Biosorption of Heavy Metals from Acid Mine Drainages onto Pig Bristles, Poultry Feathers and Crustacean Shells Industrial Biowastes

Fernando I. Ramírez-Paredes[a], Teresa Manzano-Muñoz[b], Juan C. Garcia-Prieto[b], J. Felipe Bello-Estévez[a], Galina G. Zhadan[c], Valery L. Shnyrov[c], John F. Kennedy[d] and Manuel G. Roig[a,b,*]

[a]*Department of Physical Chemistry, University of Salamanca, 37008 Salamanca, Spain*

[b]*Water Research and Development Centre (CIDTA), University of Salamanca, 37007 Salamanca, Spain*

[c]*Department of Biochemistry and Molecular Biology, University of Salamanca, 37007 Salamanca, Spain*

[d]*Chembiotech Laboratories, Advanced Science and Technology Institute, Kyrewood House, Tenbury Wells, Worcestershire, WR15 8SG, UK*

Abstract: The removal of metals ions from aqueous solutions plays an important role in water pollution control. In this study, a biosorption process for the bioremediation of heavy metal-contaminated acid mine drainages, located in Western Spain, has been developed. The process is based on the physico-chemical properties for the adsorption, ion exchange, and complexation of metal ions by biopolymers keratin and chitin from different industrial biowastes such as pig bristles, poultry feathers and crustacean shells. The selectivity for metals, the first order kinetics and yields of the corresponding biosorption processes of uranium and other metals polluting such acid mine drainages by such biosorbents are described. The biowaste rich in keratin (pig bristles) seems to show a higher biosorption capacity than that of bioresidues rich in chitin (crustacean shells). Moreover, factors such as the lower contamination by metals of acid waters, the lower influent water volume/biosorbent mass ratio, the configuration of the packed-bed reactor and the partial hydrolysis of keratin increase both the capacity and the rate of the process of metal biosorption onto the biosorbent.

Keywords: Biopolymers, chitin, keratin, biosorption, desorption, isotherms, kinetics, heavy metals, mine drainages, toxicity, industrial wastes.

1. INTRODUCTION

Mining has been part of human activity for thousands of years and abandoned mines and mining operations are present across the planet. Mines are local phenomena and their greatest impact on terrestrial and aquatic ecosystems are also essentially local, although not always, and the dispersion of heavy metals may reach regional and even global proportions. Mines generate huge volumes of rocks and slag, which must be deposited on the terrain, and they also generate bioleachates that may well end up in aquatic ecosystems. The main results of this in terms of heavy metal contamination are large, completely useless areas and the pollution of lands, lakes, rivers and coastal areas. The processes involved in the remediation or control of bioleaching from such dumps and the remediation of watercourses polluted by mine wastes are complicated and expensive.

As mentioned, the basic problem of mining is the huge volume of wastes. The procedure traditionally followed for getting rid of these has been to store them on the land, although it is evident that in most cases this will lead to contamination by acid mine drainage and heavy metal dispersion and pollution. The depositing of wastes or effluents in watercourses injects pollution directly into the ecosystem. However, under certain specific conditions, underwater dumping can reduce acid mine drainages to a considerable extent, although toxic metals may be released or recycled, or other types of impact on the environment may emerge. In view of the developing legislation addressing mining wastes and practices for distributing wastes, it is likely that sub-aquatic distribution may not always sanctioned, even when it avoids problems of terrestrial contamination.

Remediation measures for reducing the release of metals into terrestrial environments should first be applied to the source of contamination. Solid wastes deposited onto the land can be neutralised with lime or encapsulated in impermeable bases and surface covers to reduce acid drainage and the consequent diffusion of metals and their subsequent bioavailability. When a neutral or slightly alkaline pH is accomplished by the addition of lime, limestone, alkaline ash or

*Address correspondence to this author at the Department of Physical Chemistry, University of Salamanca, 37008 Salamanca, Spain;
Tel: (+34) 923294670; Fax: (+34) 923294744; E-mail: mgr@usal.es

ISSN: 1814-8085 / E-ISSN: 1927-5129/13

similar materials, the adsorption and precipitation of metals is favoured.

The remediation of mining areas, however, often surpasses the economic capacity of nearly all corporations and abundant large open deposits, soils, lakes and coastal zones have been contaminated in even the most advanced nations, with the greatest awareness about environmental problems. In view of the number of mines -both past and present- and their corresponding dumps and of zones with polluted aquatic ecosystems, remediation -or at least mitigation- is a mammoth task.

In mining operations, remediation should only be implemented while the mine is in its productive phase and is generating benefits. For abandoned sites in operational mines, retroactive measures should be attempted, such as reseeding the vegetation, the treatment of leachates, and other initiatives. The viability of open-cast metal mines depends on the fluctuations at global level of the value of the metal being extracted and on supply and demand. When an open-cast mining cut is abandoned, this does not normally mean that all the metals sought have been extracted; only those that were of economic interest up to the time of mine closure. Thus, metal mines are opened, shut down, and may be opened again if necessary by future generations, when techniques for extracting veins of less economic interest have been developed or when the economy has changed. The remediation of such deposits may facilitate or even prevent reopening. However, one question that arises in all this is what degree of remediation is desirable for metal mining cuts.

The need for innovating techniques for *in situ* treatment will increase, as will the need for research into the development of such techniques and predicting their results. Sophisticated technology for the remediation of pollution coming from mining activities is not always the best solution in the long term and simpler technologies -in particular the burgeoning biotechnology, which would be particularly interesting for less developed nations- may often be the best solution in many areas. Passive and self-maintaining biological systems could be used to treat residual mine waters and leachates. These procedures are attractive because of their low cost. The systems proposed include the use of bacteria, fungi, and biowastes able to concentrate and remove metals from mining effluents [1-5]. Some microscopic algae (*Charophytes*) have been assayed in the alkaline waters of the refining ponds of mines extracting uranium or nickel, where they biosorb several metals and modify pH to neutral values [6]. Despite this, in many cases remediation is not compatible with economic or engineering viability and all that can be done is to assess the environmental impacts while nature attempts to clean itself up.

Natural biopolymers have always existed and these macromolecules produced by the metabolism of living beings date back to 300 million years ago. The use of such polymers for ion exchange is by no means a novelty. Suffice it to mention that the book of *Exodus* (15:23-25) contains a reference to how *Jehovah* advised *Moses* to cast a tree into the bitter waters of the River *Marah* to make them sweet. For many centuries, sand and peat have been used as ion exchange materials, although it was not until 1912 that it was proposed that their capacity to remove ions was due to their acid groups.

The study of synthetic polymers, by contrast, is relatively recent and only began in the twentieth century. Although polystyrene was synthesised as early as 1880 and the first synthetic ion exchange material was produced by Harm and Rumpler [7], a generalised perception of the existence of macromolecules of this type did not emerge until the 1920s [8]. Indeed, the use of fragments of ground gramophone records as ion exchangers, by Adam and Holmes [9], did not occur until 1935. This triggered the production of synthetic exchangers in the proper sense of the term. Although most biopolymers have ion exchange properties, the truth of the matter is that they were not much used for such purposes until the semisynthetic materials for ion exchange appeared on the market. Biopolymers are now extensively used in biochemical separations, such as the fractionation of proteins, polysaccharides and nucleic acids. Indeed, most if not all these ion exchange materials are polysaccharides.

In 1931, Kullgren [10] may have been the first investigator to apply the properties of modified cellulose as an ion exchanger for analytical purposes when he observed that cellulose treated with sulphite was able to remove copper ions from water and that these ions could be recovered after acid treatment.

Thus, since the fifties of the last century cellulose with both anionic and cationic exchange properties, dextrans and other marine polysaccharides have been marketed with a huge variety of exchange groups.

More recently, a certain interest has arisen in chitin and its derivative chitosan as ion-binding agents that can be applied in a broad range of industrial uses [11].

There seems to be certain consensus concerning the tripartite nature of main polymers; proteins, nucleic acids, and polysaccharides. This is because all three are readily found in the world of living beings: animals, plants, protozoa and microorganisms.

Both proteins and polysaccharides form an almost boundless group of different macromolecules, although proteins are by far the most important. This is so because more than one type of protein is required to synthesise even the simplest polysaccharide. Since nucleic acids have such a specific function with respect to all cellular activities, they are inevitably less numerous than proteins or polysaccharides.

However, much less attention has been devoted to a whole series of biological polymers with basic molecular structures different from those of proteins, polysaccharides or nucleic acids. Furthermore, hybrid polymers are very common -examples are glycoproteins, proteoglycans and lipopolysaccharides- and are ubiquitous. In fact, in nature there are more glycoproteins than simple proteins and substitution by carbohydrate is more the rule than the exception. The hybridisation of proteins and polysaccharides with other molecules species is also quite common.

Hair and feathers are residual sub-products of industries such as tanning, pork-meat production, chicken slaughterhouses, etc. In most cases, these biomasses, almost 100% of which is made up of the most abundant protein on the planet (keratin), are burned or biodegraded in dumps and do not find use in different applied technological processes. Thus, in Spain many tonnes of hair and feathers are generated as waste every year. In the particular case of the province of Salamanca, this is surprising since more than a thousand tonnes of pig bristles are generated as residues every year at the certified brand of origin pork-meat producing factories in the town of Guijuelo.

