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Process Biochemistry 34 (1999) 391–398

PROCESS BIOCHEMISTRY

# Preparation and some properties of immobilized *Penicillium funiculosum* 258 dextranase

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Received 25 June 1998; received in revised form 18 October 1998

**Abstract**

The production of dextranase was investigated in static cultures of *Penicillium funiculosum* 258. Maximal enzyme productivity was attained at pH 8.0, with 3.5% (w/v) dextran (MW, 260 000) as carbon source, $NaNO_3$ (1%, w/v) and yeast extract (0.2%, w/v) as nitrogen source, 0.4% (w/v) $K_2HPO_4$ and 0.06% (w/v) $MgSO_4$. It was possible to increase the productivity of dextranase to 41.8 units $ml^{-1}$ in the modified medium. The enzyme was immobilized on different carriers by different techniques of immobilization. The enzyme prepared by covalent binding on chitosan using glutaraldehyde had the highest activity, the immobilized enzyme retaining 63% of its original specific activity. Compared with the free dextranase, the immobilized enzyme exhibited: a higher pH optimum, a higher optimal reaction temperature and energy of activation, a higher Michaelis constant, improved thermal stability and higher values of deactivation rate constant. The immobilized enzyme retained about 80% of the initial catalytic activity even after being used for 12 cycles. 

*Keywords:* Dextranase; Production; Immobilization; Properties

## 1. Introduction

Dextran is a collective name for polysaccharides composed almost exclusively, of the monomeric unit α-D-glucopyranose linked mainly by a $1 \rightarrow 6$ glucosidic bonds [1]. Dextranases [$(1 \rightarrow 6)$-α-D-glucan-6-glucanohydrolases, EC 3.2.1.11] are the group of hydrolytic enzymes that specifically hydrolyze the $(1 \rightarrow 6)$ linkages in dextrans.

Dextranases are widely used for theoretical and practical purposes. In medicine, dextranases are used for partial hydrolysis of native dextran in the preparation of blood substitutes [2]. Since dextran is involved in dental plaque formation, dextranases are also used in the manufacture of sophisticated dentifrices as an additive for prevention of dental caries [2,3]. Dextranases, have been applied in sugar cane mills as agents that efficiently diminish the levels of dextrans in deteriorated mill-juices with beneficial effects upon the processing rate [1,4]. The use of dextranases had also been extended to structural studies of glucose polymers and purification processes [2,4].

The objective of this paper was to investigate some factors affecting the production of dextranase from *Penicillium funiculosum* 258. Due to the potential application of the immobilized enzymes, immobilization of *P. funiculosum* 258 dextranase on different canters by different methods of immobilization were also studied. The properties of both free and immobilized enzyme were compared.

## 2. Materials and methods

### 2.1. Microorganisms

The fungal cultures used in the present study were obtained from the culture collections of the National Research Center, Dokki, Cairo, Egypt and from the Northern Regional Research Laboratory (NRRL), Peoria, Illinois, USA.

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0032-9592/99/$ - see front matter 
PII: S0032-9592(98)00127-7

contained 1.0 ml of the enzyme solution (918 U dextranase), 5.0 ml of 13.6% (w/v) acrylamide, and 4.0 ml of acetate buffer (0.05 M, pH 5.5) The amount of cross-linker monomer (*N,N*, methylene-*bis*-acrylamide) added was at the level of 2, 3, 4 and 5% of the total monomer content in separate experiments. The catalyst system consisted of the following: 0.02 ml of *N,N,N,N*, tetramethyl-ethylenediamine (TEMED) and 0.01 g of ammonium persulphate dissolved in 0.2 ml of distilled water, was finally added to the polymerization mixture. The total volume was kept to 10 ml. Finally the mixture was kept in $N_2$ atmosphere at 20°C. After polymerization, the gel was washed with acetate buffer (0.05 M, pH 5.5), cut into 1 × 1 × 1 mm fragments and kept in the same buffer at 4°C to remove the unbound enzyme.

### 2.7. Protein estimation

Protein was estimated by the method of Lowry et al. [12]. The protein content of the immobilized enzyme was calculated by subtracting the amount of unbound protein from the protein originally added.

