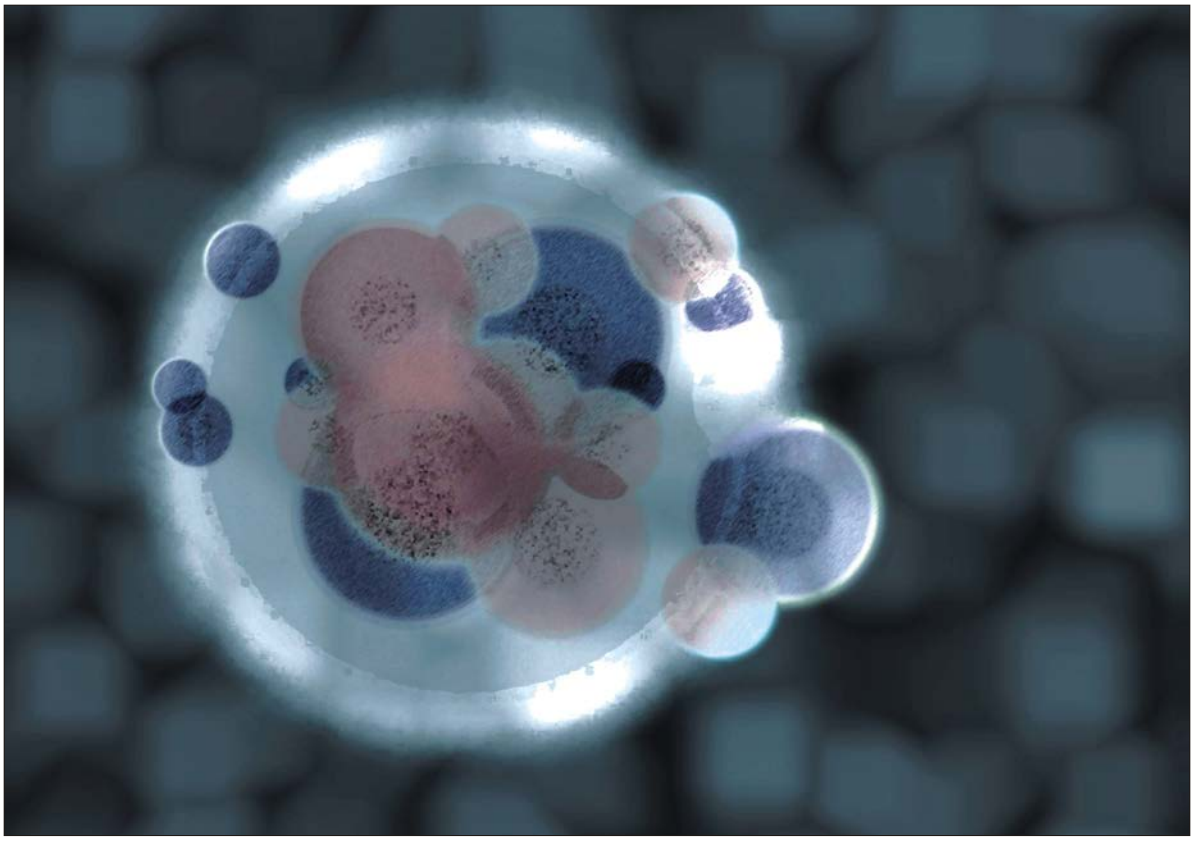

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Biosorption of Mercury by Biomass of Filamentous Algae *Spirogyra* Species

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Abstract: In this study, the filamentous algae *Spirogyra*, harvested locally, were investigated for their biosorption performance in the removal of mercury from dilute aqueous solutions. It was found that the biosorption capacities were significantly affected by solution pH, with pH 4 favoring higher mercury removal. Biosorption rate and kinetic were obtained from batch adsorption experiments. Kinetic and isotherm experiments were carried out at the optimal pH. Biosorption kinetic was found to be fast, with 90% of adsorption within 15 min and equilibrium reached at 30 min. Langmuir adsorption models was suitable for describing the short-term biosorption of mercury by the algal species. The results indicated that the biomass of *Spirogyra* is suitable for the development of efficient biosorbent for the removal and recovery of mercury from wastewater.

