

Biosorption of copper(II) by *Marrubium globosum* subsp. *globosum* leaves powder: Effect of chemical pretreatment

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Abstract

The study was aimed at determining the effect of chemical pretreatment on copper(II) biosorption by *Marrubium globosum* subsp. *globosum* leaves. The uptake capacity of the biomass was increased by chemical pretreatment when compared with the raw biomass. The results of biosorption experiments, carried out at the conditions of 50 mg l⁻¹ initial metal concentration and pH 5.5, showed that pretreating the biomass with alkali solutions (laundry detergent, sodium hydroxide and sodium bicarbonate, 0.5 M) improved the biosorption capacity of biomass (45.90, 45.78 and 43.91%, respectively) compared with raw biomass. Pretreatment with sulfuric and nitric acid solutions, 0.5 M, increased the biosorption capacity of biomass by 11.82 and 10.18%, respectively, while there was no considerable change in the biosorption capacity of biomass (0.35%) after pretreatment with formic acid solution, 0.5 M. Furthermore, sodium chloride and calcium chloride, 0.5 M, pretreatments resulted in the improvement in biosorption capacity of biomass (31.38 and 26.69%, respectively). FT-IR analysis revealed that hydroxyl and carboxyl functional groups were mainly responsible for copper(II) biosorption.

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Keywords: Biosorption; Copper; Chemical pretreatment; *Marrubium globosum* subsp. *globosum*; FT-IR

1. Introduction

Environmental pollution due to developments in technology is one of the most significant problems of this century. Among all heavy metals, copper, chromium and zinc ingestion beyond permissible quantities causes various chronic disorders in human beings [1]. Essential for human life, copper, like all heavy metals, is potentially toxic as well. The excessive intake of copper results in its accumulation in the liver causing gastrointestinal problems, kidney damage and anemia. Besides, an increase in lung cancer among exposed workers is associated with continued inhalation of copper-containing sprays [2].

The best solution to heavy metal pollution is to prevent the entrance of heavy metals into the ecosystem. Conventional technologies traditionally used for the removal of heavy metals from aqueous solutions such as chemical reduction, electrochemical treatment, ion exchange and evaporative recovery are expensive and, as a rule, difficult to apply [3,4]. Furthermore, as the

requirements for wastes purification have become more stringent, alternative treating technologies have been developed, such as the ones based on sorption methods, due to their economic, ecological and technological advantages. The optimization of sorption methods should be carried out, first of all, by choosing or developing inexpensive sorbents selective to the contaminants to be removed [5].

Different types of biomass (or sorbent of natural origin) have been studied for the last two decades and the sorption characteristics of many of them have been widely investigated [6–8]. These biomaterials present different types of functional groups, such as carboxylic, sulfates, phosphates and amino-groups, which are the binding sites for the ion exchange and complexation reactions. The choice of the biomass should be based on its origin, type and chemical composition and on the composition of the solution to be purified. The sorption properties of an adsorbent, like its capacity, can be improved/changed by several pre-treatments or modification techniques [9].

Marrubium globosum subsp. *globosum* (*M. g. ssp. globosum*) is an original plant endemic to Turkey. The cell wall composition of the plant contains a large number of complex organic components such as proteins, lipids, carbohydrate polymers (cel-

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lulose, xylane, mannan, etc.) and inorganic ions, Ca^{2+} , Mg^{2+} , etc. Lignin and cellulose, which are the main components of plants, are also known to adsorb many heavy metal ions from aqueous solution [10]. The carboxylic groups in the cell wall are acidic functional groups of biomass and these functional groups directly affect the adsorption capacity of the biomass [11]. As the strategy to manage and utilize this plant for pollution control, the use of *M. g. ssp. globosum* leaves collected as a new biosorbent material for the removal of heavy metals from aqueous solution was investigated.

