

Two types of cell immobilization performed by sol-gel derived hybrid silica nanocomposites with polyethylene oxide



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Introduction

The last decade has seen a revolution in the area of sol-gel derived biomaterials since the demonstration that these materials can be used to encapsulate biological species such as enzymes, antibodies and cells in a functional state. In particular, recent years have seen tremendous progress in the development of more “protein-friendly” sol-gel processing methods and their use for immobilization of delicate proteins (Brennan et al. 2007). Nanocomposite materials of such kind, combining inorganic and organic phases are the objects of novel nanotechnologies, because they join together the best properties of metal oxides and either polymers or biopolymers (Pomogailo, 2005; Hamano et. al. 2004; Shchipunov et. al. 2005; Chen et. al. 2004; Gill et al. 2000). Hybrids, composed of inorganic oxides covalently bonded to organic polymers, are of special interest, since the lack of interface imperfections. A sharp increase of the enzyme lifetime when applying these materials as carriers for immobilization was observed after the immobilization procedure (Shchipunov et al. 2004). Biotechnological processes employing specialized microorganisms could be an effective alternative for cleaning up contaminated waters, as they are particularly suitable for the treatment of large volumes of moderately contaminated water and can allow the re-introduction of the treated water into the environment (Di Giola et al., 2006). In particular, it has been shown that immobilized cell-packed bioreactors are very effective for this purpose (Armenante, 1992). Herein, we report on the immobilization by entrapment or chemical binding to the surface of cells, producing nitrilase and capable of degrading toxic substrates, possible constituents of waste industrial waters into sol-gel derived nanocomposite materials on the basis of silica, PEO and different natural polymers such as agar, gelatine or alginate.

Experimental part

Sol-gel transparent silica hybrid matrices with different quantity of organic compounds have been synthesized at room temperature and controlled pH conditions (pH=7). As a silicon alcoxide precursor tetraethylortosilicate (TEOS - purchased by “Merck”), has been used. The organic component is polyethylene oxide mixed with different natural organic additives - Calcium alginate, agar-agar and gelatin. The gelation time is less than a minute for all samples. No phase separation is observed before and after the gelation point.

For studying the structure of synthesized hybrids the following methods have been used: FT-IR, XRD, BET-Analysis and AFM. Whole cells from the thermophile *Bacillus sp.* UG-5B, capable of degrading different nitriles due to the action of the enzyme nitrilase have been immobilized. The suspension was with enzyme activity 0.75U/ml and concentration of 34mg cells/ml. Full entrapment of the whole cell quantity was established. In the process of chemical binding formaldehyde was applied as a cross-linker and 2g of the hybrid flakes were mixed with 5ml of cell suspension to realize the attachment. Batch processes with the three types of matrices were performed to evaluate the enzyme activity of the obtained biocatalysts. Enzyme activity was assayed by the method of Fawcett and Scott (Fawcett and Scott, 1960).

A glass laboratory reactor with a working volume of 100ml was filled with 45g of the most appropriate biocatalyst (TEOS + 5%(PEO + gelatin)) for the treatment of the model mixture of

contaminated water containing toxic compounds: p-tolunitrile, benzonitrile and fumaronitrile in concentration 60mM at 55°C.

Results and Discussion

The results from the XRD - analysis prove that all the studied hybrids have an amorphous structure. The FT-IR spectra of the synthesized hybrids 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. Probably in the samples synthesized with TEOS the inorganic and organic components interact by Hydrogen bonding, Van der Waals or electrostatic forces. From the data of BET analysis it has been established that the surface area is in the range of 100 to $325\text{ m}^2/\text{g}$. With increasing the percent of organic part, the surface area decreases.

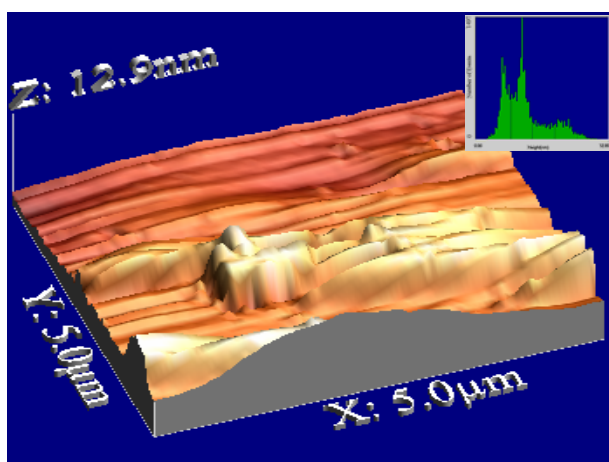


Fig. 1. AFM images and height distribution profile of surface roughness of the hybrids containing PEO and gelatin.

