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Biosorption of Cu^{2+} and Zn^{2+} by raw and autoclaved *Rocella phycopsis*

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Abstract

The behavior of Cu^{2+} and Zn^{2+} biosorption onto raw and modified *Roccella phycopsis* from aqueous solutions was studied. Modification process was applied by autoclavation at 121°C for 30 min. The effects of pH, initial metal concentration and biosorbent dosage were investigated. The maximum Cu^{2+} biosorption was achieved at pH 5.0 and the maximum biosorption capacities of 31.5 and 37.8 mg/g were recorded for raw and modified biosorbent, respectively. In the case of Zn^{2+} biosorption, maximum biosorption capacities were obtained at pH 4.0 as 29.1 and 35.3 mg/g for raw and modified biosorbent, respectively. Biosorption of Zn^{2+} and Cu^{2+} on all form of *R. phycopsis* increased much quickly with increasing initial metal concentrations from 10 to 100 mg/L. After modification process, probable changes in the surface polarity of raw and modified *R. phycopsis* were investigated by contact angle measurements. As expected, *R. phycopsis* has a polar surface and shows a highest contact angle with water, while after autoclavation water contact angle of *R. phycopsis* was significantly decreased from 47.5° to 34.4°.

Key words: biosorption; *Roccella phycopsis*; polarity; contact angle measurement

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Introduction

Considerable attention has been paid to the removal of heavy metals from industrial wastewaters because of their dangerous to living organisms. Various physicochemical and biological techniques have been applied to remove heavy metals from wastewaters. Biological treatment based on living or non-living organisms, may reduce the level of toxic metals to environmentally acceptable limits in a cost-effective manner. The major advantages of biological treatment over conventional treatment methods also include high efficiency of metal removal from dilute solution, minimization of chemical and/or biological sludge, no additional nutrient requirement and regeneration of biosorbent (Volesky, 2001; Jana and Edita, 2005).

Biosorption is a mode of non-active metal uptake by biological materials, such as adsorption, ion exchange, coordination and complexation. Biological cell walls contain large quantity of polysaccharides and proteins offered many functional groups, such as carboxyl, hydroxyl, sulphate, phosphate and amino groups, which can bind metal ions. The performance of a biosorbent depends on its surface characteristics, which can be changed by modification process. The use of raw and modified biosorbents for metal ions removal has gained importance in recent years (Greene and Darnall, 1990; Crist et al., 1992). The difference in the surface of raw and modified biosorbent is most important in biosorption mechanism. Among the surface characters, the chemical structure, the hydrophobicity/hydrophilicity and polarity should be investigated.

High metal-binding capacities of several biological materials have already been identified in literature. Among the biosorbents, marine algae, bacteria, yeasts and fungi were studied extensively. Phytotechnologies, with an increasing development during the last two decades, involve the use of plants for metal removal (Loppi and Dominicis, 1996; Miretzky et al., 2004). Lichens are one of the most valuable biomonitors of heavy metal pollution and can be used as sensitive indicators. Carreras and Pignata (2007) suggested that *U. amblyoclada* has a stronger ability to bind Pb^{2+} than to Cu^{2+}, probably as a result of its greater affinity for the lichen cell wall. In addition, Chettri et al. (1998) observed that the uptake of metals followed the sequence Pb > Cu > Zn in two lichen species. It has been demonstrated that the attraction of metals to the lichen surface structure is largely due to the secondary metabolites of lichens, such as usnic acid and parietin, which can binding metals (Purvis et al., 1996).

In the present study, Cu^{2+} and Zn^{2+} biosorption potential of raw and modified *R. phycopsis* was investigated. The optimum conditions required for biosorption were determined. The changes in the surface properties of *R. phycopsis* after modification was also evaluated through FT-IR and contact angle measurements.

