Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2017.21050

82 (2017) 234–240
July

The investigation of Cu(II) removal by biocomposites synthesized with the immobilization of *R. delemar* and *Candida* microorganisms

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Received 14 January 2017; Accepted 16 June 2017

ABSTRACT

In this study, Cu(II) adsorption using the biocomposites prepared by the immobilization of chitosan polymer and *Candida yeasts (Candida lipolytica, Candida membranaefaciens, Candida tropicalis, Candida utilis)* and *Rhizopusdelemar* fungus as a microorganism on chitosan was examined. In the parameter ranges used, it was identified that the temperature was 25–55°C, pH was 2–7, the concentration was 10–100 mg/L, the optimum pH value was 4.0, the temperature was 25°C and the maximum capacity was 10.06 mg/g for the chitosan-*Candida utilis* biocomposite. The experimental equilibrium data were adjusted to the Langmuir isotherm model of chitosan and biocomposites and the kinetic data were adjusted to the pseudo-first-order kinetics model. It was determined that the adsorption process operating according to enthalpy, entropy, and Gibbs free energy exchange thermodynamic parameters was exothermic and voluntary. The FTIR, SEM analyses and characterization studies of the chitosan-*Candida utilis* biocomposite were carried out before and after adsorption. It is thought that with this study conducted, new chitosan-*Candida* biocomposites will become an alternative for the removal of heavy metals.

Keywords: Chitosan; *Candida species*; *R. delemar*; Cu(II); Adsorption

1. Introduction

The presence of heavy metals in wastewater released after industrial activities has become an important issue recently since it threatens the environment and human health [1]. Although some heavy metals play an important role in the human metabolic system, they cause serious health problems in the food chain due to their accumulation in living tissues in high concentrations [2]. In addition to classical chemical and physical methods used for the removal of heavy metals, other alternative methods are ion exchange, chemical precipitation, reserve osmosis, membrane separation, electrochemical techniques and biosorption [3]. Recently, biological materials used for the removal of metal ions of non-living microorganisms from industrial wastewater have attracted attention due to their performance, cost efficiency and environmentally compatible sorbents [4]. Bacteria used for the biosorption of heavy metals from aqueous solutions such as *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa,* etc. are easily reproducible biomasses which are fungi and yeasts and they can be also manipulated and modified genetically. Fungal adsorbents are *Aspergillus niger*, *Rhizopus arrhizus*, *Saccharomyces cerevisiae*, *Lentinus edodes*, etc. [5]. The removal of single Fe(III), Cr(III), Co(II), Cu(II), Zn(II), Cd(II), Ag(II), Pb(II) ions with the dead *G. thermodenitrificans* biomass from industrial wastewaters was examined in the study of Chatterjee et al. (2010) and the highest biosorption capacity was observed for Fe(III) ion [6]. Yeast and fungal cells can be immobilized on a porous or non-porous support with various methods. The most common ones among these methods are immobilization and adsorption of living cells [7]. Lidija et al. examined the immobilization of *Candida rugosa* lipase on silica SBA-15 and zirconia support [8]. In general, the immobilization which begins with the adsorption of a cell to a solid surface causes adhesion with weak reversibility among cells after a certain period of time due to the fact that the van der Waalsforces defeat electrostatic repulsive forces. Then, the

