

## Bio-mobilization of Heavy Metals from Contaminated Sediments by Acidophilic Microbial Consortia

Francesca Beolchini\*, Viviana Fonti, Antonio Dell'Anno, Francesco Vegliò<sup>a</sup>

Dept. of Marine Sciences (DiSMar), Polytechnic University of Marche, Ancona, Italy.

<sup>a</sup>Dept. of Chemistry, Chemical Engineering and Materials, University of L'Aquila, Italy

\*Via Breccie Bianche, 60131 Ancona, f.beolchini@univpm.it

This work deals with bioremediation of dredged sediments contaminated by heavy metals. Consortia of acidophilic bacteria were added to sediments collected from the Ancona harbor (Adriatic Sea) in order to test their ability to remove heavy metals and thus to obtain valuable material suitable for different purposes (e.g. building industries or beaches nourishment). The efficiency of different bacterial consortia was compared. The simultaneous addition of autotrophic Fe/S oxidizing bacteria and heterotrophic Fe reducing bacteria to the sediment provided the highest heavy metal removal (>90% for Cu, Cd, Hg and Zn). These findings lead to hypothesize that the two metabolically different microorganisms, cooperating through a mutual substrate supply, can enhance their performance in the removal of heavy metals from the sediment. Mobilization yields in treatments based either on Fe/S oxidizing strains or on Fe reducing bacteria were similar: heavy metal extraction yields typically ranged from 40% to 50%. The additional advantage of the new bioaugmentation approach proposed here is that it is independent from the availability of sulfur. These results open new perspectives for the bioremediation technology for the removal of heavy metals from highly contaminated sediments.

### 1. Introduction

Marine sediments act as a sink for organic and inorganic pollutants coming from the water column. Sediments from high human impact areas, like harbors, can therefore represent a source of contaminants and a threat for environment and human health. Periodic dredging activities are necessary to maintain the navigation depth and the large volumes of dredged polluted materials have to be adequately managed and disposed of. Heavy metal contamination is particularly dangerous because of the persistency and toxicity, so that pollution of sediments by heavy metals is still an unsolved environmental problem all over the world. In the last decade, a particular attention has been paid to identify the most effective treatments for the decontamination of the polluted sediment deposits and the re-use of the treated sediments, either in building industries or in beaches nourishment (Krause and McDonnell, 2000).

A promising alternative to the common technologies is based on bioremediation strategies, in which processes are biologically mediated. Heavy metals cannot be degraded, unlike organic pollutants, but only removed or transformed in their less toxic forms, by changing their oxide-reduction state, and in consequence their chemical

properties (van Hullebusch et al., 2005; Tabak et al., 2005). In the case of heavy metals and other inorganic pollutants, bioremediation strategies can only be aimed at increasing their solubility (bio-mobilization) in water or increasing their stability (bioimmobilization) in the solid matrix.

Several bioremediation studies have typically focused their attention on the ability of Fe/S oxidizing bacteria to bio-mobilize metals from polluted sediments: bioleaching, in fact, is characterized by a consolidated application in bio-hydrometallurgy and is considered highly promising for the mobilization of metal containing or metal-contaminated solids, e.g. ores, sludge, soils and sediments (Blais et al., 1992; Vegliò et al., 2000; Beolchini et al., 2009).

In the present work, different microbial consortia were studied with the aim to apply bioleaching on marine dredged polluted sediments. Involved microorganisms were Fe/S oxidizing bacteria, chemolithotrophic microorganisms able to oxidize ferrous ion, elemental sulfur and reduced sulfur compounds, and Fe reducing bacteria, acidophilic chemo-heterotrophic microorganisms.

## 2. Materials and methods

### 2.1. Sediment

Sediment samples were collected from the Ancona harbor (Adriatic Sea). Total organic matter content (ca 0.5% w/w) was determined as the difference between the dry weight (60°C, 24 h) of the sediment and the weight of the residue after combustion for 2 h at 450°C. Before combustion, sediment samples were treated with 10% HCl to remove carbonates.

An X-ray diffraction analysis (Siemens D-500 diffractometer) revealed that ca 90% of the sediment was composed of silicates and carbonates, ca 5% by iron and manganese oxides, and less than 5% by sulfides.