### 2.8. Properties of the immobilized enzyme

#### 2.8.1. Effect of pH

The effect of pH on the activity of free and immobilized dextranase was studied in acetate buffer (0.05 M, pH 5–6) and citrate-phosphate buffer (0.05 M, pH 6.5–7.5).

#### 2.8.2. Thermal stability

Enzyme samples were incubated in 0.05 M acetate buffer (pH 5.7) at 80°C for 15–300 min before enzyme activity assays.

#### 2.8.3. Operational stability

A total of 1 g of chitosan immobilized dextranase (wet) containing about 64.34 U of *P. funiculosum* dextranase was incubated with 10 ml of 1.0% (w/v) dextran (MW 260 000) in acetate buffer (0.05 M, pH 5.7) at 80°C for 60 min. At the end of the reaction, the immobilized enzyme was collected by centrifugation at (2500 × *g*) for 5 min, washed with distilled water and resuspended in 10 ml of freshly prepared substrate to start a new cycle. The supernatant was assayed for reducing sugars.

All results reported are the means of at least three separate experiments.

## 3. Results and discussion

### 3.1. Screening of some fungal strains for the production of extracellular dextranase

Among 25 fungal isolates investigated for the production of exuracellular dextranase, only seven isolates were able to produce the extracellular enzyme (data not shown). These seven isolates were grown on the basal medium for different incubation periods (i.e., 4, 7, and 10 days) in both shaken and static cultures (Table 1). The local fungal shrain *P. funiculosum* 258 (at the 7th day of growth) in static culture showed the highest enzyme yield (13.5 U $ml^{-1}$ culture filtrate). The culture filtrate of this strain was free of mycotoxins especially ochratoxins (A, B, and C). *P. funiculosum* 258 was therefore selected for further investigations.

### 3.2. Culture conditions maximizing dextranase production by P. funiculosum 258

The effect of the initial pH of the culture medium on the production of extracellular dexuranase was investigated over a pH range of 4 to 9. The results indicated that maximal enzyme yield was attained at initial pH 8.0. These results are similar to those reported by Tsuru et al. [6]. In contrast, Galvez-Mariscal and Lopez-Munguia [4] found no difference in the enzyme productivity of *Paecilomyces lilacinus*, over a pH range of 5.4 to 7.0.

Table 1
Dextranase activity of the culture filtrates of the fungal isolates at different incubation periods

| Fungal culture | Dextranase activity (U $ml^{-1}$), at different incubation periods | | | | | |
|---|---|---|---|---|---|---|
| | Surface culture | | | Shaken culture | | |
| | 4 days | 7 days | 10 days | 4 days | 7 days | 10 days |
| *Mucor sp.* | 00.41 | 00.44 | 0.02 | 00.00 | 0.01 | 0.01 |
| *Paecilomyces lilacinus* 895 | 00.31 | 00.75 | 0.83 | 01.08 | 0.99 | 0.97 |
| *P. funiculosum* 52 | 2.14 | 08.20 | 4.69 | 03.09 | 0.46 | 0.10 |
| *P. funiculosum* 37 | 11.06 | 11.93 | 6.56 | 02.53 | 0.55 | 0.31 |
| *P. funiculosum* 258 | 07.85 | 13.51 | 6.13 | 10.73 | 2.53 | 0.51 |
| *Penicillium piscarium* 1075 | 00.08 | 00.68 | 0.85 | 00.10 | 0.26 | 0.38 |
| *Scopulariopsis brevicaulis* | 00.20 | 00.82 | 2.54 | 00.59 | 0.59 | 0.66 |

Table 4
Immobilization of *P. funiculosum* dextranase by physical adsorption

| Carrier | Enzyme added (U g carrier$^{-1}$) A | Unbound enzyme (U g carrier $^{1}$) B | Immobilized enzyme (U g carrier$^{-1}$) I | Immobilization yield (%) $=(I/A-B)\times 100$ |
|---|---|---|---|---|
| Chitin | 918 | 186.70 | 295.9 | 40.44 |
| Chitosan | 918 | 85.96 | 511.25 | 61.44 |
| Colloidal chitin | 918 | 141.80 | 358.00 | 46.12 |
| Tannin-chitosan | 918 | 59.14 | 192.86 | 23.36 |
| Tannin-sepharose | 918 | 183.58 | 234.18 | 34.24 |

### 3.3. Immobilization of *P. funiculosum* 258 dextranase

The culture filtrate from the optimized medium was precipitated with 96% methanol and this partially purified enzyme (specific activity 90 U mg$^{-1}$ protein) was used for enzyme immobilization.

