Research and Development of Sol-Gel Silica Hybrids for Obtaining of Hybrid Biomaterials

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INTRODUCTION

The sol-gel silica and silica hybrid biomaterials have attracted considerable attention recently mainly due to their advantages: the mild experimental conditions of the synthetic method and the possibility to obtain many new materials with nanoscaled structure and extraordinary behaviours (Ogoshi T. et. al. 2005, Hamano Y. et. al. 2004, Schubert U. et. al. 2003). Silica hybrids are excellent matrices for immobilization of inorganic and organic compounds and various types of enzymes, whole cells, antibodies, polysaccharides, phospholipids, and other biomolecules. Micro algae, fungi, filamentous yeasts and bacterial cells were immobilized and applied in different biotechnological processes (Shchipunov Y. et. al. 2005, Chen J. et. al. 2004). The cell immobilization is concerned as a viable alternative to conventional microbial fermentations (Ramakrishna and Praksham 1999). This process eliminates most of the constraints faced with free cells due to the considerably higher stability and activity the immobilized systems (Miyake-Nakayama et al., 2006, Viggiani et al., 2006, Nagazawa T. et al., 1989). Another field of application of the silica hybrid biomaterials is medicine. Nanomaterials are in a solid amorphous or crystalline state. In recent years, hybrid nanomaterials have attracted considerable attention as potential drug delivery devices in view of their applications in the controlled release of drugs (K. Soppimath et al., 2001; C. Muller-Goymann, 2004). They are able to absorb and/or encapsulate drugs, protecting them against chemical and enzymatic degradation and ensuring their controlled release in human body.That is why, they are also attractive as carriers for development of drug delivery systems of controlled release.
The aim of the present work is to investigate hybrid matrices synthesized on the basis of different precursors and organic materials by the sol-gel method for immobilization of bacterial cells for a biodegradation process of toxic organocyanide substrates and fungal spores for α-galactosidase production as well as to prove that the synthesized sol-gel silica biomaterials could be also applied in medicine.

MATERIALS AND METHODS

For hybrid materials synthesis different types of inorganic silica precursors and organic materials have been used. The main inorganic precursor is tetraethylortosilicate (TEOS). 0.1 N HCl and phosphate buffer with pH=7.00±0.02 at 20^{o}C were also used in the synthesis. A small amount of 0.1 N HCl is introduced in order to increase hydrolysis rate (pH~1, 5). The inorganic-organic hybrid matrices have been prepared by substituting part of the inorganic precursor with organic constituents. Thus synthesized matrices were applied as carriers for immobilization.
Bacillus sp. UG-5B, a moderate thermophile and the mesophilic fungus *Humicola lutea* 120-5 were used in this study.

The nitrilase activity was assayed by measuring the ammonia released by the nitrilase action according to the method of Fawcett and Scott (1960). As substrate tolunitrile was tested. The fungal strain *Humicola lutea* 120-5, registered in the National bank for industrial microorganisms and cell cultures: 391, Sofia, Bulgaria was used in this study too.
As a nutrient medium soy meal waste extract (5% dry content) was used. The washed and dried sol-gel particles with the entrapped spores or vegetative cells were cultivated in 500 ml Erlenmayer flasks with 50 ml mediun in a rotary shaker (220 rpm) at 30°C. After 120 h the particles with the immobilized mycelium were transferred into fresh medium at 144-h and 216–h intervals during the repeated batch use.
The next object of this study was to synthesize SiO_2-MMA gel matrices and to develop the delivery systems of model drug Ibuprofen (IBP).
The SiO_2-MMA/(IBP) system of modified drug release have been studied.

RESULTS AND DISCUSSION

The results from the XRD - analysis prove that all the studied hybrids have an amorphous structure. At the same time the sharpening of the amorphous halo indicates that some processes of ordering are carried out.

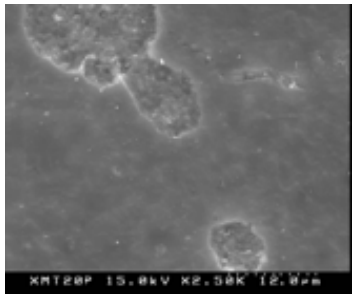

Fig. 1. SEM image of hybrid MTES-5PEO

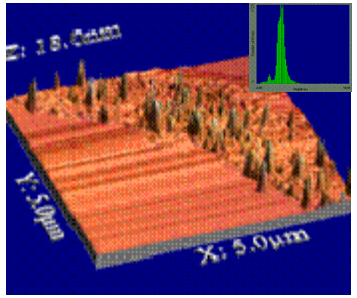

Fig. 2. AFM image and Roughness of hybrid MTES-5PEO

The FT-IR spectra of synthesized inorganic-organic materials show that in all samples bands at 1080 cm^{-1}, 790 cm^{-1} and 480 cm^{-1} are observed. They are assigned to ν_{as}, ν_s and δ of Si-O-Si vibrations, but at the same time these bands can be related to the presence of Si-O-C, C-O-C and Si-C bonds. The band at 960 cm^{-1} is due to a stretching Si-OH vibration. The band at 1439 cm^{-1} is assigned to C-O-H vibrations. The characteristic bands at around 3450 cm^{-1} and at 1620 cm^{-1} assigned to H-O-H vibration can also be detected. These bonds in the samples with MTES are in a narrower range compared to IR spectra of samples with TEOS. From the data of BET analysis it has been established that the pore size is in the range of 1 to 1, 8nm. With increasing the percent of organic part, the pore size decreases.
The presence of a heterogeneous structure with well-defined nano units is clearly seen from the AFM studies (Fig. 1, 2). The average size of nanoparticles on the sample surface is about 30 nanometers and the formation of their self-organized structures can be observed.
The enzyme nitrilase (EC.3.5.5.1) catalyzes the direct hydrolysis of nitrile compounds to carboxylic acid and ammonia. Bacterial nitrile-metabolizing enzymes can be applied in chemical synthesis and

used as whole cells in immobilized biocatalysts for detoxification of organic pollutants because of their effectiveness.