Among the possible technologies to which this type of protein residue is directly amenable is the removal (recovery) of metals from polluting wastewaters. In this sense, Michelsen *et al*. [12] reported that the hair of cattle and pigs in the tanning industry was selectively able to remove mercury from wastewaters. Additionally, the elution of mercury from these biomasses using solutions of HCl and NaCl allowed the element to be recovered and concentrated in small volumes.

Chicken feathers comprise an intricate network of keratin fibres that are stable and insoluble in water and that have a large surface area. They are also an abundant bioresidue. Ishikawa and Suyama [13] observed that these proteins accumulate precious metal cations such as Au^{3+}, Pt^{2+} and Pd^{2+}, in some cases at percentages as high as 17%. It also appears that the simultaneous presence of certain metals (Na, Fe or Cu) is not related to any decrease in the sorption capacity of this biomaterial. Accordingly, keratin from feathers and hair seems to be a promising material for the control of the pollution of waters by metals.

The residues from the seafood industry have traditionally been sent to dumps or released directly into the ocean. However, this material has also proved to be useful for a whole series of applied processes. It is composed of proteins, calcium carbonate, and large amounts of chitin (N-acetyl-polyglucosamine).

Chitin is an uncharged polysaccharide that, owing to its N-acetyl and hydroxyl groups, has a strong capacity for metal binding. It forms almost 80% of the shells of arthropods and approximately 30% of the shells of crustaceans. Of all animal polysaccharides, only chitin is found in sufficient abundance and can be isolated with sufficient ease for use in commercial practice.

It has been reported that both chitin and deacylated chitin (chitosan) are able to remove arsenic from waters [14]. Chui *et al*. [15] compared both products (chitin and chitosan) as a function of their capacity to adsorb metals such as Cu^{2+}, Cr^{3+} and Ni^{2+}, observing that chitin adsorbed Cu^{2+} and Cr^{3+} at percentages very similar to chitosan, although it was less efficient in the case of Ni^{2+}.

McKay [16] showed that chitosan has a similar adsorption capacity for metal ions to those of certain commercial products, such as the ion exchange resins *Chelex 100* and *Amberlite XE-318*. Likewise, it has been reported that the metal recovery values of chitin are lower than those of chitosan. Despite this, kinetic and design data for the binding of metal ions by chitosan are still lacking.

With regards to these two biopolymers, Knorr [17] studied extraction from the shells of *Cancer magister, Paralithoides carnschatica* and *Pandalus borealis*. Coughlin *et al*. [18] assayed the use of chitin and

chitosan to remove metal ions from wastewaters from the chrome- and nickel-plating industries. Van Daele and Thome [19] studied the applications of chitosan for the decontamination of rivers affected by high PCB levels, undoubtedly one of the types of pollution best studied recently owing to the strong impact of these chlorine derivatives on living organisms. Onsoeyen and Skaugrud [20] reported that the interaction of metals with chitosan are complex and are probably dominated by adsorption, ion exchange and chelation. Piron and Domard [21] established a series of parameters on studying the adsorption of uranyl ions by chitosan; they concluded that the binding of these ions occurred mainly through complexes with the amino groups of the chitosan instead of through electrostatic interactions. They also established a 6.5-7.5 optimum pH for the adsorption of uranyl. At this pH, there is a sufficient number of deprotonated amino groups and no carbonate ions, which although able to strongly complex uranyl ions at the same time inhibit their binding to chitosan.

In comparison with the above, Salah-Azab and Peterson [22] established an order of yield of different biosorbents as regards the elimination of 100 ppm of cadmium: human hair, 40%; bone, 93%; peach pit, 35%; walnut husk, 45%; peanut shell, 45%; orange peel, 80%, compost, 95%; *Zygorhynchus*, 95%; *Rhizophus*, 98%; *Mucor ramanianus*, 95%; *Penicillium*, 70%; *Aspergillus terreus*, 78%; ion exchange resin, 7%; activated carbon, 65%. Treatment with bases increased the efficiency of some of the biosorbents by more than 50%.

Anionic metal complexes are very effectively bound by biomass types containing an abundance of amine groups. Readily available chitinous materials such as acid-washed *Ucides* shells (AWUS) provided food sorption of anionic gold-cyanide ($Au(CN)_2^-$), selenate (SeO_4^{2-}), chromate (CrO_4^{2-}) and vanadate (VO_4^{3-}) at low pH. Equilibrium biosorption uptakes by AWUS reached 0.17 mmol Au g^{-1} AWUS (pH 3.4), 0.15 mmol Se g^{-1} (pH 3.0), 0.54 mmol Cr g^{-1} (pH 2.0) and 0.79 mmol V g^{-1} (pH 2.5). Increased ionic strength suppressed the primary anion uptake since chloride ions competed for biosorbent protonated sites and higher ionic strength reduced the activity of ions in solution. The biosorption mechanism was suspected to involve electrostatic attraction [23].

A binary biosorption system of chromate and vanadate with acid-washed crab shells was studied by Niu and Volesky [24] at pH 2.5 and 0.1 M NaCl. AWCS showed a higher affinity for vanadate than for chromate at a concentration ratio of vanadium to chromium ranging from 0 to 1.5 in the solution. The presence of chromate did not affect vanadate uptake within the concentration range examined. However, chromium uptake was reduced to 40% in the presence of vanadate. The results indicated that vanadate could be selectively separated from the vanadate-chromate mixture with the AWCS material. Based on the model parameters regressed from the respective mono-metal systems, the multi-component Langmuir model developed predicted the interference of vanadate in chromate uptake reasonably well. In addition, the mono-vanadium Langmuir model predicted vanadium uptake in the chromium and vanadium binary system very well [24].

Removal of arsenate (As (V)) by biosorption with acid-washed crab shells is very sensitive to solution pH, because changes in water pH not only affect the charged functional groups on crab shells but also the speciation of arsenate in solution. Increasing ionic strength of the solution negatively affects arsenic uptake. Arsenic biosorption was mainly through arsenate binding on the amide groups in the crab shells [25].

Summarizing, after introducing the environmental problem of acid mine drainages and possible remediation measures, and the hystorical background of different biopolymers with regards to biosorption of metals from polluted waters, the focuss of this work is on the feasibility study of some industrial and abundant biowastes, such as pig bristles, poultry feathers and crustacean shells, which major components are the biopolymers keratin and quitin, for the efficient removal of soluble metals from acid mine drainages.

2. MATERIALS AND METHODS

2.1. Reagents

The reagents sulphuric acid, 35% hydrochloric acid (Panreac, Barcelona, Spain), sodium hydroxide, hexahydrated uranyl nitrate ($UO_2(NO_3)_2.6H_2O$) (Merck, Darmstadt, Germany), arsenazo III, powdered lead (Fluka Chimie Gmbh, Buchs, Switzerland), Triton X-100 (Sigma Chem Co), Hypl 2002 (Grace Service Chemicals Gmbh, Heidelberg, Germany) and Milli-Q ultra-pure water (Millipore Spain, Madrid) were obtained from the sources indicated.

2.2. Biosorbents

The biosorbents -wastes donated by different industries- were as follows: 300 kg of *Iberian* breed pig bristles (*Iberian* = cross between *Sus scrofa fernus* and *Sus mediterraneus*) donated by the *Maguisa* slaughter industry in Guijuelo (Salamanca, Spain); 5 kg of red partridge feathers (*Alectoris rufa*) donated by *Aves Vázquez* S.A. (Avila, Spain), and 25 kg of river crab shells (*Procambarus clarkii*) donated by *Alfocan* S.A. (Sevilla, Spain). All these biomasses were conserved at room temperature, except in the case of the river crab shells, which were conserved at –10 ºC. Once it had been decided which biomasses were to be used for each line of experimentation, they were subjected to the different processes prior to biosorption and were conserved under refrigeration at 4 ºC.

2.3. Preparation of Pig Bristles

This biomass was used either directly (i.e., without previous treatment) or after a prior treatment. In the latter case, since the hair supplied was damp and contained remains of fat and organic matter, it was washed four times with 0.3 M NaOH and with soap and abundant water until the bad odour and greasy aspect had disappeared (2 days). Following this, it was washed again with abundant water to remove the remains of the soap and reduce the pH from alkaline to 6.0. Finally, the water was removed from the hair mechanically and it was dried in an oven at 34-40 ºC over 24 hours.

2.4. Acid Hydrolysis of Keratin from Pig Bristles

Partial hydrolysis of the hair by dynamic incubation (15.1 cm^3 g^{-1} hair) in a packed-bed reactor with 8 M HCl (pH=0) with recirculation for 16.5 h was performed. After the hydrolytic treatment, the hair was washed repeatedly with ultrapure water until an optimum pH of 3.4 was obtained.

2.5. Preparation of Crustacean Shells

In some cases, dry samples -without any previous treatment- were ground with a view to obtaining powdered biomass. In others, the shells were subjected to a washing process with abundant soap for 3 hours in order to remove the remains of the biological material adhering to the walls of the shells, after which they were subjected to a very short grinding process aimed at increasing the contact surface area of the biosorbent with the water.

