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(54) Title: BIOSORPTION SYSTEM PRODUCED FROM BIOFILMS SUPPORTED ON FAUJASITE (FAU) ZEOLITE, PROCESS OBTAINING IT AND ITS USAGE FOR REMOVAL OF HEXAVALENT CHROMIUM (CR (VI))

(57) Abstract: The present invention refers to a biosorption system composed of a bacterial biofilm supported in synthetic zeolites, for usage in various types of industry for the removal of hexavalent chromium, through the retention of metal ions in the biofilm, in solutions with concentrations between 50 and 250 mg_{Cr}/L, process for obtaining it and respective usages. This process consists in obtaining a bacterial biofilm of *Arthrobacter viscosus*, supported on a faujasite (FAU) zeolite. The biofilm promotes the reduction of Cr (VI) to Cr (III) and, subsequently, Cr (III) is fixed in the zeolite by ion exchange. Several characterization procedures, like spectroscopic techniques (FTIR and ICP-AES), surface analysis (XRD and SEM) and thermal analysis (TGA) reveal that the biosorption process does not modify the morphology or the structure of the FAU zeolite. The biosorption system, and respective fixation process of hexavalent chromium in faujasite (FAU) zeolites, may be applicable to the treatment of industrial, mining or agriculture wastewater, for hexavalent chromium removal.

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Description

BIOSORPTION SYSTEM PRODUCED FROM BIOFILMS SUPPORTED ON FAUJASITE (FAU) ZEOLITE, PROCESS OBTAINING IT AND ITS USAGE FOR REMOVAL OF HEXAVALENT CHROMIUM (Cr (VI))

FIELD OF THE INVENTION

[1] The present invention is included within the domain of liquid effluents treatment, like for example the treatment of industrial, mining or agriculture wastewater by means of the removal of hexavalent chromium through its fixation in a faujasite zeolite.

SUMMARY OF THE INVENTION

[2] The present invention refers to a biosorption system composed of a bacterial biofilm supported in synthetic zeolites, for usage in various types of industry for the removal of hexavalent chromium, through the retention of metal ions in the biofilm, in solutions with concentrations between 50 and 250 mg_{Cr}/L, process for obtaining it and respective usages.

[3] This process consists in obtaining a bacterial biofilm of *Arthrobacter viscosus*, supported on a faujasite (FAU) zeolite.

[4] The biofilm promotes the reduction of Cr (VI) to Cr (III) and, subsequently, Cr (III) is fixed in the zeolite by ion exchange.

[5] Several characterization procedures like spectroscopic techniques (FTIR and ICP-AES), surface analysis (XRD and SEM) and thermal analysis (TGA) reveal that the biosorption process does not modify the morphology or the structure of the FAU zeolite.

[6] This process defines a low cost technology, appropriate for locally very active small companies producing liquid effluents with low heavy metal concentrations.

BACKGROUND OF THE INVENTION

[7] Nowadays, pollution control is one of the major concerns of the scientific and environmentalist communities. The main pollutants are the gases responsible for greenhouse effect, heavy metals, organic and inorganic effluents, and volatile organic compounds, VOC's. Chromium (Cr), in particular, is a toxic pollutant present in wastewater of many industries, as for example metallurgies, mining installations and tanning facilities. The soils are contaminated and, as a consequence, water sources and groundwater. There are numerous processes for removal of heavy metals in liquid solution, as for example chemical precipitation, ion exchange, membrane filtration, reverse osmosis and activated carbon adsorption [1]. These processes are quite expensive and not really efficient to be applied to low concentration solutions, so that

small industries, with tight budgets, may have some difficulties in the accomplishment of environmental legislation regarding the emission of liquids.

[8] Activated carbon adsorption is considered a competitive and efficient process for heavy metal removal in low concentrations. Nevertheless, the utilization of activated carbon is quite expensive and alternative sorbents and technologies are needed [2, 3]. In this perspective, the zeolites present a great potential for heavy metals removal from industrial wastewater. The zeolites are composed of SiO_4 and AlO_4 tetrahedrons bond by the oxygen atoms of the vertexes. The existence of a negative structural charge, due to the AlO_4^{5-} tetrahedrons, promotes a strong affinity for metal ions, defining the sorbent properties to these supports. Sodium, potassium and other positively charged exchangeable ions occupy the defined channels within the three-dimensional structure and can be replaced by heavy metals [4].

