O7-1	**Encapsulated bacterial and algal cells involved in bioremediation processes**

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INTRODUCTION

Industrial activities have been increasing during recent years and many water bodies suffer from the accumulation of organic chemical wastes and heavy metals. Ways to protect the environment, animals and humans have to be sought. The physical and chemical methods used at present can be efficiently replaced by biological, involving biomaterials in the processes of pollutants removal (Wang 2009). Reports exist on the use of microorganisms-algae (Tien 2002), fungi, yeasts and bacteria (Iqbal 2007) in the detoxification of waste waters. Encapsulation of cells in hybrid materials for biochemical conversions, and treating different wastes by natural populations of bacteria is useful (Yañez-Ocampo 2009). The hybrid materials are derived using the sol-gel method at low temperature, using both organic and inorganic components combined in such a way that can lead to dramatically enhanced mechanical, thermal and chemical properties (Kickelbick 2007) serving manifold applications.
Some bacterial strains produce enzymes capable of catalyzing processes of degradation of cyanogroup-containing compounds (Gupta 2010). The encapsulation of such microbial cells in suitable carriers makes possible the obtained biocatalysts to be used for an efficient treatment of toxic organocyanides polluted industrial effluents (Martinkova 2010).
Algae hybrid materials can be successfully applied for biosorption in the treatment of metal contaminated water.
In this study we report on the preparation of hybrid materials encapsulated cells applied in biodegradation and biosorption processes.

MATERIALS AND METHODS

Sol-gel hybrid materials

The inorganic-organic hybrid materials were prepared by substituting part of the inorganic precursor tetraethylortosilicate (TEOS) with organic components: Polyethylene oxide (PEO), algal hetero-polysaccharide (APS) and gelatin. The APS was isolated from microalga Dixionella grisea, strain UTEX LB 2320 with MW 5-7 kD. Sol -gel transparent silica hybrid matrix with 5 wt % of organic compound were synthesized at room temperature. The quantity of sol-gel precursor solution is 19 ml. After prehydrolysis of the initial mixture, the organic component in a quantity 1 g was added, followed by homogenization of the solution and phosphate buffer for raising the pH to 7.2±0.02 at 20 °C was added to the to keep cell vitality. The drying procedure was carried out overnight.

Bacterial and algal cells

The bacterial strain used in the present study was *Bacillus sp.* UG5B (NBIMCC-Bulgaria №8021/2001) with nitrile degrading activity. It was cultivated at pH 7.5 and 55°C. Cells were separated from the culture medium by centrifugation and re-suspended in a phosphate buffer solution (0,06M, pH 7.2 at 20°C). Cell suspension with 35 mg/ml dry cells and enzyme activity of 1.4 U/mg protein was used in the immobilization. A model mixture of fumaronitrile, 4-cyanopyridin and p-tolunitrile in concentration 30mM was used as a substrate. The enzyme activity was measured by the ammonia released due to nitrilase action according to the phenol-hypocloride method.
The cyanobacterium *Synechocystis salina* was isolated from a soil sample collected in Livingston Island, the South Shetland Archipelago. Cells were grown in a modified Brody and Emerson medium where 200 mg/l EDTA and 2 g/l $NaHCO_3$ were added. *S. salina* was intensively cultivated at 30 °C. The hybrid matrices with incorporated *S. salina* cells were added separately to 30 ml $CuSO_4.5H_2O$ (25 μg/ml Cu^{2+}) and to 30 ml $CdCl_2.2H_2O$ (23 μg/ml Cd^{2+}) aqueous salt solutions. After incubation for 3 days they were removed and the residual heavy metal concentrations in the solutions were determined using Atomic Emission Spectrometer.

RESULTS AND DISCUSSION

Batch experiments were carried out with the obtained biocatalysts of the hybrid materials with bacterial cells inside to follow the enzyme activity and operational stability. Batch reusability was proved for the three different immobilized preparations and kept for 20 cycles of operation- each with fresh substrate medium-from 39% to 43% from the initial activity for the ones with PEO, gelatin and APS, respectively.
Next experiments were performed in a column bioreactor with 50g of the three types of biocatalysts separately. A continuous degradation process carried out for 3 hours lead to a 68mM degraded substrates (Fig. 2) for the matrix, containing APS.
The most effective biocatalyst was filled in the bioreactor and after finishing the first step of 4 hours the solution released was put again in the reactor for a second treatment of the not degraded substrates for another 5 hours. At the first step a degradation of 42 % was achieved.
Operation of the system was at 45ml.h^{-1} at 60 °C. In this way the overall conversion realized was 92% (149 mM), showing a good effectiveness of the developed process.

