Bioaccumulation of heavy metals and detoxification

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Heavy metals (HMs) pollution

- Soil heavy metal pollution mainly refers to the deposition of heavy metals, such as mercury, cadmium, lead, chromium, and other bio-toxic heavy elements in the soil, resulting in concentrations that exceed background values.
- Metals are not biodegradable, and through biological amplification, their concentrations can be increased thousands of times, with significant effects on human health.
- In recent years, discharges of large volumes of heavy metals from industrial activity and mining, with final deposition in the soil, have led to increases in soil heavy metal concentrations.
- Wastewater effluents from mines and metal refineries are often contaminated with heavy metal ions, so they pose hazards to human and environmental health.
- Widespread use of pesticides and fertilizers may also have led to an increase in soil heavy metal concentrations

Heavy metals remediation (HMR)

- Conventional technologies to remove heavy metal ions are well-established, but the most popular methods have drawbacks:
  - chemical precipitation generates sludge waste,
  - activated carbon and ion exchange resins are made from unsustainable non-renewable resources.
- Advantages commonly associated with these conventional methods include rapid processing time, controllability, resilience to high concentrations of HMs, ease of operation, and well understood molecular basis.
- However, traditional methods are often expensive, complicated, frequently cause secondary pollution, and significantly alter the soil structure, among other limitations and deficiencies.
- Using microbial biomass (bacteria, fungi, algae) as the platform for heavy metal ion removal is an alternative method.
- Bioaccumulation and biosorption can be used and proposes that it can be developed for bioextractive applications—the removal and recovery of heavy metal ions for downstream purification and refining, rather than disposal.

