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INVESTIGATIONS ON CARRIER SYSTEMS FOR ALPHA-AMYLASE ENZYME IMMOBILIZATION

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ABSTRACT

α-Amylase enzyme was immobilized onto gelatin carrier system. Chromium (III) acetate and potassium chromium (III) sulfate were used as crosslinking agents. Effect of carrier system composition on immobilization was investigated by combining different proportions of carboxymethylcellulose or polyacrylamide with gelatin. Considerable relative activity enhancements were achieved (41% for carboxymethylcellulose-gelatin and 25% for polyacrylamide-gelatin). Reusability's of immobilized enzymes were also investigated. Activities were stable at least for 20 uses.

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INTRODUCTION

α–Amylase (EC 3.2.1.1) catalyze the hydrolysis of α–1,4 glucosidic linkages in polysaccharides of three or more α–1,4-linked D-glucose units to produce maltose and larger oligosaccharides. α–Amylase converts starch to dextrin with sufficient hydrolysis. This makes the product soluble and not susceptible to gelling upon cooling. Glucoamylase may further break down dextrin to syrups of high glucose content at this point (1).

Gelatin (G) is a water soluble protein resulting from the partial hydrolysis of collagen and it is abundant in the animal kingdom. It has primarily hydroxyl, carboxyl and amino reactive groups. Gelatin reacts with some metal ions to form insoluble complexes. Carboxymethylcellulose (CMC) is the most widely used water soluble cellulose derivative; it also has hydroxyl and carboxyl functional groups. It is an inexpensive material specially used in detergents, foods, textile, pharmaceuticals, etc. Carboxymethylcellulose forms insoluble salts in water when reacted with some metal ions. Polyacrylamide (PAA) is a well-known support material in enzyme immobilization. It is a high molecular weight water soluble polymer (2-5).

Immobilized enzymes are widely used for various biochemical and biomedical processes, in food technology and in analytical applications. There are various methods used for the immobilization of enzymes. These procedures are divided into two broad categories, chemical and physical methods. A large variety of support materials, which may be inorganic or organic in structure, have been used for the attachment of enzymes (6,7).

Selection of support material is more critical in food technology than other applications of immobilization. In this study, gelatin, carboxymethylcellulose and polyacrylamide were chosen as support materials. All of them have suitable chemical properties and they are

inexpensive and physiologically inert. The scope of this work was to immobilize α–amylase onto gelatin and to investigate the effect of carrier system composition on relative activity.

EXPERIMENTAL

Materials

α–Amylase (1,4-α–D-glucanohydrolyse) lyophilized from porcine pancreas was purchased from Merck (Germany).

Soluble starch (extra pure) was the product of Merck (Germany) and used as substrate.

Carboxymethylcellulose was purchased from Sigma Chemical Co. (USA).

Gelatin was obtained from Croda Gelatin Co. (UK)

Polyacrylamide was supplied from Aldrich (USA) having an average molecular weight of about 5-6 million.

Polyester film precoated with photographic gelatin (100 μm) were obtained from Dupont De Nemours (Luxembourg).

Chemicals used in preparation of buffers and crosslinking agents were purchased from Merck (Germany).

Methods

Immobilization of α–amylase

Gelatin (7.5%), carboxymethylcellulose (7.5%) and polyacrylamide (3%) solutions were prepared by dissolving in phosphate buffer (pH 6.9, 20 mmol dm^{-3}). CMC-G or PAA-G solutions were mixed in different ratios to

obtain carrier systems. α–Amylase solution 0.3 cm^3 (30 U) and appropriate amounts of crosslinkers were added to the carrier system solutions at 32 °C to obtain a final volume of 10 cm^3. The solution was stirred for 1 minute and 0.1 cm^3 aliquots were taken and placed on polyester film strips. The strips were allowed to rest for 24 hours. All the immobilized samples were washed 3 times with phosphate buffer to release the unbounded enzyme.

Determination of α–amylase activity

Free and immobilized enzyme activities were determined according to the Bernfield method (8). Substrate was added to the solution after 2 minutes preincubation. The reaction mixture consisted of immobilized or free enzyme, substrate (starch) and phosphate buffer (pH 6.9, 20 mmol dm^{-3}) was incubated for 10 minutes at 25 °C. Then 2 cm^3 of 3,5-dinitrosalicylic acid was added to reaction mixtures and the solutions were heated for 5 minutes in boiling water bath to terminate the reaction. The activities were determined from the amount of reducing sugar produced. The absorbencies were measured at 546 nm by using Spectronic 20 D Milton Roy model spectrophotometer.

The activity of free and immobilized enzyme was calculated using the equation;

Volume Activity = 4950 δE (U/l)

(U/min) = (U/l) (l/min)

%Relative activities were calculated according to the following formula;

ra : {activity of complex / (total activity of free enzyme used for coupling - activity loss by enzyme leakage)}

ma : maximum value of ra in series of experiments

%ra : ra x 100 **%ma** : ra x 100 / ma

RESULTS AND DISCUSSION

In our previous works we immobilized α–amylase onto gelatin by using crosslinkers chromium (III) acetate (CA), potassium chromium (III) sulfate (PCS) and chromium (III) sulfate (CS). In those studies we obtained best results with potassium chromium (III) sulfate and chromium (III) acetate among chromium salts, so in this investigation we decided to use PCS and CA (9).

In our introductory tests we could manage to obtain mechanically stable immobilized samples by using 0.001 mol dm^{-3} PCS and 0.01 mol dm^{-3} CA and decided to use these crosslinker concentrations throughout the study. Relative activities obtained for a carrier system composed of pure gelatin with PCS and CA were found to be 12% and 11% respectively.

