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Bioremediation of Toxic Metals and Recovery of Target Metals from Actual Wastewater using Algal Sorbents

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The use of biological material is an emerging and environmentally friendly technology with great prospects to effectively clean up toxic metals at low concentrations and possible recovery for re-use in industry. The living biomass accumulates high levels of metals but possible recovery without cell disruption seems almost impossible. Biosorption is a term often used to refer to the treatment of wastewater containing heavy metals using dead biomass. This study utilised algal samples collected from a eutrophic dam and isolated pure strains for use in biosorption experiments. Two species *Chlorella vulgaris* and *Chlamydomonas reinhardtii* were identified and tested for their ability to remove and or reduce the concentration of metals in simulated and industrial wastewater. The algal species were initially tested for their biosorption potential for removal/recovery of Lanthanum (La) and Thallium (Tl) in single metallic studies. In addition, test algae was characterised before and after adsorption using Surface Electron Microscope (SEM). *Chlamydomonas reinhardtii* showed the highest sorption capacity (q_{max}) of 143 mg/g compared to *Chlorella vulgaris* with a q_{max} of 75 mg/g for removal of La. Both species showed a similar q_{max} for removal of Tl at 1000mg/g but *Chlamydomonas reinhardtii* had a higher affinity (b) of 1.67 L/g. Wastewater from the mine beneficiary plant showed complete removal of Cobalt from an initial concentration of 0.316 mg/L by *C. vulgaris*. Industrial water from smelter clave tailings showed Tl as the highest heavy metal concentration of 2.386 mg/L which was reduced to 0.021 mg/L using *C. vulgaris.* Potassium was reduced significantly by *C.reinhardtii* from 2036 to 744.7 mg/L. Smelter return water had the highest level of Cr at 135.56 mg/L and was reduced to 45.67mg/L by *C.vulgaris*. The tested algal sorbents showed potential for the treatment of actual wastewater with the best adsorbent performance from *C. vulgaris.*

1. Introduction

For many decades, toxicity of metals has been recognised due to documented evidence in plants, animals and or humans (Wang and Chen, 2009). The various metal compounds are usually released into the environment directly or indirectly with the main culprit being man due to the various industrial activities. Unlike toxic heavy metals which are relatively abundant, some metals are scarce and unevenly distributed in the earth crust. Rare Earth Metals (REM) are ranked by as one of the most critical elements with many emerging technologies but with a diminishing supply (European Commission, 2010). REM are used in many applications such as green energy technologies, nuclear oil, automobile industry, electronics. Lanthanum is one of the most abundant and commonly used REM in industrial processes. Removal and recovery of REM from wastewater and mine tailings remains largely unexplored (Binnemans, et al., 2013). Tl is generally considered as a mobile element in aqueous environments usually emitted as a by-product of industrial processes like refining, cement industry and coal powered plants (Peter and Viraraghavan, 2005). In the past, Tl was used as a poison for rodents and insecticides, treatment of ring worms, tuberculosis, malaria and veneral disease but was discontinued due to toxicity. Recently, Tl is used in industries for the manufacture of scintillation counters, low temperature thermometers, electronic devices, optical systems, special glass, oxidation of hydro carbons and olefins, dyes, mineralogical separation, pigments, imitation jewelry and fireworks among others (Vaněk, et al., 2011).Thallium poisoning in humans mainly occurs through ingestion of contaminated food, inhalation or and through the skin (Urík, et al., 2010).

Conventional treatment technologies such as sand filtration, GAC adsorption, precipitation-sedimentation, flotation, ion-exchange, and electrochemical deposition systems have been used for decades for the removal of toxic heavy metals wastewater. These technologies seem to operate relatively well for effluents containing higher metal concentrations ≥ 100 mg/L. The common failure of adsorption processes is the lack of selectivity for the target metals and the high cost of operation due to high energy requirements (Fu and Wang, 2011). Biosorption is a proposed alternative technology with relatively abundant waste biomass. The main challenge is to select the most efficient biosorbents from the relatively abundant biomass. Micro-algae have a wide distribution of species found mostly in freshwater habitats but only a few have been investigated for their biosorption potential. This study investigated the bioremediation potential of La and Tl for the treatment of wastewater.

