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Equilibrium and Spectroscopic Studies on Biosorption of Mercury by Algae Biomass

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Abstract: Mercury has been used in many industries and the removal of mercury ions from waste waters is significant. Biosorption equilibrium of mercury ions to algae biomass was studied in a batch system with respect to temperature and initial metal ion concentration. Langmuir isotherm models was applied to experimental equilibrium data of mercury biosorption. The maximum adsorption of mercury ions on algae biomass was observed at pH 4.0. The biosorption of mercury ions by the algae biomass increased as the initial concentration of the mercury ions increased in the biosorption medium. Biosorption equilibrium was established in about 60 min and the equilibrium was well described by the Langmuir biosorption isotherms. The biomass could be regenerated using 0.1 M HCl, with up to 98% recovery, which allowed the reuse of the biomass in two biosorption-desorption cycles without any considerable loss of biosorption capacity. The functional groups involved in mercury biosorption were identified using spectroscopy analysis. Spectroscopic analysis of algal biomass revealed the presence of amino, carboxyl, hydroxyl and carbonyl groups, which were responsible for biosorption of mercury ions.

Key words: Mercury, algae, isotherm, Langmuir, spectroscopic

INTRODUCTION

Mercury has received considerable attention from the environmental engineers due to its high toxicity, a tendency to bio-accumulation and difficulties in its control. Mercury contamination of the environment is caused by both natural and man-made sources. Natural sources include volcanic action and erosion of mercury-containing sediments. Some of the ways in which humans contaminate the environment with mercury include: mining, transporting and processing mercury ores; dumping of industrial wastes (such as chloralkali) into rivers and lakes; combustion of fossil fuels (e.g., the mercury content of coal is approx. 1 mg kg⁻¹), pulp and paper, paint; use of mercury compounds as seed dressings in agriculture and exhaust from metal smelters. The major effects of mercury poisoning manifest as neurological and renal disturbances as it can easily pass the blood-brain barrier, effect the brain and impairment of pulmonary function and kidney, chest pain and dyspnoea. The main techniques utilized for treatment of mercury bearing waste streams include precipitation, evaporation, adsorption, ion exchange, membrane processing and solvent extraction sulphide precipitation, ion exchanger, alum and iron-coagulation, adsorption on activated carbon, reverse osmosis and lime softening

(Patterson, 1977). These methods have been found to be limited, since they often involve high capital and operational costs and may also be associated with the generation of secondary wastes which present treatment problems (Ajmal *et al.*, 2000; Wong *et al.*, 2000). For example, precipitation limits required by regulatory standards and produce wastes difficult to treat; on the other hand, ion exchange and adsorption processes are very effective, but require expensive materials and difficult plant management. In this respect, the search for a new, economical and effective heavy metal adsorbent is focused on biomaterials, such as bacterial and algal biomasses (Bailey *et al.*, 1999). Biosorption utilizes the ability of biological materials to accumulate heavy metals from waste streams by either metabolically mediated, or purely physico-chemical pathways of uptake. Using microorganisms as biosorbents for heavy metals offers a potential alternative to existing methods for detoxification and for recovery of these components from industrial waste waters (Gavrilescu, 2004). The special surface properties of microorganisms enable them to adsorb different kinds of pollutants from solutions (Saglam, 2002). Adsorption has been an effective separation process for a wide variety of applications. Since commercial adsorbents is expensive, an alternative inexpensive adsorbent able to drastically reduce the cost of an

