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Removal of Cu(II) ions from water using thermally-treated horn–hoof powder as biosorbent

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ABSTRACT

In this work, thermally-treated "horn–hoof" powder was studied as biosorbent to remove copper (II) ions from aqueous solutions. Adsorption of copper onto horn–hoof powder was tested in batch process at different experimental conditions. The effect of the initial metal ion concentration, biosorbent dosage, contact time, pH and temperature were investigated with respect to the biosorption characteristics of Cu(II) ion on the horn–hoof powder. The biosorbent characterization was carried out by Fourier transform infrared spectroscopy, mini-cell electrophoresis, BET and particle sizing analysis. According to the tests, the adsorption equilibrium time was reached within 48 h and the optimum adsorption of Cu(II) ions took place at the pH value of 5.5. The Langmuir adsorption isotherm was successfully applied to the biosorption process of Cu(II) ions onto horn–hoof powder. The thermodynamic and kinetic data suggested that the biosorption process is an endothermic process involving chemical reactions and following a pseudo-second-order model.

Keywords: Biosorbent; Copper (II); Horn–hoof powder

1. Introduction

Heavy metal pollution of water from different industrial wastes, e.g. metallurgy, microelectronics, tannery and chemical manufacturing [1], represents a serious environmental problem. Most of the heavy metals used in industrial processes, in fact, are extremely harmful to humans, animals and plants mainly because they are non-degradable, persistent and therefore they can accumulate in the body [2–5].

Cadmium, for example, is derived from smelting, metal plating, cadmium–nickel battery, phosphate fertilizers, mining, pigments and sewage sludge [6]. Its toxic effects include acute and chronic metabolic disorders, renal damages, emphysema, hypertension and testicular atrophy [7]. Lead is particularly hazardous because it disperses throughout the body immediately and causes toxic effects on the nervous system, kidneys and blood, damaging its red cells [8]. One of the major contaminants of water is copper. Copper is often found in high concentrations near mines, landfills and waste disposal sites and it is one of the major

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contaminants coming from electrical, electroplating and metal finishing industries [7]. Copper toxicity can cause itching, dermatization and keratinization of the hands and soles of feet [9,10] in humans after exposure. Moreover, copper can cause severe gastro-intestinal irritation and possible changes in the liver and kidney functionalities above its bio-recommended limits [11]. Copper is also toxic to aquatic organisms even at very small concentrations in natural water [12].

Numerous techniques are currently available to remove heavy metals from industrial wastewater, such as chemical precipitation and filtration, electrochemical treatments, ion exchange, reverse osmosis, adsorption and biosorption [7].

Adsorption processes on activated carbon are effective for removal of heavy metals like copper. Adsorption characteristics of activated carbon are determined by its pore structure and surface chemistry. Activated carbon has large internal surface area, a porous structure and high degree of surface reactivity due to the existence of active sites at the edges of aromatic planes. Heteroatoms, such as oxygen, hydrogen or nitrogen also have a strong influence on the mechanisms of the adsorption process [13].

Since environmental protection is an important global issue, biosorption has become a promising and attractive method to remove heavy metals from aqueous solutions by inexpensive biomaterials like bacteria [14], yeast [15], fungi [16], algae [17], chitin [18] and chitosan [19]. In recent years, products taken from agriculture and forests, like rice husk [20], neem leaves [1], *Acacia* bark [7], cork biomass [21] and *Cedrus deodara* sawdust [22] were used as biosorbents. The main advantages of these materials are the reusability, low operating cost, no production of secondary compounds, improved selectivity for specific metals of interest, biodegradability and biocompatibility.

Among them one of the most abundant agricultural waste biomass is represented by horn and hooves of animals from butchery, currently used only as surfactant foams for fire extinguishers and slow-release nitrogen fertilizer. Horn and hooves are composed of a core of bone covered by a thick keratin layer. In the hoof tissue, keratin molecules having different biomechanical properties and molecular weights, with different degrees of hardness and sulphur concentration, are expressed. Keratin characteristics of horn and hooves were previously studied: a high amount of α-helix structures, low sulphur content and low cystine amount are the typical properties of horn–hoof proteins [23]. Moreover, it was demonstrated that many polarisable and ionisable groups of keratin proteins can bind metal ions [24], so it appears of interest to apply this material for the heavy-metal adsorption from water. It is reported that the good adsorption capacity of the keratin nanofibres' membranes for Cu(II) ions is also maintained with the co-existence of other metals like Co(II) and Ni(II) and that the adsorption of metal ions by keratin nanofibre membranes followed the order Cu(II) > Ni(II) > Co(II) [25].

In this work, horn–hoof scales were thermally-treated and then ground to powder. The thermally-treated horn–hoof powder was studied as biosorbent to remove copper (II) ions from water, with the aim to investigate the possible application of this material in water depuration. Adsorption of copper onto horn–hoof powder was tested in batch process at different experimental conditions of pH, temperature, initial metal concentration and biosorbent/liquid ratio (biosorbents dosage). The adsorption capacities and the metal removal efficiencies were calculated, the kinetic and equilibrium isotherm models, and the thermodynamics parameters related with the adsorption process were performed.

