Biorecovery of Heavy Metals Using Vermiculite for Sediment and Water Protection

P-BE9

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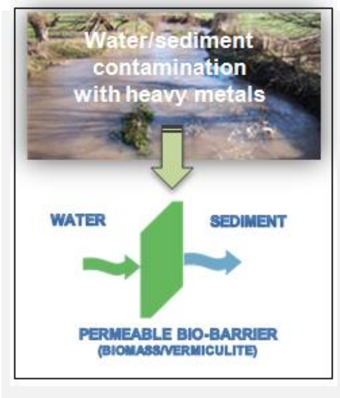


The release of heavy metals in aquatic ecosystems is a matter of great concern due to their toxicity and accumulation in biota. Bottom sediments can act as sink of these pollutants. Several remediation technologies have been applied in order to treat wastewater and contaminated sediments. In this study, a permeable bio-barrier composed by low cost biomaterials was tested for water treatment and sediment protection against metal adsorption. The novelty of this work entails the combination of bacterial biosorption properties with the adsorption capacity of a natural clay. The results of preliminary continuous column experiments reveal the ability of vermiculite to entrap Cu ions, and highlight that metal adsorption can be enhanced by the presence of a *Pseudomonas putida* biofilm attached to the vermiculite surface.

Introduction

The contamination of superficial water bodies by heavy metal released from anthropogenic sources is a matter of the highest concern to public health and environment. Heavy metals are persistent pollutants and their mobility in soils depends on pH and on their speciation, possible becoming bioavailable and therefore harmful to living organisms[1, 2].In the last decades, several treatment techniques have been applied with the purpose of remediating contaminated soil and superficial water.In the field of adsorption and ion exchange processes, natural clays such as vermiculite have been studied for the removal of heavy metals from water [3, 4]. Natural clays have the advantage of being widely available at low cost. Their basic structure is formed by tetrahedral and octahedral sheets, which are mainly composed of silicon and aluminum oxides, respectively [3]. Among biological and biosorptive treatment approaches, numerous biological materials such as bacteria, have been explored for heavy metal removal [5, 6]. In this study, a natural bio-barrier, consisting of a bacterial biofilm supported on vermiculite, is proposed as a low-cost technology for effective water remediation and sediment protection against metal adsorption.

Methods

Toxicity assays

Pseudomonas putida and *Streptococcus equisimilis* bacteria were obtained from the Spanish Type Culture Collection of the University of Valencia. Cadmium ($CdSO_4.8/3H_2O$, Riedel-de Haën), copper ($Cu(NO_3)_2 \cdot 3H_2O$, Panreac) and zinc ($Zn(NO_3)_2 \cdot 6H_2O$, Panreac) were the selectedmetals for the toxicological assays. A culture medium with 10 g/L of tryptone; 5 g/L of yeast extract and 5 g/L of NaCl (Luria Bertani medium) was used for *P. putida* growth, for 24 h at 26°C. *S. equisimilis* bacteriawere grown in a culture medium of heart brain infusion (Oxoid CM1135) for 24 h at 37 ºC. The growth behavior of the microorganisms in the presence of different concentrations of Cd, Cu and Zn (5-100 mg/L) in the culture media was investigated. The growth curves of *P. putida* and *S. equisimilis* were determined by measuring the Optical Density (OD) at 620 λ (PG Instruments T60-UV Visible Spectrophotometer) during 24h. The maximum specific growth rate of the cells was measured by the increase in biomass over time and it was determined from the exponential phase of each assay.

Biofilm preparation and open system assays

Previously to open system studies, the formation of a *P. putida* biofilm supported on vermiculite clay (Sigma-Aldrich) was performed in a batch system. Luria Bertani medium was used for *Pseudomonas putida* growth, for 24 h at 26°C. The cells of 500 mL of bacterial suspension were then harvested by centrifugation at 7000 rpm for 15 min and resuspended in 200 mL of diluted Luria Bertani medium (1 g/L of tryptone; 0.5 g/L of yeast extract and 0.5 g/L of NaCl). 12 g of sterilized vermiculite was added to *P. putida* suspension and kept at 26 ºC for 72 h with moderate stirring in an incubator in order to create a biofilm on the clay surface.

