Biosorptive removal of Hg(II) ions by *Rhizopus oligosporus* produced from corn-processing wastewater

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In this study, corn processing wastewater was used as a new low-cost substrate to produce *Rhizopus oligosporus*. Dried biomass of *R. oligosporus* was evaluated as a biosorbent for treatment of synthetically contaminated waters with Hg(II) ions. The biosorption process was carried out in a batch process and the effects of contact time (1 to 48 h), initial pH (2.0 to 7.0), initial metal ion concentration (20 to 100 mg/L) and temperature (20 to 38°C) on the biosorption were investigated. Hg(II) ions concentration were measured with ICP-MS. The maximum adsorption capacity was determined at pH 6.0. The isothermal data of dried fungal biomass could be described well by the Langmuir equations and the Langmuir monolayer capacity had a mean value of 33.33 mg/g. Experimental results indicated that the pseudo-second order reaction model provided the best description of the data with a correlation coefficient 0.99 for different initial metal concentrations. This result indicates that chemical sorption might be the basic mechanism in this system.

**Key words:** Biosorption, corn-processing wastewater, mercury, *Rhizopus oligosporus*.

**INTRODUCTION**

Heavy metal ion pollution has become widespread throughout the world as a result of industrialization, which significantly threatens the ecosystem, especially the people’s health due to their severe toxicity. In order to minimize the impacts of metals contaminated wastewaters need to be treated before discharge to water bodies.

Environmental mercury levels have increased considerably in recent years. The direct anthropogenic sources of mercury in water bodies are related to numerous industrial applications (e.g. chloro-alkali productions, pharmaceutical and cosmetic preparations, electrical instruments, pulp and paper industries etc) and many products of common use (e.g. thermometers, batteries, medical drugs, etc) (Li and Bai, 2005; Natale et al., 2006).

Mercury is one of the priority pollutants listed by the USEPA as it can easily pass the blood-brain barrier and affect the fetal brain (Zabihi et al., 2010). High concentrations of Hg(II) causes impairment of pulmonary function and kidney, chest pain and dyspnoea (Inbaraj and Sulochana, 2006; Yavuz, 2006; Inbaraj et al., 2009; Rao et al., 2009). The illness, which came to be known as Minamata disease, was caused by mercury poisoning as a result of eating contaminated fish. Mercury has very high tendency for binding to proteins and it mainly affects the renal and nervous systems (Byrne and Mazycz, 2009). Mercury removal from wastewaters needs to achieve very low levels for all these reasons.

Metal-sorption by various types of biomaterials like metabolically inactive dried biomass of algae, bacteria and fungi can find useful application for removing metals from solution because of their unique chemical composition (Chen and Wang, 2008; Ozsoy et al., 2008). Chatterjee et al. (2010), investigated the metal binding capacity of the thermophilic bacteria *Geobacillus thermodenitrificans*. According to this study, bacterial biomass reduced the concentration of Fe$^{3+}$ (91.31%), Cr$^{3+}$...
obtain the efficacy of dry fungal biomass as biosorbent and reduce operational costs of biosorption processes and water as a substrate for cultivating the fungal biomass to investigate possible usage of corn-processing waste for removal of Hg (II) ions from aqueous solution. Therefore, cultivation cost is the most important factor or to produce these biosorbent. Hence, to lower the cost of biosorption, microorganisms such as carbon, nitrogen and trace elements are required and stored at 4° C until further use. HNO3 and NaOH were added to the spore suspension as a cryoprotectant for ultra-low frozen storage at –75°C in 2 mL cryo-vials for future use as a bioreactor inoculum (Jasti et al., 2006).

The inocula were used as a seed in laboratory-scale continuous attached growth tank reactors using corn-processing wastewater as organic substrate. The fungi were growing in the form of attached mycelia and harvested from the bioreactor by natural sloughing off the attachment surface and subsequent gravity settling. The mycelia were washed with deionised water and dried at 65°C for 24 h. The dried fungi pellets were ground and sieved (0.5 mm < diameter).

### BIOSORPTION EXPERIMENTS

Hg(II) ions were selected for biosorption tests due to the high toxicity. The effects of contact time, initial pH, initial metal ion concentration and temperature on biosorption efficiency were examined through a series of shaker flask tests. After determining the optimum conditions, a series of biosorption tests were conducted to determine isotherms for Hg(II) ions.

