BIOENCAPSULATION OF WHOLE CELLS WITH CIS-EPOXYSUCCINATE HYDROLASE ACTIVITY IN SODIUM ALGINATE-CELLULOSE SULFATE-POLY(METHYLENE-CO-GUANIDINE) CAPSULES USING HIGHLY CONTROLLED IMMOBILIZATION PROCESS

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Bioencapsulation has been used as an immobilization technique for biosystems in a number of applications including biotechnological (Štefuca et al. 1991), pharmaceutical (Chang 1998), environmental (Stormo et al. 1992) etc.

Capsules made of high viscosity sodium alginate (SA) and cellulose sulfate (CS) as polyanions and the polycation poly(methylene-co-guanidine) (PMCG) in the presence of $CaCl_2$ as gelling and NaCl as anti-gelling agents in the polycation solution (SA-CS/PMCG capsule) have shown a high potential for encapsulation of islets of Langerhans (Lacík et al. 1998). Formation of uniform capsules achieved by employing a newly-designed multiloop reactor (Anilkumar et al. 2001) for continuous capsule production, is driven by electrostatic interactions under mild and physiological conditions and, importantly, the encapsulation process is very fast, which makes this process friendly for an encapsulating biological systems.

Nocardia tartaricans bacterial cells with cis-epoxysuccinate (CES) hydrolase activity are able to catalyse single step and stereospecific biohydrolysis of CES yielding enantiomerically pure L-(+)-tartrate (Miura et al. 1977) which is, as acid, used in the food industry, pharmaceutical industry, chemical analysis, textile industry and cosmetics (Changmao Biochem: http://www.cmbec.com/en/product3.htm). Additionally, this strain is easy to grow and handle (Davies et al. 1989) and therefore it is well suited as a model for bioencapsulation.

The main objective of presented work (Bu*f*ko et al. 2004). was to encapsulate the whole-cell biocatalyst into SA-CS/PMCG capsules. The storage stability, time of total biotransformation, purity and yield of product as well as the viability of encapsulated cells in capsules is compared with cells entrapped in calcium pectate gel (CPG) beads. Additionally, this work was focused on the encapsulation process aimed at the fast production of highly uniform batches of capsules in terms of shape, size and membrane thickness. This approach can be deemed as an important impulse to the field of immobilized biotechnology, which requires well-defined immobilization systems for a proper understanding of the performance of encapsulated biosystems.

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MATERIALS AND METHODS

Immobilization of cells:

Encapsulation. The cells were obtained as reported earlier (Vikartovská et al. 2004), followed by lyophilization. Polyanion solution (PA) was prepared from 0.8% (w/v) SA and 0.8% (w/v) CS in 0.9% (w/v) NaCl at pH 7.0. Selected mass of lyophilized cells of *N. tartaricans*, with an initial activity of CES hydrolase of 12.4 U/mg, was suspended in PA solution to obtain concentrations from 7 to 9 mg/ml. Drops of PA solution at a flow rate of about 1.0 ml/min were air-stripped into the stream of polycation (PC) solution consisting of 1.8% (w/v) PMCG, 1.0% (w/v) CaCl2 and 0.9% (w/v) NaCl at pH 7.0 continuously flowing in the multiloop reactor (Anilkumar et al. 2001) at a flow rate of 45 ml/min, providing a reaction time of 100 s. The reaction was quenched by collection of capsules at the exit of the reactor in batches of 0.9% NaCl. 1 M CES has been used as a storage solution for immobilized cells (Rosenberg et al. 1999).

Entrapment. A commercial high-performance bead generator with open 13 nozzle system and feed pump Var E (Nisco Engineering AG, Zürich, Switzerland) was used for production of CPG beads (Vikartovská et al. 2004), followed by a two-stage hardening procedure as reported previously (Kurillová et al. 2000).

Capsule size and membrane thickness:

Values for two perpendicular diameters and four membrane thicknesses at positions 90° apart were taken for each capsule using capsule imaging provided

by optical microscope equipped with a digital camera (Bučko et al. 2004). The camera was interfaced to a PC operating with image analysis software. Average diameter, membrane thickness and standard deviation of both parameters were obtained by analysis of 20 randomly selected capsules from each capsule batch.

Biotransformations and analytical techniques:

A wet, either SA-CS/PMCG capsules or CPG beads with cells were placed in 10 ml flasks with fresh substrate. The biotransformation of CES to L-(+)-tartrate in batch-wise mode was carried in water shaking bath. The course of the biotransformation as well as purity and yield of product was followed by three complementary techniques, namely reversed-phase high-performance liquid chromatography (RP-HPLC), optical rotation and electrospray ionization mass spectrometry (ESI-MS) (Bučko et al. 2004). One unit of activity of CES hydrolase was defined as the amount of enzyme capable of generating 1 Ÿmol of disodium L-(+)-tartrate per hour under experimental conditions. Viability of biomass in SA-CS/PMCG capsules and CPG beads was determined using bioluminometry as reported previously (Navrátil et al. 2000).