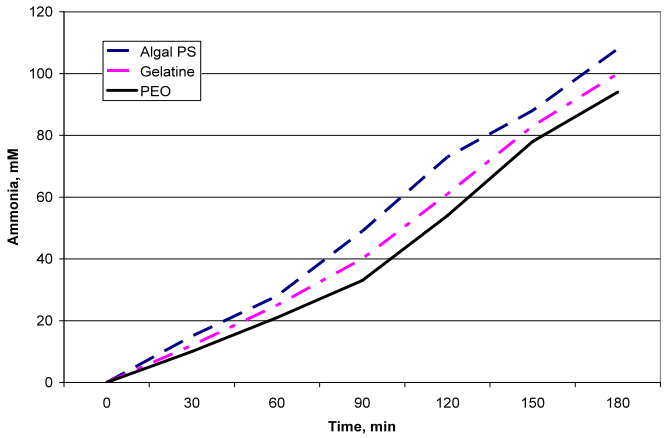

Figure 1: Quantity of ammonia released – corresponding to the quantity of degraded substrate

Immobilized cell bioreactors have been used due to the stability and effectiveness of a continuous biodegradation process (Soares 2010).

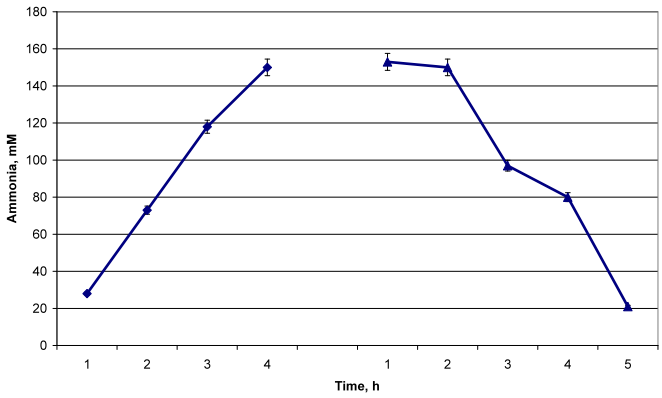

Figure 2: A two-step biodegradation process with biocatalysts on the basis of TEOS and APS

The obtained hybrids were used for immobilization of *S. salina* cells as sorbents of heavy metal ions. The results obtained from ICP-AES analyses for the residual heavy metal concentration showed different decrease in their content depending on the type of metal ions.

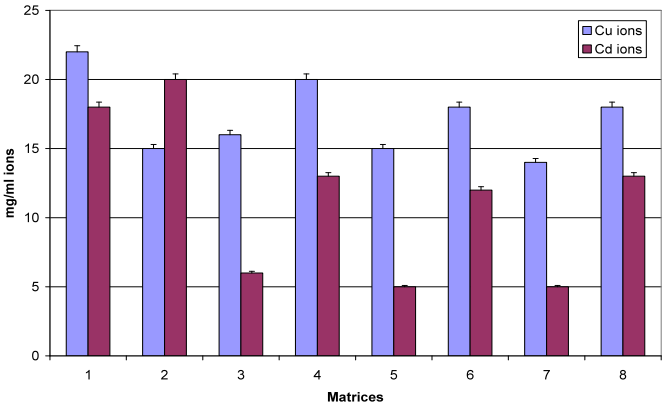

Figure 3: Residual concentration of metal ions in the salt solution after 72h incubation

The accumulation of copper (15.2%) by the biosorbents containing native non-irradiated cells (column 1) increased after UV-B treatment of the cells (column 2). More significant is the change in the quantity of Cd ions depending on UV-B treatment of algal cells (Fig. 3). Addition of an organic part in the matirx enhanced the bioaccumulation of Copper (3.66 mg/g biomass) and that of Cadmium (5.87 mg/g biomass) in matrices containing vital non-irradiated cells (columns 1, 3, 5, 7). The results indicated that considerable enhancement of the bioaccumulation capacity of viable *S. salina* cells for both metals could be achieved mostly by the addition of APS due to both its pore forming properties and effective heavy metal binding capacity (columns 1, 2).

CONCLUSIONS

The hybrid sol-gel matrices proved to be effective for the entrapment of the two types of cells. The newly isolated from Antarctic cyanobacterium *Synechocystis salina* was successfully incorporated in hybrid matrices used for heavy metal ions sorption. The capability of cells to absorb both metals can be enhanced by their treatment with UV-B prior to immobilization in the matrices and by the addition of organic part in the hybrids.
The obtained biocatalyst used for biodegradation of organo-cyanides showed an efficient way for treatment of the industrial effluents, realizing almost full degradation (92%) in the two-step biodegradation process in the bioreactor. These encapsulated biomaterials can be involved in bioremediation processes - decreasing the negative effects of heavy metals and other toxic pollutants and reducing their distribution throughout the environment.

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