adsorption system has always been searched (Tarley and Arruda, 2004). It can mainly be used for treatment of wastewater with low or medium initial heavy metal concentration. Other advantages of biosorption are that it avoids the generation of toxic sludge and can be used under a broad range of operating conditions (pH, temperature, metal concentration, presence of other ions in the solution, etc.). These demands led to increasing interest in biosorption. Algae can sequester heavy metal ions by the same adsorption and absorption mechanisms as other microbial biomass (Davis *et al.*, 2003; Tatykostodes *et al.*, 2003; Wagner-Dobler *et al.*, 2000). The mechanism of binding metal ions by inactivated algal biomass may depend on the species and ionic charges of metal ion, the algal organism, the chemical composition of the metal ion solution and other external environmental factors such as pH and temperature. The binding of most metals to microorganisms by biosorption is observed to enhance as temperature is increased (Gavrilescu, 2004). The most appropriate method for assessing biosorbent capacity is the derivation of a whole adsorption isotherm. The adsorption isotherm is the equilibrium between the concentration in the fluid phase and the concentration in adsorbent particles (Ho *et al.*, 2002). The purpose of the present study is to evaluate the biosorption of the algae biomass for mercury from aqueous solutions. The system variables studied include biosorptive time, pH and temperature. Equilibrium isotherms were obtained from batch adsorption experiments. The isotherm constants for the Langmuir has been determined. FTIR analysis confirmed that C = O, -NH, -OH and -SO₃ responsible for the binding of the metal ions.

MATERIALS AND METHODS

Algae biomass: The algae biomass was collected from Golab darreh river, Tehran, Iran in spring and autumn seasons. The biomass was maintained in modified Bold's basal medium (Table 1). After 20 days of growth under illumination cycle 12/12 at the average intensity of 3000 lux at 25°C, the biomass was picked out and transferred to another stages. Before use of algae, it was washed with distilled water to remove culture media and was kept on a filter paper to reduce the water content. The biomass dried at 60°C for 24 h and milled to a gritty consistency. The biomass was sieve to select particles smaller than 1 mm (powdered biomass) in size for use and stored in a dry cabinet.

Table 1: Formulation of modified Bold's basal medium

Ingredient	Concentration (mg L ⁻¹)
NaNO ₃	95.20
CaCl ₂ . 2H ₂ O	25.00
MgSO ₄ .H ₂ O	75.00
K ₂ HPO ₄	75.00
KH ₂ PO ₄	175.00
NaCl	25.00
FeSO ₄ .H ₂ O	4.98
H ₂ SO ₄	0.001
H ₃ BO ₃	11.42
EDTA	50.00
KOH	31.00
ZnSO ₄ .7H ₂ O	8.82
MnCl ₂ . 7H ₂ O	14.40
MoO ₄ Na ₂ .2H ₂ O	0.71
CuSO ₄ .5H ₂ O	1.57
CoCl ₂	0.49

Preparation of synthetic sample: The stock solutions of mercury ion were prepared in distilled water using mercury chloride(HgCl₂). All working solutions were prepared by diluting the stock solution with distilled water to the needed concentration.

Analysis of mercury ions: All the glass and plastic wares used were kept in 1.0 N HNO₃ solution overnight and then thoroughly rinsed with deionized water. Mercury stock solution (1000 ppm) were prepared from dissolve chloride mercuric in mixture nitric acid and water. Working standard were prepared daily from the stock solution by serial dilution and stored in polyethylene bottles. Mercury contents were determined by flameless cold vapor adsorption spectroscopy by using a flow injection system which linked to an atomic adsorption spectrophotometer (UNICAM, model 929, UK). To determine soluble mercury contents, 5 mL of samples were routinely oxidized by adding 0.01 volume of 65% HNO₃. Ionic mercury was then reduced with NaBH₄ (4 g L⁻¹) to metallic mercury, which was volatilized by the carrier gas argon and detected at 253 nm by the atomic adsorption spectrophotometer (Lenore and Arnold, 1989). If necessary, samples were diluted so that they contained less than 100 µg L⁻¹ of mercury. To determine total mercury concentrations, 7 mL samples were pretreated by oxidizing them with 3 mL of 65% HNO₃ for 2 h at 140°C.

Batch biosorption studies: Experiments were conducted in 250 mL Erlenmeyer flasks containing mercury synthetic solutions. Flasks were agitated on a shaker at 100 rpm constant shaking rate for 80 min to ensure equilibrium was reached. Samples were taken before mixing the biosorbent solution and mercury ion bearing solution and at pre-determined time intervals (0, 5, 10, 15, 30, 60 and 80 min) for the residual metal ion concentration in the solution. Before analysis the samples were centrifuged at 4000 rpm for 3 min and the supernatant fraction was

analyzed for the remaining mercury ions. All experiments were carried out at least twice. Values used in calculations were the arithmetic averages of the experimental data.

