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Immobilization of a whole-cell epoxide-hydrolyzing biocatalyst in sodium alginate−cellulose sulfate−poly(methylene-*co*-guanidine) capsules using a controlled encapsulation process

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Abstract

A whole-cell epoxide-hydrolyzing biocatalyst designed as uniform polyelectrolyte complex capsules was developed. *Nocardia tartaricans* bacterial cells with *cis*-epoxysuccinate (CES) hydrolase activity were used as a model microbial strain. Stereospecific hydrolysis of CES catalyzed by CES hydrolase in encapsulated cells yields enantiomerically pure L-(+)-tartrate. An air-stripping device with multiloop reactor was used for controlled production of capsules containing bacterial cells with the standard deviation in diameter below 4% and in membrane thickness below 7%. Capsules formed by polyelectrolyte complexation of sodium alginate and cellulose sulfate as polyanions, poly(methylene-*co*-guanidine) as polycation, $CaCl_2$ and NaCl (SA-CS/PMCG) provide a favorable microenvironment for encapsulated cells. Biotransformation was monitored by RP-HPLC, electrospray ionization mass spectrometry and optical rotation. The results are discussed in view of data previously obtained by cell entrapment in hardened calcium pectate gelled beads (CPG). Encapsulation of the whole-cell biocatalyst in SA-CS/PMCG capsules leads to (i) about two-fold increase, from 91.5 to 208.2 U/mg, in the CES hydrolase activity, (ii) a decreased time required for total biotransformation, from 5.5 to 3 h, and (iii) a significantly improved relative increase in CES hydrolase activity during 51 days of storage, from about 3-fold to about 20-fold, compared to the CPG beads.

Keywords: Bioencapsulation; Immobilization; Capsule; Sodium alginate; Cellulose sulfate; Poly(methylene-co-guanidine); Biocatalyst; L-(+)-Tartaric acid; *cis*-Epoxysuccinate hydrolase; *Nocardia tartaricans*

1. Introduction

Bioencapsulation has been commonly used as an immobilization technique for biosystems in a number of applications including biotechnological [1–5], pharmaceutical and therapeutical [3,6,7], environmental [8] and dairy-product industries [5]. In particular, it has been used with immobilized whole-cells and enzymes as industrial biocatalysts for production of fine chemicals via biotransformations [9–11]. Chiral substances are the main products of biocatalytic routes [11], while hydrolases are the most widely used enzymes in biotransformations [11,12].

Epoxide hydrolases (EC 3.3.2.3) are versatile biocatalysts for the asymmetric hydrolysis of epoxides requiring neither cofactors, prosthetic group nor metal ions for their activity [13]. The industrial synthesis of L- and meso-tartaric acids was the first application of an epoxide hydrolase catalysed epoxide hydrolysis [14]. *Nocardia tartaricans* cells with *cis*-epoxysuccinate (CES) hydrolase activity are able to catalyse single step and stereospecific hydrolysis of disodium CES yielding enantiomerically pure disodium L-(+)-tartrate. Iso-

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lation and purification of CES hydrolase from *N. tartaricans* was patented in 1977 [15]. Since then, despite the attractiveness of epoxide hydrolases, CES hydrolase has not been isolated and feasible approaches for the microbial production of L-(+)-tartaric acid from CES are solely based on whole cells possessing CES hydrolase activity and not the isolated pure enzyme CES hydrolase. The possible difficulties encountered with the isolation of pure epoxide hydrolases have been described by Kroutil et al. [16]. The epoxide hydrolase, acting on 2-methyl-2-pentyloxirane or (±)-*cis*-2-heptene oxide, was isolated from *Nocardia sp*. EH1 in a low yield and a partially purified enzyme of limited stability was obtained.

L-(+)-Tartaric acid is widely used in the food industry, pharmaceutical industry, chemical analysis, textile industry and cosmetics [17]. The biotechnological production of enantiomerically pure L-(+)-tartaric acid from CES is preferable to the chemical conversion of the racemic DL-form to pure L-form [15]. Enantiomeric purity of L-(+)-tartaric acid is important for highly enantioselective processes such as Sharpless epoxidation [18].

