Adsorption of Cu and Zn on calcium alginate immobilized Penicillium sp.

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Calcium alginate has been used for immobilization of Penicillium sp. (PS). The bio granules thus prepared by the immobilization technique, i.e. immobilized PS (IPS), are then used for Cu and Zn sorption from waste water. Various sorption parameters are studied to establish the optimum sorption conditions. The study shows that 5.0 pH and 200 min contact time are the optimum values for both Cu and Zn sorption. The activation energy for Cu sorption by IPS is found to be 19.4 kJ/mole and Zn sorption on IPS is found to be temperature independent. The sorption process obeys Freundlich sorption isotherm in both the cases. The sorption of Cu and Zn by IPS follows pseudo-first order reaction kinetics and is found to be diffusion controlled in nature. The dependence of the rate constant on different sorption parameters is observed and these dependence factors are included in the final rate expressions.

Keywords: Calcium alginate, Dependence factors, Immobilization, Penicillium sp., Sorption parameters

The heavy metal pollution in natural water is mainly due to acid mine drainage and waste water released by different metallurgical industries. As ions of heavy metals like Cu, Ni, Co, Zn, Pb and Cd are toxic to the living system, it is required to remove the same from the waste water before disposal. Since heavy metals are present in trace amount in the waste water, the so-called conventional methods like lime precipitation technique, reverse osmosis, solvent extraction, ion exchange, evaporation and electro dialysis are inefficient and economically not viable. Sorption technology may provide an attractive alternative path for the removal and recovery of heavy metals from waste water. Various forms of chemical sorbents and materials of biological origin or bio sorbents have been found to be effective metal remover from the waste water. Sorption of heavy metals by bio sorbents or bio sorption is proven to be quite effective for the removal of metal ions from contaminated water in a cost effective and environmentally friendly manner. Peat moss, yeast, algae, fungi and bacteria are some of the microorganisms capable of sorbing toxic heavy metal ions from dilute aqueous solution. The free microbial cells have generally small particle size and low mechanical strength. So, in order to retain its ability to adsorb metals during continuous industrial process, it is important to utilize an appropriate immobilization technique. Use of immobilized biomass is also a suitable technique for the treatment of waste water as it does not require the costly solid–liquid separation and it offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow system.

The best example of large scale use of this technique is the activated sludge process. The treatment of waste water containing heavy metal ions can be carried out either through fixed or fluidized bed reactor using immobilized biomass. Different compounds such as agricultural waste, polyurathane and polysulfone are usually used for crosslinking of the biomass. Generally, biopolymers extracted from different microorganisms are selective, efficient and inexpensive. Alginate is one of the most common biopolymers used for the complexation of divalent heavy metal ions from the dilute aqueous stream. Alginate is the ammonium or alkali salt of alginic acid and consists of linear, unbranched chains of 1,4 linked β – D – manuronic and α – L – guluronic acid residues. The length, proportion and sequence of the acid units present in the biopolymer vary with the source from which it is extracted. Alginate becomes a negatively charged anionic polymer on acidification with the formation of –COO groups and these –COO groups are the main metal chelators. After reaction with calcium salts

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alginate can form gels so that it can retain its shape and size unchanged. Calcium ions can be bound to the polymer by simple ionic bridging with two carboxyl groups. These calcium ions can be readily exchanged with the heavy metal ions in the waste water.

The metal removal capacity of calcium alginate immobilized materials has already been tested. Heavy metal sorption by calcium alginate beads from Laminaria digitata was studied by Papageorgiou et al. Orange and banana peel cellulose immobilized calcium alginate beads for the removal of Cu\(^{2+}\), Pb\(^{2+}\) and Zn\(^{2+}\) from an aqueous solution was tested by Lal et al. Arsenic and lanthanum can also be removed from the aqueous solution by using bio polymeric crosslinked and iron oxide loaded calcium alginate beads. An innovative sorbent, i.e. calcium alginate with iron oxide has also been synthesized for the removal of multiple contaminants like As\(^{5+}\) and Cu\(^{2+}\).