2. Materials and methods

2.1. Preparation of the biosorbent

Agricultural fertilizer "horn–hoof" (scales) was kindly supplied by the Profoam s.r.l. (Italy). Copper(II) sulphate pentahydrate ($CuSO_4{\cdot}5H_2O$), sodium hydroxide, hydrochloric acid, dithiothreitol, urea, Tris–HCl, Na_2EDTA and trichloroacetic acid were purchased from Sigma–Aldrich (Italy). Novex IEF Gel, NuPAGENovex4–12% Bis-Tris Pre-Cast Mini Gels and buffer solutions were purchased from Invitrogen (Italy).

The scales of horn–hoof were thermally-treated at 200˚C by muffle for 1 h. After the treatment, the sample was ground to powder using mortar and pestle and it was put through solubility test (2% in weight of sample in distilled water for 24 h under agitation at 20˚C). The powder was filtered using filter paper and dried in oven at 105˚C until constant weight.

2.2. Characterization of the biosorbent

The mean particle size distribution of the thermally-treated hoof–horn powder was determined using a Particle Sizing Systems (Model 780 AccuSizer, USA). The sample was suspended into ultrapure water, and a 0.01% polysorbate 80 solution was used as surfactant to prevent aggregation.

Particle size was expressed as undersize cumulative percentages, and the population dispersity (span) was referred as span and calculated as in the following equation:

$$\text{Span} = \frac{(d_{90} - d_{10})}{d_{50}} \tag{1}$$

where d_{90}, d_{10} and d_{50} are the mean diameters at the 90, 10 and 50% of the population distribution, respectively. Surface area determination was performed by means of a SA3100 Surface Area Analyzer (Beckman Coulter, UK) according to the BET method using N_2 as adsorbate gas (European Pharmacopoeia 7th edition §2.9 Pharmaceutical technical procedures. §2.9.26 Specific surface area by gas adsorption). An accurately weighed amount of powder (2.5–3 g) was introduced in a 9 mL glass sample tube and degassed under vacuum for 1 h at 90°C. The measurement was carried out in three replicates.

XCell *SureLock*$^{\text{TM}}$ Mini-Cell Electrophoresis was used to determine the isoelectric point (IEF) and the molecular weight of the powder's protein. An amount of 150 mg of horn–hoof powder was extracted with 9.5 mL of dithiothreitol/urea extraction solution [26]. One-dimensional gel-electrophoresis was performed using NuPAGENovex 4–12% Bis-Tris Pre-Cast Mini Gels, suitable for proteins with molecular weights from 200 to 2.5 kDa. This method eliminates the problems associated with the traditional Laemmli system [27]: improved gel stability, better protein stability during the run and higher protein capacity. The gel was stained with Coomassie Blue in order to evaluate the molecular weight distribution of the proteins.

Moreover, IEF gel-electrophoresis was performed using Novex IEF Pre-Cast Gels, suitable for proteins with IEF in the range of 3–10. The gel was fixed in 12% TCA for 30 min and stained with Coomassie Blue in order to evaluate the IEF of the biosorbent.

The powders (native horn–hoof powder, thermally-treated powder and thermally-treated powder after Cu(II) ion adsorption) were characterised by means of Fourier Transform Infrared Spectroscopy (FT-IR; Thermo Nicolet Nexus) with attenuated total reflection technique. Before analysis, samples were dried at 105°C for 1 h in order to remove the adsorbed water. The spectra were acquired in the range of 4,000–650 cm^{-1}, using 100 scans and a gain of 8.0. After the acquisition, the spectra were baseline corrected and smoothed with a nine-point Savitsky–Golay function.

In order to study the protein secondary structure, the amide I band was subjected to a curve-fitting carried out with ORIGIN 8.1 software (OriginLab Corporation, MA, USA) using Marquardt's method [28].

2.3. Batch adsorption tests

The stock solution containing 1 g/L of Cu(II) was prepared by dissolving an appropriate amount of copper sulphate in distilled water. The stock solution was diluted conveniently in order to explore the effect of initial metal ion concentration, contact time, biosorbent dosage, pH and temperature on the adsorption capacity of horn–hoof.

Batch adsorption tests were carried out in triplicate by shaking specified amount of powder in 10 mL of the metal solution at the desired concentration at varying pH and temperature, for 48 h. The sample was then let to settle and the Cu(II) residual concentration in the supernatant was analysed by an inductively coupled plasma—optical emission spectrometer (ICP-OES, Optima 7000 DV Perkin-Elmer). The adsorption capacity was evaluated as follows:

$$q_t = \frac{(C_0 - C_t)V}{m} \tag{2}$$

where q_t (mg/g) is the adsorption capacity of the horn–hoof powder at a given time t; C_0 and C_t (mg/L) are the initial metal concentration and at time t, respectively; V (L) is the volume of the Cu(II) solutions; and m (g) is the mass of the biosorbent.

