Construction of Inorganic and Hybrid Biosorbents for Heavy Metal Ions Removal

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Abstract: Hybrid biosorbents, synthesized by the sol-gel method on the basis of Tetraethylorthosilicate (TEOS) proved to be efficient for the removal of the heavy metal ions from aqueous solutions. The potential use of immobilized in TEOS algal cells of the red microalga Porphyridium cruentum and its products – low and high molecular heteropolysaccharides to remove Cu(II), Cd(II) and Ni(II) ions was evaluated. A laboratory bioreactor was involved in this process. Sol-gel sorbent with TEOS only was used as a control system to the hybrid biosorbents. Their structural characterization was performed using different methods. The maximum adsorption capacities were registered for the biosorbents with immobilized algal biomass and for the preparations with TEOS and high molecular algal heteropolysaccharide: for Cu(II), Cd(II) and Ni(II), they were 18.771 and 21.715 mg Cd·g^{-1} adsorbent; 16.662 and 17.545 mg Ni·g^{-1} adsorbent and 40.633 and 34.431 mg Cu·g^{-1} adsorbent, respectively. The adsorption of toxic Cu(II), Zn(II) and Ni(II) ions in the four types of sorbents proved to be effective. High percent of these ions removal was obtained during the first hours of the adsorption process.

Keywords: Hybrid biosorbents, Algae, Heteropolysaccharide, Heavy metal ions.

Introduction

Decontamination of heavy metals in water around industrial plants has been a challenge for a long time. Presence of heavy metals even in trace amounts is toxic and detrimental to both flora and fauna. Finding a way for their removal is a serious task nowadays. Several methods have been used for the removal of heavy metal ions from aqueous wastes (chemical precipitation, ion exchange, adsorption on activated carbon, membrane technologies) [23]. When the metal ion concentration is below 50 mg·l^{-1} the technologies have been directed towards biosorption [1]. The mechanism of metal biosorption is a complicated process influenced by different factors and consists of two steps. In the first step metal ions are adsorbed to the cell surface by interactions between metals and the functional groups situated on the surface of the cells. All metal ions, before reaching the cell membrane and cytoplasm, get attached to the cell wall. The various polysaccharides and proteins with a number of active

sites are responsible for binding of metal ions. First step is passive biosorption and is metabolism independent. In the second step, due to active biosorption, metals penetrate the cell membrane and enter into the cell [6]. Metal uptake by non-living matter is mainly passive [25].

Heavy metal biosorption capacity of algae had proved to be high because of the algal cell wall, which is composed of a fiber-like structure and an amorphous embedding matrix of various polysaccharides [16]. Polysaccharides are essential constituents of all living organisms and perform a variety functions. The red microalgae are surrounded by a cell wall of polysaccharides. It consists of oligosaccharide structures, acidic building units, sulfated groups attached to the backbone and uronic acids [12, 13].

Presently, sol-gel chemistry offers new possibilities for the promising encapsulation of biomolecules and other organic substances [17]. Organic-inorganic hybrids are a relatively new type of composites with valuable mechanical, optical, electrical and thermal properties. A simple method to obtain organic-inorganic hybrids is mixing an organic polymer with silicon alkoxides such as tetraethoxysilane (TEOS) followed by sol-gel reaction. Such hybrids are promising materials for various applications: as biocatalysts and as materials for immobilization of different cells and enzymes [14]. Hybrid varieties based on silicates are attracting, because of their chemical stability, durability, biocompatibility, non-toxicity, low cost and applicability. Considerable attention has been drawn on problems and future prospects of sol-gel matrix for biotechnological applications [9]. The mild conditions associated with these bio-inspired syntheses allow the formation of hybrids in which both organic and inorganic phases are mixed at a molecular level. Even fragile bio-species such as enzymes and whole cells of yeasts, bacteria, fungi and algae can be trapped within the solid network, opening new possibilities in the field of biotechnology and bioremediation [4, 18].

The precise mechanism of different reactions was influenced by the effect of the immobilization on the sorption capacity of biosorbents [10, 11, 24].

The aim of the present study was to create different types of biosorbents, using the sol-gel technology and to test their uptake of heavy metals capacity in an aqueous solution using a laboratory bioreactor.

Materials and methods

Strain
The culture of the red microalga *Porphyridium cruentum* (Rhodophyta, strain Vischer 1936/10z strain A 126) from the algal collection of the Institute of Botany at the Chech Academy of Sciences. *P. cruentum* was intensively cultivated for 96 h in Brody Emerson modified medium at light intensity of 260 $\mu E \cdot m^{-2} \cdot s^{-1}$ and temperature 27 °C. Suspension was aerated with 1% CO_2.