All sorption tests were conducted using single reagent grade metal to minimize the variability of metal concentrations and to avoid competitive adsorption of mixed metals on the biosorbent. 100 mL of metal solution was added to each of flask containing 0.1 g (dry weight) of R. oligosporus. The flasks were placed on an orbital shaker table running at 150 rpm at 30±1°C (except the temperature experiments) until equilibrium was reached. The residual concentration of each metal in the aqueous phase (obtained by centrifugation) was determined using Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). All tests were conducted in triplicate.

The concentrations of the Hg(II) ions the in aqueous phase were used to determine the adsorption capacity of R. oligosporus by mass balance. Equilibrium sorption isotherms were determined.

The solid-phase concentration of Hg(II) ions at equilibrium, qeq (mg/g) was calculated as follows:

\[
q_{eq} = \frac{[(C_0 - C_{eq})V]}{x}
\]  

where C0 and Ceq are the initial and equilibrium concentrations of Hg(II) ions (mg/L), V volume of solution and x the mass of sorbent (g).

### ICP-MS ANALYSIS

Measurements were performed with a Hewlett Packard 4500 Series ICP-MS using external calibration. The instrument was calibrated before each measurement. Operating parameters are summarized in Table 1.

### EQUILIBRIUM ISOTHERMS AND KINETICS OF ADSORPTION

The Langmuir isotherm was used to describe observed sorption phenomena. The Langmuir isotherm applies to adsorption on completely homogenous surface with negligible interaction between adsorbed molecules (Langmuir, 1918; Ho, 2004; Yu et al., 2001). The linear form of the equation can be written as:

### MATERIALS AND METHODS

#### CHEMICALS

A 1 g/L stock solution of Hg(II) ions was prepared with single reagent grade metal solution (Claritas, Fisher Company) in deionized water. The metal solution was diluted to appropriate concentrations as needed and stored at 4°C until further use. HNO3 and NaOH were obtained from Fisher Company and used for pH value adjustment.

#### CULTIVATION OF RHIZOPUS OLGOSPORUS

This research comprised two phases. Fungus was grown on corn-processing wastewater and then harvested, dried and used for biosorption of Hg(II) from aqueous solutions.

R. oligosporus was obtained from American Type Culture Collection (Rockville, MD). The culture was rehydrated and revived in yeast-malt (YM) nutrient broth at 24°C. The culture was transferred on to numerous potato dextrose agar (PDA) plates and incubated at room temperature (24°C) for 7 days. Then fungal sporangiospores were harvested from the surface of PDA plates into sterile distilled water containing 0.85% (w/v) saline solution (NaCl) and 0.5% (v/v) of Tween 80. The harvested cultures were diluted further to achieve a spore count of 10^6 to 10^7 spores/mL, determined by haemocytometer counts. Glycerin (20%; v/v) was added to the spore suspension as a cryoprotectant for ultra-low frozen storage at –75°C in 2 mL cryo-vials for future use as a bioreactor inoculum (Jasti et al., 2006).

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### BIOHARNESS RESULTS

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<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Rf power</td>
<td>1200 W</td>
</tr>
<tr>
<td>Rf matching</td>
<td>2 V</td>
</tr>
<tr>
<td>Sample depth</td>
<td>7.8 mm</td>
</tr>
<tr>
<td>Plasma gas flow</td>
<td>16 L/min</td>
</tr>
<tr>
<td>Auxiliary gas flow</td>
<td>1.4 L/min</td>
</tr>
<tr>
<td>Carrier gas flow</td>
<td>1.0 L/min</td>
</tr>
<tr>
<td>Acquisition time</td>
<td>22.83 s</td>
</tr>
<tr>
<td>Resolution</td>
<td>300</td>
</tr>
</tbody>
</table>

(80.80%), Co^{2+} (79.71%), Cu^{2+} (57.14%), Zn^{2+} (55.14%), Cd^{2+} (49.02%), Ag^{+} (43.25%) and Pb^{2+} (36.86%) at different optimum pH within 720 min (Luo et al., 2010), were investigated biosorption of cadmium (II) from aqueous solutions by industrial fungus Rhizopus cohnii. Researchers reported that the maximum uptake of Cd^{2+} from aqueous solutions by industrial fungus Rhizopus cohnii is 80.80%.

