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Biosorption of cobalt and copper from hydrometallurgical solutions mediated by *Pseudomona spp*

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Pseudomonas spp was isolated from mine water and tailings samples collected from mining effluents and mine dumps in the Gauteng and Northern provinces of South Africa respectively. Adsorption properties of *Pseudomonas spp* were then tested for the removal of Cu and Co initially from their synthetic sulphate solutions, subsequently from effluents derived from metallurgical operations. The effects of experimental conditions such as pH, temperature, time, volume and metal concentration on the efficiency of the biosorption process were studied. Absorption of 45% Cu (pH 6, 37°C, 24 hours) and 40% Co (pH 6, 37°C, 24 hours) was observed from solutions with low concentrations (0.07 M). A maximum of 73% and 65% of Cu and Co respectively were recovered from the mine water samples derived from effluents.

Introduction

Metal accumulation in the environment as a result of mining and metallurgical activities is arising as a matter of serious concern. The removal and recovery of metals from hydrometallurgical solutions and waste water prior to their release to the environment are important in preserving the ecosystem as well as from an economical perspective. There are several technologies used to recover metals such as Cu, Co, Zn, Hg and many others. These include among others, ion exchange, precipitation, filtration, electrochemical treatment, electrowinning, reverse osmosis and reduction. These technologies, however, tend to be excessively expensive when the metal concentrations are less than 100 mg/l (Bueno *et al.*, 2008). Since microorganisms have developed survival strategies in metal polluted habitats, their different microbial detoxifying mechanisms such as bioaccumulation, biotransformation, biomineralization or biosorption can be applied either *in situ* or *ex situ*. Bacteria also make excellent biosorbents because of their high surface to volume ratios and a high content of potentially active chermosorption sites such as teichoic acid in their cell walls (Beveridge, 1989).

Biosorption of metals is one of the most promising technologies involved in the removal of toxic substances from industrial wastes and has been receiving a great deal of attention in recent years, not only because of its scientific novelty but also because of its potential application in industry (Wase and Forster, 1997; Bailey *et al*., 1999; Kumar *et al*., 2006). It is also a cost-effective technology since it does not require high starting costs.

Metal ions uptake by biosorption is complex and may involve the contribution of diffusion, adsorption, chelation, complexaton, coordination or microprecipitation mechanisms, depending on the specific biomass or substrate (Veglio and Beolchini, 1997). Therefore this biological phenomenon can be affected by many chemical and physical variables such as pH, ionic strength, biomass concentration and presence of different heavy metals in solution. All these factors have to be investigated in order to understand how this phenomenon takes place and to optimize operating conditions.

Pseudomonas spp, on the other hand, is a widespread bacteria and was observed in the early history of microbiology. The name *Pseudomonas* meaning 'false unit' was derived from Greek *pseudo* (*ψευδο* 'false') and monas (*μονάς* / *μονάδα* 'a single unit'). The term 'monad' was used in the early history of microbiology to denote single-celled organisms. This genus was first defined in 1894 as a genus of Gram negative, rod-shaped and polar-flagellated bacteria (Wikipedia, 2008-09-06) It has been used in bioremediation processes because of its ability to bind to metal ions. The extent of binding is dependent on the nature of binding, metal chemistry and metal affinity of binding sites (Brady and Tobin 1994).Copper and cobalt were chosen for biosorption studies to their wide use in industry and potential pollution impact. Mining companies have become increasingly aware of the potential of microbiological approaches for recovering base and precious metals from low-grade ores, and for bioremediating acid mine drainage and mine tailings. There are two strategies: biomining and bosorption where microorganisms are used to recover metals in solution (e.g. acid mine drainage) by precipitation of the metal, or complexing or absorption with cellular molecules. Biopyrometallurgy then enables recovery of these metals with the microbial biomass contributing as fuel.

Materials and methods

Isolation of microorganisms

Samples of water and soil were collected from mining effluents and mine dumps in the Gauteng and Northern provinces of South Africa respectively. These samples were kept in cool conditions for about 12 hours before isolation could be done.

