and sampling can stop. Biosorption capacity can then be calculated according to Equation 4 [27].

$$q = \frac{V(C_0 - C_f)}{S} \tag{4}$$

where

q = biosorption capacity, (mg metal/g VSS);

C_0 = initial metal concentration (mg metal/L);

C_f = final metal concentration (mg metal/L);

S = biosorbent (biomass) used (g);

V = volume of metallic solution (L).

The data at equilibrium (concentration and biosorption capacity) can be adjusted to established adsorption models. A correlation factor can be calculated by lineal regression to determine which model fits best to the experimental values. The most commonly used models in the literature are the Langmuir and Freundlich models.

4.4. Continuous studies

Continuous studies can be carried out in bioreactors of all shapes and sizes, but the most commonly used configuration is the anaerobic packed bed reactor (APBR). Generally, wastewater flows upward through the reactor bed, and the use of a material support prevents from biomass losses and enhances bed stability. Environmental conditions depend upon the type of biomass used. Figure 3 shows the schematic diagram of an APBR used for the biosorption of heavy metals [28]. Bioreactors startup times are varied, and the parameters commonly measured during operation are pH, chemical oxygen demand (COD), substrate consumption, methane formation, VFAs formation, volatile suspended solids (VSS). Recirculation of the effluent can be added to the reactors configuration to enhance biomass growth before biosorption takes place.

The COD values are a measure of the organic load of wastewaters. When both the influent and effluent points are sampled, the COD analysis provides a quantifiable measurement of the removal efficiency of organic matter in the bioreactor. The most common COD method involves digestion of the sample at 120°C followed by a colorimetric analysis. The procedure

is thoroughly described in the Standard Methods for the Examination of Water and Wastewater [26].

VFAs concentrations are indicative of the acidogenic activity of anaerobic biomass. Total VFAs can be analyzed by a simple titration method (using hydrochloric acid and sodium hydroxide) proposed by [29] Powell and Archer (1989). Specific VFAs, such as acetic acid, propionic acid, or butyric acid can be analyzed by HPLC. For the determination of substrate consumption, most methods are relatively simple and involve colorimetric techniques. A method utilized for glucose concentration is the DNS (3,5-dinitro-salicyclic acid) method, where the free sugar reduces the DNS reagent at high temperature, resulting in the formation of a colored product that absorbs light at 540 nm [30].

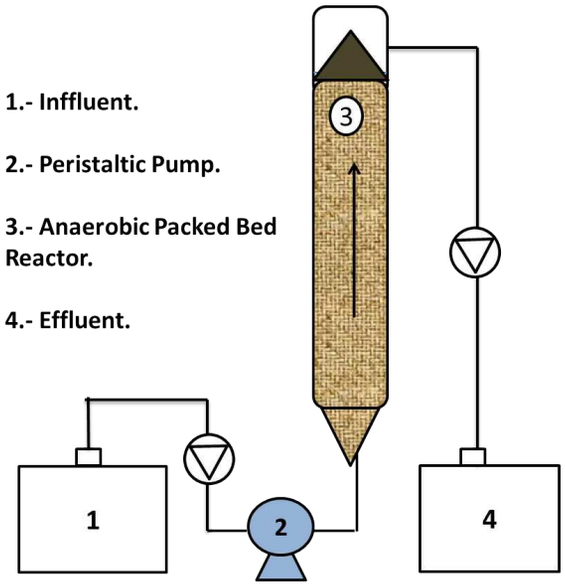

Figure 3. Example of an APBR used for heavy metals biosorption.