[9] Several research works presented in the last years, refer the utilization of natural zeolites in effluent treatment. The removal of heavy metals from wastewater was studied, using clinoptilolite, the most abundant natural zeolite. The results for ion exchanged ranged between 1.6 mg/g for Pb^{2+} and 0.0 mg/g for Cr^{3+} [5]. This issue is complicated when the ion is Cr (VI), as it appears in chromate or dichromate form, i.e. negatively charged and with a high characteristic dimension, as it is normally quite hydrated. One way to solve the problem of Cr^{6+} removal in solution, is the combination of bacterial biosorption with the ion exchange ability of the zeolite.

[10] Biosorption is the fixation of metals through biological materials without active uptake and can be considered as a collective term for a number of passive accumulation processes, which may include ion exchange, coordination, complexation, adsorption and microprecipitation [6]. Other authors [7] refer that biosorption is the ability of biological materials to accumulate heavy metals from waste streams by either metabolically mediation or by purely physical-chemical uptake pathways.

[11] Bacteria are quite adequate for biosorption due to their ability to fix metal ions, adaptability to natural environments and low cost. *Arthrobacter viscosus* is a good exopolysaccharide producer, which, by itself, would allow foreseeing good qualities for support adhesion and for metal ions entrapment [8]. The system, described herewith, combine the biosorption properties of the microorganism with the characteristics of the zeolites, such as ion exchange ability and shape selectivity.

[12] Among the heavy metals that may be removed from liquid solutions by biosorption, chromium demands special attention due to its several oxidation states. The process herein described allows the removal of chromium from $K_2Cr_2O_7$ liquid solutions with different initial concentrations. A reduction of $Cr_2O_7^{2-}$ is performed by the biofilm. The metabolic reduction has been studied and modeled for different pure bacterial cultures [9]. The *Arthrobacter viscosus* bacterium supported on the zeolite

reduces Cr (VI) to Cr (III) and the Cr (III) is retained in the zeolite by ion exchange.

[13] Recent patents refer the utilization of biosorbents in the removal of heavy metals of effluents, as for example the usage of ceramic substrates covered with chitosan (US2003150802). However, chitosan is not efficient in Cr removal. FAU zeolites have been used as support for adsorption, mainly for separation of compounds in gaseous effluents, like car exhaust (JP2000202282, JP9192486, EP1356862, and US6350428, among others), but their application as a biofilm support for chromium removal is not known. Patent US2004124150, 'Hexavalent chromium removal from aqueous media using ferrous-form zeolite materials' suggest the removal of Cr through the contact with the zeolite, but does not take the biological path and does not offer a solution for the used zeolite. The present invention aims solving this problem, compensating the gaps regarding current solutions for hexavalent chromium removal from effluents.

DESCRIPTION OF THE INVENTION

[14] The present invention proposes a biosorption system consisting of a biofilm of *Arthrobacter viscosus* supported on a FAU zeolite for removing heavy metal ions from current environment pollutants.

[15] The biofilm supported by the FAU promotes the existence of a negative structural charge matrix revealing a strong affinity for metal cations, conferring excellent adsorption properties to these supports. Positively charged exchangeable ions, as for example Na and K, occupy the channels within the three-dimensional structure, which can be replaced by heavy metals.

[16] On the other hand, bacteria are quite adequate for heavy metals biosorption since they possess good capacity for fixing metal ions. The exopolysaccharide producer *Arthrobacter viscosus*, in particular, reveals excellent qualities for support adhesion and for metal ions entrapment, conferring to the biofilm - zeolite system properties, such as ion exchange and shape selectivity.

[17] Therefore, dichromate ions are entrapped by the supported biofilm, starting from $K_2Cr_2O_7$ solutions of low concentration (concentrations between 50 mg_{Cr}/L and 250 mg_{Cr}/L). The zeolite presents a high surface area (500-700 m^2/g^{-1}), but most of it is internal. The novelty of this method resides in the fact that the *Arthrobacter viscosus* bacteria, supported in the zeolite, reduces Cr (VI) to Cr (III), allowing a subsequent fixation of the cation inside the matrix. The resulting structure reveals highly adequate for application in environmental catalysis due to its activity and selectivity.

[18] It is foreseen that this pollution control method may have a highly positive cost effective component, since the final product, that is, the matrix loaded with the metallic ion, presents adequate activity and selectivity for its application in environmental catalysis. In this way, the final product of this process of Cr (VI) removal, may be reintroduced in the industrial process instead of being released in the environment.