2.6. Preparation of Partridge Feathers

The feathers were first washed to remove any remains of blood. In some cases, following this brief washing procedure, they were subjected to hydrolysis with 7.2 M HCl.

2.7. Bioreactors

Several packed-bed bioreactors were constructed from cylindrical methacrylate tubes with the following geometry: 3.5 cm internal diameter; 10.4 cm height; a diameter/height ratio of 0.34 and 100 cm^3 capacity. Each bioreactor had a thermostatted jacket to maintain temperature constant during the experiments. A peristaltic pump maintained a constant recirculation flow (20 cm^3 min^{-1}) along the reactor circuit.

In each methacrylate bioreactor, the corresponding mass of biosorbent (finely cut to a particle size of ≤ 0.125 cm^3 or in powdered form) was packed between two plastic grids to prevent the escape of biosorbent, operating with the acid drainage water in continuous mode with recirculation. Entry of the acid water into the reactor was through the base and the exit was from the top, both ends being connected to a stirred tank of 0.5-4.0 dm^3 capacity. This tank served for sample collection and analysis of the physico-chemical parameters of the water such as pH, specific conductivity, etc.

Several stirred tank reactors with different capacities (0.5, 1.0, 10, 25 and 100 dm^3) were also used in batch mode, in which the metal-contaminated acid waters were placed in dynamic contact with the different biosorbents.

2.8. Acid Mine Drainages

After samples of the water from the mine cuts had been taken, they were transported to the lab and stored at room temperature in PVC recipients, previously washed with 2 N hydrochloric acid. The samples were from the following mines: 1) the *Feli* or *Alabancos* mine in the mining district of Barruecopardo-La Fregeneda (La Fregeneda, Salamanca), with the minerals casiterite (SnO_2), wolframite [$(Fe,Mn)WO_4$], arsenopyrite (FeAsS), pyrite (FeS_2), and lepidolite [$K_2Li_3Al_4Si_7O_{21}(OH,F)_3$]; 2) The *Merladet* mine in the mining district of Barruecopardo-La Fregeneda (Barruecopardo, Salamanca), with the minerals scheelite ($CaWO_4$), wolframite, arsenopyrite, and pyrite; 3) The *Terrubias* mine in the mining district of Morille-Martinamor (San Pedro de Rozados,

Salamanca), with the minerals scheelite, wolframite, and casiterite; 4) The *Alegría* mine in the mining district of Morille-Martinamor (Morille, Salamanca), with the minerals scheelite, wolframite and casiterite; 5) the *Dorinda* mine in the mining district of Granito de Ricobayo (Villalcampo, Zamora), with the mineral casiterite; 6) The ENUSA *Faith* mine in the mining district of Ciudad Rodrigo (Saelices El Chico, Salamanca), with the minerals pitchblende [Complex], pyrite and marcasite (FeS_2).

2.9. Instruments

The instruments and apparatus used in the present work were as follows: a CRISON micropH 2001 pH-meter, a Branson 2200 ultrasound device, Centrolit "Selecta P" and Centromix centrifuges, a Moulinex blender, Mettler Toledo AB204 and Precisa 600C balances, a WTB Binder drying oven, GILSON Minipuls 2 and Ismatec peristaltic pumps, a WTW Profilab multiparametric probe, a GEM Biochemical Inc. Bacterial Systems "BG-1" ecotoxicity device, a UV-Visible Hitachi 150-20 spectrophotometer, an ICP (Emission Spectroscopy by Induction-Coupled Plasma) spectrophotometer for quantitative analysis of metals and other chemical elements (Central Analysis Services of the University of Salamanca), and an atomic absorption spectrophotometer (kindly facilitated by the Department of Analytical Chemistry, Nutrition and Food Sciences of the University of Salamanca).

2.10. Physico-Chemical, Chemical and Radiological Analysis of the Acid Mine Drainages

The physico-chemical parameters determined (some *in situ* and others at the laboratory) in the mine drainage were temperature, pH, specific conductivity, dissolved oxygen, turbidity, chemical oxygen demand (COD), and biochemical oxygen demand (BOD), following the usual methods (Standard Methods) for the analysis of waters and waste waters (APHA, AWWA, WPCF) [26].

Qualitative chemical analysis of the waters was accomplished by obtaining dry residues from the samples (dried in an oven at 98 ºC) or by progressively increasing the pH of the waters to produce a selective chemical precipitation. The dry residues and precipitates obtained were subjected to chemical analysis by X-ray dispersive energy (EDAX).

Quantitative chemical analyses of the water samples were carried out using ICP (Emission Spectroscopy by Induction-Coupled Plasma) spectroscopy at the Central Analysis Services of the University of Salamanca (Spain).

With a view to checking the possible radioactivity of some water samples, replicate determinations were made at the Environmental Radioactivity Laboratory of the University of Salamanca of the α and β radioactivities of acid waters samples from cut 1 of the ENUSA *Faith* uranium mine. Standard methods (APHA, AWWA, WPCF) [26], improved by Moron *et al*. [27] and Sill [28], were used.

2.11. Ecotoxicological Analysis of the AMDs

According to the bioluminescence test employing *Photobacterium phosphoreum* (Ames test), a liquid sample is considered to be toxic when an inhibition of the luminescence of the bacteria greater than 20% occurs (toxicity threshold) in an analysis carried out at 15ºC in 15 min. Quantification of the toxicity of the samples is carried out according to the EC_{50} (maximum effective concentration: the dilution value of the sample for which 50% inhibition of luminescence occurs) according to the ANFORT T-90-320 directive.

2.12. Chemical Analysis of Metals

Analysis of the metals in these acid supernatant solutions was accomplished by flame atomic absorption spectrophotometry, with the exception of Al, which was analysed by UV-Vis spectrophotometry, following the eriochromocyanine-R method and performing the analysis in continuous mode by flow injection analysis (FIA).

UO_2^{2+} Cation

A dual-channel merge-point flow injection (FIA) system was used. The carrier was a stream of 3.6 M HCl in the presence of 1% Triton X-100. The flow rate of the carrier and reagent streams was 2.0 cm^3 min^{-1}.

Analysis of the uranyl cation in the acid mine drainage was accomplished using a rapid and sensitive method [29] that avoids manipulation in order to achieve the reduction of U(VI) to U(IV) upon inserting a lead- reducing minicolumn in the system [30].

2.13. Statistical Fitting of Data

The fitting of the kinetic and equilibrium data on biosorption to different equations and models was done using the SIMFIT statistical package, authored by Prof. W.G. Bardsley of the University of Manchester (UK).

3. RESULTS AND DISCUSSION

3.1. Biosorption of Metals from Acid Mine Drainages onto Pig Hair (Keratin) in a Continuous Stirred Tank Reactor

3.1.1. Acid Drainages from Merladet Mine with Low Level of Metal Pollution

A metal biosorption experiment was carried out, incubating well washed and neutralised pig bristles (1.0 g) with 200 cm^3 metal-contaminated water samples (pH=4.0) from the *Merladet* mine (Barruecopardo, Salamanca) at 20 ºC in a discontinuous stirred-tank reactor (120 r.p.m); i.e., not very contaminated waters (9.37 ppm Al; 3.55 ppm Zn; 3.32 ppm Mn; 0.65 ppm Cu; 0.09 ppm Cu).

In parallel, a blank experiment was performed (with acid mine water but without sorbent) and aliquot samples of 5 cm^3 of the water were taken at different operation times. To each of these samples, a further 5 cm^3 of a 0.01 M solution of H_2SO_4 was added immediately in order to maintain pH low and avoid precipitates.

The results of the above experiments are shown in Figures **1** and **2** and Table **1**, in which the good capacity of clean pig bristles to biosorb metals efficiently and rapidly can be seen. The following conclusions can be drawn:

1) Regarding the biosorption selectivity and capacity of the different metals by the biomass, it may be seen that the percentages of elimination at the end of the first hour of operation were Cu (98% elimination; i.e., 0.13 mg Cu g^{-1} biosorbent), Zn (87% elimination; 0.62 mg Zn g^{-1} biosorbent); Mn (56% elimination; 0.36 mg Mn g^{-1} biosorbent) and Ni (26%; 0.13 mg Ni g^{-1} biosorbent). After one week of batch incubation, the percentages of biosorption of the metals were: Ni (100% elimination; 0.51 mg Ni g^{-1} biosorbent); Cu (99% elimination; 0.13 mg Cu g^{-1} biosorbent); Zn (97% elimination; 0.67 mg Zn g^{-1} biosorbent) and Mn (63% elimination; 0.41 mg Mn g^{-1} biosorbent) (Figure **1**).

