The next step in the bioremediation of heavy metal polluted water: development of suitable microbial-sorbent

E Fosso-Kankeu[1*], AF Mulaba-Bafubiandi[1], G Tlou[2] and L Piater[2]

[1] *Minerals Processing and Technology Research Center, Department of Extraction Metallurgy, School of Mining, Metallurgy and Chemical Engineering Faculty of Engineering and the Built Environment, University of Johannesburg, PO Box 32142 Braamfontein, South Africa, [*]E-mail: elvisf@uj.ac.za*

[2] *Department of Biochemistry, Faculty of Science, University of Johannesburg, PO Box 524, Auckland Park 2006, South Africa*

Abstract

In this study needful changes are inflicted on potential microbial sorbent in order to facilitate it use industrially for remediation of heavy metal pollution of water sources. Bacillaceae bacterium isolated from mining areas was transformed with *cnr* gene coding for nickel and cobalt resistance. Nickel recovery ability of the wild strain was then improved and prediction of adsorption behaviour made possible.

Keywords: bioremediation, adsorption behaviour, cloning, Bacillaceae bacterium, nickel, mine water

Introduction

Metal uptake by microorganisms mainly occurs through physico-chemical mechanism (passive mechanism), however higher concentration of metal ions in solution could inhibit the microorganism and therefore affect metal uptake (Gupta et al., 2000; Tsezos et al., 2006; Wang and Chen, 2006). Some microorganisms have inherent ability to resist to certain metals, but do not necessarily have the characteristics of suitable biosorbents (Legatzki et al., 2003). Bacillaceae bacterium which has demonstrated potency to uptake nickel from solution has however shown some limitations when exposed to relatively high concentration of nickel (Fosso-Kankeu et al., 2011). - Slower growth or total inhibition of growth, - reduction of the biomass surface available for metal uptake, - impossibility to predict biosorbent capacity. The *cnrYXHCBA* operon originally present in the plasmid pMOL28 can mediate resistance to nickel and cobalt (Grass et al., 2000). In previous work, the pMOL28-encoded nickel resistance gene was cloned in *Pseudomonas* spp and the conjugated species showed increased resistance to nickel (Sidiqui et al., 1989). Bacteria with metal resistance trait could be suitable biosorbent for recovery of residual metal in metallurgical solutions.

This study aims to develop microbial sorbent with improved nickel absorption capacity and predictable behaviour in bioremediation processes.

Methodology

Bacterial strains and plasmids

The plasmid pECD312 (kindly offered by Dr C Grosse from Institut fur Biologie/Mikrobiologie of Martin-Luther-Universitat Halle-Wittenberg in

Germany) was isolated from *Escherichia coli* EC232 and used for transformation of Bacillaceae bacterium (isolated from mining effluent around Johannesburg).

Molecular cloning experiment

Electrocompetent cells were prepared according to a modified high-osmolarity protocol and transformed by administration of a single electrical pulse in the presence of pECD312.

Characterization of transformants

Transformed cells were screened on agar plate containing nickel, then the presence of insert ascertained by normal PCR and gene sequencing.

Metal biosorption experiment

BIOMASS PREPARATION

Wildtype and transformed strains of the Bacillaceae bacterium, were respectively grown on simple nutrient agar or supplemented with ampicillin (100 µg/mL) and kept in the refrigerator at a temperature of between 4°C and 8°C. Single colonies of the strains were inoculated into 200 mL nutrient broth ('Lab-Lemco' powder: 1.0 g/L; yeast extract 2.0 g/L; peptone 5.0 g/L; sodium chloride 5.0 g/L; pH 7.4 ± 0.2 at 25°C; Merck Chemicals, SA) in sterile Erlenmeyer flasks (the flask containing the transformed strain was supplemented with ampicillin up to the final concentration of 100 µg/mL) and incubated overnight at 37°C in a shaking incubator (150 rpm). Microbial biomasses were prepared as described by Fosso-Kankeu et al. (2011) and suspended in appropriate volumes of sterile distilled water for the adsorption experiments discussed in the next section.

METAL BIOSORPTION EXPERIMENT

Metal and cell solutions were mixed under conditions described by Fosso-Kankeu et al. (2011). The mixture was incubated at 37°C in a shaking incubator (150 rpm) and 5 mL of the mixture was collected at intervals of 20, 60, 120, 300 and 480 min. The samples were centrifuged at 8000 rpm for 15 min and the amount of residual metal in solution was determined in the supernatant as described in the following section.

QUANTIFICATION OF METAL AND EXPERIMENTAL PROCEDURES

The concentration of residual metal ions in the supernatant was determined using an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). All experiments were done in triplicate with an abiotic control (without microorganisms) that assisted in accounting for metal loss due to precipitation. The error discrepancy between replicates was less than 10%.