As Penicillium sp. is a waste material of the antibiotic industries, in the present study calcium alginate is used to immobilize Penicillium sp. (PS) for the removal of heavy metals like Cu and Zn from waste water in a cost effective manner. Using immobilized PS (IPS) beads, effect of various sorption parameters such as pH, temperature, concentration of metal ions and biomass concentrations has been studied.

**Experimental Procedure**

**Penicillium sp. (PS)**

A PS strain was procured from Maharashtra Association for Cultivation of Science (MACS), Pune and was routinely maintained in potato dextrose agar slants. For sorption purpose, the strain was grown in a medium comprising NH\(_4\)NO\(_3\) 3.0 g/dm\(^3\), KH\(_2\)PO\(_4\) 1.0 g/dm\(^3\), MgSO\(_4\)\(\cdot\)7H\(_2\)O 0.5 g/dm\(^3\) and sucrose 50.0 g/dm\(^3\) in conical flasks at room temperature. After 72 h the grown culture was sterilized by autoclaving, filtered through nylon mesh and washed with 2 dm\(^3\) of distilled water. Excess water was removed by pressing it with filter paper. Biomass thus harvested was dried at 70°C, ground and used for sorption studies.

**Immobilization of PS**

Sodium alginate was used for immobilization of PS. 0.1 dm\(^3\) of 4% sodium alginate solution in distilled water was prepared by shaking manually. Then 0.5 dm\(^3\) suspension of 10% powdered PS in distilled water was added to it. Both of them were mixed by agitation. After mixing, the solution was drawn through hyperdermic needles and was added dropwise to a stirred solution of 1M CaCl\(_2\). After stirring continuously for 24 h the beads thus formed i.e. calcium alginate were kept in fresh CaCl\(_2\) solution. Before sorption studies, the beads were dried by suction for 10 min. The required amount of beads was dropped into a measuring cylinder containing fixed volume of 0.1 M CaCl\(_2\) solution. The increase in volume of CaCl\(_2\) solution by the addition of PS-alginate beads was considered to be the volume of the PS-alginate beads. The average weight of one bead of calcium alginate immobilized PS was found to be 0.01g.

**Sorbates**

Stock solutions (1g/dm\(^3\)) of Cu and Zn were prepared using respective sulphate salts. For sorption purposes, the metal solutions were prepared by diluting the above stock solution.

**Sorption isotherm studies and metal ion analyses**

For sorption experiments, known quantities of calcium alginate beads were taken in a glass reactor. The pH of the metal solution was adjusted by adding N/10 H\(_2\)SO\(_4\) acid. The pH of the solution was measured by pH meter. The whole mixture in the glass reactor was agitated by means of a mechanical stirrer at a constant agitation speed of 350 rpm. To study temperature effect, sorption studies were carried out in a thermostat where the temperature variation was ±0.5°C. Samples were collected at regular time intervals and equal volume of original solution was replaced to maintain the volume constant. Due corrections were made during the interpretation of sorption results due to addition of metal solution. Unless or otherwise specified, the sorption experiments were carried out under the following conditions : time 540 min, pH 6.0, temperature 30°C, metal ion concentration 20 mg/dm\(^3\) and pulp density (% w/v) 0.135.

The metal ions were analyzed by 3100-Perkin Elmer atomic absorption spectrophotometer.

**Results and Discussion**

**Optimization of sorption parameters**

Sorption experiments have been carried out for 12 h to find out the optimum contact time. For both Cu and Zn sorption, the equilibrium isotherm consists of three regions, such as (i) rapid initial phase (ii) deceleration and (iii) a plateau corresponding to the
equilibrium condition (Fig. 1). In Cu and Zn system, the equilibrium is achieved after 200 min. The kinetics of metal uptake is initially very fast due to availability of binding sites and as the same is depleted, the reaction rate drop down considerably. In both the cases, it is observed that the initial faster phase constitutes almost 80% of the total sorption. The pH of the solution is varied from 2 to 6. The pH 5.0 is found to be the optimum pH for the Cu and Zn uptake.

