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Robust enzyme immobilizates for industrial isomalt production

Frank Hellmers [a], Ralf Takors [b,*], Oliver Thum [a,*]

[a] Evonik Creavis GmbH, Paul-Baumann-Str. 1, 45772 Marl, Germany
[b] Universität Stuttgart, Institut für Bioverfahrenstechnik, Allmandring 31, 70569 Stuttgart, Germany

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ABSTRACT

The isomerization of sucrose to isomaltulose for the production of isomalt using the enzyme sucrose isomerase (SI) [EC5.4.99.11] is a known industrial process preferably using immobilized biocatalysts. We describe a novel immobilization method that has been developed to overcome disadvantages of current immobilization techniques and to meet todayís industrial requirements. Current methods are primarily very process specific and often suffer from scale-up limitations, thus preventing their industrial use. We demonstrate, that industrially already established processes can be replaced by cost-effective formulation technologies comprising of only a few process steps for producing the heterogeneous biocatalyst. Feasibility studies simulating industrial process conditions show that the granules provide good activity and sucrose conversion enabling their application in a fixed-bed reactor.

1. Introduction

Sugar alcohols, also known as polyol sweetener, are increasingly important for sugar replacement and low caloric human nutrition. In contrast to high potency sweetener, Polyol sweetener can provide bulk to the product, resulting in application properties similar to sucrose [1]. Especially the polyol sweetener Isomalt. commercially known as Palatinit (current tradename released by Südzucker AG), has attracted the food industry in the last decades due to several advantages, such as no contribution to the formation of caries, its low glycemic index and relatively small caloric value [2] For these reasons Isomalt is beneficial for patients suffering from diabetes. Recent studies suggest that Isomalt has even prebiotic properties, promoting the growth of *Bifido* bacteriae [3]. Isomalt is obtained by hydrogenation of the keto group of isomaltulose, yielding an equimolar mixture of the diastereomers 1,6-GPS (6-*O*-alpha-D-glucopyranosyl-D-sorbitol) and 1,1-GPM (1-*O*-alpha-D-glucopyranosyl-D-mannitol). Isomaltulose is produced exclusively by biocatalysis, was first described by Weidenhagen and Lorenz [4], and intensive toxicological studies revealed no adverse health effects [5].

The production of isomaltulose from sucrose is catalyzed by the enzyme sucrose isomerase (SI) [EC 5.499.11], also named isomaltulose synthase or sucrose α-glucosyltransferase, usually with a yield of 82–85% isomaltulose [6]. SI catalyzes the rearrangement of the glycosidic α (1–>2) – linkage between glucose and fructose in sucrose molecules into an α (1–>6) – linkage forming isomaltulose. Besides isomaltulose, the byproducts trehalulose, fructose, glucose and small amounts of oligosaccharides are formed, as shown in Fig. 1. Today, a variety of bacteria are known to be capable of forming isomaltulose from sucrose, but to our knowledge only the SI containing strain *Protaminobacter rubrum* is used for the commercial production.

As resting whole cells containing SI can be used in this biotransformation, our industrial isomerization process makes use of immobilized cells in a continuous packed-bed reactor. In such processes cells are entrapped in a calcium alginate matrix, yielding relatively low enzyme cost contributions due to long enzyme half-life of up to 8500 h [6,8]. However, to achieve this performance under industrial conditions several modifications have to be introduced. The major disadvantage of the use of calcium alginate is its rapid dissolution in the presence of only moderate concentrations of chelation agents and/or certain cations (for example buffered solutions) [9]. To obtain sufficient stability against disintegration or mechanical stress internal cross-linking with glutaraldehyde or polyethylene imine can be applied [6] at the cost of reduced enzyme activity [8] and the use of harmful chemicals.

* Corresponding authors.
E-mail addresses: takors@ibvt.uni-stuttgart.de (R. Takors), oliver.thum@evonik.com (O. Thum).

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Table 1
Overview on published immobilization strategies for SI.

Method	material	Strain	Ref.
Binding on carrier	Ionic binding: DEAE-Cellulose; polystyrole	*P. rubrum, E. rhapontici, S. plymuthica*	[11]
	Covalent binding: Sepharose 4 B CL	Isolated enzyme from *P. rubrum*	[12]
	Ionic binding stabilized by aldehyde crosslinking	Isolated enzyme from *P. rubrum*	[13]
Entrapment	Alginate, $CaCl_2$	*Erwinia rhapontici NCPPB 1578*	[14]
	Alginate, $CaCl_2$, stabilized by PEI and glutaraldehyde	*P. rubrum*	[6]
	Alginate, $CaCl_2$	*Erwinia* sp.	[15]
	Alginate, $CaCl_2$; DEAE-Cellulose; Polyacrylamide; K-Carrageen; Bone-Char; Xanthan; Agar-Agar	*P. rubrum, E. rhapontici, S. plymuthica, S. marcescens, E. carotovora var. atroseptica*	[16]
	Alginate, $CaCl_2$	*P. rubrum*	[17]
	Chitosane	*S. plymuthica*	[18]
Cross-linking	Tannine, polyethylenimine, glutaraldehyde	*P. rubrum*	[19]
	Glutaraldehyde	*E. rhapontici*	[16]
Ultrafiltration/membranes	Enzyme Membrane Reactor	*P. rubrum*	[12]
	Hollow-fibre bioreactor	*S. plymuthica*	[20]

Whereas there are numerous, universal immobilizations strategies reported [10], some even applied for SI (see Table 1), none managed to fulfil the dual demand of achieving long-term stability together with high specific activity to allow its industrial application without using potentially harmful substances.