### 3.4. Immobilization by physical adsorption

Immobilization of *P. funiculosum* dextranase by physical adsorption was employed on different carriers including: chitin, colloidal chitin, chitosan, tannin-chitosan and tannin-sepharose (Table 4). The immobilized enzymes prepared by physical adsorption on chitosan had the highest activity (511.25 U g$^{-1}$ carrier) and the highest immobilization yield (61.39%).

### 3.5. Immobilization by ionic binding

The data on the immobilization of *P. funiculosum* 258 dextranase by ionic binding (Table 5) showed a low immobilization yield of enzymes immobilized on dowex 1 × 4 (8.8%), DEAE-cellulose (11.9%), and ecteola-cellulose (14%). On the other hand enzymes immobilized on amberlite IR-120 and dowex 50W showed no activity. This inhibition of the immobilized enzymes cannot be attributed to enzyme denaturation during the immobilization process, because the eluted enzymes from these carriers with 0.25 M NaCl showed a specific activity similar to that of the free enzyme. Therefore, this apparent inhibition may be due to the involvement of the fixation process to the active sites of the enzyme. Similar results were reported for *Bacillus acidopullulyticus* pullulanase immobilized by ionic binding on amberltie IR-45 [14].

### 3.6. Immobilization by covalent binding

Data on the immobilization of *P. funiculosum* 258 dextranase by covalent binding indicated good immobilization yield (Table 6). The enzyme immobilized on chitosan had the highest immobilized activity (599 U g$^{-1}$ carrier) and the highest immobilization yield (80.93%). The immobilized activity reached for *P. funiculosum* 258 dextranase by covalent binding on chitosan was higher than those reported by Madhu [15] (150 U g$^{-1}$ bentonite matrix) and Kuboki et al. [3] (106 U g$^{-1}$ hydroxyapatite).

### 3.7. Immobilization by entrapment

The results of immobilization of *P. funiculosum* 258 dextranase by entrapment in polyacrylamide (Table 7) showed that increasing the level of crosslinking monomer (*N,N*, methylene-*bis*-acrylamide) from 2 to 5% resulted in a gradual decrease of both the entrapped activity (from 359.4 to 97 U 10 ml$^{-1}$ gel) and the immobilization yield (from 47 to 12%). This may be

Table 5
Immobilization of *P. funiculosum* dexhanase by ionic binding

| Carrier | Added enzyme (U g carrier$^{-1}$) A | Unbound enzyme (U g carrier $^{1}$) B | Immobilized enzyme (U g carrier $^{1}$) I | Immobilization yield (%) $=(I/A-B)\times 100$ |
|---|---|---|---|---|
| Amberlite IR-120 ($H^+$) | 895 | 174.90 | 00.00 | 00.00 |
| Dowex 1 × 4 (Cl ) | 895 | 307.28 | 51.89 | 8.82 |
| DEAE-cellulose DE-52 | 895 | 00.00 | 00.00 | 11.90 |
| Dowex 50W | 895 | 208.74 | 81.73 | 00.00 |
| Ecteola-cellulose | 895 | 107.63 | 110.66 | 14.00 |