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1 Experiment

1.1 Biosorbent preparation

Samples of *R. phycopsis* were collected from the coast of Gedikkaya Hill (Giresun, Turkey). The biosorbent was washed with tap and deionized water to eliminate impurities. After drying at 60°C for 12 hr, it was crushed and ground in an analytical mill (IKA A10, Daigger Company, USA). To modify the raw biosorbent, autoclavation was applied for 30 min at 121°C. Afterwards, the material was rinsed thoroughly with deionized water and dried in an oven at 60°C overnight. The raw and modified *R. phycopsis* were used as biosorbent for the removal of Cu^{2+} and Zn^{2+}.

1.2 Characteristics of biosorbent

1.2.1 Scanning electron microscopy

The dried raw and modified *R. phycopsis* samples were coated with gold under reduced pressure and the scanning electron microscopy (SEM) micrographs were obtained using a scanning electron microscope JEOL (JSM 5600LV, Tokyo, Japan).

1.2.2 Swelling ratio

Swelling properties of *R. phycopsis* samples were determined by gravimetric process. Dried lichen samples (0.1 g) were carefully weighed before being placed in 50 mL vials containing buffer solutions with a pH range 1.0–7.0. The vials were placed in a waterbath (37°C) for 4 hrs. The samples were removed every 15 min and weighed. The swelling ratio (S) of *R. phycopsis* can be calculated using Eq. (1):

$$S = \frac{(W_S - W_0)}{W_0} \times 100\% \quad (1)$$

where, W_0 and W_S are the weights of samples before and after uptake of water, respectively.

1.2.3 Fourier transform infrared spectroscopy

Fourier transform infrared spectra (FT-IR) of the dried raw and modified *R. phycopsis* samples were obtained using PerkinElmer Paragon 1000 (USA). The samples were mixed with KBr and pressed into a tablet form, and then spectrum was recorded.

1.2.4 Contact angle measurements

Contact angles of raw, modified and metal adsorbed *R. phycopsis* to water and diiodomethane test liquids were measured using a digital optical contact angle meter CAM 200 (KSV Instruments Ltd., Helsinki, Finland). The sessile drop was formed on the samples by a Hamilton microsyringe contrivence. Then the left and right contact angles were automatically calculated from the digitalized system. Contact angle of test liquids, Lifschitz-van der Waals and polar components of samples were calculated according to Eqs. (2) and (3) by CAM 200 software package operated under Windows 98.

$$\gamma_l(1 + \cos\theta) = 2((\gamma_l^P \gamma_s^P)^{1/2} + (\gamma_l^{LW} \gamma_s^P)^{1/2}) \quad (2)$$

Rearranged Eq. (2):

$$\frac{\gamma_l(1 + \cos\theta)}{(\gamma_l^{LW})^{1/2}} = (\gamma_s^P)^{1/2}\left(\frac{(\gamma_l^P)^{1/2}}{(\gamma_l^{LW})^{1/2}}\right) + (\gamma_S^{LW})^{1/2} \quad (3)$$

where, θ is the contact angle, γ_l is liquid surface tension and γ_s is the solid surface tension, or free energy. LW and P refer the dispersive (Lifschitz-van der Waals) and polar components of each, respectively. The form of the equation is of the type $y = mx + b$. The slope of the $(\gamma_l^{LW})^{1/2}/(\gamma_l^{P})^{1/2}$ vs. γ_l $(1+ \cos \theta)/(1+\gamma_l^{LW})^{1/2}$ graph is equal to $(\gamma_s^{P})^{1/2}$ and the y-intercept is $(\gamma_s^{LW})^{1/2}$. The total free surface energy is merely the sum of its two component forces.

1.3 Biosorption studies

The Cu^{2+} and Zn^{2+} stock solutions were prepared by dissolving $CuCl_2 \cdot 5H_2O$ or $ZnSO_4 \cdot 2H_2O$ (analytical grade, Merck) into distilled water. The sorption experiments were performed by batch method where samples of 0.1 g of biosorbent were equilibrated with 50 mL of 100 mg/L metal solution under an intermittent stirring. The biosorbent was removed from the solution after centrifuging. The residual metal concentration in the solution was analyzed using atomic adsorption spectrometer (Model-933, GBC Scientific Equipment, Melbourne).