The metal solution was diluted to appropriate concentrations as needed and stored at 4°C until further use. HNO3 and NaOH were obtained from Fisher Company and used for pH value adjustment.

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\[
\frac{C_{eq}}{q_{eq}} = \frac{1}{bq_{max}} + \frac{C_{eq}}{q_{max}} \quad \text{(2)}
\]

where \(C_{eq}\) is the equilibrium concentration of Hg(II) ions, \(q_{eq}\) is the amount of adsorption at equilibrium, \(q_{max}\) is the mono layer capacity, and \(b\) is an equilibrium constant of Langmuir. The Freundlich isotherm (empirical model adsorption in aqueous systems) was also tested with our experimental data. The linear form of the equation can be written as:

\[
\ln q_{eq} = \ln K_f + \frac{1}{n} \ln C_{eq} \quad \text{(3)}
\]

where \(K_f\) is the measure of sorption capacity, \(1/n\) is sorption intensity (Freundlich, 1926; Simaratananamongkol and Thiravetyan, 2010).

In order to analyse the sorption kinetics of Hg(II) ions, pseudo-first order and pseudo-second order kinetic models were applied to data. A simple pseudo first-order equation due to Lagergren was used by different researchers (Ho and McKay, 1998; Bhattacharyya and Sharma, 2005):

\[
\ln (q_{eq} - q_t) = \ln q_{eq} - \frac{k_{ad}}{2.303t} \quad \text{(4)}
\]

where \(q_t\) and \(q_{eq}\) are the amount of adsorption at equilibrium and at time \(t\), respectively, and \(k_{ad}\) is the rate constant of the pseudo first-order adsorption process. A plot of \(\ln (q_{eq} - q_t)\) vs. \(t\) would provide a straight line for first order adsorption kinetics, which allows computation of the adsorption rate constant, \(k_{ad}\).

Ho's second-order rate equation which has been called pseudo-second order kinetic expression has also been applied widely (Ho, 2005; Ho and McKay, 2000). The linear form of the kinetic rate equations can be written as follows:

\[
\frac{t}{q_t} = \frac{1}{kq_{eq}^2} + \frac{1}{q_{eq}} \quad \text{(5)}
\]

where \(k\) is the rate constant of sorption (dm\(^3\)/mg min), \(q_e\) is the amount of metal ion sorbed at equilibrium (mg/g), and \(q_t\) is the amount of metal ion sorbed at time \(t\) (mg/g). The constants can be determined experimentally by plotting of \(t/q_t\) against \(t\).

**Thermodynamics of biosorption**

The thermodynamic parameters such as free energy (\(\Delta G\)), enthalpy (\(\Delta H\)) and entropy changes (\(\Delta S\)) for the adsorption process can be determined using the following equations:

\[
\Delta G = -RT \ln b \quad \text{(6)}
\]

where \(R\) is gas constant (8.314 J/mol K), \(b\) is Langmuir constant and \(T\) is absolute temperature.

The relationship between \(\Delta G\), \(\Delta H\) and \(\Delta S\) can be expressed by the following equations:

\[
\Delta G = \Delta H - T\Delta S \quad \text{(7)}
\]

Equation (7) can be written as:

\[
-RT \ln b = \Delta H - T\Delta S \quad \text{(8)}
\]

Or

\[
\ln b = \frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad \text{(9)}
\]

where the values of \(\Delta H\) and \(\Delta S\) can be determined from the slope and the intercept of the plot between \(\ln b\) versus \(1/T\) (Southichak et al., 2006).

**RESULTS AND DISCUSSION**

**The effect of contact time**

Figure 1 shows the effect of contact time on adsorption of Hg(II) ions (100 mg/L) to the dried R. oligosporus biomass. As seen there, Hg(II) ions adsorption capacity increases during the first 6 h than the levels of equilibrium adsorption capacity. For these reasons, 6 h contact time was used for further experiments.

**The effect of the initial pH**

The pH of metal solutions has been identified as the major parameter controlling metal sorption processes. This is partly due to the fact that protons are strong competing sorbates and partly to the fact that the solution pH influenced the ionization of functional groups of the biosorbent and the chemical speciation of metal ions in solution (Fiol et al., 2006).