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cells connect to the solid surface with hydrophobic interaction, polar interaction, hydrogen bonds and the interaction of various specific molecules [9]. The microorganism immobilization method can be implemented successfully in recent years in the alcohol production (ethanol, butanol and isopropanol), organic acid production (malic acid, citric acid, lactic acid and glauconic acid), biotransformation of steroids in wastewater treatment and food applications (beer and wine) [10]. Bacteria can be immobilized on the solid matrix for high-speed metal removal and reusable systems [11]. With this combined action (adsorption and immobilization), studies were conducted on the removal of lead, cadmium and chromium with the immobilization of bacteria such as *Escherichia coli, Streptococcus esquisimilis* and *Bacillus coagulans*on activated carbon [12]. Kartikumar et al. examined the immobilization of *Candida cylindracealipase* (CCL) with chitosan (CH), polylactic acid (PLA), polyvinyl alcohol (PVA), the polymer film they prepared and with this immobilization they investigated the applications of N-acrylation reactions and the activity and stabilization of the polymer film [13]. Monvisade et al. analyzed the adsorption of the composite (chitosan-MMT) prepared by adding clay (montmorillonite) to chitosan in cationic dyes in their study [14]. Godjevargov et al. investigated Cu(II), Ni(II), Zn(II), Pb(II) adsorption from the aqueous solution of polyacrylonitrile porous spheres. The adsorption capacity of the chitosan-*Candida utilis* biocomponent was found to be 10.06 mg/g at pH 4 in the study of the adsorption capacity of Cu(II) in the aqueous solution of spheres obtained by modifying PAN with hydrosilamine and sodium hydroxide at a maximum of 1.07 mg/g at pH 4 [15]. The immobilized biocomposite materials, which are an alternative method of the removal of heavy metals from aqueous media or wastewaters, are also used. In this study, the adsorption of the biocomposite materials prepared with chitosan and microorganisms for Cu(II) ions was examined and it was considered that it would become an alternative for the treatment of waters with high heavy metal pollution.

2. Material and methods

2.1. Reagents

The reagents used in the experiments were Sigma-Aldrich branded, and *Candida* yeast and fungus strains were ATCC® 201377™ *Candida membranafiens, ATCC® 9950™ Candida utilis,* ATCC® 13803™ *Candida tropicalis,* ATCC® 9733™ *Candida lypolitica* yeast, *Rhizopus delemar* NRLL® 2872™ fungus strains provided from Ankara University, Department of Biology.

2.2. Immobilization experiments (Immobilization of microorganisms on chitosan)

1 g of chitosan was added to 3 g of wet dry microorganism and mixed under room conditions. *Candida yeasts (Candida lipolytica, Candida membranaefaciens, Candida tropicalis, Candida utilis)* as a microorganism and *Rhizopusdelemar* fungus were dissolved in distilled water and 1.000 mg/L of stock solutions were prepared at pH 4.0 chitosan polymers added the desired amount to the solutions as a total 1.0 g weighing dry chitosan. The mixture was shaken for 12 h and then the mixture was filtered. This mixture was dried in an oven at 25°C and adsorption biocomposites were obtained. Supernatant was collected to asses the amount of released back into the washing solution and then the batch system experiments were continued. It is thought that this study, which has not been used previously in the literature for the *Candida utilis* biocomponent, will become an alternative for wastewater treatment.

2.3. Batch reactor experiments

The optimum reproduction conditions of the microorganisms were determined to be 25°C and pH 4. The microorganisms were left to reproduce for 4 d at the stirring rate of 95 rpm. Then, each microorganism was stored in the refrigerator by being centrifuged. The adsorbents used in the experiments are biocomposite materials formed with chitosan and the immobilization of microorganisms on chitosan. Experimental studies were examined in the batch system. Then, a certain amount was taken from the samples and centrifuged and the adsorption values were measured by using UV spectrometry at 460 nm. The adsorption of Cu(II) solutions prepared at a concentration of 10–100 mg/L was examined for 10–240 min. The maximum adsorption effect of Cu(II) solutions at the concentration of 100 ppm between 25–55°C was measured to identify thermodynamic parameters, and the amount of adsorption was calculated [16]:

$$q_e = \frac{\left(C_o - C_e\right)V}{m} \tag{1}$$

where C_0 and C_e are the initial and the equilibrium Cu(II) concentrations (mg/L), V is the volume of solution (L) and m is the amount of adsorbent (g).