Media preparation

Pseudomonas agar base powder (24.20g) was weighed and suspended in 500 ml of distilled water. 5 ml of glycerol was added and the mixture was boiled for about 2 minutes in a microwave to dissolve the mixture completely. The media was sterilized by autoclaving using the HUXLEY HL 341 speedy autoclave at 121°C for 15 minutes. The medium was allowed to cool to about 50°C and a vial of *Pseudomonas* supplement (SR 103) was aseptically added. The mixture was mixed well and poured into Petri dishes and then allowed to cool for 3 hours.

Dilutions (sample preparation)

Each sample was mixed thoroughly using a vortex mixer and diluted with a phosphate buffer to 10^{-1}(9 ml of phosphate buffer to 1 ml sample) and 10^{-2} (9 ml of phosphate buffer to 1 ml of

10^{-1} sample). These were then spread on the media and incubated in the Scientific Series incubator. After 24 hours, some colonies were observed in the media and the number of colony forming units in each sample was calculated using the formula below.

$$\text{Number of CFU/ ml} = \frac{\text{Colonies counted}}{\text{Volume plated (ml) x total dilution used}}$$

After notable growth was observed, the microorganisms were transferred into nutrient broth and incubated for 24 hours then concentrated by centrifugation and the obtained solid material was washed with deionized water. From this a glycerol stock culture was made for further experiments.

Test solutions

The metal solutions were divided into three categories i.e. single metal system (Co or Cu), binary metal (Co and Cu mixed system and multi element system (Co and/ Cu plus impurities in varying ratios). The concentrations of Cu (II) and Co (II) were 0.07 M, 0.33 M and 0.66 M and their ratios were varied depending on the category in testing.

Batch biosorption experiments

The factors that affect the adsorption rate were examined in a batch system. All experiments were carried out with microorganism suspention in Erlenmeyer flasks in an incubator at 37±0.5°C with constant shaking to elucidate optimum conditions (contact time, pH, initial metal concentration and bacterial dose). The contents of the flasks were filtered and the filtrates were diluted (10 ml was pipetted into 100 ml volumetric flask and filled to the mark with distilled water.) This was done in order to get lower concentrations, which could be analysed by flame absorption spectrometry to obtain residual metal concentrations. The metal adsorbed by biomass was calculated as:

$$\%\ \text{Metal adsorbed by biomass} = \frac{C_i - C_f}{C_i} \times 100 - MAS$$

where C_i is the initial metal concentration, C_f is the final metal concentration and MAS is the total amount of metal adsorbed by other co-existing possible biomass contaminants or metal precipitated from the solution.

Bacterial interaction with metal species

Interaction of the bacteria with the different categories of metal species was studied using the scanning electron microscope (SEM), model SEM Joel JSM 5600.

Recovery from mine water samples

Mine water samples were collected from mining effluents and mine dumps in the Gauteng province of South Africa. These samples were assayed to determine the presence of copper and cobalt ions. *Pseudomonas spp* isolated from metallurgical operation effluents was then used to recover these metals from their solutions. This was done by spiking 100 ml of the water with 20 ml of *Pseudomonas spp*.

Results and discussion

Isolation of bacteria

The isolation of bacteria using *Pseudomonas* agar base yielded positive results meaning that there blue-green colonies of *Pseudomonas spp* were observed.

Effect of biomass (bacteria) and metal ion concentration

In the first stage of batch biosorption experiments on *Pseudomonas spp*., the combined effects of the amount of biomass (bacteria) and metal ion concentration were examined. Three different amounts were used (50×10^4 CFU/ml , 100×10^4 CFU/ml and 150×10^4 CFU/ml) i.e. 1:1, 1: 3 and 3:1 ratios and 0.07, 0.33 and 0.66 M solutions of both Cu and Co sulphate solutions. These results are illustrated in Figure 1.

Low concentrations of both copper and cobalt sulphate solutions are absorbed more than higher concentrations and the 3:1 (bacteria: metal ion) ratio absorbs more than 1:1 and 1:3 ratios.

