CHAPTER 3

Biosorption
A methodological approach to investigate the pH effect on biosorption process: experimental and modeling procedures

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Abstract

A methodological approach to study and model equilibrium of heavy metals in biosorption processes has been proposed and discussed. Four cases of copper biosorption are here reported and discussed as examples of application: biosorption of copper onto \textit{Sphaerotilus natans}, \textit{Rhizopus oligosporus}, calcium alginate and olive mill residues (OMR) have been here described and discussed. Several empirical and semi-empirical models have been proposed and summarised, to consider the pH effect on the heavy metal uptake. The proposed models, originated from Langmuir isotherm, may be useful to fit experimental data avoiding pH control during biosorption tests and simply monitoring its equilibrium value. The adsorption isotherms were built considering experimental procedures at constant pH (in standard manner) and in pH edge conditions. Both empirical and semi-empirical models were able to fit these experimental results. The pH-edge experimental procedure coupled with the proposed pH-related models is proposed as useful tools to investigate and model biosorption processes with single heavy metals in solution.

\textit{Keywords:} biosorption, heavy metals, equilibrium, modelling, pH-effect

1. INTRODUCTION

Biosorption is an innovative technology aimed at the removal of toxic metals from polluted streams by using inactive and dead biomasses. Metals entrapment is due to chemico-physical interactions with active groups present on the cell wall: carboxylic, phosphate, sulfate, amino, amide and hydroxyl groups are the most commonly found, according to the biosorbent nature (Cox et al., 1999; Plette et al., 1995; Veglio’ and Beolchini, 1997). Considering its mechanism, biosorption is affected by several factors such as pH, simultaneous presence of other metals, kind of biosorbent material. In any case, the development of a model in agreement with experimental data is fundamental in

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order to simulate and predict biosorption courses. Equilibrium models are essential, considering that they represent the first step encountered in the development of kinetic models to be applied in unit operations typical of chemical engineering, such as fixed bed adsorption columns (Veglio’ et al., 1999) and membrane adsorbers (Veglio’ et al., 2000; Beolchini et al., 2002). In the case of sorption equilibrium data obtained at different pH, the effect of pH on heavy metal biosorption cannot be considered by simple empirical and physical equations such as Freundlich and Langmuir models (Esposito et al., 2001, 2002). These adsorption isotherms can be used to fit only experimental data obtained at constant equilibrium pH (Esposito et al., 2001). Consequently, more complex models have to be considered, with pH as a further independent variable. In the present work, some empirical and semi-empirical models are presented to describe copper sorption equilibrium by four different biosorbents: *Sphaerotilus natans*, *Rhizopus oligosporus*, calcium alginate and olive mill solid residues.

2. MATERIALS AND METHODS

2.1 Biosorbents

*Sphaerotilus natans* is a Gram-negative bacterium isolated from the waste streams of a water purification plant. Further details for biomass cultivation and separation can be found elsewhere (Esposito et al., 2001).

*Rhizopus oligosporus* has been supplied by C.R.A.B. (Consorzio per le Ricerche Applicate alla Biotecnologia, Avezzano, Italy).

Calcium alginate beads were prepared as described in Veglio’ et al. (2002).

Olive mill residues (OMR) were the solid residues of oil production, provided by an olive mill in Abruzzo, Italy. Before their use, the olive mill solid wastes were ground and sieved at 400-1000 µm. Further details can be found elsewhere (Veglio’ et al., in press).

2.2 Equilibrium tests

Equilibrium biosorption tests were realised with two experimental procedures: sorption test at constant pH (noted in the following as standard method – STD) and pH edge tests. In both cases a selected amount of lyophilised biomass (0.1 g) was placed in a shaken flask with a known volume of distilled water: the biomass was re-hydrated for 1 h. A selected volume of a copper solution (prepared by dissolving CuSO₄ in distilled water, 1 g/L) was added to the shaken flask maintaining the liquid total volume at 100 mL. The shaken flasks were then placed in a shaker at 250 min⁻¹ at room temperature (25°C) and heavy metal uptake was monitored sampling the solution and measuring the copper concentration by atomic absorption spectrophotometer (Varian Spectra 2000): the heavy metal uptake and the concentration in the solid phase \( q \) (mg of Cu²⁺ per g of biomass) was estimated by material balance. In the first series of adsorption tests (STD) the pH was continuously monitored and controlled by adding HCl 0.1 M or NaOH 0.1 M until the equilibrium conditions were reached (after 1 h): each isotherm (at constant pH) was obtained increasing the initial copper concentration in different shaken flasks. The second series of tests (pH-edge tests) were carried out as STD tests, but pH was changed from low to high values (from about pH 3 to 5, adding NaOH 0.1M or 1M) in each shaken flask and *vice versa* (from pH 5 to 3, adding HCl 0.1 N or 1 M): in this manner, the total amount of copper is constant for each test and the copper in solution is monitored after that each pH change has been induced. In both cases, particular care was paid to have negligible dilution by alkali or acid solutions during the pH control. After each pH change, an
equilibration time of 60 min was used before the collection of the liquid sample. The \( q \) values were calculated also considering the copper collected during the sampling procedure in order to avoid a propagation of this systematic error. Most of the biosorption tests were replicated twice and the c.v. values ranged from 2 to 5%.

2.3 Analytical determinations

Copper concentration in the liquid phase was determined by atomic absorption spectrophotometer (Varian Spectra 2000). All samples were diluted with HNO\(_3\) at pH 2 and stored at 4°C before the analysis.

2.4 Mathematical models

The proposed empirical and semi-empirical models used to fit equilibrium data are summarised in the following equations: (Veglio’ et al., 2002; Pagnanelli et al., in press):

Model 1a: \[ q = \left( \alpha_1 \cdot pH + \alpha_2 \right) \frac{C_{eq}}{\alpha_3 + C_{eq}} \] (1a)

Model 1b: \[ q = \left( \alpha_1 \cdot pH^{\alpha_2} \right) \frac{C_{eq}}{\alpha_3 + C_{eq}} \] (1b)

Model 1c: \[ q = \left( \alpha_1 \cdot e^{-\alpha_2 \cdot pH} \right) \frac{C_{eq}}{\alpha_3 + C_{eq}} \] (1c)

Model 1d: \[ q = \frac{\alpha_1 \cdot pH + \alpha_2}{\alpha_3 + pH} \frac{C_{eq}}{(\alpha_4 \cdot pH + \alpha_5) + C_{eq}} \] (1d)

Model 2a: \[ q = \frac{\alpha_1 \cdot e^{\alpha_2 \cdot pH}}{1 - \frac{\alpha_1}{\alpha_3} \left( 1 - e^{\alpha_2 \cdot pH} \right)} \frac{C_{eq}}{\alpha_4 + C_{eq}} \] (2a)

Model 2b: \[ q = \frac{\alpha_1 \cdot e^{\alpha_2 \cdot pH}}{1 - \frac{\alpha_1}{\alpha_3} \left( 1 - e^{\alpha_2 \cdot pH} \right)} \frac{C_{eq}}{(\alpha_4 \cdot pH + \alpha_5) + C_{eq}} \] (2b)

Model 3a: \[ q = \frac{\alpha_1}{1 + \frac{10^{-pH}}{\alpha_2}} \frac{C_{eq}}{\alpha_3 + C_{eq}} \] (3a)

Model 3b: \[ q = \frac{\alpha_1}{1 + \frac{10^{-pH}}{\alpha_2}} \frac{C_{eq}}{\alpha_3 \left( 1 + \frac{10^{-pH}}{\alpha_2} \right) + C_{eq}} \] (3b)

Model 3c: \[ q = \frac{\alpha_1}{1 + \frac{10^{-pH}}{\alpha_2}} \frac{C_{eq}}{\alpha_3 \left( 1 + \frac{10^{-pH}}{\alpha_4} \right) + C_{eq}} \] (3c)

All these equations have been built considering Langmuir model as a reference model, introducing the pH effect in the two parameters \( q_{max} \) and \( K_s \) (Esposito et al., 2001): in fact at constant pH all the equations degenerate in the classical Langmuir model.
The empirical models reported in equations (1a), (1b), (1c) and (1d) were named Model 1a, 1b, 1c and 1d, respectively (Pagnanelli et al., in press; Veglio’ et al., 2002); in equations (2a) and (2b) two different versions of the logistic equation coupled with Langmuir model have been shown (Veglio’ et al., 2002): both models were named Model 2a and 2b; the equations (3a), (3b) and (3c) have been originated from non-competitive biosorption models (Esposito et al., 2002; Veglio’ et al., 2002) with some empirical changes introduced considering the obtained experimental data (these were named Model 3a, 3b and 3c respectively). A detailed description of the non-competitive mechanism between H+ ions and the metal can be found elsewhere (Esposito et al., 2002).

4. EXPERIMENTAL RESULTS AND DISCUSSION

Obviously each equilibrium model gives different results according to the experimental system; in fact, as well known, the empirical models are in general built on specific experimental results. Each model previously described was tested on different experimental systems. Some examples are shown in Figures 1 to 4. Figure 1 shows models 2a (empirical) and 3b (non competitive semi-empirical) fitting to copper sorption equilibrium data by *Sphaerotilus natans*. Figure 2 shows results obtained in the study of equilibrium sorption by *Rhizopus oligosporus*. In particular, Fig. 2a shows Langmuir model parameters dependence on pH, as estimated considering separately each test performed at constant pH, while Fig. 2b shows model 2a (empirical) fitting, performed on all tests. Figure 3 shows the semi-empirical model (equation 3b) application for copper biosorption by calcium alginate. Figure 4 reports the empirical fitting (model 1a) of copper sorption equilibrium by olive mill residues.

![Figure 1. Empirical (equation 2a) and non-competitive semi-empirical (equation 3b) models for copper biosorption by *Sphaerotilus natans* with 1 g/L biomass concentration (points represent experimental data, obtained in pH edge tests)
Figure 2. Copper biosorption by *Rhizopus oligosporus*. Langmuir parameter dependence on pH (a) and sorption isotherms (b). In the isotherms, points represent experimental data (pH edge tests), while lines have been calculated by Model 3a (equation 3a).
Figure 3. Semi-empirical model (equation 3b) application for copper biosorption by calcium alginate at pH 4: STD (standard tests – 5 mL of beads; pH = 4.0; Temperature 22°C); Test 1 (pH-edge test - 5 mL of beads; pH = 3.9; Temperature 22°C); Test 2 (pH-edge test - 5 mL of beads; pH = 3.8; Temperature 22°C); Test 3 (pH-edge test - 10 mL of beads; pH = 3.8; Temperature 22°C) (Veglio’ et al., 2002)

Figure 4. Sorption isotherms in the case of copper sorption by olive mill residues (biosorbent 1 g/L, standard test) Continuous lines have been calculated by equation 3a
Models showed in Figures 1 to 4 are the ones characterised by the best agreement between experimental and calculated specific uptakes. The data fitting was performed by a non-linear regression method, in order to evaluate the adjustable parameters of each model ($\alpha_i; i = 1, p$) by minimizing the sum of the squared deviations of experimental from calculated values of $q$ (Himmelblau, 1978). The model validity is indicated by the following statistical parameters (Himmelblau, 1978; Montgomery, 1991):

i) the parameter standard error;

ii) the model residual variance ($s^2_{res}$), calculated as

$$S^2_{res} = \sum_{i=1}^{n} (q_{exp} - q_{ical})^2 \over n - p$$

where $n$ is the total number of experimental points, $p$ is the number of estimated parameters. Table 1 shows as example the obtained results for Model 2a fitting to copper sorption by different biosorbents. Other results can be found elsewhere (Esposito et al., 2002; Veglio’ et al., in press; Pagnanelli et al., in press).

The performances of the selected models were also compared by using an F-Test (not reported here) (Montgomery, 1991; Veglio’ et al., 2002). This statistical tool permits to evaluate if there is a difference in the accuracy of the investigated tested models. Considering the results of the F-tests and the other statistical parameters previously reported, it was possible to select the best model. For example, in the case of copper biosorption by calcium alginate the best models are N°3a and 3b because they have the lowest $s^2_{res}$ values, they have a physical meaning and they are able to describe the experimental results with the lowest number of adjustable parameters ($p = 3$). An analogous discussion was performed for each biosorption system and the best models have been individuated and reported in Figures 1 to 4.

**Table 1.** Empirical model (Model 2a) fitting in the case of copper sorption by different biosorbents: adjustable parameters ($\pm$ standard error), degree of freedom (d.f.), regression coefficient ($R^2$), model residual variance

<table>
<thead>
<tr>
<th>Biosorbent</th>
<th>$X$ (g/L)</th>
<th>$\alpha_1$ (mg/g)</th>
<th>$\alpha_2$ (mg/g)</th>
<th>$\alpha_3$ (mg/g)</th>
<th>$\alpha_4$ (mg/L)</th>
<th>d.f.</th>
<th>$R^2$</th>
<th>$S_r^2$ (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sphaerotilus natans</strong></td>
<td>0.5</td>
<td>0.00037±0.00009</td>
<td>2.6±0.5</td>
<td>100±10</td>
<td>1.8±0.3</td>
<td>24</td>
<td>0.986</td>
<td>11.205</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.022±0.002</td>
<td>1.6±0.2</td>
<td>90±10</td>
<td>5.5±0.7</td>
<td>30</td>
<td>0.995</td>
<td>2.331</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>0.0045±0.0005</td>
<td>1.9±0.2</td>
<td>68±8</td>
<td>3.9±0.7</td>
<td>23</td>
<td>0.988</td>
<td>5.852</td>
</tr>
<tr>
<td><strong>Rhizopus oligosporus</strong></td>
<td>1</td>
<td>1.3±0.8</td>
<td>1.1±0.2</td>
<td>240±70</td>
<td>125±10</td>
<td>36</td>
<td>0.990</td>
<td>56.5</td>
</tr>
<tr>
<td><strong>Calcium alginate</strong></td>
<td>1.0</td>
<td>0.2±0.2</td>
<td>1.8±0.4</td>
<td>14.9±0.6</td>
<td>480±50</td>
<td>4</td>
<td>0.96</td>
<td>9.63</td>
</tr>
</tbody>
</table>

A further important aspect comes out from the analysis of Figure 3 (and from other results with other biosorbents, not reported here): a comparison among the different adsorption equilibrium data highlights that pH-edge tests give similar results obtained in standard adsorption tests. In this way, it is possible to conclude that pH edge tests can be applied in equilibrium studies with several advantages from the practical experimental point of view (saving biosorbent material and laboratory time to maintain pH constant during the building of an adsorption isotherm).
4. CONCLUSIONS

In this paper, equilibrium models for biosorption in single metal systems are described and applied for several biosorbent materials. pH was included as independent variable in the equilibrium models, in order to have more flexible models, suitable for data fitting also in the case of not constant pH. In this way, pH edge tests (Veglio’ et al., in press) coupled with the proposed pH-related models result to be an effective tool procedure for the study of biosorption equilibrium.

5. REFERENCES


A model for the copper biosorption in dried leaves


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Abstract

We report on biosorption of copper ions by dried leaves. Sorption experiments with lettuce leaves (L. sativa), mainly composed of cellulose, and corn husks (Z. mays), mainly composed of lignin, showed that the sorptive performance of L. sativa is better than all other plant biomasses studied earlier and that Z. mays presents a very weak sorptive capacity. Electron Paramagnetic Resonance (EPR) spectra revealed that the copper ion occupies a site with axial symmetry inside the biomass. Fourier Transform Infrared (FTIR) absorption spectra indicated that the presence of copper affects CH₂, CO and OH bonds of the biomass structure. These results allowed us to conclude that copper ions sorbed by dried leaves occupy the sites located between two glucose rings of cellulose fibers.

Keywords: biosorption, plant biomass, copper, sorption sites, cellulose, lignin, EPR, FTIR

1. INTRODUCTION

The biosorption, that is, the capacity of biomasses to retain metallic ions from solutions, is widely known [1, 2], but few studies propose sorption models that could explain this phenomenon. From our previous results of the sorption of copper by dried leaves using Atomic Absorption Spectroscopy (AAS), Electron Paramagnetic Resonance (EPR) and Fourier Transform Infrared Spectroscopy (FTIR) we concluded that: (i) the sorption sites are similar in all plant leaves studied [3]; (ii) after sorption, the Cu²⁺ ion is incorporated in the biomass in a site with axial symmetry [3]; (iii) the sorption is more efficient in the fibre residuals than in the complete leaves [3]; and (iv) the presence of copper inside the biomass affects C bonds in carbon rings but the Cu ion does not substitute any other ion of the biomass [4].

The aim of this work is to investigate the site responsible for the sorption in dried plant leaves in order to propose a model for the sorption sites. Since the fibres are more effective in sorption than the complete leaves, this site must be located near fibre macromolecules, such as lignin or cellulose.

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2. MATERIALS PREPARATION AND EXPERIMENTAL METHODS

The sorption of copper ions was studied by using CuSO₄ solutions prepared with a Carlo Erba Atomic Absorption standard solution. The biomasses were prepared with dried leaves of lettuce (Lactuca sativa) and corn husks (Zea mays). Biomasses were sun dried during 4 weeks then dried at 35°C during 7 days. After grounding in a domestic blender to a size of about 1mm the biomasses were dried at 70°C during 24h and washed in a water solution with pH=4 using a drop of diluted HCl solution. Chemicals used were analytical grade Aldrich products.

The sorption experiments were done as described in [5] (equilibrium experiments): 100mg biomass were contacted with 50mL CuSO₄ solution, with Cu initial concentrations varying from 20µg/mL to 200µg/mL. The pH of the solution was controlled and adjusted to 4.0. After 1h of contact, the solution was filtered and the Cu final concentration was determined. The Cu concentrations of the initial and final solutions were determined by Atomic Absorption Spectrometry (AAS) in a CG-AA-7000 equipment, following traditional procedures [6].

The metal uptakes q were determined from the initial and final Cu concentration (Cᵢ and Cₖ, respectively) in a solution of volume V, and with a mass M of biomass, as described in [7]:

\[ q = \frac{V(C_i - C_f)}{M} \]  

For each biomass the uptake results were fitted using the Langmuir sorption model [8,9]:

\[ q = q_o b C_f / (1 + b C_f) \]

where \( q_o \) and b are the characteristic parameters of the Langmuir isotherm: \( q_o \) represents the saturation uptake for high equilibrium concentrations and b is related to the affinity of the metal ion with the biomass structure, which defines the inclination of the isotherm for low equilibrium concentrations.

For EPR measurements copper-charged samples of L.sativa were dried and inserted in quartz tubes in a custom-build spectrometer for spectra recording. The powder-like spectrum of Cu²⁺ in the biomass was simulated for comparison with the spectrum obtained experimentally. Details of the sample preparation and of the EPR spectra recording and simulation are given in [4], as well as an overview of the EPR theory.

For FTIR studies natural and copper-charged samples of L.sativa were prepared as pellets using KBr (Graseby Specac LTD.) as substratum. Spectra were recorded with a BOMEM-DA8 FTIR spectrometer and deconvoluted. Details of the sample preparation, FTIR measurements and analysis are given in [4], as well as an overview of the FTIR theory.

3. RESULTS AND DISCUSSION

3.1 Sorption isotherms

Figure 1 shows the sorption isotherms constructed by fitting Langmuir-type curves to the uptake values measured for L. sativa - lettuce (L) and Z. mays - corn husk (Z), compared with those reported for M.truncata fibres (F) and complete leaves (C) from [4].

Table 1 shows the adjusted values of \( q_o \) and b for the biomasses studied, as well as the correlation coefficient \( \chi^2 \) for the adjusted curves.
Figure 1. Sorption isotherms for *L.*sativa (L-□), *M.*truncata fibres (F-○) [4], *M.*truncata complete leaves (C-X) [4] and *Z.*mays (Z-●).

Table 1. Values for *q*<sub>o</sub>, b and *χ*<sup>2</sup> for the biomasses showed in Figure 1

<table>
<thead>
<tr>
<th>biomass</th>
<th><em>q</em>&lt;sub&gt;o&lt;/sub&gt; (mg/g)</th>
<th>b (L/mg)</th>
<th><em>χ</em>&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>*L.*sativa</td>
<td>33 ± 1</td>
<td>0.22 ± 0.04</td>
<td>11</td>
</tr>
<tr>
<td>*M.*truncata (fibers)</td>
<td>26 ± 4</td>
<td>0.04 ± 0.02</td>
<td>10</td>
</tr>
<tr>
<td>*M.*truncata (complete)</td>
<td>21 ± 1</td>
<td>0.02 ± 0.03</td>
<td>9</td>
</tr>
<tr>
<td>*Z.*mays</td>
<td>6 ± 3</td>
<td>0.04 ± 0.01</td>
<td>7</td>
</tr>
</tbody>
</table>

It can be seen that the sorption capacity of the *Z.*mays is much smaller than the sorption capacity of all other biomasses, and that *L.*sativa biomass presents the highest sorption uptake capacity. Since *Z.*mays is formed mainly by lignin while *L.*sativa is mainly composed of cellulose, we conclude that the cellulose is the macromolecule responsible for the metal sorption in dried leaves.

### 3.2 EPR spectra

Figure 2 shows the EPR spectrum for copper-charged *L.*sativa and the simulated spectrum obtained by assuming that the Cu ion is located in a site of nearly axial symmetry. For the simulation, the following spin Hamiltonian *H* was used:

\[
H = \beta S g B + S A I
\]

where the first term is the electron Zeeman and the second the hyperfine interaction. The symbols *g* and *A* denote the *g*-tensor and the hyperfine tensor, respectively, *β* is the Bohr Magneton and *S* and *I* are the electron and nuclear spin, respectively. For the analysis of the EPR spectrum of Cu<sup>2+</sup>, *S* = ½ and *I* = 3/2, with 100% natural abundance due to the two isotopes ⁶³Cu and ⁶⁵Cu, has been taken. The best simulation of the EPR spectrum for Cu<sup>2+</sup> in *L.*sativa could be obtained assuming axial *g* and *A* tensors with values of *g*<sub>||</sub> = 2.33 and *g*<sub>⊥</sub> = 2.11 and *A*<sub>||</sub> = 110 G and *A*<sub>⊥</sub> = 50 G, respectively.
Figure 2. Experimental (A) and simulated (B) EPR spectra for copper-charged \textit{L. sativa}.

From these results, in particular the \(g\) and \(A\) tensors symmetry, we conclude that the \(\text{Cu}^{2+}\) ion is incorporated in an axial site of the biomass structure. A similar result was found earlier for other biomasses [4].

3.3 FTIR spectra

Figure 3 shows the FTIR absorption spectra for natural (A) and copper-charged (B) \textit{L. sativa}, normalized to account for thickness differences in the prepared samples. It can be seen that the presence of copper modifies the infrared absorption of the sample in the region between 800 cm\(^{-1}\) and 1800 cm\(^{-1}\). This region is amplified and deconvoluted in Figures 4A (natural \textit{L. sativa}) and 4B (copper-charged \textit{L. sativa}).
Figure 4. FTIR absorption spectra and deconvolution for natural (A) and copper-charged (B) *L. sativa*

The absorption peaks shown in Figures 4A and 4B can be assigned to vibration modes of molecular bonds of the glucose rings ramifications of cellulose [10]. This assignment is shown in Table 2.

Table 2. Assignment of FTIR absorption peaks (Figure 4) to cellulose molecular vibrations.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Wavenumber (cm⁻¹)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>880</td>
<td>C-H out-of-plane deformation</td>
</tr>
<tr>
<td>2</td>
<td>1010</td>
<td>C-O stretching coupled to ring modes</td>
</tr>
<tr>
<td>3</td>
<td>1055</td>
<td>C-O stretching coupled to ring modes</td>
</tr>
<tr>
<td>4</td>
<td>1120</td>
<td>C-O stretching coupled to ring modes</td>
</tr>
<tr>
<td>5</td>
<td>1160</td>
<td>C-O-C stretching</td>
</tr>
<tr>
<td>6</td>
<td>1245</td>
<td>C-O stretching</td>
</tr>
<tr>
<td>7</td>
<td>1325</td>
<td>ring breathing with C-O stretching</td>
</tr>
<tr>
<td>8</td>
<td>1385</td>
<td>CH bending</td>
</tr>
<tr>
<td>9</td>
<td>1450</td>
<td>CH₂ symmetrical bending</td>
</tr>
<tr>
<td>10</td>
<td>1525</td>
<td>C=C in carbon rings</td>
</tr>
<tr>
<td>11</td>
<td>1645</td>
<td>C=C stretching or O-H bending</td>
</tr>
<tr>
<td>12</td>
<td>1745</td>
<td>C=O stretching</td>
</tr>
</tbody>
</table>

It can be seen that the presence of copper causes changes in the peaks 8 and 9 (CH₂ vibrations); 2, 4 and 7 (CO modes); and 11 (OH vibration). No absorption peaks were created or extinguished by the presence of the copper in the biomass indicating that no molecular bonds were formed or destroyed after the sorption of the metal ion.

It is worthy to note that our investigation was limited to the mid-IR region (500-2000 cm⁻¹). In this region we can observe practically all-internal molecular vibrations and, thus, the characteristics of organic molecules. Nevertheless, absorption peaks originated from vibrational modes of ionic species like copper would be located at lower frequencies (below 500 cm⁻¹) and cannot be observed in samples made with KBr substrata.

Similar changes in the absorption peaks of dried plants charged with metallic ions were seen in other works that studied copper sorption in other biomasses [3,4] or other metals sorption in *L. sativa* [11].
4. DISCUSSION

The results presented above allow us to conclude that cellulose is the macromolecule responsible for the copper sorption in biomasses prepared from dried plant leaves. Cellulose is a polymer composed of glucose monomers, joined by the sharing of an oxygen atom, which can rotate around the molecular long axis, creating hydrogen bonds between two monomers, depending on their relative positions. Figure 5 illustrates part of the cellulose chain.

![Figure 5. Part of the cellulose chain showing glucose monomers and their bonds with neighbors](image)

Our EPR spectra revealed that the copper ion is located in a site with strong axial symmetry and FTIR spectra showed that the presence of the copper ion affects CH₂, CO and OH bonds of cellulose and that no molecular bonds were created or destroyed in the process. CH₂ and OH are present in the ramification and CO in the glucose rings of cellulose. OH bonds can also come from H₂O molecules present in the structure.

To fit the information obtained, we propose the model shown in Figure 6, where the copper ion, hydrated with 4 H₂O molecules, is located near two of the CH₂-OH cellulose ramifications, from two different glucose chains or from the same chain, folded in this region. CO bonds of the glucose ring near the ramification are also affected by the presence of the metal ion.

In the solution, the copper ions are hydrated with 6 H₂O molecules, in an octahedral symmetry; after sorption in the biomass, the ion looses two of the H₂O molecules, situated in an axial position, in order to accommodate inside the biomass structure. The four remaining hydration molecules are located in a plane orthogonal to the axis formed by copper and the CH₂-OH ramifications, explaining the axial symmetry of the copper ion neighborhood found in the EPR studies.

![Figure 6. Model for the site of a copper ion (solid circle at the center) after biossorption by L. sativa](image)

Brown and Kevan [12] report an EPR study in copper-containing clays where the spectra obtained are similar to ours and are explained with the presence of a 4-hydrated copper ion in an intermediate layer between the crystal layers.
In a recent work, Boutreau and col. [13] used computational methods to show that, in the minimum energy situation, the copper ion affects O-H and ramification bonds of glucose, in agreement with our infrared results.

5. CONCLUSION

Sorption isotherms, EPR spectra and FTIR absorption spectra of the biosorption of copper ions in *L.sativa* (lettuce leaves) and *Z.mays* (corn husk) biomasses led us to conclude that cellulose is responsible for biosorption in dried leaves, and that, after sorption, a hydrated copper ion is located near two glucose rings of the cellulose structure, in a site near the glucose ramification and with axial symmetry neighborhood.

Dried lettuce leaves showed to be an efficient raw material in regards to metal biosorption and could be used in the treatment of metal-loaded disposal waters by means of, e.g., continuous-flow sorption columns [9]. Since there is a huge waste disposal of salad leaves in the food distribution centers of all big cities, the use of this biomass for metal sorption could help the depollution of contaminated waters and will be a way to alleviate the problems of unsold leaves disposal.

ACKNOWLEDGEMENTS

We wish to thank Dr. Marilene Marinho Nogueira and Ms. Zabelê Dantas Moura, from the Depto. Fisiologia Vegetal (UFMG) for the discussions on the plant composition, Ms. Zenaide Souza Vasconcelos, from the Depto. Engenharia Química (UFMG), for the AAS determinations, and our colleague Dr. Roberto L. Moreira for his help with the infrared analysis. This work was supported by the Brazilian financing agencies CNPq and FAPEMIG.

REFERENCES

Agar-plate screening of effective metal biosorbents among yeast

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Abstract

The use of microbial cells as biosorbents for heavy metals offers a potential alternative to existing methods for decontamination or recovery of heavy metals from environment. Yeasts can be successfully used in metal sorption. An agar-plate screening method was developed for a rapid isolation of heavy metal-accumulating microorganisms and preliminary estimation of their biosorption capacity. In present investigation a variety of pink-coloured and pigment-less yeast cultures isolated from different habitats (67 strains) were screened for accumulation of zinc, copper, lead, chromium and cobalt ions using agar-plate method. According to the agar-plate screening data, the best copper and zinc accumulation capacity was found for pink-coloured yeast \textit{Rhodotorula mucilaginosa}, \textit{Rhodotorula aurantiaca}, \textit{Rhodotorula glutinis} and pigmented-less yeast \textit{Candida krusei}, \textit{Williopsis californica}.

Keywords: metal, biosorption, yeast, agar-plate screening

1. INTRODUCTION

An increase of environmental heavy metals pollution over last years has led researchers to search for the efficient methods for the treatment of heavy metals using biosorbents. Among the most perspective groups of microorganisms which have the ability to sorb heavy metals such as copper, zinc, lead, cobalt, chromium are the yeasts [1-7]. Yeasts are easy to grow, produce high yields of biomass and at the same time can be manipulated genetically and morphologically [2, 7]. It is known, that the yeasts can remove heavy metals and radionuclides from aqueous solutions and soils in substantial quantities [4, 8]. The yeast is capable to grow at rather high concentration of heavy metals. The resistance of the yeast to heavy metals varied considerably with metal and yeast genera [9-11].

The aim of this study was an extended screening of the most widespread species of yeast for their metal sorption ability using agar-plate screening method based on the visualization and interpretation of the metal distribution between agar and colonies by chemical precipitation with hydrogen sulphide [12]. The best biosorbents found among tested yeasts were studied further as alive population in submerged culture for their resistance to zinc and copper and metal accumulation.
2. MATERIALS AND METHODS

2.1 Microorganisms

There was used a variety of yeast cultures from the collection of yeasts of the Industrial Microorganisms Physiology Department, Institute of Microbiology and Virology of National Academy of Sciences of Ukraine, Kiev, isolated from diverse habitats such as soil, water, plants, human gastrointestinal tract and from industrial processes. A total of 67 yeast cultures comprising species of genera *Debaryomyces, Kluyveromyces, Saccharomyces, Williopsis, Candida, Cryptococcus, Rhodotorula, Sporobolomyces* were screened for metal sorption property.

2.2 Biomass preparation

The yeast biomass was grown in the medium of the following composition (g/l): (NH₄)₂SO₄ – 3.0; K₂HPO₄ – 0.1; KH₂PO₄ – 1.0; MgSO₄ – 0.7; NaCl – 0.5; glucose – 10.0; extract of yeasts 1%; pH was 6.8. For inoculation, the yeast cultures were grown 24h in the above medium with agar (20 g/l). For agar-plate screening method we used the above medium containing 5mM Cu²⁺, Co²⁺, Cr⁶⁺ and 10mM Pb²⁺, Zn²⁺ and 10 g/l agar. Metals were used as salts Cu(NO₃)₂, CoCl₂, K₂Cr₂O₇, Pb(NO₃)₂, ZnCl₂ that were added to the agar from a 100mM stock solutions after cooling the stock down to 55°C.

2.3 An agar-plate screening method for a rapid isolation of heavy metal-accumulating yeasts

The agar-plates were inoculated by punctual inoculation with yeast strains (4-8 colonies in one plate). The plates were incubated for 48h at 28°C and 60% relative humidity until the biggest colonies reached about 4 mm in diameter in the dark to allow the yeasts to accumulate the metal. After this 48h they were exposed to gaseous H₂S for 10 min in a desiccator [12]. H₂S was generated by reacting 3g Na₂S with the stoichiometric amount of 10% HCl. After incubation the metal became visualized. The main optical effects are the staining of colonies due to accumulated and precipitated metal and the formation of light haloes around the colonies within the uniformly darkened agar as a result of the diffusion of dissolved metal towards the organism. The main parameter, indicating metal accumulation ability of yeast, was diameter of a halo. The plates were inspected using light microscopy. Forming the light haloes around the yeast colonies was detected and yeasts of interest were taken from the plates for further investigation.

2.4 Determination of metal ion accumulation and metal resistance of the best-found biosorbents in submerged culture

To estimate the metal resistance of selected isolates, the test tubes containing 9.9 ml of medium mentioned before and comprising Cu²⁺ or Zn²⁺ in concentration 5-500 mg/l were inoculated with 0.1 ml of cell suspension (10⁹ cells/ml) of the yeast cultures grown for 24h. The cultures in tubes were grown aerobically at 28°C on a shaker during four days. The criterion of resistance of yeast cultures was yield of biomass, measured as optical density at 540 nm and recalculated as biomass dry weight, at different initial metal concentrations compared to non-metal control variant of medium. Initial and final (after yeast growth) values of medium pH were estimated. Metal concentration was measured using AAS in liquid medium after yeast biomass separation.
Table 1. The ability of yeasts to form light haloes around the colonies. Mean values ±SEM, n=3

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<thead>
<tr>
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<th>Strains</th>
<th>The light haloes, mm</th>
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<th>Pb²⁺</th>
<th>Co²⁺</th>
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3 RESULTS AND DISCUSSION

A total of 67 different strains were used in the study of accumulation of metals (copper, lead, zinc, cobalt and chromium). Our agar-plate screening data demonstrated very high inter- and intraspecific variations in metal accumulating capacity evaluated as light haloes in agar for studied yeasts (Table 1). Different isolates of the same species showed remarkable differences in metal accumulation from total inactivity to median and high activity for at least three studied metals (e.g., *K. lactis* 383, 1891, 1892 versus *K. lactis* 327, or *W. californica* 250, 258 versus *W. californica* 248). Among all investigated strains the ability to sorb copper was found to be the best for genera of *Rhodotorula* and *Debaryomyces*, *Williopsis*, lead for genera of *Rhodotorula*, *Cryptococcus*, *Kluyveromyces*, *Debaryomyces*, zinc for genera of *Rhodotorula* and *Cryptococcus*, *Williopsis*, cobalt for genera of *Rhodotorula* and *Debaryomyces*, chromium for genera of *Rhodotorula* and *Saccharomyces* (Table 1). Toxic metal sorption capacity varied widely among different yeast strains. As control cultures, that have been studied previously [3], we used strains of...
pigment-less *Saccharomyces cerevisiae* 1968 (industrial strain), pink *Rhodotorula* sp. 4 and black *Cryptococcus* sp. WT as examples of yeasts with different cell wall composition and with known metal sorption ability. Ability to accumulate heavy metals was observed for both pigmented and pigmented-less strains of yeast. The one-way ANOVA analysis of the data obtained (Table 1) showed that for all studied metal species the diameters of light haloes did not differ significantly for pigment-less yeast strains compared to pink ones (P-values>0.1 for all metal ions). However the best uptake capacity was shown by some pink-pigmented yeasts of genera *Rhodotorula*.

**Table 2. Growth of yeasts at different concentration of copper. *Metal-free control. Data are mean values ±SEM, n=3***

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<tr>
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<td>1.6±0.1</td>
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<td><em>R. minuta</em> 1342</td>
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**Table 3. Growth of yeasts at different concentration of zinc. *Metal-free control. Data are mean values ±SEM, n=3***

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</table>
Biosorption

The sorption ability of metals depends on structure of cell wall of yeasts. The fungal (yeast) cell wall accounts for about 20-30% of cellular dry weight, is responsible for the shape of the cell, offers protection against mechanical damage and functions as a molecular sieve [13]. The best–studied from the Ascomycotina yeast in terms of cell wall composition and molecular architecture are currently S. cerevisiae and Candida albicans. The cell wall of the budding yeast S. cerevisiae contains four classes of components, namely chitin, β1,3-glucan, β1,6-glucan, and mannoproteins [13]. Many other yeasts cell walls contain α1,3–glucan. Other yeast’s cell wall carbohydrate polymers are protein-bound (galacto)mannan and acidic polysaccharides. Moreover, the cell walls of coloured yeasts contain different concentrations of pigments. The strains of pink-coloured yeasts (Rhodotorula) can produce very high quantity (630 µg/g DW biomass) of carotenoids pigments (beta-carotene, torulene, torularhodin) [14]. The black yeasts (e.g., Cryptococcus, Exophiala) blacken by the polymerization of dihydroxynaphthalene (DHN) into melanin (DHN-melanin) in their cell walls [15]. The main role in the processes of sorption by pigment-less yeasts is played by chitin and glucan-mannoprotein complex. Whereas for sorption by pigmented yeasts, the significant role is played by chelation properties of melamins and carotenoids. The major constituents of fungal cell walls such as chitin and melanin (for black yeasts) have significant metal binding abilities [2, 7, 9, 16]. The cell wall components of both main groups of tested yeast cultures, pigment-less Saccharomyces, Candida and Williopsis, containing chitin, glucans and mannan, and pink Rhodotorula and Sporabolomyces, containing chitin and carotenoid pigments and deprived of mannoproteins, could have active metal sorption sites [6, 7, 16]. Negative charge of yeast cell walls is formed mainly by carboxyl, hydroxyl, and phosphate groups. Composition, architecture and sorption properties of yeast cell wall can be altered by different conditions of growth, age and physiological state of cells [6, 10].

Metal sorption by yeast biomass has been studied for more than twenty years [1, 2, 4, 8, 9, 11, 17], but there is still a lack of information about a connection between sorption capacity of yeast cells and cell walls composition, as well as between sorption sites availability and physiological state of yeast cells.

As a result of agar-plate screening, 13 cultures, that demonstrated the greatest values of light haloes on the plates with copper or zinc, were selected for further investigation of their zinc and copper toxicity and their ability to sorb heavy metals from medium in submerged culture (Table 2, 3, Fig. 1, 2). The pH values of medium after yeast growth decreased from initial 6.8 to 4.3-5.7 for copper-containing media, and to 3.9-4.8 for zinc-containing media. Resistance of yeast cultures to copper and zinc, measured as biomass yield in liquid medium, varied between species and strains (Table 2, 3). The most copper-resistant yeasts were R. aurantiaca 1195 and R. mucilaginosa 1803 (Table 2). Rhodotorula strains showed also the highest resistance to zinc (Table 3). The most sensitive to zinc yeasts cultures appeared to be pigment-less yeasts (Candida, Saccharomyces and Williopsis) (Table 3).

In general, high metal resistance of pigmented yeasts compared to pigment-less suggests the protective role of yeast pigments (carotenoids, melamins) [17]. However, the ability of carotenoid pigments production by Rhodotorula cultures was altered by copper and zinc. It was found that pink cells of most Rhodotorula strains were gradually losing their colouring with increasing copper or zinc concentration and some of them completely lost the ability to form pigments at 100 mg/l (e.g. R. mucilaginosa 1803 (copper-medium) and 1776 (zinc-medium).

Sorption screening experiments on the removal of metal ions by alive yeast biomass from liquid medium showed that the best copper sorbents were R. aurantiaca 1195 and C.
krusei 61T, regardless control strains S. cerevisiae 1968, Rhodotorula sp. 4 and Cryptococcus sp. WT (Fig. 1). There were found very similar zinc sorption data that did not differ statistically for the most of tested cultures. The high zinc sorption capacities were demonstrated by R. aurantiaca 1195, R. mucilaginosa 1776, R. minuta 1342 and W. californica 248 and were similar to the values, obtained for control strains S. cerevisiae 1968 and Cryptococcus sp. WT (Fig. 2). Such variation in metal accumulation by alive yeasts could be due to the differences in mechanisms of extra- and intracellular metal sequestration due to the differences in the cell wall and extracellular matrix structure, intracellular metal transport, compartmentation, efflux of metal cations, etc. [2, 7, 11, 16, 17]. It was also revealed some weak negative correlation (Zn: R=−0.57; Cu: R=−0.3) between biomass produced by yeasts, indicating their metal tolerance, and percent of metal removal from liquid medium at initial metal concentration 50 mg/l.

![Figure 1. Cu²⁺ sorption by yeasts at initial metal concentration 50 mg l⁻¹. Data are means derived from three replicated determinations, error bars are ±SEM](image)

In terms of specific metal sorption capacity per gram of sorbing biomass, the highest specific copper sorption was found for R. aurantiaca 1195 (240 mg/g biomass) and R. mucilaginosa 1779 (255 mg/g biomass), being at least twice greater than values for all other tested cultures. The considerably higher values of specific zinc sorption (twice and threefold) were found for R. aurantiaca 1195 (182 mg/g biomass), and R. minuta 1342 (284 mg/g biomass) and Cryptococcus sp. WT (280 mg/g biomass). All mentioned above cultures were pigmented.

According to the results of both agar-plates and aqueous solutions metal sorbent screening experiments, including analysis of specific sorption capacity per gram of
biomass, *R. aurantiaca* 1195 appeared to be the most efficient universal sorbent for both copper and zinc among all tested cultures.

However, the analysis of the data of light haloes and percent of metal removal from liquid medium for zinc showed the absence of correlation between these two groups of data. Moreover, some strains (e.g., *R. mucilaginosa, C. krusei* (see Table 1)) that did not produce a light haloes around colonies in agar appeared to be efficient (40-50%) in zinc removal from liquid medium. Such difference between data from agar-plates and liquid medium screenings could be a consequence of abiotic and biotic factors. Abiotic factors could be different physico-chemical conditions in two methods affecting the changes in metal speciation and availability (and bioavailability). Biotic factors might be the differences between the growth conditions on solid and liquid medium altering metal toxicity and leading to the physiological and biochemical changes in yeast cells, including cell walls composition and sorption properties.

Nevertheless, quite high positive correlation (R=0.75) between the data of light haloes and percent of metal removal from liquid medium was found for copper. Thus, the suitability of agar-plate screening for metal biosorbents depends on metal species as it has been already discussed [12].

![Figure 2. Zn²⁺ sorption by yeasts at initial metal concentration 50 mgT⁻¹. Data are means derived from three replicated determinations, error bars are ±SEM](image-url)
4. CONCLUSION

It can be concluded that the agar-plate screening method is very useful for extended primary search for efficient metal biosorbents and it could be a great help for the initial qualification of the sorption properties of alive yeast biomass, being combined and verified with traditional methods of metal sorption from aqueous solutions. However, it should be taken into account that this method has some limitations, e.g., evaluates metal sorption capacity of biomass only indirectly and under specific conditions (surface growth on agar medium), and strongly depends on metal species and yeast strains.

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Bioremediation of chromium using *Bacillus polymyxa*

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**Abstract**

The use of a Gram positive, neutrophilic, facultative anaerobe, namely *Bacillus polymyxa* in the biosorption and the bioreduction of chromium species has been illustrated. Bioreduction of Cr (VI) and its biosorption has been monitored during bacterial growth using Cr (VI) adapted and unadapted strains. Bioremoval of both Cr (VI) and Cr (III) has been assessed with respect to time, pH, initial chromium concentration and biomass loading. The results indicate that bioreduction of Cr (VI) is feasible during growth of both adapted and unadapted strains. About 90% bioremoval of Cr (VI) could be achieved in 72 h using unadapted *Bacillus polymyxa*, while it took only 48 h using adapted bacteria for a similar amount of reduction. The bacterial metabolite is also found to be efficient in bioremoval of Cr (VI). Possible mechanisms of bioremoval of chromium species are discussed.

**Keywords:** bioremediation, chromium, *Bacillus polymyxa*, biosorption, bioreduction

1. **INTRODUCTION**

Chromium is a metal that exists in a variety of oxidation states, the most common being the +3 and the +6 forms. In the industrialized world, the use of chromium in industries like electroplating, textile, leather tanning, metallurgical metal finishing, photography, dye manufacturing, ink and pigments, power generation, and chemical manufacturing etc., is extensive, and hence it is not uncommon for the aqueous effluents from such industrial plants to have high amounts of chromium. Additionally, this can lead to the contamination of the soils or sediments that they contact. It has been estimated that more than 1,70,000 tonnes of chromium wastes are released annually in the United States of America, mainly due to industrial practices [1]. The consumption of basic chromium sulphate in the chrome tanning industries is reported to be about 50,000 tonnes per annum in India alone [2]. Hexavalent chromium (Cr (VI)) compounds are known to be toxic, mutagenic and carcinogenic, apart from being highly water-soluble. The Cr (III) form on the other hand, is innocuous and less soluble.

Several conventional methods like precipitation and ion exchange are known for the elimination of toxic heavy metals from aqueous solution, but they are of high cost and low economic viability, especially at low and variable heavy metal concentration, and thus make these processes less advantageous. Accumulation of metals by microorganisms has been known for a few decades but has received more attention in recent because of its potential application in environmental protection or recovery of precious or strategic metals. Development of new biosorbent materials from microbial biomass is an emerging
area of significant interest. This biotechnological tool has great importance, as it is both cost effective and environmental friendly.

Microbial reduction of toxic hexavalent chromium to the less soluble trivalent form represents a useful detoxification process that has been shown to be of practical importance for the removal of chromium from industrial wastewaters [3-4]. The unique ability of bacteria to reduce Cr (VI) may provide a possible means for cleaning up hazardous Cr (VI) wastes. The earliest reports pertained to a microorganism, Pseudomonas chromatophila, isolated from industrial sewage, which could use chromate or dichromate as a terminal electron acceptor during anaerobic respiration [5]. Subsequently, a number of microorganisms have been identified, which are capable of reducing hexavalent chromium, under different conditions [6-7]. It thus becomes imperative to devise suitable strategies to detoxify the Cr containing wastewater. Keeping these objectives in mind, the present investigation was taken up. The thrust of the present investigation has been directed towards the assessment of the potential of a Gram positive, heterotrophic, neutrophilic, facultative anaerobic soil bacterium, namely Bacillus polymyxa, for the bioremoval of Cr (VI) and Cr (III).

2. EXPERIMENTAL

2.1 Bacterial strain

A pure strain of Bacillus polymyxa NCIM 2539 was obtained from the National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India and used for all the studies. The bacterium was cultured in the modified Bromfield medium [8]. The pH of the medium was adjusted to 7.

2.2 Reagents

Analytical grade reagents namely potassium dichromate (K₂Cr₂O₇) and chromium nitrate (Cr(NO₃)₃.9H₂O) were procured from Qualigens, Mumbai, India and Loba Chemie Ltd., Mumbai, India, respectively and used as the hexavalent and trivalent forms of chromium respectively.

Nitric acid and potassium hydroxide were used as pH modifiers. All the reagents used in this study were of analytical reagent grade and were made up in deionised double distilled water of conductivity < 1.5 µmho

2.3 Analytical estimation of Cr (VI) and Cr (III)

A 0.25% w/v solution of diphenyl carbazide was prepared in 50% acetone. 15 ml each of the sample solution, containing various concentrations of Cr (VI) were pipetted out into 25ml standard flasks. To this 2ml of 3M H₂SO₄ was added followed by 1ml of diphenyl carbazide and the total volume was made upto 25 ml using deionised double distilled water such that the final concentrations were in the range 0.15 to 0.3 ppm. The intensity of the colour complex formed was measured using a Shimadzu model UV-260 uv-visible spectrophotometer. The absorbance was measured against a reagent blank at 540-nm wavelength maximum. A linear plot was obtained indicating adherence to the Beer-Lambert's law in the concentration range studied.

The total chromium was analysed using a Thermo Jarrell Ash Video 11E atomic absorption spectrophotometer. The instrumental parameters were set as per the specifications provided [9]. The concentration of Cr (III) was obtained as the difference
Biosorption between the values of the total chromium content and the amount of Cr (VI) estimated as per the procedures highlighted above.

2.4 **Bacterial cell count**

The bacterial cell count was enumerated by microscopic counting using a Petroff-Hausser counter under a phase contrast Leitz microscope.

2.5 **Bioremoval and bioreduction studies**

The bioremoval of Cr (VI) and Cr (III) was studied using

1. Metabolite devoid of cells
2. Cells

The bioremoval of Cr (VI) and its subsequent reduction to Cr (III) was monitored

1. During growth studies with unadapted strain
2. During growth studies with the bacterial strain adapted to 2 ppm Cr (VI)

The detailed procedures are described elsewhere [10].

2.6 **Biosorption studies**

Biosorption studies of both Cr (VI) and Cr (III) were studied, using the modified Bromfield medium grown cells, as a function of various parameters such as time, pH, amount of wet biomass and chromium concentration.

2.6.1 **Kinetics of biosorption**

The cell pellet was dispersed in 100 ml of 2 ppm Cr (VI) or Cr(III). Ten such flasks were agitated in a Remi rotary shaker at 240 rpm at 30°C ± 2°C and at regular time intervals centrifugation was done and the supernatant was analysed for the residual chromium content. The final pH was also noted. A blank was maintained without adding the Cr (VI) or Cr(III) solution.

Another experiment was performed using the cells, which were adapted to 2 ppm Cr (VI). In this, the adapted cells were dispersed in 100 ml of 2ppm of Cr (VI) and agitated as per the same conditions given above. At regular time intervals, the cells were separated by centrifugation and the supernatant was analysed for Cr (VI) content. All these experiments were carried out at natural pH of the respective solutions, namely 5.75 in the case of 2 ppm Cr (VI) and 5.45 in the case of 2 ppm Cr (III)

2.6.2 **Effect of pH on the biosorption process**

The stability of 2 ppm Cr (VI) and 2 ppm Cr (III) in the pH range from 2 to 7 was studied. Nitric acid and potassium hydroxide were used as pH regulators. Around 0.3 g of the wet biomass was dispersed in 100ml of the solution containing 2 ppm Cr (VI) or Cr (III). Eight such flasks were maintained at different pH values ranging from 2 to 7. After an equilibration period of 48 h in the case of Cr (VI) and 25 h in the case of Cr (III), the solutions were centrifuged and the supernatant was analysed for the residual concentrations of the chromium ions. The final pH values have been plotted.

2.6.3 **Effect of wet biomass loading**

A fully-grown culture was centrifuged and different weights of the biomass ranging from 0.1g to 1.5 g were dispersed in solutions containing the desired amount of Cr ion under consideration. The solutions were adjusted to the optimum pH, corresponding to
maximum biosorption of the Cr ion. These values were 2 for Cr (VI) and natural pH (5.45) for Cr (III). The flasks were equilibrated in a Remi rotary shaker at 240 rpm, the temperature being maintained as 30°C ± 2°C, for the desired time period, namely, 48 h for Cr (VI) and 25 h for Cr (III). The solutions were later centrifuged and the metal ion concentrations were determined using the procedures described earlier.

2.6.4 Biosorption isotherms

A known amount of the wet biomass (1.9g) was dispersed in a desired concentration ranging from 2 to 25 ppm of either Cr (III) or Cr (VI). In all these cases the initial pH was adjusted to that of the optimum value namely 2 for the Cr (VI) system and 5.45 for the Cr (III) system. The flasks were equilibrated for their respective time periods (48 h for Cr (VI) and 25 h for Cr (III)), at the end of which the residual concentrations were determined.

3. RESULTS AND DISCUSSION

3.1 Bioremoval of Cr (VI) during the growth of Bacillus polymyxa (unadapted strain)

The decrease in the concentration of Cr (VI) during the growth of the bacteria was monitored. The bioreduction of Cr (VI) to Cr (III) and the biosorption process contribute to the total bioremoval. The bioremoval of 2 ppm Cr (VI) was examined during the growth of Bacillus polymyxa and the results are depicted in Figure 1. In these experiments, both the Cr (VI) and Cr (III) contents were monitored as a function of time. The percentage removal of Cr (VI) steadily increases with increase in time upto 72 h, wherein nearly 90% removal is achieved. Beyond that period no further improvement is observed. The amount Cr (III) obtained by bioreduction, closely parallels the bioremoval trend of Cr (VI). Nearly 65% reduction is effected in 72 h and thereafter a saturation value is attained. The amount of Cr biosorbed marginally increases with time and reaches a maximum value of about 25% in 54 h. A further increase in the growth period does not improve the biosorption efficiency. These results highlight that significant bioremoval of Cr (VI) can be achieved during the growth of Bacillus polymyxa. Many bacteria have been reported to be able to detoxify Cr (VI) by reducing it to Cr (III). The prevention of the adverse effect of Cr (VI) on its growth is achieved by either reduction or accumulation inside the bacterium or adsorption of Cr (VI) on its surface [11 - 13].

The bioremoval of 5 ppm Cr (VI) is shown in Figure 2. It is worthy of observation that the kinetics of this experiment is much slower, when compared to that with 2 ppm Cr (VI). The reason for this could be due to the increase in toxicity. In 24 h, only 15% bioremoval is obtained, mainly contributed by means of reduction to Cr (III). The bioremoval efficiency steadily increases with increase in time upto about 160 h and thereafter attains a saturation value of about 85%. It is noteworthy that the bioreduction efficiency is about 73% during this period. The contribution due to biosorption is only about 10% with respect to Cr uptake. This trend is similar to that observed with 2 ppm Cr (VI) (Figure 1).
3.2 Bioremoval studies using cells adapted to Cr (VI)

Figure 3 depicts the percentage bioremoval of 2 ppm Cr (VI) using the *Bacillus polymyxa* strain, which has been adapted to 2 ppm Cr (VI) concentration. The percentage bioremoval steeply increases with increase in time up to about 24 h and thereafter a slight decrease in the rate is observed. The percentage bioremoval is 62% in 24 h while the bioreduction is about 60%. The contribution of biosorption is only about 2% in 24 h and marginally increases to 8% between 48 h and 72 h. In 48 h complete bioremoval of Cr (VI) is effected, of which 92% is bioreduced to Cr (III) and 8% is biosorbed.

A comparison of Figures 1 and 3 reveals that the adapted cell is more efficient for the bioremoval process. The time taken for 90% bioremoval is 72 h in the case of the unadapted strain, whereas the adapted one takes only around 48 h to achieve comparable results. Moreover, it is also evident that the adapted strain is more efficient in bringing about the reduction of Cr (VI) to Cr (III). For example, the percentage bioreduction with the adapted strain is about 1.5 times better than the unadapted one. It is also pertinent to observe that the biosorption efficiency of the adapted strain is much lower than that of the unadapted one. It is thus apparent that the bioreduction mechanism is a major contributing factor to the overall bioremoval process.

Figure 4 shows the bioremoval of 5 ppm Cr (VI) as a function of time, using a strain initially adapted to 2 ppm Cr (VI). The percentage bioremoval increases with increase in time up to about 165 h and thereafter attains a saturation value. The maximum bioremoval obtained is around 90%. A similar trend is observed with respect to the bioreduction to Cr (III) and 70% reduction is achieved in about 165 h. On the other hand, only about 20% biosorption is achieved during this time period. As observed in the case of unadapted strain (Figure 2), bioreduction contributes a significant extent to the bioremoval process.

