

Biosorption of metals from gold mine wastewaters by *Penicillium simplicissimum* immobilized on zeolite: Kinetic, equilibrium and thermodynamic studies

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Abstract A biosorbent based on zeolite and *Penicillium simplicissimum* (heat-killed fungal biomass) was developed for the clean up of metals from gold mine wastewaters. With an initial concentration of 500 mg L⁻¹ at pH 3 - 4 for a single component system, 99% adsorption was observed for: Cu²⁺, Co²⁺, Cr³⁺, Fe²⁺, Ni²⁺, Zn²⁺, Hg²⁺. Immobilisation of fungi on zeolite yielded higher biomass, showing the potential of this study towards remediation of polluted mine sites. Desorption results showed that the adsorbent could be reusable.

Key Words biosorbent, *Penicillium simplicissimum*, heavy metals, wastewaters, acid mine drainage, chemisorption

Introduction

Wastes generated by the mining industry contain high concentrations of metals and metalloids which can be mobilised, resulting in leaching into groundwater and surface water. Most of these heavy metals are highly toxic and are not biodegradable: As such, they must be removed from the polluted streams in order to meet increasingly stringent environmental quality standards. Conventional chemical and some physical methods are often expensive, generating toxic and non eco-friendly products. At times, these are ineffective when the metal concentration is very high. Such methods include: ion exchange, adsorption on charcoal and activated carbon, chemical precipitation, reverse osmosis and solvent extraction (Ahluwalia 2007).

Biosorption has been used with success in the last decades because of its advantages which are: ecofriendly nature, reusability of biomaterial, short operation time, selectivity for specific metals of interest, no production of secondary compounds which might be toxic, low operating cost (Tobin *et al.* 1984). Several adsorbents have been developed and used world-wide for the removal of pollutants from wastewaters but the challenge of achieving high adsorption efficiencies still remains as well as the development of cheaper materials for the adsorption of heavy metals from polluted sites.

In this study, a biosorbent based on zeolite and *Penicillium simplicissimum* was developed to clean up heavy metals from gold mine wastewaters. Several chemical processes may be involved in biosorption, including adsorption, ion exchange, co-ordination and covalent bonding. The main chemical groups in biomass which are able to uptake metals are hydroxyl, thiol, carboxyl, phos-

phate and amino groups (Quintillas *et al.* 2009). Zeolite was selected as a support material in this study due to its capacity for immobilising micro-organisms and its large surface area.

Materials and methods

Fungal biomass preparation

Penicillium simplicissimum (originally isolated from gold mine tailings) was maintained on the following solid media: 39 g L⁻¹ Potato Dextrose Agar (PDA) and 50 g L⁻¹ Malt Extract Agar (MEA). For experimental purposes, cultures were grown at 25°C in liquid medium at pH 4, comprising the following: (NH₄)₂SO₄, KCl, MgSO₄·7H₂O, EDTA-Fe, ZnSO₄·7H₂O, MnSO₄·H₂O, CaCl₂·2H₂O, K₂HPO₄, yeast, glucose in 1 L of sterilised deionised water. Zeolite (1 g) was added into the medium, and the mixture was inoculated after autoclaving. All chemicals used were from Merck. The Zeolite used in this work, purchased from Sigma Aldrich (South Africa), was in a powder form with 150 Å of pore diameter. The immobilized biomass was separated from the broth by filtration and washed with deionised water.

Biosorption studies

Batch biosorption assays were carried out in heat-killed biomass for Co, Cu, Fe, Hg, Cr, Ni, U and Zn metals (single and multi components). The effects of initial metal concentrations were assessed at pH 3 in the range 50 - 500 mg L⁻¹ while the contact time was in the range 0 - 180 min. The process was monitored at temperatures between 25 and 60°C. A ratio of 1 g biomass: 50 mL metal solution was used. The concentration of metal ions remaining in solution was analyzed using the multi-element Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Spectro, Ger-

many). Langmuir and Freundlich isotherms) were used to fit the experimental data (Guangyu *et al.* 2003). These isotherm equations are given in (tab. 1).

It is well known that the Langmuir model is usually used with an ideal assumption of a monolayer adsorption surface (Langmuir 1918). The Freundlich model is appropriate for the description of multilayer adsorption with interaction between adsorbed molecules (Freundlich 1906). If the value of *n* is between 1 and 10, this refers to a beneficial isotherm.

Kinetics of metal ion sorption governs the rate, which determines the residence time and it is one of the important characteristics defining the efficiency of an adsorbent (Krishnan *et al.* 2003). The kinetic models used included: the pseudo first-order model, the pseudo second-order model and the intraparticle diffusion model and are given in (tab.2), (Lagergren 1898; Ho *et al.* 1999).