The objectives of this study were to determine the effect of chemical pretreatment conditions on removal of copper (in form of Cu^{2+}) by *M. g. ssp. globosum* biomass and to assess the changes, formed after the pretreatments, in the functional groups present in the biomass using Fourier Transform Infrared (FT-IR) spectrometer technique. Recently, numerous studies on metal biosorption using FT-IR technique have been reported [12–20]. In these studies, only the IR spectra of raw biomass and metal loaded biomass have been compared, combining with the results of FT-IR spectra analysis to offer reasonable explanation for the biosorption phenomenon between biomass and heavy metals. The differences between the spectra have been attributed to the presence of metal ions. However, the effect of pH on functional groups and their functionality has not been investigated with FT-IR studies. Since it is not considered in FT-IR studies, changes observed after the comparison of spectra lead the researchers to think that the main result of changes occurred in functional groups may be due to the metal binding, which make the peaks in FT-IR spectra shift or disappear. It is necessary to note that pH effect can change the results before biosorption observations, while the change still remains constant after biosorption. For example; Southichak et al. [21], who studied the effect of pretreatment on functional groups present in reed (*Phragmites australis*) biosorbent, suggested that adsorption performance of the biomass was affected not only by the reagent used for pretreatment, but also by the pH condition during the pretreatment process. In the present study, FT-IR technique was applied to determine the effect of chemical pretreatment and pH on functional groups present in *M. g. ssp. globosum* leaves.

2. Material and methods

2.1. The biomass

Leaves of plant used in this work were collected from Isparta Davraz Mountain, Turkey. The biomass was washed with distilled water several times to remove soil-associated particles, dried in an oven at 60°C for 2 h and then stored in a desiccator. The dried biomass was sieved into a size ranging from 125 to $250\ \mu\text{m}$ and retained in a desiccator until use for the biosorption process.

2.2. Chemicals and measurements

All chemicals used in this study were of analytical grade and solutions were prepared using double distilled water. Stock

solution of Cu^{2+} was prepared by dissolving solid copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in distilled water. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) solutions were used to adjust the solution pH. The pH was measured using HI 9321 pH meter (Hanna Instrument). The metal ion concentration was measured by a spectrophotometer (Hach DR 2000) with bicinchoninate method at 560 nm wavelength.

2.3. Pretreatment of biomass

To study the effect of chemical pretreatment on Cu^{2+} uptake capacity of the biomass, an amount of dried biomass (1 g, for each procedure) was subjected to pretreatment with various solutions: (a) pretreatment with alkali solutions (sodium hydroxide (NaOH), sodium bicarbonate (NaHCO_3); 0.5 M each), (b) pretreatment with acidic solutions (sulfuric acid (H_2SO_4), nitric acid (HNO_3), formic acid (CH_2O_2); 0.5 M each), (c) pretreatment with sodium chloride (NaCl) and calcium chloride (CaCl_2) solutions; 0.5 M both, and (d) pretreatment with commercial laundry detergent solution prepared by dissolving 2 g of the laundry detergent in distilled water. The raw biomass was allowed to contact 2 l of such solutions for 2 h by stirring the mixture in a beaker placed on a magnetic stirrer at 60°C and 150 rpm. The biomass was washed with double distilled water after each pretreatment procedure until a neutral pH (6.8–7.2) was measured, dried in an oven at 60°C for 2 h, sieved again into a particle size ranging from 125 to $250\ \mu\text{m}$, and then stored in a desiccator until use for the following studies.

2.4. Biosorption studies

Cu^{2+} uptake with pretreated biomass was studied in batch reactors. The optimum conditions (initial metal ion concentration, initial pH, equilibrium time and initial temperature) for Cu^{2+} biosorption by *M. g. ssp. globosum* were obtained in our previous studies. All tests were carried out in vials placed in an orbital shaker operated at 150 rpm and 25°C . 0.125 g (dried weight) of the pretreated biomass was kept in contact with 25 ml Cu^{2+} solution of $50\ \text{mg l}^{-1}$, whose pH was adjusted to 5.5, for 2 h. Control samples (not including metal bearing solution but pretreated biomass and distilled water, whose pH was adjusted to 5.5) were also tested simultaneously in metal uptake experiments in order to compare the FT-IR spectra of pretreated/unloaded biomass and pretreated/ Cu -loaded biomass. The mixtures were filtered with $0.45\ \mu\text{m}$ filter paper (Millipore) to extract the biomass from the liquid phase, and the biomass was dried in an oven at 60°C for 2 h and stored in a desiccator until use for FT-IR studies.

Metal uptake was determined according to the following equation:

$$q_e = \frac{(C_0 - C_f) \times V}{M} \quad (1)$$

where q_e is the amount of metal ions biosorbed on the biomass at equilibrium (mg g^{-1}); C_0 is the initial metal ion concentration in solution (mg l^{-1}); C_f is the effluent metal ion concentration (mg l^{-1}); M is the dry weight of raw and pretreated biomass

in the reaction mixture (g) and V is the volume of the reaction mixture (l).