Consequent to adaptation, there is a marginal improvement in the percentage bioremoval, though the percentage bioreduction achieved is very similar with and without adaptation. On the contrary, there is an improvement in the percentage biosorption from 10% to 20% after adaptation (Figures 2 and 4).
3.3 Bioremoval studies in the presence of metabolite

The bioremoval of Cr (VI) was next investigated using the metabolite devoid of cells. From Figure 5 it is evident that there is a steep rise in the bioremoval efficiency upto 10 h and thereafter there is only a marginal improvement, for the two concentrations studied. On a comparative basis, the bioremoval efficiency is marginally better when a lower concentration of 2 ppm Cr (VI) is used. Over 80% bioremoval is achieved in about 10 h using 2 ppm Cr(VI), while it takes almost 48 h for a similar amount of removal to be effected using 5 ppm Cr(VI). Figure 6 depicts the bioremoval of Cr (VI) using the metabolite derived during the growth of the strain adapted to 2 ppm Cr (VI). The bioremoval efficiency is better than in the previous case (Figure 5) and complete removal is possible in 24 h. With respect to 5 ppm, nearly 36 h are required to bring about 90% removal. Metabolites contain organic acids, proteins and the exo-polysaccharides secreted by Bacillus polymyxa during its growth.
metabolic products can facilitate the removal of Cr (VI). These results corroborate the results of the earlier studies, wherein adaptation enhances the bioremoval efficiency.

3.4 Bioremoval studies on Cr (VI) and Cr (III) using cells

3.4.1 Kinetics of bioremoval

The percentage biosorption of Cr (VI) and Cr (III) as a function of time is shown in Figure 7. In this experiment, the initial concentration was 2 ppm for both Cr (VI) and Cr (III). The pH of the system for Cr (VI) and Cr (III) was 5.7 and 5.5 respectively. The amount of wet biomass was around 0.3 g. It is evident that the kinetics of the biosorption process is very slow and the maximum amount of Cr (VI) biosorbed is only about 18% after 48 h. A further increase in the biosorption time up to 100 h does not improve the uptake. Hence the time of equilibration was fixed at 48 h in all further experiments using Cr (VI). It is evident that the amount of Cr (III) biosorbed steeply increases with increase in time up to 24 h and thereafter attains a saturation value of about 75%. It is noteworthy that there is a preferential biosorption of Cr (III) by the cells vis-à-vis Cr (VI). The equilibration time in all the subsequent experiments using Cr (III), was fixed at 24 h.

Such slow kinetics can be associated with the intracellular uptake of Cr (VI). There are two possible reasons for the low uptake:

1. K₂Cr₂O₇ around natural pH exists in the form of HCrO₄⁻ [14]. The bacterial cell wall is negatively charged and hence interaction with Cr (VI) is not favorable due to electrostatic forces of repulsion.
2. Cr (VI) is mutagenic and carcinogenic [15-16]. The bacteria are capable of sensing the toxicity of Cr (VI) and hence accumulate very less quantity of the metal. Reduction is possible only around very low pH values in the range of 2-3, and at natural pH, the bacteria are incapable of reduction, to overcome the adverse effect of the hexavalent salt.

3.4.2 Effect of pH

The cells obtained by centrifuging were washed and later dispersed in solutions of 2 ppm Cr (VI) or Cr (III). The pH values range from 1.5 to 7 in the case of Cr (VI) and pH 2-6 for Cr (III). The time of equilibration was fixed at 48 h for Cr (VI) and 24 h for Cr (III), and the final pH values were noted. The percentage bioremoval of Cr (VI) as a function of pH is depicted in Figure 8. The Cr (VI) bioremoval shows a marginal increase from pH 1.5 to 2 and thereafter continuously decreases with increase of pH. The maximum bioremoval of about 75% is observed at pH 2, while at pH 7 it is significantly reduced to 15%.

No precipitate of Cr (VI) was observed in the pH range studied. Cr (VI) is a strong oxidising agent and exists as monohydrogen chromate (HCrO₄⁻) and chromate (CrO₄²⁻) ion in solution and has a high solubility in water [17].

The following observations are noteworthy:

1. A variation in the pH values was observed between the initial and final readings. The shift was marginal in the pH range of 2 – 3 but was prominent at other pH values.
2. The bioreduction of Cr (VI) to Cr (III) is more pronounced in the acidic pH range and is significantly reduced at pH 7.
3. The percentage biosorption of Cr (VI) is about 22% and increases to about 30% in the pH range 2–3 and thereafter further increases, and at pH 7, it is enhanced to about 45%. 

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4. A comparison of the bioreduction and biosorption processes reveals that bioreduction contributes to a greater extent to the overall bioremoval below pH 3, while biosorption is more dominant beyond that pH.

5. The decrease in the percentage bioremoval and biosorption below pH 2 may be attributed to the production of Cr (III) and also the effect of the hydronium ions, which will then compete for the binding sites.

The effect of pH on the biosorption of Cr (III) is also shown in Figure 8. It is evident that the maximum uptake occurs at the natural pH (~6) of the system. The drop in the Cr (III) uptake at lower pH values can be attributed to the competition of H⁺ for the binding sites. As can be seen from the figure, there is hardly any significant uptake of Cr (III) up to pH 4. As the pH is increased, more negative sites are exposed and hence the biosorption increases. This can be visualized in the pH region of 4 to 6, wherein the biosorption increases from 12% to about 80%. After the maximum value, precipitation was observed beyond pH 6 and hence no experiment was conducted beyond pH 6. A comparison of the bioremoval results of Cr (VI) and Cr (III) reveals that Cr (VI) uptake is much higher than that of Cr (III) below pH 5. On the contrary, above pH 5, the trend is reversed with Cr (III) showing higher uptake. It is interesting to observe that Cr (VI) biosorption shows a maximum of about 75% at pH 2, while Cr (III) exhibits a maximum (79%) at pH 6.

![Figure 7. Kinetics of bioremoval of Cr (III) and Cr (VI)](image)

![Figure 8. Effect of pH on the bioremoval of Cr (III) and Cr (VI)](image)

3.4.3 Effect of biomass loading on Cr (VI) uptake

The effect of biomass loading on the bioremoval of Cr (VI) is shown in Figure 9a. In these experiments, the pH was fixed at 2, initial Cr (VI) concentration at 2 ppm and the equilibration time at 48 h. In the same figure, the specific uptake expressed as mg chromium/g biosorbent is also portrayed. It is readily evident that the percentage bioremoval steadily increases with increase in the biomass loading up to 0.5 g and thereafter there is only a marginal increase. Over 90% Cr (VI) bioremoval is achieved with 0.5 g, while at 0.92 g almost 100% bioremoval takes place. As can be expected, the specific uptake of Cr (VI) continuously decreases with increase in the amount of biomass. For example, the specific uptake is the highest at 0.03 g biomass, namely 12 mg/g and steeply decreases and is negligible with 0.92 g biomass loading. The trends observed are in good agreement with those reported earlier [18-20].
The contributions of the bioreduction and biosorption components to the bioremoval process have also been determined and these results are depicted in Figure 9b. The experimental conditions are the same as those pertaining to Figure 9a. The percentage bioreduction is found to increase with increase in the biomass loading. The percentage bioreduction is about 45% with 0.5 g biomass, while almost 100% bioreduction is achieved with 0.92 g biomass. It may be recalled that the results of the growth studies carried out in the presence of Cr (VI) also revealed the significant contribution of bioreduction to the bioremoval process (Figure 1). The percentage biosorption increases with increase in the biomass loading up to 0.5 g and subsequently decreases (Figure 9b). It is thus apparent that the biosorption process is more effective at lower biomass loading, whereas the bioreduction process is more efficient when the amount of biomass is increased. The decrease in the biosorption capacity at higher biomass loading can be attributed to the "screening effect", wherein cell–cell interaction inhibits the metal uptake from the solution. Similar results have been reported by other workers [14, 18].

The effect of increasing the amount of the wet biomass on the uptake of Cr (III) with other conditions like the pH, time etc, being maintained as outlined earlier, is depicted in Figure 10. The specific uptake capacity of Cr (III) is also shown in this figure. The percentage uptake steeply increases to about 85% with increase in the amount of wet biomass up to 0.4 g and thereafter there is only marginal improvement to about 90% at 0.95 g biomass. The specific uptake continues to decrease with the increase in the amount of biomass, from about 5.25 mg Cr (III) per gram at 0.9 g biomass to about 1.8 mg/g at 0.85 g biomass. As explained earlier with respect to biosorption of Cr (VI), a higher amount of the biomass results in the "screening effect" and consequently Cr (III) uptake is diminished.

3.5 Biosorption isotherms

The biosorption isotherm for Cr (VI) at pH 2, carried out at a temperature of 28°C ± 3°C is shown in Figure 11. In this experiment, the wet weight of biomass (substrate) used was 1 g and the equilibration time was fixed at 48 h. The adsorption density steadily increases with increase in equilibrium concentration up to about 7 ppm and thereafter attains a saturation value. The biosorption isotherm of Cr (III) is also depicted in Figure 11. The amount adsorbed steeply increases with increase in the equilibrium concentration of Cr (III) up to 15 ppm and subsequently, there is a decrease in the slope of the isotherm, tending to saturation coverage. Both the isotherms exhibit Langmuirian behaviour and resemble the type L2 of the Giles classification [21]. A comparison of the biosorption...
isotherms of Cr (VI) and Cr (III) with respect to the bacterial cells highlights that saturation coverage is attained at a much lower concentration for Cr (VI) vis-à-vis Cr (III). This is understandable as Cr (VI) is toxic, while Cr (III) is not.

**Figure 10. Effect of biomass loading on Cr (III) uptake**  
**Figure 11. Biosorption isotherms for Cr (VI) and Cr (III)**

### 3.6 Biosorption mechanisms

Biosorption mechanisms involving living cells include both metabolism dependent and metabolism independent processes. Further, there is a potential for biologically altering the valency state of the metal through the bioreduction mechanism and in some cases biodegradation of organometallic complexes has been achieved. Clearly, metabolism independent metal binding to cell walls and external surfaces is the only mechanism present in the case of non-living biomass. Metabolism independent uptake essentially involves adsorption processes such as ionic, chemical and physical adsorption. A variety of ligands located on the fungal walls are known to be involved in metal chelation [22]. These include carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups. Metal ions could be adsorbed by complexing with negatively charged reaction sites on the cell surfaces [23]. The relative importance of each functional group is often difficult to resolve. The microbial cell walls are rich in polysaccharides and glycoproteins such as glucans, chitin, chitosan, mannana and phosphomannans. These polymers are abundant sources of the above-mentioned metal binding ligands.

The FTIR spectral results confirmed the presence of functional groups such as NH, NH$_2$, CONH, OH, CO, PO, POC, CH$_2$, CH$_3$, and COO$^-$ groups [10]. It has been reported that different types of functional groups present on the cell wall such as carboxyl, amino, phosphate, hydroxyl are implicated in metal binding [24]. Cr ions could be adsorbed by complexing with the negatively charged reaction sites on the cell surfaces. Similar findings have been reported by other workers [23]. Furthermore, the chromium uptake is compounded by the complex solution chemistry of Cr (III) and Cr (VI) as a function of pH

### 4. CONCLUSIONS

From the results of the present investigation, the following conclusions can be drawn:

1. Both bioreduction and biosorption contribute towards the bioremoval of Cr (VI) during the growth of *Bacillus polymyxa*. Nearly 90% of 2ppm Cr (VI) is removed in 72 h, 65% by reduction to Cr (III) and 25% by biosorption.
2. Bioremoval studies using an adapted strain show a greater efficiency namely 90% bioremoval is effected in 48 h.

3. Complete bioremoval of Cr (VI) was achieved at pH 2 using bacterial cells under optimum conditions. The bioreduction of Cr (VI) to Cr (III) was observed in the pH range of 1.5 to 4.

4. About 90% uptake of Cr (III) could be obtained at a natural pH of 5.5.

5. The biosorption isotherms for both Cr (III) and Cr (VI) follow Langmuirian behaviour.

REFERENCES

**Biosorption and bioaccumulation of heavy metals by bacteria isolated from contaminated sites of Karachi, Pakistan**

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**Abstract**

Resistance to toxic heavy metals, their accumulation and biosorption by bacteria is a widespread phenomenon that could be exploited for the improvement of the environment. This study describes investigation of metal resistant bacteria isolated from metal contaminated industrial sites in Karachi, Pakistan. Biosorption and bioaccumulation were studied with reference to the cadmium, copper, and chromium. The isolates were identified by 16s rRNA gene analysis and by API kits. The *Pseudomonas* species were more prevalent, showing multiple metal resistances to copper, chromium, cadmium, nickel, zinc and cobalt salts. Maximum accumulation and biosorption of cadmium, copper and chromium was found in CMG64, CMG462, CMG463, and CMG480 respectively. The biosorption and bioaccumulation were periodically monitored and the metal concentrations were estimated by atomic absorption spectrophotometer (AAS) and by enzyme assays. The localizations or deposition of heavy metal inside/outside of the cell surface were further confirmed by transmission electron microscopy (TEM) and by energy dispersive x-ray analysis (EDX). These microbes are good candidates for bioremediation purposes.

**1. INTRODUCTION**

Prevalence of heavy metals in effluent is a major cause of environmental damages. The most prevalent ones include barium, cadmium, chromium, copper, iron, lead, manganese, nickel and zinc. In bacteria toxic heavy metal ion resistance systems have been reviewed over the last decade (1-5). The resistance mechanisms against all these heavy metals are highly specific. There is no general mechanism for resistance to all heavy metals. Microorganisms can physically remove heavy metals from solution through either bioaccumulation or biosorption (6-8). Bioaccumulation plays an important role in the detoxification of hazardous heavy metals. The uptake of metal ions onto the cell surfaces and their subsequent translocation into the cell are well known natural processes but are highly specific (9). Microbial cells can intracellularly and extracellularly accumulate both essential and non-essential metals such as chromium, cadmium, copper, nickel, lead, iron, germanium, silver and zinc. Several species of bacteria have been reported for the accumulation/uptake of various metal e.g., *Citrobacter* species accumulated cadmium and uranium (10-12), *Pseudomonas syringae* accumulated copper (13), *Pseudomonas stutzeri* accumulated germanium (14) etc.

Biosorption does not consume cellular energy. Positively charged metal ions are sequestered primarily through the adsorption of metals to the negative ionic groups on cell
Biosorption surfaces (8), such as the polysaccharide coating found on most forms of bacteria, or other extracellular structures such as capsules or slime. Metallic cations are attracted to the negatively charged sites at the surface of the cell (15). Anionic ligands such as phosphoryl, carboxyl, sulphydryl, and hydroxyl group of membrane proteins also involve in metal binding to the cell surface (16).

These processes are applied to clean the effluents, contaminated ground waters and soils. For the development of this technology microorganism especially bacteria are of great importance. They have the ability to reduce the toxicity of metals; this ability of bacteria can be harnessed in biotechnological applications for the removal/control of excess metal in various environments such as industrial and other wastes.

2. MATERIALS AND METHODS

2.1 Bacterial strains and growth media

CMG64, CMG462, CMG463 and CMG480, local isolates, were used in this study. Nutrient broth (Oxoid) was used as a starter medium. Maximum tolerable concentrations of various heavy metals were estimated in tris-minimal medium (5). Reduction experiment was performed in acetate minimal medium (AMM) as described earlier (8).

2.2 Identification of bacterial strains

The isolates (CMG462, CMG463 and CMG480) were identified using partial 16S rRNA gene analysis. A small colony of each was resuspended in 0.1 ml of sterile, de-ionised water (SDW), mixed and heated at 70°C (10 min) for cell lysis. Crude lysate (0.2 µl) was added to SDW (0.0198 ml) and used as a template in a polymerase chain reaction using the eubacterial 16S targeted PCR primers (pA and pH') as designed by Edwards (17). These are known to amplify a 1,536 base pair (approx. 1.5 kb) length of 16S rDNA. The reaction mixture for amplification was as published by Bruce et al, (1992). For PCR MJ thermalcycler (MJ Research Inc., USA) was used under tube temperature control and using 30 cycles of the following program: 94°C for 40 sec, 55°C for 1 min, 72°C for 2 min and a final 10 min extension at 72°C. PCR products were cleaned using Sephacryl S400 columns (Pharmacia, Sweden), and partially sequenced using 16S sequencing primer 943 reverse (Lane et al, 1985) by Alta Biosciences (University of Birmingham, UK). Sequences (up to 650 bp) were analysed using ADVANCED BLAST software to access the EMBL database, Heidelberg, Germany (Web ref: HTTP://www.ncbi.nlm.nih.gov/cgi-bin/Blast). Netscape browser interface was used. The isolate CMG64 was identified by API- kit.

2.3 Maximum tolerable concentration of heavy metal salts

To determine the maximum tolerable concentration (MTC) of heavy metal salts such as CuSO4, NiCl2, Pb(CH3OO)2, ZnSO4, Cr2O7, CoCl2, and CdCl2, bacterial culture were streaked on tris mineral medium plates supplemented with variable salt concentrations. The plates were then incubated at 37°C and growth was observed after 24-48 h.

2.4 Accumulation of heavy metals

The accumulation of heavy metals was assessed by growing bacterial cultures in tris minimal broth. The 50ml tris minimal broth in 250ml flask supplemented with variable concentrations of metal salts such as cadmium, copper and chromate were inoculated with 1 ml of overnight grown culture and incubated at 37°C in shaker incubator (100rpm). Each
day samples were collected for estimation of copper using copper assay and total cell protein content by protein assay using protein test kit (Sigma: TPRO 562).

2.5 Copper and chromate assay

Copper was assayed by a method described by Macaskie (18) and modified by Qureshi et al (19) to increase the sensitivity of a 1 ml reaction by 20 folds. For determination of Cr(VI) the mixture contained 400 µl of 20 mM MOPS-NaOH buffer (pH7), 327 µl distilled water, 33 µl of 3M H₂SO₄, 40 µl of 0.25% diphenyl carbazide (DPC) solution and culture supernatant or standard solution (200 µl). The absorbance was determined immediately at 540 nm (1.5 ml cuvettes, 1 cm path length). DPC solution comprised 0.25% wt/vol diphenylcarbazide in 0.1M H₂SO₄ in AnalaR acetone (20).

2.6 Transmission Electron Microscopy and Energy Dispersive X-ray Analysis

Bacterial pellets were harvested after 48 h, washed with distilled water and fixed by immediate resuspension in 2.5% vol/vol glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2, 60 min). The cells were dehydrated in an ethanol series (70, 90, 100, 100, and 100% ethanol: 15 min each), twice with propylene oxide (15min) and then in a mixture of propylene oxide/epoxy resin (1:1; 45min). Samples were then embedded in epoxy resin under vacuum in plastic moulds (20 min) and left to polymerize at atmospheric pressure (24 h, 60°C). Sections (70 nm) were cut, collected on copper grids/aluminium grids and examined by transmission electron microscopy. For energy dispersive X-ray analysis (EDX) thicker sections (200-300 nm) were cut and examined by scanning transmission electron microscopy (JEOL JEM-100CXII) using a LINK ISIS X-ray analyzer to determine elemental distribution in/on and around the cells.

3. RESULTS AND DISCUSSIONS

Three isolates were homologous to strains of Pseudomonas (Table 1) CMG462 and CMG463 were identified as P. stutzeri. These pseudomonads were 99% similar to the matching sequences, while CMG64 was identified as Pseudomonas aeruginosa. The fourth one a tannery isolate gave a high homology to an isolate that was identified as "cucurbit yellow vine disease bacterium", an enterobacterial plant pathogen. This isolate was 97% similar to reference sequence on the EMBL database (Table 1).

Table 1. Source of bacterial isolates

<table>
<thead>
<tr>
<th>Strain Code</th>
<th>Bacteria</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMG462</td>
<td>Pseudomonas stutzeri</td>
<td>(Foundry Soil, Karachi Shipyard and Engineering works)</td>
</tr>
<tr>
<td>CMG463</td>
<td>Pseudomonas stutzeri</td>
<td>(Foundry Soil, Karachi Shipyard and Engineering work)</td>
</tr>
<tr>
<td>CMG64</td>
<td>Pseudomonas aeruginosa</td>
<td>Korangi Industrial Area, Sector 7-A, Karachi</td>
</tr>
<tr>
<td>CMG480</td>
<td>Cucurbit yellow vine disease bacterium</td>
<td>Korangi Tannery Air, Sector 7-A, Karachi</td>
</tr>
</tbody>
</table>

The metal resistance were studied in tris-based medium because the complexation with heavy metals is minimum therefore the shown metal concentration is approximately the free metal concentration (5).
All the isolates of this study were originated from various metal contaminated sites of Karachi, Pakistan and showed multiple metal resistances (Table 2). The isolates CMG462, CMG463 and CMG64 showed resistance against cadmium chloride up to 2 mM whereas CMG462 and CMG463 exhibited highest resistance against copper i.e., 8 mM and 10 mM respectively with respect to other tested heavy metal salts. The multiple metal resistance ability suggested the prior exposure of the isolates to these metals, which are present in the sampling sites; this phenomenon of multiple metal resistances has been reported by many workers (21-22). One of the potential metal resistance mechanisms is accumulation or uptake of metal by bacterial cell. Metal accumulation of cadmium by CMG64 and copper by CMG462 and CMG463 was studied. Results (Table 3) show 40% of cadmium accumulation by CMG64, whereas CMG462 and CMG463 accumulated copper 90.7% and 97.4% respectively. Accumulation might be due to the presence of extracellular components such as proteins or polysaccharide. Falla and Bloch (23) have reported that polysaccharide-producing strains are active metal accumulators. It is reported that the resistant strain have well developed mechanisms to prevent the shock, such as in Pseudomonas which accumulates metal in the periplasmic space that prevents the entrance of an excess amount of metal into the cytoplasm (24).

Table 2. Maximum tolerable concentrations (MTC) of different metals

<table>
<thead>
<tr>
<th>Strain Code</th>
<th>CdCl₂</th>
<th>CuSO₄</th>
<th>ZnCl₂</th>
<th>Cr₂O₄</th>
<th>CoCl₂</th>
<th>NiCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMG462</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>1.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>CMG463</td>
<td>2</td>
<td>10</td>
<td>0.5</td>
<td>1.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>CMG64</td>
<td>2</td>
<td>1.5</td>
<td>2.5</td>
<td>-</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CMG480</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

Analysis of cell sections using transmission electron microscopy with energy dispersive X-ray analysis showed metal accumulation in these strains. Intracellular accumulation of cadmium in CMG64 was confirmed by TEM, which showed dark precipitates when cells were grown in the presence of 0.1 mM cadmium while no precipitates were observed in absence of cadmium. CMG462 and CMG463 showed accumulation/removal of copper from media while growing at 1 mM CuSO₄. The concentration of copper removal was estimated by copper assay and calculated by copper standard curve. The intracellular/extracellular accumulation or the localization of copper was observed under electron microscope.

The localization of copper was determined in stained and unstained cells (Figure 3). In CMG462 it was observed that the dark patches in side the cells i.e., in the cytoplasm and the darkly stained outer membrane was seen in the cells grown in presence of copper (Figure 3A, 3C). The EDX analysis also revealed the presence of copper inside the cell as well as at cell edges or the outer cell membrane (Figure 4). In contrast with CMG463, the copper was bound with the cells extracellularly which was clearly observed in unstained cells (Figure 3E). In EDX analysis the copper at the outer surface was extremely low or undetectable while it was detectable intracellularly (Figure 4). Accumulation of copper has been observed in several species of Pseudomonas as well as copper resistant Rhizobium loti (12, 25).
CMG462 and CMG463 *P. stutzeri* strains, were also evaluated for their ability to reduce and remove Cr(VI). Since Cr-stress can lead to resistance which may be associated with cellular exclusion of Cr(VI). The potential of the CMG462 and CMG463 strains were evaluated in parallel with another strain i.e., CMG480 which had been isolated from Korangi tannery air environment. All strains grew aerobically in 100 µM Cr(VI)-supplemented minimal medium, the doubling time was generally 4-6 h but reduced little Cr(VI). These strains grew aerobically in 100 µM Cr(VI)-supplemented acetate medium the doubling time was generally 4-6 h but reduced little Cr(VI). Anaerobic growth at the expense of Cr(VI) as the electron acceptor was negligible, similar phenomenon has been reported earlier (26-27) but Cr(VI) was reduced and removed efficiently. These cultures were essentially resting cell suspensions. This was observed only after a lag of 31 h.

Cr(VI) reduction aerobically was low in accordance with the provision of O2 as the primary electron acceptor and electron "sink". CMG462, CMG463 and CMG480 showed a comparable loss of Cr(VI) after 192 h. In contrast Cr(VI) reduction was apparent in these strains anaerobically; the best strains were CMG463 and CMG480 in terms of total Cr(VI) removed (88% and 76.08%, respectively) (Table 4).

This study suggested that there are two mechanisms of chromate resistance and biosorption. *P. stutzeri* CMG462 showed some electron opaque material at the outer cell surface but the localized concentration of this was below the sensitivity of the EDX technique. This suggested that the Cr trivalent precipitates dispersed in the medium more as compared with the cell surface adsorption. But these precipitates uniformly adsorbed at the cell surface (Figure 1), whereas in CMG480, the extracellular dark precipitates were attached with the cell surfaces when grown in presence of chromate (Figure 1E). It is suggested that bacteria have excellent nucleation sites for fine-grained mineral formation due to their high surface area to volume ratio (28) and the presence of electronegative surface functional groups for e.g., carboxyl group, phosphoryl and hydroxyl groups, the electron opaque material (Figure 1C) deposited by CMG480, identified as containing Cr and P by EDX. Similar results were found with *B. pumulis* strain which deposits Cr(VI) extracellularly (8). CMG463 showed occasional intracellular deposits of electron opaque material which gave a positive result for Cr and also for P by EDX. Analysis of the cell surface and background resin around the cells gave no detectable Cr (Figure 2). Detection of intracellular Cr suggests that anionic CrO4^{2-} or its reduced species Cr^{3+} enter the cell but for a waste remediation process a cell surface-localized deposit is preferable because it

### Table 3. Accumulation of metals in tris-minimal medium

<table>
<thead>
<tr>
<th>Strain Code</th>
<th>Metal salt</th>
<th>Conc. (mM)</th>
<th>Metal Accumulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMG64</td>
<td>CdCl₂</td>
<td>0.1</td>
<td>40.0</td>
</tr>
<tr>
<td>CMG462</td>
<td>CuSO₄</td>
<td>1.0</td>
<td>90.7</td>
</tr>
<tr>
<td>CMG463</td>
<td>CuSO₄</td>
<td>1.0</td>
<td>97.4</td>
</tr>
</tbody>
</table>

### Table 4. Aerobic and anaerobic reduction rate observed in acetate medium

<table>
<thead>
<tr>
<th>Cell Incubation</th>
<th>Strain Code</th>
<th>Residual Cr(VI) (µM)</th>
<th>Loss of Cr(VI) (µM)</th>
<th>Rate of reduction (nmol/mg protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobically</td>
<td>CMG462</td>
<td>87 ± 1</td>
<td>14</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>CMG463</td>
<td>85 ± 1</td>
<td>15</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>CMG480</td>
<td>91.18 ± 4.86</td>
<td>8.82</td>
<td>0.13</td>
</tr>
<tr>
<td>Anaerobically</td>
<td>CMG462</td>
<td>42 ± 3</td>
<td>58</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>CMG463</td>
<td>12 ± 3</td>
<td>88</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>CMG480</td>
<td>23.92 ± 7.18</td>
<td>76.08</td>
<td>4.29</td>
</tr>
</tbody>
</table>
may be possible to remove this and conserve the biomass for re-use. These results do suggest that these microbes are good candidates for exploitation of decontamination of cadmium, chromium and copper contaminated sites.

Figure 1. Transmission electron micrographs (TEM). Unstrained cells of CMG462 (A, B), CMG463 (C, D) and CMG480 (E, F)

Bacterial strains grown in presence (A, C, E) and in absence (B, D, F) of Cr(VI) in medium
Figure 2. Energy dispersive X-ray analysis (EDX) of electron opaque areas. CMG462 (A), CMG463 (B), CMG480 (C) and background control (D)
Figure 3. Transmission electron micrographs (TEM) of bacterial strains grown in presence (A, C, E, G) and absence of copper (B, D, F, H)

A, B, E, F: unstrained cells;
C, D, G, H: stained cells;
A, B, C, D: CMG462;
E, F, G, H: CMG463
Figure 4. Energy Depressive X-ray Analysis (EDX) in presence of 1 mM CuSO$_4$ of electron opaque material of CMG462 (A, B) and CMG463 (C, D); analysis inside the cells (A, C); analysis at cell surface (B, D) and control without metal (E)

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Biosorption

Biosorption equilibria with *Spirogyra insignis*

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**Abstract**

The recovery of heavy metals from different dissolutions by algae has successfully been demonstrated. We have studied the biosorption process of cadmium, nickel, zinc, copper and lead using the filamentous green alga *Spirogyra insignis* as biomass. The most favourable sorption conditions using different pH and biomass concentration were set for monometallic solutions. This allowed to determine the sorption isotherms, as for monometallic as bimetallic systems. Both the sorption maximum capacity of the biomass and the equilibrium constant of the reaction between the biomass and the metal were determined by Lagmuir’s model. Finally, the biosorption process was simulated with a computer program, checking these results with those obtained experimentally.

**Keywords:** biosorption, algae, bimetallic systems, cadmium, nickel, zinc, copper, *Spirogyra insignis*

1. **INTRODUCTION**

Biosorption is a novel technique that uses dead biomass for the recovery of heavy metals from aqueous solutions and a clear alternative to the conventional methods for the treatment of contaminated effluents.

Research and development of new biosorbent materials are especially focus on biomass made from algae. The fundamental reason is its high sorption capacity and its availability almost unlimited [1]. Nevertheless, most publications in biosorption in recent years have mainly been devoted to other biomass (fungi and bacteria) more than to algae.

It is more and more accepted that algae form a homogeneous group within the vegetal kingdom. They are divided among different evolutionary via, completely independent each other. A huge simplification leads to the following classification: a "red branch" with the red algae (rhodophyta), a "grey branch" with the grey algae (phaeophyceans), among them, and a "green branch" that contains beside the green algae (chlorophyta), mosses, ferns and several plants. The wall cell of each one of these algae is different and, therefore, the material responsible for the sorption is substantially different.

Research works in the literature have principally been orientated to studies with brown algae [2-7] whereas both green [8-10] and red [11] algae have been used in less extension. On this base, the aim of the present work was to determine the sorption
capacities of different heavy metals by *Spirogyra insignis*, a green alga used as biomass. Until now, there has not been mention of the sorption behaviour of this alga in the literature.

Our study was carried out as with monometallic as with bimetallic systems, these last ones near to the actual situation in industrial effluents [12]. On the other hand, having in mind the idea of foreseeing the behaviour of a given biomass in the presence of a contaminated effluent, there has been an attempt to model mathematically the biosorption system tested by means of a computer program of chemical speciation.

2. MATERIALS AND METHODS

The biomass used came from sweet water, collected from the Valmayor reservoir (Madrid, Spain) and was constituted in 99% by *Spirogyra insignis*. This alga grows very easily in stagnant or slowly flowing waters. It forms filamentous colonies where each one of the cells conserves its identity. The filamentous, without branches, are constituted by the joint of numerous cylindrical cells, which keep united each other by a viscous pectin layer that covers them superficially. Each cell has only one nucleus and several chloroplasts with ribbon and S form, which adapt to the membrane as rolled spiral ribbons. The membrane is of cellulosic nature. More than 300 species of *Spyrogiras* have been described, in all cases from low pH waters [13].

The tests were initiated from the frozen alga, which proceeded from the biomass collected in the reservoir. After several washes with distilled water, the liquid supernatant was eliminated with a peristaltic Watson Marlow pump model 503S. The remaining pulp, enriched with the alga, was centrifuged at 5000 rpm for 30 minutes in an Eppendorf centrifuge model 5804. Then, the biomass was dried in an oven at 60ºC up to reach constant weight. Later, the biomass was ground to a particle size less than 0.5 mm.

The experiments were performed with synthetic solutions of 1000 mg/L of Cd$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, Pb$^{2+}$ and Zn$^{2+}$, made from the corresponding sulphate salts, except for lead where nitrate was used, by dilution in distilled water. In all cases, chemically pure reagents were employed. The pH adjustment of the solutions was carried out with sulphuric or nitric acid, according with each case, 1% v/v diluted. 1 g/L NaOH was used as basic reagent. The pH values were tested between 2 and 6, depending on the metal, but always below the hydroxide precipitation pH. In all tests, the biomass concentration and the initial metal concentration were kept constant at 1 g/L and 50 mg/L, respectively.

Tests were carried out in 100 mL Erlenmeyer flasks set on a multiple stirring Selecta Multimatic plate model 5S. Periodically, aliquots were taken out of the mixture to follow the biosorption process. These samples were centrifuged at 5000 rpm and, on the supernatant liquid, the pH was measured and the metallic concentration was determined by atomic absorption spectrometry.

3. RESULTS AND DISCUSSION

3.1 Influence of pH

The pH influences the biosorption process in two ways [14]. On the one hand, on the total charge of the biosorbent, since protons have the chance to be either adsorbed or released. This behaviour would be affected by the functional groups on the cell wall of the alga, establishing the equilibrium state as a function of the pH value of the medium. On the other hand, the pH value affects the solubility of the metallic ion in solution. For
instance, at the metal concentration used, the hydroxide precipitation pH values are: 8.5 (Cd), 5.9 (Cu), 7.9 (Ni), 5.9 (Pb) and 7.5 (Zn).

In order to study the effect of this variable and to determine its optimum value, for which the sorption capacity of the biomass is maximum (q, expressed in mg or mmol of metal/g of biomass), a series of tests were carried out by modifying the pH. The results obtained are shown in Figure 1.

![Figure 1. Effect of pH on biosorption of metals by Spirogyra insignis](image)

A general trend was observed: at pH 2 the amount of metal adsorbed was negligible, increasing biosorption with higher pH values. This suggests that, at low pH values, there is a preferential biosorption by protons versus metal ions on the active sites of the biomass. Thus, the sorption capacity values are kept constant above pH 4, or even decrease, as in the case of copper. Bearing in mind that the solubility of metal hydroxides is not only described by the precipitation pH, before reaching such pH value, hydroxo metal-ion complexes of the type \([\text{Me(OH)}_n]^{n-}\) can be present. This could justify the evolution of q versus pH. The highest sorption capacity was set between 4 and 6, depending on the metal (6 for Zn, Cd and Ni, 4 for Cu and 5 for Pb). These results agree with others given in the literature for another kinds of algae [15].

### 3.2 Influence of biomass concentration

The biomass concentration is another important variable that can affect the amount of metal retained. For a given equilibrium concentration, the biomass adsorbs more metallic ions at lower than at higher cell densities [16]. It has been suggested that electrostatic interactions between cells must be a significant factor on the biomass concentration dependence with respect to metal sorption. In this sense, by decreasing the biomass concentration in suspension, for a given metal concentration, an increase of both the metal/biosorbent ratio and the metal retention by sorbent unit is observed, as long as there is not saturation. High biomass concentrations can exert a shell effect protecting the active sites against the metal. As a result, the amount of metal adsorbed by biomass unit is lower.

In these tests the initial metal concentration in solution (50 mg/L) and the pH value (the optimum for each metal, according with the previous section) were kept constant. The biomass concentrations tested were: 0.5, 1.0 and 2.0, and the results obtained are shown in Figure 2.

As can be observed, the amount of metal retained by the biomass presents a maximum at 1.0 g/L, except for lead. This confirms the loss of efficiency for high concentrations of biosorbent. The same can be said for low concentrations, probably due to the weak electrostatic attraction forces with biomass concentrations so low. However, this is not
Biosorption observed for lead since, as it will later be described, is the metal with higher affinity for the biomass. Anyway, differences are small and could also be due to experimental errors typical of this kind of tests.

![Figure 2. Effect of biomass concentration on biosorption of metals by *Spirogyra insignis*](image)

### 3.3 Sorption isotherms

**Monometallic systems**

The isotherm represents the apparent solute sorption versus its equilibrium concentration at a given temperature. At low concentrations, which is the case for many practical sorption situations, the isotherms present two main forms, classified by Giles et al. [17] in two types: type L, which is concave with respect to the concentration axis, and type S, or sigmoidal, which is firstly convex and then concave respect to the same axis. A type L isotherm, with a horizontal part well defined, is associated to the sorption of a monolayer of solute with a minimum competition for the solvent.

With the aim of determining the sorption isotherms related with *Spirogyra insignis*, five series of tests were conducted with monometallic solutions varying the initial metal concentration between 10 and 150 mg/L. The pH values and biomass concentration were maintained at optimum, as indicated previously.

In all cases, type L isotherms were recorded, with a rapid increase in the sorption values with low equilibrium concentrations, reaching a stable maximum value with higher equilibrium concentrations. Using the linear Lagmuir’s model, which supposes the adsorption of a monolayer on the sorbent, and is represented by the following equation:

\[
\frac{C_e}{q} = \frac{C_e}{q_{\text{max}}} + \frac{K}{q_{\text{max}}}
\]

The results shown in Table 1 were obtained. \(q_{\text{max}}\) data correspond to the maximum sorption value for high equilibrium concentrations. \(b\) is the equilibrium constant for the interaction between the metal and the biomass, and \(K\) is its inverse value. Therefore, both parameters measure the biomass affinity for the metal cation. \(R^2\) is the fitting coefficient expressing the degree of linear fitting of the experimental results to equation (1); a value close to one reflects a good fitting of the data obtained to Lagmuir’s equation.

From the comparison of \(q_{\text{max}}\) data, the maximum sorption values for the five metals are very similar; the lower \(K\) value for lead reveals a higher affinity of the biomass for this metal, followed by cadmium, copper and zinc. Nickel and zinc present the same affinity.
Table 1. Langmuir parameters for adsorption isotherms

<table>
<thead>
<tr>
<th>pH</th>
<th>Biomass (g/L)</th>
<th>q_{max} (mmol/g)</th>
<th>K (mmol/L)</th>
<th>B (L/mmol)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>6 1</td>
<td>0.20</td>
<td>0.07</td>
<td>13.6</td>
<td>0.99</td>
</tr>
<tr>
<td>Nickel</td>
<td>6 1</td>
<td>0.30</td>
<td>0.39</td>
<td>2.57</td>
<td>0.97</td>
</tr>
<tr>
<td>Zinc</td>
<td>6 1</td>
<td>0.32</td>
<td>0.39</td>
<td>2.58</td>
<td>0.95</td>
</tr>
<tr>
<td>Copper</td>
<td>4 1</td>
<td>0.25</td>
<td>0.13</td>
<td>7.86</td>
<td>0.98</td>
</tr>
<tr>
<td>Lead</td>
<td>5 0.5</td>
<td>0.27</td>
<td>0.01</td>
<td>73.13</td>
<td>0.99</td>
</tr>
</tbody>
</table>

**Bimetallic systems**

Industrial effluents use to have more than one metal ion in solution. The retention of a metal by dead biomass is significantly affected by the presence of other cations in solution [18]. This is due to the fact that many functional groups on the cell wall and membrane are non-specific and then there is a competition of the different cations for the binding sites. This leads to a lower retention of a given metal in mixed solutions than when it is alone. The decrease in the metal retention by the presence of other ones depends on the nature and concentration of the other cations, decreasing the metal retention when increasing other cations concentration.

In this section the biomass sorption capacity from bimetallic solutions within the system: Zn-Cd-Ni was analyzed. The experiments were carried out with a biomass concentration of 1 g/L and a pH value of 6. The metal concentrations tested were: 0, 10, 25, 50, 100 and 150 mg/L, testing for each metal all the concentrations of the other metal.

The most appropriated way of studying the sorption of two metals together is by fitting the experimental data to a mathematic model [19] able to generate several parameters that help to evaluate quantitatively the influence of the presence of one metal on the sorption capacity of the other. In this way, Langmuir’s equation of the binary type was used, which establishes the equilibrium between two metals (M_1 and M_2) and the species resulting from the sorption by the biomass (B), B-M_1 and B-M_2:

\[
\begin{align*}
B + M_1 & \Leftrightarrow B-M_1 \\
K_1 &= \frac{k_{-1}}{k_1} = \frac{[B][M_1]}{[B-M_1]} \\
B + M_2 & \Leftrightarrow B-M_2 \\
K_2 &= \frac{k_{-2}}{k_2} = \frac{[B][M_2]}{[B-M_2]}
\end{align*}
\]

Whose final expression is as follows:

\[
q(M_i) = \frac{q_{max} C_r(M_i)}{1 + \frac{1}{K_1} C_r(M_i) + \frac{1}{K_2} C_r(M_2)}
\]

A high value of K for M_2 (K_2) versus M_1 (K_1) means a higher affinity of the biosorbent for M_1 than M_2, since high values of K are associated to a high value of the ratio between desorption and sorption amount. These two parameters, together with q_{max}, allow quantifying the biosorption process.

Several authors have worked during the last years with this type of binary systems although their number is relatively limited. Biosorption modelling considering two metals systems can be carried out using either empirical equations or chemico-physical mechanistic models [20]. Empirical models are more or less simple mathematical...
equations with some adjustable parameters, which can be fitted to the experimental data. On the other hand, the mechanistic models provide a major understanding of the chemical and physical aspects involved in the biosorption of heavy metals. These models are not simple mathematical equations and are based on a series of hypothesised chemico-physical reactions among active sites of the biomass and ions in solution. Any case, mechanistic models require a wide and deep experimental investigation and its application to real systems is difficult. The experimental data obtained in the present work were fitted according to the empirical modelling approach.

Table 2 collects all these parameters for the three bimetallic systems under study, which were obtained through a computing treatment of the experimental data by using the MATLAB program. It can be seen, similarly to monometallic systems, that the biomass affinity is higher towards Cd than to Zn and Ni. Of the three metals studied, nickel presents a higher K value and, therefore, less affinity for the biomass. With respect to the maximum sorption capacity, it is higher in those systems with Cd, which is in agreement with the higher affinity of the biomass for this element, within the trimetallic system used.

<table>
<thead>
<tr>
<th>System</th>
<th>( K_1 ) (mmol/L)</th>
<th>( K_2 ) (mmol/L)</th>
<th>( Q_{\max} ) (mmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni - Cd</td>
<td>0.23</td>
<td>0.07</td>
<td>0.28</td>
</tr>
<tr>
<td>Zn - Cd</td>
<td>0.12</td>
<td>0.06</td>
<td>0.27</td>
</tr>
<tr>
<td>Zn - Ni</td>
<td>0.08</td>
<td>0.16</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Using the same computer program, equation (4) could be represented by sorption isotherms surfaces in three dimensions, recording in X and Y axes the equilibrium concentrations of the two metals, and in Z-axis the sorption capacity either of one of the two metals or the sum of both. Figures 3, 4 and 5 are an example of the power of the program and show the equilibrium conditions for the systems Cd-Ni, Zn-Ni and Ni-Cd, respectively. These three cases have been choosing to show the influence of the metal with the less biomass affinity (Ni) on the sorption of the two others (Cd and Zn), and reciprocally the influence of the metal with the highest affinity (Cd) on the metal with the less one (Ni).

It can be observed as Ni does not exert a clear influence on the sorption of Cd and Zn, since for high Ni concentrations there is not significant decrease in the q values of Cd and Zn (Figures 3 and 4). On the contrary, Cd provokes an outstanding decrease on the sorption levels of Ni (Figure 5).
3.4 Simulation of the biosorption process

The last stage of this study consisted in the simulation of the data corresponding to the equilibrium conditions of monometallic systems by means of a computer program of chemical speciation, PHREEQC 6.2 [21]. Behind the comparison of the experimental data with those obtained from the model is the possibility of foreseeing the behaviour of the biomass theoretically.

For this, the surface reactions between the biomass and each one of the metals were incorporated to the database of the program, with its corresponding equilibrium constant determined, as commented before, by the b constant of Langmuir’s model. Such reactions are:

\[ B + Me^{+2} = BMe^{+2} \]
\[ (q_{max} - q) Ce = q \]
\[ Spiroins + Cd^{+2} = SpiroinsCd^{+2} \]
\[ log_k 4.1332 # (k = 13,590) \]
\[ Spiroins + Zn^{+2} = SpiroinsZn^{+2} \]
Biosorption

\[ \log_k 3.4121 \quad \# (k = 2,583) \]

\[ \text{Spiroins} + \text{Ni}^{2+} = \text{SpiroinsNi}^{2+} \quad (8) \]

\[ \log_k 3.4103 \quad \# (k = 2,572) \]

\[ \text{Spiroins} + \text{Cu}^{2+} = \text{SpiroinsCu}^{2+} \quad (9) \]

\[ \log_k 3.7414 \quad \# (k = 5,513) \]

\[ \text{Spiroins} + \text{Pb}^{2+} = \text{SpiroinsPb}^{2+} \quad (10) \]

To run the program it was necessary to specify the conditions of the dissolutions to deal with (pH, temperature, equilibrium metal concentration, ionic species, etc.), besides the own characteristics of the biomass (\(q_{\text{max}}\), specific area and weight used). Table 3 collects both the experimental data and those obtained with the computer program. \(q_{\text{max}}-q\) values are related to the active sites of the biomass that remained unoccupied during the equilibrium, which does not necessarily mean the amount of metal in dissolution; this would only happen in the case of saturation of the biomass.

**Table 3. Comparison between experimental and program values**

<table>
<thead>
<tr>
<th></th>
<th>(\text{Ci} (\text{mg/g}))</th>
<th>(\text{Ce} (\text{mmol/L}))</th>
<th>Experimental</th>
<th>Program</th>
<th>Experimental</th>
<th>Program</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(q) (mmol/g)</td>
<td>(q_{\text{max}}-q) (mmol/g)</td>
<td>(q) (mmol/g)</td>
<td>(q_{\text{max}}-q) (mmol/g)</td>
<td>(q) (%)</td>
<td>(q_{\text{max}}-q) (%)</td>
</tr>
<tr>
<td><strong>Cadmium</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>0.04</td>
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<td>0.05</td>
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</tr>
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<td>0.18</td>
<td>0.02</td>
<td>0.18</td>
<td>0.02</td>
<td>89</td>
</tr>
<tr>
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<td>1.07</td>
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<td>0.00</td>
<td>0.18</td>
<td>0.02</td>
<td>97.9</td>
</tr>
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<td></td>
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</tr>
<tr>
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</tr>
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<td>0.05</td>
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<td></td>
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<tr>
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<td>25</td>
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<tr>
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<tr>
<td>150</td>
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<tr>
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<td>25</td>
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</tr>
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<td>150</td>
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<td>0.00</td>
<td>0.25</td>
<td>0.00</td>
<td>99.2</td>
</tr>
</tbody>
</table>
It can be checked that, in general, there is a remarkable similarity between the experimental data and those obtained with the program. The value with the worse correlation is the one corresponding to the lowest initial metal concentration. This can be due to a higher competition of protons for the active sites on the cell wall of the alga, since the presence of the metallic species in dissolution was, in this case, little significant. By increasing the metal concentration, the difference between the experimental and calculated values is about 10%. This demonstrates the validity of the computer program used in order to simulate this type of processes.

4. CONCLUSIONS

In the sorption process of Cd, Ni, Zn, Cu and Pb by *Spirogyra insignis* the optimum sorption pH was set between 4 and 6 depending on the metal and the optimum biomass concentration was reached at 1 g/L, practically in all cases. The $q_{\text{max}}$ values were very similar for all five metals, being lead the metal with higher affinity for the biomass. The fitting of the experimental data to Langmuir’s model was excellent. In multimeatallic systems, Ni did not exert a remarkable influence on Cd and Zn sorption. On the contrary, Cd provoked a significant decrease on Ni sorption levels. Finally, the computer treatment of the data revealed an outstanding similarity between experimental data and those obtained through the computer program. Definitively, the biomass used in this study is a very efficient biosorbent for the recovery of metals from residual effluents.

ACKNOWLEDGMENTS

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REFERENCES

Biosorption of $^{226}$Ra and Ba by *Sargassum* sp.

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**Abstract**

The goal of this work was to investigate the removal of $^{226}$Ra from a radioactive solution and to verify if barium can be an useful model for radium-226 biosorption since both elements are chemically similar. The non-living biomass utilized in this study was obtained from *Sargassum* sp. The biosorption kinetics experiments were carried out at pH 3.5 and the specific metal uptake (Ba) or radionuclide activity ($^{226}$Ra) and efficiency of removal were the parameters evaluated. *Sargassum* sp. biomass showed an efficient removal of $^{226}$Ra from solution (~99%) and the behavior of barium biosorption was close to that of $^{226}$Ra, showing that it can be used as a model for this radionuclide in this process.

1. **INTRODUCTION**

Biosorption comprises binding of metals to the biomass by a process, which does not involve metabolic energy or transport, although this process may also occur simultaneously where live biomass is used [1]. Biosorption of heavy metals or radionuclides by microbial cells or biomass in general has been recognized as a potential alternative to existing technologies for removal of these contaminants from polluted waters [2]. This phenomenon is generally described as retention of ions from solution by microbial cells and this metal uptake is normally very efficient and frequently selective [3].

According to the International Atomic Energy Agency (IAEA), radioactive waste is any material that contains a concentration of radionuclides greater than those deemed safe by national authorities, and for which no use is foreseen. Based on this definition and on the anthropogenic radioecological effects, the percentage of radionuclides removed from effluents, waters deriving from nuclear installations and acid mine drainage must be maximized.

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Despite a great number of studies of biosorption applied to remove toxic metals from industrial effluents, mainly from mining activities, only few studies have been done focusing biosorption of radionuclides \([4, 5]\).

Uranium and thorium are natural sources of radionuclides \(^{226}\)Ra and \(^{228}\)Ra, respectively. Mining activities and hydrometallurgical processing of ores and minerals of these elements can result in wastes and effluents containing both radionuclides. Effluents and/or residual waters from nuclear installations have ultra-diluted concentrations of radionuclides along with other diverse chemical species. Most of the time, the concentration of these diverse chemical species is much higher to the radionuclide concentrations. Therefore, the biosorbents must show elevated selectivity and removal efficiency (above 99\%) of the present radioactive species.

The main objective of this study was to investigate the biosorption of \(^{226}\)Ra and to compare its behavior with barium, a chemically similar element, utilizing \(Sargassum\) sp., a brown seaweed that has been utilized in several studies of biosorption \([6, 7]\).

### 2. MATERIALS AND METHODS

#### 2.1 Biomass

The brown seaweed \(Sargassum\) sp. was collected at coast of São Sebastião – SP, Brazil. The biomass was washed four times with 500 ml of water purified to 18 MΩ in a 1 L beaker. The system was kept agitated and heated to 50°C in a magnetic agitator for a total of 12 hours. The changes in washing waters were performed every 3 hours, and the last operation of solid-liquid separation was accomplished through vacuum filtration. The washed biomass was sun dried and maintained at room temperature. For biosorption experiments the algae was chopped in pieces with size around 1 mm and 3 mm, washed for 2 hours in hydrochloric acid 0.1 mol L\(^{-1}\) and after that it was separated in 2 fractions and washed in ultra-pure water up to pH value of 3.50 or 5.00.

#### 2.2 Working solutions

a) \(\text{Ba}^{2+}\)

The reagent \(\text{BaCl}_2\cdot 2\text{H}_2\text{O}\) was used to prepare the stock standard solution at 1 g L\(^{-1}\) of Ba. From this stock solution, were prepared by appropriated dilution working solutions in concentrations ranged from 0.1 mg to 10 mg L\(^{-1}\) of Ba. These working solutions were prepared at pH 3.50 and 5.00 using HCl for pH adjustments.

b) Radioactive \(^{226}\)Ra

The radionuclide \(^{226}\)Ra working solutions were prepared in the following nominal activity concentrations (Bq L\(^{-1}\)): 900; 1,125; 1,350; 1,575; 1,800; 2,025; 2,250; 4,500; 22,500 e 45,000, adjusted to pH 3.00 or 5.00 with LiOH solution during the biosorption experiments.

#### 2.3 Biosorption experiments

Biosorption experiments were carried out by using 0.1 g of biomass in sealed 125 mL Erlenmeyers and 20 mL of working solutions of Ba or \(^{226}\)Ra, at initial concentrations (Ci) of 0.1 to 10 mg L\(^{-1}\) and 900 to 45,000 Bq L\(^{-1}\), respectively. The flasks were incubated in a shaker at 28°C, 250 rpm for 180 minutes (Ba\(^{2+}\)) and 240 minutes (Ra\(^{2+}\)) as final contact time. The pH values were adjusted to 3.50 or 5.00 every 30 minutes, whenever necessary. The experiments were performed in duplicate for each condition; water purified to 18 MΩ
at above mentioned pH values was used as a control. Due to adsorption of $^{226}$Ra on glass walls (physical adsorption, 13% at pH 3.50 and 36% at pH 5.00) the actual $C_i$ and $C_f$ (final concentration at equilibrium) values for $^{226}$Ra standard solutions were determined and considered in the final results.

The following formula was used to calculate the accumulated $\text{Ba}^{2+}$ mass or concentration of the accumulated activity for $^{226}\text{Ra}^{2+}$ [8]:

$$q = \frac{V \times (C_i - C_f)}{b}$$

(1)

where:

$q$ is the mass (mg) of metal or activity (Bq) of radionuclide by 1g of dry biosorbent;

$V$ is the volume of the test solution (L);

$C_i$ and $C_f$ are respectively, the initial and the equilibrium concentrations of the metal (mg L$^{-1}$) or radionuclide (Bq L$^{-1}$) in solution;

$b$ is the mass of dry biosorbent (g).

2.4 Chemical and radiometric determinations

Barium was determined by ICP-AES, using a Jarrell-Ash model AtomComp 975. The radionuclide $^{226}$Ra activity was determined using a proportional alpha and beta meter, run with an ultra-low background gas, model ESM-EBERLINE FHT 770T.

3. RESULTS AND DISCUSSION

3.1 Biosorption kinetics

Figures 1 and 2 show, respectively, the kinetics of Ba and $^{226}$Ra biosorption by Sargassum sp. As it can be seen high affinities were observed between $\text{Ba}^{2+}$ (a Class A metal) and the linkage sites at the Sargassum sp. cell walls. This fact agrees with the Class A metal ions preference to linkage sites that contain oxygen, which is the case of several oxygenated groups such as alginic acid, fucoidan, agars and carrageenans, present in algae cell walls, especially marine algae. This responds to the hydrophilic properties of the algae, making it susceptible to ionic exchange [9, 10, 11, 12].

Figure 1. Biosorption kinetics of Ba by Sargassum sp. at pH 3.50 and initial concentration of 0.094 mg L$^{-1}$
Comparing both kinetics it is evident that Sargassum sp. showed a higher efficiency of biosorption of $^{226}$Ra than that observed for Ba. Group II elements, which belong to Class A ions, bind to radicals containing oxygen through ionic exchange mechanisms. The ionic exchange selectivity for these elements has the following qualitative prevalence:

- Selectivity increases with the increase of ion charge
- Selectivity increases with the decrease of ion radius (hydrated)
- Selectivity increases with the increase of ion polarization

Therefore, the ranking of affinity of these elements for strong cationic resins containing sulfonic radicals (RSO3 -) are:

\[ \text{Group II: } \text{Ra}^{2+}_{\text{aq}} > \text{Ba}^{2+}_{\text{aq}} > \text{Sr}^{2+}_{\text{aq}} > \text{Ca}^{2+}_{\text{aq}} > \text{Mg}^{2+}_{\text{aq}}. \]

Besides, solubility product ($K_{sp}$) is another parameter that can be used to evaluate the affinity of radium and barium for these radicals. The $K_{sp}$ values of barium sulfate and radium sulfate are $1.08 \times 10^{-10}$ and $3.66 \times 10^{-11}$, respectively [13], that is, radium has a higher affinity than barium for sulfate containing radicals, such as the fucoidan present in Sargassum sp. cell wall. However the difference in $K_{sp}$ values is not much significant, which become barium a competitor ion with radium for these radicals; this is not true for calcium, for example, which has a much higher $K_{sp}$ ($3.14 \times 10^{-5}$) [14]. So, barium can be used as a model for radium biosorption experiments.