2.4 Adsorption isotherms

The correlation between the amount of the substances adsorbed by the adsorbent at constant temperature and equilibrium pressure is called the adsorption isotherm. The distribution of metal ions between the liquid-solid phases in adsorption processes can be explained by the Langmuir isotherm model [Eq. (2)] and Freundlich isotherm models [Eq. (3)].

$$q_e = \frac{Q_{\max} b C_e}{1 + b C_e} \tag{2}$$

$$q_e = K_F C_e^{\frac{1}{n}} \tag{3}$$

where q_e is the amount of Cu(II) ions adsorbed at equilibrium (mg/g), Q_{max} is the maximum adsorption capacity of the adsorbent (mg/g), b is the Langmuir constant related to the affinity of Cu(II) ions to the adsorbent (mg/L), K_F is the equilibrium constant and $1/n_f$ is the Freundlich constant, C_e is the equilibrium concentration of Cu(II) ions in the solution (mg/L), n is the adsorption degree. The Langmuir isotherm model is used to explain single-layer homogeneous adsorption and the Freundlich isotherm model is used to explain heterogeneous adsorption [17].

2.5. Adsorption kinetics

The equations used to determine the adsorption kinetics are as follows.

The pseudo-first-order kinetics model is as follows:

$$\log\frac{(q_e-q)}{q_e}=-\frac{k_{1,ad}t}{2.303} \tag{4}$$

The pseudo-second-order kinetics model is expressed as follows and is

$$\frac{t}{qt}=\frac{1}{k_2q_e^2}+\frac{t}{q_e} \tag{5}$$

given with this correlation [18], where q_e is the equilibrium adsorption capacity (mg/g), q_t is the certain time instant adsorption capacity (mg/g), k_1 is the first-order rate constant (1 / min), k_2 is the second-order rate constant (g/mg min).

2.6 Adsorption thermodynamics

The thermodynamic parameters of enthalpy, entropy and free energy exchanges for the adsorption process are indicated in Eq. (6) [19].

$$\Delta G^0=\Delta H^0-T\Delta S^0 \tag{6}$$

where $\Delta G°$ is the free energy change (kJ/mol), $\Delta H°$ is the enthalpy change (kJ/mol), $\Delta S°$ is the entropy change (kJ/mol K), T is the absolute temperature (Kelvin), R is the gas constant (8.314 J/mol K).

3. Results and discussion

3.1. Adsorption kinetics

The contact time for Cu(II) adsorption was examined at the constant initial Cu(II) concentration for chitosan and chitosan-microorganism biocomposites. While Cu(II) adsorption was fast at the beginning, it decreased over time. This decrease indicates that the adsorption on the surface is single-layer [20]. Fig. 1a is obtained when t is plotted against Log(q_e-q_t) and Fig. 1b is obtained when t is plotted against t/q_t. When the experimental q_e and correlation values were examined, it was observed that the biocomposites were compatible with the pseudo-first-order kinetics model. The constants for the adsorption kinetics model are presented in Table 1 for the concentration of 100 mg/L Cu(II).