Effect of biosorption time: Batch biosorption tests were done at different biosorption time at the initial concentration of mercury ion 80 mg L^{-1} and algae biomass 0.4 g . The temperature was controlled with a water bath at the temperature of 25°C for all studies.

Equilibrium studies: A volume of 50 mL of mercury ion solution with a concentration in the range $1\text{-}100 \text{ mg L}^{-1}$ was placed in a 125 mL conical flask. An accurately weighed algae biomass sample 0.4 g with particle size 1 mm was then added to the solution. A series of such conical flasks was then shaken at a constant speed of 100 rpm in a shaking water bath with temperatures 25°C . After shaking the flasks for 30 min , the algae biomass was separated by filtration through a $0.45 \mu\text{m}$ membrane filter to remove particulates and the filtrate was analyzed for the remaining mercury ion concentration by atomic absorption spectrophotometer.

Equilibrium parameters of biosorption: Equilibrium data, commonly known as adsorption isotherms, are basic requirements for the design of adsorption systems. Classical adsorption models (Langmuir) was used to describe the equilibrium between adsorbed metal ions on the algal cell (q_{eq}) and metal ions in solution (C_{eq}) at a constant temperature. The Langmuir equation which is valid for monolayer sorption onto a surface with a finite number of identical sites is given by Eq. (1).

$$1/q_{\text{eq}} = 1/(x_{\text{m}} \cdot b \cdot C_{\text{eq}}) + 1/X_{\text{m}}$$

Where, X_{m} and b are Langmuir constants, indicative of maximum adsorption capacity and a measure of adsorption energy, respectively. q_{e} is the metal adsorption in mg g^{-1} of dry weight biomass and C_{e} is the equilibrium mercury concentration ($\mu\text{g L}^{-1}$) (Langmuir, 1916). The Freundlich isotherm is also more widely used but provides no information on the monolayer adsorption capacity, in contrast to the Langmuir model (Langmuir, 1916; Freundlich, 1906).

Desorption and reuse: In order to determine the reusability of the algae, consecutive adsorption-desorption cycles were repeated three times by using the same biosorbent. Desorption of mercury was performed by 10 mM HCl solution. The algae loaded with mercury were placed in the desorption medium and stirred at 100 rpm for 60 min at 25°C . The mercury concentrations in

the aqueous phase were determined by using an atomic absorption spectrometer as described above. Desorption ratio was calculated from the amount of mercury adsorbed on the immobilized preparations and the mercury concentration in the adsorption medium.

Infrared spectroscopy: In order to determine the functional groups responsible for mercury biosorption, IR spectroscopy was used.

RESULTS AND DISCUSSION

In this study, mercury removal by inactivated algae biomass was investigated. The data from batch studies provided fundamental information in terms of optimum pH, optimum temperature for maximum removal of mercury from the solution. Although a large number of publications have recently suggested using living and nonliving algae for accumulation and removing heavy metals from polluted water, there seems to be a few study which reports all the equilibrium and spectroscopic studies of mercury biosorption by dried algae biomass, in a batch system in a wide range of mercury concentration. It was shown that in practical applications of algae biosorption that pH could be adjusted to be more favorable to the removal of the ions of interest. Earlier studies have indicated that pH is an important parameter effecting biosorption of heavy metal ions (Gupta *et al.*, 2001; Rezaee *et al.*, 2005). Figure 2 shown the mercury removal by algae biomass as a function of pH in different times. The maximum adsorption takes place at pH 4. The cell wall of the algae contains a large number of surface functional groups. The pH dependence of metal adsorption can largely be related to type and ionic state of these functional groups and also on the metal chemistry in solution. The positively charged hydrogen ions may also compete with metal ions for binding on the ligands on the cell wall (Kaewsarn, 2002). At lower pH, the higher concentration of the hydrogen ions effectively leads to fewer ligands being available for the binding of the metal ions. Increased pH (i.e., fewer H^+ ions) results in more ligands being available for metal ion binding and hence biosorption is enhanced. The typical dependence of metal uptake on pH suggests that the weak acidic carboxyl groups R-COO^- (apparent pK_a in the range $3.5\text{-}5.0$) of algal cell wall constituents as the probable biosorption sites (Sheng *et al.*, 2004). Similar results were also obtained for the carboxyl groups in the biomass of the freshwater algae *Chlorella pyrenoidosa* and *Cyanidium caldarium* (Wong *et al.*, 2000). The sulfonic acid R-SO_3^- (apparent pK_a in the range $1.0\text{-}2.5$) of fucoidan in the brown algal cell wall may play a