The effect of pH on the biosorption of metal ions was carried out in the range that would not be influenced by the metal precipitation. The suitable pH ranges for Cu^{2+} and Zn^{2+} was selected as 1.0–6.0 and 1.0–7.0, respectively (Churchill et al., 1995; Dönmez and Aksu, 2002). The solution pH had been adjusted to the desired value with concentrated NaOH. To determine the effect of initial metal concentration on biosorption capacity of *R. phycopsis*, a concentration range of 10–100 mg/L for Cu^{2+} and Zn^{2+} was used. The effect of the biosorbent dosage (0.01–0.40 g/L) on biosorption of heavy metal ions was studied.

The amount of metal biosorbed by *R. phycopsis* was calculated as following Eq. (4):

$$Q = \frac{(C_i - C_f)V}{m} \quad (4)$$

where, Q (mg metal/g biosorbent) is the amount of metal uptake, C_i (mg/L) is the initial concentration metals in the solution, C_f (mg/L) is the final (equilibrium) concentration of metals in the solution, V (mL) is the volume of liquid sample and m (mg) is the amount of the added biosorbent on the dry forms.

2 Results and discussion

2.1 Characteristics of biosorbents

2.1.1 Surface properties

SEM micrographs of raw and modified *R. phycopsis* are presented in Fig. 1. The magnification view of modified *R. phycopsis* shows a ramified surface structure of lichen formed by modification. An explanation of the ramified surface is the deterioration in the integrity of cortex layer by pressure and heat treatment risen from autoclavation. After disintegration of cortex layer, a mesh of hair-like