To evaluate the possible prevention ofsediment contamination with Cu, the efficiency of a vermiculite permeable barrier (VPB) and of a *P. putida* biofilm supported vermiculite (VPB-Bio), was evaluated in open system assays. The fresh sediment was collected from Dosolo canal (Bologna, Italy). The continuous experiments were performed in acrylic columns at lab-scale (internal diameter= 3.5 cm, height = 33 cm), with an internal volume of 318 cm^3. Three columns were used in these assays: one column was filled with a bottom layer with 96 $cm^3$of vermiculite (VPB) and a top layer with192 $cm^3$of fresh sediment; the second column was filled likewise but with a bottom layerof biofilm supported on vermiculite (VPB-Bio), and the third column with a single layer of fresh sediment (V=192 cm^3) to perform as a control experiment.12 L of Cu solution with an initial concentration of 10 mg/L, was continuouslypassed through each column upwards with a flow rate of 0.5 L/h for 24 h.Samples were taken periodically, centrifuged and the concentration of Cu in the supernatant was analyzed by ICP-OES (Optima 8000, Perkin Elmer).

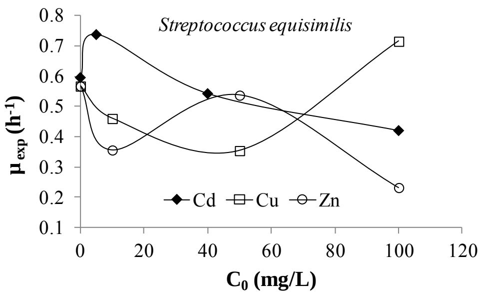
The starting vermiculite VPB and VPB-Bio samples, taken after the open experiments, were analyzed by Scanning Electron Microscope (SEM – Leica Cambridge S360) in order to evaluate the morphology of vermiculite and the biofilm formation.

Results

Toxicity assays

In order to evaluate the influence of the presence of different metals in the growth behavior of *S. equisimilis* and *P. putida*, several toxicological assays were performed.

In Figure 1 are presented the maximum specific growth rates for *S. equisimilis* and *P. putida* in the presence of different initial metals concentrations in the respective culture medium.As it can be observed, the specific growth rate of *S. equisimilis* is higher than that obtained for the control (0 mg/L of metals) in the presence of cadmium with initial concentration of 10 mg/L. In culture medium with zinc, the specific growth rate with an initial concentration of 50 mg/L of this metal is very similar to that obtained in the absence of metal, whereas for Cu there is an enhancement on the growth of the bacteria in the presence of this metal with the highest initial concentration, 100 mg/L. For the rest of concentration values of the different metals, it is observed a decrease in the specific growth rate, between 30 % for cadmium and 60 % for zinc for an initial concentration of 100 mg/L.Analyzing these results, it can be concluded that the presence of metals such as cadmium, copper and zinc, in certain doses, can perform a positive stimulation on the growth of *S. equisimilis*.Regarding the growth behavior of *P. putida*, it can be seen thatgenerally the maximum specific growth rate decreases when the initial concentration of Cu and Zn increase. The maximum decrease in the specific growth rate, 21 % for Cu and 33 % for Zn, was attained in the presence of the highest concentration of metal, 100 mg/L.Comparing the behavior of microbial growth in the presence of metals, it is possible to verify that *P. putida* is less sensitive to the presence of different concentrations of Zn or Cu than *S. equisimilis*.



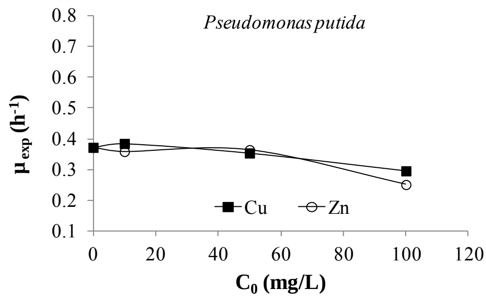


Figure 1. Maximum specific growth rates of*S. equisimilis* and*P. putida* at different metals concentrations in culture medium.

Open system assays

In columns experiments, vermiculite (VPB) and *P. putida* biofilm supported on vermiculite (VPB-Bio) were tested to assesstheir efficacy for metals capture from contaminated water and for sediment protection against metals adsorption.Figure 2 presents the concentration of Cu along time in the outflow of each column (see *Methods* section).After 24 hof experiment, the highest concentration of Cu in the outflow (6.5 mg/L) was found in the control experiment (just with sediment), whereas the lowest concentration (2.8 mg/L) was achieved in the experiment performed with the biofilm supported in vermiculite (VPB-Bio). Comparing the control experiment and the assay performed with VPB, it is clear that the presence of a bottom layer of vermiculite led to a significant decrease of the final Cu concentration in the outflow. On the other hand, the presence of a bio-barrier consisting of a biofilm of *P. putida* supported on vermiculite (VPB-Bio) allowed a reduction of about 37 % in Cu concentration in the column outflow in comparison with the experiment with a barrier of vermiculite (VPB).From these preliminary results, it is possible to state that cupper adsorption in the barrier is increased by the presence of the bacterium biofilm.

