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Biosorption of heavy metals from aqueous solutions using keratin biomaterials

Helan Zhang

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Supervisor

Cristina Palet Ballús

Departament de Química

Facultat de Ciències

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Helan Zhang

Supervisor

Prof. Cristina Palet Ballús

Full professor of Analytical Chemistry
Group leader in *Centre Grup de Tècniques de Separació en Química* (GTS), in *Universitat Autònoma de Barcelona* (UAB)

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Abstract

Abstract

Biosorption is an effective technique for the removal of heavy metals from wastewaters resulting from human and industrial activities. Waste biomaterials are considered ideal alternatives as novel biosorbents due to the low relatively cost-effective ratio and the high efficient adsorption capacities for heavy metals. A vast array of low-cost waste biomaterials from large-scales industrial operations have been successfully employed as biosorbents for the removal of heavy metals from wastewaters. As a kind of abundant biological resources, keratin biomaterials are investigated currently for this purpose, which show high capacity of biosorption of heavy metals due to their high contents of carboxyl, hydroxyl, amino and sulfur-containing functional groups. This thesis is concerned with the use of keratin biomaterials, specifically human hair, dog hair, chicken feathers and degreased wool, as biosorbents for the removal of heavy metals from aqueous solutions.

First of all, the removal of heavy metals from aqueous solutions by different kinds of human hair was investigated. It was found that the bleached and dyed hair showed the best biosorption capacities for all checked heavy metal ions. Based on these preliminary results, two human hair samples including native hair (**H1**) and bleached-dyed hair (**H2**) were selected to do further study. A systematic comparison of biosorption performance for removing Cr(III), Ni(II), Co(II), Mn(II), Cu(II), Zn(II), Cd(II) and Pb(II) ions from aqueous solution under different conditions, was provided. The **H2** (bleached-dyed hair) showed better biosorption capacity than **H1** (in native). It could be attributed to the higher surface area and more number of sulfonate groups, which is confirmed by the SEM and FT-IR analysis, respectively. The biosorption capacities of heavy metals followed the order Cu(II) > Pb(II) > Cr(III) > Zn(II) > Cd(II) > Ni(II) > Co(II) > Mn(II) for **H2**, and Cu(II) > Cr(III) > Pb(II) for **H1**, and the biosorption of the rest of the heavy metals for **H1** was not significant. The pseudo-second order kinetic model is more likely to predict kinetic behavior of the

metal biosorption process for whole contact time range, with the chemical sorption being the rate-controlling step. The maximum biosorption capacity of **H1** and **H2** for Pb(II) by Langmuir isotherm model were 1.7×10^{-5} and 23.2×10^{-5} mol/g at 295 K, respectively. The effect of sulfonate groups on the human hair surface can enhance the biosorption capacity of human hair as well as the elution efficiency of EDTA solution as eluent. The regenerated human hair samples with EDTA and rinsing later with water, showed good biosorption capacities of their reuse. So, the human hair is considered as a potential biosorbent for the removal of heavy metals from aqueous solutions.

Based on the above results, we can confirm that **H2** (bleached-dyed hair) shows the best biosorption capacity, which inspire us to properly chemically modify the human hair to enhance its biosorption capacity. So, native human hair was treated by a proper oxidation method, and its biosorption performance was evaluated and compared with the native one. The chemical composition and morphology of human hair were analyzed by FR-IR and SEM respectively, after the oxidation treatment as well as after the metal biosorption process. The treated human hair showed better biosorption capacity for heavy metals comparing with the untreated one, as expected, due to the higher surface area after the treatment, and more sulfonate groups present on the surface, which have been formed through the oxidation reaction. The parameters affecting the biosorption of heavy metals, such as contact time, initial aqueous pH value, biosorbent dosage and initial metal concentration have been investigated. According to the kinetics and isotherm studies, the rate limiting step of the biosorption process might be the chemical sorption due to the formation of chemical bonds between the metal ions and the functional groups of the treated human hair in monolayer coverage. The maximum biosorption capacity of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) by Langmuir isotherm model at 295 K are 9.47×10^{-5}, 5.57×10^{-5}, 3.77×10^{-5}, 3.61×10^{-5} mol/g, respectively. Negative standard Gibb's energy indicates that the heavy metal biosorption process is thermodynamically feasible and spontaneous nature. The sulfonate groups on the

treated human hair formed in the oxidation treatment, not only enhance the biosorption capacity, but also assist the desorption of metal ions by using EDTA. In addition, the elution using EDTA is beneficial to its reuse. Thus, the human hair treated by the oxidation method exhibits satisfactory biosorption capacity and can be used as an effective biosorbent for the removal of heavy metals from aqueous solutions.

Four common waste keratin biomaterials including human hair, dog hair, chicken feathers and degreased wool were used as biosorbents for the removal of heavy metals from aqueous solutions. The biosorption process was carried out under different conditions. The total biosorption capacity of the four biosorbents generally followed the order degreased wool > chicken feathers > human hair > dog hair. This is probably due to the partial oxidation of the degreased wool and the high surface area of chicken feathers, which are confirmed by FT-IR and SEM, respectively. Kinetic results indicated that chemical sorption is the basic mechanism in the biosorption processes. The experimental data fitted very well to the Langmuir isotherm model and the biosorption process is thermodynamically feasible and spontaneous nature, as indicated previously with the human hair case. These four keratin biomaterials regenerated with EDTA and rinsed with deionized water exhibit good biosorption capacity, especially for the chicken feathers (low cystine content) and the degreased wool (oxidized keratin fiber). The four waste keratin biomaterials, being cheap and easily available materials, could be an alternative to more costly adsorbents used nowadays for the heavy metals removal in wastewaters treatment processes.

Based on these results, it can be said that keratin biomaterials can be used as potential biosorbents for the removal of heavy metals, and that the human hair treated with an oxidation procedure can enhance its biosorption capacity. Therefore, the other keratin biomaterials including dog hair, chicken feathers and degreased wool were also treated by using the same chemical modification method. The treated dog hair shows better biosorption capacity than the native one, with similar behavior to the treated human hair. However, the biosorption capacities of chicken feathers and

degreased wool are not improved by this method, which is probably due to the condition of modification (especially for the wool that has been degreased previously) and the properties of the keratin biomaterials themselves. Therefore, further characterization of the treated dog hair is followed. Corresponding biosorption experiments with the treated dog hair were carried out to investigate the effect of different parameters, such as contact time, pH of the solution and biosorbent dosage, as in the previous cases of study. Kinetic and isotherm models were also used to fit the experimental data, the results indicate that the chemical sorption is the rate limiting step in the biosorption process. The maximum biosorption capacity of the treated dog hair for Pb(II) by the Langmuir isotherm model is 5.79×10^{-5} mol/g at 295 K. The treated dog hair shows a good reusability after simple elution with EDTA and rinsing with deionized water.

In conclusion, the waste keratin biomaterials can be used as low-cost and effective biosorbents to remove heavy metals from aqueous solutions. In general, the oxidation treatment can significantly enhance the biosorption capacities of the keratin biomaterials. But the different conditions of the oxidation treatment for each keratin biomaterial should be considered for the proper modification in each case. These kinds of keratin biomaterials are expected to be applied in large scale to deal with the industrial wastewaters in near future.

Resum

Resum

La biosorció és una tècnica eficaç per a l'eliminació de metalls pesants de les aigües residuals resultants de l'activitat humana, principalment de les activitats industrials actuals. Els biomaterials provinents de residus es consideren alternatives ideals com a nous biosorbentes donada la baixa relació del seu cost amb la seva eficàcia i alta capacitat d'adsorció per a metalls pesants. Una àmplia gamma de biomaterials de baix cost provinents de residus, alguns dels quals provenen de processos d'operacions industrials a gran escala, s'han emprat amb èxit com a biosorbents aplicats a l'eliminació de metalls pesants, principalment d'aigües residuals. Com a tipus abundant de recurs biològic, els biomaterials amb base de queratina s'investiguen actualment per a aquest propòsit. Aquests biomaterials mostren una alta capacitat de biosorció de metalls pesants a causa del seu alt contingut en grups funcionals carboxil, hidroxil, amino, així com grups que contenen sofre. Aquesta tesi té a veure amb l'ús de biomaterials de queratina, especialment cabell humà, pèl de gos, plomes de pollastre i llana desgreixada, com a materials biosorbents emprats per a l'eliminació de metalls pesants de solucions aquoses.

En primer lloc, es va investigar l'eliminació de metalls pesants de solucions aquoses emprant diferents tipus de cabell humà. Es va trobar que el pèl tenyit mostra les millors capacitats de biosorció per a tots els ions de metalls pesants triats. Sobre la base d'aquest resultat preliminar, es van seleccionar dues mostres de cabell humà, incloent cabell natural (**H1**) i cabell tenyit (**H2**) per mirar de comprovar-ne la seva eficàcia com a biosorbents de metalls pesants. De manera que es realitza una comparació sistemàtica del procés de biosorció per a l'eliminació d'ions com Cr (III), Ni (II), Co (II), Mn (II), Cu (II), Zn (II), Cd (II) i Pb (II) sota diferents condicions. El cabell **H2** (cabell tenyit) va mostrar una millor capacitat de biosorció que el cabell **H1** (cabell natural). Aquest comportament, es podria atribuir a la major àrea superficial del primer, així com a la presència de més grups sulfonat (arrel del procés de tinció), fet que és confirmat per l'anàlisi SEM i FT-IR, respectivament. Les capacitats de

biosorció dels metalls pesants segueix l'ordre següent: Cu(II) > Pb(II) > Cr(III) > Zn(II) > Cd(II) > Ni(II) > Co(II) > Mn(II) per al cabell **H2**, i Cu(II) > Cr(III) > Pb(II) per **H1**, de manera que la biosorció de la resta dels metalls pesants per **H1** no va ser significativa. El model de cinètica de pseudo-segon ordre és el que millor s'ajusta i prediu el comportament cinètic de biosorció dels sistemes, per a tot el temps de contacte aplicat, sent la reacció química de sorció l'etapa que controla la velocitat del procés. La capacitat de biosorció màxima de Pb(II) per **H1** i **H2**, segons el model de l'isoterma de Langmuir és de $1{,}7\times10^{-5}$ i $23{,}2\times10^{-5}$ mol/g a 295 K, respectivament. L'efecte dels grups sulfonat en la superfície del cabell (**H2**) pot millorar la seva capacitat de biosorció, així com l'eficiència de la següent elució amb EDTA. Les mostres de cabell humà regenerades amb EDTA mostren una bona capacitat de biosorció quan es reutilitzen. Així, el cabell humà es considera un potencial biosorbent per a l'eliminació de metalls pesants de mostres aquoses.

Basant-nos en els resultats anteriors, s'arriba a la conclusió que el cabell **H2** (cabell tenyit) mostra la millor capacitat de biosorció, de manera que es pot pensar en mirar de modificar químicament el cabell humà, de forma adequada, per tal de millorar la seva capacitat de biosorció original. Així, el cabell humà natiu va ser tractat per un mètode d'oxidació adient, i es va avaluar i comparar el seu rendiment de biosorció respecte el cabell natiu. La composició química i la morfologia del cabell humà després del tractament d'oxidació es van comprovar per anàlisis de FT-IR i SEM, respectivament, així com després d'emprar el biomaterial tractat en el procés de biosorció de metalls pesants. El cabell humà tractat va mostrar una millor capacitat de biosorció dels metalls pesants respecte al cabell natiu, tal i com era d'esperar, a causa de la major àrea de superfície obtinguda després del tractament, i de la presència de més grups sulfonat en la superfície del biomaterial, els quals s'han format a través de la reacció d'oxidació. S'han investigat els paràmetres que afecten a la biosorció dels metalls pesants, com ara temps de contacte, el valor inicial de pH de la solució, la dosi de biosorbent i la concentració inicial dels metalls. D'acord als estudis amb els models cinètics i les isotermes, el pas limitant del procés de biosorció sembla ser la reacció

química de sorció que és la causant de la formació d'enllaços químics entre els ions metàl·lics i els grups funcionals del cabell humà tractat, en la monocapa de la superfície del biomaterial. La màxima capacitat de biosorció del cabell humà tractat per Cr (III), Cu (II), Cd (II) i Pb (II) segons el model de la isoterma de Langmuir a 295 K és $9,47\times10^{-5}$, $5,57\times10^{-5}$, $3,77\times10^{-5}$ i $3,61\text{x}10^{-5}$ mol/g, respectivament. L'energia de Gibbs estàndard de signe negatiu indica que el procés de biosorció de metalls pesants és termodinàmicament factible i de naturalesa espontània. Els grups sulfonat del cabell humà tractat (formats en el tractament d'oxidació), no només milloren la capacitat de biosorció, sinó que també ajuden a la desorció dels ions metàl·lics estudiats mitjançant l'ús de solucions d'EDTA. A més, l'elució amb EDTA és beneficiosa per a la reutilització del biomaterial. Per tant, el cabell humà tractat pel mètode d'oxidació exhibeix una capacitat de biosorció de metalls pesants satisfactòria, de manera que es pot utilitzar com a biosorbent eficaç per a l'eliminació d' aquests a partir de solucions aquoses.

Es van utilitzar quatre biomaterials comuns de rebuig de base de queratina, incloent el cabell humà, el pèl de gos, les plomes de pollastre i la llana desgreixada com biosorbents per a l'eliminació de metalls pesants de solucions aquoses. El procés de biosorció es va dur a terme en diferents condicions. La capacitat total de biosorció dels quatre biosorbents generalment segueix el següent ordre: la llana desgreixada > les plomes de pollastre > el cabell humà > el pèl de gos. Aquest comportament és probablement a causa de l'oxidació parcial de la llana original arrel del procés de desgreix, i de l'àrea de superfície alta de les plomes de pollastre, fets que es van confirmar per FT-IR i SEM, respectivament. Els resultats dels estudis cinètics del procés de biosorció amb aquests materials de queratina indiquen que la sorció química és el mecanisme bàsic del procés. Les dades experimentals s'ajusten molt bé al model de la isoterma de Langmuir, i el procés de biosorció és termodinàmicament factible i de naturalesa espontània, tal i com ja s'ha indicat anteriorment en el cas del cabell humà. Aquests quatre biomaterials de queratina regenerats amb EDTA i rentats amb aigua destil·lada mostren una bona capacitat de biosorció, especialment pel cas de la

ploma del pollastre (amb baix contingut de cistina) i per la llana desgreixada (que es tracta de fibra de queratina prèviament oxidada en el procés de desgreixat). Els quatre residus de biomaterials de queratina, sent materials barats i fàcilment disponibles, podrien ser una alternativa adient als adsorbents més costosos utilitzats avui en dia per a l'eliminació de metalls pesants en els processos de tractament d'aigües residuals.

Sobre la base d'aquests resultats, es pot afirmar que els biomaterials de queratina assajats es poden utilitzar com a potencials biosorbents per a l'eliminació de metalls pesants, i que el cabell humà tractat amb un procediment d'oxidació pot millorar la seva capacitat de biosorció. Per tant, els altres biomaterials de queratina, incloent el pèl de gos, les plomes de pollastre i la llana desgreixada es van tractar també utilitzant el mateix mètode de modificació química. El pèl de gos tractat mostra una millor capacitat de biosorció que el pèl de gos natiu, de manera similar al cas del cabell humà. No obstant, les capacitats de biosorció de les plomes de pollastre i de la llana desgreixada no milloren després del tractament d'oxidació, probablement degut a la condició de material modificat (especialment pel cas de la llana que ha estat desgreixada previament) i degut a la naturalesa i a les propietats dels biomaterials en si mateixos. Per tant, es continua amb la caracterització del pèl de gos tractat. Es preparen els corresponents experiments de biosorció del pèl de gos tractat per tal d'investigar els efectes de diversos paràmetres com ara els temps de contacte, el pH inicial de la solució aquosa i la dosi de biosorbent. També s'utilitzen models cinètics i d'isotermes per tal d'ajustar les dades experimentals obtingudes, tal que els resultats obtinguts indiquen que la sorció química és l'etapa limitant de velocitat del procés de biosorció, i la capacitat màxima de biosorció del pèl de gos tractat per Pb (II), segons el model de la isoterma de Langmuir és de $5{,}79\times10^{-5}$ mol/g a 295 K. El pèl de gos tractat, també es pot reutilitzar després de l'elució amb EDTA i del rentat amb aigua destil·lada, com ja s'havia vist anteriorment.

En conclusió, els biomaterials de residu de base de queratina poden ser utilitzats com a biosorbents de baix cost i alta eficàcia per a l'eliminació de metalls pesants de solucions aquoses. En general, el tractament d'oxidació pot millorar

significativament la capacitat de biosorció d’aquests biomaterials. Cal considerar les millors condicions pel tractament d'oxidació per cada tipus de biomaterial de queratina, per tal d’assolir la millor modificació i eficàcia d’aquest. S'espera que aquests tipus de biomaterials de queratina puguin ser aplicats a gran escala per fer front a la descontaminació de metalls pesants de les aigües residuals industrials en un futur proper.

Contents

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List of Symbols

FT-IR	Fourier transform infrared spectroscopy
SEM	Scanning electron microscopy
ICP-MS	Inductively coupled plasma mass spectrometry
H1	Human hair (native) from 27-year old woman
H2	Human hair (bleached-dyed) from 45-year old woman
UH	Untreated human hair (native)
TH	Treated human hair (chemically-modified)
MTH	Metal loaded-treated human hair
q_t	Amount of adsorbed metal on the biosorbent at time t(mmol/g)
q_e	Amount of metal ions uptake at equilibrium (mmol/g)
C_e	Equilibrium concentration (mmol/L)
C_i	Initial concentration (mmol/L)
C_f	Final concentration (mmol/L)
W	Weight of biosorbent (g)
k_1	Pseudo-first order adsorption rate constant (min^{-1})
k_2	Pseudo-second order adsorption rate constant (g $mmol^{-1}min^{-1}$)
k_3	Weber-Morris intraparticle diffusion rate constant (mmol g^{-1} $min^{-1/2}$)
k_F	Freundlich adsorption constant
n	Freundlich adsorption constant
Q_o	The saturated amount of adsorbed metal unit of adsorbent (mol/g)
ΔG^0	Gibbs free energy change (J/mol)
R	Gas law constant (J/mol K)
b	Langmuir adsorption constant (L/mol)
K_L	Langmuir adsorption constant (L/g)
R^2	Correlation coefficient
T	Solution temperature (˚C, K)

Introduction

1 Introduction

1.1 Heavy metals pollution

Earth's surface comprises of 70% water is the most valuable natural resource existing on our planet. However, less than 3% of it is fresh, and most of it is in polar ice or too deep underground to be harvested. The amount of available fresh water in lakes, rivers and reservoirs is less than a quarter of 1% of the total fresh water quantity.[1] The life on the earth would not exist without water. Consequently nearly 2.2 billion people in more than 62 countries are lacking sufficient water supplies. Although this fact is widely recognized, pollution of water resources is a common problem being faced today.[2] To a large degree, intensifying environment pollution and deteriorating of ecosystems anthropologically, caused by mainly industrial activities, have seriously threatened human existence and development.[3] Consequently, a wide variety of toxic inorganic and organic chemicals are discharged to the environment as industrial wastes, causing serious water pollution. Industrial waste usually contains many pollutants, such as heavy metal ions, dye, phenols (including other organic compounds), inorganic anions, and pesticides (which are toxic to many living lifeforms and organisms), etc..[4, 5]

Among toxic substances, heavy metals pollution of wastewater is the most strongly connected to human life.[6] Heavy metal pollution occurs directly by effluent outfalls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soil/ground water systems and from the atmosphere via rain water.[7, 8] Unfortunately, many aquatic environments face metal concentrations that exceed water criteria designed to protect the environment, animals and humans. Heavy metals are defined as metals with a specific weight usually more than 5.0 g/cm^3, which is five times than water. Most heavy metals are transition elements with incompletely

filled *d* orbitals. These *d* orbitals provide heavy metal cations in solution with the ability to form complex compounds which may or may not be redox-active. Heavy metal cations play an important role as trace elements in sophisticated biochemical reaction, though some of them are essential for many organisms. Therefore, heavy metal ions are extremely harmful to human, animals, and plants because of their accumulation (over at different limiting concentration levels depending of the metal), toxicity, no biodegradable nature and causing various diseases and disorders.[9] The toxicity of heavy metals occurs even in low concentration of about 1.0-10 mg/L, which are classified as follows.[2]

Table 1.1. Classification of heavy metals based on toxicity.

Heavy metals	Toxicity
Fe, Mo, Mn	Low toxicity
Zn, Ni, Cu, V, Co, W, Cr	Average toxicity
As, Ag, Sb, Cd, Hg, Pb, U	High toxicity

Based on that, the removal of heavy metals from wastewaters has attracted attention from a long time by researchers, for the protection of public health and environment, so, nowadays they should be removed before discharge.[10]

1.2 The removal of heavy metals

Various techniques have been employed for the treatment of heavy metal pollutions in different wastewaters, which usually include chemical precipitation (hydroxides, sulfides, etc.), membrane filtration (reverse osmosis, nanofiltration, etc.), electrochemical treatment, solvent extraction, ion exchange and adsorption.[11] Table 1.2 compares selective techniques used.[12] Chemical precipitation and electrochemical treatment are ineffective, especially when metal ion concentration in aqueous solution is among 1 to 100 mg/L, and also produce large quantity of sludge required to treat with great difficulty. Membrane filtration, solvent extraction and ion exchange are extremely expensive when treating large amount of wastewaters containing heavy metal at low concentration levels, so they cannot be used at large scale.[13] Therefore, most of these

techniques have some disadvantages such as complicated treatment process, high cost, and energy use. Adsorption has considered ideal alternatives for such situations due to ease of operation and cost-effective process.[14] The main properties of the adsorbent generally are strong affinity and high loading capacity, also for the removal of heavy metals. In addition, adsorption is sometimes reversible and the adsorbent can be regenerated by suitable desorption process.[15]

Table 1.2. Removal of metal ions from wastewaters.

Method	Advantage	Disadvantage
Chemical precipitation	Simple Inexpensive Most of metals can be removed	Large amounts of sludge produced Disposal problems
Chemical coagulation	Sludge settling Dewatering	High cost Large consumption of chemicals
Ion-exchange	High regeneration of materials Metal selective	High cost Less number of metal ions removed
Electrochemical methods	Metal selective No consumption of chemicals Pure metals can be achieved	High capital cost High running cost Initial solution pH and Current density
Adsorption using activated carbon	Most of metals can be removed High efficiency (>99%)	Cost of activated carbon No regeneration Performance depends upon adsorbent
Adsorption using natural zeolite	Most of metals can be removed Relatively less costly materials	Low efficiency
Membrane process and ultra filteration	Less solid waste produced Less chemical consumption High efficiency (>95% for single metal)	High initial and running cost Low flow rates Removal (%) decrease with the presence of other metals

Adsorption take place in three definable steps[16]: (1) bulk solution transport which moves the adsorbate through the bulk liquid by means of advection and dispersion, to the fixed film boundary layer surrounding the adsorbent media; (2) diffusive transport which moves the adsorbate across the fixed film boundary; and (3) bounding processes

which act to attach the adsorbate to the media surface of the adsorbent. Adsorption onto solid adsorbents has great environmental significance, since they can effectively remove pollutants from both aqueous and gaseous streams.[17]

Adsorption results as a consequence of an unbalance of surface forces or surface energy, which includes both physical and chemical mechanisms.[18] Physical sorption mechanisms result from molecular condensation in the capillaries of a solid (used as adsorbent), the process is reversible, and the system has a low heat of adsorption. While chemical sorption mechanisms involve the formation of ion complexes and the formation of chemical bonds between the adsorbate and the adsorbent (a solid), it requires high energy, and can be irreversible. Due to its relatively irreversibility, monolayer is expected to form in chemical sorptions while multilayer is encountered in physical sorption. In an adsorption system, both sorts are expected to occur, therefore, upon analysis, properties of both appear. However, most adsorption separation processes depend on physical adsorption.[19]

Over the last few decades adsorption has gained importance as a purification and separation process on an industrial scale. Various materials have been developed as adsorbents for the removal of heavy metals. In particular, activated carbon has been traditionally used as a good adsorbent for the removal of heavy metal contaminants.[20, 21] Its usefulness derives mainly from its large micropore and mesopore volumes and the resulting high surface area, high adsorptive capacity, and degree of surface reactivity.[22] However, activated carbon is relatively expensive, and it is difficult to elute metal ions from it and recycle the activated carbon because of strong interaction between them. Moreover, the application of expensive synthetic materials also has similar problems like technical or economic constraints.[23] Therefore, the main research focus is searching for low-cost, effective and regenerable adsorbent materials which are capable of removing heavy metals from wastewaters.[24] In general, an adsorbent can be assumed as "low cost" if it requires little processing, is abundant in nature, or is a by-product or waste material from industry.[7] To date, hundreds of studies on the use of low-cost

adsorbents have been published. Agricultural wastes, industrial byproducts and wastes, and natural substances have been studied as low-cost adsorbents for the heavy metal wastewater treatment. Several reviews are available that discuss the use of low-cost adsorbents for the treatment of heavy metals wastewater. Babel and Kurniawan reviewed the use of low-cost adsorbents for heavy metals uptake from contaminated water, including chitosan, zeolites, clay, peat moss, fly ash, coal, natural oxide, industrial waste and so on.[25] Sud et al. reviewed agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solution.[26] Wan Ngah and Hanafiah reviewed the removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents.[27]

1.2.1 Biosorption of heavy metals

In recent years, applying biotechnology in controlling and removing metal pollution has been paid much attention, and gradually becomes hot topic in the field of metal pollution control because of its potential application.[28] Alternative process is the biosorption, which can be defined as the removal of metal or metalloid species, compounds and particulates from solution by various certain natural materials of biological origin.[29]

According to the metal classification by Pearson[30] as well as by Nieboer and Richardson[31], metal affinity for ligands is supposed and illustrated in Table 1.3.[32] The symbol R represents an alkyl radical such as CH_2-, CH_3CH_2-, etc. Class A metal ions preferred to bind the ligands of I through oxygen. Class B metal ions show high affinity for III types of ligands, but also form strong binding with the ligands with II type of ligands. Borderline metal ions could bind these three types of ligands with different preference.

According to the Hard and Soft Acid Base Principle (HSAB principle), hard ions which bind F^- strongly, such as Na^+, Ca^{2+}, Mg^{2+} could form stable bonds with OH^-, HPO_4^{2+}, CO_3^{2+}, R-COO^- and =C=O which are oxygen-containing ligands. Contrast to hard ions, soft ions, for example, heavy metal ions such as Hg^{2+} and Pb^{2+} form strong

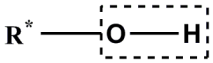
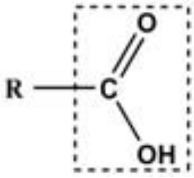
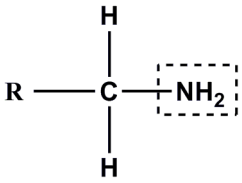
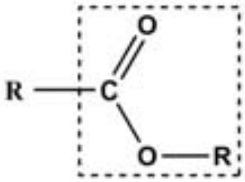
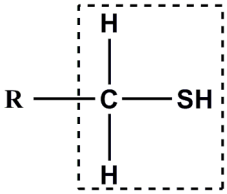
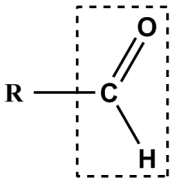
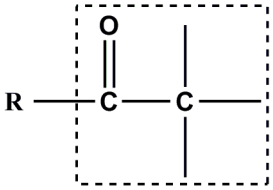
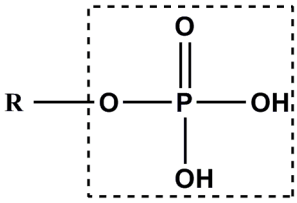
bond with CN^-, $R\text{-}S^-$, $\text{-}SH^-$, NH_2^- and imidazole, which are groups containing nitrogen and sulfur atoms. Hard ions mainly show ionic nature of binding, whereas soft ions binding exhibit a more covalent degree.[28] Borderline or intermediate metal ions such as Zn^{2+} and Co^{2+} are less toxic, and can act as hard or soft ions depending on the nature of the chemical groups or ligands present and depending on their speciation in liquid phase.

Table 1.3. The ligands present in biological systems and classes of metals.[28]

Ligand class	Ligands	Metal classes
I: Ligands Preferred to Class A	F^-, O^{2-}, OH^-, CO_3^{2-}, SO_4^{2-}, $ROSO_3^-$, NO_3^-, HPO_4^{2-}, PO_4^{3-}, ROH, $RCOO^-$, C=O, ROR	Class A: Li, Be, Na, Mg, K, Ca, Sc, Rb, Sr, Y, Cs, Ba, La, Fr, Ra, Ac, Al. Lanthanides, Actinides
II: Other Important Ligands	Cl^-, Br^-, N_3^-, NO_2^-, SO_3^{2-}, NH_3, N_2, RNH_2, R_2NH, R_3N, =N-, -CO-N-R, O_2, O_2^-, O_2^{2+}	Borderline ions: Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Cd, In, Sn, Sb, As
III: Ligands preferred to Class B	H^-, I^-, R^-, CN^-, CO, S^{2-}, RS^-, R_2S, R_3As	Class B: Rh, Pd, Ag, Lr, Pt, Au, Hg, Tl, Pb, Bi

Metal biosorption by biomass mainly depend on the functional groups. The most important of these groups including carbonyl (ketone), carboxyl, sulfhydryl (thiol), sulfonate, thioether, amine, secondary amine, amide, imine imidazole, phosphonate and phosphodiester are summarized by Volesky.[33] The relevant structural formula, pK_a, HSAB classification, ligand atom, as well as occurrence in selected biomolecules were offered. Table 1.4 offers some representative functional groups and classes of organic compounds in biomass. The symbol R is shorthand for residue, and its placement in a formula indicates that what is attached at that site varies from one compound to another.[34]

Table 1.4. The representative functional groups and classes of organic compounds in biomass.[33]

Formula of functional group	Name	Class of compounds
	Hydroxyl	Alcohols, carbohydrates
	Carboxyl	Fatty acids, proteins, organic acids
	Amino	Proteins, nucleic acids
	Ester	Lipids
	Sulfhydryl	Cysteine (amino acid), proteins
	Carbonyl, terminal end	Aldehydes, polysaccharides
	Carbonyl, internal	Ketones, polysaccharides
	Phosphate	DNA, RNA, ATP

The biosorption of heavy metals by the biomaterials might be attributed that these biomaterials, mainly composed of polysaccharides, proteins and lipids have carboxyl,

hydroxyl, sulfhydryl, phosphate, carbonyl and amino groups, can bind metal ions involving valance forces through sharing or exchange of electrons between biosorbent and metal ions (see Figure 1.1).[35] However, these materials can also passively bind metal ions via various physicochemical mechanisms. Mechanisms responsible for biosorption, although understood to a limited extent, may be one or a combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and/or microprecipitation. There are some evidence to confirm that the O-, N-, S-, or P- containing groups participate directly in binding a certain metals.[3, 8, 36]

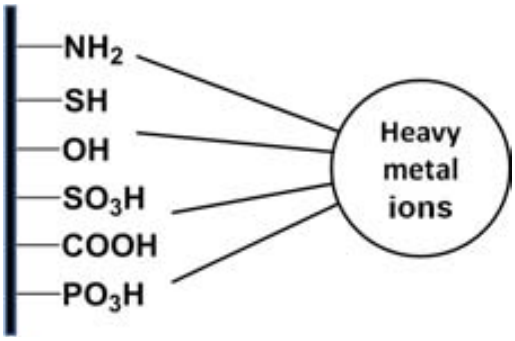


Figure 1.1. Possible binding modes between functional groups and heavy metal ions.

A large quantity of materials and biomass types has been investigated as biosorbents for the removal of heavy metals, for their metal binding capacity under various conditions. Waste biomaterials are considered ideal alternatives as low-cost biosorbents for the removal of heavy metals from low strength wastewaters due to the relatively cost-effective reason and quite efficient adsorption capacities. A vast array of low-cost waste biomaterials from large-scale industrial operations have also successfully been employed as biosorbents for the removal of heavy metals from aqueous solution and wastewaters.[37] Although many biomaterials can bind metal ions, only those with sufficiently high metal binding capacity and selectivity for heavy metals are suitable for use in a full-scale biosorption process.[38]

Among the various biosorbents investigated, cellulosic waste materials are the most abundant for the removal of heavy metals, which include agricultural waste materials[39,26] (wheat based waste material,[40,12] rice husk,[41]), waste products from timber industry[7] (bark,[42] wood, sawdust[43,44]). Cellulosic waste materials usually are composed

of lignin and cellulose as major constituents and may also include other polar functional groups of lignin, which includes alcohols, aldehydes, ketones, carboxylic, phenolic and other groups. These groups have the ability to extent to bind heavy metals by donation of an electron pair from these groups to complexes with the metal ion in solution. Chitin is the second most abundant as an excellent biosorbent for the removal of heavy metals after cellulose. The most abundant natural source of chitin is usually from shell or exoskeleton of animal such as crustaceans, arthropods and mollusks. Chitin is a polymer composed of N-acetyl-D-glucosamine or specifically 2-acetamido-2-deoxy-β-D-glucose through a β(1-4) linkage residue.[45] The adsorption behavior of chitin is primarily attributed to the high hydrophilicity of the polymer, the presence of a large number of functional groups (acetamide, primary amino, hydroxyl) and the high chemical reactivity of these functional groups. However, more important than chitin is chitosan, which was prepared from chitin by partial deacetylation. Yang and Zall reported that the biosorption capacity of chitosan chelates is greater five to six times of metals amount than chitin due to the free amino groups exposed during deacetylation.[46] Although chitosan can dissolve in acidic medium, the use of a cross-linking agent such as glutaraldehyde or the use of some modification process like coating, can diminish the dissolution properties of chitosan (which is an improvement for its use as biosorbent). Recently, chitosan composites and derivatives have been developed to adsorb heavy metals from wastewater due to their low costs and high contents of amino and hydroxyl functional groups.[47,48] The chitosan/ceramic alumina composites,[49] chitosan/magnetite composites,[50] chitosan/cellulose composites,[51,52] ethylenediamine-modified magnetic chitosan nanoparticles,[53] crosslinked chitosan resin modified by L-lysine[54] have all been used to remove heavy metal ions from wastewater. As a kind of abundant biological resources, keratin biomaterials are currently being investigated as biosorbents for the removal of heavy metal ions from water and wastewater, which are relatively abundant and inexpensive materials. Various keratin biomaterials, such as wool, feather, hair and horn can be used as effective biosorbents, either directly or after activation, to remove

heavy metals due to their high contents of carboxyl, hydroxyl, amino and sulfur-containing functional groups. These biomaterials have showed significant potentials of removing metal ions from aqueous solution.

Considered the importance of keratin biomaterials as biosorbents for the removal of heavy metals from wastewater, an overview of summarized information concerning the utilization of keratinous materials based on recent publications is presented in the following part and their biosorption performance, influencing factors of the heavy metal biosorption process, the favorable conditions and the biosorption mechanism, etc., are here discussed.

1.3 Removal of heavy metals by keratin biomaterials: an overview

1.3.1 The structure and chemical composition of keratins

Keratins are highly specialized fibrous proteins known as scleroproteins. All keratins are produced in epithelial cells in higher vertebrates (reptiles, birds and mammals) and in humans. Keratins are classified as heterogeneous proteins due to the amino acid structure and composition. These proteins are resistant to physical and chemical environmental factors. They are insoluble in water, weak acids and alkalis, organic solvents, and are insensitive to the attack of common proteolytic enzymes such as trypsin or pepsin. Keratin is a high molecular weight polymer containing polypeptide chains formed by the condensation of L-amino acids as shown by Figure 1.2.[55]

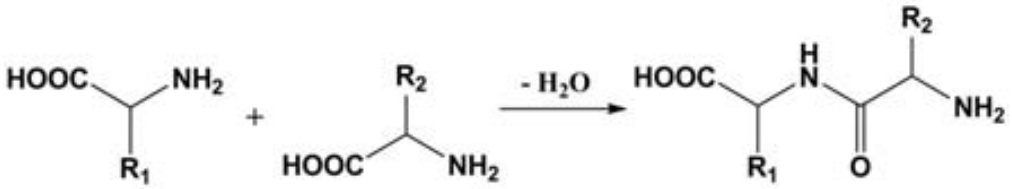


Figure 1.2. The condensation reaction of amino acids.[55]

The bond that forms upon condensation which links the amino acids is called the peptide bond. A number of these condensation reactions will ultimately produce a polypeptide chain. The R_1 and R_2 group signifies the side chains of the amino acid residues corresponding to 18 different compositions (Table 1.5).[58]

Table 1.5. Amino acid composition of whole human hair.[58]

Amino acid	Whole hair (μmol/g)
Cysteic acid	32
Aspartic acid and asparagines	399
Threonine	554
Serine	967
Glutamic acid and glutamine	916
Proline	588
Glycine	437
Alanine	347
Valine	405
Half-cystine	1435
Methionine	13
Isoleucine	174
Leucine	457
Tyrosine	158
Phenylalanine	124
Lysine	196
Histidine	62
Arginine	466

Keratin is a structural protein and is typified by sulfur content. As shown in the Table 1.5, besides serine and glycine, keratins exhibit the presence of a large concentration of the sulfur-containing diamino acid cystine that largely contributes to the stability of the fiber. A high cystine (Figure 1.3) content is the most important property that differentiates keratins from other structural proteins.[56, 57]

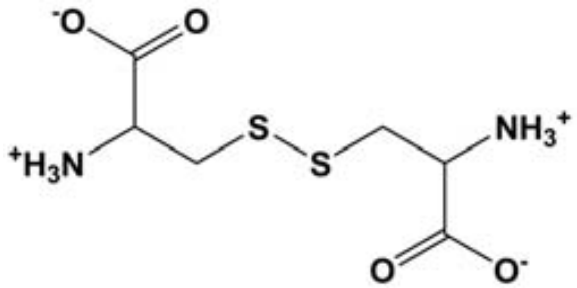


Figure 1.3. Molecular structure of the amino acid cystine.[56, 57]

Generally, polypeptide chains in keratins can occur in two major configurations: α-helix and β-sheet, also known as a pleated sheet. X-ray studies of hair keratin show an

α-helical conformation of the polypeptide chain. The polypeptide chain is a spiral coil in the α-helix. Intracellular hydrogen bonds that are the principal interactions make hair elastic. X-ray diffraction in hair also shows that individual polypeptide chains in the α-helix are right-handed and form a left-handed superhelix stabilised by hydrogen bonds running parallel to the main axis and by disulfide bridges formed by cystine radicals between adjacent chains. It can be seen in the Figure 1.4, microfibrils visible under an electron microscope form macrofibrils visible under a light microscope.[58] Both protofibrils and microfibrils are fixed by disulfide bridges, which determine their stiffness and resistance to microbial degradation. Individual microfibrils and macrofibrils are embedded in an anamorphic matrix. The matrix surrounding microfibrils contains low-molecular keratins with high cystine content. Conversely, microfibrils are formed by high-molecular and low-cystine keratins. The matrix surrounding macrofibrils contains little keratin and mostly has non-keratin proteins. β-keratin occurs in reptiles and birds in scales, claws, beaks and feathers and cuticle hair. It lacks cystine and has a high content of serine, glycin, proline, alanine and low levels of lysine, histidine and tryptophan. Feather keratin occurs in both configuration systems of the polypeptide chain: α-helix and β-sheet, which is composed of β-conformation proteins in 1/3 and α-keratin in 2/3. Like in α–keratin in hair, microfibrils of α-keratin in feathers are immersed in the matrix. Unlike hair keratin, feather keratin does not stretch and is not elastic.

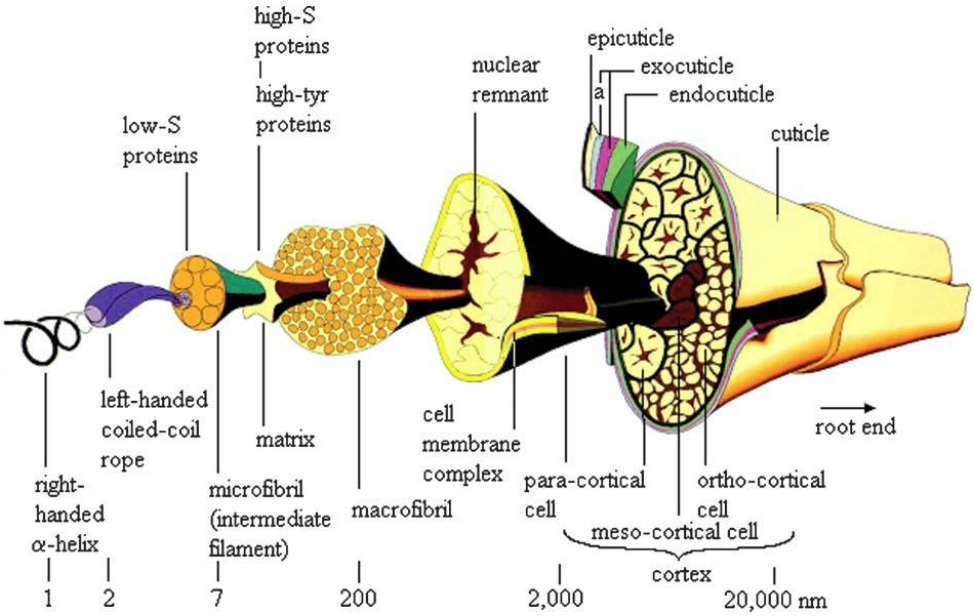


Figure 1.4. Structure of the α-keratin fibre under various magnifications.[58]

Most notably, keratin biomaterials possess a much higher content cysteine residues in their non-helical domains and thus form tougher and more durable structures via intermolecular disulfide bond formation.[59] In addition, keratin biomaterials are insoluble in water and contain some primary functional groups involving carboxyl (R-COOH), hydroxyl (R-OH), amino (R-NH_2) and sulfhydryl (R-SH), which leads to the keratin biomaterials used as good biosorbents for removal of heavy metal ions from wastewater. Among the functional groups, the sulfur atoms can more readily coordinate to the heavy metal ions, as shown by Dąbrowski et al.[60] Therefore, these functional groups allows for the strong attraction of metal ions to keratinous materials, which makes the keratinous materials are deal biosorbent for removal heavy metals from wastewater.

1.3.2 Removal of heavy metals by keratin biomaterials

Keratin is the major component of wool, hair, feathers, horn, nail. Moreover, keratin wastes such as feathers, horns, and nails from butchery, human hair from barber, poor quality raw wools from sheep breeding and some by-products from textile industry, account worldwide for more than four million tons per year.[61] In other words, keratinous materials are easily available in the local place at negligible price. As regards the use of keratinous materials, wool, feather and hair have been used to adsorb metal ions from

solution in their native state and with suitable chemical pretreatment, the adsorption capacity for these ions can be significantly enhanced.

1.3.2.1 Removal of heavy metals by wool

Among various waste keratin biomaterials, wool is abundant. Wool keratin is attracting an increasing amount of research interests, as it is an effective biosorbent and can be used for concentrating, removing, and recovering toxic, precious, radioactive or other metal ions from natural water supplies or industrial wastewater. This is because the protein fiber on wool surface has polar an ionisable groups on the side chain of amino acid residues, which can strongly bind charged species such as metal ions. The rate and the extent of uptake was reported to depend on various factors such as the kind of metal and its valence state, the solution concentration, pH, time, temperature, etc..[37]

Various researches on wool keratin for removal of heavy metals have been done recently. The native wool protein,[62] chemically-modified wool fibers and regenerated wool protein[63] have been used to remove metal ions from aqueous solution. In 1968, the mohair has been used for the uptake of Cu(II) by Guthrie and Laurie.[64] Freeland et al. investigated the raw wool for the removing Hg(II), Cu(II), and Ni(II), and it was found that the adsorption of metal ions by wool is strongly inhibited by sodium chloride.[65] They also studied wool modified with polethylenimine (PEI) for Hg(II) adsorption with good adsorption capacity.[66] Masri and Friedman also investigated raw wool modified by the addition of various functional groups for removing metal ions, especially those containing sulphur.[67] They suggested that the sulfhydryl group could increase the adsorption capacity due to the formation of metal mercaptide. Edgar and Simpson reported that the adsorption of Al(III) by wool from water/alcohol mixtures.[68] The adsorption of Al(II) ions by wool keratin is increased in the presence of alcohols. Shinoda and Kikyotani used special resin prepared from waste wool fiber as adsorbent for recovering Hg(II) from wastewater.[63] A similar study for Hg(II) sorption using waste wool discarded from wool manufacturing processes was also conducted, it was note that the waste wool can be used for the removal of Hg(II) from industrial effluents,

particularly those from the chlor-alkali industry. The amount of Hg(II) sorbed at the keratin protein (wool) reaches 90% of sorption (from solutions containing 100 ppm of Hg(II)).[69]

In 1992, a study with the wool fibers to remove Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), and Pb(II) from aqueous solution was conducted by Devrim and Hayri.[70] It was reported that a fiber diffusion is the rate-controlling step in the adsorption process. It was also suggested that wool is a potential adsorbent for removing toxic metal ions from contaminated waters.

Freddi et al. reported that the removal of Cu(II) and Ag(I) ions is possible using wool modified by treatment with tannic acid (TA) and by acylation with ethylenediaminetetraacetic (EDTA) dianhydride.[71] Modification of wool fibers was effective in enhancing their intrinsic ability to absorb and bind Ag(I) and Cu(II), where the extent of metal uptake was dependent on the number of available binding sites introduced into the fiber matrix with the modifying agents. The acylation technique appeared particularly attractive because metal cations were effectively adsorbed over a wider pH range, especially in the acidic and neutral regions. Wool-Ag, wool-EDTA-Ag, and wool-TA-Ag complexes showed lower levels of metal release at acidic pH and better antimicrobial activity against bacteria than the different wool-Cu complexes. Afterwards, Paola et al. explored the interaction between the binding sites of this two chemically modified wool fibers and metal cations (i.e. for Co(II) and Cu(II)) through an IR spectra elucidation.[72]

Dakiky et al. reported the selective adsorption of Cr(VI) from industrial wastewater using several abundantly available low-cost adsorbents including wool, olive cake, sawdust, pine needles, almond shells, cactus leaves and coal.[73] Among the selected adsorbents, natural wool from sheep was the best one for the selective removal of Cr(VI) at acidic pH and with quite high amounts of adsorbent, at 30°C. It was also found that the kinetics of the Cr(VI) adsorption on the wool was followed the pseudo-first order rate mechanism. Wool was found to fit better to the Langmuir isotherm, which is based

on the formation of layers on the active sites. The Gibbs free energy obtained for wool as an adsorbent showed that it has the largest capacity and affinity for the selective removal of the metal.

The removal of Cu(II), Co(II), Ni(II), Zn(II) and Cr(VI) from their aqueous solutions using low-class native wool or wool wastes was explored by El-Sayed and coworkers.[74] It was reported that the sorption efficiency of the aforementioned metals by wool is in the following order: Cu(II) > Co(II) = Ni(II) > Zn(II) > Cr(VI). Meanwhile, the authors also investigated the effect of the modification of wool on the sorption of Cu(II). The results indicated that modified wool is better than both native and oxidized wool in removing Cu(II) from the effluent. Wool could be reused for many times for copper sorption after elution with dilute hydrochloric acid. The efficiency of wool to remove traces of metals from water effluents gives the possibility of using wool fibers as filters for potable water in areas where water is contaminated with these metals.

The effect of the contact time, temperature and pH on the uptake of Cu(II) by wool was investigated by Sheffield and Doyle.[75] It was found that the uptake of Cu(II) can be improved by longer heating times and higher temperatures, 1 hour near the boil gives optimal results. It was also observed that Cu(II) uptake increased linearly with pH up to a pH of around 6.0. However, at higher pH value, precipitation of solid copper salts will be formed.

Wool fiber can adsorb not only heavy metal cations but also metal nanoparticles. Richardson and Johnston reported that monodisperse gold nanocrystals can be adsorbed and chemically bound to Merino wool fibers.[76] It was found that the nanocrystalline gold was bound through the nitrogen of the amino groups in the surface of the gold to the keratin of the fibers, which was confirmed by surface analysis of X-ray photoelectron spectroscopy.

Park and coworkers reported that the removal of Cu(II) from aqueous solution using nanofibrous membrane of wool keratose/silk fibroin blend.[77] It was found that

wool keratose is preferred for removing metal ions. It can be attributed to more amino acids having polar side residues in the wool keratose than in the silk fiber. However, the use of only wool keratose for preparation of nanofibrous membrane can be hardly performed due to its low electrospinnability as well as its structural instability in water. It was also indicated that blending the wool keratose with silk fiber can improve those characteristics, and also its adsorption capacity for Cu(II) is improved compared with only the silk fibroin nanofibrous membrane. Furthermore, the adsorption capacity of wool keratose/silk fibroin nanofibrous membrane was maintained after several cycling process of adsorption and desorption. This indicated that this membrane is a promising material for removing and recovering heavy metal ions in water.

Monier and coworkers reported that the graft copolymerization of ethyl acrylate (EA) onto natural wool fiber (wool-g-PIAH) as adsorbent could be used for the removal of Cu(II), Hg(II) and Ni(II) metal ions from aqueous solutions.[78] It was shown that the adsorption affinity of this fiber for Cu(II) was higher than Hg(II) and Ni(II). The adsorption kinetics followed the pseudo-second order equation and the equilibrium date can be described well by the Langmuir isotherm. It was also indicated that regeneration of the fibers obtained was achieved by using EDTA with efficiency of greater than 85%. At the same time, the authors also investigated a similar wool fiber (wool-g-PCAH) as adsorbent for the removal of Hg(II), Cu(II) and Co(II).[79] It was pointed out that Hg(II) showed higher adsorption affinity towards the studied fibers relative to Cu(II) and Co(II). It was further mentioned that this wool is a good regeneration fiber and its efficiency is greater than 80%. Thus, indicating that feasible improvements in the uptake properties encourage efforts for wool-g-PIAH and wool-g-PCAH obtained to be applied for water and wastewater treatment.

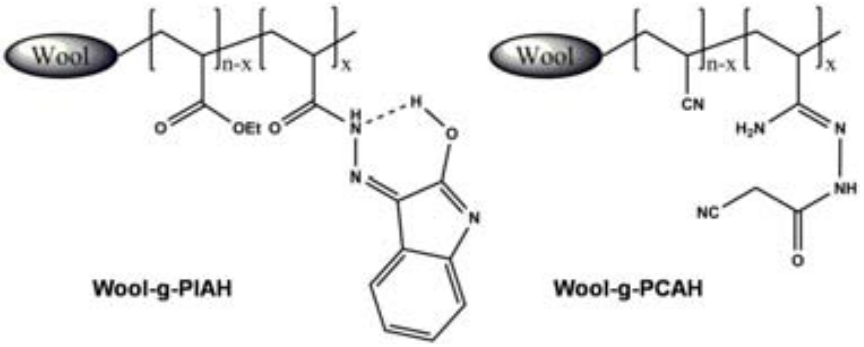


Figure 1.5. Structure of wool-g-PIAH and wool-g-PCAH.

An investigation on metal binding properties of novel wool powders was conducted.[80] It was found that the optimum pH for binding of Cu(II) and Cd(II) was in the range 6.0-8.0, while Co(II) was pH 8.0. It was pointed out that the Co(II) with weak binding affinity for wool fiber, showed considerably enhanced binding, suggesting that similar trends may be possible with other weak binding metal ions. It was also reported that the wool powder showed significantly higher metal ion loading capacity than the commercial cation exchange resins.

A comparative study on the removal of Co(II) from aqueous solution was also carried out using two wool powders derived from wool in native and wool treated with sodium salt of dichoroisocyanuric acid (DCCA).[81] It was shown that binding of Co(II) to wool powders is heavily dependent on the pH value and the chemical nature of buffer. The optimum sorption of Co(II) by both powders occurred at pH 8.0 in phosphate buffer and pH 10.0 in ammonium sulphate buffer. It was also found that coarse wool powders showed more efficient for removing and recovering Co(II) from solution in comparison with untreated wool native fiber and the fine wool powders.

Aluigi et al. report that keratin/PEO nanofibers were produced by electrospinning the blend aqueous solutions, keratin was extracted by sulphitolysis from wool, and blended with PEO in aqueous solution.[82] Due to the sorption property of keratin materials, author also reported the adsorption of Cu(II) by keratin/PA6 blend nanofibers.[83] It was found that keratin rich nanofibers showed adsorption performances better than conventional wool fabric and generally, the adsorption capacity increases with

increasing the keratin content. It was pointed out that the maximum adsorption capacities of this nanofiber (~103.5 mg/g), which are better than that when using the commercial activated carbon (~50 mg/g). Kinetics of the adsorption fitted better with a pseudo-second order rate equation. The IR analysis demonstrated the formation of complexes between Cu(II) ions and keratin that involve the terminal free carboxyl groups of the protein.

Another similar research evaluated the adsorption of heavy metals onto the wool-derived keratin nanofiber membranes.[84] It was demonstrated that an adsorption capacity for Cu(II) is greater than for Ni(II), and Co(II) through dynamic adsorption tests for the Cu(II), Ni(II), and Co(II) metal ion removal from aqueous solution. It was also reported that the keratin nanofiber membranes maintained good adsorption performances for Cu(II) ions in the competitive conditions. As regards the Cu(II) adsorption from non-competitive condition, the experimental data had a good fitness with both Langmuir and Freundlich isotherm models. The maximum adsorption capacity obtained from the Langmuir model was about 11 mg/g, the adsorption of Cu(II) ions on keratin nanofiber membranes was a multilayer process and occurred by ion exchange reactions.

One of the first reports on the application of cashmere guard hair fiber (a specific kind of wool) for the separation and waste management of metal ions was recorded by Patil and coworkers.[85] The authors investigated the adsorption behavior of cashmere guard hair fiber and its powder towards Zn(II) and Cr(VI) ion species and compared their results with other protein fiber powder sorbents. It was found that the adsorption of Zn(II) and Cr(VI) from aqueous solutions was governed by ionic interactions over different pH ranges. When the solution pH value is at 8 and 2, the cashmere guard hair, wool and silk powders show largest adsorption yield to Zn(II) and Cr(VI), respectively. The absorption performance of the powder sorbents towards Zn(II) ions (silk powder > wool powder > cashmere guard hair powder) showed an opposite trend to Cr(VI) ions (cashmere guard hair powder > wool powder > silk power). Thus, cashmere guard hair

powder may serve as an economical alternative to wool and silk powders for removal of heavy toxic metal ions from water for environment protection applications.

In the latest research, Mariwaki and coworkers investigated the removal of Pb(II) by using keratin colloidal solution obtained from wool.[86] It was also found that the Pb-keratin aggregate was easily decomposed via the addition of nitric acid, which enabled the recovery of Pb(II). However, aggregation did not occur in solutions with Pb(II) concentrations below 0.10 mM. Therefore, a keratin colloidal solution encapsulated in a dialysis cellulose tube can be used to remove Pb(II) from 0.10 mM solutions, which enabled the removal of 95 % of the Pb(II).

Overall, researchers came to understand that waste wool samples in general, which are available in large quantities and at virtually little cost, offer a real potential to treatment the wastewater prior to 1990. After 1990, wool keratin was mixed with other components to enhance the sorption performance, and the kinetics and isotherm models have been intensively investigated. Table 1.6 summarizes the biosorption capacity of the metal ions investigated on wool keratins, which were discussed above. These results showed that wool hold great potential to remove heavy metal species from industrial wastewaters.

Table 1.6. Literature update of research related to the removal of heavy metals using wool.

Biosorbent	Year	Metal ions	Kinetic and isotherm models	Amount of metal sorbed (mmol/g)	REF
Wool(natural)	1992	Ni(II) Cu(II) Zn(II) Cd(II) Hg(II) Pb(II)	-	-	70
Treated wool	2001	Ag(I)[a], Cu(II)	-	1.78[a]	71
wool	2002	Cr(VI)	Pseudo-firstorder, Langmuir	0.663	73
Treated wool	2003	Cu(II) Co(II)	-	-	72
Wool in native Reduced wool Oxidized wool	2004	Cu(II) Co(II) Ni(II) Zn(II) Cr(VI)	-		74
wool	2005	Cu(II)	-	0.165	75
wool	2007	Au	-	-	76
Nanofibrous membrane of wool/silk	2007	Cu(II)	-	0.045	77
Wool grafted PIAH chelating fibers	2010	Cu(II) Hg(II) Ni(II)	Pseudo-first order Pseudo-second order Langmuir, Freundlich Tempkin	2.24 0.246 0.796	78
Wool grafted PCAH chelating fibers	2010	Hg(II) Cu(II) Co(II)	Pseudo-first order Pseudo-second order Langmuir, Freundlich Tempkin	0.769 1.740 1.240	79
Wool fiber[a] Wool powder[b]	2010	Co(II) Cu(II) Cd(II)	Langmuir	0.0039[a], 0.0077[b] 0.0097[a], 0.0091[b] 0.0093[a], 0.0086[b]	80
Wool powder	2010	Co(II)	Langmuir	53.78	81
Keratin(wool) /PA6 blend nanofibers	2011	Cu(II)	Pseudo-first order Pseudo-second order Freundlich	1.63	83
Keratin (wool) nanofiber membrane	2012	Cu(II) Ni(II) Co(II)	Pseudo-first order Pseudo-second order Intraparticle diffusion Langmuir, Freundlich Dubinin-Radushkevi	0.173 - -	84
Wool powders	2012	Zn(II), Cr(VI)	-	-	85
Keratin (wool) colloidal solution	2013	Pb(II)	Langmuir, Freundlich	0.209	86

1.3.2.2 Removal of heavy metals by feathers

An estimated 15 million tons of chicken feathers are available globally each year as a by-product of meat manufacture.[87] The raw material is tough and chemically resistant. Currently the feathers are disposed of in landfill, burned or processed to make a low-grade animal feedstock. These methods are environmentally unsound and are restricted. Actually, feathers consist of about 91% keratin, 1.3% fat, and 7.9% water, which mean they would be used as potential biosorbents for the removal of heavy metal ions.

Banat and coworkers examined and compared the ability of different keratin-composed biosorbent (chicken feathers, hair and horn) for the removal of heavy metals from aqueous solutions.[88] Among of them, Chicken feathers are effective biosorbents and showed a higher Cu(II) ion uptake than Zn(II). It was indicated that an equilibrium time of 8 h was needed for the adsorption of Cu(II) or Zn(II) by chicken feathers. Intraparticle diffusion was involved in the adsorption processes but was not the rate-controlling step. Freundlich isotherm model was found to be applicable to the adsorption of Cu(II) and Zn(II) and provides an indication of the adsorption capacity of the chicken feather. The affinity of chicken feathers towards Cu(II) ion adsorption was better than that towards Zn(II).

The efficiency of Cu(II) and Zn(II) removal from wastewater by chicken feather was investigated by Al-Asheh et al..[89] The authors compared the efficiency of Cu(II) and Zn(II) removal from wastewater with untreated native chicken feathers, chicken feathers treated with alkaline solution and chicken feathers treated with anionic-surfactant. It was found that the chicken feathers treated with an alkaline solution adsorb more metal ions than those treated with an anionic surfactant, followed by the untreated chicken feathers. Authors explained this behavior because of the treatment with alkaline solution could increase the porosity of the keratin structure, which can increase the accessibility of the fiber for metal ions to easily diffuse into the active sites of the fibers. The treatment with anionic-surfactant can increase the negative

charges on the sorbent surface to improve the sorption capacity for metal ions. Alkaline solution of 0.2 N NaOH as found to be the best treatment for chicken feathers for the sorption process. The authors suggested that most of sorption occurs on the surface during the first 20 min of the sorption process. The Freundlich isotherm model was found to represent the equilibrium results, of all adsorbents toward Cu(II) and Zn(II), reasonably well.

Kar and Misra also investigated the biosorption of heavy metals from solutions using chicken feather.[90] They found that chicken feathers are a nano-porous and intrinsic keratin fiber, which have demonstrated a good capacity for the removal of Cu(II), Pb(II), Cr(VI), Hg(II) and U(II) from diluted solutions, especially after an alkaline ultrasonic treatment of the keratin fiber which resulted in an increase in metals uptake. In the natural pH range (pH 5.0–7.0) the keratin protein fibers have unique selectivity for Pb(II) and Cu(II), and at acidic pH for Cr(VI) and Hg(II). Adsorption of heavy metals using both batch and column operations was conducted. The surface hydrophobicity of keratin protein fibers makes mixing difficult in aqueous solutions, thereby limiting adsorbate-adsorbent contact and the metal uptake. The metal uptake is increased significantly by the column mode due to the nonexistence of difficulties such as mixing. Multiple elution and adsorption tests indicated that it is possible to reuse keratin fiber as a biosorbent for a number of cycles.

In another study, alkaline treatment of chicken feathers with NaOH or NaOCl and immobilization of resultant hydrolyzates on silica for the removal of metals including Ca(II), Mg(II), Fe(II) and Mn(II) ions from waters is presented.[91] The investigations showed good metal removal efficiency, and the adsorption capacity was determined to be > 90% for Ca(II), and for Fe(II) and Mg(II) above 70% at pH 6.0 to 9.0 within 20-30min. The sorbents could be regenerated with NaCl solution. More interestingly, these cheap regenerable sorbents were applied successfully for treating industrial water in different Egyptian industrial areas.

Guadalupe et al. reported that kinetics and equilibrium studies of Pb removal from

aqueous solutions by chicken feathers.[92] Under different experimental conditions (pH, temperature, and metal concentration), the results showed that the maximum Pb(II) sorption capacity was obtained at 30 ºC and at pH 5.0. Pseudo-second order kinetic and Freundlich isotherm models are suitable to represent the experimental data obtained in their investigation. Most of the metal removal occurs on the external surface of the sorbent during the first hours of contact. This trend is similar to that obtained by other studies. The authors suggested that the Pb(II) sorption by using chicken feathers may be controlled by a chemical sorption process.

The removal of Zn(II) ions from aqueous solutions by using raw chicken feathers was evaluated in both batch and continuous conditions.[93] It was reported that Zn(II) sorption is fast and may occur in the external surface of chicken feathers through kinetic study. On the other hand, from sorption isotherms results, author found that chemical sorption may play an essential role in the process. For batch experiments, the pseudo-second order kinetic and Sips isotherm[a] are the most appropriate models for fitting Zn(II) sorption experimental data. In the continuous system using a packed bed column, breakthrough curves were obtained for different conditions of feed flow rate, pH and metal concentration. The characteristics of these breakthrough curves are dependent of the column operating conditions especially of pH and feed flow rate. Finally, Thomas model[b] has shown an appropriate performance in the modeling of Zn(II) breakthrough curves obtained at operating conditions tested in this study. In summary, the design parameters obtained from both batch and continuous experiments could be further employed in the optimization and implementation of sorption processes for wastewaters treatment for Zn(II) removal using raw chicken feathers.

Another feathers, duck feathers were used as biosorbents for the removal of metal cations (Cu(II) and Zn(II)).[94] Author compared the adsorption of metal cations (Cu(II) and Zn(II)) by unmodified and tannic acid (TA)-modified feathers at different

[a] Sips isotherm is an isotherm model that incorporates the features of both Langmuir and Freundlich

[b] Thomas model is simple to use in the design of fixed bed columns and allows a quick and easy interpretation of sorption results.

conditions (as a function of fiber weight gain, temperature and pH of the initial metal aqueous solution). The influence of the presence of anions in the solution on the adsorption of metal cations was also studied. The uptake of Cu(II) from chloride was higher and faster than that from nitrate. The desorption study indicated that the feather-TA-metal complexes exhibited higher stability for metal cations than the feather-metal complexes. All these experiments reveal that TA-modified feather fibers have good adsorption to metal cations and can be used as metal adsorbent in wastewater treatment.