Recovery effectiveness

Isotherm and reaction kinetic: The adsorption capacity at equilibrium was determined by the following equation:

$$q_e\,(\text{mg/g}) = \frac{(C_o - C_e)V}{m} \quad (1)$$

where:

q_e is the adsorption capacity in mg/g ; C_o is the initial concentration of metal ions in solution (mg/L); C_e is the equilibrium of metal ions (mg/L); m is the biomass (g); V is the volume of the solution (L)

Adsorbed nickel was localised on biomass using Transmission Electron Microscope.

Results and discussion

Examination of successful cloning

Bacillus species have been reported to be good expression hosts especially for large plasmids (Lam et al., 1998; Miwa et al., 2000; Schallmey et al., 2004). The large (~20 kb) plasmid pECD312 isolated from EC 232 was used to transform an electrocompetent Bacillaceae bacterium. The transformed cells were evaluated for a nickel resistance phenotype by exposing the cells to relatively high concentrations of nickel. It was observed that the transformants grew slowly (longer lag phase) and were inhibited at 3 mM nickel concentration (Figure 1b), while the wildtype strain was totally inhibited at a nickel concentration of 1.5 mM (Figure 1a), indicating that expression of the *cnr* determinant in the transformed Bacillaceae bacterium led to higher tolerance to nickel.

The nickel resistance observed as a result of transforming other wildtype strains with the *cnr* determinant (this study) has also been demonstrated elsewhere and the mechanism identified was ascribed to a resistance nodulation cell division (RND)-driven transenvelope efflux systems encoded by the *cnr* determinant (Marrero et al., 2007; Nies and Silver, 1995).

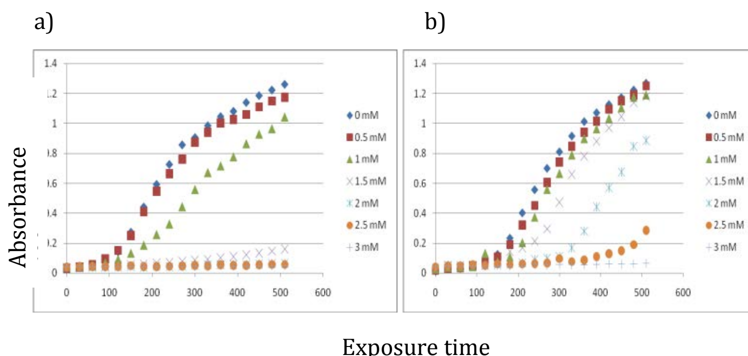


Figure 1 *Nickel tolerance by a) wild strain and b) transformed strain, of Bacillaceae bacteria*

PCR results showed that the cnr insert in the plasmid was specifically amplified, confirming that nickel tolerance resulted from expression of the gene of interest. The gene sequencing decoded was exactly the one expected.

Nickel uptake as a function of initial concentration and time

Nickel uptake by wild strain increased (~25%) with time only when exposed to low Ci of nickel. However cloned strain showed better uptake at higher initial

concentration, removing up to 37% more nickel than the wild strain at Ci equal to 150 mg/L, certainly due to better tolerance of nickel. Improvement of nickel uptake following cloning was also observed by Marrero et al. (2007) with *E. coli* strain bearing *ncr*A gene.

Prediction of adsorption behaviour (Langmuir isotherm)

The Langmuir model is applicable to saturable adsorption mechanisms that can allow determination of the equilibrium constant. The equilibrium constant can be derived from the following equation:

$$C_e/q_e = 1/kq_m + C_e/q_m$$

where q_e is the amount of heavy metal adsorbed onto the unit amount of the biomass (mg/g); C_e is the equilibrium concentration (mg/L); q_m is a complete monolayer (mg/g) and k is and adsorption equilibrium constant related to the strength of the binding site.

However according to the results, only the pattern exhibited by the transformed strain during adsorption of nickel could fit the Langmuir model (Figure 2); this was confirmed as the correlation coefficient of the Langmuir equation for nickel adsorption by the transformed strain (0.991) was much closer to the unit as compared to the adsorption by the wildtype strain (0.875), showing that the adsorption pattern of the transform strain fitted better the model. These results imply that nickel adsorption by the transformed Bacillaceae bacterium will increase as long as the binding sites are free.

