Journal of Geochemical Exploration 156 (2015) 34–43

Contents lists available at ScienceDirect

Journal of Geochemical Exploration

journal homepage: www.elsevier.com/locate/jgeoexp

A zeoponic system modified with *Penicillium simplicissimum* for the removal of trace elements from aqueous solutions and gold mine leachates

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ARTICLE INFO

Article history:
Received 6 August 2014
Accepted 23 April 2015
Available online 4 May 2015

Keywords:
Biomass
Biosorbent
Mine leachates
Trace elements
Penicillium simplicissimum

ABSTRACT

The aim of this research was to develop a biosorbent system with high metal loading capacity based on an inactive (heat-killed) fungal biomass, *Penicillium simplicissimum*, supported on zeolite for the removal of trace elements from gold mine leachates. The effects of pH, initial metal ions concentration, contact time and temperature on the adsorption of Cu, Co, Cr, Hg, Fe, Ni, Zn, and U were studied in batch mode. The re-usability of the biosorbent was also investigated. The growth of fungi (*P. simplicissimum*) on zeolite yielded 600 mg g^{-1} of biomass (10-fold higher than the free *P. simplicissimum*) at pH 4. The maximum uptake of metal ions was higher and constant (40–50 mg g^{-1}) in the inactive fungal biomass from pH 2 to 7. The uptake of U and Hg increased significantly in the zeolite-*P. simplicissimum* system due to the presence of the N–H, S–H and COO^- functional groups on the cell wall surface of the biomass. The thermodynamic constants ΔG, ΔH and ΔS showed that adsorption was feasible and spontaneous. Consistent adsorption/desorption potential of the biosorbent in repetitive cycles portrayed the re-usability potential of the adsorbent.
Zeolite-*P. simplicissimum* biosorbent removed 97% of the metals from the real gold mine leachates. *Penicillium* sp. immobilisation enhanced the potential of metals removal and makes it an attractive bioremediation agent.

1. Introduction

Waste generated by the mining industry usually contains high concentrations of toxic metals and metalloids that can be mobilised, resulting in pollution of soils, surface and groundwater (Nehdi and Tariq, 2007; Yanga et al., 2009).

The utilization of several biological materials such as microbial cells (specifically bacteria, algae, yeasts and fungi) for the removal and recovery of metals from wastewater has received growing interest due to the high surface to volume ratio, large availability of active adsorption sites, rapid kinetics of adsorption–desorption and little cost (Abbas et al., 2014; Das et al., 2007). However, the large scale implementation of in situ attenuation and remediation schemes remains to be extensively explored. Several studies on bioremediation have been reported (Chen et al., 2005; Kumar et al., 2011; Pazos et al., 2010; Quintelas et al., 2009; Zhang et al., 2011). The structural polymers in the cell walls of microorganisms contain functional groups such as carboxyl (COO^-), phosphoryl ($R–PO_3$) and amino (RN–H) groups that largely influence adsorption (Kulczycki et al., 2002). Compared to other methods, biosorption is a more promising and less expensive way for cleaning up contaminated water (Ahemad, 2012). The passive metal uptake is rapid, reversible and often independent of physical conditions such as pH and ionic strength (Issazadeh et al., 2013). It is relatively nonspecific with respect to the metal species (Gadd, 1990).

The simultaneous use of zeolites and bacteria or fungi is a recent topic of research and only few studies are reported. Quintelas et al. (2009, 2013) reported that the *Escherichia coli* bio-film on modified zeolite is a very promising way to remove metal ions from effluents due to the fact that both components have an affinity for metal cations. Pazos et al. (2010) demonstrated the efficiency of a zeolite–bacteria system while Holan and Volesky (1994) explained that immobilised microbial cells offer many advantages including better reusability, high biomass loading and minimum clogging in continuous flow systems.