Similarly, the temperature of the solution is varied from 30°C to 60°C. The Cu sorption by IPS is found to be maximum at 60°C, whereas Zn sorption by IPS is temperature independent. The activation energy for Cu sorption is found to be 19.4 kJ/mole, which is temperature independent. The activation energy for metal uptake is initially very fast due to availability of binding sites and as the same is depleted, the reaction rate drop down considerably. In both the cases, it is observed that the initial faster phase constitutes almost 80% of the total sorption. The pH of the solution is varied from 2 to 6. The pH 5.0 is found to be the optimum pH for the Cu and Zn uptake.

The effect of pulp density and initial metal ion concentration on Cu and Zn sorption by IPS are shown in Table 1. It is observed that the percentage of Cu and Zn sorption increases with increase in pulp density, whereas metal loading shows a reverse trend. Similarly, the percentage of Cu and Zn sorption decreases and loading increases with the increase in metal ion concentration.

Sorption isotherm model

Sorption of Cu and Zn by IPS bead is due to surface phenomena. Therefore, sorption data would follow either Freundlich or Langmuir isotherm models. The variation of sorption with sorbate concentration at a given constant temperature can be expressed by the following empirical equation known as Freundlich sorption isotherm:

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

(1)

According to Langmuir, sorption kinetics increases with the rise in concentration up to a stage, where the entire surface of the sorbents is completely covered by uni molecular layer of sorbates. It can be expressed mathematically by the following equation known as Langmuir sorption isotherm:

\[ X/M = b C_e/(1/a + C_e) \]  

(2)

where, \( X \) is the amount of metal ion sorbed (mg); \( M \), the mass of the sorbent (g); \( C_e \), the equilibrium metal ion concentration (mg/dm\(^3\)); \( K \) and \( n \), the Freundlich constants; and \( a \) and \( b \), the Langmuir constants.

In case of Freundlich isotherm model, a straight line would be obtained by plotting a graph between ln \( X/M \) and ln \( C_e \). Similarly, a graph plotted between \( C_e/X/M \) and \( C_e \) would give a straight line for Langmuir isotherm model. The slope \( 1/n \) and intercept ln \( K \) values are shown in Tables 2 and 3. From the coefficient of determination values, it is evident that the Cu and Zn sorption by IPS followed Freundlich sorption isotherm model. By using \( 1/n \) and ln \( K \) values, the theoretical \( C_e \) is calculated in each case. There is a good agreement between the theoretical and experimentally observed equilibrium concentrations as shown in Tables 2 and 3.

Table 1—Effect of pulp density and metal ion concentration on % of metal sorption and loading at pH 6.0, time 120 min and temperature 30°C

<table>
<thead>
<tr>
<th>Metal ion concentration</th>
<th>Pulp density % (w/v)</th>
<th>% of sorption</th>
<th>Loading, mg/g of dry biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/dm(^3)</td>
<td></td>
<td>Cu</td>
<td>Zn</td>
</tr>
<tr>
<td>20</td>
<td>0.07</td>
<td>16.02</td>
<td>8.05</td>
</tr>
<tr>
<td>20</td>
<td>0.14</td>
<td>25.47</td>
<td>9.01</td>
</tr>
<tr>
<td>20</td>
<td>0.35</td>
<td>49.68</td>
<td>9.64</td>
</tr>
<tr>
<td>20</td>
<td>0.7</td>
<td>50.7</td>
<td>15.83</td>
</tr>
<tr>
<td>10</td>
<td>0.14</td>
<td>25.57</td>
<td>12.79</td>
</tr>
<tr>
<td>30</td>
<td>0.14</td>
<td>18.07</td>
<td>8.57</td>
</tr>
</tbody>
</table>

Evaluation of rate equation

An attempt is also made to determine the order of sorption reaction using integral method. The coefficient of determination values for both 1\(^{st}\) and 2\(^{nd}\) order equations indicate that the sorption of Cu and Zn by IPS obeys 1\(^{st}\) order rate expression. The slope of the graph plotted between ln \( [M^{2+}]_i/[M^{2+}]_e \) and \( t \) gives \( k \), the rate constant of sorption reaction.