Herein we report a novel and robust immobilization strategy for *P. rubrum* cells expressing SI that yields particles with good specific activity without the use of harmful chemicals such as polyethylene imine or glutaraldehyde. Moreover, the process is based on standard unit operations such as granulation and thus can be easily scaled-up to relevant industrial application.

2. Experimental

2.1. Biomass production

P. rubrum (CBS574.77) cells were produced according to lit [21]. in 20L scale using a Sartorius Biostat D fermenter. Aliquots were stored at −80 °C and thawed prior to use. To take into account any activity loss due to storage a sample of the used aliquot of free cells was always included as reference in the activity assays. To evaluate the effect of drying on the enzyme activity wet cells were dried by placing 1 mL of wet biomass onto a petri dish and placing the dish into an oven (Heraeus B12/UB12 Function Line) for 5 h at different temperatures. Cells were rehydrated in 1 mL of water prior to use.

2.2. Carrier materials

The following carriers have been used in this study:

Carrier	Chemical composition	Particle size [mm]	Specific surface [m^2/g]	Supplier
Sipernat Exp 4432-1	Precipitated silicic acid	0.4–1.25	450	Evonik Industries AG
Sipernat Exp 4441-1	Precipitated silicic acid	0.4–1.25	294	Evonik Industries AG
Sipernat Exp 4056-1	Precipitated silicic acid	0.4–1.25	175	Evonik Industries AG
Sipernat 320	Precipitated silicic acid	0.4–1.25	180	Evonik Industries AG
Duolite A568	Crosslinked phenol-formaldehyde polycondensate	0.15–0.85	150–200	Dow Deutschland GmbH
Lewatit VP OC 1600	Polymethylmethacrylat (PMMA)	0.32–0.45	130	Lanxess AG
Eupergit C250 L	Copolymer of methacrylamide, N,N′-methylen-bis(acrylamide) and a monomer carrying oxirane groups	0.1–0.25	150–200	Evonik Industries AG

2.3. Binder materials

The following Eudragit-type binders (Evonik Industries AG, Essen, Germany) have been used in this study:

Eudragit NE: Ethyl Acrylate Methyl Methacrylate dispersion, containing 1.5% Nonoxynol 100 as an emulsifier

Eudragit NM: Ethyl Acrylate Methyl Methacrylate dispersion, containing 0.7% macrogol stearyl as an emulsifier

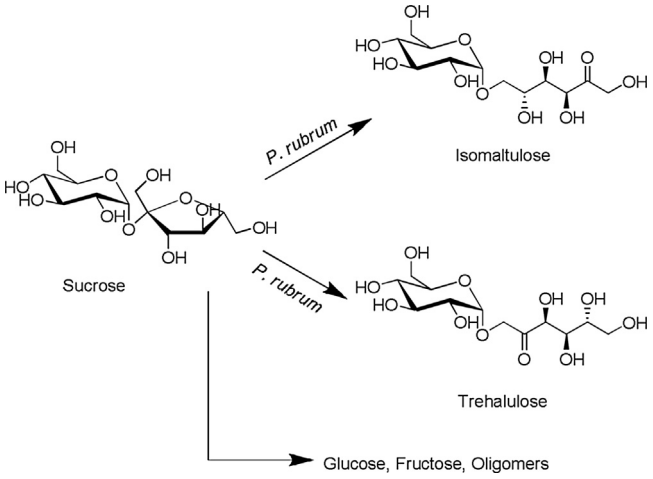

Fig. 1. Isomerisation of sucrose to isomaltulose and formation of the side products trehalulose, glucose, fructose and various types of oligomers [7].

Eudragit RL: Aminoalkyl methacrylate copolymers, copolymer ratio of 1:2:0.1 for fast release profile

Eudragit RS: Aminoalkyl methacrylate copolymers, copolymer ratio of 1:2:0.1 for fast release profile.

2.4. Cell adsorption on carrier

1 g of the corresponding carrier material was transferred into 15 mL falcon tubes. 4 mL of 0.1 M potassium phosphate buffer solution (pH = 6.0) as well as 1 mL of a cell suspension of known biomass concentration were added. The mixture was incubated at rt for 24 h on a rotary shaker (Certomat R, B. Braun, Melsungen, Germany) at 150 rpm.

Afterwards the supernatant was separated and transferred to a fresh falcon tube. The carrier was washed by incubating twice with each 4 mL of 0.1 M potassium phosphate buffer solution (pH = 6.0) for 10 min at 150 rpm. The washing fractions were added to the