Another similar research was carried out to compare the phenomenon of Pb(II)[95] and Cu(II)[96] adsorption by chicken and duck feathers from aqueous solution. It was reported that chicken and duck feathers were treated with the tannic acid (TA), which can form insoluble products with keratin by tanning reaction and increase the chemical and physical stability of the proteins. These works have demonstrated the use of Box–Behnken design for determining the optimum process conditions leading to the maximum percentage removal of lead from aqueous solutions.

Table 1.7 gives the summary of previously research work done by various researchers using feathers for the removal of heavy metals, which have been mentioned above. It is clear that feathers (chicken and duck feathers) are promising biosorbents for the removal of the heavy metals due to their nano-porous structure and abundant functional groups, especially after a proper chemical treatment of the feathers which results in a multi-fold increase in metals uptake.

Table 1.7. Summary of previously research workusing feathers for the removal of heavy metals.

Biosorbent	Year	Metal ions	Kinetic and isotherm models	Amount of metal sorbed (mmol/g)	REF
CF (natural)	2002	Cu(II)	Intraparticle diffusion	0.186	88
		Zn(II)	Freundlich	0.0980	
CF (natural)[a]	2003	Cu(II), Zn(II)	Freundlich		89
Treated CF				0.186[a], 0.098[a]	
(alkaline solution[b] and				0.391[b], 0.231[b]	
anionic-surfactant[c])				0.295[c], 0.163[c]	
CF treated with alkaline in ultrasonic process	2004	Cu(II)	-	-	90
		Pb(II)		0.0260	
		Hg(II)		-	
		U(II)		-	
		Cr(III)		-	
NaOH-treated[a]and	2005	Ca(II)	Freundlich	0.362[a],0.345[b]	91
NaOCl-treated[b]		Mg(II)		0.394[a], 0.387[b]	
CF-immobilized silica		Fe(II)		0.243[a], 0.233[b]	
		Mn(II)		0.241[a], 0.236[b]	
CF washed with solution (deionized water and ethanol, 5:1 proportion)	2008	Pb(II)	Pseudo-first order	0.0470	92
			Pseudo-second order		
			Freundlich		
			Langmuir		
CF	2011	Zn(II)	Pseudo-first order	0.111	93
			Pseudo-second order		
			Freundlich, Langmuir		
			Sips		
DF(natural)[a]	2007	Cu(II), Zn(II)		0.210[a], 0.270[a]	94
Treated DF (tannic acid)[b]				0.460[b], 0.690[b]	
Treated CF(tannic acid)	2011	Pb(II)	-	1.71	95
Treated DF(tannic acid)				1.42	
Treated CF(tannic acid)	2012	Cu(II)	-	0.391	96
Treated DF(tannic acid)				0.382	

1.3.2.3 Removal of heavy metals by hair

Just a few research investigations have been reported in the literature related to the biosorption of heavy metals by hair from aqueous solutions. Pace and Michelsen used different hair samples including human and hog, in general from cattle and tannery to remove Hg(II).[97] They found that the adsorption capacity of tannery hair for Hg(II) is better than others, probably due to the crystalline regions in cattle hair are changed to be

amorphous during the swelling in the tannery dehairing process. Moreover, the adsorption capacity of tannery hair for Hg(II) was comparable with those given by ion exchange resins (Dowex 50W-×8 and Dowex 1-×8). Kulkarni and Rane reported a maximum adsorption capacity of 41.6 and 50.5 mg/g^{-1} of Hg(II) by tannery hair pretreated in alkali and alkaline Na_2S solution, respectively.[98]

Fergusson and coworkers studied the sorption of Cu(II), Mn(II), Zn(II) and As(III) from aqueous solution by using human hair.[99] At an equilibrium metal concentration of 0.3 μg/mL, the sorptions are relatively low for Mn and As, and higher for Zn and Cu. The author considered that the greater sorption of Cu(II) probably is due to its greater potential binding capacity and stability, specially of the Cu-S interaction, and also due to the greater electrostatic interactions between Cu(II) and hair.

Adsorption capacities of untreated hair, inorganic acid treated hair and alkaline treated hair are compared by Tan and coworkers.[100] Among the pretreatment processes checked, hair with an alkaline treatment was more effective for the adsorption of Cu(II) than others. A favorable pretreatment of the human hair in a 0.1 N NaOH / 0.1 N Na_2S solution for 20 min gave the best adsorption capacity for Cu(II). The adsorption capacity was found to increase as pH increased from 1.0 to 5.0. Moreover, the uptake capacity was significantly affected by the presence of other metal ions. Anionic effect was found to be more pronounced for a mixed ions system than for a single ion system.

The use of readily available natural materials as adsorbents of heavy metals from industrial wastewaters was investigated by Asubiojo and Ajelabi.[101] The natural adsorbents also contain human hair. Two modes of removal including column and batch experiments were carried out and their adsorption efficiency were compared. It was found that, in terms of the efficiency of heavy metal scavenging (heavy metal adsorbed per unit weight of adsorbent) it follows: bagasse and human hair > corn cob and peanut skin > wheat bran > paddy husk. Moreover, efficiency of heavy metal removal by the natural adsorbents increased with column height and decreases with the increase on particle size of adsorbent and residual heavy metal concentration in the effluent.

Fawzi and coworkers examined and compared the ability of different keratin-composed biosorbents (chicken feathers, hair and horn) for the removal of heavy metal ions from aqueous solutions.[88] Among of them, hair is an effective biosorbent and show showed a higher Zn(II) ion uptake over Cu(II). The absolute amount of metal adsorbed increased with an increase in the initial metal concentration and in the pH of the aqueous initial solution. Intraparticle diffusion was involved in the adsorption processes but was not the rate-controlling step.

The studies by Jung et al. showed that perm-lotion-treated human hair can be efficient in the removal of Pb(II) and Cu(II) from diluted aqueous solution.[102] The maximum metal removal at pH 4.2 was over than 90% for both metals. This paper confirmed the ability of human hair to adsorb heavy metals due to the presence of reduced disulfide bonds. The perm-lotion-treated hair satisfactorily removed heavy metals from s solutions with high concentrations.

Although hair, especially human hair has shown excellent adsorption efficiency for the removal of heavy metals, the research based on human hair is still rare (Table 1.8). More importantly, the kinetic and isotherm studies on the biosorption by using human hair are quite rare. However, there is still big room to enhance the adsorption ability though the chemical modification of human hair (as it has been done with other keratin biomaterial, here presented).

Table 1.8. Summary of previously research work using hair for the removal of heavy metals.

Biosorbent	Year	Metal ions	Kinetic and isotherm models	Amount of metal sorbed (mmol/g)	REF
Hair (human, hog, cattle and tannery hair)	1975	Hg(II)	-	-	97
Human hair (natural)	1983	Cu(II)	-	0.551×10^{-3}	99
		Mn(II)		0.020×10^{-3}	
		Zn(II)		0.153×10^{-3}	
		As(III)		0.134×10^{-5}	
Treated human hair	1985	Hg(II)	-	3.33	100
		Hg(I)		2.00	
		Ag(I)		1.32	
		Cu(II)		0.463	
		Cu(I)		0.337	
		Pb(II)		0.436	
		Cr(III)		0.021	
		Cr(VI)		0.233	
		Cd(II)		0.592	
		Ni(II)		0.176	
Human hair (natural)	2009	Fe(II)	-	0.551×10^{-3}	101
		Pb(II)		0.020×10^{-3}	
		Zn(II)		0.153×10^{-3}	
Human hair (natural)	2002	Cu(II)	Intraparticle diffusion	0.087	88
		Zn(II)	Freundlich	0.131	
Perm-lotion-treated human hair	2014	Pb(II)	-	0.110	102
		Cu(II)		0.141	

1.3.3 Conclusions

Biosorption is a relatively new process that has shown significant contribution for the removal of contaminants from aqueous effluents. Keratin biomaterials (wool, feather and hair) have received increasing attention for the heavy metal removal due to their good performance, low cost and large available quantities. The functional groups present in the surface of the keratin biomaterials such as carboxyl, hydroxyl, amino and sulfur-containing groups etc. have some affinity in general for heavy metals. Based on the literatures, different keratin biomaterials as biosorbents are used for the removal of heavy metals. Information reviewed is mainly focusing on the biosorption performance of such biosorbents, their possible pretreatment and modification, their regeneration/

reuse, about the modeling of the heavy metal biosorption process (even kinetics and isotherm models), together with an evaluation of their potential application and their future in the market.

The pretreatment and modification of the natural keratin biomaterials aiming to improve their biosorption capacity was introduced and evaluated. Keratin-rich nanofiber and keratin colloidal solution also have good biosorption abilities. Although chemical modified keratin biomaterials or some keratin-rich materials having specific structure and properties can enhance the adsorption of heavy metals, the cost of chemicals used and methods for the modification also have to be taken into consideration in order to actually produce "low-cost" biosorbents in the real applications. In conclusion, the widespread keratin biomaterials in industries for wastewaters treatment applications are strongly recommended due to their local availability, technical feasibility, engineering applicability, and cost effectiveness. In spite of this, the process of biosorption requires further investigation in the direction of modeling, regeneration of biosorbent, recovery of metal ions, and modification of the keratin biomaterials for the practical application.

Most of the reported studies are performed in the batch process, which gives a platform for the further designing of continuous flow systems with possible industrial applications at the commercial level. Further research is to be carried out to make the process economic viable at industrial scale with focus on metal recovery and regeneration of keratin biomaterials, especially in the case that they are modified with some specific pretreatment.

Therefore, in the future, one trend is to use hybrid technology for the pollutants.[103] Another trend is to develop the commercial biosorbents, and to improve the biosorption process including the proper immobilization of biomaterials, improvement of their regeneration and reuse, improvement on their possible pretreatment and modification, optimization of biosorption process under different conditions and for different case studies (feed samples), etc.[28]

Comparing with other keratin biomaterials, hair has been used as biosorbent for the

removal of heavy metals in wastewaters since 1970s', even the study on this biosorption process is rear. There are still many unknown area for further research, such as the quantification relationship between surface structure of biosorbent, functional groups present on the surface of biosorbent, batch and column conditions, and the biosorption capacity which will help in predicting and selecting biosorbents in the application of real wastewater treatment. Chemical treatment has shown expressive benefit to enhance the biosorption ability for other keratin biomaterials, such as wool and feather. The chemical modified human hair has been proved qualitatively, which has been proved to be a better biosorbent than natural hair. A widespread research on hair as biosorbent is needed not only to understand the relationship between hair structure and its absorption performance, but also to push the abundant and cheap materials for real applications.

1.4 Research main objectives

Based on the studies mentioned above, the main objective of this research was to investigate heavy metal removal from aqueous solutions by using various keratin biomaterials (especially human hair) under different experimental conditions. For that reason, biosorption of heavy metals from aqueous solution is here investigated under various experimental conditions such as contact time, pH of solution, biosorbent dosage and initial metal concentration to achieve the optimized conditions. Furthermore, the kinetics and isotherm models (different models involved) are carefully checked to understand the biosorption process.

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Methodology

2 Methodology

2.1 Chemicals

All the chemicals used in this work were of analytical grade. Stock solutions of separate heavy metal ions, such as Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) were prepared by dissolving their nitric salts (> 99%, all from Panreac, Spain) in deionized water. A 1000 ppm stock solutions of each metal ion were first prepared, which were then diluted to the aqueous feed initial metal concentration for each experiment. Sodium hydroxide (> 98%, from Panreac, Spain) and nitric acid (> 70%, from JT-Baker, Spain) were alternatively used for the pH adjustment of the feed initial aqueous solution prior commencing the biosorption experiments. Ethylenediaminetetraacetic acid (EDTA) solution was prepared by dissolving its sodium salt (Ethylenediaminetetraacetic acid disodium salt, $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, from Panreac, Spain) in deionized water. Deionized water purified with a milli-Q Gradient system from Millipore Corporation was used to prepare all aqueous solutions. pH standard buffer solutions were used to calibrate an Omega 300 pH meter (Crison instruments, S.A., Spain) for the measurement of the initial and final aqueous pH values in all the experiments.

2.2 Biosorbents and treatment of biosorbents

2.2.1 Biosorbents

As a kind of abundant biological resources, keratin biomaterials can be used as biosorbents for the removal of heavy metal ions from aqueous wastes, either used directly or after properly activation pre-treatment. Such keratin biomaterials have intricate networks of stable, water-insoluble fibers with high surface areas and with

abundant functional groups (carboxyl, hydroxyl, amino and sulfonate groups) on their surface which give them such sorbent properties.[1] Keratin is the major component of wool, hair, feather, horn and nail. In the present work, different kind of keratin biomaterials including human hair, dog hair, chicken feather and wool were selected as biosorbents for the removal of heavy metals from aqueous solution.

Hair samples were obtained from local barbershops (in Terrassa city). Human hair samples were washed with commercial detergent, rinsed several times with deionized water and then left to dry at room temperature (22±1 ºC). The hair was cut to an approximate length < 5 mm by using scissors (Figure 2.1). The small hair size < 5 mm was used in the batch biosorption experiments.

Figure 2.1 Human hair and human hair cut at 1-2 mm.

Dog hair was obtained from some colleagues of the research laboratory. Dog hair samples (Figure 2.2) were also washed with commercial detergent, rinsed several times with deionized water and then left to dry at room temperature (22±1 ºC). An electrical grinding machine was used to make the dog hair much looser than the initial one (corresponding equipment description is specified in Table 2.2). The loose dog hair was used in the batch biosorption experiments.

Figure 2.2. Dog hair and oxidized dog hair

Table 2.1. Coffee Grinder description.

Equipment	Coffee Grinder
Model	MO-3250
Company and Country	Sonifer, Murcia, Spain
Laboratory of analysis	Centre Grup de Tècniques de Separació en Química, GTS (UAB, Barcelona, Spain)
Image	

Chicken feathers (Figure 2.3), collected from poultry farms were washed with commercial detergent, rinsed several times with deionized water and then left to dry at room temperature (22±1 ºC). After cleaning, they can be directly used in the batch biosorption experiments (previous studies run in the same research group, GTS, concluded that there was no influence on the size of the chicken feathers onto the biosorption of heavy metals from aqueous solution).[2]

Figure 2.3. Chicken feathers

The wool sample used is flock degreased wool yarn (Figure 2.4) kindly provided by a textile company. So, to remove impurities from the degreased wool sample, when received, it was washed with commercial detergent degreased wool, when received, was washed with commercial detergent, rinsed several times with deionized water and then left to dry at room temperature (22±1 ºC). It can be separated by scissors to weigh and was directly used in the batch biosorption experiments.

Figure 2.4. Degreased wool

2.2.2 Chemical treatment of biosorbents

Keratin biomaterials were chemically pre-treated in order to try to improve their biosorption properties for the heavy metal uptake. The treatment processes of the biomaterials (human hair, dog hair, chicken feathers and wool) were performed as follows: 20.0 g of washed, dried biomaterial was weighted out and soaked in 400 ml of the treatment reagent (H_2O_2, originally at 35% in water, from Sigma-Aldrich, Germany) of known concentration (10% H_2O_2) and pH (pH 9.0 is the most efficient in comparison with others, when different pH were assayed 5.0, 7.0 and 9.0).[3] After a given soaking time (5 h for human hair, 3 h for dog hair and wool, and 1 h for chicken feathers), the solution was filtered. The keratin biomaterials were accurately separated and they were washed many times with deionized water. In the case of the human hair, to minimize any loss of the treated hair, at each washing step was separated by using centrifuge (Alvarez Redondo, DIGICRN CE007, USA), and after that the liquid was decanted. For the other keratin biomaterials, the liquid was decanted directly without the need of the centrifugation step. Finally, the treated and cleaned biomaterials were dried at room temperature.

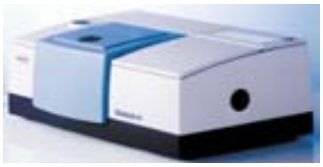
2.3 Characterization

2.3.1 Fourier transform infrared spectrometer

Fourier transform infrared spectrometer (FT-IR) is commonly used to identify compounds or the purity of a material.[4] In this study, a FT-IR (Tensor 27, Bruker, Germany) was used to determine the structural changes of biosorbent samples. Samples

less than 1 mg were analyzed. The spectrum was recorded in the range of 600-4000 cm^{-1} with 16 scans and with a resolution of 4 cm^{-1}. The equipment description is specified in Table 2.2.

Table 2.2. Fourier transform infrared spectrometer equipment.

Equipment	FTIR
Model	Tensor 27
Company and Country	Bruker, Germany
Laboratory of analysis	Service of Analytical Chemistry (SAQ, UAB, Barcelona, Spain)
Image	

2.3.2 Scanning electron microscopy

Scanning electron microscopy (SEM) images were used to demonstrate the surface topography of the different biosorbent samples (human hair, dog hair, chicken feathers and degreased wool).[5] The scanning electron microscopy (SEM ZEISS EVO® MA 10, Oberkochen, Germany) is a powerful tool to analyze the morphological structure of the biosorbent materials. Sampling preparation is simple and the biosorbent material used sputter-coating arrangement is relatively stable to the penetrating electron beam. Equipment description is specified in Table 2.3.

Table 2.3. Scanning electron microscopy equipment description.

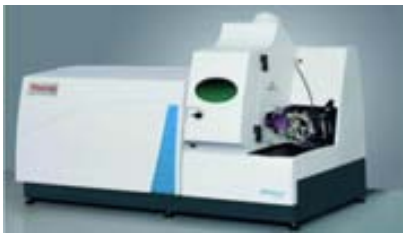
Equipment	SEM
Models	ZEISS EVO MA 10
Company and Country	Carl Zeiss, Germany
Laboratory of analysis	Service of microscopy (UAB, Barcelona, Spain)
Image	

2.3.3 Inductively coupled plasma mass spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) is an analytical technique

used for elemental determinations, so it can be used to analyze the heavy metal ions.[6] ICP-MS is the most sensitive technique for trace elements in solution. Typical detection limits for ICP-MS are around 0.01-1 µg/L (ppb) in solution. Sample preparation usually consists of simply diluting the sample in 2% nitric acid, properly. XSeries 2 ICP-MS instrument was used to perform samples analysis. The equipment description is specified in Table 2.4.

Table 2.4. ICP-MS equipment description.

Equipment	ICP-MS
Model	VG Plasma Quad ExCell and XSeries 2
Company and Country	Thermo Scientifics, USA
Laboratory	Centre Grup de Tècniques de Separació en Química, GTS (UAB, Barcelona, Spain)
Image	

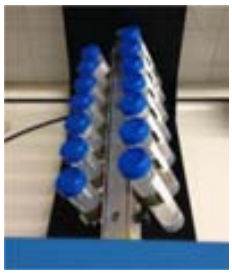
2.4 Procedure

2.4.1 Batch biosorption experiments

The biosorption experiments for the removal of heavy metal ions from aqueous solution by using keratin biomaterials as biosorbents (human hair, dog hair, chicken feathers and degreased wool) were carried out under batch operation mode at a constant temperature of 22±1 ˚C. In all sets of experiments, 0.100 g of biosorbent was accurately weighted in 50 mL plastic extraction tubes, and 10 mL of the corresponding heavy metal aqueous solution was added. The system was properly shaken on a rotary mixer at 25 rpm, during the desired time. The equipment description is specified in Table 2.5. After that, the two phases were separated firstly by decantation and later the liquid was filtered through 0.22 μm Millipore filters (Millex-GS, Millipore, Ireland). Finally, after the filtration step, the metal concentration in the remaining aqueous solution was

determined by ICP-MS, as indicated previously. The operational process of biosorption was shown schematically in Figure 2.5.

Table 2.5. Rotary Mixer description.

Equipment	Rotary Mixer
Model	CE 2000 ABT-4
Company and Country	SBS Instruments SA, Barcelona, Spain
Laboratory of analysis	Centre Grup de Tècniques de Separació en Química, GTS (UAB, Barcelona, Spain)
Image	

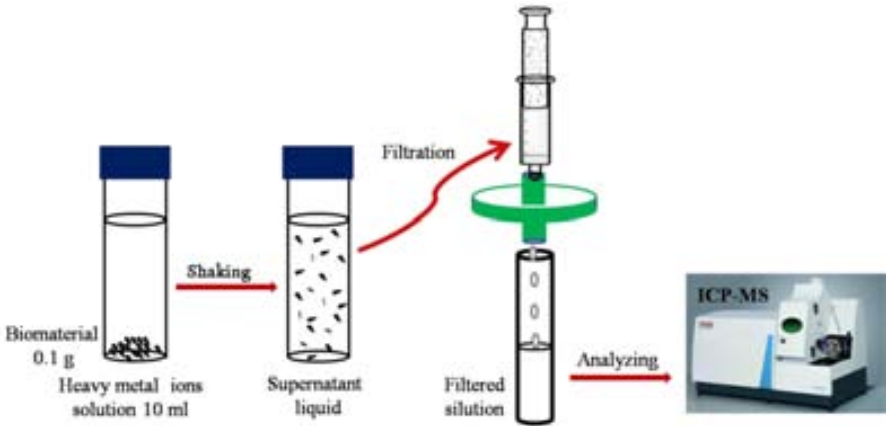


Figure 2.5. Schematic operational process of the biosorption step.

The biosorption percentage of each metal ion in each biosorption experiment was calculated using the following equation (1), which indicates the biosorption efficacy:

$$\%biosorption = \frac{C_i - C_f}{C_i} \times 100 \qquad (1)$$

where C_i and C_f are the initial and the final concentration (later at an specific time) of each metal ion in the aqueous solution, respectively (in mmol/L).

The metal uptake (q_t, in mmol/g) is the concentration of adsorbed metal ion per unit of mass of biosorbent (in mmol/g) at time t, which was calculated using the

equation (2):

$$q_t(mmol/g) = \frac{(C_i - C_f) \times V}{W} \quad (2)$$

where V is the total volume of the solution (in L), and W is the total amount of biosorbent used (in g).

The amount of metal adsorbed, q_e (in mmol/g), is the concentration of adsorbed metal ion per unit of mass of biosorbent (in mmol/g) at the equilibrium, which was obtained as follows:

$$q_e(mmol/g) = \frac{(C_i - C_e) \times V}{W} \quad (3)$$

where C_e is the equilibrium concentration of each metal ion in the aqueous solution (in units of mmol/L).

In this study, all the experiments were carried out in duplicate and the average values are presented.

2.4.1.1 Kinetic study

For the kinetic studies, 0.1 g of biosorbents (human hair, dog hair, chicken feathers and wool) were contacted separately with 10 mL of either multiple- or single- metal aqueous solution by a rotary mixer during 5, 10, 20, 30 and 45 min and 1, 2, 3, 4, 6, 12, 24, 48 and 72 h. At each predetermined interval of time, the aqueous and the solid phases were separated by filtration (with Millipore filters) and the concentration of metal ions in the final aqueous solution was analyzed by ICP-MS, as indicated.

2.4.1.2 Isotherm study

Biosorption isotherm studies were conducted as usual at 22±1 ˚C in single-metal (Pb(II) was selected as a target) aqueous solution varying the initial metal concentration from 0.5×10^{-3} to 6.0 mmol/L. So, 10 mL of single-metal aqueous solution at pH 4.0 was agitated by a rotary mixer with 0.1 g of each biosorbent (human hair, dog hair, chicken feathers or wool) for 24 h (which is enough to reach the biosorption equilibrium). Meanwhile, this can be used to determine the effect of initial metal

concentration onto the biosorbents efficacy and to evaluate the corresponding biosorption thermodynamic parameters (using different isotherm modeling systems).

2.4.1.3 Influence of the initial pH

The effect of initial pH on the equilibrium uptake of metal ions was investigated in the pH range from 1.0 to 6.0 in a multiple heavy metal aqueous solution (higher pH values are not evaluated to avoid possible metal hydrolysis and precipitation). The pH was adjusted using 0.1 mol/L NaOH and 0.1 mol/L HNO_3 solutions, alternatively. In this study, each initial metal concentration is 0.18 mmol/L in the multiple-metal aqueous solution. 10 mL of this solution, at each selected pH, was agitated by a rotary mixer with 0.1 g of each biosorbent sample (human hair, dog hair, chicken feathers or wool) for 24 h, which is more than sufficient time to reach equilibrium. The temperature was kept constant at 22±1 ˚C.

2.4.1.4 Influence of the biosorbent dosage

Influence of the biosorbent dosage for each keratin biomaterial chosen (human hair, dog hair, chicken feathers or degreased wool) on the removal of metal ions was investigated in the range of 0.01 to 0.2 g of each biosorbent. Experiments are all conducted at pH 4.0, and other conditions were kept equal as mentioned previously.

2.4.1.5 Influence of the initial metal concentration

The initial metal concentration was changed in the range 0.5×10^{-3} - 0.5 mmol/L for eight metals included in multiple-metal system and 0.5×10^{-3} - 6 mmol/L for Pb(II) in single-metal system, while keeping the other parameters, such as pH (4.0), biosorbent dosage (0.1 g), the contact time (24 h) and metal solution volume (10 mL), constant.

2.4.2 Desorption, regeneration and reuse

Desorption experiments were performed just for the case of Pb(II), after the corresponding biosorption step by using all the selected keratin biomaterials (human hair, dog hair, chicken feathers and degreased wool), and the centrifugation and filtration steps (by Millipore filters). Each biosorbent material containing the adsorbed

Pb(II) was contacted and stirred with 10 mL of 0.1 mol/L HNO_3 or 10 mL of 0.1 mol/L EDTA, separately.[7] After 24 h of stirring (with the rotary mixer) at room temperature (22±1 ˚C), the aqueous and the solid phases were properly separated (by centrifugation and filtration as usual) and the Pb(II) content of the final solutions were analyzed by ICP-MS, as indicated. Desorption percentage can be calculated using the equation:

$$\%\,\text{desorption} = \frac{amount \quad of \quad Pb(II) \quad desorbed}{amount \quad of \quad Pb(II) \quad adsorbed} \qquad (4)$$

In the use of HNO_3 and EDTA solutions as eluents, H^+ and EDTA are the responsible to the metal desorption. Therefore, the reuse of keratin biomaterials in biosorption after elution of adsorbed metal ions will require H^+ and EDTA to be removed from the biosorbent surface. Two methods are used to regenerate the biosorbents after eluting the adsorbed metal ions. One method is washing the biosorbents with deionized water. In the other method, the biosorbents after eluting the adsorbed metal ions by using EDTA, are firstly rinsed with HNO_3 solution, and then are washed with deionized water. The biosorbents regenerated by these two methods are dried in an oven at 40 ˚C. In addition, another regenerated keratin biomaterials after elution by using EDTA, are firstly rinsed with HNO_3 solution, and then are washed with deionized water and dried. These three different regenerated keratin biomaterials obtained from these two methods are employed to adsorb heavy metals again.

2.5 Biosorption kinetic models

Biosorption equilibria studies are important to determine the efficacy of the biosorption process when using different keratinous materials and for multiple- and single-heavy metal systems. Furthermore, it is also interesting to identify the biosorption mechanism in such systems. So, in order to investigate the mechanism of biosorption and the potential rate controlling steps such as mass transport and chemical reaction processes, different kinetic models have been used to fit the experimental data. Many attempts have been made to formulate a general expression describing the kinetics of sorption onto solid surfaces in liquid-solid phase sorption systems.[8] These

kinetic models included the pseudo-first order equation, the pseudo-second order equation and the Weber-Morris intraparticle diffusion model. Biosorption kinetic studies were carried out in batch mode, as indicated previously.

2.5.1 Pseudo-first order rate equation

The pseudo-first order equation was first represented by Lagergren for the sorption of oxalic acid and malonic acid onto charcoal.[9] The pseudo-first order kinetics equation has been most widely used for the adsorption of an adsorbate from aqueous solution, such as for metal ions, dyestuffs, and contaminating organic compounds.[10] This model based on the solid capacity considers that the rate of occupation of adsorption sites is proportional to the number of unoccupied sites. The pseudo-first order rate expression is generally expressed as follows:

$$\frac{dq}{dt} = k_1(q_e - q) \qquad (5)$$

Integrating (5) between the limits, t = 0 to t = t and q_0 = 0 to q_t = q_t, the Eq. (6) is obtained:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \qquad (6)$$

where, q_e and q_t are the concentration of biosorbed metal ion per unit of biosorbent mass (in mmol/g) at the equilibrium and at time t (min), respectively, and k_1 (in min^{-1}) is the rate constant of the pseudo-first order equation. Linear plots of $\log(q_e\text{-}q_t)$ versus t indicate the applicability of this kinetic model.[11] However, to adjust Eq. (6) to the experimental data, the value of q_e must be pre-estimated by extrapolating the experimental data to t = ∞.

The pseudo-first order equation has been adequately employed to describe sorption reactions, however, it is typically expressed in a range of reaction only. The insufficiency of the pseudo-first order model to fit the kinetics data could possibly be due to the limitations of the boundary layer controlling the sorption process.[12]

2.5.2 Pseudo-second order rate equation

The pseudo-second order model is derived on the basis of the sorption capacity of

the solid phase.[13] Early applied second-order rate equations in solid-liquid systems described reactions between soil and soil minerals.[14] This model assumes that the rate of occupation of adsorption sites is proportional to the square of the number of unoccupied sites. It can be expressed as

$$\frac{dq}{dt} = k_2(q_e - q)^2 \qquad (7)$$

Integration of Eq. (7) with the boundary conditions $t = 0$ to $t = t$ and $q_0 = 0$ to $q_t = q_t$, the Eq. (8), which corresponds to the integrated rate law for a second-order reaction, is obtained:

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \qquad (8)$$

Eq. (8) can be stated in the linear form as Eq. (9):

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (9)$$

where, q_e and $q_t$are the concentration of adsorbed metal ion per unit of mass biosorbent (in mmol/g) at the equilibrium and at time t (min), respectively, and k_2 (in min^{-1}) is the rate constant of the pseudo-second order equation. The plot of t/q_t versus t should give a straight line if second-order kinetics are applicable, and q_e and k_2 can be determined from the slope and intercept of the plot, respectively. It is important to notice that for the application of this model the experimental estimation of q_e is not necessary.[15] The pseudo-second order equation can predicts the behavior over the whole range of time studied, and is in agreement with chemical sorption as the rate controlling step.

2.5.3 Weber-Morris intraparticle diffusion model

The overall rates of metal biosorption from aqueous solutions onto porous biosorbent surface have been frequently evaluated by biosorption kinetic models alone such as the pseudo-first and pseudo-second order models. However, the pseudo-first order and pseudo-second order rate equations cannot identify the diffusion mechanisms during the biosorption process, so the intraparticle diffusion equation as described by Weber and Morris could be applied in the determination of the intraparticle diffusion

rate constant, k_3, and the boundary resistance, k_d.[16, 17] Theoretical treatments of intraparticle diffusion yield rather complex mathematical relationships which differ in form as functions of the geometry of the biosorbent particles.[18] A functional relationship which is common in most solid-liquid treatments for the intraparticle diffusion in such systems, is that uptake (q_t) varies almost proportionately with the half-power of time, $t^{1/2}$, rather than with t; and a nearly linear variation in the adsorbed quantity with $t^{1/2}$ is predicted for large initial fraction of reactions controlled by intraparticle diffusion rates.[19] This model is expressed by the following equation (10):

$$q_t = k_3 t^{1/2} + k_d \qquad (10)$$

where k_3 is the intraparticle diffusion rate constant (mmol/(g min$^{1/2}$)), k_d is the intercept that gives an idea about the thickness of the boundary layer, and q_t is the concentration of adsorbed metal ion per unit of mass biosorbent (in mmol/g) at time t (min). According to Weber and Morris intraparticle diffusion model, if the rate-limiting step is the intraparticle diffusion, a plot of solute adsorbed (q_t) against the square root of the contact time (t) should yield a straight line passing through the origin (when using the corresponding experimental data).

2.6 Biosorption isotherm models

A biosorption isotherm is an expression that shows the relationship between the amount of biosorbate adsorbed per unit weight of biosorbent (q_e, mmol/g) and the concentration of biosorbate in the bulk solution (C_e, mmol/L) at a given temperature and under equilibrium conditions.[20] Biosorption equilibrium is established when the amount of biosorbate being adsorbed is equal to the amount being desorbed from the biosorbent. At this stage, the equilibrium concentrations in both phases are constant. The equilibrium biosorption isotherms are very useful in giving information on biosorption mechanisms, surface properties and affinity of an biosorbent towards some biosorbates, such as heavy metal ions.[21]

In order to investigate the biosorption isotherm of the heavy metals on to the

keratin biomaterials, two most common isotherm models are analyzed. These included Freundlich and Langmuir isotherms.

2.6.1 Freundlich isotherm model

Freundlich isotherm gives the relationship between equilibrium liquid and solid phase capacity based on the multilayer adsorption on heterogeneous surface. This isotherm is derived from the assumption that the adsorption sites are distributed exponentially with respect to the heat of adsorption. It also assumes that the stronger adsorption sites are occupied first and the binding strength decreases with the increasing degree of site occupation. The Freundlich model[22] is given by:

$$q_e = k_F C_e^{1/n} \qquad (11)$$

The equation is conveniently used in the linear form by taking the logarithm of both sides as:

$$\log q_e = \log k_F + \frac{1}{n} \log C_e \qquad (12)$$

where C_e is the equilibrium concentration data of the metal ion in the residual solution (in mol/L), q_e is the equilibrium concentration of the adsorbed metal ion per unit of mass of sorbent (in mol/g), k_F represents the adsorption capacity and n is related to the adsorption intensity (unit less). If the value of n is greater than unity, this is an indication of a favorable adsorption.[23] A plot of logq_e versus logC_e will give a straight line of slope $1/n$ and intercept k_F, when the liquid-solid system follows this model.[20]

2.6.2 Langmuir isotherm model

Langmuir isotherm model is probably the most widely applied in many adsorption studies.[24] Langmuir isotherm assumes that the enthalpy of the adsorption process is independent of the amount of sorbate, while Freundlich isotherm can be derived assuming a logarithmic decrease in the enthalpy of adsorption with the increase in the fraction of occupied sites. A basic assumption of the Langmuir theory is that (i) the solid surface presents a finite number of identical sites which are energetically uniform; (ii) there are no interactions between sorbate species, meaning that the amount of adsorbate

has no influence on the rate of its adsorption; and (iii) a monolayer is formed when the solid surface reaches saturation. Therefore, the model applies to adsorption on completely homogenous surface with negligible interaction between adsorbed molecules. It was used successfully in many monolayer adsorption processes, and it also assumes no transmigration of adsorbate in the plane of adsorbent surface.[25] It is given by Eq. (13).

$$q_e = \frac{Q_0 b C_e}{1 + b C_e} \tag{13}$$

where Q_0 (mol/g) is the saturation concentration of the adsorbed metal ion per unit of mass of adsorbent to form a complete monolayer on the surface bound at high C_e, and b is the ratio of adsorption/desorption rates (in L/mol). Q_0 represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in cases where the sorbent did not reach its full saturation in real experiments.[11] Furthermore, $K_L = Q_0 b$, where K_L is a factor related to the heat of adsorption and the affinity to the binding site. The Langmuir equation can be described by its linearized form:

$$\frac{C_e}{q_e} = \frac{b}{K_L} C_e + \frac{1}{K_L} \tag{14}$$

So, a plot of C_e/q_e versus C_e gives a straight line with the slope equal to b/K_L and the intercept equal to $1/K_L$. Langmuir model suggests monolayer coverage of metal ions on the adsorbent surface. It incorporates two easily interpretable constant: q_{max}, which corresponds to the highest possible adsorption capacity, and coefficient b, which is related to the affinity between the adsorbent and the adsorbate. Low values of b are reflected in the steep initial slope of an adsorption isotherm, indicating a desirable high affinity. Thus, for evaluating 'good' adsorbents in general, one is looking for a high q_{max} and a low steep initial sorption isotherm slope (low b).[26]

2.6.3 Thermodynamic parameter

In order to determine the thermodynamic feasibility on the thermal effects of the

sorption, Gibb's free energy (ΔG^0) was calculated. The ΔG^0 is the fundamental criterion to determine if a process occurs spontaneously. For a given temperature, a phenomenon is considered to be spontaneous if the ΔG^0 has a negative value.[27] According to the estimated Langmuir sorption/desorption affinity coefficient *b*, the standard Gibb's free energy (ΔG^0) of the biosorption process can be evaluated by using following Eq. (15)

$$\Delta G^0 = -RT \ln b \qquad (15)$$

where *b* is the Langmuir equilibrium affinity coefficient shown in equation (14), *R* is the universal gas constant (8.314 J/mol K) and *T* is the absolute temperature (295 K).[28] Generally, the change in free energy for physisorption processes is between -20 and 0 kJ/mol, but for chemisorptions processes is in the range of -80 to -400 kJ/mol.[29]

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Results and discussion

3 Results and discussion

3.1 Biosorption of heavy metals by human hair

The current section describes the biosorption of multiple heavy metals from aqueous solutions by using human hair. First of all, the removal of heavy metals from aqueous solution by using different kinds of human hair samples was investigated. Based on the preliminary results of their biosorption efficiency, two biosorbent samples including natural hair (**No. 21**, 27-year old woman, marked as **H1**) and bleached-dyed hair (**No. 32**, 45-year old woman, marked as **H2**) were selected to do further study. So, a systematic comparison of biosorption performance, based on the biosorption capacities of the two human hair samples (**H1** and **H2**) for removing Cr(III), Ni(II), Co(II), Mn(II), Cu(II), Zn(II), Cd(II) and Pb(II) ions under different conditions, was also checked. The parameters influencing the metal uptake including the contact time, the pH of aqueous solution and the initial metal concentration were analyzed. FT-IR and SEM analysis were followed to better characterize the different behavior of such hair samples. The biosorption kinetics of heavy metals onto the human hair was modeled by using the pseudo-first order, pseudo-second order kinetic models and the Weber-Morris intraparticle diffusion model. The equilibrium data of the Pb(II) biosorption has been also correlated with both Freundlich and Langmuir isotherm models. In addition, metal ions adsorbed by the human hair samples could be eluted with EDTA and HNO_3 solution. The biosption capacities of regenerated hair samples are examined.

3.1.1 Comparison biosorption efficiency and FT-IR of different human hairs

The removal of metal ions Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) from multiple-metal aqueous solutions by using thirty-two different kinds of human hair samples as biosorbents was investigated. The selected thirty-two human hair

samples (from different individuals) were labeled by numbers, which is shown in Table 3.1. Thirty-two different human hair samples are labeled from **No. 1** to **No. 32**. Figure 3.1 shows the biosorption percentage of the eight metal ions onto the human hair samples. The different hair samples showed different biosorption capacities for the different metal ions. In general, the biosorption capacity of metal ions by using female's hair is greater than the male's hair. Human hair can effectively bind a number of heavy metals, with different binding affinity of the biosorbents for each metal ions studied, following in order of decreasing affinity: Pb(II) ≈ Cr(III) ≈ Cu(II) > Cd(II) ≈ Zn(II) > Ni(II) > Co(II) > Mn(II). Those differences could be explained by the type and concentration of the surface functional groups responsible for the interaction with the metal ions. So the different hair samples here, used as heavy metal biosorbents, with corresponding binding sites, have different affinity for each metal ion.

Table 3.1. Information of the origin of all hair samples, and their labeled numbers.

Hair No.	The information of hair samples	Hair No.	The information of hair samples
1	Male (3-year old)	17	Female (45-year old)
2	Male (27-year old)	18	Female (9-year old)
3	Female (12-year old)	19	Female (15-year old)
4	Male (11-year old)	20	Female (26-year old)
5	Male (44-year old,)	21	Female (27-year old)
6	Male (27-year old)	22	Female (35-year old)
7	Male (6-year old)	23	Male (33-year old)
8	Male (12-year old)	24	Female (10-year old)
9	Male (50-year old)	25	Female (40-year old)
10	Male (27-year old)	26	Female (28-year old)
11	Male (44-year old)	27	Female (17-year old)
12	Male (35-year old)	28	Female (38-year old)
13	Male (30-year old)	29	Female (13-year old)
14	Male (47-year old)	30	Female (60-year old)
15	Male (6-year old)	31	Female (40-year old, bleached-dyed hair)
16	Female (40-year old, dyed partially)	32	Female (45-year old, bleached-dyed hair)

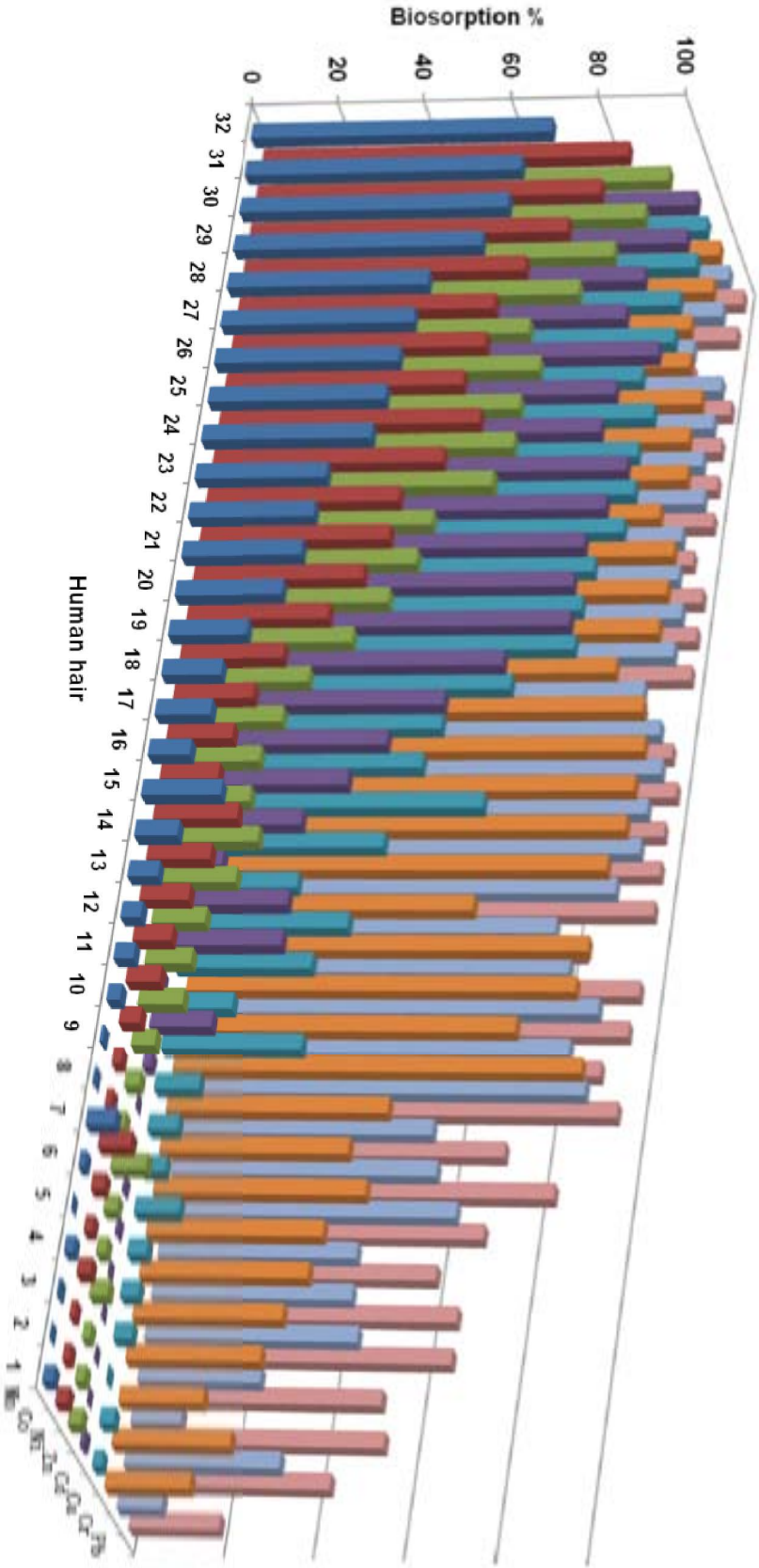

Figure 3.1. Biosorption percentage of the different kinds of human hair samples for Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) in multiple-metal system. The initial metal concentration is 0.1mmol/L, the initial aqueous pH is 4.0, the amount of biosorbent is 0.1 g in 10 mL of metal solution. Experimental errors found were less than 5% in biosorption percentage.

Fourier transform infrared (FT-IR) spectroscopy is a powerful technique that can provide rapid and specific chemical information at the molecular level.[1] According to the order of biosorption capacity, four representative hair samples including **No.1** (in native, 3-year old boy), **No.13** (in native, 30-year old man), **No.21** (in native, 27-year old woman) and **No.32** (bleached-dyed, 45-year old woman) were selected to be characterized by the FT-IR. FT-IR spectra of the four human hair samples are shown in Figure 3.2. The peak located at 3275 cm^{-1} is a mode arising from N-H stretching, which is sensitive to hydrogen bonding and it is essential in the structuring of water and holding protein together. The two weak bands located at 2960 cm^{-1} and 2920 cm^{-1}could be assigned as modes arising from amino acid bands of $-CH_3$ and $-CH_2-$ asymmetric and symmetric modes, respectively. The peaks located at 1632 cm^{-1} (Amide I), 1520 cm^{-1} (Amide II) and 1241 cm^{-1} (Amide III) are related to typical keratin fiber amino acids. The weak broad shoulder bands in the 1180-1030 cm^{-1} region are related to the cystine and its oxidation products which includes cysteic acid, cystine monoxide, and cystine dioxide as well as sulfonates.[2] Furthermore, it is worth noting that there are some differences in this region. The small peaks observed at 1040 cm^{-1} and 1175 cm^{-1} are probably related to the oxidation products of cystine, which are gradually formed eventually in the human hair which biosorption capacity increases. The human hair (**No. 1**) having the worst biosorption capacity is from the male of 3 years old, whose hair is really natural and the extent of oxidation is the lowest one, as expected (see Figure 3.2 (b)). The human hair (**No. 32**) having the best biosorption capacity is from the female of 45 years old, whose hair is bleached and dyed (submitted to an external and forced oxidation process), and the extent of oxidation is the highest one (see Figure 3.2 (b)). The other two human hair samples characterized by FT-IR (**No. 13** and **No. 21**) showed relatively weak intensity for the corresponding oxidation peaks. Most likely, this oxidation of human hair in native is due to environmental factors such as sunlight (exposure to ozone), chlorinated water and frequent shampooing causing partial natural oxidation of the hair surface.[3] In general, the hair of female suffering from these factors

is more frequent than male that lead to them oxidation easily (the use of more specific products for cleaning and softening the female hair can be the main reason for that behavior). This corresponds with the experimental results found that the biosorption capacity of female's hair is greater than male's hair. The FT-IR analysis indicates that there are potential metal binding functional groups (including hydroxyl, amino, carboxyl and sulfur-containing) on the four biosorbents characterized by FT-IR, and the oxidation products of cystine can enhance the biosorption capacity of the human hair.

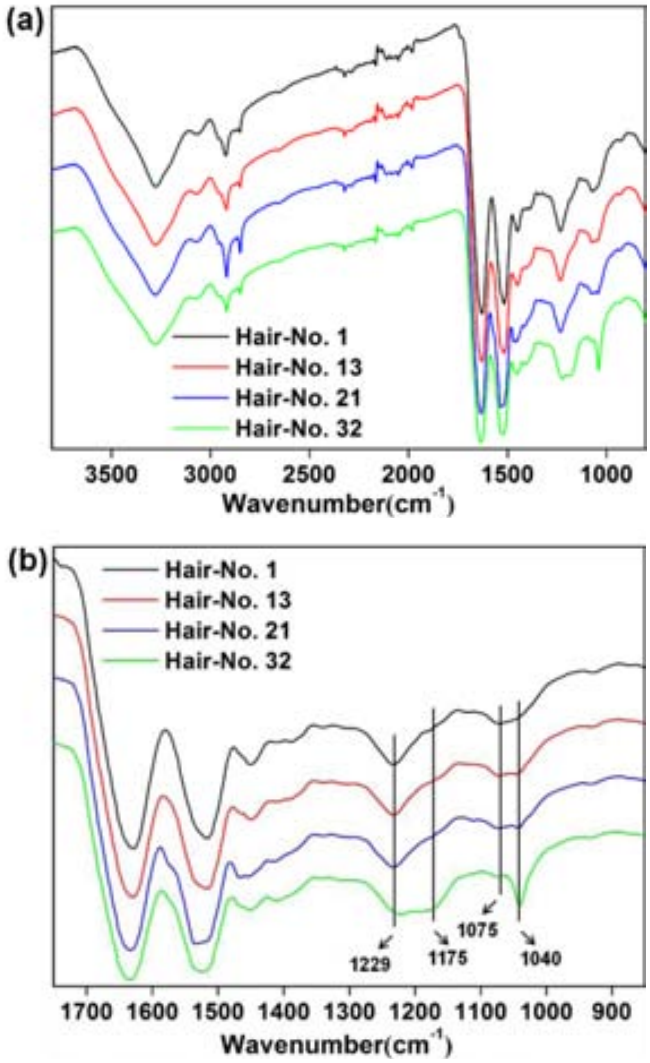

Figure 3. 2. FT-IR spectra of the four human hairs: (a) full-scan spectra, and (b) partial-scan spectra of human hairs (partial-scan spectra correspondto the cystine acid bands region).

According to the above studies, the untreated hair from human hair sample **No. 21** (in native, 27-year old woman, marked as **H1**) and the treated hair from human hair

sample **No. 32** (bleached-dyed, 45-year old woman, marked as **H2**) were selected to study their behavior on the heavy metal biosorption by comparison. Different experimental parameters were checked, as the initial aqueous pH, contact time and initial metal ion concentration. The biomaterials have been characterized prior and after their use as biosorbents by FT-IR and SEM, and experimental data are adjusted to kinetic models and isotherms.

3.1.2 Effect of the initial aqueous solution pH

The pH of the heavy metal aqueous solution is one of the most important chemical parameter affecting biosorption process.[4] The pH of solution has both influences on the metal aqueous speciation and on the chemical functional groups of the biosorbent surface speciation. The effect of the initial pH on the biosorption capacity was studied in the range of pH 1.0 to 6.0 at an initial metal concentration in solution of 0.18 mmol/L (for each metal ion). As shown in Figure 3.3, in both hair systems the metal uptake is strongly dependent on the pH value of the aqueous solutions. At an initial pH value lower than 3.0, little biosorption occurs for **H1** and **H2**, especially at pH 1.0 almost no biosorption is observed. A sharply increase in the biosorption capacity take place in the pH range between 3.0 and 5.0 for all heavy metal ions. Above pH 5.0, biosorption of metal ions is found to be relatively constant. The low biosorption capacity at pH values below than 3.0 is attributed to the high concentration of H^+ ions. In this situation, the concentration of H^+ ions far exceeds the metal ions concentration in the solution and hence H^+ compete with them for reaching the hair surface binding sites, caused by repulsive forces between protons and metal ions.[5] With decreasing the acidity of the solution, so increasing the pH value, those functional groups on the hair surface become deprotonated, resulting in an increase of the negative charge density and consequently facilitating the binding of heavy metal ions (together with a less competence with the less amount of H^+). So, the reduction of protons can efficiently decrease the competition between metal ions and H^+ ions on the biosorbent surface and increase the biosorption metal capacity of the hair.

Although biosorption process for **H1** and **H2** show similar trend with the pH, there are some clear differences. For the removal of Pb(II), Cr(III) and Cu(II), **H2** is more sensitive to the pH values than **H1**. On the other hand, the biosorption capacity of **H1** to the rest of metal ions under study is low even at pH around 5.0 and 6.0. In contrast, the bleached and dyed hair (**H2**) shows a better biosorption capacity compared with **H1** for all the metal ions at pH 5.0 and 6.0 probably due to the presence of new active sulfonate groups (which at those pH are deprotonated, so having higher metal binding affinity). Also, probably the increase of the surface area produced during the treatment applied to **H2** increases the metal biosorption efficiency in such conditions. This behavior can be explained since the sulfonate groups created on **H2** surface (after bleaching and dyeing process) makes it more sensitive to pH change.

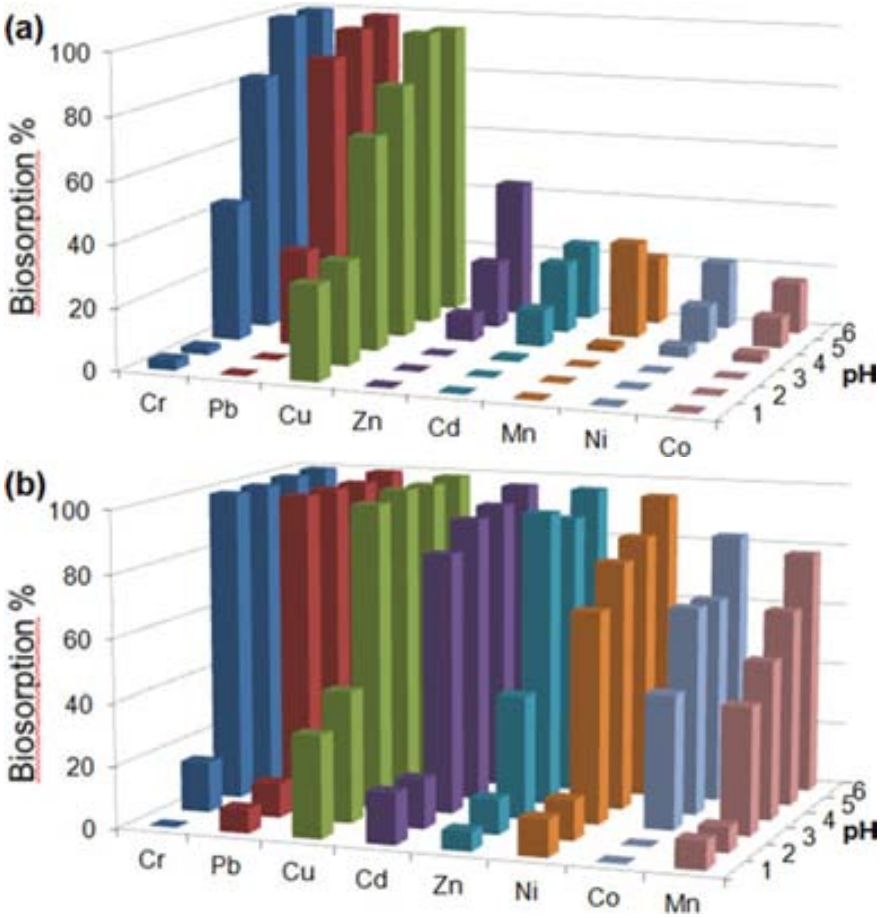

Figure 3.3. Effect of the initial pH on the biosorption of **H1** (a) and **H2** (b) for Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) in multiple-metal system. The initial concentration is 0.18 mmol/L, the contact time is 24 h, the biosorbent is 0,1 g. Experimental errors found were less than 2% in biosorption percentage.

3.1.3 Effect of the contact time

The contact time is one of the important parameters to be controlled for successful usage of biosorbents in practice. Figure 3.4 shows the biosorption percentage of the eight metal ions (Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II)) onto **H1** and **H2** at different contact time (5, 10, 20, 30, and 45 minutes, and 1, 2, 3, 4, 6, 12, 24, 48 and 72 hours). From the results shown in Figure 3.4, it is clear that the two different hair samples (**H1** and **H2**) show different biosorption capacities for the different metal ions under study. In general, **H2** reaches the equilibrium in less than 1 h being better biosorption efficacy than **H1**. For **H1**, even after 48 h of contact time between the hair and the heavy metal ion solutions, the system did not reach a maximum uptake value, so no equilibrium state for most of the heavy metals studiedis reached for **H1**, even at 48 h. For **H1**, only Cu(II), Cr(III) and Pb(II) have high biosorption percentages over this time, around 90%. Percentages of biosorption of Cr(III), Cu(II) and Pb(II) by using **H2** sample reached over 95% in less than 30 min. This may be caused by the higher surface area and the more abundant and available functional groups (especially sulfonate ones) with **H2**. Both **H1** and **H2** show better biosorption capacity to Cr(III), Cu(II) and Pb(II) compared with the rest of metal ions in aqueous solution which have an uptake capacity that decreases as follow: Cd(II) > Zn(II) > Ni(II) > Co(II) > Mn(II), and getting always higher biosorption percentages when using **H2** as biosorbent. This different behavior is probably due to the stronger interactions between Cr(III), Cu(II) and Pb(II) ions and the function groups on the hair samples (especially for **H2** human hair sample, the biosorption percentages of metal ions are higher than 90% at the equilibrium). Given that, the biosorption capacities of the Cd(II), Zn(II), Ni(II), Co(II) and Mn(II) ions onto **H1** are very low (the percentage of biosorption lower than 40% even for longer contact time i.e. 72 h). So, the kinetic analysis is not considered for the sorption of these metal ions onto **H1** sample. In contrast, the percentage of biosorption, even for short contact times, is significant for all metal ions onto **H2** sample (Cd(II) (90%), Zn(II) (92%), Ni(II) (75%), Co(II) (63%) and

Mn(II) (49%)), and for this reason all the set of eight heavy metals were considered for modeling the kinetics of biosorption onto **H2**. In brief, it is worth to note that **H2** sample is a better biosorbent relative to **H1**.

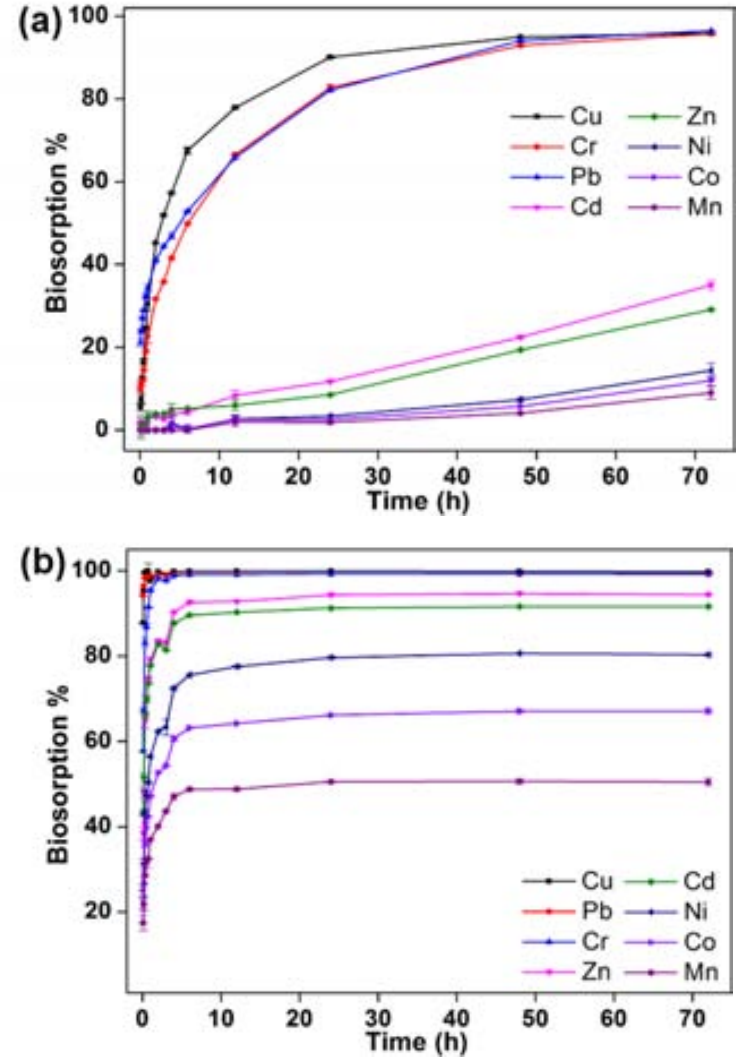

Figure 3.4. The biosorption percentages of **H1** (a) and **H2** (b) for Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) in multiple-metal system at different contact time. The initial concentration is 0.18 mmol/L of each metal ion, the pH is 4.0, and the biosorbent is 0.1 g.

3.1.4 Effect of the initial metal concentration

The initial metal concentration provides an important driving force to overcome all mass transfer resistance of metal ions between the aqueous and solid phase.[6] In this sense, the effect of the initial metal concentration on the biosorption was evaluated in the range from 0.5×10^{-3} to 0.5 mmol/L. Corresponding results are shown in Figure 3.5 and 3.6. It is found that the equilibrium biosorption capacity of **H1** and **H2** increased

with increasing the initial metal concentration due to an increase of the mass-transfer driving force since increasing the initial metal concentration (Figure 3.5).[7] It can also be observed that the saturated biosorption capacities of **H1** and **H2** were reached at 0.05 mmol/L and 0.2 mmol/L, respectively, for the metal ions including Mn(II), Co(II), Ni(II), Zn(II) and Cd(II). However, the equilibrium biosorption capacities of **H1** and **H2** continuously increased with increasing the initial metal concentration for Cr(III), Cu(II) and Pb(II), which is attributed that the functional groups of the hair samples have greater affinity for the three metal ions (Cr(III), Cu(II) and Pb(II)) than the rest of metal ions in the multiple-metal system.[8]

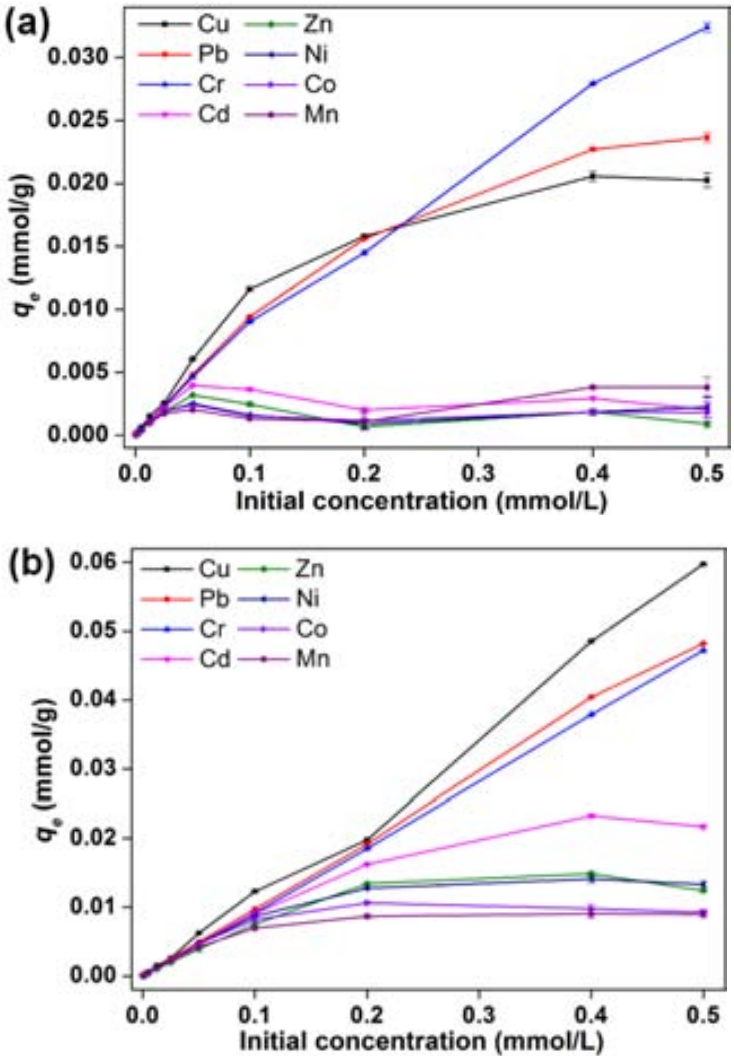

Figure 3.5. The equilibrium biosorption capacities (q_e) of **H1** (a) and **H2** (b) for Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) in multiple-metal system at different initial metal concentration. The contact time is 24 h, the pH is 4.0, and the biosorbent is 0.1 g.

