Silica composite materials with algal polysaccharide used for immobilization of procaryotic and eucaryotic cells

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Introduction

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 cell systems.
The enzyme nitrilase (EC.3.5.5.1) catalyzes the direct hydrolysis of nitrile compounds to carboxylic acid and ammonia. Bacterial nitrile-metabolizing enzymes are applied in chemical synthesis (Nagasawa *et al*., 1989) and used as whole cells in immobilized biocatalysts for detoxification of organic pollutants because of their effectiveness. This is due to the considerably higher stability and activity the immobilized systems retain for a long period of time (Miyake-Nakayama *et al*., 2006, Viggiani *et al.*, 2006).
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 (Kotwal *et al*., 1998, Rezende *et al*., 2005). For the development of hybrid nanocomposites by the sol-gel method a mixing of inorganic precursors with polymer organic molecules should take place and during the setting between inorganic and organic components covalent chemical bonds, hydrogen bonds and electrostatic interactions can appear (Shchipunov *et al*., 2005).
The aim of the present work is to investigate a new type of hybrid matrix synthesized on the basis of algal polysaccharide by the sol-gel method for immobilization of *Bacillus sp*.UG-5B cells for a biodegradation process of three different toxic organocyanide substrates and fungal spores of *Humicola lutea* 120-5 for α-galactosidase production.

Materials and Methods

Microorganisms: Bacillus sp. UG-5B, a moderate thermophile and the mesophilic fungus *Humicola lutea* 120-5 were used in this study.
Enzyme assays: The nitrilase activity was assayed by measuring the ammonia released by the nitrilase action according to the method of Fawcett and Scott (1960). As substrates o-, m- and p-tolunitrile were tested.
α-Galactosidase activity was assayed by the method of Dey *et al*., (1993).
Immobilization: Cell suspension from both strains (5ml) is mixed with 40 ml of the gel mixture before gelation. The gelation time is less than a minute. No phase separation is observed. Hybrid gels with entrapped cells are obtained and after drying the gels are washed before enzyme activity assay.
Synthesis of nanobiomaterials: The hybrids have been prepared at room temperature as films. Silicon precursors tetrraethylortosilicate (TEOS) and methyltriethoxysilane (MTES) and as organic part algal heteropolysacharide have been used. A poly-step sol-gel procedure is used at strictly controlled conditions in order to obtain the desired nanostructred materials.

The cells of *Bacillus sp*. UG-5B and *Humicola lutea* 120-5 were observed by scanning electron microscopy (SEM), (Dobreva *et al*., 1998).

Results and Discussion

Hydrolyzing activity of the nitrilase from *Bacillus sp*. UG-5B was established towards aliphatic, carboxylic and heterocyclic nitriles. This enzyme possesses a broad substrate specifity. The enzyme activity towards benzonitrile was assumed as 100%. The other results for the nitrilase activity are calculated towards the activity (0.5 U/mg protein) of the cell suspension. As it can be seen (Table 1) highest enzyme activity was shown towards p-tolunitrile for both free and entrapped cells compared to o- and m-tolunitrile. The influence of the concentration of the substrates was also shown. It is evident that the increase in the concentration up to 40 mM did not lead to increase in the nitrilase activity. Higher concentrations of these toxic substrates probably affect negatively the cells and their enzyme systems. Certain differences were established between free and entrapped cells which can be devoted to the formation of a barrier around the entrapped cells in the cases when the enzyme activity is higher than free cells. This barrier acts as a defence towards the toxic effect of these substrates. The same fact was proved by Graham *et al*., (2000) who stated the immobilization inside a gel lead to additional stabilization of the cells towards high concentration of organocyanide substances. By immobilization an increase of the cells tolerance towards substrate and product inhibition was also achieved. For the remaining biocatalysts where the enzyme activity of free cells is higher than the entrapped ones it can be suggested that the formation of barrier may serve as a hindrance for the penetration of the substrate inside the cells.
Table 1 represents the relative nitrilase activity of free and entrapped in the hybrid matrix cells, using three different substrates in three concentrations. The influence of the type of substrate and its concentration is followed.

Table 1. Relative nitrilase activity in relation to the type of substrate and its concentration

Substrate, Concentration	Relative enzyme activity of free cells, %	Relative enzyme activity of entrapped cells, %
20 mM o-tolunitrile	14	18
30 mM o-tolunitrile	8	16
40 mM o-tolunitrile	10	6
20 mM m-tolunitrile	30	90
30 mM m-tolunitrile	20	15
40 mM m-tolunitrile	9	14
20 mM p-tolunitrile	100	105
30 mM p-tolunitrile	30	45
40 mM p-tolunitrile	28	22

The SEM images present the two types of microorganisms incorporated to the hybrid matrix (Fig.1, Fig. 2).