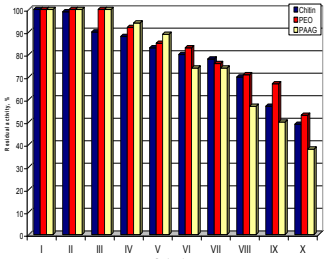

Fig. 3. Operational stability of immobilized bacterial cells in hybrid matrices with different organic content

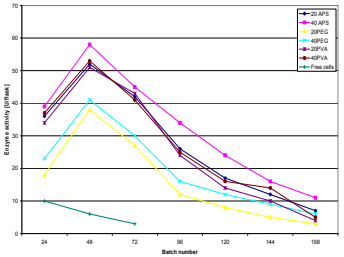

Fig. 4. α-Galactosidase production by *H. lutea* cells, immobilized in different matrices during repeated batch shake flask cultivation.

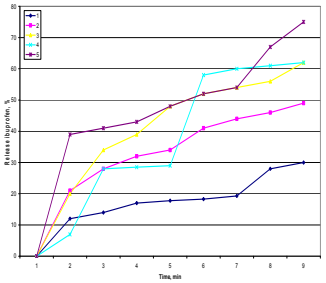

Fig. 5. In vitro dissolution profiles of SiO_2-MMA/IBP models: (1, 3) in 0.1M HCl; (4, 5) in water and (2) pure IBP in HCl

The operational stability of the obtained biocatalysts was followed to find a good stability for 10 reaction cycles. The enzyme activity was retained up to 54% for the matrix, containing PEO (Fig.3).

The α-galactosidases (α-D-galactoside galactohydrolase, EC 3.2.1.22) catalyzed the hydrolysys of α-1, 6-linked galactosyl residues in oligosaccharides and galactomannans.

Fungal α-galactosidases have a wide application in biotechnology, including the nutritional improvement of legumes based foods and fodders. Fig. 4 presents the a-galactosidase production (U/flask) by *H. lutea* mycelium immobilized in a sol-gel matrix with a weight of 1.0 and-2.0 g/flask as well as by free cells during repeated use in sequential 144-h batch cultures. The use of 2.0 g pieces containing immobilized mycelium resulted in higher enzyme yield. The a-galactosidase level: rapidly increased during the first three reincubaions reaching a maximal value of 52 U/flask in the second batch, which is about two-fold higher than the activity in the control sample (24 U/flask or 100 % obtained in 144 h of free cell fermentation). When the weight of the carrier was lower (1.0 g/ flask) the a-galactosidase yields in the batches were smaller as compared to the experiments with higher carrier content. In this case the maximal enzyme level (39 U/flask or 162%) was also reached in the second cycle. The increase of *a*-galactosidase production in the medium with a greater weight of the carrier at equal starting quantities of entrapped spores (6 ml, 10^{10} spores/ ml) may result in an increase in the diffusion surface and consequently a better nutrient and oxygen supply as well as enzyme excretion throughout the matrix.

The main absorption peaks of Ibuprofen are at 1710 cm^{-1} caused by the carbonyl stretching vibration and at 2955 cm^{-1} - by the hydroxyl groups.

The SiO_2-MMA/IBP system shows an effective and prolonged drug release (around 8 hours) in H_2O and 0.1M HCl (Fig. 5). The IBP release from the sample with 5% MMA is insufficient in 0.1M HCL. In contrast, IBP of the system with 20% MMA dissolves at a relatively uniform rate in both media - 0.1 M HCl and water and the total amount released attains about 75% from the initial concentration.

CONCLUSIONS

The present study offers new possibilities for application of sol-gel hybrid materials:

Studying the silica hybrid nanocomposites we have established a strong dependence between chemical composition, structure and properties of the synthesized biomaterials. The investigated matrix appeared to be very suitable for incorporation of fungal spores. They are entrapped in the hybrid matrix and continue their development as a mycelium which attaches to the surface, while the biosynthetic capability is preserved in both strains used for bioencapsulation, and the values for the enzyme titer exceeded three-fold the α-galactosidase activity of the free cells, while nitrilase half-life of activity is retained for 10 operational cycles, showing good degradation capability of the toxic substrates subjected to biodegradation.

The hybrids are homogeneous, amorphous nanocomposites of low and medial roughness.

These nanomaterials are able to adsorb drugs protecting them against chemical and enzymatic degradation and ensuring their controlled release in human body to increase drug availability at the diseased site. The focus is on drug successfully crossing the barriers existing in the gastrointestinal tract such as induced hydrolysis, enzymatic degradation and bacterial fermentation. The formulated hybrids/IBP adsorbates show a prolonged drug release probably because of drug/carriers interactions, in that way they offer a great potential for more effective therapeutics. The synthesized nanocomposites are perspective to develop drug systems of modified release.

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