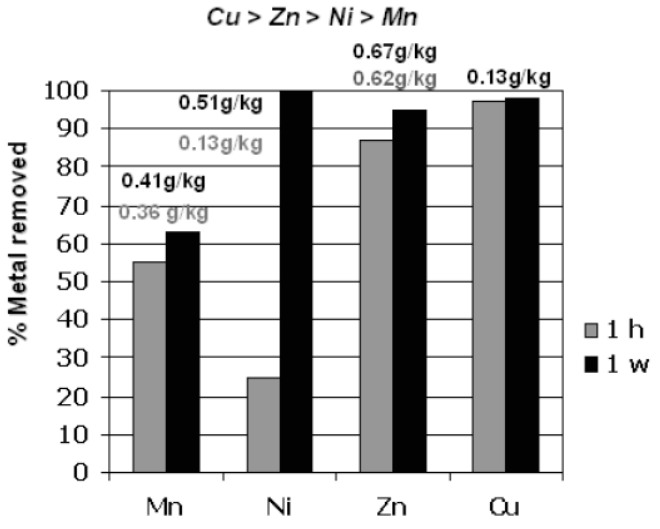

Figure 1: Metal selectivity and bioadsorption capacity of metals on pig bristles after 1 hour and 1 week incubation in a stirred tank reactor in discontinuous mode (120 rpm) of 200 cm^3 of acid drainage from the *Merladet* mine (Barruecopardo, Salamanca) with 1.00 g of the biosorbent.

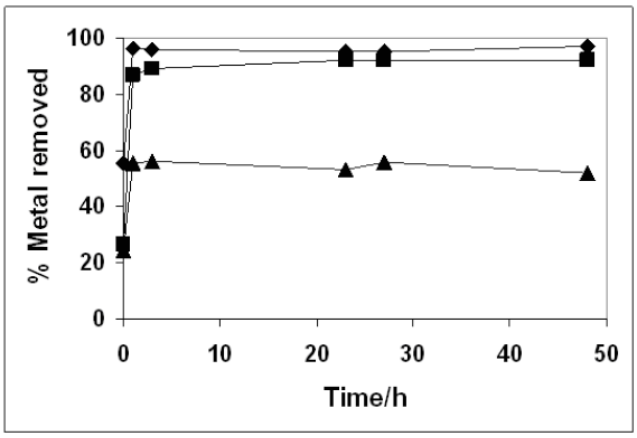

Figure 2: Batch biosorption kinetics of metal (Cu ♦, Zn ■, Mn ▲) ions from *Merladet* mine drainage (Barruecopardo, Salamanca) on 1.00 g of clean pig bristles.

2) The kinetics of biosorption by keratin from pig bristles of the metals dissolved in acid mine water was fast (k = 0.52 (Ni), 0.68 (Mn), 0.85 (Zn) and 1.02 (Cu) h^{-1}) and was of first order with respect to the concentration of the metal ($[Metal]_{solution} = Ae^{-kt} + C$), equilibrium being reached in the first 60-120 min (Figure **2**). This points to a rapid process of adsorption of the metal onto the biomass surface.

3.1.2. Acid Drainages from Faith Mine with Intermediate Level of Metal Pollution

Influent Acid Drainage/Biosorbent Ratio = 300 cm^3 g^{-1}

Having checked the efficient elimination of metals from the acid water from the *Merladet* mine by biosorption onto previously washed pig bristles, it was of interest to use the same treatment on acid waters with much high levels of metal pollution that were also toxic and radioactive. These were water samples from the *Faith* mine (uranium extraction) run by ENUSA and BERKELEY Resources Ltd. companies at Saelices el Chico (Salamanca, Spain).

Table 1: Metal Biosorption Selectivity, Kinetics (k) and Capacity (% Bound Metal) of the Biosorbents

	Cu k (h^{-1}) %	Zn k (h^{-1}) %	Ni k (h^{-1}) %	Mn k (h^{-1}) %	Al k (h^{-1}) %	Fe k (h^{-1}) %	U k (h^{-1}) %
Keratin1	1.02 98	0.85 87	0.52 26	0.68 56			
Keratin2	1.74 97	2.4 38					
Keratin3	■ 100		0.37 29				0.19 93
Keratin4	2.29 100		▲ 44	2.01 11	0.37 8	19.2 38	
Keratin5	5.31 100		1.59 51	16.5 13	♦ 48	22.1 38	0.40 83
Keratin6	0.91 31						2.4 20
Chitin1	● 90	0.15 40	0.12 28	4.01 19			0.40 83
Chitin2	0.25 100	0.29 74	0.29 51	0.59 24			
Chitin3							21.7 83

Keratin1 = Keratin from pig hair + Acid drainage from *Merladet* mine (stirred tank).
Keratin2 = Keratin from pig hair + Acid drainage from *Faith* mine (300 cm^3 g^{-1}, stirred tank).
Keratin3 = Keratin from pig hair + Acid drainage from *Faith* mine (30 cm^3 g^{-1}, stirred tank).
Keratin4 = Keratin from pig hair + Acid drainage from *Faith* mine (packed-bed reactor).
Keratin5 = Hydrolysed keratin from pig hair + Acid drainage from *Faith* mine (packed-bed reactor) Keratin6 = Hydrolysed keratin from bird feathers + Acid drainage from *Faith* mine (packed-bed reactor).
Chitin1 = Chitin from crustacean shells + Acid drainage from *Faith* mine (packed-bed reactor).
Chitin2 = Regenerated chitin from crustacean shells + Acid drainage from *Faith* mine (packed-bed reactor).
Chitin3 = Chitin from powdered crustacean shells + Acid drainage from *Faith* mine (stirred tank).
■ Biphasic kinetics: $k_1 = 0.858\ h^{-1}$, $k_2 = 959\ h^{-1}$.
▲ Biphasic kinetics: $k_1 = 7.44\ h^{-1}$, $k_2 = 0.251\ h^{-1}$.
♦ Biphasic kinetics: $k_1 = 0.113\ h^{-1}$, $k_2 = 9.12\ h^{-1}$.
● Biphasic kinetics: $k_1 = 17.12\ h^{-1}$, $k_2 = 0.076\ h^{-1}$.

The waters from this mine have much higher levels of heavy metals (114 ppm Mn; 90 ppm Al; 25.2 ppm Zn; 6.3 ppm Fe; 4.7 ppm Ni; 1.22 ppm Cu) than those of the *Merladet* mine and also contain significant levels of uranium (42 ppm). The reactor used in this case was a stirred-tank reactor (120 r.p.m) in which 16.7 g of pig bristles were incubated with 3.0 dm^3 of acid water. The operation temperature was 27 ºC and pH was 3.2. Aliquot samples of 10 cm^3 were added to 10 cm^3 of 0.01 M H_2SO_4 at times of 0, 1 min, 15 min, 30 min, 1 h, 3h, 6h, 13h, 15h, 18h, 18.5h, 24h, 25.5h and 97.5 h.

It was observed that under the experimental conditions described, only in the cases of Cu and Zn was there a significant degree of biosorption of the metal onto the clean pig bristles. In the cases of Al and Fe, the percentages of metal biosorbed onto the bristles were not directly detectable, although the desorption experiment carried out later on showed their desorption from the bristles.

In comparison with the results obtained with the water from the *Merladet* mine, it was seen that only Cu had a similar level of biosorption; that is, by 24 h elimination was 97% for Cu (0.35 mg Cu g^{-1} biosorbent), the percentage of elimination of Zn being substantially lower (38%; 2.9 mg Zn g^{-1} biosorbent). The biosorption kinetics of both metals by keratin from pig hair was of first order with respect to the concentration of the metal ($[Metal]_{solution} = Ae^{-kt} + C$ (k = 1.74 h^{-1}; $t_{1/2}$= 23.9 min for Cu, k = 2.4 h^{-1}, $t_{1/2}$=17.3 min for Zn) (Table **1**), equilibrium being reached in the first 3 hours of operation.

Influent Acid Drainage/Biosorbent Ratio = 30 cm^3 g^{-1}

With a view to checking the greater biosorption of metals from acid waters of the *Faith* mine by clean pig bristles, the above experiment was repeated but decreasing the volume of water to be treated/mass of biosorbent ratio, which in this case was 30 cm^3 water g^{-1} pig bristles. To accomplish this, the bioreactor employed was of the stirred-tank type (120 r.p.m.) for the treatment of 500 cm^3 of acid water (pH 3.2), incubating at 20 ºC with 16.7 g of pig bristles. Sampling was carried out at different times, extracting aliquots of 3.5 cm^3 from the reactor, which were then added to 3.5 cm^3 of 0.01M H_2SO_4.

It was observed that only in the cases of U, Cu and Ni did any significant degree of biosorption occur on the residues of clean pig bristles. Surprisingly, for the other metals no biosorption process was observed under the experimental conditions of the assays, although the biosorption of Al, Mn, Fe and Zn was expected, in logical consonance with the desorption experiments that were later carried out with the biosorbent (*vide infra*). This removal of metals from the drainage reached 100% in the case of Cu (0.04 mg Cu

g^{-1} biosorbent); 93% U (1.1 mg U g^{-1} sorbent) and 29.0% Ni (0.15 mg Ni g^{-1} sorbent).