Polysaccharide
The algal heteropolysaccharide used in this study was isolated from the cell walls of the alga. It contains 9% sulphates. The main monosaccharides in its composition are xylose, galactose and glucose. Glucuronic acid and semi-esterified sulphate groups determine the acidic properties of the polymer [2]. After intensive cultivation of the algae, the cell suspension is subjected to centrifugation for 30 min at 6000 g. Cells are removed and the supernatant is precipitated with ethyl alcohol in a ratio 1:2. During precipitation two fractions are distinguished (one with MW = 2×10^6 Da; and the other with MW = 6×10^6 Da). After this

operation, the precipitated fractions are collected. Dissolving in distilled water on a magnetic stirrer is the next step. To estimate the molecular weight of the heteropolysaccharide fractions, dextrans of different size and standards were used [22]. The eluted heteropolysaccharides were dialyzed for 24 h at 4 °C against water, freeze dried and five wt. % of both types of fractions were introduced for the preparation of the sorbents.

Hybrids preparation

The organically modified silica materials were prepared using sol-gel technique at room temperature. Preparation of the silica network is connected with hydrolysis of TEOS in the presence of distilled water and 1N HCl solution, as an initiator of the process. Three component mixtures were stirred for 1 h to full hydrolysis of the silicate precursor. The molar ratio was kept constant for all samples (TEOS:H_2O = 1:5,4). HCl solution (2 ml) was added to each mixture for accelerating of the hydrolysis process.

Biosorbent construction

Four types of biosorbents were tested. 1-inorganic sol-gel sorbent on the basis of TEOS; 2-hybrid sol-gel sorbent with TEOS and algal biomass of *Porphyridium cruentum* (concentration 26×10^6 cells); 3-TEOS + 5 wt. % algal heteropolysaccharide MW = 2×10^6 Da; 4-TEOS + 5 wt. % algal heteropolysaccharide MW = 6×10^6 Da. Experiments for evaluation of the adsorption capacity were conducted at previously optimized conditions (pH = 7 and at 25 °C).

Laboratory bioreactor performance

A laboratory bioreactor with a working volume of 100 ml with glass walls forming a cylinder was used (Fig. 1). The experiments in the bioreactor were executed under the following experimental conditions: process temperature 25 °C maintained by a water jacket, pH = 7.0 and aqueous solution passing through it, containing the heavy metal ions with a definite concentration. 40 g of any type of the sorbents was introduced in the reactor.

Methods for structural characterization

FT-IR (*Fourier transforming infra-red spectrometry*). Infrared spectra were obtained using a MATSON 7000 Fourier Transforming Infra-Red spectrometer. Pellets of ca. 2 mg of hybrid samples were mixed with 200 mg of spectroscopic grade KBr.

BET (*Brunauer-Emmett-Teller*) *analysis.* The N_2 adsorption at 77 K was utilized to determine the specific surface areas and porosities of the prepared hybrids. A gas adsorption manometry apparatus was used for the N_2 adsorption experiments. The BET equation was used for the calculation of the specific surface area.

SEM (*Scanning electron microscopy*). Structural details were obtained using a JEOL JSM- 5510 Scanning Electron Microscope (SEM). An JEOL JFC-1200 fine sputter coater was used to sputter the fractured surfaces with gold so as to enhance their conductivity.

ICP-OES (*Inductively coupled plasma with optical emission spectrometer*). Prodigy High Dispersion ICP-OES, Teledyne Leemans Labs was used for the heavy metals concentrations determination.

