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Immobilization of α-amylase on poly(vinylamine) and poly(vinylformamide) supports and its performance

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ABSTRACT

α-Amylase was immobilized on six poly(vinylamines) and three poly(vinylformamides) hydrogels polymerized using various techniques and crosslinkers. The enzyme was covalently bound to the supports using glutaraldehyde as a spacer. The immobilization procedure was optimized involving such factors as temperature, pH, time, sequence of reactions, and kind of carrier employed. Results of the immobilization were evaluated based on analyses of the enzyme activity and stability prior and after immobilization, as well as on the immobilization yield and stability. Highly active biocomposite preparations were designed which provided their multiple application for starch hydrolysis. The selection of a carrier was essential for the activity and stability of immobilized α-amylase. Poly(*N*-vinylformamide) crosslinked with divinylbenzene in form of spherical beads obtained in a suspension polymerization appeared to be a superior carrier for α-amylase.

1. Introduction

Amylases play essential role as hydrolyzing enzymes widely used in food, fermentation, textile, and paper industry [1]. α-Amylase (1,4-α-D-glucan-glucanhydrolase, EC. 3.2.1.1) is an endo-acting, widely distributed enzyme that hydrolyzes the α-1,4-glycosidic bonds, by-passing α-1,6-glycosidic linkages in starch and related substrates. Amylases are available from various sources, including plants, animals and microorganisms [2–5]. Their immobilization on water insoluble carriers seems to be the most promising way to obtain more stable products for multiple use [6–14].

There are several methods for immobilizing insoluble enzymes. For that purpose, enzymes are enveloped into a gel matrix, encapsulated, incorporated into emulsions and membranes, bound to a support by either adsorption, coordination or covalent binding. In fact, the binding of an enzyme to a support is most common. Therefore, the selection of a suitable carrier for a given enzyme and a way of fixing enzyme to it are key problems. Glutaraldehyde renders the highest enzyme stability when bound to a support provided the support has amino moieties on its surface [15–18]. The amination introducing such groups to the support most commonly involved carcinogenic ethyleneimine. Use of ethylenediamine was an alternative approach. Fortunately, few years ago the precursor of polyvinylamine, PVAm, i.e. *N*-vinylformamide, NVF, became commercially available. Its polymer, poly(*N*-vinylformamide), PNVF, can be easily hydrolyzed to PVAm making the amination unnecessary. In this work, PVAm and PNVF have been applied as carriers for enzymes. These carriers have been produced in form of spherical beads suitable also for packing in columns where hydrodynamical factor should be considered.

Effect of the immobilization depends on several parameters such as surface area, accessibility of the surface for enzymes, number of activated functional groups on the support, availability for binding the functional groups on the protein, spacer used, distance between the bound enzyme and the surface of the support, and the steric orientation of the active centre. Mono- or multipoint binding of an enzyme to the support, the chemical affinity of protein to the material of the support are further factors influencing immobilization [19–21].

In this paper, immobilization of highly active, thermostable α-amylase on poly(vinylamine) (PVAm) and poly(*N*-vinylformamide) supports is described. Glutaraldehyde was used as a spacer.

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Table 1
Origin of immobilizing hydrogels.

Sample	Polymer	Monomer	Crosslinker	Polymerization type	Hydrolysing or rinsing agent
PVAm-1	PVAm	NVF	NMBA	Miniemulsion	KOH
PVAm-2	PVAm	NVF	NMBA	Miniemulsion	HCl
PVAm-3	PVAm	NVF	3A2H	In mass	KOH
PVAm-4	PVAm	NVF	DVB	In mass	HCl
PVAm-5	PNVF	NVF	DVB	In mass	NH_3[a]
PVAm-6	PNVF	NVF	DVB	Suspension	NH_3[a]
PVAm-7	PNVF	NVF	DVB	Suspension	None
PVAm-8	PVAm	NVF	DVB	Suspension	NaOH
PVAm-9	PVAm	NVF	DVB	Suspension	HCl

[a] Crosslinked poly(*N*-vinylformamide) was yielded.

2. Materials and methods

2.1. Materials

α-Amylase SPEZME® PRIME 107-05127-001 (Genencor International, USA) from a genetically modified strain of *Geobacillus stearothermophilus*, had 7163 U/g at pH 5.0–6.5 at 35–50 °C.

Hydrogels: poly(vinylamines) (PVAm) were prepared from *N*-vinylformamide (NVF) which was polymerized using three different crosslinkers. Six of so prepared poly(*N*-vinylformamides), PNVFs, were submitted to either base or acid hydrolyses, respectively, while in three cases the support was provided by PNVFs (Table 1). All the carriers were activated with glutaraldehyde (GA) functioning as a spacer between support and the enzymatic protein [22].

PVAm-1 and PVAm-2 supports were derived from PNVFs polymerized in microemulsion, crosslinked with *N*,*N*′-methylenebisacrylamide (NMBA), and hydrolyzed either by aqueous KOH or hydrochloric acid.