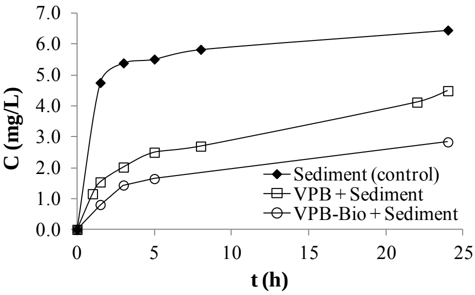


Figure 2. Cu concentration in the column outflow along time for each open system assay. Initial concentration of Cu in the influx: 10 mg/L.

With the purpose of evaluating the morphology of the starting vermiculite and to assess the extension of the biofilm in VPB-Bio sample at the end of the experiment, SEM images were taken and are shown in Figure 3.

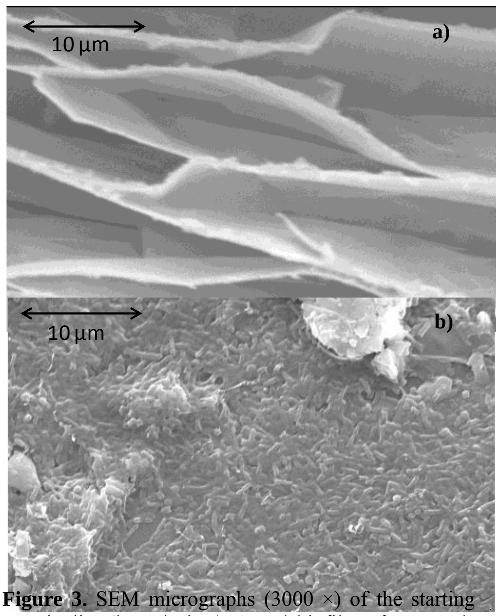


Figure 3. SEM micrographs (3000 ×) of the starting vermiculite (lateral-view)**(a)** and biofilm of *P. putida* supported on vermiculite (top-view) **(b)**.

The lateral-view SEM image of Figure 3**a)**shows the general overview of the surface morphology of the starting vermiculite. Vermiculite particles exhibit a very well defined structure such as layers stacked one over another, which is characteristic of this type of phyllosilicates. In Figure 3**b)**(top-view),it can be observed a high coverage of the vermiculite surface with a biofilm of *P. putida.* A considerable production of exopolysaccharide, that provides a good adhesion to the support, can also be seen. SEM analysis shows that the cells of *P. putida* were rod-shaped with anaverage length of approximately 0.5–2.0 µm.

Conclusions

The toxicological assays performed revealed that both bacteria are able to grow in the presence of metals such as Cd, Cu and Zn, for *S. equisimilis*, and Cu and Zn, for *P. putida* in a concentration range of 5-100 mg/L. The comparison between the growth behavior of each bacterium in the presence of metals reveals that the specific growth rate of *P. putida* is less influenced by the presence of different concentrations of Zn or Cu than that of *S. equisimilis*.

The preliminary results of the open system experiments emphasizethe good performance of vermiculite for metals entrapment and clearly confirm that the presence of a *P. putida* biofilm enhances the capture of metals from water.

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References

[1] M.A. Hashim, S. Mukhopadhyay, J.N. Sahu, B. Sengupta, *Journal of Environmental Management*, 92 (2011) 2355.

[2] D. Di Giuseppe, L. VittoriAntisari, C. Ferronato, G. Bianchini, *Chemie der Erde – Geochemistry*, in press, http://dx.doi.org/10.1016/j.chemer.2014.02.004.

[3] E. Padilla-Ortega, R. Leyva-Ramos, J. Mendoza-Barron, *Applied Clay Science*, 88–89 (2014) 10.

[4] M. Malandrino, O. Abollino, S. Buoso, A. Giacomino, C. La Gioia, E. Mentasti, *Chemosphere*, 82 (2011) 169.

[5] B. Silva, H. Figueiredo, O.S.G.P. Soares, M.F.R. Pereira, J.L. Figueiredo, A.E. Lewandowska, M.A. Bañares, I.C. Neves, T. Tavares, *Applied Catalysis B: Environmental,* 117–118 (2012) 406.

[6] C. Quintelas, Z. Rocha, B. Silva, B. Fonseca, H. Figueiredo, T. Tavares, *Chemical Engineering Journal,* 152 (2009) 110.