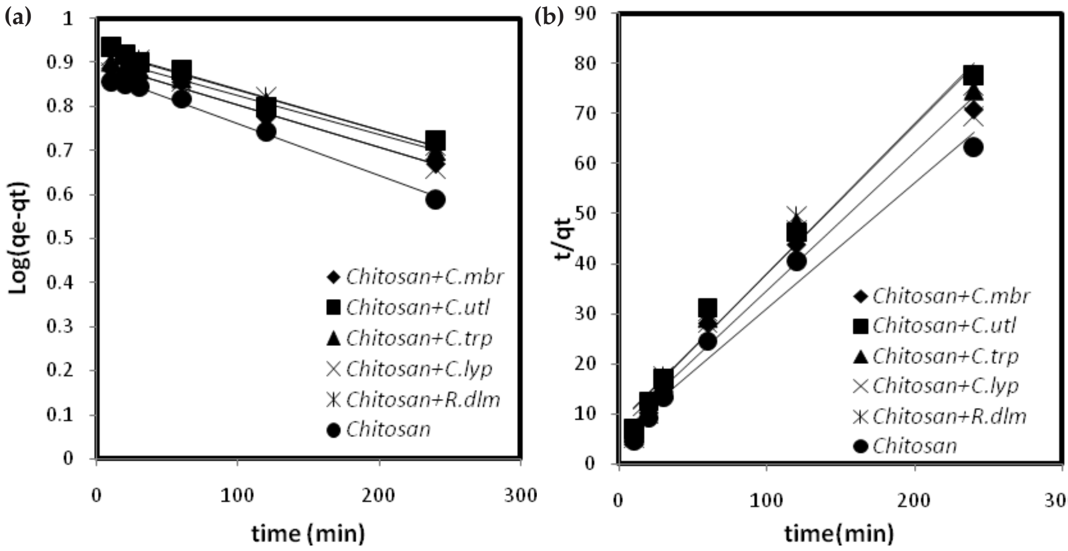

Fig. 1. (a) Pseudo-first-order kinetics model (b) Pseudo-second-order kinetics model for Cu(II) adsorption.

Table 1
First- and second-order kinetics model parameters for Cu(II)

25°C	Experimental data		First-order kinetics model			Second-order kinetics model		
	C_e (mg/L)	q_e (mg/g)	q_e (mg/g)	k_1 (1/min)	R^2	q_e (mg/g)	k_2 (g/mg min)	R^2
Chitosan-*C.mbr*	18.67	9.61	8.91	0.0113	0.991	3.57	0.0122	0.979
Chitosan-*C.utl*	14.16	10.06	9.55	0.0123	0.973	3.44	0.0111	0.964
Chitosan-*C.trp*	17.16	9.76	9.18	0.0023	0.998	3.38	0.0119	0.997
Chitosan-*C.lyp*	19.51	9.41	8.92	0.0043	0.978	3.59	0.0113	0.965
Chitosan-R.*dlm*	16.16	9.71	9.49	0.0103	0.998	3.37	0.0106	0.972
Chitosan	24.51	8.83	8.56	0.0083	0.994	3.98	0.0106	0.976

3.2. Adsorption isotherms

According to the experimental data of chitosan and chitosan-microorganism biocomposites, the Langmuir adsorption isotherm (Fig. 2a) is more compatible. The surface of chitosan and biocomposites is homogeneous and can be explained by the presence of bonding sites that enable single-layer adsorption [21].

R^2 values are an important parameter for the selectivity of the adsorption process, and if this value is $R^2 > 1$, it is inconvenient for adsorption, if it is $0 < R^2 < 1$, it provides convenience for adsorption [22]. The adsorption of Cu(II) ion on biocomposite materials occurs with the immobilization of metal ions in the micro environment on sorption zones on the adsorbent surface with electrostatic interaction, and chemical adsorption is more dominant in the experiments conducted at the temperature of 25°C [23]. The Langmuir isotherm model indicates that the energy on the surface is uniformly distributed and there is no transmigration on the surface of the biocomposite. Adsorption occurs in the specific regions of the biocomposites and all regions are similar to each other and the biocomposite material does not chemically interact with metal ions [24]. Chitosan-microorganism biocomposites for Cu(II) indicated a higher removal only when compared to chitosan (Table 2). Although the concentration of metal ions in the biocomposite micro environments remained constant, adsorption capacities of the microorganisms and Cu(II) removal increased when compared to chitosan. This results from the fact that yeasts and fungi, which are among the most known examples of the microorganism family,are resistant to metal ions in the micro environment on a cellular basis and these ions accumulate in the cell [25].