### 2.2. Microorganisms

A consortium of chemo-litho-autotrophic Fe/S oxidizing bacteria (i.e., *Acidithiobacillus thiooxidans*, *At. ferrooxidans* and *Leptospirillum ferrooxidans*) were isolated from acid mine drainage by Professor Groudev group (Department of Engineering and Geoecology, University of Mining and Geology “Saint Ivan Rilski”, Sofia, Bulgaria).

This consortia was grown in 9K liquid medium (Silverman and Lundgren, 1959). The acidophilic heterotrophic *Acidiphilium cryptum* 2390 strain was provided by German National Resource Centre for Biological Material (DSMZ) and grown in his optimal medium M-269 ([www.dmsz.de](http://www.dmsz.de)).

### 2.3. Bioleaching experiments

Tests were conducted on dry sediment (2% w/v) at room temperature, in 100 mL Erlenmeyer flasks, filled with 50 mL culture medium and with 150 rpm rotary shaking. The inoculum (5 mL) was obtained from cultures in exponential growth. Control tests were also performed, by suspending sediment in artificial sterilized sea water. All tests were performed in triplicate.

Different acidophilic microbial consortia were tested: (i) autotrophic Fe/S oxidizing bacteria (*At. thiooxidans*, *At. ferrooxidans* and *L. ferrooxidans*), (ii) heterotrophic Fe

reducing bacteria (*A. cryptum*), and (iii) a mixed culture containing both autotrophic and heterotrophic strains. In the first set of treatments, sediment was suspended into a modified 9K medium, without iron source and in the presence of sulfur at different concentration: 0.0, 1.25 and 5 g/L. The medium for the second set of treatments was the optimal growth medium for *A. cryptum*, modified for the concentration of glucose: two alternative levels were tested (i.e. 1% and 10% of the optimal glucose concentration, respectively). Finally, in the third set of treatments experiments were conducted in the 9K medium, without iron source but with glucose 0.01 g/L. For this group of experiments autotrophic and the heterotrophic strains were added with a volumetric ratio of autotrophic:heterotrophic at three levels: 10:1, 1:1 and 1:10. All of the experiments were carried out at pH 2.

#### **2.4. Analytical methods**

Total heavy metal content was determined by an acid digestion, at 150°C for 90 min, with fluoridric acid and “aqua regia” (i.e. HCl:HNO<sub>3</sub> = 3:1); the obtained extract was suspended in 10% boric acid and analyzed by atomic absorption spectrophotometry (Varian SpectrAA 400 PLUS, atomizator GTA96, graphite furnace), for cadmium, and by inductively coupled plasma-atomic emission spectrometry (Jobin Yvon JY 24) for the other metals.

Metals distribution in different geochemical fractions were determined by means of sequential selective extraction (Quevauviller et al., 1997).

The total bacterial number was determined by epifluorescence microscopy after staining with Acridine orange (Danovaro et al., 2002).

### **3. Results and Discussion**

#### **3.1. Heavy metal content**

Metal and metalloid content in the sediment was as follows: 410 ± 10 ppm for Cu, 500 ± 20 ppm for Zn, 1.2 ± 0.1 ppm for Cd, 5.4 ± 0.3 ppm for Hg, 105 ± 6 ppm for Ni, 21 ± 2 lg ppm for As, 76 ± 4 ppm for Pb and 141 ± 8 ppm for Cr. With the exception of lead, arsenic and chromium, all the considered heavy metals were upper than Italian safety limit. In particular, Cu was 6 times higher than the highest accepted levels for the use and displacement of the dredged sediments.

Heavy metal and metalloid partition in sediment can be ordered in terms of decreasing mobility as follows: Cu, Zn, Cd, Hg, Pb, Ni, As and Cr (data not shown).

#### **3.2. Bioremediation experiments**

Growth curves and metal extraction yields of the different bioaugmentation experiments are illustrated in figure 2, 3 and 4. Biological treatments based only on Fe/S-oxidizing strains showed a close correlation between the of elemental sulfur concentration and the bacterial abundance and the heavy metal solubilization: for this set of treatments, the highest extraction yields were observed in the presence of sulfur 5 g/L (Fig.1).