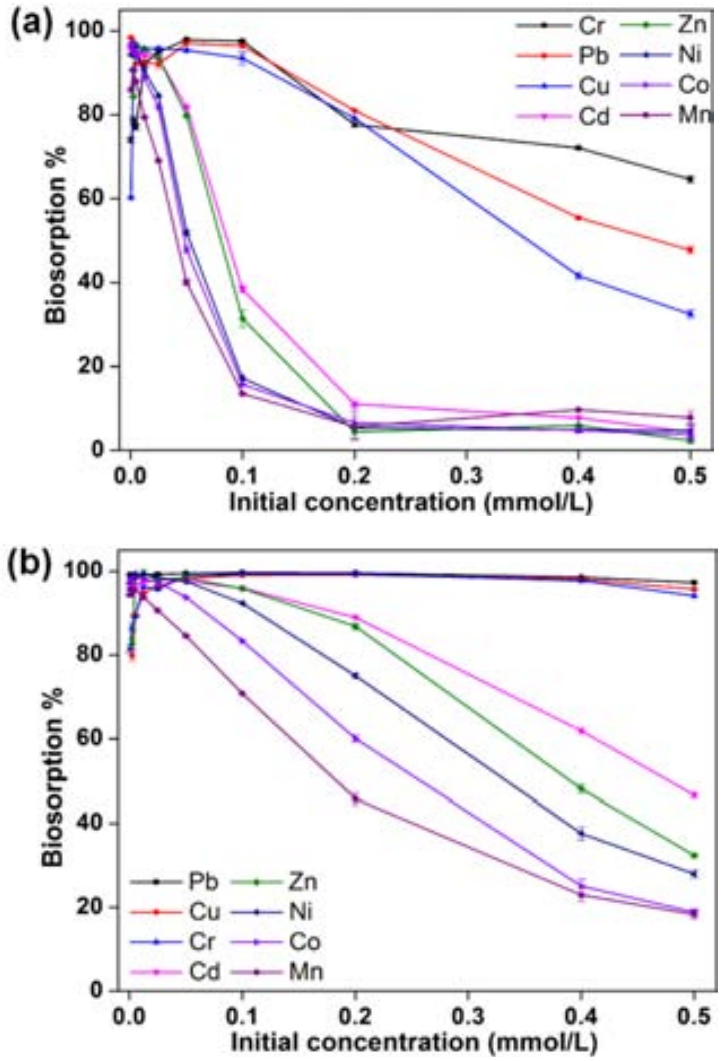

Figure 3.6. The equilibrium biosorption percentages of **H1** (a) and **H2** (b) for Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) in multiple-metal system at different initial metal concentration. The contact time is 24 h, the pH is 4.0, and the biosorbent is 0.1 g.

The equilibrium biosorption of **H1** and **H2** was relatively effective for all metal ions at low metal concentration due to the sufficient metal binding sites on the biosorbent that lead to more opportunities for their metal uptake (Figure 3.6).[9] A steeper slope of the curve was observed at high concentration, the biosorption percentages of **H1** and **H2** for metal ions start to decrease, which indicates that the availability of the biosorption sites are insufficient to remove metal ions at relatively high metal concentrations. However, the biosorption percentages of the both hair samples for Pb(II), Cr(III) and Cu(II) are still much higher at high metal concentrations than the rest of

metal ions in multiple-metal system, especially for the biosorption percentage of **H2**, a slight decline is seen at 0.5 mmol/L. Thus, **H2** shows better biosorption capacity in a wider range of initial metal concentrations than **H1**, likewise, probably due to more active metal binding sites available on **H2** compared with **H1**. However, both hair samples are effective for the removal of metals at low metal concentrations.[10]

3.1.5 FTIR and SEM characterization

In order to elucidate the functional groups of the biosorbent surface which could be responsible for the metal binding in the biosorption process, the FT-IR analysis was carried out to indentify them.[11] In this time, just two different hair samples (**H1** and **H2**, as indicated preciously) before and after biosorption were characterized by FT-IR. As it is shown in Figure 3.7 and Table 3.2, infrared absorption bands at 1040, 1075, 1175, and 1229 cm^{-1} all correspond to different products of cystine oxidation. The other peaks at 1241, 1454, 1518, and 1637 cm^{-1} are related to chemical groups of the typical amino acid constituents of human hair. It is seen that there are no significant differences with other human hair reported previously in the literature.[12] Comparing **H1** and **H2**, before and after heavy metal biosorption process, there are some differences in the range of 1000 to 1300 cm^{-1} (as can be seen in Figure 3.7 (b)). For **H2** sample, the intensity of the peaks at 1041 and 1175 $cm^{-1}$are higher, as well as the profile became sharper compared with **H1** sample, probably due to the greater presence of sulfonate ($R\text{-}SO_2O^-$) in **H2** sample as a result of the oxidation reactions produced during bleaching and dyeing treatments. This process corresponds to the oxidation reaction which is shown in Figure 3.8.[13] The presence of anionic functional groups in the surface of the biosorbent is an important factor worth considering since biosorption of metals in their cationic form can be promoted, so increasing the biosorption capacity of this biomaterial to adsorb metal ions from waters, as reported previously.

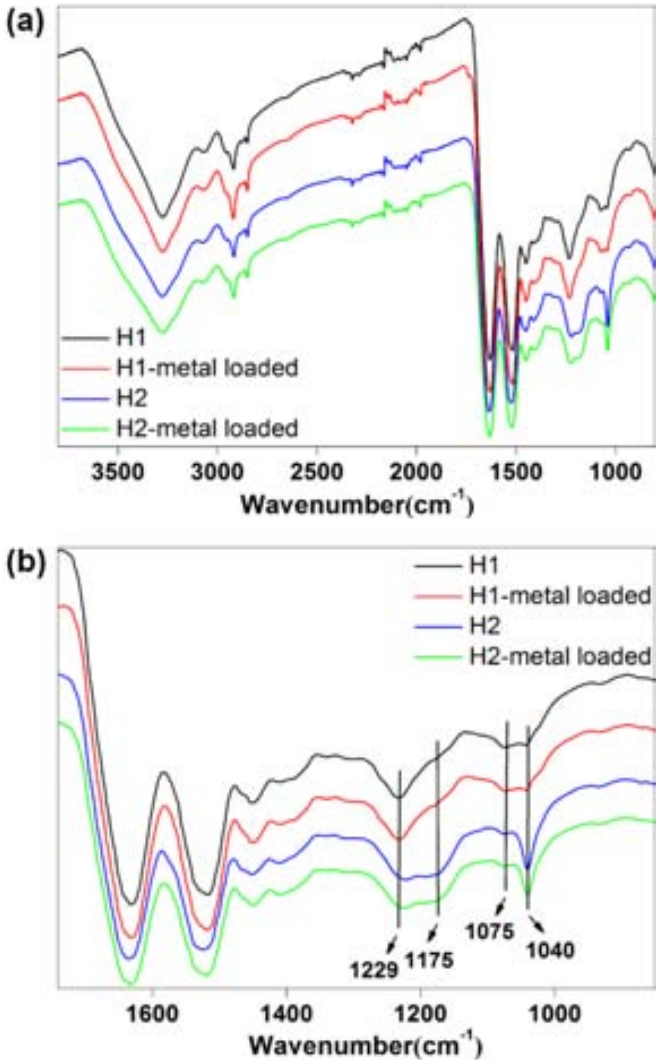

Figure 3.7. FT-IR spectra of human hair samples **H1** and **H2**, before and after their use: (a) full-scan spectra, and (b) partial-scan spectra of human hair samples (partial-scan spectra correspond to the cystine acid bands region).

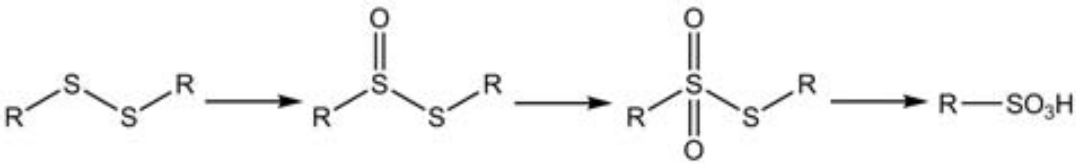

Figure 3.8. Scheme of the S-S cleavage mechanism for the bleaching and dyeing process.[13]

Table 3.2. Human hair FT-IR spectral bands and assignments.

Assignments	Wavenumbers(cm^{-1})				
		H1		**H2**	
	literature	Before biosorption	After biosorption	Before biosorption	After biosorption
NH stretching	3295	3275	3275	3276	3280
C-H stretching	2958	2957	2957	2961	2961
	2927	2920	2920	2920	2920
Amide I,80% C=O stretchand small contribution from NH bend	1637	1633	1634	1632	1636
Amide II, 60% C-N stretch plus 40% N-H in plane bend	1518	1517	1518	1524	1519
CH_2 and CH_3 bending modes	1454	1451	1450	1451	1452
Amide III (N-H stretch)	1241	1233	1232(sh)	-	-
Cystine dioxide ($R-SO_2-S-R$)	1229	1233(s)	1232(s)	1222(w)	1225(w)
Sulfonate, S-O asym. stretch	1175	1175(sh)	1175(sh)	1175(m)	1175(m)
Cystine monoxide (R-SO-S-R)	1075	1074(w)	1073(w)	1075(sh)	1074(sh)
Sulfonate, S-O sym. stretch	1040	1044(w)	1043(w)	1041(s)	1041(s)

Apart from chemical structure, the morphology of a biomass also plays an important role in the biosorption process.[14] In fact, the morphology of human hair can be easily changed or damaged by internal or external factors. So, SEM micrographs were obtained from **H1** and **H2,** before and after the metal biosorption process (Figure 3.9). As it can be seen, the surface topography of **H1** and **H2** is slightly different (Figure 3.9 (a) and (c), respectively). The bleached and dyed hair **H2**, showed more "jagged" or "chipped" appearance, probably due to those treatments (corresponding to the bleaching and dyeing processes). The rougher surface of **H2** results in an increase of the surface area, which acts by promoting the biosorption of metal ions, and supports the higher biosorption capacity obtained for **H2** compared with **H1** (Figure 3.6). Furthermore, the

biosorption of metal ions onto the human hair did not change significantly the morphology of **H1** and **H2** as it can be seen by comparison of Figures 3.9 (a) and (b) (for **H1**), and 3.9 (c) and (d) (for **H2**).

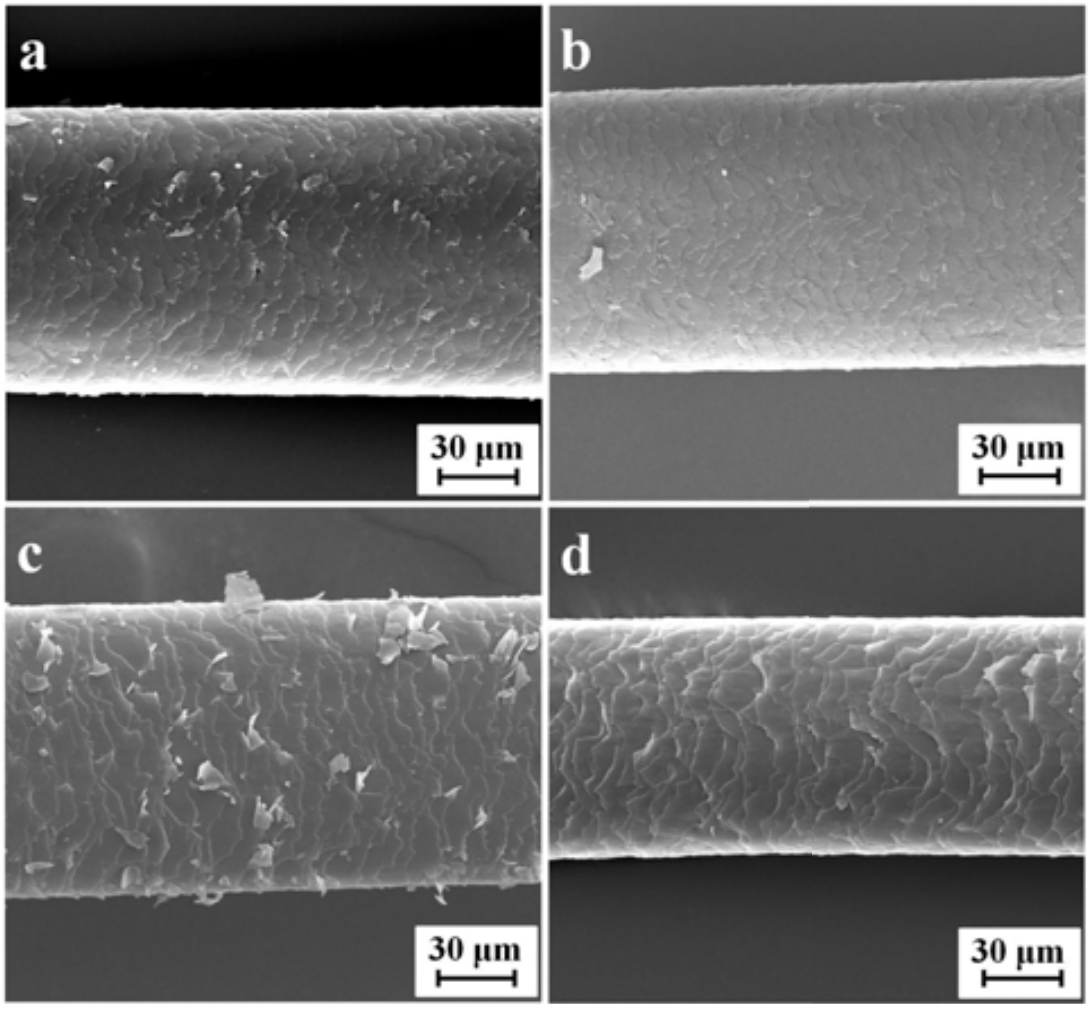

Figure 3.9. Scanning electron microscopy (SEM) micrographs of the human hair samples (**H1** and **H2**). a: **H1** before biosorption; b: **H1** after biosorption; c: **H2** before biosorption; d: **H2** after biosorption.

FTIR and SEM characterization results showed that **H2** sample has a higher surface area as well as more quantity of active sulfonate groups. This can explain the better biosorption efficacy of **H2** for metal ions relative to **H1**, as expected. The fact that no morphological differences are found before and after the biosorption process pointed out that these samples could be reusable, so it can be a practical biosorbent system for further real applications.

3.1.6 Biosorption kinetics modeling

Biosorption kinetics depends on the biosorbate (metal ions) and biosorbent (human hair) interactions and also on the operating conditions. In order to investigate the potential rate-controlling steps of such biosystem, which include mass transport and physical-chemical interactions, kinetic models have been exploited to fit the experimental data and to describe the biosorption behavior.[15] In this sense, the pseudo-first and pseudo-second order kinetic models, and the Weber-Morris intraparticle diffusion model are tested.

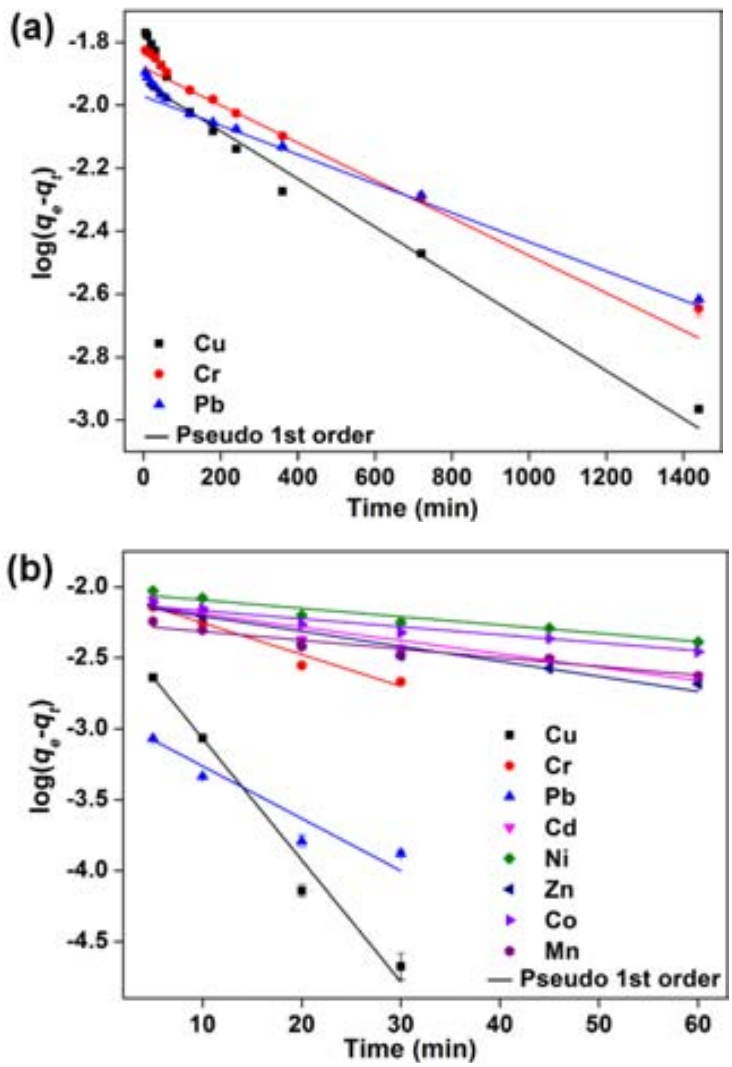

Figure 3.10. Pseudo-first order sorption kinetics of metal ions on the hair samples at different contact time. (a) **H1** and (b) **H2**.

The plots of log(q_e-q_t) vs. t for the pseudo-first order model (see 2.5.1 Pseudo-first

order rate equation Eq. (6)) are shown in Figure 3.10. The rate constants (k_1), the R^2 and the q_e values are given in Table 3.3. Generally, the pseudo-first order equation does not fit well for the whole range of contact time, only it is applicable on the initial time of the sorption process.[16] So, the initial rapid step, where biosorption was fast, has been chosen and the experimental data of this step were fitted by the pseudo-first order model. The range time was different depending on the hair. For **H1,** the range time was less than 24 h when determining Cu(II), Cr(III) and Pb(II) (the rest of the metals were not checked with **H1**, as indicated previously). For **H2,** the range time was less than 30 min for Cu(II), Cr(III) and Pb(II), and less than 1 h for the rest of the metals (Cd(II), Ni(II), Zn(II), Co(II) and Mn(II)). For both hair systems, correlation coefficients are found to be above 0.87, but the calculated q_e is not equal to experimental q_e, suggesting the insufficiency of the pseudo-first order model to fit the experimental data for the initial period of time examined in each case, and underestimate the experimental q_e values. A reason for these differences in the q_e values is that there is a time lag, possibly due to the boundary layer on the surface of the biomaterial or to the external resistance at the surface controlling the beginning of the sorption process.[17]

Table 3.3. Metal biosorption kinetic constants of the Pseudo-first and Pseudo-second models.

Metal	Hair	Pseudo-first order			Pseudo-second order		
		$k_1\times10^3$ (min^{-1})	$q_e\times10^2$(mmol/g)	R^2	k_2 (g/mmol min)	$q_e\times10^2$ (mmol/g)	R^2
Cu(II)	H1	1.886[a]	1.393	0.9505	0.3701	1.863	0.9996
	H2	193.2[b]	0.5400	0.9694	382.3	1.876	1.000
Cr(III)	H1	1.316[a]	1.395	0.9849	0.2578	1.732	0.9937
	H2	50.53[b]	0.8960	0.9487	20.74	1.729	1.000
Pb(II)	H1	1.110[a]	1.153	0.9840	0.3867	1.663	0.9923
	H2	76.07[b]	0.1040	0.8708	343.5	1.681	1.000
Cd(II)	H2	21.77[c]	0.7900	0.9154	5.937	1.542	0.9999
Ni(II)	H2	14.26[c]	0.9320	0.9324	2.862	1.365	0.9999
Zn(II)	H2	22.57[c]	0.7330	0.9257	6.627	1.261	0.9999
Co(II)	H2	14.13[c]	0.7870	0.9408	3.340	1.176	0.9999
Mn(II)	H2	14.80[c]	0.5640	0.9221	5.787	0.8750	0.9999

a: 24 h, b: 30 min, c: 1 h

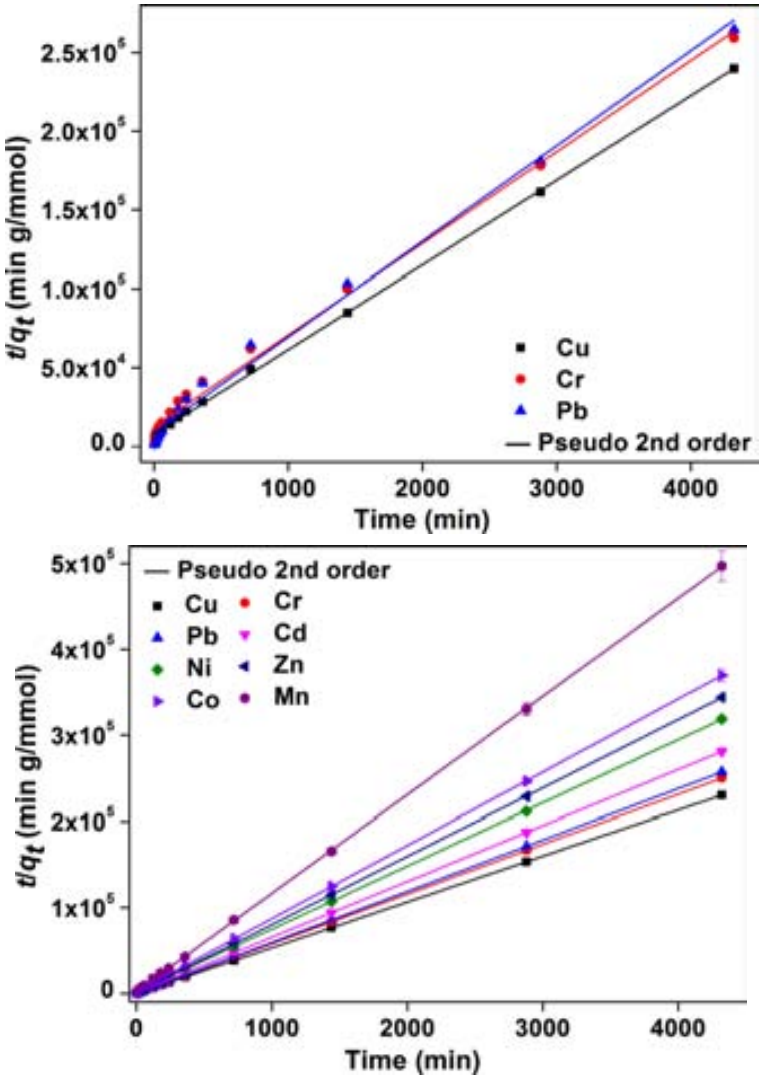

Figure 3.11. Pseudo-second order sorption kinetics of metal ions on the hair samples at different contact time: (a) **H1** and (b) **H2**.

The plots of t/q_t vs. t for the pseudo-second order model (see 2.5.2 Pseudo-second order rate equation Eq. (9)) are shown in Figure 3.11. The q_e and k_2 values are calculated from slope and intercept of this plots, respectively. Table 3.3 shows that the correlation coefficients (> 0.99) of the pseudo-second order kinetic model are greater than the pseudo-first order kinetics. Also, the calculated q_e values agree very well with the experimental data. This model provides the better prediction of the biosorption kinetics for whole range of contact time studied and the good regression curves showed in Figure 3.12 (continuous curves), for **H1** and **H2**, respectively in all periods of time. These indicate that chemical sorption due to the formation of chemical bonds between

the metal and biosorbent in a monolayer onto the surface is the rate-controlling step.[18, 19]

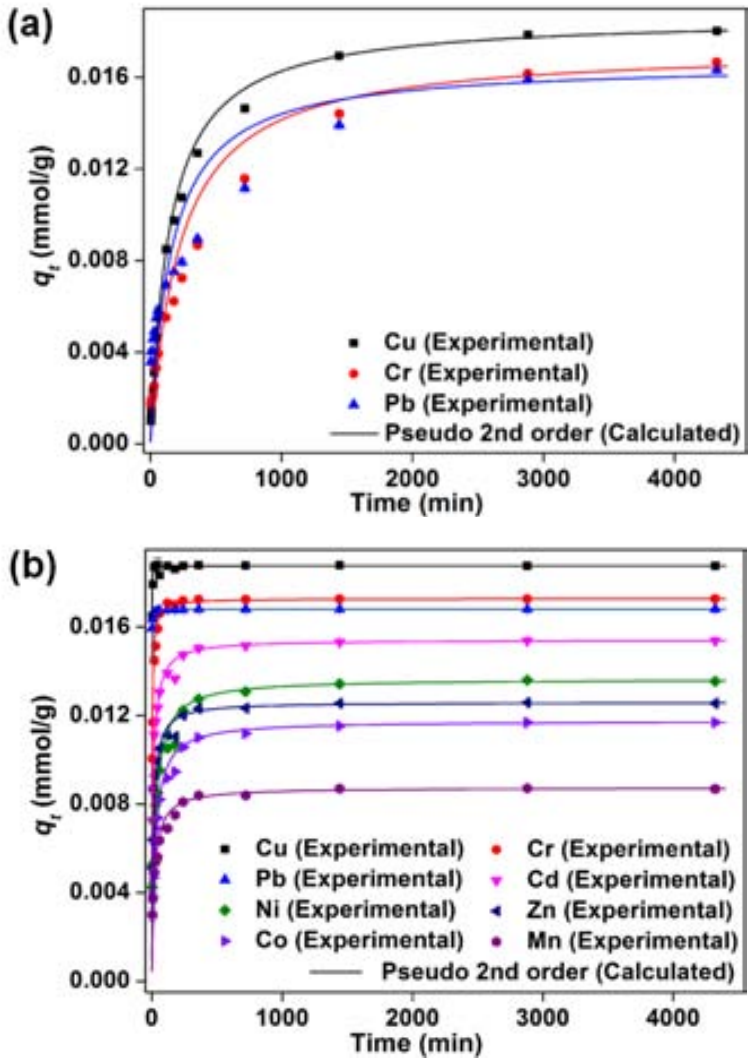

Figure 3.12. Experimental (•) and calculated (—) biosorption capacity values (q_t) adjusted by following the pseudo-second equation: (a) **H1** and (b) **H2**.

The metal ions are most likely transported from the bulk of the solution onto the solid phase through an intraparticle diffusion process, which is often the rate-limiting step in many adsorption processes. The possibility of intraparticle diffusion was explored by using Weber-Morris intraparticle diffusion model (see 2.5.3 Weber-Morris intraparticle diffusion model Eq. (10)).

According to Weber and Morris, if the rate-limiting step is intraparticle diffusion, a plot of solute adsorbed (q_t) against the square root of the contact time (t) should yield a straight line passing through the origin. Mathematical formulations representing the

diffusion and adsorption are not generally solvable analytically, except for some simple cases.[20] In this case, these plots have the same general features that can be divided into multi-linearity correlations (Figure 3.13), which indicates that three steps occur during the whole biosorption process.[21] The first linear portion can be attributed to the transport of metal species from the solution to the external surface of the biosorbent through film diffusion, or the diffusion of the metal species on the boundary layer.[22] In this step, the transport of metal species for the **H2** is faster than the **H1**, probably due to the higher surface area and more surfonate groups on the **H2** compared with **H1**. Especially for the effect of surfonate groups, which can make the biosorbent surface more hydrophilic, and accordingly enhance the transport of metal species from the solution to the external surface of **H2**. The second linear portion describes the gradual biosorption where the intraparticle diffusion is rate-limiting. The third stage was attributed to the final equilibrium where the intraparticle diffusion starts to slow down due to extremely low biosorbate concentration left in solution. Moreover, it can be observed that the larger slope is represented for higher metal concentration. Both the film diffusion and intraparticle diffusion were simultaneously operating during the biosorption process and both enhanced with the increase of initial concentration.

From the above modelization analysis, it can be concluded that film diffusion controlled the early stage of biosorption process while in the later stage, chemical interaction between biosorbate and biosorbent become the limiting mechanism. High surface area and more hydrophilic surface of **H2** can enhance the transport of metal species from the solution to the external surface, which leads to the better biosorption capacity of **H2** compared with **H1**.

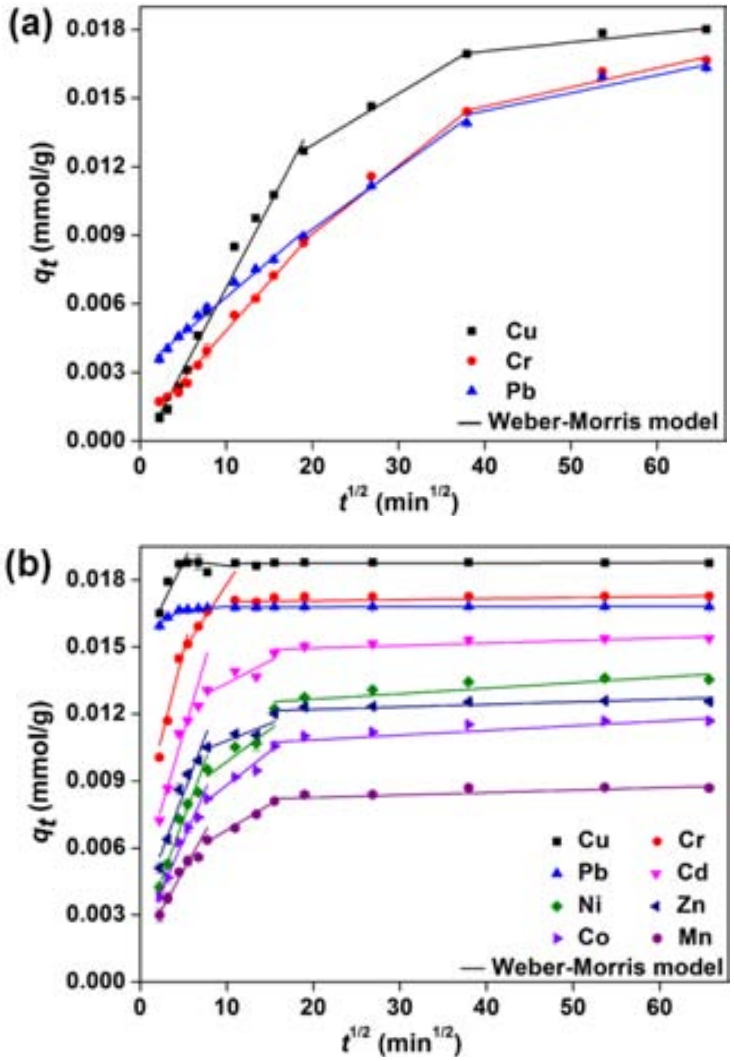

Figure 3.13. Weber-Morris intraparticle diffusion kinetic model of metal ions on hair samples at different contact time. (a) **H1** and (b) **H2**.

3.1.7 Biosorption isotherm modeling

The adsorption isotherm indicates how the adsorbed molecules distribute between the liquid and the solid phase when the adsorption process reaches an equilibrium state.[23] The removal of Pb(II) by **H1** and **H2** has been studied at different initial concentration levels to determine the biosorption isotherms and corresponding thermodynamic parameters. Freundlich[24] (see 2.6.1 Freundlich isotherm model Eq. (12)) and Langmuir[25] (see 2.6.2 Langmuir isotherm model Eq. (14)) isotherm models were selected to fit the experimental data. All the constants and correlation coefficients obtained by the models are summarized in Table 3.4. The Freundlich isotherm gives the

relationship between equilibrium liquid and solid phase capacity based on the multilayer adsorption in a heterogeneous surface. The Langmuir isotherm assumes that a maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface, that the energy of adsorption is constant, and there is no transmigration of adsorbate in the plane of the surface.[15]

Table 3.4. Freundlich and Langmuir isotherms constants for the biosorption of Pb(II) by **H1** and **H2**.

	constant	H1/Pb(II)	H2/Pb(II)
Freundlich	$K_F \times 10^3$	0.3291	420.5
	n	2.6448	1.55099
	R^2	0.66622	0.77848
Langmuir	$Q_0 \times 10^5$ (mol/g)	1.69617	23.2261
	$b \times 10^{-4}$ (L/mol)	12.2091	2.5590
	K_L (L/g)	2.07087	5.94354
	R^2	0.99918	0.99814
	$-\Delta G^0$ (kJ/mol)	28.726	24.894

On the basis of regression analysis of the experimental data on the biosorption of metal ions on **H1** and **H2**, it may be inferred that the biosorption behavior of metal ions on **H1** and **H2** is good agreement with Langmuir isotherm model with high correlation coefficient values (> 0.99). Langmuir isotherm model shows that the biosorption process rely on a specific sites sorption mechanism where biosorbate molecules occupy specific sites on the biosorbent in monolayer coverage way. In Figure 3.14 experimental data and calculated data by the Langmuir isotherm model are represented showing good correlation between them. Taking on board the Langmuir equation, the maximum biosorption capacities of **H1** and **H2** calculated for Pb(II) at 295 K were 1.7×10^{-5} and 23.2×10^{-5} mol/g, respectively.

In addition, from the estimated Langmuir adsorption constant, the standard Gibb's free energy (ΔG^0, see 2.6.3 Thermodynamic parameter Eq. (15) $\Delta G^0 = -RT\ln b$) of the biosorption process can be evaluated. The standard Gibb's free energy (ΔG^0) values of **H1** and **H2** are shown in Table 3.4. The negative ΔG^0 values indicate that the

biosorption of metal ions into human hair is thermodynamically feasible and of spontaneous nature.[26]

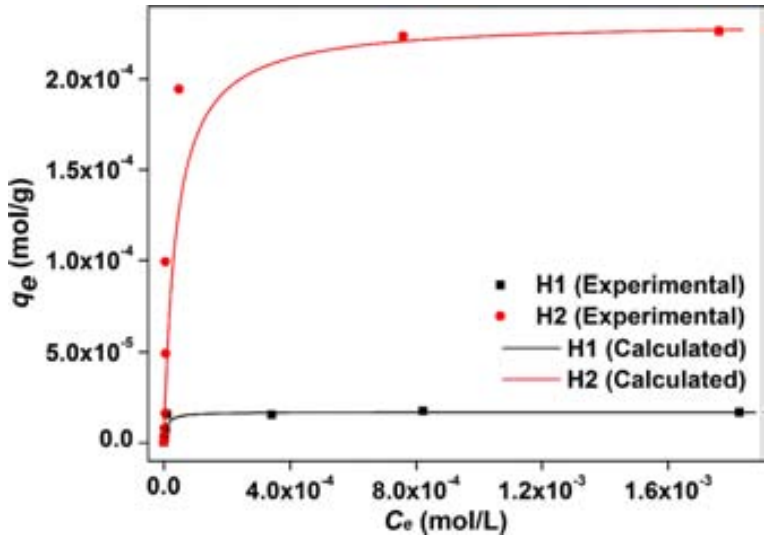

Figure 3.14. Experimental (•) and calculated (—) values adjusted by following the Langmuir isotherm model for the adsorption of Pb(II) using **H1** and **H2**.

3.1.8 Desorption, regeneration and reuse studies

Desorption of Pb(II) adsorbed onto the biomaterials (**H1** and **H2**) and the biosorption capacities of the regenerated biomaterials are investigated. The M^{2+}-H^{+} ion exchange is easily reversible, depending upon pH, which means Pb(II) can be recovered with an acid such as HNO_3.[27] EDTA solution also can be used for the recovery of Pb(II) bound to the biosorbents because of the high value of the conditional formation constant of Pb(II)-EDTA complex.[28] So, two eluents including EDTA and HNO_3 solutions are screened for their potential to desorb Pb(II) ions from metal-loaded **H1** and **H2** (Table 3.5). The solution of 0.1 M HNO_3 performed reasonably well compared with the EDTA solution at the same concentration, and the elution efficiencies of HNO_3 solution for the loaded **H1** and **H2** were 87% and 95%, respectively.

However, for **H2** (bleached and dyed hair), the elution efficiency of EDTA is better compared with **H1** (native one). The sulfonate groups formed in the bleaching and dyeing process of human hair (**H2**) could assist the transport of EDTA molecules from the solution to the external surface of it, making EDTA able to combine with Pb(II).

Therefore, the sulfonate groups on the human hair could not only promote metal ions to move from the solution to the external surface of it in the biosorption process, but also ensure the EDTA has more opportunities to combine with Pb(II) in the desorption process.

Table 3.5. The elution of adsorbed Pb(II) by using EDTA and HNO_3 solutions and the biosorption of the regenerated human hair samples (**H1** and **H2**).

Biosorbent		**H1**	**H2**
1st biosorption, Pb(II) adsorbed (%)		94 ± 1	97 ± 1
1st desorption	Pb(II) elution efficiency (%) using EDTA (0.1 mol/L)	57 ± 1	90 ± 1
	Pb(II) elution efficiency (%) using HNO_3 (0.1 mol/L)	87 ± 1	95 ± 6
2nd biosorption, Pb(II) adsorbed (%)	Regeneration by using EDTA elution	35 ± 5	88 ± 1
	Regeneration by using HNO_3 elution	0	50 ± 1
	Regeneration by using EDTA elution and rinsing with HNO_3	20 ± 1	72± 1

After the desorption process, the regenerated hair samples are washed several times with deionized water, dried in an oven at 40 ˚C, and then check their performance in the biosorption of fresh Pb(II) aqueous sample. The results collected in Table 3.5, which show that the removal percentages of the regenerated hair samples are dependent on the elution methods. When using EDTA as eluent and deionized water for rinsing the used biomaterials, the best biosorption of Pb(II) can be found in second time, especially for **H2**. Although HNO_3 is a very powerful metal eluent, it shows negative effects for the "reuse" of hair samples and result in a decrease of metal uptake capacity in second time. Probably the acidic environment after the desorption step with nitric acid leads to the ionization state of functional groups on the biomaterials surface, so becoming a competitive media for the next metal biosorption step. To confirm this concept, another set of samples are eluted by using EDTA first, rinsed with HNO_3 solution, and later washed with deionized water and also dried. The biosorption capacities of the regenerated human hair samples by this method also decrease in the second time. It is clear that the desorption of metal adsorbed onto the biomaterials by using HNO_3 can

affect their "reuse".

3.1.9 Conclusions

The biosorption of Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) from their multiple aqueous solution by different hair systems were investigated. The **H2** (bleached-dyed hair) showed better biosorption capacity than **H1** (untreated one). The biosorption capacities of the heavy metals followed the order Cu(II) > Pb(II) > Cr(III) > Zn(II) > Cd(II) > Ni(II) > Co(II) > Mn(II) for **H2**, and Cu(II) > Cr(III) > Pb(II) for **H1**, being not significant the biosorption of the rest of the heavy metal ions for **H1**. The operating parameters, such as the contact time, the pH of the aqueous solution and the initial metal concentration strongly affect the efficacy of removing the selected heavy metals under study. **H2** shows higher (>95%) and faster biosorption rates than **H1**. The optimum pH value is 4.0. The pseudo-second order kinetic model is more likely to predict kinetic behavior of biosorption for whole range of the contact time with chemical sorption being the rate-controlling step. The equilibrium biosorption is better correlated with Langmuir isotherm model compared with Freundlich model, and Langmuir suggests monolayer coverage of metal ions on the human hair. The negative standard Gibb's free energy (ΔG^0) indicates the feasibility and spontaneous nature of the biosorption process.

The better biosorption capacity of **H2** could be attributed to the higher surface area and more surfonate groups formed during bleaching and dyeing treatments relative to **H1**, which is confirmed by the FT-IR and SEM studies. The sulfonate groups of such hair also enhance the elution efficiency of EDTA. The regenerated human hair samples with EDTA exhibit good biosorption capacities of their reuse. So, the human hair is considered as a potential biosorbent for the removal of heavy metal ions. Based on the biosorption capacity of **H2** (bleached and dyed hair), the similar modification is studied in the next research work.

3.2 Biosorption of heavy metals by chemically-modified human hair

From the above part we can come to the conclusion that **H2** (oxidized hair) shows the best biosorption capacity. This conclusion can provide a reference for the possible modification of human hair. So, the human hair (in native) is treated with an oxidation method and its performance in the removal of metal ions from aqueous solution is evaluated, which is presented in this part. The interactions between metal ions and the functional groups on the chemically-modified biosorbent surface are confirmed again by FT-IR, its morphology is also observed, after the chemical modification and after the biosorption processes, by SEM. The effects of several important parameters such as the contact time (from 5 min to 72 h), the aqueous initial pH value (ranged from 1.0 to 6.0), the biosorbent dosage (between 0.01 and 0.2 g), and the initial metal concentration (from 0.005 to 4 mmol/L for several ones), are also checked. Three kinetic models including pseudo-first order, pseudo-second order and Weber-Morris intraparticle diffusion are used again to characterize the biosorption process, for a good understanding of the biosorption mechanism. Furthermore, Freundlich and Langmuir isotherm models are also tested in the biosorption process. Recovery of the adsorbed Pb(II) ions and reuse of the treated human hair are investigated for the viewpoint of practical application for the industrial effluents treatment.

3.2.1 The chemical treatment of human hair

According to the previous study, **H2** (the oxidized hair) shows better biosorption capacity than **H1** (in native). So, chemical modification through an oxidation method is used for the purpose of enhancing the biosorption capacity of heavy metals of the native human hair. The oxidation of human hair (as a keratin biomaterial) can usually take place with hydrogen peroxide in acid or alkaline medium by attacking the disulfide bond of keratin.[29] As a result of this oxidation reaction, sulfonate groups are formed, which will be new functional groups of the human hair. In alkaline medium the oxidation process is much more effective.[30] Moreover, during the oxidation process, other proteins in the human hair are also oxidized which leads to cell membrane damage

causing the cortex and the cuticle to open and separate.[31] So, with this knowledge, the main objective of this chemical pretreatment is to activate the human hair with new metal binding sites on the surface and also to increase its specific surface area. Finally, the chemical oxidation was run with a H2O2 solution at 10% and at pH 9.0.

3.2.2 Comparison of biosorption efficacy between untreated and treated human hairs

The biosorption capacities of the untreated and treated human hair samples for removing eight metal ions (Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II)) were determined. The obtained results are shown in Figure 3.15. As seen in Figure 3.15, the metal biosorption capacity of the treated human hair is significantly higher than the untreated human hair. Moreover, the affinity of both hair samples for Cr(III), Cu(II), and Pb(II) is greater than that for the rest of metal ions in solution, which can be explained by the stronger interactions between the functional groups (especially sulfonate ones) of the biosorbent and these three metal ions, as indicated previously. The metal biosorption onto the treated human hair follows in the order of Cr(III) > Pb(II) > Cu(II) > Cd(II) > Ni(II) > Co(II) > Mn(II) > Zn(II).

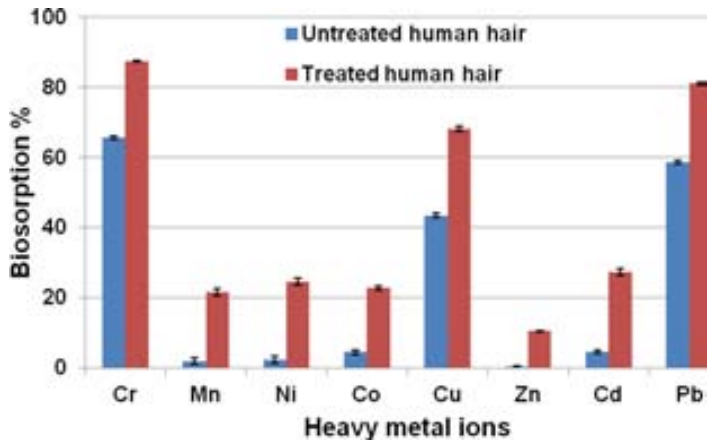

Figure 3.15. Comparison of biosorption capacity between untreated and treated human hair in multiple-metal system. The initial metal concentration is 0.1 mmol/L, the contact time is 24 h, the pH is 4.0, and the biosorbent is 0.1 g.

Taking into account such results, four metal ions including Cr(III), Cu(II), Cd(II)

and Pb(II) were selected to check the biosorption behavior under different conditions and to elucidate the biosorption mechanism of the chemically-modified human hair in subsequent experiments. Firstly, the functional groups and morphology of the human hair surface in the chemical modification and after biosorption processes are checked.

3.2.3 FT-IR and SEM characterization

In order to understand how metal ions bind to the biosorbent, it is essential to identify the functional groups of its surface as they could be the responsible for such metal binding. So, the untreated, treated and metal loaded-treated hair samples are discriminated by using Fourier transform infrared (FT-IR) spectroscopy, as can be seen from the infrared spectra collected in Figure 3.16. The full-scan spectra of human hair samples (Figure 3.16 (a)) display their corresponding infrared peaks. The broad and medium intensity band ranging from 3000 to 3600 cm^{-1} is indicative of the stretches of the bonds belonging to the carboxylic acid (-COOH), the alcohol (-OH) and the amino acid ($-NH_2$) groups. The peaks located at 1632 cm^{-1} (Amide I), 1520 cm^{-1} (Amide II) and 1241 cm^{-1} (Amide III) are related to typical human hair amino acids. The peaks at 1041, 1075, 1180, and 1229 cm^{-1} all correspond to different products of cystine oxidation in human hair, and their peak assignments are shown in Table 3.6. Carefully comparing the spectra of the three different human hair samples, it can be seen some differences between them, as expected due to the oxidation process first (treated human hair), and also due to the metal binding after the later biosorption process (metal loaded-treated human hair), especially in the region from 850 to 1750 cm^{-1} (see in Figure 3.16 (b)). The intensity of the peaks at 1041 and 1180 cm^{-1} increased after chemical modification, which means that conversion of cystine to cysteic acid, cystine monoxide, and cystine dioxide as well as to sulfonate occurs during the oxidation treatment process. The weak broad shoulder between approximately 1000-1130 cm^{-1} in the untreated human hair infrared spectra is probably due to environmental factors, such as sunlight, chlorinated water and frequent shampooing causing partial oxidation of the hair surface.[29] The FT-IR spectra from treated human hair before and after the metal

biosorption are very similar, indicating that the main functional groups on treated human hair did not change significantly during the metal biosorption process, which can be an indication of the possible reuse of such biomaterial. However, the slightly differences found around 1400 cm^{-1}, and some red shift of the emission spectra (from 3277.2 cm^{-1} to 3274.8 cm^{-1}, from 1526.8 cm^{-1} to 1519.7 cm^{-1}, and from 1078.4 cm^{-1} to 1074.9 cm^{-1}), before and after the biosorption process, probably can be related to the presence of the metal ions on the hair surface.[32] Based on the FT-IR spectra changes, as seen in Figure 3.16, some chemical functional groups (including hydroxyl, amino, carboxyl and sulfonate groups) of the human hair surface could act as important biosorption sites for metal ions.

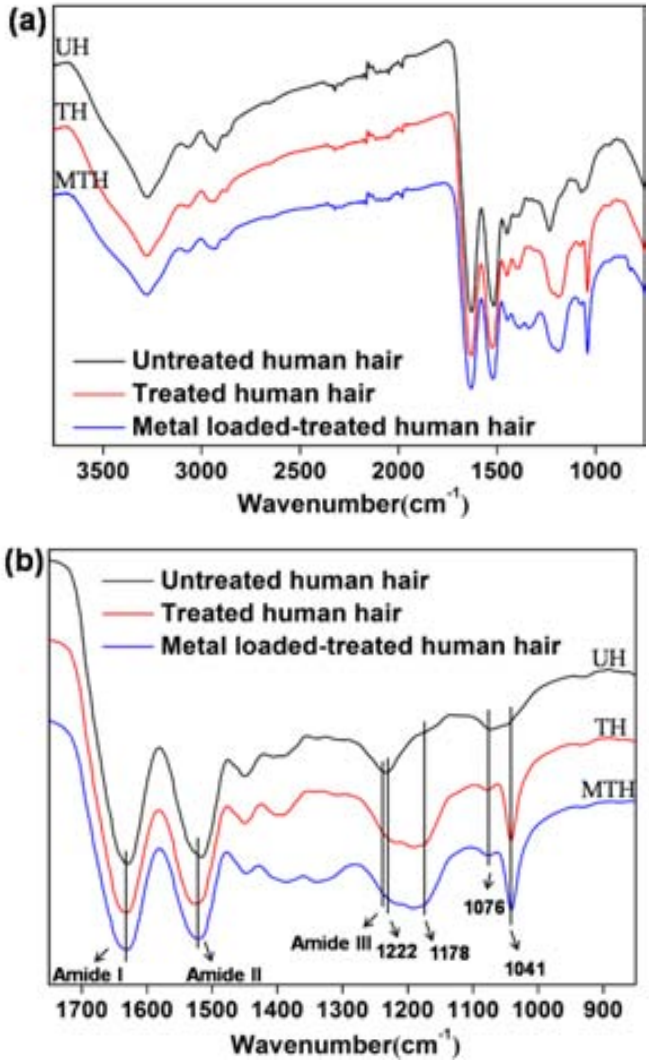

Figure 3.16. FT-IR spectra of human hair.UH: untreated human hair, TH: treated human hair, MTH: metal loaded-treated human hair: (a) full-scan spectra, and (b) partial-scan spectra.

Table 3.6. FT-IR peak assignments of the oxidation products of the cystine.[1]

Assignments	Wave number (cm^{-1})			
	literature	Current work		
		Untreated	Treated	Metal loaded-treated
Cystine dioxide ($R-SO_2-S-R$)	1229	1222	1221	1220
Sulfonate, S-O asym. stretch	1175	1178	1190	1181
Cystine monoxide (R-SO-S-R)	1075	1076	1078	1080
Sulfonate, S-O sym. stretch	1040	1041	1042	1042

Scanning electron microscope (SEM) technique was applied to concern about the alteration of the human hair surface morphologies in the different cases of study. Figure 3.17 shows the SEM micrographs of the untreated, treated, and metal-loaded treated human hair. It is observed that each cuticle scale of human hair is uniquely shaped. Some have smooth rounded edges and others with jagged-like edge, overlapping each other as they ascend along the length of the fiber towards the tip (Figure 3.17). The surface topography of the untreated and the treated human hair have some difference (as can be seen in Figure 3.17(a) and (b), for comparison).The majority of the cuticle scales of the treated human hair fiber represent more 'jagged' appearance, probably due to the oxidation treatment. After metal biosorption, the surface appears to be somewhat smoother comparing with the chemically-modifiedhair prior its use, suggesting that the cuticle scales are closed through the metal biosorption process, probably due to the acidic water media of the initial aqueous solution (see Figure 3.17(b') and (c')).

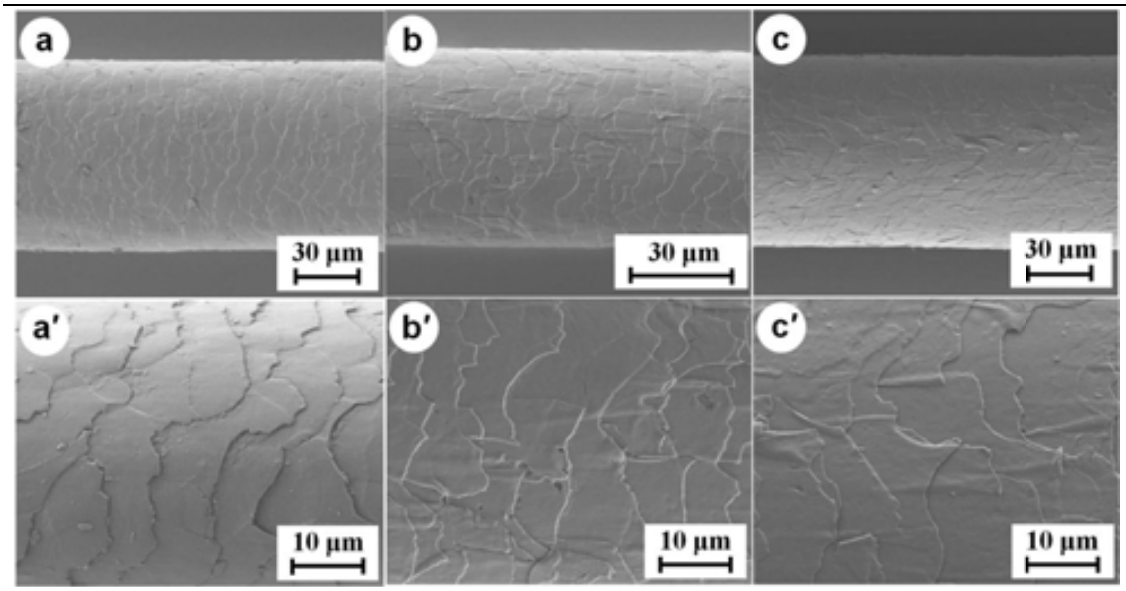

Figure 3.17. Scanning electron microscopy (SEM) micrographs of the human hair: (a) and (a') correspond to the untreated human hair; (b) and (b') correspond to the treated human hair; and (c) and (c') correspond to the metal loaded-treated human hair.

3.2.4 Effect of the initial aqueous solution pH

The pH level of the aqueous solution has been shown to be an important variable for the metal biosorption process, due to the metal speciation and also to the speciation of the chemical functional groups present on the biosorbents surface. So, the effect of the initial aqueous solution pH on the removal efficacy of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) was studied between pH 1.0 and 6.0 in multiple-metal system (Figure 3.18). As observed from the results in Figure 3.18, the biosorption of metal ions increases significantly with the increase of the aqueous initial pH value. This behavior can be explained by the competition between the proton and the metal ions for the same binding site on the surface of the treated human hair. At low pH values, the surface of the biosorbent would also be surrounded by H^+ ions, which decreases the Cr(III), Cu(II), Cd(II) and Pb(II) ions interaction with the surface binding sites. As the pH increases, the basic forms of the chemical functional groups overall the hair surface predominate, increasing the surface negative charge, so the metal biosorption increases significantly.[33] However, when the pH is around 5, the partial hydrolysis of metal ions (specially for Cu, the rest of metals could occur at pH higher than 5), resulting in the formation of $M\,OH^{(m-1)+}$ and/or $M\,(OH)_m$ affect the biosorption capability of human

hair.[34] The biosorption percentage found at pH up to 5.0 can be also related to the precipitation of the metals. Therefore, the pH 4 was selected as optimal condition in the subsequent experiments (to avoid this possible and uncontrolled precipitation effect).

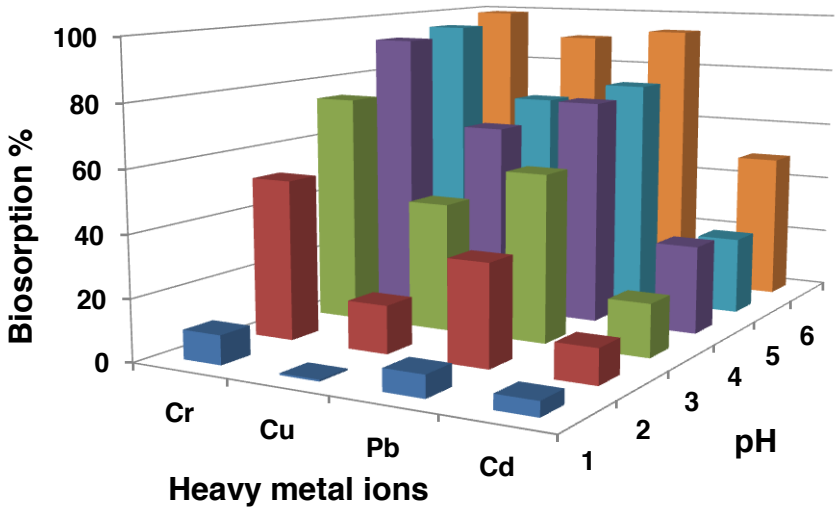

Figure 3.18. Effect of the initial pH on the biosorption of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) in the multiple-metal system. The initial metal concentration is 0.18 mmol/L, the contact time is 24 h, and the biosorbent is 0.1 g. Experimental errors found were less than 2% in biosorption percentage.

3.2.5 Effect of the biosorbent dosage

The influence of the biosorbent dosage on the removal efficacy of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) ions is studied in the range of 0.01- 0.2 g of biosorbent in a multiple-metal system (Figure 3.19). It is observed that the biosorption percentage of the indicated metal ions increases with the increase of the biosorbent dosage.[27] This can be explained with the increase in the surface area of biosorbent when increasing its amount, which in turn increases the metal binding sites, as indicated previously in the case of **H2** hair sample (see 3.1 Biosorption of heavy metals by human hair).[35] Taking into account the adsorbed metal ion (mmol) per unit weight of biosorbent, for Cr(III) and Pb(II), it significantly decrease by increasing the biosorbent dosage. This can be explained due to the fact that at high biosorbent dosage level, the available metal ions (Cr(III) and Pb(II)) in the aqueous solution are insufficient to cover all the biosorbent sites due to the corroborated high affinity of these two metals (as can be seen from results collected in Figures 3.15 and 3.19). In the case

of Cu(II) and Cd(II), at high biosorbent dosage level, there is enough metal ions, related to their biosorption affinity for the binding sites, to be adsorbed by the biosorbent. So, the metal uptake (mmol/g) for Cu(II) and Cd(II) increase through increasing the biosorbent dosage. Thus, as mentioned for **H2**, the functional groups of the treated human hair surface have greater affinity for Cr(III), Pb(II) than for Cu(II) and Cd(II).

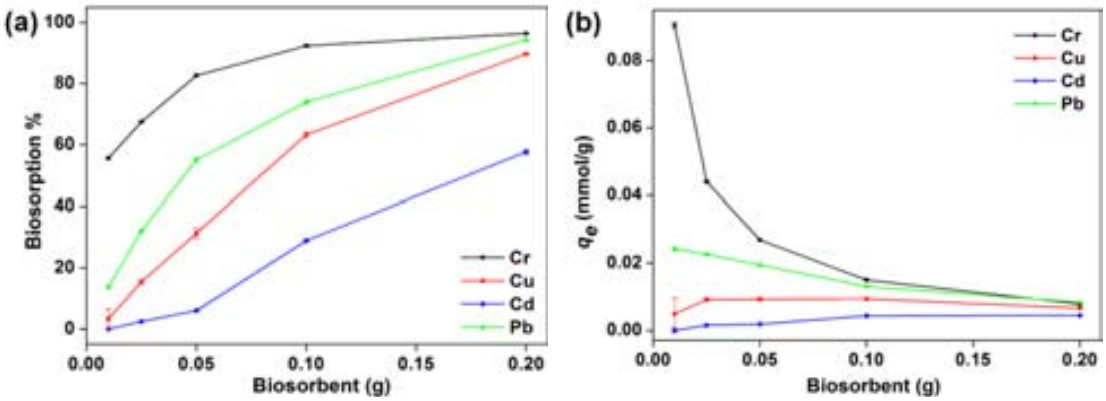

Figure 3.19. Effect of the biosorbent dosage on the biosorption of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) in the multiple-metal system. The initial metal concentration is 0.18 mmol/L, the contact time is 24 h, and the initial pH is 4.0.

3.2.6 Effect of the contact time

The contact time with aqueous metal solution is one of the key parameters for the successful usage of biosorbent in practice. Single- and multiple-metal aqueous systems (at pH = 4) of Cr(III), Cu(II), Cd(II) and Pb(II) are contacted with the treated human hair (0.1 g) during 5, 10, 20, 30, 45 minutes, and 1, 2, 3, 4, 6, 12, 24, 48 and 72 hours. Results plotted in Figure 3.20 show the biosorption of the treated human hair for removing Cr(III), Cu(II), Cd(II) and Pb(II) ions from both, single- and multiple-metal systems as a function of time. The curves indicate that the rate of the metal biosorption is high in the initial step of the process, especially for the single-metal system (which needs only few minutes to reach the maximum metal biosorption capacity), compared with the multiple-metal system. This behavior can be attributed to the effect of the competition between the heavy metal ions, in the later case. The biosorption increases until the equilibrium is reached in both systems.[36] From the data shown in Figure 3.21, it can be seen that compared with that of multiple-metal system, biosorption capacity of

individual metal ions is greater, i.e. the biosorption percentage of the treated human hair for Cd(II) is outstanding with a difference from 29% up to 86%, respectively. Therefore, the selectivity order is Cr(III) > Pb(II) > Cu(II) > Cd(II), which corresponds to a biosorption efficacy in the single-metal system of 98%, 96%, 95%, and 86%, respectively. So, in comparison with single-metal system, biosorption capacity of metal ions are greater than in the multiple-metal system.[37]

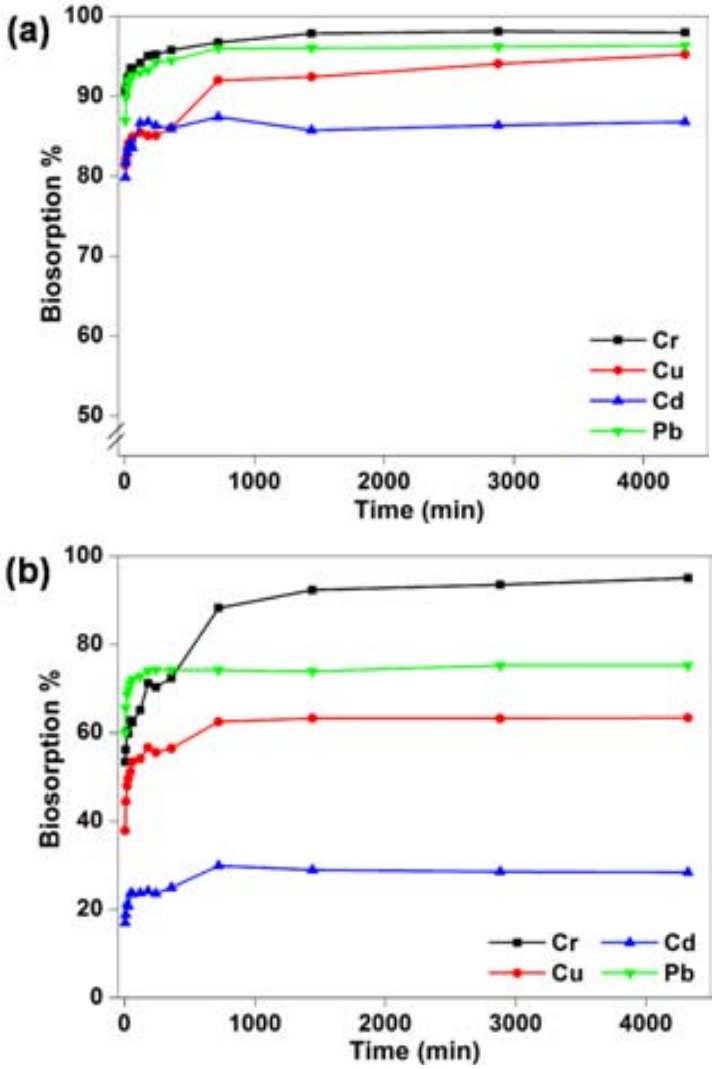

Figure 3.20. The biosorption percentage of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) at different contact time. (a): multiple-metal system, (b): single-metal system. The initial metal concentration is 0.18mmol/L, the pH is 4.0, and the biosorbent is 0.1 g.

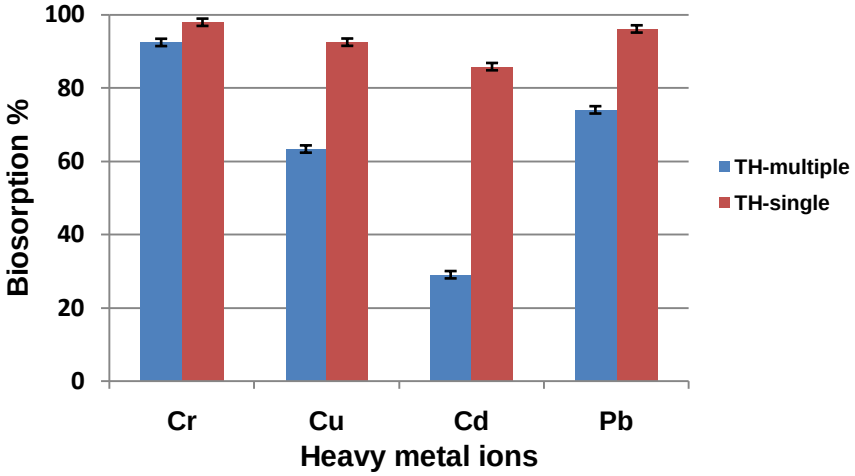

Figure 3.21. Comparison of biosorption for Cr(III), Cu(II), Cd(II) and Pb(II) by the treated human hair from single- and multiple-metal systems. The initial metal concentration is 0.18 mmol/L, at pH 4.0, and the biosorbent is 0.1 g, after 72 h.