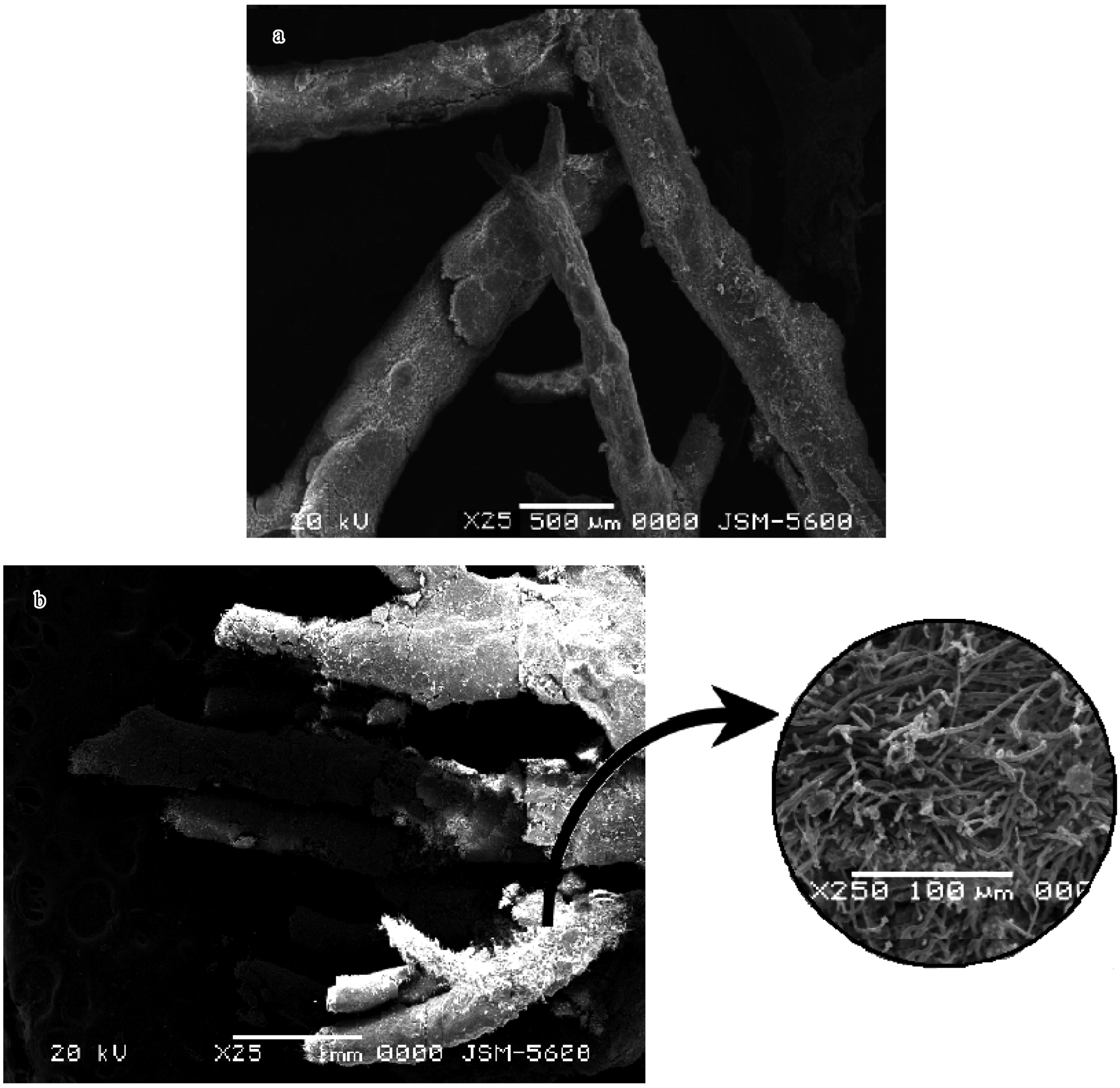

Fig. 1 SEM micrographs of raw (a) and modified (b) *Roccella phycopsis*.

threads called thallus appeared and caused a ramified structure. This new formation should be considered as a factor providing an increase in surface area for biosorption process.

2.1.2 Swelling ratio

The swelling ratios of *R. phycopsis* in pH range 1.0–7.0 are shown in Fig. 2. The maximum swelling ratio (69%) of the biosorbent was obtained at pH 4.0 and the minimum value (7.9%) was obtained at pH 2.0. The ionize groups of the biosorbent such as carboxyl, hydroxyl and amine introduced positive and negative charges into the structure, and should be caused more water uptake. The swelling ratio of *R. phycopsis* was very fast at pH range 4.0–6.0, reaching its maximum in initial minutes, which, however, reaches the equilibrium with a longer contact time.

2.1.3 FT-IR spectra

Lichen cell components such as polysaccharides, proteins and lipids have been shown to affinity metal ions. Changes in the functional groups and surface properties of *R. phycopsis* after autoclavation were confirmed by FT-IR spectra (Fig. 3). The FT-IR spectra of both raw and modified *R. phycopsis* have a peak at a frequency level of 3400–3000 cm^{-1} representing –OH stretching of carboxylic groups and –NH groups. The peaks observed in both spectra between 1000–1800 cm^{-1} representing N–H bending, $–CH_3$ wagging and C–OH stretching vibrations, respectively, are due to the several functional groups present on the cell wall. The bands observed in FT-IR spectra of modified *R. phycopsis* at 2253 cm^{-1} could be assigned to –CH stretch, and this peak was not seen for raw counterparts. This observation could be explained by

