Stability of biocatalysts synthesized by sol-gel method containing lactic acid

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Introduction

A new field in the sol-gel technology is the synthesis of nanocomposite hybrid materials simultaneously containing organic and inorganic components. Such kind of materials is a subject of intensive investigations because they represent significant interest for the structural chemistry for studying their physical and chemical properties and potential applications in electronics, optics, microbiology, medicine and pharmacology (Carturan et al., 1998, Kang et al., 2004, Ogoshi et al., 2005) Inorganic-organic hybrid materials are increasingly important due to their extraordinary properties, which arise from the synergism between the properties of the components (Hamano Y. et al., 2004, Schubert et al., 2003).
The most popular precursors used for the synthesis of silicate hybrid materials assuring SiO_2 introduction are: TEOS, TMOS, MTES and others (Shchipunov et al., 2005).
One of the most interesting and important problems in the study of hybrid nanocomposite materials is their structure analysis. Following the correlation between its evolution and the composition-structure-property dependence, as well as the processes of aggregation and development of self-organized or self-assembled structures. Up to now there is not enough information about these structures and the processes occurring during their formation are very important (Chen et al., 2004, Sgarbi et al., 2004, Tan et al., 2005, Shchipunov et al., 2005).
For the synthesis of hybrid materials the choice of the type and quantity of precursors and the organic components is also of importance, as well as the conditions for their synthesis (Valle et al., 2006).
Immobilization of cells can offer solution to difficulties that appear during a biodegradation process by increasing the retension of catalytic activity, protection of cells against leaching and substrate inhibitory effects, ease in separation as well as long-term stability. Bioremediation using cells has been widely investigated for a number of toxic chemicals such as phenol, nonyphenol (Soares et al., 2006), acetonitrile (Dias et al., 2000), polychlorinated biphenyls (Adebusoye et al., 2008). The industrial involvement of nitriles may result in a great amount of synthetic nitriles released and accumulated into marine, freshwater or soil environments. Higher concentrations of nitriles can cause harmful effects on human health being toxic, mutagenic and carcinogenic so an urgent need of their removal exists.
The main advantages of viable immobilized cells are higher reaction rates due to increased cell densities; possibility of regenerating the biocatalytic activity; easier control of processes; long term stabilization; reusability of the biocatalyst; higher specific product yields (Karandikar et al., 2006).
In our previous works we have compared pure silica matrices with organic-inorganic hybrid matrices obtained by using different inorganic precursors (TEOS, TMOS, ETMS, MTES) and organic compounds such as: agar-agar, polyacrylamide gel (PAAG), PEG, PVA and AA (Chernev et al., 2005).

Material and methods

Sol-gel transparent silica and hybrid matrices with different quantity of organic compounds were synthesized at room temperature and controlled pH conditions pH~1.5 and pH=7. The inorganic-organic hybrid materials were prepared by substituting part of the inorganic precursor tetraethylortosilicate (TEOS) with lactic acid (5 and 20 %). A poly-step sol-gel procedure was used at strictly controlled conditions in order to obtain the desired nanostructred materials. In all cases the ratio TEOS/H_2O was kept constant and equal to 1. No alcohol was added as a co solvent. A small amount of 0.1 N HCl was introduced to increase hydrolysis rate (pH~1.5) and phosphate buffer with pH=7.00±0.02 at 20 ^{0}C was used for pH adjustment.

As a substrate for measuring the hydrolyzing activity of nitrilase, crotonitrile (10mM) and 4-chlorobutyronitrile (10mM) in a mixture were used.

For structure investigation of the synthesized hybrids the following methods have been used: XRD (X-ray PW1730/10 diffractometer, in the 2θ range of 50–800, Cu-Kα radiation), FT-IR (IR-MATSON 7000–FTIR spectrometer), Surface Area Analyzers (BET-Analysis - Gemini 2370 V5.00) and Atomic Force Microscopy (AFM - NanoScope Tapping Mode^{TM}).