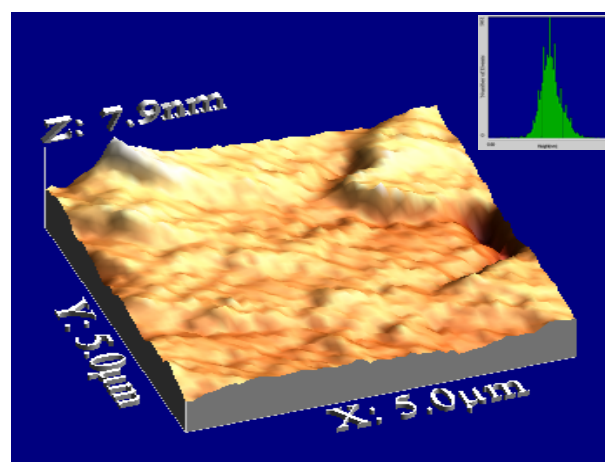


Fig. 2. AFM images and height distribution profile of surface roughness of the hybrids containing PEO and Ca alginate.

A different design of the surface structure of the hybrids containing different natural organic additives was established by AFM. In the synthesized samples the average size of nanoparticles on the surface are about 6 - 12 nm and the dimensions of their self - assembled aggregates are about 20 - 50 nm (Fig. 1, 2, 3). A rough structure is clearly seen.

The sol-gel derived hybrid silica nanocomposites were employed as immobilization supports at batch processes and in a continuous process in a bioreactor. Highest nitrilase activity was registered for the biocatalysts synthesized with PEO+gelatin and immobilization procedure-chemical binding at a batch process, assumed as 100%. The enzyme activity with the same type of matrix at entrapment was almost the same-98%. For the biocatalysts, synthesized on the basis of TEOS and PEO+agar, as well as for these with PEO+alginate, the values for the enzyme activity in a batch process were 86% and 70%, respectively at entrapment and 90% and 75% at chemical binding (Fig. 4). A laboratory column bioreactor was set up and tested for the treatment of water, artificially contaminated with a complex mixture of three organo-cyanide compounds. The matrix, containing PEO+gelatin which gave the best results in the batch process was applied in a biodegradation in the

bioreactor although all three organic components have proved to be usable as immobilization carriers. The immobilization within gelatin involves both physical entrapment and covalent crosslinking, each one playing its role (Alteriis *et al.*, 1988). Probably these features lead to efficient immobilization using gelatin as an organic constituent in the hybrid matrix and accomplishment of a biodegradation process for the mixture of p-tolunitrile, benzonitrile and fumaronitrile (60 mM).

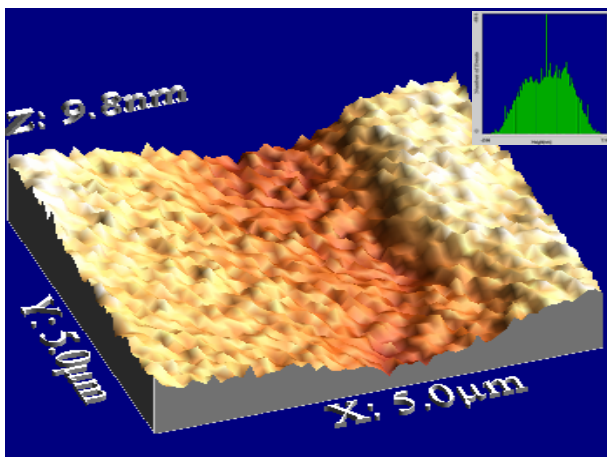


Fig. 3. AFM images and height distribution profile of surface roughness of the hybrids containing PEO and agar.