Results and discussion

Adsorption capacity of the created biosorbents was estimated together with elucidation of the structure of the synthesized sol-gel hybrid materials using different structural methods. All experiments were carried out in the bioreactor. Flow rate applied was 0.75 ml·min^{-1} at a continuous mode.
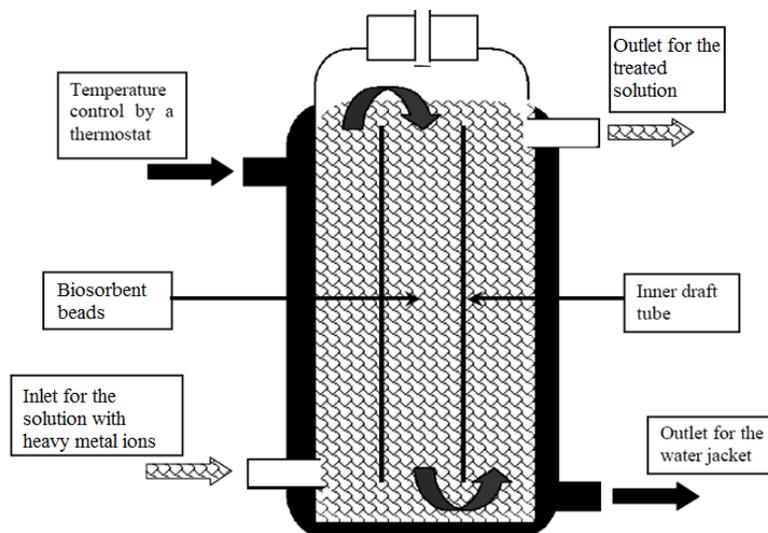
Fig. 1 Schematic presentation of the laboratory bioreactor

Structural investigations

The results of BET analysis proved that the pore diameter size is about 2 nm, and the specific surface area is in the range from 156 to 400 m^2·g^{-1}, depending on the hybrid chemical composition. With introduction of the heteropolysacharide the surface area increases creating larger space for the adsorption of metal ions (Fig. 2).
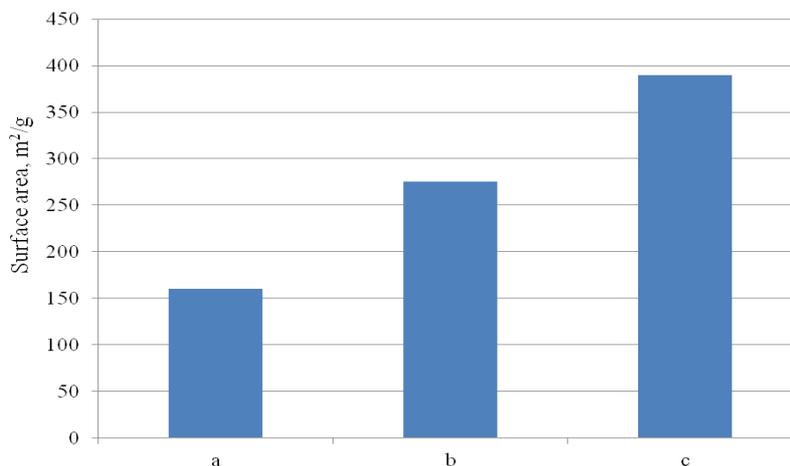
Fig. 2 Surface area of: a) pure SiO$_2$; b) SiO$_2$ and low molecular polysaccharide; c) SiO$_2$ and high molecular polysaccharide.

The FT-IR spectra of synthesized hybrids show characteristic peaks at around 1080 cm^{-1}, 790 cm^{-1} and 460 cm^{-1} attributed to the SiO$_2$ network (Fig. 3). They are assigned to v_{as}, v_s and δ of Si-O-Si vibrations, but at the same time the band at 1080 cm^{-1} can be related to the presence of Si-O-C, C-O-C and Si-C bonds. The presence of bands at 950 cm^{-1} is attributed

to Si-OH groups. The characteristic bands at around 3460 cm^{-1} and at 1630 cm^{-1} assigned to H-O-H vibration can also be detected. At the same time the bands around 3460 cm^{-1} can be associated with v (OH) ring stretching vibrations. Absence of bands in the region between 645 cm^{-1} and 720 cm^{-1} indicates that the silica precursor used is fully hydrolyzed. Bands around 2936 cm^{-1} and 2860 cm^{-1} refer to C-H absorption. These include CH, CH$_2$ and CH$_3$ stretching and bending vibrations. The region 1000-1500 cm^{-1} represents various configurations of C, O and H bonds in the polysaccharide structure. More detailed is the band at 1378 cm^{-1}, assigned to δ_s CH$_3$ groups. The region between 1200 cm^{-1} to 900 cm^{-1} is associated with "fingerprint" region for polysaccharides.
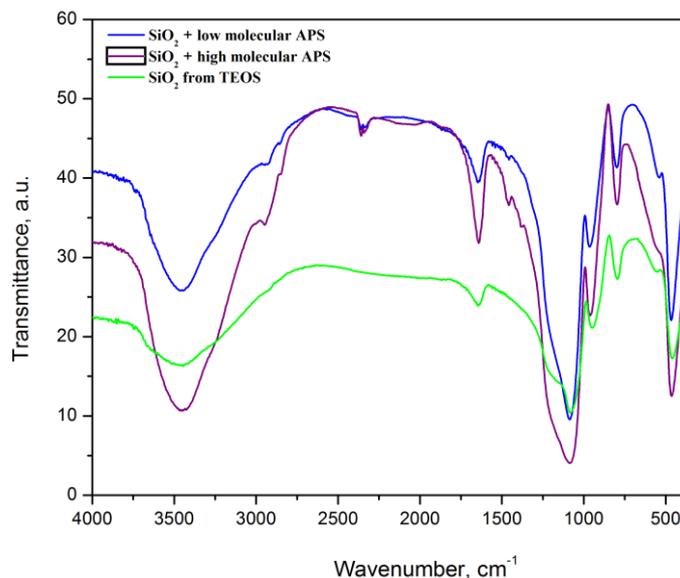
Fig. 3 FT-IR spectra of the synthesized hybrids

