OPTIMIZATION OF THE PRODUCTION OF CHITIN FROM TENEBRIO MOLITOR AND ITS APPLICATION FOR BIOSORPTION OF METALSFROM INDUSTRIAL WASTEWATERS

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Abstract - The optimization of the extraction process ofthe high content of chitin from the exoskeletons of Tenebrio molitorL. beetles has been developed. Once extracted, chitin has been characterized physicochemically and spectrally (FTIR, X-ray difraction). Finally, the bioadsorption of metals from industrial wastewater by the action of the previously obtained chitin was studied, providing data that demonstrate that the biosorption of metals by this chitin can represent an alternative, low cost and competitive technology for the removal/recovery of metals from different industrial wastewaters.

Keywords - Chitin, Tenebrio Molitor, Extraction, Metals, Biosorption, Kinetics, FTIR, X-ray Spectra.

I. INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) has identified edible insectssuch as mealworms, crickets, and grasshoppers as a potential future food resource, which may alleviate food shortage and delay global warming [1].The number of speciesappropriate for human consumption is reported to beapproximately two thousand species worldwide [2], from which the chemical composition of 236 specieshas been published [3]. Commonmealworm, Tenebrio molitor L.(Coleoptera:Tenebrionidae) is commercially produced as edible insect product. Common mealworm is among the most economical species for the production of protein-rich food and animal feed. The nutritional composition of edible mealworm larvae is protein (47–49%), fat (38–43%), fiber (6–7%), nitrogen-free extract (0.26%), and ash (2–3%) based on dry matter [4], [5].

Chitin (poly (β-(1→4)-N-acetyl-D-glucosamine) isan uncharged polysaccharide of animal origin, with a strong metal-binding potential due to its N-acetyl and hydroxyl groups, which constitutes approximately 80% of the exoskeleton of arthropods compared to a composition slightly lower than 13% in the exoskeleton of crustaceans [6]. Of all animal polysaccharides, only chitin is found in sufficient abundance and can be easily isolated to allow commercial exploitation.

The current market for chitin involves obtaining it from the byproducts of crustacean industries, leading to expensive purification processes to obtain high purity chitins as a consequence of its degradation due to unhygienic conditions and the transport processes inherent in the management of this type of by-products. However, the development of the industrial production of the insect Tenebrio molitor provides the opportunity to obtain this polysaccharide from the adult beetle, after the exhaustion of sexual activity in its reproductive cycle, being obtained at the time of sacrifice and minimizing, thus, the effects derived from this degradation.

The emerging and innovative phenomenon of insect breeding, which currently arises as a result of the search for solutions to the global protein deficit as a result of the growth of the world population, provides the possibility of obtaining subsidiary large amounts of chitin, which, on the other hand, can provide commercial profit to adult beetles once their reproductive function has been fulfilled. This subsidiary use of the chitin of adult insects in different applications will drastically reduce the amount of beetles as by-products or waste of difficult management.

The current situation of the terrestrial and aquatic ecosystems presents great problems derived from the industrial development, finding numerous aquifers and superficial waters with high concentrations of metals as consequence of exploitations as the mining or other types of industries that generate large amounts of metallized wastewaters, such as acid mine and other drainages .

In this field, biopolymers of natural origin, such as chitin/chitosan from industrial byproducts of the American crab, are able of removing/recovering metals from acid mine drainages [7], [8].

Currently, as a consequence of the development of the industrial production of insects for its introduction into the food chain, new viable alternatives for the production of high purity chitin with a clear mid-term projection in all types of applications are emerging.

The optimization of the processes to obtain chitin from insects such as Tenebrio molitor(Tm) and the evaluation of the capacity of the chitin obtained as a metal bioadsorbent in wastewater treatment, would provide an integral solution to waste management and to the improvement of heavy metal removal processes in industrial wastewaters, aquifers and surface waters.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Commercial chitin was purchased from Merck-Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The samples of the adult of Mealworm Beetle (Tenebrio molitor L.) were donated by the company Meal Food Europe (Doñinos de Salamanca, Spain).
These Tm beetles after beingwashed 2–3 times in distilled water, were dried and kept clean in the refrigerator at 4°C.