3.2.7 Effect of the initial metal concentration

The initial metal concentration in the solution is also an important parameter which affects to the driving force of the biosorption systems.[38] The effect of the initial metal concentration is determined in single metal systems. Corresponding results are shown in Figure 3.22. It is found that the biosorption capacity of the treated human hair increases with the increase of the initial metal concentration. This concentration level is increased up to no remarkable changes are observed on the metal uptake. In this case, the surface of the treated human hair does not have free sites for the metal uptake, being fairly saturated, as can be seen from Figure 3.22 (a).[39] Probably, as the concentration increased, a higher probability of collision between adsorbate (biosorbate) and adsorbent (biosorbent) surface occurs, which should overcome the mass transfer resistance between the aqueous and the biosorbent phase.[40] Taking into account the biosorption percentages (from Figure 3.22 (b)) of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II), all over 90% at lower metal ion concentration level (< 0.1 mmol/L), this probably means that sufficient sorption sites are available for the biosorption of each heavy metal ion (in the single-metal system). However, a steeper slope of the curve is observed at higher concentration level (> 0.18 mmol/L), where it looks like the amount of the metal ion is relatively high compared to the availability of

the sorption sites of the treated human hair, as mentioned previously for **H2** sample.

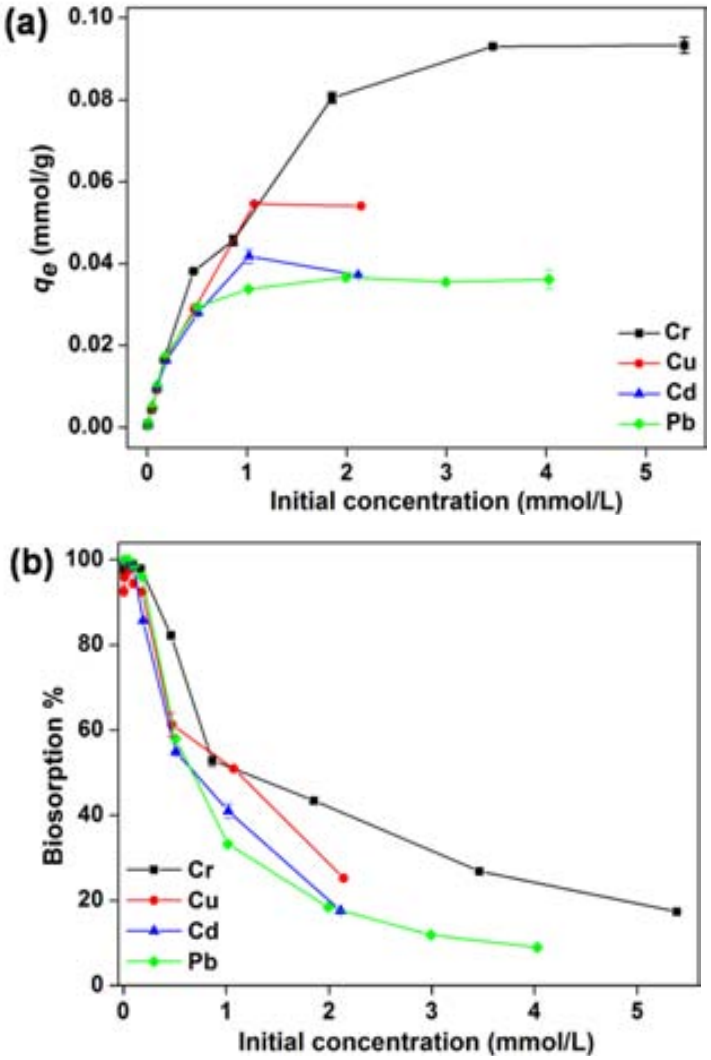

Figure 3. 22. Biosorption percentage of single-metal systems with treated human hair at different initial metal concentration. The contact time is 24 h, the initial pH is 4.0, and the biosorbent is 0.1g.

3.2.8 Biosorption kinetics modeling

As mentioned, kinetic models are used to fit the experimental data in order to investigate the mechanism of the metal biosorption process. So, it is important to estimate which is the possible rate controlling steps in biosorption process, such as mass transport, chemical reaction and intraparticle diffusion processes.[15] Many attempts had been made to formulate a general expression describing the kinetics liquid-solid phase sorption systems.[16] In the present case, the kinetic models including the pseudo-first

order equation[41], the pseudo-second order equation[18] and the Weber-Morris intraparticle diffusion model are applied.

The plots of log(q_e-q_t) vs. t of each metal ion (in the single- and multiple-metal systems) for the pseudo-first order model (see 2.5.1 Pseudo-first order rate equation Eq. (6)) are shown in Figure 3.23 (a). As it is properly shown, only the data corresponding to the first 30 and 60 min for the single- and multiple-metal systems, respectively, are adjusted since after this period the experimental data deviated considerably from those theoretical.[42] The pseudo-first order rate constant (k_1) and q_e determined from the model are presented in Table 3.7. For the four metal ions in single and multiple systems, the q_e estimated by this model differs significantly of those measured experimentally, so suggesting that the biosorption doesn't follow a pseudo first-order reaction.[17]

Table 3.7. Pseudo-first and pseudo-second order metal equations parameters for the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) ions by the treated human hair in the single- and multiple-metal systems.

Metal		Cr(III)		Cu(II)		Cd(II)		Pb(II)	
system		single	multiple	single	multiple	single	multiple	single	multiple
Pseudo first order	$k_1 \times 10^3$ (min^{-1})	9.212[a]	4.192[b]	6.909[a]	14.970[b]	26.254[a]	12.943[b]	26.783[a]	27.337[b]
	q_e (mmol/g)	0.00132	0.00655	0.00263	0.00346	0.00151	0.00190	0.00173	0.00233
	R^2	0.93265	0.73429	0.99053	0.87939	0.91361	0.85924	0.83812	0.90298
Pseudo second order	k_2 (g/mmol min)	15.406	1.5068	3.8279	6.7457	38.1796	16.1676	17.7525	16.6435
	q_e (mmol/g)	0.01655	0.01554	0.01738	0.00945	0.0165	0.00429	0.0174	0.01330
	R^2	0.99999	0.99933	0.99985	0.99991	0.99998	0.99966	1	0.99997

a): 30 min, b): 1 h

A straight line is obtained in all cases in all the time range (Figure 3.23 (b)) by plotting t/q against t for the pseudo-second order model (see 2.5.2 Pseudo-second order rate equation Eq. (9)), and the rate constant (k_2) and q_e values are determined from these plots. Both parameters and the correspondent correlation coefficients are also presented in Table 3.7. The pseudo-second order model shows the good fit to the experimental data with high correlation coefficients (R^2> 0.999) for four metal ions in both systems (single- and multiple-metal), and theoretical values of q_e mostly agree with the

experimental data (Figure 3.24). Both facts suggest that the pseudo-second order model is more likely to predict kinetic behavior for the whole range of time studied, which relies on the assumption that the rate limiting step might be chemical sorption due to the formation of chemical bonds between the metal ions and the functional groups of the treated human hair, as it was the case of **H2**.[43] The equilibrium biosorption capacities for Cr(III), Cu(II), Cd(II), and Pb(II) were 0.0166, 0.0174, 0.0165, and 0.0174 mmol/g, respectively in the single-metal system, and 0.0155, 0.0095, 0.0043, and 0.0133 mmol/g, respectively in the multiple-metal system.

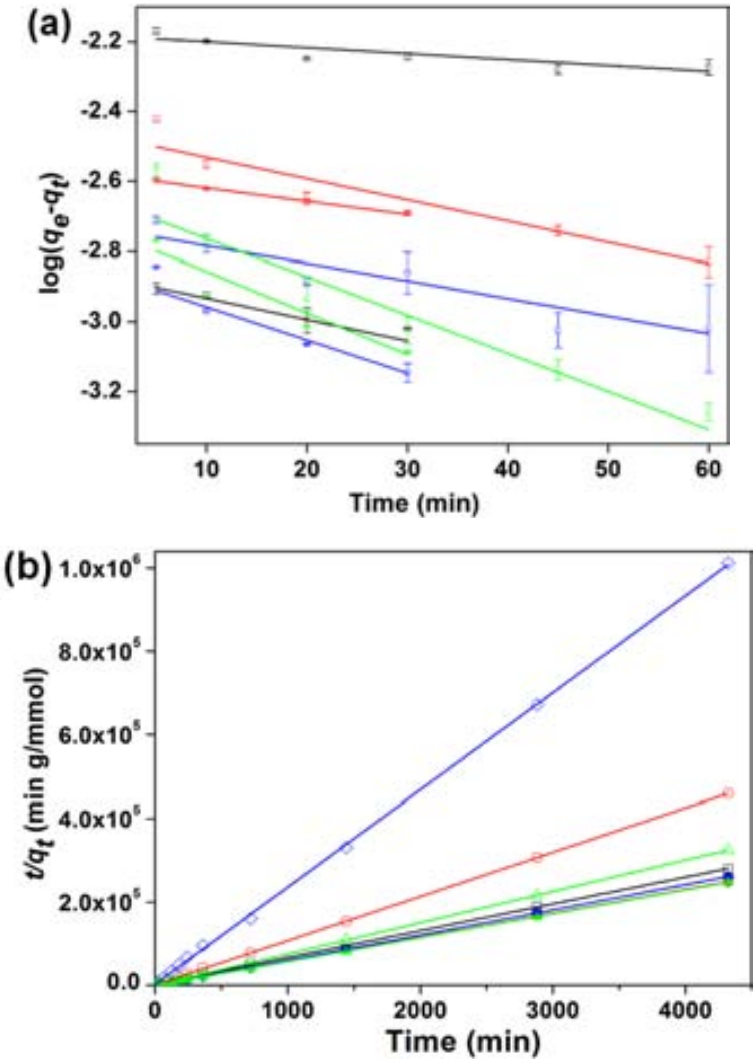

Figure 3.23. Biosorption kinetic models applied to the biosorption of metal ions by the treated human hair: (a) Pseudo-first order model, (b) Pseudo-second order model. (■ Cr/single, □ Cr/multiple; ● Cu/ single, ○ Cu/multiple; ◆ Cd/single, ◇ Cd/multiple; ▲ Pb/single, △ Pb/multiple; — the linearization of the Pseudo1st order and Pseudo 2nd order models)

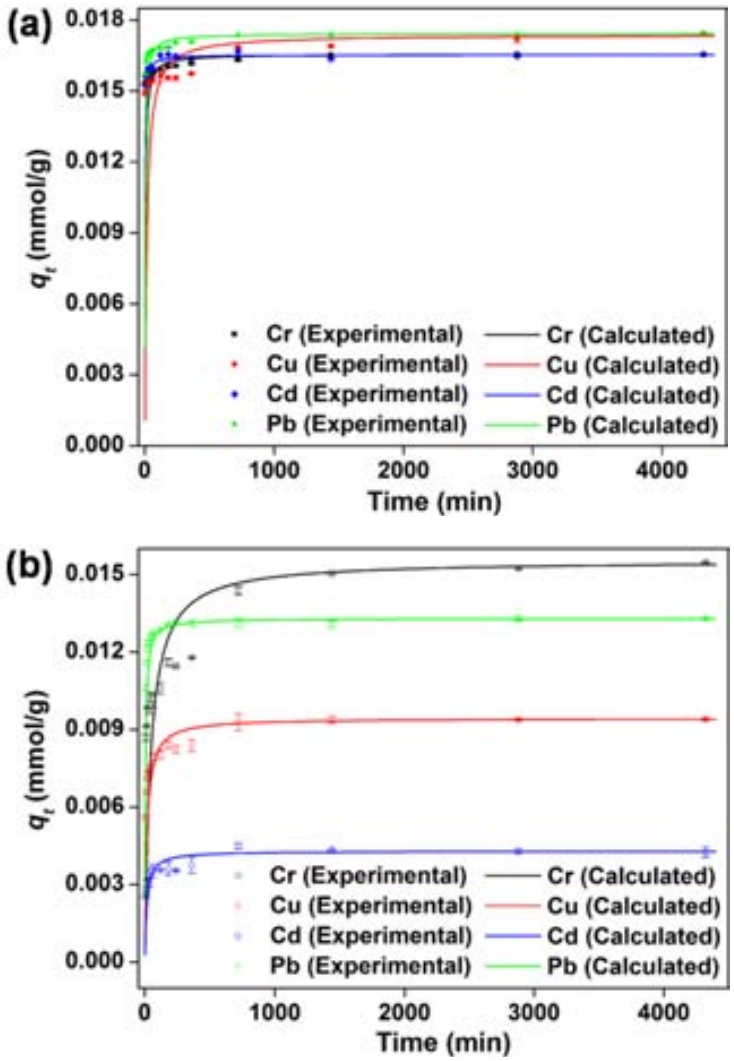

Figure 3. 24. Experimental (•) and calculated (—) values adjusted by following the pseudo-second order model equation: (a) in the single-metal system, and (b) in the multiple-metal systems.

As the pseudo-first and pseudo-second order models cannot provide information about the diffusion mechanism controlling the metal biosorption process onto the treated human hair. Weber-Morris intraparticle diffusion model (see 2.5.3 Weber-Morris intraparticle diffusion model Eq. (10)) is also adjusted.[44] The plots of q_t versus $t^{1/2}$ are shown in the Figure 3. 25. In this case, these plots can be divided into multi-linear correlations, which indicate that the biosorption process take place in three steps and it is not controlled only by intraparticle diffusion mechanism.[45] As for **H2**, the first stage corresponds to the sharper stage, where the metal ions move from the solution to the external surface of the biosorbent, through the film diffusion, or the boundary layer

diffusion.[22] In the present case, the sulfonate groups formed in the oxidation treatment could assist the achievement of this step. For each metal ion, this step is faster in single-metal system than in multiple-metal system. The second step describes the gradual biosorption onto the surface of the treated human hair, where the intraparticle diffusion is the rate-limiting.[20] The third stage corresponds to the final biosorption equilibrium where the intraparticle diffusion starts to slow down due to extremely low metal ion concentration left in solution. The presence of these three stages in the plots (Figure 3. 25) suggests that the film diffusion and intraparticle diffusion were simultaneously controlling the biosorption process and both are enhanced with the increase of the initial metal concentration.

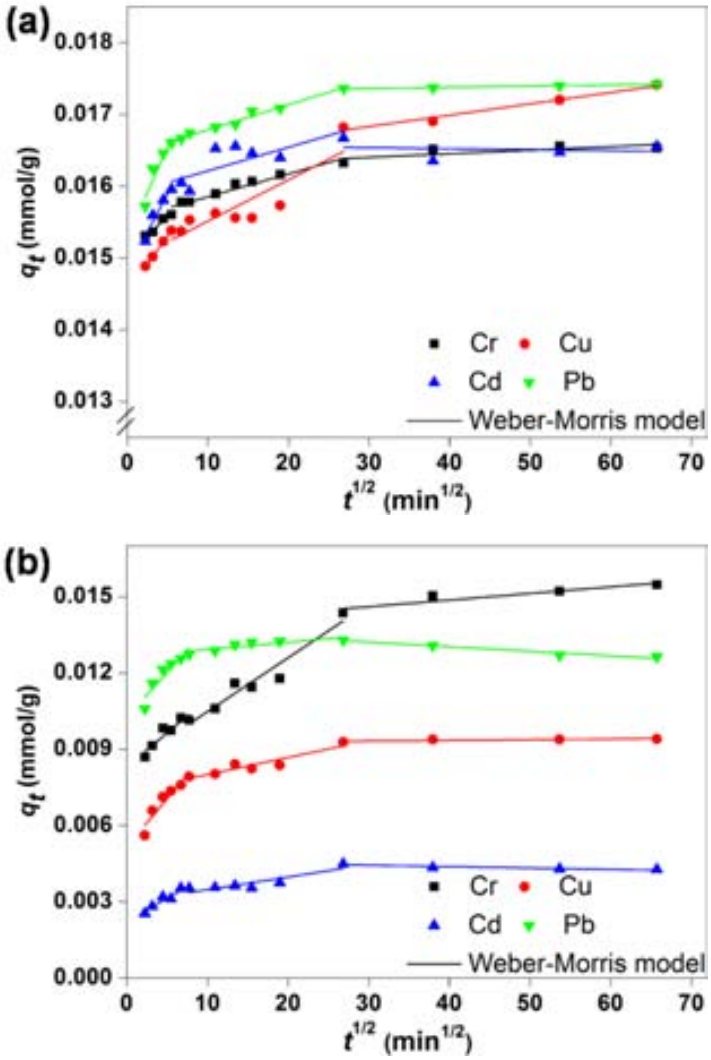

Figure. 3.25. Weber-Morris intraparticle diffusion model applied for the metal biosorption onto the treated human hair: (a) in the single-metal system, and (b) in the multiple-metal system.

To sum up, the modelization analysis suggests that the film diffusion control the early stage of the biosorption process, while in the later stage the chemical sorption play an even greater role in the removal of heavy metals, as it was also stated for **H2**.

3.2.9 Biosorption isotherm modeling

Biosorption isotherms at the equilibrium should be checked to be able to understand the mechanism of each sorption system from a physicochemical point of view. The adsorption capacity of an adsorbent can be also described by the equilibrium sorption isotherm, which is characterized by some specific constants whose values give information about the affinity between the liquid-solid sorption systems. This can be also applied to the present biosorption system, by using the treated human hair in the single-metal system.

In the present study, the same isotherm models are selected to fit the experimental data as previously, which are namely Freundlich[24] (see 2.6.1. Freundlich isotherm model Eq. (12)) and Langmuir[25] (see 2.6.2 Langmuir isotherm model Eq. (14)) isotherm models. Logq_e versus logC_e and C_e/q_e versus C_e are calculated and compared with the experimental data, respectively. Also, all the constants and correlation coefficients obtained for each model are summarized in Table 3.8.

Table 3.8. Freundlich and Langmuir isotherms constants for the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) ions by the treated human hair.

Isotherm model	constant	Cr	Cu	Cd	Pb
Freundlich	$K_F \times 10^3$	1.56	2.87	0.546	0.247
	n	2.29621	1.89703	2.86287	3.62726
	R^2	0.86464	0.84023	0.92914	0.86069
Langmuir	$Q_0 \times 10^5$ (mol/g)	9.47168	5.56652	3.77063	3.61032
	$b \times 10^{-4}$ (L/mol)	1.0676	2.0630	8.6384	8.0364
	K_L (L/g)	1.01115	1.14837	3.25722	2.90141
	R^2	0.99115	0.99047	0.9952	0.99995
	$-\Delta G^0$ (kJ/mol)	22.750	24.366	27.878	27.701

Regarding the corresponding correlation coefficient values obtained, we can conclude that the Langmuir isotherm fits the data better than the Freundlich isotherm

showing that the biosorption process relies on a specific sites sorption mechanism where biosorbate molecules in the monolayer of the surface occupy specific sites on the biosorbent. In Figure 3.26, experimental and calculated data for the Langmuir isotherm model are represented, showing good correlation between them. Taking on board the Langmuir equation, the maximum biosorption capacity of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) at 295 K are 9.47×10^{-5}, 5.57×10^{-5}, 3.77×10^{-5}, 3.61×10^{-5} mol/g, respectively.

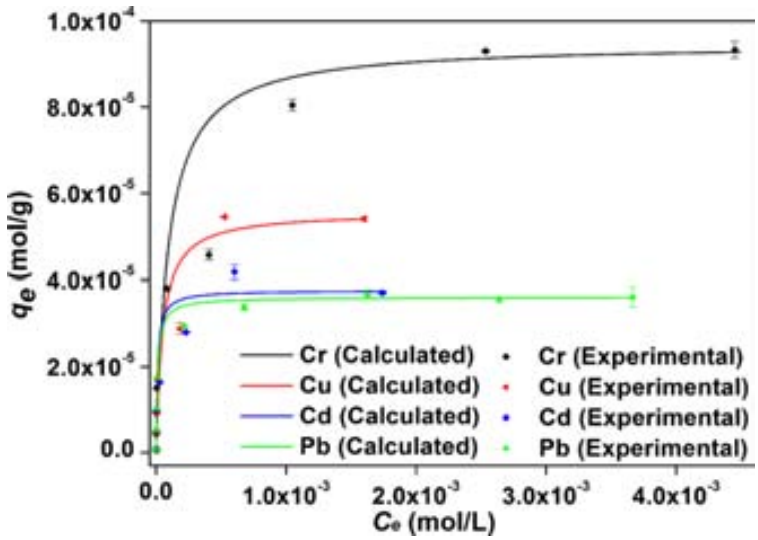

Figure 3.26. Experimental (•) and calculated (—) values adjusted by using the Langmuir isotherm model for the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) ions by the treated human hair.

In addition, from the estimated Langmuir adsorption constant *b*, the standard Gibb's free energy (ΔG^0) of the biosorption process can be evaluated by using the equation (ΔG^0, see 2.6.3. Thermodynamic parameter Eq. (15) $\Delta G^0 = -RT \ln b$). The standard Gibb's free energy (ΔG^0) values are shown in Table 3.8. The negative ΔG^0 values indicates that the biosorption of metals into the treated human hair is thermodynamically feasible and of spontaneous nature, as expected when comparing with **H2**.[10]

3.2.10 Desorption, regeneration and reuse studies

Recovery of the adsorbed heavy metals and reuse of the biosorbent are of significance from the viewpoint of practical application. As indicated previously, two

eluents including EDTA and HNO_3 solutions are screened for their potential to desorb Pb(II) ions from metal adsorbed treated human hair (corresponding results are collected in Table 3.9). Both eluents can effectively desorb the heavy metals from the metal loaded-treated human hair, even the elution efficiency of EDTA solution is slightly better than the HNO_3 solution. These results are similar to previous ones. The sulfonate groups formed in the treatment process can assist EDTA to be able to combine with Pb(II), as indicated previously with **H2** sample (also oxidized by blenching and dyeing).

The reuse of regenerated human hair for removal of heavy metals is investigated. Two regeneration methods used previously are also studied: one is rinsing with deionized water, the other is rinsing with HNO_3 firstly and then with deionized water (this just in the case of EDTA as eluent). After regeneration, the samples are used to adsorb Pb(II) ions from fresh aqueous solutions. The results show that the treated human hair regenerated with EDTA and rinsing with water exhibited higher metal biosorption capacity than the other options. As before, the ionization state of functional groups on the biosorbent surface have a negative effect for its reuse, which formed in the elution or rinsing when using HNO_3 processes.

Table 3.9. The elution of adsorbed Pb(II) by using EDTA and HNO_3 solution and the biosorption capacity of the regenerated human hair.

Biosorbent		The treated human hair
1st biosorption, Pb(II) adsorbed (%)		89 ± 1
1st desorption	Pb(II) elution efficiency (%) using EDTA (0.1 M)	89 ± 1
	Pb(II) elution efficiency (%) using HNO_3 (0.1 M)	85 ± 1
2nd biosorption, Pb(II) adsorbed (%)	Regeneration by using EDTA elution	87 ±2
	Regeneration by using HNO_3 elution	38 ±4
	Regeneration by using EDTA elution and rinsing with HNO_3	75 ± 1

3.2.11 Conclusions

As expected, the treated human hair with an oxidation modification shows good biosorption capacity for the removal of heavy metals. FT-IR analysis of different

samples confirm that abundant metal binding groups such as carboxyl, hydroxyl, amino and sulfonate groups are responsible for the metal removal. The sulfonate groups formed in the oxidation modification process play a great role in the removal of heavy metals. Biosorption has been investigated following the influence of various parameters such as pH of solution, biosorbent dosage, contact time and initial metal concentration. Biosorption of heavy metals is highly dependent on the pH of the solution, and the optimum biosorption capacity is determined at pH 4.0. Increase in the mass of the biosorbent leads to an increase in the metal removal, owing to corresponding increase in the number of metal binding sites. In addition, the treated human hair shows higher biosorption capacity in the multiple-metal system in terms of total biosorption capacity. According to the kinetic study, the biosorption of metal ions onto the treated human hair follows well the pseudo-second order kinetic model, which is in agreement with the chemical sorption being the rate controlling step. The biosorption equilibrium of metal ions is better fitted with Langmuir isotherm model compared to Freundlich model. Langmuir model suggests monolayer coverage of metal ions on the treated human hair, as previously. The maximum biosorption capacities of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) at 295 K were 9.47×10^{-5}, 5.57×10^{-5}, 3.77×10^{-5}, 3.61×10^{-5} mol/g, respectively. The calculated standard Gibb's free energy (ΔG^0) indicates the thermodynamically feasible and spontaneous nature of the biosorption process. Desorption experiments prove that 0.1 mol/L EDTA and HNO_3 solutions are efficient eluents for the recovery of Pb(II) from the treated human hair. Furthermore, the treated human hair regenerated with EDTA show the best biosorption efficiency when reusing.

Taking into consideration present findings, it can be stated that the treated human hair (chemically-modified by an oxidation reaction process) is an effective and low-cost biosorbent for the removal of heavy metals from aqueous solutions. The oxidation treatment would be quite useful in modifying the keratin biomaterial to enhance the removal of heavy metals from contaminated effluents.

3.3 Comparison of the keratin biomaterials for the removal of heavy metals

It is well known that the waste keratin biomaterials can be applied for the treatment of wastewaters as low cost biosorbents, even at low contamination levels. In this part, four common waste keratin biomaterials including human hair, dog hair, chicken feathers and degreased wool were used as biosorbents for the removal of heavy metals from aqueous solutions. The biosorption processes are carried out in batch experiments, as indicated in previous cases. The effect of contact time (from 5 min to 72 h), the initial aqueous pH value (ranged from 1.0 to 6.0), and the biosorbent dosage (0.01- 0.2 g) on the biosorption of heavy metals are investigated. The functional groups and the surface morphology of the biosorbents are confirmed before and after the metal biosorption process by FT-IR SEM. Kinetic models including pseudo-first order, pseudo-second order kinetics and Weber-Morris intraparticle diffusion models are used to characterize the biosorption process (as it has been done previously). Furthermore, biosorption isotherms of Pb(II) with the different keratin biomaterials are determined and correlated with common isotherm equations such as Freundlich and Langmuir models.

The keratin biomaterials including human hair, dog hair, chicken feathers and degreased wool were evaluated as biosorbents for the removal of heavy metals from a multiple-metal aqueous solution containing eight metal ions (Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II)). The results are shown in Figure 3.27. As seen from the figure, the four different keratin biomaterials show different biosorption capacities for the different metal ions. In general, these four keratin biomaterials exhibit better biosorption capacities for Cr(III), Cu(II) and Pb(II) compared with the rest of metal ions present in the initial aqueous solution, which have an uptake capacities that decreases as follow: Cd(II) > Zn(II) > Ni(II) ≈ Co(II) > Mn(II). This can be explained by the different affinity of metal ions for the metal binding donor atoms present in all the biosorbents. The total biosorption capacity of the four biosorbents followed the

order degreased wool > chicken feathers > human hair > dog hair. Finally, four metal ions including Cr(III), Cu(II), Cd(II) and Pb(II) were selected to check the biosorption behavior of the four keratin biomaterials in subsequent experiments (under different conditions). Firstly, the characterization of the four keratin biomaterials before and after biosorption process is followed by FT-IR and SEM, as indicated.

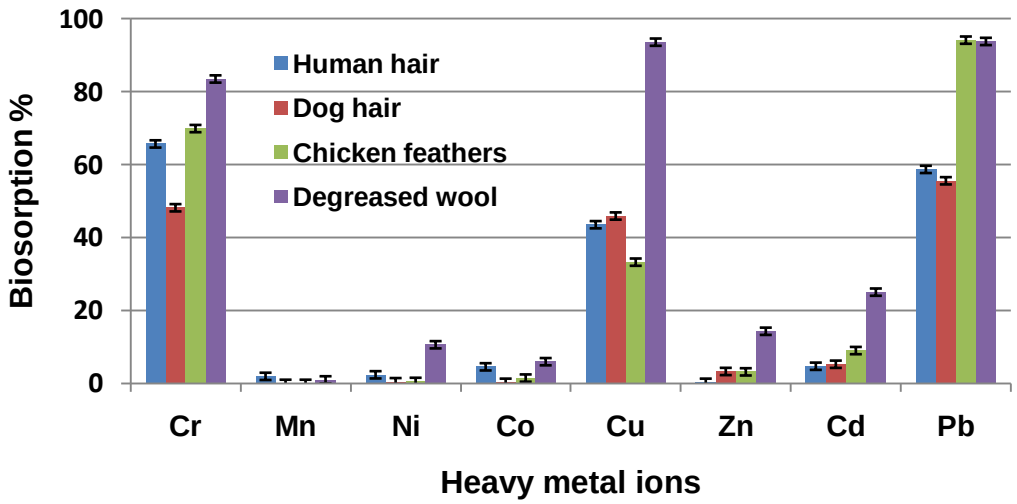

Figure 3.27. Biosorption of human hair, dog hair, chicken feather and degreased wool for Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) from multiple-metal aqueous system. The initial metal concentration is 0.1 mmol/L, the contact time is 24 h, the pH is 4.0, and the biosorbent is 0.1 g.

3.3.1 FT-IR and SEM characterization

The FT-IR analysis is carried out to identify the functional groups in the different biosorbents that might be involved in the biosorption process, as previously. A comparison of FT-IR spectra of these four biosorbents before and after biosorption is shown in Figure 3.28 (a)-(d). The wavenumbers and approximate assignments of the vibrational modes for the FT-IR spectra are listed in Table 3.10.[45]

From the FT-IR analysis of the biosorbents before biosorption, there are some findings. The peak located at 3275 cm^{-1} is a mode arising from N-H stretching and sensitive to hydrogen bonding, which is essential in the structuring of water and holding protein together. The two weak bands located at 2960 cm^{-1} and 2920 cm^{-1} could be assigned as modes arising from amino acid bands of $-CH_3$ and $-CH_2-$ asymmetric and symmetric modes, respectively.[12] The peaks located at 1632 cm^{-1} (Amide I), 1520 cm^{-1}

(Amide II) and 1241 cm^{-1} (Amide III) are related to common keratin fiber amino acids. The Amide I is a complex band with a coupling between carbonyl stretching modes, high contribution of -C=O stretch and a small contribution from NH bend. The Amide II is a mixed mode made up of C-N stretch and the N-H in plane bends. The Amide III includes C-N stretching, N-H in-plane bends, C=O stretching and O=C=N bending.[24] A weak broad shoulder bands in approximately 1180-1030 cm^{-1} region is related to the cystine and its oxidation products which includes cysteic acid, cystine monoxide, and cystine dioxide as well as sulfonate. Besides some environmental factors, such as sunlight, chlorinated water and frequent shampooing causing partial oxidation of the keratin biomaterial surface, partial oxidation of the degreased wool caused by the degreasing process is the important reason.[29] This has resulted in the better biosorption capacity of the degrease wool compared with other keratin biomaterials. So, the FT-IR spectra analysis indicates that there are potential metal binding functional groups (including hydroxyl, amino, carboxyl and sulfur-contianing groups) on the four biosorbents surfaces, sulfonate groups formed in the oxidation process also can enhance of the biosorption capacity as previously indicated.

Table 3.10. FT-IR spectral bands assignments for human hair, dog hair, chicken feathers and degreased wool.

Assignments	Wavenumbers(cm^{-1})			
	Human hair	Dog hair	Chicken feathers	Degreased wool
NH stretching	3280	3273	3268	3272
C-H stretching, $-CH_3$ and $-CH_2-$ asymmetric and symmetric modes	2957	2957	2961	2961
	2924	2926	2922	2930
Amide I, 80% C=O stretch and small contribution from NH bend	1630	1629	1624	1629
Amide II, C-N stretching/N-H bending	1517	1516	1534	1515
Amide III, complex vibration contains N-H bending, C-N stretching, C=O stretching, and O=C=N bending	1233	1232	1235	1234
Cystine	1180 - 1030			

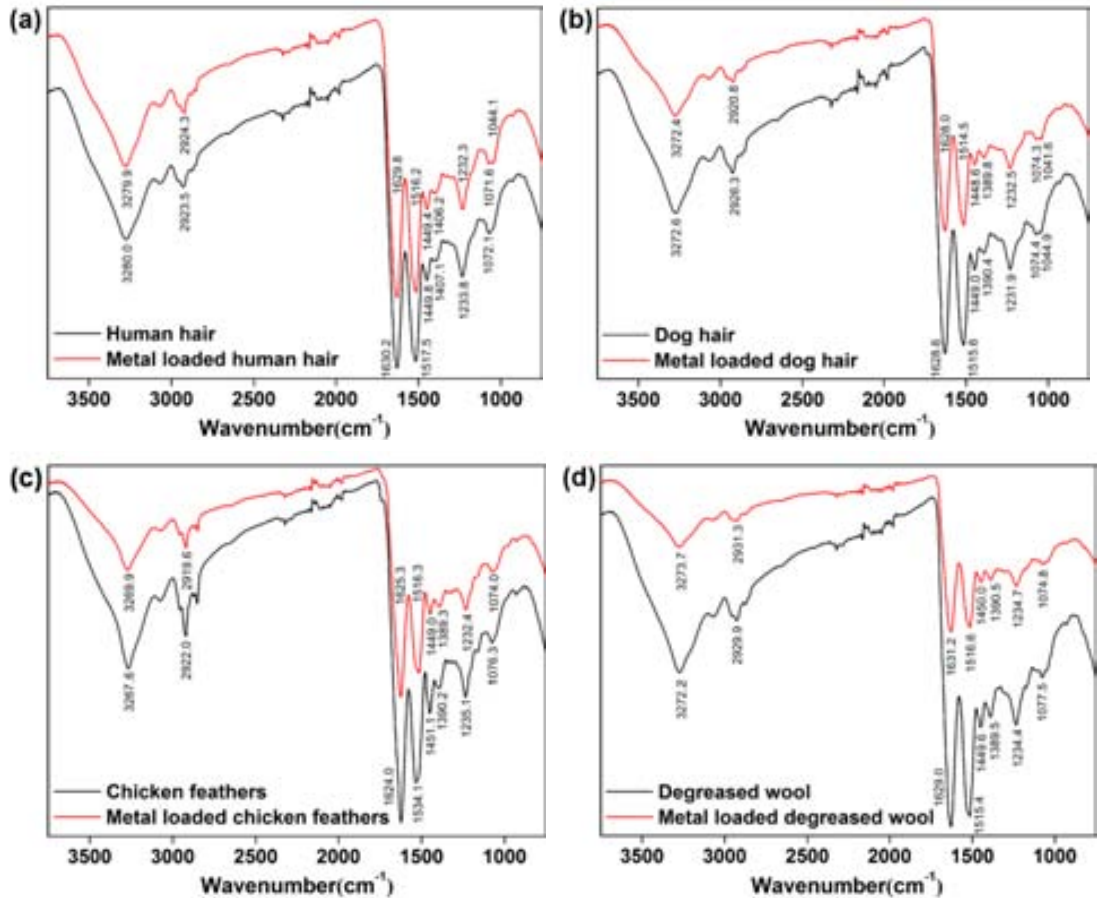

Figure 3.28. FT-IR spectra of human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d).

The four metal-loaded biosorbents are also discriminated. The FT-IR spectra from four biosorbents before and after biosorption are very similar, indicating that the main functional groups on the biosorbents did not change significantly during the biosorption process (which can be an indication of the possible reuse of such biomaterials). However, the four metal-loaded biosorbents show small differences in the FT-IR spectra. For instance, at the wavenumber 1072.1 cm^{-1} the cystine monoxide (R-SO-S-R) can be assigned for the human hair. This weak peak has a small red shift (from 1072.1 cm^{-1} to 1071.6 cm^{-1}) and was of greater intensity after the metal biosorption process. The same changes also happened to the other three keratin biosorbents. Moreover, more slightly shifts of the peaksare found in other functional groups which can be seen in the spectra collected in Figure 3.28 (a)-(d). Based on the shifts in the wavenumbers of different peaks from the FT-IR spectra of the metal-loaded keratin biomaterials, it can be deduced

that hydroxyl, amino, carboxyl and sulfur containing functional groups could act as the biosorption sites for the removal of metal ions.

The surface morphologies of the four keratin biomaterials under study are observed by Scanning electron microscope (SEM) before and after the metal biosorption process. It is also shown in Figure 3.29 (a)-(d). As can be seen, there are no significant differences on the surface morphology of the four biosorbents before and after the metal biosorption process. This result suggests that the four biosorbents are relatively stable under the biosorption process, which is favorable in terms of their reuse. The surface morphologies of the keratin fibers including human hair, dog hair and degreased wool are very similar, which appears to be somewhat smoother after the biosorption process comparing with them before biosorption, suggesting that the cuticle scales are closed through the biosorption, probably due to the acidic initial aqueous media (see Figure 3.29 (a2), (b2) and (d2) and compared with Figure 3.29 (a1), (b1) and (d1), respectively). In the case of the chicken feathers a little bit rough appearance after biosorption process is seen (Figure 3.29 (c2) compared with (c1)), probably due to the relative lower stability compared with the other biosorbents.[46] This may be caused by a lower cystine content of chicken feathers that contributes to this lower stability compared with the other biosorbents.[29] In addition, according to the comparison of the keratin fiber size, it can be observed that chicken feathers have higher surface area compared with other three keratin biomaterials, which lead to a relatively good biosorption capacity.

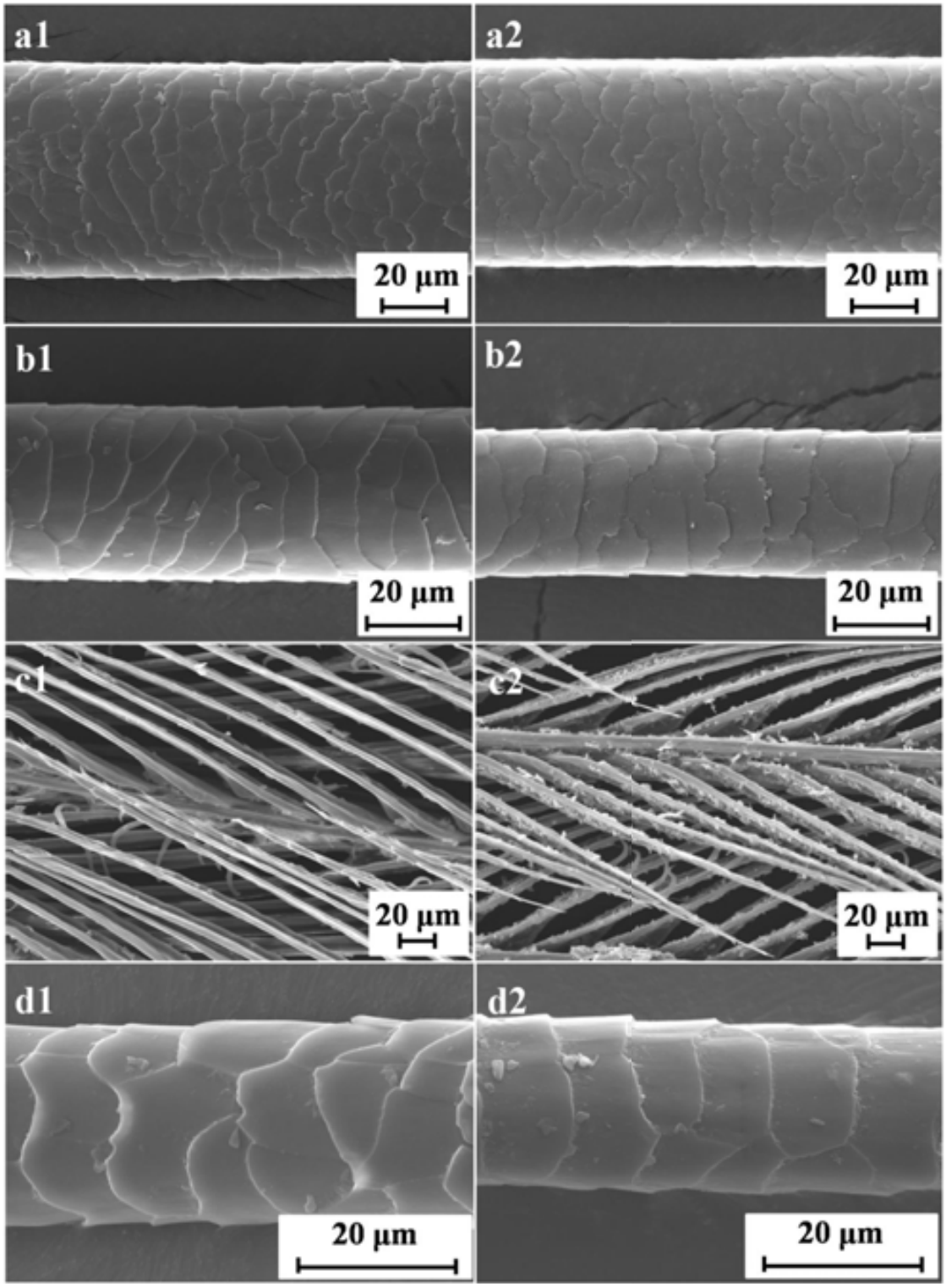

Figure 3.29. Scanning electron microscopy (SEM) micrographs of the biosorbents: (a1) and (a2) correspond to the human hair and metal loaded human hair; (b1) and (b2) correspond to the dog hair and metal loaded dog hair; (c1) and (c2) correspond to the chicken feathers and metal loaded chicken feathers; and (d1) and (d2) correspond to the degreased wool and metal loaded degreased wool.

3.3.2 Effect of the initial aqueous solution pH

As seen before, the initial aqueous pH is a key parameter in metal biosorption process, for both the metal aqueous speciation and the surface functional groups speciation of the keratin biomaterials.[47] As usual, batch biosorption experiments are carried out in the pH range of 1.0 to 6.0 in a multiple-metal system (collected results are shown in Figure 3.30 (a)-(d)). As shown in Figure 3.30 (a)-(d), the biosorption of metal ions increases significantly with the increase of the aqueous pH value. As in the previous cases presented, at lower pH values, metal ions had to compete with a large number of protons for the metal binding sites on the biosorbent surface. Therefore, the biosorption capacities of metal ions are very low for all four biosorbents. As pH increased, the concentration of proton decreases, and the positive metal ions can be easily adsorbed by the available binding sites on the biosorbent surface. Above pH 4.0,

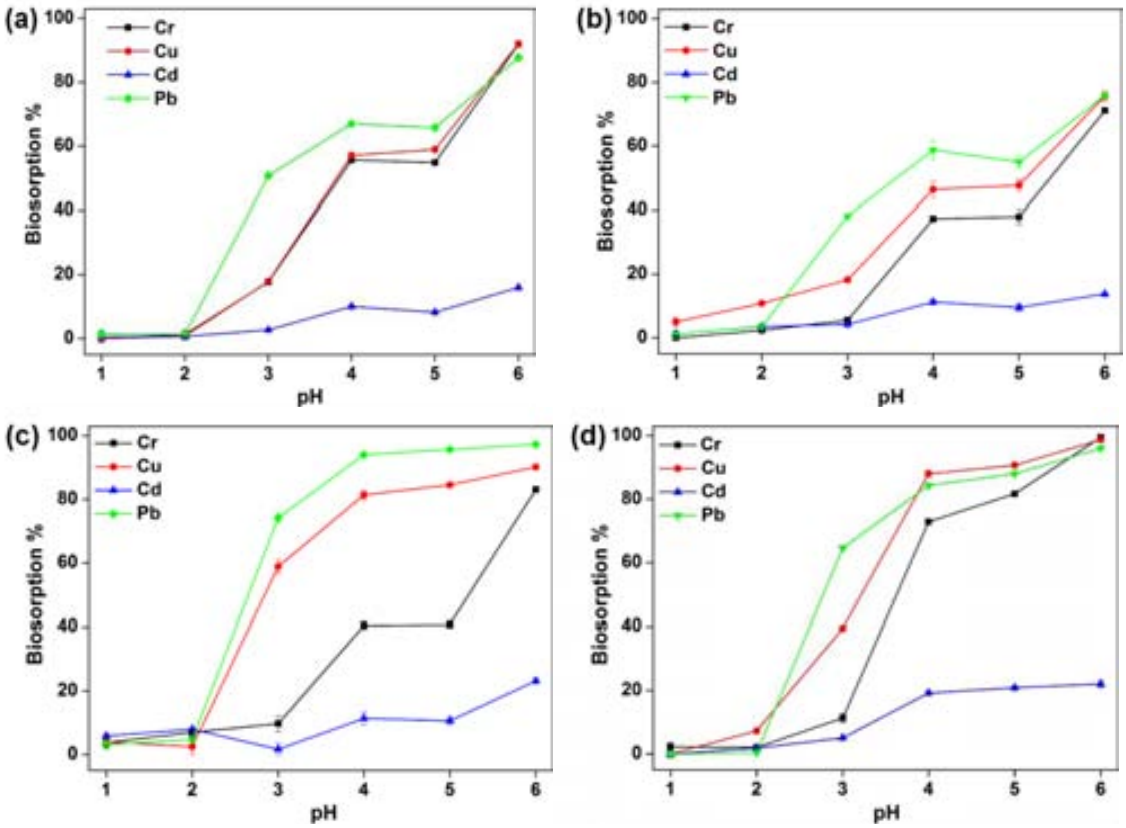

Figure 3.30. Effect of the initial pH on the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) by human hair (a), dog hair (b), chicken feathers (c), and degreased wool (d) from the multiple-metal system. The initial concentration is 0.18 mmol/L, the contact time is 24 h, and the biosorbent is 0.1 g.

biosorption of metal ions was found to be relatively constant, probably due to metal speciation with the partial hydrolysis of metal ions (especially for Cu(II), for the rest of the metal ions it can occur at pH higher than 5.0).[48] Therefore, the pH 4.0 was selected as optimal condition in the subsequent experiments for all biomaterials.

3.3.3 Effect of the biosorbent dosage

The removal of the avy metal ions from multiple-metal aqueous solution (0.18 mmol/L) was significantly depending on the amount of biosorbent, as seen previously. The effect of biosorbent dosage on the biosorption percentage of metal ions for the different keratin biomaterials is shown in Figure 3. 31 (a)-(d). The biosorption percentage of metal ions increases with the increase of biosorbent dosage, probably due to the increase of the total surface area of each biosorbent when increasing its amount,

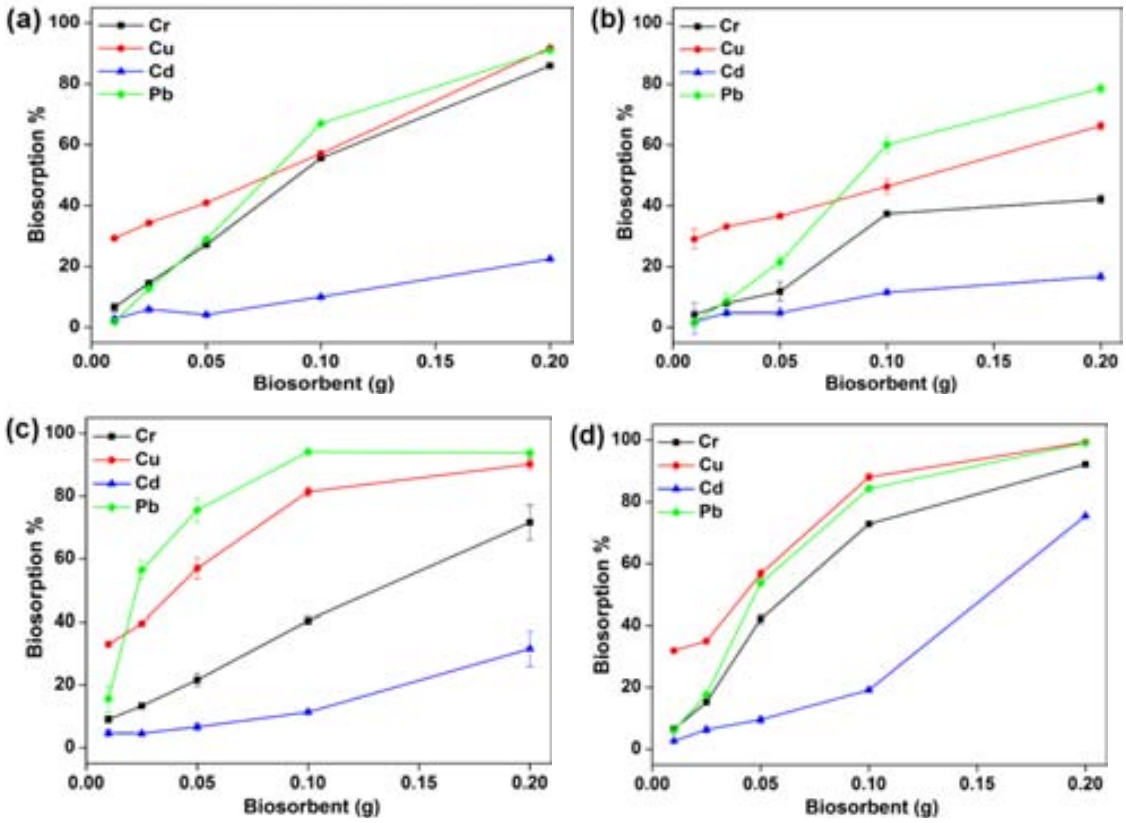

Figure 3. 31. The biosorption percentage of Cr(III), Cu(II), Cd(II) and Pb(II) in the multiple-metal system by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d). The initial metal concentration is 0.18 mmol/L, the contact time is 24 h, and the initial pH is 4.0.

which can be related to a greater number of available metal binding sites.[49] There is a slight sharper increase in the biosorption percentage with increasing biosorbent dosage for Pb(II) ions compared with other metal ions (being Cr(III) closer, Cu(II) and Cd(II) the lowest one). Figure 3.32 (a)-(d) shows the adsorbed metal ion (mmol) per unit weight of biosorbent, where Cu(II) uptake shows a reverse trend to the biosorption percentage. This can be explained due to the fact that at high biosorbent dosage level, the available Cu(II) ions in the aqueous solution are insufficient to cover all the biosorbent sites, meanwhile other metal ions have opportunity to cover the biosorbent sites (related with the different affinities of the biomaterials for each heavy metal ion). So, the adsorbed Pb(II) (mmol) per unit weight of biosorbent increased at first, then decreased slowly with increasing biosorbent dosage, which corresponds to a shaper increase in percentage removal for Pb(II) ions.[23]

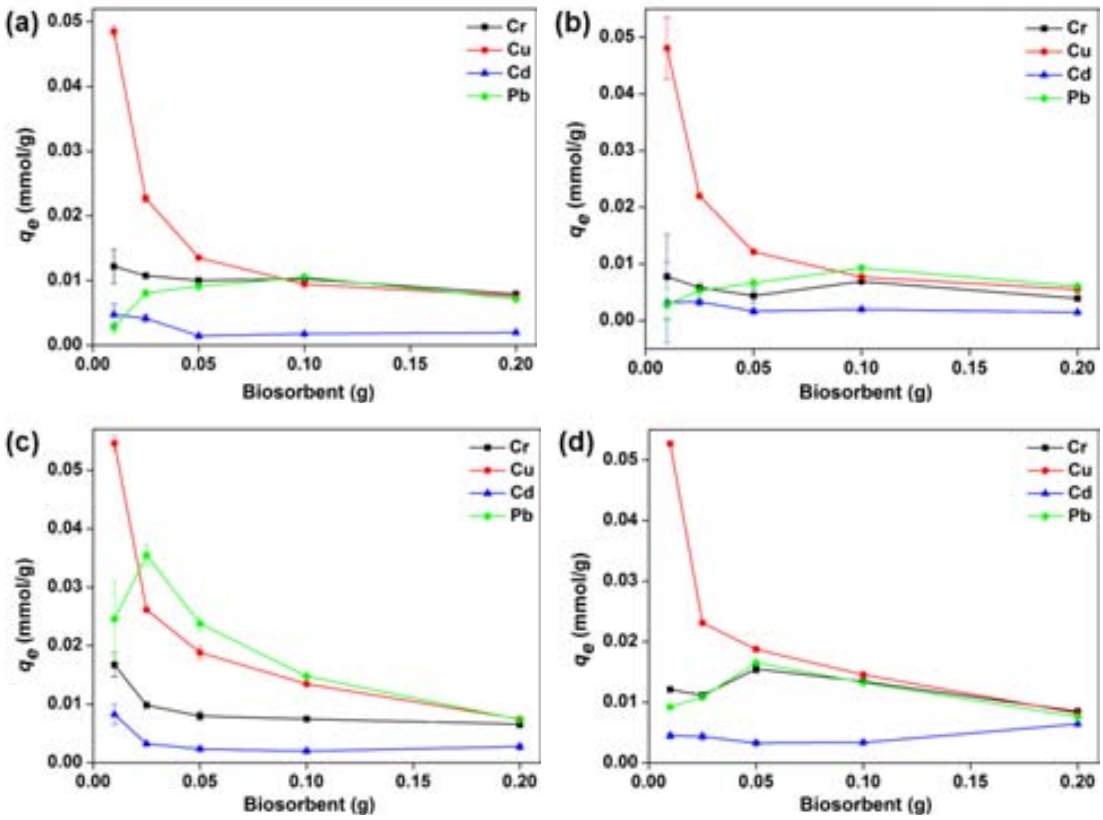

Figure 3.32. The adsorbed metal ion per unit weight of biosorbent for Cr(III), Cu(II), Cd(II) and Pb(II) in the multiple-metal system by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d). The initial metal concentration is 0.18 mmol/L, the initial pH is 4.0, and 24 h of contact time.

These four keratin biomaterials have great biosorption capacities for Cr(III), Cu(II) and Pb(II) ions and there is a competition between metal ions in their biosorption process. The four biosorbents show a preferential binding capacity for Cu(II) ions compared with the other three metal ions in the mixture. The biosorption capacity and removal percentage are equally important in adsorption experiments because both factors usually take part in deciding the adsorption performance of a given adsorbent.[50] The biosorbent dosage of 0.1 g was selected for the further biosorption studies because it showed both relatively high biosorption percentage (%) and high biosorption capacity (mmol/g) for all four biosorbents.

3.3.4 Effect of the contact time

Multiple-metal aqueous system (at pH = 4) of Cr(III), Cu(II), Cd(II) and Pb(II) and single-metal aqueous system of Pb(II) were contacted with the keratin biomaterials (0.1 g of human hair, dog hair, chicken feathers and degreased wool) during 5, 10, 20, 30, 45 minutes, and 1, 2, 3, 4, 6, 12, 24 48 and 72 hours. Results plotted in Figure 3.33 (a)-(d) show the biosorption percentage in each biosorbent case for the removal of Cr(III), Cu(II), Cd(II) and Pb(II) ions from the multiple-metal aqueous solutions. Different biosorbents require different time interval to reach the equilibrium. It is observed that in all cases the biosorption percentage of metal ions increases with the increase of the time until the equilibrium is reached. In general, the biosorption of metal ions consisted of two steps. An initial rapid step where the rate of biosorption is high, and a second slower step where the equilibrium uptake achieves. Moreover, the biosorption process of the multiple-metal system by the four keratin biomaterials is different. The biosorption percentage of Cr(III), Cu(II) and Pb(II) by human hair and dog hair increases gradually with increasing contact time, reaching nearly equilibrium at around 24 h. For the degreased wool only 6 h are needed to reach the equilibrium. In the case of the chicken feathers, the removal of Cu(II) and Pb(II) ions increases sharply with time, and reaches the equilibrium after 30 minutes, being necessary 48 h for the Cr(III) case. All the biosorbents show low removal of Cd(II), even at long time of the biosorption

process, and their maximum of biosorption percentage is about 20%. So, in the subsequent kinetic analysis Cd(II) is not considered for the biosorption modeling onto the four biosorbents.

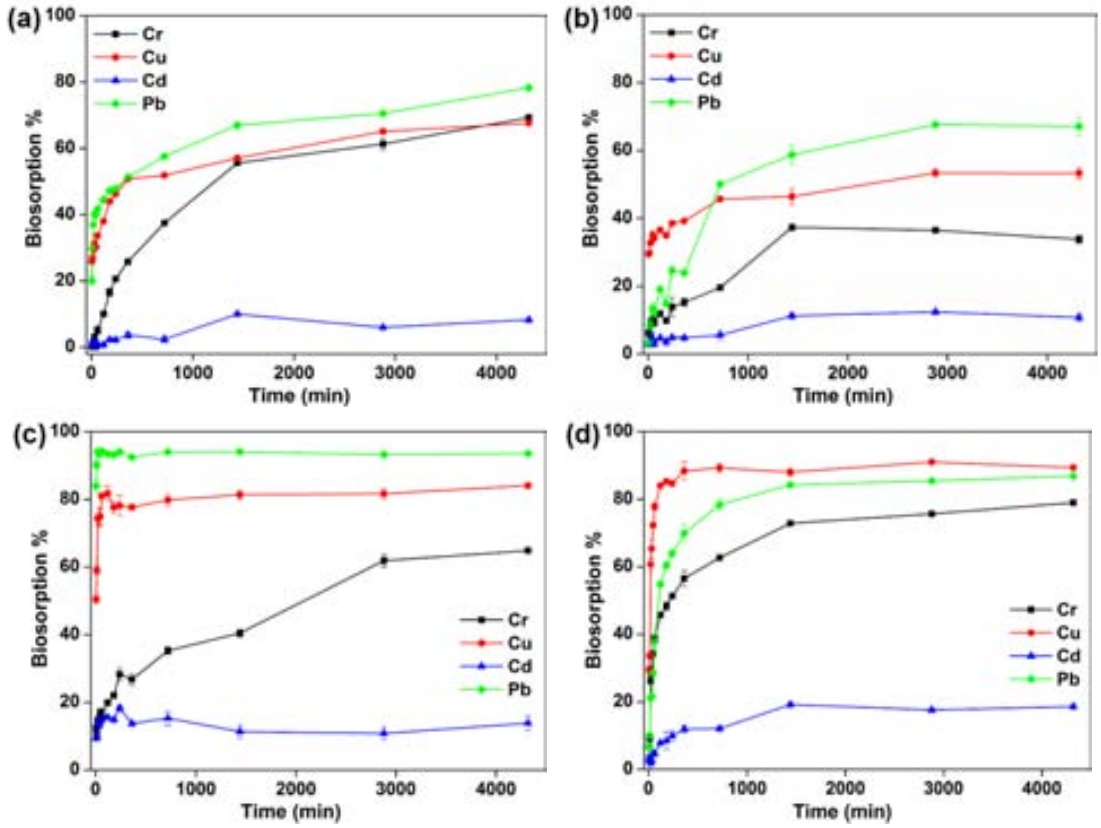

Figure 3.33. The biosorption percentage of Cr(III), Cu(II), Cd(II) and Pb(II) by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d) from multiple-metal system at different contact time. The initial metal ionsconcentration is 0.18mmol/L, the pH is 4.0, and the biosorbent is 0.1 g.

Effect of the contact time is also studied in the single-metal system. The biosorption of Pb(II) by human hair, dog hair, chicken feathers and degreased wool is shown in Figure 3.34. It is observed that an equilibrium time of 24 h is needed for the biosorption of Pb(II) by human hair and dog hair, while 30 and 5 min are enough to attain equilibrium when degreased wool and chicken feathers are used, respectively. The experimental results indicate that the biosorption percentage of Pb(II) by the four biosorbents is greater than 90 % in all cases, and followed the upper order as follows: chicken feathers > degreased wool > human hair > dog hair. This is probably due to the high surface area of the chicken feathers and the partial oxidation of the degreased wool,

which are confirmed by SEM and FT-IR studies, respectively.

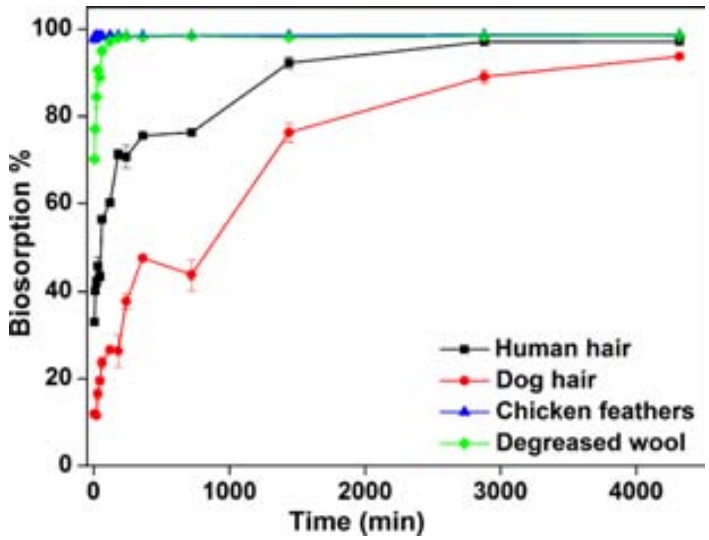

Figure 3.34. Biosorption percentage of Pb(II) by human hair, dog hair, chicken feathers and degreased wool from single-metal system at different contact time. The initial metal concentration is 0.18 mmol/L, the pH is 4.0, and the biosorbent is 0.1 g.

According to the comparison of the biosorption capacities of Pb(II) by the four keratin biosorbents from single- and multiple-metal systems (Figure 3.35), it can be seen that the presence of other metal ions affects the biosorption of Pb(II) onto the biosorbents, as expected, due to metal ion competition to the binding sites of the biosorbent surface.

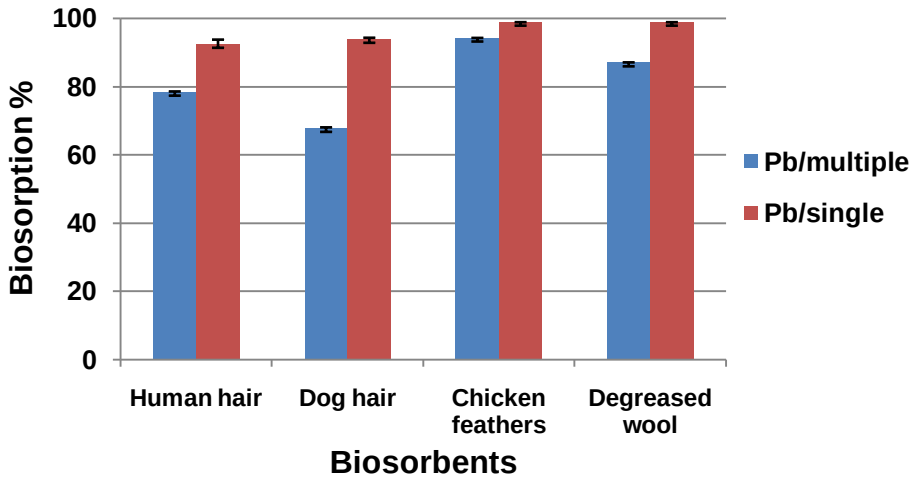

Figure 3.35. Biosorption of Pb(II) by human hair, dog hair, chicken feathers and degreased wool from single- and multiple-metal systems. The initial metal concentration is 0.18 mmol/L, the pH is 4.0, and the biosorbent is 0.1 g.

3.3.5 Biosorption kinetics modeling

Adsorption of a given solute on a solid is a fairly complex mechanism. Indeed, the speed of adsorption is strongly influenced by several parameters such as the status of the solid matrix that has generally heterogeneous reactive sites, and the physical-chemical conditions under which the adsorption takes place.[11] A study of kinetics of adsorption is desirable as it provides information about the mechanism of adsorption, which involved the potential rate controlling steps such as mass transport, pore diffusion and chemical reaction processes. In the present study, the biosorption data are analyzed by using three simple kinetic models for the four keratin biomaterials, which include the pseudo-first order and the pseudo-second order kinetic models and the Weber-Morris intraparticle diffusion model.