Key words: Biosorption, mercury, algae, *Spirogyra*, heavy metals

INTRODUCTION

Heavy metal pollution is an environmental problem of worldwide concern (Sheng *et al*., 2004). The heavy metal mercury is among the most common pollutant found in industrial effluents. The major sources of mercury pollution in the aquatic environment are industries such as chloralkali, paint, pulp and paper, oil refining, electrical, rubber, processing, fertilizer, pharmaceutical and battery manufacturing (Manohar, 2002). Mercury is generally considered to be one of the most toxic metals found in the environment (Namasivayam and Kabdirvelu, 1999). The major effects of mercury poisoning manifest as neurological and renal disturbances as it can easily pass the blood-brain barrier. Hence it is essential to remove mercury from wastewaters before its transport and cycling into the environment. Various treatment technologies such as precipitation, ion exchange, Biological and adsorption have been employed to remove metal pollutants from aqueous solutions. One of the promising techniques for the removal of metals is the use of living or nonliving organisms and their derivatives. A wide variety of microorganisms (both living and nonviable) have been found to be capable of sequestering trace levels of metal ions from dilute aqueous solutions. The nonviable forms have been proposed as potential sorbents, since these are essentially dead materials, which require no nutrition 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 than living cells in sequestering metallic elements. Over the past two decades, much effort has been directed at identifying readily available biomass which, in its nonliving state, is capable of effectively removing heavy metals (Vitic and Giovannettil, 2001). It has been demonstrated that biosorption is a potential alternative to traditional treatment processes of metal ions removal (Volesky, 1990). Biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions. Biomass exhibits this property, acting just as a chemical substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria which was found responsible for this phenomenon. Opposite to biosorption is metabolically driven active bioaccumulation by living cells. That is an altogether different phenomenon requiring a different approach for its exploration. The phenomenon of biosorption has been described in a wide range of non-living biomass like bark (Alves *et al*., 1993); lignin (Srivastava *et al*., 1994) and peanut hulls (Periasamy and Namasivayam, 1994) as well as of living biomass like fungi (Lewis and Kri, 1988; Matheickal and Yu, 1996; Fourest *et al*., 1994), bacteria (Scott and Palmer, 1990; Grappelli *et al*., 1992; Churchill *et al*., 1995; Andreoni *et al*., 2003), yeast (Huang *et al*., 1990; Volesky *et al*., 1993), moss (Lee and Low, 1989; Low and Lee, 1991), aquatic plants (Srivastav *et al*., 1994) and algae (Xue *et al*., 1988; Yu *et al*., 1999). Biosorption utilizes the ability of

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biological materials to accumulate heavy metals from waste streams by either metabolically mediated, or purely physico-chemical pathways of uptake (Fourest *et al*., 1994). Some of the biomass types come as a waste by-product of large-scale industrial fermentations (the mold *Rhizopus* or the bacterium *Bacillus subtilis*). Other metal-binding biomass types, certain abundant seaweeds (particularly brown algae e.g., *Sargassum*, *Ecklonia*), can be readily collected from the oceans. These biomass types, serving as a basis for metal biosorption processes, can accumulate in excess of 25% of their dry weight in deposited heavy metals: Pb, Cd, U, Cu, Zn, even Cr and others . Research on biosorption is revealing that it is sometimes a complex phenomenon where the metallic species could be deposited in the solid biosorbent through different sorption processes of ion exchange, complexation, chelation, microprecipitation, etc. The biotechnologies based on the action of actively growing algal cells can solve some of these diffculties by means of cell adsorption and absorption (Perez-Rama *et al*., 2002; Terry and Stone, 2002) and biologically produced alkaline conditions (Rose *et al*., 1998; Sternberg and Dorn, 2002). Most of the research on the assessment of heavy metal removal by living algae has been performed in batch cultures, where high pH values can be attained during comparatively long exposition times (about 7-13 days) (Sternberg and Dorn, 2002). The biosorption capability of algae has been attributed mainly to the cell wall, which is composed of a fiber-like structure and an amorphous embedding matrix of various polysaccharides. The purpose of present study is to evaluate the biosorption capacity of the algae *Spirogyra* species for mercury from aqueous solutions. The adsorption capacities were evaluated from equilibrium adsorption isotherms and the results indicated that the algae is a suitable material for the development of high capacity biosorbent for mercury removal.