Once the startup stage is complete, heavy metals can be fed to the bioreactor to initiate the biosorption stage. A plot of C/C_0 versus time is known as a rupture curve, where C_o is the inlet concentration and C is the outlet concentration. Rupture curves provide information about the quality of a biosorbent in terms of the breakthrough time, saturation time, and retention capacities. The breakthrough time, t_b, is defined as the time in which the outlet concentration is equal to a maximum permissible value (usually 10% of the inlet concentration or lower). Saturation time, t_s, is the time in which the column is completely saturated by the metallic ions. Metallic retention capacity, Q_{ads}, can be calculated according to the following equation:

$$Q_{ads} = \frac{C_0 F}{m_s}\int_{t=t_0}^{t=t_s}\left(1-\frac{C}{C_o}\right)\mathrm{d}t \quad \Rightarrow \quad Q_{ads} = \frac{F}{m_s}\int_{t=t_0}^{t=t_s} C_{ads}\,\mathrm{d}t \tag{5}$$

where

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Q_{ads}: Retention capacity [mg/gVSS];

C_{ads}: C_o-C [mg/L];

t_0: Initial time [d];

t_s: Saturation time [d];

F: Volumetric flow [L/d].

Removal efficiency can also be determined simply by calculating the total metallic load and final metallic retention.

4.5. Bed characterization

Bed characterization in anaerobic reactors is usually achieved by the following techniques: fraction of solids, Gram staining, microscopic observation via optical microscopy or scanning electron microscopy (SEM), X-ray diffraction (XRD), and energy dispersive spectroscopy (EDS). These analyses supply plenty of information about the morphology and structure of the microorganisms and extracellular polymeric substances of the biofilm. XRD and EDS are especially helpful when a material support is utilized since these analyses provide the elemental composition of the different solid phases of the bioreactor bed.

5. Sulfate-reducing process and metal bioprecipitation

The microbial sulfate-reducing process (SRP) has been utilized as a potential tool for heavy metals removal during the final steps of wastewater treatments and effluent recovery of several industries. Under anaerobic conditions, sulfate-reducing bacteria (SRB) reduce sulfate to sulfur, which reacts with the metallic ions, resulting in the formation of metallic sulfurs. Metallic sulfurs are universally identified because of their low solubility in aqueous systems, making the sulfate-reducing process an effective alternative for wastewater treatment [31]. Furthermore, selective recovery of economically important metals is also possible [32]. The sulfate-reducing process is successfully applied in the removal of metallic ions and sulfates in acid mine drainage (AMD), and can be useful in the removal of the remaining metals in industrial wastewaters [31].

During the SRP, sulfate ions $\left(SO_4^{2-}\right)$ are enzymatically reduced to sulfur (H_2S, HS-, and S^{2-}) to obtain the energy required for the growth and maintenance of SRB. In order for this process to take place, cells carry out an enzymatic oxidation of organic matter (electron donor) to carbon dioxide and water [33, 34]. The SRP is strictly an anaerobic process, since it can only occur in the absence of electron acceptors with high redox potential such as oxygen or nitrate [35]. The SRP for heavy metals removal is based on the formation of metallic sulfurs with low solubility and the neutralization of water as a result of the alkalinity produced during the microbial oxidation of the electron donors [36]. This phenomenon has been defined as bioprecipitation [37], and can be described by the following equations [38].

Formation of sulfur and alkalinity (sulfidogenic oxidation) is defined by Equation 6, where CH_2O represents the electron donor:

$$2\ CH_2O + SO_4^{2-} \rightarrow H_2S + 2\ HCO_3^- \tag{6}$$

When H_2 is used as electron donor, the reaction generates hydroxide ions:

$$8H_2 + 2SO_4^{2-} \rightarrow H_2S + HS^- + 5H_2O + 3OH^- \tag{7}$$

The formation of biogenic sulfur (H_2S, HS-, S_2-) enhances precipitation of dissolved metals, where M2+ represents metallic ions such as: Zn2+, Cu2+, Ni2+, Co2+, Fe2+, Hg2+, Pb2+, Cd2+, o Ag+:

$$H_2S + M^{2+} \rightarrow MS_{(s)} + 2\ H^+ \tag{8}$$

The precipitation of metallic ions releases protons which acidify the water. Consequently, it is necessary to reduce the excess of sulfate to compensate acidity. The alkalinity of the hydroxide ions or bicarbonate produced during the sulfidogenic oxidation neutralizes the acidity of water.