The SEM micrographs of the obtained materials are shown on Fig. 4. Difference in their surface is visible. Smooth surface due to formation of homogeneous silica network is presented. Incorporation of low, high molecular polysaccharide component and algal biomass leads to formation of particles all over the surface and underneath. The size of formed particles varies between 50 nm and 0.1 μm. As a result of cross linkage of organic chains with inorganic network, formation of big pores and particles of porous inhomogeneous structure is observed. The created nanoscaled formations could be assigned to the surface structure of the particles building the aggregates registered by SEM.

All values measured were evaluated on the basis of heavy metals adsorption experimental data obtained from the four supports (1-inorganic sol-gel sorbent on the basis of TEOS; 2-hybrid sol-gel sorbent with TEOS and algal biomass of *Porphyridium cruentum*; 3-TEOS + 5 wt. % algal polysaccharide MW = 2 × 10^6 Da; 4-TEOS + 5 wt. % algal polysaccharide MW = 6 × 10^6 Da.

The experimental data on studied adsorption processes of heavy metal ions removal by the four chosen adsorbents are presented in Table 1.
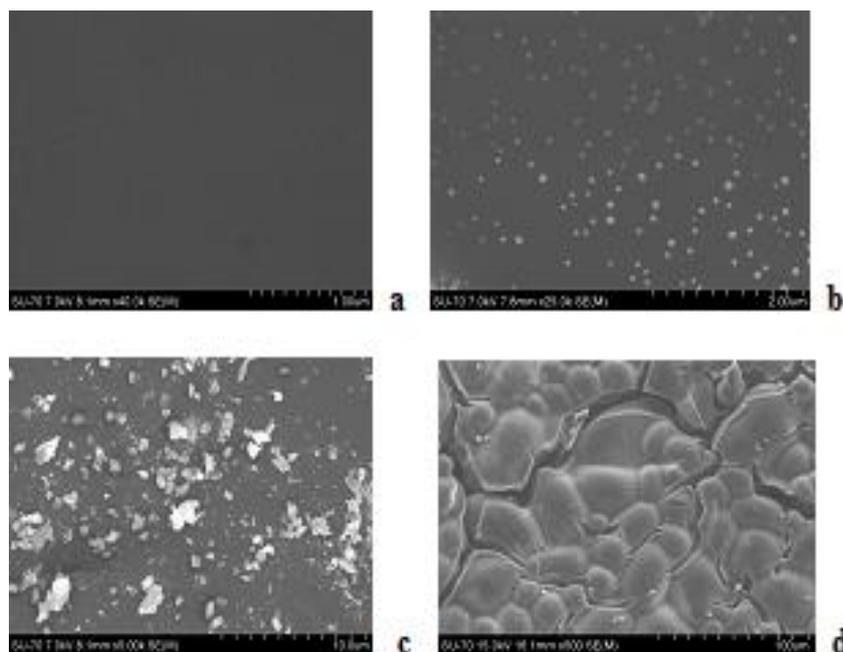
Fig. 4 SEM images of: a) pure SiO_2; b) SiO_2 and low molecular polysaccharide;
c) SiO_2 and high molecular polysaccharide; d) SiO_2 and algal cell mass.