MATERIALS AND METHODS

Isolation and cultivation: Fresh algal biomass was collected from a river-basin in Tehran, Golab dareh, Iran in spring and autumn seasons. The collected algae were washed with distilled water to remove dirt and was kept on a filter paper to reduce the water content. The algae was identified, *Spirogyra* species, by standard biological methods. All plastic and glassware used for the growing of *Spirogyra* species was previously cleaned for 24 h with 1 N HNO_3 solution and then rinsed with distilled water. The algae *Spirogyra* was cultures in modified Bold's basal medium (Table 1) supplied with filtered air (Sartrius filter with a pore-size of 0.2 μm), maintained at 25°C and illuminated at 4000 lux light intensity provided by fluorescent lamps with a light/dark cycle of 16/8 h for 20 days. The algal cells at the log phase were harvested by centrifugation at 7000 rpm for 20 min at 4°C. The biomass was sieved to select particles smaller than 1 mm in size and stored in a dry cabinet. The biomass dried at 60°C for 24 h and milled to a gritty consistency.

Preparation of synthetic wastewater: A stock solution of mercury with concentration of 10 mg L^{-1} was prepared in distilled water by mercury chloride. All working solutions of varing concentrations were obtained by diluting the stock solution with distilled water.

Analysis of mercury: All the glass and plastic wares used were kept in 1.0 N HNO_3 solution overnight and then thoroughly rinsed with deionized water. Mercury stock solution (1000 ppm) were prepared from dissolve chloride mercuric in mixture nitric acide 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 adsoption 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% HNO_3. Ionic mercury was then reduced with $NaBH_4$ (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. 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% HNO_3 for 2 h at 140°C.

Batch adsorption experiments: Sorption studies were conducted in 100 mL conical flasks at solutions with pH (4-8.5). In the experiment to investigate the effect of pH, mercury solutions at various initial pH were prepared using 0.1 mol L^{-1} HNO_3 or 0.1 mol L^{-1} NaOH. The final solution pH was measured with an ORION 525A pH meter. Dry *Spirogyra* biomass, 1 g L^{-1}, were thoroughly mixed individually with 50 mL of mercury solution, 1000 μg L^{-1}, at temperatures 4, 25 and 37°C. Samples were taken at pre-determined time intervals (1, 5, 10, 20, 40, 60 and 80 min) and were filtered through whattman filter paper. The filters were analyzed for residual mercury concentration in the solution. The flasks were agitated at 100 rpm for 6 h. The mercury concentrations were measured using a flow injection system, which linked to an atomic adsorption spectrophotometer (UNICAM, model 929, UK).

Adsorption isotherms: Batch sorption experiments were carried out in 100 mL conical flask at 25°C on a rotary shaker for 40 min. The dry biomass, 1 g L^{-1}, was thoroughly mixed with 50 mL of mercury solution. The isotherm studies were performed by varying the initial mercury concentrations (1000, 2000, 3000, 4000, 5000 and 6000 μg L^{-1}) at pH of 4.0. The pH value was adjusted using 0.1 M HCl or NaOH before addition of biomass and was maintained throughout the experiment. After shaking the flasks for 40 min, the reaction mixture was analyzed for residual mercury concentration. The Langmuir sorption model was applied for adsorption equilibrium (Gupta *et al*., 2001).