Only few studies on metal removal based on *Penicillium simplicissimum* supported on zeolite has been encountered in literature. This study sought to take advantage of the natural occurrence of this fungal biomass in mine tailings sites in Johannesburg, South Africa and the abundance of zeolitic material in the country, making such a biophysical system viable. To this end, this study was aimed at

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http://dx.doi.org/10.1016/j.gexplo.2015.04.012
0375-6742/

investigating the effectiveness of zeolite as an immobilisation matrix for *P. simplicissimum* for the removal of trace metals from aqueous solutions and mine leachates.

2. Material and methods

2.1. Natural zeolite

The natural zeolite used in this study, white fine powder (particle size <2 mm), was purchased from Sigma-Aldrich (South Africa). The zeolite has the surface area of 0.692 $m^2\ g^{-1}$, cation exchange capacity of 61.06 meq/100 g and a pore diameter of 150 Å. The chemical composition mainly consisted of (%): SiO_2 (40.6), Al_2O_3 (32.92), Fe_2O_3 (0.01), FeO (0.08), MnO (0.01), MgO (0.06), CaO (0.03), Na_2O (19.92), K_2O (0.25), TiO_2 (0.02), P_2O5 (0.01), and H_2O (6.1).

2.2. Synthesis of zeolite-P. simplicissimum

2.2.1. Preparation of the basal medium

A strain of *P. simplicissimum* commercially available was used for the synthesis of zeolite-*P. simplicissimum*. The same species can be isolated in the gold tailings dams and used for heavy metals bioremediation. The fungi was grown in a basal medium prepared as follow: $(NH_4)_2SO_4$: 1.15 g, KCl: 0.67 g, $MgSO_4 \cdot 7H_2O$: 0.5 g, Fe-EDTA: 5 mg, $ZnSO_4 \cdot 7H_2O$: 4.4 mg, $CaCl_2 \cdot 2H_2O$: 44.5 mg, K_2HPO_4 (100 mg L^{-1} phosphorous): 0.56 g, yeast extract: 50 mg, and glucose: 10 g, in 1 L of reverse osmosis de-ionised sterilised water.

After dissolving the above contents by heating, the pH of the medium was measured before autoclaving at 121 °C at 1000 Pa for 15 min. For experimental purposes, the pH of the basal medium (prepared as indicated above) was adjusted at pH 4 to 7 using 1 M NaOH or 1 M HCl. Twenty millilitre of the medium was distributed in 50 Schott bottles of 100 mL and zeolite (1 g) was added into the medium. All chemicals used were of analytical grade from Merck.

2.2.2. Preparation of homogenate

The pure culture of *P. simplicissimum* used in this study was obtained from the School of Molecular and Cell biology (Wits University). The strain was maintained on the following solid media: 39 g L^{-1} potato dextrose agar (PDA) and 50 g L^{-1} malt extract agar (MEA) and stored at 4 °C. A homogenate was prepared using 0.05% Tween 80.

Spores were obtained from 250 ml flasks containing 17 g of a doughy medium composed.

2.2.3. Spore inoculum preparation and growth experiments

Two drops of homogenate was inoculated under a sterilised laminar flux into the Schott bottles containing 20 mL of autoclaved medium broth and 1 g of zeolite. The fungus was cultivated on a rotary shaker (120 rpm) at 25 °C for 1 to 20 days. The harvested biomass was separated from the broth by filtration and washed with de-ionised water and then oven dried at 60 °C for 24 h. The mass of the harvest was recorded, this permit to obtain the growth curve of the fungus with days. The experiment was done in triplicate and a mean was calculated. The fungal strain was identified by using standard identification techniques such as colony morphology and microscopic examination under light microscope.