The metal removal efficiency was calculated using the following equation:

$$\text{Removal efficiency (\%)} = \frac{C_0 - C_e}{C_0} \times 100 \tag{3}$$

where C_e (mg/L) is the equilibrium concentration.

3. Results and discussion

3.1. Biosorbent characterization

The thermally-treated horn–hoof powder was characterised in term of morphology, particles size, specific surface area, protein molecular weight distribution and IEF.

The morphology of the biosorbent is shown in Fig. 1. The powder used to assay the bioadsorbant ability of the thermally-treated horn–hoof presented a broad monodisperse distribution (PI = 3.14) with volume weighted mean diameter of 161.19 μm and a specific surface area of 0.245 m^2/g.

Fig. 2 reports the molecular weights distribution of the thermally-treated horn–hoof proteins compared with the electrophoretical separation pattern of keratin extracted from the non-treated horn–hoof.

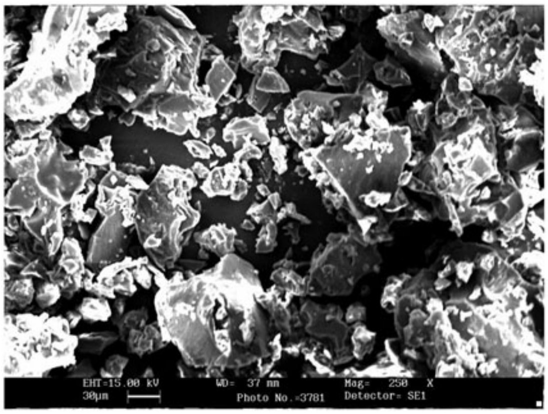

Fig. 1. SEM micrograph of horn–hoof thermally-treated powder (250x magnification).

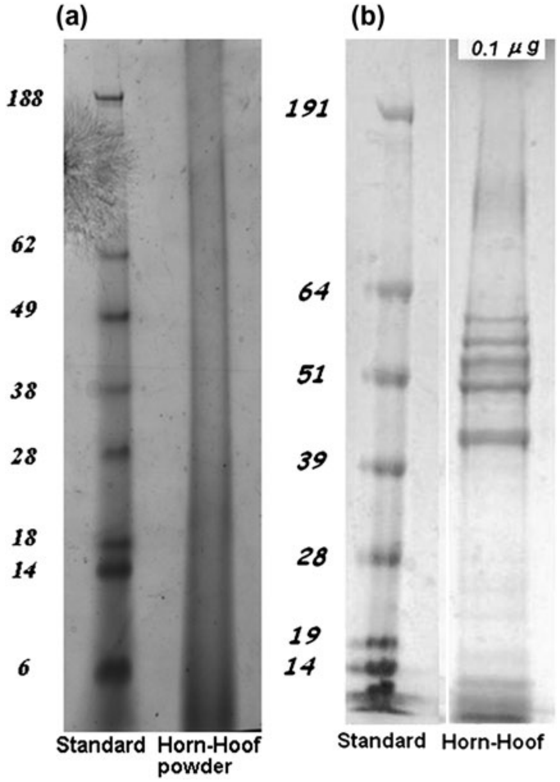

Fig. 2. Horn–hoof gel electrophoresis: molecular weights distribution of thermally-treated (a) and non- treated (b) horn–hoof.

As can be seen, the powder protein shows very low molecular weights (<6,000 Da) probably because the high temperature used during the preparation of the biosorbent caused denaturation of the horn–hoof proteins. On the contrary, the electrophoretical separation pattern of the non-treated horn–hoof protein showed different molecular weight bands between 60,000 and 45,000 Da, typical of the low-sulphur α-keratins, probably related to the different degrees of tissue hardness in accordance with the function of the origin tissue [29].

In order to select the pH value to carry out the biosorption tests, the biosorbent was characterised on the basis of the IEF. After the electrophoresis, the horn–hoof powder keratin showed an IEF between 5.2 and 5.5.

FTIR spectra of starting horn–hoof and thermally-treated horn–hoof powders before and after the Cu(II) ions adsorption process are reported in Fig. 3(a). All spectra showed the characteristic adsorption bands of peptide bonds (–CONH–) of proteins and peptides. Particularly, the amide A band (in the range 3,700–3,000 cm^{-1}) is due to the stretching vibration of N–H bonds, the amide I band (1,700–1,600 cm^{-1}) is related to the stretching vibration of the carbonyl groups, the amide II (1,500–1,510 cm^{-1}) is mainly due to the in-plane N–H bending with some contribution of C–N stretching vibrations and finally, the amide III (1,300–1,220 cm^{-1}) is a very complex band dependent on the nature of side chains and hydrogen bonding [30]. The adsorption peaks in the range of 3,000–2,800 cm^{-1} are related to the stretching vibrations of methylene groups. As shown in Fig. 3(b) and (c), the thermally-treated powders showed a broadening of the amide A and amide I bands towards higher wavenumbers with respect to the starting material. This behaviour could be due to a higher amount of free NH_2 and COOH terminal groups as a consequence of the high degradation induced by the thermal treatment.