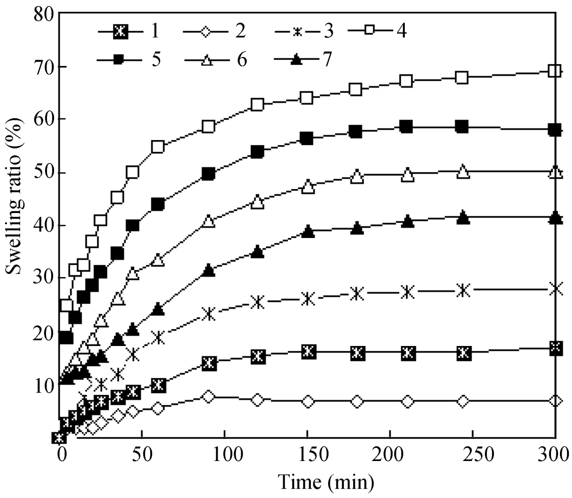

Fig. 2 Swelling ratio of *R. phycopsis* at different pH values.

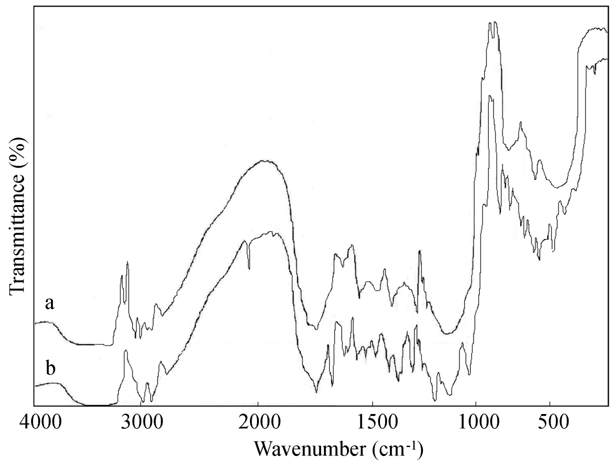

Fig. 3 FT-IR spectra of of raw (line a) and modified (line b) *Roccella phycopsis*.

the new formation of organic residues after autoclavation. On the other hand, there was clear decrease in the intensity of C–N–C band at 540–470 cm^{-1}, representing scissoring of some components.

2.1.4 Contact angle measurements

Contact angle measurement is a method applied for surface analysis related to polarity and wettability of materials. The results of the contact angle measurements of water and diiodomethane on the raw, modified and metal adsorbed biosorbent surfaces are presented in Table 1. The lowest contact angles were obtained with water, whereas diiodomethane gave the highest contact angles. The wettability of the biosorbent surfaces can be examined by comparing these contact angles since water and diiodomethane were used as the test liquids to evaluate the hydrophilic and hydrophobic interactions, respectively. As expected, *R. phycopsis* has a polar surface and shows a lower contact angle compared to diiodomethane. As shown in Table 1, the water contact angle of *R. phycopsis* was significantly decreased from 47.5° to 34.4° after autoclavation. This may due to the increase density of polar functional groups on the biosorbent surfaces after

Table 1 Contact angle measurements, polarity and Lifschitz-van der Waals components (γ_s^{LW}) of samples

Samples	Contact angle (°)		Polarity (%)	γ_s^{LW} (mN/m^2)
	Water (γ = 71.3)	Diiodomethane (γ = 50.8)		
Raw biosorbent	45.7	46.6	17.06	32.78
Modified biosorbent	34.4	41.7	22.18	45.04
Cu^{2+} adsorbed-modified biosorbent	54.9	48.9	11.89	21.37
Zn^{2+} adsorbed-modified biosorbent	51.7	49.1	15.43	20.71

modification process. The tissue and cell components of *R. phycopsis* should be disintegrated by heat and pressure rised from autoclavation. The modification may also degrade the polysaccharide compounds of the cell wall, and produce additional functional sites. Therefore, new components may be resulted an increase in the polar groups, and then the contact angle of water on modified biosorbent was decreased. This result indicated that the polarity and wettability of the surfaces of modified biosorbent significantly increased with modification process. The re-organization and new formations of lichen surface structure after autoclavation was corrected by FT-IR analysis. Also the Lifschitz-van der Waals component of the surface free energy of modified biosorbent (γ_s^{LW}) was increased from 32.78 to 45.04 mN/m^2 with modification.