The pseudo-first order kinetic model has been most widely used for the adsorption of an adsorbate from an aqueous solution throughout the years, such as metal ions, dyestuffs, and contaminating organic compounds. This model based on solid capacity considers that the rate of occupation of adsorption sites is proportional to the number of unoccupied sites. In general, the pseudo-first order equation could be adequately employed to describe reactions. However, it is typically expressed in a range of reaction only.[16]

In the present case of the biosorption of Cr(III), Cu(II) and Pb(II) ions by different keratin biomaterials, according to the effect of contact time on the biosorption, the equilibrium time is less than 20 min when chicken feathers are used for the biosorption of Pb(II). So the pseudo-first order model cannot be used to characterize the biosorption process in the Pb(II)/chicken feathers, neither in multiple nor in single systems. The plots of the linearized form of the pseudo-first order model (see 2.5.1 Pseudo-first order rate equation Eq. (6)) for the rest of metal/biosorbent systems are shown in Figure 3.36 (a)-(d). The k_1 value, q_e calculated and correlation coefficient is given in Table 3.9.

As shown in Figure 3.36 and Table 3.9, the pseudo-first order equation does not fit well over the entire range of contact time investigated, which is generally applicable

only over the initial time of the sorption process. The calculated q_e by this model differs significantly of those measured experimentally, which is suggesting the insufficiency of the pseudo-first order model to fit the experimental data. An exception was noted for the Cr(III)/human hair system (Figure 3.36 (a)), where the pseudo-first order equation fits well in the whole range of time with high correlation coefficient (0.9472), and the calculated q_e is close to the experimental one (just slightly smaller). A reason for these slightly differences in the q_e values is that there is a time lag, possibly due to the boundary layer at the surface of the biosorbent or the external resistance at the surface controlling the beginning of the sorption process, as mentioned previously. So, like most cases in the literature, this model generally underestimate the q_e values.[16]

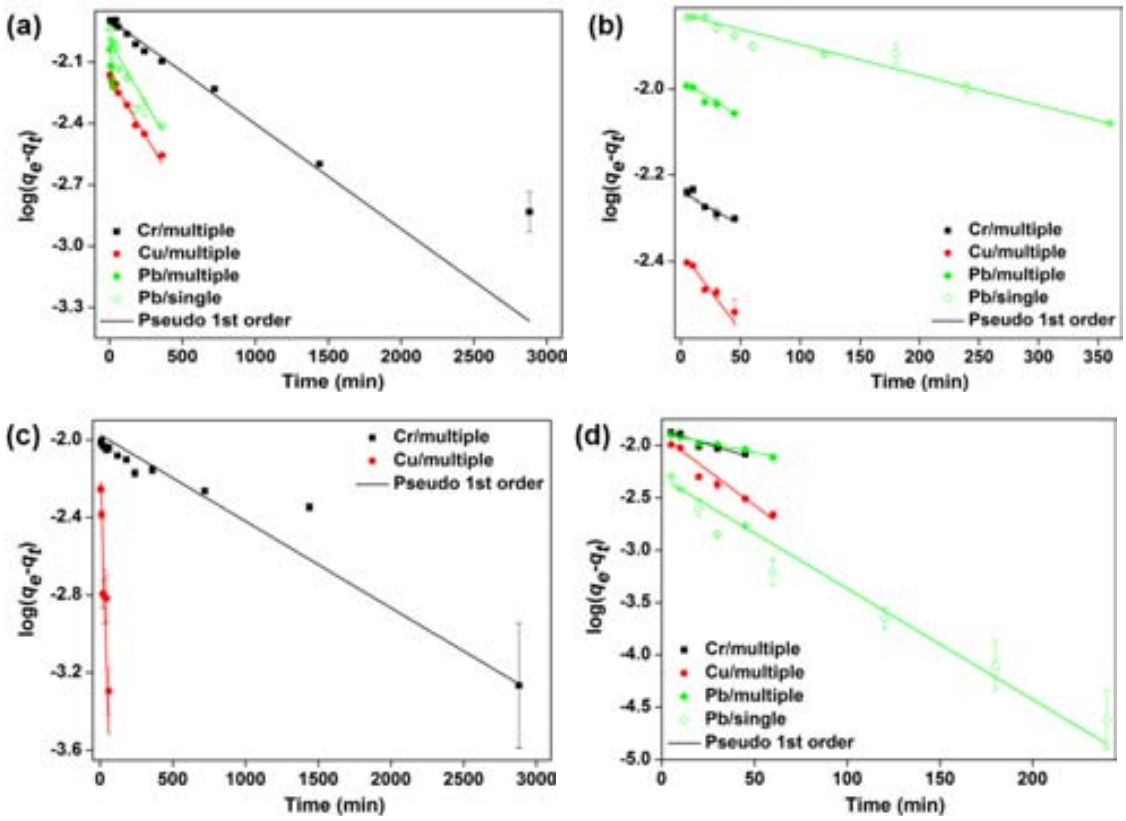

Figure 3.36. The pseudo-first order adsorption kinetics plots for the biosorption of metal ions by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d).

The pseudo-second order model is derived on the basis of the sorption capacity of the solid phase. This model assumes that the rate of occupation of adsorption sites is

proportional to the square of the number of unoccupied sites. The plots t/q_t versus t, for the pseudo-second order model (see 2.5.2 Pseudo-second order rate equation Eq. (9)) applied to the biosorption of Cr(III), Cu(II) and Pb(II) with the different keratin biomaterials, are shown in Figure 3.37 (a)-(d). The k_2 value, q_e calculated and correlation coefficient is given in Table 3.9. Except for the biosorption of Cr(III) by human hair, the pseudo-second kinetic model showed the best fit to the experimental data with the highest correlation coefficients ($R^2 > 0.95$). In addition, as shown in Table 3.11, the pseudo-second order calculated q_e values agree most with the experimental data. Thus, these results suggest that the rate limiting step might be the chemical sorption as previously. Chemical sorption could occur by the functional groups on the biosorbent surface with the metal ions as valence forces through sharing or exchange electrons.

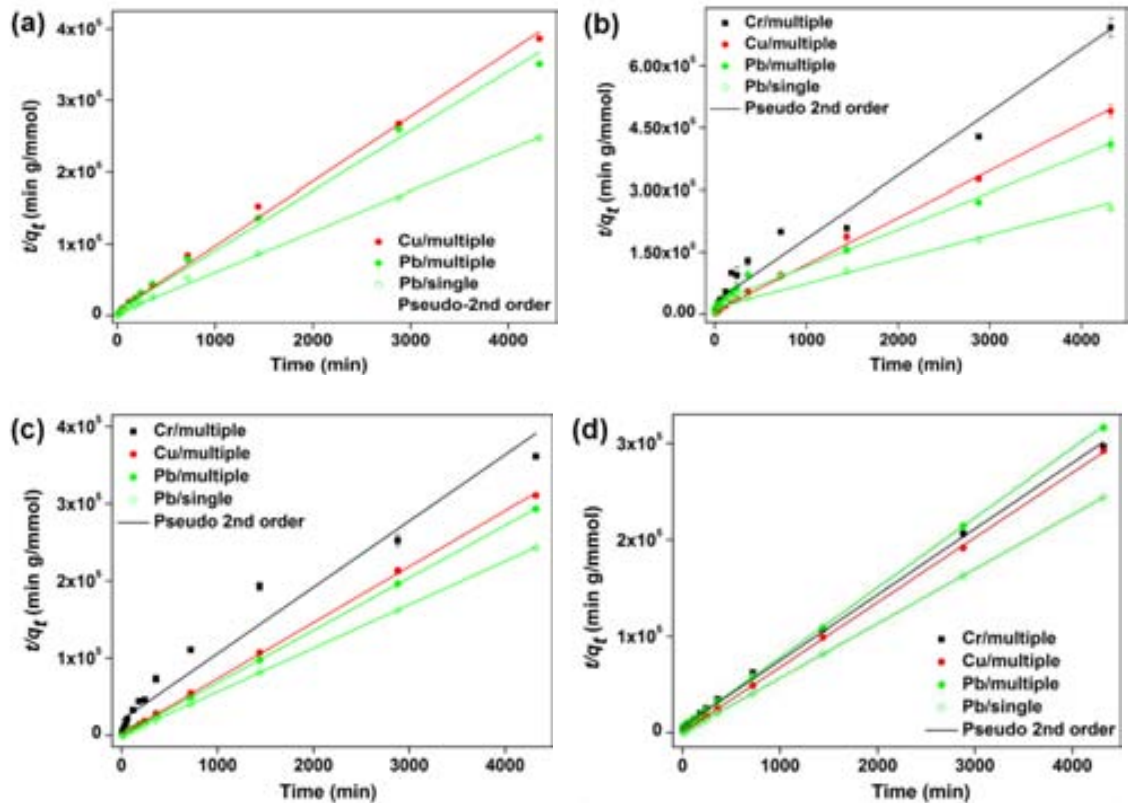

Figure 3.37. The pseudo-second order adsorption kinetics plots for the biosorption of metal ions by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d).

Table 3.11. Biosorption kinetic constants for the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) by human hair, dog hair, chicken feathers and degreased wool.

Biosorbent	Metal	Experimental $q_{max}\times10^2$ (mmol/g)	Pseudo-first order			Pseudo-second order		
			$k_1\times10^3$ (min^{-1})	$q_e\times10^2$ (mmol/g)	R^2	k_2(g/mmol min)	$q_e\times10^2$ (mmol/g)	R^2
Human hair	Cr/multiple	1.278	8045.1[f]	1.177	0.94722	-	-	-
	Cu/multiple	1.118	2.53[e]	0.671	0.98167	1.059	1.115	0.99616
	Pb/multiple	1.231	15.89[a]	0.937	0.866	0.8839	1.207	0.99373
	Pb/single	1.747	3.11[e]	1.021	0.89417	0.8632	1.767	0.99835
Dog hair	Cr/multiple	0.689	3.87[b]	5.88	0.85959	0.6701	0.673	0.97138
	Cu/multiple	0.883	6.72[b]	0.408	0.93641	1.831	0.886	0.99734
	Pb/multiple	1.064	3.8[b]	1.034	0.91988	0.2847	1.137	0.98477
	Pb/single	1.685	1.53[c]	1.47	0.95016	0.2003	1.745	0.97092
Chicken feathers	Cr/multiple	1.196	0.9086[f]	0.972	0.93951	0.3089	1.209	0.95992
	Cu/multiple	1.390	37.47[c]	0.543	0.8354	5.691	1.38	0.99969
	Pb/multiple	1.481	-	-	-	365.39	1.474	0.99999
	Pb/single	1.775	-	-	-	124.74	1.775	1
Degreased wool	Cr/multiple	1.457	10.59[c]	1.347	0.90169	0.7180	1.466	0.99846
	Cu/multiple	1.505	28.17[c]	1.088	0.9414	6.3072	1.489	0.99986
	Pb/multiple	1.366	8.41[c]	1.298	0.95928	0.8826	1.389	0.99991
	Pb/single	1.770	23.08[d]	0.401	0.9501	25.084	1.770	1

a): 30 min, b): 45 min, c) 1 h, d): 3 h, e): 6 h, f): 72 h

According to the kinetic studies of pseudo-first order and pseudo-second order models, the Cr(III)/human hair system was found to fit better to the pseudo-first order equation, while other metal/ biosorbent systems fit better to the pseudo-second order equation. For all the metal/biosorbent systems, the theoretical biosorption capacity (q_t) data calculated by the agreeable kinetic models and the experimental ones are shown in Figure 3.38, where it can be seen that those values are really close each others.

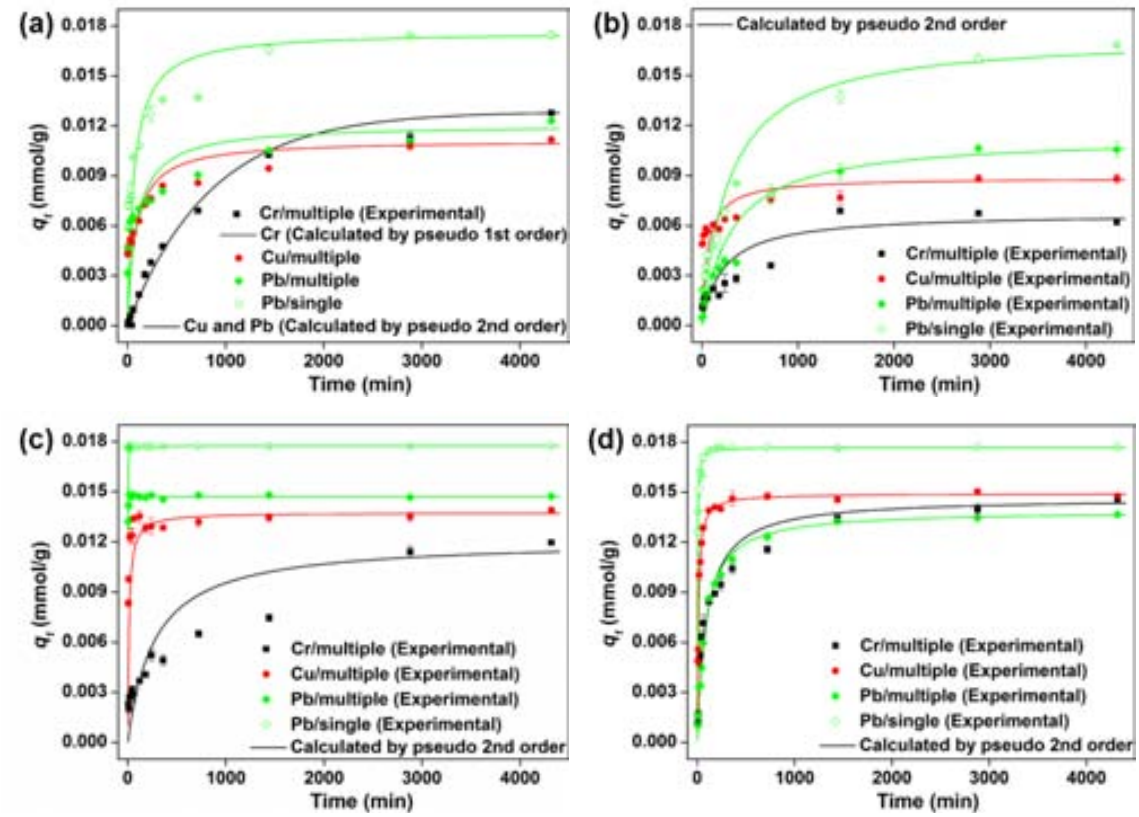

Figure 3.38. Experimental (•) and calculated (—) values adjusted by following the agreeable kinetic model equation: (a) human hair, (b) dog hair, (c) chicken feather and (d) degreased wool.

The intraparticle diffusion equation as described by Weber and Morris[51] may be applied in the determination of the intraparticle diffusion rate constant, k_3, and the boundary resistance, k_d. The plots of q_t versus $t^{1/2}$ for the Weber-Morris intraparticle diffusion model (see 2.5.3 Weber-Morris intraparticle diffusion model Eq. (10)) are shown in Figure 3.39 (a)-(d). From the figure, it was noted that the biosorption process tends to be followed by two or more steps. Before arriving the final biosorption equilibrium, the film diffusion where the metal ions move from the solution to the external surface of biosorbent, and the intraparticle diffusion together control this initial stage of the biosorption process.[52] However, the time required for arriving the final biosorption equilibrium, is totally different for each biosorbent used. For instance, metal ions can be quickly adsorbed onto the chicken feathers and degreased wool compared with the other two biosorbents (human hair and dog hair), especially in Pb(II)/chicken feathers system. This is due to the fact that the mentioned lower cystine content of

chicken feathers makes them more hydrophilic and due to higher surface area compared with the other keratin biomaterials, and due to partial oxidation in the case of degreased wool. They can assist the metal transport from the solution to the external surface of biosorbent. Further, the linear plots do not pass through the origin and this indicates that intraparticle diffusion is not only the rate controlling step, as previously.[53]

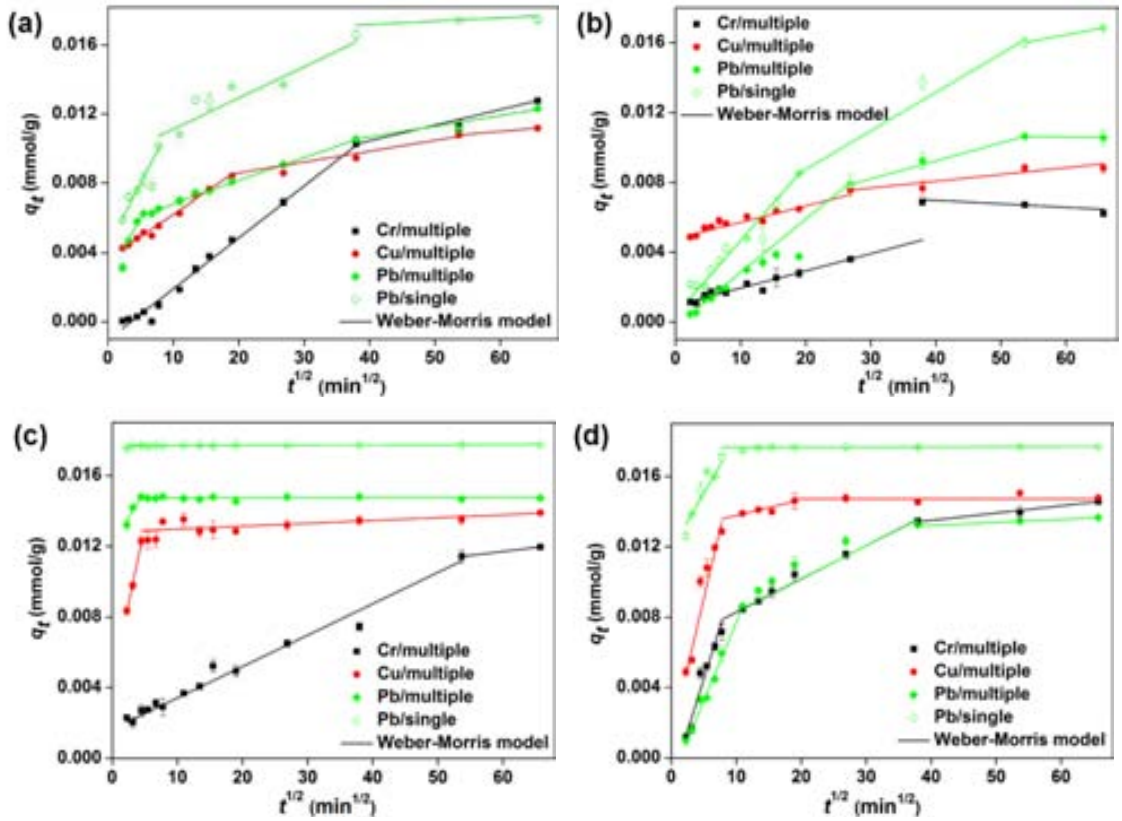

Figure 3.39. Weber-Morris intraparticle diffusion kinetic model applied for the biosorption of metal ions onto human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d).

3.3.6 Biosorption isotherm modeling

The empirical Freundlich equation, based on the multilayer adsorption on heterogeneous surface, can be derived assuming a logarithmic decrease in the enthalpy of adsorption with the increase in the fraction of occupied sites. While Langmuir isotherm assumes that enthalpy of adsorption is independent of the amount adsorbed. A basic assumption of the Langmuir theory is that sorption takes place at specific homogeneous sites within the sorbent, no further sorption can take place at that sites. The Langmuir equation is valid for monolayer sorption on to a surface a finite number

of identical sites. It incorporates two easily interpretable constants: q_{max}, which corresponds to the highest possible adsorbate uptake; and coefficient b, which is related to the affinity between the adsorbent and the adsorbate. Low values of $b$are reflected in the steep initial slope of a adsorption isotherm, indicating a desirable high affinity. Thus, for 'good' adsorbents in general, one is looking for a high q_{max} and a steep initial adsorption isotherm slope (low b).[23]

The biosorption of Pb(II) by human hair, dog hair, chicken feathers and degreased wool is determined in a proper initial metal concentration range at pH 4. The obtained results are analyzed by the well-known Freundlich[24] (see 2.6.1 Freundlich isotherm model Eq. (12)) and Langmuir[25] (see 2.6.2 Langmuir isotherm model Eq. (14)) isotherm models.

The Freundlich and Langmuir parameters, with the respective correlation coefficients R^2, are presented in Table 3.12. It is observed that the equilibrium data are very well represented by the Langmuir isotherm equation with high correlation coefficients (R^2> 0.98) when compared to the Freundlich equation. The Langmuir plots are also represented in Figure 3.40, and the good agreement with the experimental data suggests that the metal ions adsorbed form a monolayer coverage on the biosorbent surface. From the Table 3.12, it is observed that the maximum biosorption capacities of the four biosorbents follows the order chicken feathers (3.87×10^{-5} mol/g) > degreased wool (3.40×10^{-5} mol/g) > human hair (2.43×10^{-5} mol/g) > dog hair (2.07×10^{-5} mol/g) for the Pb(II) at 295K. Also a desirable affinity between the sorbent and the sorbate depending on b value is observed, which follows the order degreased wool > chicken feathers > human hair > dog hair. Both constants usually take part in determining the adsorption efficacy of a given adsorbent. It is quite obvious that chicken feathers are the best biosorbents for the removal of Pb(II) among the four biosorbents.

Table 3. 12. Freundlich a nd La ngmuir i sotherms constants for t he bi osorption o f P b(II) by human hair, dog hair, chicken feathers and degreased wool.

	constant	Human hair	Dog hair	Chicken feathers	Degreased wool
Freundlich	$K_F \times 10^4$	4.89	3.50	8.38	9.0
	n	2.51212	2.33918	2.5227	2.45592
	R^2	0.75742	0.84984	0.67664	0.65263
Langmuir	$Q_0 \times 10^5$ (mol/g)	2.4281	2.06544	3.8719	3.4049
	$b \times 10^{-4}$ (L/mol)	4.4757	0.9155	8.8388	12.0861
	K_L (L/g)	1.087	0.189	3.422	4.115
	R^2	0.99899	0.98991	0.99982	0.99989
	$-\Delta G^0$ (kJ/mol)	26.265	22.373	27.934	28.702

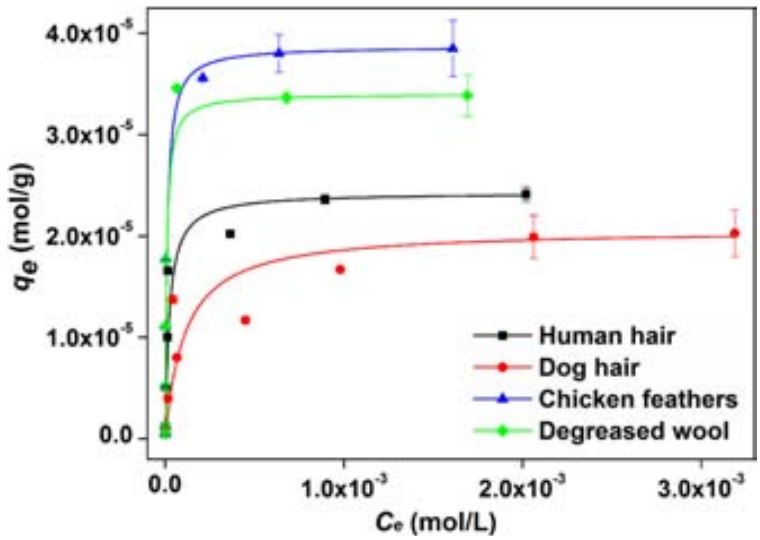

Figure 3. 40. Experimental (•) a nd ca lculated (—) v alues adjusted by usi ng t he La ngmuir isotherm model for the biosorption of Pb(II) by using human hair, dog hair, chicken feathers and degreased wool.

In addition, from the estimated Langmuir adsorption constant, the standard Gibb's free energy (ΔG^0, see 2.6.3 Thermodynamic parameter Eq. (15), $\Delta G^0 = -RT\ln b$) of the biosorption process can be evaluated. The standard Gibb's free energy (ΔG^0) values are shown in Table 3.10. The negative ΔG^0 values indicated that the biosorption of metals onto the four keratin biomaterials is thermodynamically feasible and of spontaneous nature, as expected from previous case studies.

3.3.7 Desorption, regeneration and reuse studies

Desorption results for all the Pb(II) loaded keratin biomaterials with EDTA and HNO_3 solutions as eluents are collected in Table 3.13. As seen previously, for native human hair (the same with **H1**), the elution efficiency of HNO_3 solution is higher than the EDTA, being 76% and 48%, respectively. On the contrary, the recovery of Pb(II) from degreased wool with EDTA is better than that with HNO_3. The wool samples here used are degreased ones, being oxidized under this degreased process which leads to an efficient desorption with EDTA. This result is similar to the treated human hair as indicated previously. In the case of chicken feathers, the elution efficiency of EDTA is more effective as well as HNO_3. This can be explained that the lower content of cystine in chicken feathers leads to a lower stability and higher hydrophilicity comparing with the other keratin fibers.

Following the same procedures of regeneration and reuse as before, it is found that the regeneration of these four biosorbents with EDTA and rinsing with deionized water is favorable to reuse them for the biosorption of metal ions. The results also indicate that the second biosorption capacities of these four biosorbents, after their regeneration with EDTA decrease compared with the first time. The human and dog hairs are deprived of a half of their biosorption capacities, and the biosorption capacities of chicken feathers and degreased wool also decreased from 97% to around 70%.

Table 3.13. The elution of adsorbed Pb(II) by using EDTA and HNO_3 solutions.

Biosorbent	Metal adsorbed (%)	Pb(II) elution efficiency (%) using EDTA (0.1 M)	Pb(II) elution efficiency (%) using HNO_3 (0.1 M)
Human hair	80 ± 1	48 ± 1	76 ± 1
Dog hair	89 ± 2	62 ± 2	61 ± 1
Chicken feathers	98 ± 1	100 ± 2	100 ± 1
Degreased wool	97 ± 1	88 ± 4	67 ± 1

3.3.8 Conclusions

In this part, four keratin biomaterials including human hair, dog hair, chicken feathers and degreased wool have been used successfully for the removal of heavy

metals from aqueous solutions. The FT-IR analysis reveals that carbonyl, hydroxyl, amino and sulfur-containing groups as the major functional groups that act as biosorption sites for metal ions in such keratin biomaterials. Biosorption capacity is affected by various parameters such as the pH of the initial aqueous solution, the biosorbent dosage and the contact time. The aqueous pH is found to be a critical parameter in the biosorption processes, with an optimum pH being 4.0 in all the cases. The increase in the biosorbent dosage leads to an increase in the metal removal owing to the corresponding increase in the number of metal binding sites. In addition, the four keratin biomaterials exhibit good biosorption capacities in multiple-metal system in terms of total biosorption capabilities. In general, the experimental data fits well the pseudo-second order kinetic model. Even in the case of Cr(III) biosorption by human hair, the experimental data fits well the pseudo-first order kinetic model during the whole biosorption process. Kinetic results of biosorption indicate that chemical sorption is the basic mechanism in these systems. Equilibrium data fits very well in a Langmuir isotherm equation, confirming the monolayer sorption of the metal ions onto the biosorbent surface. The maximum biosorption capacities of Pb(II) by human hair, dog hair, chicken feathers and degreased wool are obtained as follows 2.43×10^{-5}, 2.07×10^{-5}, 3.87×10^{-5}, and 3.40×10^{-5} mol/g, respectively, at optimum pH (4.0) and temperature (295 K). The calculated standard Gibb's free energy (ΔG^{0}) indicates the thermodynamically feasible and spontaneous nature of the biosorption process. These four keratin biomaterials regenerated with EDTA rinsing with deionized water exhibit good biosorption capacities, even though the corresponding second biosorption capacities of these four regenerated biosorbents decrease compared with them used for a first time.

Taking into consideration present findings, these four keratin biomaterials, being cheap and easily available materials, could be an alternative to more costly adsorbents used really for heavy metals removal in wastewater treatment processes.

3.4 The chemically-modified keratin biomaterials as biosorbents for the removal of heavy metals

From the above parts, it was found that the keratin biomaterials can be used as potential biosorbents for the removal of heavy metals. Also, it has been seen that proper chemical treatment of the human hair (oxidation) can enhance the biosorption capacity of the native hair. So, the same chemical modification method is used to treat the keratin biomaterials used, including dog hair, chicken feathers and degreased wool.

The biosorption percentage results of heavy metals using the selected chemically-modified keratin biomaterials are shown in Figure 3.41. As seen from the figure, the metal biosorption percentages when using treated human and dog hair samples are significantly better than the untreated ones (see Figure 3.41 (a) and (b)). In the case of the chicken feathers and the degreased wool, this chemical modification did not enhance the biosorption capacities of them (see Figure 3.41 (c) and (d)). Moreover, the affinity of all type of keratin biomaterials for Cr(III), Cu(II) and Pb(II) is greater than that for the rest of metal ions in solution, even in the case of treated human and dog hair a significant increase on their affinity to the rest of the heavy metal ions checked is observed.

In the case of chicken feathers, the mentioned lower cystine content compared with the other keratin biomaterials, give them too low stability to bear the chemical oxidation conditions. Probably almost no modification was undergoing. For degreased wool, this oxidation condition is not effective to oxidize the cystine of degreased wool, although the partial oxidation occurred in the previously degreasing process (it can be confirm by FT-IR, in the 3.3.1 FT-IR and SEM characterization).

Therefore, like the treated human hair, the treated dog shows the better biosorption capacity than the untreated one. Furthermore, the chemical modification did not improve the biosorption capacities of chicken feather and degreased wool.

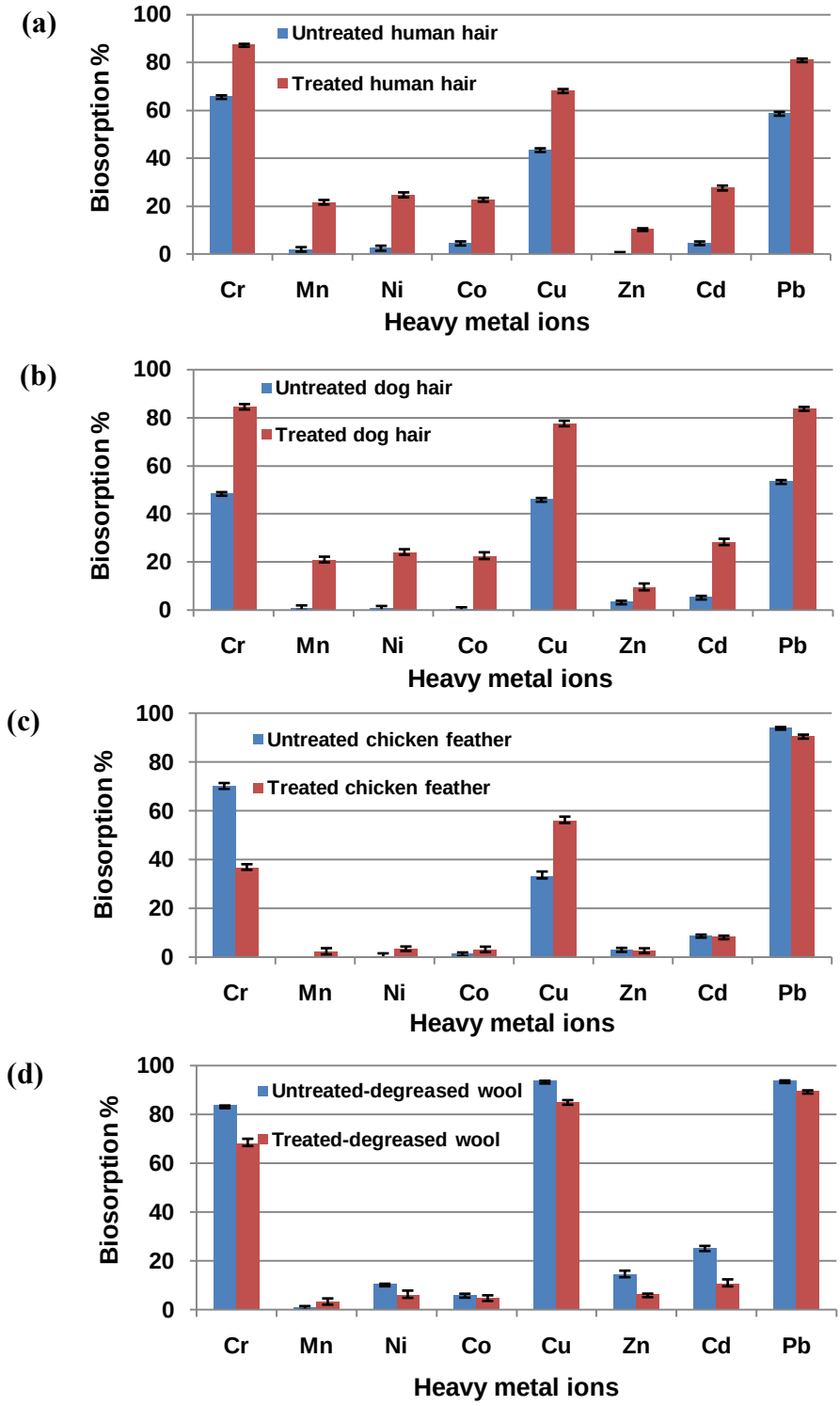

Figure 3.41. Comparison of the biosorption capacity between untreated and treated biosorbents in multiple-metal system: (a): human hair; (b): dog hair; (c): chicken feathers; (d): degreased wool. The initial metal concentration is 0.1 mmol/L, the contact time is 24 h, the pH is 4.0, and the biosorbent is 0.1 g.

Subsequently, four metal ions including Cr(III), Cu(II), Cd(II) and Pb(II) are selected from those metals to better known the biosorption mechanism with chemically-treated dog hair. Therefore, the influences of different experimental parameters on metal uptake, such as contact time, pH of the solution and biosorbent dosage are investigated with the chemically-modified dog hair. Also, the kinetic study is included for a good understanding of the reaction mechanism, and the Langmuir and Freundlich models are tested to determine the adsorption isotherms. The desorption, regeneration and reuse of the treated dog hair are also studied in the same way as previously.

3.4.1 Biosorption of heavy metals by chemically modified dog hair

3.4.1.1 FT-IR and SEM characterization

The untreated, treated and metal loaded-treated dog hairs are discriminated by Fourier transform infrared (FT-IR) spectroscopy, as can be seen from the infrared spectra collected in Figure 3.42. The full-scan spectra of dog hairs (Figure 3.42 (a)) display their corresponding infrared peaks, which contain the typical hair fiber amino acids bands including Amide I, Amide II and Amide III. The peaks at 1041, 1075, 1170 and 1230 cm^{-1} all corresponding to different products of cystine oxidation in dog hair, the intensity of the peaks at 1041 and 1170 cm^{-1} increases after chemical pretreatment. The FT-IR spectra of the treated dog hair and metal loaded-treated dog hair are very similar, indicating that the main functional groups on the treated dog hair did not change during the biosorption process.

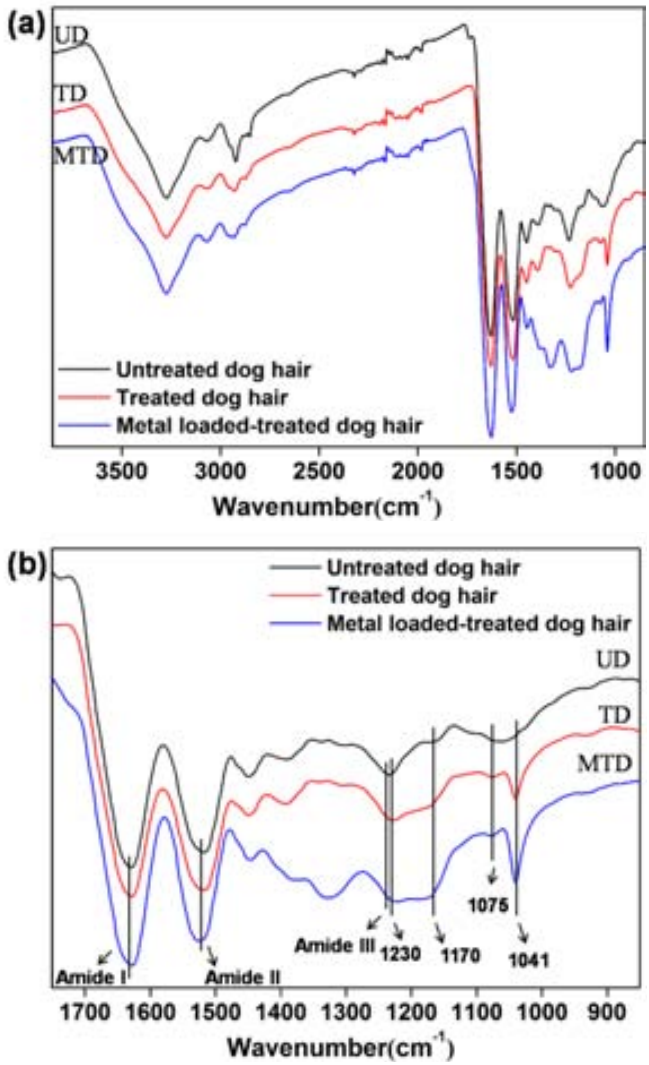

Figure 3. 42. FT-IR spectra of dog hair. UD: untreated dog hair, TD: treated dog hair, MTD: metal loaded-treated dog hair. (a) full-scan spectra, and (b) partial-scan spectra.

Scanning electron microscope (SEM) technique is also applied to concern about the alteration of the dog hair surface morphologies in the different cases of study. Figure 3.43 shows the SEM micrographs of the untreated (a), treated (b) and metal loaded-treated (c) dog hair. It is observed that the majority of the cuticle scales of the treated dog hair represent more jogged appearance than the untreated dog hair, probably due to the chemical oxidation treatment (see Figure 3.43 (a) and (b)). However, the surface of the metal loaded-treated dog hair appears to be smoother comparing prior its use, probably due to the acidic conditions of the ion metal aqueous media (see Figure 3. 43 (b) and (c)).

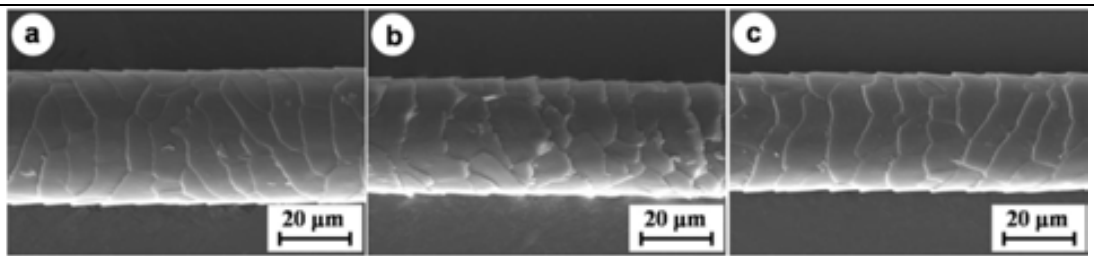

Figure 3.43. Scanning electron microscopy (SEM) micrographs of the dog hair: (a) correspond to the untreated dog hair; (b) correspond to the treated dog hair; and (c) correspond to the metal loaded-treated dog hair.

3.4.1.2 Effect of the initial aqueous solution pH

The effect of the pH of the metal aqueous solution on the biosorption efficacy of the treated dog hair for Cr(III), Cu(II), Cd(II) and Pb(II) ions is investigated. The initial pH is ranged from pH 1.0 to 6.0 in multiple-metal system. As observed from the results collected in Figure 3.44, the metal biosorption percentage increases when increasing the pH value. At very low pH values, metal ions adsorption is found to be very low, probably due to the competition between H^+ and metal ions for the adsorption sites, where the adsorbent surface acquired a positive charges and hence inducing repulsive forces, as previous case studies behaviour.[54] Increasing in the aqueous pH, the negative charge density on the biosorbent surface increases due to deprotonation of the binding sites and thus metal biosorption increases.[39] Over pH 5.0, metal hydroxylation can occur. Therefore, the pH 4.0 was selected as optimal condition in the subsequent experiments.

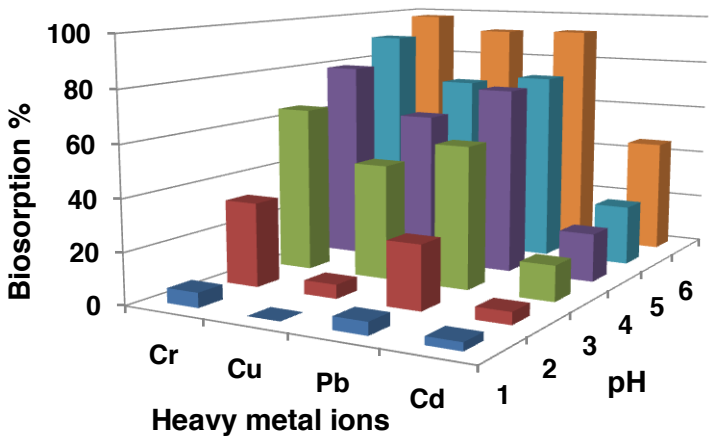

Figure 3.44. Effect of the initial pH on the biosorption of the treated dog hair for Cr(III), Cu(II), Cd(II) and Pb(II) in the multiple-metal system. The initial metal concentration is 0.18 mmol/L, the contact time is 24 h, and the biosorbent is 0.1 g. Experimental errors found were less than 2% in biosorption percentage

3.4.1.3 Effect of the biosorbent dosage

The influence of the biosorbent dosage on the removal efficiency for Cr(III), Cu(II), Cd(II) and Pb(II) ions is studied in the range of 0.01 – 0.2 g of biosorbent in multiple-metal system (Figure 3.45). It is apparent that the biosorption percentage of heavy metals increases rapidly with the increase in the biosorbent dosage. This can be related to the availability of more binding sites for complexation of metal ions.[55] However, the biosorption of Cr(III) and Pb(II) ions, in mmols per unit weight of biosorbent shows a reverse trend to the percentage biosorption.[38] When increasing the biosorbent dosage from 0.01 to 0.2 g, q_e of Cr(III) and Pb(II) ions significantly decrease from 0.026 to 0.0077 mmol/g and from 0.031 to 0.0083 mmol/g, respectively. It can be related to the availability of metal ions (Cr(III) and Pb(II)) in the aqueous solution, which could be insufficient to cover all the biosorbent sites at high biosorbent dosage levels. Meanwhile Cu(II) and Cd(II) ions have opportunity to cover the biosorbent sites. So, the metal binding sites on the treated dog hair have stronger affinity for Cr(III) and Pb(II) than Cu(II) and Cd(II).

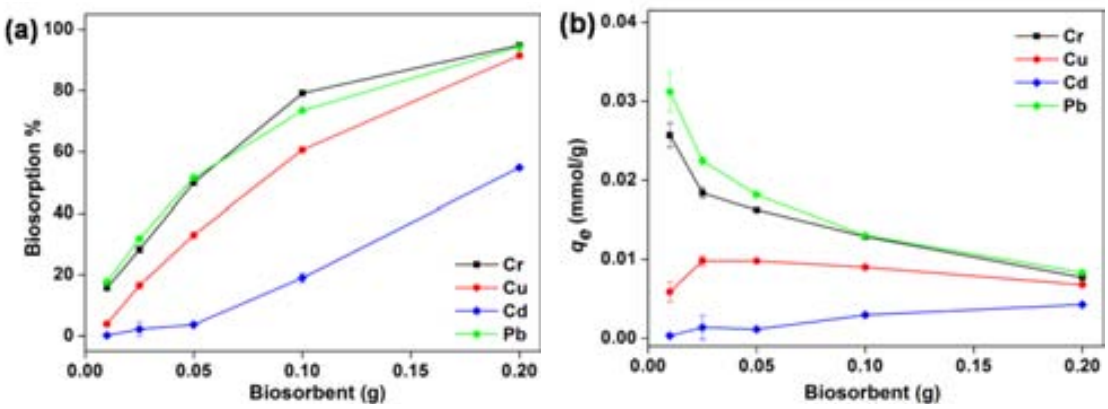

Figure 3.45. Effect of the biosorbent dosage on the biosorption of the treated dog hair for Cr(III), Cu(II), Cd(II) and Pb(II) ions in the multiple-metal system. The initial metal concentration is 0.18 mmol/L, the contact time is 24 h, and the initial pH is 4.0.

3.4.1.4 Effect of the contact time

Figure 3.46 shows the effect of contact time on the biosorption of the treated dog hair for the removal of Cr(III), Cu(II), Cd(II) and Pb(II) ions from single- and multiple-metal systems. As expected in both cases, the percentage of metal biosorption

increases with the increase of the contact time between the metal aqueous solution and biosorbent. The biosorption is fast at the initial time period, especially in singe-metal system, then it goes slower until the equilibrium uptake is achieved. For the removal of Cd(II), Cu(II) and Pb(II), it needs 3 h to reach the equilibrium. However, the removal of Cr(II) increases gradually up to 72 h in the multiple-metal system and 48 h in the single-metal system, when the equilibrium is reached.

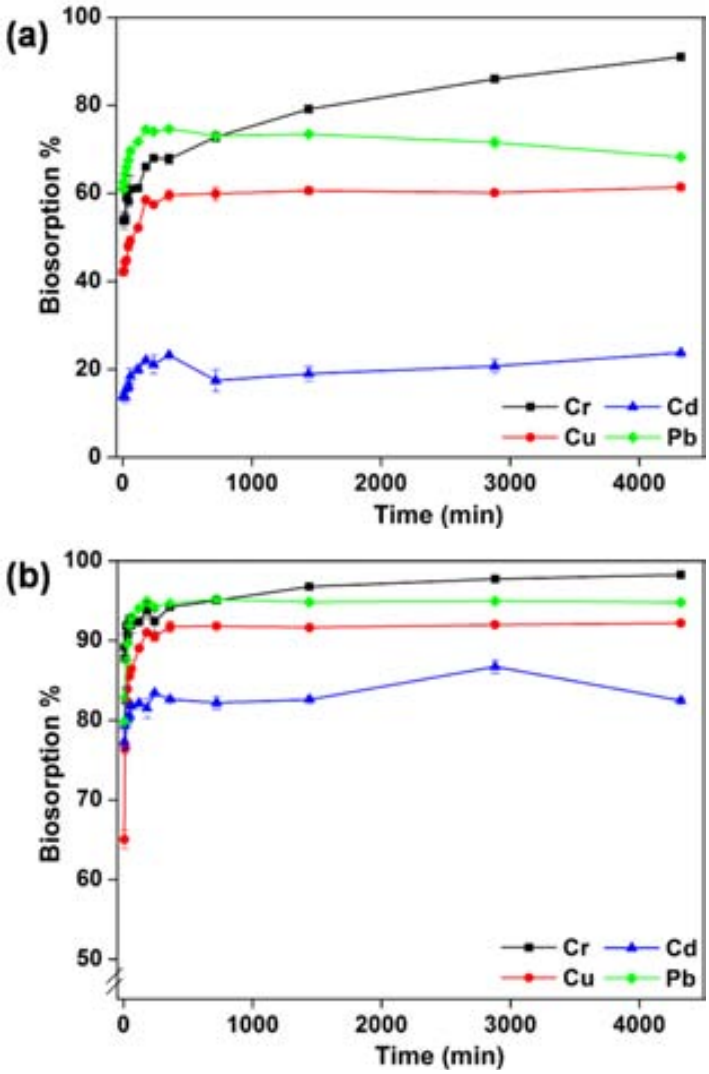

Figure 3.46. Biosorption percentage the treated dog hair for Cr(III), Cu(II), Cd(II) and Pb(II) at different contact time. (a): single-metal system, (b): multiple-metal system. The initial metal concentration is 0.18 mmol/L, the pH is 4.0, and the biosorbent is 0.1 g.

Figure 3.47 shows the comparison of the maximum metal biosorption percentage using the treated dog hair for Cr(III), Cu(II), Cd(II) and Pb(II) ions from single- and

multiple-metal systems. It can be seen that in the single-metal system, for the biosorption capacity of the individual metal ion solutions, higher biosorption capacities are found than the multiple-metal system (especially for the Cd(II) case, with a variation of 24% to 87% in the multiple- and single-metal systems, respectively), as expected due to the metal competition for the binding sites of the treated dog hair.

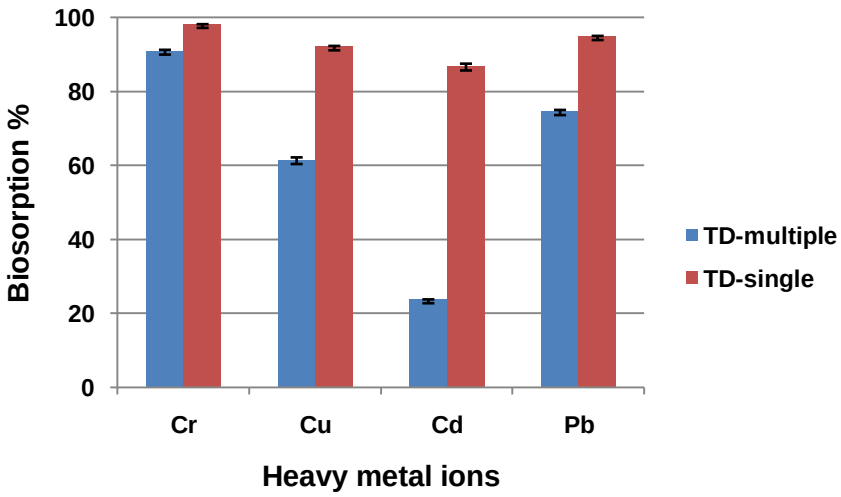

Figure 3.47. Comparison of biosorption the treated dog hair for Cr(III), Cu(II), Cd(II) and Pb(II) from multiple- and single-metal systems. The initial metal concentration is 0.18 mmol/L, the initial pH is 4.0, and the biosorbent is 0.1 g, after 72 h.

3.4.1.5 Biosorption kinetics modeling

The kinetic models including the pseudo-first order equation (see 2.5.1 Pseudo-first order rate equation Eq. (6)), pseudo-second order equation (see 2.5.2 Pseudo-second order rate equation Eq. (9)) and the Weber-Morris intraparticle diffusion model (see 2.5.3 Weber-Morris intraparticle diffusion model Eq. (10)) were tested for the present biosorption process, as before. The rate constants (k), correlation coefficient (R), and the calculated q_e are shown in Table 3.14. From linear test regression presented in Figure 3.48, it can be concluded that the experimental data fitted well the pseudo-second order kinetics with high correlation coefficients (R^2> 0.99), which indicated that chemical sorption is the rate controlling step, as expected. The calculated q_t showed a good correlation with the experimental q_t values for all four metal ions in both system (see Figure 3.49).[56] The equilibrium biosorption capacities of the treated

dog hair for Cr(III), Cu(II), Cd(II), and Pb(II) ions were 0.0166, 0.0169, 0.0160, and 0.0172 mmol/g, respectively in single-metal system, and 0.0146, 0.0091, 0.0035, and 0.0130 mmol/g, respectively in multiple-metal system.

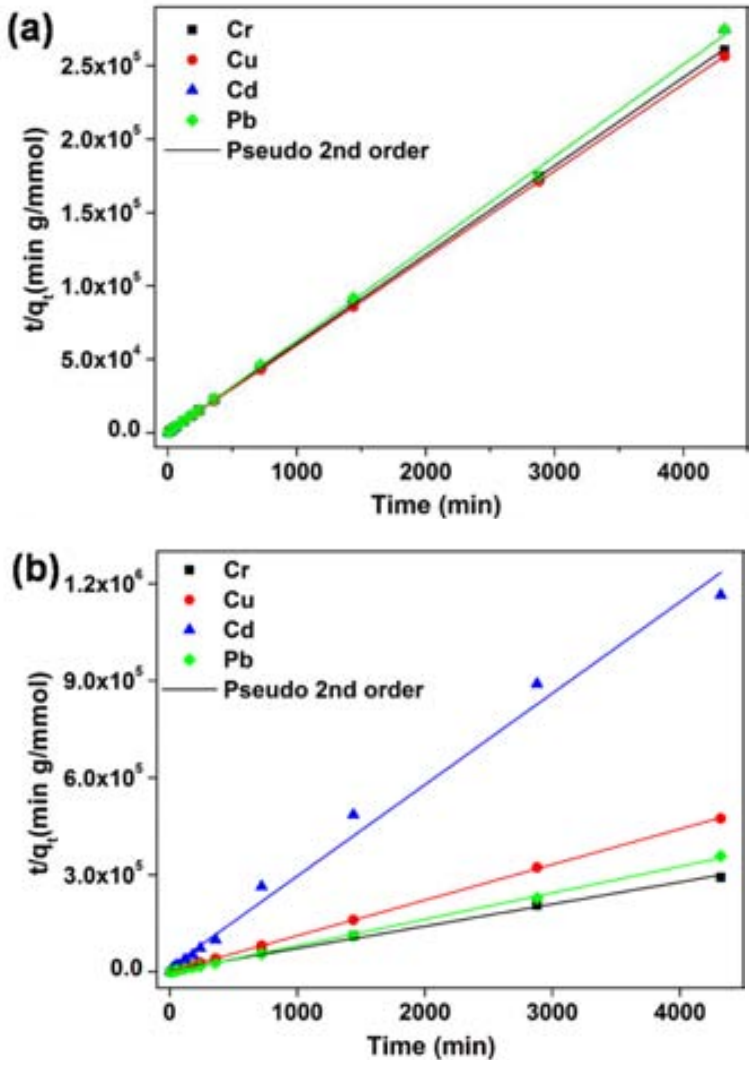

Figure 3.48. The pseudo-second order adsorption kinetics plots for the biosorption of metal ions by the treated dog hair at different contact time. (a): single-metal system, (b): multiple-single system.

Table 3.14. Biosorption kinetic constants in the multiple- and single-metal systems for Cr(III), Cu(II), Cd(II) and Pb(II) for both, Pseudo-second order models.

Metal		Cr(III)		Cu(II)		Cd(II)		Pb(II)	
system		single	multiple	single	multiple	single	multiple	single	multiple
q_e (mmol/g, experimental)		0.01658	0.01482	0.01686	0.00912	0.01654	0.00371	0.01721	0.01317
Pseudo-second order	k_2(g/mmol min)	7.46	1.13	17.50	8.90	126.46	5.93	67.00	53.41
	q_e (mmol/g)	0.01657	0.01464	0.01686	0.00909	0.0160	0.00354	0.0172	0.0130
	R^2	0.99996	0.99685	1	0.99988	0.99928	0.99001	1	0.99906

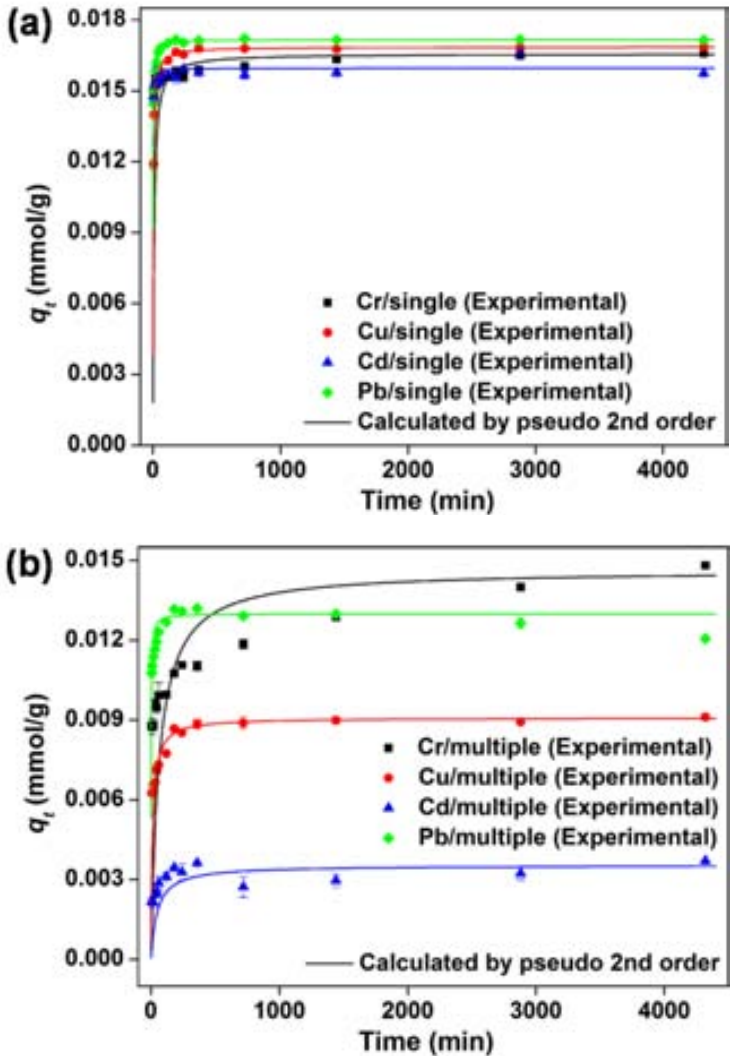

Figure 3. 49. Experimental (•) a nd c alculated (—) v alues adjusted by f ollowing t he pseudo-second o rder m odel equation: (a) i n t he si ngle-metal sy stem, a nd (b) i n t he multiple-metal system.

3.4.1.6 Biosorption isotherm modeling

The re moval o f P b(II) by t he treated do g ha ir has b een s tudied at the di fferent initial metal concentration t o determine t he biosorption isotherms a nd thermodynamic

parameters. Two well known adsorption isotherm models were again tested in the present study namely Freundlich[24] (see 2.6.1 Freundlich isotherm model Eq. (12)) and Langmuir[25] (see 2.6.2 Langmuir isotherm model Eq. (14)). The constant and correlation coefficient values obtained by the models are summarized in Table 3.15 as follows:

Table 3.15. Freundlich and Langmuir isotherms constants for the biosorption of Pb(II) by the treated dog hair.

	constant	Pb(II)
Freundlich	$K_F \times 10^3$	0.9103
	n	2.687
	R^2	0.90886
Langmuir	$Q_0 \times 10^5$ (mol/g)	5.792
	$b \times 10^{-4}$ (L/mol)	8.892
	K_L (L/g)	5.150
	R^2	0.99962
	$-\Delta G^0$ (kJ/mol)	27.949

It can be seen that the correlation coefficient R^2 is close to the unity for Langmuir model, indicating monolayer coverage by the Pb(II) ions on the treated dog hair surface, as found before. In Figure 3.50 experimental and calculated data for the Langmuir isotherm model are represented showing good correlation between them. The maximum biosorption capacity of the treated dog hair for Pb(II) at 295 K were 5.79×10^{-5} mol/g. The standard Gibb's free energy (ΔG^0, see 2.6.3 Thermodynamic parameter Eq. (15) $\Delta G^0 = -RT \ln b$) value is also shown in Table 3.16. The negative ΔG^0 values indicated that the biosorption of metal ions onto the treated human dog is thermodynamically feasible and spontaneous nature, as for all the previous cases.

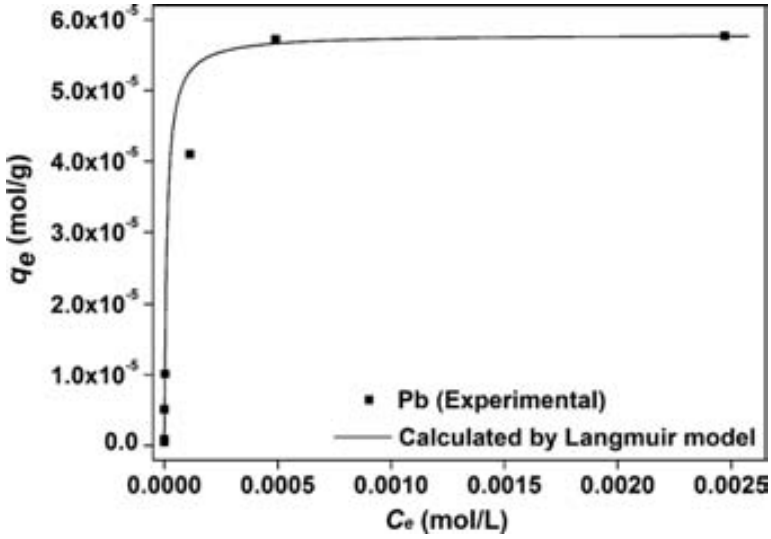

Figure 3.50. Experimental (•) and calculated (—) values adjusted by the Langmuir isotherm model for the biosorption of Pb(II) by using the treated dog hair.

3.4.1.7 Desorption, regeneration and reuse studies

The adsorbed Pb(II) ions are eluted from the treated dog hair using two eluents (EDTA and HNO_3 solutions). The results indicate that both solutions are able to effectively elute the adsorbed Pb(II) ions (both near 100%). In addition, the elution efficiency of the treated dog hair with EDTA is better compared with native one (about 60%) as previously indicated.

The EDTA eluted sample was showing the best biosorption capacity for Pb(II) when it was used a second time, which supports the previous results. Even though, the biosorption capacity of the treated dog hair regenerated with EDTA decreased compared with the first time use, which decreased from 95% to 75%.

3.4.2 Conclusions

Comparing the biosorption capacity of the four treated keratin biomaterials including human hair, dog hair, chicken feathers and degreased wool, it was found that the treated human hair and the treated dog hair show better biosorption capacities comparing with the untreated ones, respectively. However, theselected chemical modification does not improve the biosorption capacities of chicken feathers and degreased wool, which is related to the condition of the modification reaction and the

properties of the keratin biomaterials themselves, as mentioned (high surface area for chicken feathers and partial oxidation of the wool when degreased).

Since the treated dog hair shows the better biosorption capacity than the native one, its biosorption mechanism is characterized, investigating the effects of various parameters such as contact time, pH of the solution and biosorbent dosage, as in previous cases. Biosorption of heavy metals is also highly dependent on the pH of the solution, being the optimum biosorption capacity determined at pH 4.0. The increase in the mass of the biosorbent leads to an increase in the metal removal, related to the corresponding increase in the number of metal binding sites in the biosorbent surface. In addition, the treated dog hair shows higher biosorption capacity in the multiple-metal system in terms of total biosorption capacity, as expected. According to the kinetic study, the biosorption of heavy metals onto the treated dog hair followed well also the pseudo-second order kinetic model and it is in agreement with the chemical sorption being the rate controlling step. Biosorption at equilibrium is better correlated with Langmuir isotherm model compared to Freundlich model, as previous cases. The maximum biosorption capacity of the treated dog hair for Pb(II) by the Langmuir isotherm model is 5.79×10^{-5} mol/g at 295 K. The calculated standard Gibb's free energy (ΔG^0) also indicates the thermodynamically feasible and spontaneous nature of the biosorption process in this case (as previously). EDTA and HNO_3 can elute Pb(II) ions from the metal loaded-treated dog hair, and the regeneration of the treated dog hair with EDTA is more beneficial to its reuse for the biosorption of lead than nitric acid.

Taking into consideration present findings, the oxidation treatment would be quite useful in modifying the hair fibers to enhance the removal of heavy metal ions from contaminated effluents by using the treated dog hair, as it is the case for the previous human hair system.

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Conclusions

4 Conclusions

In this study, different kinds of keratin biomaterials have been tested as heavy metal biosorbents and their biosorption performance has been examined using batch adsorption methods. It can be stated that all the common keratin biomaterials here checked (human hair, dog hair, chicken feathers and degreased wool) show satisfactory biosorption heavy metal capacity, so they can be considered as effective biosorbents for the removal of heavy metal ions from aqueous solutions. The chemical oxidation treatment applied has been demonstrated to be useful in the modification of the hair fibers (i.e. human and dog hairs) to enhance the removal of heavy metal ions from contaminated effluents comparing with the native ones.