The difference between the uptake capacity of pretreated and raw biomass was determined according to the following equation:

$$\Delta q_e = \frac{q_{e,2} - q_{e,1}}{q_{e,1}} \times 100 \quad (2)$$

where $q_{e,2}$ and $q_{e,1}$ is the amount of metal ions biosorbed on the pretreated biomass and raw biomass at equilibrium (mg g^{-1}), respectively.

2.5. FT-IR analysis

Infrared spectra of the raw and the pretreated biomass were performed with a Fourier Transform Infrared (FT-IR) spectrometer (Perkin-Elmer FT-IR BX-II). 5 mg of dried biomass was mixed and ground with 150 mg of potassium bromide (KBr, Spectranal) in an agate mortar. The translucent disks were prepared by pressing the ground material with the aid of 8-ton pressure bench press. The tablet was immediately analyzed with a spectrophotometer in the range of $4000\text{--}600\text{ cm}^{-1}$ with a resolution of 4 cm^{-1} .

3. Results and discussion

3.1. Effect of chemical pretreatment

Changes in the biosorption capacity of the biomass which was chemically pretreated with different ways were investigated. The effect of chemical pretreatment on the biosorption of Cu^{2+} ions by *M. g. ssp. globosum* leaves is shown in Table 1. Data obtained from pretreatment studies showed that alkali pretreatments of biomass with laundry detergent, NaOH and NaHCO_3 resulted in an improvement in biosorption capacities compared with raw biomass. As seen from Table 1, Δq_e values (%) for laundry detergent, NaOH and NaHCO_3 and were found as 45.90, 45.78 and 43.91%, respectively.

In the case of pretreatment with acidic solutions of H_2SO_4 and HNO_3 , biosorption capacity of the biomass slightly increased in comparison with that of raw biomass (8.54 mg g^{-1}) whereas CH_2O_2 pretreatment did not stimulate the biosorption of Cu^{2+} . Δq_e values obtained in this study were 11.82% for H_2SO_4 , 10.18% for HNO_3 and 0.35 for CH_2O_2 .

Pretreatment of biomass with NaCl and CaCl_2 resulted in an increase in biosorption capacity in comparison with raw biomass by 31.38 and 26.69%, respectively. These pre-treatments were performed for the conversion of the active binding sites from the H^+ to the Na^+ or Ca^{2+} form. This substitution may favor the biosorption of copper, because, due to the size of the ions, it should be easier to exchange copper for sodium or calcium than for H^+ .

Some researchers reported that alkali pretreatments of biomass enhanced the biosorption capacity in comparison with raw biomass [5,22–24]. Pretreatments with sodium hydroxide, detergent and other alkali solutions may result in a rupture on the surface wall of the biomass and form additional functional

Table 1
Effect of chemical pretreatment on the Cu^{2+} sorption capacity of *M. g. ssp. globosum* leaves (initial metal concentration: 50 mg l^{-1} , initial pH: 5.5, mixing time: 2 h, solid/liquid ratio: 0.125 g/25 ml)

	Raw biomass		Pretreatment methods							
			Acidic solutions			Alkali solutions			Salt solutions	
	q_e (mg g^{-1})	Δq_e (%)	0.5 M H_2SO_4	0.5 M HNO_3	0.5 M CH_2O_2	Laundry detergent	0.5 M NaOH	0.5 M NaHCO_3	0.5 M NaCl	0.5 M CaCl_2
q_e (mg g^{-1})	8.54		9.55	9.41	8.57	12.46	12.45	12.29	11.22	10.82
Δq_e (%)	0		11.82	10.18	0.35	45.90	45.78	43.91	31.38	26.69
Weight loss of biomass after pretreatment (%)	0		24	27	24	50	48	30	20	26

groups in terms of metal binding sites. Remaining alkalinity can cause hydrolysis of various metals. Thus, an improvement in biosorption capacity of biomass may appear [25]. Yan and Viraraghavan [26] stated that the increase in metal biosorption after pretreating the biomass could be due to the destroying of autolytic enzymes causing putrefaction of biomass and the removal of lipids and proteins as well as polysaccharides that mask binding sites.

