Bioremediation of heavy metal contaminated aqueous solution by using red algae *Porphyra leucosticta*

Jianjun Ye, Henglin Xiao, Benlin Xiao, Weisheng Xu, Linxia Gao and Gan Lin

**ABSTRACT**

Bioremediation is an effective process for the removal and recovery of heavy metal ions from aqueous solutions. In this study, red algae *Porphyra leucosticta* was examined to remove Cd(II) and Pb(II) ions from wastewater through biological enrichment and biological precipitation. The experimental parameters that affect the bioremediation process such as pH, contact time and biomass dosage were studied. The maximum bioremediation capacity of metal ions was 31.45 mg/g for Cd(II) and 36.63 mg/g for Pb(II) at biomass dosage 15 g/L, pH 8.0 and contact time 120 minutes containing initial 10.0 mg/L of Cd(II) and 10.0 mg/L of Pb(II) solution. Red algae *Porphyra leucosticta* biomass was efficient at removing metal ions of 10.0 mg/L of Cd(II) and 10.0 mg/L of Pb(II) solution with bioremediation efficiency of 70% for Cd(II) and 90% for Pb(II) in optimal conditions. At the same time, the removal capacity for real industrial effluent was gained at 75% for 7.6 mg/L Cd(II) and 95% for 8.9 mg/L Pb(II). In conclusion, it is demonstrated that red algae *Porphyra leucosticta* is a promising, efficient, cheap and biodegradable sorbent biomaterial for reducing heavy metal pollution in the environment and wastewater.

**Key words** | bioremediation, heavy metals, Langmuir isotherm, *Porphyra leucosticta*, wastewater

**INTRODUCTION**

Heavy metals are major environmental pollutants throughout many countries and they are toxic at very low concentrations (Ekmekyapar et al. 2006). Many industrial activities such as metal plating, fertilizer production and mining introduce heavy metals to the environment (Bayramo & Arıca 2008). Heavy metal toxicity can be observed in a variety of syndromes including renal dysfunction, hypertension, hepatic injury, lung damage, cancer and the potential to modify the DNA transcription process.

Increases in heavy metal discharging have accelerated the search to develop efficient and attractive treatment methods for their removal from water. Bioremediation is one of the most promising technologies in recent years (Anjana et al. 2007). Bioremediation is cheaper and more environmentally friendly than other traditional wastewater treatment technologies. In recent decades, different biomass types, including bacteria, fungi, and algae have been studied extensively with the purpose of identifying highly efficient metal removal biosorbents (Pardo et al. 2003). So far, many of the studies on metal bioremediation using seaweeds have largely been restricted to brown seaweeds (Khodaverdiloo & Samadi 2011), while only a few studies have paid attention to green and red seaweed species until now (Lodeiro et al. 2006; Salehi et al. 2014).

Except for Naik et al. (2011) and Maurya & Mittal (2014), there are very few studies concerning bioremediation of heavy metal contaminated aqueous solution by using red algae *Porphyra eucosticta* until now. To obtain more knowledge on the heavy metal removal capacity of *Porphyra eucosticta*, this study investigated the experimental parameters affecting the bioremediation process such as pH, contact time and biomass dosage of *Porphyra eucosticta*. In addition, field application of *Porphyra leucosticta* was checked in removing Cd(II) and Pb(II) ions from real aqueous solution and industrial wastewater.

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MATERIALS AND METHODS

Biomass preparation

Samples of the red algae Porphyra leucosticta were acquired from the East Sea at Lianjiang beach, Fujian, China in June 2013. The collected red algae Porphyra leucosticta was rinsed thoroughly with distilled water in order to remove any adhering debris. Samples were placed in heater ovens and dried at 80 °C for 48 hours, then ground and sieved to a particle size of 300–400 μm. The biomass powder was stored in airtight polyethylene bottles until required.

Reagents and equipment

Standard stock metal solutions (analytical grade) containing 500 mg/L of Cd(II) and Pb(II) (as nitrate) separately were purchased from Fuzhou Zhixin Limited Company (China). Working solutions were prepared by diluting the stock solution with distilled water. The metal concentrations of solutions were examined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (HK-2000, Huake Beijing Tiancheng Technology Co., Ltd, China).

Determination of optimum biomass dosage, pH determination and contact time

In order to investigate the optimum algal biomass dosage for heavy metal bioremediation, biomass dosages in the range 1–30 g/L were added to different flasks containing 10.0 mg/L of Pb(II) solution. Flasks were shaken for 120 minutes at 150 rpm and 30 °C. The metal concentrations of solutions were examined using ICP-AES and the percentage of bioremediation was calculated according to the following equation (Amudaa et al. 2009):

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

The Ci and Cf are the initial and final metal ion concentrations (mg/L), respectively.