The biosorption capacities of the native and treated selected keratin biomaterials for heavy metal ions have been checked at different pH values of the initial aqueous solution, varying the biosorbent dosage, checking different contact time periods, and also varying the initial metal concentration (in single and multiple-metal systems). The experimental data were characterized by the kinetics and isotherm models.The main conclusions are presented in the following.

(1). Characterization of the keratin biomaterials by FT-IR and SEM

FT-IR analysis has been carried out to identify the functional groups of the biosorbents surface. It is found that those keratin biomaterials contain their typical amino acids including Amide I, Amide II and Amide III and cystine. Thus, it seems that this type of functional group is likely to participate in the metal binding. With the chemically modified biomaterials (especially for human and dog hairs), sulphate groups have been characterized as the product of cystine oxidation, which probably are responsible to the binding interaction of the biomaterials with heavy metal ions, so

increasing the biosorption efficiency comparing with the native biomaterials.

SEM technique has been applied to concern about the alteration of the biosorbent surface morphology in the different cases of study. It was found that each cuticle scale of hair fiber is uniquely shaped. Some have smooth rounded edges and others with jagged-like edge, overlapping each other as they ascend along the length of the fiber towards the tip. The majority of the cuticle scales of the treated hair fibers represent more 'jagged' appearance, probably due to the oxidation treatment. After metal biosorption, the surface appears to be somewhat smoother comparing with the hair prior its use, suggesting that the cuticle scales are closed through biosorption, probably due to the acidic water media. Chicken feathers are shown in opposite sense of the hair fibers, so, the surface of chicken feathers present a little bit rough appearance after biosorption process, probably due to their relative bad stability compared with the other biosorbents. This may be caused by a lower cystine content of chicken feathers which contributes to a worse stability compared with the other three biosorbents (human and dog hairs, and degreased wool).

(2). Effect of the initial aqueous solution pH

Changes in the initial aqueous solution pH affects to the chemistry of the aqueous systems and significantly affects biosorption performance, due to different metal and functional groups speciation in the liquid phase and in the surface of the biosorbent, respectively. The biosorption of metal ions increases significantly while increasing the pH value of solutions.On the one hand, it is likely that the type and ionic state of the functional groups on the biosorbent at these pH values determine the extent of the sorption. On the other hand, H^+ ions may compete with metal ion for the functional groups of the biosorbent at low pH values. Around pH 5.0, the partial hydrolysis of metals will affect their biosorption. Therefore, the pH 4.0 was selected as optimum condition in this work (in all case studies), where the metal ions are present in their free ionic form.

(3). Effect of biosorbent dosage

The increase of biosorbent dosage essentially increases the metal binding sites number, which leads to an increase of the removal percentage of metal ions. For Cr(III) and Pb(II), the adsorbed metal ion (mmol) per unit weight (g) of biosorbent significantly decrease by increasing the biosorbent dosage. The metal uptake (mmol/g) for Cu(II) and Cd(II) are basically stable with the increase of biosorbent dosage, that means that the biosorption quantity of Cu(II) and Cd(II) increase through increasing the biosorbent dosage. This can be explained due to the fact that at high biosorbent dosage level, the available metal ions (Cu(II) and Pb(II)) in the aqueous solution are insufficient to cover all the biosorbent sites, even these biosorbent sites were able to adsorb Cu(II) and Cd(II) ions. So, it can be deduced that functional groups of the biosorbent surface have stronger affinity for Cr(III), Pb(II) than for Cu(II) and Cd(II).

(4). Effect of the contact time between biomaterials and heavy metals

In all cases, the removal percentage of heavy metals increases with the increase of contact time till the equilibrium is attained. In general, all the plots showed that the rate of the biosorption of metal ions consisted on two steps: an initial step with rapid biosorption and a second slower step where equilibrium uptake is achieved.

(5). Effect of the initial metal concentration

Regarding the influence of the initial metal concentration of metal ions, the equilibrium sorption capacity of biosorbents increased when increasing the initial metal concentration up to be saturated. So, at this point, the biosorbent surface does not display free sites for metal uptake, being saturated. High metal concentration levels provide important driving forces to overcome all mass-transfer resistance of metal ions between the aqueous and the solid phase, in all the case studies.

(6). Biosorption kinetics modeling

The kinetic data obtained at different contact time, and kinetic models including pseudo-first order, pseudo-second order and Weber-Morris intraparticle diffusion

models were used to characterize the biosorption process. The experimental data of all system fitted well the pseudo-second order kinetic modelwith extremely high correlation coefficients R^2, except for the case of the biosorption of Cr(III) by human hair from multiple-metal system. Kinetic results of biosorption indicated that chemical sorption was the basic mechanism in those systems, for all keratin biomaterials, untreated or treated ones. The intraparticle diffusion analysis of the biosorption showed that the biosorption process proceeds by surface adsorption in the earlier stages and by intraparticle diffusion at later stages, both are enhanced with the increase of the initial metal concentration.

(7). Biosorption isotherm modeling

The experimental data obtained at different initial metal concentration level were adjusted to the Langmuir model (based on monolayer adsorption of solute) and the Freundlich model (developed for heterogeneous surfaces). The isotherm data were obtained at different initial metal concentrations of single-metal systems for some of the heavy metals properly selected. In all the cases of metal biosorption by using the tested keratin biomaterials, the experimental data fitted reasonably well Langmuir isotherm, indicating mono layer coverage by the metal ions on the biosorbent. The thermodynamic parameter Gibb's free energy (ΔG^0) has a negative value in all metal biosorption systems checked, which indicates the feasibility and spontaneous nature of the biosorption processes (for all the keratin biomaterials).

(8). Desorption, regeneration and reuse studies

Recovery of the biosorbed heavy metals and the reuse of the keratin biomaterials are of significance from the viewpoint of practical application for the treatment of wastewaters. This aspect was investigated through desorption of Pb(II) ions and the biosorption capacity of the regenerated keratin biomaterials in a second time. It was found that Pb(II) ions adsorbed by the keratin biomaterials could be eluted with EDTA or HNO_3. These biosorbents regenerated with EDTA exhibited higher metal second biosorption capacity than the ones regenerated using HNO_3. That may be due to some

traces of protons from HNO_3 on the regenerated biosorbents, probably related to the ionization state of their functional groups.

Future perspectives

Biosorption is being demonstrated to be an useful alternative to conventional systems for the removal of heavy metal ions from wastewaters, as industrial effluents. The development of the biosorption process requires further investigation in the direction of modeling, of the regeneration and reuse of the biosorbent material, and of testing immobilized raw biomass (in continuous mode), first with synthetic samples and later with real industrial contaminated effluent samples.

Chemical modification procedure, in general, improved the adsorption capacity of adsorbents probably due to higher number of active binding sites after the modification, together with the formation of new functional groups that favor metal uptake (such as sulfonate groups, In the case of chicken feathers and degreased wool, other modification reactions can be explored in order to improve their capability). Although chemically modified hair fibers can enhance the adsorption of heavy metal ions, the cost of the chemicals and methods of modification used also have to be taken into consideration in order to produce a real 'low-cost' biosorbent. Therefore, exploring the cheaper and the versatile method as well as built up whole-cost-evaluation system should be considered in near future.

Furthermore, the extraction of the keratin from the original biomaterials can be explored in order to be able to prepare biocompatible membrane films or fibers (at nanoscale level). Such new systems can be tested in order to improve the stability of the raw materials, so to improve the reuse of them. Again, exploring the cheaper and the versatile method of the keratin extraction and the membrane and/or fibers preparation, as well as built up whole-cost-evaluation system should be considered in near future.

Annex I

Article submitted

Removal of heavy metal ions from aqueous solutions using chemically treated human hair: Characterization, modeling and optimization of the parameters affecting the biosorption step

Helan Zhang[a], Fernando Carrillo[b,c], Cristina Palet[a,‡]

[a] *Centre Grup de Tècniques de Separació en Química, Unitat de Química Analítica, Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Catalunya, Spain*

[b] *INTEXTER – Universitat Politècnica de Catalunya (UPC), Colom 15, 08222 Terrassa, Spain*

[c] *Departament d'Enginyeria Química, EET – Universitat Politècnica de Catalunya (UPC), Colom 1, 08222 Terrassa, Spain*

Abstract: In this study, first of all, the removal of heavy metals from aqueous solution by different kinds of human hair was evaluated. It was found that the bleached and dyed hair showed the best biosorption capacity for all checked heavy metal ions. It could be attributed to the higher surface area and more number of sulfonate groups, which is confirmed by the SEM and FT-IR analysis, respectively. Based on these preliminary results, native human hair was treated by a proper oxidation method, and its biosorption performance was evaluated and compared with the native one. The results indicated that the treated human hair showed better biosorption capacity for heavy metals comparing with the untreated one, as expected, due to the higher surface area after the treatment, and more sulfonate groups present on the surface, which have been formed through the oxidation reaction. The parameters affecting the biosorption of heavy metals, such as contact time, initial aqueous pH value, biosorbent dosage and initial metal concentration have been investigated. Three kinetic models including pseudo-first order, pseudo-second order and Weber-Morris intraparticle diffusion were used to characterize the biosorption process in both multiple- and single-metal systems. Furthermore, the equilibrium data fitted reasonably well Langmuir adsorption isotherm. According to the evaluation using the Langmuir equation, the saturated sorption capacities of Cr(III), Cu(II), Cd(II) and Pb(II) into the treated human hair at 295 K were 9.47×10^{-5}, 5.57×10^{-5}, 3.77×10^{-5}, 3.61×10^{-5} mol/g, respectively. Negative standard Gibb's energy indicated that the heavy metal biosorption process is thermodynamically feasible and spontaneous nature. The treated human hair showed satisfactory biosorption capacity and can be considered as an effective biosorbent for the removal of heavy metal ions from aqueous media.

Keywords: Chemically treated human hair; Heavy metal; Kinetics; Isotherms; Biosorption.

[‡]Corresponding author. Tel.: +0034 935813475; fax: +0034 935811985.
e-mail address: cristina.palet@uab.cat.

1. Introduction

Intensifying environment pollution and deteriorating of ecosystems anthropologically, caused by mainly industrial activities, have seriously threatened human existence and development. Compared with other pollutants, such as dye, phenols (including other organic compounds), inorganic anions, and pesticides (which are toxic to many living lifeforms and organisms), etc., heavy metal pollution of wastewater is most strongly connected to human life. Heavy metal ions are considered extremely harmful to human, aquatic organisms, and other life forms because of their toxicity, accumulation and non biodegradable nature, which causing various diseases and disorders.[1] Recently, the removal of heavy metal ions from wastewaters has attracted attention for the protection of public health and environment.[2] Conventional methods for removing heavy metal ions including chemical precipitation, flotation, ion exchange, evaporation and membrane processes are practical and cost-effective only with high strength wastewater (which contains high concentration levels of pollutants), and they are ineffective when applied to low strength aqueous effluents with heavy metal ion concentrations less than 100 ppm.[3] Adsorption techniques currently play an important role in the removal of heavy metal ions from wastewater, offering considerable advantages like the low-cost, availability, profitability, easy for operation and efficiency.[4] Various materials have been developed as adsorbents for the removal of heavy metal ions. In particular, activated carbon is frequently used as an adsorbent due to its high surface area, high adsorption capacity, and high degree of surface reactivity.[4] However, activated carbon is relatively expensive and it is difficult to recycle by eluting the heavy metal ions because of the strong interaction between activated carbon and heavy metal ions, the application of expensive synthetic materials also exist similar problems like technical or economic constraints. Hence, it is imperative to find alternate low-cost sorbent material to replace high cost activated carbon or expensive synthetic materials for water treatment. Waste biogenic materials are considered ideal alternative biosorbents for the removal of heavy metal ions from low strength wastewater due to the relatively good cost-effective adsorption capacity.[5] Accordingly, various biogenic materials including chitosan derivatives,[6] agricultural waste materials,[7] chicken feather,[8, 9] cork waste,[10, 11] rubber leaf powder,[12] chemically modified plant wastes,[13] and soybean stalk,[14] among others, have been proposed and applied as biosorbent to effectively remove heavy metal ions. The good biosorption properties of these biogenic materials are attributed to the presence of abundant metal binding functional groups on these materials, such as carbonyl, carboxyl, hydroxyl, sulphate, sulfur, phosphate, amido and amino groups.[15, 16]

Among natural resources, keratinous materials can be used as biosorbents, either directly or after activation, to remove heavy metal ions due to their intricate networks characterized by

high stability, insolubility in water, high surface area containing many carboxyl, amido, and sulfur functional groups.[17] Also, keratin is an abundant nonfood protein; in fact, it is the major component of wool, hair, horn, nail and feathers. Moreover, keratin wastes such as feathers, horns, nails from butchery,[18] human hair from barber, poor quality raw wools from sheep breeding and some by-products from textile industry, account worldwide for more than four millions tons per year.[19] As regards the use of keratinous materials, several examples have been reported recently, especially using modified keratinous materials. Al-Asheh et al.[20] compared adsorption capacities between inactivated and chemically activated chicken feather as biosorbent for removing heavy metal ions (i.e. Cu(II) and Zn(II)) from wastewaters. They pointed out that treatment of chicken feather with alkaline solutions improve the biosorption of metal ions compared to those treated with an anionic surfactant, this followed by the untreated chicken feather. In addition to this an alkaline ultrasonic treatment of chicken feather improves the heavy metal uptake due to the simultaneous chemical and cavitation effects which increase the surface area and the amount of hydroxyl functional groups.[8] On the other hand, Park et al. prepared wool and silk blend nanofibrous membranes by electrospinning, which exhibited an excellent performance as a heavy metal ions adsorbents.[21] The Aluigi research group successfully prepared keratin-rich nanofibre mats by electrospinning wool keratin/polyamide blends. This material shows good adsorption capacity to Cu(II) ion from waters and the adsorption capacity increases with increasing the specific surface area of the nanofiber mats.[22]

Keratinous-composed human hair contains more cystine relative to other biomass, which is considered a good biosorbent for metal ions due to the presence of potential metal binding functional groups including hydroxyl, amino, carboxyl and sulfur-containing groups. However, the human hair used as biosorbent has been rarely studied.[9, 23] In this sense, human hair is presented in this work as a biosorbent for the removal of heavy metals. According to the comparison of the biosorption capacities of different kinds of human hair samples, the bleached and dyed hair showed the best biosorption capacity for all checked heavy metal ions. So, native human hair was treated by a similar method, and its biosorption performance was evaluated and compared with the native one. Environmental parameters affecting the biosorption process such the pH value, the biosorbent dosage, the contact time and the initial concentration of metal were studied. Also, FT-IR and SEM analysis were conducted for the structural and morphological characterization of the biosorbent after the treatment and after the subsequent the heavy metal biosorption process. The kinetic and isotherm data experimentally obtained were correlated with the established kinetic models (pseudo-first order, pseudo-second order and Weber-Morris intraparticle diffusion model) and with the well-known thermodynamic models (Freundlich and Langmuir) and a proper comparison between them was performed.

2. Experimental

2.1. Chemicals

All the chemicals used in this work were of analytical grade. Stock solutions of separate heavy metal ions, such as Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) were prepared by dissolving their nitric salts (> 99%, all from Panreac, Spain) in deionized water. A 1000 ppm stock solution of metal ions was first prepared, which was then diluted to the initial heavy metal concentration for each experiment. Sodium hydroxide (> 98%, from Panreac, Spain) and nitric acid (> 70%, from JT-Baker, Spain) were alternatively used for the pH adjustment of the initial aqueous solution prior commencing the biosorption experiments. In all the experiments, the initial pH was measured, and usually the final pH was also checked, using an Omega 300 pH meter (Crison instruments, S.A., Spain).

2.2. Biosorbent

Different kinds of human hair provided by local barbershops were obtained from different people. The human hair samples were washed with common laboratory detergent, rinsed several times with deionized water (purified with a milli-Q Gradient system from Millipore Corporation) and then left to dry at room temperature (22±1 ˚C). The hair was cut to an approximate length of 1-2 mm by using scissors.

2.3. Chemical treatment of human hair

The treatment process of the human hair was carried out as follows: 20.0 g of the untreated human hair (from the different individuals about 13 years old boy) was weighted out and soaked in 400 ml of the pretreatment reagent of known concentration (10% H_2O_2, originally at 35% in water, from Sigma-Aldrich, Germany) and at adjusted pH (pH 9.0 gives better biosorption results in comparison with others, when different pH are assayed 5.0, 7.0 and 9.0).[24] After a given soaking time (5 h), the solution was filtered. The hair separated from the solution was washed many times with deionised water. To minimize any loss of the treated hair, at each washing step the hair was separated by centrifugation, and after that the liquid was decanted. Finally, the treated and cleaned hair was dried at room temperature.

2.4. Characterization of human hair

Structural characterization of the human hair was carried out to analyze any chemical change produced in the samples after the oxidative treatment and/or after the biosorption of heavy metals. The identification of the functional groups on the untreated and treated human hair was performed by using Fourier transform infrared spectrometer (FT-IR) (Tensor 27, Bruker, Germany). The spectrum was recorded in the range of 600–4000 cm^{-1} with 16 scans and

a resolution of 4 cm^{-1}. On the other hand, the surface morphology of the human hair samples (untreated and treated) was observed by scanning electron microscope (SEM ZEISS EVO® MA 10, Oberkochen, Germany). The sample used sputter-coating arrangement.

2.5. Heavy metal ions biosorption experiments

The uptake of the heavy metal ions onto the hair systems was carried out by batch experiments at a constant temperature (22±1 ˚C) on a rotary mixer (CE 2000 ABT-4, SBS Instruments SA, Barcelona, Spain) at 25 rpm. In all sets of experiments, 0.1 g of human hair (untreated or treated) was weighted in 50 mL plastic extraction tubes, later on 10 mL of heavy metal ions aqueous solution was added and the finally system was shaken for a certain period of time. Usually, the concentration of each heavy metal ions was 0.10 mmol/L (for multiple metal system, containing eight metal ions) and 0.18 mmol/L (in the single-metal systems and also in the multiple-metal system containing four ions). The pH was adjusted at 4.0, otherwise specified. The initial pH of the multiple-heavy metal aqueous solution was varied within the range 1.0 to 6.0 (higher pH values were not considered to avoid precipitation of metal hydroxides). To study the effect of the biosorbent dosage on metal uptake, its mass was varied from 0.01 to 0.2 g. The effect of the initial metal ion concentration on biosorption isotherms was studied in single-metal systems with 0.1 g of treated human hair. A range of initial metal ion concentrations from 0.5×10^{-3} to 2.0 mmol/L was used. In all cases, after agitation, the two phases were separated by decantation and the liquid was filtered through 0.22 μm syringe filter (CN-CA Syringe Filter, HEAION, USA). After that, the heavy metal concentration in the remaining aqueous solution was determined by an inductively coupled plasma optical emission spectrophotometer with mass detector, ICP-MS (XSERIES 2 ICP-MS, Thermo Scientific, USA).

The percent removal of metal ions was calculated as follows:

$$\%\ biosorption = \frac{C_i - C_f}{C_i} \times 100 \qquad (1)$$

where C_i and C_f are the initial and the final concentration of heavy metal in the aqueous phase solution, respectively (in mmol/L).

The adsorption capacity of the biosorbent (q_t), is the concentration of biosorbed metal ion per unit of mass of biosorbent (in mmol/g) at any time t, which was calculated using the equation 2.

$$q_t(mmol/g) = \frac{(C_i - C_f) \times V}{W} \qquad (2)$$

where V is the total volume of the solution (in L), W is the amount of biosorbent (in g), C_i and C_f are the initial and the final concentrations of heavy metal in the aqueous solution (each given in

units of mmol/L), respectively.

The adsorption equilibrium capacity of the biosorbent (q_e), is the concentration of biosorbed metal ion per unit of mass of biosorbent (in mmol/g) at the equilibrium, which was obtained as follows:

$$q_e(mmol/g) = \frac{(C_i - C_e) \times V}{W} \quad (3)$$

where V is the total volume of the solution (in L), W is the amount of biosorbent (in g), C_i and C_e are the initial and equilibrium concentrations of each heavy metal in the aqueous solution (each given in units of mmol/L), respectively.

2.6. Desorption, regeneration and reuse

Desorption experiments were performed just for the case of Pb(II), after the corresponding biosorption step by using untreated and treated human hair samples, and later the centrifugation and filtration steps. Each biosorbent material containing the adsorbed Pb(II) was contacted and stirred with 10 mL of 0.1 mol/L HNO_3 or 10 mL of 0.1 mol/L EDTA, separately. After 24 h of stirring (with the rotary mixer) at room temperature (22±1 ˚C), the aqueous and the solid phases were properly separated (by centrifugation and filtration as usual) and the Pb(II) content of the final solutions were analyzed by ICP-MS, as indicated. Desorption percentage can be calculated using the equation:

$$\%\,\text{desorption} = \frac{amount \quad of \quad Pb(II) \quad desorbed}{amount \quad of \quad Pb(II) \quad adsorbed} \quad (4)$$

Reuse of both hair samples in a second biosorption step, after elution of the adsorbed metal ions (with nitric acid or EDTA) will require the cleaning of H^+ and EDTA from the surface of the biomaterial. Two methods are used to clean or regenerate the biosorbent, after eluting the adsorbed metal ions. One method includes washing the biosorbents with deionized water. The other method, just for the case that EDTA is used for the metal elution from the biosorbent, firstly implies rinsing with HNO_3 solution and then washing with deionized water. After this cleaning or regeneration step, the biosorbents regenerated by these two methods are dried in an oven at 40 ˚C. In addition, another regenerated hair samples after elution by using EDTA, are firstly rinsed with HNO_3 solution, and then are washed with deionized water and dried. These three different regenerated human hair samples obtained from these two methods are employed to adsorb heavy metals again.

All batch biosorption experiments were carried out in duplicates and the results were reported as their average in the corresponding figures (experimental errors found were less than 2% and 0.0025 mmol/g in the biosorption percentage and the biosorption capacity, respectively)

3. Results and discussion

3.1. Comparison biosorption efficiency and FT-IR of different human hairs

The removal of metal ions Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) from multiple-metal aqueous solutions by using thirty-two different kinds of human hair samples as biosorbents was investigated. The selected thirty-two human hair samples (from different individuals) were labeled by numbers, which is shown in Table 1. Thirty-two different human hair samples are labeled from **No. 1** to **No. 32**. Figure 1 shows the biosorption percentage of the eight metal ions onto all the human hair samples. The different hair samples showed different biosorption capacities for the different metal ions. In general, the biosorption capacity of metal ions by using female's hair is greater than the male's hair. Human hair can effectively bind a number of heavy metals, with different binding affinity of those biosorbents for each metal ions studied, following in order of decreasing affinity: Pb(II) ≈ Cr(III) ≈ Cu(II) > Cd(II) ≈ Zn(II) > Ni(II) > Co(II) > Mn(II). Those differences can be explained by the type and concentration of the surface functional groups responsible for the interaction with the metal ions. So, the different hair samples for such binding sites used as biosorbents have different affinity of each metal ion.

Table 1. Information of the origin of the hair samples and labeled number.

Hair No.	The information of hair samples	Hair No.	The information of hair samples
1	Male (3-year old)	17	Female (45-year old)
2	Male (27-year old)	18	Female (9-year old)
3	Female (12-year old)	19	Female (15-year old)
4	Male (11-year old)	20	Female (26-year old)
5	Male (44-year old,)	21	Female (27-year old)
6	Male (27-year old)	22	Female (35-year old)
7	Male (6-year old)	23	Male (33-year old)
8	Male (12-year old)	24	Female (10-year old)
9	Male (50-year old)	25	Female (40-year old)
10	Male (27-year old)	26	Female (28-year old)
11	Male (44-year old)	27	Female (17-year old)
12	Male (35-year old)	28	Female (38-year old)
13	Male (30-year old)	29	Female (13-year old)
14	Male (47-year old)	30	Female (60-year old)
15	Male (6-year old)	31	Female (40-year old, dyed hair)
16	Female (40-year old, dyed partially)	32	Female (45-year old, dyed hair)

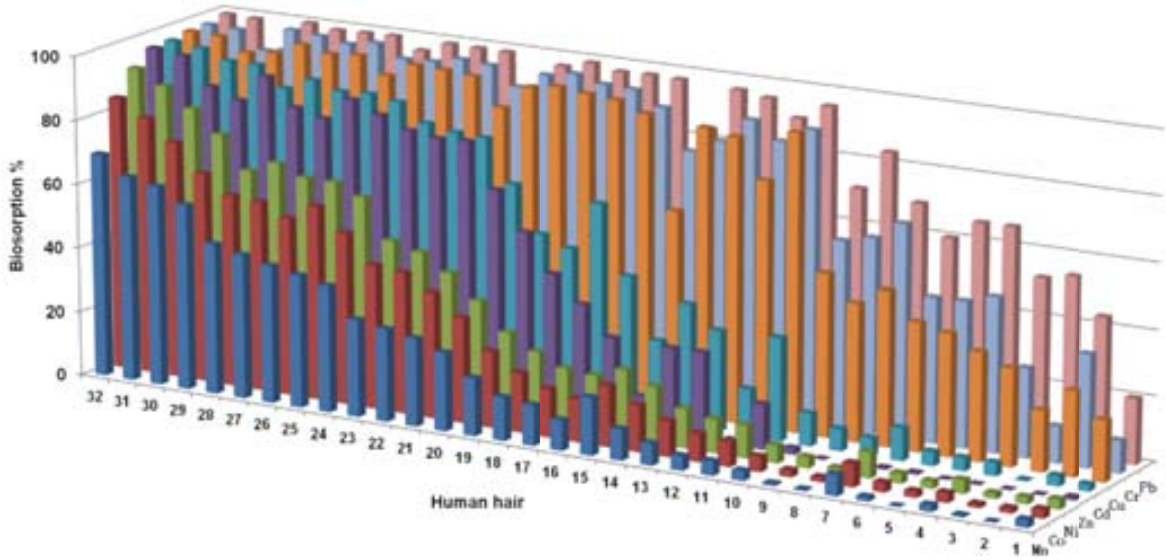

Figure 1. Biosorption percentage of the different kinds of human hair for Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) in multiple-metal system. The initial metal concentration is 0.1 mmol/L, the initial pH is adjusted at 4.0, and the biosorbent dosage is 0.1 g in 10 mL of the initial solution.

Fourier transform infrared (FT-IR) spectroscopy is a powerful technique that can provide rapid and specific chemical information at the molecular level.[25] According to the order of biosorption capacity, four representative hair samples including **No.1** (in native, 3-year old boy), **No.13** (in native, 30-year old man), **No.21** (in native, 27-year old woman) and **No.32** (bleached-dyed, 45-year old woman) were selected to be characterized by the FT-IR. FT-IR spectra of the four human hair samples are shown in Figure 2. The peak located at 3275 cm^{-1} is a mode arising from N-H stretching, which is sensitive to hydrogen bonding and it is essential in the structuring of water and holding protein together. The two weak bands located at 2960 cm^{-1} and 2920 cm^{-1} could be assigned as modes arising from amino acid bands of $-CH_3$ and $-CH_2-$ asymmetric and symmetric modes, respectively. The peaks located at 1632 cm^{-1} (Amide I), 1520 cm^{-1} (Amide II) and 1241 cm^{-1} (Amide III) are related to typical keratin fiber amino acids. The weak broad shoulder bands in the 1180-1030 cm^{-1} region are related to the cystine and its oxidation products which include cysteic acid, cystine monoxide, and cystine dioxide as well as sulfonates. Furthermore, it is worth noting that there are some differences in this region. The small peaks observed at 1040 cm^{-1} and 1175 cm^{-1} are probably related to the oxidation products of cystine, which are gradually formed eventually in the human hair which biosorption capacity increases. The human hair (**No. 1**) having the worst biosorption capacity is from the male of 3 years old, whose hair is really natural and the extent of oxidation is the lowest one, as expected (see Figure 2 (b)). The human hair (**No. 32**) having the best biosorption capacity is from the female of 45 years old, whose hair is bleached and dyed (submitted to an external and forced oxidation process), and the extent of oxidation is the highest one (see Figure 2 (b)). The other

two human hair samples characterized by FT-IR (**No. 13** and **No. 21**) showed relatively weak intensity for the corresponding oxidation peaks. Most likely, this oxidation of human hair in native is due to environmental factors such as sunlight (exposure to ozone), chlorinated water and frequent shampooing causing partial natural oxidation of the hair surface. In general, the hair of female suffering from these factors is more frequent than male that lead to them oxidation easily (the use of more specific products for cleaning and softening the female hair can be the main reason for that behavior). This corresponds with the experimental results found that the biosorption capacity of female's hair is greater than male's hair. The FT-IR analysis indicates that there are potential metal binding functional groups (including hydroxyl, amino, carboxyl and sulfur-containing) on the four biosorbents characterized by FT-IR, and the oxidation products of cystine can enhance the biosorption capacity of the human hair.

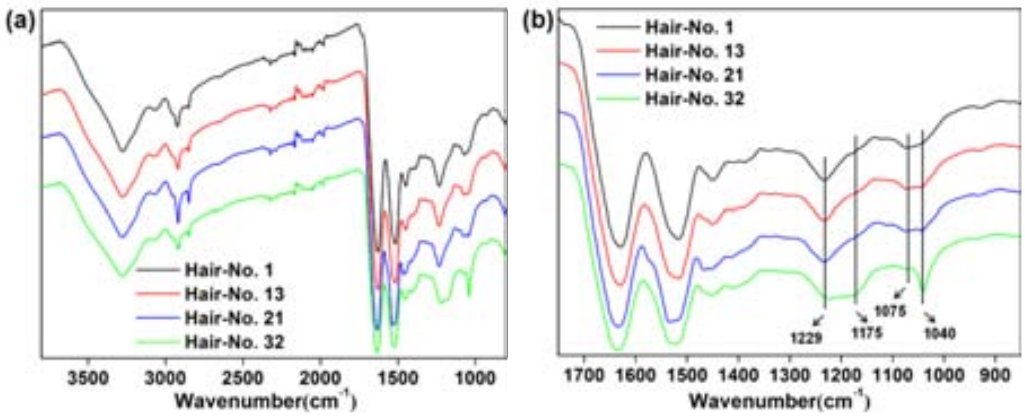

Figure 2. FT-IR spectra of the four human hairs. (a) full-scan spectra, and (b) partial spectra of human hair, corresponding to the cystine acid bands region.

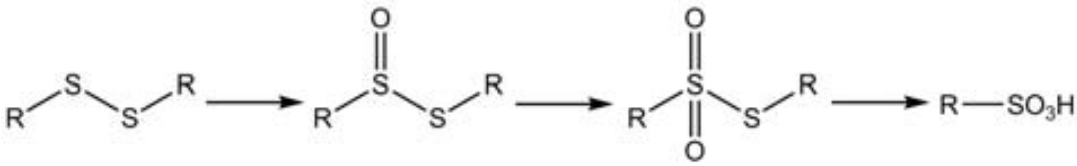

Figure 3. Scheme of the S-S cleavage mechanism for the bleaching process.

This conclusion can provide reference for the possible modification of human hair. So, the human hair is treated with an oxidation method and its performance in the removal of metal ions from aqueous solution was evaluated in the following experiments.

3.2. The chemical treatment of human hair

Based on the previous study, chemical modification through an oxidation method is used for the purpose of enhancing the biosorption capacity of heavy metals of the native human hair. The oxidation of human hair (as a keratin biomaterial) can usually take place with hydrogen peroxide in acid or alkaline medium by attacking the disulfide bond of keratin. As a result of

this oxidation reaction, sulfonate groups are formed, which will be new functional groups of the human hair. In alkaline medium the oxidation process is much more effective.[24] Moreover, during the oxidation process, other proteins in the human hair are also oxidized which leads to cell membrane damage causing the cortex and the cuticle to open and separate. So, with this knowledge, the main objective of this chemical pretreatment is to activate the human hair with new metal binding sites on the surface and also to increase its specific surface area.

3.3. Comparison of the biosorption efficacy between untreated and treated human hairs

The biosorption capacities of the untreated and treated human hair samples for removing eight metal ions (Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II)) were determined. The obtained results are shown in Figure 4. As seen in Figure 4, the metal biosorption capacity of the treated human hair is significantly higher than the untreated human hair. Moreover, the affinity of both hair samples for Cr(III), Cu(II), and Pb(II) is greater than that for the rest of metal ions in solution, which can be explained by the stronger interactions between the functional groups (especially sulfonate ones) of the biosorbent and these three metal ions. The metal biosorption onto the treated human hair follows in the order of Cr(III) > Pb(II) > Cu(II) > Cd(II) > Ni(II) > Co(II) > Mn(II) > Zn(II).

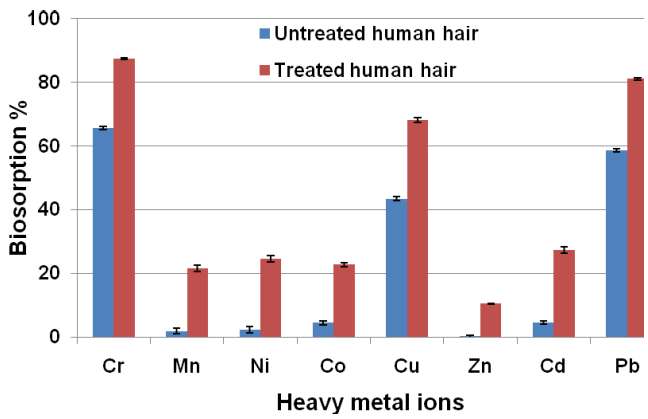

Figure 4. Comparison of biosorption between untreated and treated human hair in multiple-metal system. The initial metal ions concentration is 0.1 mmol/L, the contact time is 24 h, the pH is 4.0, the biosorbent is 0.1 g.

Taking into account such results, four metal ions including Cr(III), Cu(II), Cd(II) and Pb(II) were selected to check the biosorption behavior in different conditions and the biosorption mechanism of the chemically-modified human hair in subsequent experiments. Firstly, the functional groups and morphology of the human hair surface in the chemical modification and biosorption processes are checked.

3.4. FT-IR and SEM Characterization

In order to understand how metal ions bind to the biosorbent, it is essential to identify the functional groups of its surface as they could be the responsible for such metal binding. So, the untreated, treated and metal loaded-treated hair samples are discriminated by using Fourier transform infrared (FT-IR) spectroscopy, as can be seen from the infrared spectra collected in Figure 5. The full-scan spectra of human hair samples (Figure 5(a)) display their corresponding infrared peaks. The broad and medium intensity band ranging from 3000 to 3600 cm^{-1} is indicative of the stretches of the bonds belonging to the carboxylic acid (-COOH), the alcohol (-OH) and the amino acid ($-NH_2$) groups. The peaks located at 1632 cm^{-1} (Amide I), 1520 cm^{-1} (Amide II) and 1241 cm^{-1} (Amide III) are related to typical human hair amino acids. The peaks at 1041, 1075, 1180, and 1229 cm^{-1} all correspond to different products of cystine oxidation in human hair, and their peak assignments are shown in Table 2. Carefully comparing the spectra of the three different human hair samples, it can be seen some differences between them, as expected due to the oxidation process first (treated human hair), and also due to the metal binding after the later biosorption process (metal loaded-treated human hair), especially in the region from 850 to 1750 cm^{-1} (see in Figure 5(b)). The intensity of the peaks at 1041 and 1180 cm^{-1} increased after chemical modification, which means that conversion of cystine to cysteic acid, cystine monoxide, and cystine dioxide as well as to sulfonate occurs during the oxidation treatment process. The weak broad shoulder between approximately 1000-1130 cm^{-1} in the untreated human hair infrared spectra is probably due to environmental factors, such as sunlight, chlorinated water and frequent shampooing causing partial oxidation of the hair surface.[26] The FT-IR spectra from treated human hair before and after the metal biosorption are very similar, indicating that the main functional groups on treated human hair did not change significantly during the metal biosorption process, which can be an indication of the possible reuse of such biomaterial. However, the slightly differences found around 1400 cm^{-1}, and some red shift of the emission spectra (from 3277.2 cm^{-1} to 3274.8 cm^{-1}, from 1526.8 cm^{-1} to 1519.7 cm^{-1}, and from 1078.4 cm^{-1} to 1074.9 cm^{-1}), before and after the biosorption process, probably can be related to the presence of the metal ions on the hair surface.[16] Based on the FT-IR spectra changes, as seen in Figure 5, some chemical functional groups (including hydroxyl, amino, carboxyl and sulfonate groups) of the human hair surface could act as important biosorption sites for metal ions.

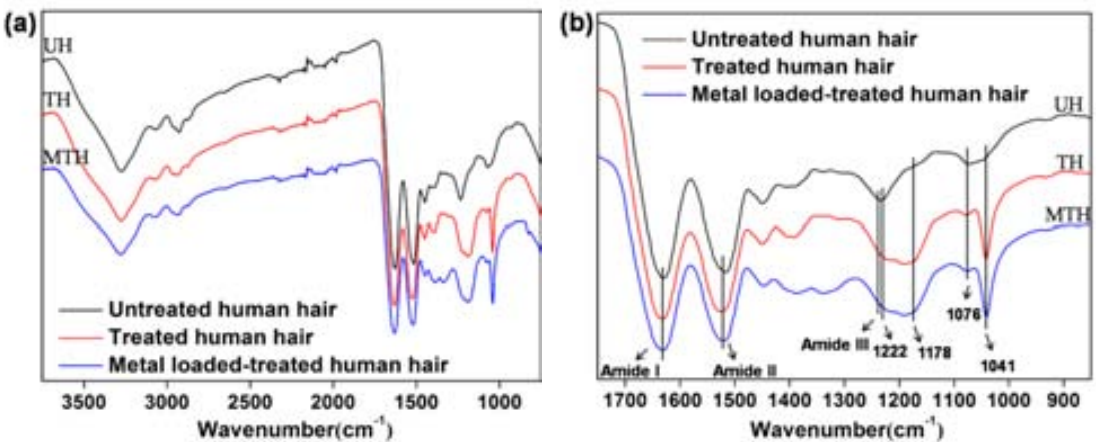

Figure 5. FT-IR spectra of human hair. UH: untreated human hair, TH: treated human hair, MTH: metal loaded-treated human hair. (a) full-scan spectra, and (b) spectra in the range 850 up to 1750 cm^{-1}.

Table 2. FT-IR peak assignments of the oxidation products of the cystine.

Assignments	Wave number (cm^{-1})			
	literature	Current work		
		Untreated	Treated	Metal loaded-treated
Cystine dioxide (R-SO_2-S-R)	1229	1222	1221	1220
Sulfonate, S-O asym, stretch	1175	1178	1180	1181
Cystine monoxide (R-SO-S-R)	1075	1076	1078	1080
Sulfonate, S-O sym, stretch	1040	1041	1042	1042

Scanning electron microscope (SEM) technique was applied to concern about the alteration of the human hair surface morphologies in the different cases of study. Figure 6 shows the SEM micrographs of the untreated, treated, and metal-loaded treated human hair. It is observed that each cuticle scale of human hair is uniquely shaped. Some have smooth rounded edges and others with jagged-like edge, overlapping each other as they ascend along the length of the fiber towards the tip (Figure 6). The surface topography of the untreated and the treated human hair have some difference (as can be seen in Figure 6 (a) and (b), for comparison).The majority of the cuticle scales of the treated human hair fiber represent more 'jagged' appearance, probably due to the oxidation treatment. After metal biosorption, the surface appears to be somewhat smoother comparing with the chemically-modifiedhair prior its use, suggesting that the cuticle scales are closed through the metal biosorption process, probably due to the acidic water media of the initial aqueous solution (see Figure 6 (b') and (c')).

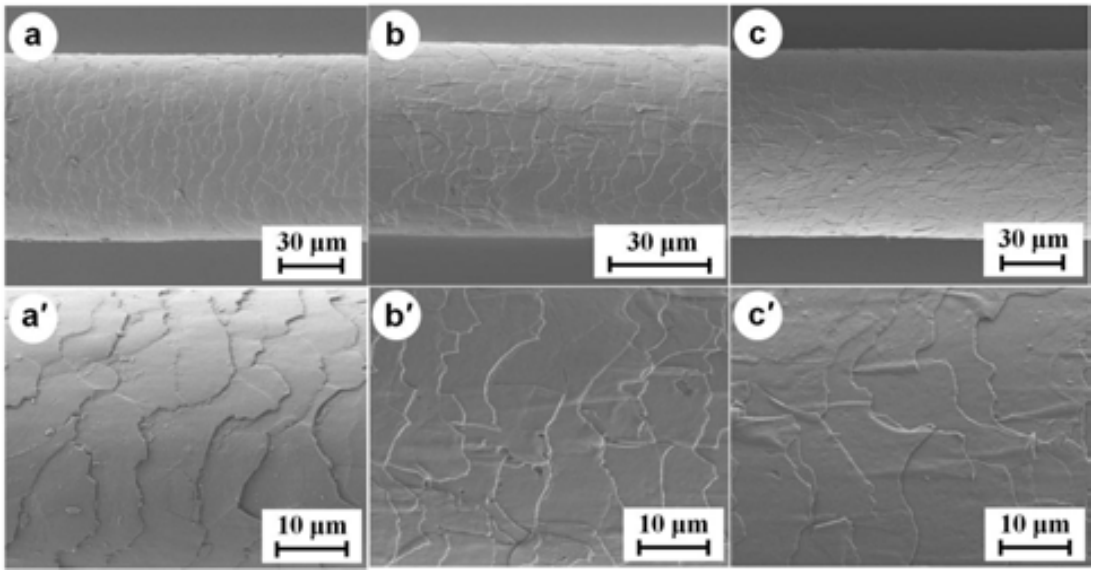

Figure 6. Scanning electron microscopy (SEM) micrographs of the human hair: (a) and (a') correspond to the untreated human hair; (b) and (b') correspond to the treated human hair; and (c) and (c') correspond to the metal loaded-treated human hair.

3.5. Effect of the initial pH

The pH level of the aqueous solution is an important variable for the metal biosorption process, due to the metal speciation and also to the speciation of the chemical functional groups present on the biosorbent surface. So, the effect of the aqueous solution pH on the removal efficacy of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) was studied between pH 1.0 and 6.0 in multiple-metal system (Figure 7). As observed from the results in Figure 7, the biosorption of metal ions increases significantly with the increase of the aqueous initial pH value. This behavior can be explained by the competition between the proton and the metal ions for the same binding site on the surface of the treated human hair. At low pH values, the surface of the biosorbent would also be surrounded by H^+ ions, which decreases the Cr(III), Cu(II), Cd(II) and Pb(II) ions interaction with the surface binding sites.[27] As the pH increases, the basic forms of the chemical functional groups overall the hair surface predominate, increasing the surface negative charge, so the metal biosorption increases significantly.[28] However, when the pH is around 5, the partial hydrolysis of metal ions (specially for Cu, the rest of metals could occur at pH higher than 5), resulting in the formation of $MOH^{(m-1)+}$ and/or $M(OH)_m$ affect the biosorption capability of human hair. The biosorption percentage found at pH up to 5.0 can be also related to the precipitation of the metals. Therefore, the pH 4 was selected as optimal condition in the subsequent experiments (to avoid this possible and uncontrolled precipitation effect).

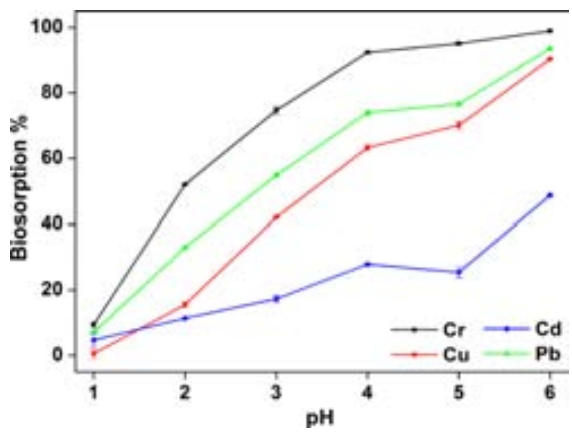

Figure 7. Effect of the pH on the biosorption of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) in the multiple-metal system. The initial concentration is 0.18 mmol/L, the contact time is 24 h, the biosorbent is 0.1 g.

3.6. Effect of the biosorbent dosage

The influence of the biosorbent dosage on the removal efficacy of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) ions is studied in the range of 0.01- 0.2 g of biosorbent in a multiple-metal system (Figure 8). It is observed that the biosorption percentage of the indicated metal ions increases with the increase of the biosorbent dosage. This can be explained with the increase in the surface area of biosorbent when increasing its amount, which in turn increases the metal binding sites.[29]

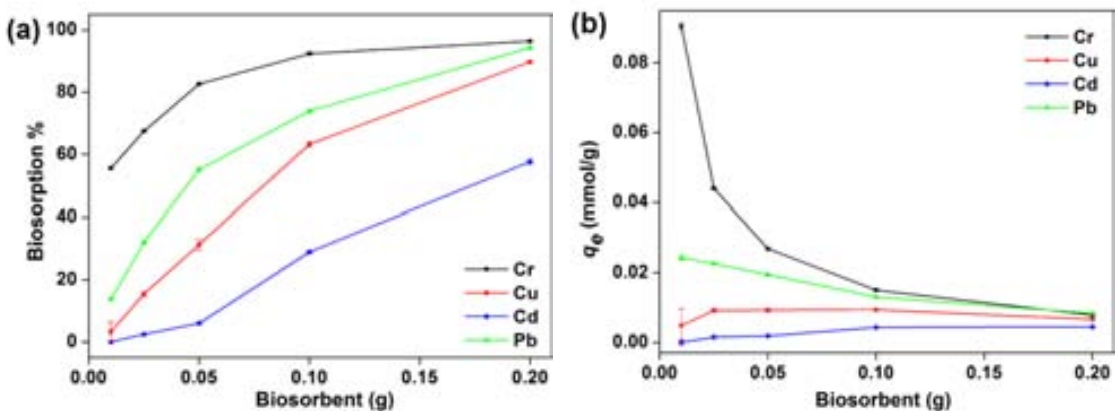

Figure 8. Effect of the biosorbent dosage on the biosorption of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) in the multiple-metal system. The initial metal ions concentration is 0.18 mmol/L, the contact time is 24 h, and the initial pH is 4.0.

Taking into account the adsorbed metal ion (mmol) per unit weight of biosorbent, for Cr(III) and Pb(II) significantly decrease by increasing the biosorbent dosage. This can be explained due to the fact that at high biosorbent dosage level, the available metal ions (Cr(III) and Pb(II)) in the aqueous solution are insufficient to cover all the biosorbent sites due to the corroborated high affinity of these two metals (as can be seen from results collected in Figures 4 and 7). In

the case of Cu(II) and Cd(II), at high biosorbent dosage level, there is enough metal ions, related to their biosorption affinity for the binding sites, to be adsorbed by the biosorbent. So, the metal uptake (mmol/g) for Cu(II) and Cd(II) increase through increasing the biosorbent dosage. Thus, as mentioned, the functional groups of the treated human hair surface have greater affinity for Cr(III), Pb(II) than for Cu(II) and Cd(II).

3.7. Effect of the contact time

The contact time with aqueous metal solution is one of the key parameters for the successful usage of biosorbent in practice. Multiple- and single-metal aqueous systems (at pH = 4) of Cr(III), Cu(II), Cd(II) and Pb(II) are contacted with the treated human hair (0.1 g) during 5, 10, 20, 30, 45 minutes, and 1, 2, 3, 4, 6, 12, 24, 48 and 72 hours. Results plotted in Figure 9 show the biosorption of the treated human hair for removing Cr(III), Cu(II), Cd(II) and Pb(II) ions from multiple- and single-metal systems as a function of time. The curves indicate that the rate of the metal biosorption is high in the initial step of the process, especially for the single-metal system (which needs only few minutes to reach the maximum metal biosorption capacity), compared with the multiple-metal system. This behavior can be attributed to the effect of the competition between the heavy metal ions, in the later case. The biosorption increases until the equilibrium is reached in both systems. From the data shown in Figure 10, it can be seen that compared with that of multiple-metal system, biosorption capacity of individual metal ion was increased, the biosorption percentage of the treated human hair for Cd(II) is outstanding with an increase from 29% up to 86%. Therefore, the selectivity order is Cr(III) > Pb(II) > Cu(II) > Cd(II), which corresponds to a biosorption efficacy in the single-metal system of 98%, 96%, 95%, and 86%, respectively. So, in comparison with single-metal system, total biosorption capacity of metal ions increases in the multiple-metal system.

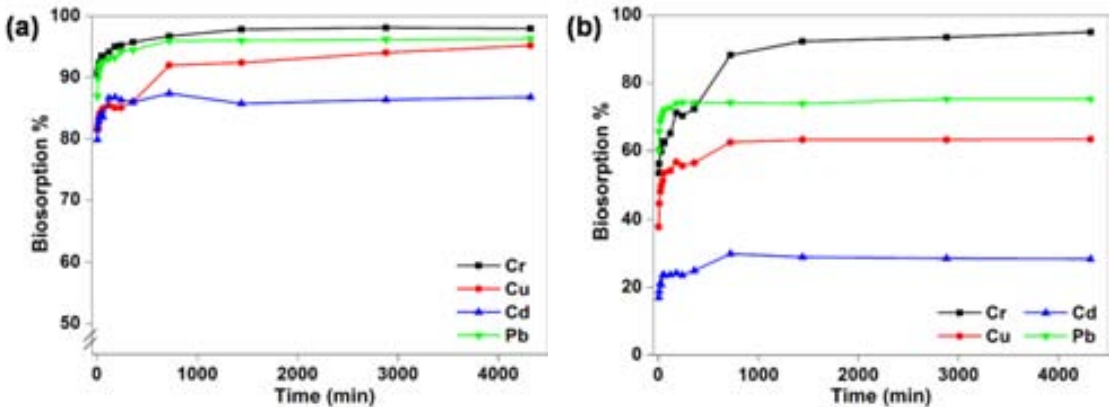

Figure 9. Biosorption percentage of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) at different contact time. (a) single-metal system, and (b) multiple-metal system. The initial metal concentration is 0.18mmol/L, the initial pH is 4.0, and the biosorbent is 0.1 g.

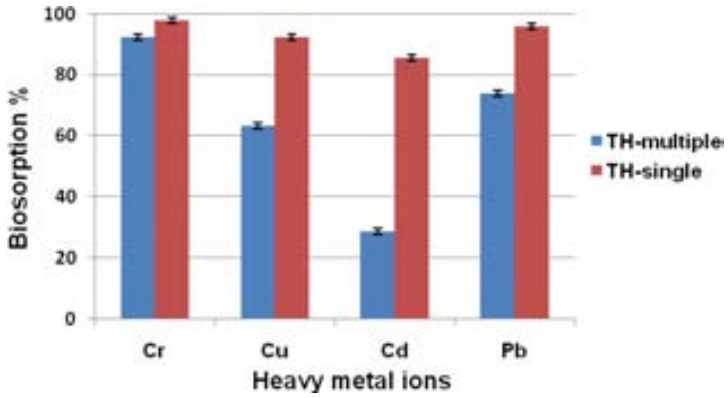

Figure 10. Comparison of the biosorption for Cr(III), Cu(II), Cd(II) and Pb(II) by the treated human hair from single- and multiple-metal systems. The initial metal concentration is 0.18 mmol/L, the pH is 4.0, and the biosorbent is 0.1 g, after 72 h.

3.8. Effect of the initial metal concentration

The initial metal concentration in the solution is also an important parameter which affects to the driving force of the biosorption systems.[30] The effect of the initial metal concentration is determined in single metal systems. Corresponding results are shown in Figure 11. It is found that the biosorption capacity of the treated human hair increases with the increase of the initial metal concentration. This concentration level is increased up to no remarkable changes are observed on the metal uptake. In this case, the surface of the treated human hair does not have free sites for the metal uptake, being fairly saturated, as can be seen from Figure 11 (a). Probably, as the concentration increased, a higher probability of collision between adsorbate (biosorbate) and adsorbent (biosorbent) surface occurs, which should overcome the mass transfer resistance between the aqueous and the biosorbent phase. Taking into account the biosorption percentages (from Figure 11 (b)) of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II), all over 90% at lower metal ion concentration level (< 0.1 mmol/L), this probably means that sufficient sorption sites are available for the biosorption of each heavy metal ion (in the single-metal system). However, a steeper slope of the curve is observed at higher concentration level (> 0.18 mmol/L), where it looks like the amount of the metal ion is relatively high compared to the availability of the sorption sites of the treated human hair.

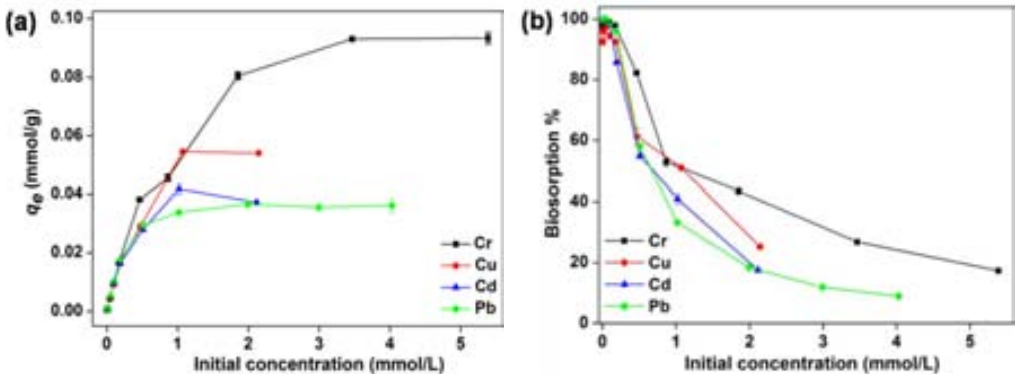

Figure 11. The equilibrium biosorption of the treated human hair at different initial metal concentration in the single-metal systems. The contact time is 24 h, the pH is 4.0, and the biosorbent is 0.1g.

3.9. Biosorption kinetics modeling

Kinetic models are used to fit the experimental data in order to investigate the mechanism of the metal biosorption process onto the chemically modified human hair system. So, it is important to determine the possible rate controlling steps, such as mass transport, chemical reaction and intraparticle diffusion processes, in such biosystem. Many attempts had been made to formulate a general expression describing the kinetics liquid-solid phase sorption systems.[31] In the present case, the kinetic models including the pseudo-first order equation,[32] the pseudo-second order equation and the Weber-Morris intraparticle diffusion model are applied, which are given by equations (5), (6), and (7), respectively:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \qquad (5)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (6)$$

$$q_t = k_3 t^{1/2} + k_d \qquad (7)$$

where, q_e and q_t are the concentration of biosorbed metal ion per unit of mass biosorbent (in mmol/g) at the equilibrium and at time t (min), respectively, and k_1 (in min^{-1}) is the rate constant of the pseudo-first order equation, k_2 (in g/(mmol·min)) is the rate constant of the pseudo-second order equation, k_3 is the intraparticle diffusion rate constant (in mmol/(g $min^{1/2}$)) and k_d is the intercept that gives an idea about the thickness of the boundary layer.

The plots of $\log(q_e-q_t)$ vs. t of each metal ion (for the single- and multiple-metal systems) for the pseudo-first order model are shown in Figure 12(a). As it is properly shown, only the data corresponding to the first 30 and 60 min for the single- and multiple-metal systems, respectively, are adjusted since after this period the experimental data deviated considerably from those theoretical. The pseudo-first order rate constant (k_1) and q_e determined from the model are presented in Table 3. For the four metal ions in single and multiple systems, the q_e estimated by this model differs significantly of those measured

experimentally, so suggesting that the biosorption doesn't follow a pseudo first-order reaction.

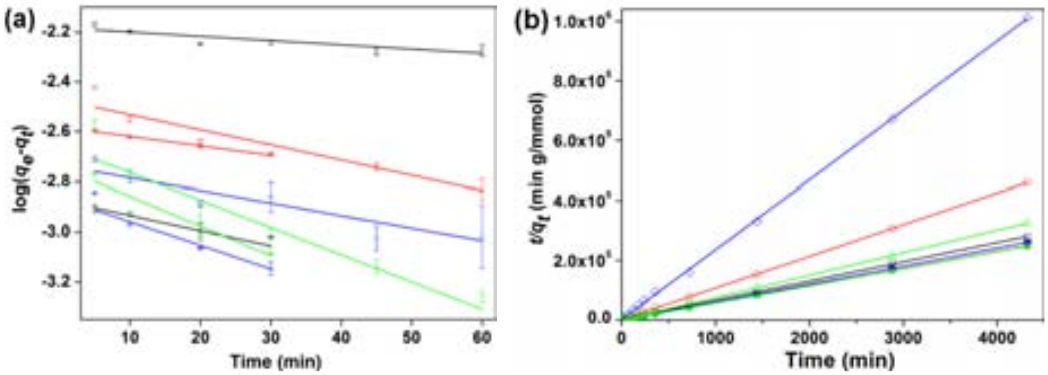

Figure 12. Biosorption kinetic models applied to the biosorption of metal ions by the treated human hair: (a) Pseudo-first order model, (b) Pseudo-second order model. (■ Cr/single, □ Cr/multiple; ● Cu/ single, ○ Cu/multiple; ◆ Cd/single, ◇ Cd/multiple; ▲ Pb/single, △ Pb/multiple; —— the linearization of the Pseudo1st order and Pseudo 2nd order models)

A straight line is obtained in all cases in all the time range (Figure 12 (b)) by plotting t/q against t for the pseudo-second order model, and the rate constant (k_2) and q_e values are determined from these plots. Both parameters and the correspondent correlation coefficients are also presented in Table 3. The pseudo-second order model shows the best fit to the experimental data with high correlation coefficients ($R^2 > 0.999$) for four metal ions in both systems (single- and multiple-metal), and theoretical values of q_e mostly agree with the experimental data (Figure 13, continuous lines). Both facts suggest that the pseudo-second order model is more likely to predict kinetic behavior for the whole range of time studied, which relies on the assumption that the rate limiting step might be the chemical sorption due to the formation of chemical bonds between the metal and the biosorbent in a monolayer onto its surface.[33, 34] The equilibrium biosorption capacities for Cr(III), Cu(II), Cd(II), and Pb(II) were 0.0166, 0.0174, 0.0165, and 0.0174 mmol/g, respectively in the single-metal system, and 0.0155, 0.0095, 0.0043, and 0.0133 mmol/g, respectively in the multiple-metal system.

Table 3. Pseudo-first and pseudo-second order metal equations parameters for the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) ions by the treated human hair in the single- and multiple-metal systems.

Metal		Cr(III)		Cu(II)		Cd(II)		Pb(II)	
system		single	multiple	single	multiple	single	multiple	single	multiple
Pseudo first order	$k_1 \times 10^3$ (min^{-1})	9.212[a]	4.192[b]	6.909[a]	14.970[b]	26.254[a]	12.943[b]	26.783[a]	27.337[b]
	q_e (mmol/g)	0.00132	0.00655	0.00263	0.00346	0.00151	0.00190	0.00173	0.00233
	R^2	0.93265	0.73429	0.99053	0.87939	0.91361	0.85924	0.83812	0.90298
Pseudo second order	k_2 (g/mmol min)	15.406	1.5068	3.8279	6.7457	38.1796	16.1676	17.7525	16.6435
	q_e (mmol/g)	0.01655	0.01554	0.01738	0.00945	0.0165	0.00429	0.0174	0.01330
	R^2	0.99999	0.99933	0.99985	0.99991	0.99998	0.99966	1	0.99997

a): 30 min, b): 1 h

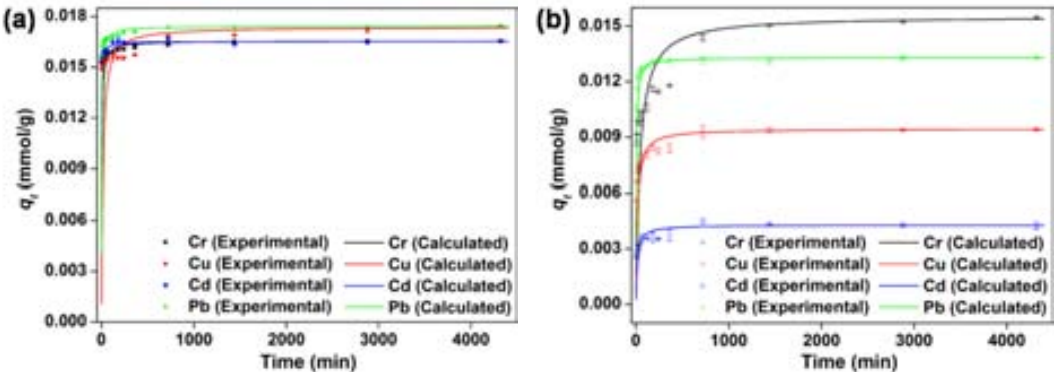

Figure 13. Experimental (•) and calculated (—) values adjusted by following the pseudo-second order model equation: (a) in the single-metal system, and (b) in the multiple-metal systems.

The pseudo-first and pseudo-second order models cannot provide information about the diffusion mechanism controlling the metal biosorption process onto the treated human hair. So, Weber-Morris intraparticle diffusion model is adjusted.[35] The plots of q_t versus $t^{1/2}$ are shown in the Figure 14. Unlike some simple cases, mathematical formulations representing the diffusion and the sorption processes are generally solvable analytically. In this case, these plots can be divided into multi-linear correlations, which indicate that the biosorption process take place in three steps and it is not controlled only by intraparticle diffusion mechanism. The first stage corresponds to the sharper stage, where the metal ions move from the solution to the external surface of the biosorbent, through the film diffusion or the boundary layer diffusion.[36] The sulfonate groups formed in the oxidation treatment could assist the achievement of this step. For each metal ion, this step is faster in single-metal system than in multiple-metal system. The second step describes the gradual biosorption onto the surface of the treated human hair, where the intraparticle diffusion is the rate-limiting.[37] The third stage corresponds to the final biosorption equilibrium where the intraparticle diffusion starts to slow down due to extremely low metal ion concentration left in solution. The presence of these three stages in the plots (Figure 14) suggests that the film diffusion and intraparticle diffusion were simultaneously controlling the biosorption process.

To sum up, the modelization analysis suggests that the film diffusion control the early stage of the biosorption process, while in the later stage the chemical sorption play an even greater role in the metal ion removal.

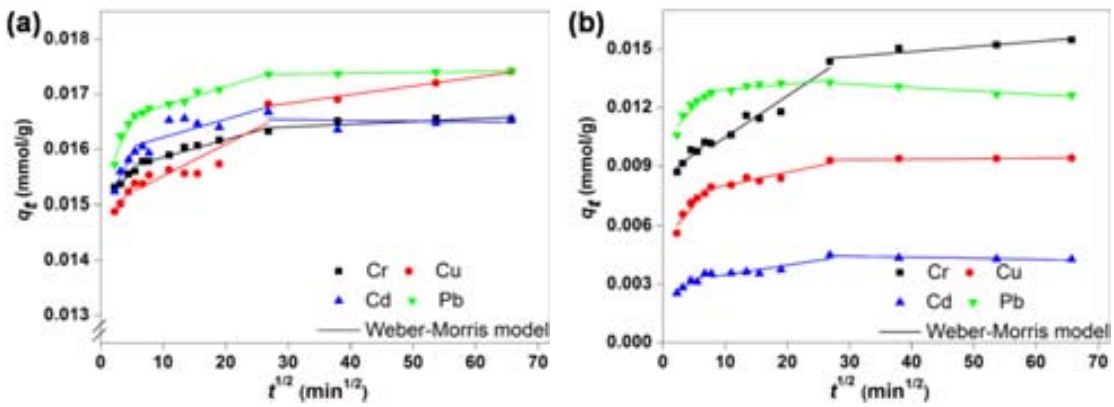

Figure 14. Weber-Morris intraparticle diffusion kinetic model applied for the metal ions biosorption onto the treated human hair, with time: (a) in the single-metal system, and (b) in the multiple-metal systems.