The kinetics of the biosorption of U and of Ni by keratin from pig bristles are of first order with respect to the metal concentration ($[Metal]_{solution} = Ae^{-kt} + C$), the kinetic parameters being as follows: Uranium (k = 0.194 h^{-1}, $t_{1/2}$ = 3.57 h, A = 31.32 ppm, C = 4.80 ppm), biosorption equilibrium being reached in 24 h; Ni (k = 0.367 h^{-1}, $t_{1/2}$ = 1.89 h, A = 1.505 ppm, C = 6.582 ppm), equilibrium being reached in the first 15 hours of operation. However, the overall biosorption kinetics of Cu by keratin fitted a biphasic process with an integrated rate equation of the type $[Metal]_{solution} = A_1e^{-k_1t} + A_2e^{-k_2t}$ ((k_1 = 0.858 h^{-1}, k_2 = 959 h^{-1}, A_1 = 0.60 ppm, A_2 = 0.62 ppm), equilibrium being reached in the first 6 hours of operation. In this case, it appears that a rapid phase of biosorption of the metal onto sites class 2 of the biosorbent with high affinity for the metal or more accessible to the acid water occurs simultaneously with another stage of biosorption of the metal onto sites class 1 of the biosorbent with lower affinity for the metal or less accessible to the water (mass transfer limitations) (Table **1**).

3.1.3. Desorption of Metals Biosorbed from Keratin by Different Eluents

The following step of the work consisted of studying the recovery of the metals that could have remained adsorbed on the 16.7 g of the pig bristles from the previous experiments.

0.4 M Na_2CO_3 as Eluent

To perform this desorption, 675 cm^3 of an aqueous solution of 0.4 M Na_2CO_3 (pH 9.9) at 25 ºC, free of metals, was used as eluent. In a bioreactor identical to that employed in the previous experiments on biosorption, the mass of biosorbent loaded with metals (obtained from the first biosorption experiment carried out with acid waters from the *Faith* mine) was placed in contact with the eluent solution, taking aliquot samples of 19 cm^3 of the solution at preset times, to which 10 cm^3 of 0.01M H_2SO_4 was added immediately. Following this, the concentration of the different metals in each solution removed was measured.

Partial desorption of Al, Fe, Cu and Zn from the biosorbent to the 675 cm^3 of the eluent solution was observed after 95 hours of dynamic incubation. That is, under the experimental conditions of the assay 12.7 mg Al, 0.80 mg Fe, 0.24 mg Cu and 0.16 mg Zn were desorbed from the 16.7 g of wet residue of pig bristles to the 675 cm^3 of 0.4 M Na_2CO_3.

Bearing in mind that the wet biosorbent represents the same mass of biosorbent as that used in the adsorption experiment, it would be possible to propose a percentage of desorption due to the action of the 0.4M sodium carbonate of 0.34 mg Cu/5.84 mg Cu (= 5.8%); 0.16 mg Zn/48.4 mg Zn (= 0.3%). In other word, the efficiency of the 0.4 M Na_2CO_3 aqueous solution as an eluent should be questioned.

The desorption kinetics of Zn and Cu from the biosorbent to the eluent fit the following first-order rate equation: $[Metal]_{eluent} = B(1-e^{-kt})$, with rapid kinetics for Zn (k = 175.8 h^{-1}, B = 0.16 ppm) and slow kinetics for Cu (k = $5.96.10^{-3}$ h^{-1}, B = 1.15 ppm). For Fe and Al, the overall desorption kinetics fitted a biphasic process with the following integrated rate equation of the sum-of-exponentials type: $[Metal]_{eluent} = B_1(1-e^{-k_1t}) + B_2(1-e^{-k_2t})$, the desorption kinetics of Fe being rapid (k_1 = 50.7 h^{-1}, k_2 = $98.9.10^{-5}$ h^{-1}. B_1 = 0.81 ppm, B_2= 38.5 ppm) but slower in the case of Al (k_1 = 10.1 h^{-1}, k_2 = 0.128 h^{-1}, B_1 = 8.65 ppm, B_2 = 12.5 ppm). That is, a rapid desorption of the metal from sites on the biosorbent with low affinity for the metal or more accessible to the eluent occurs simultaneously with another slower step of desorption of the metal from the biosorbent with greater affinity for the metal or less accessible to the eluent (mass transfer limitations).

0.2 M HCl as Eluent

The possible recovery -by desorption- of the metals that may have remained adsorbed on the 16.7 grams of pig bristles in the second biosorption experiment performed with acid waters from the *Faith* mine was studied.

It was logical to expect that keratin from bristles would display cation exchange capacity and that in very acid medium it would exchange free H^+ from the aqueous solution for the different metal cations adsorbed onto the biomass that would be released into the solution. Accordingly, in an initial experiment on desorption, 8.0 g of bristles was incubated at pH 1.0 at 20 ºC with 150 cm^3 of 0.2 M HCl (sufficient to cover the bristles completely) and subjected to orbital shaking. At different times, 1 cm^3 aliquot samples were removed from the eluent solution and added to 4 cm^3 of ultrapure H_2O, after which the concentration of metals desorbed was measured. As may be seen in Figure **3**, a solubilisation, by desorption from the biosorbent, of 0.15 mg Al g^{-1} biosorbent, 0.11 mg Mn g^{-1} biosorbent, 0.04 mg Fe g^{-1} biosorbent, and 0.02 mg Zn g^{-1} biosorbent was obtained, while Cu and Ni were not desorbed.

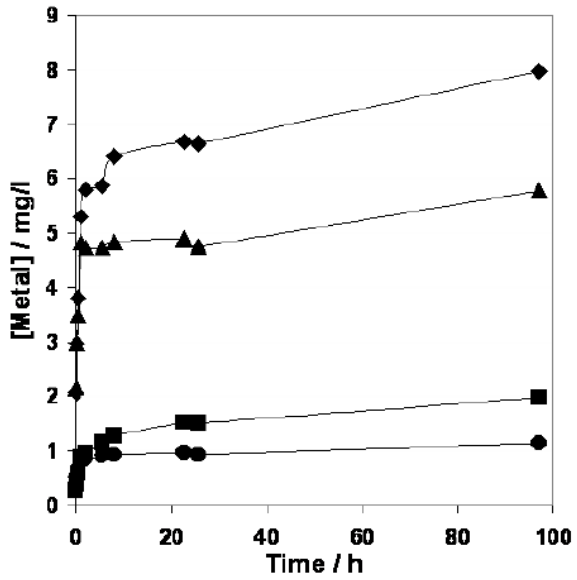

Figure 3: Desorption of Al (◆), Mn (▲), Fe (■) and Zn (●) by elution of the loaded pig bristle biosorbent with 0.2 M HCl.

The desorption kinetics of Mn and Zn from the biosorbent to the eluent fitted the following first-order integrated rate equation $[Metal]_{eluent} = B(1-e^{-kt})$, with kinetic parameters for Mn (k = 3.76 h^{-1}, B = 4.91 ppm) and for Zn (k = 3.58 h^{-1}, B = 0.94 ppm). As occurred with the 0.4 M Na_2CO_3 eluent, for Fe and Al the overall kinetics of desorption fitted a biphasic process, with the following integrated rate equation of the sum-of-integrals type: $[Metal]_{eluent} = B_1(1-e^{-k_1 t}) + B_2(1-e^{-k_2 t})$, with the kinetic parameters for Fe (k_1 = 1.94 h^{-1}, k_2 = 3.19.10^{-2} h^{-1}, B_1 = 0.97 ppm, B_2 = 1.05 ppm) and for Al (k_1 = 2.32.10^{-2} h^{-1}, k_2 = 2.88 h^{-1}, B_1 = 2.62 ppm, B_2 = 5.62 ppm). In other words, a rapid stage of desorption of the metals from sites on the biosorbent with low affinity for the metal or more accessible to the eluent occurs simultaneously with another slower step of desorption of the metal from sites on the biosorbent with greater affinity for the metal or less accessible to the eluent (limitations to mass transfer).

0.4 M Na_2CO_3 (pH=1.8) as Eluent

Another variant of desorption explored was that carried out using 0.4 M Na_2CO_3 as eluent, previously acidified to pH 1.8 with concentrated H_2SO_4. In this case, the remaining 8.00 g of pig bristles from the previous experiment on biosorption with acid water from the *Faith* mine (*vide supra*) were submerged in 150 cm^3 of an acidified aqueous solution of 0.4 M Na_2CO_3 (sufficient to cover the bristles completely), and then subjected to orbital shaking. At preset times, 1 cm^3 samples were taken and added to 4 cm^3 of 0.01 M H_2SO_4 (in order to maintain pH low and avoid precipitates).

As may be seen from Figure **4**, a solubilisation of some metals, due to desorption from the biosorbent, of 0.10 mg of Al g^{-1} biosorbent, 0.059 mg Mn g^{-1} biosorbent, 0.017 mg Fe g^{-1} biosorbent and 0.015 mg Zn g^{-1} biosorbent was confirmed. Cu and Ni were not desorbed. These results, therefore, are similar to those obtained previously using 0.2 M HCl as eluent.

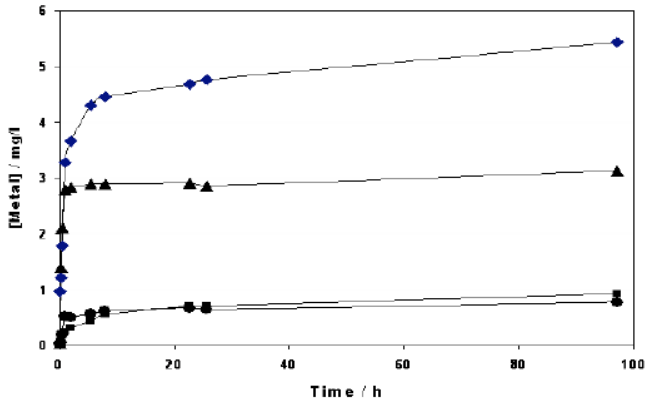

Figure 4: Desorption of Al (◆), Mn (▲), Fe (■) and Zn (●) by elution of the loaded pig bristle biosorbent with 0.4 M Na_2CO_3 (pH 1.8).