Batch biosorption studies were repeated at different pH values in the range of 2.0 to 7.0 to establish the effect of pH on the biosorption of Hg(II) ions onto the dried R. oligosporus biomass. Figure 2 indicates that Hg(II) ions biosorption was greatly influenced by pH. Biosorption of mercury at pH 2.0 was very low. This can be explained by the fact that at this acidic pH, the proton concentration is high and the studied metals are present in solution as free cation, so protons can compete with metal cations for surface sites (Fiol et al., 2006). However biosorption increased with increasing pH and then reached a plateau value around pH 6.0. Higher values were not used because precipitation of mercury ions becomes significant at pH 8.0 as the solubility product of Hg(OH)\(_2\) would predict (Byrne and Mazycz, 2009).

Similarly, Liu et al. (2009) reported that mercury (II) adsorption by thymine-based sorbent was pH sensitive. And, when the pH was 4.0, more than 80% of Hg (II) was removed by thymine sorbent; the removal efficiency reached 100% in the pH range of 7.0 to 10.0.

**The effect of temperature**

The effect of temperature on the Hg(II) ions removal is
Figure 1. The effect of contact time on adsorption of Hg(II) ions (100 mg/L) to the dried *R. oligosporus* biomass (Biosorbent dosage 1 g/L; pH:6.0; 30°C).

Figure 2. The effects of the initial pH on adsorption of Hg(II) ions (100 mg/L) to the dried *R. oligosporus* biomass (Biosorbent dosage 1 g/L; contact time 6 h; 30°C).

illustrated in Figure 3. The maximal adsorption capacity (33.33 mg/g) was found at 30 ± 1°C. While an increasing trend of adsorption was observed with increasing temperature from 20 ± 1 to 30 ± 1°C, adsorption capacity of dried *R. oligosporus* biomass decreased with temperatures higher than 30 ± 1°C.

**The effect of the initial metal concentrations**

Biosorption capacities of the dried *R. oligosporus* biomass for the Hg(II) ions were studied as a function of the initial Hg(II) ions concentration between 20 and 100 mg/L in the biosorption medium (Figure 4). Although, percent of adsorption (%) decreased, equilibrium sorption capacity of the dried *R. oligosporus* biomass increased with increasing initial Hg(II) ion concentration. A similar type of trend was reported for the removal of Hg(II) from aqueous solution by sorption on activated carbon prepared from agricultural by-product/waste by Rao et al. (2009). These results may be explained to be due to the increase in the number of ions competing for the available binding sites and also because of the lack of active sites on the biomass at higher concentrations.
Equilibrium isotherms and kinetics of adsorption

Two equilibrium models were employed to investigate the sorption isotherm for the dried *R. oligosporus* biomass: The Langmuir and Freundlich isotherm equations. The correlation coefficient of Freundlich isotherm ($R^2$) was 0.9689 (Figure 5). In this study, the Langmuir model was the best-fit isotherm for adsorption of Hg(II) to the dried *R. oligosporus* biomass. Langmuir isotherm model parameters, $q_{\text{max}}$ and $b$, were estimated from the intercept and slope of $C_{\text{eq}}/q_{\text{eq}}$ versus $C_{\text{eq}}$, according to Equation (2) and obtained as 33.33 (mg/g) and 0.485 (L/g), respectively. The correlation coefficient of the Langmuir isotherm ($R^2$) was 0.9944 (Figure 6).

Kinetic studies were carried out for biosorption of Hg(II) as a function of contact time at various initial Hg(II) concentrations ranging from 20 to 100 mg/L. Experimental results indicated that the pseudo-second order reaction model provided the best description of the data with a correlation coefficient 0.99 for different initial metal concentrations (Figure 7). The fit of the experimental data to this model suggest that the process controlling the rate may be a chemical sorption involving valence forces through sharing or exchange the electrons between fungi and sorbate (Martinez et al., 2006).

The values which were derived for the reaction rate constant for pseudo-second order equations, are shown at Table 2. The results also indicated that the adsorption system studied follows a pseudo-second order kinetic model at all time intervals and pseudo-second order rate constants were affected by initial Hg(II) ions concentration. Similarly, Zabihi et al. (2009) showed that pseudo-
The Freundlich adsorption isotherms for Hg(II) adsorption by dried *R. oligosporus* biomass (Biosorbent dosage 1 g/L; pH 6.0; initial metal concentration of 20-100 mg/L; 30°C).