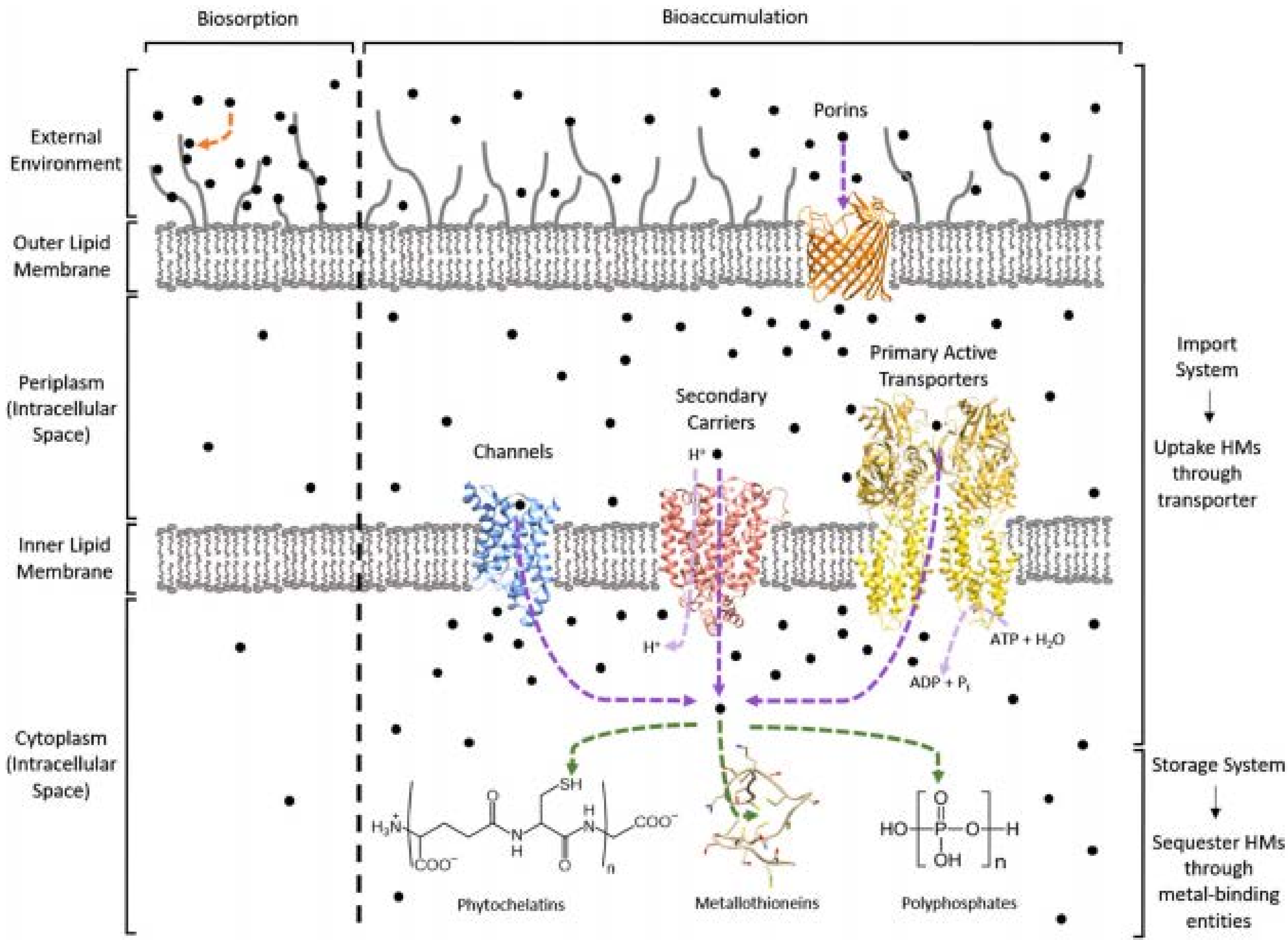
Biosorption

- Biosorption is the adsorption of particles to a biological matrix using physical interactions (electrostatic forces), chemical interactions (ion or proton displacement), complexation, or chelation.
- At neutral pH, the extracellular surface of microorganisms contains anionic moieties that provide binding sites for cationic HMs.
- Many ions in the cell surface functional groups, such as nitrogen, oxygen, sulfur, and phosphorus, can be complexed with metal ions.
- In addition, phosphoric acid anions and carboxyl anionic groups on the surface of the microbial cell wall are negatively charged, and most heavy metal surfaces carry a cationic group that interacts with the cell wall.
- Researchers have also engineered microorganisms to have recombinant metal-binding proteins and peptides on the extracellular surface, thus improving the capacity and specificity of these microbial biosorbents.
- This area has seen remarkable progress and has leveraged molecular biotechnology.
- However, since it is based on adsorption, it encounters challenges like:
  - the susceptibility to variations in pH and ionic strength that exists in heterogeneous wastewater effluents.
  - Biosorbents also have limited lifespans as they often use dead biomass that degrades over time, and because fouling renders the binding sites unavailable.

Bioaccumulation

- Bioaccumulation is a metabolically-active process where microorganisms uptake HMs into their intracellular space using importer complexes that create a translocation pathway through the lipid bilayer (i.e., import system).
- Once inside the intracellular space, the HMs can be sequestered by proteins and peptide ligands (i.e., storage system).
- The bioaccumulative capacity of a biomass for a target HM is a measure of performance commonly reported as μmol_x or mg_x per g dry weight, where x is the HM.
- The organism that will accumulate heavy metals should have:
  - tolerance to one or more metals at higher concentrations,
  - must exhibit enhanced transformational abilities, changing toxic chemicals to harmless forms that allows the organism to lessen the toxic effect of the metal, and at the same time, keep the metal contained.
- Bioaccumulation requires the host cell to be alive, which imposes unique challenges: nutrient feeds for sustaining and propagating biomass, level of aeration to accommodate aerobic/anaerobic needs.

Bio-HMR technology overview using a Gram-negative bacterium



Latest techniques for HM bioremediation

- **Heavy metal removal using biofilm:** There are several reports on the application of biofilms for the removal of heavy metals.
- Biofilms have very high tolerance against toxic inorganic elements even at a concentration that is lethal.
- Biofilms mechanisms of bioremediation could either be via biosorbent or by exopolymeric substances present in biofilms which contain molecules with surfactant or emulsifer properties.
- **Immobilized biosorption of HMs:** The use of encapsulated biomass enhances biosorption performance, and increases its physical and chemical stability.
- Immobilizations of microbial biomass in polymeric matrixes confer rigidity and heat resistivity with optimum porosity for practical applications.
- **Microbial genetic engineering:** With the advanced in genetic engineering, microbes are engineered with desired characteristics such as ability to tolerate metal stress, overexpression of metal-chelating proteins and peptides, and ability of metal accumulation.
- Engineered *Chlamydomonas reinhardtii* generated significant increase in tolerance to Cd toxicity and its accumulation.

Microbial Detoxification of Heavy Metal

- Certain metals like Cu, Zn, Co, and Fe are essential for survival and growth of microbes.
- However, these metals can exhibit toxicity at higher concentrations and may inactivate protein molecules.
- Although some metals such as Al, Cd, Hg and Pb remain with unknown biological functions, yet they accumulate within cells and may:
  - affect enzyme selectivity
  - deactivate cellular functions
  - damage the DNA structure
  - may result in cell death
- In response to metals in the environment, microorganisms have developed ingenious mechanisms of metal resistance and detoxifcation.
- The major mechanical means to resist heavy metals by microorganism are metal-organic complexion, metal effux pumps, demethylation, intracellular and extracellular metal sequestration, exclusion by permeability barrier, and production of metal chelators like metallothioneins and biosurfactants.
- The mechanism involves several procedures, together with electrostatic interaction, ion exchange, precipitation, redox process, and surface complexation.

Intracellular Sequestration

- Intracellular sequestration is the complexation of metal ions by various compounds in the cell cytoplasm.
- Cadmium-tolerant *Pseudomonas putida* strain possessed the ability of intracellular sequestration of copper, cadmium, and zinc ions with the help of cysteine-rich low molecular weight proteins.
- Also, intracellular sequestration of cadmium ions by glutathione was revealed in *Rhizobium leguminosarum* cells.