Reference is made to the fact that the catalysts promoting the total oxidation of the volatile organic compounds, VOC, are defined in two catagories: transition metals oxides (usually chromium, cobalt, copper, nickel and manganesium) or noble metals (typically platinum or palladium). The high cost of noble metals promotes the interest regarding their substitution by transition metals. Therefore, the final product of the re-mediation process proposed herewith, opposing to the products obtained due pre-cipitation and other classical treatment of effluents, reveals a strong potential for recycling with an ecological applicability.

[19] This process defines a low cost technology appropriate for locally very active small companies, producing liquid effluents with low heavy metal concentrations. These companies have tight budgets and are not especially motivated in treating their wastes with the conventional pollution reducing technology, because of the high cost involved and for not being always efficient when applied to low concentration effluents.

DETAILED DESCRIPTION OF THE INVENTION

[20] The preparation of the biosorption system is carried out in such a way, that the FAU zeolites form a support matrix to the biofilm of *Arthrobacter viscosus*, retaining Cr ions, through inoculation in culture medium containing the mentioned bacteria, in batch experiments, and by adsorption of Cr ions to this support, according to the following:

[21] **1. Preparation of the faujasite (FAU) zeolites**

[22] The zeolites were obtained from W.R. Grace and were, prior to use, calcined at 500 ^{o}C during 8 hours under a dry air stream.

[23] **2. Preparation of the biofilm supported in FAU zeolites**

[24] The preparation of the biosorbent is carried out trough the growing of the *Arthrobacter viscosus* bacteria in presence of pre-treated zeolites.

[25] The *Arthrobacter viscosus* bacteria was obtained from the Spanish Type Culture Collection of the University of Valencia.

[26] For the microorganism growth, a medium with 5 g/L of peptone, 3 g/L of malt extract, 3 g/L of yeast extract and 10 g/L of glucose is prepared, previously sterilized at 120 ^{o}C for 20 min. The Erlenmeyer flasks are kept at 28 ºC, in moderate stirring.

[27] **3. Adsorption tests of Cr in the biofilm - zeolite**

[28] Adsorption tests are carried out, using 1.0 g of the NaY or NaX zeolite with 150 mL of the different dichromate solutions with a concentration between 50 and 250 mg $_{Cr}$/L and 15 mL of *Arthrobacter viscosus*culture medium in 250 mL Erlenmeyer flasks at 28 ºC, in moderate stirring.

[29] Aqueous chromium solutions were prepared by dilution of $K_2Cr_2O_7$ (Aldrich) in distilled water.

[30] The matrix obtained through centrifugation at 5000 rpm, is subsequently calcined

at 500 °C during 6 hours under a dry air stream in order to remove the organic matter of the *Arthrobacter viscosus* bacterium, for a later catalytical utilization.

[31] The biosorption system was tested under conditions of low Cr concentration, samples (1 mL) were systematically taken, and, after centrifugation, analyzed by atomic absorption spectrophotometry (AAS) for metal determination.

[32] The material obtained in this way, after the biosorption process, is identified by the designation Cr_n zeolite, where *n* represents the initial concentration of chromium in the solution to be treated.

[33] The total chemical analysis of the zeolite, after biosorption, quantifies the entrapped metal.

[34] The obtained data show a maximum efficiency of Cr removal of about 20%, realized through the usage of the described biosorption systems.

[35] **Table 1. Chemical analysis of the zeolite samples**

	Si/Al	Cr content (total w %)	Metal quant./ cel. unit
NaY	2.88	—	—
NaX	1.63	—	—
Cr_{100}-NaY	2.88	0.14	0.45
Cr_{100}-NaX	1.63	0.19	0.67

[36] No significant variations were found in the diffraction patterns relatively to the structure of the NaY and NaX zeolites, before and after the biosorption.

[37] Through thermal analysis (TGA) was verified that the biosorption systems of zeolites, containing the biofilm, present the same type of performance than the original support.

[38] Data obtained trough spectroscopy FTIR show that in the systems, spectra are dominated by strong zeolite bands, namely from 3,700-3,300 cm^{-1} and from 1,300-450 cm^{-1}, showing that the structural characteristics of the zeolites remain unaltered.