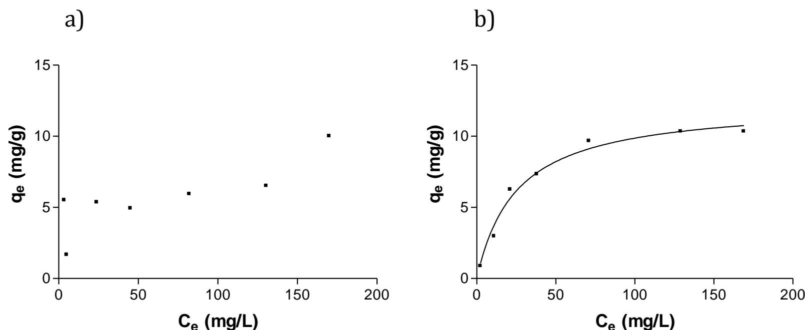


Figure 2 *Nickel adsorption by a) wild strain and b) cloned strain, of Bacillaceae bacteria*

Prediction of adsorption behaviour (Pseudo second order kinetic)

The correlation coefficients (R^2) (Table 1) values are close to 1, and the calculated values of the equilibrium adsorption capacity (q_e) close to experimental values are further contributing facts that the adsorption of Ni^{2+} obeys to the pseudo second-order kinetic. The equilibrium constant (k) was found to decrease with the increase of equilibrium adsorption capacity, however Ni^{2+} adsorption rate increase constantly at initial concentration between 40 and 200 mg/L when using

transformed strain while in the case of nickel adsorption by wildtype strain the constant increase was observed from 20 mg/L to 100 mg/L initial concentration, implying that inhibition of the wildtype strain at higher concentrations of nickel reduced its capacity as biosorbent of Ni^{2+}.

Recovery of nickel from mine water

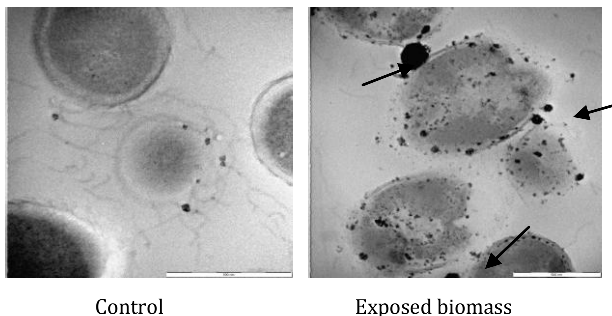
The use of wildtype and transformed strains of a Bacillaceae bacterium for recovery of Ni^{2+} from mine water showed that both strains could removed around 80 % of the Ni^{2+} content, confirming the results obtained with the synthetic solutions whereby at lower (less than 20 mg/L) initial concentrations, the microbial sorbents do not perform to their fullest capacities, probably because of the low mass transfer; the transformed strain could then perform better than the wildtype strain when recovering nickel from solution containing more than 40 mg/L as shown in Figure. 3.

Table 1 *Pseudo second-order kinetic parameters for the adsorption of Ni^{2+} on microbial sorbents*

Ci mg/L	Experimental and calculated parameters per strain							
	Wild strain				Cloned strain			
	q_e exp. (mg/g)	K (g/mg/min)	R^2	q_e cal. (mg/g)	q_e exp. (mg/g)	K (g/mg/min)	R^2	q_e cal. (mg/g)
5	1.67	0	1	1.67	0.867	na	0.996	0.86
20	5.5	0.0092	0.9907	5.513	2.967	0.224	0.9998	2.99
40	5.357	0.007	0.9911	5.45	6.261	0.006	0.992	6.44
60	4.933	0.007	0.996	5.15	7.333	0.0035	0.986	7.62
100	5.933	0.012	0.9987	6.083	9.667	0.0027	0.9941	10.33
150	6.5	0.044	0.9997	6.51	10.333	0.0037	0.9941	10.69
200	10	0.145	1	10.02	10.333	0.004	0.9957	10.67

na: not available

The q_e for cloned strain is higher than for wild strain at higher concentration of nickel.



Control Exposed biomass

Figure 3. *Visualization of Nickel on Bacillaceae bacterium cell surface using Transmission Electron Microscope*

Localisation of nickel on biomass

Visualization of unloaded (not exposed to nickel) and loaded (exposed to nickel) biomasses with Transmission Electron Microscope allowed to locate nickel attachment sites on cell surface.

This showed that the cells in the control (Figure 3.A) did not have any Ni^{2+} ion attached to them while the cells exposed adsorbed nickel on the surface (Figure. 3.B), confirming that nickel recovery occurs mainly as a result of passive binding or physicochemical interaction with active groups on cell surface.

Conclusions

Bacillaceae bacterium was successfully cloned with the *cnrYXHCBA* determinant and showed improved tolerance to relatively high concentration of nickel. The cloned strain was suitable biosorbent at relatively higher initial concentrations of nickel. Furthermore, the cloned strain exhibited characteristics suitable for Langmuir and pseudo second-order kinetic models studies allowing prediction of its behaviour as biosorbent.

Acknowledgements

The authors are grateful to Dr TG Barnard of the Water and Health Research Centre of the University of Johannesburg for providing the facilities, Dr C Grosse of the Institut fur Biologie/Mikrobiologie of Martin-Luther-Universitat Halle-Wittenberg in Germany for providing the plasmid pECD312; CF van der Merwe and A Hall of the Laboratory for Microscopy and Microanalysis of the University of Pretoria for the TEM analysis. The NRF and Commonwealth for sponsoring through academic scholarships.

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