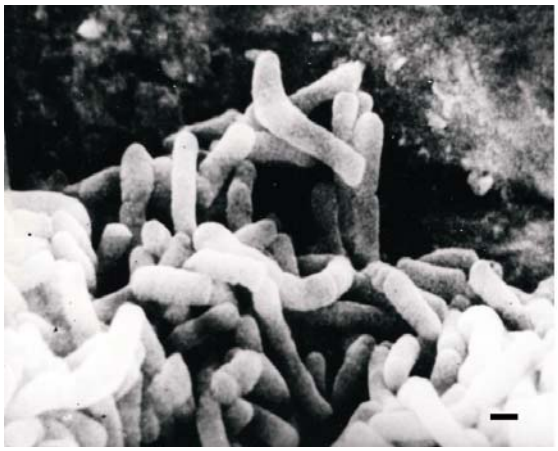

Fig. 1. SEM image of *Bacillus sp.* UG-5B cells, Bar=0.5µm

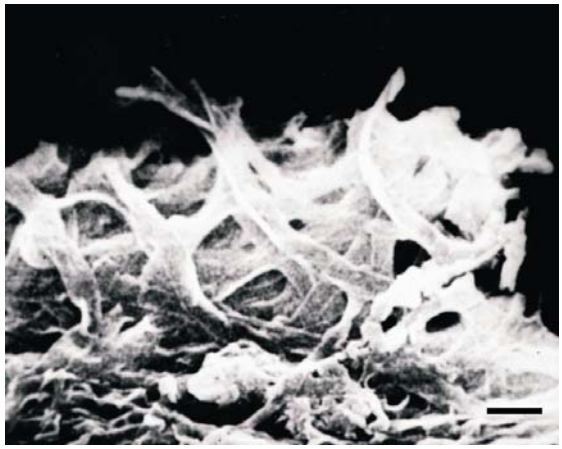

Fig. 2. SEM of mycelium of *H.lutea* 120-5, Bar=5µm

α-Galactosidase production by immobilized in the same matrix *H. lutea* 120-5 as compared to free cells (Fig. 3) during batch shake flasks fermentation with equal amount of spores (5.0 ml) with a density of 10^5 and 10^{10} spores per ml was investigated. A rapid increase in the enzyme titer after 24 h and 48 h for free and entrapped spores respectively was observed and a peak was found at 120 h. The maximum activity obtained with immobilized mycelium (4.9 U/flask) using spore suspension with a density of 10^5 spores per ml was 65% lower than that registered with free mycelium (14 U/flask – 100%). In the case of 10^{10} spore suspension density the enzyme titer of immobilized cells was greater which corresponded to 55% compared to free cells (23 U/flask). The particles formed after 120 h of batch cultivation when the maximal value for the enzyme activity was reached using both spore suspension densities were ready for re-use by means of their transfer into fresh medium at every 120 h.

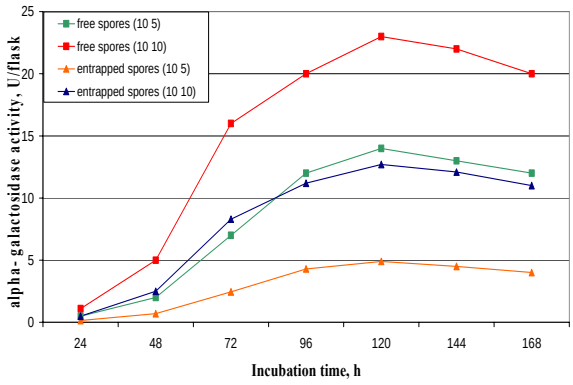

Fig. 3. α-Galactosidase activity in batch fermentation with free and entrapped in hybrid matrix spores of *H. lutea* 120-5

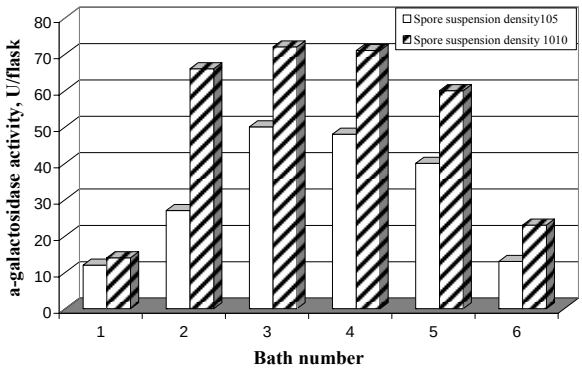

Fig.4 α-Galactosidase production by immobilized *H. lutea* mycelium during repeated use in sequential 120 h batch cultures in shake flasks

The enzyme titer quickly increased with repetition of the reaction cycles (Fig. 4) and reached maximum levels (50 and 72 U/flask) in the case of 10^5 and 10^{10} spore suspension density respectively in the third batch. The half-life of enzyme production was four reaction cycles. Consequently significant improvement (from 2 to 3-fold) in the yield of the immobilized system for

the period of 20 days was registered while in the case with free cells the enzyme activity decreased rapidly due to the cell lysis.
These results are in accordance with our previous results concerning acid proteinase and acid phosphatase production by immobilized *H. lutea* cells. (Aleksieva *et al*., 1998, Aleksieva *et al*., 2003).

Conclusions

Finally we can say that the synthesized matrix on the basis of TEOS and algal polysaccharide was successfully used in the immobilization of *Bacillus sp*. UG-5B for treatment of substances with cyanide groups in their composition. The investigated matrix also 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 (Fig.2), while the biosynthetic capability is preserved and the values for the enzyme titer exceeded three-fold the α-galactosidase activity of the free cells.

Acknowledgement

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