Storage stability. The storage stability over 51 days of storing the immobilized cells in 1 M substrate CES at 6°C was assessed by biotransformation measurements monitoring the production of L-(+)-tartrate using the RP-HPLC technique.

RESULTS AND DISCUSSION

Encapsulation of N. tartaricans cells:

Figure 1 depicts the encapsulated *N. tartaricans* cells in SA-CS/PMCG capsules after 21 days storage in substrate (Bučko et al. 2004). The capsule uniformity in size and membrane thickness is documented by the statistical analysis for capsules with the encapsulated cells given in Table 1. Figure 1 also illustrates that bacterial cells settle down to the bottom of the capsule core, which has been a regular observations

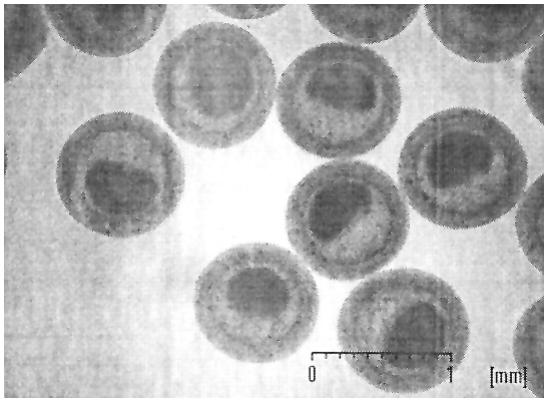

Figure 1. Detail of SA-CS/PMCG

Table 1. Statistical analysis of capsule diameter and membrane thickness

Concentration of biomass in polyanion (mg/ml)	Capsule diameter (mm)	Membrane thickness (mm)
0	0.79±0.01	0.13±0.01
7	0.75±0.03	0.16±0.01
8	0.91±0.02	0.17±0.01
9	0.83±0.03	0.15±0.01

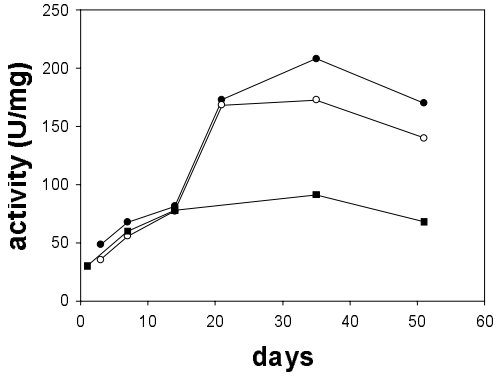

Figure 2. Storage stability of N. tartaricans capsules containing N. tartaricans cells in SA-CS/PMCG capsules and in cells CPG beads.Capsules contained 7 mg/ml (■) and 9 mg/ml (●) cells, CPG beads contained 8 mg/ml (○) cells

accompanying encapsulation of cells in the SA-CS/PMCG capsules.

Performance of immobilized cells in biotransformations:

Storage stability. The activity of CES hydrolase reaches the maximum value 35 days after encapsulation in SA-CS/PMCG capsules, i.e., 208.2 and 172.8 U/mg. The evolution of storage stability for the cells entrapped in the CPG beads, given in Figure 2, show that the maximum of CES hydrolase activity, reached after 35 days is roughly half that obtained with the SA-CS/PMCG capsules and equals to 91.5 U/mg determined by HPLC (Bučko et al. 2004).

Time of total biotransformation. For the highest enzyme activity in encapsulated cells of 208.2 U/mg, only 3 hours are needed for completion of biotransformation. The latter value is about half the time 5.5 hours observed for the complete biotransformation using cells entrapped in CPG beads at the highest CES hydrolase activity of 91.5 U/mg.

Viability of immobilized cells. Since the immobilized cells lost their viability after 14 days after encapsulation or 1 day after entrapment, the biosynthesis of CES hydrolase de novo can be excluded as the reason of increased enzyme activity as shown in Figure 2.

Purity and yield of product. Three complementary analytical techniques showed with excellent agreement that 96% yield of pure L-(+)-tartrate was obtained from biotransformation using SA-CS/PMCG capsules, which was very similar to 94% achieved by entrapment in CPG beads.

CONCLUSION

This work was focused on applying an advanced encapsulation protocol providing the uniform capsules used for encapsulation of the model microbial strain *N. tartaricans* cells with the CES hydrolase activity. The performance of encapsulated cells in SA-CS/PMCG capsules was indeed improved when compared to that of the CPG entrapped cells, expressed in enzyme activity and time of total biotransformation. Whether or not this observation is connected with the capsule uniformity remains to be answered, when the other reasons for different performance between encapsulated and entrapped cells may include effects on permeabilization of the cells, the accessibility of the enzyme by substrate and the differences in the immobilization protocols. Regardless of this, using the well-defined uniform immobilization materials should become a merit in the field of biotechnology, engineered in order to assist in understanding and modeling the performance of the immobilized biosystems.

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