Extracellular Sequestration

- Extracellular sequestration is the accumulation of metal ions by cellular components in the periplasm or complexation of metal ions as insoluble compounds.
- Copper-resistant *Pseudomonas syringae* strains produced copper-inducible proteins CopA, CopB (periplasmic proteins), and CopC (outer membrane protein) which bind copper ions.

Reduction of Heavy Metal Ions

- Microbial cells can convert metal ion from one oxidation state to another, hence reducing their harmfulness.
- Bacteria use metals and metalloids as electron donors or acceptors for energy generation.
- Metals in the oxidized form could serve as terminal acceptors of electrons during anaerobic respiration of bacteria.
- Reduction of metal ions through enzymatic activity could result in formation of less toxic form of mercury and chromium.
- *Geobacter sulfurreducens* and *G. metallireducens* have the ability to decrease chromium (Cr) from the very lethal Cr (VI) to less toxic Cr (III).

Metal efflux pumps

- Bacteria can eject metal ions from the cytoplasm to sequester the metal within the periplasm.
- Zinc ions can cross from the cytoplasm by effux system where they are accumulated in the periplasm of *Synechocystis*.

Demethylation & Volatilization

Demethylation: In mercury-resistant bacteria, organomercurial lyase converts methyl mercury to Hg(II), which is one hundred-fold less toxic than methyl mercury.

Volatilization: mechanisms involve turning metal ions into a volatile state.

- This is only possible with Se and Hg, which have volatile states.
- Mercury-resistant bacteria utilizes the MerA enzyme to reduce Hg(II) to the volatile form Hg(0).
- The reduction of Se(V) to elemental Se(0) has been employed to remediate contaminated waters and soils.

Extracellular Barrier of Preventing Metal Entry into Microbial Cell

- Microbial plasma membrane, cell wall, or capsule could prevent metal ions from entering the cell. Bacteria can adsorb metal ions by ionizable groups of the cell wall (amino, carboxyl, phosphate, and hydroxyl groups) .
- *Pseudomonas aeruginosa* biofilm cells show higher resistance to ions of copper, lead, and zinc than planktonic cells, while cells located at the periphery of the biofilm were killed.
- Extracellular polymers of biofilm accumulated metal ions and then protect bacterial cells inside the biofilm.

Questions

- Write an essay on use of microorganisms for heavy metals remediation.
- What are the mechanisms used by microorganisms for detoxification of heavy metals? How their ability used for heavy metal remediation?
- Write short notes on:
  - Bioaccumulation
  - Biosorption
  - Heavy metal detoxification by microorganisms

Bioleaching, or microbial ore leaching

- **Bioleaching, or microbial ore leaching:** is a process used to extract metals from their ores using bacterial micro-organisms.
- The bacteria feed on nutrients in the minerals, causing the metal to separate from its ore.
- The metals commonly extracted using this process include gold, silver, zinc, copper, lead, arsenic, antimony, nickel, molybdenum cobalt, and uranium.
- Bioleaching is performed mostly by iron and sulfide oxidizing bacteria, or acid producing fungus.
- Some of the types of bacteria used in this process include *Leptospirillum ferrooxidans*, *Thiobacillus ferrooxidans*, and some species of *Sulfolobus*, and *Sulfobacillus*.

...Bioleaching, or microbial ore leaching

- For example, bacteria catalyse the breakdown of the mineral pyrite (FeS_2) by oxidising the sulfur and metal (in this case ferrous iron, (Fe^{2+}) using oxygen. This yields soluble products that can be further purified and refined to yield the desired metal.

Pyrite leaching (FeS_2): In the first step, disulfide is spontaneously oxidized to thiosulfate by ferric ion (Fe^{3+}), which in turn is reduced to give ferrous ion (Fe^{2+}):

(1) $FeS_2 + 6Fe^{3+} + 3H_2O \longrightarrow 7Fe^{2+} + S_2O_3^{2-} + 6H^+$ (spontaneous)

The ferrus ion is then oxidized by bacteria using oxygen:

(2) $4Fe^{2+} + O_2 + 4H^+ \longrightarrow 4Fe^{3+} + 2H_2O$ (iron oxidizers)

Thiosulfate is also oxidized by bacteria to give sulfate:

(3) $S_2O_3^{2-} + 2O_2 + H_2O \longrightarrow 2SO_4^{2-} + 2H^+$ (sulfur oxidizers)

The ferric ion produced in reaction (2) oxidized more sulfide as in reaction (1), closing the cycle and given the net reaction:

(4) $2FeS_2 + 7O_2 + 2H_2O \longrightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+$

The net products of the reaction are soluble ferrous sulfate and sulfuric acid.

...Bioleaching, or microbial ore leaching

- Using micro-organisms helps to reduce production costs, minimize environmental pollution, compared to conventional leaching processes that use cyanide, and to efficiently extract metals, even when their concentration in the ore is low.
- This process is growing in popularity, as the bacteria can grow naturally in mining environments, and can also be easily cultivated and recycled.