An interesting result was obtained with the water contact angles of metal adsorbed-modified *R. phycopsis*. As can be seen in Table 1, after biosorption process contact angles of water on the modified biosorbent was increased and as expected, the polarity and LW components were decreased according to initial values. This may be contributed by screening the polar functional sites on the biosorbent surface after biosorption mechanism (Sharma and Agarwal, 2001; Brack et al., 2003)

2.2 Biosorption studies

The pH of the aqueous medium is perhaps the most important parameter in the biosorption of metal ions. Figure 4 shows the biosorption of Cu^{2+} and Zn^{2+} as a function of pH. The maximum Cu^{2+} biosorption capacities were achieved at pH 5.0 and was 31.5 for raw and 37.8 mg/g for modified *R. phycopsis*. In the case of Zn^{2+} biosorption, maximum biosorption capacities were obtained at pH 4.0 as 29.1 for raw and 35.3 mg/g for modified *R. phycopsis*. A sharp increase in metal uptake was observed with increasing pH from 2.0 to 5.0. This results can be explained using the fact that with increasing pH, the ligands in *R. phycopsis* would be exposed, increasing the attraction of metal ions with positive charge and allowing the biosorption on the cell wall surface. Carboxylic groups are the main functionalities involved in metal binding reactions in biological materials. Nevertheless, a smaller amount of functional groups such as sulphonic, N– and S–containing groups from proteins may also be important for metal ion binding (Kapoor and Viraraghavan, 1998; Vilar et al. 2005). The presence of carboxyl and hydroxyl groups on the surface of *R. phycopsis* were demonstrated

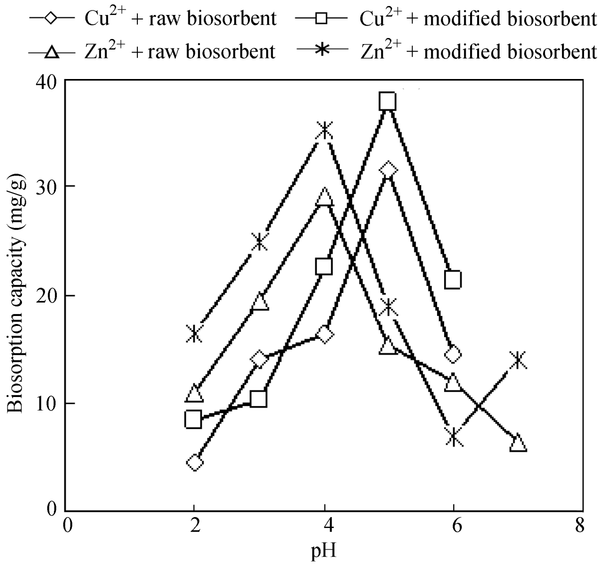

Fig. 4 Effect of pH on Cu^{2+} and Zn^{2+} biosorption. Initial metal concentration: 100 mg/L, temperature: 25°C.

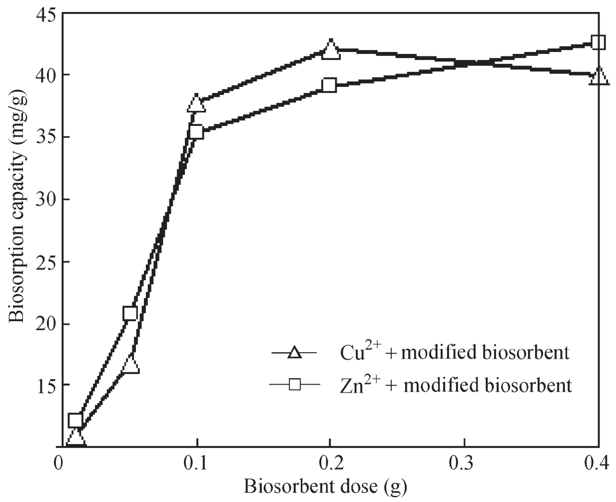

Fig. 5 Effect of biosorbent dose on Cu^{2+} and Zn^{2+} biosorption. Initial metal concentration: 100 mg/L, temperature: 25°C.