Moreover, the amide I band shape of thermally-treated horn–hoof before the adsorption process is different from that of the same powder subjected to adsorption process. This suggested slight conformational rearrangements induced by Cu(II) complexation.

The keratin supermolecular changes induced by the interactions with copper cations were studied by analysis of the amide I band shape that is known to be sensible to the secondary structure of the protein [31]. In particular, the amide I bands of thermally-treated horn–hoof powder before and after Cu ions adsorption were resolved into Gaussian bands whose number was defined in the second order derivative of the spectra (Fig. 4).

In Table 1, the positions of the Gaussian bands and the related secondary structure assignments and area percentage (calculated respect to the amide I total peak area) were reported. The thermally-treated horn–hoof powder is characterised by different secondary structures, namely random coil (1,687–1,671 cm^{-1}), α-helix (1,651–1,657 cm^{-1}) and β-sheet (1,621–1,631 cm^{-1}) [32].

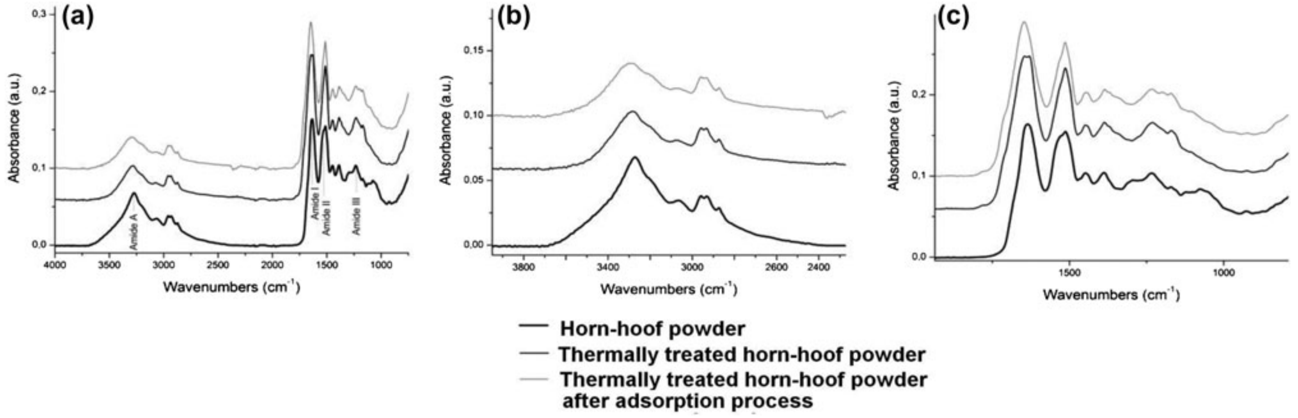

Fig. 3. FT-IR spectra of starting horn–hoof (a), thermally-treated horn–hoof before the adsorption (b) and thermally-treated horn–hoof after Cu(II) adsorption (pH 5.5; $T = 20$°C; $t = 48$ h $C_0 = 200$ mg/L).

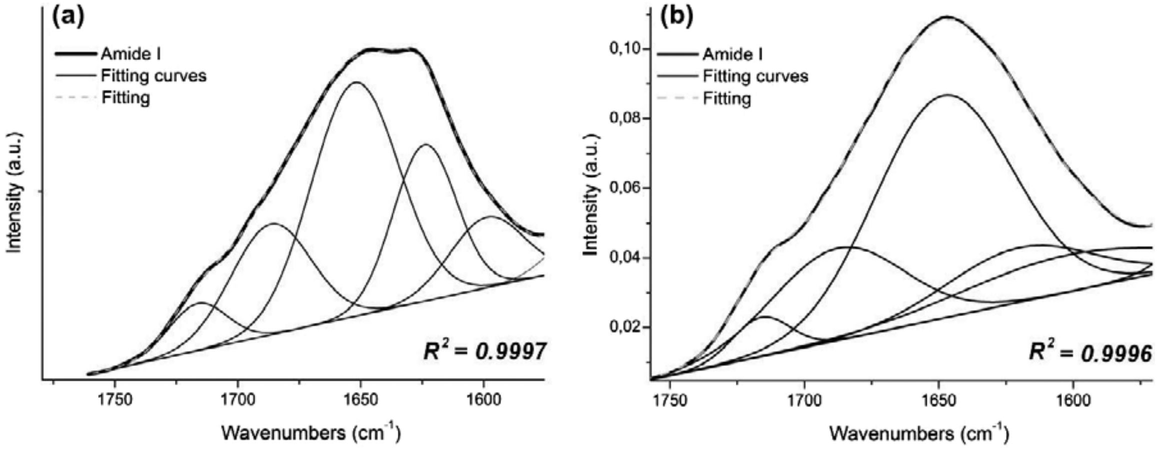

Fig. 4. Resolved amide I of thermally-treated horn–hoof powder before adsorption (a) and after adsorption (b).