Results and Discussion

The results from the XRD - analysis proved that all the studied hybrids had an amorphous structure (fig. 1). The results showed that with the increase of concentration of the lactic acid the intensity of curves decreases.

The FT-IR spectra of synthesized inorganic-organic materials are shown (fig. 2). The bands at 1080 cm^{-1}, 790 cm^{-1} and 480 cm^{-1} are observed in the IR spectra of all studied samples. 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 bands at 1439 cm^{-1} is assigned to C-O-H vibrations. The characteristic bands at around 3450 cm^{-1} and at 1640 cm^{-1} assigned to H-O-H vibration can also be detected.

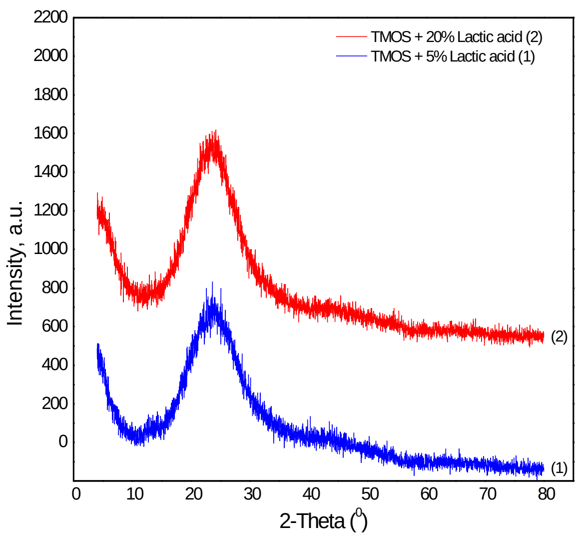

Fig.1 XRD patterns of hybrid nanomaterials containing lactic acid

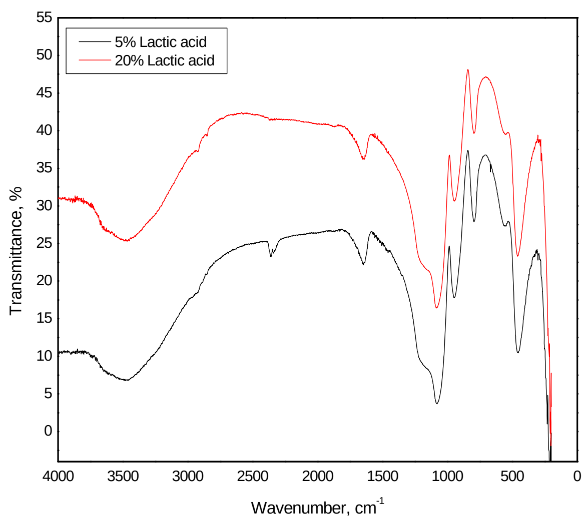

Fig.2 FT-IR spectra of hybrid nanomaterials containing lactic acid

From the data of BET analysis it was established that the surface area is in the range of 70 to 290 m^2/g. The results clearly show that with increasing the percent of the organic part, the surface area decreases.

A self-organized nanostructure was observed by AFM. In the samples synthesized with TEOS and 5% lactic acid the average size of nanoparticles of the surface is about 3-6 nm and the dimensions of their aggregates are about 25-37 nm (fig. 3). When the samples contained 20% lactic acid the average size of nanoparticles of the surface is about 4-8 nm and the dimensions of their aggregates are about 28-39 nm (fig. 4). In the obtained hybrids the nanobuilding blocks are made up of nanounits of SiO_x groups and that Van der Walls and Hydrogen bonding or electrostatic interactions between the nano-building blocks exist.