The desorption kinetics of Mn and Zn from the biosorbent to the eluent fitted the following first-order integrated rate equation $[Metal]_{eluent} = B(1-e^{-kt})$, with kinetic parameters for Mn, k = 2.35 h^{-1}, B = 2.94 ppm, and for Zn, k = 0.92 h^{-1}, B = 0.66 ppm.

As happened with the 0.4 M Na_2CO_3 (pH 9.9) and 0.2 M HCl eluents, for Fe and Al the overall desorption kinetics fitted a biphasic process, with the following sum-of-exponentials-type integrated rate equation: $[Metal]_{eluent} = B_1(1- e^{-k_1 t}) + B_2(1- e^{-k_2 t})$, with kinetic parameters for Fe, k_1 = 0.569 h^{-1}, k_2 = 3.76.10^{-2} h^{-1}, B_1 = 0.38 ppm, B_2 = 0.57 ppm, and for Al, k_1 = 2.76.10^{-2} h^{-1}, k_2 = 1.40 h^{-1}, B_1 = 1.48 ppm, B_2 = 4.04 ppm. Again, a rapid desorption of the metal from low-affinity sites on the biosorbent or more accessible to the eluent occurs simultaneously with another slower stage of desorption of the metal from sites on the biosorbent with greater affinity for the metal or less accessible to the eluent (limitations to mass transfer).

It is thus concluded that the 0.2 M HCl and 0.4 M Na_2CO_3 eluents at acid pH are more efficient than the 0.4 M Na_2CO_3 solution at alkaline pH.

3.2. Biosorption of Metals from *Faith* Mine Drainage onto Keratin in a Packed-Bed Reactor

Since the previous experiments had been carried out in stirred-tank reactors, in which efficient stirring

and intimate water-biosorbent contact were mediated by the mechanical impediments that the biosorbent itself exerted on the magnetic stirring bars, it was decided to use a different packed-bed flow reactor in which it would be possible to improve the contact between the biosorbent and the metal-polluted acid mine drainage.

The biosorbent consisted of 17.9 g of pre-washed pig bristles cut into random lengths of approximately 0.5 cm that were packed into a 100 cm^3- capacity cylindrical reactor. In the recirculation circuit, including a stirred tank, there was 240 cm^3 of mine water (pH 3.2) under a flow rate of 20 cm^3 min^{-1}. Each 5 cm^3 sample extracted was added to a further 5 cm^3 of 0.01 M H_2SO_4.

Thus, our first aim was to check whether a packed-bed reactor configuration would favour greater biosorption of metals from the acid waters of the *Faith* mine onto clean pig bristles.

A significant biosorption of 100% Cu was observed (0.01 mg Cu g^{-1} biosorbent); 44% Ni (0.15 mg Ni g^{-1} biosorbent); 38% Fe (0.11 mg Fe g^{-1} biosorbent); 11% Mn (0.78 mg Mn g^{-1} biosorbent) and 8% Al (0.38 mg Al g^{-1} biosorbent) onto the keratin.

With the exception of Ni, the biosorption kinetics of all the metals fitted a first-order integrated rate equation with respect to the concentration of the type $[Metal]_{solution} = Ae^{-kt} + C$, with the following kinetic parameters: Fe (k = 19.24 h^{-1}, $t_{1/2}$ = 2.2 min, A = 2.01 ppm, C = 3.20 ppm), adsorption equilibrium being reached in 0.50 h; Cu (k = 2.29 h^{-1}, $t_{1/2}$ = 0.30 h, A = 0.695 ppm, C = 0.048 ppm), adsorption equilibrium being reached in 8.00 h; Mn (k = 2.01 h^{-1}, $t_{1/2}$ = 0.35 h, A = 10.17 ppm, C = 10.37 ppm), adsorption equilibrium being reached in 24 h and Al (k = 0.372 h^{-1}, $t_{1/2}$ = 1.86 h, A = 5.87 ppm, C = 80.23 ppm), adsorption equilibrium being reached in 6.00 h (Table **1**).

However, the overall biosorption kinetics of Ni by keratin fitted a biphasic process with an integrated rate equation of the type $[Metal]_{solution} = A_1e^{-k_1 t} + A_2e^{-k_2 t} + C$ (k_1 = 7.44 h^{-1}, k_2 = 0.251 h^{-1}, A_1 = 0.864 ppm, A_2 = 1.55 ppm), adsorption equilibrium being reached in the first 13 hours of operation. In this case, a rapid process of biosorption of the metals onto sites class 1 of the biosorbent with high affinity for the metal or more accessible to the acid water seems to occur simultaneously with another slower biosorption of the metal on sites class 2 with lower affinity for the metal or less accessible to the water (limitations to mass transfer) (Table **1**).

On comparing the results of the above experiment with those obtained with the stirred-tank reactor, it was observed that the packed-bed configuration not only eliminated, by biosorption, 100% of the Cu present in the waters but also that it elicited an increase in the removal of Ni (from 29% up to 44%). Furthermore, a significant biosorption of Fe, Mn and Al was observed, which was not found when the stirred-tank reactor was used. Accordingly, the packed-bed reactor seems to provide a greater and better biosorption of the metals from the acid water onto the keratin (from pig bristles).

3.3. Biosorption of Metals from *Faith* Mine Drainages on Hydrolysed Keratin in a Packed-Bed Reactor

With a view to improving the biosorption capacity of the packed-bed reactor, it was decided to increase the number of primary carboxyl and amino groups on the keratin from bristles, hydrolysing the –CONH-bonds of their polypeptide chains to afford $–COO^+$ + ^+H_3N-, functional groups with affinity for the metals present in the waters and protonated or not, depending on the pH of the water. Thus, 17.9 g of bristles was hydrolysed and incubated with 240 cm^3 of acid water from the *Faith* mine in the packed-bed reactor configuration.

A greater sorption of metals onto the partially hydrolysed keratin than onto the native keratin was observed, this being 100% for Cu (0.01mg Cu g^{-1} biosorbent), 83% for U (1.0 mg U g^{-1} biosorbent), 81% for Fe (0.15 mg Fe g^{-1} biosorbent), 50.9% for Ni (0.16 mg Ni g^{-1} biosorbent), 48.4% for Al (2.2 mg Al g^{-1} biosorbent) and 12.9% for Mn (0.73 mg Mn g^{-1} biosorbent) as compared with 100% Cu, 44% Ni, 38% Fe, 11% Mn and 8% Al of the observed biosorption onto native keratin (pig bristles).

With the exception of Al, the biosorption of all the metals fitted a first-order integrated rate equation with respect to the concentration of metal of the type $[Metal]_{solution} = Ae^{-kt} + C$, with the following kinetic parameters: Fe (k = 22.14 h^{-1}, $t_{1/2}$ = 1.9 min, A = 2.36 ppm, C = 0.84 ppm), equilibrium being reached at 0.50 h; Mn (k = 16.45 h^{-1}, $t_{1/2}$ = 2.5 min, A = 11.6 ppm, C = 89.6 ppm), equilibrium being reached at 1.0 h; Cu (k = 5.31 h^{-1}, $t_{1/2}$ = 7.8 min, A = 0.53 ppm, C = 0.032 ppm), equilibrium being reached at 0.5 h; Ni (k = 1.59 h^{-1}, $t_{1/2}$ = 26.1 min, A = 2.19 ppm, C = 3.06 ppm), equilibrium being reached at 10.0 h; U (k = 0.40 h^{-1}, $t_{1/2}$ = 1.73 h, A = 35.33 ppm, C = 7.12 ppm), equilibrium being reached

at 10.0 h. However, the overall biosorption kinetics of Al by keratin fitted a biphasic process with an integrated rate equation of the type $[Metal]_{solution} = A_1e^{-k_1 t} + A_2e^{-k_2 t} + C$ (k_1 = 0.113 h^{-1}, k_2 = 9.12 h^{-1}, A_1 = 13.39 ppm, A_2 = 25.13 ppm), equilibrium being attained in the first 48 h of operation. In this case, a rapid biosorption step on sites class 2 of the biosorbent with high affinity for the metal or more accessible to the acid water seems to occur simultaneously with another slower biosorption step onto sites class 1 of the biosorbent with less affinity for the metal or more accessible to the water (mass transfer limitations). On comparing these kinetic parameters with those of the biosorption experiment carried out on native non-hydrolysed keratin, a higher rate of the biosorption process of metals onto hydrolysed keratin was observed (higher k values) (Table **1**).

3.4. Biosorption of Metals from from *Faith* Mine Drainages onto Partridge Feathers (Hydrolysed Keratin)

As with the case of keratin from pig bristles, keratin from red partridge feathers (10.27 g) was subjected to partial hydrolysis using strong acid (8M HCl for 16 h) with a view to favouring the generation of the greatest number of carboxyl and amino groups with affinity for the metals. This industrial waste is an abundant and cheap sub-product of the bird meat industry.