Yeasts and fungi can reproduce in media containing heavy metal pollution with acidic pH. Thus, the adjustment of the ambient pH plays an important role in conducting experimental studies. This is because the pH of Cu(II) solution is related to the condition of functional groups on the biocomposite surface and different results are obtained for each biocomposite. The mobility of H^+ ion at low pH is higher when compared to Cu(II) ion. This decreases the adsorption of Cu(II). If the pH of the solution increases, Cu(II) ion will be adsorbed more to the solid surface since there will be less H^+ ion in the environment. pH is one of the important factors affecting adsorption for different biocomposite materials. The pH of the solution has an effect on metal-binding regions of the biocomposite surface and changes the physicochemical characteristics of the metal [26]. When the pH change range is examined, it is observed that as the pH change between 3.85 and 4.3 in Fig. 3 increases, the

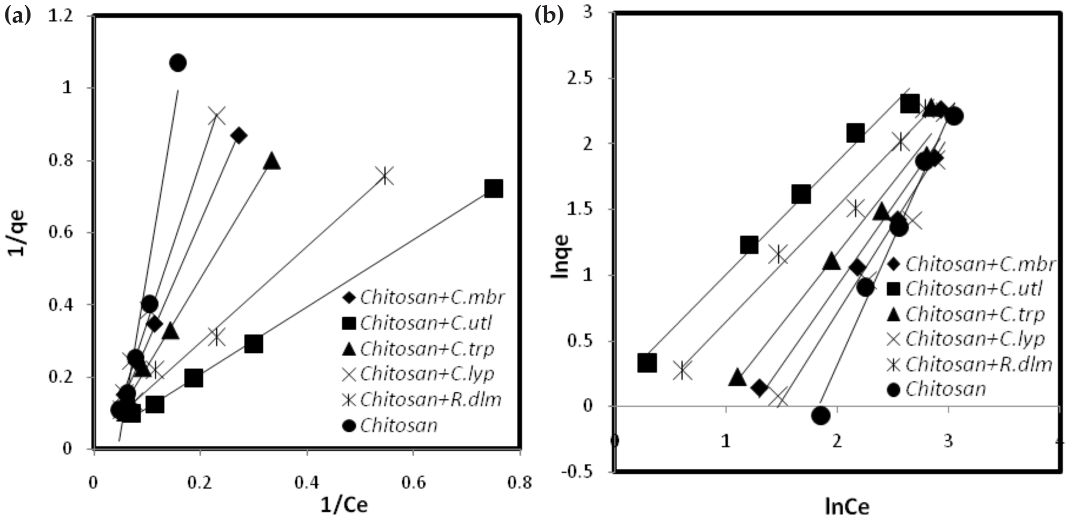

Fig. 2. (a) Graphic of the Langmuir isotherms ($1/q_e - 1/C_e$) (b) Graphic of the Freundlich isotherms ($\text{In}q_e - \text{In}C_e$).

Table 2
The comparison of the Langmuir and Freundlich isotherm parameters for Cu(II)

	Langmuir			Freundlich		
Cu(II)	Q_e (mg/g)	b (mg/L)	R^2	K_F (g/L)	n_f	R^2
Chitosan+C.mbr	19.62	0.051	0.996	0.73	1.19	0.987
Chitosan+C.utl	43.47	0.023	0.999	1.15	1.16	0.988
Chitosan+C.trp	19.61	0.051	0.995	0.81	1.19	0.987
Chitosan+C.lyp	30.31	0.032	0.993	0.48	1.01	0.992
Chitosan+R.dlm	31.25	0.033	0.998	0.79	1.13	0.984
Chitosan	14.48	0.069	0.990	0.32	1.00	0.983

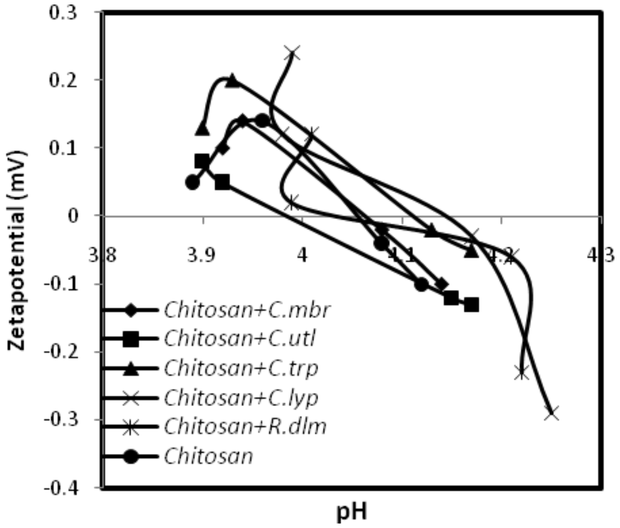

Fig. 3. Relation between the pH of chitosan and the microorganism and zeta potential.