The effect of the initial concentration of Co (II) and Cu (II)

The effect of the initial concentration of Co (II) and Cu (II) ions is illustrated in Figure 1, i.e. metallic uptake as a function of the equilibrium concentration. The results demonstrate a decrease in metallic uptake with an increase in the equilibrium concentration. This observation is linked to the metallic concentration gradient with respect to the active and available sites of the *Pseudomonas sp*, which means that metal uptake decreases with an increase in initial ion concentration. For example, the highest removal percentages are observed in the 0.07 M concentration solutions for both Cu^{2+} and Co^{2+} i.e. 45% and 40% respectively. This could be attributed to the microbial tolerance of the bacterial species. For example, the bacteria thrive better in the case of low concentration levels of cobalt than in high concentrations. As time progresses the die off is not as bad as in the highly concentrated solutions. Different biosorbents used on the uptaking of various heavy metals yielded similar observations (Kumar *et al*., 2006; Fiol *et al*., 2006; Abu Al-Rub *et al*., 2006, Pan *et al*., 2006).

The effect of biomass concentration

As illustrated in Table I and Figure 1, an increase in the amount of adsorbent, which in this case are bacteria (biomass), results in the increase in metal absorption efficiency (removal efficiency). This behaviour is due to an increase in binding sites as the bacterial species increase (surface area), (Brady and Tobin 1994).

Table I
The effect of biomass (bacteria) and metal ion concentration on metal extraction

Solution concentration	Amount of bacteria (× 104 CFU/ ml)	Amount of solution (ml)	Ratio	Removal efficiency (%)	
				Cu	Co
0.07 M	50	150	1 : 3	26	21
	100	100	1 : 1	38	31
	150	50	3 : 1	45	40
0.33 M	50	150	1 : 3	22	19
	100	100	1 : 1	33	22
	150	50	3 : 1	38	29
0.66M	50	150	1 : 3	19	18
	100	100	1 : 1	24	21
	150	50	3 : 1	31	25

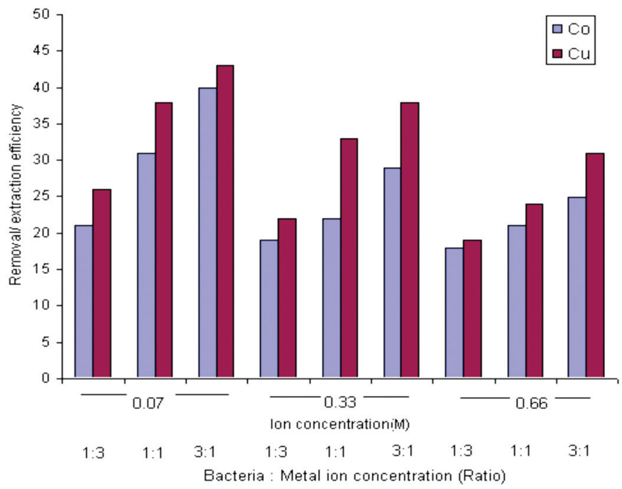

Figure 1. The combined effect of biomass (bacteria) and metal ion concentration on metal extraction

Table II
Showing the effect of co-cations on metal extraction (3:1 biomass:solution ratio)

Solution	Concentration of solution (M)	Ratio CO:CU	% Metal extracted	
			CU	CO
Cu/Co SO_4	0.07:0.07	1:1	43	31
Cu/Co SO_4	0.07:0.33	1:5	41	26
Co/$CuSO_4$	0.33:0.07	5:1	36	38
Cu/Co SO_4	0.07:0.66	1:9	44	21
Co/$CuSO_4$	0.66: 0.07	9:1	32	40

The effect of co-cations on metal extraction

Many researchers have explored the feasibility of this approach (Tsezos, 1983). However, the majority of published work on biosorption is concerned with one metal. Very little information is available for binary and multimetal biosorption systems (Gonzalez Davila, 1990). Since biosorption is foreseen to be implemented in the fields of water treatment and hydrometallurgy where complex multicomponent metal systems are common, more work is required. The copper and cobalt sulphate solutions were further mixed to try and mimic or be as close to reality as possible and to see the effect each cation has on the other. This was done by varying the concentrations of the two cation solutions and the observations were as follows:

Mixing of the two cations does affect the rate at which biosorption occurs. Table II depicts the effect the two cations have on each other. It is notable especially when comparing the single element matrices in Table I with binary element system in Table II that mixing of the two cations results in an enhancement in the amount of copper absorbed by the bacteria. The more dilute combinations showed better absorption than concentrated solutions. These results are illustrated graphically in Figure 2.