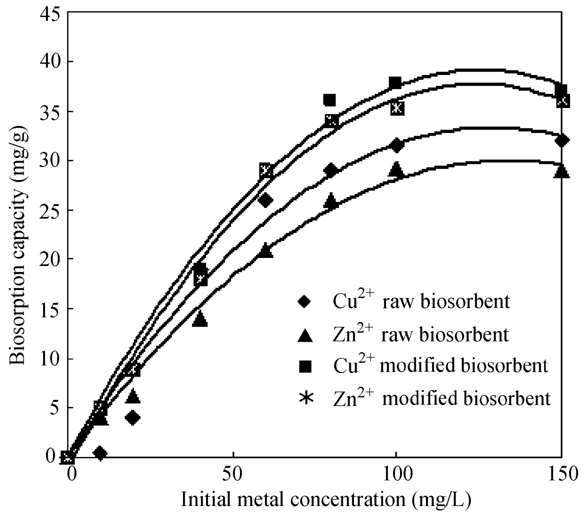

Fig. 6 Effect of initial metal concentration on Cu^{2+} and Zn^{2+} biosorption. Temperature: 25°C.

by FT-IR spectra. As a consequence of this chemical functionality, there will be a more or less broad range of affinities for the metal ions. Furthermore, above pH 6.0 the biosorption of metals decreased probably because of chemical precipitation (Kapoor and Viraraghavan, 1998).

A significant difference in biosorption capacities of raw and modified *R. phycopsis* is shown in Fig. 4. The bisorption capacities of Cu^{2+} and Zn^{2+} ions onto the biosorbent were 1.20- and 1.22 times higher than raw counterparts, respectively. Modification of biosorbent by autoclavation results a re-organization of cell wall so the additional binding sites occurred. Heat and pressure treatment degrade some components of cell wall. By heating the compounds in cell wall, new binding sites formed (Churchill et al. 1995; Donmez and Aksu, 2002; Yu et al., 1999). The formation of new binding sites could be affirmed by FT-IR analysis. The bands of –CH stretching at 2253 cm^{-1} only observed in FT-IR spectra of modified *R. phycopsis*, and not in raw counterparts. On the other hand, there was a clear decrease in the intensity of the C–N–C band at 540–470 cm^{-1} in FT-IR spectra of raw biosorbent, representing the scissoring of some components and the formation of new organic residues. In addition, the denaturation of proteins located in cell wall by treatment and structural changes was occurred. All these cumulative effects of modification explain the increase of biosorption capacity.

The effect of biosorbent dosage on the Cu^{2+} and Zn^{2+} biosorption was determined at initial metal concentration of 100 mg/L (Fig. 5). At equilibrium, the biosorption of Cu^{2+} and Zn^{2+} increased 72.75% and 76.5% with increasing the biosorbent dosage from 0.01 to 0.40 g. The increase in biosorption rate was due to the increase in the available sorption surface and sites.

The effect of initial metal concentration on the biosorption capacity was investigated at pH 4.0 for Zn^{2+} and pH 5.0 for Cu^{2+}. As shown in Fig. 6, the biosorption of Zn^{2+} and Cu^{2+} increased much quickly with increasing initial metal concentration from 10 to 100 mg/L. A higher initial concentration provides an important driving force to overcome all mass transfer resistances between the metal solution and algal cell wall, thus the biosorption capacity increases. In addition, the number of collisions between metal ions and biosorbent increases with increasing initial metal concentration, and thus the biosorption process enhances (Aksu and Tezer, 2005).