2. Materials and Methods

2.2 Algal Collection and Identification

The planktonic algae were collected from a fresh water dam in Hartbeespoort dam in North West province in South Africa. The algal samples were isolated using streak plating technique and analysed using molecular methods. DNA extraction from the algal samples was performed with the CTAB method (Murray and Thompson, 1980). The Internal transcribed spacer (ITS) and 18S ribosomal RNA gene (rRNA) were used in this study. ITS1 and ITS2 primers were used to amplify the ITS region in the forward and reverse directions, respectively. Phylogenetic analysis of sequences was checked for similarity using a basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/).

2.3 Algal Culture

The pure colonies were picked up by loop and allowed to grow in tubes and vials containing AF-6 media (Andersen, et al., 2005) as starter cultures and stock cultures. The algae was cultured under controlled conditions using algal lights (Osram L 36W/77 Floura) at 23°C for a 12 hours day light. Starter cultures were used as back up and were maintained under illumination in static flasks. The stock cultures were further inoculated into 1000 mL and 2000 mL volumetric flasks to increase the volume of cultured sample and sub-cultured twice a month. The algae was harvested, centrifuged, cleaned twice with de-ioned water and dried at 50°C in the oven over night and stored for biosorption experiments.

2.3 Equilibrium and Kinetic Experiments

For preparation of an aqueous mixture of bi-metallic, initial concentration of 25-150 mg/L for La and 150-800 mg/L for Tl was used. The varying concentrations were measured into 100 mL volumetric flask for each metal, and then mixed to make a volume of 300 mL for the 2 metals. The mixture was then divided into 2 volumes of 150 mL each into Erlenmeyer flasks to make duplicates. The pH was adjusted to optimum at 6 before the start of the experiment and a constant biomass of 0.08 g was used. The experimental sets were stirred on the magnetic stirrer at room temperature and a constant speed of 350 rpm. Equilibrium and kinetic studies were carried out con-currently. The preceding samples were taken at pre- determined time interval between 5-1440 minutes. The sample volume of 10 mL each was centrifuged for 10 minutes at 6000 rpm and the supernatant analysed using ICP.

2.4 Characterisation using Scanning Electron Microscope (SEM)

The morphology of the biosorbents before and after biosorption was identified using a Scanning Electron Microscope (SEM, JOEL JSM 5800LV, Tokyo, Japan). The wet algal samples were prepared using traditional chemical methods before embedding for SEM. Each sample was dehydrated in ethanol (30%, 50%, 70%, 90%, and 100%) for 10 minutes. The samples were dried at critical point from liquid carbon dioxide, mount on the stub and sputtered with gold ready for identification. After sample preparation, SEM was used to obtain images at different magnification before and after adsorption.

2.5. Recovery of Metals by Tested Algae

The algae were tested for their re-usability for the recovery for Lanthanum. The adsorption experiment was carried for 2 hours with the initial concentration of 100 mg/L Lanthanum and 0.1g of biomass. The sample was centrifuged and the supernatant analysed for metal analysis. The metal laden biomass was rinsed twice in deionised water and 0.1 M HNO3 used as the eluent for 2 hours. The supernatant was also analysed for metal recovery. The same procedure was repeated for the other 2 cycles.

2.4 Biosorption using Actual Industrial Samples

Industrial wastewater samples were collected from 2 industries in South Africa, namely Dilakong chrome mine, and Manganese metal company. The industrial samples were initially analysed using ICP to determine the metal types and concentration available before biosorption experiments.100 mL of waste samples were then contacted with 0.1g of algal biomass in 250 mL of Erlenmeyer flasks and stirred on the magnetic stirrer at 240 rpm for 24 hours. The samples were filtered and the filtrate analysed using ICP.

3. Results and Discussion

3.1 Equilibrium Modelling For Single Metals

Empirical models of Langmuir and Freundlich models were used to quantitatively describe sorption potential of algae using linear regression. The following equations (Eq.) were used to quantitatively describe the adsorption data;

$$C_e/q_e = C_e/q_{\max} + 1/bq_{\max} \tag{1}$$

$$\log\ q_e = \log\ k + 1/n \log\ C_e \tag{2}$$

where q_e is the adsorbed metal at equilibrium (mg/g), $q_{\max}$ is the maximum amount of metal sorbed (mg/g), b is a constant related to the energy of sorption, C_e is the equilibrium metal ion concentration (mg/L), k is biosorption equilibrium constant and n is a constant indicative of biosorption intensity.