According to Arrhenius equation, \( k \) should be dependent only on temperature. But experimentally it is observed that the rate constant also depends on other parameters like pH, bead and metal ion concentration apart from temperature. The dependence of \( k \) on other parameters requires modification of rate equation.
\[ R = -\frac{d[M^{2+}]}{dt} = k[M^{2+}] = k'[H^+]^{n_1}[\text{Beads}]^{n_2}[M^{2+}] \] \quad \ldots (3)

where \( k = k'[H^+]^{n_1}[\text{Beads}]^{n_2} \); \( k' \), the modified reaction rate constant; \( n_1 \), the dependence factor for pH; and \( n_2 \), the dependence factor for beads concentration.

Hence,

\[ \log k = \log k' + n_1 \log[H^+] + n_2 \log[\text{Beads}] \] \quad \ldots (4)

For pH variation experiments, beads concentration is kept constant. Therefore, \( n_1 \), i.e. dependence of \( k \) on pH is observed from the negative of the slope of a graph plotted between \( \log k \) and \( \log[H^+] \) or \( -\log[H^+] \). The dependence factors for Cu and Zn sorption on pH are found to be -1.41 and -0.014 respectively (Fig. 2). Similarly, the dependence of \( k \) on IPS beads concentration can be determined from the slope of a graph plotted between \( \log k \) and \( \log[\text{Beads}] \). The dependence of \( k \) on IPS concentration is found to be 1.21 and 0.93 for Cu and Zn sorption respectively (Fig. 3).

\[ \text{Rate} = -\frac{d[Cu^{2+}]}{dt} = k'[H^+]^{1.41}[\text{Beads}]^{1.21}[Cu^{2+}] \] \quad \ldots (5)

where \( k' \) is the modified reaction rate constant.

### Table 2—\(1/n\) and lnK values of Freundlich sorption isotherm model for Cu sorption by IPS

<table>
<thead>
<tr>
<th>Sorption condition</th>
<th>Cu (initial), mg/dm(^3)</th>
<th>Amount of sorbent, g</th>
<th>(1/n)</th>
<th>lnK</th>
<th>(R^2)</th>
<th>(C_e) (Th)</th>
<th>(C_e) (Exp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(pH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.77</td>
<td>19.9</td>
<td>0.27</td>
<td>-3.90</td>
<td>11.9</td>
<td>0.93</td>
<td>15.1</td>
<td>14.8</td>
</tr>
<tr>
<td>3.09</td>
<td>20.5</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td>16.3</td>
<td>16.8</td>
</tr>
<tr>
<td>2.52</td>
<td>20.5</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td>18.8</td>
<td>18.4</td>
</tr>
<tr>
<td>Conc. of Cu ion, mg/dm(^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.02</td>
<td>10.0</td>
<td>0.27</td>
<td>0.65</td>
<td>-0.6</td>
<td>0.89</td>
<td>6.7</td>
<td>7.4</td>
</tr>
<tr>
<td>20.8</td>
<td>20.8</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td>14.2</td>
<td>14.8</td>
</tr>
<tr>
<td>32.5</td>
<td>32.5</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td>24.9</td>
<td>25.5</td>
</tr>
<tr>
<td>Sorbent amount, g</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.67</td>
<td>20.7</td>
<td>0.67</td>
<td>0.78</td>
<td>-0.7</td>
<td>0.93</td>
<td>10.3</td>
<td>10.4</td>
</tr>
<tr>
<td>0.27</td>
<td>19.9</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td>13.3</td>
<td>14.8</td>
</tr>
<tr>
<td>0.14</td>
<td>20.0</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td>17.2</td>
<td>16.8</td>
</tr>
</tbody>
</table>