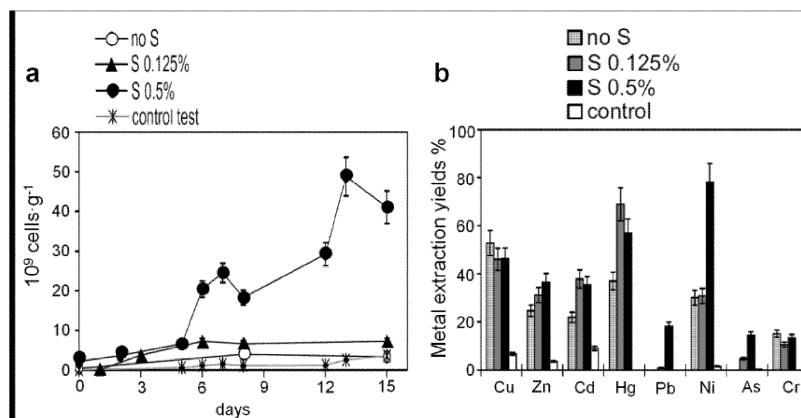


Figure 1: Cell growth (a) and metal extraction yields at the end of the treatment (b) with chemoautotrophic Fe/S oxidizing bacteria, in the presence of different concentration of elemental sulfur (S).

As already observed by Chen and Lin (2001), the addition of elemental sulfur enhanced Fe/S oxidizing bacteria metabolism and could promote the acidification of the medium, increasing heavy metal solubilization. As expected, no changes in heavy-metal mobilization were observed in controls.

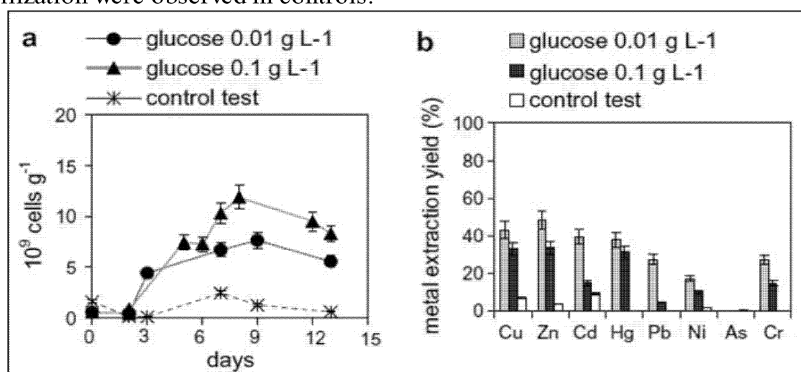


Figure 2: Cell growth (a) and metal extraction yields at the end of the treatment (b), with heterotrophic Fe reducing bacteria, in the presence of different concentration of glucose.

Experiments conducted only with *A. cryptum* strain displayed an efficiency of heavy-metal mobilization always <50% (Fig.2), and probably associated with the chemical action of the acid medium. The obtained yields were comparable to those observed for treatments based on autotrophic Fe/S-oxidizing bacteria in the absence of elemental sulfur (Fig.1). It can be noticed that the highest heavy-metal extraction yields were observed at the lowest glucose concentration and metal solubilization did not increase with increasing glucose concentration (Fig.2).

Flasks inoculated with autotrophic Fe/S oxidizing bacteria and heterotrophic acidophilic strain were characterized by significantly higher than those reported for the other treatments (Fig.3); the efficiency of metal removal increased significantly, reaching extraction yields >90% for Cu, Cd, Hg and Zn. With the except of Zn, no significant differences in heavy metal extraction yields were observed among experimental systems containing autotrophic and heterotrophic bacteria inoculated in different proportion.

These results suggest that the presence of a mixed consortium of heterotrophic and autotrophic bacterial strains may determine a higher heavy-metal extraction efficiency due to a coupled and synergistic metabolism. In fact in the absence of elemental sulfur the presence of *A. cryptum* may enhance the metal bioleaching activity of Fe/S-oxidizing strain (Fournier et al., 1998). At the same time the growth of autotrophic bacteria and the release of labile organic material from their metabolism seems to be enough for the heterotrophic needs of the Fe-reducing bacteria. Overall data reported in the present work may open new perspectives for the application of bioleaching technologies to remediation purposes based on mutual interactions among bacterial strains displaying different metabolic pathways and requirements.