Table 1. Changes in heavy metals ions concentrations with time for the four different sorbents

Sorbents	Heavy metals ions	Time							
		30 min	60 min	90 min	2 h	12 h	24 h	72 h	120 h
SiO$_2$	Cu^{2+} mg·g^{-1} sorbent	1.08	1.865	2.672	3.68	5.183	5.273	5.439	5.35
	Ni^{2+} mg·g^{-1} sorbent	0.427	0.559	0.797	1.144	1.339	1.792	1.47	1.465
	Cd^{2+} mg·g^{-1} sorbent	0.661	1.986	2.057	2.399	2.632	2.861	3.441	3.457
TEOS + Algal cells	Cu^{2+} mg·g^{-1} sorbent	4.623	9.617	12.275	19.533	31.523	34.633	40.087	40.633
	Ni^{2+} mg·g^{-1} sorbent	2.682	4.879	7.164	9.678	12.789	14.468	16.662	16.436
	Cd^{2+} mg·g^{-1} sorbent	4.886	6.949	9.973	12.88	14.664	16.627	18.771	15.536
TEOS + high molecular PS	Cu^{2+} mg·g^{-1} sorbent	2.581	6.383	10.531	15.531	19.286	30.288	33.283	34.431
	Ni^{2+} mg·g^{-1} sorbent	3.732	5.688	8.554	10.694	13.755	15.745	17.545	17.433
	Cd^{2+} mg·g^{-1} sorbent	2.832	4.905	8.919	10.147	13.657	17.767	21.715	16.616
TEOS + Low molecular PS	Cu^{2+} mg·g^{-1} sorbent	2.22	6.28	9.286	13.185	19.286	23.54	28.059	25.043
	Ni^{2+} mg·g^{-1} sorbent	0.7	3.542	5.75	7.646	10.2	12.592	15.836	15.211
	Cd^{2+} mg·g^{-1} sorbent	3.531	5.588	7.628	13.765	15.138	17.683	18.563	14.618

Highest adsorption capacities were monitored for the adsorbent with algal cells and high molecular algal polysaccharide. These results are not unexpected, having in mind that the representatives of *Rhodophyta*, to which the red algal strain *Porphyridium cruentum* is attained, are potentially excellent heavy metal biosorbents because they contain the largest amount of amorphous embedding matrix polysaccharides with their well known metal binding ability [7]. *Porphyridium sp.* contains 7.6-14.6% sulphate, 7.8-10% uronic acids and 1-2% protein. It has been hypothesized that sulfate is the bioactive group of the red microalgal polysaccharides [3]. In addition, the mucilaginous layers that cover algal cells can bind metal ions due to the presence of uronic acids [20]. The heteropolysaccharide of *Porphyridium cruentum* contains also a certain quantity of proteins, additionally supplying active groups for binding.

Algal cell walls are freely permeable to low molecular-weight constituents such as water, gases and ions. Algal cell walls contain pores about 3-5 nm wide to allow the passage of low-molecular-weight substances.

It must also be pointed that the amount of heavy metal adsorbed is highest for the copper ions and reached its maximum when the second support (TEOS and Algal cells) was applied. The picture changed for Ni and Cd ions where best results were observed for the third support (TEOS and High molecular polysaccharide). These results have their explanation, stepping on the facts that algal cell wall constituents have been implicated as being responsible for metal binding in interaction of heavy metals with the algal cells in aqueous solutions where both electrostatic attraction and the formation of complexes play their role [21]. For the third type of biosorbent, the dominant role of algal polysaccharides in accumulation of metals was proved in these microalgae and is considered to be correlated with the ability of polysaccharides to form gels [19], the ion exchange properties of the sulfated polysaccharides; the degree of sulfatation of the molecule and the presence of uronic, glucuronic and galacturonic acids [8]. Not less is the influence of the supports structure and their ability to provide more active sites for metal ion acceptance and fixation [5, 15].

Following the process mechanism, uptake capacity and physical characteristics of the created sorbents, many configurations could be suggested for multiple use and in a continuous mode. Application aspects of creation of new types of sorbents are aiming at processes optimization and reaching effective bioremediation.

Conclusion

This study evaluated the possibility of using four sol-gel derived sorbents of different kind for heavy metal ions adsorption. Highest uptake capacity was registered for the hybrid biosorbent, synthesized by the sol-gel method on the basis of TEOS and red algal cells suspension (most promising support for Cu ions adsorption). Together with the hybrid sorbent with high molecular red algal polisaccharide in its composition, they were considered the best supports for Cu, Ni and Cd ions removal. High percentages of Cu, Ni and Cd ions adsorption were obtained during the first hours of the adsorption process (up to 10 h). These results suggested that the chemical adsorption controlled the overall process. The adsorption process, therefore, involved valence forces as a result of sharing or exchanging electrons between the hybrid matrix and the different ions.

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