$$HCO_3^- + H^+ \rightarrow CO_{2(g)} + H_2O \tag{9}$$

$$OH^- + H^+ \rightarrow H_2O \tag{10}$$

5.1. Advantages of the sulfate reducing process in wastewater treatment

The SRP is a valuable biotechnological tool for heavy metals removal in mining lixiviates and industrial effluents. It is considered potentially superior to other biological processes due to its capacity to produce alkalinity, neutralize the pH of acidic water, and simultaneously remove organic matter, sulfates and heavy metals [39, 32, 40, and 38]. Furthermore, recent studies of the SRP have revealed potential immobilization for metalloids (arsenic), radioactive isotopes (uranium), and cyanides [41, 42, and 43]. The SRP has also shown applications in organic matter removal and degradation of xenobiotic and toxic compounds [44].

The most commonly known advantages of the SRP are the low formation of metallic sulfur sludge (small volume and low solubility) compared to hydroxide precipitation and the recovery of economically important metals and precipitated metallic ions [45]. Recently, some methods have been implemented to selectively recover metals through pH and sulfur control [33].

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5.2. Toxicity of metals

It has been reported that metals are inhibitory agents for anaerobic microorganisms, including SRB [46, 47]. The inhibition is mostly due to the capacity of metals to deactivate enzymes by reacting with other sulfhydryl groups (-SH) and replacing the metals that constitute the active sites, such as Cu(II), Zn(II), Co(II), Ni(II). The deactivation of enzymes implies a negative impact on bacterial growth and activity [48]. There are some discrepancies in the literature with regard to the inhibitory levels of heavy metals over SRB because the majority of experiments are carried out at different environmental conditions [49].

Biogenic sulfur (produced during the SRP) forms complexes insoluble with heavy metals, resulting in the precipitation of metallic sulfur and, in turn, a toxicity reduction [46]. Sulfur inhibition may be decreased by precipitating sulfur with iron [50]. Several studies have focused on the use of SRP for the precipitation of metallic sulfurs within the same reactors where the sulfate-reducing activity takes place. However, this method might increase the inhibition of SRB [51].

To reduce inhibitory effects and increase pH in anaerobic reactors, a portion of the wastewater can be recycled and mixed with the influent. The remaining sulfur in the recirculating effluent reacts with heavy metals and causes precipitation of metallic ions before they get in contact with the anaerobic sludge [52]. The search of new strains tolerant to sulfurs or the special designs of bioreactors can help to prevent the toxic effect of heavy metals on SRB [53].

Another problem associated with heavy metal precipitation within the reactor is that metallic sulfurs are deposited on the biomass, and the contaminated sediments generate an increase in volume [54]. Moreover, contrary to general belief that only soluble metallic ions cause inhibition, it has been proven that metallic sulfurs affect the metabolic activity of SRB. Metallic sulfurs are not toxic, but they block substrate and nutrients access into the cells by forming a barrier on the cellular walls of SRB [47]. A proper alternative to separate the biological process from the precipitation is to use a two-step process, where metallic precipitation is isolated from the biological process [54].

5.3. Selective precipitation of heavy metals

Metallic sulfurs are generally highly insoluble at neutral pH, whereas some compounds, such as CuS, are insoluble at pH values as low as 2. The great advantage of precipitation is the possibility for selective recovery of metallic sulfurs. It has been shown that each metal precipitates at a unique sulfur concentration S^{2-}, or potential (pS), directly related to the solubility of the metallic sulfur formed. Controlling these concentrations within a precipitator can be carried out using pH electrodes and sulfide ion selective electrodes (pS electrode). The unique quality of the potential level (pS) of each metal has been successfully applied as a controlling parameter for the selective precipitation of metals and formation of pure metallic sulfurs suitable for reutilization. The success of the precipitation process depends not only on the heavy metal removal from the soluble phase but also on its separation from the liquid phase. Thus, solid–liquid separation processes (for instance, sedimentation and filtration) are of great importance for a successful removal [55].