Data analysis

In order to compare the validity of each model, the normalized standard deviation ($\Delta q(\%)$) was calculated using the following equation:

$$\Delta q(\%) = 100 \sqrt{\frac{\sum_{i=1}^n \left[\frac{q_{exp} - q_{calc}}{q_{exp}} \right]^2}{n - 1}} \quad (1)$$

where: q_{exp} is the experimental metal ion uptake, q_{calc} the calculated amount of metal ions adsorbed and *n* is the number of experimental data. Curve Expert 1.37 free ware program was used in all calculations with the confidence level set at 95%.

Desorption studies

Batch desorption tests of metals (regeneration of the biosorbent) was conducted using different concentrations of HNO_3 (0.5 – 7 mol L^{-1}). The mixture was agitated in 250 mL bottles at 150 rpm for 12 h using a mechanical automated shaker. The solution was then filtered using a Whatman No. 41 filter paper. The concentration of the metal ions in the filtrate was measured using the ICP-OES. The desorption efficiency was calculated using the following equation:

Desorption efficiency:

$$\frac{\text{Amount of metal ions desorbed (mg/L)}}{\text{Amount of metal ions adsorbed (mg/L)}} \times 100\% \quad (2)$$

Results and discussion

Characterization of the biomass

The growth of the fungus showed a 10-fold increase in biomass when immobilized on zeolite (600 mg g^{-1}) at pH 4 (fig. 1).

Maximum biomass was obtained 5 days after inoculation at pH 4. Infrared spectra of the biomass in the range of 500 – 4000 cm^{-1} confirmed the presence of functional groups with lone pairs of electron that are available to bind to the positively divalent metal ions. These include: hydroxyl, carbonyl, carboxyl, amide, amine, imidazole, phosphate groups. It was pointed that more compounds were released after 10 days of inoculation.

The results obtained for the CEC and the elemental analysis of the biomass are given in (tab. 3). The % of C was high in the biomass; these results

Table 1 Isotherm models used in this work and their parameters.

Isotherms	Equations	Parameters
Langmuir	$C_e/q_e = 1/q_m \cdot b + C_e/q_m$	q_e : amount adsorbed at time t (mg g^{-1}); b : Langmuir constant related to the Energy of adsorption (L mg^{-1}), C_e : concentration at equilibrium (mg L^{-1}) and q_m : maximum amount adsorbed required to saturate a unit mass of adsorbent (mol Kg^{-1}).
Freundlich	$q_e = K_f C_e^{1/n}$	K_f : the Freundlich constant for a heterogeneous adsorbent (L mg^{-1}); $1/n$: the heterogeneity factor

Table 2 Kinetic models used in this work and their parameters.

Kinetic models	Equations	Parameters
Pseudo first-order	$\frac{dq_t}{dt} = k_1 (q_e - q_t)$	q_e and q_t : amount of metal ions adsorbed (mg g^{-1}) at equilibrium and at any time t respectively; k_1 : rate constant (min^{-1}) of pseudo first-order adsorption
Pseudo second-order	$\frac{dq_t}{dt} = k_2 (q_e - q_t)^2$	k_2 : rate constant of pseudo second-order adsorption (g $mg^{-1} min^{-1}$)
Intraparticle diffusion model	$q_t = k_{id} t^{1/2} + C$	k_{id} : initial rate constant (mg $g^{-1} min^{-1/2}$) of intraparticle diffusion; t : time of sorption duration (min), C (unitless) gives information about the boundary layer thickness.

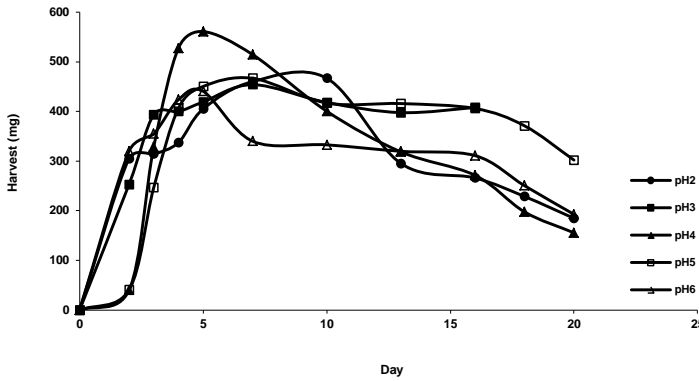


Figure 1 Growth curve of *Penicillium simplicissimum* on natural zeolite.