Table 1
Amide I curve-fitting

Thermally-treated horn–hoof powder before adsorption			Thermally-treated horn–hoof powder after adsorption		
Band position (cm^{-1})	Band assignment	Content (%)	Band position (cm^{-1})	Band assignment	Content (%)
1,716	Terminal COOH	6	1,716	Terminal COOH	4
1,687	Random coil	19	1,688	Random coil	20
1,652	α-helix	43	1,648	α-helix	50
1,624	β-sheet	21	1,620	β-sheet	13
1,599	His ring vibration	11	1,600	His ring vibration	13

As it can be seen the random coil, α-helix and β-sheet content of the thermally-treated horn–hoof powder before and after the adsorption was similar. Nevertheless, the α-helix and β-sheet band positions of the powder after Cu(II) adsorption were shifted towards lower wavenumbers, suggesting that the interactions of the protein with Cu(II) influences mainly the α-helix and β-sheet structures than random coil structures.

3.2. Adsorption process

3.2.1. Effect of pH

It is known that pH is one of the most important parameters influencing the metal ion biosorption, because of the competition between the metal and the H$^+$ ions for the active biosorption sites [33]. Moreover, the pH dependency on the metal ion uptake by the biomasses can also be due to association–dissociation of certain functional groups, such as the carboxylic groups [33,34].

The effect of pH on the biosorption onto horn–hoof powder was studied in the range of values from 2 to 10 by using a biosorbent dosage of 10 g/L, an equilibrium time of 48 h and an initial metal ion concentration of 10 mg/L (initial pH 5), to which any metal precipitation related with the pH correction occurs. The pH was corrected using HCl 1 N and NaOH 1 N according to the cases. The results are reported in Fig. 5.

The maximum biosorption was observed at pH 5.5; therefore all the following tests were carried out at this pH value. At lower pH values the uptake of the metal ions onto the powder decreased because of the positive charge of the binding active sites that competes strongly with Cu(II) ions. Decrease in biosorption at higher pH values (pH > 5) could be related to the formation of soluble hydroxylated complexes of the metal ions [7].

3.2.2. Effect of biosorbent dosage

The biosorbent dosage is an important parameter that influences the adsorption capacity of the material for a given initial metal concentration. The effect of horn–hoof powder mass on the kinetic of copper removal was studied using an initial metal ion concentration of 100 mg/L and an equilibrium time of 48 h. Fig. 6 shows that the percentage of the metal removal increased with the biosorbent loading up to 50 g/L. In particular, the removal efficiency increased by increasing the biosorbent dose up to a maximum of 94%, whereas the adsorption capacity decreased. This can be explained with the fact that the more biosorbent mass increased, the more biosorption sites were available to bind the metal ions dissolved in solution. Moreover, the removal efficiency and the adsorption capacity already reached satisfactory levels using a dosage of 10 g/L, so this solid/liquid ratio was taken as the optimum biosorbent dosage for the following experiments.

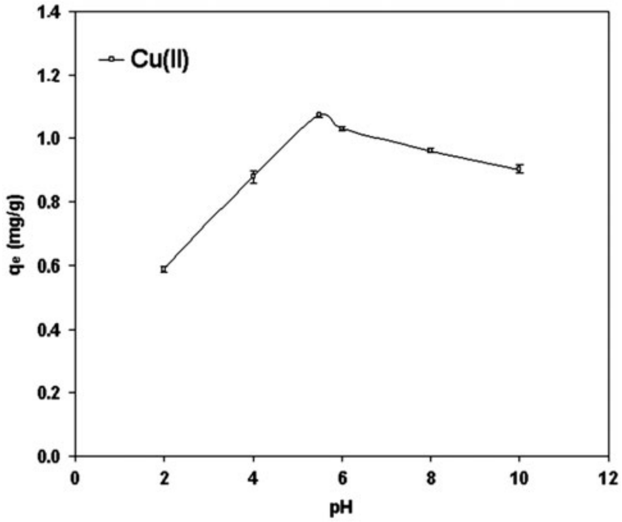

Fig. 5. Effect of pH on the biosorption of Cu(II) onto thermally-treated horn–hoof powder.

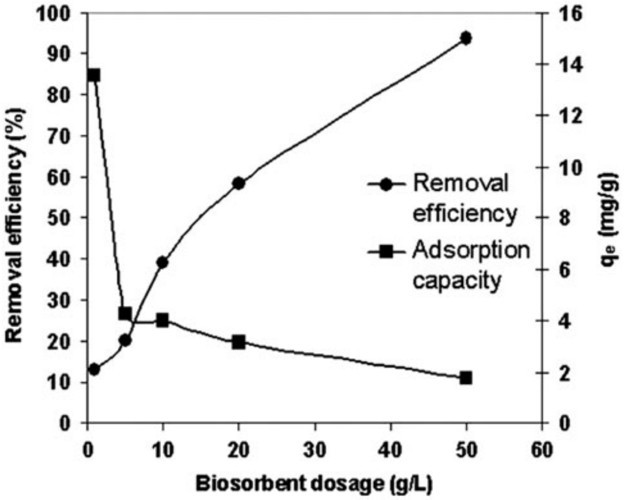

Fig. 6. Effect of the biosorbent dosage on the removal efficiency and adsorption capacity (q_e) of Cu(II) onto thermally-treated horn–hoof powder.