The 5:1 and 9:1 copper: cobalt ratio (0.33:0.07 and 0.66: 0.07 respectively) in Table II and Figure 2 illustrates an almost comparable recovery of copper and cobalt, with cobalt being favoured. This could be due to the fact that the cobalt is more dilute than the copper and thus binds easily to the surfaces of the bacteria.

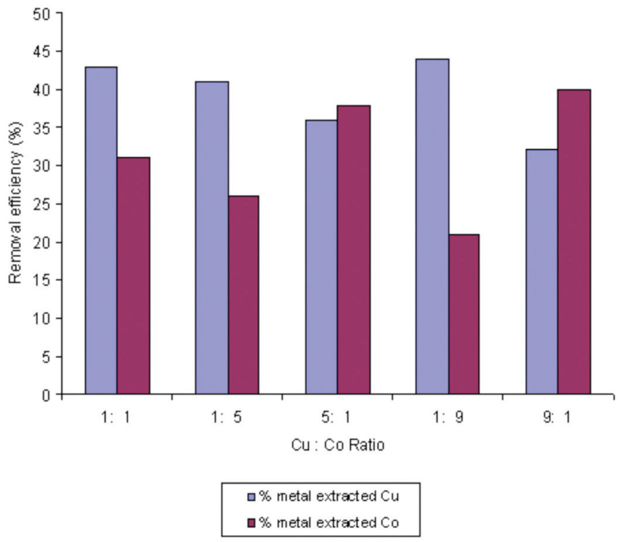

Figure 2. The effect of co-cations on metal extraction

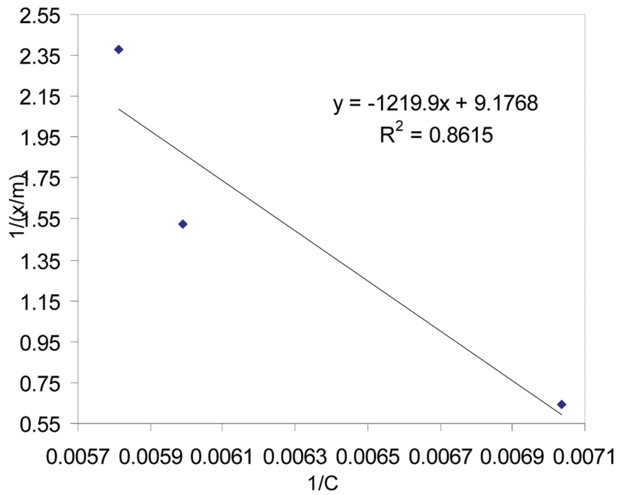

Figure 3. Langmuir isotherm

The data fitted well on both Langmuir and Fruendlich equations but the correlation was much higher on the Fruendlich as expected, since Freundlich approach gives an equation that includes the heterogeneity of the surface of the ion exchanger and the exponential distribution of active sites and their energies.

As seen from Figure 3, when 1/ (x/m) is plotted against 1/c, the Langmuir model fits the data quite well; (1/ab) is the intersection of the line with the y-axis.

As observed in Figure 3 and 4 both models fit the data well. The Freundlich equation gave a plot with higher correlation as compared to the Langmuir equation. The Freundlich gave an R^2 value of 0.9622 whereas the Langmuir had an R^2 value of 0.8615. Therefore the Fruendlich plot is favoured over the Langmuir.

The effect of contamination on metal extraction (for a 3:1 biomass: solution ratio)

The 3: 1 bacteria: solution ratio was further examined for contaminant tolerance abilities. The contaminants used were silicon dioxide and iron chloride.