5.4. Types of reactors used for the sulfate-reducing process

Biomass is retained within bioreactors according to the adherence properties of cells. Thus, bioreactors can be classified into two groups [56]: fluidized bed reactors and fixed bed reactors. In a fixed bed reactor, biomass is retained either by the formation of biofilms on static or suspended inert materials or by the obstruction of biological particles on packing materials (Figure 4). A biofilm is defined as a complex structure constituted by cells and extracellular products in elongated or granular forms [57]. In fluidized bed reactors (or free bed reactors), biomass is retained by forming biological particles of high density and sedimentability: granules. Methanogenic granular sludge and sulfate-reducing sludge are composed of microbial aggregates that grow by mutual bonding of bacterial cells in the absence of a support material [58].

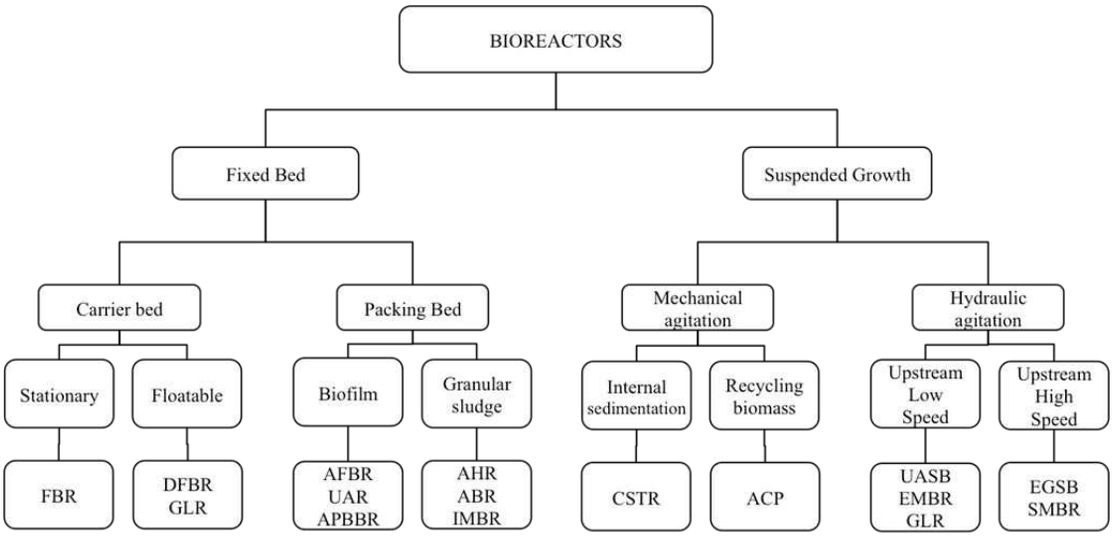

Figure 4. Anaerobic reactors used in sulfate-reducing applications.

Numerous literature studies have applied multiple reactors designs of the sulfate-reducing process for the treatment of water with high concentrations of sulfates and heavy metals. Some of these designs include batch reactors (BR), sequencing batch reactors (SBR), continuously stirred tank reactors (CSTR), anaerobic contact processes (ACP), anaerobic baffled reactors (ABR), anaerobic filter reactors (AFR), fluidized bed reactors (FBR), gas lift reactors (GLR), anaerobic hybrid reactors (AHR), membrane bioreactors (MBR), and upflow anaerobic sludge blanket reactors (UASB) [38].

6. Biosorption models

The kinetic model of a microbial process is defined as: the verbal or mathematical correlation between velocities and concentrations of reagents products, inserted into mass balances for the prediction of substrate conversion level and individual yields at specific operating conditions [59].