Reagents hydrochloric acid, sodium hydroxide (Panreac, Barcelona, Spain), inorganic salts(Merck-Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and Milli-Q ultra-pure water (Millipore Spain, Madrid) were obtained from the sources indicated. All chemicals were analytical or higher grade.

2.2. Physicochemical characterization of Tm beetle

An initial characterization of the raw material was carried out, being measured the moisture (stove at 105ºC ± 3ºC for 24h), ash (muffle at 550ºC ± 5ºC for 2h)and metal (by ICP-OES and ICP-MS of the ashes obtained after calcination at 550ºC and microwave digestión) contents in the untreated beetle were determined.

2.3. Extraction of chitinfrom Tenebrio molitor

Treatments for extracting chitin from Tm exoskeletons were: 1) Deproteinization (DP) with 2 M NaOH for 24 hours. The mass (after removing soluble protein in alkaline medium) is reduced up to 41.0%. 2) Demineralization and deproteinization (DMDP) (2M HCl for 24 hours, washings until pH neutralization and 2 M NaOH for 24 hours). The mass (after removing soluble mineral and protein components) is reduced up to 31.6%. 3) Deproteinization and demineralization (DPDM) (2M NaOH for 24 hours, washings until pH neutralization and 2 M HCl for 24 hours). In the three cases, the remaining chitin was triturated (T) and sieved at > 0.8 mm (S).

2.4. Spectral characterization of extracted chitin

To perform the infrared spectra of chitin, a spectrophotometer Perkin Elmer Spectrum One, coupled to a Total Attenuated Reflectance (ATR) equipment, from the Department of Inorganic Chemistry of the University of Salamanca (Spain)was used. The scans were performed from 350 to 4000 cm^{-1} wavenumber with a resolution of 4 cm^{-1},using the KBr pellet technique, 100 scans average to improve the signal/noise ratio.

An automatic Philips X'pert MPD diffractometer from the department of Tissue Engineering of the Institute of Biofunctional Studies, Complutense University of Madrid (Spain), was also used. The equipment has a goniometer PW3050 (-2) and the generator power was set at 45kV and 40mA. The measurements were made at room temperature with CuK 1 radiation (wavelength 1.54056Å) with graphite monochromator and in confocalized geometry (Bragg-Berntano). The size and duration of step (2θ) of the measurements were set at 0.040 ° and in 1 second. The angular range studied was 5° - 40° (2θ).
FTIR and X-ray diffraction spectra of Tm exoskeleton samples after DMDPTS processes and of commercial samples of chitin from shrimp shells (Merck-Sigma-Aldrich, ref. C9213) were obtained.

2.5. Performance and kinetics of metals biosorption by the extracted chitin

The performance and kinetics of bioadsorption of soluble metals from synthetic and real wastewaters by the chitin extracted from Tenebrio molitor has been studied at pH 5.0 in batch and in packed bed bioreactors. At the end of the kinetic essays, the bioadsorbed metals per gram of each chitin sample (Tenebrio exoskeleton without pretreatment, after DMDPTS and after DPTS) were analyzed in the ashes of each calcined sample of biosorbent.

2.6. Statistical Fitting of Data

The fitting of the kinetic and equilibrium data on biosorption to different equations and models was doneusing the SIMFIT statistical package, authored by Prof.W.G. Bardsley of the University of Manchester (UK)(http://simfit.usal.es/).

III. RESULTS AND DISCUSSION

3.1. Physicochemical composition of Tenebrio molitor beetles.

The Tm beetles supplied by Mealfood Europe are characterized by being exo-skeletons in good condition, clean, whole and dry. With them, initial analyzes were carried out to know their composition (Table 1). The found concentrations of endogenous metals of Tenebrio molitor in their adult stage coincide with the bibliographic data [9].

Humidity (%) 16; Ashes (%) 4.1; K (mg/kg) 7160; Mg (mg/kg) 1710; Na (mg/kg) 1150; Ca (mg/kg) 625; Fe (mg/kg) 109; Zn (mg/kg) 68; Cu (mg/kg) 21; Mn (mg/kg) 11; Sr (mg/kg) 1.6; Ba (mg/kg) 0.7; Ni (µg/kg) 3.5; Pb (µg/kg) 0.7; Cd (µg/kg) 0.1

Table 1: Physicochemical composition of Tenebrio molitor beetles.