Table 1: Equilibrium adsorption parameter for Lanthanum using tested algae

Algal species	Langmuir constants			Freundlich constants		
	q_{max} (mg/g)	b (L/g)	R^2	n	K	R^2
Chlamydomonas reinhardtii	143	0.18	0.99	3.35	4.72	0.89
Chlorella vulgaris	75	0.25	0.91	3.41	3.66	0.78

Table 2: Equilibrium adsorption parameter for Thallium using tested algae

Algal species	Langmuir constants			Freundlich constants		
	q_{max} (mg/g)	b (L/g)	R^2	n	K	R^2
Chlamydomonas reinhardtii	1000	1.67	0.99	1.85	9.9	0.73
Chlorella vulgaris	1000	1.11	0.99	2.13	9.84	0.72

Preliminary tests were carried out to determine adsorption potential for Lanthanum and Thallium using selected species. Generally the linearized Langmuir model performed better than the Freundlich model with a higher correlation co-efficient, Tables 1 and 2. *Chlamydomonas reinhardtii* showed the highest sorption capacity ($q_{\max}$) at 142 mg/g and lowest affinity (b) of 0.18 L/g, Table 1. Both species showed a high $q_{\max}$ for Tl adsorption at 1000 mg/g but *Chlamydomonas reinhardtii* showed a higher affinity at 1.67 L/g.

4. Kinetic Modelling of Binary Systems

The experimental data from the kinetic studies of a binary system of La and Tl was simulated using AQUASIM (Version 2). A non-linear Langmuir-Hinshelwood (L-H) was used to describe the sorption behaviour of metallic ions on the adsorbent given by the following equation.

$$-{}^{dc}\!/_{dt} = {}^{qC}\!/_{K+C} \tag{3}$$

The assumption of L-H equation is that complete degradation occurs which does not seem to be the case with biosorption. When the active surface binding sites become saturated, then no further reduction occurs. The L-H kinetics was modified to the following equation;

$$-{}^{dc}\!/_{dt} = {}^{qC}\!/_{K+C}\left[A_o - \frac{(C_o - C)}{R_c}\right] \tag{4}$$

Where q is the rate reaction constant (1/h), K is the adsorption equilibrium constant (mg/L), A_o is the initial surface area (m^2/g), and R_c is the reduction capacity (mg.g)/ (L.m^2).

Table 3: Parameter estimations for Lanthanum adsorption

C_0 (*mg/L*)	q (*1/h*)	K (*mg/L*)	R_c (*mg.g)/(L.m^2*)
25	9.949	0.00725	3.965
50	1.0987	9.2955	0.6766
100	0.01511	8.9921	0.00608

Table 4: Parameter estimations for Thallium adsorption

C_0 (*mg/L*)	q (*1/h*)	K (*mg/L*)	R_c (*mg.g)/(L.m^2*)
150	0.0006	9.9071	0.0074
250	0.0004	9.9990	0.0062
500	0.0001	1.0000	0.0026

Experimental data was used for model optimisation and parameter estimations were carried out for variables q, K and R_c to determine the most important parameters influencing the prediction. An increase in La concentration with time resulted in decrease in rate of reaction (q) and reduction capacity (R_c). The results for La adsorption implied that as the concentration increases, the binding sites get saturated and therefore the rate of reaction also decreases, Table 3. Tl adsorption showed that q and R_c increased with an increase in concentration, Table 4. The results for Tl adsorption indicated an antagonistic effect on biosorption with an increase in time.

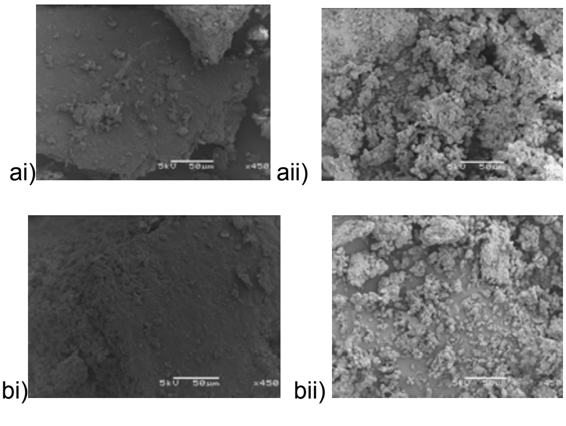

Figure 1: SEM micrographs a, b (i) are biomass before adsorption and a, b, (ii) after adsorption for Chlamydomonas reinhardtii and Chlorella vulgaris respectively.