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The complexity of the kinetic models used to describe the changes within the cell during a microbial transformation can be very broad. Several kinetic models proposed in the literature are summarized in Table 1 [60].

Kinetic model	Definition
Unstructured – non segregated	Biomass is considered the only component. An average cell is representative of the microbial consortium.
Metabolic	Metabolic pathways are described as a network of reactions using a simplified reaction scheme. Stoichiometric relations are defined.
Sructured (or Cell)	Biomass is considered to be constituted by several species. Intracellular components are taken into account.
Segregated	The distribution of a property is considered in the description of the biomass.

Table 1. Classification of kinetic models.

The simplest models, unstructured-non segregated models, have been used for numerous engineering troubleshooting applications. However, in order to have a better system description it is necessary to use models that take into account complex reaction schemes, i.e., models that take into account the metabolic pathways of each microorganism.

A mathematical model is the abstract representation of a specific aspect of reality. Its structure is composed of two parts. The first part corresponds to all those characteristic aspects of an idealized reality, and the second part refers simply to the existing relationships between the aforementioned elements [61].

The order of reaction is an experimental magnitude dependent of the way in which velocity relates to concentration [62]. Any typical reaction in nature will occur at a rate dependent of certain factors, the reaction rate is indicated by a constant value (k). It is found that reaction rates are related to the reaction order according to the following mathematical expression [63]:

$$-\frac{dA}{dt} = k\left[\mathrm{A}\right]^{\mathrm{n}} \tag{11}$$

where

n = reaction order;

k = rate constant;

A = concentration of component A;

t = time.

This equation is integrated for every order of reaction (zero order, first order, second order, pseudo first order, and pseudo second order) as follows:

- *Zero order reaction:*

Differential equation:

$$-\frac{dA}{dt} = kA^{\circ} \tag{12}$$

Separating variables:

$$\int dA = k\int dt \tag{13}$$

Solving the integral:

$$A = kt + C \tag{14}$$

- *First-order reaction:*

Differential equation:

$$-\frac{dA}{dt} = kA^{1} \tag{15}$$

Separating variables:

$$\int \frac{dA}{A} = k\int dt \tag{16}$$

Solving the integral:

$$LnA = kt + C \tag{17}$$

- *Second-order reaction:*

Differential equation:

$$-\frac{dA}{dt} = kA^{2} \tag{18}$$

Separating variables:

$$\int \frac{dA}{A^{2}} = k\int dt \tag{19}$$

Solving the integral:

$$\frac{1}{A} = kt + C \tag{20}$$

where C: Integration constant

- *Pseudo first-order reaction:*

Differential equation:

$$\frac{dx}{dt} = k_2(C_{A0} - x)b \tag{21}$$

Solving the equation:

$$k = bk_2 = \frac{1}{t}\left[\frac{1}{C_A} - \frac{1}{C_{A0}}\right] \tag{22}$$

- *Pseudo second-order reaction:*

$$\frac{dx}{dt} = k_2(C_{A0} - x)^2 b \tag{23}$$

Solving the equation:

$$k = bk_2 = \frac{1}{t}\left[\frac{1}{C_A} - \frac{1}{C_{A0}}\right]^2 \tag{24}$$

where

C_A = amount of metal adsorbed (mg/L)

C_{Ao} = initial concentration (mg/L)

t = time (min)

k = equation constant (mg/L-min)

b = initial concentration of component b, constant throughout the reaction time.

If the lineal model properly fits the experimental values (i.e., a correlation factor, R^2, close to 1) the adsorption process can be described as chemisorption [64].

The development of biosorption systems is dependent of many factors including: temperature, pH, biosorption capacities and selectivities, recovery efficiency, and resistance to other components or operating conditions. Nevertheless, most biosorption studies focus on the measurement of the biosorption capacities of biomass [65]. The quantification of the sorbate–biosorbent interactions is fundamental for the evaluation of the biosorption capacity. Due to the similarity between the biosorption process and the adsorption process, biosorption

capacity can be analyzed by sorption isotherms. Sorption isotherms are model equations that represent the behavior of experimental data.

