BIOREMEDIATION OF WATER SYSTEMS POLLUTED WITH MERCURY: USE OF *MER* OPERON

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INTRODUCTION

In recent years, the mercury concentration has increased in nature due to anthropological activity. One of the most responsible activity is cloralcaline industry.
There are currently four technologies for treating mercury- contaminated waters: precipitation/ coprecipitation, adsorption, membrane filtration and bioremediation. Technologies that don´t use biological systems have high economic costs and undermine the environment. Bioremediation is presented as an alternative. One technique is the development of packed- bed bioreactors with bacteria containing the *mer* operon (figure 1). The proteins which encodes for reduce Hg(II) to Hg(0), a less toxic form that can be retained (figure 2).

OBJECTIVES

A. Analyze the NaCl concentration effects in effluents from cloralcaline industries in a bioreactor (figure 3).
B. Analyze the mercury concentration effects in from cloralcaline industries in a bioreactor (figure 4).
C. Analysze monospecie and multispecie mercury-reducing biofilm (figures 5 and 6).

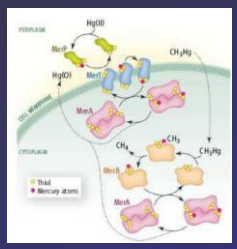

Figure 1. Running of proteins encoded by *mer* operon.

Figure 2. Mercury droplets formed in a microbial biofilm.

MATERIALS AND METHODS

The strains used were: *Pseudomonas putida* Spi3 (A, B, C), *Pseudomonas putida KT2442::mer-73* (C), *Citrobacter freundii* Bro62 (C), *Pseudomonas aeruginosa* Bro12 (C), *P.putida* Elb2 (C), *Sphingomonas sp.*Spi7 (C), *Pseudomonas stutzeri* Ibu8 (C) and *Citrobacter freundii* Bro62 (C).
A. Wastewater to determine the NaCl effect contained 1mg/l Hg and increasing concentrations up to 24g/l NaCl.
B. To determine the concentration of mercury itself, the wastewater used had 10/NaCl and different mercury concentrations: 1mg/l, 2mg/l, 3,5mg/l, 5mg/l and 9mg/l.
C. To determine the reduction efficiency according to the biofilm composition, the wastewater had the following concentrations: ECI 1 (3,5mg/l Hg and 6,0 g/l Cl), ECI 2 (7,0mg/l Hg and 6,0 g/l Cl), ECI 3 (7,0 mg/l Hg and 12,0 g/l Cl) and ECI 4 (10,0mg/l Hg and 7,3 g/l Cl) .

RESULTS

A. Hg concentrations in the outflow range between 20 and 80µg/l and the efficiency of metal removal is between 92% and 98% (Figure 3).
B. The removal of mercury is not affected by the metal in inflow concentrations up to 7mg / l Hg. The retention efficiency at concentrations of 1 mg / l Hg is 95%, whereas when is 7mg/l are reaching efficiencies of 99.2% (Figure 4).
C. Bioreactors inoculated *with Pseudomonas putida* KT2442 :: mer-73 show a decrease in the number of cells in the first 10 days, falling to 0 days after the change to wastewater ECI 2 (Figure 5). Bioreactors inoculated with *Citrobacter freundii* Bro62 have a retention efficiency of Hg (II) high and stable in ECI1, ECI2 and ECI3 tanks, which results in low concentrations of mercury in the effluent. Hg retention is 98%. The number of cells is also stable. After a few days of the change in water ECI 4, mercury retention efficiency decreases as the number of cells. In bioreactors with multispecies biofilm, are observed extremely low mercury concentrations in outflow wastewater ECI 4, which corresponds to a 99% withholding.
Regarding the composition of the multispecies biofilm, Bro62 is the dominant strain for bioremediation of high concentrations. As the selective pressure increases, diversity decreases. But when the pressure decreases, the initial diversity is restored.

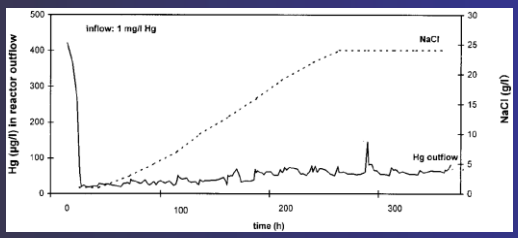

Figure 3. Removal of mercury from wastewater containing 1mg/l Hg in presence of different concentrations of NaCl by *Pseudomonas putida* Spi3 biofilm.