	CEC (meq 100g ⁻¹)	C (%)	H (%)	N (%)	S (%)
Zeolite-Fungi	82.50	0.388	2.295	0.254	0.102
Natural zeolite	61.06	0.219	2.209	0.193	0.090

Table 3 Elemental analysis of the biomaterial.

confirm the presence of organic compounds released by the fungi as revealed by with the IR spectra (not shown in this paper).

The effect of pH

The effect of pH on the adsorption of heavy metals on the natural zeolite and the zeolite-fungi was studied in the pH range 2 to 7. The results are shown in figure 2 and 3.

The results showed an increase in adsorption capacity with the zeolite - fungi (40 – 50 mg/g) compare to the natural zeolite (2 – 10 mg/g). The adsorption rate of metal ions studied on zeolite-fungi was constant for the all range of pH. This high adsorption capacity for the metals indicates that adsorption of these metals is independent of the change in pH. This adsorbent is effective in the

removal of metal at low pH values, which makes it an effective adsorbent in the remediation of acid mine drainage.

Biosorption isotherms

Adsorption constants of Langmuir and Freundlich equation and correlation coefficient (R²) were calculated and are represented in (tab. 4).

The high adsorption capacity was obtained with Cr (III) (525 mmol kg⁻¹). The decreasing sequence of uptake values by the biosorbent is Cr(III) > Cu(II) > Co(II) > Fe(II) > Zn(II) > Ni(II) > U(VI) > Hg(II). The correlation coefficients show that the biosorption process is better defined by Freundlich demonstrating the heterogeneous character of the adsorption surface of the biomass. Only Ni and Co have high correlation coefficients

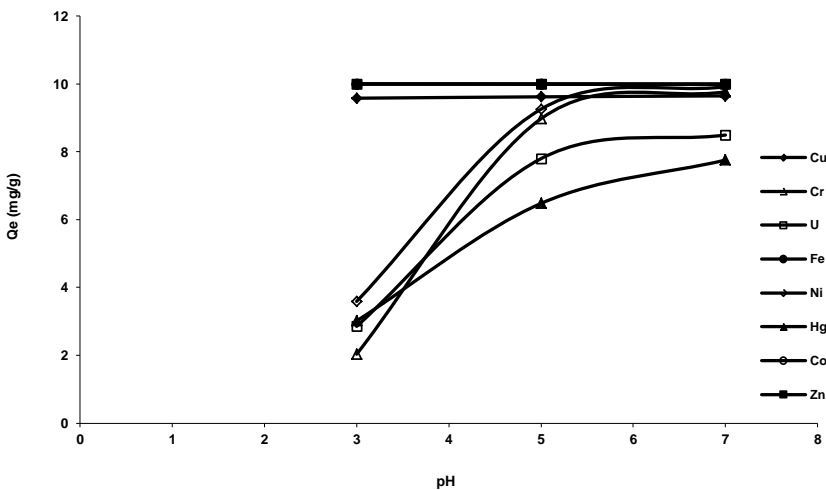


Figure 2 Plots of the adsorption capacity versus pH on natural zeolite of heavy metals in single component solutions, C_i= 100 mg L⁻¹.

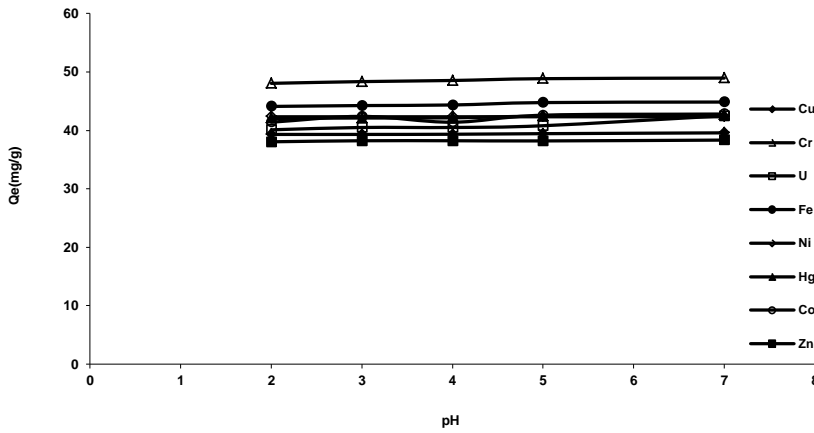


Figure 3 Plots of the adsorption capacity versus pH on zeolite-fungi of heavy metals in multi-component solutions, $C_i = 100 \text{ mg L}^{-1}$.