$$1/q_e = 1/(X_m b C_e) + 1/X_m$$

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 (μg L^{-1}). In the adsorption isotherm experiments, the pH was set as that in the kinetic experiments. The same amount of biomass was added to the solutions with varying metal concentrations. All flasks were shaken at 100 rpm at room temperature. The initial and final concentrations were determined using atomic adsorption spectrophotometer (UNICAM, model 929, UK). Biosorption of the metal ions (q) in the sorption system was calculated using the mass balance:

$$q = \frac{V(Ci.Ce)}{W}$$

Where V is the solution volume, W is the amount of biomass and Ci and Ce are the initial and final (or equilibrium) metal concentrations, respectively. The Langmuir sorption isotherm was used to fit the experimental biosorption data,

$$qe = \frac{q\max bCe}{1+bCe}$$

Where qmax and b are Langmuir constants, which reflect the maximum metal sorption capacity and the affinity between the metal ion and biosorbent (Sheng *et al*., 2004).

Biosorption/Desorption: 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.

Viability study of mercury-treated *Spirogyra*: The toxicity of various mercury concentrations to algal cells was studied. After mercury biosorption process, 1 mL algal cell suspension was harvested, washed twice with sterilized deionized water and resuspended in sterilized modified Bold's basal medium. The algal cells at a biomass concentration of 30 mg mL^{-1} were then incubated aseptically under the same culture condition as described above for two weeks. The algal cells without any mercury treatment were used as the control. The cell number during the 2-week growth period was monitored regularly with the growth evaluation.

RESULTS AND DISCUSSION

Cultivation: According to the results presented here, the culture media that enable an adequate growth and development for the algae *Spirogyra* species such as Bold's basal medium, could affect the removal rate and the efficiency of wastewater treatment when algae are used for this purpose. The Bold's basal medium has high capacity to chelate heavy metals, due to the content of EDTA, which concentration is one order of magnitude higher than the total amount of metallic ions added as micronutrients, thus leaving only a small fraction as free ions. The free ion activity of one bivalent metal increased in the presence of a second bivalent metal; similarly, such activity increased by up to two orders of magnitude in the two and three-metal mixtures. The dry weight of the biomass was determined gravimetrically at several concentrations after drying at 105°C for 24 h.

Effect of agitation time: The results of mercury removal (Fig. 1) 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.

Effect of PH: Earlier studies have indicated that pH is an important parameter effecting biosorption of heavy metal ions (Gupta *et al*., 2001; Rezaee *et al*., 2005). The maximum adsorption takes place at pH 4. The cell wall of *Spirogyra* species 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

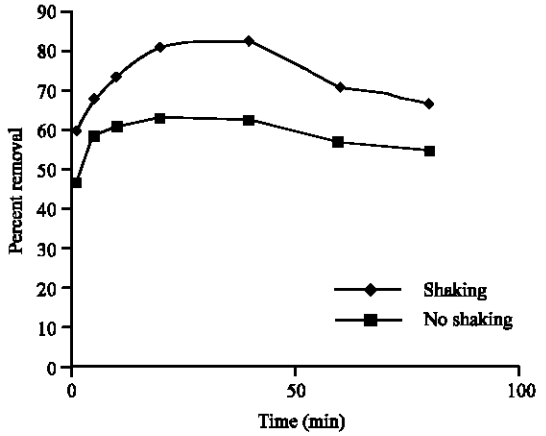

Fig. 1: Effect of agitation time on the mercury removal (temperature: 25°C, pH 8.5, algal dose: 150 mg/50 mL, agitation: 100 rpm)

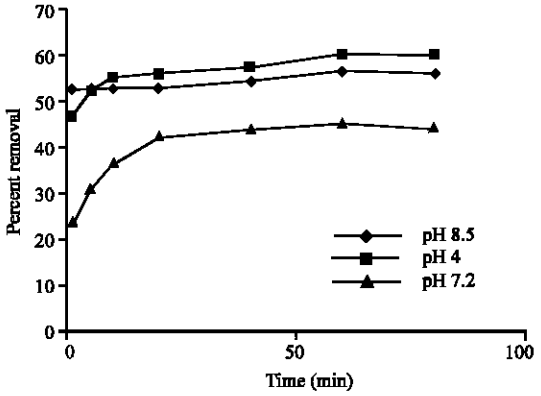

Fig. 2: Effect of pH on the mercury removal (temperature: 25°C, pH 8.5, algal dose: 150 mg/50 mL, agitation: 100 rpm)

functional groups and also on the metal chemistry in solution (Fig. 2). 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 p*Ka* in the range 3.5-5.0) of algal cell wall constituents as the probable biosorption sites. A good correlation between the degree of blocking of -COO- groups by esterification in *Sargassum fluitans* and the corresponding decrease in metal uptake has been reported (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-4(apparent p*Ka* in the