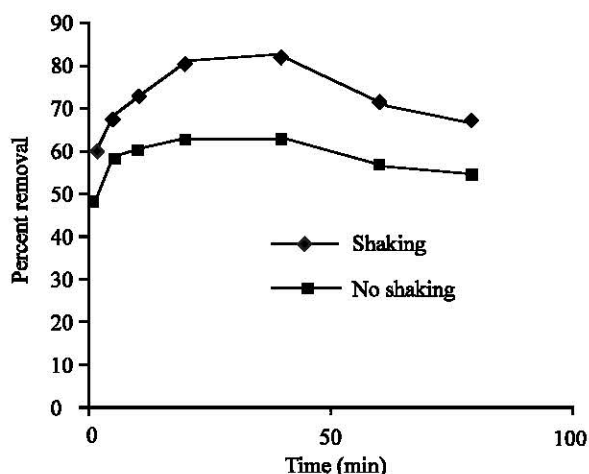


Fig. 1: Effect of agitation time on the mercury removal (temperature: 25°C, pH 8.5, algal biomass: 0.4 g, agitation: 100 rpm)

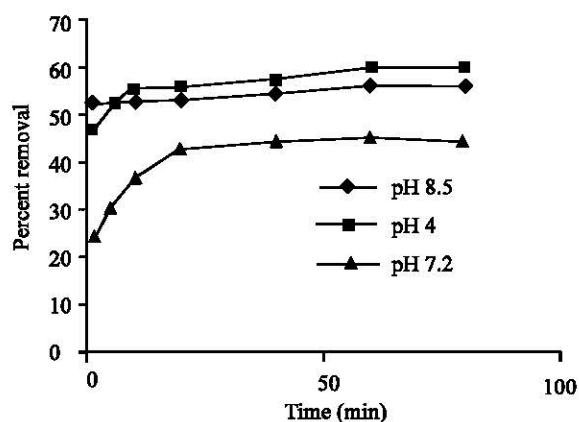


Fig. 2: Effect of pH on the mercury removal (temperature: 25°C, pH 8.5, algal biomass: 0.4 g, agitation: 100 rpm)

secondary role in biosorption. Hydroxyl groups are also present in all polysaccharides but are less abundant. It can become negatively charged at high pH, thereby contributing to metal removal at high pH. In a study on the biosorption performances of two pretreated Australian brown marine algae, the sharpest increase in the uptake of lead and copper was obtained between pH 2 and 3, with the pH effect becoming less significant beyond 3.0 for both metal ions (Fourest *et al.*, 1994). Similarly, uptake of copper by a calcium-alginate-based ion exchange resin remained unchanged with pH ranging from 4.0 to 5.5. Since the biosorption performances of these pretreated sorbents were less pH-dependent than the sorbents in this work at higher pH (e.g., pH >4.0), this may imply that the importance of the functional groups involved in metal

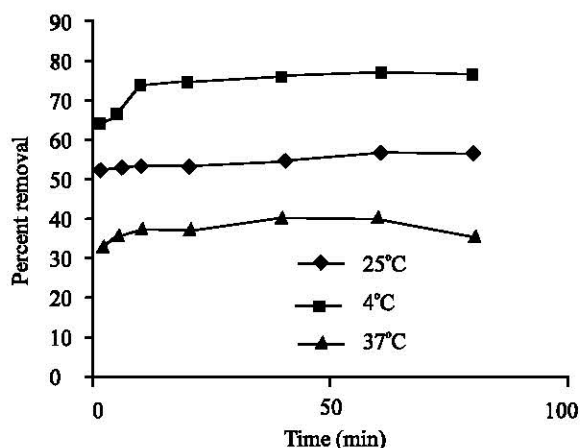


Fig. 3: Effect temperature on the mercury removal (mercury concentration: 80 mg L⁻¹, pH 4.0, algal biomass: 0.4 g)