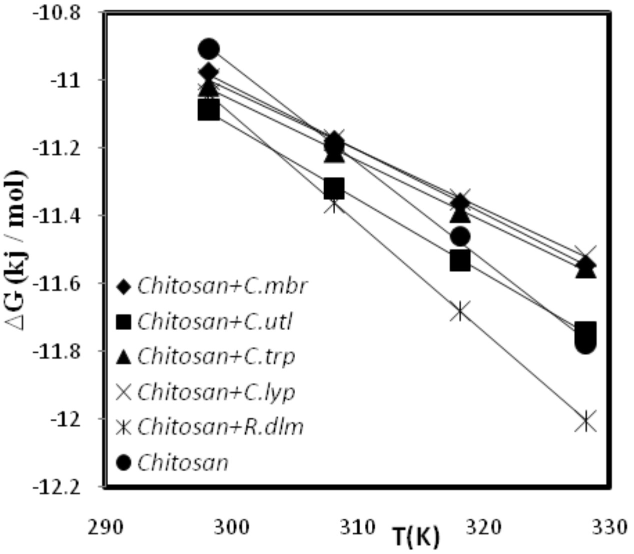

Fig. 4. The changes in the Gibbs free energy with temperature.

removal of Cu(II) ions decreases. This is because, with the increase in pH, decreasing H^+ ions in the medium decrease the electrostatic interaction between Cu(II) ions and the composite surface.

3.3. Thermodynamic parameters

As it is seen from Fig. 4, the change in the Gibbs free energy, enthalpy and entropy equilibrium constant with temperature was calculated by plotting T against ΔG. $\Delta G < 0$ results from the fact that the concentration around the biocomposite is higher than the concentration in the biocomposite. Negative ΔG values indicate that Cu(II) is spontaneously adsorbed on chitosan and chitosan-micro organism biocomposites [27]. Positive ΔS values result from the increase in randomness and affinity at the solid-liquid interface during adsorption. The fact that the ΔH value is negative in this spontaneous case for the equilibrium condition indicates that the interaction on the surface is exothermic (Table 3). In the studies, the Cu(II) adsorption of the biocomposite prepared by using only chitosan is lower when compared to the others (Table 3). Due to the multilayer adsorption on the chitosan surface, desorption occurs on the surface after a certain time. Since ΔH and ΔS are greater than zero, chitosan-micro organism biocomposites are convenient for adsorption. The fact that ΔG is negative indicates that adsorption develops spontaneously at increasing temperatures, and the entropy effect also takes place as a driving force [28].

In this study, the Cu(II) ion adsorption of the biocomposite materials prepared using chitosan and chitosan-micro organism from the aqueous solution was examined. Spontaneous adsorption abilities of the microorganisms increased the adsorption capacity of the newly created biocomposite material. It was observed that Cu(II) adsorption with the biocomposites prepared from the mixture of microorganisms and chitosan was higher when compared to chitosan.