To examine the effect of carrier composition on relative activity immobilization experiments were performed for PAA-G proportions (w/w), 0.053, 0.111, 0.177, 0.250, 0.333 for CMC-G ratios (w/w), 0.053, 0.111, 0.177, 0.250, 0.333. In these experiments parameters other than carrier composition were kept constant. Carrier composition changes affected relative activity similarly. They had a positive effect on relative activity for low ratios and negative effect for high ratios. Optimum ratios were found to be 0.177 for PAA-G and 0.177 for CMC-G. Results are presented in Figures 1 and 2.

As seen from Figures 1 and 2 increasing PAA-G or CMC-G ratios at first stages increased relative activity. This can be explained by loosening of the carrier matrices (easier diffusion) with respect to pure G. Further increase of PAA, CMC content possibly caused increased enzyme binding with PAA, CMC (enzyme inactivation) which resulted with poorer relative activity yields. Maximum relative activities were obtained as 25% with PAA-G and 41% with CMC-G carrier systems.

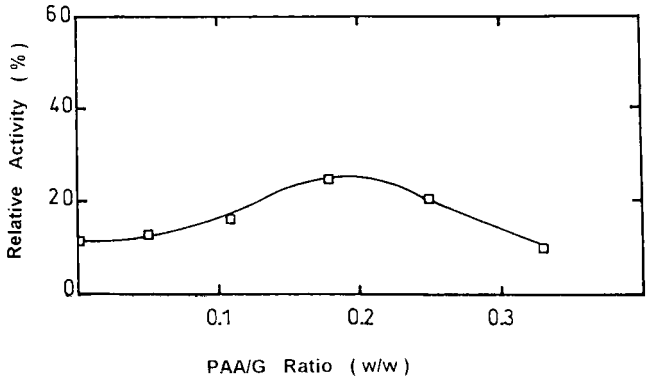

Figure 1. Effect of Polyacrylamide - Gelatin ratios on Immobilized α–Amylase activity (Chromium (III) Acetate: 0.01 mol dm^{-3})

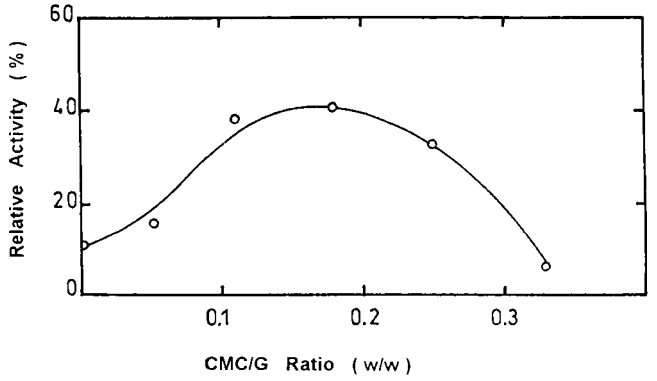

Figure 2. Effect of Carboxymethylcellulose-Gelatin Ratios on Immobilized α-Amylase activity (Potassium Chromium (III) Sulfate: 0.001 mol dm^{-3})

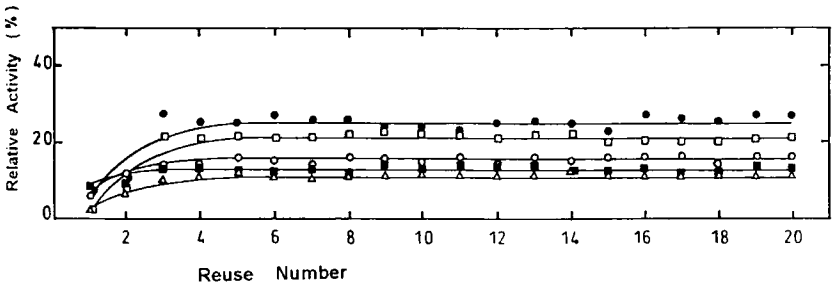

Figure 3. Reuseability of Immobilized α-Amylase with 0.01 mol dm^{-3} Chromium (III) Acetate for Polyacrylamide / Gelatin (w/w: △ :0.000, ■ :0.053, ○ :0.111 ● :0.177, □ :0.250) System

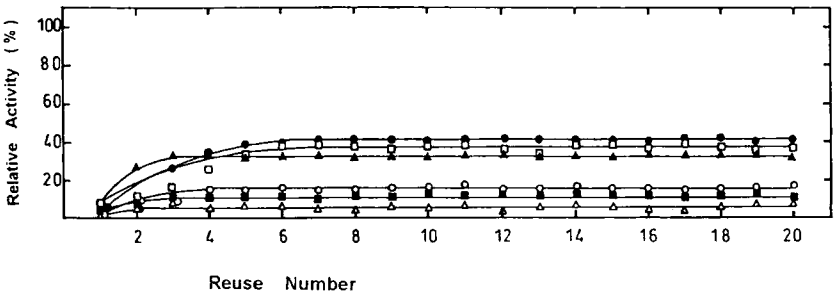

Figure 4. Reuseability of Immobilized α-Amylase with 0.001 mol dm^{-3} Potassium Chromium (III) Sulfate for Carboxymethylcellulose/Gelatin (w/w: ■ :0.000, ○ :0.053 □ :0.111, ● :0.177, ▲ :0.250, △ :0.333) System

Reusability of immobilized enzymes was tested for different carrier system compositions. Experiments were continued for 20 uses which lasted for 40 days. Results are given in Figures 3 and 4.

As shown in Figures 3 and 4 immobilized enzymes obtained for all carrier compositions can be used 20 times without considerable activity loss.

In this study, effect of carrier composition on α–amylase immobilization was investigated. By using PAA 14% and by using CMC 29% relative activity enhancements were achieved. Immobilized samples obtained were stable and could be used at least for 20 times in 40 days without considerable activity loss.

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