Similar results obtained in the former studies by some researchers showed that pretreating a biomass with an acidic solution can cause an improvement in biosorption capacity of heavy metals [9,27–29]. For example, pretreatment of *N. crassa* [15] and *A. flavus* [22] with acetic acid resulted in positive effect on the biosorption of copper(II) and lead(II). However, some researchers reported that acidic pretreatment little or significantly reduced the biosorption capacity of biomass [26,19]. The differences between the obtained results may originate from the different type of biomass used in the studies, different interactions between pretreated biomass and metal species or different properties of functional groups formed after pretreatment. In this study, an increase in biosorption capacity observed after pretreatment with H_2SO_4 and HNO_3 , which cause protonation of biomass surface, may be attributed to the exchange of bound hydrogen ions with heavy metal ions.

The differences in the biosorption capacity of the biomass pretreated with sodium and calcium chlorides may originate from the different charge of the cations. Similar results were reported by Chubar et al. [5], who showed that Cu^{2+} biosorption by cork biomass was more efficient after $NaCl$ pretreatment. The explanation they offered is that the interaction of the biomass binding sites with the divalent calcium ions is stronger than that of the monovalent sodium cations, thus hindering the biosorp-

tion of copper and decreasing the correspondent capacity of the calcium form of the biomass.

Comparing the results presented in Table 1, one can notice that pretreatment of biomass with solutions caused the weight loss of biomass compared with the initial weight of biomass (1 g) after each pretreatment procedure. Results obtained in this study showed that the highest weight losses were found after pretreatment with alkali solutions. It should also be noted that the highest biosorption capacities were obtained after alkali pretreatments. When an acid is involved in the pretreatment, the H^+ ions alter the cross-linkage, causing dissolution of organic solids. In the case of pretreatment with an alkali solution such as $NaOH$, hydrolysis reactions can occur, causing high dissolution of organic substances from the biomass. The hydrolysis reactions can lead to the formation of more carboxylic ($-COOH$), carboxylate ($-COO^-$), and alcohol ($-OH$) groups in the pretreated biomass, which enhances the cationic biosorption [27].

3.2. FT-IR analysis

It was observed after biosorption studies that the most efficient chemical pretreatment method was alkali pretreatment. Thus, we turned our attention to the alkali pretreated biomass in FT-IR studies. The IR spectra of raw biomass (RB), alkali pretreated biomass ($PB_{det.}$, PB_{NaOH} and PB_{NaHCO_3}), control sample biomass ($CSB_{det.}$, CSB_{NaOH} and CSB_{NaHCO_3}) and Cu-loaded biomass ($LB_{det.}$, LB_{NaOH} and LB_{NaHCO_3}) were compared in order to determine the effect of pretreatment with alkali solutions on the functional groups present in the biomass and the findings were presented in Figs. 1–3. The spectrum of raw biomass exhibits a broad band between 3000 and 3750 cm^{-1} due to the presence of hydroxyl groups on the biomass surface [30]. The stretching of the hydroxyl groups bound to methyl

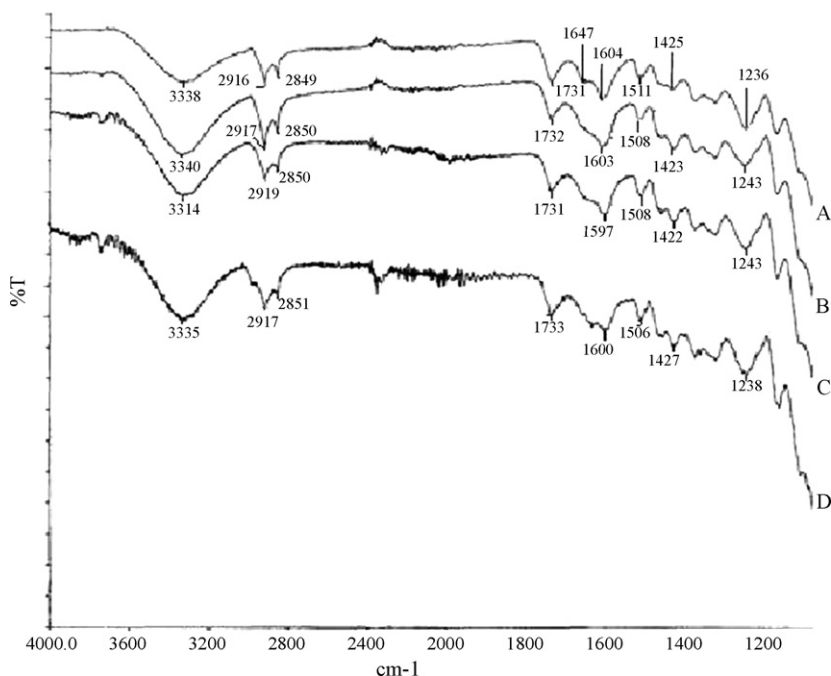


Fig. 1. IR spectra of RB (A), $PB_{det.}$ (B), $CSB_{det.}$ (C) and $LB_{det.}$ (D).