The optimum pH for heavy metal bioremediation was investigated by adding 15 g/L algal biomass to heavy metal solutions adjusted to various pH (3–10) using 0.1 M NaOH or 0.1 M HCl. Flasks were shaken for 2 hours at 150 rpm and 30 °C. The samples were analyzed by ICP-AES and the percentage of bioremediation was calculated according to the following equation:

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

A quantity of 0.75 g biomass was added to 50 mL metal solutions adjusted to pH 8 (Oliveira et al. 2011). Flasks were shaken at 150 rpm and 30 °C. Samples were taken 10, 20, 40, 60, 80, 100, 120, 140, 160, and 180 minutes later and the residual heavy metal contents were analyzed.

Industrial effluent sampling

Effluent samples were collected from outfalls in Fujian Jianou Fertilizer Company during working hours. The collected samples were filtered through glass filters and stored at 4 °C in the dark, and used within 24 hours. Table 1 indicates the physico-chemical properties of the effluent.

Batch bioremediation procedure

Batch bioremediation of Cd(II) and Pb(II) contaminated effluent was carried out in stoppered conical flasks at the optimum pH, biomass dosage and contact time: biomass dosage 15 g/L, contact time 120 minutes, pH 8.0, temperature 30 °C and 150 rpm. The contents of the solution were filtered through filter paper and the filtrate was analyzed for heavy metal concentration.

The bioremediation isotherms expressed the surface properties and affinity of the biosorbent and can also be used to compare the biosorptive capacities of the biosorbent for different pollutants (Vijayaraghavan et al. 2005). In this study, the Langmuir isotherm model was found to fit experimental data. This model assumes that the absorption process takes place at a specific monolayer absorption surface. The attraction between molecules decreases when getting further from the sorption surface. The Langmuir isotherm can be defined according to the following equation:

\[ \frac{C_e}{q_e} = \frac{C_i}{q_m} + \frac{1}{K_L q_m} \]

Table 1 | Physico-chemical properties of the industrial effluent collected from Fujian Jianou Fertilizer Company

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.6 ± 0.1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>30.1 ± 0.5</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>48.2 ± 2.1</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>30.9 ± 1.4</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>6.03 ± 0.17</td>
</tr>
<tr>
<td>Hardness (mg/L)</td>
<td>131.2 ± 3.4</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>589.4 ± 21.0</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>41.3 ± 1.3</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>5.1 ± 0.18</td>
</tr>
</tbody>
</table>

Values are means ± SE for three samples (n–3). COD: chemical oxygen demand; BOD: biochemical oxygen demand; TDS: total dissolved solids; TSS: total suspended solids; DO: dissolved oxygen.
where $q_e$ is the equilibrium metal ion concentration adsorbed on the biosorbent (mg/g), $C_e$ is the equilibrium metal ion concentration in the solution (mg/L), $q_m$ is the monolayer bioremediation capacity of the adsorbent (mg/g), and $K_L$ is the Langmuir bioremediation constant (L/mg) relating to the free energy of the bioremediation process (Pabst et al. 2010).

Statistical analyzes

Data were presented as the mean of three replications and were analyzed statistically using Student’s $t$-test for independent samples. Statements of significant differences were based on $P < 0.05$ (Bahadir et al. 2007).

RESULTS AND DISCUSSION

Effect of biomass dosage

The effect of biomass dosages on the bioremediation of heavy metal ions Cd(II) and Pb(II) is shown in Figure 1. The bioremediation efficiency was proportional to the biomass dosage. The maximum bioremediation (70–90%) was attained when the dosage was 15 g/L or more. This is expected because, at the higher dosage of biosorbent in the solution, there is greater availability of exchangeable sites for the ions in the surface area (Chatterjee et al. 2010). The bioremediation efficiency was almost the same when biomass dosages were higher than 15 g/L. This may be attributed to a partial aggregation and saturation of biomass which reduces effective surface areas for heavy metal bioremediation (Wang & Qin 2006). Therefore, the optimum biomass dosage was selected as 15 g/L for further study.