3.2.3. Effect of contact time and biosorption kinetic

It is important to establish the relationship between contact time and adsorption capacity of the biosorbent for planning batch experiments. Therefore, the effect of the contact time on the Cu(II) biosorption onto horn–hoof powder was studied using an initial metal concentration of 100 mg/L. As it can be seen in Fig. 7, the adsorption capacity considerably increased with increasing the contact time and reached a constant value of 5 mg/g after 72 h. However, the equilibrium was already reached after 48 h, which is the time used to carry out all the batch experiments.

The experimental data were fitted to the Lagergren's pseudo-first-order and pseudo-second-order models in order to clarify the biosorption kinetics of Cu(II) ions onto horn–hoof powder.

The linear pseudo-first-order equation according Lagergren [35] can be written in the following form:

$$\log(q_e - q) = \log q_e - \frac{K_1}{2.303}t \tag{4}$$

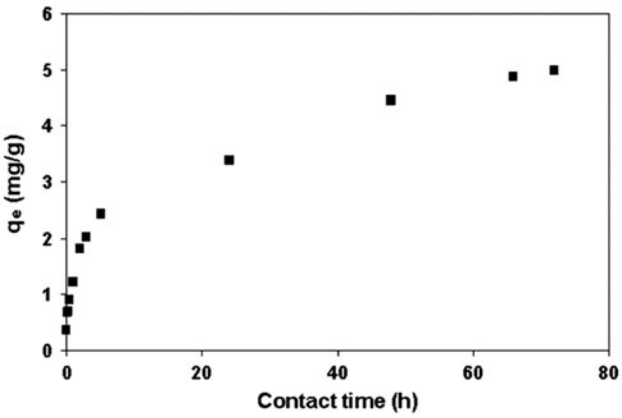

Fig. 7. Kinetic of Cu(II) biosorption onto thermally-treated horn–hoof powder.

where q_e and q_t (mg/g) are the amounts of Cu(II) ions adsorbed at the equilibrium time and t (min), respectively, and K_1 is the rate constant of the equation (min^{-1}). The K_1 constant can be determined experimentally by plotting of log (q_e–q_t) vs. t.

The linear pseudo-second-order equation is given in the following form [36]:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_2} t \tag{5}$$

where K_2 is the rate constant of the equation (g/mg min) and q_e and q_t (mg/g) are the amounts of metal ions adsorbed at the equilibrium time and t (min) time, respectively.

The parameters of pseudo-first-order and pseudo-second-order models, performed using an initial metal ion concentration of 100 mg/L, a biosorbent dosage of 10 g/L and a pH of 5.5, are reported in Table 2.

As a result, a good sound of correlation was obtained for the pseudo-second-order kinetic model, which indicates that the biosorption of Cu(II) metal ions onto thermally-treated horn–hoof powder follows the pseudo-second-order rate. This shows that the overall rate of the process of metal adsorption appeared to be controlled by a chemical process.

3.2.4. Effect of the temperature and biosorption thermodynamic

The experiments were carried out using an initial metal ion concentration of 100 mg/L, a pH of 5.5, a biosorbent dosage of 10 g/L and an equilibrium time of 48 h. In order to study the effect of the temperature on the Cu(II) biosorption onto the horn–hoof powder, four different temperatures were selected, namely 20, 30, 40 and 50℃. The results shown in Fig. 8 indicate that the adsorption capacity and the removal efficiency increased with increasing the temperature from 20 to 50℃, presenting a plateau from 30 to 40℃. This is a common adsorption capacity behaviour related to adsorbents which follows the Langmuir adsorption isotherm model; practically, the adsorption capacities increases with increasing the initial metal ions concentration (because the driving force for the mass transfer increases with increasing the initial metal ions concentration) until a plateau was reached corresponding to the saturation of the adsorption sites of the adsorbent. The plateau indicates the maximum adsorption capacity of the adsorbent.

Thermodynamic parameters such as the Gibbs free energy change (ΔG), enthalpy (ΔH) and entropy (ΔS) change can be used for the characterization of temperature effect. The aforementioned thermodynamic parameters can be calculated using the Gibbs Eq. (6) and Van't Hoff Eq. (7):

$$\Delta G = -RT \ln K_D \tag{6}$$

$$\ln k_D = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{7}$$

where $K_D = q_e/C_e$, R is the molar gas constant (8.314 J/mol K) and T is the absolute temperature [37,38]. ΔH and ΔS were obtained from the slope and intercept of linear plot of $\ln K_D$ against $1/T$, respectively.