Table 4 Langmuir and Freundlich constants and correlation coefficients.

Metal ions	Langmuir parameters				Freundlich parameters			
	q_{\max} (mol/kg)	b 10^2 (L/mg)	R^2	$\Delta q(\%)$	K_f (L/g)	n	R^2	$\Delta q(\%)$
¹ Cu ²⁺	0.483	86.17	0.868	93.2	1.019	7.671	0.969	12.4
² Co ²⁺	0.427	58.75	0.999	15.1	1.094	7.229	0.910	42.3
³ Cr ³⁺	0.525	12.26	0.606	78.2	1.055	7.591	0.969	8.4
⁴ Fe ²⁺	0.421	69.76	0.401	65.7	1.068	7.971	0.914	16.7
⁵ Hg ²⁺	0.128	43.47	0.517	80.9	1.050	4.037	0.974	8.9
⁶ Ni ²⁺	0.268	37.85	0.997	17.1	1.028	4.298	0.949	15.2
⁷ UO ₂ ²⁺	0.169	22.03	0.731	92.5	1.023	4.250	0.908	18.9
⁸ Zn ²⁺	0.298	41.42	0.700	93.2	1.027	7.392	0.931	28.5

Detection limit (mg l^{-1}): ¹ 7.41×10^{-4} ; ² 1.05×10^{-4} ; ³ 2×10^{-4} ; ⁴ 1.18×10^{-4} ; ⁵ 2.65×10^{-4} ; ⁶ 3.88×10^{-4} ; ⁷ 1.52×10^{-2} ; ⁸ 4.85×10^{-4}

for the Langmuir model, implying a monolayer coverage process. The good fit of Freundlich isotherm to an adsorption system means there is almost no limit to the amount adsorbed. The magnitude of the Freundlich parameters K_f gives the quantitative information on the relative adsorption affinity towards the adsorbed cation. The value of $1/n$ less than unity is an indication that significant adsorption takes place at low concen-

tration but the increase in the amount adsorbed with concentration becomes less significant at higher concentrations and vice versa (Teng, Hsieh 1998).

Biosorption kinetics

The estimated model and related statistics parameters are reported in tab. 5.

Based on the linear regression ($R^2 > 0.99$) val-

Table 5 Kinetic models at 298 K and pH 3; R^2 - Correlation coefficient.

Metal ions	Pseudo first-order parameters			Pseudo second-order parameters			Intraparticle diffusion parameters		
	q_e	K_1 /[min ⁻¹]	R^2	q_e	K_2 /[g mg ⁻¹ min ⁻¹]	R^2	K_{id} /[mg g min]	C	R^2
Cu ²⁺	0.022	0.075	0.715	0.168	0.062	0.999	0.005	0.028	0.829
Co ²⁺	0.014	0.072	0.618	0.182	0.0574	0.998	0.006	0.031	0.928
Cr ³⁺	0.034	0.065	0.794	0.206	0.0505	1.000	0.007	0.034	0.842
Fe ²⁺	0.012	0.011	0.466	0.193	0.054	1.000	0.006	0.032	0.936
Hg ²⁺	0.072	0.029	0.751	0.0305	0.526	0.992	0.002	0.004	0.865
Ni ²⁺	0.002	0.02	0.507	0.183	0.057	1.000	0.006	0.031	0.928
UO ₂ ²⁺	0.033	0.019	0.846	0.0174	0.425	0.773	0.007	0.002	0.982
Zn ²⁺	0.005	0.014	0.625	0.164	0.063	0.999	0.005	0.028	0.927

ues of the metal ions, it is observed that the sorption of the metals studied followed the pseudo second-order kinetic model except for U. The biosorption of U followed the intraparticle diffusion model rather than the pseudo second-order. The pseudo second-order is based on the assumption that sorption follows a second order mechanism, with chemisorption as the rate limiting step. Adsorption at such low pH regimes shows potential for the remediation of acid mine drainage (AMD) impacted water.