3.4. FTIR and SEM analyses

3.4.1. FTIR

When the FTIR spectrum is examined, it is seen that the peak interval observed after adsorption for chitosan (Fig. 5a) and chitosan-*Candida utilis* biocomposite (Fig. 5b) gives higher peaks when compared to the peak interval observed before adsorption. It is considered that the hydroxyl (–OH) bond which is the characteristic peak of large peak chitosan at 3082.13-878.4 cm^{-1} wavelength for chitosan-*Candida utilis* before adsorption (Fig. 5b) belongs to the peak amide N–H bending peak at 1600 cm^{-1} wavelength and peak at 1076.2–1110.9 cm^{-1} wavelength belongs to the asymmetric bending peak of CH_2 which is the characteristic peak of chitosan [29]. It is observed that the chitosan-*Candida utilis* biocomposite gives a higher peak after adsorption when compared to chitosan. This can be explained by the fact that it results from the interaction of the most functional group with Cu(II) ions with the adsorption process and that the affinity of functional groups in the structure of *Candida utilis* is high against Cu(II) ions [30].

3.4.2. SEM

The SEM images are presented in Fig. 6 for chitosan-*Candida utilis* biocomposites before and after adsorption. It is observed that the chitosan-*Candida utilis* biocomposite is porous before adsorption. The SEM analyses showed that aggregates and porous structure after the biocomposite synthesis occurred according to chitosan-*Candida utilis* and aggregates and porous structures of the biocomposite decreased after the adsorption of Cu(II) (Fig. 6) [31]. As it can be understood from the experimental results, thanks to the increasing surface area with the pore size, the Cu(II) adsorption values were determined to be higher for the chitosan-*Candida utilis* biocomposite. The chitosan-*Candida utilis* biocomposite

Table 3

Thermodynamic parameters for Cu(II)

Cu(II)	ΔG (kJ/mol)					
T (K)	Chitosan+C.*mbr*	Chitosan+C.*utl*	Chitosan+C.*trp*	Chitosan+C.*lyp*	Chitosan+R.dlm	Chitosan
298.15	–10.97	–11.08	–11.01	–10.99	–11.03	–10.91
308.15	–11.17	–11.31	–11.21	–11.17	–11.36	–11.19
318.15	–11.36	–11.53	–11.38	–11.35	–11.67	–11.45
328.15	–11.54	–11.74	–11.55	–11.51	–12.01	–11.77
	ΔH(kJ/mol)					
	Chitosan+C.*mbr*	Chitosan+C.*utl*	Chitosan+C.*trp*	Chitosan+C.*lyp*	Chitosan+R.dlm	Chitosan
	–5.36	–4.58	–5.66	–5.81	–1.45	–2.32
	TΔS(kJ/mol)					
T (K)	Chitosan+C.*mbr*	Chitosan+C.*utl*	Chitosan+C.*trp*	Chitosan+C.*lyp*	Chitosan+R.dlm	Chitosan
298.15	5.61	6.51	5.35	5.19	9.58	8.57
308.15	5.79	6.72	5.53	5.36	9.91	8.85
318.15	5.98	6.94	5.71	5.38	10.22	9.14
328.15	6.17	7.16	5.89	5.71	10.54	9.43

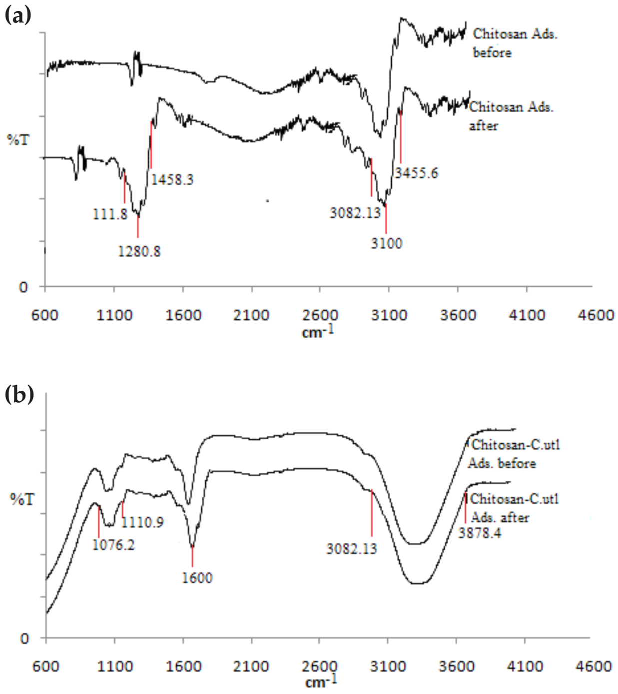

Fig. 5. (a) Chitosan before and after adsorption (b) Chitosan-*Candida utilis* biocomposite before and after adsorption.