N. tartaricans is a bacterial strain which is easy to grow and handle [19]. Therefore, it is well suited as a model biocatalyst for encapsulation. Previously, covalent linking [20] and ionic binding [21] methods were used for immobilization of biocatalysts with epoxide hydrolase activities. Entrapment was used for immobilization of *N. tartaricans* cells [22–24] and *Corynebacterium* sp. cells [25], both having CES hydrolase activity. Using these entrapment techniques, the microenvironment of immobilized cells can be characterized as a dense polymer network.

It is desirable to employ capsules for immobilization of bacterial cells and to compare their behavior to the previously studied immobilization techniques [22–24]. 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 [26,27]. Specifically, a great attention has been paid to the uniformity of capsule batches, which naturally is of enormous importance for correct reading of the response of any encapsulated biosystems. The capsule uniformity was achieved by employing a newly designed multiloop reactor [28] for continuous capsule production. Moreover, capsule formation is driven by electrostatic interactions under mild and physiological conditions and, importantly, the encapsulation process is very fast ranging from a few tens of seconds maximally up to a few minutes, which makes this process friendly for an encapsulating biological systems. Therefore, this type of capsules possesses all the attributes required for rigorous studies into performance of encapsulated bacterial cells in the desired bioransformation of disodium CES to disodium L-(+)-tartrate.

The main objective of the present paper is, in conjunction to our previously published work on entrapment of *N. tartaricans* cells in the calcium pectate gelled (CPG) beads [22–24], to encapsulate the whole-cell biocatalyst into SA-CS/PMCG capsules. The CES hydrolase activity, time of total biotransformation, storage stability, purity and yield of product as well as the viability of encapsulated cells in both systems is compared. Additionally, this work is 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.

2. Materials and methods

2.1. Microorganisms and cultivation conditions

Nocardia tartaricans ATCC 31191 cells were grown aerobically at 30 °C for 24 h in 50 ml sterile medium containing 0.3% (w/v) meat extract Fluka (Buchs, Switzerland), 1% (w/v) peptone Fluka (Buchs, Switzerland), 1% (w/v) disodium *cis*-epoxysuccinate (CES) and salts 0.3% (w/v) NaCl, 0.3% (w/v) KH_2PO_4, 0.2% (w/v) $MgSO_4{\cdot}7H_2O$, 0.001% (w/v) $FeSO_4{\cdot}7H_2O$, 0.003% (w/v) $CaCl_2{\cdot}2H_2O$, 0.003% (w/v) $MnSO_4{\cdot}4H_2O$. Under sterile conditions, eight aliquots of inoculum, each of 5 ml, were withdrawn and transferred into eight 250 ml flasks containing 100 ml of the above described medium and cultivation proceeded for additional 30 h at 30 °C. Cells were collected by centrifugation, thoroughly washed with distilled water to remove residuals from culture medium, lyophilized and stored at −5 °C.

2.2. Chemicals

High viscosity sodium alginate (SA) was kindly donated from ISP Alginates (Girvan, Ayrshire, UK); cellulose sulfate (CS), sodium salt was from Acros Organics (New Jersey, NJ, USA); poly(methylene-*co*-guanidine) hydrochloride (PMCG) from Scientific Polymer Products Inc. (Ontario, NY, USA) supplied as 35% aqueous solution was lyophilized; potassium pectate was prepared from commercial apple pectin from Pectin-Fabrik (Smiřice, Czech Republic) by Institute of Chemistry of SAS (Bratislava, Slovakia, http://www.saccharides/products/catalog.pdf), polyethyleneimine, under the trade name Sedipur CL 930, was purchased from BASF (Ludwigshafen, Germany); glutaraldehyde, 50% aqueous solution was supplied from Fluka (Buchs, Switzerland); disodium *cis*-epoxysuccinate (CES) was prepared and purity was determined as reported previously [24]. All inorganic salts used for preparation of media and solutions were of analytical grade.

2.3. Characterization of polymers

Polyelectrolytes used for capsule manufacturing were characterized in terms of molecular weight (SA, CS and