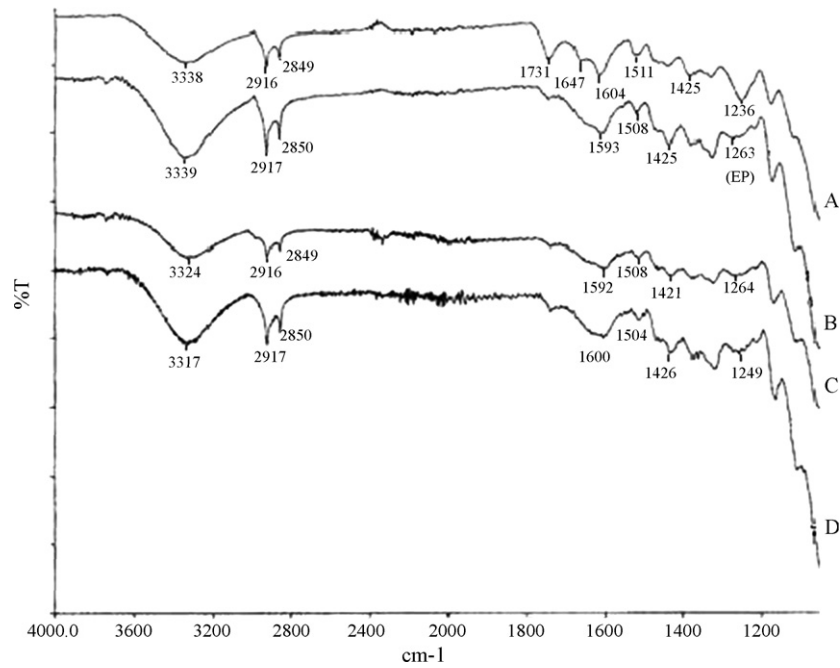


Fig. 2. IR spectra of RB (A), PB_{NaOH} (B), CSB_{NaOH} (C) and LB_{NaOH} (D); EP: extra peak.

radicals presented a signal between 2916 and 2849 cm^{-1} . These groups are present on the lignin structure [31]. The peaks located at 1731 and 1647 cm^{-1} are characteristics of carbonyl group stretching from carboxylic acids. These groups can be conjugated or non-conjugated to aromatic rings (1731 and 1647 cm^{-1} , respectively). The peak at 1604 cm^{-1} is due to the asymmetric stretching vibration of carboxylate [32]. The peak at 1511 cm^{-1} is a constant value for all the lignin esters [37]. The IR peak at 1425 cm^{-1} may be due to the symmetrical bending vibration of alkane bonds ($-\text{CH}_2$). The absorption peak at 1236 cm^{-1} could

be due to C–O, C–H or C–C stretching vibrations of carboxyl groups ($-\text{COOH}$) [33]. The region below 1000 cm^{-1} is the ‘fingerprint zone’ and the absorption can not clearly be assigned to any particular vibration because they correspond to complex interacting vibration systems.

The relevance of a shift in the spectra is that there is an effect of chemical pretreatment, pH or metal biosorption on functional groups. If the spectral changes are considered in the order of spectra of A, B, C and D (firstly A to B, next B to C, last C to D; A: spectra of RB, B: spectra of $\text{PB}_{\text{det.}}$, PB_{NaOH} and $\text{PB}_{\text{NaHCO}_3}$,