Thermodynamics of biosorption

Activation energy (E_a), standard Gibbs' free energy (ΔG°) and enthalpy change (ΔH°) calculated from the experimental data are given in (tab. 6). The equilibrium regime is defined by the distribution coefficient K_d ($L \text{ mol}^{-1}$) calculated as:

$$K_d = \frac{C_o - C_e}{C_e} \cdot \frac{V}{M} \quad (3)$$

where: C_o and C_e (mol L^{-1}) represent the initial concentration and equilibrium concentration, respectively, and V/M ($L \text{ kg}^{-1}$) is the ratio of the solution volume to the mass of adsorbent. The experimental data obtained at different temperatures were used in calculating the thermodynamic parameters E_a , ΔG° and ΔH° using the following equations:

$$\Delta G^\circ = -RT \cdot \ln K_d \quad (4)$$

$$\ln K_d = (1/R)(\Delta S^\circ + \Delta H^\circ/T) \quad (5)$$

The negative values of the standard free energy change, ΔG° , imply that the biosorption process was spontaneous. These results validate the feasibility of the biosorption. The positive values obtained for ΔH° , except for Fe and Co, demonstrate that the biosorption process was exothermic. This indicates that higher temperature led to a decrease in biosorption. The rate of biosorption also decreases with an increase of temperature.

The values of activation energy (E_a) obtained in tab. 6 show that most of these metals adsorb onto the biosorbent by chemisorption ($40 < E_a <$

800 kJ mol^{-1}) reaction, with the exception of Cu, Co and Ni which adsorb by a physisorption ($5 < E_a < 40 \text{ kJ mol}^{-1}$) type of reaction (Nollet *et al.* 2003).

Most of the metals studied have negative values for activation energy which could be attributed to their preference to bind to low energy active sites.

Desorption

The results for the effect of nitric acid in the removal of metals are presented in fig. 4.

Desorption studies help to elucidate the nature of adsorption and recycling of the spent adsorbent and the metal ions. The desorption results showed an optimum desorption (> 90%) at about $1 \text{ mol L}^{-1} \text{ HNO}_3$ for the metals studied except for U^{6+} with 60% desorption. Uranium metal ion forms strong complex with the phosphate group of the biosorbent. Sodium carbonate solution may be used to remove the uranium; it is well known that carbonate ions have high affinity for the uranyl ion, therefore the energy formation of uranyl carbonate complex is greater than the uranyl phosphate complex. Further experiments have to be done with the sodium carbonate to remove the uranium from the biosorbent.

Conclusions

The biosorbent displayed good adsorption of metals even at low pH values and as such can be used efficiently in areas contaminated by AMD with high metals concentration.

The metal loaded in the biomass can potentially be desorbed in order to regenerate the biosorbent and possibly reclaim valuable metals.

Adsorption kinetics is important in establishing the time zones and effective lifetime of adsorbents and shed information on the need for regeneration. Thermodynamic results are essential in determining the surface-metal reaction mechanisms.

Acknowledgements

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Table 6 Different parameters E_a , ΔG° and ΔH° calculated from the adsorption rates.

Metal ion	ΔE_a (kJ/mol)	ΔH° (kJ/mol)	ΔG° (kJ/mol)			
			25°C	30°C	40°C	60°C
Co^{2+}	17.49	104.4	-18.66	-19.31	-17.09	-19.29
Cr^{3+}	-80.57	-481.0	-6.232	-6.019	-22.78	-16.58
Cu^{2+}	-6.424	-38.32	-1.513	-0.436	-1.833	-2.484
Fe^{2+}	109.9	651.1	-25.96	-21.64	-22.24	-16.89
Hg^{2+}	-75.84	-452.4	0.986	0.795	-5.200	-7.767
Ni^{2+}	-27.91	-166.6	-5.860	-5.768	-6.896	-9.978
UO_2^{2+}	-120.4	-718.9	-5.689	-6.342	-12.75	-20.64
Zn^{2+}	-113.2	-675.7	-7.373	-19.65	-19.47	-21.72

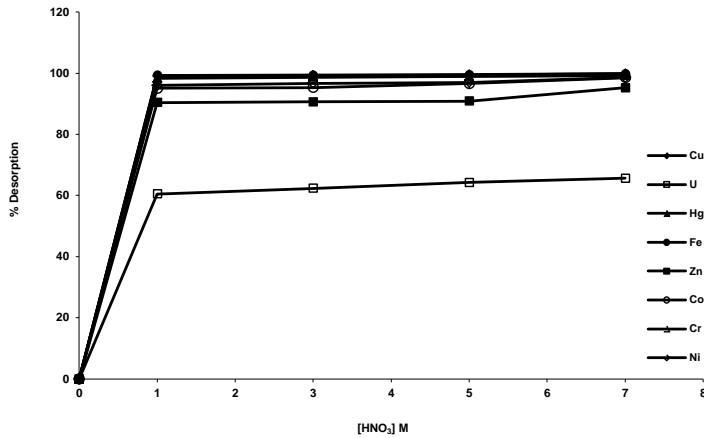


Figure 4 Desorption of metal with HNO₃ solution.

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