### Table 3—\(1/n\) and lnK values of Freundlich sorption isotherm model for Zn sorption by IPS

<table>
<thead>
<tr>
<th>Sorption condition</th>
<th>Zn (initial), mg/dm(^3)</th>
<th>Amount of sorbent, g</th>
<th>(1/n)</th>
<th>lnK</th>
<th>(R^2)</th>
<th>(C_e) (Th)</th>
<th>(C_e) (Exp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(pH)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.77</td>
<td>19.9</td>
<td>0.27</td>
<td>-40.9</td>
<td>122.1</td>
<td>0.99</td>
<td>19.7</td>
<td>19.2</td>
</tr>
<tr>
<td>3.09</td>
<td>20.5</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td>19.7</td>
<td>19.2</td>
</tr>
<tr>
<td>2.52</td>
<td>20.5</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
<td>20.1</td>
<td>19.5</td>
</tr>
<tr>
<td>Conc. of Zn ion, mg/dm(^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10.08</td>
<td>10.1</td>
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<td>0.8</td>
<td>-1.9</td>
<td>0.81</td>
<td>9.2</td>
<td>8.8</td>
</tr>
<tr>
<td>20.89</td>
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<td></td>
<td></td>
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<td>19.2</td>
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<td></td>
<td></td>
<td>28.1</td>
<td>28.9</td>
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<tr>
<td>Sorbent amount, g</td>
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<td></td>
</tr>
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<td>1.34</td>
<td>14.1</td>
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<td>20.5</td>
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<td>18.7</td>
</tr>
<tr>
<td>0.14</td>
<td>20.4</td>
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<td></td>
<td></td>
<td></td>
<td>19.1</td>
<td>18.8</td>
</tr>
</tbody>
</table>

Fig. 2—Evaluation of dependence factors for pH
The Arrhenius equation is used to get the energy of activation \(25\). By plotting a graph between \(\ln k'\) and \(1/T\), \(A\) and \(E\) were calculated from the intercept and slope respectively, and are found to be \(A = 4.53\) and \(E = 19.4\) kJ/mole.

By substituting these values in Eq. (5), the final rate expression becomes

\[
\text{Rate} = 4.53 \left[H^+\right]^{1.41}[\text{Beads}]^{1.21}[\text{Cu}^{2+}]e^{-19.4RT} \quad \ldots (6)
\]

Similarly, the final rate expression for Zn sorption by IPS is found to be

\[
\text{Rate} = k'[H^+]^{-0.014}[\text{Beads}]^{0.93}[\text{Zn}^{2+}] \quad \ldots (7)
\]

**Conclusion**

The use of immobilized *Penicillium sp.* (IPS) is found to be suitable for the removal of metal ions like Cu and Zn from waste water. The rate of sorption of Cu depends on all the four parameters studied, whereas Zn sorption is independent of temperature. The metal loading for Cu and Zn are found to be 4.57 and 2.36 mg/g respectively under the conditions, such as time 200 min, pH 6.0, temperature 30°C, pulp density 0.07% (w/v), solution volume 0.2 dm\(^3\) and initial metal concentration 20 mg/dm\(^3\). The moderate activation energy suggests the sorption process to be diffusion controlled. The sorption process obeys Freundlich sorption isotherm in both the cases. The sorption of Cu and Zn by IPS follows pseudo-first order reaction kinetics and the final rate expressions are established.

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The author expresses her deep sense of gratitude and indebtedness to Dr. G Roy Chaudhury, Scientist, Institute of Minerals and Materials Technology (IMMT), Bhubaneswar, for his constant help and inspiration.

**References**