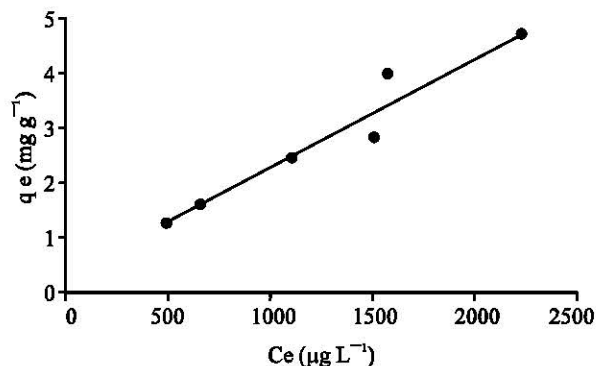


Fig. 4: Langmuir plot for adsorption of mercury (mercury concentration: 80 mg L⁻¹, pH 4, agitation time: 40 min, algal biomass: 0.4 g)

biosorption at higher pH was reduced as a result of the pretreatment procedures. The results of mercury removal by algae biomass as a function of time have been shown in Fig. 1. Biosorption can be accomplished with agitation by increasing the contact time up to 40 min. The removal increases with agitation time and attains equilibrium in 20-40 min and maximum percent removal 81.1 and 62.97 for the shaking and no shaking, respectively. The results indicates that agitation would require for the complete removal of mercury. In order to examine the biosorption potential of the algal biomass and to ensure that the mercury exist in their ionic states during biosorption, the pH in subsequent kinetic and isotherm experiments were controlled at 4.0. The mercury efficiency obtained at there temperature (4, 25 and 37°C) using algae. The removal percent was able to reach as high as 76% at 4°C, while it only reached 39.65 and 56.47% at 37 and 25°C, respectively, (Fig. 3). The temperature of the adsorption

Table 2: Langmuir constants

R^2	X_m (mg g^{-1})	b (L mg^{-1})	Mercury concentration (mg L^{-1})	R_L
0.9407	156.75	3.18	1.5	0.17
			2	0.13
			3	0.09
			4	0.07
			5	0.05
			6	0.04

medium could be important for energy- dependent mechanisms in metal biosorption by Microorganisms. Energy-independent mechanisms are less likely to be affected by temperature since the process responsible for biosorption is largely physicochemical in nature. The adsorption of mercury by inactivated algae appears to be temperature dependent over the temperature tested (4, 25 and 37°C). In this study the removal decreased with increase of temperature. The langmuir adsorption model was used for mathematical description of the biosorption of mercury to dried algae and isotherm constants was evaluated to compare the biosorptive capacity of the dried algae for the mercury ion. It was seen that the adsorption equilibrium data fitted very well to the langmuir model. The mercury sorption isotherm followed the langmuir model as shown by high value of the correlation coefficient (r^2 (given in Table 2 along with the langmuir constants. The linear plot of q_e versus C_e shows that the adsorption obeys Langmuir isotherm model (Fig. 4). X_m and were determined from the slop and intercept of the plot and are presented in Table 2. The essential characteristics of a langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L which is defined by:

$$R_L = 1 / (1 + bC_0)$$

Where, C_0 is the Initial metal ion concentration (mg L^{-1}) and b is the langmuir constant(L mg^{-1}). mathematical calculations hat the parameter, R_L , indicates the shape of the isotherm as follows:

R_L Value	Type of isotherm
$R_L > 1$	Unfavorable
$R_L = 1$	Linear
$0 < R_L < 1$	Favorable
$R_L = 0$	Irreversible