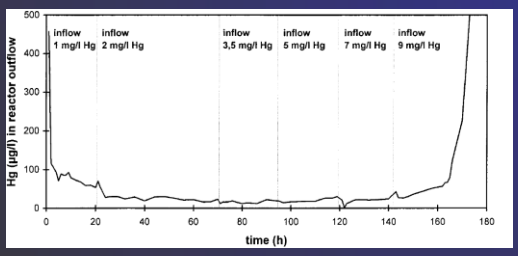

Figure 4. Removal of different concentrations of mercury from wastewater containig 10g/l NaCl by *Pseudomonas putida* Spi3 biofilm.

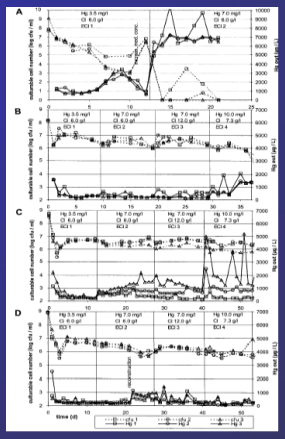

Figure 5. Cultibable cell numbers and mercury concentrations in the bioreactor effluent. (A) *P. putida KT2442::mer-73* monospecies bioreactor . (B) *C. freundii* Bro62 monospecies bioreactor . (C) *P. aeruginosa* Bro12 monospecies bioreactor. (D) multispecies bioreactor.

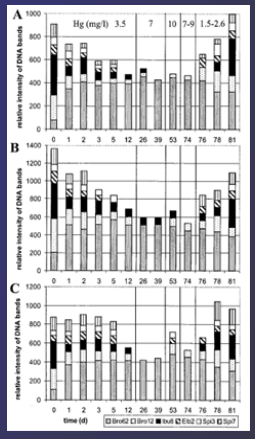

Figure 6. Time course analyses of strain abundance in the multispecies bioreactor. (A) replica 1, (B) replica 2 i (C) replica 3.

DISCUSSION

A. NaCl is one of the most important co-pollutants that interferes with the reductase activity. A big decrease in reduction activity when subject the packed bed to concentrations of 24g/l NaCl is not observed. Therefore, the concentrations of salts produced by chloralcaline industries are not inhibitory of the resistant microbiota to the mercury used to delete it.

B. Regarding the level of resistance to mercury, we can say that the inhibitory concentrations are about to 9mg / l with a bioreactor operating with a biofilm formed by *Pseudomonas putida* Psi3. However, the resistance of biofilms to certain concentrations of mercury depends on the composition of this. Biofilm formed by several species obtains good results with retention concentrations of 10mg / l Hg.

C. In terms of productivity, multispecies biofilms are more effective than monospecies biofilms. In comparing the data obtained monospecies and multispecies biofilm, those that consist of various species have better stability when subjected to changes in mercury concentrations. Also achieve good levels of retention close to 100%.
The diversity of the bacterial community decreases when the pressure increases mercury. When the pressure becomes to decrease, diversity recovers. The reappearance of the strains showed that they did not die simply states that were below detectable limits.

REFERENCES

Canstein, H. Von, Li, Y., Timmis, K.N., Wagner-Döbler, I. (1999). *Removal of Mercury form Chloralkali Electrolysis wastewater by Mercury-Resistant Pseudomonas putida Strain.* Applied and Environmental Microbiology, 62(12): 5279-5284.
Canstein, H. Von, Kelly, S., Li, Y., Wagner-Döbler, I. (2002). *Species Diversity Improves the Efficiency of Mercury-Reducing Biofilms under Changing Environmental Conditions.* Applied and Environmental Microbiology, 68(6):2829.
Nascimiento A.M.A, Chartone-Souza, E. (2003). *Operon mer: Bacterial resistance to mercury and potencial for bioremediation of contaminated environments.* Genetic Molecular Reserch, 2(1):92-101.
Wagner-Döbler, I. (2003). *Pilot plant for bioremediation of mercury-containig industrial wastewater*. Applied Microbiology and Biotechnology, 62:124-133.
Wagner-Döbler, I. *Bioremediation of Mercury: Current Research and Industrial Applications*. Norfolk, UK; Caister Academic Press, 2013.