### 3.2 Biosorption isotherms

The isotherms of biosorption are curves that describe the equilibrium between the metal in solution and the biosorbent at a constant temperature. These curves are extensively used in the studies for comparison of biosorption performance of different biosorbents. Figures 3 and 4 show the biosorption equilibrium isotherm for Ba and $^{226}$Ra, respectively.
Figure 3. Ba biosorption equilibrium isotherms at pH = 5.00, for Sargassum sp.

Figure 4. $^{226}$Ra biosorption equilibrium isotherms at pH = 5.00, for Sargassum sp.

The results obtained from the Langmuir fitting showed a maximum biosorption coefficient ($q_{\text{max}}$) of around 150 mg g$^{-1}$ for Ba and 10,000 Bq g$^{-1}$ for $^{226}$Ra. For others biosorbents tested in our laboratory, such as the microalgae Monoraphidium sp., Penicillium sp. (filamentous fungi) and the yeast Saccharomyces cerevisiae, these values (data not shown) were significantly lower than those obtained with Sargassum sp.

4. CONCLUSION

Radium bioaccumulation has not been studied frequently, and with rare exceptions, few scientists tried to develop bioprocesses that employ some biological systems with potential for radium removal [4]. It has been demonstrated the superiority of biosorbents
compared with traditional adsorbents such as natural zeolites, manganese zeolites, zircon salts, Bio-rex exchange ions resin and activated carbon [15].

For example, radium sorption values ranged from 80 to 130 Bq g⁻¹ are reported for traditional sorbents whereas for biomass present in activated sludge the values ranged from 1,500 to 7,700 Bq g⁻¹ depending on the initial concentration of radium and pH. In our work a maximum biosorption coefficient of 10,000 Bq g⁻¹ has been obtained for Sargassum sp., which means practically 100% of ²²⁶Ra removal at pH 3.50 as well as pH 5.00. This value is lower compared to the data reported before [15], but different conditions were used in that research, such as initial radium concentration and pH values which ranged from 7.00 to 10.00.

In our work barium biosorption data indicated that it can be used as an “indicator” of the biosorption of ²²⁶Ra, since similar behavior was found in the assays carried out, despite some differences in the kinetics experiments in which ²²⁶Ra was more specific and more efficient for Sargassum sp. biosorption activity.

The results obtained in this work are useful to define biosorption capacity of Sargassum sp. for ²²⁶Ra and will help to evaluate the potentiality of the process for its technological application.

REFERENCES
Biosorption of arsenic and heavy metals on a ceramic-based biomass. Batch equilibrium experiments with Cu\textsuperscript{2+} model solutions

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Abstract

The aim of this work was to study the suitability of a new ceramic-based biomass for the sorption of heavy metals from aqueous solutions. The biosorbents (Biocer) used here is consisting of a strain of \textit{Bacillus sphaericus} immobilised in a ceramic material.

In the first batch equilibrium experiments for the sorption of Cu\textsuperscript{2+} in model solutions was obtained a maximum sorption capacity of 6 mg Cu\textsuperscript{2+}/g Biocer material (dry mass).

A yield of up to 85\% of the quantitative copper sorption was achieved after 60 minutes. Subsequently, the sorption rate of copper is relatively slow; it is fitting to a 1\textsuperscript{st} order reaction. For practical use, a regeneration of the sorbens material is of major economical importance. Desorption experiments with 0.005 M citric acid resulted in an almost completely recovery of copper after a single regeneration. The Biocer material showed also a really good regeneration behaviour of recently 5 to 6 sorption/desorption circles without any loss of its loading capacity or its stability.

As a result, the material shows a good suitability not only for the decontamination of solutions with a low metal content, but also possibly for the recycling of economically valuable metals.

1. INTRODUCTION

Pit waters and seepage from mine tailings or dumps are containing a huge potential of harmful substances like arsenic and heavy metals. In the former uranium mining areas in Germany, especially uranium and arsenic are contaminants of enhanced environmental attention. The wastewaters from the flooded pits there as well as seepage waters from the large spoiled piles are containing a high future contamination potential.

Many approaches were developed and tested to solve this problem; one of them is the use of biosorption for the treatment of mine drainage waters [1-5]. In the last years, several sorption experiments with different biomaterials were successfully implemented

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Biosorption

Bacillus sphaericus, the Bacillus strain used here, is known for its excellent sorption capacity of uranium and other heavy metals [23,24]. In this context, a new biosorption material, called Biocer, was developed combining the good sorption properties of the bacteria with mechanical stability by means of the sol-gel technology [25]. The name Biocer means here the combination of a biological component with ceramic material. The vegetative cells are firmly bound in this ceramic material, whereas harmful metal ions can diffuse through the pores of the ceramics and then they become sorbed on the cell walls.

With an optimised grain size, the Biocer material shall later be used in column reactors for the treatment of contaminated waters. Such reactors may be applied as small decentral constructions for especially low concentrations of harmful metal ions and arsenic. The Biocer material can also be used for the post-processing of discharge of conventional treatment units in order to keep the limits of environmental contaminants into the surface water.

In this work, first biosorption experiments with this Biocer material were carried out with synthetic solutions as model investigations in order to obtain first equilibrium and kinetic data about the removal of metal ions and to get first results about the stability of the biosorbing material.

2. MATERIALS AND METHODS

2.1 Material

The Biocer material was supplied by "Kallies Feinchemie AG" Sebnitz (Germany). In these experiments was used a grain size of the biocer material of 90-710 µm.

Before starting the experiments, the material was conditioned with physiological sodium chloride solution or 0.1% sodium perchlorate solution. Therefore, 0.1 g of the material was four times washed for 15 min with 3 ml of the salt solution. After conditioning, the Biocer material was suspended in the metal containing solutions to perform the biosorption experiments.

2.2 Biosorption experiments

The measurement of the sorption isotherms for copper(II) was carried out in batch experiments in closed test tubings. 1% of the Biocer material (w/v) was added to the copper solutions, and all was mixed in an overhead shaker for 2 hours. The concentration of the copper solutions used ranged from 0.1-250 mg/l and they were prepared by dilution of a stock solution (1 g/l Cu by dissolving of CuSO₄ in deionised water). The pH of the experimental solutions was adjusted to pH 3 and pH 5 by adding HNO₃ and NaOH as required. Finally, the metal content of the sample supernatants was analysed by atomic absorption spectroscopy (AAS, Perkin Elmer 4100).

2.3 Desorption experiments

The desorption experiments of copper were carried out directly after the end of the sorption experiments. Therefore, the Biocer material was separated from the metal solution, and suspended in a 0.005 M citric acid adjusted to pH 3.2. The desorption experiments were carried out by mixing in an overhead shaker over a time of 20 h.

For a new sorption cycle, the Biocer material was conditioned again 3-4 times with a physiological sodium chloride solution, as described in point 2.1, and further used as described above.
2.4 Kinetic sorption experiments

Kinetic experiments of the biosorption of copper were carried out with 1 g Biocer in 100 ml copper solution at a pH of 5.3. Up to 10 samples were taken in different time intervals, each 5 ml.

3. RESULTS AND DISCUSSION

3.1 Solution chemistry of Cu²⁺

For a better understanding of the sorption behaviour of the Cu²⁺ on the Biocer material, at first a few important items of the solution chemistry of CuSO₄ shall be shortly outlined. The copper sulfate has a good solubility in aqueous solutions, and the Cu exists there in form of its dissociated bluish [Cu(H₂O)₄]²⁺ ions. The solutions react weakly acid, and at a concentration of 250 mg/l was measured a pH of 5.1. For this copper concentration, the solubility product is achieved at a pH of 5.8. This means, that at a pH higher than 5.8 the copper is already precipitated in different species. Additionally, in the literature is described the existence of undissolved copper species like Antlerite [Cu₃SO₄(OH)₄] and Brochanite [Cu₄SO₄(OH)₆] in the pH range of 4,5-6 [26]. By this reasons, a precipitation of different copper species in the biosorption experiments under the conditions described above should be avoided.

3.2 Sorption isotherms of Cu²⁺

The Biocer material is nearly completely neutralising weak acid solutions. After adding the Biocer material, an increase of the pH was measured in the copper solutions depending on the copper concentrations. The pH increased from the initially adjusted pH 3 to pH 5.2 in a 250 mg/l Cu solution, and to pH 6.3 in a solution of 1 mg/l Cu. In copper solutions initially adjusted to pH 5, the pH was increasing to 5.1 for 250 mg/l Cu and to pH 7.4 for 1 mg/l Cu. At the final pH of the solutions exists a high probability for the formation of precipitated copper species under these conditions.

![Sorption isotherms for Cu²⁺ in solutions of various initial pH](image)

**Figure 1. Sorption isotherms for Cu²⁺ in solutions of various initial pH**
For this reason, additional samples were analysed parallel to the sorption experiments. A variance coefficient of the measurement data of 25% was calculated for the low concentrated copper solutions with an enhanced final pH of 6.3 and 7.4, resp., after a reaction time of 2 h. At a final pH of 5.5, a variance coefficient of less than 10% was calculated.

In Fig. 1 are shown the sorption isotherms of copper at two different initial pH values of the solutions. An increase of the sorption capacity with an increasing pH is clearly recognisable (Fig. 1).

The cell surfaces of the immobilised *Bacillus* strain contain a number of functional groups like hydroxy and carboxy groups, which are able to bind protons and metal ions coordinatively and to dissociate them in a sophisticated equilibrium. This is resulting in a strong pH dependency of the biosorption processes. The metal ions are easily replacing the protons at higher pH values. Furthermore, other binding mechanisms (complex or chelate bindings) are also involved in the biosorption process.

**Sorption model**

The Langmuir model was used to fit the experimental data (eq.(1)). The model is basing on the assumption of a confined number of binding places on the surface of the sorbens [27,28].

\[
\frac{c_{eq}}{a_q} = \frac{1}{q_{max}} \cdot c_{eq} + \frac{b}{q_{max}}
\]  

(1)

Figure 2. Linearised $Cu^{2+}$ isotherm, initial pH 5

Figure 3. Linearised $Cu^{2+}$ isotherm, initial pH 3

As a result, the Langmuir equation can be used to fit the experimental biosorption data obtained with the Biocer material. So, the copper ions seem to be bound nearly exclusively on the surface groups of the biomass, and they are easily exchangeable. Under the given experimental conditions, precipitation processes as well as a longer lasting incorporation into the cells, resulting in a lower exchangeability of the copper ions, seem not to occur.

Fig. 4 shows the sorption behaviour of the Biocer material at various $Cu^{2+}$ concentrations of the solutions. Up to a concentration of 50 mg/l, the $Cu^{2+}$ is sorbed in an average amount of 85% in the equilibrium experiments. At $Cu^{2+}$ concentrations above, a
decrease of the yield of the sorbed Cu$^{2+}$ was observed. In the equilibrium biosorption experiments was used a concentration of the Biocer material of 1% (Pt. 2.2.). This is resulting in a specific sorption capacity of this material of up to 6 mg Cu/g Biocer.

3.3 Desorption experiments

For practical use, a regeneration of the sorbens material is of major economic importance. Experiments desorbing the copper(II) bound to the Biocer material were carried out with 0.1M NaOH and 0.5 M citric acid. Under these experimental conditions, a complete desorption, but also a partial destruction of the Biocer material was observed. Obviously, a pH of 3 is the lower limit of pH stability for this kind of material. Below this pH limit, a repeated sorption of copper is impossible. Probably, the Biocer material is then directly destructed.

In further optimising experiments was found out, that the Cu$^{2+}$ can be nearly quantitatively desorbed without destructing the Biocer material by using a 0.005 M citric acid at a pH of 3.2 (Fig.4).

Figure 4. Sorption and desorption behaviour of the Biocer material at various Cu$^{2+}$ concentrations

Fig. 4 shows the amount of desorbed copper compared to the sorbed amount. At all loading concentrations used, a nearly quantitatively desorption of the bound Cu$^{2+}$ was observed (Fig. 4). So, the optimised experimental conditions of the regeneration with 0.005 M citric acid at a pH of 3.2 seem to be really good suited for a nearly quantitatively removal of the sorbed Cu$^{2+}$ ions from the Biocer material.

3.4 Regeneration behaviour of the Biocer material

After the successful experiments of the desorption of Cu$^{2+}$ from the Biocer material, the loading behaviour in various sorption/ desorption cycles was investigated under various concentrations of the Cu$^{2+}$ solutions (Fig.5).

In all sorption cycles was achieved a stable sorption/ desorption behaviour of the Biocer material, without a substantial loss of its sorption properties or the stability of the material (Fig. 5). So, a constant use of the Biocer material at least over 5 to 6 regeneration cycles seems to be feasible. The material seems to withstand several regeneration cycles without any loss of its loading capacity or its stability. The runoff of the metal concentrates in a regeneration cycle can be possibly used for further recovery processes.
3.5 Sorption kinetics of the Biocer material

In further experiments, the sorption kinetics of the Biocer material was investigated (Fig. 6). The equilibrium sorption capacity of 85% of the dissolved Cu$^{2+}$ is achieved after 1 hour (Fig. 6). After that, the sorption process of the Cu$^{2+}$ is only slightly increasing and approaching its maximum.

The results show, that the sorption process of the Cu$^{2+}$ ions needs a time of around an hour for the diffusion through the porous ceramic material and until the Cu$^{2+}$ is bound to the cell walls in a steady state equilibrium.

In order to obtain closer predications about the kinetics, the reaction order of the biosorption process was graphically determined. The reaction rate is calculated by eq. (2), where $a_q^*$ is the loading of the Biocer material, calculated from the difference of the maximum loading $a_{qs}$ and the loading $a_{qt}$ versus the time $t$. 

Figure 5. Multiple regeneration cycles of the Biocer material at various Cu$^{2+}$ concentrations (pH 5)

Figure 6. Sorption kinetics of the Biocer material with Cu$^{2+}$ in aqueous solution
By integration of eq. (2) was obtained the linearised eq. (3), with which the experimental data were fitted (Fig. 7). Fig. 7 shows, that the biosorption process of Cu\(^{2+}\) at the Biocer material obeys a reaction of 1\(^{st}\) order.

\[
\frac{d a_q^*}{dt} = k \cdot a_q^* \tag{2}
\]

\[
\ln a_q^* = \ln a_q^{*0} - k \cdot t \tag{3}
\]

Figure 7. Biosorption of Cu\(^{2+}\) on the Biocer material as a 1\(^{st}\) order reaction

Further experiments shall investigate the influence of mass transfer processes onto the sorption kinetics.

4. CONCLUSIONS

The Biocer material investigated here was good suited for the removal of copper from aqueous solutions. In batch experiments, first measurements have shown a good sorption capacity as well as an almost complete regeneration of the material with citric acid.

The Biocer material seems to withstand several regeneration cycles without any loss of its loading capacity or its stability. The runoff of the metal concentrates in a regeneration cycle can be possibly used for further rewinning processes. So, the material is good suited not only for the decontamination of waters with a low metal content, but also possibly for the recycling of economically valuable metals.

In future experiments shall be investigated in particular the mass transport behaviour of metal ions at the Biocer material, as well as the biosorption of ions like arsenate and arsenite because of their significance as contaminants in practice.

Especially interesting will also be the performance of the Biocer material in column experiments to investigate its practical application. It is expected to obtain excellent sorption results in the column investigations.

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Biosorption of chromium (VI) by marine algal biomass

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Abstract
The ability of local brown seaweeds *Sargassum* sp. and *Padina* sp. to remove Cr(VI) anions from solution was examined. Batch studies showed a higher maximum uptake capacity for *Padina* sp (1.04 mmol/g) than *Sargassum* sp. (0.60 mmol/g). The biosorption of chromium was pH dependent; higher uptake was observed as the pH decreased, with the optimal uptake occurring at about pH 2. Over an initial Cr(VI) concentration of 0.38 mmol/L to 10.6 mmol/L, the equilibrium adsorption data could be modeled according to the Freundlich and Langmuir adsorption isotherms. Both the algal biomass achieved about 90% of the maximum uptake capacity in 6-7 hours and attained equilibrium in 10 hours. Apart from the removal of Cr(VI) from solution by the biosorbent, a complete reduction of Cr(VI) to Cr(III) at low concentration occurred during the biosorption process. This offers an advantage over conventional physical/chemical treatment methods where chromium reduction and removal are accomplished separately.

1. INTRODUCTION
The widespread use of heavy metals in various industries has created environmental problems due to their hazardous nature and non-biodegradability. Chromium in industrial effluent is of major concern; the heavy metal is primarily present in industrial effluent as anion in the hexavalent form as chromate (CrO$_4^{2-}$) and dichromate (Cr$_2$O$_7^{2-}$) ions [1]. Compared with the relatively less harmful and mobile Cr(III), the hexavalent form of chromium is considered more toxic[2].

Conventionally, chemical precipitation, coagulation and flocculation, reverse osmosis and ion-exchange are some of the method commonly used in chromium treatment [3]. Chromium removal may involve several steps: the reduction of the Cr(VI) to trivalent form, the precipitation of Cr(III) as a metal hydroxide at high pH, followed by the settling and disposal of the dewatered sludge [4]. However, these processes have many drawbacks, including the generation of toxic sludge and high operational cost and incomplete reduction of Cr(VI).

To overcome these shortcomings, biosorption appears to be an alternative. The use of cheap and abundant biomass (either living or dead) has attracted much attention in the past decades. The biosorption process does not generate toxic chemical sludge, yet it is cost

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effective especially for the treatment of low concentration metal-bearing wastewater. The use of algal biomass for the chromium-containing wastewater treatment has been reported. A maximum removal of about $14.7 \times 10^3$ mg metal/kg of dry weight biomass by filamentous algae *Spirogyra* species was reported by Gupta et. al. (2001). *Chlorella vulgaris* [5-6], *Scenedesmus obliquus* [6], *Synechocystis sp.* [6] and many other species of algal biomass have also been investigated for their biosorptive ability for chromium. Marine algal biomass, *Sargassum sp.* and *Padina sp.* are investigated in this study in view of their promising results for the biosorption of various heavy metals [7-10]. The biosorption of chromium by *Padina sp.* has not been previously reported.

Factors affecting the biosorption efficiency were investigated in this study. These included a kinetic study on biosorption and reduction of chromate, as well as the effect of pH on the removal of chromium. Equilibrium studies using the Langmuir and Freundlich adsorption isotherms were also examined.

2. MATERIALS AND METHODS

2.1 Preparation of the biomass

The brown algal biomass *Sargassum sp.* and *Padina sp.* were harvested locally, and rinsed with copious quantity of water to remove all attached materials. The seaweed was sun-dried and ground into particles of various sizes; biomass of particle size 212-500µm was used in this study. The biomass was then washed with deionised distilled water and dried at 60°C overnight and stored in a dry cabinet before use.

2.2 Kinetic study

A kinetic study was conducted in order to determine the time required for the biosorption process to reach equilibrium. The biomass (6.0g) was added to 2L chromate solution (1 mM) prepared using K$_2$Cr$_2$O$_7$ (Merck Chemical). The pH of the solution was adjusted to pH 2, 4 or 6 initially using HCl or NaOH solutions, and was monitored continuously. A 4-mL sample was taken at predetermined time interval; the samples were filtered using a Whatman Autovial, with 0.45µm PTFE filter. The total chromium and Cr(VI) were analysed using ICP-ES and a colorimetric method 3500-Cr D respectively [11]. The Cr(III) concentration was obtained from the difference between these two analyses.

2.3 pH effect on chromium sorption

150 mg of the biomass was added to each conical flask containing 50 mL of chromate (Cr(VI)) solution and Cr(III) solution (prepared from Cr(NO$_3$)$_3$.9H$_2$O), with initial concentration of 1 mM and pH over the range 1 to 8 and 1 to 5.5 (using NaOH or HCl). The mixtures were agitated on a rotary shaker at 150 rpm at ambient temperature (24 ± 1°C). After 12 hours of agitation, the supernatant of the solution was filtered and analysed for total chromium, Cr(VI), and Cr(III) concentration. The final pH of the solution was measured using ORION-420A. The total chromium uptake was calculated using the equation below:

$$Q = (C_i - C_f) \frac{V}{m} \quad (2.1)$$

where $C_i$ and $C_f$ are the initial and final total chromium concentration, $V$ is the volume of the solution (L) and $m$ is the amount of biomass (g).
2.4 Analysis of the adsorption isotherms

150 mg of biomass was contacted with 50 mL of chromate solution at an initial concentration ranging from 0.4-10.6 mmol/L, and adjusted initially to pH 2. The mixtures were agitated on a rotary shaker at 150 rpm at ambient temperature (24 ± 1°C). The pH was adjusted from time to time. The supernatant was filtered after 12 hours using a Whatman Autovial, with a 0.45µm PTFE filter, and analysed for total chromium concentration.

Two commonly used adsorption models, the Langmuir and Freundlich isotherms were used to evaluate the experimental data. The Langmuir isotherm has the general form:

\[ Q = \frac{Q_{\text{max}} b C_e}{1 + b C_e} \]  

(2.2)

where \( Q \) = total chromium uptake; \( Q_{\text{max}} \) = maximum uptake of total chromium; \( b \) = affinity of sorbate for the sorbent and \( C_e \) = the equilibrium or final concentration of chromate solution.

The Freundlich isotherm has the general form:

\[ Q = K C_e^{1/n} \]  

(2.3)

where \( Q \) = total chromium uptake, \( K \) = biosorption equilibrium uptake and is indicative of the biosorptive uptake capacity, \( n \) = biosorption equilibrium constant, and \( C_e \) = the equilibrium or final concentration of chromate solution.

3. RESULTS AND DISCUSSION

3.1 Kinetic study

The kinetics of sorption describing the solute uptake rate which in turn governs the time of sorption reaction is one of the important characteristics defining the efficiency of sorption [12]. In order to determine the time required for the biosorption process to reach equilibrium, a kinetic study was carried out. From the experimental results (Figure 1a-b), both biomass achieved about 90% of the maximum uptake capacity in 6-7 hours. No further removal was observed after 10 hours. Similar results have also been reported by Luis et. al. [13]. Several studies on chromium uptake had assumed that an equilibrium is attained within 6 hours of biosorption [13, 14]. In our work, a contact time of 12 hours was used to ensure that the system has reached equilibrium. Kinetic studies at pH 4 and 6 were also conducted under the same condition. At higher pH, less time was needed for the sorption process to reach equilibrium (data not shown).

An examination of the results showed that the removal of the hexavalent chromium is a coupled process of sorption and reduction at a different rate. Complete reduction of Cr(VI) to Cr(III) was observed during the biosorption process at pH 2. Padina sp. reduced chromate at a faster rate than Sargassum sp., where complete reduction was achieved within 8 hours. Indeed, chromate in the solution was reduced as soon as the seaweed was in contact with the solution (see Figure 2). It is clear that the instantaneous reduction is faster for Padina sp. than for Sargassum sp.; at 15 minutes, the ratio of Cr(VI):Cr(III) was 9.2:1 and 16.8:1 respectively. To reduce the ratio of Cr(VI):Cr(III) to 1.5, Padina sp. takes 3 hours whereas Sargassum sp. takes about 7 hours. Both the reduction processes occur until all the chromate was reduced to the trivalent form. This is in contrast with results reported by Hayashi et. al. (2001), who found that the Cr(VI)/Cr(III) ratio in the solution increased gradually from 1.5 to 2.5 over the time interval 30 to 150 minutes. The increase
in Cr(VI)/Cr(III) ratio was attributed to the possible sorption of Cr$^{3+}$ species by the biomass.

Figure 1: Progress of biosorption and reduction of (a) Padina sp. (b) Sargassum sp. (Biomass dosage = 3.0 g/L, Initial metal concentration = 1 mM, pH = 2, Chromium was present initially as Cr(VI))

Figure 2. Reduction in Cr(VI):Cr(III) during biosorption by Padina sp. and Sargassum sp. (Biomass dosage = 3.0 g/L, Initial metal concentration = 1 mM, pH = 2)

To examine the effect of pH on chromium uptake by the seaweed, Cr (VI) and Cr(III) (i.e., K$_2$Cr$_2$O$_7$ and (Cr(NO$_3$)$_3$.9H$_2$O) were used in order to compare the binding characteristic of both biomass to chromium with different oxidation state. Cr(III) uptake was favored at a higher pH (see Figure 3a-b). At pH 2, the uptake of Cr(III) was lower compared with that of the system with chromium present initially as Cr(VI). Thus, the gradually decrease in the Cr(VI):Cr(III) ratio (at pH 2) noted earlier maybe due to the less significant uptake of Cr(III). It is noteworthy that the Padina sp. showed comparable reduction ability with that of treated Sargassum biomass reported in Luis et al. [13].

3.2 pH effects on the biosorption

In the pH effects, the biomass was contacted with chromate solution with different pH. The uptake of chromium increases with a decrease in pH up to 2, and the uptake decrease at pH 1. The results indicate that pH 2 is the optimum for the biosorption of
chromium (Figure 3a-b), where the total chromium sorbed onto the biomass is about 62% and 50% for Padina sp. and Sargassum sp. respectively. Luis et al. [13], Kratochvil et al. [14], Gupta et al. [15] and Niu et al. [16] have earlier noted a similar optimum for chromium biosorption at pH 2. The similar trend in the effect of pH on the biosorption of chromium has been reported for a variety of biomass including Rhizopus nigricans [3], Scenedesmus obliquus, Synechocystis sp. [6], Chlorella vulgaris, Clodophara crispata, Rhizopus arrhizus, Saccharomyces cerevisiae, Zoogloea ramigera [17] and dried activated sludge [18].

It is well known that pH is an important parameter that influences the biosorption process [19]. Interactions between the metal ions and the functional groups of the biomass Sargassum sp. and Padina sp. depend not only on the nature of the biosorbent but also on the solution chemistry of the metals to be removed [3]. In this study, the solution chemistry of chromium is predicted using a chemical speciation prediction program, MINEQL (Figure 4). At pH 2, HCrO$_4^-$ is the predominant species of chromium in solution. Depending on the extent of protonation on the seaweed, the chromate anions, HCrO$_4^-$ and Cr$_2$O$_7^{2-}$ that are likely to be present in the solution would be attracted to the positively charged functional group on the seaweed. A decrease in pH will result in a more positive charge on the surface of the seaweed.

![Figure 3](image3.png)

**Figure 3.** pH effects on the removal of Cr(III) and Cr(VI). (a) Padina sp. (b) Sargassum sp. (Biomass dosage = 3.0 g/L, Initial metal concentration = 1 mM, pH = 2, Contact time = 12h)

![Figure 4](image4.png)

**Figure 4.** Speciation of Cr(VI) predicted by MINEQL (Cr(VI) concentration = 1mM)
SOH + H⁺ → SOH₂⁺  
SOH → SO⁻ + H⁺  
(at low pH)

(at high pH)

The increase in the biosorption of Cr(VI) at the lower pH thus suggests that the negatively charged chromium species bind through electrostatic attraction to the positively charged functional groups on the biomass surface [3, 6,15,18].

The existence of the optimum pH was explained by the desorption of Cr(III) from the biomass at low pH and the effect of pH on the reduction potential of Cr(VI) in aqueous solutions [14]. Figure 5 shows that total removal of chromate by reduction occurred at lower pH, especially at pH 1-1.5. At these pH, the reduction of anionic Cr(VI) species to Cr(III) dominate the system. Further more, the sorption of Cr³⁺ is also not favored because positively charge hydrogen ions will compete with metal ions for the ligands on the cell wall of biomass This is also supported by the experimental results where the uptake of Cr(III) by the biomass at pH 1 is less than 20% of total chromium removal (Figure 3a-b).

The Cr(VI) reduction during chromate biosorption has also been observed by the other workers. The reduction of chromate is greater at a lower pH. Figure 5 shows that the reduction of chromate is greatest at pH 1-2 in the Padina sp. system, and at pH 1-1.5 in the Sargassum sp. system. The observations that the reduction of chromate is lower in higher pH is also consistent with the calculations by Kratochvil et. al. [14] using the Nernst equation which showed that the redox potential of chromate was greater at lower pH.

Figure 5. Cr(VI) removal by Padina sp. and Sargassum sp. as a function of equilibrium pH. (Biomass dosage = 3.0 g/L, Initial metal concentration = 1 mM, Contact time = 12h)

3.3 Langmuir and Freundlich adsorption isotherm

The adsorption isotherms of the biomass are illustrated in Figures 6-7. The experimental results are plotted using linearized Langmuir and Freundlich adsorption isotherms over a concentration range of 0.4-10.6 mmol/L. As shown in Table 1, the experimental data are consistent with both the isotherms.

Langmuir model is a theoretical model for monolayer adsorption while the Freundlich model allows for multilayer adsorption at heterogeneous surfaces. The conformity of the experimental results to both the models shows that the sorption of chromate onto the biomass is complex and may involve more than one mechanism [12].
physical interpretation thus may not be drawn; the sorption isotherms do not necessarily reflect the adsorption mechanisms involved [20-21].

Table 1. Freundlich and Langmuir model regression constants of the biomass

<table>
<thead>
<tr>
<th>Type of biomass</th>
<th>Freundlich isotherm constants</th>
<th>Langmuir isotherm constants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>n</td>
</tr>
<tr>
<td>Padina sp.</td>
<td>0.329</td>
<td>1.912</td>
</tr>
<tr>
<td>Sargassum sp.</td>
<td>0.221</td>
<td>2.228</td>
</tr>
</tbody>
</table>

The Langmuir parameters, \( Q_{\text{max}} \) and \( b \) represent the maximum uptake and the affinity between the sorbate and sorbent respectively. A high value of \( b \) indicates a (desirable) steep beginning of the isotherm, which reflects the high affinity of the biosorbent for the sorbate [7]. In comparison with Sargassum sp., Padina sp. has a higher uptake capacity although its affinity constant, \( b \) is marginally lower than Sargassum sp.

From the Freundlich Isotherm, the adsorption capacity, \( K \) of Padina sp. and Sargassum sp. is 0.329 and 0.221 respectively. Again, it is shown that the ability of Padina sp. to adsorb chromium ion is greater than Sargassum sp. In both cases, the intensity of adsorption, \( n \), is greater than one, thus indicating that the adsorption of the chromium ion is favorable [3, 12].

Figure 6. Freundlich isotherm over a concentration range of 0.4-10.6 mmol/L (pH=2.0; Biomass dosage=3g/L)

Figure 7. Langmuir isotherm over a concentration range of 0.4-10.6 mmol/L (pH=2.0; Biomass dosage=3g/L)

Compared with H\(^+\)-protonated and Ca-treated Sargassum biomass [13-14], the uptake of chromium by both the biomass reported in this study is lower. This may due to the lower extent of protonation in the untreated biomass. As reported, the unique mixture of polysaccharides (mainly alginate and fucoidan) is largely responsible for the excellent metal sequestering ability of the brown algae. Carboxylate groups of alginate have been identified as the main metal binding site. Besides that, other negatively charged functional groups such as the sulphonate groups of fucoidan may also contribute to heavy metal complexation [7]. Even though the functional groups will be protonated at lower pH, it is believed that pre-treatment with acids will enhance the protonation, and thus increase the uptake of heavy metal.
4. CONCLUSIONS

The removal of chromium (VI) is a coupled process with biosorption and reduction. The process is pH dependent, and an optimum uptake occurs at pH 2. This is related to the speciation of chromium in the solution and the extent of protonation on the biomass. As most electroplating units discharge chromium in acidic solution [13, 22], minimal pH adjustment is required when biosorption is applied. Results from equilibrium studies showed that the Langmuir and Freundlich isotherms fitted the data well. Compared with Sargassum sp., Padina sp. was found to have higher adsorption capacity and reduction ability for chromium.

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Biosorption of heavy metal ions from aqueous solutions by local seaweeds

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Abstract

The uptake capacities of the biomass of four seaweeds Sargassum sp., Padina sp., Ulva sp., and Gracillaria sp. (collected from Singapore coasts) for heavy metal ions (lead, copper, cadmium, nickel and zinc) were evaluated in this study. The metal ion removal rates were very rapid, with 90% of adsorption taking place within 10-60 min. The adsorption capacities of the biomass were strongly dependent on equilibrium solution pH; higher pH favored metal ion removal. Equilibrium experiments were carried out at pH 5.0 (for lead and copper) and pH 5.5 (for cadmium, zinc and nickel). Sargassum sp. and Padina sp. were found to have the highest potential in the removal of lead, copper, cadmium, nickel and zinc ions. The maximum uptake capacities ranged from 0.61 to 1.16mmol/g for Sargassum sp. and 0.63 to 1.25mmol/g for Padina sp.

1. INTRODUCTION

Heavy metal contamination is of worldwide environmental concern especially in developing countries. Conventional methods for removing heavy metals from industrial effluents (e.g. precipitation and sludge separation, chemical oxidation or reduction, ion exchange, reverse osmosis, membrane separation, electrochemical treatment and evaporation) are often ineffective and costly when applied to dilute effluents. A good sorbent to remove heavy metal should be both effective and inexpensive. Biosorption shows promise of fulfilling these requirements.

Since biosorbents are essentially dead materials, no nutrition is needed to maintain the biomass. Problems associated with metal toxicity in living biomass and the need to provide suitable growth condition also do not arise. Indeed, many early studies have shown that nonliving biomass may be even more effective in sequestering metallic elements than living cells [1, 2].

The first major challenge for biosorption is the selection of the most promising types of biomass from an extremely large pool of readily available and inexpensive biomaterials. Many types of biomass in non-living form have been studied for their heavy metal uptake capacities and suitability for use as bases for biosorbent development. These include bacteria [3], fungi [4], yeast [5], fresh water algae [1] and marine algae [6, 7]. Several have focused on marine algae due to its easy availability and high uptake capacity

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The capacities of a few brown algae have been found to be much higher than those of other types of biomass, and even activated carbon, natural zeolite and synthetic ion exchange resins [4, 10]. For divalent heavy metal ions, *Sargassum* spp. [11, 12], *Rhizopus* spp. [12, 13], *Durvillaea* spp. [14, 15], *Ecklonia* spp. [14,15] and *Lessonia* spp. [15] showed very good biosorption performances among the marine algae studied.

This study compares the metal biosorption performances of several local seaweeds which are abundant in Singapore seashores. This includes two brown seaweeds (*Sargassum* sp., *Padina* sp.), a green seaweed (*Ulva* sp) and a red seaweed (*Gracillaria* sp.). The choice of heavy metals (i.e. lead, copper, cadmium, zinc and nickel) was made with regard to their common industrial use and potential pollution impact.

2. MATERIALS AND METHODS

*Preparation of biomass and chemicals*

The raw biomass of *Sargassum* sp., *Padina* sp., *Ulva* sp., *Gracillaria* sp. were collected from the Singapore coasts. The sun-dried biomass was ground to various particle sizes, from which particles of 500-800 µm were used in this work. The biomass were washed with DI water and dried at about 60°C overnight before use in the experiments.

The stock metal solutions at various concentrations were prepared by dissolving lead nitrate, copper nitrate, cadmium sulfate, nickel nitrate and zinc nitrate respectively. All metal salts were of reagent grades.

*Batch adsorption experiments*

In the equilibrium experiments to study the pH effect, metal solutions at various initial pH were prepared. pH was adjusted using 0.1mol/l HNO₃ or 0.1mol/l NaOH. The biomass was added into the conical flasks containing the metal solutions. The flasks were agitated at 200 rpm for 6 hours. The experiments were conducted at room temperature (22±1°C). The final solution pH was measured with an ORION 525A pH meter. The metal concentrations were analysed using an inductively coupled plasma emission spectrophotometer (ICP-ES) (Perkin-Elmer Optima 3000).

In the kinetic experiments, the initial pH was adjusted to the selected optimum value before the biomass was added to the solutions while stirring. The pH was measured at 20-30 minute intervals and adjusted accordingly. Samples were taken at periodic time intervals and the metal concentrations were analyzed.

In the isotherm experiments, the solution pH was adjusted as in the kinetic experiments. The same amount of biomass was added to the solutions at various metal concentrations. All bottles were shaken at 200 rpm at room temperature. The initial and final metal concentrations were determined using the ICP-ES.

Biosorption metal uptake (q) was calculated from the sorption system mass balance:

$$ q = V(C_i - C_f)/S $$

where V is the volume of the solution, S is the amount of biomass, and $C_i$ and $C_f$ are the initial and final metal concentrations respectively.

The Langmuir adsorption isotherm was used to fit the experimental data:

$$ q = (q_{\text{max}} \cdot C_f)/(b^{-1} + C_f) $$

$q_{\text{max}}$ and $b$ are Langmuir constants, which reflect the maximum metal adsorption capacity and affinity between metal ion and biosorbent.
3. RESULTS AND DISCUSSION

Effect of solution pH

Figure 1 summarizes the results obtained using the chemical equilibrium program MINEQL [16] for the lead nitrate, copper nitrate, cadmium sulfate, nickel nitrate and zinc nitrate systems. All data sets were calculated considering the carbonate system naturally in equilibrium with atmospheric carbon dioxide ($P_{CO2}=10^{-3.5} \text{ atm}$). For the lead nitrate system, $Pb^{2+}$ is the dominant species present at lower pH. In this experimental system (maximum concentration of 2.0 mmol/l), $Pb^{2+}$ remains the dominant species up to about pH 5.5. At pH higher than 5.5, solid lead hydroxide is thermodynamically the most stable phase. In addition, the effect of the influence of low concentrations of sodium and nitrate (present from pH adjustment during the sorption experiment) on the speciation was negligible.

The same approach was applied to calculate the other four metal ion solution systems at a concentration of 2.0mmol/l. $Cu^{2+}$ is the dominant species present up to pH 5.2 in the copper nitrate system. For the cadmium sulfate, zinc nitrate and nickel nitrate systems, the free metallic ions species are dominant at pH lower than 6.0.

It is well documented that solution pH is an important parameter affecting biosorption of heavy metal ions [5, 17, 18]. Heavy metal ions (lead, copper, cadmium, nickel and zinc) adsorption by the seaweeds (Sargassum sp., Padina sp., Ulva sp., Gracillaria sp.) as a function of pH was studied. Only results for lead are shown here (Figure 2). On the whole, the uptake of metal ions increased sharply from pH 2 to 4.5; beyond pH 4.5, its increasing effect on uptake was reduced. These finding are in agreement with results reported earlier [17, 18].

The pH dependence of metal uptake is largely related to the surface functional groups (mainly carboxylic) [9] in the biomass cell wall and also on the metal chemistry in solution. Since the metals are present in their free ionic form (Figure 1) at pH less than 4.5, the sharp increase in metal adsorption from pH 2 to 4.5 cannot be explained by the change in metal speciation. This implies that the type and ionic state of the cell wall functional groups at these pHs determine the extent of sorption. As positively charged ions, hydrogen ions may compete with metal ions for the ligands on the cell wall. At lower pH, the concentration of hydrogen ions is higher, which leads to less ligands availability.
on the biomass for metal ions sorption. As the pH is increased, more ligands are available for metal ions, thus resulting in an enhanced metal ion removal.

In order to ensure that the metal ions were in their free ionic states during biosorption processes, the pH of the following kinetic and isotherm experiments were controlled at 5.0 for lead and copper, and at 5.5 for cadmium, zinc and nickel.

**Determination of equilibrium time**

Kinetic experiments were carried out to determine the equilibrium time required for the uptake of metal ions by different biomass. Only results for lead are shown here (Figure 3). In general, a two-stage kinetic behavior is seen: very rapid initial sorption for a few minutes, followed by a long period of a much slower uptake (especially for Padina-metal and Sargassum-metal systems). It is known that biomass cell walls are heterogeneous [9]. Various functional groups serve as adsorption sites that differ both with respect to the strength of the metal sorptive bond and the rate of adsorption on these sites. Hence this results in the classification of fast and slow uptake rates for the same metal ions. The results show that equilibrium times needed for the different metal-biomass systems ranged from 1 to 3 hours, with 90% of the total adsorbed metal ions occurring within 10-60 minutes. The contact time for the following isotherm experiments was set at 6 hours in order to assure the uptake equilibrium.

![Figure 3. Kinetic experiments of Lead uptake (m = 1.0 g/l, C_o = 1 mmol/l, pH = 5.0)](image)

**Adsorption Equilibrium**

Isotherm experimental results are shown in Figure 4a-4e. In all cases favorable isotherms are observed and the data could be modeled according to the Langmuir adsorption isotherm. Table 1 shows the maximum adsorption capacity (q_max) and affinity constant (b). Among the biomass screened, Sargassum sp. and Padina sp. are identified to be good biosorbents for removal of all the metal ions investigated.

The equilibrium isotherms of Pb^{2+} on the studied biomass in their non-pretreated forms are shown in Figure 4a. The maximum adsorption capacities of Padina sp., Sargassum sp., Ulva sp., and Gracillaria sp. for lead were 1.16, 1.14, 0.83, 0.41 mmol/g respectively at the final concentration of about 1.0mmol/l. Table 1 shows that Padina sp. and Sargassum sp. exhibited the highest capacity and adsorption affinity (initial slope) with the maximum adsorption capacities at 1.25 and 1.16 mmol/g respectively. Ulva sp. sequestered more lead at higher residual concentrations than Gracillaria sp., while its affinity for lead is less than that of Gracillaria sp.
The sorption performance of the sorbent is manifested through the two Langmuir parameters: the maximum adsorption capacity $q_{\text{max}}$; and the affinity constant $b$. In biosorption, both a high $q_{\text{max}}$ and $b$ are desired. In the uptake of lead by *Ulva* sp., it can be
seen that although the seaweed possessed the highest $q_{\text{max}}$, unfortunately it has the lowest affinity constant amongst all the biosorbents (see Table 1).

The biosorption potentials of the four seaweeds for the other four metal ions (Cu$^{2+}$, Cd$^{2+}$, Zn$^{2+}$ and Ni$^{2+}$) were further evaluated as shown in Figure 4b-4e and Table 1. The general shapes of isotherms were similar to that of lead. However, the adsorption capacities and affinities were significantly different. In general, the brown seaweeds (Padina sp. and Sargassum sp.) showed a better performance than the green seaweed (Ulva sp.) and red seaweed (Gracillaria sp.) for removal of all the metals investigated; for the same seaweed studied, the uptake capacity of lead was the highest, followed by copper, cadmium, zinc and nickel.

<table>
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<th>Table 1. Parameters for Langmuir isotherms</th>
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<td>$q_{\text{max}}$ (mmol/g)</td>
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<td>Padina sp.</td>
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<td>Sargassum sp.</td>
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<th>Cu</th>
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<td>$q_{\text{max}}$ (mmol/g)</td>
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<td>Padina sp.</td>
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<td>Sargassum sp.</td>
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<td>Ulva sp.</td>
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<td>$q_{\text{max}}$ (mmol/g)</td>
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<td>Padina sp.</td>
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<td>Sargassum sp.</td>
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<td>$q_{\text{max}}$ (mmol/g)</td>
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<td>Padina sp.</td>
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<td>Sargassum sp.</td>
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<td>Ulva sp.</td>
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<td>$q_{\text{max}}$ (mmol/g)</td>
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<td>Sargassum sp.</td>
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<td>Ulva sp.</td>
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These uptake differences in isotherms may be related to the compositional differences among the algal biomass, as well as the binding mechanisms involved for different heavy metal ions. The biomass cell walls are known to be heterogeneous. For instance, the cell wall of brown algae contain algin, fucoidan and cellulose, while in most green algae, the outer part of the cell wall consists mainly of pectic substances and cellulose [9]. The mixture of polysaccharides, mainly alginate and fucoidan, is largely responsible for the excellent metal sequestering ability of the brown algae [1]. This may explain the reason that the biosorption performances of brown algae generally are superior to those of green algae. The alginate responsible for metal sorption is present in a gel form in the cell walls [6]. The cell walls of algae are often porous in their structure, which allows molecules and ions to pass freely through [19]. In addition to the porosity of the algal cell wall structure, the cell constituents can provide an array of ligands and functional groups, which bind metallic ions [9]. Numerous metal-binding mechanisms have been postulated in biosorption. These include chemisorption by ion exchange, complexation, coordination, chelation, physical adsorption and microprecipitation [20]. Oxidation/reduction reactions also may be involved [21]. Due to the complexity of the composition of the biomaterial, it is likely that some of these mechanisms are acting simultaneously to varying degrees.
Several studies have indicated a dominant role of ion exchange for metal biosorption [16, 22]. Further studies are needed in understanding the interaction behaviors between the biomass and heavy metal ions.

The binding strength of various divalent ions sequestered also is important in the uptake process [9]. The general affinity sequence for *Padina* sp. is Pb>Cu>Cd>Zn>Ni; and for *Sargassum* sp. is Pb>Zn>Cd>Cu>Ni. Both these (and especially the latter) differ from that for the alginates extracted from another brown seaweed (*Laminaria digitata*) Pb>Cd>Ni>Zn [23].

### 4. CONCLUSION

In this study, kinetic and batch experiments were investigated for the biosorption of lead, copper, cadmium, zinc and nickel by four local seaweeds *Sargassum* sp., *Padina* sp., *Ulva* sp., and *Gracillaria* sp.. Metal removal was significantly dependent on pH. The metal ion uptake increased sharply from pH 2 to 4.5; beyond pH 4.5, its increasing effect was diminished. Results also showed that most of the adsorption capacities could be achieved in a very short time; 90% of the total adsorbed metal ions was achieved within 10-60 minutes.

The general affinity sequence for *Padina* sp. is Pb>Cu>Cd>Zn>Ni; and for *Sargassum* sp. Pb>Zn>Cd>Cu>Ni. Overall, the brown seaweeds (*Sargassum* sp. and *Padina* sp.) showed better overall biosorption performance when compared with the green and red seaweeds (*Ulva* sp. and *Gracillaria* sp. respectively). In the biosorption studies with single metal ion, the maximum uptake capacities for lead, copper, cadmium, nickel and zinc were 1.16, 0.99, 0.76, 0.61 and 0.50 mmol/g respectively for *Sargassum* sp. and 1.25, 1.14, 0.75, 0.63 and 0.81 mmol/g respectively for *Padina* sp., which are higher than or comparable to those of most other types of biomass reported in the literature. The results suggest that these brown seaweeds could be used to develop high capacity biosorbent materials for the removal of heavy metal ions from aqueous solutions.

Since this study served to identify local marine biomass that may be used in heavy metal removal, no pretreatment methods were applied. Pretreatment may be expected to enhance the biosorption behavior [9].

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Biosorption of heavy metals onto an olive pomace: adsorbent characterisation and equilibrium modelling

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Abstract

In this study an agricultural waste, an olive pomace, was used to remove heavy metals from aqueous solutions at different equilibrium pH. The adsorbent material was preliminary characterised from a physico-chemical point of view by size distribution analysis, SEM analysis, CEC, potentiometric titration and IR analyses. The acid-base properties of the functional groups on the adsorbent were then modelled by a model accounting for the functional heterogeneity. The modelling of the potentiometric data coupled with other experimental findings evidenced the presence of two main kinds of active sites: carboxylic and phenolic groups. Equilibrium tests carried out using different heavy metals at different equilibrium pH evidenced the positive effect of increasing pH on the specific adsorption capacity of the adsorbent. Moreover a general affinity series was observed in agreement with that reported in literature for other adsorbent materials (both inorganic and biological): Pb>Cu>Cd. This affinity series can be explained considering the HSAB theory of Pearson for complexes formation and it suggests a complexation reaction among metals and active sites.

1. INTRODUCTION

Heavy metal contamination is caused by different kinds of industrial, mining and military activities. Heavy metal ions are not biodegradable pollutants and tend to accumulate in the living organism, causing various diseases and disorders.

Traditional treatment processes of contaminated wastewaters (ion exchange, precipitation and evaporation) present different technical and economical constraints about the fulfilling of law regulations.

Biosorption is an alternative technology to remove heavy metal from dilute aqueous solutions based on the property of certain kinds of inactive and dead biomasses to bind...
and accumulate these pollutants. Biosorbents generally used for these purposes are wastes coming from agricultural and industrial activities or specially propagated biomasses of fungi, yeast and bacteria. The main advantage in biosorption is cost-effectiveness being based on the use of "low cost" materials. Olive pomace is one of these possible adsorbents that is abundant especially in countries of the Mediterranean area. In particular Italy is one of the major producing countries of olive oil; the manufacturing of olive oil yields an oily phase (30%), a solid residue (20%), and an aqueous phase (50%). The solid residue (pomace) is actually swallowed by means of controlled spreading on agricultural soil; a little amount is also used as natural fertilizer, combustible and added to animal food. An alternative use can be as low cost natural adsorbent (about 50 $/ton against 4500 $/ton of a granular activated carbon) [1].

At now the main enterprises concerning a wider application of biosorption technology in large-scale plants are related to product performance optimisation and continuous process development. Both these aspects are strictly connected to the understanding of the physico-chemical mechanisms involved in the interactions among active sites on solid adsorbent and ionic species in solution. Mathematical modelling is then a fundamental step in this paper both as understanding means and also as the base for dynamic process design. In particular considering pH as one of the most influencing operative condition in biosorption of heavy metals, the effect of this factor is studied both as acid-base behaviour of the adsorbent and as a factor affecting the equilibrium uptake of different heavy metals.

The aim of this work is then twofold:

- modellistic and interpretative: characterizing the adsorbent and representing its acid-base behaviour
- technological and applicative: exploring the capacity of olive pomace in heavy metal removal from aqueous solutions in different operative conditions

2. MATERIALS AND METHODS

2.1 Olive pomace

Pomace was collected as pressed and sunny dried disks from an olive oil production plant in Italy. Pomace was grounded and particle size distribution was determined by an automatic sieve. Pomace samples were washed by distilled water: 20 g/L biomass suspensions were kept at 250 rpm in a shaker for 90 minutes at 25°C; solid-liquid separation was performed by centrifugation. After three successive washings solid samples were dried and used for titration and heavy metal biosorption [1].

2.2 Potentiometric titration

Pomace suspensions were preliminary acidified (6 mg in 20 mL of H2O acidificated with HCl 0.1 M, standard solution) and then potentiometrically titrated by successive additions of NaOH 0.1 M, standard solution.

2.3 Equilibrium tests

Equilibrium biosorption of Pb(II), Cd(II), Cu(II) was determined by using 10 g/L pomace suspensions in which different initial metal concentrations were added. Samples were kept at constant pH and temperature under magnetic stirring until equilibrium conditions were reached (90 minutes). Sold-liquid separation was performed by centrifugation and the metal concentration was determined by an Inductively Coupled Plasma Spectrophotometer (ICP). For each sample, a blank test without biomass was
performed to determine the initial metal concentration by ICP and to avoid confusion between biosorption and possible metal precipitation.

3. RESULTS AND DISCUSSION

3.1 Adsorbent characterisation

Olive pomace is a very heterogeneous matrix both from a morphological and a functional point of view. The morphological heterogeneity was evidenced experimentally by SEM analyses (here not reported) which show the presence of particles with different shape and a wide range of dimensions. This is also evidenced by the distribution of particle size of a grounded sample (Fig. 1).

![Figure 1. Size distribution of pomace particles expressed as % weight](image)

A part from the different classes evidenced in the histogram two main types of particles come out from grinding: a type A with lower dimensions (<500µm) and a type B with larger dimensions (>500µm). This classification was chosen according to the different aspect of particles over and below 500µm. In particular type A is characterised by a dark brown colour and is preferentially made up of the cellulose, residual fats and polyphenolic substances of olive pulp, while type B fraction is dark yellow and composed of lignin fragments of olive seeds [2]. The functional heterogeneity associated to this morphological difference was put in evidence by the potentiometric titration data of olive pomace mix after grinding compared with those of a blank without biomass and of the two fractions (Fig. 2).

The analysis of the experimental results shows that:

- acidic active site concentration is larger in the A fraction with lower dimensions then in the B one, so that morphological and functional heterogeneity results strictly related according to the different fractions composition already mentioned;
- in any case trends are rather flat testifying the heterogeneity of the matrix and the impossibility of distinguishing separated groups with well separated acid pK.
According to this last observation titration modelling of this material cannot be performed assuming the presence of few distinct sites. A continuous approach was necessary requiring the introduction of an affinity distribution for the logarithm of the protonation equilibrium constant of the active sites determining the acid-base behaviour of the system. This approach was developed for representing the interaction among ionic species in aqueous solutions with humic substances [3].

The continuous approach here considered relates the total fraction of protonated sites \( \theta_{T,H} \) on the adsorbent given by the following general integral:

\[
\theta_{T,H} = \frac{[LH]}{[L] + [LH]} = \int_{\log K_H} \theta_{L,H}(K_{H,H}) f(\log K_H) \log K_H
\]

(1)

where \( L \) is the free active site with an affinity constant \( K_H \) whose logarithm is distributed according to a certain affinity distribution \( f(\log K_H) \) over a specified range \( \Delta \log K_H \).

This integral can be solved to obtain an analytical expression for \( \theta_{T,H} \) choosing the proper local isotherm \( \theta_{L,H} \) and \( f(\log K_H) \) distribution. In the literature there are different expressions obtained using different kinds of distributions for \( f(\log K_H) \) [4] and a Langmuir type model (eq. 2) as local isotherm

\[
\theta_{L,H} = \frac{K_{H[H]}}{1 + K_{H[H]}}
\]

(2)

where \( K_{H[H]} \) is the affinity equilibrium constant among active site \( L \) and protons

\[
K_{H[H]} = \frac{[L][H]}{[L][H]}
\]

(3)

Experimental data were firstly reported as negative charge for gram of adsorbent \( Q_H \) mmol/g) as a function of pH by applying the charge balance in the system

\[
Q_H = \sum_i \left( \frac{L_i}{m} \right)^V = \left( \frac{[H^+]}{m} \left[ Na^+ \right] - \left[ OH^- \right] - \left[ Cl^- \right] \right)^V
\]

(4)
where $V(L)$ is the suspension volume during titration, $m \text{ (g)}$ is the amount of biomass, $[\text{Na}^+]$ is the sodium concentration added in solution as sodium hydroxide (titrant) and $[\text{Cl}^-]$ is due to the initial addition of HCl to the biomass suspension to lower the initial pH (see section 2.2).

The heterogeneity analysis of experimental data was then used to have preliminary information about the number of active site types and the shape of the log$K_H$ distribution [5]. Two main kinds of acidic sites were assumed to represent experimental data of $Q_H$ versus pH considering a Sips distribution for each one

$$f(\log K_H) = \frac{\ln(10) \sin(m\pi)}{\pi \left( \frac{K_H}{K^*_H} \right)^{m} + 2 \cos(m\pi) + \left( \frac{K_H}{K^*_H} \right)^m}$$

(5)

where $\tilde{K}_H$ is the median value of the distribution and $m \text{ (0<m<1)}$ is a parameter related to the shape of the distribution and in particular as $m$ decreases the distribution becomes larger (for $m=1$ it is a Dirac distribution meaning a homogeneous type of site).