R_L values between 0 and 1 at different concentrations indicate favorable adsorption of mercury on to algae biomass (Table 2). In this study it is assumed that the algal particles are spherical and only surface adsorption is occurring. When the biomass is employed as a free cell suspension in a well-agitated batch system, all the cell

wall binding sites are made readily available for metal uptake so the effect of external film diffusion on biosorption rate can be assumed not significant and ignored in any engineering analysis. It can be assumed that measured concentrations are equal to cell surface concentrations in this case. From experimental data, it was shown that the initial biosorption rate is proportional to the first power of the initial metal ion concentration at lower bulk metal ion concentrations and at higher metal ion concentrations, the rate becomes independent of initial metal ion concentration. Two important physicochemical aspects the evaluation of the sorption process as a unit operation are the equilibrium of sorption and the kinetics. Biosorption equilibrium is established when the concentration of metal in a bulk solution is in dynamic balance with that of the interface (Kaewsarn, 2002). Many studies have shown that at low metal ion concentrations, the mass of the metal ion accumulated (per unit of cell mass) is directly proportional to the concentration of the ion in solution. The amount of mercury ions adsorbed per unit mass of the biosorbent (i.e., biosorption capacity) increased with the initial concentration of metal ions, as expected. In order to reach the plateau values which represent saturation of the active sites on the biosorbent, in other words to obtain the maximum biosorption of algae mass for mercury ions, the initial concentration was increased up to 1 mg L^{-1} . Several explanations for biosorption of heavy metal on the microbial cells have been previously reported. For example, the biosorption of heavy metal ions occurred on the cell surface or within the cell wall matrix. The algae cell walls have a negative charge due to the arrangement of the carboxyle, phosphate and other groups of the cell walls components. The phosphate containing teichoic acid in the cell wall of the algae is primarily responsible for metal binding. The biosorption on the algae cell surface is a result of the complexation reaction between heavy metal ions and the charged constituents of the cell wall components. The desorption of mercury from the biosorbents, the algae, was studied in a batch system. The metal ions taken onto biosorbents were eluted with 10 mM HCl. More than 97% of the adsorbed metal ions were desorbed from the biosorbents. In order to show the reusability of the biosorbents, an adsorption desorption cycle of metal ions was repeated three times using the same preparations. The adsorption capacities for all the biosorbents did not noticeably change (only a maximum 3% change was observed with the tested biosorbent) during the repeated adsorption-desorption operations. These results showed that the algae could be repeatedly used in heavy metal adsorption studies without detectable losses in their initial adsorption capacities. In order to determine the functional groups responsible for mercury biosorption, IR spectroscopy was used. In

general, the IR spectra for biosorbent has intense peaks at a frequency level of 3300-3500 cm^{-1} representing -OH groups stretching vibration. The bands at 1000-1260 cm^{-1} were due to the -C-O stretching of ether, ester, alcoholic and carboxylic acid groups. The bands at 2915, 1517, 1522 and 1054 cm^{-1} representing C-H stretching, N-H bending, -NH₂ wagging and C-OH stretching, respectively, are due to the several functional groups present on algae biomass. Some of the functional groups wave number shift to different extents after contact with metal solutions, such as C=O from 1635 to 1650 cm^{-1} , -NH from 1416 to 1417 cm^{-1} , -OH from 1635 to 3355 cm^{-1} and -SO₃ from 1248 to 1251 cm^{-1} . The spectral analysis before and after metal binding indicated that these functional groups involved in metal biosorption. According to the IR analysis, the functional groups involved in metal biosorption included C=O, -NH, -OH and -SO₃. Sulfonate groups did not play a major role in the binding of mercury ions. The study also indicated that algae biomass used to develop high capacity biosorbent materials for the removal and recovery of mercury ion and other metal ions from dilute industrial wastewater streams. Algae biomass was chosen as biosorbent in this experiment. Firstly, as the algae biomass, the physical characterization contains abundant floristic fiber and protein and properties the functional groups such as carboxyl, hydroxy and amidogen, etc. are in existence, which make biosorptive processes possible. Secondly, the adsorbent of algae biomass obtained from natural basins, furthermore, their yield is vastness. The study also indicated that algae biomass can be used to develop high capacity biosorbent materials for the removal and recovery of mercury ion and other heavy metal ions from dilute industrial wastewater streams.

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