The Langmuir adsorption isotherms for Hg(II) adsorption by dried *R. oligosporus* biomass (Biosorbent dosage 1 g/L; pH 6.0; initial metal concentration of 20 to 100 mg/L; 30°C).

second order kinetic model is better in explaining the adsorption kinetics of the mercury adsorption on to carbonaceous sorbents derived from walnut shell.

Thermodynamic parameters

The change in Gibbs free energy (ΔG) for biosorption of Hg(II) ions onto dried *R. oligosporus* biomass were calculated from Equation (6). ΔH and ΔS values were also determined from the slope and the intercept of the plot between ln b versus 1/T (Figure 8).

Thermodynamic parameters calculated as a function of temperature are listed in Table 3. The negative value of ΔH confirms that the process is exothermic. The negative values of ΔG and ΔS indicate that nature of adsorption process is spontaneous and randomness at the solid/liquid interface during the biosorption of Hg(II) ions on dried *R. oligosporus* biomass is decreasing.

Fungal biomass is a complex material containing protein, lipid and polysaccharides (cellulose, chitin) as major constituents. Therefore, biosorption may occur at the polar functional groups of cellulose and chitin, which include hydroxyl, amino and carboxyl groups as chemical bonding agents.

Conclusion

The maximum Hg(II) adsorption capacity *R. oligosporus*
Figure 7. The plots of pseudo-second order kinetics with respect to different initial Hg(II) ion concentrations (Biosorbent dosage 1g/L; pH 6.0; 30 °C).

Table 2. Pseudo-second order reaction rate regression results.

<table>
<thead>
<tr>
<th>Initial metal conc. (mg/L)</th>
<th>Rate constant (L/ mg min)</th>
<th>(q_{\text{exp}}) (mg/g)</th>
<th>(q_{\text{cal}}) (mg/g)</th>
<th>Correlation coeff. ((R^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>(26.1 \times 10^{-3})</td>
<td>19.93</td>
<td>20.92</td>
<td>0.9995</td>
</tr>
<tr>
<td>40</td>
<td>(7.5 \times 10^{-3})</td>
<td>27.76</td>
<td>30.86</td>
<td>0.9925</td>
</tr>
<tr>
<td>60</td>
<td>(6.5 \times 10^{-3})</td>
<td>28.72</td>
<td>32.36</td>
<td>0.9927</td>
</tr>
<tr>
<td>80</td>
<td>(4.9 \times 10^{-3})</td>
<td>31.77</td>
<td>35.84</td>
<td>0.9902</td>
</tr>
<tr>
<td>100</td>
<td>(4.6 \times 10^{-3})</td>
<td>33.18</td>
<td>38.46</td>
<td>0.9953</td>
</tr>
</tbody>
</table>

Conc. = Concentration; coeff. = coefficient.

Figure 8. Plot of \(\ln b\) versus \(1/T\).

Biomass for Hg(II) has been found to be 33.33 mg/g at an biosorbent dose of 1 g/L in 6 h of contact time with initial Hg(II) concentration of 100 mg/L and optimum pH of 6.0. Experimental results indicated that the pseudo-second order reaction model provided the best description of the data with a correlation coefficient 0.99 for different initial metal concentrations. The fit of the experimental data to this model suggests that the process controlling the rate may be a chemical sorption.

With the advantage of high adsorption capacity, the
dried *Rhizopus oligosporus* biomass has the potential to be used as an effective and economic biosorbent material for the removal of Hg(II) ions from wastewater streams. These results also show that it is possible to use food-processing wastewater as a substrate for cultivating the fungal biomass to reduce operational costs of biosorption processes.

**REFERENCES**


**Table 3.** Entropy and gibbs free energy change for the biosorption of Hg(II) ions on dried *R. oligosporus* biomass.

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>ΔH (KJ/mol)</th>
<th>ΔS (KJ/mol K)</th>
<th>ΔG (KJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>303</td>
<td>-178.53</td>
<td>-0.49</td>
<td>-28.95</td>
</tr>
<tr>
<td>308</td>
<td></td>
<td></td>
<td>-26.71</td>
</tr>
<tr>
<td>311</td>
<td></td>
<td></td>
<td>-24.96</td>
</tr>
</tbody>
</table>

ΔH, Enthalpy; ΔS, entropy; ΔG, gibbs free energy change.