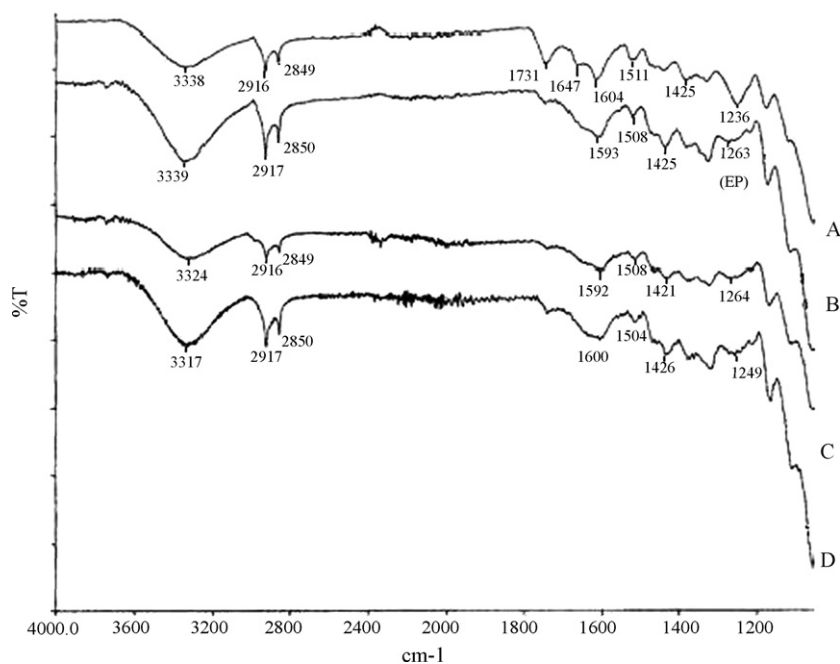


Fig. 3. IR spectra of RB (A), $\text{PB}_{\text{NaHCO}_3}$ (B), $\text{CSB}_{\text{NaHCO}_3}$ (C) and $\text{LB}_{\text{NaHCO}_3}$ (D); EP: extra peak.

C: spectra of CSB_{det.}, CSB_{NaOH} and CSB_{NaHCO₃}, D: spectra of LB_{det.}, LB_{NaOH} and LB_{NaHCO₃}), the cause of spectral changes observed in peaks may be easily understood. Resolution of the device used for FT-IR analysis was 4 cm⁻¹, which means that a shift of 4 cm⁻¹ (or less than 4 cm⁻¹) may mainly result from any effect (chemical pretreatment, pH or metal binding on functional groups) in the solution environment or may be due to the resolution of the device. Thus, we turned our attention to the shift of more than 4 cm⁻¹ even if all the wave number of each peak was given.

Comparison of raw biomass (RB) with detergent-pretreated biomass (PB_{det.}), detergent-pretreated control sample biomass (CSB_{det.}) and detergent-pretreated Cu-loaded biomass (LB_{det.}) is shown in Fig. 1. The peak at 3338 cm⁻¹ appeared in raw biomass (RB) shifted to 3324 cm⁻¹ as a result of the effect of pH on detergent-pretreated control sample biomass (CSB_{det.}), and the latter peak was observed at 3335 cm⁻¹ in detergent-pretreated Cu-loaded biomass (LB_{det.}). The IR peak at 1647 cm⁻¹, observed in the RB, changed into low intensity peak in detergent-pretreated biomass (PB_{det.}) and the intensity of this peak became higher in the IR spectra of CSB_{det.} compared with that of PB_{det.} In addition to this result, this peak disappeared in the presence of Cu²⁺. The peak at 1236 cm⁻¹ in the RB shifted to a wave number of 1243 cm⁻¹ compared with PB_{det.} and CSB_{det.}, and was observed at 1238 cm⁻¹ in the spectra of LB_{det.}

In the case of NaOH-pretreated biomass (Fig. 2), the absorption peak at 3338 cm⁻¹ present in RB was observed at 3324 and 3317 cm⁻¹ in the spectra of NaOH-pretreated control sample biomass (CSB_{NaOH}) and NaOH-pretreated Cu-loaded biomass (LB_{NaOH}), respectively, while there was no change in the spectra of NaOH-pretreated biomass (PB_{NaOH}). The peak at 1731 cm⁻¹ located in RB changed into low intensity peak in PB_{NaOH}, CSB_{NaOH} and LB_{NaOH}. The peak at 1647 cm⁻¹ appeared in RB disappeared as seen in PB_{NaOH} and this situation continued for CSB_{NaOH} and LB_{NaOH}. The peak observed in RB shifted from 1604 to 1593 cm⁻¹ after NaOH pretreatment but shifted again from 1592 (CSB_{NaOH}) to 1600 cm⁻¹ in the presence of Cu²⁺. An extra peak appearance was observed at 1263 cm⁻¹ in PB_{NaOH} after pretreatment and this peak shifted to 1249 cm⁻¹ after Cu²⁺ biosorption. Similar to the disappearance of the peaks at 1647 cm⁻¹, the peak at 1237 cm⁻¹ in RB disappeared after NaOH pretreatment.