indicates that pores have been completely filled after adsorption and this suggests that Cu(II) adsorption has taken place. It is considered that the bonds between *Candida utilis* and chitosan rupture for the chitosan-*Candida utilis* biocomposite before adsorption and Cu(II) adsorption takes place in these regions [32].

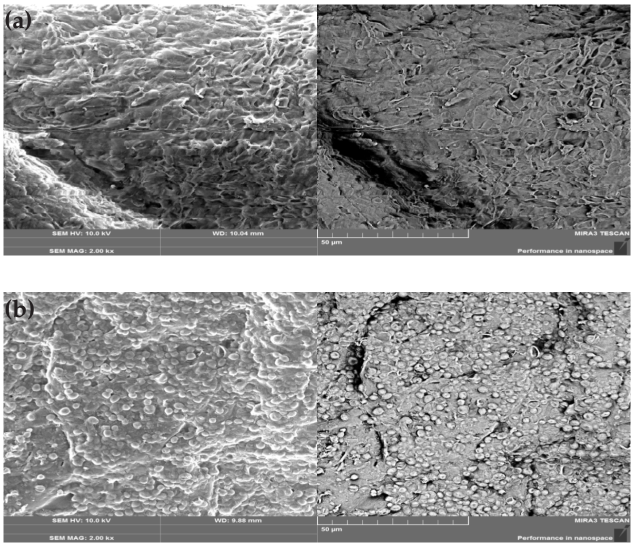

Fig. 6. (a) Chitosan-*Candida utilis*-biocomposite before adsorption (b) Chitosan-Candida utilis biocomposite after adsorption.

4. Conclusion

In this study, Cu(II) adsorption using the biocomposite materials prepared with chitosan and microorganisms was examined. Spontaneous adsorption abilities of the microorganisms increased the adsorption capacity of the newly created biocomposite material. It was observed that the experimental data were compatible with the Langmuir and Freundlich isotherms at different concentrations and the kinetic parameters were calculated under optimum conditions by using the pseudo-first and second-order kinetics models. However, when the condition was examined for each of chitosan and chitosan-micro organism biocomposites used in the experiment, it was observed that it was more compatible with the Langmuir isotherm model. Cu(II) removal for chitosan-microorganism biocomposites is higher when compared to only chitosan. The Gibbs free energy was calculated with regard to the change in the enthalpy and entropy equilibrium constant with temperature for thermodynamic parameters. Negative ΔG values indicate that Cu(II) is spontaneously adsorbed on chitosan and chitosan-microorganism biocomposites. The Cu(II) adsorption of the biocomposite prepared by using only chitosan was found to be lower when compared to the others in the studies. It is considered that with this study conducted new biocomposite materials will become an alternative for the removal of heavy metals and this study will become an example study for the production of new biocomposite materials due to the fact that there is a limited number of similar studies in the literature.

Symbols

b — Equilibrium Langmuir constant
C_a — The amount of the substance kept in the unit mass of the adsorbent (mg/g)
C_e — The concentration of the substance remaining in the solution after adsorption (mg/dm^3)
$k_{1.\text{ad}}$ — First-order rate constant (min^{-1})
k_2 — Second-order rate constant (g mg^{-1} min^{-1})
K_F — Adsorption capacity calculated experimentally
Q_e — The amount of the substance adsorbed on the unit adsorbent (mg/g)
R — Gas constant (8.314 J/mol K)
T — Absolute temperature (Kelvin)
$\Delta G°$ — Free energy change (kJ/mol)
$\Delta H°$ — Enthalpy change (kJ/mol)
$\Delta S°$ — Entropy change (kJ/mol K)

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