Bioremediation of marine environments polluted with mercury

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Introduction

Mercury is one of the most toxic heavy metals that exist, and the environmental pollution caused by it is rising, especially in recent years, due to several anthropogenic and natural sources. It has been demonstrated that several organic species of mercury (especially methyl-mercury) tend to accumulate in the tissues of living organisms, causing many problems. Mercury, as all other heavy metals, cannot be degraded by any chemical or biological pathway, which leads to their accumulation through the food chain by depredation of contaminated organisms, process known as biomagnification.

The aim of this work is to describe the chemical transformations and how they influence the development of the most toxic mercury compounds, and to describe microbial resistance mechanisms that can reduce mercury toxicity, especially biosorption, bioaccumulation and volatilization of soluble mercury compounds, which have proved to be the most effective bioremediation methods.

Mercury cycle

Mercury is emitted to the atmosphere in its gaseous form (Hg^0), where it is oxidized to liquid form and precipitates to water environments. The ionic form can be methylated mainly by sulfate-reducing bacteria to methyl mercury, which accumulates in the cell structures.

Some microorganisms have developed resistance mechanisms to mercury, such as biosorption, bioaccumulation or volatilization by *mer* operon. These mechanisms allow to hydrolyze methyl link, reduce, or adsorb these toxic forms of mercury, thus reducing its toxicity. Dissolved organic matter or chemical photoreduction reactions can also reduce the soluble Hg fraction in oceans.

Oxidation	Oxygenic conditions
	Photochemical reactions
Reduction	*Mer* operon (MerA)
	Photochemical reactions
Methylation	Anoxic conditions (sediment)
	Microorganisms: Methanogens/Sulfate-reducing/Iron-reducing
Demethylation	*Mer* operon (MerB)
	Oxidative demethylation

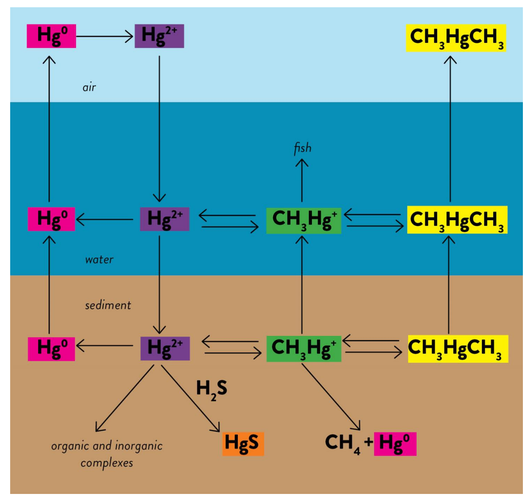

Figure 1. Global mercury cycle: This figure shows all chemical transformations of mercury in the aquatic environment, including atmospheric oxidation, methylation/demethylation, combined with organic compounds and reduction. (Madigan et.al., 2009)

Biosorption and bioaccumulation

- **Biosorption**: Passive adsorption of the mercury ions to bacterial biomass, or materials derived from it.
- **Bioaccumulation:** Process by which metal ions are collected exclusively by viable cells and may include adsorption mechanisms, intracellular accumulation or bioprecipitation.

Feature	Biosorption	Bioaccumulation
Metal affinity	High under favorable conditions	Toxicity will affect cell viability
rate of metal uptake	Usually rapid, a few seconds	Usually slower than biosorption
Selectivity	Variety of ligands involved, hence poor	Better than biosorption
Temperature tolerance	Within a modest range	Inhibited by low/high temperatures
Vaersatility	May be affected by other anions. pH dependent	ATP (nutrients) needed

Hg volatilization by *mer* operon

- Regulatory proteins: MerR/MerD. Regulate the transcription of other proteins
- Detection and transport proteins: MerT/MerP and/or MerC/MerF
- Reduction and demethylation:

  MerB: ($CH_3Hg^- \rightarrow Hg^{2+}$)

  MerA: ($Hg^{2+} \rightarrow Hg^0$)

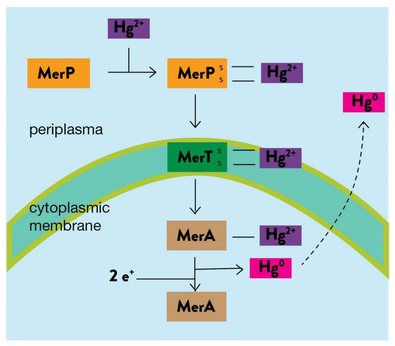

Figure 2. Reduction of Hg^{2+} by mer operon. The figure shows how the different proteins of the mer operon interact with mercury: (1) MerP: Detects Hg^{2+} in the extracellular space; (2) MerT is a trans membrane protein able to recognise MerP- Hg complex. It translocate Hg^{2+} into cytoplasm and binds it to MerA; (3) MerA: Reduces Hg^{2+} to Hg^0 with two additional electrons. Hg^0 is able to cross the cytoplasmic membrane by diffusion and finally leaves the aquatic environment to the atmosphere. (Madigan et.al., 2009)

Volatilization of Hg by *P.aeruginosa* PU21

Objectives

- Evaluate the ability of *P. aeruginosa* PU21 to volatilize Hg^{2+}.
- Evaluate how the stage of the culture influence mercury volatilization.