By this way

- considering the relation among fraction of protonated sites $\theta_{T,H}$, negative charge on the adsorbent $Q_H$ and maximum negative charge $Q_{\text{Max}}$

$$Q_H = Q_{\text{Max}} \left( 1 - \theta_{T,H} \right)$$

(6)

- extending the general Langmuir-Freundlich expression to two kinds of active sites, the following expression can be obtained relating the negative charge on the adsorbent $Q_H$ with the hydrogen ion concentration in solution

$$Q_H = \frac{Q_{\text{Max},1}}{1 + (\tilde{K}_{H,1}[H])^m_1} + \frac{Q_{\text{Max},2}}{1 + (\tilde{K}_{H,2}[H])^m_2}$$

(7)

A non-linear regression of the experimental data was then performed to determine the adjustable parameters characteristic of each site, which define concentration $Q_{\text{Max},i}$ (mmol/g) and shape of the related affinity distribution ($\tilde{K}_H$ and $m_i$). In order to find significative values of the parameter, the range was restricted during non linear regression: $\tilde{K}_H$ varied from 2 to 12, that is the range of pH studied during the titration, $m$ varied from 0 to 1 in accordance with possible value of the Sips distribution and an estimate of $Q_{\text{Max}}$ was obtained by the graphic method of Gran [6]. Experimental data and model curve were reported in Figure 3 along with the affinity distribution for the two sites obtained putting the adjustable parameters (Table 1) in the Sips distribution to give an idea of the dispersion of the acid-base properties due to adsorbent heterogeneity. The goodness of the model evidenced by the agreement among experimental and predicted data is also enforced considering the obtained values of the adjustable parameters. In particular the total concentration of active sites is in agreement with the preliminary estimate made by Gran’s method (0.67 mmol/g), and the equilibrium acidic constants are realistic considering the component of olive pomace.

On the base of the parameters values and from the literature analysis it is then reasonable to assume that these two kinds of functional groups are carboxylic and phenolic. Also the analysis of the IR spectrum on solid phase (here not reported) can be used to obtain some confirms about the nature of these sites. In fact even though this spectrum resembles the high heterogeneity of the pomace, some characteristic peaks can
be evidenced. In particular different peaks in the C=O stretching region (two peaks at 1704 and 1643 cm⁻¹) are in agreement with the presence of the carboxylic groups and also confirm the heterogeneity of this functional group that is present both in fat and polyphenol compounds. The characteristic peaks of phenolic groups are those related to OH stretching which here cannot be distinguished by the other kinds of OH stretching typical of the cellulosic matrix (a large band around 3000 cm⁻¹). Moreover a peak at 1507 cm⁻¹ can be assigned to the stretching of C-C bound in aromatic ring present in polyphenols and lignin as confirmed by the comparison of the pomace spectrum with those of natural standard of lignin and cellulose which respectively present and do not present this specific peak (data here not reported).

![Figure 3. Experimental data and model curve for potentiometric titration; affinity distribution calculated by Sips equation (eq. 5) using the adjustable parameters reported in Table 1](image)

**Table 1. Adjustable parameters for potentiometric titration modelling**

<table>
<thead>
<tr>
<th>Site</th>
<th>( Q_{max,i} ) (mmol/g)</th>
<th>( \log K_{H_i} )</th>
<th>( m_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>0.07</td>
<td>4.7</td>
<td>0.90</td>
</tr>
<tr>
<td>Site 2</td>
<td>0.70</td>
<td>9.0</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**3.2 Equilibrium tests**

Experimental data for Pb(II), Cd(II), Cu(II) biosorption onto olive pomace at different equilibrium pH (3, 4 and 5) were reported in Figures 5-7.

The analysis of these experimental data outlines some interesting results and in particular:
- the effect of equilibrium pH on adsorption performances;
- the effect of metal speciation onto adsorbent affinity.

As for the effect of equilibrium pH onto adsorption performances it is evident that increasing the hydrogen ion concentration from pH 5 to 3 maximum metal uptake decreases. This is due to the competition among metals and hydrogen ions for active sites.
on the adsorbent. In fact the acid-base properties evidenced by potentiometric titration data of a pomace suspension show the presence on the adsorbent of two main kinds of weakly acidic active sites probably carboxylic and phenolic ones. As pH increases the active sites dissociate themselves becoming negatively charged and then able to bind positive metallic species in solution.

Figure 5. Copper biosorption onto olive pomace at different equilibrium pH

Figure 6. Cadmium biosorption onto olive pomace at different equilibrium pH
Figure 7. Lead biosorption onto olive pomace at different equilibrium pH

As for the adsorbent affinity the comparison among metal specific maximum uptakes (the plateau of the isotherm) at the different equilibrium pH shows that lead removal is larger than copper that is larger than cadmium. This experimental result (affinity order: Pb>Cu>Cd) can be explained considering different interpretations.

Considering the logarithmic values of the metal first hydrolysis constant (1) the order is LogK_{Pb}=-7.71 > LogK_{Cu}=-8.00 > LogK_{Cd}=-10.80 [7].

\[ HO + H + Me^{2+} \rightarrow Me(OH)^+ + H^+ \]  \hspace{1cm} (8)

This correlation between metal acidic property and its uptake seems to be even more important than the specific functional groups present on the adsorbent surface; in other words, metal speciation predominates on adsorbent characteristics. This observation is strongly enforced considering that the same experimental behaviour was observed using different biological and inorganic matrices [8]. This experimental result can be explained considering the analogy between the reaction of metal hydrolysis (eq. 8) and the reaction between metal and active site (eq. 9):

\[ S + H + Me^{2+} \rightarrow SMe^+ + H^+ \]  \hspace{1cm} (9)

where Me is the heavy metal and SH is the active site in the protonated form. In both reactions (8-9) the bond of hydrogen is broken (HO-H and S-H), a H^+ ion is released and substitute by a metal (HO-Me and S-Me).

From this point of view, it is logical that if a heavy metal is very acidic (because of its charge to mass ratio) then it will react more easily with a protonated site with respect to a weaker acidic heavy metal.

The observed affinity order can be also explained considering the Hard Soft Acid Base theory of Pearson (HSAB) for complex formation which classifies different species as acid and bases arranged in a specific scale of hardness and says that hard bases react preferentially with hard acid and soft with soft ones [9]. Assuming the presence of hard bases as functional active groups on the adsorbent (such as carboxylic and phenolic ones) the hardness of the metals as acids reacting with the bases follows the order Pb > Cu > Cd. The existence of this particular affinity series can then help in the identification of the operating mechanism.
4. CONCLUSIONS

This paper evidenced the possibility of using olive pomace, an agricultural waste, as adsorbent for heavy metal pollutants in aqueous solutions. In particular the effect of equilibrium pH was studied both regarding the acid-base behaviour of the active functional groups on the adsorbent and regarding the depression of the metal specific uptake for decreasing pH values.

REFERENCES

Biosorption of Hg by vegetal biomasses

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Abstract

The Hg sorption capacities as well as the evolution of these capacities during sorption/desorption cumulative cycles were tested for dried algae (\textit{Sargassum} sp), rich in alginates, dried lettuce leaves (\textit{Lactuca sativa}), rich in cellulose fibers, and corn husk (\textit{Zea mays}), rich in lignin. The \textit{Sargassum} biomass showed the more efficient uptake, and after three cycles of acidic desorption treatment the seaweed biomass still presented a very good sorptive capacity (20mg Hg/g(biomass)). The lettuce biomass presented sorptive capacity of 22 mg Hg/g(biomass) for saturation concentrations in the first sorption cycle. After 3 sorption/desorption cycles the biomass was degraded and showed no more the same sorption efficiency. The corn husk biomass presented a sorption capacity of 8,6mg Hg/g(biomass) in the first sorption cycle, a small value if compared to the other biomasses. FTIR spectra indicated that after sorption the metal is located in similar sites in all the biomasses studied, that is, near the ramifications of glucose rings of the alginate, cellulose or lignin fibers of seaweed, lettuce leaves or corn husk, respectively.

Keywords: biosorption, mercury, FTIR, \textit{Sargassum}, \textit{L. sativa}, \textit{Z. mays}, sorption, desorption

1. INTRODUCTION

Mercury has been used in gold mining in Brazil since gold was first discovered in the Amazon basin in the 18th century. In the last ten years, between 1,000-2,000 tons of this highly poisonous liquid metal have been released into the environment. Concern over the contamination of the Amazon River and of local populations is escalating with hundreds of thousands of people living in the region thought to be at risk. Mercury pollution is one of the most serious environmental problems related to this activity [1]. Solutions for the problem have received little attention from researchers and governments. Clean-up technologies, which are capable of treating large volumes of soil, water or sediments contaminated with relatively low levels of mercury in a cost-effective way, are urgently needed and an integrated approach to mercury problem is necessary [2].

Bioremediation of toxic metals by biosorption as an alternative technology for the metal removal of industrial and mining waste has received much attention recently [3].
The first major challenge for the biosorption field is to select the most promising types of biomass from an extremely large pool of readily available and inexpensive biomaterials.

The aim of this study is to evaluate the Hg sorption efficiency of inexpensive biomasses as well as the evolution of these efficiencies during sorption/desorption cumulative cycles. The Hg sorption capacity was tested for biomasses composed of dried sargassum algae (Sargassum sp), dried lettuce leaves (Lactuca sativa) and corn husk (Zea mays). At the same time, the modifications of the structure of biomasses caused by the presence of mercury ions were studied using Fourier Transform Infrared Spectroscopy (FTIR).

2. MATERIALS AND METHODS

2.1 Biomass preparation

For biosorption experiments the biomasses of Sargassum sp. and corn husk (Z. mays) were washed with distilled water and dried in the oven overnight at 60°C. Then, they were chopped in pieces of size around 0.3-0.6 cm, washed with 6 N HCl (Merck), rinsed three times with distilled water and oven dried for 24 hours at 60°C.

Lettuce leaves (L. sativa) were sun dried during 4 weeks, then oven dried at 35°C for 7 days and at 70°C for 24h. After grounding in a domestic blender to a size of about 1 mm, the biomass was dried at 70°C during 24h and washed in an aqueous solution with pH=4 using a drop of diluted HCl solution (Aldrich).

2.2 Batch sorption experiments

Batch kinetics experiments were conducted at room temperature (25°C), in a rotary shaker, using 250 mL Erlenmeyer flasks. The Hg(II) solution was prepared in distilled water using HgCl2 (ACS-QM). Approximately 100 mg of dried biomass was combined with 50 mL of the metal solutions and the flasks were placed on the shaker for 6 h. The pH of the solutions before and during the sorption experiments was adjusted to 5.0 with NaOH and/or HNO3 solutions (Merck) in a pHmeter DMPH (Digimed).

2.3 Analysis of mercury

The total concentration of mercury in the liquid samples was determined by Atomic Absorption Spectrometry (AAS) in the Perkin-Elmer Flow Injection Hydride Generation Atomic Absorption Spectrometers, models AA403 coupled to MHS-20 and AAS FIMS-100.

2.4 FTIR spectra

For Fourier Transform Infrared Spectroscopy (FTIR) studies, natural and Hg-charged samples of the biomasses were prepared as pellets using KBr (Graseby Specac LTD.) as a substratum. Spectra were recorded with a BOMEM-DA8 FTIR spectrometer and deconvoluted using Peakfit™ 4.00 software. Details of the sample preparation and of the spectra recording and deconvolution are given in [4], as well as an overview of the FTIR theory.

2.5 Data evaluation

Uptakes of mercury were determined from the difference of metal concentrations in the initial and final solutions and the biosorption coefficient (q) was calculated as [5]:
\[ q = (C_i - C_f)V / M \]  

Where \( q \) is the amount of metal ion adsorbed onto the biosorbent (mg/g), \( C_i \) and \( C_f \) are the concentrations of the metal ions in the initial solution (mg/L) and after biosorption, respectively. \( V \) is the volume of the aqueous phase (L) and \( M \) is the amount of biosorbent (g). For each biomass the uptake results were fitted using the Langmuir sorption model [6]:

\[ q = q_o bC_f / (1 + bC_f) \]

where \( q_o \) and \( b \) are the characteristic parameters of the Langmuir isotherm: \( q_o \) represents the saturation uptake for high equilibrium concentrations and \( b \) is related to the affinity of the metal ion with the biomass structure, which defines the inclination of the isotherm for low equilibrium concentrations.

2.6 Desorption experiments

Desorption of Hg(II) was performed with 50 mL of HNO₃ solution, 0.2M (Merck). The biomasses loaded with Hg(II) ions were placed in this desorption solution and stirred at 200 rpm for 3 hours at room temperature. Then the mercury concentration in the supernatants was analyzed using AAS. The sorbent samples regenerated were washed with distilled water and then reused for a new adsorption process. The loading and regeneration cycle was repeated three times.

3. RESULTS AND DISCUSSION

3.1 Comparative sorption of different biomasses

Figures 1 to 3 show the sorption isotherms for *Sargassum* sp., *L. sativa* and *Z. mays*, respectively, for 3 sorption/desorption cycles. The vertical axis scale (sorption uptake \( q \)) was made the same for the three curves, in order to facilitate the comparison of uptake values.

During the three sorption cycles studied, *Sargassum* biomass showed the more efficient uptake. In the concentration range covered by the experiments it was not possible to attain the biomass saturation condition. For this reason the curves were not fitted using Langmuir equation (2), and the results are presented as smooth curves joining the average of the experimental points. Although the sorptive capacity of the biomass degrades after each sorption/desorption cycle, after three cycles the biomass still presents a very good sorption efficiency, with a \( q \) value of about 20 mg (Hg)/g (biomass) for the higher values of metal concentration studied.

*L. sativa* presented also a good sorptive capacity, with \( q_o = 22\pm2 \) mg (Hg)/g (biomass) in the first sorption cycle. For the second cycle few data were obtained and it was not possible to make a Langmuir fit. Due to a very low mechanical resistance, after 3 cycles the sorptive capacity is degraded and the maximum uptake is about half the original one.

*Z. mays* presented \( q_o = 8.6\pm0.8 \) mg(Hg)/g (biomass) in the first cycle, and the maximum uptake decreased to about half this value after 3 cycles. Data present a larger dispersion than for the other biomasses, due to the lower uptake values. Even if the sorptive capacity of corn husk is less effective than the one found for the other biomasses, it is worthy to note that this biomass has a bigger density than the others studied, and that this can compensate the low value of \( q_o \) if the sorption is planned to be done in a column of fixed size.
Figure 1. Biosorption isotherms of dried sargassum algae (*Sargassum* sp) biomass in 3 cycles of Hg sorption/desorption

Figure 2. Biosorption isotherms of dried lettuce leaves (*Lactuca sativa*) biomass in 3 cycles of Hg sorption/desorption

Figure 3. Biosorption isotherms of corn husk (*Zea mays*) biomass in 3 cycles of Hg sorption/desorption

3.2 FTIR spectra

Figure 4 shows the FTIR absorption spectra for natural (A) and Hg-charged (B) *Sargassum*, natural (C) and Hg-charged (D) *L. sativa*, and natural (E) and Hg-charged (F) *Zea mays*, in the region between 800 cm\(^{-1}\) and 1800 cm\(^{-1}\). The spectra were normalized to
Biosorption

take account for thickness differences in the prepared samples. It can be seen that the presence of mercury modifies the infrared absorption of all the samples in similar ways.

Figure 4. FTIR absorption spectra and deconvolution for natural (A) and Hg-charged (B) Sargassum, natural (C) and Hg-charged (D) L. sativa, and natural (E) and Hg-charged (F) Zea mays

a. on Sargassum samples, we can note a modification after Hg sorption on the peak with wavenumber 1520 cm\(^{-1}\), assigned to COO\(^-\) bonds vibrations [7]: in spectrum A (natural) the height of this peak is equal to the height of its right and left neighbors, whereas in spectrum B (Hg-charged) the height of this peak is smaller than its neighbors height. We can also note modifications in the peak with wavenumber 1160 cm\(^{-1}\), assigned to CO bonds vibrations [7]: in spectrum A its height is smaller than its right neighbor height, whereas in spectrum B the heights are approximately the same. COO\(^-\) is present in the ramification of alginate, main component of dried seaweed, and CO bonds are present in the alginate rings, near the ramification.

b. on lettuce samples (L. sativa), peaks modified by the sorption of mercury have wavenumbers 1400 cm\(^{-1}\), assigned to CH bonds vibrations; 1160 cm\(^{-1}\), assigned to C-
O-C bonds vibrations, and 1115 cm\(^{-1}\), assigned to CO bonds vibrations [8]: in spectrum C (natural) the CH peak height is approximately 2/3 of the height of its left neighbor, while in spectrum D (Hg-charged) heights are almost equal; meanwhile, C-O-C and CO peaks heights change from half the height of the left neighbor in spectrum C to about 1/3 the height of left neighbor in spectrum D. CH is present in the ramification of cellulose, the main component of dried lettuce leaves, and CO and C-O-C bonds are present in glucose rings of cellulose, near the ramification.

c. on Z. mays samples, the sorption of mercury causes smaller modifications on infrared absorption peaks, due to the smaller quantity of metal retained by the biomass. Even though, we can note a small modification on the peak with wavenumber 1170 cm\(^{-1}\), assigned to C-O-C bonds vibrations [8]: in spectrum F (Hg-charged) this peak is smaller as compared with its neighbors than it was in spectrum E (natural). C-O-C bonds are present in glucose rings of lignin, main component of corn husk. We note also the disappearance of a peak with wavenumber 1610 cm\(^{-1}\), which can be assigned to OH bonds of water present in some samples [9].

No absorption peaks that could be assigned to bonds of the biomass structure components were created or extinguished by the presence of the mercury in the biomass, indicating that no molecular bonds of the structure were formed or destroyed after the sorption of the metal ion.

It is worthy to note that in our study, the absorption peaks have wavenumbers localized between 800 cm\(^{-1}\) and 2000 cm\(^{-1}\). These peaks can be associated to the bond vibrations of the organic molecules of the biomass. Absorption peaks associated to bond vibrations of mercury bonds would have a very low wavenumber, and would not be seen in our spectra.

4. CONCLUSIONS

The metals biosorption depends strongly on the nature of the biosorbent. It appears from the results of this study that the differences in the cell wall constituents in the sargassum biomass tested make the marked difference in the Hg(II) removal from solutions. Among the biomasses studied, Sargassum sp. far displays the best performance, followed by lettuce leaves (Lactuca sativa) and corn husk (Zea mays).

The presence of the sorbed metal affects the bonds of ramifications of the structures, as well as the bonds of the carbon rings, near the ramifications. Similar features about the absorption peaks were seen in another work that studied copper sorption in L. sativa [10] where it was concluded that, after sorption, a hydrated copper ion is located near two glucose rings of the cellulose structure, in a site near the glucose ramification and with axial symmetry neighborhood. We thus conclude that the sorption mechanism of mercury is similar to the one for copper sorption. Also, our results agree with observations in [11] for Hg sorption on steam activated and sulphurised activated carbons prepared from bagasse pith. The authors proposed that in acidic medium Hg(OH)\(^+\) species present in the solution may be bond to COOH groups of the carbon materials.

Finally, we conclude that, between the tested biomasses, Sargassum is the more efficient for mercury uptake and can be utilized mainly in coastal regions where it can be easily found. L. sativa also presented a good sorptive capacity but a very low mechanical resistance and this brings difficulties in its use on sorption columns. Corn husk (Z. mays) biomass is a less effective sorbent than the other biomasses. However, this biomass is very abundant in developing countries with mercury pollution problems, and its density is
higher than the density of the other biomasses studied. These factors can compensate the low value of Hg uptake, if the sorption is planned to be done in a column of fixed size.

Biosorption studies have been conducted by several researchers using live or inactivated microbial systems and surface-modified adsorbents for removal of mercury from wastewater and synthetic solutions [12-15]. Such preparations offer advantages in terms of high biosorption capacities, mechanical strength and durability, handling and ease of scale up. Nevertheless, all these adsorbents are expensive and require several preparation steps. So, for scale up applications of mercury removing in remote areas, it is important to attain efforts on obtaining inexpensive and abundant biomasses with mechanical strength and durability, and good mercury sorption capacity. The biomasses proposed in this study answer to these requirements and present a novel option to large scale Hg decontamination.

The structural information about sorption sites based on infrared absorption analyses, presented in this study, can also help in the understanding of the Hg sorption phenomenon and in the search of other convenient biomasses.

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REFERENCES


Biosorption of lead in aquatic environment by *Mucor rouxii* biomass

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Abstract

Kinetics and nature of biosorption process for the removal of lead ion from aqueous solution by fungal biomass *Mucor rouxii* were studied. Temperature, pH, residence time, metal ion and biomass concentration had been found to influence the biosorption process. At equilibrium sorption process followed Langmuir isotherm model. Biosorption of lead ion by *Mucor rouxii* biomass is a fast process requiring less than 20 min to achieve more than 90% of adsorption.

1. INTRODUCTION

Physico-chemical processes usually remove heavy metals present in industrial wastewater before discharging into water system. Physico-chemical processes in use for heavy metal removal from wastewater include precipitation, coagulation, reduction, ion exchange, and membrane technology. All these processes are either costly or less effective. A search for low cost and easily available adsorbant has led to the investigation of materials of agricultural and biological origin including microorganism. Microorganism such as bacteria, fungi, yeast and algae [1] can remove heavy metal from aqueous solution in substantial quantities. Microbial biomass has been studied by several researchers [2-7]. Metal uptake by non-living biomass involves different types of adsorption processes. Biosorption is affected by various physical and chemical factors such as pH, temp, contact time, metal and biomass concentration etc [8]. The biosorption processes consist of two steps, (1) initial rapid process, followed by (2) slower second step [9]. In slower process the metal uptake can be due to number of mechanism including covalent bonding, surface precipitation, redox reaction [10,11]. During adsorption a rapid equilibrium is established between the adsorbed metal ion on the cell (Q) and unadsorbed metal ion in solution (C₀) and can be represented by either Freundlich or Langmuir adsorption isotherms. [12-14]. The present work aims to evaluate Pb biosorption by the fungus *Mucor rouxii* MTCC-386, study the adsorption isotherm and evaluate the kinetics of the reaction [15].

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2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Microorganism

*Mucor rouxii* (MTCC 386) was obtained from Institute of Microbial Technology Chandigarh, India and maintained on potato dextrose agar slants and subcultured at regular intervals of 30 days.

2.1.2 Chemicals

Chemicals were obtained from E. Merck, Germany and agar powder was purchased from Hi- Media, India.

2.2 Methods

2.2.1 Production of *Mucor rouxii* biomass

*Mucor rouxii* was grown in potato dextrose broth in 250-ml Erlenmeyer flasks containing 50-ml medium and inoculated with mycelia of *M. rouxii* grown previously. The flasks were incubated at 30°C in a rotary shaker with continuous shaking (120 rpm) for 80h. Mycelia were collected by filtration, washed with deionised water, dried at 80°C, pulverized and kept in desiccators for adsorption studies.

2.2.2 Metal solution

Stock solution of lead 1000 mg L⁻¹ was prepared by dissolving its nitrate salt into deionized water [16].

2.2.3 Metal sorption studies

A batch equilibrium method was used to determine the sorption of Pb ion by *Mucor rouxii* dried biomass. A set of 250-ml Erlenmeyer flasks each containing 50 ml of metal solution at varying concentrations (10-100 mg L⁻¹) were taken. Dried biomass (0.5 gm) was added in each of the flasks, which were incubated for 4 h at 30°C on a rotary shaker at 120 rpm. pH was kept at 5.5 in each experiment. Biomass was separated by centrifugation and Pb²⁺ in the supernatant was analyzed by atomic absorption spectrometer (Varian Spectra AA55). Metal adsorbed by biomass was obtained from the following formula:

\[ Q = \frac{V(C_i-C_f)}{1000 M} \]  

where \( Q \) is the specific metal uptake (mg metal/ g biosorbent), \( V \) is the volume of metal solution (mL), and \( C_i \) and \( C_f \) are the initial and final metal concentration in the solution (mg metal L⁻¹) and \( M \) is the dry weight of the biomass (gm). The metal sorption ability of the biomass was determined by the above-mentioned procedure, in all the experiments unless stated otherwise [17].

Data obtained with respect to the effect of initial metal concentration on metal biosorption was applied to the widely used Langmuir equation of adsorption isotherm (Fig. 2).

2.2.4 Reaction kinetics

To examine metal biosorption kinetics, 0.5 gm of dry biomass was contacted with 50 ml of metal solution in 100-ml Erlenmeyer flasks. Flasks were incubated on a rotary
shaker at 30°C. Samples of metal solutions were withdrawn from each flask at different time intervals (0-200 minutes) and metal content was analyzed by using the same formula (I).

3. RESULTS AND DISCUSSION

3.1 Adsorption isotherm and biosorption characteristic

In the biosorption studies of metal by *M. rouxii*, adsorption isotherm was studied under Langmuir and Freundlich adsorption isotherms. Plot of Log Ce/Q vs Log Ce gave the straight line with correlation value 0.96 (Fig. 1), which indicates that the adsorption process obey the Langmuir equation.

\[
\frac{C_e}{Q} = \frac{C_e}{Q_{\text{max}}} + b \cdot Q_{\text{max}}
\]

(II)

where \(C_e\) be the equilibrium metal concentration at a fixed temperature and pH. \(Q_{\text{max}}\) be the maximum value of adsorption and \(b\) be the affinity of biomass for the metal.

Extrapolation of log \(C_e/Q\) to Log \(C_e\) gave the value of Langmuir constant \(b\). The values of \(b\) and \(Q_{\text{max}}\) were 81.23mg g-1 and 0.12 respectively. \(Q_{\text{max}}\) be the amount of adsorbate to form a complete monolayer on the adsorbate surface. Equilibrium sorption isotherm studies showed (Fig. 2) that metal uptake by M. rouxii was a chemically equilibrated and saturable mechanism. Thus there was an increase in metal uptake as long as binding sites were free [20, 21]. As the experimental data fit in the Langmuir model it indicates that biosorption of metals in the present study is characterized by a monolayer single type phenomenon with no interaction between sorbed metals [22]. Langmuir constant \(b\) is related to energy of adsorption through the Arrhenius equation, which also gives an indication of the affinity of the metal for binding sites on the biosorbent [23].

![Figure 1. Adsorption isotherm](image)

3.2 Kinetics study

Kinetic experiments were necessary to determine the required time to reach the equilibrium condition [18]. Metal uptake data when plotted as a function of time (Fig. 2) at pH 5.5, showed that uptake was rapid in first 20 min of contact, and time required for attaining equilibrium was below 30 min. It could also be seen that the rate of uptake during the entire course of biosorption was independent of initial metal ion concentration.
used. Thus, it is likely that kinetics of the process was influenced only by the step of metal transfer from solution to the binding sites [19].

Figure 2. Kinetics of metal adsorption

4. CONCLUSION

From the above study it can be stated that the Mucor rouxii biomass can adsorb lead ion from solution and this process is very fast. The equilibrium of the process reaches very quickly. It follows Langmuir adsorption isotherm. This biosorbent may provide a new technology of removal of lead ion from wastewater.

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Cadmium(II) biosorption by *Aeromonas caviae*: kinetic modeling

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Abstract

The removal and depletion of cadmium from aqueous solutions by sorption on *Aeromonas caviae* particles was investigated in a well-stirred batch reactor. Kinetic experiments were performed at various initial bulk concentrations, biomass loads and temperatures, in order to investigate the probable mechanism of the process. It was seen that the sorption capacity is appreciably high for most experimental conditions, so *Aeromonas caviae* may be considered as a suitable sorbent for this application. A detailed analysis was conducted testing several chemical reaction and diffusion (external or intraparticle) kinetic models in order to identify a suitable kinetic model. In the present paper, the results obtained when using the so-called Ritchie 2nd order equation and also a pseudo 2nd order rate expression are given, with promising fitting of the experimental data.

**Keywords:** biosorption, modeling, cadmium, metal removal, wastewater

1. INTRODUCTION

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Cadmium is one of the most toxic metals contaminating the environment, as it is widely used in rechargeable nickel-cadmium batteries, pigments, stabilisers, coatings, and alloys.

The relevant E.U. Directive, as well as the U.S. E.P.A., have set the maximum contaminant level (MCL) for Cd(II) cations in domestic water supplies to be 5 µg L⁻¹ (Directive 98/36/EC). The commonly used treatment methods to remove Cd(II) ions from wastewaters include chemical precipitation, ion exchange, reverse osmosis and membrane processes. However, biosorption, the uptake of heavy metals by dead biomass, has gained credibility during recent years as it offers a technically feasible and economical approach [1,2]. Several biological materials investigated for heavy metals removal include bacteria, yeasts, algae and fungi [3,7].

The present study aims in examining biosorption by using biomass of *Aeromonas caviae*. Despite the fact that this microorganism is often present in groundwater and general in aquatic environments, little attention seems to have been given to its resistance to heavy metals [8]. The purpose of selecting this bacterium for studying biosorption was,

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apart from its originality, to assess the possibility of utilizing a waste biomass for heavy metal removal. Equilibrium and kinetic analyses not only allow the estimation of sorption rates, but also lead to suitable rate expressions characteristic of possible reaction mechanisms [9,10].

Sorption kinetics may be controlled by several independent processes that can act in series or in parallel [11]. These processes fall in one of the following general categories: (i) bulk diffusion, (ii) external mass transfer (film diffusion), (iii) chemical reaction (chemisorption) and (iv) intraparticle diffusion. For sufficiently high agitation speed in the reaction vessel the bulk diffusion step can be safely ignored since then sorption onto sorbent particles is decoupled from mass transfer in the bulk mixture.

Apart from that, it is quite common that more than one processes can contribute in the system performance at the same time. In this case, the extensive interrelationships between the various equations make the overall kinetic model exceedingly complicated to evaluate. A rather simplifying approach to circumvent this problem is to assume that each one of the co-current processes dominate over the others (i.e. the rate controlling step) at specific time regimes of the process and then study them independently [12].

In order to identify the most appropriate mechanism for a process, several models apparently must be checked for suitability and consistency in a broad range of the system parameters. The model selection criteria proposed by Ho et al., [11] concerning sorption of pollutants in aqueous systems were used herein, as a general guideline. According to this, several reaction-based and diffusion-based models were tested in simulating our data. The finally chosen kinetic models are those, which not only fit closely the data, but also represent reasonable sorption mechanisms.

2. MATERIALS AND METHODS

2.1 Biomass and grown condition

_Aeromonas caviae_, a Gram-negative bacteria isolated from the raw water wells sample by enrichment culture technique. Culture units of microorganism were identified by Dr. J.M. Tobin (School of Biological Sciences, Dublin City University). The strain was grown at 29°C in a rotating shaker for 24 h in liquid medium containing: yeast extract (0.5% w/v), tryptone (1% w/v), NaCl (0.5% w/v) and FeSO₄ 7H₂O (0.2 g L⁻¹). The produced biomass was separated by centrifugation at 3000 rpm, washed several times by a solution of NaCl (0.9% w/v), sterilized and stored.

2.2 Biosorption experiments

Batch biosorption experiments were carried out at different: biomass feed (0.5, 1 and 2 g L⁻¹), initial cadmium concentration (5 and 50 mg L⁻¹), temperature (20, 40 and 60°C). The experiments were performed in an Erlenmeyer flask at a 180-rpm agitation speed (Heidolph type, RZR 2102). This speed was selected to ensure that the effect of external film diffusion on biosorption rate is not significant and can be ignored in any analysis. The initial pH of the solution was adjusted to optimum value of 7, as it was determined during preliminary experiments, i.e. from the precipitation value, as metal hydroxide according to the aqueous speciation. For the equilibrium experiments, ample time (~2 h) was allowed after the beginning of adsorption to ensure that the experiments were concluded. For the kinetic study of cadmium adsorption, 2 mL samples were acquired at selected time intervals using a 10 mL syringe. Due to experimental constraints the sampling interval
Biosorption

was no less than 2 min. The residual concentration of cadmium in all samples was
analyzed by atomic absorption spectrophotometry (AAS, Perkin-Elmer, model 2360).

3. RESULTS AND DISCUSSION

3.1 Equilibrium experiments

Experimental adsorption isotherms of cadmium ions obtained at several biomass loads
and temperatures are presented in Figures 1a and 1b, respectively. For each isotherm the
initial metal concentration was varied, while the biomass load and temperature was kept
constant.

![Equilibrium Langmuir model to cadmium biosorption](image)

The results of fitting the Langmuir and the Freundlich models to these data are
presented in Table 1.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Freundlich constants</th>
<th>Langmuir constants</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (°C)</td>
<td>Biomass load (g.L⁻¹)</td>
<td>K_f (g.L⁻¹)</td>
</tr>
<tr>
<td>20</td>
<td>0.5</td>
<td>20.37</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>10.85</td>
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<td>11.68</td>
</tr>
<tr>
<td>60</td>
<td>1</td>
<td>27.66</td>
</tr>
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</table>

The higher values of the correlation coefficient (r²), show that the Langmuir equation
is very suitable for describing the biosorption equilibrium of Cd(II) by \textit{A.caviae} in the
studied concentration range. The magnitude of the biosorption capacity, q_{max}, spans a
range of values (68.17 to 175.11 mg.g⁻¹) comparable to other types of sorbent reported
earlier [13].
In addition, the large values of $b$ clearly imply that \textit{A. caviae} exhibits a high affinity for cadmium ions. The biosorption capacity, $q_{\text{max}}$, decreased with the increasing biomass load, indicating a poorer biomass utilisation (low efficiency). This may be attributed to a possible aggregation of solids occurring at higher biomass loads, capable of reducing the effective sorption area. The value of $q_{\text{max}}$ (175.11 mg g$^{-1}$) obtained at 40°C appears to be higher in comparison with the uptakes obtained at the other temperatures. However, one should withhold judgement until experimental information for higher $C_{eq}$ values is acquired.

3.2 Kinetic experiments

Figure 2 presents the remaining concentration of cadmium in the bulk solution as a function of time at different experimental conditions. Unless differently stated, runs were performed at 20°C and with 1 g L$^{-1}$ biomass load. The key role of the initial metal concentration is apparent. That is, the adsorption capacity is markedly enhanced, at a higher initial concentration of Cd (II), since the initial concentration provides an important driving force to overcome mass transfer resistance of Cd (II) between the aqueous and solid phase.

![Figure 2](image)

**Figure 2.** Cadmium biosorption kinetics by \textit{Aeromonas caviae} obtained at initial cadmium concentrations of: (a) 5 mg L$^{-1}$; (b) 50 mg L$^{-1}$ (pH: 7, agitation speed: 180 rpm)

The qualitative resemblance among experimental data is considerable despite the different experimental conditions. Overall, there is a monotonous decreasing trend with time. The very steep descent at the beginning of sorption is succeeded by a less rapid decay down to about 20-30 minutes. From then on, the Cd (II) concentration gradually levels-off and remains almost constant till the end of the experiment (120 min). Thus, the major part of adsorption takes place within the first 30 minutes of the process. Moreover, the time required to reach the final equilibrium is practically the same for all experimental conditions. It is evident that adjusting the temperature or the biomass load in the examined range of values can lead to a comparable metal removal.

In order to examine further whether the sorption of cadmium follows a mechanism of electrostatic or chemical nature, some biosorption experiments were performed by adding various concentrations of a nitrate salt. The impact of the presence of dissolved nitrate ions on the kinetics of Cd(II) biosorption is shown in Fig. 3. It appears that as the dosage of salt increases both the sorption capacity and sorption rate of Cd(II) ions decrease. This
Biosorption

The effect is more intensive with the higher initial metal concentration (50 mg L$^{-1}$). The added ionic background alters both the equilibrium and the kinetic behavior of the sorbate/sorbent system. The effect of the ionic strength may be explained as the outcome of the competition between sodium and cadmium cations during electrostatical binding to the biomass.

![Figure 3. Biosorption kinetic curves obtained for different concentrations of NaNO$_3$ at 20°C](image)

3.3 Kinetic modeling

The relatively short duration of the present experiments, apart from the process advantages, is a first indication that adsorption of cadmium ions on A. caviae is a chemical reaction rather than a diffusion controlled process [11]. From surface titration experiments and analyzing IR spectrum, it was illustrated that the cell wall contains two or more main functional groups (carboxyl and phosphate) responsible for the uptake of heavy metals [14]. These types of groups one capable of removing metallic ions, usually cations, from aqueous solutions through the application of different mechanisms, such as cell surface sorption (complexation, surface precipitation etc) [5].

From the chemical reaction category (chemisorption), the best fit for the data sets of this study is achieved by a 2$^{nd}$ order-type chemical reactions. The solution of the standard 2$^{nd}$ order reaction based on a constant stoichiometry of one metal ion per binding site, is [12]:

$$C_t = \frac{C_o}{1 - \frac{C_o}{C_e} \exp(-k_2C_o t)}$$  \hspace{1cm} (1)

where $k_2$ is the reaction rate constant [L*(mg$^{-1}$ of metal)*min$^{-1}$]. This adsorption model has been very effective in describing the kinetics of adsorption of gases on solids [12].

Figure 3 shows that equation (1) clearly fails to capture the steep concentration gradient of the early removal stage. This is a direct indication that adsorption on solids from a liquid phase is a different process than adsorption from a gas phase where traditionally the remaining bulk concentration dictates the kinetics [12].
Figure 4. Cadmium biosorption kinetics obtained at initial cadmium concentrations of (a) 5 and (b) 50 mg L\(^{-1}\)

If the rate of sorption depends not on bulk concentration but on uptake by the sorbent this can be described by the so-called Ritchie 2nd order equation according to which one metal ion occupies two binding sites [15]:

\[
q_t = q_e \left(1 - \frac{1}{1 + k_2 t}\right)
\]  

(2)

where \(q_t\) and \(q_e\) are the amounts of adsorbed metal ions on the biosorbent at time \(t\) and at equilibrium, respectively (mg g\(^{-1}\)) and \(k_2\) is the reaction rate constant [min\(^{-1}\)]. When in the above treatment it is not necessarily \(q_e\) that dictates the sorbate uptake then a pseudo 2nd order rate expression is more appropriate [11]:

\[
t = \frac{q_t}{k_m q_m^2} + \frac{1}{q_m}
\]

(3)

where \(k_m\) is the reaction rate constant [g of biomass*(mg\(^{-1}\) of metal)*min\(^{-1}\)] and \(q_m\) is a numerically determined parameter which under ideal 2nd order rate control corresponds to \(q_e\).

However, equation (2) and (3) provide a quite suitable description of data for advancing time (Figures 4, 5). It is noteworthy that both models adequately capture the rapid rate of adsorption during the first minutes of the experiments. This already implies that the metal uptake by the sorbent is a satisfactory rate-controlling parameter under a 2nd order reaction mechanism.

Table 2 displays the numerically best-fit values of the rate parameters of equations (2) and (3). The predicted equilibrium sorption capacities are quite close to the experimental values for both models. Nevertheless, the rate constant of the pseudo 2nd order model, \(k_m\), is monotonously correlated with changes in the biomass load and in the bulk concentration, features that have been encountered in the past regarding biosorption [15]. On the contrary, the rate constant of the Ritchie 2nd order equation, \(k_2\), fluctuates beyond any physical reasoning. In addition, equation (3) exhibits better fitting statistics. Despite the goodness of fit for sorption at 40 and 60°C, the reaction rate constant of both models varies randomly with temperature. Preliminary calculations using the Arrhenius model between two temperatures every time gave activation energies always below 10 kJ/mol which is far less than what is expected for reaction controlled sorption processes [11]. The
morphological changes of the biomass surface at different temperatures and the dependence of sorption capacity on temperature may be blamed for this irregularity.

Figure 5. Comparison of experimental uptake curves against theoretical predictions based on the Ritchie 2nd order equation (equation 2) at initial cadmium concentrations of: a) 5 mg L\(^{-1}\); b) 50 mg L\(^{-1}\) (pH:7)

Figure 6. Comparison of experimental uptake curves against theoretical predictions based on the pseudo 2nd order equation (equation 3) at initial cadmium concentrations of: a) 5 mg L\(^{-1}\); b) 50 mg L\(^{-1}\) (pH:7)
Table 2. Kinetically determined parameters and comparison with equilibrium sorption capacities

<table>
<thead>
<tr>
<th>Co (mg L⁻¹)</th>
<th>Conditions</th>
<th>Equilibrium</th>
<th>Pseudo 2nd order eqn</th>
<th>Ritchie 2nd order eqn</th>
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<tr>
<td>T (°C)</td>
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<td>qₑq (mg g⁻¹)</td>
<td>qₑq (mg g⁻¹)</td>
<td>kₘ (mg g⁻¹ min⁻¹)</td>
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<td></td>
<td>40 1</td>
<td>36.20</td>
<td>41.67</td>
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<td></td>
<td>60 1</td>
<td>45.87</td>
<td>43.47</td>
<td>0.132</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

Dead cells of *Aeromonas caviae* showed a high biosorption capacity for cadmium(II) ions comparing with other types of biosorbent. The present results demonstrate that temperature, initial metal concentration and biomass load highly affect the uptake capacity of the biosorbent.

The Freundlich and Langmuir adsorption models were tested for the mathematical description of the biosorption equilibrium of Cd(II) ions to *A. caviae* of various temperatures and biomass loads. The calculated isotherm constants were used to assess the biosorptive capacity of the biomass. The obtained results showed that the adsorption equilibrium data fitted fairly well to the Langmuir model in the examined concentration range.

The suitability of the pseudo 2nd order chemical reaction for the sorption of Cd(II) ions onto biomass is also presented. This kinetic model may effectively describe the largest part of the process. The obtained kinetic expression is of a great practical value for technological applications, since kinetic modelling successfully replaces time- and material-consuming experiments, necessary for process equipment design.

ACKNOWLEDGEMENTS

Many thanks are due to Dr. John M. Tobin (School of Biological Sciences, Dublin City University) for his help with the microbiological identification of the microorganism used.

REFERENCES

Chromium uptake by pretreated cells of *Aeromonas hydrophila* isolated from textile effluents

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Abstract

Effluent discharges from textile and dyestuff industries create serious risk of pollution to the environment. This is due to the presence of various types of pollutants including inorganic compounds and polymers, organic products and also heavy metals. Even though more attention was focused on colour, the presence of heavy metals should not be discounted due to its toxic effects through long-term accumulation. In this study, a locally isolated bacteria from wastewater discharge of a textile-based manufacturing industry identified as *Aeromonas hydrophila* was used as biosorbent to remove chromium species from simulated solution. Four types of pretreatment were used; autoclaving, 50%v/v acetone, 0.1N HCL and UV. The treated bacteria, plus the viable but non-culturable (VBNC) cells was then assessed for Cr (III) uptake ability using the shake-flask technique at pH 4.5. The application of Langmuir adsorption isotherm showed that the autoclaved-acid treated cells gave the highest uptake capacity compared to others with a q_{max} value of 26.42 mg/g cell dry wt at around 1 hour of equilibration time. Pretreatment of the cells showed a slightly higher surface area for the autoclaved-acid treated and autoclaved-acetone treated cells compared to the UV and untreated cells respectively.

*Keywords:* uptake, chromium, pretreatment, textile, *Aeromonas*

1. INTRODUCTION

The discharge of heavy metals into the environment especially from the industrial and agricultural source is of main concern lately. Heavy metal contamination exists in aqueous waste streams of many industries, such as metal plating facilities, textile industries and tanneries [1]. Simply, any industrial activity that utilizes metals in some part of its operation would have a metal disposal problem. This is because heavy metals tends to form toxic, carcinogenic or mutagenic compounds even in very low concentrations [2] which can accumulate through the food chain, leading to serious ecological and health problems [3,4].

Chromium (Cr) for instance is often present in the wastewaters of metal finishing parts, electroplating and textile industries, frequently at ppb level. In the textile industry, Cr compounds are contained in Cr complex dyes where it was introduced to the dyestuff
molecules during the dyeing process i.e. chromate treatment in the textile industry. From all the manufacturing processes, Cr can seep into the environment directly via the effluents or indirectly via a slower release as solid wastes [2].

Chromium is found either as Cr(II), Cr(III) and Cr(VI) in water. The divalent state is unstable with respect to evolution of hydrogen, trivalent state has broad stability while hexavalent chromium occurs under strong oxidizing conditions [5] which accounts for it being more toxic to microorganisms such as bacteria [6] and fungi [7]. Cr(VI) is normally present in its chromate (CrO$_4^{2-}$) or dichromate (Cr$_2$O$_7^{2-}$) forms. Biological membranes are impermeable to Cr(III) but permits the penetration of Cr(VI) into the cell cytoplasmic region where it can be reduced and precipitated there as Cr(III) which readily forms insoluble hydroxide compounds at pH 7.5 [5,8].

Amongst the proven methods for removing Cr from industrial wastes solution includes chemical precipitation, chemical oxidation or reduction, filtration, electrochemical treatment, membrane and evaporation technology [1,9-12]. The use of ion-exchange resins has its limitations in terms of low selectivity in metals recovered and is expensive. This makes the natural biological metal-microbe interactions such as biosorption, bioprecipitation, biodegradation and bioaccumulation an interesting yet feasible alternative treatment processes. Amongst the mechanisms suggested for the interactions are methylation, chelation, adsorption/absorption, complexation and redox reaction [13]. A number of researchers have reported the use of fungus [14], seaweed [15,16], algae [17], cone biomass [18] and bacteria [19] for the biosorption of chromium from simulated or real industrial effluents.

In this study, the biosorption of Cr by the Gram-negative bacterial cells of *A. hydrophila*, which was isolated from a Cr-laden textile effluent, was investigated. The effect of physical and chemical treatment on the cells Cr-uptake ability was elucidated using the simplest mode of Langmuir and Freundlich sorption isotherms i.e. without taking into considerations the effect of pH and the ion-exchange situation in the sorption process. Possible changes in the overall surface area of the bacterial cells due to the various treatments were also assessed.

2. MATERIALS AND METHODS

Unless otherwise stated, all experiments were performed in duplicates. Glasswares used were washed with 10% v/v HNO$_3$ and rinsed with deionized water. All reagents used were of analytical grade.

2.1 Bacteria

*A. hydrophila* used in this study was isolated from a local textile-based industrial effluent and was identified using the API20NE kit (Biomerieux, France). It was grown in nutrient broth (Merck, Germany) until early stationary phase at 200rpm in a Certomat-R, B.Braun orbital shaker at 30°C.

2.2 Pretreatment of the cells

*A. hydrophila* cells grown to early stationary phase was harvested by centrifugation at 9000rpm, 10mins and 0°C (SIGMA 2K-15, B. Braun). The cell pellet obtained was resuspended in 5mL distilled deionized water (UHQII, Elgastat) and was then termed as VBNC (viable but non-culturable) cells.

The VBNC cells were also made non-living by autoclaving at 121°C, 101.325 kPa for 15 minutes (Fedegari, Italy) and by exposing to UV-irradiation. The autoclaved cells was
then subjected to treatment using 0.1N HCL and 50%v/v acetone respectively and termed as HA and HAt cells respectively. The cell pellet was washed twice using distilled deionised water before final suspension in the respective medium.

2.3 Specific surface area determination

The effect of pretreatment was further investigated using the methylene blue adsorption method [20].

A stock solution (1mM, 0.01 to 0.14mL) of methylene blue (C₁₆H₁₈ClN₃S·xH₂O, 319.86gmol⁻¹) was added to 20mg dry wt. of VBNC, UV-treated, HA and HAt cells suspension each in a 25mL polyethylene centrifuge tube. The volume was made up to 20mL using deionized water. Final methylene blue concentrations prepared ranged from 0.5 to 7µM. The mixture was then shaken in a rotary water bath shaker at 100rpm, 30°C for 4 hours. Centrifugation (SIGMA 2K-15, B.Braun) at 8225rpm, 10mins and 0°C was carried out to separate the cells from the mixture. The filtrate was analyzed spectrophotometrically (SPECTRONIC 21-D) at 661nm to determine the residual concentrations of methylene blue in solution. The amount of methylene blue adsorbed by the cells was determined based on the difference between the initial and residual methylene blue concentrations.

In determining the surface area of A. hydrophila cells, it is assumed that a complete monolayer of methylene blue was formed at the bacterial surface when the adsorption profile reached a plateau. To assess the reaction, the Langmuir adsorption isotherm was applied:

\[ R = \frac{T_m K C_{eq}}{1 + K C_{eq}} \]  \hspace{1cm} (1)

where R is the amount of adsorbed methylene blue (µmol/g), C_{eq} is the concentration of methylene blue at equilibrium (µmol/L), K is a constant related to the energy of adsorption (mg/L) and T_m is the amount of methylene blue needed to form a complete monolayer on the bacterial surface (µmol/g). The T_m value was then used to determine the bacterial surface area, S (µm²) according to the following equation:

\[ S = \frac{T_m N_A}{\sigma} \]  \hspace{1cm} (2)

where N_A is the Avogadro no. (6.02 x 10^{23} molecules per mol) and \( \sigma \) is total area of methylene blue (0.55 x 10^{-18} m²) when a complete monolayer was formed [20].

2.4 Chromium uptake experiment

The chromium uptake experiment was carried out in 6, 250mL Erlenmeyer flasks. A. hydrophila, 20 mg cell dry wt. were mixed with 25mL of Cr(III) solution prepared by dissolving CrCl₃·6H₂O in distilled deionized water. The final concentrations of the Cr(III) solutions used ranged between 5-200 mg/L. The pH of the Cr solution was adjusted to 4.5 (WTW, Germany) using 0.1N HCL or 0.1N NaOH. The mixture was then shaken (Certomat-R, B.Braun) for 12 hours, 100rpm and 30°C. It was then centrifuged at 9000rpm, 5mins and 0°C and the filtrate collected was analyzed for Cr using AAS (Philips PU9100X). The amount of Cr removed was determined by the difference between initial and residual concentrations.

A time-course study of the biosorption process was also investigated using living (VBNC) and non-living cells of A. hydrophila. The bacterial cell, 20 mg cell dry wt. were contacted with 25mL of Cr(III) solution in 6, 250mL Erlenmeyer flasks. Different
concentrations of Cr(III) were used i.e. 150mg/L and 50mg/L for the VBNC and HAt cells respectively. The cell suspension was shaken at 100rpm, room temperature with incubation times ranging between 1-12 hours. It was then centrifuged at 9000rpm, 5mins and 0°C and the filtrate collected was analyzed for Cr using AAS (Philips PU9100X).

3. RESULTS AND DISCUSSIONS

3.1 Bacteria

In this study, *A. hydrophila* was used as the biosorbent to remove Cr species from solution. It was originally isolated from a local textile-based (batek) manufacturing effluent using procedure suggested by Greenberg *et al.*, 1985. Identification of the bacteria was made using the API20NE kit (Biomerieux, France) which gave between 99.7-99.9% positive results as *A. hydrophila*. This Gram-negative bacterium whose normal habitat include soil and water [21] is a member of the family *Vibrionaceae*. It is one of the frequently isolated species from drinking water due to its ability to withstand the chlorination procedure, thus recolonizing the water distribution networks [22].

3.2 Specific surface area determination

Methylene blue adsorption method was used to determine the specific surface area of pretreated cells of *A. hydrophila* namely VBNC, autoclaved, autoclaved-acid treated (HA) and autoclaved-acetone treated (HAt) cells. The basis of using this methodology for a Gram-negative bacterium has been reported elsewhere [23]. Table 1 shows profiles obtained from the Langmuir adsorption analysis:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VBNC</td>
</tr>
<tr>
<td>Regression analysis, $R^2$</td>
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</tr>
<tr>
<td>$R$, Lµmol⁻¹ (adsorbed MB)</td>
<td>1.367</td>
</tr>
<tr>
<td>$K$, µM (energy of adsorption)</td>
<td>1.339</td>
</tr>
<tr>
<td>$S$, m²·mg⁻¹ (bacterial surface area)</td>
<td>0.020</td>
</tr>
</tbody>
</table>

*VBNC-viable but non-culturable,
HA- autoclaved and acid-treated,
HAt- autoclaved and acetone treated

Based on table 1, it was noted that the surface area for HA and HAt cells were quite similar i.e. 0.026 and 0.024 m²·mg⁻¹ respectively which might indicate the role of autoclaving in creating a larger surface area for the bacterial cell. UV-treatment did not impose any significant effect on the overall surface area based on values obtained for the VBNC cell.

Zakaria, Z.A. [24] has reported the disappearance of an extracellular polymeric substance (EPS) from *Thiobacillus ferrooxidans* upon autoclaving, which has resulted in a 2-fold increase in the overall specific surface area measured as compared to the acid-treated cells only. Acidic treatment was not expected to cause significant physical modification on the cell, but rather towards the overall charges of the cell wall; by protonation of potential binding sites especially from the amino acid fraction of the bacteria [25] and to yield pure amino sugars such as D-glucosamine which could aid in the metal binding process [14]. Acetone treatment used did not cause significant changes to
the physical properties of the bacterial cell surface, which contradicts the findings of Bai and Abraham [14] who worked with the fungus, *Rhizopus nigricans*. This could be due to different cell wall properties for both fungus and bacteria respectively. The acetone treatment effect was more pronounced in *Rhizopus nigricans* due to the significant removal of lipids and proteins; these components are found in larger quantities in fungus as compared to bacteria.

### 3.3 Dynamics of chromium biosorption

The time course experiment of chromium biosorption by *A. hydrophila* cells is shown in Figure 1.

![Figure 1. Dynamics of chromium biosorption by *A. hydrophila*.](image)

Two types of *A. hydrophila* cells namely VBNC and HAt contacted with different concentrations of Cr (III) i.e. 150mg/L and 50mg/L respectively were used in the time course study. A higher concentration of Cr (III) was used for the VBNC cells based on the possibilities of a two-phase metal removal by these cells [26] hence, the ability to remove higher amounts of the metal. This was clearly indicated from the Cr uptake profiles shown in figure 1 where the VBNC cells showed much higher capacity to remove Cr from solution with a maximum uptake (q_{max}) value of 135.75mg/g cell dry wt. compared to 32.75mg/g cell dry wt. for the HAt cells.

The HAt cells showed a favourable uptake profile where most of the metal were removed after 1 hour at a pH of around 4.5. This is comparable with the findings of other researchers; *Ecklonia* algae biomass – 12 hours, pH 4.0 [15] *Sargassum* sp. – 6 hours, pH 4.0 [16]. However, this was not the case for the VBNC cells where a saturation condition was not observed even after 12 hours. This could be attributed to the two-step metal-bacteria interaction as suggested by Huang, *et al* [26]. The first step involving surface binding or extracellular association is rapid whilst the second step involving intracellular metal uptake is slow and might be the rate-limiting step. This is an energy consuming process and can be explained as follows. As there exist a difference in pH inside the
cytoplasmic membrane (pH 6.0) and the external environment (pH 4.0), a natural proton motive force exists which can play a role in ATP synthesis [27]. The ATP synthesized could well be the energy source required for the translocation of chromium.

### 3.4 Biosorption of chromium

Figure 2 shows the Cr (III) biosorption profiles by pretreated cells of *A. hydrophila*.

![Figure 2: Profiles for the biosorption of Cr (III) by pretreated cells of *A. hydrophila*.](image)

From figure 2, the non-living cells of *A. hydrophila* (HAt, HA and UV-treated) showed a good agreement to the Langmuir adsorption isotherm used as opposed to the living cells (VBNC). The inability to describe the uptake phenomenon by the VBNC cells might be attributed to the different types of metal deposition on the bacterial surface i.e. multilayer-type compared to the suggested monolayer-type of metal deposition. Based on figure 2, the HAt cells showed the highest capacity to remove Cr (III) from solution with $q_{\text{max}}$ of 26.42 mg/g cell dry wt. This was followed by the HA and UV-treated cells with $q_{\text{max}}$ values of 20.50 and 18.60 mg/g cell dry wt. respectively. This finding is in agreement with results from the specific surface area determination work which further supported the assumption that a larger surface area would lead to higher metal binding capacity [14, 28, 29]. The Langmuir parameters were analyzed using the nonlinear binding method and are summarized in Table 2:

<table>
<thead>
<tr>
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<th>$q_{\text{max}}$ (mg/g)</th>
<th>$b$ (mg/L)</th>
<th>$R^2$</th>
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<td>29.33</td>
<td>0.055</td>
<td>0.992</td>
</tr>
<tr>
<td>HA</td>
<td>29.94</td>
<td>0.013</td>
<td>0.983</td>
</tr>
<tr>
<td>UV-treated</td>
<td>20.41</td>
<td>0.062</td>
<td>0.862</td>
</tr>
</tbody>
</table>

In elucidating the biosorption process of heavy metal ions, metal speciation especially its hydrolysis in water is an important factor to be considered. The metal speciation is closely related to the solution’s pH, hence, the overall valence of ionic charges which is
considered as two of the most crucial factor in biosorption together with biomass concentrations [15].