The Langmuir and Freundlich equations are two of the most utilized adsorption models. These models are described by the following equations [1]:

6.1. Langmuir model

$$q_e = \frac{q_{\max} b C_e}{1 + b C_e} \tag{25}$$

$$\frac{1}{q_e} = \left(\frac{1}{b q_{\max}}\right)\left(\frac{1}{C_e}\right) + \left(\frac{1}{q_{\max}}\right) \tag{26}$$

where

q_e: biosorption capacity at equlibrium (mg/g VSS).

q_{max}: máximum biosorption capacity (mg/g VSS).

C_e: metallic concentration at equilibrium (mg/L).

b: affinity coefficient between the sorbate and the biosorbent (L/mg).

q_e and C_e are obtained at the equilibrium point, whereas q_{max} and b can be determined graphically by a plot of $(1/q_e)$ versus $(1/C_e)$.

6.2. Freundlich model

$$q_e = k C_e^{1/n} \tag{27}$$

$$\mathrm{Ln}(q_e) = \frac{1}{n}\mathrm{Ln}(C_e) + \mathrm{Ln}(k) \tag{28}$$

where

q_e: biosorption capacity at equilibrium;

C_e: metallic concentration at equilibrium;

k,n: Freundlich constants.

The parameters k and n can be graphically determined from a plot of $\mathrm{Ln}(q_e)$ versus $\mathrm{Ln}(C_e)$.

7. Conclusion

Environmental pollution is one of the main problems of our society. Heavy metals constitute a major group of contaminants characterized by having a density five times greater than that

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of water. One of the main sources of heavy metals pollution is the acid mine drainage (AMD) generated by mining industries. The AMD is an acid lixiviate that may contain high concentrations of sulfates, iron, calcium, zinc, manganese, aluminum, copper, and other types of toxic elements such as arsenic and lead. In México, several regions have been affected due to the presence of heavy metals in wastewaters, which generates the necessity of implementing economic and efficient remediation techniques. The review focuses on biological methods and the advantages they offer over conventional treatments. One particular alternative studied in recent years is biosorption – based on the ability of biomass to bind and concentrate heavy metals – because of its economic nature and high removal efficiencies in dilute wastewaters.

Biological technologies provide plenty of advantages and can be just as effective and economic as other technologies (Table 2). However, it is of upmost importance to continue with scientific research to acquire an improved understanding of the bioremediation processes and optimize industrial applications.

Method	**Advantages**	**Disadvantages**
Chemical precipitation	-Low cost -Simple operation	-Excessive formation of sludge -Slow or insufficient precipitation
Reduction	-No residual sludge generation	-Formation of toxic gaseous products -Difficult handling of reagents
Ultrafiltration	-Requires small operating space -High separation	-High operating costs -Membrane clogging
Reverse osmosis	-High efficiency -Removal of other ionic compounds	-High operating and maintenance costs
Electrodialysis	-High efficiency	- High energy requirements (high pressures) -Clogging
Adsorption-Ionic Exchange	-Simple operation -High adsorption capacity	-Low selectivities - pH sensitive
Phytoremediation	-Eco-friendly technology and low cost -Removes other types of pollutants	-Depends on the growing conditions. Long remediation times. It requires expensive agricultural equipment.
Microbial bioremediation	-Eco-friendly technology and low cost -Generates no toxic waste (CO_2, H_2O)	-Limited pollutants range -Microbes need proper growing conditions.
Biosorption	-Low cost -Minimum formation of residual sludge -Potential for metal recovery -Simple operation -Effective in diluted solutions.	-Toxic effects on living biomass. -Constant nutrient supply for biomass growth.

Table 2. A comparison between the existing methods for heavy metals removal.

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