5. Characterisation of Test Algae using Surface Electron Microscope (SEM)

Surface electron microscope was used in identification of the morphology of the surface on the cell wall of the algae before and after biosorption. The SEM micrographs before adsorption appeared more compact whereas after adsorption, the walls were unconsolidated and rough, Figure 1.

6. Treatment of Actual Industrial Wastewater

Industrial wastewater samples were subjected to some tested algal species to check for feasibility of application in the removal of toxic metals. Wastewater from mine beneficiary plant had Na as the highest light metal concentration which was most removed by *C. vulgaris.* Cobalt was completely reduced to zero from an initial concentration of 0.316 mg/L by *C. vulgaris*, Figure 3. Industrial water from smelter clave tailings showed Tl as the highest heavy metal concentration of 2.386 mg/L which was best reduced to 0.021 mg/L using *C. vulgaris.* Potassium was reduced significantly by *C.reinhardtii* from 2036 to 744.7 mg/L, Figure 3.

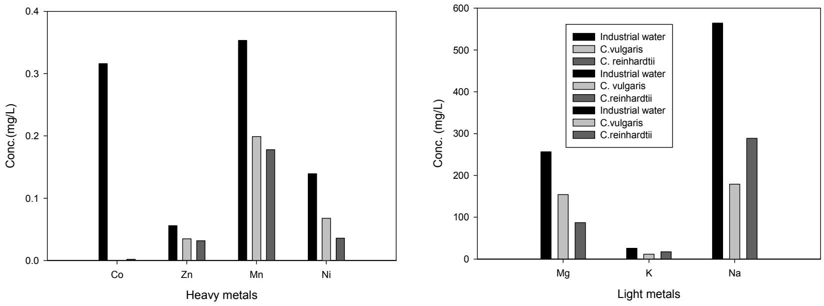

Figure 2: Treatment of industrial wastewater from mine beneficiary plant using 2 tested algal species

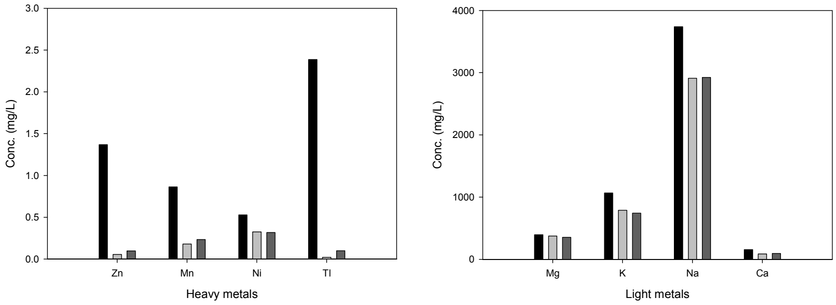

Figure 3: Treatment of industrial wastewater from smelter clave tailings using 2 tested algal specie

7. The Removal/ Recovery of Tested Metals

The rate of removal and recovery was highest for La at 94.04 and 90.92% respectively using *Desmodesmus multivariabilis*, Figure 4. The removal rate was generally in the order La >Tl >Cd and recovery in the order La >Cd> Tl for all the 3 tested metals.

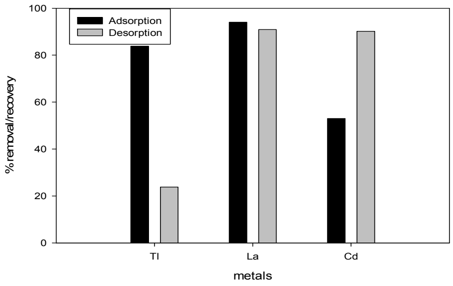

Figure 4: Adsorption/Desorption efficiency for Tl, La and Cd using D. multivariabilis

8. Conclusion

Both species showed high potential for removal of La and Tl with *Chlamydomonas reinhar*dtii having the highest sorption capacity. The Langmuir model performed better than the Freundlich model with a higher correlation coefficient of ≤ 0.99. The rate of reaction decreased with increase in concentration for La unlike Tl. There's need for further research into understanding the behaviour of Tl as it significantly reduces in the first few minutes and then desorbs back into the system with time. In the treatment of actual wastewater, the tested biosorbents showed great potential with the best performance from *Chlorella vulgaris*.

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