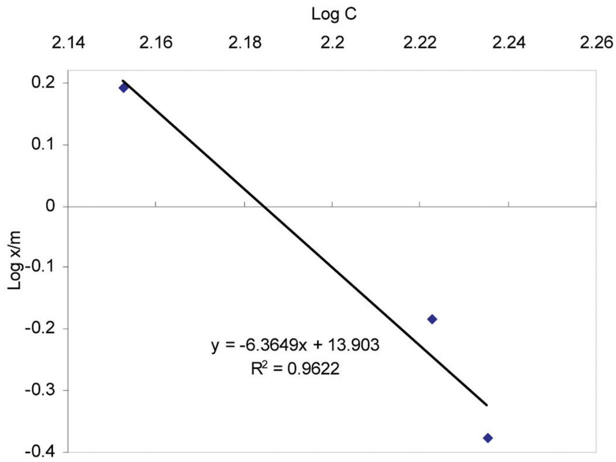

Figure 4. Freundlich Plot

It is observed that the presence of any contaminant decreases the copper and cobalt biosorption by *Pseudomonas spp*. From the results in Table III it is observed that contamination in single element species does not result in major decreases in extraction of metal ions. SiO_2, however, decreases ion extraction more when compared to $FeCl_2$. The same trend is observed in the binary element category. When both contaminants exist further reduction in the extracted metal ions is observed, especially in the case where the ratio of the contaminants to solution is higher (2:1). This is the same case in both single metal and binary metal categories.

It is observed that in all conditions that there is better adsorption of Cu (II) than that of Co (II). This may be accounted for by the nature of the two metal ions. The charges on both Cu (II) and Co(II) ions are the same, but they have different hydrated radii; Cu(II) has a lower hydrated radius than Co(II). Adsorption of an ion by an ion exchanger largely depends on the hydrated radius. Similar observations on an adsorbent called clinoptilolite were observed by Erdem, who concluded that the larger the diameter, the slower its mobility and thus the less likely its exchange will be (Erdem *et al*; 2004). It appears that for ease in adsorption the water molecules surrounding the cation should be fewer.

Interaction of bacteria with metal ions

Interactions of the micro-organisms with the metal species were examined using scanning electron microscopy (SEM), model SEM Joel JSM 5600) and transmission electron microscopy, as illustrated in Figure 5 and 6. The SEM micrograph revealed attachment of metal ions on the cell wall of the Pseudomonad and the same observation is confirmed by the darkish accumulations observed in the cell wall of the bacterium in the TEM image.

The interaction of the Pseudomonas rods with the multi-element matrix is illustrated in Figures 5 and 6. Figure 6 shows the bioaccumulation of the metal ions on the cell walls of the bacteria.

Recovery from mine water

The metal removal concept was then applied on mine water sampled from Nigel town and Table IV shows the recovery of copper and cobalt from the mine water.

Table III
The effect of contamination using silicon dioxide and iron chloride on metal extraction (for a 3:1 biomass: solution ratio)

Solution	Solution: contaminants ratios	% Metal extracted	
		Cu	Co
SiO_2 contaminant only, on single element species			
Cu : Si	1:1 1:2 2:1	29 22 35	N/A
Co : Si	1:1 1:2 2:1	N/A	22 19 28
$FeCl_2$ contaminant only, on single element species			
Cu : Fe	1:1 1:2 2:1	33 26 37	N/A
Co : Fe	1:1 1:2 2:1	N/A	24 20 28
$FeCl_2$ and SiO_2 contaminants on single element species			
Cu : (Si : Fe)	1:1 1:2 2:1	23 19 26	N/A
Co : (Si : Fe)	1:1 1:2 2:1	N/A	19 14 22
SiO_2 contaminant only, on binary element speices			
(Cu/Co) : Si	1:1 1:2 2:1	28 24 36	23 16 28
$FeCl_3$ contaminant only, on binary element species			
(Cu/Co) : Fe	1:1 1:2 2:1	33 28 40	24 19 34
$FeCl_3$ and SiO_2 contaminant on binary element species			
(Cu/Co) : (Fe: Si)	1:1 1:2 2:1	26 19 31	18 12 24