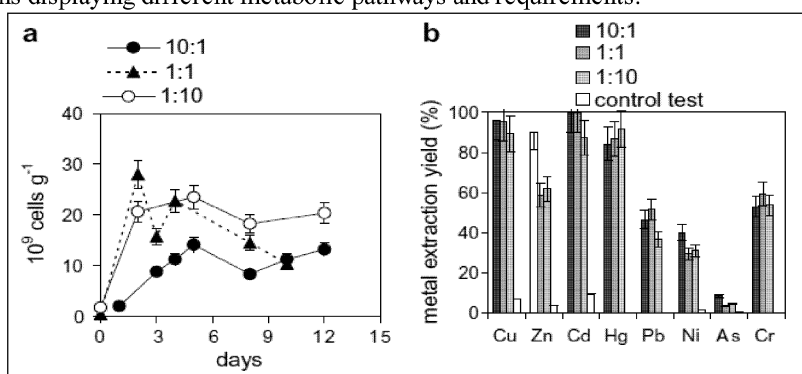


Figure 3: Cell growth (a) and metal extraction yields (b) in the microcosms inoculated with a mixed consortium of autotrophic and heterotrophic bacteria in the ratio 10:1 (circles); 1:1 (stars) and 1:10 (triangles), respectively.

Further investigations are needed to fully understand the potential application of this technology, to evaluate the efficiency of this bioremediation approach to different kinds of contaminated sediments and the extent by which these treatments can be further optimized.

## References

- Beolchini F., Fonti V., De Michelis I., Ferella F., Centofanti M. and Veglio F., 2009, Biotechnologies for the valorisation of spent refinery catalysts and recovery of Ni, V and Mo, Chemical Engineering Transactions, 17, 415-420.
- Blais J.F., Auclair J.C., and Tyagi R.D., 1992, Cooperation between two Thiobacillus strains for heavy metal removal from municipal sludge, J. Microbiol. 38, 181-187.

- Chen S.Y., and Lin J.G., 2001, Effect of substrate concentration on bioleaching of metal-contaminated sediment, *J. Hazard. Mat.* 82, 77-89.
- Danovaro R., Manini E. and Dell'Anno A., 2002. Higher abundance of bacteria than of viruses in deep Mediterranean sediments. *Applied and Environmental Microbiology*, 68, 1468-1472.
- Fournier D., Lemieux R., and Couillard D., 1998, Essential interactions between *Thiobacillus ferrooxidans* and heterotrophic microorganisms during a wastewater sludge bioleaching process, *Environ. Pollut.* 101, 303-309.
- Garbaciak S.Jr., and Miller J.A., 1995, Dredging, remediation, and Containment of Contaminated Sediments: Field demonstrations of sediment treatment technologies by the USEPA's Assessment and Remediation of Contaminated Sediments (ARCS) program, Eds. Demars K.R., Richardson G.N., Yong R.N and Chaney R.C., American Society for Testing and Materials, Philadelphia.
- Krause P.R., and McDonnell K.A., 2000, *The Beneficial Reuse of Dredged Material for Upland Disposal*, CA, U.S.A., Harding Lawson Associates Novato.
- Quevauviller P., Rauret G., Lopez-Sanchez J.F., Rubio R., Ure A., and Muntau H., 1997, The certification of the extractable contents (mass fractions) of Cd, Cr, Ni, Pb and Zn in sediment following a three-step sequential extraction procedure, Report EUR 17554 EN European Commission, Bruxelles.
- Rulkens W.H., and Bruning H., 2005, Remediation of Contaminated Sediments: Finding Achievable Risk Reduction Solutions. Proceedings of the Third International Conference on Remediation of Contaminated Sediments, edited by R.F. Olfenbuttel and P.J. White. Battelle Press, Columbus, OH.
- Silverman M.P., and Lundgren D.G., 1959, Studies on the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields, *J. Bacteriol.* 77, 642-647.
- Tabak H.H., Lens P., van Hullebusch E.D., and Dejonghe W., 2005, Developments in bioremediation of soils and sediments polluted with metals and radionuclides. 1. Microbial processes and mechanisms affecting bioremediation of metal contamination and influencing metal toxicity and transport, *Rev. Environ. Sci. Biotechnol.* 4, 115-156.
- van Hullebusch E.D, Lens P., and Tabak H.H., 2005, Developments in bioremediation of soils and sediments polluted with metals and radionuclides. 3. Influence of chemical speciation and bioavailability on contaminants immobilization/mobilization bio-processes, *Rev. Environ. Sci. Biotechnol.* 4, 185-212.
- Vegliò F., Beolchini F., Nardini A., and Toro L., 2000, Bioleaching of a pyrrhotite ore by sulfooxidans strain: kinetic analysis, *Chem. Eng. Sci.* 55, 783-95.