3.2. Extraction of chitinfrom Tenebrio molitor

According to literature, the extraction of chitin from other sources of chitin extraction in different raw materials has to be carried out in two clearly differentiated stages: a) Demineralization process (DM) in acid medium. b) Deproteinization process (DP) in alkaline médium [10], [11]. After studying the order of application of the two stages in the processes of extraction of chitinfrom the exoskeletons of Tm, ie only deproteinization (DP), demineralization and deproteinization (DMDP) and deproteinization and demineralization (DPDM), it was concluded that the higher percent in chitin, 33%, was obtained after DMDP treatment, percentage higher than the 18.2-25.2%.reflected in [12]. The mass (after removing protein and soluble mineral components by DMDP treatment) is reduced up to 30.0% (Fig. 1).



Fig. 1. Chitin obtained from Tm beetles before (left) and after DMDP treatments (right).

3.3. Spectral characterization of extracted chitin

The chitin obtained after DMDP treatment of Tm exoskeletons was characterized by FTIR spectroscopy , its spectrum being compared with that of a commercial purified chitin obtained from shrimp shells (Merck-Sigma Aldrich, ref. C9213) (Fig. 2).

The vibration bands at 3250 and 3400 cm^{-1} corresponding to the -OH and -NH groups respectively, characteristic of alcohols, amines and amides present in the structure of chitosan, can be tested in all spectra. The tensión bands at 2850 and 2930 cm^{-1} belong to the methyl and / or methylene, at 1651 cm^{-1} appear tension bands corresponding to amide of type I, at 1577 cm^{-1} to amide of type II and at 1377 cm^{-1} the bands of $-CH_2$. Next, the characteristic bands of the polysaccharides are distinguished (at 1050 cm^{-1} of primary alcohol and at 1077 cm^{-1} of secondary alcohol).

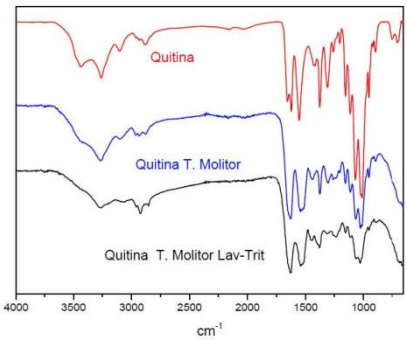


Fig. 2. FTIR spectra of three samples of chitin, two from Tenebrio molitor exoskeletons (in blue) and one from shrimp shells (Merck-Sigma Aldrich, reference C9213)(in red).

The same samples of chitin from Tenebrio molitor and the commercial chitin from Sigma Chemical Co. (as control) were subjected to X-ray diffraction, obtaining the spectra corresponding to chitin (Fig. 3), showing the homologous two crystalline peaks at approximately 2□ = 9.0 and 19.4 characteristic of chitin as a substantially crystalline substance.In this way, it was verified that the degree of purity of chitin obtained from Tenebrio molitor exoskeleton by the DMDP procedure is similar to that of Sigma's commercial chitin.

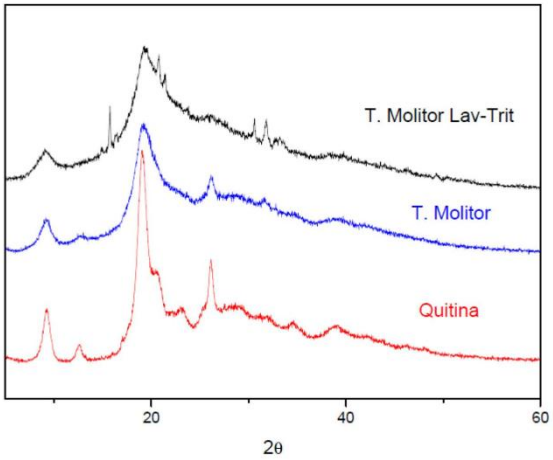


Fig. 3. X-ray diffraction spectra ofthree samples of chitin, two from Tenebrio molitor exoskeletons (in blue) and one from shrimp shells (Merck-Sigma Aldrich, reference C9213)(in red).

3.4. Removal kinetics of 6 heavy metals from synthetic waters by biosorption on DMDP chitin from Tenebrio molitor

The performance and kinetics of removal of heavy metals from an aqueous solution, pH 5.0,doped with 23 ppm Cd, 23 ppm Cu, 21 ppm Ni, 10 ppm Pb, 21 ppm U and 21 ppm Zn, were studied in batch reactors, at 25 ºC, with gentle dynamic contact between the solution and chitin extracted from Tenebrio molitor and trapped in a porous nylon bag.