Table 2
Parameters of pseudo-first-order and pseudo-second-order models for Cu(II) biosorption onto thermally-treated horn–hoof powder

	Cu(II) biosorption parameters			
	q_e (mg/g)	K_1 (min^{-1})	K_2 (g/mg min)	R^2
Lagergren first-order	4.28	0.000921	–	0.9409
Pseudo-second-order	5.00	–	0.000941	0.9879

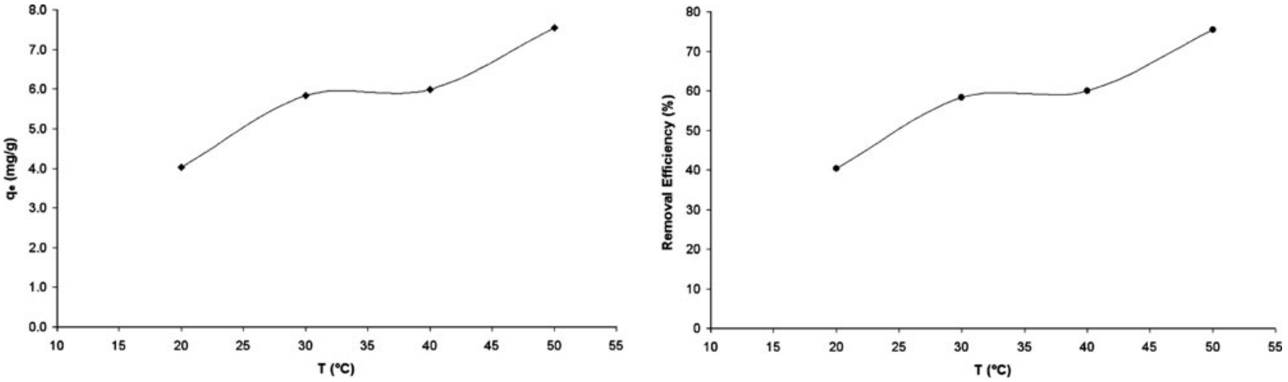

Fig. 8. Effect of the temperature on the adsorption capacity and removal efficiency of Cu(II) onto thermally-treated horn–hoof powder.

The adsorption enthalpy is positive indicating that the adsorption process is endothermic in nature; moreover, its value (36.34 kJ/mol) is higher than 20 kJ/mol and this means that the process is chemical adsorption [39].

The changes in free energy (ΔG) are positive suggesting that the adsorption process is not spontaneous; moreover, the free energy values decrease from 6.6 to 3.2 kJ/mol with increasing the temperature from 20 to 50°C, therefore, the adsorption process is more feasible at higher temperature.

3.3. Biosorption isotherm models

The distribution of divalent cations between the liquid phase and the adsorbent is a measure of the equilibrium condition in the adsorption process and can generally be expressed in terms of the two most popular isotherm theories: the Langmuir [40] and the Freundlich [41] isotherms.

The Langmuir isotherm model is based on the assumption of homogeneous adsorption and its equation can be written in the following form:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \tag{8}$$

where q_e (mg/g) is the equilibrium metal ion concentration on the biosorbent, q_m (mg/g) is the maximum adsorption capacity of the biosorbent, C_e (mg/L) is the equilibrium metal ion concentration in the solution and K_L (L/mg) is the Langmuir adsorption constant related to the free energy of adsorption.

The Freundlich isotherms model assumes a heterogeneous adsorption surface and its equation can be as the following:

$$q_e = K_f C_e^{1/n} \tag{9}$$

where q_e (mg/g) is the equilibrium metal ion concentration on the biosorbent, K_f is a constant relating the biosorption capacity, C_e (mg/L) is the equilibrium metal ion concentration in the solution and $1/n$ is an

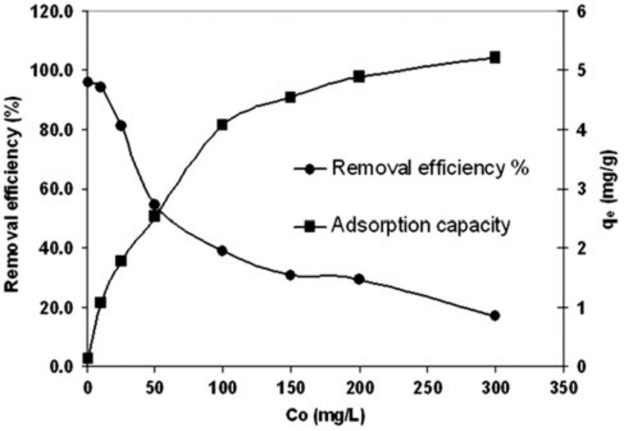

Fig. 9. Effect of the initial metal ions concentration on the adsorption capacity and removal efficiency of Cu(II) onto thermally-treated horn–hoof powder.

Table 3
Adsorption isotherm coefficients of Langmuir and Freundlich models applied to Cu(II) biosorption onto thermally-treated horn–hoof powder

	Langmuir			Freundlich		
Metal	q_m	K_L (L/mg)	R^2	K_f	n	R^2
Cu(II)	5.3	0.088	0.992	0.72	2.44	0.922

Table 4
Comparison of biosorption capacity (mg/g) of thermally-treated horn–hoof powder for Cu(II) on different adsorbent in the literature

Sorbent	q_m (mg/g)	pH	Refs.
Acacia leucocephala bark powder	147.1	6.0	[7]
Tobacco dust	36	4–5	[41]
Rice husk	31.85	5–6	[42]
Newspaper pulp	30	5.5	[43]
ZeoAds	23.25	–	[44]
Cork biomass	20	4.5–5.4	[45]
Modified peanut husk	10.15	4	[46]
Sawdust	6.58	4	[46]
Phosphate rock (francolite)	4.88	–	[47]
Horn–hoof powder	5.3	5.5	Present study

empirical parameter relating the biosorption intensity, which varies with the heterogeneity of the material.