In this study, pH of the mixture was adjusted to 4.5 where most of the chromium is expected to exist as Cr$^{3+}$ and CrOH$^{2+}$ as shown in Equation 3 [15].

$$\text{Cr}^{3+} + \text{H}_2\text{O} \leftrightarrow \text{CrOH}^{2+} + \text{H}^+ \quad (3)$$

This might suggest the predominance of two-types of chromium removal mechanism i.e. ion-exchange and direct electrostatic interaction. For a bacterial species, most of the metal-binding properties can be attributed to the amino acid fraction of the bacterial cell wall emphasizing on potential binding sites such as carboxylate, amino and sulfhydryl groups. At pH 4.5, most of the carboxylic group would be deprotonated (pK, 1.8-2.9) resulting in negatively charged carboxylate ions which could serve as potential binding sites for the positively charged chromium ions. The involvement of amino groups was not taken into consideration due to the predicted repulsive action with the positively charged chromium ions while the contribution of sulfhydryl might not be significant due to its low distribution in a Gram-negative bacteria (*Thiobacillus ferrooxidans*, unpublished data). The possibilities of ion-exchange should not be discounted either especially between CrOH$^{2+}$ and H$^+$ from the carboxyl groups. This assumption was made based on the condition of CrOH$^{2+}$ being the dominant species (~74%) at pH of around 4.0 [15]. Kratochvil et al. [16] have reported the equilibrium constant for equation 3 to be at pK of 3.82, approximating the presence of 50% of the total Cr in solution as CrOH$^{2+}$. However, due to limitation on the scope of this study, further elucidation on the ion-exchange process between Cr and biomass binding sites was not carried out.

4. CONCLUSION

Based on this study, it can be concluded that *A. hydrophila* might serve as a useful biosorbent to remove Cr species from solution. Acetone treatment of the autoclaved cells has resulted in the highest Cr uptake capacity (26.42 mg/g cell dry wt.) with an overall surface area of 0.024m$^2$/mg compared to the untreated bacterial cell. However, more work needs to be carried out in order to apply the *A. hydrophila* cells for the treatment of Cr from industrial effluent.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Science, Technology and Innovation for funding the project and the NSF scholarship award to Zainul Akmar Zakaria.

REFERENCES


Copper ion adsorbed on chitosan beads: Physico-chemical characterization

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Abstract

Beads prepared from chitosan having 89% of degree of deacetylation, polydispersity index around 1.2 and molecular weight $1 \times 10^6$ were used to study physico-chemical characterization of copper ion adsorption from aqueous solution. Kinetics of metal adsorption on chitosan beads had been found to follow first order rate law. More than 90% of the copper ion was adsorbed on the beads within 50 min. Temperature and pH of the solution influenced the rate of adsorption process. Desorption of copper ion from chitosan beads by changing pH to ~1 with sulfuric acid showed that this process also follows first order.

1. INTRODUCTION

Disposal of toxic heavy metals into the environment is increasing due to rapid industrialization. Copper ion is coming into wastewater due to its extensive use in electrical industries, antifouling paints and as fungicides. Present method for the removal of Cu (II) is to precipitate it as hydroxide by lime treatment. But this conventional procedure becomes less effective when metal ion concentration is in the range of 100 ppm [1]. This leads to research for the development of new technology for removal of toxic metals from wastewater [2] One of these technologies is the use of biopolymer for the removal of heavy metals from wastewater. Chitosan, polymer of β-1,4 glucosamine, due its wide availability, eco-friendly nature and capability of lowering transition metal ion concentration to ppb level through chelation [3] finds an alternative procedure for the treatment of wastewater. Metal binding capacity of chitosan depends on its physico-chemical characteristics. Most of the researches [4-7] have been carried out with chitosan powder. In the present investigation we describe the adsorption and desorption kinetics of Cu (II) ions on chitosan in bead form. Influence of temperature and pH on adsorption process has also been reported.

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2. EXPERIMENTAL

2.1. Preparation of chitosan

Chitin was isolated from prawn shells by the method described by Hackmann [8] and deacetylated to chitosan by treatment with 50% NaOH (Chitin:NaOH; 1:20) for 7 h at 120°C. Crude chitosan was purified by dissolving in 7% AcOH and precipitated out by adjusting pH to 8.5 with 1 (N) KOH. Chitosan thus obtained was washed first with water followed by ether.

2.2 Characterization of chitosan

The degree of deacetylation and weight average molecular weight and chitosan were determined by first derivative of UV spectroscopy [9] and intrinsic viscosity [10] respectively. Polydispersity was determined by light scattering method. FTIR spectra of chitosan were taken in Shimadzu spectrophotometer. Specific rotation was measured using Perkin Elmer Polarimeter (model 241 MC).

2.3 Preparation of chitosan beads

Chitosan beads were prepared by drop wise addition of 3% (w/v) solution of chitosan in 7% (v/v) AcOH to alkali coagulating mixture [H2O:MeOH:NaOH; 4:5:1 (w/w)] [11]. Beads were collected by filtration; washed with water to neutral pH and then conditioned by treatment with 0.1 (M) (NH4)2SO4 [12].

2.4 Preparation and estimation of Cu (II) solution

1-liter stock solution (1000 mg L⁻¹) of Cu (II) was prepared from analytical grade CuSO4·5H2O and diluted to working metal ion concentration. Initial pH was adjusted with dilute H2SO4. Cu (II) ion concentration was determined by atomic absorption spectrophotometer (Perkin Elmer model no 2380).

2.5 Adsorption of Cu (II) ion on chitosan beads

The sorption experiments were carried out in batch process. 20 ml of CuSO4·5 H2O solution containing 26.5 mg L⁻¹ Cu(II) were taken in different 250 ml Erlenmeyer flasks. 1 gm of chitosan beads having water content 96.4% was added to each flask. Flasks were agitated gently at 30°C and 10 ml solution was taken out from duplicate flasks at different time intervals. Solution was filtered and diluted to its atomic absorption analysis. The experiments were performed at two different pHs (3.0 and 5.0).

Experimental tests were also performed under different operating conditions: temperature 10-50°C and initial Cu (II) ion concentration (75-305 mg L⁻¹).

2.6 Desorption of Cu (II) ion from beads

Desorption of Cu (II) ion from chitosan beads were performed immediately after the adsorption experiments. Beads were collected by filtration; washed with water and suspended in 20 ml of 0.1 (M) NH4SO4 and H2SO4 at pH 1.0 and gently agitated for 24 h. Beads were separated by filtration and Cu (II) ion concentration in the filtrate was estimated. Beads were reconditioned as described and reused.
3. RESULTS AND DISCUSSION

Properties of the chitosan used for the preparation of beads have been presented in Table I, which shows that chitosan was highly deacetylated and polydisperse.

<table>
<thead>
<tr>
<th>Degree of deacetylation (%)</th>
<th>Molecular weight (×10^5)</th>
<th>Polydispersity index</th>
<th>Average molecular size (nm)</th>
<th>Ash content (%)</th>
<th>Moisture content (%)</th>
<th>Specific rotation [α_d]</th>
</tr>
</thead>
<tbody>
<tr>
<td>89.71</td>
<td>10</td>
<td>1.205</td>
<td>1448.6</td>
<td>0.6</td>
<td>5.12</td>
<td>-21°</td>
</tr>
</tbody>
</table>

Co FTIR result (Fig. 1) shows that the chitosan used in this study was similar to that purchased from Sigma USA.

Rate of adsorption of Cu (II) ion on chitosan beads at two different pHs is presented in the Fig 2a. The adsorption process during initial 1 min is presented in Fig. 2b.

It appears from the figures that pH of the solution influenced the adsorption process and higher adsorption was noted with pH 5.0 than 3.0. Better adsorption of Cu (II) ion noted at pH 5.0 may be due to protonation of –NH_2 groups of chitosan at lower pH inhibits chelation of metal ion by chitosan. Adsorption of Cu (II) ion on chitosan beads was very fast during the first minute, rate of adsorption gradually retreated down and 90% adsorption of Cu (II) ion was completed within 50 min. Initial high rate adsorption may be due to large availability of free amino groups of chitosan as well as high copper ion concentration. Fig. 3 represents the influence of temperature on sorption of Cu (II) by chitosan beads. The rate of adsorption increased with increase in temperature and maximum adsorption was noted at 30°C and after that no further increase was observed.

Order of adsorption of Cu (II) by chitosan beads was calculated by plotting log (residual Cu (II) ion mg L^-1 in solution) against time and presented in fig. 4.

![Figure 1. Co FT IR spectra of chitosan](image-url)
Figure 2a. Cu (II) adsorption

Figure 2b. Cu (II) adsorption in the first min

Figure 3. Effect of temperature on adsorption

Figure 4. Adsorption kinetics of Cu (II) ion on chitosan beads
Since 50% of adsorption took place within first min, kinetics was studied only for that period. Straight-line curve obtained in this respect indicates that this adsorption process followed first order kinetics.

There are different equations including Langmuir and Freundlich which described relationship between the amount adsorbed (X/m) and equilibrium concentration (C_e). Plot of log X/m vs. log C_e (Fig. 5) gave a straight line indicating that it followed Freundlich’s adsorption isotherm.

The desorption of Cu (II) from chitosan beads with time is presented in Fig. 6, which shows that this process was also very fast and almost 80% of desorption took place within first 60 min. This may be due to ready elution of copper ion from the outer surface of the beads. Removal of copper ion which goes inside of the porous beads due to capillary action takes some times to elute.

The plot of Log [Cu (II) mgL^-1 in solution] with time shows linearity (Fig. 7), indicates desorption follows first order kinetics.

![Figure 5. Adsorption isotherm](image1)

![Figure 6. Cu (II) desorption](image2)
Figure 7. Kinetics of Cu (II) desorption

Amount of Cu (II) adsorbed and desorbed per gm of chitosan are presented in the Table 2.

<table>
<thead>
<tr>
<th>Metal ion present initially in 20 ml of solution (mg)</th>
<th>Metal ion present finally in 20 ml of solution (mg)</th>
<th>(% of metal ion removed)</th>
<th>Chitosan content per gm of beads (g)</th>
<th>Amount of metal ion adsorbed by per gm of chitosan (mg)</th>
<th>Amount of metal ion desorbed per gram of chitosan (mg)</th>
<th>(% of metal ion desorbed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.53</td>
<td>0.016</td>
<td>96.98</td>
<td>0.0358</td>
<td>14.0000</td>
<td>13.7003</td>
<td>97.85</td>
</tr>
</tbody>
</table>

It is clear from the table that in this experiment around 97% of Cu (II) ion in the solution was removed by chitosan treatment and approximately 98% of could be desorbed from the beads. It was observed that beads could be reused five more times without affecting its efficacy.

4. CONCLUSIONS

It may be concluded from the present study
1. Chitosan beads can remove more than 96% of Cu (II) ion from solution by sorption.
2. Optimum pH and temperature in this respect being 5.0 and 30°C respectively.
3. Sorption process is very fast during 0 to 10 min.
4. Cu (II) ion can be disorbed from the beads by changing pH and thus facilitates repeated use.
5. Both sorption and desorption process follows first order rate law.
6. Adsorption of Cu (II) on chitosan follows Freundlich isotherm model.
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Development of a process for biosorptive removal of mercury from aqueous solutions

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Abstract

Mercury biosorption studies with fungal cultures revealed that optimum pH for biosorption was 8 and culture PK2 possessed highest efficiency. Metal loading capacity for PK2 was 76 mg/g. Effect of varying biomass concentration, time, and mercury concentration on biosorption by PK2 was checked. It was found that biosorbent concentration of 0.2 g, contact time of 60 min. and mercury concentration of 40 mg/L were optimal parameters. Pre-treatment with dimethyl sulfoxide (100%), hydrochloric acid (1M), sodium carbonate (1M) resulted in an increase in the biosorption efficiency, while ethanol (absolute), triton X-100 (1%), and ammonium sulphate treatment decreased the efficiency. It was observed that mercury uptake values could be fitted in the Freundlich and Langmuir isotherm models. The biosorbent beads of PK2 biomass were prepared by a proprietary process. The beads (1 g, pre-treated with dimethyl sulfoxide) were packed in glass columns (length 5.5 cm, internal diameter 1 cm) and a solution containing mercury (100 mg/L) was passed in an up-flow mode. It was observed that adsorption efficiency dropped below 60% and 50% after passing 10 and 35 bed volumes, respectively. However, a removal efficiency of 40% could be sustained beyond 100 bed volumes of mercury solution.

1. INTRODUCTION

Mercury is toxic in its metallic (including gaseous), ionic and organic (monomethyl, dimethyl and phenyl) forms, and has long been recognised as an environmental hazard. Its use in industry is widespread, especially in the production of chlorine, caustic soda, thermometers, certain pharmaceutical drugs, pesticides and agricultural products, electrical equipments such as batteries, metal switches, fluorescence lamps, etc. Although there has been a belated shift from the polluting mercury cell technology to the membrane cells, mercury pollution in India is still very high. India does not produce mercury and relies completely on imports. Between 1998-2001, the annual mercury imports for mercury cell plants stood at 170-190 tonnes, which is 10% of the global mercury consumption. Assuming that these mercury cell plants and other industries release only

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50% of the mercury, the total mercury pollution load generated per year will be about 125 tonnes of elemental mercury [1]. This is about five times more than the total mercury compounds dumped into the Minamata Bay in Japan in 36 years, equivalent to five Minamata disasters.

Current technologies for mercury removal from wastewater are costly and ineffective to achieve desired permissible limits for discharge. Hence, there is a need for development of cost-effective green technology. Biosorption provides a good alternative for the removal of metals from solution. Algae have been shown to accumulate [2-3] and volatilise mercury [4-5]. A process based on bioaccumulation of Hg [6] by genetically modified, mercury-resistant *Pseudomonas putida*, *Aeromonas hydrophila* and natural consortia has been developed in a bench-scale column.

This paper describes studies on the uptake of mercury by fungal biomass. The study carried out will be helpful in developing full-scale biosorption process to clean up mercury pollution of water and wastewater.

2. MATERIALS AND METHODS

Biosorption studies were carried out with fungal cultures isolated in our laboratory through extensive screening for metal biosorbents. Fungal isolates *Aspergillus niger* (501), *Alternaria alternata*, PK1, PK2, *Aspergillus niger* (502), *Aspergillus fumigatus*, *Fusarium oxysporum* (NCIM-718), *Absidia blakesleena* (NCIM-889), *Actinomucor* sp. (NCIM-1183) and *Absidia corymbifera* (NCIM-1233) were grown in bulk quantity in Sabouraud medium. After 5 days of incubation at room temperature (28±5°C) biomass was harvested by filtration. After washing the biomass with deionised water, it was dried in an oven at 60°C for 24 h. Dry biomass was powdered in a blender (particle size 0.1-0.2 mm) and then used in biosorption experiments. Standard protocol [7] was used for screening of cultures. The dried and finely powdered biomass obtained by above procedure was conditioned to pH 4, 5, 6, 7, and 8 by contacting with distilled water of respective pH. Metal solution was prepared by dissolving appropriate quantity of HgCl₂ in water so as to get final concentration of 1 mM. The pH of the Hg solution was adjusted to 4, 5, 6, 7, and 8 separately. The dried biomass (0.2 g) conditioned to different pH was then contacted with 100 mL metal solution of same pH in a 250 mL Erlenmeyer flasks. The flasks were kept on shaker (120 rpm) for 30 min. A culture showing maximum mercury biosorption was selected for further studies.

The mercury loading capacity or cumulative biosorption by the selected culture was determined by contacting the dried, pH conditioned (pH 8) biomass powder (1 g) several times with fresh batches of 100 mL mercury solution (200 mg/L, pH 8) till the biomass was saturated with mercury ions.

To check the effect of biomass concentration, mercury solution (100 mL, 200 mg/L of Hg, pH 8) was contacted with varying amounts of dried, pH-conditioned biomass (pH 8) of the culture in the range of 0.1% to 1% (w/v).

Rate of mercury uptake by the culture was studied by contacting the dried biomass (pH 8, 0.2 g) with 100 mL mercury solution (200 mL, pH 8), for various time intervals (10 - 80 min.).

To check the effect of mercury concentration on biosorption, the culture biomass (0.2 g, and pH 8) was contacted with different concentrations (20, 40, 60, 80, 120, 160, 200 mg/L) of mercury solution (100 mL, pH 8) for 1 hour.
In order to check the effect of different pretreatments, freshly harvested biomass (10 g wet weight) was treated with 50 mL of the following solutions for 30 min.: sodium carbonate (1 M), sodium hydroxide (1 M), hydrochloric acid (1 M), urea (1 M), ammonium sulphate (1 M), triton-X 100 (1%), dimethyl sulfoxide (100%), and ethanol (absolute). Biomass was separated by filtration and washed several times with deionised water to remove traces of adhering chemicals. It was then dried at 60°C, powdered and used in mercury biosorption experiments.

Biosorbent beads were prepared by mixing the biomass with a polymeric matrix from waste poultry feathers by a proprietary process [15]. A solution containing 100 mg L−1 mercury (pH 8) was passed in upflow mode through a glass column (length 5.5 cm, internal diameter 1 cm) containing 1 g biosorbent beads (pre-treated with dimethyl sulfoxide). The void volume of the column after packing the biosorbent was 2 mL. Solution flow rate was adjusted to 0.2 mL min⁻¹ using a programmable peristaltic pump (Ismatec, Switzerland, model MCP 552).

In all the above experiments, after contacting with mercury solutions, the residual mercury content in solutions/filtrates were analysed for using an atomic absorption spectrometer (ATI-UNICAM, UK, model 929). The experiments were carried out in duplicates and appropriate experimental controls were run simultaneously.

3. RESULTS AND DISCUSSION

3.1 Biosorption efficiency of the fungal cultures

The data given in Table 1 show mercury sorbed (%) by the different fungal biomass at pH 8. Sorption of Hg²⁺ by the different fungal biomass was observed to vary according to the pH and found to occur over a pH range of 6-8. Mercury precipitation was not observed at pH 8. The optimum pH for sorption by all the cultures was 8. Biomass of culture PK2 was the most efficient in removing mercury from solutions; hence it was used at pH 8 in further experiments. The pH of metal solution influences metal biosorption by changing surface properties of biomass and metal speciation [8]. Most investigators have shown that a pH range of 4.0-8.0 is optimal for metal uptake [9]. At acidic pH metal uptake was less, which increased, as the pH was increased up to 8. As the pH level was increased, more negatively charged ligands would be available for binding positively charged metal ions.

Table 1. Mercury sorbed (%) by the fungal biomass

<table>
<thead>
<tr>
<th>Culture</th>
<th>Mercury sorbed at pH 8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. niger (Culture no. 501)</td>
<td>50</td>
</tr>
<tr>
<td>Alternaria alternata (NCIM-718)</td>
<td>33</td>
</tr>
<tr>
<td>Culture PK1</td>
<td>40</td>
</tr>
<tr>
<td>Culture PK2</td>
<td>53</td>
</tr>
<tr>
<td>A. niger (Culture no. 502)</td>
<td>42</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>03</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>39</td>
</tr>
<tr>
<td>Absidia blakesleana (NCIM-889)</td>
<td>05</td>
</tr>
<tr>
<td>Actinomucor sp. (NCIM-1183)</td>
<td>26</td>
</tr>
<tr>
<td>Absidia corymbifera (NCIM-1233)</td>
<td>02</td>
</tr>
</tbody>
</table>

3.2 Metal loading capacity of PK2 biomass

It was found that mercury-loading capacity of PK2 was 76 mg/g. The economics of metal biosorption process are more dependent upon metal loading capacity of a biosorbent
rather than its percent biosorption efficiency. Metal loading capacity gives more realistic 
figure as compared to percent metal uptake from solutions or the specific uptake values 
based on calculations [10].

3.3 Effect of biomass concentration

It was observed that amount of PK2 biosorbent required for maximum mercury 
biosorption was 0.2% (Figure 1). Further increase in the biosorbent concentration had not 
increased the biosorption efficiency. So 0.2% was considered as optimum biomass 
concentration for maximum \( \text{Hg}^{2+} \) uptake.

3.4 Rate of mercury uptake

Mercury uptake increased gradually with time upto 60 min. of contact, following 
which there was no change in the sorption capacity (Figure 2).

3.5 Effect of mercury concentration

Initially there was increase in the efficiency of sorption as the concentration of 
mercury increased, but after 40 mg/L considerable change was not observed in biosorption 
efficiency, which remained almost stationary up to 200 mg/L.

3.6 Biomass pre-treatment

Chemical pre-treatment to biomass result in unmasking or exposing the metal binding 
groups, adding new metal binding groups or modifying the existing ones and alterations in 
charge density on the surface. Such pre-treatments might be useful in improving the metal 
biosorption.

Sodium carbonate, hydrochloric acid, and dimethyl sulfoxide treatment resulted in 
significant increase in biosorption efficiency of PK2. Maximum increase was due to 
dimethyl sulfoxide treatment (Table 2). The increase may be a result of change in charge 
density on the surface. Ethanol, ammonium sulphate, triton X-100 treatments resulted in 
reduced biosorption, probably as a result of denaturation, changes in surface charges or 
leaching of low molecular weight compounds [11-12].

Table 2. Effect of chemical pre-treatments to biomass

<table>
<thead>
<tr>
<th>Biomass pre-treatment</th>
<th>Mercury sorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>44</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>62</td>
</tr>
<tr>
<td>Ethanol</td>
<td>24</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>78</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>18</td>
</tr>
</tbody>
</table>
### 3.7 Adsorption isotherm

Adsorption isotherm is an important tool that indicates relative affinities of biosorbents for a particular metal. It also helps in understanding the type of interaction that takes place between metal ions and microbial surfaces such as physical adsorption, nucleation or multilayer adsorption, etc. The metal uptake value (Q) was calculated using the equation:

$$ Q = \frac{V(C_i - C_f)}{1000m} $$  \hspace{1cm} (1)

where Q is the metal uptake (mg g\(^{-1}\) biosorbent), V the volume of metal solution (mL), C\(_i\) the initial concentration of metal in solution (mg L\(^{-1}\)), C\(_f\) is final concentration of metal in solution (mg L\(^{-1}\)) and m is the mass of biosorbent (g). Figure 3 shows the isotherm for mercury biosorption. It could be seen that specific metal uptake (Q) increased with the increase in initial mercury concentration up to 160 mg L\(^{-1}\). Above this concentration, the metal uptake remained constant indicating saturation of the binding sites. From the Q value obtained, adsorption isotherms were plotted according to Freundlich and Langmuir equations:

$$ \ln Q = \ln k + \left(\frac{1}{n}\right) \ln C_{eq} $$ \hspace{1cm} (Freundlich equation)  \hspace{1cm} (2)

$$ \frac{C_{eq}}{Q} = \frac{1}{bQ_{max}} + \frac{C_{eq}}{Q_{max}} $$ \hspace{1cm} (Langmuir equation)  \hspace{1cm} (3)

where, C\(_{eq}\) is the liquid phase concentration of the metal (mg L\(^{-1}\)), b the Langmuir constant, Q the metal uptake (mg g\(^{-1}\) biosorbent) and Q\(_{max}\) the maximum metal uptake (mg g\(^{-1}\) biosorbent). The mercury uptake values could be fitted to the widely accepted Freundlich (Figure 4) and Langmuir model (Figure 5).

Adsorption isotherm studies suggested that mercury biosorption by PK2 was a physical interaction characterised by monolayer adsorption onto heterogeneous surfaces at constant adsorption energy according to the basic assumptions of the Langmuir and Freundlich models [13-14].

### 3.8 Breakthrough curve

Based on the data breakthrough curve was plotted (Figure 6). It was observed that 10 and 35 bed volumes could be passed through the column before the mercury removal efficiency dropped below 60% and 50% respectively. The efficiency of the column remained above 40% even after passing more than 100 bed volumes of mercury solution.

### 4. CONCLUSION

The biomass of culture PK2 is a good biosorbent material for mercury removal. Although the residual mercury concentration in the column experiments performed using PK2 biosorbent beads was above the permissible limit, it is possible to improve the efficiency of the system further by employing a battery of columns.
**Biosorption**

![Graph](image1)

**Figure 3. Adsorption isotherm for mercury sorption**

![Graph](image2)

**Figure 4. Freundlich isotherm for Hg sorption**

![Graph](image3)

**Figure 5. Langmuir isotherm for Hg biosorption**

![Graph](image4)

**Figure 6. Breakthrough curve for Hg sorption**

**REFERENCES**

Effects of ionic strength, background electrolytes and heavy metals on the biosorption of hexavalent chromium by *Ecklonia* biomass

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\(^b\) Division of Environmental and Chemical Engineering, Chonbuk National University 664-14, 1 Ga, Duckjin-dong, Duckjin-ku, Jeonju, Chonbuk, 561-756, Korea

Abstract

Brown seaweed, *Ecklonia*, biomass was used to remove Cr(VI) from wastewater. Previously, we found that the Cr(VI) was not really removed by adsorption to the biomass but reduced to Cr(III) on the surface of biomass. In this study, the effects of ionic strength and background electrolytes of the solution on the Cr(VI) reduction rates were evaluated. Although the increase of ionic strength slightly inhibited the Cr(VI) reduction, the rate equation proposed in our previous study was applicable over a wide range of ionic strengths with different background electrolytes. The presence of other heavy metals such as Cr(III), Ni(II), and Zn(II) did not nearly affect the Cr(VI) reduction, while it caused the slight reduction of the uptake of total Cr at equilibrium. In addition, Cr(VI)-Ni(II) binary system was analyzed using the competitive Langmuir model.

1. INTRODUCTION

Chromium in aquatic environments is known to be very toxic and classified by the US EPA in their list of human carcinogens (Group A) \[1\]. The major source of chromium is the wastewater from electroplating and metal-finishing industries \[2\]. Of its several oxidation states (e.g., di-, tri-, penta-, and hexa-), trivalent and hexavalent chromium are principal forms found in industrial effluents \[3\]. It is interesting that these two forms of chromium exhibit very different toxicity and mobility. Cr(III) is relatively insoluble over pH 5 in aqueous systems and exhibits little or no toxicity. In contrast, Cr(VI) usually exists as highly soluble and highly toxic chromate anions (HCrO\(_4^-\) or CrO\(_4^{2-}\)), and is a suspected carcinogen and mutagen. The conventional treatment method of chromium-containing wastewater is based on precipitation of the hydroxide form of Cr(III). Initially chromium is present as Cr(VI) which is then converted to Cr(III) by reaction with reducing chemicals, followed by precipitation. However, chromium precipitation produces a large amount of chemical sludge which is a major disadvantage of this method in addition to costs associated with its chemical reduction. It is generally considered that ion
Biosorption exchange method can minimize the sludge generation. However, due to the high costs of synthetic resins, its application for the wastewater treatment has been limited [4].

Recently, biomaterials such as microbial biomass (fermentation wastes) and seaweeds have been examined as an adsorbent alternative to synthetic ion exchange resin considering the economics [4-9]. Several types of seaweeds were found to successfully remove chromium species [4, 9]. However, the mechanisms involved in Cr removal and factors affecting the Cr removal efficiency have not been studied in detail. In our previous studies, we characterized the removal mechanism of Cr(VI) by *Ecklonia* biomass by using various experimental systems and techniques. It was found that Cr(VI) was removed through redox reaction by biomass. The rate equation was also established, which was applicable over a wide range of pH, [Cr(VI)]₀, [B]₀, and temperature. However, in general, industrial wastewater does not contain only chromium but also many other ions that may affect the removal efficiency of chromium by biomass. Therefore, it is necessary to examine the effects of these ions on the Cr(VI) reduction for the practical application to the actual wastewater.

In this study, we examined the Cr(VI) reduction rates according to ionic strength, background electrolytes, and other heavy metals such as Cr(III), Ni(II) and Zn(II). In addition, Cr(VI)-Ni(II) binary system was analyzed using the competitive Langmuir model.

2. MATERIALS AND METHODS

2.1 Biosorbent

The brown macro-alga, *Ecklonia* was collected from the seashore of Pohang, South Korea and sun-dried. After cutting into approximately 5-mm size particles, the *Ecklonia* biomass was contacted with 1 M H₂SO₄ for 24 h, by which ions originally in the biomass were expected to be replaced with proton. The biomass was then washed several times with double-deionized water and dried at 80°C for 24 h. The resulting dried biomass was used in all experiments.

2.2 Biosorption experiments

The sorption of chromium was investigated using batch kinetic and equilibrium experiments. All of experiments were carried out with 5 g l⁻¹ of biomass concentration. Flasks were mixed at 200 rpm on a shaker at room temperature. During the experiment, the solution pH was adjusted to the desired value by the incremental addition of concentrated H₂SO₄ or NaOH. Control experiment was conducted at 100 mg l⁻¹ of initial Cr(VI) concentration, and pH 2. For kinetic experiments, samples were intermittently removed from the flasks in order to analyze the Cr(VI) concentration. For equilibrium experiments related to Cr(VI), all trials were conducted until Cr(VI) was completely removed from the solution. Depending on both the solution pH and initial concentration of Cr(VI), the contact time required for the complete removal of Cr(VI) from the solution was ranged from hours to weeks. While, equilibrium experiments with only Ni(II) were conducted for 12 hours which was sufficient to reach the equilibrium state.

2.3 Analytical methods

The concentration of Cr(VI) in the liquid samples was determined colorimetrically by reaction with 1,5-diphenylcarbazide in the acid solution. The absorbance of the resulting red-violet sample was measured at 540 nm using a spectrophotometer (Spectronic 21,
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Milton Roy Co., USA). To determine the total concentration of chromium, all chromium was converted into the hexavalent state by oxidation with potassium permanganate. Thereafter, the oxidized chromium was analyzed by the above-mentioned method for Cr(VI) analysis. Since chromium in solution is mostly in hexavalent or trivalent states, the concentration of Cr(III) can be obtained from the concentration difference between the total and hexavalent chromium. The analytical method of chromium is detailed elsewhere [10]. The concentration of Ni(II) was determined in atomic absorption spectrophotometer (Model SpectrAA.800, Varian) at the wavelength of 232 nm.

3. RESULTS AND DISCUSSION

3.1 Effect of background electrolyte

The dependence of reduction rates on the background electrolyte was examined by using three monovalent (Li⁺, Na⁺, K⁺) and two divalent (Mg²⁺, Ca²⁺) cations. The concentration of each cation was 0.1 M, and common anion was chloride. Fig. 1 shows the time courses of [Cr(VI)] change. There was almost no difference in the reduction rate of Cr(VI) according to the change of cations except Ca²⁺.

![Figure 1. Effect of various cations on the reduction of Cr(VI) by Ecklonia biomass at pH 2](image)

Effects of cations on the reduction of Cr(VI) was further evaluated by calculating the reduction rates (redox reaction between Cr(VI) and biomass). The high magnitude of correction factors reaffirmed that the removal of Cr(VI) by Ecklonia biomass followed the oxidation/reduction reaction. The reduction rate of control experiment was 0.0118 mmol l⁻¹ h⁻¹. For monovalent cations, there was only a 0.0002 unit difference between the maximum and minimum value of reduction rates. This implies that the presence of monovalent cations did almost not affect the reduction of Cr(VI). However, divalent cations caused the decrease in the reduction rate of Cr(VI). Especially, Ca²⁺ significantly reduced the reduction rate of Cr(VI) (about 26%). It has been known that alkali-metal and alkaline-earth cations can bind to negatively charged groups of biomass primarily by electrostatic interaction. However, mono- or divalent light metals do not bind on the biomass at low pH, such as 2.0, and may not greatly affect the reduction of Cr(VI). It is also reasonably safe to presume that the conformational change of biomass did not occur at cationic concentration lower than those tested in our experiment. The decrease of the
reduction rate by Ca$^{2+}$ can be explained as follows: Ca$^{2+}$ forms insoluble complexes with OH$^{-}$ and SO$_4^{2-}$ on the surface of the biomass which might hinder the contact between Cr(VI) ions and the biomass.

The effect of anions on the reduction rate of Cr(VI) was also examined by using various background electrolytes (Cl$^{-}$, NO$_3^{-}$, CO$_3^{2-}$, SO$_4^{2-}$, HPO$_4^{2-}$). The concentration of each anion was 0.1 M, and common cation was sodium ion. Figure 2 shows the time course of Cr(VI) change by the reduction.

![Figure 2. Effect of various anions on the reduction of Cr(VI) by Ecklonia biomass at pH 2](image)

The reduction rate constants varied up to 21% according to anions added. For the redox reaction between Cr(VI) and biomass, the intermediate of Cr(VI)-biomass has to be formed. However, the high concentration of anions inhibits the formation of Cr(VI)-biomass intermediate, competitively. This inhibition is exaggerated by the increase of the electrostatic force of anions. The order of electrostatic force of anions added in this experiments is Cl$^{-}$ < NO$_3^{-}$ < CO$_3^{2-}$, SO$_4^{2-}$, HPO$_4^{2-}$, while the order of reduction rates measured in this experiment was Cl$^{-}$ > NO$_3^{-}$ > CO$_3^{2-}$ > SO$_4^{2-}$, HPO$_4^{2-}$.

### 3.2 Effect of ionic strength

Changes in ionic strength of the solution can cause conformational modification of the functional groups of biomass. It was suspected that these conformational changes would make the reactive functional groups either more or less accessible to Cr(VI), thereby altering the reduction rate. The Cr(VI) reduction by the biomass was studied in the ionic strength range 0.01-1M of NaCl. Time course of [Cr(VI)] change did not display a difference when the ionic strengths were below 0.1 M (Figure 3). However, significant differences were observed above 0.5 M of NaCl. The reduction rates at 0.5 M and 1.0 M of NaCl decreased to 29% and 41% of the control, respectively. These results suggest that conformational changes in the functional groups of biomass at ionic strength below 0.1M of NaCl did not greatly affect the reduction rate of Cr(VI). High ionic strength of the solution such as over 0.5 M definitely affected the reduction rate of Cr(VI) by Ecklonia biomass but this may be ignored in actual process since the ionic strength of usual wastewater would be lower than 0.1 M. In a separate experiment, it was found that many other factors such as pH and temperature gave much greater impacts (data not shown).
Figure 3. Effect of ionic strength on the reduction of Cr(VI) by *Ecklonia* biomass at pH 2

3.3 Effect of other heavy metals

The reduction of Cr(VI) by the biomass ultimately results in the formation of Cr(III) which possibly affects the Cr(VI) reduction by binding functional groups on the surface of biomass. It is presumed that the binding of Cr(III) makes the biomass more resistant to oxidation by Cr(VI). In addition to Cr(III), other heavy metals coexisting in wastewater can also be bound on the surface of biomass, resulting in the decrease of reduction rate. The effects of other heavy metal on the reduction rate of Cr(VI) were estimated with a series of experiments added 500 mg l⁻¹ of Cr(III), Ni(II), and Zn(II), respectively. As can be seen in Figure 4, there was almost no change in time course of [Cr(VI)] at each solution and, therefore, no significant difference in the reduction rate of Cr(VI). Similar to the ionic strength, other heavy metals possibly affect the reduction rate of Cr(VI) but the effects may be insignificant compared to other factors such as pH and temperature. It is likely that the functional groups responsible for the reduction of Cr(VI) are not the same groups responsible for cationic heavy metal binding. Therefore, the presence of other heavy metals may be ignored in the actual application of biosorption for Cr(VI) detoxification.
3.4 Cr(VI)-Ni(II) binary system

At equilibrium state, since Cr(VI) completely reduced into Cr(III) by biomass, chromium exists only in the form of trivalent chromium in the solution. As mentioned above, other cationic heavy metals did not much affect the reduction rate of Cr(VI). However, at the equilibrium state, they can affect the binding efficiency of Cr(III). Ni(II) was chosen to determine the effect of co-existing heavy metal on the uptake of chromium. The maximum uptake of total Cr was about 8 times of the maximum uptake of Ni(II) in each single system. For each metal ion in single system, the individual adsorption data was fitted by mono-component Langmuir model, and high magnitude of correction factors guaranteed the validity of this model. The individual Langmuir constants, $q_{\text{max}}$ and $b$, of total Cr and Ni(II) were determined as, 107.5 mg g$^{-1}$ and 0.0131 l mg$^{-1}$, 13.73 mg g$^{-1}$ and 0.0085 l mg$^{-1}$, respectively. Figures 5 and 6 show the adsorption isotherms of total Cr and Ni(II) in the binary system at pH 3, respectively.

![Figure 5. The isotherms of total Cr at various initial concentration of Ni(II). Lines were predicted by the comparative Langmuir model](image1)

![Figure 6. The isotherms of Ni(II) at various initial concentration of Cr(VI). Lines were predicted by the comparative Langmuir model](image2)
To express the simultaneous biosorption phenomena of total Cr and Ni(II) in the binary system, the competitive Langmuir model was used with constants obtained from isotherms of the single metal system. In a two-metal system at an equilibrium, the uptake of one metal decreases as the other metal concentration increases. However, in the Cr(VI)-Ni(II) system, the competitive Langmuir model showed poor performance in estimating the uptake of total Cr, while much better performance in estimating the uptake of Ni(II). The model gave us under-estimated values for the uptake of total Cr, which implies that the Ni(II) present in the solution did not properly compete with the total Cr on the biomass. On the contrary, the uptake of Ni(II) was completely inhibited by the competitive adsorption of Cr(III). The incomplete competition between Ni(II) and adsorbed total Cr suggested that the uptake of total Cr is slightly inhibited by Ni(II) because of the strong binding of chromium [especially Cr(III)] with the biomass, while the uptake of Ni(II) is completely inhibited by the competitive adsorption of reduced Cr(III).

4. CONCLUSIONS

In our previous report, we derived a rate equation applicable for the reduction of Cr(VI) by Ecklonia biomass over a wide range of pH, [Cr(VI)]o, [B]o, and temperature (data not shown). In this study, we found that this rate equation is applicable for over a range of ionic strength and different background electrolytes. Other heavy metals such as Cr(III), Ni(II), or Zn(II) did not nearly affect the reduction rate of Cr(VI), but caused the slight decrease of the uptake of total Cr on the biomass. Therefore, in actual wastewater treatment system, we may ignore the presence of other metals in the detoxification of Cr(IV). Although various parameters of actual wastewaters probably affect the reduction rate of Cr(VI) by Ecklonia biomass, these effects are relatively small compared to pH or temperature. In conclusion, the Ecklonia biomass is a good candidate for a biosorbent in the Cr(VI) detoxification process, and the scale up may be accomplished using the rate equation previously derived.

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Evaluation of silver recovery from photographic waste by

*Thiobacillus ferrooxidans* and chitin

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Abstract

Chitin was able to adsorb both silver ion and silver-thiosulphate complexes at their optimum pH of system 7.0-8.0 and 2.0, respectively. Silver ion was probably adsorbed by ion exchanging and/or co-ordination, whereas silver-thiosulphate complexes were adsorbed at only pH 2.0 by electrostatic interaction. Sodium acetate in the fixer did not affect to silver-thiosulphate adsorption due to its low ionic strength, but sodium thiosulphate let free thiosulphate to form strong silver-thiosulphate complexes, which could not be adsorbed by chitin. Adsorption isotherm showed that the maximum adsorption capacity ($Q_{\text{max}}$) and the affinity ($b$) of chitin to adsorb silver ion ($Q_{\text{max}} = 4.67$ mg Ag/g; $b = 0.565$) and silver-thiosulphate complexes ($Q_{\text{max}} = 4.37$ mg Ag/g; $b = 0.518$) were slightly different. These constants implied that silver ion and silver-thiosulphate complexes were adsorbed onto the same functional groups: acetylamino and amino groups. The biooxidation of silver-thiosulphate by *Thiobacillus ferrooxidans* was able to degrade thiosulphate. The biooxidation process also changed silver-thiosulphate complexes to silver ion as adsorption occurring at system pH of 7.0. The advantage of using *T. ferrooxidans* and chitin is the achievement of higher purity silver.

Keywords: silver, photographic waste, biooxidation, chitin, adsorption

1. INTRODUCTION

Silver is a precious metal. It is also classified as a hazardous substance [1]. Film development causes photographic waste of spent fixer and rinse water containing 1,000-10,000 mg Ag/L and 50-200 mg Ag/L, respectively, in the form of silver-thiosulphate complexes [2]. Therefore, it should be recovered completely for worth usefulness and environmental issues. Electrolysis, metallic replacement, and precipitation are used to recover the silver waste, but they are not suitable for recovery of silver at low concentrations (< 100 mg/L) [3]. Moreover, metallic replacement and precipitation produce impure sludge that requires refining through further treatment. The ion-exchange method can regain silver at low concentrations, but costs of the ion-exchanger and

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maintenance are high [4]. Therefore, the production of low-cost alternatives has been brought into focus.

Chitin is a cheap biopolymer that is directly extracted in large quantities from crab and shrimp shells, and seafood wastes. The structure of chitin is similar to the structure of cellulose. It is a completely substituted polysaccharide carrying acetyl-amino and amino groups per glucose ring, which the portion of amino groups is less than 50%. Chitin is an alternative adsorbent due to its content of acetyl-amino and amino groups for chelating metal ions [5] and its stability in acid conditions [5,6]. Chitin has been studied for the adsorption of anionic complexes such as Cr(VI) [5-7], Mo(VI) [8], and of cationic ions such as Cd\(^{2+}\) [9,10] and Cu\(^{2+}\) [11]. The adsorption of silver in the forms of Ag\(^{+}\), Ag(NH\(_3\))\(^{2+}\), Ag(SCN)\(_3\)\(^{-}\) and Ag(S\(_2\)O\(_3\))\(_2\)\(^{3-}\) by fungi that contain chitin and chitosan in the cell wall has been studied [3,12], but the use of the biomass as an adsorbent has had problems because the content of these polymers is able to change during growth of mycelia, which can account for the variations in metal uptake capacity [3,12,13].

In addition, thiosulphate in the photographic waste causes impurity to silver and reduced silver adsorption. Therefore, thiosulphate should be degraded to sulphur and sulphite by Thiobacillus sp., according to Equation (1) [3,14,15]. Moreover, Thiobacillus sp. is tolerant of the toxicity of silver at high concentration [16].

\[
\text{Thiobacillus sp.} \\
\begin{array}{c}
\text{S}_2\text{O}_3^{2-} \\
\rightarrow
\end{array} \\
\text{SO}_3^{2-} + \text{S}^0
\] (1)

Therefore, the aim of this research is to study the adsorption of silver ion and silver-thiosulphate complexes by chitin. The effect of fixer composition on silver adsorption was also studied. Adsorption isotherm of silver ion and silver-thiosulphate by chitin were then compared. Finally, biooxidation of silver-thiosulphate by T. ferrooxidans before silver adsorption by chitin was also examined.

2 MATERIALS AND METHODS

2.1 Chitin

Seafresh Chitosan (Lab) Co., Ltd., Thailand provided chitin as powder. The particle size was 0.5-1.0 mm. The degree of deacetylation (% DD) was approximately 50%. In these experiments, chitin was used as received, without any further treatment.

2.2 Silver solutions and photographic waste

Silver-thiosulphate solution was prepared by adding 10 ml of silver standard 1,000 mg Ag/L in 2% nitric acid (Scharlau Chemie) to a 500-ml volumetric flask and then adjusting the volume with 0.0020 M sodium thiosulphate (Na\(_2\)S\(_2\)O\(_3\)) (Carlo Erba) to obtain 20 mg Ag/L of silver-thiosulphate complexes. The solution of silver ion (20 mg Ag/L) was also obtained by adjusting the volume with de-ionised water. The initial pHs of the solutions were adjusted to the desired value ± 0.05 pH units by nitric acid and sodium hydroxide.

Fixer and rinse water was obtained from a KODAK photographic laboratory that uses Kodak 3000 as the fixer. The composition of Kodak 3000 is shown in Table 1.
Table 1. Composition of Kodak 3000

<table>
<thead>
<tr>
<th>Composition</th>
<th>Amount in 1000 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodiumthiosulphate</td>
<td>40-45 g</td>
</tr>
<tr>
<td>Sodiumacetate</td>
<td>40-45 g</td>
</tr>
<tr>
<td>Sodiumbisulphite</td>
<td>1-5 g</td>
</tr>
<tr>
<td>Boric acid</td>
<td>1-5 ml</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>1-5 ml</td>
</tr>
</tbody>
</table>

2.3 Adsorption experiments

Batch adsorption experiments were conducted in 15-ml dark bottles. Experiments were carried out by adding 0.2000 g of chitin to 10 ml of 20 mg Ag/L silver-thiosulphate solution, and then shaking at 150 rpm, 30°C for 2 hours without attempting to control the pH during the course of the experiment. Adsorption isotherms were studied by varying adsorbent dosage in the range of 0.25%-2.00% (w/v). A sample was collected by filtration with Whatman no. 4. The remaining silver and changing pH were detected by the Inductive Couple Plasma Spectroscopy (ICP) (JY-124, France) at 328 nm and a pH meter (Mettler Delta 340, USA), respectively. The thiosulphate concentration was examined by iodometric titration [3,17].

The percentage of silver adsorption was calculated according to Equation (2). The silver adsorption isotherm was analysed by the Langmuir models as in Equations (3) [18,19].

\[
Silver \text{ Adsorption (\%)} = \frac{C_i - C_f}{C_i} \times 100
\]

Langmuir model:

\[
Q_e = \frac{Q_{max} b C_e}{1 + b C_e} = \frac{Q_{max}}{\sqrt{b} + C_e}
\]

where  
\(C_i\) is the initial concentration of silver in the solution (mg Ag/L),  
\(C_f\) is the residual concentration of silver in the solution (mg Ag/L),  
\(C_e\) is the residual concentration of silver in the solution at equilibrium (mg Ag/L),  
\(Q_e\) is the silver adsorption at equilibrium (mg Ag/g),  
\(Q_{max}\) is the maximum adsorption of silver (mg Ag/g),  
\(b\) is the affinity coefficient (L/mg).

2.5 Thiosulphate biooxidation

*T. ferrooxidans* DSM 583 was incubated in 9K medium [20] shaken at 150 rpm, 30°C for 15 hours. A 10% v/v of an inoculum was transferred to 250-ml Erlenmeyer flask containing 150 ml of silver-thiosulphate solution, 5 mg Ag/L within 0.0020 M thiosulphate, shaken at 150 rpm, 30°C for 5 days. The thiosulphate residue and pH chaining were determined by iodometric titration and the pH meter.
3. RESULTS AND DISCUSSION

3.1 Effect of initial pH

Under non-precipitation conditions pH ≤ 10, the effect of initial pH to adsorb silver ion (positive charge) and silver-thiosulphate complexes (negative charge) by chitin was investigated to obtain the optimum pH and adsorption mechanism. Figure 1 (a) showed that 97% of silver ion was adsorbed since initial pH of 6-10, which the pHs of system became to approximate pH of 7.0-8.0 (Figure 1 (b)). The adsorption of silver ion was decreasing to 40% at the initial pH of 2.0. Whereas, silver-thiosulphate complexes was adsorbed to 98% and the adsorption was occurred at only pH 2.0. The system pH of both was changeless at the initial pH 2.0 as shown in Figure 1 (b). The change in solution pH from the initial value is still unclear, but the rise in pH has been attributed to the adsorption of protons from water by the amine group [4,21]. The drop in pH at a pH greater than 9 could be attributed to the reverse process, that is, the release of protons in the high pH conditions [4,21].

![Figure 1. Effect of initial pH of solution for adsorption of silver ion and silver-thiosulphate complexes by chitin](image)

Figure 1. Effect of initial pH of solution for adsorption of silver ion and silver-thiosulphate complexes by chitin
The adsorption of silver ion and silver-thiosulphate complexes was pH dependent, because the $pK_a$ of chitin-chitosan is 6.0-6.5 [18,22]. The acetylamino and amino groups of chitin (Figure 2) were protonated at the low pH; it supported silver-thiosulphate adsorption by electrostatic interaction. The release of protons was occurred in the high pH conditions, it might support the adsorption of silver ion by ion exchanging [23] and/or coordination between lone pair electrons of nitrogen and oxygen and silver ion [9,24].

**Figure 2. Acetylamino group and amino group of chitin structure**

Additionally, the results of thiosulphate residue (Figure 3) indicated that there are 10% of thiosulphate was decomposed at initial pH of 2.0, according to Equation 4. While other 40% of thiosulphate were disappeared due to silver was adsorbed in the form of silver-thiosulphate complexes.

$$2H^+ + S_2O_3^{2-} \rightarrow [HS_2O_3^-] \rightarrow HSO_3^- + S^{\circ} \rightarrow SO_2 + S^{\circ} + H_2O \quad (4)$$

**Figure 3. Thiosulphate residual at the initial pH of solution**

### 3.2 Effect of sodium acetate and sodium thiosulphate

Sodium acetate and sodium thiosulphate are the main composition of Kodak 3000 (Table 1). Therefore, the effect of silver adsorption by chitin was studied by adding an amount of sodium acetate or sodium thiosulphate into silver-thiosulphate solution. Figure 4 clearly shows that sodium acetate did not affect silver adsorption, because it cannot form any complexes with silver, and it has too little ionic strength to interfere the silver adsorption onto chitin surface. Whereas, sodium thiosulphate gave thiosulphate to form silver-thiosulphate complexes, which an increasing of thiosulphate caused more stable of
silver-thiosulphate complexes as shown in Table 2. The stable complexes were more difficult to be adsorbed by chitin as the adsorption of silver-thiosulphate from rinse water containing 0.089 M thiosulphate was only 76% as shown in Table 3.

Table 2. Complex formation equilibria for Ag(I) and thiosulphate [2]

<table>
<thead>
<tr>
<th>Equilibrium</th>
<th>pK (0.1 mol L⁻¹ ionic strength)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Ag}^+ + \text{S}_2\text{O}_3^{2-} \rightleftharpoons \text{AgS}_2\text{O}_3^-$</td>
<td>6.93</td>
</tr>
<tr>
<td>$\text{Ag}^+ + 2 \text{S}_2\text{O}_3^{2-} \rightleftharpoons \text{Ag(S}_2\text{O}_3)_2^{3-}$</td>
<td>12.72</td>
</tr>
<tr>
<td>$\text{Ag}^+ + 3 \text{S}_2\text{O}_3^{2-} \rightleftharpoons \text{Ag(S}_2\text{O}_3)_3^{5-}$</td>
<td>14.78</td>
</tr>
<tr>
<td>$2\text{Ag}^+ + 4 \text{S}_2\text{O}_3^{2-} \rightleftharpoons \text{Ag}_2(\text{S}_2\text{O}_3)_4^{6-}$</td>
<td>28.23</td>
</tr>
<tr>
<td>$3\text{Ag}^+ + 5 \text{S}_2\text{O}_3^{2-} \rightleftharpoons \text{Ag}_3(\text{S}_2\text{O}_3)_5^{7-}$</td>
<td>42.58</td>
</tr>
<tr>
<td>$6\text{Ag}^+ + 8 \text{S}_2\text{O}_3^{2-} \rightleftharpoons \text{Ag}_6(\text{S}_2\text{O}_3)_8^{10-}$</td>
<td>85.23</td>
</tr>
</tbody>
</table>

Figure 4. Effect of sodium acetate and sodium thiosulphate to silver adsorption

Table 3. Adsorption of silver-thiosulphate obtained from standard solution and rinse water

<table>
<thead>
<tr>
<th>Adsorbent</th>
<th>Silver adsorption (%) Standard solution ($C_i = 20$ mg Ag/L)</th>
<th>Rinse water ($C_i = 20.1$ mg Ag/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin powder</td>
<td>96.53</td>
<td>75.85</td>
</tr>
<tr>
<td>Thiosulphate concentration</td>
<td>0.0020 M</td>
<td>0.0089 M</td>
</tr>
</tbody>
</table>

3.3 Adsorption isotherm

The adsorption isotherm of silver ion and silver-thiosulphate complexes was studied at 30°C under their initial optimum pH of 6.0 and 2.0, respectively. The maximum adsorption capacity ($Q_{max}$) of chitin to adsorb silver ion and silver-thiosulphate complexes was 4.67 and 4.37 mg Ag/g, respectively. The $b$ constants were 0.565 and 0.518, respectively (Figure 4). These indicated that chitin had the efficiency to adsorb silver ion a few higher than silver-thiosulphate complexes. This implied that silver ion and silver-thiosulphate complexes were adsorbed onto the same functional groups of chitin: acetylamino and amino groups. Since the effect of pH (Figure 1) suggested that silver-
thiosulphate was adsorbed onto the protonated groups at low pH by electrostatic interaction, whereas silver ion was adsorbed by ion exchanging [23] and/or co-ordination [9,24].

![Graph](image)

**Figure 4.** Adsorption isotherm of silver ion and silver-thiosulphate complexes by chitin

### 3.4 Biooxidation of thiosulphate

Recovery of silver from photographic waste still had interference from high thiosulphate concentration in rinse water (Table 3). Therefore, biooxidation of silver-thiosulphate by *Thiobacillus ferrooxidans* for degradation of thiosulphate was studied. The silver recovery from photographic waste by biooxidation with *T. ferrooxidans* and then adsorption by chitin was studied in this research. The result confirmed that *T. ferrooxidans* was able to degrade all of 0.0020 M thiosulphate in 5 days without silver precipitation. Silver-thiosulphate complexes were changed to silver ion as adsorption occurs better at pH 6 than pH 2.0. The advantage of biooxidation process is the reduction of the organic impurity from the solution. That will support silver purification processes.

### 4. CONCLUSIONS

Chitin was able to adsorb both silver ion and silver-thiosulphate complexes. The optimum pH of system to adsorb silver ion was 7.0-8.0, because it provided the silver ion adsorption by ion exchanging and/or co-ordination. Silver-thiosulphate complexes were adsorbed at only pH 2.0 because this system induced the chitin protonation that supported electrostatic interaction, and also reduced the stability of the silver complexes. Sodium acetate in the fixer did not affect silver-thiosulphate adsorption, but sodium thiosulphate could interfere the silver adsorption. Adsorption isotherm showed that the maximum adsorption capacity and the affinity of chitin to adsorb silver ion and silver-thiosulphate complexes were slightly different. This implied that silver ion and silver-thiosulphate complexes were adsorbed onto the same functional groups. The biooxidation of silver-thiosulphate by *T. ferrooxidans* degraded thiosulphate and increased the purity of silver. In view of the usefulness, the cooperation between biooxidation and adsorption by chitin should be studied further for application to recovery silver from photographic waste.
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Influence of the treatment of fungal biomass on sorption properties for lead and mercury uptake

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Abstract

Aspergillus niger biomass, obtained as a by-product of fermentation processes, has been tested for lead and mercury sorption. Biomass was produced by two different ways of citric acid production: (a) surface growth, and (b) submerged growth. Their sorption properties have been compared to biomass subjected to an alkaline treatment. The influence of the pH has been studied to select the optimum pH for lead and mercury and pre-select the best sorbent samples. Optimum sorption performances for Pb were obtained with surface-grown biomass, which was submitted to alkaline treatment, while with Hg biomass produced by submerged culture was preferred. For lead the experimental pH was set to pH 3, while for mercury the experimental pH was fixed to pH 6. Experiments were continued on selected sorbents by determination of sorption isotherms and uptake kinetics. The influence of experimental parameters (particle size, sorbent dosage, metal concentration) has been checked on sorption kinetics to evaluate the best operating conditions. In the case of lead sorption properties the excellent sorption properties characterized by fast sorption kinetics and high sorption capacities (close to 600 mg Pb g⁻¹) may be explained by the release of a cell component or a by-product of culture procedure (residue of growing media or sub-product of the fermentation process) that induces lead precipitation at long contact time.