[39] In conclusion, it is possible to verify that the biofilm of *Arthrobacter viscosus* supported in X or Y zeolites presents a removal capacity of Cr ions contained in weak solutions and, therefore, they are a suitable solution for usage in bio-remediation.

[40] The reduction of Cr (VI) to Cr (III) is promoted by the biofilm, being Cr (III) retained in the zeolite by ion exchange.

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Claims

[1] Biosorption system produced from biofilms supported in faujasite (FAU) zeolites, characterized for being produced from biofilms with the *Arthrobacter viscosus* bacterium supported in faujasite (FAU) zeolites and dichromate solutions.

[2] Process for obtaining the biosorption system according to the previous claim, characterized for making a bacterial culture of *Arthrobacter viscosus* grow in the presence of pre-treated zeolites and for promoting the contact of this biosorbent with a hexavalent chromium solution.

[3] Process for obtaining the biosorption system according to claim 2, characterized for performing a pre-treatment of the FAU zeolites, by their calcination at 500 °C during 6 hours under a dry air stream.

[4] Process for obtaining the biosorption system according to claim 3, characterized for comprising the growth of the *Arthrobacter viscosus* bacterium in the presence of the pre-treated zeolites and in an adjusted and sterilized growth medium at 120°C during 20 min.

[5] Process for obtaining the biosorption system according to claim 3, characterized for comprising the contact of the biosorbents, prepared according to claim 4, with hexavalent chromium solutions with concentrations between 50 and 250 mg_{Cr}/L, prepared from potassium dichromate.

[6] Process for obtaining the biosorption system according to the previous claim, characterized because the contact between the biosorbents is performed at a temperature of 28°C, in medium stirring.

[7] Process for obtaining the biosorption system according to claim 1, characterized because the recovery of the solid matrix is performed by centrifugation at 5000 rpm.

[8] Process for obtaining the biosorption system according to claim 1, characterized because the recovery of the solid matrix, for later catalytic application, is performed by the removal of organic material of the *Arthrobacter viscosus* bacterium, by calcination of the same at 500°C during 6 hours under a dry air stream.

[9] Utilization of the biosorption system produced from biofilms supported in faujasite (FAU) zeolite, according to the previous claims, characterized for being applicable in industrial, mining and agriculture wastewater treatment, for hexavalent chromium removal.

[10] Utilization of the biosorption system produced from biofilms supported in faujasite (FAU) zeolite, according to the previous claim, characterized for

being applicable for hexavalent chromium fixation in a matrix and its subsequent reuse or catalytical utilization.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C02F3/34 C02F3/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C02F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE COMPENDEX [Online] ENGINEERING INFORMATION, INC., NEW YORK, NY, US; LAMEIRAS S ET AL: "Bacterial biofilm supported on granular activated carbon and on natural zeolites- An application to wastewater treatment" XP002414622 Database accession no. E2005389377074 abstract & CHISA INT. CONGR. CHEM. PROCESS ENG.; CHISA 2004 - 16TH INTERNATIONAL CONGRESS OF CHEMICAL AND PROCESS ENGINEERING; CHISA 2004 - 16TH INTERNATIONAL CONGRESS OF CHEMICAL AND PROCESS ENGINEERING 2004, 2004, pages 2929-2931, ----- -/--	1-10

[X] Further documents are listed in the continuation of Box C.

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International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 607 636 A2 (GRACE W R & CO [US]) 27 July 1994 (1994-07-27) abstract page 3, line 5 - line 10 page 4, line 9 - line 10 page 5, line 34 - line 40 page 5, line 50 - page 6, line 5 page 7, line 49 - page 8, line 3 page 8, line 46	
A	GB 2 312 893 A (HOLDING COMPANY BELGIE NV [BE]) 12 November 1997 (1997-11-12) abstract page 4, paragraph 3 - page 5, paragraph 4	
P,X	FIGUEIREDO ET AL: "Oxidation catalysts prepared from biosorbents supported on zeolites" APPLIED CATALYSIS B: ENVIRONMENTAL, ELSEVIER, vol. 66, no. 3-4, 20 July 2006 (2006-07-20), pages 274-280, XP005456290 ISSN: 0926-3373 abstract page 275, right-hand column, paragraph 2 - page 276, left-hand column, paragraph 1 page 280, left-hand column, paragraph 2	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2006/052792

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0607636 A2	27-07-1994	CA 2111398 A1	22-06-1994
		US 5403809 A	04-04-1995
GB 2312893 A	12-11-1997	NONE	