Effect of solution pH

One of the important factors affecting the bioremediation of metal ions is the acidity of the solutions. The effect of pH can be explained by the availability of negatively charged groups of the biosorbent surface, which is necessary for the sorption of metals to proceed. Solution pH affects the cell wall metal binding sites and the metal ion chemistry of the water environment. Various authors have shown that solution pH greatly influences metal bioremediation by algal biomass (Aoki & Kamei 2006). To examine the effect of pH on bioremediation efficiency, several experiments were performed at different pH ranges of 3–10 as shown in Figure 2. The absorption capacity progressively increases until the pH reached 8, where the maximum bioremediation efficiency was obtained at 72% for Cd(II) and 91% for Pb(II). Then as pH increased, the bioremediation of metal ions decreased. Therefore, all further bioremediation experiments were carried out at pH 8.

The phenomenon of protonation and deprotonation that occur on the surface area of the biomass explains the relationship between the uptake of metals and solution pH (Costodes et al. 2005). The algae contained a high content of carboxyl groups, which suggests that the bioremediation process could be affected by changes of the solution pH. As carboxyl groups are acidic, at low pH they will be protonated due to the high concentration of the positively charged sites.
charged H\(^+\) ions, thereby limiting availability for binding heavy metals. The increase of the bioremediation up to pH 8 could be associated with deprotonation of carboxyl or other negatively charged groups leading to the electrostatic attraction of positively charged heavy metals (Wang & Qin 2006). The reduction in the bioremediation efficiency at higher pH may be attributed to the formation of hydroxide precipitation complexes, which decrease the dissolved metal concentration in solution.

**Effects of contact time**

The contact time was also evaluated as one of the most important factors affecting bioremediation efficiency (Mata et al. 2008). Figure 3 shows the bioremediation efficiency of metal ions by algal biomass at various contact times for the aquatic solution. The bioremediation efficiency significantly increased with contact time when the contact time was less than 120 minutes. Therefore, the optimum contact time was selected as 120 minutes for further experiments. Many bioremediation studies have also indicated that the majority of tested algae attained maximum metal ion bioremediation between 30 and 120 minutes (Amini et al. 2009).

**Bioremediation isotherm model**

Figure 4 indicates the linear relationship between the amounts (mg) of heavy metal ions absorbed per unit mass (g) and the concentration of heavy metal ions remaining in solution (mg/L). The correlation coefficients (R\(^2\)) ranged from 0.9719 to 0.9764 for metal bioremediation (Table 2).

Table 2 | Langmuir isotherm constants for the biosorption of Cd(II) and Pb(II) on algal biomass

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>Biosorbent</th>
<th>(q_m) (mg/g)</th>
<th>(K_L) (L/mg)</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd(II)</td>
<td>Porphyra leucosticta</td>
<td>31.45</td>
<td>0.0199</td>
<td>0.9764</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>Porphyra leucosticta</td>
<td>36.63</td>
<td>0.0298</td>
<td>0.9719</td>
</tr>
</tbody>
</table>

This high R\(^2\) values indicated that the equilibrium fitted to the Langmuir model. As shown in Table 2, maximum bioremediation capacities \(q_m\) were 31.45 mg/g for Cd(II) and 36.63 mg/g for Pb(II). Therefore, it showed considerable potential for the removal of metal ions from aqueous solutions for Cd(II) and Pb(II).

**Bioremediation efficiency of removing metal ions from industrial effluent**

After bioremediation cycles, the bioremediation efficiency of removing metal ions from industrial effluent ranged from 75 to 95% in optimum conditions (Table 3).

Table 3 | Removal percentage for Cd(II) and Pb(II) in effluent from fertilizer producing company

<table>
<thead>
<tr>
<th>Heavy metal ions</th>
<th>Original contents (mg/L)</th>
<th>Final contents (mg/L)</th>
<th>Removal percentage(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd(II)</td>
<td>7.6</td>
<td>1.9 ± 0.1</td>
<td>75 ± 1.3</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>8.9</td>
<td>0.445 ± 0.06</td>
<td>95 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE for three samples (n = 3).
CONCLUSIONS

This study focused on how the bioremediation parameters of pH, biomass dosage and contact time affected the bioremediation efficiency of metal ions Cd(II) and Pb(II) contaminated aqueous solution by using red algae Porphyra leucosticta. The algal biomass Porphyra leucosticta demonstrated a high capacity of metal bioremediation, highlighting its potential for treating chemical effluents. The bioremediation efficiency of removing of metal ions from industrial effluent was 75% for Cd(II) and 95% for Pb(II). According to these results, the algal biomass can be used as an effective and alternative biomass for the removal of heavy metal ions from industrial effluents due to its high bioremediation capacity.

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