3.9. Biosorption isotherm modeling

Sorption isotherms at equilibrium are very important data to understand the mechanism of each sorption system from a physicochemical point of view. The sorption capacity of a sorbent can be also described by the equilibrium sorption isotherm, which is characterized by some specific constants whose value gives information about the affinity between the liquid-solid sorption systems.

In the present study, two isotherm models were selected to fit the experimental data, which are namely Freundlich and Langmuir isotherm models.[31] The linear forms of the Freundlich and Langmuir isotherms are presented by the following equations, (8) and (9), respectively:

$$\log q_e = \log k_F + \frac{1}{n}\log C_e \qquad (8)$$

$$\frac{C_e}{q_e} = \frac{b}{K_L}C_e + \frac{1}{K_L} \qquad (9)$$

where C_e is the equilibrium concentration of the metal ion in the residual solution (in mol/L), q_e is the equilibrium concentration of the sorbed per unit of mass of sorbent (in mol/g), k_F and n are Freundlich constants, $K_L = Q_0 b$, where Q_0 and b are the Langmuir constants, corresponding to the saturation concentration of the sorbed metal ion per unit of mass of sorbent (in mol/g) and the ratio of sorption/desorption rates (in L/mol), respectively.

For both models and following equations (8) and (9), log q_e versus log C_e and C_e/q_e versus C_e are calculated and compared with the experimental data, respectively. Also, all the constants and correlation coefficients obtained for each model are summarized in Table 4.

Regarding the corresponding correlation coefficient values obtained, we can conclude that the Langmuir isotherm fits the data better than the Freundlich isotherm showing that the biosorption process relies on a specific sites sorption mechanism where biosorbate molecules in

the monolayer of the surface occupy specific sites on the biosorbent. In Figure 15, experimental and calculated data for the Langmuir isotherm model are represented, and exhibit good correlation between them. Taking on board the Langmuir equation, the maximum biosorption capacity of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) at 295 K are 9.47×10^{-5}, 5.57×10^{-5}, 3.77×10^{-5}, 3.61×10^{-5} mol/g, respectively.

Table 4. Freundlich and Langmuir isotherms constants for the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) ions by the treated human hair.

Isotherm model	constant	Cr	Cu	Cd	Pb
Freundlich	$K_F\times10^3$	1.56	2.87	0.546	0.247
	n	2.29621	1.89703	2.86287	3.62726
	R^2	0.86464	0.84023	0.92914	0.86069
Langmuir	$Q_0\times10^5$ (mol/g)	9.47168	5.56652	3.77063	3.61032
	$b\times10^{-4}$ (L/mol)	1.0676	2.0630	8.6384	8.0364
	K_L (L/g)	1.01115	1.14837	3.25722	2.90141
	R^2	0.99115	0.99047	0.9952	0.99995
	$-\Delta G^0$ (kJ/mol)	22.750	24.366	27.878	27.701

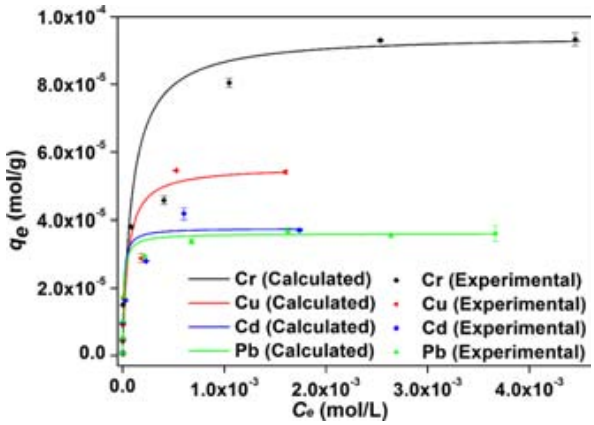

Figure 15. Experimental (•) and calculated (——) values adjusted by using the Langmuir isotherm model for the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) ions by the treated human hair.

In addition, from the estimated Langmuir adsorption/desorption constant, the standard Gibb's free energy (ΔG^0) of the biosorption process can be evaluated by using the following equation (9)

$$\Delta G^0 = -RT\ln b \qquad (10)$$

where b is the Langmuir equilibrium constant shown in equation (9), R is the universal gas constant (8.314 J/mol K) and T is the absolute temperature (K). The standard Gibb's free energy (ΔG^0) values are shown in Table 4. The negative ΔG^0 values indicated that the biosorption of metals into human hair is thermodynamically feasible and of spontaneous nature.[38]

3.10. Desorption, regeneration and reuse studies

Recovery of the adsorbed heavy metals and reuse of the biosorbent are of significance from the viewpoint of practical application. As indicated previously, two eluents including EDTA and HNO_3 solutions are screened for their potential to desorb Pb(II) ions from metal adsorbed treated human hair (corresponding results are collected in Table 5). Both eluents can effectively desorb the heavy metal ions from the metal loaded-treated human hair, even the elution efficiency of EDTA solution is slightly better than the HNO_3 solution. The sulfonate groups formed in the treatment process can assist EDTA to be able to combine with Pb(II).

The reuse of regenerated human hair for the possible continuous removal of heavy metals is investigated. After the desorption process, the hair samples are washed several times (following two different methods of cleaning), dried in an oven at 40 ˚C, and then their performance in a second biosorption step of fresh Pb(II) aqueous sample is checked. The results collected in Table 5, which show that the metal removal percentages (in the second biosorption step) of the regenerated hair samples are dependent on the elution methods. When using EDTA as eluent and deionized water for rinsing the used biomaterials, the best biosorption of Pb(II) can be found in second time. Although HNO_3 is a very powerful metal eluent, it shows negative effects for the "reuse" of hair samples and result in a decrease of metal uptake capacity in second time. Probably the acidic environment after the desorption step with nitric acid leads to the ionization state of functional groups on the biomaterials surface, so becoming a competitive media for the next metal biosorption step. To confirm this concept, another set of samples are eluted by using EDTA first, rinsed with HNO_3 solution, and later washed with deionized water and also dried (second chleaning or regeneration method). The biosorption capacities of the regenerated human hair samples by this method also decrease in the second time. It is clear that the desorption of metal adsorbed onto the biomaterials by using HNO_3 can affect their "reuse".

Table 5. The elution of adsorbed Pb(II) by using EDTA and HNO_3 solution and the biosorption capacity of the regenerated human hair.

Biosorbent		The treated human hair
1st biosorption, Pb(II) adsorbed (%)		89 ± 1
1st desorption	Pb(II) elution efficiency (%) using EDTA (0.1 M)	89 ± 1
	Pb(II) elution efficiency (%) using HNO_3 (0.1 M)	85 ± 1
2nd biosorption, Pb(II) adsorbed (%)	Regeneration by using EDTA elution	87 ±2
	Regeneration by using HNO_3 elution	38 ±4
	Regeneration by using EDTA elution and rinsing with HNO_3	75 ± 1

4. Conclusions

As expected, the treated human hair with oxidation modification shows good biosorption capacity for the removal of heavy metals. FT-IR analysis of different samples confirm that abundant metal binding groups such as carboxyl, hydroxyl, amino and sulfonate groups are responsible for the metal removal. The sulfonate groups formed in the oxidation modification process play a great role in the removal of heavy metals. Biosorption has been investigated following the influence of various parameters such as the initial pH of the aqueous solution, the biosorbent dosage, the contact time and the initial metal concentration. Biosorption of heavy metals is highly dependent on the pH of the solution, and the optimum biosorption capacity is determined at pH 4.0. Increase in the mass of the biosorbent leads to an increase in the metal removal, owing to corresponding increase in the number of metal binding sites. In addition, the treated human hair shows higher biosorption capacity in the multiple-metal system in terms of total biosorption capacity. According to the kinetic study, the biosorption of metal ions onto the treated human hair follows well the pseudo-second order kinetic model, which is in agreement with the chemical sorption being the rate limiting step. The biosorption equilibrium of metal ions is better fitted with Langmuir isotherm model compared to Freundlich model. Langmuir model suggests monolayer coverage of metal ions on the treated human hair. The maximum biosorption capacities of the treated human hair for Cr(III), Cu(II), Cd(II) and Pb(II) at 295 K were 9.47×10^{-5}, 5.57×10^{-5}, 3.77×10^{-5}, 3.61×10^{-5} mol/g, respectively. The calculated standard Gibb's free energy (ΔG^{0}) indicates the thermodynamically feasible and spontaneous nature of the biosorption process. Desorption experiments prove that 0.1 mol/L EDTA and HNO_3 solutions are efficient eluents for the recovery of Pb(II) from the treated human hair. Furthermore, the treated human hair regenerated with EDTA show the best biosorption efficiency when reusing. Taking into consideration present findings, it can be stated that the treated human hair (chemically-modified by an oxidation reaction process) is an effective and low-cost biosorbent for the removal of heavy metals from aqueous solutions. The oxidation treatment would be quite useful in modifying keratin biomaterials to enhance the removal of heavy metals from contaminated effluents.

Acknowledgements

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Annex II

Article submitted

Comparison between different keratin biomaterials for the removal of heavy metals from aqueous solutions

Helan Zhang[a], Fernando Carrillo[b,c], Montserrat López-Mesas[a], Cristina Palet[a,§]

[a] *Centre Grup de Tècniques de Separació en Química, Unitat de Química Analítica, Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Catalunya, Spain*

[b] *INTEXTER – Universitat Politècnica de Catalunya (UPC), Colom 15, 08222 Terrassa, Spain*

[c] *Departament d'Enginyeria Química, EET – Universitat Politècnica de Catalunya (UPC), Colom 1, 08222 Terrassa, Spain*

Abstract: It is well known that waste keratin biomaterials can be applied for the treatment of wastewaters as low cost biosorbents, even at low concentration levels. In this study, four common waste keratin biomaterials including human hair, dog hair, chicken feathers and degreased wool were used as biosorbents for the removal of heavy metal ions from aqueous solutions. The biosorption processes are carried out in batch processes, and the effect of contact time (5 min to 72 h), initial pH value (1.0-6.0) and biosorbent dosage (0.01-0.2 g) on the biosorption are investigated. The functional groups and the surface morphology of the biosorbents are confirmed before and after the metal biosorption process by Fourier transform infrared (FT-IR) spectroscopy and Scanning electron microscopy (SEM). For multiple-metal system, biosorption capacity of individual metal ion was reduced in the presence of other metal ions, but the total biosorption capacity increased. The total biosorption capacity of the tested four biosorbents followed the order degreased wool > chicken feathers > human hair > dog hair. Kinetic models including pseudo-first order, pseudo-second order and Weber-Morris intraparticle diffusion models are used to characterize the biosorption process. The experimental data fitted well the pseudo-second order kinetics except the biosorption of Cr(III) by human hair from multiple-metal system. Furthermore, biosorption isotherms of Pb(II) with different keratin biomaterials are determined and correlated with common isotherm equations such as Freundlich and Langmuir models, and results revealed that Langmuir model fitted much better than Freundlich model. The maximum biosorption capacities of Pb(II) onto human hair, dog hair, chicken feather and degreased wool are 2.43×10^{-5}, 2.07×10^{-5}, 3.87×10^{-5}, and 3.40×10^{-5} mol/g, respectively at 295K. Negative standard Gibb's energy indicated that the heavy metal biosorption process is thermodynamically feasible and spontaneous nature. It can be concluded that the four keratinous materials could be a good adsorbent for the metal ions from aqueous

[§]Corresponding author. Tel.: +0034 935813475; fax: +0034 935811985.
e-mail address: cristina.palet@uab.cat.

media.

Keywords: Keratin biomaterials; Heavy metal; Kinetics; Isotherm; Biosorption.

1. Introduction

A wide variety of toxic inorganic and organic chemicals are discharged to the environment as industrial wastes, causing serious water pollution. Industrial waste usually contains many pollutants, such as heavy metals, dye, phenols (including other organic compounds), inorganic anions, and pesticides (which are toxic to many living lifeforms and organisms), etc..[1, 2] Among toxic substances, heavy metal pollution is one of the most important environmental problems today. Heavy metal ions are extremely harmful to human, animals, and plants because of their toxicity, accumulation, no biodegradable nature and causing various diseases and disorders.[3] In this situation, the removal of heavy metals from wastewaters has attracted attention for the protection of public health and environment, and they must be removed before discharge.

Various techniques have been employed for the treatment of heavy metal pollutions, which usually include chemical precipitation (hydroxides, sulfides, etc.), membrane filtration (reverse osmosis, nanofiltration, etc.), electrochemical treatment, solvent extraction, ion exchange, evaporation and adsorption on activated carbon.[4] However, these methods existe disadvantages such as ineffective, especially when metal ion concentration in aqueous solution is among 1 to 100 mg/L, produce large quantity of sludge required to treat with great difficulty, also extremely expensive when treating large amount of water and wastewater containing heavy metal in low concentration.[5]

In recent years, applying biotechnology in controlling and removing metal pollution has been paid much attention, and gradually becomes hot topic in the field of heavy metal pollution control because of its potential application.[6] Alternative process is biosorption, it can be defined as the removal of metal or metalloid species, compounds and particulates from solution by various certain natural materials of biological origin.[7] A large quantity of materials has been investigated as biosorbents for the removal of heavy metal ions. Waste biomaterials are considered ideal alternatives as low-cost biosorbents for the removal of heavy metals from low strength wastewater due to the relatively cost-effective reason and efficient adsorption capacities.[8] Among the various biosorbents, cellulosic waste materials are the most abundant biosorbents for the removal of heavy metals, which include agricultural waste materials[9, 10] (wheat based waste material, rice husk), waste products[11] (bark, wood, sawdust) from timber industry. Cellulosic waste materials usually are composed of lignin and cellulose as major constituents and may also include other polar functional groups of lignin, which includes alcohols, aldehydes, ketones, carboxylic, phenolic and other groups. These groups have the ability to extent to bind heavy metals by donation of an electron pair from these groups to

complexes with the metal ion in solution. Chitin is the second most abundant as an excellent biosorbent for the removal of heavy metals after cellulose. The most abundant natural source of chitin is usually from shell or exoskeleton of animal such as crustaceans, arthropods and mollusks. Chitin is a polymer composed of N-acetyl-D-glucosamine or specifically 2-acetamido-2-deoxy-β-D-glucose through a β(1-4) linkage residue.[12] The adsorption behavior of chitin is primarily attributed to the high hydrophilicity of the polymer, the presence of a large number of functional groups (acetamido, primary amino, hydroxyl) and the high chemical reactivity of these functional groups. As a kind of abundant biological resources, keratinous materials are currently being investigated as a biosorbent for the removal of heavy metal ions from water and wastewater, which are relatively abundant and inexpensive materials. Various keratin biomaterials, such as wool, feather, hair and horn can be used as effective biosorbents, either directly or after activation, to remove heavy metals due to their high contents of carboxyl, hydroxyl, amino and sulfur-containing functional groups. These materials have showed significant potentials of removing metal ions from aqueous solutions.

Considered the importance of keratinous materials as biosorbents for the removal of heavy metals from wastewater, The aim of this work is to assess the performance of the human hair, dog hair, chicken feather and degreased wool for the removal of heavy metals from aqueous solutions, and to study the influence of the operating conditions such as the solution pH, the biosorbent dosage, the contact time. The experimental kinetic data were evaluated by applying the pseudo-first and pseudo-second order kinetic expressions and the Weber-Morris intraparticle diffusion model. The sorption equilibrium modeling was performed for removal of Pb(II) at different initial concentration by the Freundlich and Langmuir isotherm models.

2. Experimental

2.1. Chemicals

All the chemicals used in this work were of analytical grade. Stock solutions of separate heavy metal ions, such as Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) were prepared by dissolving their nitric salts (> 99%, all from Panreac, Spain) in deionized water. A 1000 ppm stock solution of metal ions was first prepared, which was then diluted to the initial heavy metal concentration for each experiment. Sodium hydroxide (> 98%, from Panreac, Spain) and nitric acid (> 70%, from JT-Baker, Spain) were alternatively used for the pH adjustment of the initial aqueous solution prior commencing the biosorption experiments. In all the experiments, the initial pH was measured, and usually the final pH was also checked, using an Omega 300 pH meter (Crison instruments, S.A., Spain)

2.2. Biosorbents

The native hair sample was obtained from local barbershops (in Terrassa city), which was washed with commercial detergent, rinsed several times with deionized water and then left to dry at room temperature (22±1 ºC). The hair was cut to an approximate length < 5 mm by using scissors. The small hair size < 5 mm was used in the batch biosorption experiments.

Dog hair was obtained from some colleagues of the research laboratory, which was also washed with commercial detergent, rinsed several times with deionized water and then left to dry at room temperature (22±1 ºC). An electrical grinding machine was used to make the dog hair much looser than the initial one. The loose dog hair was used in the batch biosorption experiments.

Chicken feathers collected from poultry farms were washed with commercial detergent, rinsed several times with deionized water and then left to dry at room temperature (22±1 ºC). After cleaning, they can be directly used in the batch biosorption experiments.

The wool sample used is flock degreased wool yarn kindly provided by a textile company. So, to remove impurities from the degreased wool sample, when received, it was washed with commercial detergent degreased wool, when received, was washed with commercial detergent, rinsed several times with deionized water and then left to dry at room temperature (22±1 ºC). It can be separated by scissors to weigh and was directly used in the batch biosorption experiments.

2.3. Characterization of biosorbents

Structural characterization of these four keratinous materials were carried out to analyze any chemical change produced in the samples after the oxidative pretreatment and/or after the biosorption of heavy metals. The identification of the functional groups on the untreated and treated human hair was performed by using Fourier transform infrared spectrometer (FT-IR) (Tensor 27, Bruker, Germany). The spectrum was recorded in the range of 600–4000 cm^{-1} with 16 scans and a resolution of 4 cm^{-1}. On the other hand, the surface morphology of these four keratinous materials was observed by scanning electron microscope (SEM ZEISS EVO® MA 10, Oberkochen, Germany). The sample used sputter-coating arrangement.

2.4. Batch biosorption experiments

The biosorption step for removing heavy metal ions from the aqueous solution by using biosorbent (human hair, dog hair, chicken feather and wool) was carried out under batch operation mode at a constant temperature of 22±1 ˚C. In all sets of experiments, 0.100 g of biosorbent was accurately weighted in 50 mL plastic extraction tubes, and 10 mL of the heavy

metal aqueous solution was added. The system was properly shaken on a rotary mixer (CE 2000 ABT-4, SBS Instruments SA, Barcelona, Spain) at 25 rpm, during the desired time. After that, the two phases were separated by decantation and the liquid was filtered through 0.22 μm Millipore filters (Millex-GS, Millipore, Ireland). Finally, after the filtration step, the metal concentration in the remaining aqueous solutions was determined by an inductively coupled plasma optical emission spectrophotometer with mass detector, ICP-MS (XSERIES 2 ICP-MS, Thermo Scientific, USA).

The percentage of biosorption of each metal ion by each biosorbent sample was calculated using the following equation 1, which indicates the biosorption efficacy:

$$\%\ biosorption = \frac{C_i - C_f}{C_i} \times 100 \qquad (1)$$

where C_i and C_f are the initial and the final concentration of heavy metal in the aqueous phase solution, respectively (in mmol/L).

The effect of pH on the biosorption of heavy metals was analyzed over the pH range from 1.0 to 6.0 in the multiple-metal aqueous solution (high pH values are not evaluated to avoid metal hydroxides precipitation). To study the effect of the biosorbent dosage on metal uptake, its mass was varied from 0.01 to 0.2 g. Each metal concentration is 0.18 mmol/L in multiple-metal aqueous solution. 10 mL of multiple-metal aqueous solution was agitated by a ratary mixer with biosorbent samle (human hair, dog hair, chicken feather and degreased wool) for 24 h, which is more than sufficient time to reach equilibrium. The temperature was kept constant at 22±1 ˚C.

For the kinetic studies, 0.1 g biosorbent sample (human hair, dog hair, chicken feather and wool) was contacted with 10 mL of multiple- and single- metal aqueous solutions (pH 4.0) by a rotary mixer during 5, 10, 20, 30 and 45 min and 1, 2, 3, 4, 6, 12, 24, 48 and 72 h. Experiments are conducted in the same conditions as mentioned previously.

The metal uptake (q_t) is the concentration of biosorbed metal ion per unit of mass of biosorbent (in mmol/g) at time t, which was calculated using the equation 2.

$$q_t(mmol/g) = \frac{(C_i - C_f) \times V}{W} \qquad (2)$$

where V is the total volume of the solution (in L), W is the amount of biosorbent (in g), C_i and C_f are the initial and the final concentrations of heavy metal in the aqueous solution (each given in units of mmol/L), respectively.

Biosorption isotherm studies were conducted at 22±1 ˚C in single-metal system with different initial concentration (0.5×10^{-3} - 6.0 mmol/L), whereby 10 mL of Pb(II) aqueous solution (pH 4.0) was agitated by a rotary mixer with 0.1 g of biosorbent sample (human hair, dog hair, chicken feather or wool) for 24 h, which is more than sufficient time to reach

equilibrium.

The amount of metal sorbed (q_e), is the concentration of biosorbed metal ion per unit of mass of biosorbent (in mmol/g) at the equilibrium, which was obtained as follows:

$$q_e(mmol/g) = \frac{(C_i - C_e) \times V}{W} \quad (3)$$

where V is the total volume of the solution (in L), W is the amount of biosorbent (in g), C_i and C_e are the initial and equilibrium concentrations of heavy metal in the aqueous solution (each given in units of mmol/L), respectively.

2.5. Desorption, regeneration and reuse

Desorption experiments were performed just for the case of Pb(II), after the corresponding biosorption step by using these four keratin biomaterials, and the centrifugation and filtration steps (by Millipore filters). Each biosorbent material containing the adsorbed Pb(II) was contacted and stirred with 10 mL of 0.1 mol/L HNO_3 or 10 mL of 0.1 mol/L EDTA, separately. After 24 h of stirring (with the rotary mixer) at room temperature (22±1 ˚C), the aqueous and the solid phases were properly separated (by centrifugation and filtration as usual) and the Pb(II) content of the final solutions were analyzed by ICP-MS, as indicated. Desorption percentage can be calculated using the equation:

$$\%\,\text{desorption} = \frac{amount \quad of \quad Pb(II) \quad desorbed}{amount \quad of \quad Pb(II) \quad adsorbed} \quad (4)$$

Reuse of these four keratin biomaterials in biosorption after elution of adsorbed metal ions will require H^+ and EDTA to be removed from the biosorbent surface. Two methods are used to regenerate the biosorbents after eluting the adsorbed metal ions. One method is washing the biosorbents with deionized water. In the other method, the biosorbents after eluting the adsorbed metal ions by using EDTA, are firstly rinsed with HNO_3 solution, and then are washed with deionized water. The biosorbents regenerated by these two methods are dried in an oven at 40 ˚C. In addition, another regenerated hair samples after elution by using EDTA, are firstly rinsed with HNO_3 solution, and then are washed with deionized water and dried. These three different regenerated human hair samples obtained from these two methods are employed to adsorb heavy metals again.

All batch biosorption experiments were carried out in duplicates and the results were reported as their average in the corresponding figures (experimental errors found were less than 5% and 0.0025 mmol/g in the biosorption percentage and the biosorption capacity, respectively)

3. Results and discussion

3.1. Comparison of the keratin biomaterials for the removal of heavy metals

The keratin biomaterials including human hair, dog hair, chicken feathers and degreased wool were evaluated as biosorbents for the removal of heavy metals from a multiple-metal aqueous solution containing eight metal ions (Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II)). The results are shown in Figure 1. As seen from the figure, the four different keratin biomaterials show different biosorption capacities for the different metal ions. In general, these four keratin biomaterials exhibit better biosorption capacities for Cr(III), Cu(II) and Pb(II) compared with the rest of metal ions present in the initial aqueous solution, which have an uptake capacities that decreases as follow: Cd(II) > Zn(II) > Ni(II) ≈ Co(II) > Mn(II). This can be explained by the different affinity of metal ions for the metal binding donor atoms present in all the biosorbents. The total biosorption capacity of the four biosorbents followed the order degreased wool > chicken feathers > human hair > dog hair. Finally, four metal ions including Cr(III), Cu(II), Cd(II) and Pb(II) were selected to check the biosorption behavior of the four keratin biomaterials in subsequent experiments (under different conditions). Firstly, the characterization of the four keratin biomaterials before and after biosorption process is followed by FT-IR and SEM, as indicated.

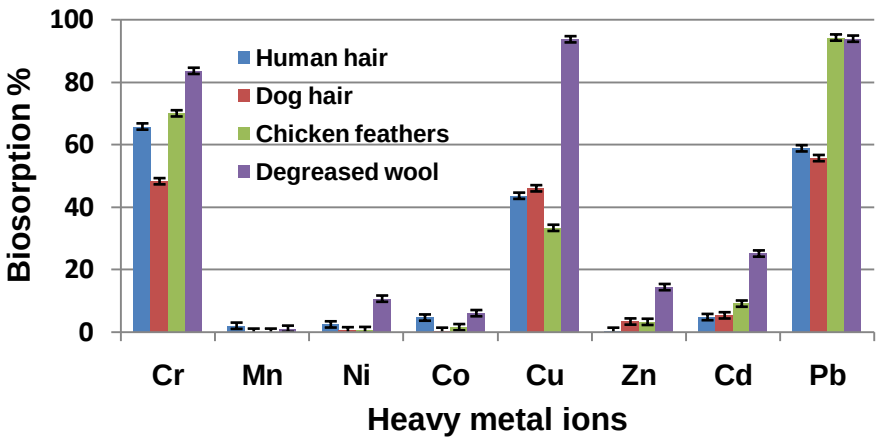


Figure 1. Biosorption of human hair, dog hair, chicken feather and degreased wool for Cr(III), Mn(II), Ni(II), Co(II), Cu(II), Zn(II), Cd(II) and Pb(II) from multiple-metal aqueous system. The initial metal concentration is 0.1 mmol/L, the contact time is 24 h, the intial pH is 4.0, the biosorbent is 0.1 g in 10 mL of the initial solution.

3.2. FT-IR and SEM Characterization

The FT-IR analysis is carried out to identify the functional groups in the different biosorbents that might be involved in the biosorption process. A comparison of FT-IR spectra of these four biosorbents before and after biosorption is shown in Figure 2 (a)-(d). The wavenumbers and approximate assignments of the vibrational modes for the FT-IR spectra are listed in Table 1.

Table 1. FT-IR spectral bands assignments for human hair, dog hair, chicken feathers and degreased wool.

Assignments	Wavenumbers(cm^{-1})			
	Human hair	Dog hair	Chicken feathers	Degreased wool
NH stretching	3280	3273	3268	3272
C-H stretching, $-CH_3$ and $-CH_2-$ asymmetric and symmetric modes	2957	2957	2961	2961
	2924	2926	2922	2930
Amide I, 80% C=O stretch and small contribution from NH bend	1630	1629	1624	1629
Amide II, C-N stretching/N-H bending	1517	1516	1534	1515
Amide III, complex vibration contains N-H bending, C-N stretching, C=O stretching, and O=C=N bending	1233	1232	1235	1234
Cystine	1180 - 1030			

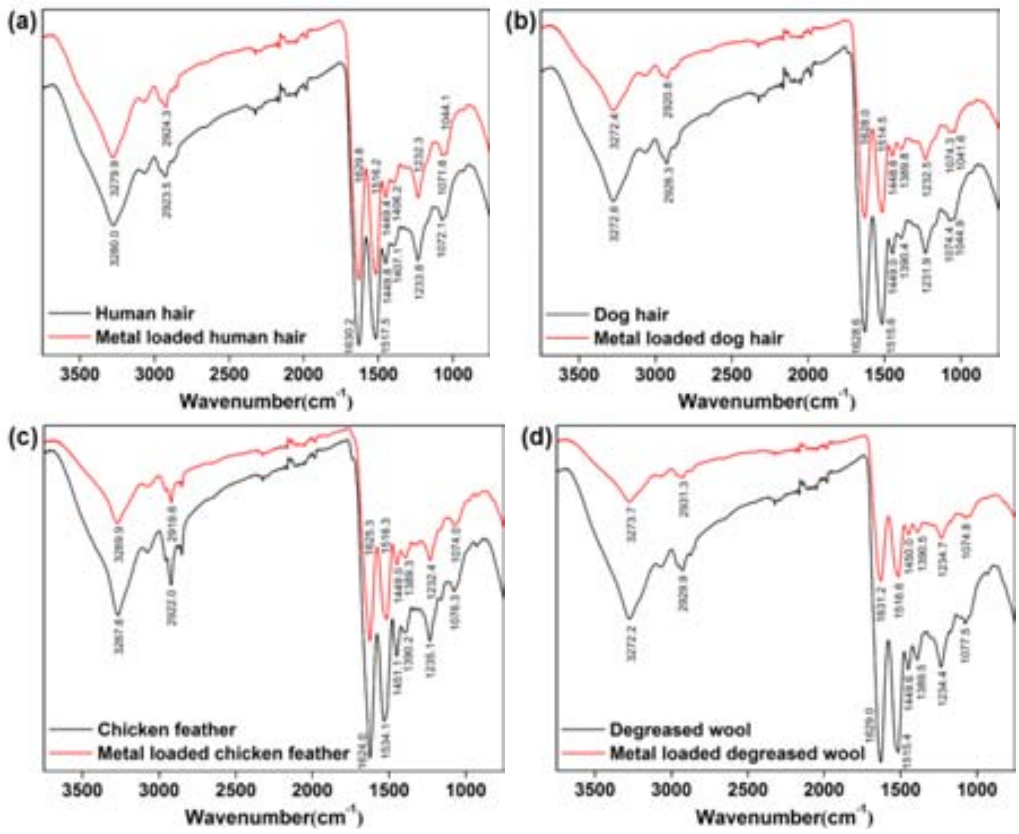


Figure. 2. FT-IR spectra of human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d).

From the FT-IR analysis of the biosorbents before biosorption, there are some findings. The peak located at 3275 cm^{-1} is a mode arising from N-H stretching and sensitive to hydrogen bonding, which is essential in the structuring of water and holding protein together. The two weak bands located at 2960 cm^{-1} and 2920 cm^{-1} could be assigned as modes arising from amino acid bands of $-CH_3$ and $-CH_2-$ asymmetric and symmetric modes, respectively.[13] The peaks

located at 1632 cm^{-1} (Amide I), 1520 cm^{-1} (Amide II) and 1241 cm^{-1} (Amide III) are related to common keratin fiber amino acids. The Amide I is a complex band with a coupling between carbonyl stretching modes, high contribution of -C=O stretch and a small contribution from NH bend. The Amide II is a mixed mode made up of C-N stretch and the N-H in plane bends. The Amide III includes C-N stretching, N-H in-plane bends, C=O stretching and O=C=N bending.[14] A weak broad shoulder bands in approximately 1180-1030 cm^{-1} region is related to the cystine and its oxidation products which includes cysteic acid, cystine monoxide, and cystine dioxide as well as sulfonate. Besides some environmental factors, such as sunlight, chlorinated water and frequent shampooing causing partial oxidation of the keratin biomaterial surface, partial oxidation of the degreased wool caused by the degreasing process is the important reason. This has resulted in the better biosorption capacity of the degrease wool compared with other keratin biomaterials. So, the FT-IR spectra analysis indicates that there are potential metal binding functional groups (including hydroxyl, amino, carboxyl and sulfur-containing groups) on the four biosorbents surfaces, sulfonate groups formed in the oxidation process also can enhance of the biosorption capacity as previously indicated.

The four metal-loaded biosorbents are also discriminated. The FT-IR spectra from four biosorbents before and after biosorption are very similar, indicating that the main functional groups on the biosorbents did not change significantly during the biosorption process (which can be an indication of the possible reuse of such biomaterials). However, the four metal-loaded biosorbents show small differences in the FT-IR spectra. For instance, at the wavenumber 1072.1 cm^{-1} the cystine monoxide (R-SO-S-R) can be assigned for the human hair. This weak peak has a small red shift (from 1072.1 cm^{-1} to 1071.6 cm^{-1}) and was of greater intensity after the metal biosorption process. The same changes also happened to the other three keratin biosorbents. Moreover, more slightly shifts of the peaks are found in other functional groups which can be seen in the spectra collected in Figure 2 (a)-(d). Based on the shifts in the wavenumbers of different peaks from the FT-IR spectra of the metal-loaded keratin biomaterials, it can be deduced that hydroxyl, amino, carboxyl and sulfur containing functional groups could act as the biosorption sites for the removal of metal ions.

The surface morphologies of the four keratin biomaterials under study are observed by Scanning electron microscope (SEM) before and after the metal biosorption process. It is also shown in Figure 3 (a)-(d). As can be seen, there are no significant differences on the surface morphology of the four biosorbents before and after the metal biosorption process. This result suggests that the four biosorbents are relatively stable under the biosorption process, which is favorable in terms of their reuse. The surface morphologies of the keratin fibers including human hair, dog hair and degreased wool are very similar, which appears to be somewhat

smoother after the biosorption process comparing with them before biosorption, suggesting that the cuticle scales are closed through the biosorption, probably due to the acidic initial aqueous media (see F igure 3 (a2), (b 2) a nd (d 2) a nd c ompared w ith F igure 3 (a1), (b 1) a nd (d 1), respectively). In the case of the chicken feathers a little bit rough appearance after biosorption process is seen (Figure 3 (c2) compared with (c1)), probably due to the relative lower stability compared with the other biosorbents. This may be caused by a lower cystine content of chicken feathers that contributes to this lower stability compared with the other biosorbents. In addition, according to the comparison of the keratin fiber size, it can be observed that chicken feathers have higher sur face a rea c ompared w ith other t hree k eratin bi omaterials, w hich l ead t o a relatively good biosorption capacity.

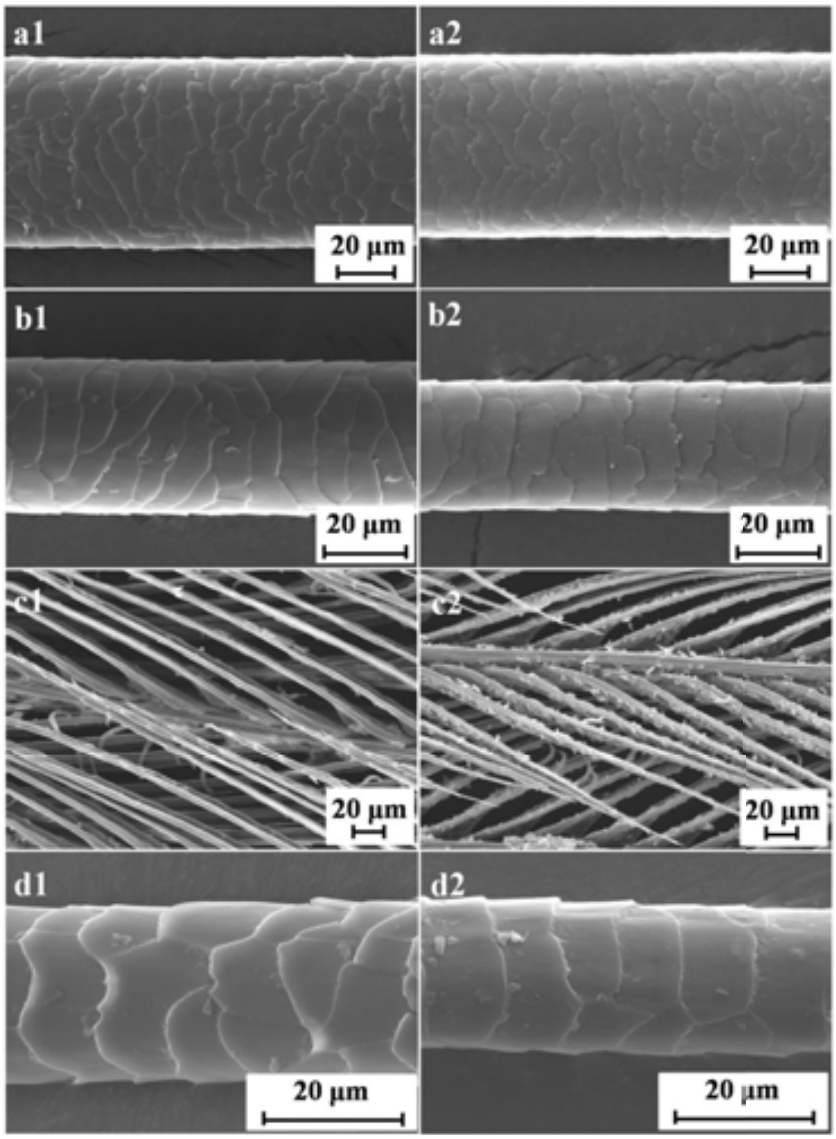


Figure 3. Scanning electron microscopy (SEM) micrographs of the biosorbents: (a1) and (a2) correspond to the human hair and metal loaded human hair; (b1) and (b2) correspond to the dog hair and metal loaded dog hair; (c1) and (c2) correspond to the chicken feathers and metal loaded chicken feathers; and (d1) and (d2) correspond to the degreased wool and metal loaded degreased wool.

3.3. Effect of the initial pH

The initial aqueous pH is a key parameter in metal biosorption process, for both the metal aqueous speciation and the surface functional groups speciation of the keratin biomaterials.[15] As usual, batch biosorption experiments are carried out in the pH range of 1.0 to 6.0 in a multiple-metal system (collected results are shown in Figure 4 (a)-(d)). As shown in Figure 4 (a)-(d), the biosorption of metal ions increases significantly with the increase of the aqueous pH value. As in the previous cases presented, at lower pH values, metal ions had to compete with a large number of protons for the metal binding sites on the biosorbent surface. Therefore, the biosorption capacities of metal ions are very low for all four biosorbents. As pH increased, the concentration of proton decreases, and the positive metal ions can be easily adsorbed by the available binding sites on the biosorbent surface. Above pH 4.0, biosorption of metal ions was found to be relatively constant, probably due to metal speciation with the partial hydrolysis of metal ions (especially for Cu(II), for the rest of the metal ions it can occur at pH higher than 5.0). Therefore, the pH 4.0 was selected as optimal condition in the subsequent experiments.

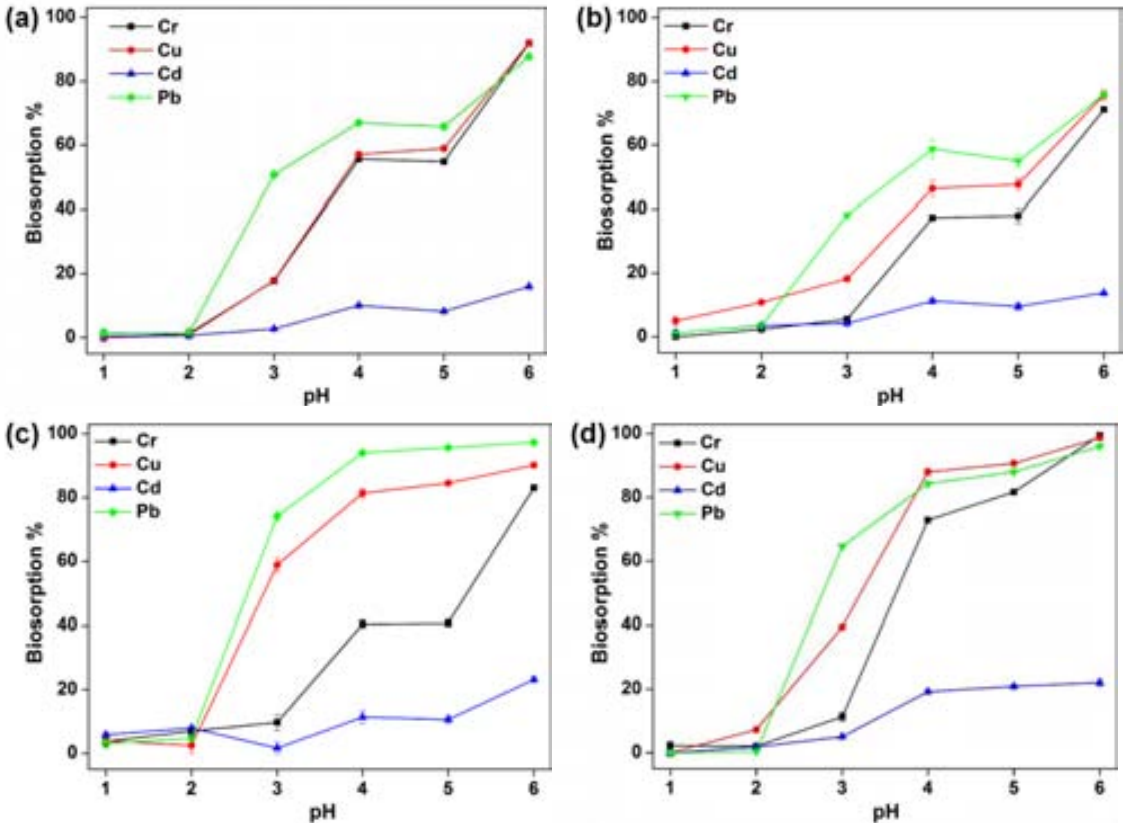


Figure 4. Effect of the initial pH on the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) by human hair (a), dog hair (b), chicken feathers (c), and degreased wool (d) from the multiple-metal system. The initial concentration is 0.18 mmol/L, the contact time is 24 h, and the initial pH is 4.0.

3.4. Effect of biosorbent dosage

The removal of heavy metal ions from multiple-metal aqueous solution (0.18 mmol/L) was

significantly depending on the amount of biosorbent, as seen previously. The effect of biosorbent dosage on the biosorption percentage of metal ions for the different keratin biomaterials is shown in Figure 5 (a)-(d). The biosorption percentage of metal ions increases with the increase of biosorbent dosage, probably due to the increase of the total surface area of each biosorbent when increasing its amount, which can be related to a greater number of available metal binding sites.[16] There is a slight sharper increase in the biosorption percentage with increasing biosorbent dosage for Pb(II) ions compared with other metal ions (being Cr(III) closer, Cu(II) and Cd(II) the lowest one). Figure 6 (a)-(d) shows the adsorbed metal ion (mmol) per unit weight of biosorbent, where Cu(II) uptake shows a reverse trend to the biosorption percentage. This can be explained due to the fact that at high biosorbent dosage level, the available Cu(II) ions in the aqueous solution are insufficient to cover all the biosorbent sites, meanwhile other metal ions have opportunity to cover the biosorbent sites (related with the different affinities of the biomaterials for each heavy metal ion).[17] So, the adsorbed Pb(II) (mmol) per unit weight of biosorbent increased at first, then decreased slowly with increasing biosorbent dosage, which corresponds to a shaper increase in percentage removal for Pb(II) ions.

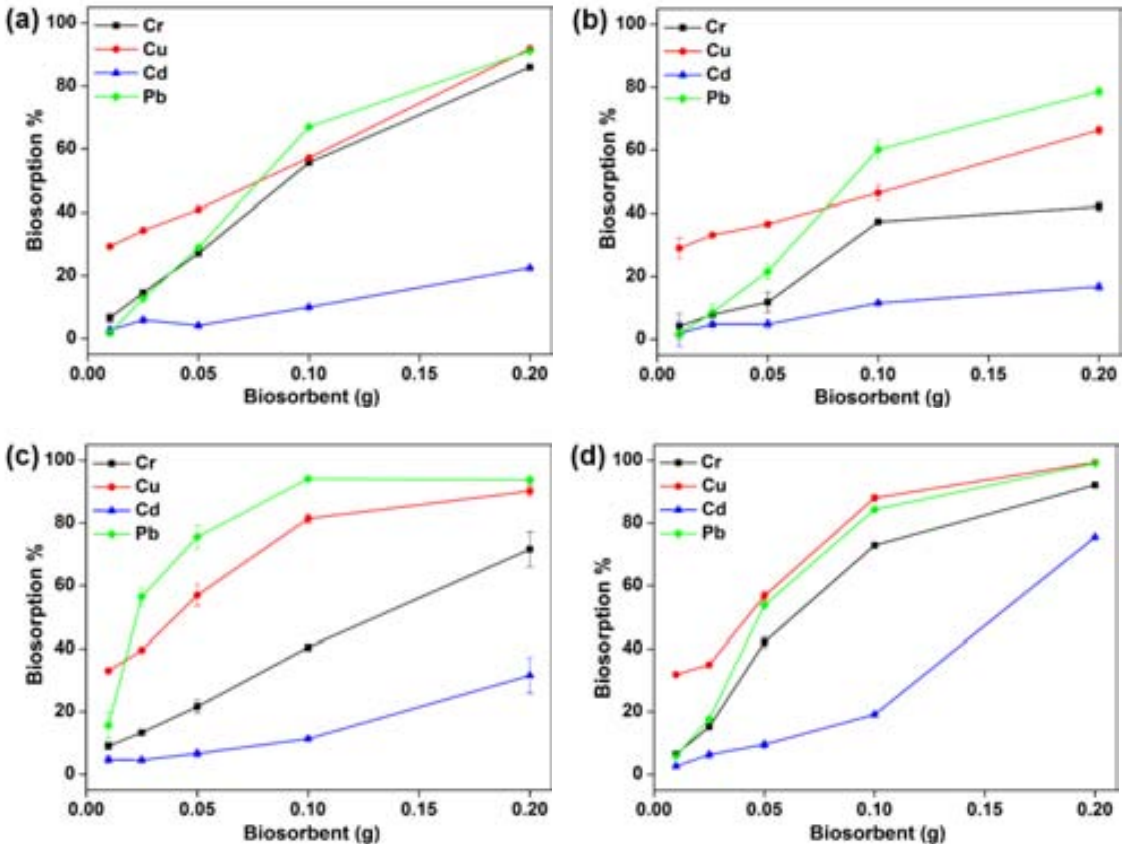


Figure. 5. The percentage of biosorption for for Cr(III), Cu(II), Cd(II) and Pb(II) in the multiple-metal system by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d). The initial metal ions concentration is 0.18 mmol/L, the contact time is 24 h, and the initial pH 4.0.

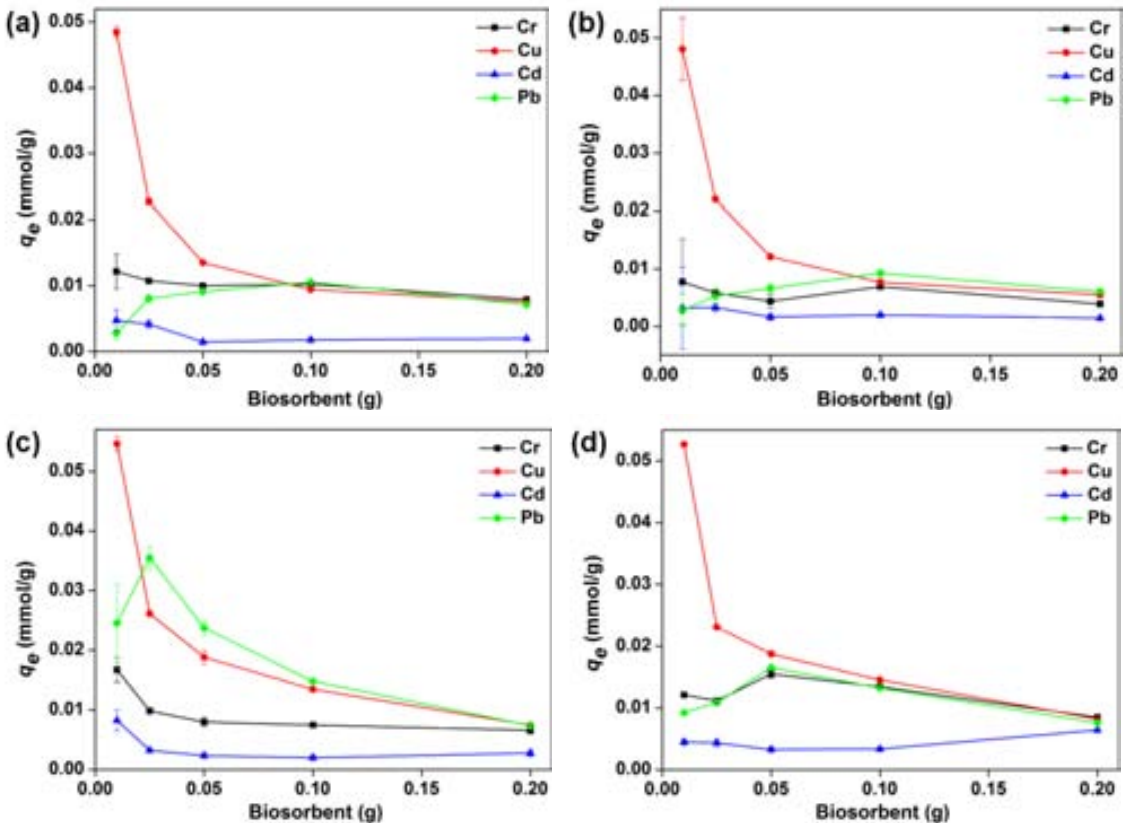


Figure 6. The adsorbed metal ion per unit weight of biosorbent for Cr(III), Cu(II), Cd(II) and Pb(II) in the multiple-metal system by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d). The initial metal ions concentration is 0.18 mmol/L, the contact time is 24 h, and the initial pH is 4.0.

These four keratin biomaterials have great biosorption capacities for Cr(III), Cu(II) and Pb(II) ions and there is a competition between metal ions in their biosorption process. The four biosorbents show a preferential binding capacity for Cu(II) ions compared with the other three metal ions in the mixture. The biosorption capacity and removal percentage are equally important in adsorption experiments because both factors usually take part in deciding the adsorption performance of a given adsorbent. The biosorbent dosage of 0.1 g was selected for the further biosorption studies because it showed both relatively high biosorption percentage (%) and high biosorption capacity (mmol/g) for all four biosorbents.

3.5. Effect of the contact time

Multiple-metal aqueous system (at pH = 4.0) of Cr(III), Cu(II), Cd(II) and Pb(II) and single-metal aqueous system of Pb(II) were contacted with the keratin biomaterials (0.1 g of human hair, dog hair, chicken feathers and degreased wool) during 5, 10, 20, 30, 45 minutes, and 1, 2, 3, 4, 6, 12, 24 48 and 72 hours. Results plotted in Figure 7 (a)-(d) show the biosorption percentage in each biosorbent case for the removal of Cr(III), Cu(II), Cd(II) and Pb(II) ions from the multiple-metal aqueous solutions. Different biosorbents require different time interval to reach the equilibrium. It is observed that in all cases the biosorption percentage of metal ions

increases with the increase of the time until the equilibrium is reached. In general, the biosorption of metal ions consisted of two steps. An initial rapid step where the rate of biosorption is high, and a second slower step where the equilibrium uptake achieves. Moreover, the biosorption process of the multiple-metal system by the four keratin biomaterials is different. The biosorption percentage of Cr(III), Cu(II) and Pb(II) by human hair and dog hair increases gradually with increasing contact time, reaching nearly equilibrium at around 24 h. For the degreased wool only 6 h are needed to reach the equilibrium. In the case of the chicken feathers, the removal of Cu(II) and Pb(II) ions increases sharply with time, and reaches the equilibrium after 30 minutes, being necessary 48 h for the Cr(III) case. All the biosorbents show low removal of Cd(II), even at long time of the biosorption process, and the maximum of biosorption percentage is about 20%. So, in the subsequent kinetic analysis Cd(II) is not considered for the biosorption modeling onto the four biosorbents.

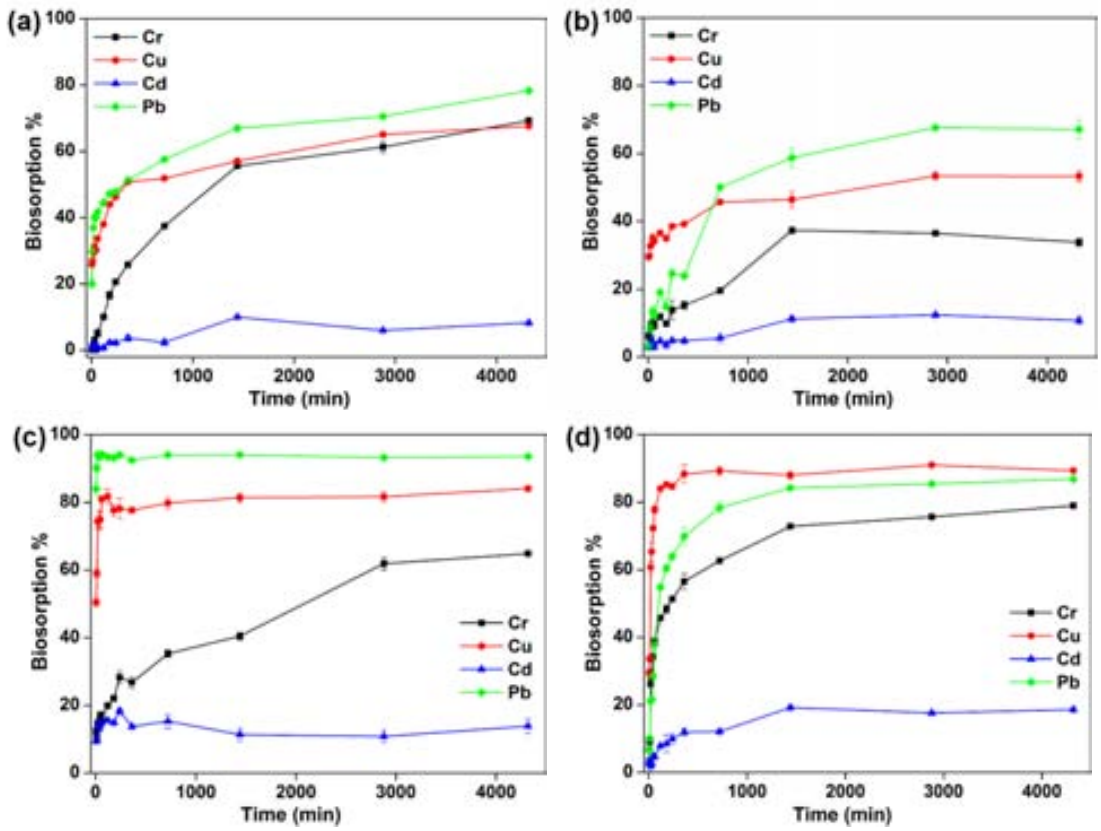


Figure 7. The biosorption percentage of Cr(III), Cu(II), Cd(II) and Pb(II) by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d) from multiple-metal system at different contact time. The initial metal ionsconcentration is 0.18mmol/L, the pH is 4.0, and the biosorbent is 0.1 g.

Effect of the contact time is also studied in the single-metal system. The biosorption of Pb(II) by human hair, dog hair, chicken feathers and degreased wool is shown in Figure 8. It is observed that an equilibrium time of 24 h is needed for the biosorption of Pb(II) by human hair

and dog hair, while 30 and 5 min are enough to attain equilibrium when degreased wool and chicken feathers are used, respectively. The experimental results indicate that the biosorption percentage of Pb(II) by the four biosorbents is greater than 90 % in all cases, and followed the upper order as follows: chicken feathers > degreased wool > human hair > dog hair. This is probably due to the high surface area of the chicken feathers and the partial oxidation of the degreased wool, which are confirmed by SEM and FT-IR studies, respectively.

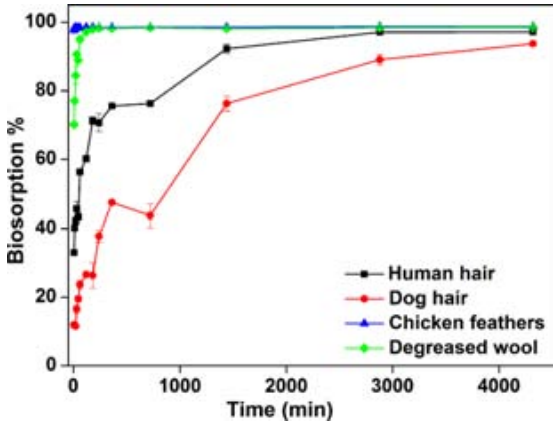


Figure 8. Biosorption percentage of Pb(II) by human hair, dog hair, chicken feathers and degreased wool from single-metal system at different contact time. The initial metal concentration is 0.18 mmol/L, the pH is 4.0, and the biosorbent is 0.1 g.

According to the comparison of the biosorption capacities of Pb(II) by the four keratin biosorbents from single- and multiple-metal systems (Figure 9), it can be seen that the presence of other metal ions affects the biosorption of Pb(II) onto the biosorbents, as expected, due to metal ion competition to the binding sites of the biosorbent surface.

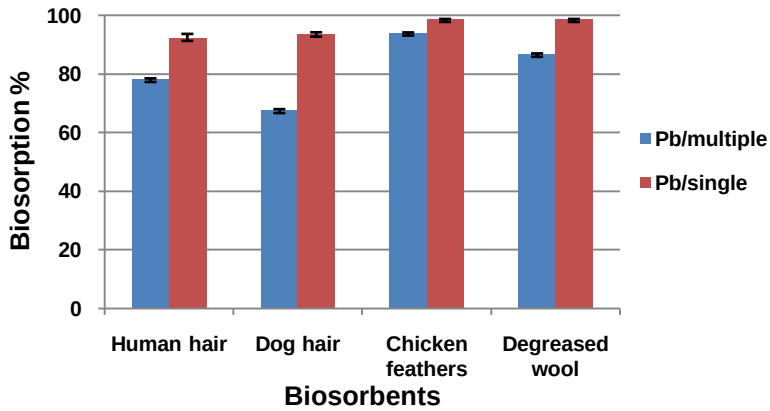


Figure 9. Biosorption of Pb(II) by human hair, dog hair, chicken feathers and degreased wool from single- and multiple-metal systems. The initial metal concentration is 0.18 mmol/L, the pH is 4.0, and the biosorbent is 0.1 g.

3.6. Biosorption kinetics modeling

Adsorption is a given solute on a solid is a fairly complex mechanism. Indeed, the speed of adsorption is strongly influenced by several parameters such as the status of the solid matrix that

has generally heterogeneous reactive sites, and the physical-chemical conditions under which the adsorption takes place.[18] A study of kinetics of adsorption is desirable as it provides information about the mechanism of adsorption, which involved the potential rate controlling steps such as mass transport, pore diffusion and chemical reaction processes. In present study, the sorption data are analyzed by using three simple kinetic models, which include the pseudo-first order and the pseudo-second order rate equations and the Weber-Morris intraparticle diffusion model.

3.7.1. Pseudo-first order rate equation

The pseudo-first order kinetic model has been most widely used for the adsorption of an adsorbate from an aqueous solution throughout the years, such as metal ions, dyestuffs, and contaminating organic compounds.[19, 20] This model based on solid capacity considers that the rate of occupation of adsorption sites is proportional to the number of unoccupied sites. The pseudo-first order rate equation is generally expressed as follows:

$$\frac{dq}{dt} = k_1(q_e - q) \qquad (5)$$

Integrating this for the boundary condition $t = 0$ to $t = t$ and $q_0 = 0$ to $q_t = q_t$, Eq. (5) may be rearranged for linearized data plotting as shown by Eq. (6)

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \qquad (6)$$

where, q_e and q_t are the concentration of biosorbed metal ion per unit of mass biosorbent (in mmol/g) at the equilibrium and at time t (min), respectively, and k_1 (in min^{-1}) is the rate constant of the pseudo-first order equation. The pseudo-first order equation has been adequately employed to describe reactions. However, it is typically expressed in a range of reaction only. In the present case of the biosorption of Cr(III), Cu(II) and Pb(II) ions by different keratin biomaterials, according to the effect of contact time on the biosorption, the equilibrium time is less than 20 min when chicken feathers are used for the biosorption of Pb(II). So the pseudo-first order model cannot be used to characterize the biosorption process in the Pb(II)/chicken feathers, neither in multiple nor in single systems. The plots of the linearized form of the pseudo-first order model for the rest of metal/biosorbent systems are shown in Figure 10 (a)-(d). The k_1 value, q_e calculated and correlation coefficient is given in Table 2.

As shown in Figure 10 and Table 2, the pseudo-first order equation does not fit well over the entire range of contact time investigated, which is generally applicable only over the initial time of the sorption process. The calculated q_e by this model differs significantly of those measured experimentally, which is suggesting the insufficiency of the pseudo-first order model to fit the experimental data. An exception was noted for the Cr(III)/human hair system (Figure 10a), where the pseudo-first order equation fits well in the whole range of time with high

correlation coefficient (0,9472), and the calculated q_e is close to experimental one (just slightly smaller). The reason for these slightly differences in the q_e values is that there is time lag, possibly due to a boundary layer at the surface of the biosorbent or the external resistance at the surface controlling the beginning of the sorption process.[21] So, like most case in the literature, this model generally underestimate the q_e values.[22]

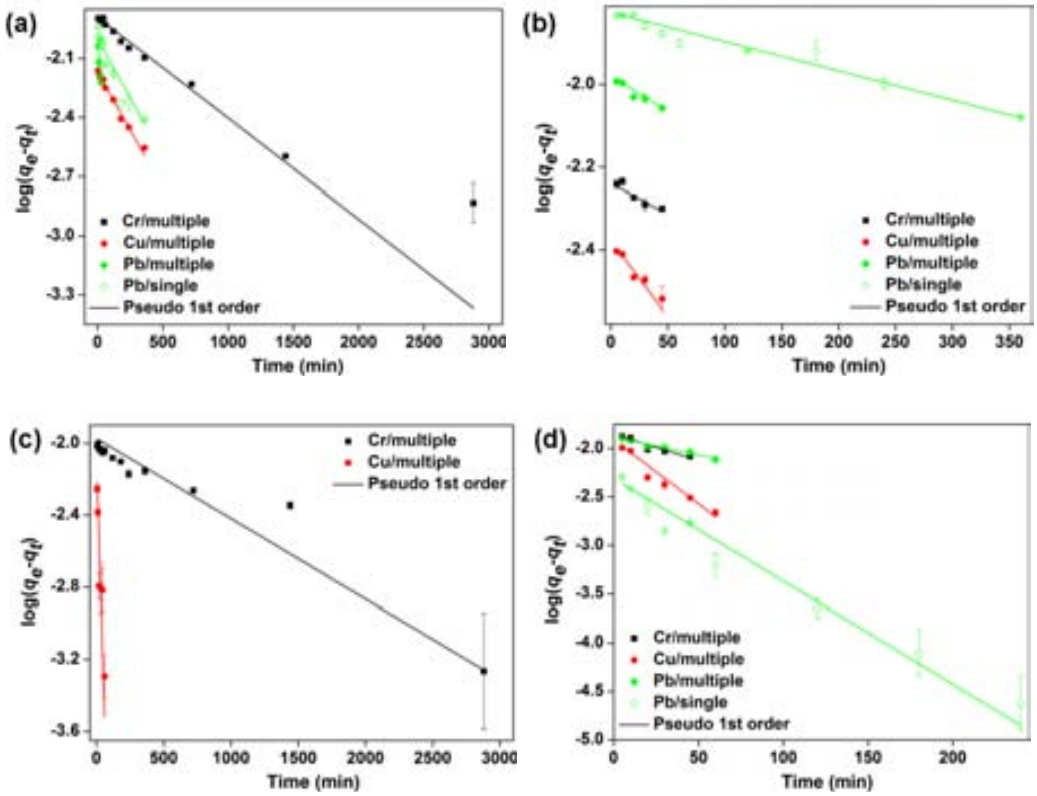


Figure 10. The pseudo-first order adsorption kinetics plots for the sorption of metal ions by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d).