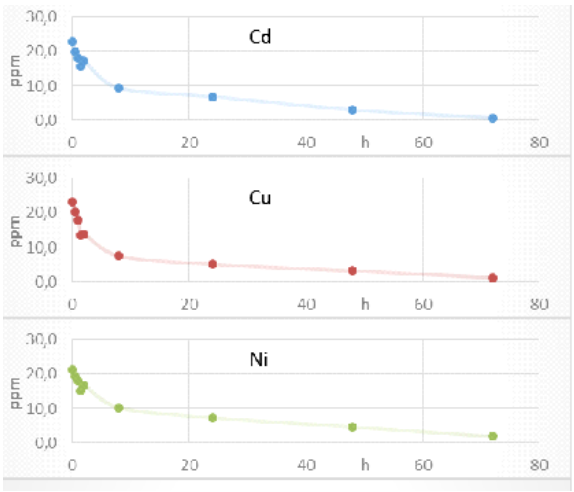
Firstly, to verify the foreseeable remarkable effect of particle size in the biosorption processes, metal biosorption tests were performed for 72 h with the intact exoskeletons of the beetles (Tm) and with triturared and sieved exoskeletons (TmTS). Furthermore, other four essays (DP, DPDM, DMDP, Chit*) for 72 h, with chitin triturated (T) and sieved at > 0.8 mm (S), were carried out to conclude which chitin extraction method produces the best metal biosorption results (Table 2).

	Cd	Cu	Ni	Pb	U	Zn
Tm	29	100	34	53		30
TmTS	61	56	36	33	63	59
DP	84	73	78	66	66	79
DPDM	0	52	0	15	76	0
DMDP	100	93	100	100	87	100
Chit*	8	78	5	88	97	5

Table 2. Performance (% metal removed) of biosorption of metals from a metalmulticomponent synthetic wastewaterby chitin from Tenebrio molitor beetles and shrimp shells before (Tm) and after being triturated and sieved (TmTS), after being deproteinized (DP), after being deproteinized and demineralized (DPDM), after being demineralized and deproteinized (DMDP). Chit* is thecommercial purified chitin from shrimp shells (Merck-Sigma Aldrich, ref C9213)

From Table 2, it was observed that the capacity to remove metals from water increases when increasing the surface area available for biosorption (Tm→TmTS) and that the efficiency of bioadsorption per TmTS follows the series U>Cd>Zn>Cu>Ni>Pb.Furthermore, the best results (87-100% metal removed) were achieved after DMDP (TS) treatment of the Tenebrio molitor exoskeletons.

The kinetics of bioadsorption of the six metals from multicomponent synthetic wastewater (23 ppm Cd, 23 ppm Cu, 21 ppm Ni, 10 ppm Pb, 21 ppm U, 21 ppm Zn) during 72 h of dynamic incubation at pH 5.0 with chitin from exoskeletons of Tenebrio molitor after the DMDPTS treatment were fitted to a biphasic first-order process with respect to the concentration of metal, with an integrated rate equation [Metal in solution] = $A_1e^{-k}{}_1t + A_2e^{-k}{}_2t$ (Fig. 4), being $k_i$and $t_{1/2}$in the following ranges:k_1 0.43 – 1.00 h^{-1}, $t_{1/2}$ 1.61 - 0.69 h for Zn and U, respectively and k_2 $5.71.10^{-3}$ – $3.02.10^{-2}$ h^{-1} for k_2, $t_{1/2}$ 121.4 – 22.9 h for U and Cd, respectively. With respect to the selectivity of the biosorbent for the different metals and their capacity to be biosorbed, the highest metal removal percentages were obtained for Cd (97.4%), Cu (94.8%), Zn (94.8%) , U (92.8%).), Pb (91.8%) and Ni (91.6%). It highlights the rapid kinetics of elimination of U from wastewater, decreasing its concentration to half of the initial one in 1 hour. For all the metals studied, its removal from the water is almost complete at 24-48 h.