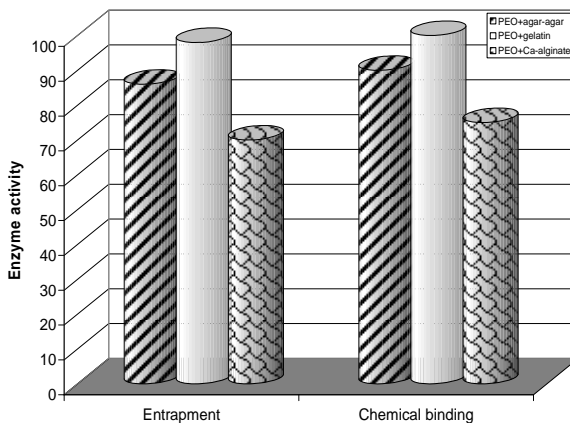


Fig. 4. Enzyme activity at batch experiments with the three type of matrices.

A biodegradation process was achieved for the period of eight hours with biocatalysts produced by entrapment in a matrix with PEO+gelatin and 47.3 mM of the substrates were degraded. In the case when chemical binding after activation was performed with the same type of hybrid matrix, better results for the efficiency of the biodegradation process were obtained (Fig. 6).

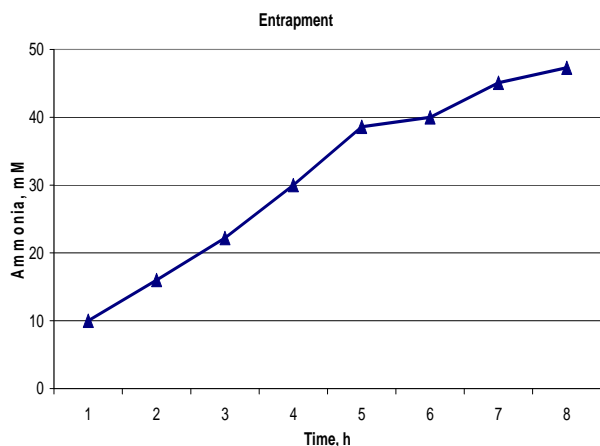


Fig. 5. Ammonia released corresponding to the quantity of degraded substrates at entrapment.

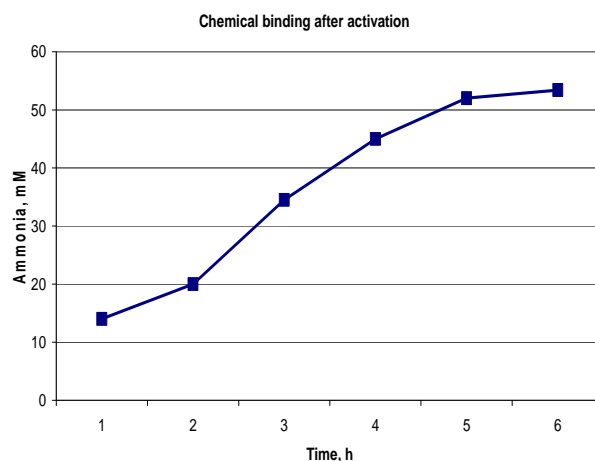


Fig. 6. Ammonia released corresponding to the quantity of degraded substrates at chemical binding.

The degradation for the mixture of substrates was faster-six hours and the quantity of degraded p-tolunitrile, benzonitrile and fumaronitrile was 53.4 mM, corresponding to 89% of degradation. Differences were established between the activity of biocatalysts produced by entrapment that can be devoted to the formation of a microenvironment around the cells. However, the cells attached to the surface at chemical binding are not covered and a direct contact between the substrate solution

and the cells without any mass transfer limitations was achieved. The statement that intrinsic catalytic activity of enzyme systems of the cells incorporated into sol-gel derived materials, produced from TEOS was not altered was again proved. Even the catalytic efficiency and long-term stability were improved (Besanger et al., 2003).

Conclusion

The synthesized hybrid nanocomposites with PEO and natural polymers agar-agar, Ca alginate and gelatin in their structure are sufficiently porous that allows the enzymatic substrates and products to diffuse from an external aqueous solution to the enzyme. The cells were immobilized firmly and not easily washed out from the silica hybrid matrix. Being immobilized, the cells are less susceptible to the toxic effect of the substrates. The porous structure, chemical and biological inertness of the hybrids, as well as their mechanical and thermal stability are major parameters for the efficiency of the immobilization process. That is why they could be applied in biodegradation processes of cyanocompounds, possible constituents of contaminated waters and soils.

Acknowledgements

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