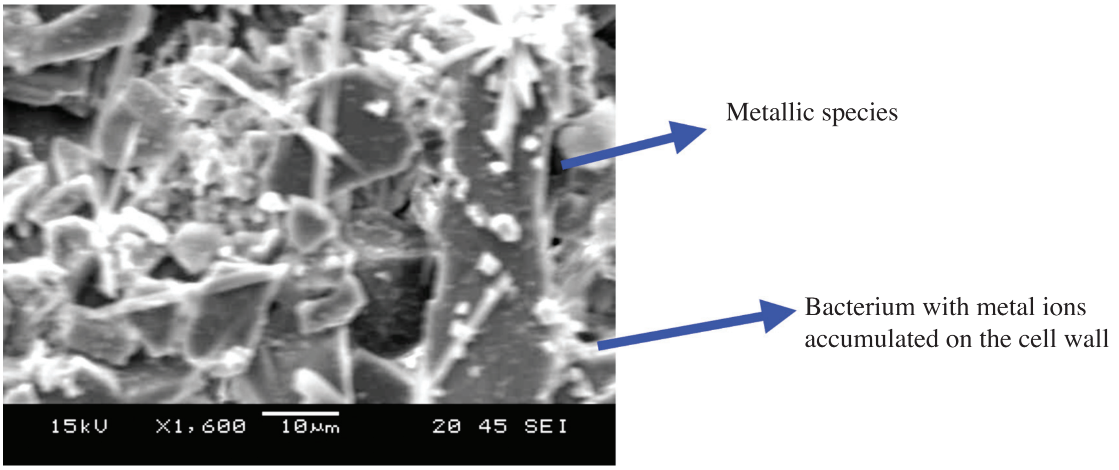

Figure 5. SEM micrograph showing bacterial interaction with metallic species (1:3 ratio)

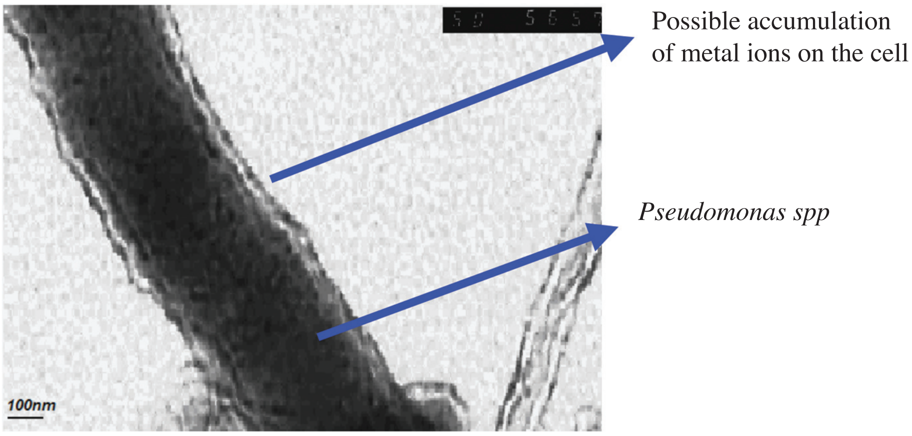

Figure 6. TEM image of metal ion accumulation on the cell wall of *Pseudomonas spp*.

Table IV
Metal recovery from mine water

Sampled site	Copper % recovery	Cobalt % recovery
1. Big dam Nigel	50	45
2. River Nigel A	42	35
3. River Nigel B	37	65
4. Stream Nigel	73	46
5. Nigel 169 (Artisanal)	61	11
6. Small dam (HVH)	29	22
7. Stream (HVH)	24	40
8. Shaft C	36	20
9. Piet Farm A	37	52
10.Piet Farm B	67	30
11. Piet Farm C	38	0
12. Piet Farm D	40	0

The results in Table IV illustrate an increased recovery efficiency of copper and cobalt. A maximum of 73% and 65% of copper and cobalt respectively was recovered. This could be attributed to the fact that the mine water samples were very dilute with concentrations ranging from 2–20 ppm of copper and from 1–8 ppm of cobalt . Other possible minor contaminants (whose effects were not considered in this study) include Fe, Ca and Mg.

Conclusion

From the results obtained thus far, it can be concluded that *Pseudomonas spp* reclaims both copper and cobalt from their sulphate synthetic solutions. It tends to remove or extract more metal ions at low concentrations and the ratio of bacteria population to solution volume being 3:1 works best. The bacteria have successfully removed up to 45% of copper from 0.07 M and up 40% from 0.07 M cobalt from cobalt sulphate solutions and 73% and 65% of copper and cobalt respectively from mine water samples. The absorption favours the removal of copper over that of cobalt, which may be accounted for by the structural differences of the two cations.

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