Microbial bioremediation to process environmental Mercury contamination in water sources of Almadén district

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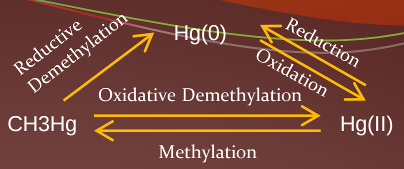

Introduction

Mercury is one of the most important environmental pollutants and the most dangerous form is Methylmercury due its large bioaccumulation capability. Toxicity is caused by its extremely high affinity to amino acid sulfhydryl groups. Anthropogenic mercury emissions are a common fact and one of the most remarkable cases can be found in Almadén district (central Spain), one of the most polluted zones on earth. To solve this problem, specially in water sources, bioremediation using microorganisms could be a satisfactory approach.

Mercury effect on microorganism communities

❖ Alteration of generational time, spore germination, N2 fixation, mineralization, respiratory activity, matter decomposition, cell morphology, DNA structure and photosynthesis [2].

❖ Mercury resistance due Hg(II) reduction and methylation [2].

❖ Mining zones like Almadén district release high amounts of sulfur in water, leading a mercury resistant community selection predominated specially by sulfate-reducing bacteria (*Desulfobacter sp., Desulfobulbus propionicus, Desulfovibrio africanus, Desulfovibrio desulfuricans*) [3].

Desulfobulbus propionicus 1pr3

➢ Non-strict anaerobic, non-motile, mesophilic, chemoorganotroph and non-sporulating Gram-negative bacteria that belongs at *Desulfobulbaceae* family [4].

➢ Propionate metabolism pathway with B12 vitamin enzyme to methylate mercury .

➢ Mercury resistant bacteria.

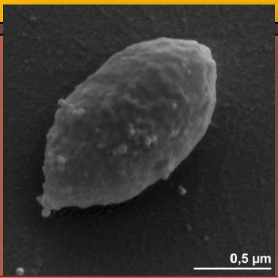

Fig.1.- *D. propionicus* 1pr3 cell. Figure extracted from Pagani, I. *et al*, 2011

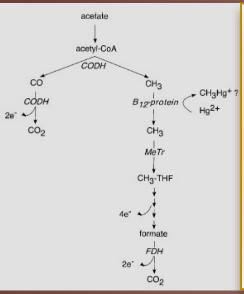

Fig.2.- Acetyl-CoA pathway scheme and proposed mercury interaction to form MeHg. Figure extracted from Ekstrom, E.B. *et al*, 2003.

Mercury Methylation

MeHg formed as an incidental side product in some methabolic reactions. Complete-oxidative genders (*Desulfosarcina, Desulfococcus or Desulfobacterium)* use Acetyl CoA pathway as a principal mercury methylation way, incomplete-oxidative genders use other pathways [5].

MeHg could be used in bioremediation but is also toxic for cells, so its concentration is controlod by demethylation, carried by *mer* genes.

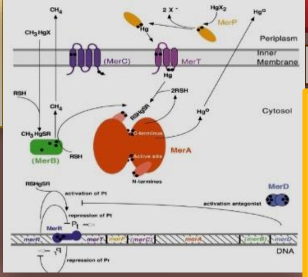

Fig.3.- Gram-negative *mer* operon scheme system. Figure extracted from Barkay, T. *et al*, 2013

Operon *mer* (*essential for activity since not all operons contain all genes)

Proteins	Category	Function
*MerA	Mercuric Reductase	Reduction of Hg(II) to Hg^0
MerB	Organomercurial Lyase	Cleaving of the methyl group of MeHg to obtain
*MerP	Periplasmatic Transport Protein	Capture and transport of mercury in the periplasm in Gram negative bacteria
*MerT	Membrane Transport Protein	Transport of Hg(II) in membrane and deliver to MerA
MerC	Membrane Transport Protein	Transport of Hg(II) in membrane and deliver to MerA
MerE	Membrane Transport Protein	Transport of organomercurial compounds in membrane and deliver to MerA
MerF	Membrane Transport Protein	Transport of Hg(II) in membrane and deliver to MerA
MerG	Periplasmatic Transport Protein	Transport of organomercurial compounds in membrane and deliver to MerA. Confers a certain organomercurial resistance.
*MerR	Regulatory Protein	Strong regulation of the *mer* operon expression
MerD	Regulatory Protein	Weak regulation of the *mer* operon expression

Based on the formation of the volatile form Hg(0).

Current applications of microbial Hg resistance directed to bioremediation Procedures

To use microorganisms to eliminate mercury from a determined environment obtaining as much as possible a clean area and controlling the mercury removed to avoid recontaminations.

Strategy	Description
Biosorption	Mercury accumulation in a bacterial biomass. Genetic enginery successful to introduce *merP* and *merT* plus a metallothionein overexpression to increase specificity and resistance [5].
Bioaccumulation	Bioaccumulation in other organisms by generation of more accumulative substances like methylmercury. An example could be phytoremediation where rhizosphere bacteria accumulate mercury in plant roots (constructed wetlands are a cheap option to implant) [6].
Operon *mer* reduction	High efficiency, specificity and biotechnological potential. Use of mercury resistant bacterial biofilms in chlor-alkaly wastewater bioreactors. Hg^0 is retained (no volatilized) where packed bed bioreactors are a useful solution to Hg^0 retention [6].
Precipitation	Precipitation of Hg(II) due the formation of HgS by its interaction with H2S or formation of other kind of insoluble mercury-sulphur complexes. Observed in some SRB and *Klebsiella pneumonia* [7].
*Biosensor construction	Highly sensitive biosensors can be constructed with regulatory gene *merR* and promoter region *merO* fused with bacterial luminicesnce genes (*lux*-CDEAB) to measure Hg(II) response [6].

* Not a bioremediation procedure but a strategy to detect Hg(II) contamination and its concentration

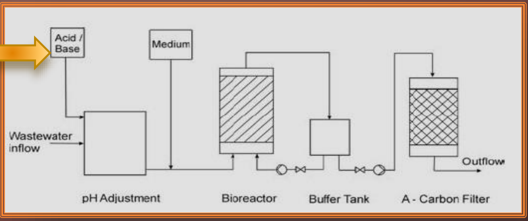

Fig. 4.- Flow scheme of the Wagner-Döbler pilot plant for a continuous treatment of mercury contaminated wastewater The instalation could remove 98% of mercury. The system treats 100 m3 per day and it contains a 1m3 bioreactor. The range of treatment is between 1 mg/l and 10 mg/ml. Figure extracted from Wagner-Döbler, 2003.

Conclusions

There is a problematic with mercury release in water reservoirs near mining areas. Is important to develop sustainable remediation techniques and the most advanced strategies are the *mer* operon based ones. For this reason, to solve Almadén district problems (also from other places) the first step is to construct an efficient bioreactor with mercury resistant microorganisms, followed by a pilot plant construction until the process will be fully optimized to make the move towards an industrial scale procedure.

References

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