Experiment design

- *mer* operon was cloned into *P. aeruginosa* PU21 with Rip64 plasmid.
- 5L fed-batch bioreactors were used.
- PMM (*Pseudomonas* minimal medium).
- $[Hg^{2+}]_{initial}$ = 2, 5, 8 and 10mg/L to induce *mer* operon
- Bypass operation: Temperature, DO, agitation and pH constant

Results:

- *P. aeruginosa* PU21 is able to reduce most of Hg within 5-10h.
- Cell death occurred on each mercury addition.
- Addition of mercury at stationary phase did not cause a considerable cell death.

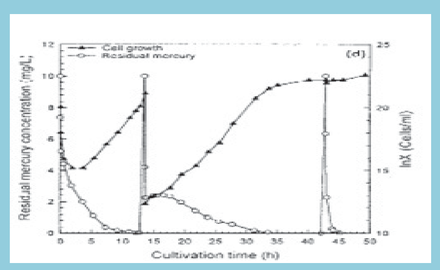

Figure 3. Volatilization of Hg^{2+} by *P.aeruginosa* PU21. The graphic shows the growth of Paeruginosa PU21 in function of the Hg^{2+} concentration in medium. In exponencial phase, when 10mg/L of Hg^{2+} are added, occurs a large cell death. When mercury is added in stationary phase, don't cause a considerable cell death (Chang and Law, 1997).

Biosorption of Hg^{2+} by *Bacillus* sp. biomass

Objectives

- Determinate the ability of *Bacillus* sp. non-living biomass to adsorb Hg^{2+}
- The effect of the initial $[Hg^{2+}]$ and pH in the process.

Experiment design:

- Biomass of *Bacillus* sp. Was autoclaved at 121ºC during 20'
- 0.25, 0.5, 1, 2.5, 5 and 10mg/L of $HgCl_2$ were added at 5 batch reactors. The amount of biomass added on each reactor was the same.
- For $[Hg^{2+}]$ of 1, 5, 10mg/L, the effect of pH was analyzed in a range between 3-9.
- Different samples were taken from each reactor at time 20, 40, 60, 90 and 120' to analyze the $[Hg^{2+}]$.

Results:

- Most of Hg^{2+} was adsorbed by bacterial biomass during the first 20'.
- An increase in absorption was observed when the metal concentration was greater.

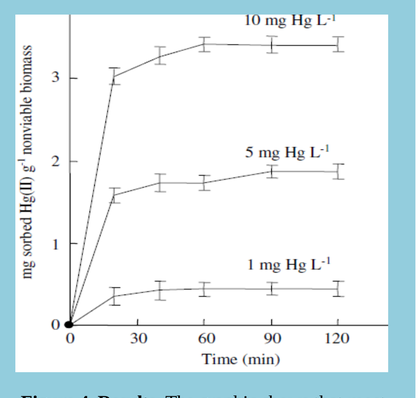

Figure 4. Results. The graphic shows that most of Hg^{2+} was adsorbed by bacterial biomass during the first 20' due to the rapid interaction between Hg^{2+} and cellular binding sites. (Green-Ruiz, 2005).

Conclusions

- Organic mercury compounds, especially methyl-mercury, are highly toxic compounds due to its powerful bioaccumulation and biomagnification.
- To decontaminate polluted environments, biological methods (especially demethylation and reduction by *mer* operon) have been proven to be the most effective.
- Different types of bacteria located in anoxic sediments (especially sulfate-reducing bacteria) play an important role in biogeochemical cycle of mercury, methylating the inorganic compounds.
- Biosorption has been demonstrated to be more effective than bioaccumulation, especially because it does not use living biomass which can be affected by mercury toxicity.
- *P. aeruginosa* PU21 is a good model microorganism to volatilize Hg^{2+} in aquatic environments, but high concentration of Hg^{2+} may cause an important cell death during its growth.
- Biosorption experiments have shown that mercury adsorption is proportional to its concentration in the medium.

References:

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