The biosorption experiment was conducted in a packed-bed reactor with the above-described biomass under dynamic incubation with 240 cm^3 of acid water from the *Faith* mine, at pH 3.2, 25º C, a significant degree of sorption only being observed in the case of Cu (30.8%, 0.0075 mg Cu g^{-1} biosorbent) and U (20.2%, 0.20 mg U g^{-1} biosorbent).

The biosorption kinetics of both metals onto hydrolysed keratin from bird feathers fitted a first-order integrated rate equation with respect to the concentration of metal of the type $[Metal]_{solution} = Ae^{-kt} + C$, with the following kinetic parameters: U (k = 2.39 h^{-1}, $t_{1/2}$ = 0.29 h, A = 5.79 ppm, C = 35.6 ppm), biosorption equilibrium being reached in 3 h; Cu (k = 0.91 h^{-1}, $t_{1/2}$ = 0.76 h, A = 0.28 ppm, C = 0.74 ppm), equilibrium being reached in 6 h (Table **1**).

3.5. Biosorption of Metals from *Faith* Mine Drainages onto Crustacean Shells (Chitin)

3.5.1. Ground Shells

To assess other materials as candidates for the biosorption of metals, we decided to evaluate the capacity of a residue rich in chitin; namely, the shells of the American river crab *Procambarus clarkii*. A 1.5-dm^3 capacity cylindrical continuous-flow recirculation reactor (flow rate of 20 cm^3 min^{-1}) was used, into which 150.4 g of chitin was packed in the form of irregular particles with a mean size of approximately 0.25 cm^2 between two plastic grids with a view to preventing the escape of the bioparticles into the flow circuit. This reactor was connected to a stirred tank of 5 dm^3 capacity. The final volume of acid mine water in the circuit was 4.4 dm^3.

Along the development of this biosorption experiment, it was observed that the pH of the waters incubated with chitin increased progressively up to pH 3.8. This would lead to partial chemical precipitation as Al hydroxide (which begins to precipitate at pH 3.5). In fact, although not by biosorption, 58% of the initial Al was eliminated in 40 h, i.e., 393g. Since the hydroxides of Cu, Ni, Zn and Mn do not begin to precipitate chemically until pH values higher than 6.0, 7.0, 7.5 and 8.7 respectively are reached, this sudden rise in the operating pH of the waters did not involve any artefact that might have affected accurate measurement of the biosorption of these metals.

Regarding the other metals, the following percentages of metal biosorbed onto the chitin residues were observed: 90% Cu (0.03 mg Cu g^{-1} biosorbent), 40.3% Zn (0.19 mg Zn g^{-1} biosorbent), 27.7% Ni (0.04 mg Ni g^{-1} biosorbent) and 19% Mn (0.49 mg Mn g^{-1} biosorbent).

The biosorption kinetics of all the metals, except Cu, fitted a first-order integrated rate equation with respect to the concentration of metal of the type $[Metal]_{solution} = Ae^{-kt} + C$, with the following kinetic parameters: Mn (k = 4.008 h^{-1}, $t_{1/2}$ = 0.17 h, A = 14.11 ppm, C = 81.72 ppm), equilibrium being reached in 16 h; Zn (k = 0.147 h^{-1}, $t_{1/2}$ = 4.71 h, A = 4.67 ppm, C = 10.05 ppm), equilibrium being reached in en 20 h; Ni (k = 0.12 h^{-1}, $t_{1/2}$ = 5.78 h, A = 0.93 ppm, C = 3.70 ppm), equilibrium being reached in en 20 h. In the case of Cu, the overall kinetics of biosorption by chitin fitted a biphasic process with a rate equation of the type $[Metal]_{solution} = A_1e^{-k_1 t} + A_2e^{-k_2 t} + C$ (k_1 = 17.12 h^{-1}, k_2 = 0.076 h^{-1}, A_1 = 0.244 ppm, A_2 = 0.532 ppm), equilibrium being reached in the first 40 h of operation. In this case, a rapid biosorption process of the metal onto sites class 1 of the biosorbent with high affinity for the metal or more accessible to the acid water appears to occur simultaneously with another slower process of biosorption of the metal onto sites class 2 of the

biosorbent with less affinity for the metal or less accessible to the acid water (mass transfer limitations) (Table **1**).

3.5.2. Powdered Shells

The next experiment was a variant of the previous one conducted on crustacean shells. In this case, the chitin was in fine powder form, aimed at achieving a greater surface area in contact with the acid water in order to check whether the adsorption of the metals dissolved in the acid waters would be increased. Additionally, working with a stirred tank reactor as a step prior to the biosorption treatment of the waters, chemical precipitation of the metals was carried out, pH being increased from 3.4 to 6.4 by controlled addition of NaOH. After removing the large mass of sludge generated (basic hydroxides and sulfates of the contaminating metals), a small volume of concentrated H_2SO_4 (18 M) was added to 2.00 dm^3 of the remaining water (almost free of metals) in order to reduce its pH to 3.2.

Once the water had been prepared, 34.17 g of powdered chitin were incubated under stirring conditions, and at each preset time 5 cm^3 aliquot samples were collected with syringes fitted with Millipore microfilters (Millex-Gs sterile 0.22 μm) in order to prevent the entry of chitin powder into the samples.

As a result of this chemical precipitation, the concentrations of all the metals -except U - were lowered in the supernatant water to minimum or undetectable values. In the case of uranium, 60% biosorption (0.35 mg U g^{-1} biosorbent) was observed. The biosorption kinetics of U fitted a first-order integrated rate equation with respect to the concentration of the metal of the type $[Metal]_{solution} = Ae^{-kt} + C$, with the following kinetic parameters: k = 21.68 h^{-1}, $t_{1/2}$ = 1.92 min, A = 5.89 ppm, C = 3.82 ppm, equilibrium being reached in 0.5 h (Table **1**).

3.5.3. Desorption of Metals Biosorbed from Chitin with 0.4 M Na_2CO_3

As in the case of keratin from pig bristles, we checked whether there would be a process of desorption of the metals sorbed onto the remains of chitin packed in the reactor by the action of the eluent (sodium carbonate) at 0.4 M. To perform this desorption, 600 cm^3 of metal-free eluent at 25 ºC (pH = 9.9) was placed in contact with the mass (113.5 g) of the metal-loaded biosorbent (from the previous experiment), taking 10 cm^3 aliquot samples of solution at preset times. To these, 10 cm^3 of 0.01 M H_2SO_4 was added immediately, after which the concentration of the different metals in each solution removed was determined.

A partial desorption of Fe (0.0087 mg g^{-1} chitin), Ni (0.0085 mg g^{-1} chitin), Mn (0.0066 mg g^{-1} chitin) and Cu (0.0022 mg g^{-1}chitin) from the biosorbent to the 600 cm^3 of eluent solution was observed after 34 hours of dynamic incubation.

Since the wet biosorbent represents the same mass of biosorbent as that used in the adsorption experiment, the percentages of desorption due to the action of 0.4 M sodium carbonate were 0.0085 mg Ni/0.04 mg Ni (= 21.3 %); 0.0022 mg Cu/0.03 mg Cu (= 7.3 %), and 0.0066 mg Mn/0.49 mg Mn (= 1.35 %). That is, the efficiency of the 0.4 M Na_2C0_3 aqueous solution as an eluent should be questioned.

At the incubation pH (9.85-9.63), all the metals should have precipitated as hydroxides, such that it should have been possible to note a decrease in their concentration in solution with time (as was the case for Al, Fe and Zn). However, considering the high ionic strength of the sodium carbonate solution it could be speculated that this saline effect, which increases the solubility of such hydroxides, would allow the desorption process for Fe, Mn, Cu and Ni. In the case of the other metals, the saline effect would not be sufficiently intense to be able to maintain the corresponding metal hydroxides in solution.

3.6. Biosorption of Metals from Acid Mine Drainages by Regenerated Chitin

In the next experiments, 106.39 g of wet chitin that had already undergone a complete sorption/desorption cycle was used to explore the biosorption of metals from the acid waters of the *Faith* mine (900 cm^3) at pH 3.8, 25º C. The following percentages of metal biosorbed on the reused (regenerated) chitin residues were observed: 100% Cu (0.0068 mg Cu g^{-1} biosorbent), 74.3% Zn (0.096 mg Zn g^{-1} biosorbent), 50.9% Ni (0.021 mg Ni g^{-1} biosorbent) y 24.1% Mn (0.20 mg Mn g^{-1} biosorbent).

The biosorption kinetics of all the metals fitted a first-order integrated rate equation with respect to the concentration of metal of the type $[Metal]_{solution} = Ae^{-kt} + C$, with the following kinetic parameters: Mn (k = 0.59 h^{-1}, $t_{1/2}$ = 1.17 h, A = 17.3 ppm, C = 75.7 ppm), Ni (k = 0.29 h^{-1}, $t_{1/2}$ = 2.39 h, A = 2.03 ppm, C = 2.61 ppm), Zn (k = 0.29 h^{-1}, $t_{1/2}$ = 2.39 h, A = 10.0 ppm, C = 4.78 ppm)

and Cu (k = 0.25 h^{-1}, $t_{1/2}$ = 2.77 h, A = 0.83 ppm, C = 0 ppm), equilibrium being reached at 24 h in all cases (Table **1**).