3.7.2. Pseudo-second order rate equation

The pseudo-second order model is derived on the basis of the sorption capacity of the solid phase.[23, 24] This model assumes that the rate of occupation of adsorption sites is proportional to the square of the number of unoccupied sites. It can be expressed as

$$\frac{dq}{dt} = k_2(q_e - q)^2 \qquad (7)$$

Integration of Eq. (7) with the boundary conditions $t = 0$ to $t = t$ and $q_0 = 0$ to $q_t = q_t$, Eq. (8) which corresponds to the integrated rate law for a second-order reaction, is obtained:

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \qquad (8)$$

Eq. (8) can be stated in the linear form as Eq. (9)

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (9)$$

where, q_e and q_t are the concentration of biosorbed metal ion per unit of mass biosorbent (in mmol/g) at the equilibrium and at time t (min), respectively, and k_2 (in min^{-1}) is the rate constant of the pseudo-second order equation. The plots t/q_t versus t for the pseudo-second order model are shown in Figure 11 (a)-(d). The k_2 value, q_e calculated and correlation coefficient is given in Table 2. Except for the biosorption of Cr(III) by human hair, the pseudo-second kinetic model showed the best fit to the experimental data with the highest correlation coefficients (R^2>0.95). In addition, as shown in Table 2, the pseudo-second order calculated q_e values agree most with the experimental data. Thus, these results suggest that the rate limiting step might be the chemical sorption. Chemical sorption could occur by the functional groups on the biosorbent surface with the metal ions as valence forces through sharing or exchange electrons.

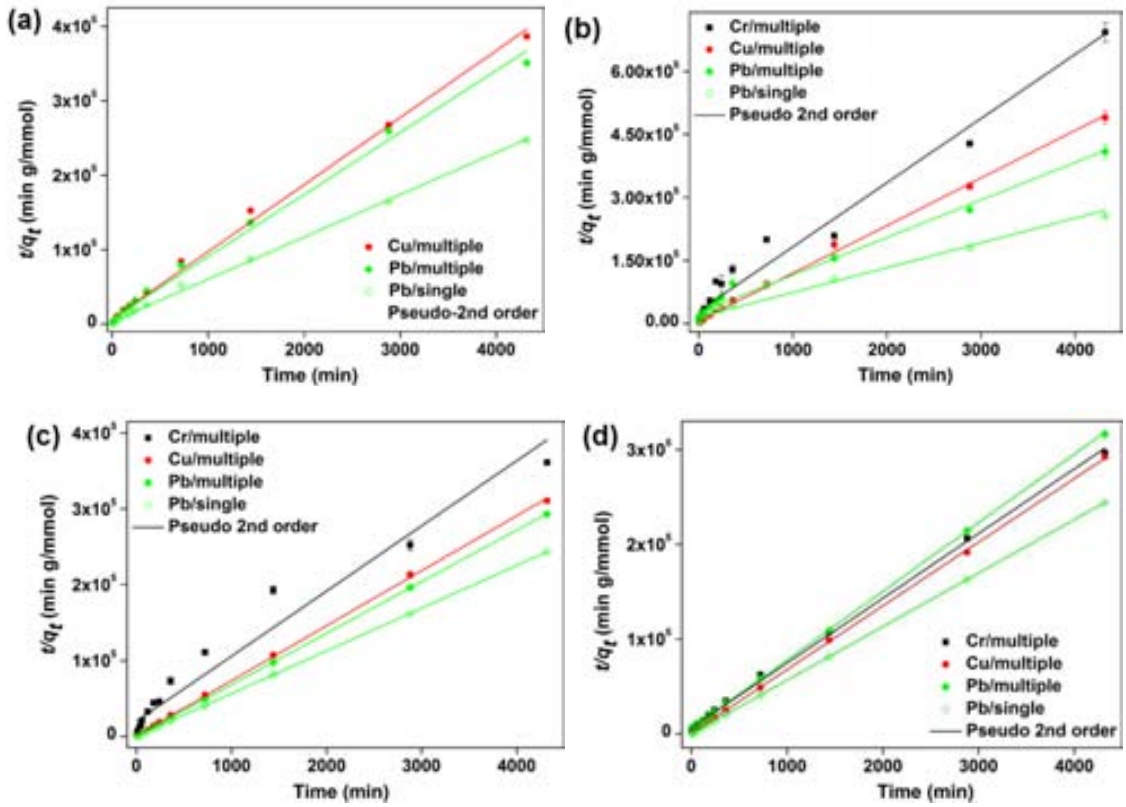


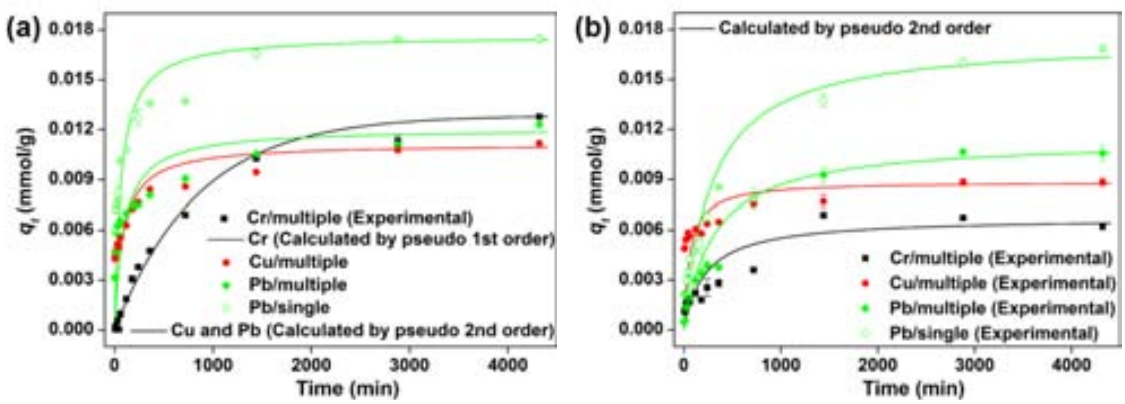
Figure. 11. The pseudo-second order adsorption kinetics plots for the sorption of metal ions by human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d).

Table 2. Biosorption kinetic constants for the biosorption of Cr(III), Cu(II), Cd(II) and Pb(II) by human hair, dog hair, chicken feathers and degreased wool.

Biosorbent	Metal	Experimental $q_{max}\times10^2$ (mmol/g)	Pseudo-first order $k_1\times10^3$ (min^{-1})	Pseudo-first order $q_e\times10^2$ (mmol/g)	Pseudo-first order R^2	Pseudo-second order k_2(g/mmol min)	Pseudo-second order $q_e\times10^2$ (mmol/g)	Pseudo-second order R^2
Human hair	Cr/multiple	1.278	8045.1[f]	1.177	0.94722	-	-	-
	Cu/multiple	1.118	2.53[e]	0.671	0.98167	1.059	1.115	0.99616
	Pb/multiple	1.231	15.89[a]	0.937	0.866	0.8839	1.207	0.99373
	Pb/single	1.747	3.11[e]	1.021	0.89417	0.8632	1.767	0.99835
Dog hair	Cr/multiple	0.689	3.87[b]	5.88	0.85959	0.6701	0.673	0.97138
	Cu/multiple	0.883	6.72[b]	0.408	0.93641	1.831	0.886	0.99734
	Pb/multiple	1.064	3.8[b]	1.034	0.91988	0.2847	1.137	0.98477
	Pb/single	1.685	1.53[c]	1.47	0.95016	0.2003	1.745	0.97092
Chicken feathers	Cr/multiple	1.196	0.9086[f]	0.972	0.93951	0.3089	1.209	0.95992
	Cu/multiple	1.390	37.47[c]	0.543	0.8354	5.691	1.38	0.99969
	Pb/multiple	1.481	-	-	-	365.39	1.474	0.99999
	Pb/single	1.775	-	-	-	124.74	1.775	1
Degreased wool	Cr/multiple	1.457	10.59[c]	1.347	0.90169	0.7180	1.466	0.99846
	Cu/multiple	1.505	28.17[c]	1.088	0.9414	6.3072	1.489	0.99986
	Pb/multiple	1.366	8.41[c]	1.298	0.95928	0.8826	1.389	0.99991
	Pb/single	1.770	23.08[d]	0.401	0.9501	25.084	1.770	1

a): 30 min, b): 45 min, c) 1 h, d): 3 h, e): 6 h, f): 72 h

According to the kinetic studies of pseudo-first order and pseudo-second order models, the Cr(III)/human hair system was found to fit better to the pseudo-first order equation, while other metal/biosorbent systems fit better to the pseudo-second order equation. For all the metal/biosorbent systems, the theoretical biosorption capacity (q_t) data calculated by the agreeable kinetic models and the experimental ones are shown in the Figure 12, where it can be seen that those values are really close each other.



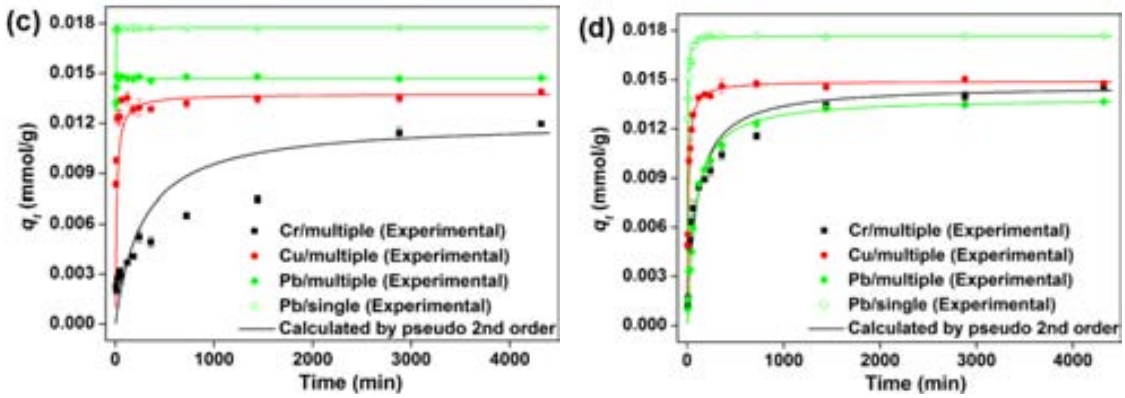


Figure 12. Experimental (.) and calculated (-) values adjusting by following the agreeable kinetic model equation: (a) human hair, (b) dog hair, (c) chicken feathers and (d) degreased wool.

2.7.3. Weber-Morris Intraparticle Diffusion Model

The overall rates of metal biosorption from aqueous solution onto biosorbent surface have been frequently evaluated by biosorption kinetic models alone such as the pseudo-first and pseudo-second order models. However, the adsorbate species are most likely transported from the bulk of the solution into the solid phase through an intraparticle diffusion process, which is often is the rate-limiting step in many adsorption processes. So, the intraparticle diffusion equation as described by Weber and Morris may be applied in the determination of the intraparticle diffusion rate constant, k_3, and the boundary resistance, k_d.[25] Theoretical treatments of intraparticle diffusion yield rather complex mathematical relationships which differ in form as functions of the geometry of the biosorbent particle.[26] A functional relationship common to most treatments of intraparticle diffusion is that uptake varies almost proportionately with the half-power of time, $t^{0.5}$, rather than t; a nearly linear variation in the quantity biosorbed with $t^{0.5}$ is predicted for large initial fraction of reactions controlled by rates of intraparticle diffusion.[27] This model is expressed as the following equation 10:

$$q_t = k_3 t^{1/2} + k_d \qquad (10)$$

where k_3 is the intraparticle diffusion rate constant (mmol/(g min$^{1/2}$)) and k_d is the intercept that gives idea about the thickness of the boundary layer, and q_t is the concentration of biosorbed metal ion per unit of mass biosorbent (in mmol/g) at time t (min).

The plots of q_t versus $t^{1/2}$ are shown in the Figure 13 (a)-(d). According to these authors, if intraparticle diffusion was involved in the sorption process, then uptake of the adsorbate should vary linearly with the square root of time and if this line passes through the origin then intraparticle diffusion is the rate-controlling factor. From the figure, it was noted that the biosorption process tends to be followed by two or more steps. Before arriving the final biosorption equilibrium, the film diffusion where the metal ions move from the solution to the external surface of biosorbent, and the intraparticle diffusion together control this initial stage of

biosorption process. However, the time required for arriving the final biosorption equilibrium, is totally different for each biosorbent used. For instance, metal ions can be quickly adsorbed onto the chicken feathers and degreased wool compared with the other two biosorbents (human hair, dog hair and degreased wool), especially in Pb(II)/chicken feathers system. This is due to the fact that the mentioned lower cystine content of chicken feathers make them more hydrophilic and higher surface area compared with the other keratin biomaterials, and partial oxidation of degreased wool. They can assist the metal transport from the solution to the external surface of biosorbent. Further, the linear plots do not pass through the origin and this indicates that intraparticle diffusion is not only rate controlling step.

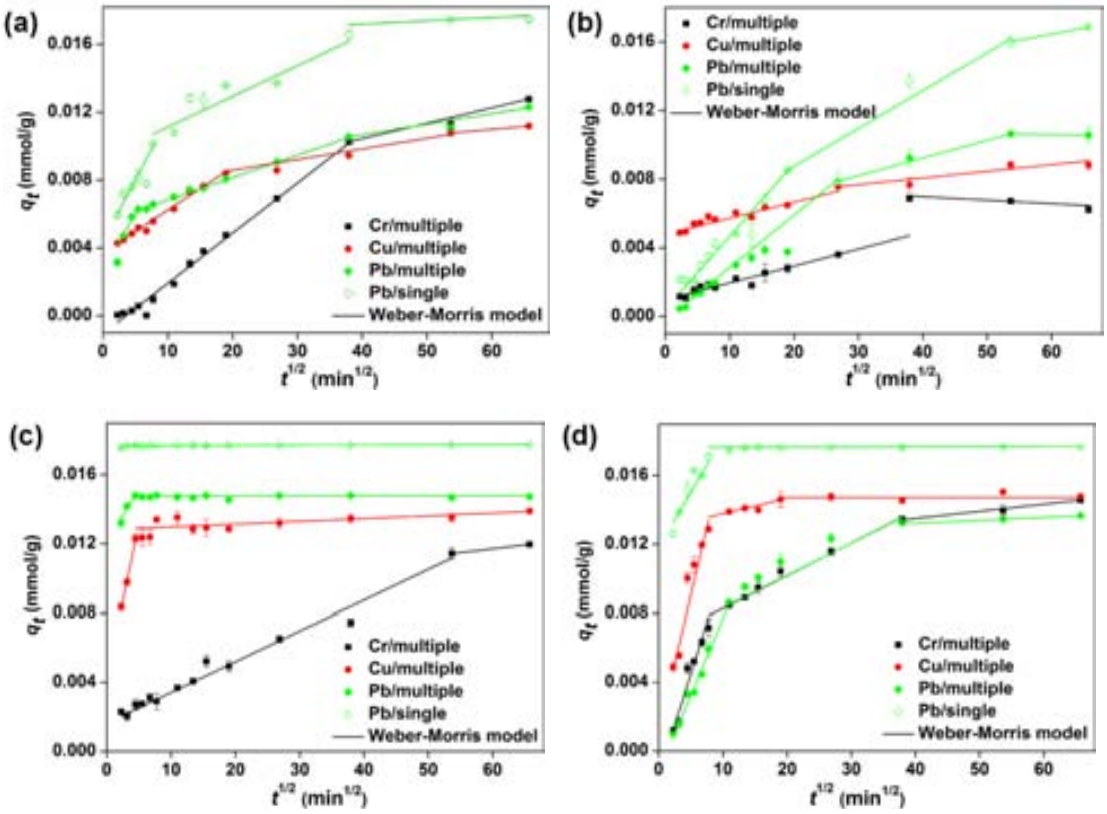


Figure 13. Weber-Morris intraparticle diffusion kinetic model applied for the metal ions biosorption onto human hair (a), dog hair (b), chicken feathers (c) and degreased wool (d).

3.8. Biosorption isotherm modeling

The equilibrium sorption studies were performed to provide the maximum metal adsorption capacities of the biosorbents. The biosorption of Pb(II) by human hair, dog hair, chicken feather and degreased wool were determined in the range of initial metal concentration at pH 4 were studied. The results obtained by the biosorption experiments were analyzed by well-known models of Freundlich and Langmuir.

The empirical Freundlich equation, based on the multilayer adsorption on heterogeneous surface, can be derived assuming a logarithmic decrease in the enthalpy of adsorption with the

increase in the fraction of occupied sites.[14] It can be given by:

$$q_e = k_F C_e^{1/n} \quad (11)$$

where C_e is the equilibrium concentration of the metal ion in the residual solution (in mol/L), q_e is the equilibrium concentration of the adsorbed per unit of mass of sorbent (in mol/g), k_F and n are Freundlich constants related to the sorption capacity and sorption intensity, respectively. The equation (11) is conveniently used in the linear form by taking the logarithm of both sides as:

$$\log q_e = \log k_F + \frac{1}{n} \log C_e \quad (12)$$

While Langmuir isotherm assumes that enthalpy of adsorption is independent of the amount adsorbed, a basic assumption of the Langmuir theory is that sorption takes place at specific homogeneous sites within the sorbent, no further sorption can take place at that sites.[28] The Langmuir equation is valid for monolayer sorption on to a surface a finite number of identical sites, which is given by equation (13).

$$q_e = \frac{Q_0 b C_e}{1 + b C_e} \quad (13)$$

Where Q_0 (mol/g) is the saturation concentration of the sorbed metal ion per unit of mass of sorbent to form a complete monolayer on the surface bound at high C_e, b is the ratio of sorption/desorption rates (in L/mol). Q_0 represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in cases where the sorbent did not reach its full saturation in experiments. $K_L = Q_0 b$, K_L is the equilibrium adsorption constant which is related to the affinity of the binding sites. The Langmuir equation can be described by the linearized form:

$$\frac{C_e}{q_e} = \frac{b}{K_L} C_e + \frac{1}{K_L} \quad (14)$$

The Freundlich and Langmuir parameters, with the respective correlation coefficients R^2, are presented in Table 3. It is observed that the equilibrium data are very well represented by the Langmuir isotherm equation with high correlation coefficient (R^2 >0.98) when compared to the Freundlich equation. The Langmuir plots are also represented in Figure 14, and the good agreement with the experimental data suggests that the metal ions adsorbed form a monolayer coverage on the biosorbent surface. From the Table 3, it is found that the maximum biosorption capacities of the four biosorbents follows the order chicken feathers (3.87×10^{-5} mol/g) > degreased wool (3.40×10^{-5} mol/g) > human hair (2.43×10^{-5} mol/g) > dog hair (2.07×10^{-5} mol/g) for the Pb(II) at 295K. And a desirable affinity between the sorbent and the sorbate depending on b value, which follows the order degreased wool > chicken feathers > human hair > dog

hair.[29] Both constants usually take part in determining the adsorption efficacy of a given adsorbent. It is obvious that chicken feather is the best biosorbent for the removal of Pb(II) among the four biosorbents.

Table 3. Freundlich and Langmuir isotherms constants for the biosorption of Pb(II) by human hair, dog hair, chicken feathers and degreased wool.

	constant	Human hair	Dog hair	Chicken feathers	Degreased wool
Freundlich	$K_F \times 10^4$	4.89	3.50	8.38	9.0
	n	2.51212	2.33918	2.5227	2.45592
	R^2	0.75742	0.84984	0.67664	0.65263
Langmuir	$Q_0 \times 10^5$ (mol/g)	2.4281	2.06544	3.8719	3.4049
	$b \times 10^{-4}$ (L/mol)	4.4757	0.9155	8.8388	12.0861
	K_L (L/g)	1.087	0.189	3.422	4.115
	R^2	0.99899	0.98991	0.99982	0.99989
	$-\Delta G^0$ (kJ/mol)	26.265	22.373	27.934	28.702

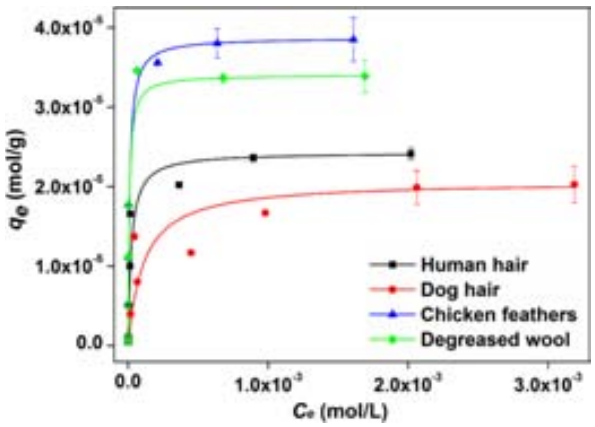


Figure 14. Experimental (•) and calculated (▬) values adjusted by using the Langmuir isotherm model for the biosorption of Pb(II) by using human hair, dog hair, chicken feathers and degreased wool.

In addition, from the estimated Langmuir adsorption/desorption constant, the standard Gibb's free energy (ΔG^0) of the biosorption process can be evaluated by using the following equation (15)

$$\Delta G^0 = -RT \ln b \qquad (15)$$

where b is the Langmuir equilibrium constant shown in equation (14), R is the universal gas constant (8.314 J/mol K) and T is the absolute temperature (K). The standard Gibb's free energy (ΔG^0) values are shown in Table 3. The negative ΔG^0 values indicated that the biosorption of metals into these four keratin biomaterials is thermodynamically feasible and of spontaneous nature.[30]

3.9. Desorption, regeneration and reuse studies

Recovery of the adsorbed heavy metals and reuse of the biosorbent are of significance of the viewpoint of practical applications.[31] Desorption results for all the Pb(II) loaded keratin biomaterials with EDTA and HNO_3 solutions as eluents are collected in Table 4. For the native human hair, the elution efficiency of HNO_3 solution is higher than the EDTA, being 76% and 48%, respectively. On the contrary, the recovery of Pb(II) from degreased wool with EDTA is better than that with HNO_3. The wool samples here used are degreased ones, being oxidized under this degreased process which leads to an efficient desorption with EDTA. In the case of chicken feathers, the elution efficiency of EDTA is more effective as well as HNO_3. This can be explained that the lower content of cystine in chicken feathers leads to a lower stability and higher hydrophilicity comparing with the other keratin fibers.

The reuse of regenerated keratin biomaterials for removal of heavy metals is investigated. Two regeneration methods used previously are also studied: one is rinsing with deionized water, the other is rinsing with HNO_3 firstly and then with deionized water (this just in the case of EDTA as eluent). After regeneration, the samples are used to adsorb Pb(II) ions from aqueous solution. It is found that the regeneration of these four biosorbents with EDTA alone is favorable to reuse them for the biosorption of metal ions. The results also indicate that the biosorption capacities of these four biosorbents regenerated by EDTA decrease compared with the first time. The human and dog hairs are deprived of half their biosorption capacities, the biosorption capacities of chicken feathers and degreased wool also decreased from 97% to 72%.

Table 4. The elution of adsorbed Pb(II) by using EDTA and HNO_3 solutions.

Biosorbent	Metal adsorbed (%)	Pb(II) elution efficiency (%) using EDTA (0.1 M)	Pb(II) elution efficiency (%) using HNO_3 (0.1 M)
Human hair	80 ± 1	48 ± 1	76 ± 1
Dog hair	89 ± 2	62 ± 2	61 ± 1
Chicken feathers	98 ± 1	100 ± 2	100 ± 1
Degreased wool	97 ± 1	88 ± 4	67 ± 1

3. Conclusions

In this part, four keratin biomaterials including human hair, dog hair, chicken feathers and degreased wool have been used successfully for the removal of heavy metals from aqueous solution. The FT-IR analysis reveals that carbonyl, hydroxyl, amino and sulfur-containing groups as the major functional groups that act as biosorption sites for metal ions in such keratin biomaterials. Biosorption capacity is affected by various parameters such as the pH of the initial aqueous solution, the biosorbent dosage and the contact time. The aqueous pH is found to be a critical parameter in the biosorption processes, with an optimum pH being 4.0 in all the cases.

The increase in the biosorbent dosage leads to an increase in the metal removal owing to the corresponding increase in the number of metal binding sites. In addition, the four keratin biomaterials exhibit good biosorption capacities in multiple-metal system in terms of total biosorption capabilities. In general, the experimental data fits well the pseudo-second order kinetic model. Even in the case of Cr(III) biosorption by human hair, the experimental data fits well the pseudo-first order kinetic model during the whole biosorption process. Kinetic results of biosorption indicate that chemical sorption is the basic mechanism in these systems. Equilibrium data fits very well in a Langmuir isotherm equation, confirming the monolayer sorption of the metal ions onto the biosorbent surface. The maximum biosorption capacities of Pb(II) by human hair, dog hair, chicken feathers and degreased wool are obtained as follows 2.43×10^{-5}, 2.07×10^{-5}, 3.87×10^{-5}, and 3.40×10^{-5} mol/g, respectively, at optimum pH (4.0) and temperature (295 K). The calculated standard Gibb's free energy (ΔG^0) indicates the thermodynamically feasible and spontaneous nature of the biosorption process. These four keratin biomaterials regenerated with EDTA alone exhibit good biosorption capacities, even though the biosorption capacities of these four regenerated biosorbents decrease compared with them used first time.

Taking into consideration present findings, these four keratin biomaterials, being cheap and easily available materials, could be an alternative to more costly adsorbents used really for heavy metals removal in wastewater treatment processes.

Acknowledgements

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Annex III

Article under preparation

Biosorption of heavy metal ions using keratin biomaterials based biosorbents – A review

Helan Zhang, Cristina Palet**

Centre Grup de Tècniques de Separació en Química, Unitat de Química Analítica, Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Catalunya, Spain

Abstract: Heavy metal pollution of wastewater is of special concern due to their toxicity, accumulation, nonbiodegradable nature and causing various disease and disorders. Biosorption is emerging as a potential alternative to the existing conventional technologies for the removal and/or recovery of toxic metal ions from aqueous solutions. The use of low-cost biosorbents has been investigated as a replacement for current costly methods of removing heavy metals from solution. Waste keratin biomaterials have gained wide attentions as effective biosorbents due to their low costs and high contents of amino and hydeoxyl functional groups. They have showed significant potentials of removing metal ions from aqueous solution. In this article, an extensive related waste keratin biomaterials including wool, feathers and hairs as biosorbents list has been compiled to provide a summary of available information on the removal of heavy metal ions from contained solution. The aims of this review article were to summarize the scattered available information in various aspects of utilization of the keratinous waste materials for heavy metal ions removal. Waste keratin biomaterials being highly efficient, low cost and renewable source of biomass can be exploited for the removal of heavy metal ions. Further these biosorbent can be modified for better efficiency and multiple reused to enhance their applicability at industrial scale.

Keywords: Heavy metal ion; waste keratin biomaterials; biosorption; hair; feathers; wool.

1. Introduction

During the past few decades, intensifying environment pollution and deteriorating of ecosystems anthropologically, caused by mainly industrial activities, have seriously threatened human existence and development.[1] Consequently, a wide variety of toxic inorganic and organic chemicals are discharged to the environment as industrial wastes, causing serious water, air, and soil pollution. Industrial waste usually contains many pollutants, such as heavy metals, dye, phenols (including other organic compounds), inorganic anions, and pesticides (which are toxic to many living lifeforms and organisms), etc..[2] Among of them, heavy metal pollution of

** Corresponding author. Tel.: +0034 935813475; fax: +0034 935811985.
E-mail address: cristina.palet@uab.cat

wastewater is most strongly connected to human life, which can be found in wastewaters originating from chemical manufacturing, painting and coating, mining, extractive metallurgy, nuclear, and other industries.[3] Heavy metal ions are extremely harmful to human, animals, and plants because of their toxicity, accumulation, no biodegradable nature and causing various diseases and disorders.[4]

The treatment methods for metals contaminated waste effluents commonly include chemical precipitation (hydroxides, sulfides, etc.), membrane filtration (reverse osmosis, nanofiltration, etc.), electrochemical treatment, solvent extraction, ion exchange and adsorption.[5] However, chemical precipitation and electrochemical treatment are ineffective, especially when metal ion concentration in aqueous solution is among 1 to 100 mg/L, and also produce large quantity of sludge required to treat with great difficulty. Membrane filtration, solvent extraction and ion exchange are extremely expensive when treating large amount of water and wastewater containing heavy metal in low concentration, they cannot be used at large scale.[6] Adsorption has considered ideal alternatives for the removal of heavy metal ions from aqueous solution.

Over the last few decades adsorption has gained importance as a purification and separation process on an industrial scale. Various materials have been developed as adsorbents for the removal of heavy metal ions. In particular, activated carbon has been traditionally used as a good adsorbent for the removal of heavy metal contaminants.[7, 8] Its usefulness derives mainly from its large micropore and mesopore volumes and the resulting high surface area, high adsorptive capacity, and degree of surface reactivity.[9] However, activated carbon is relatively expensive, and it is difficult to elute heavy metal ions from it and recycle the activated carbon because of strong interaction between them. Moreover, the application of expensive synthetic materials also has similar problems like technical or economic constraints.[10] Therefore, the main research focus is searching for low-cost, effective and regenerable adsorbent materials which are capable of removing metals from wastewater.[11] In general, an adsorbent can be assumed as “low cost” if it requires little processing, is abundant in nature, or is a by-product or waste material from industry.[3] To date, hundreds of studies on the use of low-cost adsorbents have been published. Agricultural wastes, industrial byproducts and wastes, and natural substances have been studied as low-cost adsorbents for the heavy metal wastewater treatment. In recent years, applying biotechnology in controlling and removing metal pollution has been paid much attention, and gradually becomes hot topic in the field of metal pollution control because of its potential application.[12] Alternative process is the biosorption, which can be defined as the removal of metal or metalloid species, compounds and particulates from solution by various certain natural materials of biological origin.[13] The biosorption of heavy metals by the biomaterials might be attributed that these biomaterials, mainly composed of polysaccharides,

proteins and lipids have carboxyl, hydroxyl, sulfhydryl, phosphate, carbonyl and amino groups, can bind metal ions involving valance forces through sharing or exchange of electrons between biosorbent and metal ions .[14] Waste biomaterials are considered ideal alternatives as low-cost biosorbents for the removal of heavy metals from low strength wastewater due to the relatively cost-effective reason and quite efficient adsorption capacities. A vast array of low-cost waste biomaterials from large-scale industrial operations have also successfully been employed as biosorbents for heavy metal removal from aqueous solution and wastewaters.[15] Among the various biosorbents investigated, cellulosic waste materials are the most abundant for the removal of heavy metals, which include agricultural waste materials[16,17] (wheat based waste material,[18,19] rice husk,[20]), waste products from timber industry[3] (bark[21] , wood, sawdust[22,23]). Cellulosic waste materials usually are composed of lignin and cellulose as major constituents and may also include other polar functional groups of lignin, which includes alcohols, aldehydes, ketones, carboxylic, phenolic and other groups. These groups have the ability to extent to bind heavy metals by donation of an electron pair from these groups to complexes with the metal ion in solution. Chitin is the second most abundant as an excellent biosorbent for the removal of heavy metals after cellulose. The most abundant natural source of chitin is usually from shell or exoskeleton of animal such as crustaceans, arthropods and mollusks. Chitin is a polymer composed of N-acetyl-D-glucosamine or specifically 2-acetamido-2-deoxy-β-D- glucose through a β(1-4) linkage residue.[24] The adsorption behavior of chitin is primarily attributed to the high hydrophilicity of the polymer, the presence of a large number of functional groups (acetamide, primary amino, hydroxyl) and the high chemical reactivity of these functional groups. However, more important than chitin is chitosan, which was prepared from chitin by partial deacetylation. Yang and Zall reported that the biosorption capacity of chitosan chelates is greater five to six times of metals amount than chitin due to the free amino groups exposed during deacetylation.[25] Although chitosan can dissolve in acidic medium, the use of a cross-linking agent such as glutaraldehyde or the use of some modification process like coating, can diminish the dissolution properties of chitosan (which is an improvement for its use as biosorbent). Recently, chitosan composites and derivatives have been developed to adsorb heavy metals from wastewater due to their low costs and high contents of amino and hydroxyl functional groups.[26,27] The chitosan/ceramic alumina composites,[28] chitosan/magnetite composites,[29] chitosan/cellulose composites,[30,31] ethylenediamine-modified magnetic chitosan nanoparticles,[32] crosslinked chitosan resin modified by L-lysine[33] have all been used to remove heavy metal ions from wastewater. As a kind of abundant biological resources, keratin biomaterials are currently being investigated as biosorbents for the removal of heavy metal ions from water and wastewater, which are relatively abundant and inexpensive materials. Various keratin biomaterials, such as wool, feather, hair and horn can be used as effective biosorbents,

either directly or after activation, to remove heavy metals due to their high contents of carboxyl, hydroxyl, amino and sulfhydryl functional groups. These biomaterials have showed significant potentials of removing metal ions from aqueous solution.

Considered the importance of keratin biomaterials as biosorbents for the removal of heavy metals from wastewater, an overview of summarized information concerning the utilization of keratin biomaterials based on recent publications is presented in the following part and their biosorption performance, influencing factors of the heavy metal biosorption process, the favorable conditions and the biosorption mechanism, etc., are here discussed.

Most notably, keratin biomaterials possess a much higher content cysteine residues in their non-helical domains and thus form tougher and more durable structures via intermolecular difulfide bond formation.[34]

2. Removal of heavy metals by keratin biomaterials

Keratin is the major component of wool, hair, feathers, horn, nail. Moreover, keratin wastes such as feathers, horns, and nails from butchery, human hair from barber, poor quality raw wools from sheep breeding and some by-products from textile industry, account worldwide for more than four million tons per year.[35] In other words, keratinous materials are easily available in the local place at negligible price. In addition, keratin biomaterials are insoluble in water and contain some primary functional groups involving carboxyl (R-COOH), hydroxyl (R-OH), amino (R-NH_2) and sulfhydryl (R-SH), which leads to the keratin biomaterials used as good biosorbents for removal of heavy metal ions from wastewater. Among the functional groups, the sulfur atoms can more readily coordinate to the heavy metal ions , as shown by Dąbrowski et al.[36] Therefore, keratinous materials are deal biosorbent for removal heavy metals from wastewater.

2.1. Removal of heavy metals by wool

Among various waste keratin materials, wool is the abundant. Wool keratin is attracting an increasing amount of research interests, as it is an effective biosorbent and can be used for concentrating, removing, and recovering toxic, precious, radioactive or other metal ions from natural water supplies or industrial wastewater. This is because the protein fiber on wool surface has polar an ionisable groups on the side chain of amino acid residues, which can strongly bind charged species such as metal ions. The rate and the extent of uptake was reported to depend on various factors such as the kind of metal and its valence state, the solution concentration, pH, time, temperature, etc..[15]

Various researches on wool keratin for removal of heavy metals have been done recently. The native wool protein,[37] chemically-modified wool fibres and regenerated wool protein[38] have

been used to remove metal ions from aqueous solution. In 1968, the mohair has been used for the uptake of Cu(II) by Guthrie and Laurie.[39] Freeland et al. investigated the raw wool for the removing Hg(II), Cu(II), and Ni(II), and it was found that the adsorption of metal ions by wool is strongly inhibited by sodium chloride.[40] They also studied wool modified with polethylenimine (PEI) for Hg(II) adsorption, and the adsorption capacity of the PEI-wool for Hg(II) was 330.97 mg/g.[41] Masri and Friedman also investigated raw wool modified by the addition of various functional groups for removing metal ions, specially those containing sulphur.[42] They suggested that the sulfhydryl group could increase the adsorption capacity due to the formation of metal mercaptide. Edgar and Simpson reported that the adsorption of Al(III) by wool from water/alcohol mixtures.[43] The adsorption of Al(II) ions by wool keratin is increased in the presence of alcohols. Shinoda and Kikyotani used special resin prepared from waste wool fiber as adsorbent for recovering Hg(II) from wastewater.[38] A similar study for Hg(II) sorption using waste wool discarded from wool manufacturing processes was also conducted, it was note that the waste wool can be used for the removal of Hg(II) from industrial effluents, particularly those from the chlor-alkali industry. The amount of Hg(II) sorbed at the keratin protein (wool) reaches 90% of sorption (from solutions containing 100 ppm of Hg(II)).[44]

In 1992, a study with the wool fibers to remove Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), and Pb(II) from aqueous solution was conducted by Devrim and Hayri.[45] It was reported that a fiber diffusion is the rate-controlling step in the adsorption process. It was also suggested that wool is a potential adsorbent for removing toxic metal uons from contaminated waters.

Freddi et al. reported that the removal of Cu(II) and Ag(I) ions is possible using wool modified by treatment with tannic acid (TA) and by acylation with ethylenediaminetetraacetic (EDTA) dianhydride.[46] Modification of wool fibers was effective in enhancing their intrinsic ability to absorb and bind Ag(I) and Cu(II), where the extent of metal uptake was dependent on the number of available binding sites introduced into the fiber matrix with the modifying agents. The acylation technique appeared particularly attractive because metal cations were effectively adsorbed over a wider pH range, especially in the acidic and neutral regions. Wool-Ag, wool-EDTA-Ag, and wool-TA-Ag complexes showed lower levels of metal release at acidic pH and better antimicrobial activity against bacteria than the different wool-Cu complexes. Afterwards, Paola et al. explored the interaction between the binding sites of this two chemically modified wool fibers and metal cations (i.e. for Co(II) and Cu(II)) through an IR spectra elucidation.[47]

Dakiky et al. reported the selective adsorption of Cr(VI) from industrial wastewater using several abundantly available low-cost adsorbents including wool, olive cake, sawdust, pine needles, almond shells, cactus leaves and coal.[48] Among the selected adsorbents, natural wool from sheep was the best one for the selective removal of Cr(VI) at pH 2.0 and with an adsorbent

concentration of 16 g/L at 30°C (for which the removal was 81% out of 100 ppm Cr(VI) after 2 h of stirring). It was also found that the kinetics of the Cr(VI) adsorption on the wool was followed the pseudo-first order rate mechanism. Wool was found to fit better to the Langmuir isotherm, which is based on the formation of layers on the active sites. The Gibbs free energy obtained for wool as an adsorbent showed that it has the largest capacity and affinity for the selective removal of the metal.

The removal of Cu(II), Co(II), Ni(II), Zn(II) and Cr(VI) from their aqueous solutions using low-class native wool or wool wastes was explored by El-Sayed and coworkers.[49] It was reported that the sorption efficiency of the aforementioned metals by wool is in the following order: Cu(II) > Co(II) = Ni(II) > Zn(II) > Cr(VI). Meanwhile, the authors also investigated the effect of the modification of wool on the sorption of Cu(II). The results indicated that modified wool is better than both native and oxidized wool in removing Cu(II) from the effluent. Wool could be reused for many times for copper sorption after elution with dilute hydrochloric acid. The efficiency of wool to remove traces of metals from water effluents gives the possibility of using wool fibers as filters for potable water in areas where water is contaminated with these metals.

The effect of the contact time, temperature and pH on the uptake of Cu(II) by wool was investigated by Sheffield and Doyle.[50] It was found that the uptake of Cu(II) can be improved by longer heating times and higher temperatures, 1 hour near the boil gives optimal results. It was also observed that Cu(II) uptake increased linearly with pH up to a pH of around 6.0. However, at higher pH value, precipitation of solid copper salts becomes significant.

Wool fibre can adsorb not only heavy metal cations but also metal nanoparticles. Richardson and Johnston reported that monodisperse gold nanocrystals can be adsorbed and chemically bound to Merino wool fibres.[51] It was found that the nanocrystalline gold was bound through the nitrogen of the amino groups in the surface of the gold to the keratin of the fibres, which was confirmed by surface analysis of X-ray photoelectron spectroscopy.

Park and coworkers reported that the removal of Cu(II) from aqueous solution using nanofibrous membrane of wool keratose/silk fibroin blend.[52] It was found that wool keratose is preferred for removing metal ions. It can be attributed to more amino acids having polar side residues in the wool keratose than in the silk fiber. However, the use of only wool keratose for preparation of nanofibrous membrane can be hardly performed due to its low electrospinnability as well as its structural instability in water. It was also indicated that blending the wool keratose with silk fiber can improve those characteristics, and also its adsorption capacity for Cu(II) is improved compared with only the silk fibroin nanofibrous membrane. Furthermore, the adsorption capacity of wool keratose/silk fibroin nanofibrous membrane was maintained after several cycling process of adsorption and desorption. This indicated that this membrane is a

promising material for removing and recovering heavy metal ions in water.

Monier and coworkers reported that the graft copolymerization of ethyl acrylate (EA) onto natural wool fiber (wool-g-PIAH) as adsorbent could be used for the removal of Cu(II), Hg(II) and Ni(II) metal ions from aqueous solutions.[53] It was shown that the adsorption affinity of this fiber for Cu(II) was higher than Hg(II) and Ni(II). The adsorption kinetics followed the pseudo-second order equation and the equilibrium date can be described well by the Langmuir isotherm. It was also indicated that regeneration of the fibers obtained was achieved by using EDTA with efficiency of greater than 85%. At the same time, the authors also investigated a similar wool fiber (wool-g-PCAH) as adsorbent for the removal of Hg(II), Cu(II) and Co(II).[54] It was pointed out that Hg(II) showed higher adsorption affinity towards the studied fibers relative to Cu(II) and Co(II). It was further mentioned that this wool is a good regeneration fiber and its efficiency is greater than 84%. Thus, indicating that feasible improvements in the uptake properties encourage efforts for wool-g-PIAH and wool-g-PCAH obtained to be applied for water and wastewater treatment.

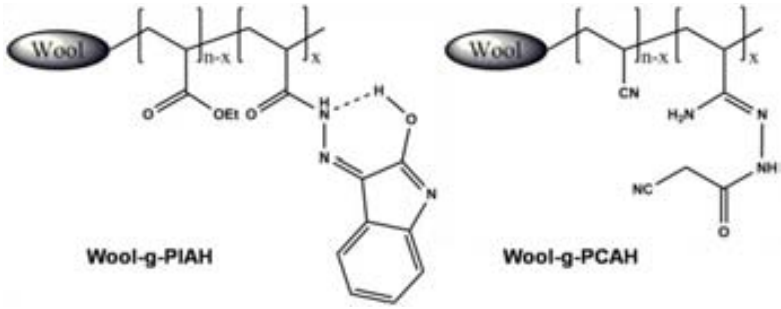

Figure 1. Structure of wool-g-PIAH and wool-g-PCAH.

An investigation on metal ion binding properties of novel wool powders was conducted.[55] It was found that the optimum pH for binding of Cu(II) and Cd(II) was in the range 6.0-8.0, while Co(II) was pH 8.0. It was pointed out that the Co(II) with weak binding affinity for wool fibre, showed considerably enhanced binding, suggesting that similar trends may be possible with other weak binding metal ions. It was also reported that the wool powder showed significantly higher metal ion loading capacity than the commercial cation exchange resins.

A comparative study on the removal of Co(II) from aqueous solution was also carried out using two wool powders derived from wool in native and wool treated with sodium salt of dichoroisocyanuric acid (DCCA).[56] It was shown that binding of Co(II) to wool powders is heavily dependent on the pH value and the chemical nature of buffer. The optimum soption of Co(II) by both powders occurred at pH 8.0 in phosphate buffer and pH 10.0 in ammonium sulphate buffer. It was also found that coarse wool powders showed more efficient for removing and recovering Co(II) from solution in comparison with untreated wool native fiber and the fine wool powders.

Aluigi et al. report that keratin/PEO nanofibres were produced by electrospinning the blend aqueous solutions, keratin was extracted by sulphitolysis from wool, and blended with PEO in aqueous solution.[57] Due to the sorption property of keratin materials, author also reported the adsorption of Cu(II) by keratin/PA6 blend nanofibres.[58] It was found that keratin rich nanofibres showed adsorption performances better than conventional wool fabric and generally, the adsorption capacity increases with increasing the keratin content. It was pointed out that the maximum adsorption capacities of this nanofibre (~103.5 mg/g), which are better than that when using the commercial activated carbon (~50 mg/g). Kinetics of the adsorption fitted better with a pseudo-second order rate equation. The IR analysis demonstrated the formation of complexes between Cu(II) ions and keratin that involve the terminal free carboxyl groups of the protein.

Another similar research evaluated the adsorption of heavy metal ions onto the wool-derived keratin nanofiber membranes.[59] It was demonstrated that an adsorption capacity for Cu(II) is greater than for Ni(II), and Co(II) through dynamic adsorption tests for the Cu(II), Ni(II), and Co(II) metal ion removal from aqueous solution. It was also reported that the keratin nanofiber membranes maintained good adsorption performances for Cu(II) ions in the competitive conditions. As regards the Cu(II) adsorption from non-competitive condition, the experimental data had a good fitness with both Langmuir and Freundlich isotherm models. The maximum adsorption capacity obtained from the Langmuir model was about 11 mg/g, the adsorption of Cu(II) ions on keratin nanofiber membranes was a multilayer process and occurred by ion exchange reactions.

One of the first reports on the application of cashmere guard hair fiber (a specific kind of wool) for the separation and waste management of metal ions was recorded by Patil and coworkers.[60] The authors investigated the adsorption behaviour of cashmere guard hair fibre and its powder towards Zn(II) and Cr(VI) ion species and compared their results with other protein fibre powder sorbents. It was found that the adsorption of Zn(II) and Cr(VI) from aqueous solutions was governed by ionic interactions over different pH ranges. When the solution pH value is at 8 and 2, the cashmere guard hair, wool and silk powders show largest adsorption yield to Zn(II) and Cr(VI), respectively. The absorption performance of the powder sorbents towards Zn(II) ions (silk powder > wool powder > cashmere guard hair powder) showed an opposite trend to Cr(VI) ions (cashmere guard hair powder > wool powder > silk power). Thus, cashmere guard hair powder may serve as an economical alternative to wool and silk powders for removal of heavy toxic metal ions from water for environment protection applications.

In the latest research, Mariwaki and coworkers investigated the removal of Pb(II) by using ketatin colloidal solution obtained from wool.[61] It was reported that the maximum Pb(II) uptake capacity of keratin in the colloidal solution was 43.3 mg/g. It was also found that the Pb-keratin

aggregate was easily decomposed via the addition of nitric acid, which enabled the recovery of Pb(II). However, aggregation did not occur in solutions with Pb(II) concentrations below 0.10 mM. Therefore, a keratin colloidal solution encapsulated in a dialysis cellulose tube can be used to remove Pb(II) from 0.10 mM solutions, which enabled the removal of 95 % of the Pb(II).

Overall, researchers came to understand that waste wool samples in general, which are available in large quantities and at virtually little cost, offer a real potential to treatment the wastewater prior to 1990. After 1990, wool keratin was mixed with other components to enhance the sorption performance, and the kinetics and isotherm models have been intensively investigated. Table 1 summarizes the biosorption capacity of the metal ions investigated on wool keratins, which were discussed above. These results showed that wool hold great potential to remove heavy metal species from industrial wastewater effluents.

Table 1. Summary of work done by various researchers using wool for the removal of heavy metals.

Biosorbent	Year	Metal ions	Kinetic and isotherm models	Amount of metal sorbed (mmol/g)	REF
Wool(natural)	1992	Ni(II) Cu(II) Zn(II) Cd(II) Hg(II) Pb(II)	-	-	45
Treated wool	2001	Ag(I)[a], Cu(II)	-	1.78[a]	46
wool	2002	Cr(VI)	Pseudo-first order, Langmuir	0.663	48
Treated wool	2003	Cu(II) Co(II)	-	-	47
Wool in native Reduced wool Oxidized wool	2004	Cu(II) Co(II) Ni(II) Zn(II) Cr(VI)	-		49
wool	2005	Cu(II)	-	0.165	50
wool	2007	Au	-	-	51
Nanofibrous membrane of wool/silk	2007	Cu(II)	-	0.045	52
Wool grafted PIAH chelating fibers	2010	Cu(II) Hg(II) Ni(II)	Pseudo-first order Pseudo-second order LangmuirFreundlich Tempkin	2.24 0.246 0.796	53
Wool grafted PCAH chelating fibers	2010	Hg(II) Cu(II) Co(II)	Pseudo-first order Pseudo-second order Langmuir, Freundlich Tempkin	0.769 1.74 1.24	54
Wool fiber[a] Wool powder[b]	2010	Co(II) Cu(II) Cd(II)	Langmuir	0.0039[a], 0.0077[b] 0.0097[a], 0.0091[b] 0.0093[a], 0.0086[b]	55
Wool powder	2010	Co(II)	Langmuir	53.78	56
Keratin(wool) /PA6 blend nanofibres	2011	Cu(II)	Pseudo-first order Pseudo-second order Freundlich	1.63	58
Keratin (wool) nanofiber membrane	2012	Cu(II) Ni(II) Co(II)	Pseudo-first order Pseudo-second order Intraparticle diffusion Langmuir, Freundlich Dubinin-Radushkevi	0.173 - -	59
Wool powders	2012	Zn(II), Cr(VI)	-	-	60
Keratin (wool) colloidal solution	2013	Pb(II)	Langmuir, Freundlich	0.209	61

2.2. Removal of heavy metals by feathers

An estimated 15 million tons of chicken feathers are available globally each year as a

by-product of meat manufacture.[62] The raw material is tough and chemically resistant. Currently the feathers are disposed of in landfill, burned or processed to make a low-grade animal feedstock. These methods are environmentally unsound and are restricted. Actually, feathers consist of about 91% keratin, 1.3% fat, and 7.9% water, which mean they would be used as potential biosorbents for the removal of heavy metal ions.

Banat and coworkers examined and compared the ability of different keratin-composed biosorbent (chicken feather, hair and horn) for the removal of heavy metal ions from aqueous solutions.[63] Among of them, Chicken feather is an effective biosorbent and showed a higher Cu(II) ion uptake than Zn(II). It was indicated that an equilibrium time of 8 h was needed for the adsorption of Cu(II) or Zn(II) by chicken feathers. Intraparticle diffusion was involved in the adsorption processes but was not the rate-controlling step. Freundlich isotherm model was found to be applicable to the adsorption of Cu(II) and Zn(II) and provides an indication of the adsorption capacity of the chicken feather. The affinity of chicken feathers towards Cu(II) ion adsorption was better than that towards Zn(II).

The efficiency of Cu(II) and Zn(II) removal from wastewater by chicken feather was investigated by Al-Asheh et al..[64] The authors compared the efficiency of Cu(II) and Zn(II) removal from wastewater with untreated native chicken feathers, chicken feathers treated with alkaline solution and chicken feathers treated with anionic-surfactant. It was found that the chicken feathers treated with an alkaline solution adsorb more metal ions than those treated with an anionic surfactant, followed by the untreated chicken feathers. Authors explained this behavior because of the treatment with alkaline solution could increase the porosity of the keratin structure, which can increase the accessibility of the fiber for metal ions to easily diffuse into the active sites of the fibers. The treatment with anionic-surfactant can increase the negative charges on the sorbent surface to improve the sorption capacity for metal positive ions. Alkaline solution of 0.2 N NaOH as found to be the best treatment for chicken feathers for the sorption process. The authors suggested that most of sorption occurs on the surface during the first 20 min of the sorption process. The Freundlich isotherm model was found to represent the equilibrium results, of all adsorbents toward Cu(II) and Zn(II), reasonably well.

Kar and Misra also investigated the biosorption of heavy metals from solutions using chicken feather.[65] They found that chicken feathers are a nano-porous and intrinsic keratin fiber, which have demonstrated a good capacity for the removal of Cu(II), Pb(II), Cr(VI), Hg(II) and U(II) from diluted solutions, especially after an alkaline ultrasonic treatment of the keratin fiber which resulted in an increase in metals uptake. In the natural pH range (pH 5.0–7.0) the keratin protein fibers have unique selectivity for Pb(II) and Cu(II), and at acidic pH for Cr(VI) and Hg(II). Adsorption of heavy metals using both batch and column operations was conducted. The surface hydrophobicity of keratin protein fibers makes mixing difficult in aqueous solutions,

thereby limiting adsorbate –adsorbent contact and the metal uptake. The metal uptake is increased significantly by the column mode due to the nonexistence of difficulties such as mixing. Multiple elution and adsorption tests indicated that it is possible to reuse keratin fiber as a biosorbent for a number of cycles.

In another study, alkaline treatment of chicken feathers with NaOH or NaOCl and immobilization of resultant hydrolyzates on silica for the removal of metals including Ca(II), Mg(II), Fe(II) and Mn(II) ions from waters is presented.[66] The investigations showed good metal removal efficiency, and the adsorption capacity was determined to be > 90% for Ca(II), Fe(II) and Mg(II) are above 70% at pH 6.0 to 9.0 within 20-30min. The sorbents could be regenerated with NaCl solution. More interestingly, these cheap regenerable sorbents were applied successfully for treating industrial water in different Egyptian industrial areas.

Guadalupe et al. reported that kinetics and equilibrium studies of Pb removal from aqueous solutions by chicken feathers.[67] Under different experimental conditions (pH, temperature, and metal concentration), the results showed that the maximum Pb(II) sorption capacity was obtained at 30 ºC and at pH 5.0. Pseudo-second order kinetic and Freundlich isotherm models are suitable to represent the experimental data obtained in their investigation. Most of the metal removal occurs on the external surface of the sorbent during the first hours of contact. This trend is similar to that obtained by other studies. The authors suggested that the Pb(II) sorption by using chicken feathers may be controlled by a chemisorptions process.

The removal of Zn(II) ions from aqueous solutions by using raw chicken feathers was evaluated in both batch and continuous conditions.[68] It was reported that Zn(II) sorption is fast and may occur in the external surface of chicken feathers through kinetic study. On the other hand, from sorption isotherms results, author found that the experimental maximum Zn(II) sorption capacity of chicken feathers is 4.31 mg/g at 30 ºC and pH 5 and that chemisorption may play an essential role in the process. For batch experiments, the pseudo-second order kinetic and Sips isotherm are the most appropriate models for fitting Zn(II) sorption experimental data. In the continuous system using a packed bed column, breakthrough curves were obtained for different conditions of feed flow rate, pH and metal concentration. The characteristics of these breakthrough curves are dependent of the column operating conditions especially of pH and feed flow rate. Finally, Thomas model has shown an appropriate performance in the modeling of Zn(II) breakthrough curves obtained at operating conditions tested in this study. In summary, the design parameters obtained from both batch and continuous experiments could be further employed in the optimization and implementation of sorption processes for wastewaters treatment for Zn(II) removal using raw chicken feathers.

Another feathers, duck feathers were used as biosorbents for the removal of metal cations (Cu(II) and Zn(II)).[69] Author compared the adsorption of metal cations (Cu(II) and Zn(II)) by

unmodified and tannic acid (TA)-modified feathers at different conditions(as a function of fiber weight gain, temperature and pH of the initial metal aqueous solution). The influence of the presence of anions in the solution on the adsorption of metal cations was also studied. The uptake of Cu(II) from chloride was higher and faster than that from nitrate. The desorption study indicated that the feather–TA–metal complexes exhibited higher stability for metal cations than the feather–metal complexes. All these experiments reveal that TA-modified feather fibers have good adsorption to metal cations and can be used as metal adsorbent in wastewater treatment.

Another similar research was carried out to compare the phenomenon of Pb(II)[70] and Cu(II)[71] adsorption by chicken and duck feathers from aqueous solution. It was reported that chicken and duck feathers were treated with the tannic acid (TA), which can form insoluble products with keratin by tanning reaction and increase the chemical and physical stability of the proteins. These works have demonstrated the use of Box–Behnken design for determining the optimum process conditions leading to the maximum percentage removal of lead from aqueous solutions.

Table 2 gives the summary of previously research work done by various researchers using feather for the removal of heavy metals, which have been mentioned above. It is clear that feather (chicken and duck feather) are promising biosorbent to the heavy metals removal due to their nano-porous structure and abundant functional groups, especially after a proper chemical treatment of the feathers fiber which results in a multi-fold increase in metals uptake.

Table 2. Summary of previously research work using feathers for the removal of heavy metals.

Biosorbent	Year	Metal ions	Kinetic and isotherm models	Amount of metal sorbed (mmol/g)	REF
CF (natural)	2002	Cu(II)	Intraparticle diffusion	0.186	63
		Zn(II)	Freundlich	0.098	
CF (natural)[a]	2003	Cu(II), Zn(II)	Freundlich	0.1859[a], 0.0977[a]	64
Treated CF (alkaline solution[b] and anionic-surfactant[c])				0.3909[b], 0.2306[b] 0.2945[c], 0.1625[c]	
CF treated with alkaline in ultrasonic process	2004	Cu(II)	-	-	65
		Pb(II)		0.0257	
		Hg(II)		-	
		U(II)		-	
		Cr(III)		-	
NaOH-treated[a] and NaOCl-treated[b] CF-immobilized silica	2005	Ca(II)	Freundlich	0.3616[a], 0.3446[b]	66
		Mg(II)		0.3944[a], 0.3867[b]	
		Fe(II)		0.2432[a], 0.2330[b]	
		Mn(II)		0.2410[a], 0.2358[b]	
CF washed with solution (deionized water and ethanol, 5:1 proportion)	2008	Pb(II)	Pseudo-first order Pseudo-second order Freundlich Langmuir	0.0473	67
CF	2011	Zn(II)	Pseudo-first order Pseudo-second order Freundlich, Langmuir Sips	0.1111	68
DF(natural)[a]	2007	Cu(II), Zn(II)		0.21[a], 0.27[a]	69
Treated DF (tannic acid)[b]				0.46[b], 0.69[b]	
Treated CF(tannic acid)	2011	Pb(II)	-	1.71	70
Treated DF(tannic acid)				1.42	
Treated CF(tannic acid)	2012	Cu(II)	-	0.39	71
Treated DF(tannic acid)				0.38	

2.3. Removal of heavy metals by hair

Just a few research investigations have been reported in the literature related to the biosorption of heavy metals by hair from aqueous solutions. Pace and Michelsen used different hair samples including human and hog, in general from cattle and tannery to remove Hg(II).[72] They found that the adsorption capacity of tannery hair for Hg(II) is better than others, probably due to the crystalline regions in cattle hair are changed to be amorphous during the swelling in the tannery dehairing process. Moreover, the adsorption capacity of tannery hair for Hg(II) was comparable with those given by ion exchange resins (Dowex 50W-×8 and Dowex 1-×8).

Kulkarni and Rane reported a maximum adsorption capacity of 41.6 and 50.5 mg/g^{-1} of Hg(II) by tannery hair pretreated in alkali and alkaline Na_2S solution, respectively.[73]

Fergusson and coworkers studied the sorption of Cu(II), Mn(II), Zn(II) and As(III) from aqueous solution by using human hair.[74] At an equilibrium metal concentration of 0.3 μg/mL, the sorptions are relatively low for Mn (1.1 μg/mL) and As (0.1 μg/mL), and higher for Zn (10 μg/mL) and Cu (35 μg/mL). The author considered that the greater sorption of Cu(II) probably is due to its greater potential binding capacity and stability, specially of the Cu-S interaction, and also due to the greater electrostatic interactions between Cu(II) and hair.

Adsorption capacities of untreated hair, inorganic acid treated hair and alkaline treated hair are compared by Tan and coworkers.[75] Among the pretreatment processes checked, hair with an alkaline treatment was more effective for the adsorption of Cu(II) than others. A favourable pretreatment of the human hair in a 0.1 N NaOH / 0.1 N Na_2S solution for 20 min gave the best adsorption capacity for Cu(II) (35.3 mg/g). The adsorption capacity was found to increase as pH increased from 1 to 5. Moreover, the uptake capacity was significantly affected by the presence of other metal ions. Anionic effect was found to be more pronounced for a mixed ions system than for a single ion system.

The use of readily available natural materials as adsorbents of heavy metals from industrial wastewaters was investigated by Asubiojo and Ajelabi.[76] The natural adsorbents also contain human hair. among others. Two modes of removal including column and batch experiments were carried out and their adsorption efficiency were compared. It was found that, in terms of the efficiency of heavy metal scavenging (heavy metal adsorbed per unit weight of adsorbent) it follows: bagasse and human hair > corn cob and peanut skin > wheat bran > paddy husk. Moreover, efficiency of heavy metal removal by the natural adsorbents increased with column height and decreases with the increase on particle size of adsorbent and residual heavy metal concentration in the effluent.

Fawzi and coworkers examined and compared the ability of different keratin-composed biosorbents (chicken feathers, hair and horn) for the removal of heavy metal ions from aqueous solutions.[63] Among of them, hair is an effective biosorbent and show showed a higher Zn(II) ion uptake over Cu(II). The absolute amount of metal adsorbed increased with an increase in the initial metal ion concentration and in the pH of the aqueous initial solution. Intraparticle diffusion was involved in the adsorption processes but was not the rate-controlling step.

The studies by Jung et al. showed that perm-lotion-treated human hair can be efficient in removal of Pb(II) and Cu(II) from aqueous solution.[77] The maximum metal removal at pH 4.2 was around 89% and 97% for a 50 ppm Cu(II) and Pb(II) initial aqueous solution, respectively. This paper confirmed the ability of human hair to adsorb heavy metal ions due to the presence of reduced disulfide bonds. The perm-lotion-treated hair satisfactorily removed heavy metal

ions from s solutions with high concentrations (i.e., 89% of Pb(II) was removed from a 120 ppm solution after three days).

Although hair, especially human hair has shown excellent adsorption efficiency for the removal of heavy metal ions, the research based on human hair is still rare (Table 3). More importantly, the kinetic and isotherms studies on the biosorption by using human hair are quite rare. However, there is still big room to enhance the adsorption ability though the chemical modification of human hair (as it has been done with other keratin biomaterial, here presented).

Table 3. Summary of previously research work using hair for the removal of heavy metals.

Biosorbent	Year	Metal ions	Kinetic and isotherm models	Amount of metal sorbed (mmol/g)	REF
Hair (human, hog, cattle and tannery hair)	1975	Hg(II)	-	-	72
Human hair (natural)	1983	Cu(II)	-	0.551×10^{-3}	74
		Mn(II)		0.020×10^{-3}	
		Zn(II)		0.153×10^{-3}	
		As(III)		0.134×10^{-5}	
Treated human hair	1985	Hg(II)	-	3.327	75
		Hg(I)		2.000	
		Ag(I)		1.315	
		Cu(II)		0.4630	
		Cu(I)		0.3370	
		Pb(II)		0.4363	
		Cr(III)		0.0212	
		Cr(VI)		0.2327	
		Cd(II)		0.5916	
		Ni(II)		0.1755	
Human hair (natural)	2009	Fe(II)	-	0.551×10^{-3}	76
		Pb(II)		0.020×10^{-3}	
		Zn(II)		0.153×10^{-3}	
Human hair (natural)	2002	Cu(II)	Intraparticle diffusion	0.087	63
		Zn(II)	Freundlich	0.131	
Perm-lotion-treated human hair	2014	Pb(II)	-	0.110	77
		Cu(II)		0.141	

3. Conclusions

Biosorption is a relatively new process that has shown significant contribution for the removal of contaminants from aqueous effluents. Keratin biomaterials (wool, feather and hair) have received increasing attention for the heavy metal removal due to their good performance, low cost and large available quantities. The functional groups present in the surface of the keratin biomaterials such as carboxyl, hydroxyl, amino, sulfide and sulphate groups etc. have

some affinity in general for heavy metal ions. Based on the literatures, different keratin biomaterials as biosorbents are used for heavy metal removal. Information reviewed mainly are focusing on the biosorption performance of such biosorbents, their possible pretreatment and/or modification, their regeneration/reuse, about the modeling of the heavy metal biosorption process (even kinetics and isotherm models), together with an evaluation of their potential application and their future in the market.

The pretreatment and modification of the natural keratin biomaterials aiming to improve their biosorption capacity was introduced and evaluated. Keratin-rich nanofibre and keratin colloidal solution also have good biosorption abilities. Although chemical modified keratin biomaterials or some keratin-rich materials having specific structure and properties can enhance the adsorption of heavy metal ions, the cost of chemicals used and methods for the modification also have to be taken into consideration in order to actually produce “low-cost” biosorbents in the real applications. In conclusion, the widespread keratin biomaterials in industries for wastewater treatment applications today are strongly recommended due to their local availability, technical feasibility, engineering applicability, and cost effectiveness. In spite of this, the process of biosorption requires further investigation in the direction of modeling, regeneration of biosorbent, recovery of metal ions, and modification of the keratin biomaterials for the practical application.

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