Equilibrium biosorption studies were carried out in the range of Cu(II) initial concentration of 1–300 mg/L using a biosorbent dosage of 10 g/L, to determine the maximum metal adsorption capacities of the biosorbent. As it can be seen in Fig. 9, the adsorption capacity increases and the removal efficiency decreases with the increase of the initial metal ions concentration.

The Langmuir and the Freundlich adsorption constants evaluated from the isotherms along with the correlation coefficients are reported in Table 3. As it can be seen, the Cu(II) biosorption data fitted the Langmuir isotherm model better than the Freundlich one, as demonstrated by the value of the coefficient of correlation (>0.99). According to the results obtained, the maximum adsorption capacity of the thermally-treated horn–hoof powder for Cu(II) was 5.3 mg/g.

On the other hand, Table 4 presents the comparison of biosorption capacity (q_m; mg/g) of horn–hoof powder for Cu(II) ions with that of various adsorbents reported in literature [7,21,42–47]. The biosorption capacity of horn–hoof powder for Cu(II) is comparable with some of the adsorbent mentioned. Therefore, the thermally-treated horn–hoof powder can be considered as a potential biosorbent for the removal of Cu(II) ions from aqueous solutions.

According to Hall et al. [48], the essential features of the Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor R_L, defined by the following equation:

$$R_L = \frac{1}{1 + K_L C_0} \tag{10}$$

where K_L is the Langmuir constant. The parameter R_L indicates if the adsorption is favourable ($0 < R_L < 1$) or unfavourable ($R_L > 1$). The value of R_L calculated for the 1.36 mg/L initial Cu(II) concentration (which is the lowest initial metal ion concentration considered) is 0.89, indicating a favourable adsorption of copper ions onto horn–hoof powder.

Finally, to relate the removal efficiency of the powder with the different parameters used in the study, the following empirical equation was elaborated:

$$\begin{aligned}\text{Removal efficiency } (\%) = {} & 1.24 \pm 0.28bd + 0.71 \pm 0.1t \\ & + 0.70 \pm 0.30T + 3.39 \\ & \pm 1.21\text{pH} - 0.30 \pm 0.03C_0\end{aligned} \tag{11}$$

where all the considered parameters resulted relevant in determining the removal efficiency ($p < 0.086$).

4. Conclusion

Thermally-treated horn–hoof powder was studied as biosorbent for Cu(II) ions removal from aqueous solutions. The initial metal ion concentration, biosorbent dosage, contact time, pH and temperature were found to have an effect on the adsorption capacity and removal efficiency of the powder. The adsorption capacity increases with the increase of the initial metal ions concentration, the contact time and the temperature, while it decreases with the increase in biosorbent dosage.

The equilibrium time of the Cu(II) biosorption was reached after 48 h and the maximum biosorption was observed at pH 5.5. The kinetic studies revealed that the biosorption process best fits the pseudo-second-order kinetic model. The Langmuir isotherm model was successfully applied to the biosorption process of Cu(II) ions onto the horn–hoof powder and it is a favourable process. According to these results, the maximum adsorption capacity of the thermally-treated horn–hoof powder for Cu(II) was 5.3 mg/g. The calculated thermodynamics parameters showed that the adsorption process of copper ions onto the thermally-treated horn–hoof powder is an endothermic process which involves chemical reactions.

Thus, the thermally-treated horn–hoof powder can be considered as a possible biosorbent for application in treatment of water solutions containing heavy metal ions, like copper.

List of Symbols

d_{90}, d_{10}, d_{50}	—	mean diameters at the 90, 10, and 50% of the population distribution
q_t	—	adsorption capacity of the horn–hoof powder at a given time t
C_0	—	initial metal concentration
C_t	—	metal concentration at time t
V	—	volume of the Cu(II) solutions
m	—	mass of the biosorbent
C_e	—	equilibrium concentration
q_e	—	amounts of Cu(II) ions adsorbed at the equilibrium time
q_t	—	amounts of Cu(II) ions adsorbed at time t
K_1	—	rate constant of the Lagergren equation
K_2	—	rate constant of the pseudo-second-order equation
ΔG	—	free energy change
ΔH	—	enthalpy change
ΔS	—	entropy change
K_D	—	ratio from q_e and C_e
R	—	molar gas constant
T	—	absolute temperature
q_m	—	maximum adsorption capacity of the biosorbent
K_L	—	Langmuir adsorption constant
K_f	—	constant of the Freundlich isotherms model
$1/n$	—	empirical parameter relating the biosorption intensity
R_L	—	dimensionless constant separation factor
bd	—	biosorbent dosage

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