Keywords: Aspergillus niger, waste biomass, sorption, lead, mercury

1. INTRODUCTION

The reinforcement of the regulations concerning industrial wastewaters has been the motive to an increasing research for developing new sorption processes. Indeed, conventional techniques such as precipitation and ion-exchange are sometimes non-competitive or unable to reach the regulation levels. There is still a need for alternative processes that should be cost-effective, and easy to manage. Biosorption that consists in using biological materials or microorganisms for the sorption of target molecules (metals,
Biosorption of dyes, pesticides) has been widely studied since the early 80’s. A great diversity of biomass material has been investigated for the sorption of metal ions from bacteria, fungi, algae [1], to agriculture waste by-products or bio-industry wastes [2]. Using dead biomass makes easier the control of the process and using waste materials reduces the cost of the material. For these reasons, fungal biomass has frequently been considered as a suitable biosorbent [3-7] since it is produced in huge amounts in the fermentation processes (citric acid production) and this biomass is poorly valorizable.

Fungal biomass may contain a wide range of functional groups including amine groups, carboxylic groups, and sulfur groups brought by cell constituents. For example fungal cell wall may be constituted by the assembling of chitin/chitosan layers (amine-rich), proteins (amino-acids), glucans [5]. Fungal biomass, especially from Mucorales group, has been considered as an alternative source for chitosan [8-10]: an aminopolysaccharide, which is commercially produced from crustacean shells, very efficient for metal recovery [11-14]. However, chitin and chitosan are strongly bound to other cell wall constituents such as glucans and proteins. While the association of chitin to proteins may be positive for sorption purpose since the amino-acids have potential sorption activity, the glucans have very weak potential for metal sorption. The immobilization of amine functions by linkage with glucans can significantly reduce the sorption activity of the material. Chemical treatments should be considered for “purifying” the biomass from this "inert" material [8-10,15-17]. The composition of the membrane is also tributary of the harvesting time and the growing conditions [18].

A preliminary study has been launched on the comparison of the sorbing potential of Aspergillus niger prepared under surface and submerged fermentation conditions used (a) as produced, and (b) after alkaline treatment. The sorption properties are investigated for lead and mercury removal from dilute solutions. First the influence of the pH is studied and these results serve to select the best sorbents for the recovery of these metals. Then the sorption properties of selected biomass are studied through sorption isotherms and uptake kinetics.

2. MATERIALS AND METHODS

2.1 Materials

Aspergillus niger biomass was supplied as an industrial waste from fermentation processes (citric acid production) by a local company (Czech Republic). Two different samples have been tested: one being obtained by a surface-growth procedure while the second sample was obtained by submerged culture. After being collected the samples were abundantly rinsed in order to remove residual compounds present in the culture media. The samples were then dried or submitted to an alkaline treatment to prepare 5 different materials. The treated samples were finally submitted to a water-rinsing step and dried. The experimental conditions for their preparation are listed in Table 1.

Typically, the treatment was performed with 100 kg (wet weight, 20-25% dry mass percentage) mixed with 500 L of sodium hydroxide solution at the appropriate concentration. After chemical treatment, the biomass was centrifuged, rinsed with water up to neutral pH and then dried in a fluidized bed dryer. The weight loss changed with the treatment. In the case of biomass B and D, which were selected for most of the experiments, the weight loss was 55% and 80%, respectively. The samples were ground and sieved before being used to prepare 4 different size fractions: G1 < 125 μm < G2 < 250 μm < G3 < 355 μm < G4 < 510 μm.
Biosorption

Lead chloride (Riedel-de-Haen, Germany) and mercury chloride (Fluka, Switzerland) have been used for the preparation of the solutions. Other reagents (hydrochloric acid, sodium hydroxide) were supplied by Carlo Erba (Italy).

Table 1. Preparation characteristics of biosorbents

<table>
<thead>
<tr>
<th>Sample</th>
<th>Culture conditions</th>
<th>Alkaline treatment (concentration)</th>
<th>Temperature</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Surface</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>Surface</td>
<td>Yes (1 M)</td>
<td>Room</td>
<td>24</td>
</tr>
<tr>
<td>C</td>
<td>Submerged</td>
<td>No</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>Submerged</td>
<td>Yes (1 M)</td>
<td>Room</td>
<td>24</td>
</tr>
<tr>
<td>E</td>
<td>Submerged</td>
<td>Yes (10 M)</td>
<td>107°C</td>
<td>6</td>
</tr>
</tbody>
</table>

2.2 Sorption procedures

The study of the influence of pH on sorption performance was carried out by mixing sorbent with metal ion solution at selected pH (controlled with HCl and NaOH) for 3 days. The final pH was measured and the residual concentration in the solution (after filtration on membrane; pore size: 1.2 µm) was determined by ICP-AES (inductively coupled plasma atomic emission spectrometry). Sorbent dosage was fixed at 300 mg L⁻¹, while initial metal concentration was 50 mg Me L⁻¹. In the case of lead, the initial pH was not raised at pH 7 to avoid lead hydroxide precipitation.

For sorption isotherms, solutions were prepared at the appropriate pH (i.e. pH 3 for lead and pH 6 for mercury) controlled with HCl or NaOH, at different initial metal concentrations, C₀ (mg Me L⁻¹), ranging between 10 and 200 mg Pb L⁻¹ and between 10 and 100 mg Hg L⁻¹. The sorbent dosage was varied using a variable mass of sorbent m: 10 and/or 20 mg in a fixed volume of solution, V, 0.1 L). After 3 days of contact the solutions were filtered and the residual concentration (Cₑ𝑞, mg Me L⁻¹) was determined by ICP-AES. The mass balance equation was used for calculating the sorption capacity, g (mg Me g⁻¹): q = V/m (C₀ – Cₑ𝑞).

For sorption kinetics, 1 L of solution (at given concentrations: 20, 50 or 100 mg Me L⁻¹) at selected pH was mixed with selected sorbent (at given sorbent dosage: 100, 200, 300 or 500 mg L⁻¹) for 3 to 4 days. Samples were regularly withdrawn and filtered on membranes and residual metal concentration was determined by ICP-AES.

3. RESULTS AND DISCUSSION

3.1 Influence of pH on sorption properties and biomass selection

A preliminary set of experiments was performed with standard conditions. This preliminary study was used to evaluate the efficiency of the different sorbents, to determine the optimum pH and to check the influence of the biomass on the pH of the solution. Results are summarized on Figures 1 and 2 for lead and mercury sorption, respectively.

The contact of the biomass with metal ion solution significantly changed the pH of the solution, regardless of the type of metal. Significant differences were observed with the different biomass. With lead, biomass C is the only sorbent that decreases the pH of the solution at weakly acidic pH (i.e. pH 4-6). Biomass D hardly increased the pH (by less than 0.5 pH unit) over the whole pH range investigated. On the opposite hand, the other samples (biomass A, C, E) significantly increased the pH of the solution, especially at pH
4: in this case the pH can be increased up to 6.5-6.7 (biomass B). Above pH 5, pH variation was less significant: the biomass had a buffering effect around pH 6. Generally at acidic pH (below pH 4) the pH of the solution was not significantly changed (less than 0.3 pH unit): the alkaline charge of the sorbent and the influence of metal sorption (uptake of OH⁻ in relation with metal sorption, proton release) were not sufficient to change the pH.

For mercury sorption, the trends are generally similar, except for biomass C (for which the pH of the solutions was also decreased). The weak buffering effect of biomass was also observed at near-neutral pH. As expected the treatment of sodium hydroxide (regardless of NaOH concentration, temperature of the treatment and contact time) significantly impacted the pH of the solution. This may be explained by an insufficient washing/rinsing of the biomass after alkaline treatment or by the presence of citric acid released from the biomass (in the case of pH decrease). It is especially significant in the case of mercury sorption for which metal sorption was negligible, except in the case of biomass E (Figure 2 shows only sorption data for biomass E, for other biomass the sorption capacities were below 10 mg Me g⁻¹). So the pH variation was only attributable to acid-base properties of the biomass.

Figure 1. pH variation during lead sorption (left panel) and sorption efficiency (right panel) in function of initial pH (Co: 50 mg Pb L⁻¹; Sorbent dosage: 300 mg L⁻¹)

Figure 2. pH variation during mercury sorption (left panel) and sorption efficiency in function of pH with biomass E (Co: 50 mg Hg L⁻¹; Sorbent dosage: 300 mg L⁻¹)
Lead sorption efficiency was only significant with biomass B, and to a lesser extent with biomass A (other biomass exhibited sorption efficiency below 20% under selected experimental conditions). Since the biomass strongly increased the pH above pH_i = 4, and in order to avoid precipitation phenomena, the initial pH was selected at pH 3. The sorption efficiency was comparable at this pH to those observed at higher pH for biomass B. For these reasons biomass B was selected for further experiments using pH 3 as the optimum pH. It appears at this stage that biomass grown under surface fermentation conditions was more favorable to lead uptake, especially when submitted to alkaline treatment.

In the case of mercury uptake, sorption efficiency was negligible in most cases, except with biomass E (submerged fermentation procedure with strongly alkaline treatment). The strong alkaline treatment may drastically change the structure of the biomass. Such a strong treatment is used for the deacetylation of chitin to prepare chitosan, which is characterized by a higher affinity for metals than the raw material. We could expect this treatment being able to remove inert material from cell walls and to transform weakly active sorption sites to strongly reactive functional groups. Less drastic treatment conditions (lower concentration of NaOH, lower contact time, lower temperature) were not able to reach the same reactivity of the biomass for mercury. In this case mercury sorption was mainly efficient at pH equal or greater to pH 4: below pH 4, the sorption efficiency drastically dropped. It is important to observe that the sorption efficiency is significantly lower than the levels reached with lead. For further experiments on mercury sorption, biomass E was selected and experiments were performed at pH 6 (in order to minimize pH variation during metal sorption procedure).

3.2 Lead sorption isotherm

Figure 3 shows lead sorption isotherm at pH 3 using biomass B. The sorption isotherm is very favorable (almost rectangular). At very low concentration (below 3-4 mg Pb L⁻¹), sorption capacity was negligible. It may be explained by a possible complexation of lead ions by biological material released from the biomass; chelated lead is then less adsorbable on biosorbents. Above this limit concentration a sharp increase in the sorption capacity was observed up to a sorption capacity of about 550-600 mg Pb g⁻¹, followed by a plateau (when the residual concentration exceeded 10 mg Pb L⁻¹).

Figure 3. Lead sorption isotherm at pH 3 using sorbent B (symbols: experimental points, lines: Langmuir modeling (q = (qm b C_{eq})/(1+ b C_{eq})) after correction of the residual concentration to take into account the non-null residual concentration due to a possible complexation of lead by dissolved compounds)
Though under selected experimental conditions (pH and metal concentration) there is no precipitation of lead under hydroxide forms, the shape of the curve (almost rectangular) may be indicative of a mixed adsorption/precipitation phenomenon. This may proceed by reaction of lead with some chelating/precipitating agents released from the biomass (phosphate used in the fermentation media; by-products from citric acid production such as oxalate ions).

### 3.3 Influence of experimental parameters on lead sorption kinetics

Figure 4 shows the influence of lead concentration at two different sorbent dosages (pH 3) using biomass B. Sorption capacities have been plotted in function of the square root of time in order to compare sorption kinetic rates: this type of curve is frequently used to evaluate the contribution of intraparticle diffusion resistance [19]. In a first section of the curve corresponding to a latency of about 1-2 hours, the sorption remained negligible, after this period the sorption capacity linearly increased with the square root of time and the slope was of the same order of magnitude for initial concentrations of 50 and 100 mg Pb L\(^{-1}\), significantly higher than those obtained at a metal concentration of 20 mg Pb L\(^{-1}\). The latency decreased with increasing sorbent dosage.

#### Figure 4. Influence of lead concentration on sorption kinetics at pH 3 at 2 sorbent dosages (SD) (Sorbent B; G1 particle size)

Figure 5 (left) shows the influence of sorbent dosage on lead sorption kinetics (initial metal concentration: 100 mg Pb L\(^{-1}\)). The same trends were observed as in Figure 4. Increasing sorbent dosage slightly decreased the slope of the plot of sorption capacities in function of the square root of time: increasing the sorbent dosage increases the excess of sorption sites (compared to metal ions). As a consequence the residual concentration strongly decreased in the initial stage of the sorption process (after the latency period), then the concentration gradient decreased and the slope of the curve also diminished.

Figure 5 (right) shows the plot of sorption capacity as a function of square root of time for different particle sizes of biomass B at pH 3. The slopes of the curves were comparable (same order of magnitude) but the latency time increased with increasing the particle size of the sorbent. The shape of the kinetic curves is a bit unusual. It confirms that the mechanism involved in the removal of lead is not a simple sorption mechanism. Actually, at long contact time a trouble can be observed in the solution indicating the occurrence of precipitation phenomena (not due to hydroxide, but that could be explained by the presence of by-products as suggested above).
To verify this hypothesis the biomass was submitted to a new washing treatment with water and acidic water (pH 3). After 2 days of contact with the “washing” solution, the solution was filtered. The biomass was used for kinetic experiments under comparable experimental conditions to those selected above (pH, metal concentration, sorbent dosage; to be able to compare). A concentrated lead solution (1 g Pb L$^{-1}$) was added to the filtrate (to a final concentration of 50 mg Pb L$^{-1}$) and the solution was mixed for 3 days in order to verify the occurrence of precipitation phenomena. Actually, the precipitation was observed in the filtrate obtained from acidic solution treatment of biomass B (pH 3) after a few minutes of contact, when the biomass was treated with demineralized water, even after 2-3 days of contact the precipitation was very low (a few percent of initial metal concentration). Using the washed biomass resulted in slower sorption kinetics (Figure 6). At equilibrium the sorption efficiency was only 50% (instead of 85%), taking into account the fraction of metal precipitated in the experiments performed with the acidic filtrate (about 35-40%, not shown): the mass balance is almost maintained.

3.4 Mercury sorption isotherm

Mercury sorption isotherms were performed at pH 6 using biomass E in presence of NaCl (0.1 M) and without salt addition (Figure 7). The addition of NaCl strongly reduced
mercury sorption and changed the type of sorption model that fitted better experimental data.

Maximum sorption exceeded 250 mg Hg g\(^{-1}\), the Freundlich equation fitted better experimental data than the Langmuir equation: the curve can be characterized by an exponential trend and it was impossible to detect the plateau in the concentration range investigated in this study.

It is important to notice that similarly to experiments on lead, a sample of biomass was submitted to a complementary washing treatment with water at the pH of sorption experiments, the filtrate was collected and completed with concentrated mercury solution (to a final concentration consistent with those selected for sorption studies). In this case, no precipitation was observed.

![Graph showing mercury sorption isotherm at pH 6 (left: without NaCl; right: with NaCl addition) using sorbent E (symbols: experimental points, lines: Langmuir or Freundlich modeling)](image)

When NaCl was added to mercury solution, the sorption capacity was significantly decreased (about two times). In this case the Langmuir equation better fitted experimental data than the Freundlich model. The theoretical maximum sorption capacity was close to 165 mg Hg g\(^{-1}\), higher than the maximum sorption capacity observed in the range of mercury concentration investigated in the present study (about 100 mg Hg g\(^{-1}\)). The addition of sodium chloride influences the ionic strength of the solution. The influence of ionic strength is usually important in the case of ion-exchange mechanism. In the case of mercury sorption at pH close to neutrality metal is expected to be adsorbed by chelation on amine groups (less sensitive to ionic strength) or ion-exchange on carboxylic functions. The influence of sodium chloride may be interpreted by either the influence of ionic strength on the ion-exchange mechanism or by a change in the speciation of mercury (formation of chloro-complexes) that may affect its adsorbability.

### 3.5 Influence of experimental parameters on mercury sorption kinetics

The influence of experimental parameters (mercury concentration, sorbent dosage) on sorption kinetics has been investigated (Figure 8). Kinetic decay curves are plotted versus time together with the plots of sorption capacities versus the square root of time. As expected, at decreasing sorbent dosage, the sorption efficiency decreased but with low sorbent dosage (100 mg L\(^{-1}\), not shown), at long contact time a partial release of mercury is observed, especially at high metal concentration and low sorbent dosage (SD: 300 mg L\(^{-1}\); but also at SD: 100 mg L\(^{-1}\), not shown). This may explain some discrepancies between the results of sorption isotherms and equilibrium points of uptake kinetics. If the time of contact is not the same it can introduces some significant differences.
The initial section of the decay curves appeared to be independent of metal concentration. This first part corresponds to a step of the process that is controlled by external diffusion, while the second part of the curve is controlled by the resistance to intraparticle diffusion. Most significant differences were observed in the second stage of the process, indicating that sorbent dosage and metal concentration parameters mainly influenced mass transfer resistance to intraparticle diffusion. Even with a sorbent dosage as high as 500 mg L\(^{-1}\), the sorption efficiency did not exceed 75-80%.

**Figure 8.** Influence of mercury concentration on sorption kinetics at pH 6 at 2 sorbent dosages (SD) (Sorbent E; G1 particle size)

Particle size hardly influenced sorption kinetics (Figure 9). Regardless of the plot system (C(t)/Co versus time or q versus t\(^{0.5}\)), the curves were very close. Decreasing the size of sorbent particles allowed increasing the slope of the kinetic curves but the differences were not very marked.

At equilibrium the sorption capacities were very close: sorption did not occur only on the surface of sorbent particles (in this case the sorption capacity would be more sensitive to the external surface area of the sorbent) but also in the whole volume of the solution. But the sorption kinetics was not strictly controlled by the mass-transfer resistance for large particle.
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Figure 9. Influence of particle size on mercury sorption kinetics at pH 6 (SD: 300 mg L\(^{-1}\); \(C_o\): 50 mg Pb L\(^{-1}\))

4. CONCLUSIONS

Waste materials (fungal biomass) from industrial fermentation processes have been successfully used for the sorption of lead and mercury after being chemically treated (alkaline treatment). Best sorbents for mercury and lead sorption were obtained after being treated with NaOH solutions with different concentration, contact time and reaction temperature. While best results were obtained at pH 3 for lead, in the case of mercury sorption may be performed at pH close to neutrality. High sorption capacities (between 1 and 2 mmol Me g\(^{-1}\)) were observed in the case of lead uptake but it appeared that a part or metal removal was due to a precipitation phenomenon probably due to a release of some sub-products or by-products of the fermentation process (phosphate, organic or inorganic; or oxalate). This illustrates the necessity to use a carefully washed material (especially industrial waste products) in order to avoid artifacts; and the care to take in the interpretation of experimental results. Sorption capacities in the case of mercury were lower, especially in the presence of NaCl that significantly reduced sorption capacities. Sorption kinetics were performed at different sorbent dosages: though the initial sorption is quite fast compared to other systems, 24 hours of contact were necessary to reach the equilibrium (due to low sorbent dosage, below 500 mg L\(^{-1}\)). Sorbent particle size has a negligible effect on equilibrium concentrations and kinetics.

REFERENCES

Lanthanum and neodymium biosorption by different cellular systems

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Abstract

Biosorption isotherms for lanthanum and neodymium were determined utilizing microalgae Monoraphidium sp., filamentous fungi Penicillium sp., algae Sargassum sp. and the yeast Saccharomyces sp. as biosorbents at pH from 2 to 5. The maximum biosorption coefficient for each isotherm was determined based on Langmuir Model fitting. A growth linear dependence with pH was observed for lanthanum biosorption (best condition achieved at pH 5), while neodymium biosorption presented little variation in the range studied. The highest biosorption coefficient was obtained using Monoraphidium sp. (1320 mg g⁻¹ for lanthanum and 1600 mg g⁻¹ for neodymium), while the Saccharomyces sp. presented values around 280 and 460 mg g⁻¹ respectively. Sargassum sp. and Penicillium sp. presented very similar biosorption for both elements (60-80 mg g⁻¹). Desorption experiments using HCl 0.24 Mol L⁻¹ showed a total recovery for the metals initially adsorbed.

1. INTRODUCTION

Due to their unique physical and chemical properties, rare-earth (RE) metals are employed to manufacture advanced materials for high technology devices. The purity grade of these elements determines its level of technological applications; so far, numerous attempts are being made to develop efficient separation and concentration processes for rare-earth (RE) metals [1]. From the 15 RE elements, lanthanum and neodymium are the most common found in monazite sands at Brazilian shores, have been used in laser sources, hydrogen absorbents, magnetic substances and fluorescent materials [2].

Solvent extraction is well known as an effective technique used for separation and concentration of these RE metals at industrial scale. However, their very similar physical and chemical properties require a large number of stages in a series of mixer-settlers to obtain high purity products, increasing costs [3].

Biosorption of metals has been extensively studied as a low cost process for removal of ionic metals from industrial effluents and wastewater. Recently, the increasing development of biosorption process has attracted attention as an alternative for...
Biosorption

concentration of RE metals [4-8]. It is based on the immobilization of these metals by chemical and physical properties of cell components, such as carboxyl groups, sulphidril groups, amino groups, sulphate and phosphate groups.

Due to the different chemical and physical constitution of each species of microorganism, the interaction with metallic ions occurs in varied forms, however it appears to be predominantly by ion-exchange mechanism if no-metabolic uptake is present [9]. Several biosorbents have been studied such as algae, microalgae, filamentous fungi, yeast and bacteria. Many metals have been investigated to be removed by biosorption processes, such as uranium, thorium, chromium, zinc, gold and many others [10-12].

Previous works have demonstrated the potential of different biomass in removing erbium and ytterbium from acid solutions [4]. The initial results obtained for lanthanum biosorption studies using Sargassum sp. [6] and neodymium on different kinds of biomass [5] also pointed out for the future use of this process as a low cost alternative method for the concentration of these metals.

2. MATERIALS AND METHODS

2.1 Organisms and culture conditions

The fungi Penicillium sp. was kindly supplied by Dr. Rubens Monti from UNESP – Araraquara – SP, Brazil. Oat flour/agar plates were inoculated with fungi spores and incubated at room temperature (32°C) for 72 h. Fungal mycelia were collected by scratching the culture surface and this biomass was washed with 100 mL boiling distilled water to remove agar residues.

Microalgae Monoraphidium sp., was kindly donated by Dr. Armando Vieira from UFSCar – São Carlos - SP, Brazil. Flask containing 500 mL of WC medium [7] was inoculated with 20 mL pre-inoculum culture of algae and incubated at 25°C for 60 days.

The Saccharomyces sp. strain was obtained a single colony from commercial Baker’s yeast. Malt extract broth (10 mL) was inoculated with this culture and incubated at 32°C for 16 h. For yeast biomass production, this previous culture was inoculated in 50 mL of malt extract broth and incubated on rotatory shaker at 150 rpm and 32°C for 16 h.

The biomass of the microorganisms used in this study was finally harvested by filtration on membrane (0.45 µm pore size) and washed twice with distilled water. The filtered biomass was then suspended in 20 mL of distilled water for further experiments.

The brown seaweed Sargassum sp. was collected from the coast of São Sebastião – SP, Brazil. The biomass was washed and sun dried for stock at room temperature. For biosorption experiments the algae was chopped in pieces with size around 0.3-0.5 cm, washed twice in distilled water and twice in hydrochloric acid 0.1 Mol L⁻¹ and after that it was washed in distilled water up to close pH 4.0.

2.2 Lanthanum and neodymium stock solutions

Lanthanum and neodymium stock solution were prepared by dissolution of the correspondent oxides (Aldrich, 99.9%) using concentrated HCl to a final concentration of 5.23 g L⁻¹ and 5.15 g L⁻¹, respectively, at pH 1.0. The metal content in solution was determined by inductively coupled plasma atomic spectroscopy (ICP-AS Thermo Jarel Ash, Trace Scan).
2.3 Biosorption assays

Biosorption experiments for isotherm determination were carried out by using 0.1 g of selected biomass in 40 mL of lanthanum or neodymium solution in concentrations ranging from 0.01 to 2.5 g L\(^{-1}\). The pulp was incubated at 30°C in a rotatory shaker and pH was adjusted every 1 h to keep it constant at 5.0 using 0.1 Mol L\(^{-1}\) NaOH or HCl. The equilibrium was reached after 24 h and samples (5 mL) were taken out and filtered on membrane (pore size 0.45 µm) for metal determination in the filtrate. Each experiment was performed in triplicate.

Metal biosorption coefficient (q) was calculated [8] according to equation (1):

\[ q = \frac{(C_i - C_f)V}{M} \]  

where \(C_i\) is the initial metal concentration (mg L\(^{-1}\)), \(C_f\) is the final metal concentration after contact with biomass (mg L\(^{-1}\)), \(V\) is the solution volume (mL) and \(M\) is the sorbent weight in dry form (g).

2.4 Desorption assays

Desorption experiments were carried out using 0.1 g of biomass previously loaded with metal (lanthanum or neodymium) at pH 5.0 as described above. The loaded biomass were drained to remove all solution remained and then mixed in 40 mL of chloridric acid 0.24 Mol L\(^{-1}\) at 30°C in an orbitary shaker for 16 hours to reach the equilibrium. Samples from supernatant were taken out for metal and pH determination. Desorption coefficient were defined as:

\[ q_d = \frac{C_f V}{M} \]  

where: \(q_d\) is the desorption coefficient (mg g\(^{-1}\)), \(V\) is the acid volume used (L) and \(M\) is the dry mass of biosorbent (g).

3. RESULTS AND DISCUSSION

3.1 Effect of pH

Considering biosorption systems similar to an ion-exchange system, where the components of the cell envelope proceed like binding sites for metal exchange, it is naturally expected that the pH has an important role in the interaction between the metal and the binding sites. The pH value affects the dissociation of chemical species in the biomass responsible for capturing the metal in solution. Indeed it can affect the speciation of the metal, changing its charge, producing complexes or associations that can favor or difficult the biosorption, once this interaction is basically electrostatic and depends on charges in both metal and binding site to be effective. Usually the increase in the pH value tends to increase the biosorption for a metallic cation. This effect can be observed for several metals and different kind of biomass.

The Figure 1 shows the pH effects on the biosorption of lanthanum and neodymium by the biomass utilized.

For lanthanum biosorption (fig. 1) it can be noted an evident linear increase in the \(q_{\text{max}}\) for *Monoraphidium* sp. and *Saccharomyces* sp. as the pH increases. For *Sargassum* sp. this effect was also detected (linear increase in the \(q_{\text{max}}\) from 10 to 80 mg g\(^{-1}\)) but it is not so evident in the graph, since the y scale was expanded to show *Monoraphidium* sp. curve. The biosorption of lanthanum by *Penicillium* sp. was not influenced by pH, since the \(q_{\text{max}}\) remains around 50 mg g\(^{-1}\) in the pH values tested. As the pH increases the
dissociation of binding sites present in the biomass favor the ion-exchange. Similar results were observed in previous work for biosorption of erbium and ytterbium (other RE metal) in the pH range from 2 to 5 for the same kind of biomass utilized in this study [4]. The increase in pH value above 5 does not imply on increase in biosorption capacity, once this condition leads to the formation of insoluble metal hydroxide.

Figure 1. Effect of pH on biosorption using Monoraphidium sp. (■), Saccharomyces sp. (□), Sargassum sp. (●) and Penicillium sp. (○)

The effect of pH value in neodymium biosorption by Saccharomyces sp., Sargassum sp. and Penicillium sp. was very similar to lanthanum biosorption (fig. 1). However, Monoraphidium sp. presents a distinct pattern, since its biosorption coefficients show, on the contrary, a little decrease as the pH increase from 2.0 to 5.0. The reasons for this behavior remain not clear until now, and new studies are being conducted to elucidate this interesting characteristic.

Despite the high q_max at pH close to 2.0 for neodymium biosorption by Monoraphidium sp., when the pH is lowered below 1 all the metal can be desorbed as it can be seen in the results of desorption presented later.

3.2 Isotherms of biosorption

The isotherms of biosorption are curves that describe the equilibrium between the metal in solution and the biosorbent at a constant temperature. These curves are extensively used for comparison of biosorption performance of different biosorbents. Figure 2 presents the biosorption isotherms for lanthanum and neodymium with the different biomass studied. For all systems the curves presented a hyperbolic shape, and after fitting using the Langmuir model, the q_max was determined, which indicates the maximum capacity of recovery of the metal for a biosorbent in these conditions.

The results obtained from the Langmuir fitting showed a wide range of values reflecting the differences in chemical composition of each biomass studied. Monoraphidium sp. was the best biomass for biosorption of both metals (q_max 1300-1600 mg g⁻¹, values calculated from curve fitting), followed by the yeast Saccharomyces sp. with maximum biosorption coefficient of 280 and 460 mg g⁻¹ for lanthanum and neodymium respectively.

Sargassum sp. and Penicillium sp. presented very similar biosorption performances (q_max around 60-80 mg g⁻¹) for both metals and lower when compared to Monoraphidium sp. and Saccharomyces sp. values. These results confirm preliminary studies of
biosorption with lanthanum and neodymium [5, 6] and pointed out that these two metals (light RE) have similar biosorption behavior than erbium and ytterbium (heavy RE) [4].

![Figure 2. Biosorption isotherms using Monoraphidium sp. (■), Saccharomyces sp. (□), Sargassum sp. (●) and Penicillium sp. (Ο) at 30°C. Continuous line represents a Langmuir curve fitting (Biosorbent concentration 0.1 g L⁻¹ and pH 5.0)](image)

3.2 Desorption of lanthanum and neodymium

Desorption consists in the shift of ion-exchange equilibrium in favor to release the metal previously loaded in the biomass. This shift can be achieved using several chemicals, but once binding groups in the biomass are known for their high selectivity for protons, small volumes of diluted mineral acids usually give complete desorption.

As the desorption equilibrium is the reverse process of biosorption, it can also be determined a desorption isotherm (at constant temperature), that can be fitted according to Langmuir model, which allows to determine the maximum coefficient of desorption ($q_{d_{max}}$).

This coefficient is very similar to $q_{max}$, but while the first represents the amount of metal bound in the biomass, $q_{d_{max}}$ indicates the amount of metal released to solution. If there is complete desorption from the biomass, $q_{max}$ should be equal to $q_{d_{max}}$.

In Table 1 are presented the maximum coefficients of biosorption ($q_{max}$) and the maximum coefficients of desorption ($q_{d_{max}}$) for lanthanum and neodymium utilizing HCl 0.24 Mol L⁻¹ for all biomass utilized.

It is possible to observe that the coefficients are very close, confirming that both metals can be removed from the biomass efficiently. The differences observed in the values showed in table 1 are due to experimental deviances, once each value in $q_{max}$ and $q_{d_{max}}$ are calculated from independent curve fitting, which explains some desorption values higher than adsorption. Despite the favorable biosorption behavior of neodymium in pH close to 2, it can be observed in table 1 that the ion exchange equilibrium can be shifted in lower pH in order to fully release the metal adsorbed. New studies are been conducted in order to establish a satisfactory explanation to the particular behavior of this RE metal, as stated above.
Table 1. Maximum coefficients of biosorption ($q_{max}$) and desorption ($qd_{max}$) for lanthanum and neodymium

<table>
<thead>
<tr>
<th>Biosorbents</th>
<th>Lanthanum</th>
<th>Neodymium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$q_{max}$ (mg g$^{-1}$)</td>
<td>$qd_{max}$ (mg g$^{-1}$)</td>
</tr>
<tr>
<td>$Monoraphidium$ sp.</td>
<td>1380.6</td>
<td>1368.4</td>
</tr>
<tr>
<td>$Saccharomyces$ sp.</td>
<td>280.2</td>
<td>273.5</td>
</tr>
<tr>
<td>$Sargassum$ sp.</td>
<td>75.3</td>
<td>80.8</td>
</tr>
<tr>
<td>$Penicillium$ sp.</td>
<td>52.9</td>
<td>55.1</td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

Recent papers have pointed out the potential of RE biosorption as an alternative process for concentration of these metals. However, there is still a lack of information about biological, chemical, physics and engineering of RE biosorption process which has to be investigated to achieve a feasible industrial alternative.

The results presented pointed out the similarities of biosorption behavior of two light RE metal (lanthanum and neodymium) with two heavy RE (erbium and ytterbium) studied in previous work. As the heavy element studied, lanthanum and neodymium are capable to be removed from solution by all the biosorbent utilized, with highest performance for microalgae $Monoraphidium$ sp.

However, the effect of pH presented some differences. While lanthanum biosorption showed in general an increase in the maximum biosorption coefficient with the increase of pH, as observed for erbium and ytterbium, it has practically no effect in the biosorption of neodymium for $Monoraphidium$ sp. More studies have to be conducted on the speciation and interaction of neodymium with this cellular system to elucidate this point.

The desorption of lanthanum and neodymium from the biomass was completely performed using hydrochloric acid and all the metal previously adsorbed was removed. This easily reversibility of the process using diluted mineral acid can be an interesting point for future industrial applications.

The data obtained in this work and the advances in biosorption studies indicate a promising alternative for these metals concentration and separation process.

ACKNOWLEDGEMENTS

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Modeling of chromium biosorption by seaweed *Sargassum* sp. biomass in fixed-bed column in series

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Abstract

The biosorption of chromium by the marine alga *Sargassum* sp. were investigated in a system with two fixed-bed columns in a series configuration (temperature = 30°C; pH = 3.5). A model that describes the dynamics of chromium ion sorption in the columns was obtained from the mass balance in the fluid phase and the biosorbent. This model considers both the axial dispersion effect in the column and the mass transfer resistance in the biosorbent. The model performance was evaluated from experimental data (breakthrough curves) obtained at pH 3.5, flow rate of 6 mL/min, feed concentration of 0.97 mmol/litre and temperature of 30°C. The results showed that the model is appropriate to represent the chromium biosorption by seaweed *Sargassum* sp. biomass in fixed-bed columns in series.

*Keywords: chromium biosorption, Sargassum, fixed-bed columns, modeling*

1. INTRODUCTION

The increase in metal consumption in industrial scale represents an important environmental issue. Chromium is present in different types of industrial effluents, being responsible for environmental pollution. Traditionally, the chromium removal is made by chemical precipitation, a conventional method for removing metals. However, this method is not completely feasible to reduce the chromium concentration to levels as low as required by environmental legislation. Biosorption processes have been proposed as an alternative method for recovering and removing metals from industrial effluents with metal concentration in range from 1-100 mg/L [1].

Most separation and purification processes that employ sorption technology use continuous-flow columns. This operating mode ensures the highest possible concentration difference driving force and avoids a subsequent solid-liquid separation process. Starting at the inlet, the saturated solid sorbent zone gradually extends throughout the column; the sorbate eventually breaking through the column. The record of the breakthrough gives
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usually a typical S-shaped breakthrough curve, whose shape and slope is the result of the equilibrium sorption isotherm relationships, the mass transfer to and throughout the sorbent in the column, and operation macroscopic fluid-flow parameters, such as axial mixing, affecting the deviation from the ideal plug-flow.

The aim of the present work was to model chromium biosorption in a system with two fixed-bed columns in a series configuration by *Sargassum* sp., a brown seaweed present in abundance on the Brazilian coast.

2. MATERIAL AND METHODS

The biomass used was the brown seaweed *Sargassum* sp. It was washed in water, rinsed with distilled water and dried in an oven at 60°C during 24 hours. The columns were packed with biomass in natural size (with leaves and thallus). Dry weight of biomass was obtained after drying at 105°C for 24 hours.

Chromium solution was prepared by dissolving CrK(SO₄)₂·12H₂O (analytical grade) in deionized water.

Continuous-flow sorption experiments were conducted in two steel columns with controlled temperature. The columns used had a height of 50 cm and a diameter of 2.8 cm. The bed length used in the experiments was 30.6 cm.

A peristaltic pump fed the chromium solution (0.97 mmol/litre) to bottom of the first column with a flow rate of 6 mL/min, and the effluent of the first column fed the bottom of the second column. The pH of the solution in the feeding tank was maintained constant at 3.5. The temperature of stream feeding solution and of the column was controlled at 30°C through a thermostatic bath.

Liquid samples of the concentration of chromium in the exit of each column were collected at pre-defined time intervals. The total concentration of chromium in liquid samples was determined by Atomic Absorption Spectroscopy (Varian SpectrAA-10 plus).

When the system reaches equilibrium, the metal concentration in the fluid phase is constant along the column and equal to the feed concentration (*C = C*= Cᵢ*). The biosorption capacity of the chromium was calculated from the experimental breakthrough curves, using the following equation:

\[
q^* = \frac{C_i^\infty Q}{1000 m_z} \int \left(1 - \frac{C}{C^\infty} \right) dt
\]

(1)

The integral represented by Eq. (1) was solved analytically by means of the polynomial approach of the term \( \left(1 - \frac{C}{C^\infty} \right) \).

The column void fraction, \( \varepsilon \), was determined by the measure of the void volume (volume of distilled water required to fill the bed), as methodology proposed by Cossich [2].

At the end of each experiment, the solution present inside the columns was removed. The exhaustion of the solution was accomplished from the bottom of the column using a minimum period of 24 hours. Afterwards, a peristaltic pump (Cole Parmer) fed the columns from a reservoir that contained a defined volume of distilled water. The necessary volume of water to fill the bed was determined initially by the difference between the volume contained in the reservoir and the volume remaining after filling the bed.

The column void fraction was calculated using the following equation:
\[ \varepsilon = \frac{V_v}{V_b} \]  

where, \( V_v \) is the bed void volume and \( V_b \) is the bed volume.

3. MODEL DESCRIPTION

In recent years, many mathematical models have been tested to represent the biosorption of different metals in fixed-bed columns [3-7].

The mathematical model for biosorption of a metal ion in a fixed bed column was obtained by means of the mass balance equations applied to an element of volume of the column in the liquid phase and in the solid phase (biosorbent).

In the model development the following hypothesis were considered:

- Isobaric and isothermic process;
- Constant physical properties;
- Superficial adsorption;
- Negligible radial dispersion.

The mass balance equation for the fluid phase is:

\[ \frac{\partial C}{\partial \tau} + \rho_b \frac{1}{\varepsilon_b} \frac{\partial q}{\partial \tau} = -u \frac{\partial C}{\partial \xi} + \frac{1}{P_e_b} \frac{\partial^2 C}{\partial \xi^2} \]  

with the following initial and boundaries conditions:

\[ C(\xi,0) = C_0 \]  

\[ \frac{\partial C}{\partial \xi} = P_e \left( C(\tau,0) - C^f \right) \quad \text{in } \xi = 0 \]  

\[ \frac{\partial C}{\partial \xi} = 0 \quad \text{in } \xi = 1 \]  

To obtain the modeling of the copper adsorption rate in the biosorbent it is assumed that the driving force for the mass transfer is linear with the concentration for the solid phase (biosorbent) and the adsorption rate is represented by the following equation:

\[ \frac{\partial q}{\partial \tau} = -Sh_a \left( q - q^* \right) \]  

with the following initial condition:

\[ q(\xi,0) = q_0 \]  

The equilibrium concentration of chromium adsorption in the algae \((q^*)\) were calculated by Langmuir isotherm model, described by following equation:

\[ q^* = \frac{q_m b C^*}{1 + b C^*} \]  

The partial differential equation system of the model was solved by the Galerkin Method on finite elements [8-9]. The concentration of the chromium in the bulk fluid phase and the biosorbent were approximated in each element using quadratic polynomials Lagrange.
The resulting equation system of application of the method was solved using the DASSL subroutine [10], whose source code is in the FORTRAN computer language. The DASSL code was used to solve a system of algebraic/differential equations.

The parameters of the model, that is, solid mass transfer \((K_s)\) and axial dispersion \((D_L)\) coefficients, had their estimated values obtained by minimizing an objective function using the Nelder and Mead method [11]. The minimized objective function was:

\[
F = \sum_{i=1}^{np} \left( C_{\text{EXP}}^{\text{out}}(K_s, D_L) - C_{\text{MOD}}^{\text{out}} \right)^2
\]

where:

- \(C_{\text{EXP}}^{\text{out}}\) - Experimental concentration of the chromium in the outlet of the column;
- \(C_{\text{MOD}}^{\text{out}}\) - Concentration of the chromium determined by the solution of the model in the outlet of the column;
- \(np\) - Number of experimental data points;

The proposed model was able to represent the chromium biosorption by \textit{Sargassum sp.} biomass in a fixed-bed column (Cossich, 2002; Cossich \textit{et al.} 2002). Now, it was used to describe the sorption dynamic of chromium in two fixed-bed columns in series.

4. RESULTS AND DISCUSSION

The results of the experimental and model simulated breakthrough curves, obtained are shown in Figure 1.

The equilibrium data of chromium(III) biosorption system by seaweed \textit{Sargassum sp.} (at pH 3.5, 30°C) and the Langmuir isotherm model curve obtained by fitting the experimental, obtained by Cossich [2], were used to perform the simulations. The Langmuir parameters used were \(q_m = 1.07\) mmol/g and \(b = 7.9\) L/mol.

The model parameters, mass transfer and axial dispersion coefficients, used to simulate the experimental breakthrough curves were \(K_s = 1.502 \times 10^{-3}\) min\(^{-1}\) and \(D_L = 1.20 \times 10^{-4}\) cm\(^2\)/min. This values were estimated by Cossich [2] in sorption experiments with only one column (with same dimensions and at the same experimental conditions), at diferents flow rates (2, 4, 6 e 8 mL/min).

As illustrated in Figure 1, the model proposed described adequately the chromium adsorption dynamics by seaweed \textit{Sargassum sp.} in two columns in series.

The first column presented a chromium(III) uptake capacity of \(q^* = 0.75\) mmol/g, while the second column presented a chromium(III) uptake capacity of \(q^* = 1.28\) mmol/g. As the biosorben mass in the column were equal and they were in equilibrium, the chromium(III) uptake capacity should be the same for both columns. This fact could be caused by changes in the solution pH. The chromium solution leaves the first column and comes in the second column with a pH higher than 3.5. In this kind of system, the capacity of chromium biosorption by biomass increases with pH [2].
5. CONCLUSIONS

In this study, the chromium removal by using seaweed biomass as biosorbent in two fixed-bed columns in a series configuration was investigated. The experimental data showed that the capacity of chromium removal was different for each column. In the first column the capacity of chromium removal was about 0.75 mmol/g, while for the second one it was about 1.28 mmol/g. The mathematical model proposed, that assumes the overall sorption rate is controlled by the mass transfer resistance in the biosorbent, represented the experimental breakthroughs.

NOMENCLATURE

- $b$: Langmuir isotherm constant (litre/mmol);
- $C$: Concentration of the copper(II) in the bulk fluid phase (mmol/litre);
- $C^*$: Equilibrium concentration of the copper(II) in the bulk fluid phase (mmol/litre);
- $C^0$: Initial concentration of the copper(II) in the bulk fluid phase (mmol/litre);
- $C^F$: Concentration of the copper(II) in the inlet in the column (mmol/litre);
- $D_L$: Axial dispersion coefficient (cm$^2$/min);
- $K_S$: Overall mass transfer coefficient in the biosorbent (min$^{-1}$);
- $L$: Length of the bed (cm);
- $m_s$: Dry weight of biomass (g);
- $q$: Concentration of copper(II) adsorption in the algae (mmol/g);
- $q^*$: Equilibrium concentration of copper(II) adsorption in the algae (mmol/g);
- $q_m$: Langmuir isotherm parameter (mmol/g);
- $\dot{Q}$: Volumetric flow rate (cm$^3$/min);
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\( t \) - Time (min);
\( u \) - Velocity (cm/min);
\( z \) - Axial coordinate in the column (cm);
\( V_v \) - Void volume (litre);
\( V_b \) - Fixed bed volume (litre).

Dimensionless group

\( Pe_b \) - Peclet number for the bed \((Lu/D_L)\);
\( Sh_m \) - Modified Sherwood Number \((K_sL/u)\);
\( \varepsilon \) - Column void fraction;
\( \rho_b \) - Fixed bed density (g/litre);
\( \tau \) - Dimensionless time coordinate \((tu/L)\);
\( \xi \) - Dimensionless axial coordinate \((z/L)\);

REFERENCES

Modelling and optimisation of copper ion uptake by *Acidithiobacillus ferrooxidans*

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**Abstract**

The copper ion uptake capacities of a living *Thiobacillus ferrooxidans* biomass were modelled and optimised thanks to the Design Of Experiment (DOE) methodology. A composite centred in a cube design was used to find out an empirical quadratic model with respect of pH, agitation speed, temperature, protein concentration and bacterial physiological state, while metal concentration was fixed at 1 g L⁻¹. An optimal copper ion uptake capacity of 74.9 mg of copper / 100 mg bacterial dry weight was found with a middle exponential growth phase biomass, for a protein concentration of 45 mg L⁻¹, a pH of 5, a temperature of 30°C and an agitation speed of 50 rpm. The influence of each parameter on fixation was finally discussed.

**Keywords:** Acidithiobacillus ferrooxidans, DOE, modelling, copper fixation

1. **INTRODUCTION**

In the wastewater treatment field, the fixation of heavy metals on biomass has been more and more investigated [1]. Particularly, *Acidithiobacillus ferrooxidans* had shown great uptake capacities of diverse metal ions: Cu²⁺ [2], Cd²⁺ [3], Cr⁶⁺ [4], UO₂²⁻ [5] or Ag⁺ [6]. Previous to consider a removal process using a biomass, its feasibility can be evaluated by studying the uptake capacities of the microorganisms. These capacities can be determined by modelling the metal ion uptake as a function of various parameters. Freundlich and Langmuir adsorption isotherms, traditionally used to describe solute-solid interaction as physi-sorption or chemi-sorption on inert surfaces, were often used to describe metal removal by died or dried biomass (microorganisms or exopolymers) [7-8]. However, for metal ion removal by a living biomass, other active processes could occur beside the simple surface adsorption (biosorption) and they should be taken into account to describe the metal removal. Uptake of metal by a micro-organism would then be a complex process including biosorption on the cell surface, bioaccumulation in the cytoplasm through non specific cation transport system or bioprecipitation of metal on the cell surface [1]. For such a complex process of fixation, Freundlich and Langmuir adsorption isotherms were no more adapted: these models were inadequate for some biosorption experiments on *Chorella fusca* [9] or on *Echerischia coli* [10]. Moreover, copper ion uptake by *A. ferrooxidans* could not be described satisfactory by such
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isotherms [2]. Consequently another type of model should be used to characterise metal ion uptake by a living biomass.

The classical well-known OVAT method (One Variable At Time) allows to study the influence of one parameter at a time, the value of the other being fixed. This method is rather long (243 trials for 5 parameters studied at 3 levels) and does not allow to take into account the interactions between the different parameters. On the other hand, Design Of Experiments (DOE) allows to simultaneously study from a minimal number of runs (32 runs for 5 parameters and 3 levels, for example) the influences of all the parameters by the way of a postulated polynomial response model [10-12]. However when a model has been determined from the experiments of a DOE, a careful analysis has to be performed for checking its adequacy and validity before using it for prediction or optimisation. This analysis, based on classical statistic tests such as t- and F- tests, is essential and too often neglected. Therefore, we describe each step of analysis through the modelling and the optimisation of copper ion uptake by a living biomass of *A. ferrooxidans*. The influence of the 5 chosen operating parameters is finally discussed.

2. MATERIALS AND METHODS

2.1 Microorganisms and medium

*Acidithiobacillus ferrooxidans* DSM583 was grown in 9K medium [13], consisted in FeSO₄·7H₂O (33 g L⁻¹), MgSO₄·7H₂O (0.4 g L⁻¹), K₂HPO₄ (0.4 g L⁻¹), and (NH₄)₂SO₄ (0.4 g L⁻¹). pH was adjusted at 1.4. The bacterial cultures were performed in a 50 L-reactor with 9K medium at 30°C and under air flow agitation. Bacterial growth was controlled from measuring the amount of substrate, Fe²⁺ ions, remaining in solution. The o-phenanthroline colorimetric method [14] associated with Fe³⁺ reduction with hydroxylamine allowed to evaluate the ratio Fe³⁺/(Fe²⁺+Fe³⁺), corresponding to the fraction of substrate still available. Once this ratio reached the desired value (65, 80 or 95%), bacterial biomass was separated by cross-flow filtration and concentrated by centrifugation at 5000 rpm. The protein concentration was then determined by the Lowry colorimetric method [15]. The bacterial dry weight concentration was 1.67 times the protein concentration [16]. Biomass was stored at 5°C for one night before utilisation.

2.2 Copper ion uptake experiments

They were carried out in 100 ml-sterile polyethylene flask reactors filled with 40 ml of metal solution (1 g L⁻¹ Cu²⁺, CuSO₄·5H₂O Normapur, Prolabo) under rotary stirring. The solutions were previously heated in the incubator at 30°C or cooled with a water-glycol mixture circulation at 5 or 17.5°C, the selected temperatures. The required amount of biomass was centrifuged at 10000 rpm and the bacterial pellet was added to the metal solution at time zero. Then pH was adjusted by adding either concentrated sodium hydroxide or sulphuric acid depending on the selected pH. After a 90-minute contact, the metal solution was filtered on 2 µm Millipore filters to separate the bacterial biomass. The metal concentration of the solution free of bacteria was determined by atomic absorption spectrophotometry. Control experiments were performed without bacteria to check that copper ion concentration in solution was constant throughout the experimental procedure.

2.2 Experimental Design Method

*Acting parameters and response choice.* First, some parameters were fixed at suitable values. The contact time was set at 90 minutes as a result of preliminary trials about the kinetics of metal fixation on the bacterium [2-4]. Actually, the equilibrium contact time
Biosorption was found to be about 15 minutes for these previous experiments, results in good agreement with literature [8,17]. The metal concentration was fixed at 1 g L⁻¹ in order to obtain the maximum amount of metal the bacterium could uptake. The acting varying parameters have been determined from previous experiments [2-4] and their ranges were chosen as large as physically possible:

* pH: pH, a well-known parameter for metal fixation on micro-organisms, has been previously shown to have a large effect on metal ion sorption. A large range from 1 to 5 was chosen. The lower limit was fixed at 1 to insure a pH avoiding any damage for the bacterium and the upper limit was fixed just under the pH corresponding to the solubility limit of copper ions.

* ω: only one work [18] described the influence of stirring, but it seemed important to study this parameter because of its action on external diffusion which is one of the fixation steps. Agitation was varied by using a rotary shaker between the minimal efficient speed, 50 rpm and the upper limit of the device, 150 rpm.

* T: temperature influence on fixation has often been studied, showing that the optimal temperature for adsorption is generally the growing temperature for most of the (living) microorganisms [17] but can also be 5°C for some of them [7]. The temperature ranged between 5 and 30°C. The lowest value corresponded to the limit allowing a reliable and accurate enough temperature control while the upper limit was set at 30°C, the growth temperature above which the bacterium could be damaged.

* φ: the physiological state of the bacterium also acted on fixation as pointed out by some previous results on the influence of the age of culture on adsorption [3-4, 19]. The physiological state of the bacterium was correlated to the ferrous oxidation activity expressed by the ratio Fe³⁺/(Fe²⁺+Fe³⁺) which varied from 65 to 95%. The lower limit was chosen in the middle of the exponential phase, which corresponded to 0.65, whereas the upper limit was fixed at the end of growth, in the stationary phase that corresponds to 0.95.

* [prot]: the protein concentration, one of the most studied parameter, has been shown to have a great influence on adsorption [8, 20]. The protein concentration range was chosen as 45-320 mg L⁻¹ in connection with the ability of protein concentration determination, but also to ensure a significant difference in metal concentration for the analysis.

The response under study, Y, was the quantity of fixed metal (in mg) by a 100 mg dry weight of bacteria (unity: mg / 100 mg dry weight).

**Design choice.** The five chosen parameters were supposed to act on the response through a non-linear way and with possible interactions. Consequently a quadratic model with interactions was postulated:

\[
Y = b_0 + \sum_i b_i X_i + \sum_{i,j} b_{ij} X_i X_j + \sum_i b_{ii} X_i^2
\]

(1)

where \(X_i\) is the varying parameter and \(b_i, b_{ij}\) the coefficients of the model.

Among all the possible optimal designs, which are available for analysing a response with a quadratic model, one of the most classical and efficient DOEs is the so-called Central Composite Design [10]. Such a design can be inscribed in a cube or in a sphere. Although the central composite design in a sphere presents the advantage of respecting rotability, a central composite design in a cube can be a better choice because the experimental region of interest is a cube what avoids any problem of extrapolation out of a sphere. Therefore we selected here a central composite design in a cube for studying the
uptake of copper ions on *A. ferrooxidans*. The corresponding 32 trials are given in Table 1. The ECHIP® software has been used to build the DOE and analyse the experimental results.

Table 1. Experiments of the composite centred in a cube DOE and experimental response (Y) for copper fixation on *A. ferrooxidans* (mg Cu$^{2+}$/100 mg bacterial dry weight)

<table>
<thead>
<tr>
<th>Exp.</th>
<th>φ = physiological state (% Fe$^{3+}$/Fe$^{2+}$+Fe$^{3+}$)</th>
<th>pH</th>
<th>ω = agitation rpm</th>
<th>T = temperature °C</th>
<th>[prot] = protein concentration (mg L$^{-1}$)</th>
<th>Y$_{Cu}$ mg / 100 mg dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>3</td>
<td>100</td>
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<tr>
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<td>150</td>
<td>5</td>
<td>318</td>
<td>6.08</td>
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<td>25</td>
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<td>5</td>
<td>50</td>
<td>5</td>
<td>45</td>
<td>1.88</td>
</tr>
</tbody>
</table>

*1 experiment repeated 5 times*

3. RESULTS

The responses Y (fixed metal per dry weight, mg / 100 mg dry weight) are recapitulated in Table 1. The control experiments showed that copper ion concentration remained unchanged by the experimental procedure in absence of bacteria, meaning that the decrease in concentration was only due to the *A. ferrooxidans* biomass.

As a first step, the coefficients of the postulated model are estimated by the way of a multi-linear regression, the so-called least-square method. The resulting model cannot be directly used for prediction but several steps of analysis have to be performed. First, the
model must be refined by suppressing the non-significant terms and then, the adequacy of the final model has to be checked from several statistical tests. Such an analysis prevents from wrong interpretations of experimental results. The model refinement and its checking are important enough to be described with some detail.

3.1 Pointing out the significance of effects and refining the response model

The results of the multi-linear regression (calculation of the model coefficients) are given under either a graphic form (Fig. 1) or a table (not shown).

Figure 1. Pareto graph: visualisation of the absolute values of each effect sorted in descendant influence. The boundary of effect significance line (determined thanks to probability of significance P) shows the limit of effect significance

Residual SD = 4.021626
Replicate SD = 3.714353
R squared = 0.974
Adjusted R squared = 0.959

The graph rapidly visualises the most important effect or the non-significant ones thanks to the boundary of effect significance. An effect is the change due to a given term in the response as the concerned variable(s) shift(s) from the low to the high limit(s). Significance is a probability assigned by a statistical test here used for checking if a coefficient has a zero value or not. A small probability means a significant result.