Many researchers have also investigated the performance of different biosorbents for the removal of various heavy metal ions, such as Ni, Cu, Pb, Zn, Cd, and Al from wastewater. Yu et al. (1999) studied the biosorption capacities of macro marine algae for Pb, Cu, and Cd ions and reported the biosorption capacity as 1.6, 1.2 and 1.2 mg/L, respectively. Munoz et al. (2005) reported a (8.5 ± 0.4) mg/g maximum Cu^{2+} adsorption capacity of algal-bacterial biosorbent at an initial Cu^{2+}concentration of 20 mg/L. Mihova and Godjevargova (2000) studied the biosorption of Cu^{2+} onto *S. cerevisiae* and *P. chrysosporium* and obtained the adsorption capacity of 3.5 mg/g and 2.5 mg/g, respectively. Sandau et al. (1996) studied the biosorption of Cu^{2+} onto *C. vulgaris* and *Spirulina platensis* and achieved the maximum Cu^{2+}adsorption capacities of 7.5 and 10 mg/g, respectively. Bina et al. (2006) studied the biosorption of Zn^{2+} and Cu^{2+} onto *Sargassum* biosorbent and observed a removal performance of 1.914 and 1.314 mg/g dry weight biosorbent, respectively. Yalçın et al. (2008) studied the effect of acid/heat-treatment on Cu^{2+} and Pb^{2+} biosorption and reported that the biosorption capacity of *Cladophora* sp. was enhanced by modification process. Compared with other biosorbents, *R. phycopsis* shows a great promise for the removal of heavy metal ions.

2.3 Adsorption isotherms

The results obtained from this study were analysed using both Freundlich and Langmuir isotherms. The Langmuir isotherm assumes that all sites have the same affinity and the secondary effects between sorbed species are negligible. The linearized Langmuir model is (Eq. (5)):

$$\frac{C_e}{Q_e} = \frac{1}{K_L} + (\frac{a_L}{K_L})C_e \quad (5)$$

where, Q_e (mg/g) is the concentration of metals in the sorbent phase viz. biosorbent, C_e (mg/L) is the equilibrium metals concentration or unadsorbed that is obtained at the end of biosorption process. K_L and a_L are the constant related to the adsorption capacity and adsorption energy, respectively.

The empirical Freundlich model also considers monomolecular layer coverage of solute by the sorbent. However, it assumes that the sorbent has a heterogeneous surface suggesting (as expected) that binding sites are not equivalent and/or independent. This model takes the following form (Eq. (6)) for a single component adsorption.

$$\ln Q_e = \ln K_f + \frac{1}{n} \ln C_e \quad (6)$$

where, K_f and n are the Freundlich constants related to the adsorption capacity and adsorption intensity of the sorbent, respectively. The Freundlich constants n and K_f (L/g) for Cu^{2+} biosorption was found as 6.45 and 7.41, respectively. And for Zn^{2+} biosorption n and K_f (L/g) constants was found as 4.78 and 5.92, respectively. Values of $n > 1$ indicates the positive co-operativity in binding and heterogeneous nature of adsorption. The magnitude of K_f showed the easy adsorption. Moreover, Freundlich model explained biosorption behaviour better than Langmuir equation as was evidenced from a higher r^2 value of Freundlich model (> 0.92) than Langmuir model (> 0.67).

3 Conclusions

The present study describes the biosorption of Cu^{2+} and Zn^{2+} onto raw and autoclaved *R. phycopsis*. The changes in surface properties of biosorbent were investigated by FT-IR and contact angle measurement. The variation of the contact angles show that the polarity of the biosorbent surface is increased with respect to the raw biosorbent. The changes in lichen surface structure after autoclavation designated by contact angle measurements was corrected by FT-IR analysis. The adsorption capacities of *R. phycopsis* were increased about 1.20- and 1.22-fold for Cu^{2+} and Zn^{2+} respectively after modification. The modification process affected the biosorption yield and metal uptake positively. The results have provided important information on the determination of changes in surface characteristics of biosorbent after modification process, and could surely be used to design a practical and economical process for wastewater treatment.

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