2.2.4. Preparation of the fungal biomass

For experimental purpose, 2 drops of *P. simplicissimum* homogenate was inoculated in 250 mL conical flask containing 100 mL of growth medium at pH 4 and 20 g of zeolite. A sample was prepared without zeolite to serve as control. Incubation was performed at a rotary shaker in a controlled room temperature (25 °C) at 120 rpm. The biomass was harvested after 2, 5, 10 and 20 days by filtration and then washed thoroughly with de-ionised water to remove the excess of the nutrient broth. The harvested biomass was then oven dried at 60 °C for 24 h, and powdered in a mortar, then stored in a desiccator and used for the subsequent experiments. The biomass harvested after 5 days was used in the adsorption study.

2.3. Analytical procedures

2.3.1. Characterization of zeolite-P. simplicissimum

FTIR spectroscopy analysis: Spectra were obtained on the FTIR, Tensor 27, Bruker, Germany. The FTIR spectra of the samples were collected in the wave number range of 4000–400 cm^{-1}.

Elemental analysis (CHNS): The amount of C, H, and N was determined using LECO CHNS-932 analyser. Samples were placed in a silver capsule and heated in the furnace at 1000 °C, where it was completely combusted. This instrument relies upon infrared detection to measure the weight percent of carbon, hydrogen, and sulphur, while nitrogen was measured using the thermal conductivity detection. A certified material was used for calibration. The data processing was performed by the software incorporated in the instrument and the results are given in percentage of carbon, nitrogen and hydrogen.

2.3.2. Preparation of metal standard solutions

Analytical grade reagents were used (Sigma-Aldrich and Merck, South Africa). The synthetic metal ion solutions (Co^{2+}, Cu^{2+}, Fe^{3+}, Cr^{3+}, Hg^{2+}, Ni^{2+}, U^{6+} (as UO_2^{2+}) and Zn^{2+}) were prepared by weighing appropriate amounts of their nitrate salts: $Cu(NO_3)_2 \cdot 3H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Hg(NO_3)_2 \cdot H_2O$, $NiSO_4 \cdot 7H_2O$, $Cr(NO_3)_3 \cdot 9H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, $UO_2(NO_3) \cdot .6H_2O$, and $Fe(NO_3)_3 \cdot 9H_2O$ that were sufficiently dried prior the preparation of standard solutions of 1000 mg L^{-1} (the ionic strength varied between 8.501e-003 and 1.982e-001). Appropriate aliquots were taken from these standards for subsequent dilution to the desired concentration level. The solutions were acidified using 1 M HNO_3 to avoid the precipitation of metals and stored in a refrigerator at 4 °C.

2.3.3. Batch equilibrium and kinetic studies

Batch adsorption was carried out using heat-killed biomass for Co, Cu, Fe, Hg, Cr, Ni, U, and Zn (in single-ion solution). A ratio of 1 g of biomass to 50 mL of metal solution was used.

The influence of pH on the uptake of metals was studied in the range of pH 2 to pH 7. The metal concentration was fixed at 100 mg L^{-1} and the mixture was shaken for 12 h at 150 rpm. The solution's pH was not controlled afterwards.

The effects of initial metal concentration were assessed at pH 3 with concentrations varying from 50 to 500 mg L^{-1}. The experiment was performed in duplicate and the mean was calculated.

The batch contact time experiments were made in a Pyrex glass vessel of 1000 mL. An aqueous solution (500 mL) was poured and agitated by using an automated shaker at 150 rpm because above this the agitation has little effect on adsorption process. A known amount of biomass (25 g) was then added into the vessel and the timing was started. The kinetic of the adsorption was assessed by varying the contact time from 0 to 180 min; the pH and initial metal concentration were fixed at 3 and 100 mg L^{-1}, respectively. At preset time intervals, the aqueous samples (5 mL) were taken and the concentration was analysed.

To determine the effect of temperature on metals adsorption, experiments were performed at 25 °C and 40 °C.

The concentration of metals remaining in solution was analysed using the multi-element Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Spectro, Germany).

The amount of metal adsorbed was calculated using the mass balance equation expression:

$$qe = \frac{(C_o - C_e)V}{M} \quad (1)$$