To refine the model, all the non-significant effects (under the boundary in Fig. 1) must be suppressed. However, such an elimination cannot be performed by simultaneously suppressing all the terms having a significance greater than 0.05. It requires a stepwise procedure by suppressing the term having the highest significance, then recalculating the new model and the new significance of the remaining terms and so on.

For the fixation of copper by *A. ferrooxidans* as a function of temperature, agitation, pH, protein concentration and physiological state, the model obtained after refining is given in next equation:
where the notation means the midrange.

### 3.2 Checking the adequacy and the validity of the model

A first very simple information about the descriptive power was obtained by plotting the fitted response vs. the experimental response (Fig. 2a).

\[
Y = 7.5653 + 0.140396 (\varphi - \bar{\varphi}) + 1.24656 (pH - \bar{pH}) + 0.500889 (T - \bar{T}) - 0.124763 ([prot] - [prot]) - 0.122381 (\varphi - \bar{\varphi})(pH - \bar{pH}) + 0.0105408 ((\varphi - \bar{\varphi})(\varphi - \bar{\varphi}) + 0.240866 (pH - \bar{pH}) (T - \bar{T}) - 0.0057464 (T - \bar{T}) - (\varphi - \bar{\varphi}) - 0.00510785 (T - \bar{T}) ([prot] - [prot]) + 9.0769 \times 10^{-8} ([prot] - [prot]) - 0.124763([prot] - [prot])
\]

where the notation means the midrange.

Figure 2. Plots testing adequacy and validity of the model: fitted values vs. experimental values (a) and residuals vs. normal scale (b)

For the ideal case this graph should be the first bisector. A too large dispersion between predicted and experimental response values would be the sign of a poor fit due either to an unsatisfactory model or to an important experimental error. Moreover, two global statistics are available to check the description power of the model fitting. The first one is the classical $R^2$ statistic which ranges from 0 to 1 and is nothing but the proportion of response variations explained by the model. This statistic is a global index of the descriptive power of the fitting but it is better to use a more realistic coefficient, the adjusted $R^2$ squared, defined from mean squares instead of raw squares and connected to the $R^2$ statistic by:

\[
R^2_{adj} = 1 - (1 - R^2) \frac{n - 1}{n - p}
\]

where $n$ is the number of experiment and $p$ the number of coefficient.

The adjusted $R^2$ squared has a value greater than 0.9 for a rather good fit while it can be even negative when the fit is very poor. In the present work for copper ion uptake, a very satisfactory value $R^2_{adj} = 0.954$ was obtained for the refined model.

The second test to check model adequacy consisted in verifying that there was no lack of fit. It is based on a comparison of the standard deviation of the residuals (residual SD, Fig. 1) and of the standard deviation of the replicates (replicate SD, Fig 1) which must have the same magnitude. Residuals are, in fact, the differences between the experimental response and the response given by the model. The classical F-test uses a statistic based on the ratio $r = [\text{residual SD}/\text{replicate SD}]^2$ which should be close enough to unity and in
practice lower than a critical value deduced by the F distribution. In this study the value of
the replicated and residual standard deviation were 3.72 and 4.02 respectively and the F-
test allowed to conclude that there was no evidence of lack of fit at the usual significance
level 5%.

Last step before accepting the model is adequate and is an accurate analysis of the
behaviour of the residuals. This last analysis is very important because it points out if the
model was only descriptive or actually predictive and highlights the possible anomalies of
the experimental investigation.

The multi-linear regression which allows to determine the model coefficients requires
the respect of a basic assumption: the experimental error must obey to a normal law with a
mean equal to zero and a constant standard deviation. Consequently the normality of
residuals, which would be nothing but the experimental errors if the postulated model was
the right one, and the independence of the residuals, both on the value of the response and
on the order of runs, must be checked.

The normality of residuals is checked by using the classical normality plot consisting
in plotting the observations under study (the residuals in this case) against a normal scale
and expecting a straight line when the observations are normally distributed (Fig. 2b) [21].
When the graph is not linear, response transformations, such as logY, lnY or Y^{1/2}, can be
tried for stabilising the variance of the response. When some points appear to be far from
the line, the corresponding runs are suspicious and should be replicated for concluding if
there was an experimental mistake or if the model was too rigid to describe the
phenomenon and has to be changed for a more complex one. For copper fixation on A.
ferrooxidans, the curve is a straight line and confirms the normality of the residuals.

The independence of residuals vs. the response value is checked by simply plotting
the residuals vs. the observations (Fig. 3a).

![Figure 3](image)

**Figure 3. Plots for checking the independence of the residuals as regards the
experimental value (a) and the run order (b)**

The residuals are equally distributed around the zero value without any dependence
on the response value. If not, all the zones of the region under study are not equivalent
because of a varying experimental error or a variable descriptive power of the model.
Finally the independence of the residuals vs. the run order of the experiments allows to
verify that no evolution of the experimental system occurs in time (Fig. 3b). Here again
the residuals are dispersed at random around zero without any dependence on the run
order.
It is only after all these checking steps that the model can be accepted as correct for describing, predicting and optimising the response within the experimental region under study.

3.3 Optimisation

Once the suitable response model has been accepted, useful visual information can be derived from it by using its graphical representation vs. the continuous factors: 2D contour-plots and 3D response surfaces (Fig. 4).

Moreover, it is possible to evaluate the optimal response in the studied field from the model and to locate it on the drawing in order to verify its robustness against possible variations of the controlled factors. A robust optimum is characterised by a rather smooth response surface around it and is always recommended for a process because of the ease to keep the response close to the optimum even if the controlled parameters vary a little.

For the present example by using the refined model, the predicted maximal mass of copper fixed by 100 mg of dry weight of bacteria should be 74.94 mg and it is expected at pH 5 and 30°C, for a 50 rpm stirring speed, with a physiological state corresponding to the middle of the exponential phase (65%), and a 45 mg/L protein concentration (Fig. 4). Under these conditions, experiment leads to 74.91 mg per 100 mg dry weight, value which is in very good agreement with the prediction. This optimum is located on the border of the studied field. Generally, in such a case, the experimental region under study should be translated and enlarged so implying to perform a new DOE. However, for the present study, such a further investigation is physically unfeasible because the variation ranges of the parameters were as wide as possible (see Material and Methods).

Analysing the response surface for the mass of copper fixed by 100 mg dry weight of bacteria provides useful information. First, a slight increase of the protein concentration leads to a sharp decrease of the response. This observation means that the protein concentration must be carefully controlled to keep up the response at its optimal value. The same conclusions can be derived for pH and temperature. On the other hand, stirring and physiological state appear to have a limited effect on fixation in the studied range so allowing a less severe control on these parameters.
4. DISCUSSION

The empirical model obtained thanks to the DOE allowed to know the influence of each of the 5 chosen parameters on copper ion uptake by a living biomass of *A. ferrooxidans*. Moreover, non-linearity or parameter interactions have been evaluated by this method, what is impossible with the common OVAT method or the Langmuir and Freundlich isotherms. Consequently, the interaction study is original compared to previous works where each parameter was analysed individually.

The most influent parameter (seen on Pareto graph, Fig. 1) for copper ion uptake by *A. ferrooxidans* was the protein concentration. Moreover, the importance of the quadratic effect of protein concentration testified of the great non-linearity of its influence on copper ion uptake. The model showed that an increase in protein concentration led to a decrease in specific copper ions fixation. This phenomenon, already observed by Sakaguchi et al [22], was probably due to the formation of aggregates of bacteria at high concentration. These aggregates decreased the contact surface between the bacteria and the copper ions and, consequently, the metal fixation. Moreover, these aggregates have been observed for *A. ferrooxidans* [3] confirming this hypothesis.

The second most influent parameter, the temperature, had different influences on metal fixation on living biomasses: either it increased with temperature with an optimal temperature close to the culture's one [17], or it decreased reaching the optimum of fixation at 5 °C [7]. Copper ion fixation on *A. ferrooxidans* increased with temperature with an optimal value at 30°C, growth temperature of the bacterium. This indicated that the metabolism had to be active for metal ion uptake. So copper ion fixation was a more complex process than simple surface biosorption, modelled by Langmuir and Freundlich isotherms.

The third influent parameter was the pH. Most of the metal ion fixation on microorganisms was maximal for pH between 4 and 6 [8, 17, 20] more rarely close to 2. Copper ion uptake by *A. ferrooxidans* increased with pH towards an optimal value at 5. This value, different to pH growth medium (1.4), was close to pH copper precipitation. The high specific copper uptake capacity of the biomass (74.9 mg / 100 mg) had to be compared with previous results obtained for Cr⁶⁺ [4] and Cd²⁺ [3]: 50.9 mg and 31 mg / 100 mg. For these metal ions, a bioprecipitation has been observed on the surface (Baillet et al. 1998) of the bacteria. Such a mechanism could explain an optimal pH at 5 where precipitates dissolution was less rapid: although no pH variation was observed in the solution, an active metabolism of the bacteria could modified locally the pH on the bacterial surface causing copper ion bioprecipitation.

Physiological state is also an influent parameter. The age of the culture was shown to be important in metal fixation [3,17,19]. The copper ion fixation was maximum in the middle of the exponential growth phase indicating that either the metabolism or the external surface were implied in copper ion fixation. The middle of exponential growth phase corresponded to the more intensive metabolism. Although the external surface of the bacteria was modified during growth (aspect, permeability or size...) which might modify the fixation process.

Finally, the less influent parameter was the agitation speed. The fixation increased when the agitation speed decreased: the uptake was better without strong turbulence. This result was in agreement with the hypothesis of bioprecipitation: a too high agitation speed would be able to separate precipitates from bacteria.
The influence of each of the 5 chosen parameters was known thanks to the DOE modelling. This technique allowed us to understand the effect of physicochemical parameters (pH, agitation speed, temperature), but also the effect of biological ones (physiological state, protein concentration). The results revealed a more complex process of fixation than the simple biosorption: the copper ion uptake by *A. ferrooxidans* need an active metabolism to reach its optimal value: 74.9 mg / 100 mg dry weight.

REFERENCES
Platinum and palladium recovery from dilute acidic solutions using sulfate reducing bacteria and chitosan derivative materials

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Abstract

The present work focuses on the study of Pd and Pt sorption using different strains of sulfate reducing bacteria, SRB, \textit{(Desulfovibrio} spp.) and the comparison of their sorption performance to that obtained using several chitosan derivatives. \textit{D. desulfuricans} was the best strain: sorption capacities as high as 190 and 90 mg Metal (Me) g\textsuperscript{-1} for Pd and Pt respectively were obtained under optimum experimental conditions at pH 3. At pH 2, for Pd sorption the maximum sorption capacities with HNO\textsubscript{3} and H\textsubscript{2}SO\textsubscript{4} solutions were comparable (close to 120 mg Pd g\textsuperscript{-1}) and higher than the levels reached with HCl solutions (less than 70 mg Pd g\textsuperscript{-1}). Sorption kinetics are very fast: the equilibrium can be reached within the first 15-30 minutes of contact. For chitosan derivatives sorption capacities under selected experimental conditions (i.e. pH 2) appear to be significantly higher than with SRB between 200 and 600 mg Me g\textsuperscript{-1}, depending on the metal and the derivative. However, compared to SRB systems the kinetics are significantly slower: Several hours of contact are necessary to reach the equilibrium.

\textit{Keywords}: chitosan, sulfate reducing bacteria, platinum, palladium, isotherms, kinetics

1. INTRODUCTION

Platinum group metals (PGM) e.g. Pd, Pt and Rh are of interest due to their high value and catalytic properties. Demand for PGM is increasing due to their widespread and often obligatory utilization in automotive catalytic converters. Currently the main large-scale industrial recovery processes are hydro- or pyro-metallurgical but these conventional technologies also generate liquors containing residual precious metals [1-2]. The use of
low-cost sorbents has been investigated for their potential to replace current costly methods of recovering heavy metals from solutions. Natural materials or waste products from industries can be economically employed. Thus research has evaluated a variety of sorbents e.g. lignin, tannin-rich materials, chitosan, microbial biomass, algae, alginate, seaweed or zeolites [3-4]. Metal sorbing biomasses can have a real potential as selective, competitive and cost-efficient sorbents for precious metal recovery [5-7]. An alternative approach is the use of enzymatic processes. Sulfate-reducing bacteria (SRB) are known to have a broad reducing metal activity coupled to hydrogenases or/and cytochromes [8-10]. This has been demonstrated in the case of reduction of Pd(II) to Pd(0) but the best reductive accumulation only occurred after a prior biosorption step which suggests a high metal uptake as a pre-requisite to enzymatic catalysis, especially in the case of Desulfovibrio desulfuricans [11-12]. The present work focuses on the optimization of sorption procedures and selection of the best strain for Pd and Pt recovery and the sorption properties will be compared to those obtained with chitosan derivatives. Indeed chitosan, which is an aminopolysaccharide produced from crustacean shell, is very efficient at removing metal ions from dilute solutions [13-17]. Sorption may involve different mechanisms including chelation (for metal cations in near-neutral solutions) or ion exchange (for metal anions in acidic solutions). The sorption properties may be enhanced by chemical modification, including amine grafting (Poly(ethyleneimine), PEI) or thiourea grafting. Sorption properties will be characterized for both SRB and chitosan derivative mediated processes by investigating the influence of the pH on sorption efficiency, and performing sorption isotherms and kinetics.

2. MATERIALS AND METHODS

2.1 SRB, growth conditions and harvesting

Desulfovibrio desulfuricans (NCIMB 8307, D.d.), Desulfovibrio vulgaris (NCIMB 8303, D.v.) and Desulfovibrio fructosivorans (DSM 2604, D.f.) were grown for 2 days anaerobically (inoculation 10% vol/vol from 24h pre-culture) in sealed bottles under a N2 atmosphere, as batch cultures in Postgate C medium at 30°C (D.d. and D.v.) or 37°C (D.f.) [18]. Harvesting was carried out by centrifugation and resting cells were obtained by centrifuging 1 L of culture and washing the cells 3 times in air with 20 mM MOPS-NaOH buffer (pH 7). Experiments were also performed with dead biomass (harvested cells were autoclaved at 120°C for 20 minutes); sorption capacities were comparable to those obtained with living biomass. Dry weight was determined by filtration and drying to constant weight.

2.2 Sorption procedures for SRB system

Pd and Pt solutions were prepared in distilled water from Na2PdCl4 and Na2PtCl6 salts, respectively, using HCl, HNO3 and H2SO4 for pH control. For sorption isotherms, known volumes of metal solutions (V, usually 10 mL) at variable initial metal concentrations (C0, 5-50 mg L⁻¹) in sterilized and sealed bottles were put in contact with a fixed amount of sorbent (m, 1.5 mg, i.e. sorbent dosage: 150 mg L⁻¹) under agitation (150 rpm) at 30°C. The pH was maintained constant. Samples were collected by filtration after 4 days of contact and analyzed using the SnCl2/HCl spectrophotometric method for Pt/Pd determination (Ceq) [19]. The mass balance equation was used for determining the sorption capacity (q, metal concentration on the biomass, mg g⁻¹ or mmol g⁻¹): q = V/m (C0-Ceq). The influence of chloride concentration was tested on biosorption performance at pH 2, using D. desulfuricans with addition of increasing amounts of NaCl. Sorption kinetics
were carried out at a metal concentration of 50 mg metal L\(^{-1}\) under agitation (150 rpm) at 30°C and pH 2 (controlled with either HNO\(_3\) or HCl) in the absence or the presence of NaCl (1 M) using D. desulfuricans. Kinetic experiments were also carried out using the same procedure with solutions containing 25 and 10 mg metal L\(^{-1}\). Samples were regularly collected by filtration and analyzed.

2.3 Preparation of chitosan derivatives

Chitosan was supplied by Aber Technologies (France) as a flaked material with a deacetylation percentage of about 87% and a molecular weight of 125,000 g mol\(^{-1}\). Chitosan gel beads were prepared using a neutralization process consisting of a two-step procedure [20]: (a) the dissolving of chitosan (4-5% w/w) in acetic acid (excess of acetic acid to completely dissolve the polymer), (b) the distribution of the viscous solution (filtered and de-bubbled) through a thin nozzle into a neutralization bath (NaOH 2M). The beads were collected after 16 hours of contact with the neutralization bath and then rinsed with several demineralized water baths up to constant pH. The size of the beads was 2.5 mm ± 0.2 mm and the water content was approximately 95%.

Chemical cross-linking of chitosan was performed by reacting 5 g of chitosan beads (dry mass) with 1.5 g of glutaraldehyde dissolved in 100 mL of demineralized water, for 24 h. For the synthesis of poly(ethyleneimine) grafted chitosan beads (PEI-GA), PEI was dissolved in dimethylacetamide (DMA). The beads were also mixed with DMA in order to exchange water with DMA and then favor the diffusion of PEI in the beads: 5 g of chitosan beads were added to 5 g (the amount was varied for some experiments using alternatively 3 g or 5 g of PEI) of PEI dissolved in 100 mL of DMA. The mixture was reacted for 24 h. The chitosan beads were then rinsed with 30 mL of DMA, and reacted for 6 h with 1.5 g of glutaraldehyde dissolved in 50 mL of DMA: glutaraldehyde is thought to establish new linkages between the amine groups of the biopolymer and some amine groups of PEI. For the synthesis of thiourea derivative chitosan beads (PEI-Th-GA), the standard procedure consists of the addition of (a) 5 g of PEI (b) 1.5 g of thiourea and (c) 5 g of chitosan to 100 mL of DMA. The components were pre-reacted for 24 h and rinsed with 30 mL of DMA before the addition of 1.5 g of glutaraldehyde dissolved in 50 mL of DMA. Following the treatments, the sorbents were extensively rinsed with demineralized water. Because of the instability of previous derivative containing thiourea, the grafting of sulfur groups was carried out according to the procedure described by Cardenas et al. (2001) with chitosan flakes. This technique uses epichlorohydrin as crosslinking agent. Chitosan (20 g) was contacted with 1 L of acetic acid/acetate buffer (pH 4.6); 40 mL of epichlorohydrin dissolved in 1 L of acetone were added, and the slurry mixed at 35°C for 36 h. Thiourea (20 g) was then added and the stirring continued for 6 h at 60°C. After that period, 46 g of thiourea were added and the system was stirred for 12 h at the same temperature. Then, 500 mL of NaOH (1 M) were added and the slurry was agitated for 4 h at 60°C. The solid product obtained (TDC) was filtered and successively washed with acetone, demineralized water and methanol. Finally, it was dried in an oven at 60°C.

2.4 Sorption procedures for chitosan derivatives system

The pH of the solutions was controlled using HCl and NaOH concentrated solutions. For sorption isotherms, known volumes of metal ion solution (150 mL) at fixed concentrations were contacted with varying sorbent quantities (from 3 to 35 mg, wet mass) at room temperature (20°C). After 3 days of agitation, the solutions were filtered through 1.2 µm membranes and the filtrates were analyzed using ICP-AES (Jobin-Yvon JY36, France). For the study of sorption kinetics, one liter of metal ion solution at fixed
Biosorption

pH was mixed with a fixed amount (100 mg) of sorbent in a jar-test agitated system. Five-milliliter samples were withdrawn at specified times, filtered through a 1.2 µm membrane and analyzed as previously specified. For the study of the influence of competitor anions, chloride ions were added in the form of NaCl. The isotherms were modeled using the Langmuir equation:

\[
q = \frac{q_m b C_{eq}}{1 + b C_{eq}}
\]

where \(q_m\) (mmol g\(^{-1}\)) and \(b\) (L mmol\(^{-1}\)) are the constants of the model.

3. RESULTS AND DISCUSSION

3.1 Selection of SRB strain and influence of the acid used for pH control

Figure 1 compares the sorption capacities at pH 2 for Pt and Pd for 3 different strains of Desulfovibrio using different acids for pH control. Maximum sorption capacities were obtained with Desulfovibrio desulfuricans, regardless of the acid used for pH control and metal. Comparing the sorption capacities for Pt and Pd on the basis of molar units, it appeared that the biomasses have a greater affinity for Pd than for Pt, regardless of the strain.

Palladium sorption was higher in sulfuric and nitric acid solutions than in hydrochloric acid media. Bacterial strains have a greater affinity for Pt in nitric and sulfuric acid solutions. These differences may be explained by differences in the composition of cell walls or/and by the effect of metal speciation. Indeed, PGM are very sensitive to the composition of the solution with respect to the formation of complexes (especially chloro-complexes), which in turn may affect the sorption mechanism (chelation versus ion-exchange) and the affinity of metal species for sorption sites. Table 1 reports the parameters of the Langmuir equation that was used for the modeling of sorption isotherms in Figure 1.

Table 1. Influence of the acid used for pH control at pH 2 on the Langmuir parameters for the modeling of Pd and Pt sorption isotherms using Desulfovibrio desulfuricans (D.d.), Desulfovibrio fructosivorans (D.f.) and Desulfovibrio vulgaris (D.v.)

<table>
<thead>
<tr>
<th>Acid</th>
<th>Bacteria</th>
<th>Pt q_m</th>
<th>Pd q_m</th>
<th>Pt b</th>
<th>Pd b</th>
<th>Pt R(^2)</th>
<th>Pd R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNO(_3)</td>
<td>D.d.</td>
<td>0.343</td>
<td>73.1</td>
<td>0.997</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
</tr>
<tr>
<td>HNO(_3)</td>
<td>D.f.</td>
<td>0.185</td>
<td>5.85</td>
<td>0.997</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
</tr>
<tr>
<td>HNO(_3)</td>
<td>D.v.</td>
<td>0.163</td>
<td>44.5</td>
<td>0.998</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
</tr>
<tr>
<td>HCl</td>
<td>D.d.</td>
<td>0.287</td>
<td>80.4</td>
<td>0.999</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
</tr>
<tr>
<td>HCl</td>
<td>D.f.</td>
<td>0.116</td>
<td>8.1</td>
<td>0.940</td>
<td>0.998</td>
<td>0.998</td>
<td>0.998</td>
</tr>
<tr>
<td>HCl</td>
<td>D.v.</td>
<td>0.195</td>
<td>93.2</td>
<td>0.998</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
</tr>
<tr>
<td>H(_2)SO(_4)</td>
<td>D.d.</td>
<td>0.228</td>
<td>62.3</td>
<td>0.998</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
</tr>
<tr>
<td>H(_2)SO(_4)</td>
<td>D.f.</td>
<td>0.118</td>
<td>21.1</td>
<td>0.972</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
</tr>
<tr>
<td>H(_2)SO(_4)</td>
<td>D.v.</td>
<td>0.118</td>
<td>36.8</td>
<td>0.995</td>
<td>0.996</td>
<td>0.996</td>
<td>0.996</td>
</tr>
</tbody>
</table>
3.2 Comparison of chitosan derivatives for Pt and Pd sorption at pH 2

Figure 2 compares Pt and Pd sorption properties at pH 2 (controlled with HCl, which is the best medium for PGM sorption, as shown in previous work) for different chitosan derivatives. Experiments were performed at low metal concentration. It is interesting to observe that sorption capacities as high as 3 mmol Me g\(^{-1}\) were obtained and that sorption isotherms were characterized by a pseudo-rectangular shape: the saturation plateau was obtained at residual concentrations as low as 0.05 mmol Me L\(^{-1}\) (especially for PEI and TDC). The Langmuir equation failed to fit experimental data at low Pt concentration in the case of TDC.
Biosorption

Figure 2. Pt and Pd sorption isotherms at pH 2 for GA, PEI and TDC chitosan derivatives

3.3 Influence of pH on sorption performance

Figure 3 shows the influence of pH on Pt and Pd sorption by *D. desulfuricans* (selected for its highest affinity for PGMs) with HNO₃, HCl and H₂SO₄ solutions. As expected, increasing the pH resulted in an increase in sorption capacities due to the weaker competitor effect of counter anions brought to the solution by the acid used for pH control. The differences between the different acidic solutions decreased at increasing the pH since the amount of acid used for pH control (and consequently the addition of counter anions) also decreased. Sorption capacities were again lower in HCl solutions. This result contrasts with other experiments performed with ion-exchange resins (chitosan sorbents) for which the control of the pH with HCl enhances the formation of chloro-complexes, readily adsorbable on protonated amine groups. It may indicate that the sorption mechanism is different to that observed with chitosan material. These results confirm the greater affinity of the sorbent for Pd than for Pt.

Figure 3. Influence of pH and acid used for pH control on Pt (left figure) and Pd (right figure) sorption capacity (Co(Pd/pt): 50 mg Metal L⁻¹; Sorbent dosage: 150 mg L⁻¹)

The influence of the pH on the sorption of Pt and Pd was also investigated. The PEI and TDC (glutaraldehyde cross-linked material) was significantly less efficient at sorbing PGMs than the other derivatives, see above). Table 2 shows the parameters of the Langmuir equation that were used for the modeling of sorption isotherms in Figure 4. In most cases, except for TDC and platinum at low metal concentration, the Langmuir equation fitted well experimental data.
Table 2. Influence of the pH (controlled with HCl) on the Langmuir parameters for the modeling of Pd and Pt sorption isotherms using GA, PEI and DTC derivatives of chitosan

<table>
<thead>
<tr>
<th>pH</th>
<th>Sorbent</th>
<th>( q_m ) (mmol Pt/g)</th>
<th>( q_m ) (mmol Pd/g)</th>
<th>( R^2 )</th>
<th>( b ) (mmol Pt/L)</th>
<th>( b ) (mmol Pd/L)</th>
<th>( R^2 )</th>
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</thead>
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<tr>
<td>1</td>
<td>PEI</td>
<td>1.11</td>
<td>27.4</td>
<td>0.984</td>
<td>1.64</td>
<td>37.85</td>
<td>0.991</td>
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<tr>
<td>2</td>
<td>PEI</td>
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<td>33.25</td>
<td>0.980</td>
<td>3.28</td>
<td>408.8</td>
<td>0.995</td>
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<tr>
<td>3</td>
<td>PEI</td>
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<td>2.68</td>
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<td>0.992</td>
</tr>
<tr>
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<td>PEI</td>
<td>2.65</td>
<td>1130</td>
<td>0.992</td>
<td>2.20</td>
<td>74.3</td>
<td>0.955</td>
</tr>
<tr>
<td>1</td>
<td>TDC</td>
<td>2.21</td>
<td>196.9</td>
<td>0.988</td>
<td>2.54</td>
<td>295.5</td>
<td>0.997</td>
</tr>
<tr>
<td>2</td>
<td>TDC</td>
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<td>0.940</td>
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<td>146.8</td>
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</tr>
<tr>
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<td>100.8</td>
<td>0.801</td>
<td>3.00</td>
<td>194.4</td>
<td>0.996</td>
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<tr>
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<td>GA</td>
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<td>52.85</td>
<td>0.978</td>
<td>2.91</td>
<td>9.48</td>
<td>0.871</td>
</tr>
</tbody>
</table>

Figure 4. Influence of pH on Pt and Pd sorption isotherms using PEI and TDC

Maximum sorption capacities were obtained at pH 2 for the PEI derivative of chitosan, with a significant decrease at pH 1 and pH 3. At pH 4, the results should be considered with caution since precipitation may occur. On the other hand, with TDC the sorption isotherms were influenced negligibly by the pH (in the range pH 1-3): the difference in maximum sorption capacities did not exceed 10-20%. This may be explained by the different mechanisms involved in metal uptake for PEI and DTC. For PEI, sorption occurs through ion-exchange on protonated amine groups; this mechanism is very sensitive to the pH of the solution, and to the presence of competitor anions. For TDC, sorption may be the combination of ion-exchange (on protonated amine groups) and chelation (on the thio groups). Chelation on sulfur compounds is less sensitive to pH and to the presence of competitor anions. This may explain the high efficiency of TDC for Pt...
and Pd sorption in a broader range of pH compared to the PEI derivative, although the sorption capacities were slightly higher for PEI than for TDC at the optimum pH (i.e. pH 2). The increase in the density of sorption sites (by grafting of supplementary amine functions) may explain this improvement in sorption properties.

3.4 Sorption kinetics

The kinetics is also an important parameter in the design of a sorption process. Figure 5 shows Pt and Pd sorption kinetics using *D. desulfuricans* at pH 2 controlled with nitric acid and hydrochloric acid (with a superimposition of kinetic curve performed in HCl media in the presence of NaCl). Sorption kinetics was little influenced by the acid used for pH control. In the case of Pd sorption, a greater contact time was necessary to reach the equilibrium. The presence of NaCl strongly decreased the efficiency of sorption, especially in the case of Pd.

![Figure 5. Influence of the acid used for pH control on Pt and Pd sorption kinetics at pH 2 using *D. desulfuricans* (C₀(Pt/Pd): 50 mg Metal L⁻¹; Sorbent dosage: 150 mg L⁻¹)](image)

In most cases, more than 90% of total sorption was achieved within the first 10 minutes of contact. Sorption kinetics was significantly faster with SRB than for chitosan derivatives (see below). The initial concentrations did not significantly influenced sorption kinetics, regardless of metal (Figure 6). The same contact time (about 10 minutes) was sufficient to achieve the same percentage of metal uptake (90%). The sorption was restricted to the external surface of the cells. The uptake was almost instantaneous (no effect of intraparticle diffusion resistance).

![Figure 6. Influence of metal concentration on Pt and Pd sorption kinetics using *D. desulfuricans* at pH 2 controlled with HCl (relative concentration decay curves: C(t)/C₀= f(t))](image)
In the case of chitosan derivatives the reaction required a greater contact time to reach equilibrium: for PEI more than 24 hours of contact were necessary to achieve the complete removal of Pd and Pt. For TDC material, at the same sorbent dosage, the equilibrium was reached within 6 hours of contact. It is difficult to compare the data since sorbent dosage (100 mg L\(^{-1}\)) was sufficient to completely remove Pt and Pd. As a consequence it is possible that the sorbents were not fully saturated, or at least that the relative saturations were different for PEI and TDC. It is not possible to conclude on the predominance of the resistance to intraparticle diffusion on the kinetic control. Other experiments have shown using similar sorbents that sorption occurred in the whole mass of the sorbent. It is thus anticipated that the diffusion of metal ions to internal sites controls the time required to achieve complete recovery of PGMs. It is important to observe that PEI was prepared with chitosan gel beads of expanded structure but with greater particle size, while TDC was obtained by chemical modification of flakes with lower porous network with small particle size.

![Figure 7. Pt and Pd sorption kinetics on PEI and TDC at pH 2 (controlled with HCl, \(C_0 (Pt/Pd): 20-25\) mg Metal L\(^{-1}\); Sorbent dosage: 100 mg L\(^{-1}\))](image)

3.5 Influence of chloride concentration on sorption performance

One purpose of this work was to develop a metal recovery technology applicable to acidic leachates obtained from solid scrap. The main characteristics of leachates from spent automotive catalysts and other wastewaters are a high concentration of chlorides and low pH, since *aqua regia* is necessary for effective PGM leaching. Hence, the influence of chloride on Pd and Pt sorption is of major concern for the evaluation of process applicability. Figure 8 compares relative sorption capacities (compared to reference performance, i.e. without NaCl addition) for the different systems with increasing concentrations of chloride. The addition of chloride strongly reduced Pt sorption capacities especially for SRB system and for PEI: when chloride concentration exceeded 0.25-0.5 M Pt sorption capacities were negligible for PEI and SRB. In the case of Pd, with PEI derivative and SRB there was also a significant decrease in sorption capacity, however it was necessary to drastically increase NaCl concentration: with SRB the sorption decrease was about 70% at a chloride concentration of 1 M; with PEI, at a 0.5 M chloride concentration the decrease did not exceed 50%. In the case of TDC, the addition of chloride decreased sorption capacities but even with a chloride concentration as a high as 2 M, the decrease of sorption capacity did not exceed 40%, regardless of metal. The grafting of supplementary amine groups (PEI) increased the number of sorption sites, and then decreases the competitor effect of chloride ions.
Figure 8. Influence of chloride concentration on Pt and Pd sorption at pH 2 (controlled with HCl; for D. desulfuricans: \(C_0(Pd/Pt): 50\) mg Metal L\(^{-1}\); Sorbent dosage: 150 mg L\(^{-1}\); for chitosan derivatives: \(C_0(Pd/Pt): 20\) mg Metal L\(^{-1}\); Sorbent dosage: 40 mg L\(^{-1}\))

In the case of thiourea grafting, new chelating groups less sensitive to the pH and the competitor effect of chloride anions, limited the influence of chloride concentration. The TDC appears to be perfectly designed for the treatment of industrial solutions containing high concentrations of chloride ions and low pH, despite the flaked conditioning that limits the use of sorbent particles in fixed-bed systems.

4. CONCLUSIONS

Sulfate-reducing bacteria (especially D. desulfuricans), and chitosan derivatives (more specifically thiourea derivative of chitosan) appear promising sorbents for the recovery of PGMs from dilute acidic solutions (in the range pH 1-3) containing chloride anions (below 0.5-1 M). Both sorbents have a preference for Pd over Pt, though more marked in the case of SRB. The SBR system is characterized by very fast sorption kinetics (equilibrium reached within 10 minutes of contact), while chitosan derivatives are characterized by slower kinetics but very high sorption capacities (up to 3 mmol Me g\(^{-1}\)). Experiments are currently performed on the sorption of Pt and Pd in multi-component systems and in the presence of other metals (typical industrial solutions).

REFERENCES
Preliminary study of lead sorption by selected sorbents

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Abstract

Lead sorption was investigated using several biosorbents, including five fungi (\textit{Aspergillus niger}, \textit{Mucor miehei}, \textit{Penicillium chrysogenum}, \textit{Rhizopus arrhizus} and \textit{Rhizopus conhii}), two algae (\textit{Ascophyllum nodosum}, \textit{Lessonia trabeculata}), one yeast (\textit{Saccharomyces cerevisiae}), alginate and chitosan. Experiments were performed with non-living microorganisms. In the present work alginate was tested as a powder. Preliminary experiments have been performed investigating the influence of the pH on lead sorption (between pH 3 and pH 6). Complementary experiments were carried out using 3 of the most efficient sorbents: alginate, \textit{A. niger} and \textit{A. nodosum}. Sorption properties were determined through isotherms and kinetics at pH 4. Maximum sorption capacities were comparable for \textit{A. niger} and \textit{A. nodosum}, tending to 130-150 mg Pb g\textsuperscript{-1}. Sorption kinetics was slightly faster for \textit{A. nodosum} than for \textit{A. niger}.

\textit{Keywords}: lead, biosorption, fungal biomass, algal biomass, isotherms, kinetics.

1. INTRODUCTION

The toxic effects of heavy metals such as mercury, cadmium or lead require the treatment of industrial wastewater prior their discharge to the environment. Though precipitation processes are efficient at removing metal ions from solutions, these techniques are sometimes inappropriate for the treatment of dilute solutions for technical or economical reasons. Moreover, the production of toxic sludge as a by-product limits the interest of these processes: the precipitation results in a single pollution transfer. For these reasons, economical and efficient processes are still necessary to develop. Biosorption processes have been recognized as promising techniques, owing to the low cost and the great diversity of these materials. Since the early 80’s many studies have focused on the use of materials of biological origin for the recovery of metal ions [1]. Living as well as

\textsuperscript{*} Authors thank the French Ministry of Foreign Office and the Concytec for the financial support under the Franco-Peruvian Program of Collaboration Raul Porras Barrenechea. E.G. thanks the European Community for financial support under Growth Program (3SPM project, Contract G1RD-CT2000-00300) for attending the IBS’03 conference.
non-living microorganisms have been used for metal recovery: algae [2-5], bacteria [6-7], fungi [8-12], and yeasts [13]. The identification of sorption sites on the cell walls of the microorganisms has been the motive for the increasing interest for metal uptake properties of the biopolymers entering in the composition of these cell walls: alginate [14], chitin and chitosan [15-16], or exopolysaccharides excreted by these microorganisms [17].

The present study focuses on a preliminary investigation of lead sorption using 5 fungi (Aspergillus niger, Mucor miehei, Penicillium chrysogenum, Rhizopus arrhizus and Rhizopus conhii), 2 algae (Ascophyllum nodosum and Lessonia trabeculata), 1 yeast (Saccharomyces cerevisiae) and 2 biopolymers (alginate and chitosan). The cell wall of selected fungi is characterized by the presence of chitin and/or chitosan material associated with proteins and other glucans. On the other hand, algal biomass may contain alginate (L. trabeculata) or fucoidan polymers (sulfate fucans, A. nodosum) [18-20]. Preliminary experiments focus on the study of the influence of the pH on 10 selected sorbents. Sorption isotherms and uptake kinetics are determined at pH 4 using alginate, A. niger and A. nodosum.

2. MATERIALS AND METHODS

2.1 Materials

The samples of Aspergillus niger, Penicillium chrysogenum, Rhizopus arrhizus, Rhizopus conhii and Mucor miehei were kindly donated by Gist Brocades (The Netherlands). They were supplied after being inactivated and dried. The particles were sieved and the fraction 125-250 µm was used for preliminary experiments. The brown alga Ascophyllum nodosum was collected on the Brittany coast while Lessonia trabeculata was collected on the Peru coast. The samples were dried and ground, the fraction 125-250 µm was used for experiments. The yeast (Saccharomyces cerevisiae) was obtained from local bakeries, as a commercial sample. Alginate was purchased from Janssen. Chitosan was supplied by ABER-Technologies (Brest-France) (Lot N° A17G28). Its characteristics were pKa = 6.2, number average molecular mass, MWn = 125,000 g mol⁻¹, weight average molecular mass, MWw = 191,000 g mol⁻¹, and deacetylation percentage = 87%. Lead solutions were prepared by dilution of an atomic absorption standard solution (1 g Pb L⁻¹) supplied by Fluka (Germany). The metal was under the form of a nitrate-salt. The pH of the solutions was controlled with molar solutions of either sulfuric acid or sodium hydroxide. The ionic strength of the solution was adjusted by adding sodium nitrate at the final concentration 0.05 M.

2.2 Sorption procedure

For the study of the influence of the pH, 200 mL of solution containing 50 mg Pb L⁻¹ was mixed with 40 mg of sorbent for 72 hours. This contact time was considered sufficient for reaching equilibrium. The pH was not controlled during sorption but its final value was measured. Samples were filtered on a filter membrane (pore size: 1.2 µm). The residual lead concentration in the filtrate was analyzed using an ICP-AES equipment (Jobin Yvon 2000, Jobin-Yvon, France). Sorption isotherms were carried out on alginate, A. niger and A. nodosum. A fixed volume of solution (V: 200 mL), which pH was controlled with H₂SO₄ and NaOH solutions at different initial concentrations (C₀, mg Pb L⁻¹: 9, 22 and 46) was mixed for 72 hours with increasing sorbent masses m (10-40 mg). After filtration, the samples were analyzed using ICP-AES equipment for the determination of the residual metal concentration, C_eq (mg Pb L⁻¹) and the mass balance equation was used for the determination of lead concentration in the sorbent, q, mg Pb g⁻¹.
q = \frac{V}{m} (C_0 - C_{eq}). Sorption kinetics was determined using 1 L of solution at the concentration 25 mg Pb L\(^{-1}\) and 200 mg of sorbent. Samples were regularly collected, filtered and analyzed for lead content by ICP-AES.

3. RESULTS AND DISCUSSION

3.1 Influence of pH on lead sorption and selection of sorbents

Experiments were carried out at pH 3, 4, 5 and 6 with low metal concentrations to avoid precipitation artifacts. This part of the study served to select the best sorbents among the ten sorbents that were tested. Table 1 shows the change in the pH of the solution, the sorption efficiency and the sorption capacity.

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>pH3</th>
<th>SE</th>
<th>SC</th>
<th>pH4</th>
<th>SE</th>
<th>SC</th>
<th>pH5</th>
<th>SE</th>
<th>SC</th>
<th>pH6</th>
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</thead>
<tbody>
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<td>44.4</td>
<td>103</td>
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<td>58.0</td>
<td>129</td>
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<td>22</td>
<td>5.7</td>
<td>17.7</td>
<td>44</td>
<td>6.0</td>
<td>21.5</td>
<td>40</td>
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</tbody>
</table>

Table 1 shows pH variation for selected sorbents. The sorbents can be classified in 3 groups: (a) whose that systematically decreased the pH of the solution (P. chrysogenum; M. miehei); (b) whose that systematically increased the pH of the solution (chitosan, A. niger); and (c) whose that increased the pH when the initial pH was below 5-5.5 and decreased the pH for pH above pH 6 (R. arrhizus, R. conhii, A. nodosum, L. trabeculata, S. cerevisiae, alginate). Even in the same kind of sorbent (fungal, algae) the pH variations were not homogeneous in value and in trend, despite similar structures and compositions. In the case of industrial fungal biomass it may be explained by the treatments used for the inactivation of the microorganisms (thermal treatment versus chemical treatment) or by the presence of impurities (residues of flocculating and filtrating material). The greatest pH variations were observed with chitosan, especially at low initial pH.

The pH significantly influenced the sorption efficiency. However, the trends and the extent of the variation depended on the sorbent. In most cases, increasing the pH increased the sorption efficiency. In the case of A. niger and R. conhii, the sorption efficiency was almost independent of the pH, while in the case of alginate, the sorption efficiency tended to decrease with decreasing the initial pH. The best sorbents were A. niger, A. nodosum, R. conhii, M. miehei, and alginate. The optimum equilibrium pH was in most cases greater than pH 5, except for alginate. Indeed, in the case of alginate the sorption was much more efficient at acidic pH. Increasing the pH resulted in a decrease of the competition of protons with lead for sorption on the sorbents, while a mild pH was much more favorable to the chelation of lead by the ligands present on the biomass, and on amine functions of
chitosan. The greatest sorption capacities were obtained at around pH 5-5.5 for A. niger, A. nodosum, R. conhii, with sorption capacities as high as 100-150 mg Pb g\(^{-1}\) (0.5-0.75 mmol Pb g\(^{-1}\)). In the case of alginate the sorption exceeded 200 mg Pb g\(^{-1}\) (1 mmol Pb g\(^{-1}\)) at pH 3-3.5, but decreased at increasing the equilibrium pH. A. niger, A. nodosum and alginate have been selected for more complete sorption studies.

3.2 Sorption isotherms

Sorption isotherms have been carried out at pH 4 as the initial pH. The pH was controlled during the sorption. Figure 1 show lead sorption isotherms using A. niger and A. nodosum. The curves were modeled using the Langmuir (solid lines) and the Freundlich (dashed lines) equations:

**Langmuir equation:**

\[
q = \frac{q_m b C_{eq}}{1 + b C_{eq}}
\]

where \(q, q_m\) are the sorption capacity and the maximum sorption capacity at monolayer coverage, respectively (mg Pb g\(^{-1}\)), \(b\) (L mg\(^{-1}\)) is the sorption affinity (proportional to the initial slope of the sorption isotherm curve).

**Freundlich equation:**

\[
q = k_F C_{eq}^{1/n}
\]

where \(k_F\) and \(n\) are the parameters of the Freundlich model.

For both A. niger and A. nodosum, the best fit of experimental data was obtained with the Langmuir equation (Figure 1, and Table 2). Though the maximum sorption capacity and the affinity coefficient varied reciprocally for A. niger and A. nodosum, and the term \(q_m b\), (i.e. the initial slope of the sorption isotherm curve) was comparable for A. niger and A. nodosum, (i.e. 97.0 and 97.8 L g\(^{-1}\), respectively). These sorbents showed comparable sorption efficacy on the basis of equilibrium performance.

In the case of alginate it was impossible to fit the experimental data with the models. Indeed, a great dispersion of sorption capacities was observed with changing the mass of the sorbent and the initial metal concentration (Figure 2). At using low initial metal concentration, the sorption capacity was very low (below 25 mg Pb g\(^{-1}\)), while increasing the initial metal concentration, significantly increased the sorption capacity. However, for intermediary initial metal concentration (\(C_0\): 22 mg Pb L\(^{-1}\)), unexpectedly, the sorption capacity decreased with increasing the residual concentration. At the highest initial metal
concentration, again, the sorption capacity did not vary continuously with increasing the residual concentration.

Table 2. Lead sorption isotherms – Parameters of Langmuir and Freundlich models.

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Langmuir Model</th>
<th>Freundlich Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>q_m</td>
<td>b</td>
</tr>
<tr>
<td>A. nodosum</td>
<td>125.4</td>
<td>0.78</td>
</tr>
<tr>
<td>A. niger</td>
<td>161.7</td>
<td>0.60</td>
</tr>
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</table>

Figure 2. Lead sorption isotherm on alginate at pH 4 (both initial concentration C_o and sorbent dosage were varied to get the distribution of the metal between the two phases)

The experimental data were also worked on with plotting the sorption capacity and the sorption efficiency versus the sorbent dosage for each initial metal concentration (Figure 3, left and right panels, respectively). At low initial metal concentration, the sorption capacity and the sorption efficiency were not controlled by the sorbent dosage. At medium initial metal concentration, as expected increasing sorbent dosage resulted in a decrease in sorption capacity, however, increasing the mass of sorbent surprisingly decreased sorption efficiency. For the highest initial metal concentration, sorption capacity was almost constant at low sorbent dosage but surprisingly decreased at the highest sorbent dosage, while sorption efficiency reached a maximum when the sorbent dosage was 150 mg alginate L^{-1}. A first attempt has been made to explain this surprising behavior of alginate in relation with the dissolving of the biopolymer. The ability of alginate to sorb metal ions is well documented but in most cases research has focused on the use of pre-formed alginate bead (gelled with calcium chloride, for example) [21], a few studies also dealt on the direct coagulation of alginate in metal ion solutions [22]. It is possible to suggest that lead sorption occurs by a dual mechanism involving the chelation of lead on functional groups of the biopolymer and by a gelation of the biopolymer, lead acting as a cross-linking agent. This ionic cross-linking / ionotropic gelation mechanism has been described by Dambies et al. in the case of chitosan gelation using molybdate as the cross-linking agent [23].

When there was an excess of polymer compared with lead, the amount of metal was not high enough to cross-link alginate chains. The biopolymer dissolved in acidic media and it was not able to form a stable network. Lead cations may be chelated to dissolved polymer chains and then they cannot be removed at filtration and the sorption efficiency decreased. When the metal concentration increased, the cross-linking with lead ions was more efficient and the biopolymer gelled with a simultaneous immobilization of metal ions.
3.3 Sorption kinetics

Though the maximum sorption capacity and affinity are important parameters in the determination of the performance of a biosorption system, it is also necessary to take into account the uptake kinetics in the selection of the optimum sorbent. Figure 4 shows lead sorption kinetics for *A. niger* and *A. nodosum*, respectively. Equilibrium was reached after 4 hours of contact in the case of *A. nodosum*, while in the case of *A. niger*, more than 90% of the total sorption was reached within the first 4 hours of contact, the sorbent continued to sorb small amounts of lead even after 24 hours of contact. It may be due to different sorption mechanisms, to diffusion limitations or to differences in the location of metal sorption. Tsezos and Volesky [24] showed in the case of uranium sorption on fungal biomass that the uptake involved different mechanisms including chelation, but also local precipitation of metal ions (in the membrane). The occurrence of successive different sorption steps could explain a longer time to be required for reaching the equilibrium. The sorption can be restricted to the external layer of the sorbent or can occur in the whole membrane: in this case the time required to penetrate, diffuse and be adsorbed can be significantly increased.

Uptake kinetics may be controlled by several mechanisms including the intrinsic sorption rate, but also resistance to diffusion. There are different steps in the mass transfer of the solute from the solution to the sorption sites: (a) bulk diffusion; (b) film mass transfer resistance; and (c) intraparticle mass transfer resistance. Providing a sufficient agitation (stirrer speed, reactor geometry) allows neglecting the bulk diffusion as the limiting step. Film diffusion and intraparticle diffusion are actually the main controlling steps. Though the separation of these two steps has no physical significance, it is possible to assume, as a simplification, that the preliminary stage (first minutes of contact) is controlled by film diffusion resistance while the later stage is mainly controlled by intraparticle diffusion resistance.

In a first approximation, the sorption kinetics (within the first minutes) may be modeled by a first-order kinetic equation [25]; and the kinetic parameter $k_a$ (min⁻¹) can be obtained by:

$$\ln \left[ \frac{C(t)}{C_0} \right] = k_a \cdot t$$  \hspace{1cm} (3)
Puranik et al. [26-27] proposed a more sophisticated and appropriate model for the description of uptake kinetics under the following assumptions: (a) the particles are assumed to be spherical (uniformity in shape and size); (b) the bulk concentration of the solute is homogeneous in the reactor (correct mixing); (c) the intraparticle diffusion is negligible (sorption located at the surface of the sorbent); (d) instantaneous sorption at the surface of the particle (fast intrinsic sorption rate); (e) limited volume of solution; and (f) isothermal sorption mechanism described by either a Langmuir or a Freundlich equation. They used several equations, including the total mass balance equation:

\[ W_s \ q(t) + C(t) = C_o \]  

where \( W_s \) is the sorbent dosage (g L\(^{-1}\)), \( q \) is the sorption capacity (mg Pb g\(^{-1}\)), \( C(t) \) is the bulk lead concentration (mg Pb L\(^{-1}\)), and \( C_o \) the initial metal concentration (mg Pb L\(^{-1}\)).

The change in the bulk concentration is proportional to the driving force for the sorption at the surface of the particle:

\[ \frac{dC(t)}{dt} = -K_m a (C(t) - C_p(t)) \]  

where \( K_m \) is the external film mass transfer coefficient (m min\(^{-1}\)), \( a \) is the specific surface area of the sorbent particles per unit volume of reactor (m\(^2\) m\(^{-3}\)) and \( C_p \) is the concentration of the metal at the liquid/sorbent interface (mg Pb L\(^{-1}\)).

Combining the mass transfer resistance equation and the mass balance equation, they established the following equation:

\[ W_s \left( \frac{dq(t)}{dt} \right) = K_m a (C(t) - C_p(t)) \]  

The differentiation of the Langmuir equation applied for lead concentration at the interface \( C_p \) gave after simplification:

\[ \frac{dq(t)}{dt} = \frac{dC_p(t)}{dt} \left[ \frac{q_m b}{(1 + b C_p(t))^2} \right] \]  

The combination of this equation with the preceding equation gave after simplification:

\[ \frac{dC_p(t)}{dt} = \frac{K_m a}{W q_m b} \left[ (C(t) - C_p(t))(1 + b C_p(t))^2 \right] \]  

Using the dimensionless variables \( C^*(t) = C(t)/C_o \); and \( C_p^*(t) = C_p(t)/C_o \), the change of metal concentration in the bulk solution with time might satisfy the system of first ordinary differential equations:

\[ \frac{dC^*(t)}{dt} = -K_m a (C^*(t) - C_p^*(t)) \] \hspace{1cm} (9)

\[ \frac{dC_p^*(t)}{dt} = \frac{K_m a}{W q_m b} \left[ (C^*(t) - C_p^*(t))(1 + b C_p^*(t))^2 \right] \] \hspace{1cm} (10)

with the boundary conditions: \( C^*(t=0) = 1 \) and \( C_p^*(t=0) = 0 \) \hspace{1cm} (11)

This system has been solved using Mathematica® package for the modeling of kinetics using the Langmuir coefficients from Table 2. The sum of the square of residuals has been minimized in order to get the optimum \( K_m a \). Assuming that the sorbent particles were not porous, and that their (wet) density \( \rho \) (kg m\(^{-3}\)) was approximately 1050,
specific surface area of the sorbent particles per unit volume of reactor, \( a \), can be calculated according to:

\[
a = \frac{6 \text{SD}}{d_p \rho} \approx 18 \text{ m}^{-1}
\]

where SD is the sorbent dosage (g L\(^{-1}\)).

Figure 4 (left panel) shows the modeling of experimental data for \( A. \) nodosum and \( A. \) niger, the kinetic parameters are given in Table 3. The sorption kinetics is quite well described by the modeling, especially in the first part of the curve. In the final stage, the residual concentration was slightly overestimated for \( A. \) nodosum. It may be due to an underestimation of the sorption capacity deduced from the Langmuir parameters. In the case of \( A. \) niger the modeling was not so good: the final concentration was dramatically underestimated and the initial part of the curve was not perfectly described as it was with \( A. \) nodosum. The worst fit of experimental data by this model may be explained by inaccurate modeling hypotheses, and especially an overestimation of the contribution of intraparticle diffusion.

Crank proposed a model whereby diffusion is controlled only by intraparticle mass transfer for well-stirred solutions of limited volume (V), assuming the solute concentration to be always uniform (initially \( C_0 \)), the sorbent sphere to be free from solute, and the external diffusion to be negligible [28]. Under these conditions, the fractional approach to equilibrium (FATE) that is the total amount of solute \( M_t \) (mg g\(^{-1}\)) in a spherical particle after time \( t \) (min), expressed as a fraction of the corresponding quantity after infinite time (\( M_\infty \)) is given by:

\[
FATE = \frac{M(t)}{M(\infty)} = 1 - \sum_{i=1}^{\infty} \frac{6 \alpha(\alpha + 1)}{(9 + 9\alpha + q_n^2\alpha^2)} \text{Exp} \left( -\frac{Dq_n^2t}{d_p^2} \right)
\]

where \( D \) is the intraparticle diffusion coefficient (m\(^2\)min\(^{-1}\)). The fractional approach to equilibrium, FATE, may be used to estimate the intraparticle diffusion coefficient \( D \), when the external diffusion coefficient is neglected. \( \alpha \) is the effective volume ratio, expressed as a function of the equilibrium partition coefficient (solid/liquid concentration ratio) and is obtained by the ratio \( C_{eq}/C_0 - C_{eq} \). \( q_n \) represent the non-zero solutions of the equation:

\[
\tan q_n = \frac{3q_n}{3 + \alpha q_n^2}
\]

The infinite terms are summed until the summation does not vary. This equation was used to determine the overall intraparticle diffusivity which best fit experimental data - minimizing the sum of the square of the differences between experimental results and calculated data. Tien pointed out that this equation is only justified when the sorption isotherm can be approximated by a linear equation [29]. However this simplified model will be sufficient to approach coefficient \( D \). Figure 4 (right panel) shows that the equation fitted well experimental data, especially for \( A. \) nodosum. The simplified equation does not take into account the sorption capacity of the sorbent, the best fit of kinetic curves in the later stage of the sorption process was thus expectable. Intraparticle diffusion coefficients were of the same order of magnitude for \( A. \) nodosum and \( A. \) niger, close to \( 4 \times 10^{-12} \text{ m}^2\text{min}^{-1} \). The Biot number was very high (between 4500 and 15000), indicating that film resistance is negligible for these sorbents.
Figure 4. Lead sorption kinetics on *A. niger* and *A. nodosum* at pH 4 (curves: modeling with external diffusion resistance model (left) and intraparticle diffusion resistance model (right))

Table 3. Lead sorption kinetics – Parameters of the kinetic models (K_m, min^{-1}; K_m, m/min; D, m^2/min) and Biot number (dimensionless)

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>First-order equation &amp; Film Resistance</th>
<th>Crank equation (Intraparticle Diffusion)</th>
<th>Biot number B_i = K_m d_p/D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K_m a* 10^3</td>
<td>K_m * 10^3</td>
<td>SSR D * 10^{12}</td>
</tr>
<tr>
<td><em>A. nod.</em></td>
<td>22.35</td>
<td>1.22</td>
<td>0.081</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>5.09</td>
<td>0.278</td>
<td>0.287</td>
</tr>
</tbody>
</table>

SSR: Sum of Square Residuals: \[ \Sigma_{i=1,n}[(f(t_i)_{exp.}-f(t_i)_{calc.})^2], \] n is the number of experimental points, \( f(t_i)_{exp.} \) and \( f(t_i)_{calc.} \) are transforms of lead concentrations at time \( t_i \) on experimental data and modeled data, respectively.