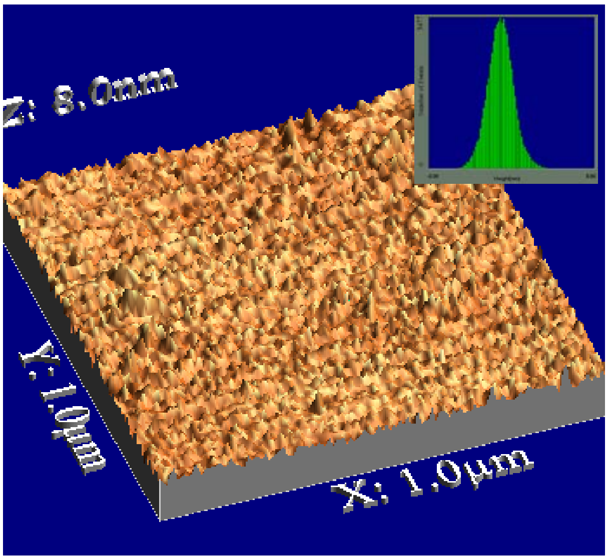

Fig. 3. AFM images of the hybrids (TEOS) containing lactic acid.

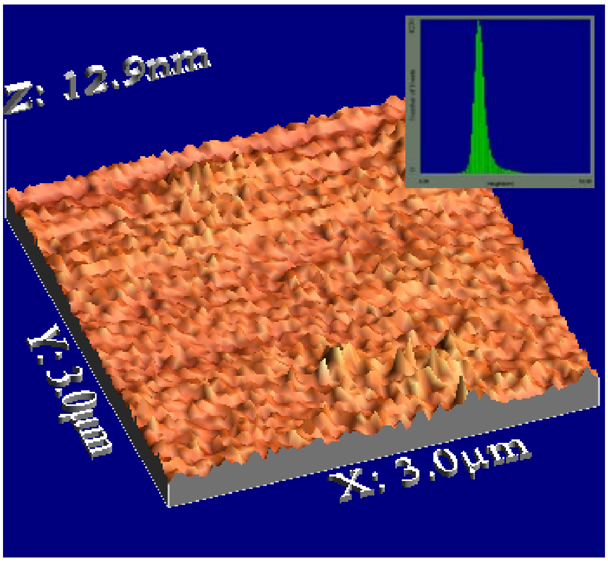

Fig. 4. AFM images of the hybrids (MTES) containing lactic acid.

Thus immobilized catalyst allowed good contact with the substrate and water phase, providing bacterial cells with oxygen. As a result the hydrolyzing activity of nitrilase showed efficiency 51% after 21 days of repeated use (7 cycles) for biodegradation using the immobilized preparations with entrapped cells, which were stable after 21 days storage (fig. 5).

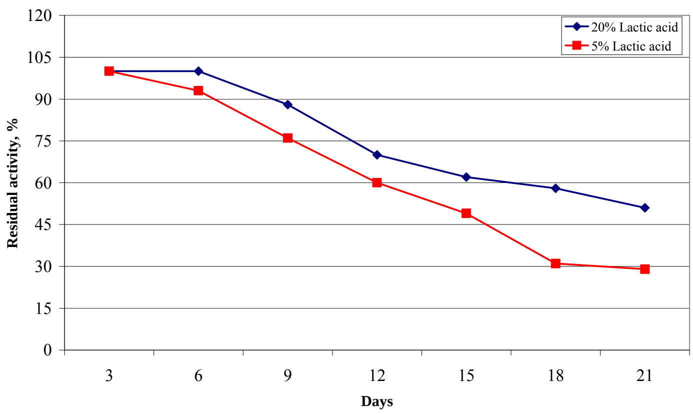

Fig.5. Operational stability of biocatalysts obtained on the bases of TEOS with 5% and 20% lactic acid

Conclusions

The hybrid containing lactic acid was synthesized using sol-gel method. The biocompatibility of the hybrid materials allows the bioactivity to be retained, enhancing the long-term stability of biocatalysts. These materials are promising for biotechnology, being inert as supports and promote high biomass density and permit the biotransformation process under toxicity and high temperature.

Acknowledgements

This work was supported by NFSR Grant NT 2-01 and NT 2-02, Republic of Bulgaria.

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