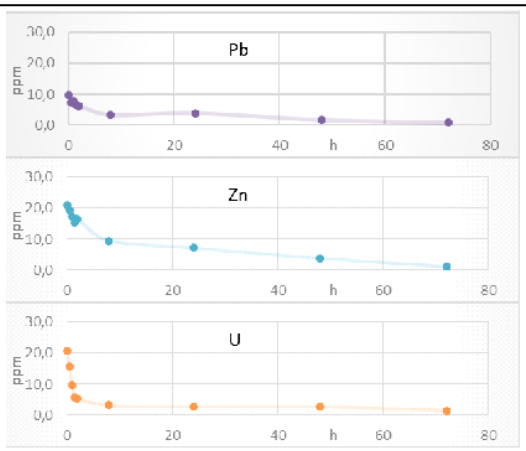


Fig. 4. Kinetics of bioadsorption of metals from synthetic wastewater doped with 6 metals (10-23 mg /L each metal) over 72 h of dynamic contact at pH 5.0 with DMDPTS chitin from Tenebrio molitor exoskeletons.

Complementing this study, the biosorbent samples,after 72 hours of biosorption runs, were calcined in muffle at 550ºC and in their corresponding ashes the amount of metal adsorbed per g of chitin used in each experiment were analyzed by ICP-OES. The results obtained forDMDP (TS) ranged between 0.8 mg Pb and 1.3 mg Cu per gram of the bioadsorbent, being 7.0 mg the global amount of metals per gram of bioadsorbent.

The kinetics of bioadsorption of metals (31 ppm Fe, 28 ppm Zn, 1.1 ppm Cu, 1.1 ppm Cr, 423 ppb Mn, 237 ppb Pb, 55 ppb Ni) from wastewater supplied by the galvanizing industry Goñabe SL (Valladolid, Spain) and carried out with exoskeletons of Tenebrio molitor after DMDPTS treatment, in batch, at pH 5.0 for 72 h, show an efficient removal of metals by bioadsorption for Cu (100%), Fe (90%), Cr (87%) and Ni (34%) but not for Zn, Mn and Pb (possibly due to its high concentration of chlorides up to 28 g/L). The biosorption kinetics of the metal were well fitted to a biphasic first-order process with respect to the concentration of metal, with an integrated rate equation [Metal in solution] = $A_1e^{-k}{}_1t + A_2e^{-k}{}_2t$,being $k_i$and $t_{1/2}$in the following ranges:k_1 2.77 – 52.8 h^{-1}, $t_{1/2}$ 0.25 h - 0.79 min for Ni and Fe, respectively and k_2 $4.09.10^{-3}$ – $8.38.10^{-2}$ h^{-1} for k_2, $t_{1/2}$ 169.4 – 8.27 h for Ni and Cu, respectively.

In these two cases, synthetic and industrial wastewaters, a rapid process of biosorption of the metals onto sites class 1 on the surface of chitin 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 on the surface of chitin with lower affinity for the metal orless accessible to the water (mass transfer limitations). With respect to the selectivity of the biosorbent for the different metals and their capacity to be biosorbed, the highest metal removal percentages were obtained forCu (100%), Fe

(89.9%), Cr (86.4%) and Ni (34.4%). For these metals, its removal from the water is almost complete at 24-48 h.

IV. CONCLUSIONS

After a simple process of demineralization (DM), deproteinization (DP) and increase of the surface area (TS) of exoskeletons of Tenebrio molitor L. beetles, the degree of purity of the obtained chitin is equal to the commercial chitin C9213 from Merck-Sigma Aldrich.

The chitin obtained after DMPDTS treatment on the exoskeletons of Tenebrio molitor L. beetles has a high biosorption performance to remove soluble metals from syntheticwastewaters (91.6 % Ni - 97.4% Cd) and industrial (34% Ni - 100% Cu).

As the specific area of this chitin increases, the performance of metals bioadsorption increases.
The kinetics of metal biosorption on chitin from T. molitor were well fitted to a biphasic first-order process with an integrated rate equation [Metal in solution] = $A_1e^{-k}{}_1t + A_2e^{-k}{}_2t$.

The above conclusions suggest that the biosorption of metals by chitin obtained from Tenebrio molitor L. beetles, provided by the company MealFood Europe S.L., can be a simple, alternative and competitive low-cost technology for the removal/recovery of soluble metals from industrial wastewaters.

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