4. CONCLUSIONS

*A. niger* and *A. nodosum* were selected for their high sorption for lead recovery from acidic solutions (around pH 4) with sorption capacities as high as 0.6-0.7 mmol Pb g^{-1}. Simple models were used for modeling sorption kinetics and evaluate the order of magnitude for mass transfer coefficients for external and intraparticle diffusion. The Biot numbers show that film diffusion can be considered as negligible. Alginate was also characterized by high sorption capacities (about 1.6-1.7 mmol Pb g^{-1}) when using high lead concentrations: it allows the gelification of the biopolymer. At low metal concentration, the amount of lead ions is not sufficient to maintain the stability of the polymer that dissolves in water and the sorption capacity strongly decreases.

REFERENCES

Regeneration of biomass after sorption of heavy metals

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Abstract

The work takes into consideration caption of heavy metals carried by freshwaters contaminated by effluents coming from industrial plants. In the case of low metal concentration and high flow of water stream is very expensive to adopt decontamination methods based on chemical precipitation or ion exchanging. Heavy metal biosorption by non-living biomass appears to be an effective technology for caption of dissolved metals, especially in very diluted streams. Considering a processing approach, the aim of this study is to verify the sorption potential of non-living biomass coming from marine seaweed. Influence of pH on sorption kinetics has been assessed before testing the desorption behaviour of cached metals by the biomass. Batch experiments were carried out bringing into contact 1 g/l of non-living biomass with a metal solution bearing a set of heavy metals. A contact period of 90 minutes between the biomass and a solution, containing Cd, Zn, Pb, Cu and Ni (10 mg/l each) + Hg (1 mg/l Hg), has been assured. It has been ascertained that the biomass extracts on average 80% of total metal content. Since the concentration of sorbed Hg was found to be well correlated to that of the other sorbed ions ($r^2 = 0.75$), mercury was assumed as an indicator of the process in sorption/desorption tests. Results show that fast sorption/de-sorption reactions occur between biomass and heavy metals solved in water. After 15 minutes contact with the metal solution (pH = 5-7), the biomass extracted on average 70% of the initial Hg concentration. After a contact period of just 5 minutes (by washing with HCl solution at pH = 2) more than 50% of sorbed Hg has been recovered. This regeneration procedure shows to be effective and the biomass can be reused for new cycles of sorption.

1. INTRODUCTION

Industrial, mineral and metallurgical processes produce large amount of heavy-metal-bearing effluents that are among the most dangerous contaminants, as they are persistent in the environment and bio-available. Mobilised metals tend to persist indefinitely in the environment, circulating and finally accumulating in the food chain, setting so a serious threat to flora, fauna and, eventually, humans.

Interest in developing a system able to reduce toxic metal concentration in waters and soils is therefore increasing.

In the case of low metal concentration and high flow of water stream is very expensive to adopt decontamination methods based on chemical precipitation or ion exchanging.
Biological treatment (based on living organisms) is a non-expensive option for effluent decontamination from low metal concentration, but it is highly affected by water temperature, pH, chemical and nutrient composition.

The use of non-living biomass (biosorption) has been widely studied in the last years. It does not require nourishing and it does not suffer from toxicity problems [5-6, 12-15].

Since non-living biomass behaves as an ion-exchange resin ([8, 16]), heavy metal bio-sorption appears to be an effective technology for treatment of dissolved metals, especially in very diluted streams. It can be applied not only to remove toxic or radioactive metals, but also to recover precious metals. For instance, many kinds of seaweed and the microbial waste, coming from fermentation processes, are abundant and economic sources of biomass.

The advantages of the bio-sorption are:
− use of low cost materials to extract metals from solution;
− ability to purify very diluted effluents and to concentrate pollutants in a small quantity of contaminated biomass.

The biomaterial can be disposed either as a toxic refuse or after incineration and eventually after metal extraction or after desorption to collect metals into a small volume liquor, that can be treated by traditional methods.

Although heavy metal bio-sorption has been extensively studied in last few years, little attention has been paid to bio-sorption of multi-component systems. In fact, evaluation of experimental results is more difficult in presence of more than one metal in the system (although it is a condition more similar to real systems): the interactions between dissolved ions and biomass surface, which has a limited number of binding sites, plays a determinant role [15].

In this work the heavy-metals uptake properties of a vegetal biomass (green algae and leaves of marine macro-phytes), coming from a very polluted wetland, were investigated with a process viewpoint.

The biomass was put in contact with a solution containing a set of metal nitrates (10 mg/l of Cd, Zn, Pb, Cu and Ni + 1 mg/l of Hg in distilled water) chosen because of their relevant presence in the wetland ad their accumulation in the living biomass [9].

2. EXPERIMENTAL

2.1 Seaweed collection

The biomass was collected on the shore of the wetland of Boi Cerbus, in south-west Sardinia (Italy), where former studies [3] and [10] found heavy contamination of the environment coming from mining and metallurgical plants. Heavy metals contamination is mainly present in sediments due to precipitation induced by the high pH (around 9) of the wetland salt water.

Collected biomass is made up of a green alga and, mainly, of leaves of marine seagrasses (Cymodocea, Zostera and Ruppia). Material was washed in tap and distilled water. All samples were dried for 15 days at room temperature (18±22°C) and then oven dried at 50°C for 24 h. The final dry weight of the biomass was about 9% of the wet one. Dried samples were ground by a blade mill (RETSCH SM2000) equipped with 4 mm outlet screen.
2.2 Experimental procedure

Sorption experiments (batch) were carried out using a solution containing metal nitrates (10 mg/l each of Cd, Zn, Pb, Cu and Ni + 1 mg/l of Hg) in distilled water.

pH was adjusted using HNO₃ and NaOH. This solution was brought into contact with 1 g/l of dry seaweed biomass and kept in agitation.

At the end of the contact period, samples were filtered and the resulting solutions were analysed for metal content. Filtered biomass samples were preserved for the desorption step.

De-sorption batch experiments were performed on the biomass coming from the sorption tests, using diluted HCl (pH adjusted by NaOH). The solution was kept agitated for a fixed contact period, and then filtered. The resulting solutions were analysed for metal content.

2.3 Metal analyses

The analyses of the metal content of Zn, Pb, Cd, Ni and Cu in the water samples were performed by using Inductively Coupled Plasma-mass Spectrometry.

The Hg content in all the experiments was analysed by AMA 254 model Atomic Absorption Spectrometer (λ = 253.65 nm).

All the analyses were carried out on liquid samples. The difference between initial metal concentration and the remaining concentration after each step of the experiments was assumed to be bound to biomass.

2.4 Influence of pH on sorption and desorption

To evaluate the influence of pH on sorption kinetics, a first set of experiments was performed at initial pH adjusted up to 5, 6, 7 and 9. For each pH value, 1,000 cm³ of metal solution were added to 1,000 mg of biomass into a magnetically stirred beaker. After 90 minutes, samples were filtered and the separated solutions were analysed for metal ions. Filtered biomass samples were conserved for the de-sorption step. In order to remove the bound metal ions from the biomaterial, filtered biomass samples were added to 1,000 cm³ of HCl solution at pH = 2 and magnetically stirred in a beaker. After 60 minutes, the samples were filtered and the separated solutions were analysed for metal content.

In the sorption tests, the concentration of sorbed Hg was found to be well correlated to that of the other sorbed ions (r² = 0.75). Therefore Hg content was assumed as an indicator of the sorption behaviour and in the next experiments the analyses were performed taking into consideration only the Hg content in the waters (notwithstanding the solution was contaminated by the set of heavy metals, as previous described).

2.6 Kinetics

Sorption experiments were performed at pH = 6, adding 500 mg of biomass to 500 cm³ of metal solution in a magnetically stirred beaker. The solution was sampled (3 cm³) after 5, 10, 15, 20, 30, 45, 60, 75 and 90 minutes from starting. The samples were filtered and the resulting solutions were analysed for Hg content. Filtered biomass samples were preserved for the de-sorption step.

In order to evaluate the de-sorption kinetic, a preliminary sorption step, under the previous described conditions for just 15 minutes contact, was carried out. 100 mg of the filtered biomass, coming from the sorption test, was added to 100 ml of HCl solution at
Biosorption

pH = 2: four samples (2 cm$^3$) were taken after 5, 15, 30 and 60 minutes after starting the desorption experiment. Water samples were filtered and analysed for Hg content.

2.7 Sorption/desorption cycles

In order to investigate the possibility to reuse the biomass for new sorption cycles, a set of experiments including 3 cycles of sorption and de-sorption was carried out. Every sorption step was performed adding 100 mg of biomass to 100 cm$^3$ of the polluted solution initially adjusted at the pH 5, 7 or 9, as previously reported. After 15 minutes rocking the biomass was filtered and separated.

Every de-sorption step was performed adding the filtered biomass to 100 cm$^3$ of HCl solution at pH = 2. After 5 minutes rocking, samples were filtered and the separated biomass preserved for a new sorption step. All the filtered solutions were analysed for Hg content.

Table 1. Overview of the batch experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sample</th>
<th>Initial pH</th>
<th>Contact time (min)</th>
<th>Analysed metal ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorption</td>
<td>1</td>
<td>5, 6, 7, 9</td>
<td>90</td>
<td>Hg, Zn, Pb, Cd, Cu, Ni</td>
</tr>
<tr>
<td>De-sorption</td>
<td>1</td>
<td>2</td>
<td>60</td>
<td>Hg, Zn, Pb, Cd, Cu, Ni</td>
</tr>
<tr>
<td>Sorption kinetic</td>
<td>2</td>
<td>6</td>
<td>90</td>
<td>Hg</td>
</tr>
<tr>
<td>De-sorption kinetic</td>
<td>3</td>
<td>2</td>
<td>60</td>
<td>Hg</td>
</tr>
<tr>
<td>Sorption cycle</td>
<td>4, 5, 6</td>
<td>5, 7, 9</td>
<td>15 + 15 + 15</td>
<td>Hg</td>
</tr>
<tr>
<td>De-sorption cycle</td>
<td>4, 5, 6</td>
<td>2</td>
<td>5 + 5 + 5</td>
<td>Hg</td>
</tr>
</tbody>
</table>

3. RESULTS AND DISCUSSION

Binding of Zn, Cd, Pb, Cu, Ni and Hg on dry seaweed is plotted in Figure 1 as a function of pH.

Sorption occurs in a very short time as a confirmation of previous works ([4, 11]) reporting that the metal (Cd, Zn, Pb and Hg) absorption equilibrium on vegetable biomass was reached in less than 80 minutes.

It can be observed that sorption behaviour appears to be efficient at pH 5÷6.

As a confrontation, sorption behaviour was performed also at pH = 9, which is the pH value of the contaminated seawater close to the site, where the seaweed were collected.

At a pH between 5 and 7 the mean sorption capacity is the 80% of total metal content: from about 70% for Ni and Hg to almost 100% for Pb.

De-sorption of the loaded biomass permits 70% recovery of bound metals. Collected seaweed shows special affinity with lead, which is the meaningful contaminant of the collected seaweed; furthermore lead is strongly bound to the biomass as resulting from sorption/de-sorption experiments shown in Table 2.

The experiments carried out at pH = 9 showed a relevant reduction of metals into the solution, probably due to their precipitation, improving the extraction of metals by the biomass. At least 40% of the total metal content is bound onto the biomass, as it can be appreciated from balance of metals coming into the leaching (HCl) solution after de-sorption at pH = 2 for 60 minutes (see Fig. 2).

At Hg concentration of 1 mg/l, in the range of pH 5÷9, activity-pH diagrams for Hg(II) species [7] show that mercury is present in solution as Hg(OH)$_2$, available for sorption onto biomass. During the leaching step by HCl solution, as the pH value
Biosorption decreases, all the Hg(II) species [HgCl\textsubscript{3}, HgCl\textsubscript{2}\textsuperscript{2-}, HgCl\textsuperscript{+}, and Hg(OH)\textsubscript{2}] appear to be soluble.

Table 2. Natural concentration, metal binding capacity (pH = 6) of the marine seaweed used for batch experiments. Metal recoveries (%) by HCl solution at pH=2 (from desorption experiments) are also shown

<table>
<thead>
<tr>
<th>Metals</th>
<th>Natural concentration into collected biomass (mg/g)</th>
<th>Binding capacity (mg/g) after 90 min at pH = 6</th>
<th>Metal recoveries (%) after 60 min desorption at pH = 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>&lt; 20.0</td>
<td>8.7</td>
<td>78.0</td>
</tr>
<tr>
<td>Zn</td>
<td>263.0</td>
<td>8.2</td>
<td>72.2</td>
</tr>
<tr>
<td>Pb</td>
<td>1011.0</td>
<td>10.9</td>
<td>44.7</td>
</tr>
<tr>
<td>Cu</td>
<td>65.0</td>
<td>9.7</td>
<td>78.2</td>
</tr>
<tr>
<td>Ni</td>
<td>35.0</td>
<td>7.5</td>
<td>68.9</td>
</tr>
<tr>
<td>Hg</td>
<td>0.1</td>
<td>0.8</td>
<td>51.4</td>
</tr>
</tbody>
</table>

Figure 1. Influence of pH on heavy metal sorption by seaweed after simultaneous feeding. Biomass (1 g/l) was stirred for 90 minutes with distilled water containing 10 mg/l of Pb, Zn, Cd, Cu and Ni + 1 mg/l of Hg as nitrates

Figure 2. Influence of sorption pH on de-sorption of heavy metals by seaweed. Biomass was stirred for 60 minutes with distilled water containing HCl (pH = 2)
In such a kind of systems metal-speciation at different pHs is very complex [1]; nevertheless Hg content has been assumed as an indicator of the sorption behaviour of the metal set, since sorption tests showed an evident correlation between Hg content and the other sorbed ions, in every tested condition. From the statistical point of view metal speciation appears to be not relevant and the adoption of Hg as an indicator shorts the time required for analyses. For this reason, even if all batch experiments were performed using solutions bearing all the metals, as previously reported, only Hg analyses were carried out on the filtered solutions coming from the sorption/de-sorption tests.

Kinetic batch experiments showed that 70% of the initial Hg content was sorbed after 15 minutes, covering 84% of the sorption capacity of the biomass achieved after 90 minutes of contact with the polluted solution (Figure 3). Metal de-sorption by HCl solution (pH = 2) for 5 minutes is enough to recover 90% of the previously bound Hg (see Figure 4).

Results of sorption/de-sorption experiments are shown in Figure 5. Contact periods of 15 minutes and 5 minutes are adopted respectively for sorption and de-sorption experiments; the cycle was repeated three times.

Results show the possibility to regenerate the biomass with HCl solution, even if the metal uptake capacity progressively decreases.

![Figure 3](image1.png)  ![Figure 4](image2.png)  ![Figure 5](image3.png)

**Figure 3.** Sorbed Hg (mg/g of biomass) (○) and pH (●) as a function of the sorption time

**Figure 4.** Time dependency of de-sorption (initial pH = 2) of the metal loaded biomass

**Figure 5.** Hg (%) sorbed on biomass after 3 cycles of sorption (S) (15 minutes contact with the polluted solution) and de-sorption (D) (5 minutes contact with the HCl solution)
4. CONCLUSIONS

Results of batch experiments show that fast sorption/de-sorption reactions occur between biomass and heavy metals (Cd, Zn, Pb, Cu, Ni and Hg) solved in water. The total metal-uptake capacity of the biomass has been found up to 0.54 mMol/g dry weight.

After a contact period of 90 minutes in a metal solution (10 mg/l of Cd, Zn, Pb, Cu and Ni + 1 mg/l of Hg) at pH between 5 and 9, the biomass extracts on average 80% of total metal content.

Since the concentration of sorbed Hg was found to be well correlated to that of the other sorbed ions ($r^2 = 0.75$) mercury was assumed as an indicator of the process in sorption/de-sorption tests.

After 15 minutes contact with the metal solution (pH = 5÷7), the seaweed biomass extracts on average 70% of the initial Hg concentration. After a desorption time of just 5 minutes with HCl solution at pH = 2 it allows to recover more than 50% of the sorbed Hg.

Regeneration of the biomass with HCl solution at pH = 2 shows to be effective and the biomass can be reused for more cycles.

This study, carried out on a laboratory-batch scale, is the first step of an experimental series devoted to point out a continuous flow sorption/de-sorption system.

Treating contaminated waters, intercepted before their influx into the water bodies, can prevent contamination of the recipient environment by heavy metals.

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Structural modeling of arsenic biosorption using X-Ray spectroscopy (XAS)

Mônica Cristina Teixeira, Graziele Duarte and Virgínia S.T. Ciminelli

Abstract

The work describes a biological route for direct immobilization of aqueous As(III) species, which is the most toxic and mobile arsenic species found in soils. Based upon the biochemical mechanisms which explain arsenic toxicity, we propose that waste biomass with a high fibrous protein content can be used for As(III) immobilisation. Our investigations demonstrated that As(III) is specifically adsorbed on the biomass and, contrary to the behaviour observed with inorganic sorbents, the lower the pH the more effective the removal. Arsenic uptake reaches values of up to 20 mg As(III)/g of biomass. Analyses by Synchrotron light techniques, such as XANES, demonstrated that arsenic is immobilised in its trivalent state, an advantage over the conventional techniques for As immobilisation, which usually require a previous oxidation stage. EXAFS analyses shows that each As atom is directly bound to three S atoms with an estimated distance of 2.26 Å. The uptake mechanism is explained in terms of the structural similarities between the As(III)-biomass complex structure and that of arsenite ions and Ars operon system encoded proteins and phytochelatins. The biological route presented here offers the perspective of a direct removal of arsenic in its reduced form.

Keywords: arsenite, sorption, bioremediation, EXAFS

1. INTRODUCTION

Arsenic and its compounds, the most usual being that of the water-soluble derived from arsenous (H₃AsO₃) and arsenic (H₃AsO₄) acids, are toxic and carcinogenic to all living organisms. The trivalent species is of great environmental concern in view of its considerably higher toxicity and mobility in soils. Recent disasters involving cases of poisoning have spawned a series of worldwide investigations towards As(III) immobilisation. Despite all work undertaken, there still remains a great lack of knowledge as regards arsenic sorption in general, especially with reference to the trivalent species.
Arsenic is found throughout the earth’s crust in small concentrations, arsenopyrite (FeAsS) being the most common arsenic mineral. In soils, As concentration may vary from 1.0 mg/Kg (apatite, fluorite, and calcite samples) to 77,000-126,000 mg/Kg (pyrite or arsenopyrite samples) [1]. Normal arsenic concentration found in soils is 6 mg/Kg [2]. Small amounts of arsenic and its compounds are used by the chemical and electronic industries to produce electronic components for laser equipment, wood preservatives, pesticides, and glasses, to name but a few of the numerous applications. Despite the many useful applications, there is a surplus of arsenic found in wastes, derived mainly from the mineral and metallurgical industries. The natural leaching of As-enriched soils and rocks for human water supplies, is yet another cause of human contamination, a good example being that of Bangladesh. Much attention has been paid to environmental contamination caused by heavy metals. Nevertheless, the understanding of the biochemical mechanisms, which are responsible for these elements’ toxicity, has not received due consideration. The toxicity of arsenic and its compounds is well established. Once ingested, arsenic provokes nausea and gastro-intestinal symptoms. The long-term exposure to this element causes skin problems like dermatitis, keratosis and cancer. From the toxicological point of view As(V) causes adverse effects to human and others living organisms due to its chemical similarity with phosphate [3, 4]. In this case, arsenic poisoning could be reverted by the administration of an excess of phosphate. The trivalent specie As(III) strongly binds to the sulphidryl (SH) active sites of some enzymes causing irreversible metabolic impairments and cellular mutagenesis [5, 6].

The wastewater produced by mining and metallurgical industries is a very important source of arsenic contamination. The conventional techniques used for As immobilisation usually require a previous oxidation stage in order to oxidize the more toxic and mobile trivalent arsenic species [7] to the pentavalent species. In the pentavalent state, arsenic acid (H$_3$AsO$_4$) species form stable complexes with soils constituents containing ferric, manganese or aluminium oxy-hydroxides, such as goethite, alumina, hematite, birnessite and gibbsite [1, 8-11]. This could explain its lower environmental mobility. On the other hand, the trivalent arsenous acid (H$_3$AsO$_3$) species are weakly bound to inorganic sorbents regardless of the pH uptake. To achieve immobilization, it is often necessary to oxidize As(III) ions to As(V).

Biosorption is usually based on unspecific ion exchange mechanisms. For instance, positively charged chemical groups present in the biomass, like the amino group, are capable binding to negatively charged ions like arsenate, arsenite, chromate, sulphate or phosphate. The poor selectivity associated with unspecific ion exchange mechanism is probably one of the main limitations for applications to complex, multicomponent systems, which characterise real systems. Arsenic binding to sulphidryl groups available in some enzymes is irreversible and very specific, which explain arsenic toxicity. This strong affinity has led us to believe that waste biomasses with a high content of fibrous protein could be used for As immobilisation. This hypothesis was tested with a waste material produced by the poultry industry. Based on the results, we present, for the first time, a highly specific biosorbent for As (III) immobilisation. This specificity is explained by the molecular structure of the adsorbed complex determined by Syncrotron light-X-Ray Spectroscopy analyses.

2. MATERIALS AND METHODS

The biomass was prepared under laboratory conditions. White chicken feathers were rinsed exhaustively with warm tap water and dried at 45 ±5°C for 24 hours. The dried material was ground and sieved to obtain a size range below 0.037 mm (400 Mesh Tyler).
Biosorption

Biomass activation was accomplished by adding a basic ammonium thioglycolate solution to the final concentration of 0.78 g/L. Treatment did not imply any mass loss. After this activation step, powdered biomass was filtered and used in the adsorption tests. All chemicals used were of analytical grade. Water was first deionised and then ultrafiltered before being used to prepare the solutions. As(III) stock solutions of 10,000 mg/L were made from AsNaO₂ (Fluka, 99.0%). The pH values were adjusted with 0.1 N HCl or NaOH solutions. Ionic strength (I) was controlled using 4 M NaCl or 0.01 M Na₃PO₄ electrolyte solutions. As(III) batch adsorption experiments were conducted at room temperature (28±3°C), by adding a known amount of biosorbent (1-10 mg/L) to each 250-ml Erlenmeyer flask containing the metal solution (100 mL). Flasks were shaken (100 rpm) for 1 hour to achieve equilibrium. As(III) semi-continuous adsorption experiments were undertaken at constant temperature (25±0.2°C) using an apparatus similar to that described by Pagnanelli et al. [12], and the same proposed procedure called "Subsequent Additions Method" (SAM). The liquid volume in the reactor was 1,000 mL, biosorbent concentration was 2 g/L, and agitation and pH values were kept constant. One hour after each metal addition, the system reaches equilibrium and a 10 mL sample is collected and analysed. Reaction suspensions were filtered through a 0.45 µm cellulose membrane, and preserved with concentrated nitric acid (5 µL) for chemical analyses. Experiments were carried out in duplicate and results were averaged.

X-Ray Absorption Near Edge Structure (XANES) and Extended X-Ray Absorption Fine Structure (EXAFS) analyses of humid biomass samples loaded with As(III) were performed using the synchrotron facilities at the Laboratório Nacional de Luz Synchrotron (LNLS), in Campinas, São Paulo. XANES and EXAFS data from the arsenic K edge (11,868 eV) were obtained at XAS workstation using beam currents of about 200 mA. All spectra were recorded at room temperature, using a Si(111) double crystal monochromator with an upstream vertical aperture of 0.6 mm. As K-edge X-ray absorption spectra were measured by monitoring the transmitted energy using a 15-element Ge detector (Canberra Industries). The energy resolution utilised were 0.8 eV at the XANES region located near the As K-edge (11855-11930 eV); 2 eV at the regions located between 11760 and 11855 eV, and 11930 and 12400 eV and 3 eV at 12400-13000 eV region. This procedure allowed us to obtain XANES and EXAFS spectra simultaneously. Counting times of 3 s were kept constant. XANES spectra were analysed using Origin 5.0 software and EXAFS collected data were analysed by using Winxas 97 software. EXAFS data fit was obtained using phase and amplitude parameters calculated with FEFF 6.01 software.

3. RESULTS AND DISCUSSION

Preliminary experimental results related to As(III) adsorption by the selected biomass are shown in Figure 1. Arsenite biosorption equilibria are achieved in less than 10 min for all experimental conditions. Results of tests performed with biomass prior to activation led to a negligible As uptake, a finding that supports the hypothesis that the reduced sulphidyl groups are responsible for As(III) adsorption.

The milling process seems to negatively contribute to As uptake. Nevertheless, all the subsequent experimental tests were performed using ground material in order to favor biosorbent’s homogeneity.

Loading capacities (8-13 mg As(III)/g of biomass) are promising and greater than those values obtained by using kaolinite and montmorillonite, 0.1 and 0.2 mg/L, respectively [13]; alumina, 0.2 mg/L [9]; goethite, 3.0 mg/L [8]. Ladeira et al. [11] reports significantly higher uptakes using thermally activated gibbsite, 25.4 mg/L. Driehaus et al. [10] and Meng et al. [14] also obtained higher arsenic adsorption capacities (20-40 mg/L)
but, in both cases, As(III) was previously oxidized to As(V). The very high value (69.2 mg of As(III) per each gram of Mo) reported by Dambies et al. [15], using a chitosan derivative biosorbent impregnated with molybdenum, can not be compared to the other results in view of the lack of information with regard to the quantities (mass) of biosorbent.

Figure 1. Milling and biomass concentration influence on As(III) uptake. Flask tests, As(III) initial concentration, 100mg/L; initial pH, 9.2; temperature, 28±3°C; pretreatment, 2h

The influence of pH on As(III) biosorption can be observed in Figure 2. The obtained results show that the lower the pH, the higher the uptake. This trend is just the opposite of that observed in As(III) uptake by inorganic sorbents, for which uptake increases with pH. During all the adsorption experiments, pH variation was less than 0.2 units. Taking into account that the pKa1 for arsenous acid is 9.2, experiments were performed at pH no higher than 8, i.e. 2.0, 5.0 and 8.0, in order to avoid or minimise the formation of anionic H₃AsO₃ species. Arsine formation during sorption experiments carried out at pH 2.0 was experimentally investigated and discarded (data not shown). A pH of 5.0 was chosen for all the subsequent experiments, as this pH value is consistent with conditions found in natural wastewater or industrial effluents.

The results found in Figure 3 are most interesting. The great majority of As sorbents described in literature are active for both the pentavalent and the trivalent species, the main difference being the relatively higher mobility of the latter. The specificity of the proposed biosorbent towards As(III) is combined with a rejection of both phosphate species (Figure 3) and arsenate (not shown here) species.

The well-described competition between arsenic and phosphate during sorptive experiments using biosorbents or resins [15-18] is not observed when the fibrous protein rich biomass is utilised. Both phosphate and arsenate molecules have the same tetraedric geometry which could explain their chemical similarity and their similar affinities for the same chemical ligands. Conversely, arsenite ion possesses a trigonal pyramidal geometry. It is possible that a steric hindrance may contribute to the rejection of the tetraedric arsenate and phosphate oxyanions.
Figure 2. Influence of pH on As(III) adsorption isotherms, SAM procedure (biomass, 2g/L; temperature, 25±1°C)

Figure 3. Influence of phosphate ions on As(III) adsorption isotherms, SAM procedure (I = 0.1; pH=5; biomass, 2g/L; temperature, 25±1°C)

X-Ray Spectroscopy analyses, X-Ray Absorption Near Edge Structure (XANES) and Extended X-Ray Absorption Fine Structure (EXAFS), may be considered state of the art when investigating adsorption at molecular levels. These are modern and very precise tools, which provide information that could not be otherwise obtained through the traditional surface analyses techniques. Among the techniques described in the present work, XANES spectra offer electronic and structural information, such as oxidation state, with regard to adsorbed ion (photoabsorbing ion). EXAFS provides information, e.g. the
coordination number and interatomic distance, about the nature and position of the ligand atoms in the coordination shell of the photoabsorbing ion (scattering atom).

The procedure involved in the analyses of the EXAFS data from arsenite-loaded biomass is illustrated in Figures 4 to 6. The As(III)-biomass EXAFS spectrum is presented in Figure 4. The averaged data from seven different spectra were converted to eV energy unit and had its background line extracted. The experimentally obtained K edge value ($E_0$) was 11,868 eV, the same obtained for the arsenite standard sample, confirming that arsenite was not oxidized by the biomass. XANES spectra (data not shown) confirmed this value and the trivalent state of arsenic atoms. The spectrum oscillations caused by all the atoms in the neighbouring coordination shells are also presented. This spectrum was submitted to Fourier Transform, thus allowing the identification of one amplified peak that corresponds to the Arsenic first coordination shell (Figure 5).

![EXAFS signal after background correction](image1)

**Figure 4. EXAFS signal after background correction**

![Fourier Transform amplitude (K=3)](image2)

**Figure 5. Fourier Transform amplitude (K=3)**
The signal obtained after submitting this data to another Fourier Transform treatment results in one spectrum that represents only the oscillations caused by the atoms in the As first coordination shell. At this point, it is possible to calculate the structural parameters such as interatomic distance between As and atoms in the first coordination shell, coordination number as well as to identify the "first neighbour" ligand. By adjusting the experimental data with the theoretical model [18] provided by the FEFF program (Figure 6) it was possible to confirm that arsenic is the scattering atom while sulphur is the retro-scattering atom. It was also possible to affirm that each arsenic atom is bound to three sulphur atoms. The final structural parameters obtained in the analyses were coordination number \( (n) = 2.5 \pm 0.4 \) and interatomic distance \( (R) = 2.26 \pm 0.01 \) Å.

![Figure 6. Back Fourier Transform, first coordination shell. Experimental and theoretical curves \((R=2.26\pm0.01 \text{ Å}, n=2.5\pm0.4)\).](image)

The structural parameters obtained during this work are quite different from those obtained by arsenic adsorption on inorganic matrices. As arsenic is adsorbed as an oxyanion, usually as a bidentate binuclear complex, the element found in the first coordination shell is always oxygen, with coordination numbers \((n)\) varying from 3.6-4 and interatomic \((R)\) distances in a range of 1.72 to 1.78 Å. The metal ligands (Fe or Al) are found in the second coordination shell with \(R\) values often greater than 3.0 Å [19-20]. The coordination number and interatomic distance obtained in the present study are, as expected, very similar to those reported in EXAFS analyses of biological As(III)/protein complexes, showing As atoms directly bound to S atoms in the first coordination shell. Each As atom is bound to the sulphur atoms coming from three different cysteine residues, \(R\) values vary from 2.20-2.25 Å [21-25]. The information provided by XAS analyses is consistent with the strong arsenic uptake reported here. The results explain that rather than adsorbed as a counter ion, or specifically adsorbed as arsenous species in the inner Helmholtz plane, As(III) undergoes a chemical reaction leading to dehydration of \(\text{H}_3\text{AsO}_3\) molecule. Based on these findings we propose the following equation to describe arsenite immobilization by fibrous protein biomass:

\[
\text{H}_3\text{AsO}_3 + 3\text{B-CysSH} \rightarrow \text{As(B-CysS)}_3 + 3\text{H}_2\text{O} \tag{1}
\]

where \(\text{B}\) represents the biomass matrix.

This adsorption mechanism is supported by the structural similarities between As(III)/biomass complex and those natural complexes formed between Arsenic atoms and
Ars Operon proteins [21, 22, 24-26], phytochelatins [23,27] or cysteine and glutathione [4, 24, 28, 29], previously identified.

Finally, it may be stressed that the immobilization phenomenon studied here involves a chemical reaction between the dissolved arsenite ions and the sulphidryl groups of biomass, strong enough to dislocate oxygen atoms from the arsenic atom first coordination shell in arsenous acid molecules. The facts explain the specificity of this tested biosorbent by the trivalent species of arsenic as well as its minor affinity for phosphate and arsenate ions.

4. CONCLUSIONS

A waste biomass with a high content of fibrous protein was shown to specifically sorb arsenic in the trivalent state, therefore dispensing with the need of a previous oxidation treatment. Sulphidyl reduced groups are shown to be the active groups involved in the arsenic biosorption. Uptake increases as pH decreases; phosphate ions do not compete with arsenite ions for the biomass’ active sites. The uptake involves an inner sphere complexation phenomenon that takes place inside the arsenic first coordination shell, with the release of water and arsenic being directly bound to the sulphidyl group. XAS analyses indicated that each arsenic atom is bound directly to three sulphur atoms from the reduced cysteine aminoacids. The arsenic/sulphur interatomic distance was found to be 2.26±0.01 Å.

ACKNOWLEDGEMENTS

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REFERENCES

Biosorption


Uranium and thorium removal by a *Pseudomonas* biomass: sorption equilibrium and mechanism of metal binding

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Abstract

A *Pseudomonas* strain was characterized to develop biosorbent for removal of uranium and thorium from nuclear waste streams. The lyophilized bacterial biomass was found extremely good for U and Th uptake. 100% of added U and Th were removed by the biomass up to an initial concentration of 100 mg l⁻¹. Actinide biosorption was rapid, as well as a high affinity and efficient process, being optimum at pH 4-5, with a maximum loading capacity of 540 mg U g⁻¹ and 430 mg Th g⁻¹ at equilibrium. Bacterial cells grown in peptone rich medium or in minimal medium showed no significant difference in U accumulation at low U concentration (100 mg l⁻¹). However, at high concentration range (1000 mg l⁻¹) minimal medium grown cells showed a significantly high metal loading. Experimental sorption data showed good conformity to Langmuir model suggesting a monolayered metal binding process. Sorption in presence of several interfering cations and anions indicates a specific U and Th binding by the biomass with significant antagonism offered only by iron (III). Transmission electron microscopy (TEM) and Energy Dispersive X ray analyses (EDXA) of metal loaded biomass revealed an intracellular U and Th sequestration possibly via an ion exchange mechanism. Nuclear Magnetic Resonance (NMR) studies showed the role of cellular phosphoryl groups in radionuclide binding. Such observations were also confirmed from X ray diffraction (XRD) patterns of the metal loaded biomass that revealed the phosphide nature of sequestered U and Th. More than 90% of biomass bound radionuclide was recovered with Na- or Ca-carbonates. Bacterial biomass immobilized in a radiation polymerized polyacrylamide matrix showed a good uranium removal potential for continuous process application. Scanning electron microscopy of immobilized bio-beads revealed a highly porous nature of the matrix with bacterial cells embedded in pore walls. The overall data strongly indicate the future potential of the biosorbent in realistic application.

1. INTRODUCTION

Radionuclide and heavy metal pollution by nuclear and other industrial activities is of paramount environmental concern [1]. In view of increasingly strict legislative requirements for the discharge of large volumes of often-low activity contaminants, for
which the conventional decontamination methods seems mostly ineffective or highly expensive, considerable recent interest has been generated in developing microbe-based remediation strategies [2, 3]. Among the several microbial processes that determine the environmental fate of metallic toxicants viz. reductive or enzymatic precipitation, solubilization, etc., biosorptive accumulation of uranium, and other radionuclides is of recent interest [4-6]. Compared to the conventional treatment methods, biomass based systems are more acceptable in being cost effective, with high efficiency of detoxification of even very dilute effluents, and minimizing the disposable sludge volume. It also offers the flexibility for developing non-destructive desorption techniques for biomass regeneration and/or quantitative metal recovery.

The passive biosorptive microbial metal removal by purely physico-chemical way seems more appropriate for bioremediation that could be regulated by the characteristics of the microorganism, targeted metal and the microenvironment of the solution [7]. Since the chemical composition of the cell wall and other surface materials are responsible for cation sequestration, cell viability and other metabolic activities effectively have no impact on biosorption. For process application, one major requirement for microbe-based biosorption is the biosorbents mechanical stability and integrity during continuous operation. Immobilization or physical entrapment of biomass in a matrix is the most suitable way to enhance its mechanical strength which imparts operational flexibility and more effective biomass utilization by providing desired particle size, enhanced cell stability, easy solid-liquid separation, biomass regeneration for reuse and recovery of metals [8].

Although biosorptive uptake of several heavy metals is well documented, studies on radionuclide sorption are relatively less. Among the few reports on radionuclide sorption, fungal and bacterial biosorbents have been tested for uranium and thorium [5, 6, 9-11]. Previous studies on microbial metal sorption by our group have identified the strains of *Pseudomonas* as a potent accumulator of metals and radionuclides [5, 6, 12-15]. The present study was undertaken to evaluate the uranium (VI) and thorium (IV) biosorption capacity of a *Pseudomonas* soil isolate. Equilibrium sorption behavior of the lyophilized biomass was characterized. Localization of metal sequestration was ascertained employing transmission electron microscopy and X ray microanalysis. Development of an immobilized biomass system was emphasized.

2. MATERIALS AND METHODS

2.1 Microorganism, growth medium and culture conditions

*Pseudomonas* sp., isolated from a garden soil was grown and maintained in Tris-minimal medium [13]. Mid exponential phase cells (culture O.D 0.6 at 600 nm) were collected by centrifugation (12000 × g, 30 min), washed thoroughly with distilled water, lyophilized and used for biosorption experiments.

2.2 Uranium and thorium biosorption experiments

Except where otherwise described, for all biosorption experiments, 50 mg (dry wt.) of lyophilized biomass was contacted with 100 ml of a 100 mg uranium or thorium L⁻¹ solution (as nitrate, UO₂(NO₃)₂·6H₂O or Th(NO₃)₂·5H₂O, Merck, Germany). Experimental details are same as described earlier [5-6]. The biosorption equilibrium uptake (q, mg metal g⁻¹ biomass dry wt.) for each sample was calculated according to the mass balance on metal ion expressed as:
Biosorption

\[ q = \frac{V(C_0 - C_e)}{M} \]

where \( V \) is the sample volume (L), \( C_0 \), the initial metal ion concentration (mg L\(^{-1}\)), \( C_e \), the equilibrium or final metal concentration (mg L\(^{-1}\)), and \( M \), the biomass dry weight (g).

For sorption kinetic studies, samples were withdrawn at timed intervals from biomass-metal mixture, centrifuged, and finally dissolved U/Th was estimated. Role of pH was studied by adjusting the initial pH of the contact solution (100 mg U / Th L\(^{-1}\)) over the pH range 2.0-8.0. Biosorption in simultaneous presence of other ions was tested in bimetallic combinations, by adding equimolar concentrations of uranium or thorium (430 \( \mu \)M Th or 420 \( \mu \)M U; equivalent to 100 mg U or Th L\(^{-1}\)) and the test cation/anion. Experimental details are same as described earlier [5-6].

2.3 Transmission electron microscopy (TEM) and X ray microanalysis

For TEM studies, ultra-thin sections of radionuclide -free and -loaded bacterial cells were used. Experimental details are same as described previously [15]. Energy dispersive X ray analysis (EDXA) of samples was done using a Link Oxford ISIS EDX system.

2.4 Desorption of sorbed metal

For desorption experiments, metal loaded biomass was contacted with respective desorbing agents (0.5 mg biomass mL\(^{-1}\)). All other conditions were same as sorption experiment.

2.5 Immobilization of biomass

Lyophilized *Pseudomonas* biomass was immobilized in radiation-polymerized polyacrylamide matrix as described earlier [16.]. The beads obtained were washed, resuspended in distilled water and stored at 0-4\(^{\circ}\)C.

2.6 Uranium sorption by immobilized biomass

Immobilized biomass (bio beads) were contacted with 100 ml uranyl nitrate (UO\(_2\)(NO\(_3\))\(_2\).6H\(_2\)O) solution (100 mg U L\(^{-1}\)) in a 250 ml conical flask (150 rpm, 30\(^{\circ}\)C, 24 h). Following contact the beads were sieved out and the solution was filtered through a millipore filter (0.25 \( \mu \)m) and analyzed for uranium content. Initial pH of all U solutions was adjusted to 4.0 with the addition of 1M NaOH or 1M HNO\(_3\). In each set biomass free polyacrylamide beads were kept as control. Dissolved uranium was estimated by the methods described earlier.

All data represents the mean of three independent experiments. Standard Deviations and error bars are indicated wherever necessary. All statistical analyses were done using Microcal Origin, Version 5.0.

3. RESULTS AND DISCUSSION

3.1 Selection of radionuclide accumulating strain

A number of metal tolerant bacterial strains were tested for their uranium accumulating capacity (Table 1). When exposed to an initial concentration of 100 mg U L\(^{-1}\), a copper resistant *Pseudomonas* sp.2 strain was found the best; accumulating a maximum of 63 mg U g\(^{-1}\) biomass dry wt followed by a cobalt resistant *Bacillus coagulans* strain with the loading capacity of 48 mg U g\(^{-1}\) dry wt. Based on this, the *Pseudomonas* sp. 2 strain was selected for further study on biosorption of uranium (VI) and thorium (IV).
Table 1. Selection of uranium accumulating bacterial strains

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>U uptake (mg g(^{-1}) dry wt.)</th>
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<tbody>
<tr>
<td><em>Pseudomonas</em> sp. 1</td>
<td>39 ± 1.4</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>45 ± 2.7</td>
</tr>
<tr>
<td><em>Bacillus coagulans</em></td>
<td>48 ± 2.9</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp. 2</td>
<td>63 ± 3.1</td>
</tr>
</tbody>
</table>

3.2 Effect of growth medium, energy source and metabolic inhibitor on uranium biosorption

To test *Pseudomonas* strain, cells were pre-grown in synthetic minimal- and enriched-medium and tested for their U sorption capacity. At low uranium concentration (100 mg L\(^{-1}\)), comparable metal sorption was observed by both enriched (63 mg g\(^{-1}\) dry wt.) and minimal (60 mg g\(^{-1}\) dry wt.) medium grown cells. However, improved (1.9-fold) uranium loading was found for minimal medium grown cells (245 mg g\(^{-1}\) dry wt.) at higher uranium concentration (1000 mg L\(^{-1}\)). The present data corroborate very well with U sorption by *P. aeruginosa* CSU strain [11]. The energy dependency of U uptake by the biomass was tested by adding glucose (as carbon/energy source) or sodium azide (as metabolic inhibitor) in uranium uptake solution (100 mg L\(^{-1}\)). An insignificant difference in U uptake indicates the metabolic independency of the tested biomass in sequestering uranium.

3.3 Time course of uranium and thorium sorption

The kinetics of uranium and thorium sorption by lyophilized biomass is shown in Fig. 1. For both radionuclides, the biomass exhibited a rapid cation uptake and more than 90% of equilibrium loading was reached within one (for Th) or ten (for U) minutes. In addition the process saturates after 2 (for U) or 4 (for Th) hours. This rapid binding of metal cations by microbial biomass is typical for radionuclide sorption as it was also shown earlier by *P. aeruginosa* [11], *Rhizopus arrhizus* [9, 10], *Mycobacterium smegmatis* [17]. The rapid cation uptake has been suggested as being essential for any good biosorbent as it allows short solution-sorbent contact time and would result in the use of much shallower contact beds of sorbent materials in column application [18].
3.4 Effect of pH on uranium and thorium biosorption

Initial solution pH plays a critical role in metal sorption by influencing both the bacterial surface chemistry as well as the chemistry of soluble metal ions. Uranium and thorium sorption by lyophilized *Pseudomonas* biomass was studied at a range of pH between 2 and 8 and 2 and 6, respectively (Fig. 2). It was observed that initial solution pH strongly affected the U and Th equilibrium loading capacity. Extreme acid condition (pH 2.0) did not favour sorption of both cations. As the pH increased, sorption of U and Th also increased and the maximum loading for both was attained at pH 4.0 and 5.0, respectively. Increase in pH beyond the optimum caused decline in sorption of respective cations. The reduced sorption at low pH could be attributed to: (i) the hydrolysis of biomass metal binding groups resulting an increased competition by $\text{H}_3\text{O}^+$, and (ii) the increased solubility and consequent reduced adsorptivity of thorium ions [19]. Furthermore, compared to the $\text{Th}^{4+}$ and $\text{Th(OH)}_2^{2+}$ ions formed at low pH that have been identified as a poor sorbate [10], the higher uptake at pH 4.0 could be correlated to the predominance of $[\text{Th}_2(\text{OH})_2]^{6+}$ and other polymerized species possessing a greater binding affinity and thus facilitating faster and enhanced metal sorption [20]. For uranium, the observed trend with regard to pH may be explained by an increasing binding affinity of monovalent uranyl species ($\text{UO}_2\text{OH}^+$, $(\text{UO}_2)_3(\text{OH})_5^{++}$) formed at higher pH (pH 4.0-5.0) over the divalent ($\text{UO}_2^{2+}$) at low pH (pH 2.0) [21].

3.5 Biosorption isotherm

The biosorptive U and Th uptake by *Pseudomonas* biomass was quantitatively evaluated by equilibrium sorption isotherms over a concentration range of 0-1200 mg L$^{-1}$ (Fig. 3). Representative isotherm curves for both cations exhibited very efficient metal binding, even at low residual concentration, and a high loading at equilibrium. The maximum sorption values obtained were 541 mg uranium g$^{-1}$ dry wt. and 430 mg thorium g$^{-1}$ dry wt. at an equilibrium concentration of 359 mg U L$^{-1}$ and 885 mg Th L$^{-1}$, respectively. Such impressive U and Th binding by the tested biomass significantly surpasses the economic threshold level (15% dry wt. basis) for practically usable biosorbents and also the previous values on Th [*R. arrhizus* (185 mg g$^{-1}$ dry wt.) [9] or *P. chrysogenum* (388 mg g$^{-1}$ dry wt.) [20]] and U [*R. arrhizus* and *Penicillium chrysogenum* (both 180 mg g$^{-1}$) [9] *P. aeruginosa* CSU (110 mg g$^{-1}$) [11], and *M. smegmatis* (44.5 mg g$^{-1}$) [17]] uptake.

![Figure 3. U (•) and Th (▲) sorption isotherm for *Pseudomonas* biomass](image)

Figure 3. U (•) and Th (▲) sorption isotherm for *Pseudomonas* biomass
Table 2. Freundlich and Langmuir constants for uranium and thorium sorption by *Pseudomonas* biomass

<table>
<thead>
<tr>
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<th>Thorium</th>
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<td><strong>Freundlich</strong></td>
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<td></td>
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<tr>
<td>(k)</td>
<td>199.00</td>
<td>159.20</td>
</tr>
<tr>
<td>(\frac{1}{n})</td>
<td>0.206</td>
<td>0.176</td>
</tr>
<tr>
<td>(r)</td>
<td>0.931</td>
<td>0.973</td>
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<tr>
<td><strong>Langmuir</strong></td>
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<td></td>
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<tr>
<td>(q_{\text{max}})</td>
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<td>476.19</td>
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<tr>
<td>(b)</td>
<td>0.0027</td>
<td>0.0009</td>
</tr>
<tr>
<td>(r)</td>
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</tbody>
</table>

The relationship between equilibrium metal uptake capacity (\(q\)) and residual metal ion concentration (\(C_e\)) was further described using the model equation of Freundlich and Langmuir. Although linearized sorption isotherm for both the metals showed a reasonably good fit to both the models, the maximum correlation coefficient (\(r\)) was obtained with Langmuir equation. Values of respective sorption constants and correlation coefficients (\(r\)) are presented in Table 2. The better fitting of Langmuir model suggests a monolayerd U and Th binding on to the biomass [22]. The asymptotic maximum adsorption capacity as predicted by the Langmuir constant ‘\(q_{\text{max}}\)’ gives a very high value for U and Th, while a desirable high affinity of the biomass for test metals are evident from the low values of other constant ‘\(b\)’.

3.6 Effect of interfering ions on uranium and thorium biosorption

Uranium and thorium sorption by *Pseudomonas* biomass was studied in the presence of equimolar amount of several competing ions (Table 3). Among the series of cations tested, a significant antagonism in U sorption was offered only by thorium (IV), iron (II and III), aluminium (III) and copper (II) while metals like cadmium (II), lead (II) silver (II), and anions like chloride (I), phosphate (II) and sulphate (II) had no effect. The order of inhibition to uranium binding by the cations was \(\text{Fe}^{3+} > \text{Th}^{4+} > \text{Fe}^{2+} > \text{Cu}^{2+} > \text{Al}^{3+}\). Iron (III), the cation considered as the most potent competitor of uranium for binding sites [11], also caused a severe decline (80%) in U loading. Noticeably, in case of thorium, except iron (III), no other tested cation showed an inhibition more than 20%. The order of inhibition by other cations was \(\text{UO}_2^{2+} > \text{Co}^{2+} > \text{Ni}^{2+} > \text{Al}^{3+} > \text{Ag}^{2+} > \text{Cu}^{2+}\). Although, uranium and thorium biosorption by the present biomass was fairly efficient in the presence of a range of cations, the role of Fe (III) in inhibiting U and Th binding imposes serious limitations in wastewater treatment by this biosorbent. Ideally, iron should be removed by pH adjustment or other methods prior to biosorption.

Table 3. Effect of interfering ions on U and Th biosorption by *Pseudomonas*

<table>
<thead>
<tr>
<th>Cations</th>
<th>Percentage of sorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uranium</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>100</td>
</tr>
<tr>
<td><strong>Cations</strong></td>
<td></td>
</tr>
<tr>
<td>(\text{Na}^+)</td>
<td>100</td>
</tr>
<tr>
<td>(\text{Ag}^{2+})</td>
<td>98</td>
</tr>
<tr>
<td>(\text{K}^+)</td>
<td>98</td>
</tr>
<tr>
<td>(\text{Ca}^{2+})</td>
<td>98</td>
</tr>
<tr>
<td>(\text{Pb}^{2+})</td>
<td>98</td>
</tr>
<tr>
<td>(\text{Cd}^{2+})</td>
<td>96</td>
</tr>
</tbody>
</table>
### Biosorption

<table>
<thead>
<tr>
<th></th>
<th>Percentage of sorption</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uranium</td>
<td>Thorian</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>78$^*$</td>
<td>83$^*$</td>
<td></td>
</tr>
<tr>
<td>Al$^{3+}$</td>
<td>82$^*$</td>
<td>85$^*$</td>
<td></td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>45$^*$</td>
<td>57$^*$</td>
<td></td>
</tr>
<tr>
<td>Fe$^{3+}$</td>
<td>20$^*$</td>
<td>60$^*$</td>
<td></td>
</tr>
<tr>
<td>Th$^{4+}$</td>
<td>37$^*$</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>UO$_2$$^{2+}$</td>
<td>-</td>
<td>92</td>
<td></td>
</tr>
</tbody>
</table>

**Anions**

- Cl$^-$: 100 100
- PO$_4$$^{3-}$: 100 -
- SO$_4$$^{2-}$: 100 100
- CO$_3$$^{2-}$: 74$^*$ 100

*Initial U/Th concentration 100 mg L$^{-1}$, pH 3.5, biomass 0.5 mg mL$^{-1}$.

* Indicates a significant difference (at 5% level) between control (U or Th alone) vs samples containing the competing ions along with U or Th. Significance is calculated using the absolute values (means) in statistical test.

#### 3.7 Transmission electron microscopy and X-ray microanalysis

Transmission electron micrographs of both uranium and thorium loaded cells revealed a dark electron opaque region throughout the cytoplasm, which indicates the intracellular sequestration of biosorbed metals (Fig. 4). Conclusive identification of the deposited elements was achieved with energy dispersive X ray analysis (EDXA). Compared to the metal free control sample, presence of specific peaks for uranium and thorium in respective metal loaded samples confirmed the presence of accumulated radionuclides (Fig. 5). The present observations on cytosolic U and Th sequestration are in conformity with similar reports on *P. aeruginosa* [23] and *M. smegmatis* [24]. The intracellular U and Th deposition even by the lyophilized cells as observed in the present study could be attributed to the increased membrane permeability of these highly toxic radionuclides [25]. Once penetrates the cell membrane, these cations are often immobilized by binding to anionic sites in the cytoplasm or by precipitation due to change in solution chemistry (e.g. pH, phosphate concentration etc.). Further analyses of U and Th loaded biomass using infrared spectroscopy and $^{31}$P NMR techniques indicated the possible involvement of cellular phosphoryl groups in sequestering the biosorbed metals. X ray powder diffraction patterns of metal-free and -loaded biomass ascertained the sequestration of U and Th as phosphate compounds.

#### 3.8 Desorption of bound uranium and thorium

Recovery of sorbed metal is one of the most important aspects of any successful biosorption process development. In the present study, although mineral acids proved quite effective (>70% recovery), maximum amount of uranium or thorium could be recovered by carbonates of sodium (98%) and calcium (93%), respectively (Table 4). Noticeably, compared to the effective desorption of uranium or thorium (>90%) from other microbial biomass by only 10 mM carbonates (sodium or calcium), the high concentrations required (1M) to remobilize the sorbed metals in the present case indicates a relatively high intensity of bonding for these element with intracellular binding ligands. The observed effectiveness of sodium carbonate over mineral acids in recovering biosorbed uranium had also been reported earlier with different bacterial and fungal biomass [24, 26, 27]. As evident from the present data, use of carbonates as desorbert seems more useful in developing a cost effective
desorption technology as they will be less destructive to the biomass over mineral acids and therefore will favour the overall process economics.

Figure 4. Transmission electron micrographs of (a) metal-free (control), (b) U- and (c) Th- loaded *Pseudomonas* cells. Magnification x 45000

Figure 5. Energy dispersive X-ray analysis of (a) metal-free (control), (b) U- and (c) Th-loaded *Pseudomonas* cells

Table 5. Recovery of sorbed U and Th by various desorbents

<table>
<thead>
<tr>
<th>Desorbents</th>
<th>Amount of metal desorbed (percent of sorbed metal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uranium</td>
</tr>
<tr>
<td>HCl (1M)</td>
<td>70</td>
</tr>
<tr>
<td>HNO₃ (1M)</td>
<td>72</td>
</tr>
<tr>
<td>H₂SO₄ (1M)</td>
<td>70</td>
</tr>
<tr>
<td>CaCO₃ (1M)</td>
<td>5</td>
</tr>
<tr>
<td>Na₂CO₃ (1M)</td>
<td>98</td>
</tr>
<tr>
<td>EDTA (0.01 M)</td>
<td>20</td>
</tr>
</tbody>
</table>
3.9 Radionuclide sorption by immobilized Pseudomonas biomass

In view of several disadvantages associated with the use of free microbial biomass in continuous effluent treatment processes [26], Pseudomonas biomass was immobilized in a radiation polymerized acrylamide matrix (bio-bead).

Figure 6. Uranium sorption kinetics by immobilized beads with (*) or without (^) Pseudomonas cells

Figure 7. Scanning electron micrographs of radiation polymerized matrix. (a) surface view of control (biomass free)- and (c) with biomass bead. (b) cross section of control- and (d) with biomass-bead

The biosorption performance of such bio-beads was evaluated using a batch reactor system. As shown in Fig. 6, the uranium sorption kinetics of the immobilized biomass was much slower and till 12h of contact no steady equilibrium state was reached. When compared with its free counterpart (ref. fig. 1), such slow kinetics seems to be a
characteristic feature of immobilized biomass resulting from mass transfer limitation in the particle interior as also observed for radium biosorption by an entrapped fungal biomass [28]. Scanning electron microscope (SEM) (Fig. 7) elucidated a desirable highly porous nature of the bio-beads with bacterial cells entrapped over the pore walls. It was important to note that lyophilized bacterial cells retained their normal shape even in immobilized state.

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