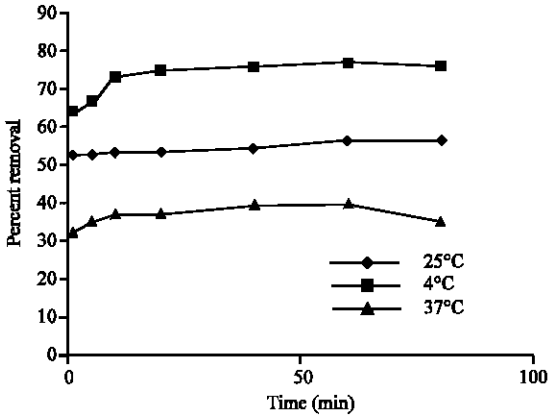

Fig. 3: Effect temperature on the mercury removal (initial concentration: 1000 μg L^{-1}, pH 4.0, algal dose: 150 mg/50 mL)

range 1.0-2.5) of fucoidan in the brown algal cell wall may play a 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 biosorption at higher pH was reduced as a result of the pretreatment procedures. In order to examine the biosorption potential of the algal *Spirogyra* 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.

Effect of temperature: 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 medium could be important for Energy-dependent mechanisms in metal biosorption by Microorgamisms. 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.

Table 1: Formulation of modified Bold's basal medium

Ingredient	Concentration (mg L^{-1})
$NaNO_3$	95.2
$CaCl_2.2H_2O$	25
$MgSO_4.H_2O$	75
K_2HPO_4	75
KH_2PO_4	175
NaCl	25
$FeSO_4.H_2O$	4.98
H_2SO_4	0.001 (mL L^{-1})
H_3BO_3	11.42
EDTA	50
KOH	31
$ZnSO_4.7H_2O$	8.82
$MnCl_2.7H_2O$	14.4
$MoO_4Na_2.2H_2O$	0.71
$CuSO_4.5H_2O$	1.57
$CoCl_2$	0.49

Adsorption isotherm: 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 1. 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}). According to it has been shown using matheother research (Namasivayam and Kabdirvelu, 1999). 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).

Desorption and reuse: 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 adsorptiondesorption 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.

Table 2: Langmuir constants

R^2	X_m(mg g^{-1})	b (l mg^{-1})	Hg(II)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

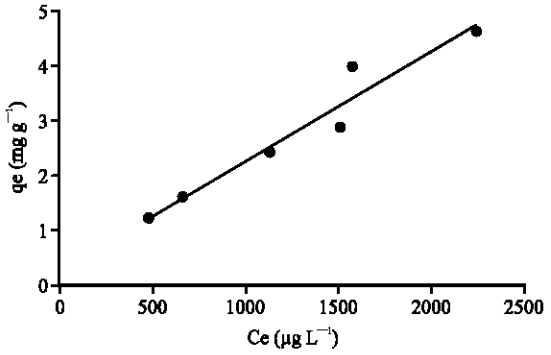

Fig. 4: Langmuir plot for adsorption of mercury (mercury concentration: 1500-6000 µg L^{-1}, pH 4, agitation time: 40 min, algal dose: 50 mg/50 mL)

CONCLUSIONS

In this study, mercury removal by inactivated *Spirogyra* species 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. It was shown that in practical applications of algal biosorption that pH could be adjusted to be more favorable to the removal of the ions of interest. Biosorption can be accomplished with agitation by increasing the contact time up to 20-40 min. The langmuir adsorption model was used for mathematical description of the biosorption of mercury to dried Spirogyra 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 study also indicated that *Spirogyra* 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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