On comparing these values with the biosorption parameters of metals onto native chitin, higher percentages of elimination of metal dissolved in acid waters and faster biosorption kinetics are seen for re-used or regenerated chitin.

4. CONCLUSION

From study of the different biowastes assayed in the present work, it may be concluded (Table **1**) that the biowaste rich in keratin (pig bristles) seems to show a slightly higher biosorption capacity than that of bioresidues rich in chitin (crustacean shells). Moreover, factors such as the lower contamination by metals of acid waters, the lower influent water volume/biosorbent mass ratio, the configuration of the packed-bed reactor and the partial hydrolysis of keratin increase both the capacity and the rate of the process of metal biosorption onto the biosorbent.

Since the technology currently used for the decontamination of metals from industrial effluents seems, at least in most cases, to be inadequate and expensive, often generating large amounts of metal-loaded sludges (which must be suitably treated or stored), biosorption seems to offer an appropriate and cheap technology, mainly due to the abundance, yield, regeneration capacity, and minimum processing required of a whole series of biosorbents generated in large amounts as industrial wastes. We thus propose the possibility of using biosorption as a secondary treatment after other types of processes as a final polishing step to further reduce the concentration of more toxic metals from mg/l (ppm) to μg/l (ppb) levels.

ACKNOWLEDGEMENTS

The authors would like to thank the Iberdrola Instituto Tecnológico and the Water Research and Development Centre (CIDTA) of the University of Salamanca for funding. They also acknowledge the excellent collaboration of the Department of Analytical Chemistry, Nutrition and Food Science of the University of Salamanca for allowing the use of atomic absorption spectrophotometers; the collaboration of the companies that provided the acid waters and biowastes used in this work - Empresa Nacional del Uranio S.A. (ENUSA) (Saelices el Chico, Salamanca), Merladet S.A. (Barruecopardo, Salamanca), Grupo Cruz Campo S.A. Finally, they thank Prof. William G. Bardsley from the University of Manchester (UK) for free use of the last version of SIMFIT software.

REFERENCES

[1] Roig MG. The biological rehabilitation of metal bearing wastewaters. In: European Commission ECSC-EC-EAEC (Ed.), Technologies for Environmental Protection. Luxembourg: European Commission 1995; 203-209.

[2] Roig MG, Manzano T, Diaz M, Pascual MJ, Paterson M, Kennedy JF. Enzymically-enhanced extraction of uranium from biologically leached solutions. Int Biodeterioration Biodegradation 1995; 95: 93-127. http://dx.doi.org/10.1016/0964-8305(95)00052-7

[3] Roig MG. Biosorption and bioaccumulation of heavy metals. In: TEMPUS PHARE European Program (FP4) (Ed.), Environmental Biochemistry and Biotechnology. Prague: European Commission 1996; 60-68.

[4] Roig MG, Manzano T, Diaz M. Biochemical process for the removal of uranium from acid mine drainages. Water Res 1997; 31: 2073-83. http://dx.doi.org/10.1016/S0043-1354(97)00036-5

[5] Macaskie LE, Yong P, Doyle TC, Roig MG, Diaz M, Manzano T. Bioremediation of uranium-bearing wastewater: biochemical and chemical factors influencing bioprocess application. Biotechnol Bioeng 1997; 53: 100-109. http://dx.doi.org/10.1002/(SICI)1097-0290(19970105)53:1<100::AID-BIT13>3.0.CO;2-S

[6] Allan RJ. Impact of mining activities on the terrestrial and aquatic environments with emphasis on mitigation and remedial resources. In: W. Salomons, U. Förstner and P. Mader (Eds.) Heavy Metals: problems and solutions, Berlin: Springer-Verlag 1195; 119-140.

[7] Harm F, Rumpler A. In 5th. Int. Congr. Pure and Applied Chemistry; 1903; 59.

[8] Staudinger H. Highly polymeric bonds 5 Report - On the constitution of poly-oxymethylene and other highly polymeric bonds. Helv Chimica Acta 1925; 8: 67-74. http://dx.doi.org/10.1002/hlca.19250080113

[9] Adams BA, Holmes EL. Absorptive properties of synthetic resins. J Soc Chem Industry 1935; 54: 1-6.

[10] Kullgren C. Vyuiiti derivcitl celuldzy pii ionexovk chromatografii kovoych ionti. Svensk Kemisk Tidskrift 1931; 43: 99-104.

[11] Kumar MNVR, Muzzarelli RAA, Muzzarelli C, Sashwa H, Domb AJ. Chitosan chemistry and pharmaceutical perspectives. Chem Rev 2004; 104: 6017-6084. http://dx.doi.org/10.1021/cr030441b

[12] Michelsen DL, Gideon JA, Griffith GP, Page JE, Kutat HL. Removal of soluble mercury from waste water by complexing techniques. Virginia Water Resources Research Center Bull 1975; 74: 1-9.

[13] Ishikawa S, Suyama K. Recovery and refining of Au by gold-cyanide ion biosorption using animal fibrous proteins. Appl Biochem Biotechnol 1998; 70: 719-28. http://dx.doi.org/10.1007/BF02920183

[14] Luong HV, Brown EJ. Removal of arsenic from contaminated water with partially deacetylated shellfish waste. National Technical Information Service PB85-214716/AS 1984; 22: 161-68.

[15] Chui VWD, Mok KWNg, Luong BP, Ma KK. Removal and recovery of copper (II), chromium (III), and nickel (II) from solutions using crude shrimp chitin packed in small columns. Environment Int 1996; 22: 463-68. http://dx.doi.org/10.1016/0160-4120(96)00034-7

[16] McKay G. Mass transfer processes during the adsorption of solutes in aqueous solutions in batch and fixed bed adsorbers. Chem Eng Res Design 1984; 62: 135-43.

[17] Knorr D. Recovery and utilization of chitin and chitosan in food processing waste management. Food Technol 1991; 45: 114-22.

[18] Coughlin RW, Deshaies MR, Davis EM. Chitosan in crab shell wastes purifies electroplating wastewater. Environ Progress 1990; 9: 35-39. http://dx.doi.org/10.1002/ep.670090116

[19] van Daele Y, Thome JP. Purification of PCB contaminated water by Chitosan: A biological test of efficiency using the common Barbel, *Barbus barbus*. Bull Environ Contam Toxicol 1986; 37: 858-65. http://dx.doi.org/10.1007/BF01607850

[20] Onsoeyen E, Skaugrud O. Metal recovery using chitosan. J Chem Technol Biotechnol 1990; 49: 395-404. http://dx.doi.org/10.1002/jctb.280490410

[21] Piron E, Domard A. Interaction between chitosan and uranyl ions. Part 1. Role of physicochemical parameters. Int J Biol Macromol 1997; 21: 327-35. http://dx.doi.org/10.1016/S0141-8130(97)00081-0

[22] Salah-Azab M, Peterson PJ. Removal of cadmium from water by the use of biological sorbents. Water Sci Technol 1989; 21: 1705-706.

[23] Niu CH, Volesky B. Characteristics of anionic metal species biosorption with waste crab shells**.** Hydrometallurgy 2003; 71: 209-15. http://dx.doi.org/10.1016/S0304-386X(03)00158-0

[24] Niu CH, Volesky B. Biosorption of chromate and vanadate species with waste crab shells. Hydrometallurgy 2006; 84: 28-36. http://dx.doi.org/10.1016/j.hydromet.2006.03.057

[25] Niu CH, Volesky B, Cleiman D. Biosorption of arsenic (V) with acid-washed crab shells. Water Res 2007; 41: 2473-78. http://dx.doi.org/10.1016/j.watres.2007.03.013

[26] APHA, AWWA, WPCF. In: Clesceri LC, Greenberg AE, Trussell RR, Franson MAH, Eds. Standard Methods for the examination of water and wastewater. Washington: American Public Health Association 1989; 275-279.

[27] Moron MC, Garcia-Tenorio R, Montaño E, Garcia León M, Madruga G. An easy method for the determination of Radium isotopes and Actinide alpha emitters from the same water sample. Appl Radiation Isotopes 1986; 37: 383-89. http://dx.doi.org/10.1016/0883-2889(86)90093-6

[28] Sill CW. Determination of ^{226}Ra in ores, nuclear wastes and environmental samples by high resolution Alpha spectrometry. Nuclear Chem Waste Management 1987; 7: 239-56. http://dx.doi.org/10.1016/0191-815X(87)90069-6

[29] Savvin SB. Analytical use of Arsenazo III. Determination of Thorium, Zirconium, Uranium and rare earth elements. Talanta 1961; 8: 673-85. http://dx.doi.org/10.1016/0039-9140(61)80164-1

[30] Perez-Pavón JL, Moreno-Cordero B, Rodriguez-Garcia E, Hernandez-Mendez J. Determination of uranium using a flow system with reagent injection. Application to the determination of uranium in ore leachates. Anal Chim Acta 1990; 230: 217-20. http://dx.doi.org/10.1016/S0003-2670(00)82786-5

Received on 19-07-2013 Accepted on 12-08-2013 Published on 27-08-2013

http://dx.doi.org/10.6000/1927-5129.2013.09.66