In the case of NaHCO₃-pretreated biomass (Fig. 3), the changes observed in the peak at 3338 cm⁻¹ were similar to those of NaOH and detergent-pretreated biomass. The effect of pH on this peak shifted the wave number to 3325 cm⁻¹ for NaHCO₃-pretreated control sample biomass (CSB_{NaHCO₃}), and the latter peak shifted again to a wave number of 3346 cm⁻¹ in the presence of Cu²⁺ (LB_{NaHCO₃}), while there was no change in the wave number for NaHCO₃-pretreated biomass (PB_{NaHCO₃}). The IR spectra of LB_{NaHCO₃} showed that two new extra peaks occurred at the wave numbers of 2980 and 2348 cm⁻¹ after Cu²⁺ biosorption but were absent in the RB. In addition to that, the peak at 1647 cm⁻¹ in the RB disappeared in the LB_{NaHCO₃} whereas there was no change in the PB_{NaHCO₃} and CSB_{NaHCO₃}. Another change observed after Cu²⁺ biosorption

was that the peak at 1604 cm⁻¹, appeared in RB, shifted to 1599 cm⁻¹.

The comparison of the spectral changes of RB, PB_{det.}, PB_{NaOH} and PB_{NaHCO₃} showed that pretreating the biomass with NaOH and detergent resulted in several changes (such as disappearance of some peaks, wave number shifting, etc.) in functional groups of the biomass surface, while NaHCO₃ pretreatment did not result in more changes than those of NaOH and detergent. The peaks at 1647 and 1236 cm⁻¹ for PB_{NaOH} (Fig. 2) and 1647 cm⁻¹ for PB_{det.} (Fig. 1) disappeared. Furthermore, the intensity of the peak at 1731 cm⁻¹ for PB_{NaOH} (Fig. 2) reduced, all of which may be due to the strong alkali effect.

It was observed that solution pH did not cause a radical structural change on biomass surface compared with that of pretreated biomass. But it should also be noted that the effective factor responsible for the wave number shifting of -OH bonds was solution pH. Erdik [34] reported that the stretching vibrations of the IR spectra shifted to a lower frequency as a result of H⁺ ions binding to the functional groups, which are H⁺ donors of the bonds (-COOH, -OH and -NH₂) present in organic compounds, while the bending vibrations shifted to a higher frequency. The reason for shifting observed in the spectra of pretreated control sample biomass may be explained by H⁺ ions binding to the functional groups.

If the spectra of Cu-loaded biomass is compared with that of pretreated control biomass, it can be seen from LB_{NaHCO₃} that there was an appearance of two new peaks at the wave number of 2980 and 2348 cm⁻¹, which indicates the stretching vibration of alkane bonds (C-H) and atmospheric CO₂, respectively, as well as disappearance of the peak at 1647 cm⁻¹. In addition to these results, the observations of hydroxyl group, carboxylic acid bonds and alkanes (symmetrical bending of CH₂) revealed that the vibrations associated with these bonds or groups were shifted by Cu²⁺ biosorption. It is well known that metal ions tend to form bonds with functional groups containing electron-donating atoms. Walter [35] reported that binding of metal on the biomass surface resulted from interaction with various anionic ligands, which react strongly with metal cations. Pradhan et al. [18] also reported that oxygen present in carboxylate ions increased anionic tendency attracting more metal cations. The nature of the specific interaction between metal ions and biomass is quite controversial due to the complex nature of the cell wall, and variable number of binding sites for metal ions [36]. In addition to that, Chen and Yang [27], who studied the enhancement of metal uptake capacity and reduction of organic leaching by chemically modified *Sargassum* biomass, suggested that the interaction between metal ions and binding sites was coordination. Similar results obtained in this study led us to think that Cu²⁺ ions may be biosorbed to binding sites by coordination.

4. Conclusions

The maximum removal efficiency of Cu²⁺ by *M. g. ssp. globosum* leaves was obtained with alkali pretreatment. Results of FT-IR analyses suggested that hydroxyl and carboxylic functional groups were mainly responsible for Cu²⁺ biosorption. The

fact that pretreatment and test pH can affect the results should be taken into consideration in pretreatment and FT-IR studies.

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