The PVAm-3 carrier represented a polyvinylamine crosslinked with (3-acryloyloxy-2-hydroxypropyl)methacrylate (3A2H). The respective PNVF was hydrolyzed in aqueous KOH [22].

PVAm-4 resulted from hydrochloric acid hydrolysis of with divinylbenzene, DVB, crosslinked PNVF, obtained in an "in mass" polymerization. It was rinsed in ammonia. The same procedure was applied to PVAm-5, and PVAm-6, which were polymerized in suspension. Unlike PNVF-5, PNVF-7 was not rinsed with NH_3 before treatment with GA. PVAm-8, and PVAm-9 supports were also prepared from poly(vinylformamide) crosslinked with divinylbenzene via free-radical polymerization in reversed suspension. PVAm-8 resulted from base-catalyzed hydrolysis with aqeous KOH, whereas PVAm-9 was hydrolyzed with hydrochloric acid [22].

In all experiments sago starch (Wah Chang International Group of Companies, Singapore) was used as a substrate.

2.2. Methods

2.2.1. α-Amylase immobilization

A support (0.1 ± 0.0001 g) was placed in 0.1 M phosphate buffer (pH 7) (25.0 mL), enzyme (1.0 mL) was added, the whole was gently agitated for 2 h followed by 24 h storage at 4–6 °C then filtered through the cellulose filter paper with 5 μm pores (Whatman). The support with immobilized enzyme was washed subsequently with 0.1 M phosphate buffer pH 7, 0.1 M phosphate buffer containing NaCl (30 g/L), 0.1 M acetate buffer pH 5.5 and, finally, with distilled water. Such product was stored in phosphate buffer pH 7 (25 mL). Prior to the use the mixture was repeatedly filtered through the cellulose filter paper whereupon in the filtrate activity of non-immobilized enzyme was determined in the enzymatic reaction.

2.2.2. α-Amylase activity

Activity of non-immobilized and immobilized enzymes was determined on enzymatic hydrolysis of starch (2 mg/1 mL buffer) carried out at 37 °C ± 1 °C and pH 7 (0.1 M phosphate buffer). Regardless either immobilized or non-immobilized, the same amount of enzyme (2 μL/1 mg starch) was applied.

At first, starch was 20 min gelatinized at 85–90 °C in phosphate buffer. After cooling to 37 °C enzyme was added on gentle stirring

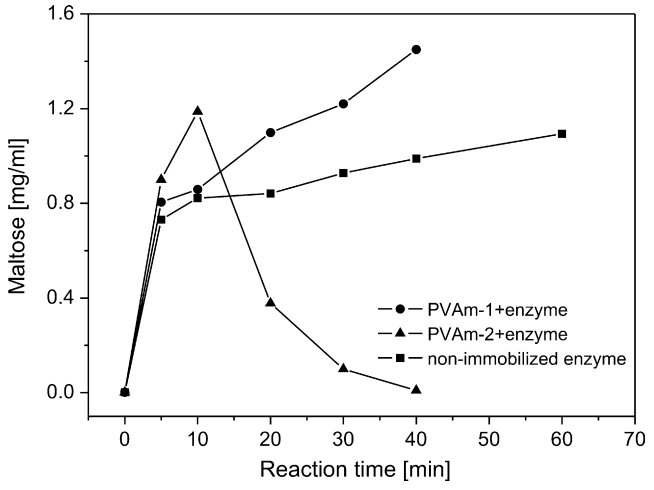

Fig. 1. Product output vs. time in starch hydrolysis catalyzed by α-amylase immobilized on PVAm-1 and PVAm-2 supports.

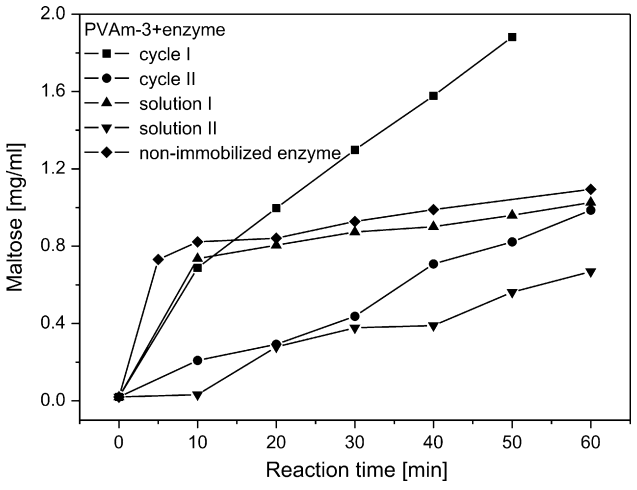

Fig. 2. Product output vs. time in starch hydrolysis catalyzed by α-amylase immobilized on PVAm-3 support.