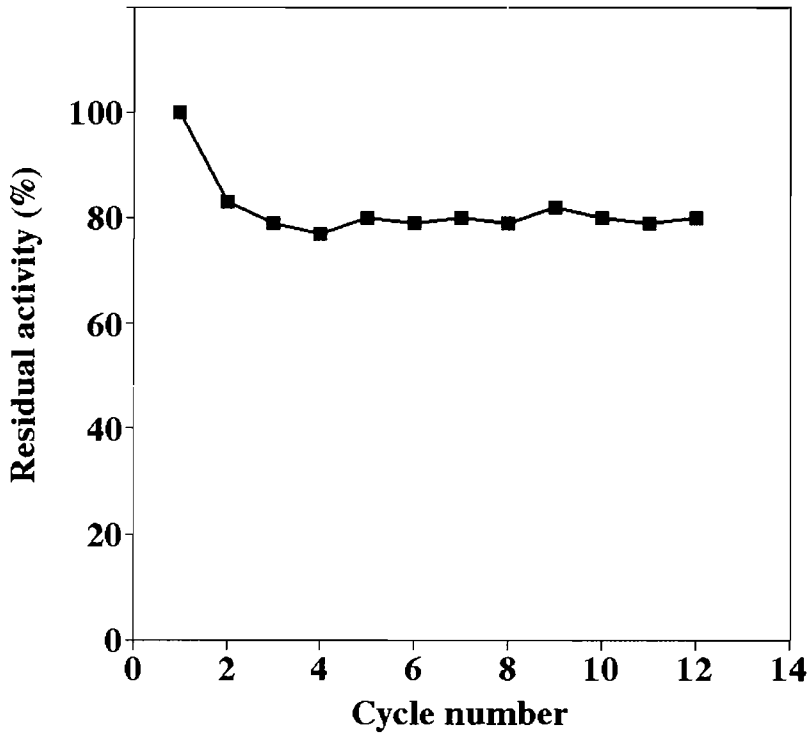


Fig. 1. Operational stability of chitosan immobilized dextranase in repeated use. The reaction mixture is described in the text.

thermal deactivation at 80°C was 2.72 and 6.0 h for the free and immobilized enzyme, respectively (Table 8). The deactivation rate constant at 80°C were $0.83 \times 10^{-3}$ and $1.83 \times 10^{3}$ $min^{-1}$ in the same order.

The calculated value of the kinetic constant $K_m$ of the immobilized enzyme (28.6 μM) for dextran (MW, 260 000) was similar to that of the free enzyme (25 μM). This small increase of the $K_m$ value after immobilization may be due to mass transfer resistance of the substrate into the porous carrier (chitosan). This was particularly so with a high molecular weight substrate like dextran. On the other hand, Gottschalk and Jaenicke [20] reported that immobilization of the enzyme by covalent binding (as the present case) would lead to a decrease in the flexibility of the enzyme molecule. Also, it could be elucidated that the substrate had a low accessibility to the enzyme active site. Consequently, the maximum rate of the reaction catalyzed by the immobilized enzyme was lower than the free form. The increase of the $K_m$ value after dextranase immobilization was similarly reported [18,21,22].

Table 8
Properties of free and chitosan immobilized dextranase

| Property | Free enzyme | Immobilized enzyme |
|---|---|---|
| Specific activity (U mg protein$^{-1}$) | 90.0 | 56.7 |
| Optimum pH of the reaction | 5.5 | 6.0 |
| Optimum reaction temperature | 60.0 | 80.0 |
| Activation energy (kcal mol$^{-1}$) | 2.14 | 6.68 |
| Deactivation rate constant at 80°C ($\times 10^{-3}$ min$^{-1}$) | 0.85 | 1.80 |
| Half-life value at 80°C (h) | 2.40 | 6.0 |
| $K_m$ value (μM) for dextran (260 000) | 25.0 | 28.0 |
| $V_{max}$ (U mg protein$^{-1}$) | 222.2 | 222.2 |

The operational stability of the immobilized *P. funiculosum* 258 dextranase was evaluated in a repeated batch process (Fig. 1). The results indicated that after the second cycle, there was a rapid drop in the concentration of reducing sugars released (17%). This rapid drop in activity was possible due to the release of untightly bound enzyme from the carrier. Similar results were reported for immobilized chitinase [8] and pullulanase [14]. After the third cycle, the immobilized enzyme was stable and no appreciable decrease of immobilized enzyme activity was observed for more than ten cycles. The immobilized enzyme was able to maintain a good yield of reducing sugars (1.3–1.5 mg ml$^{-1}$) as high as 81% of the initial catalytic activity after 12 cycles. The operational stability of *P. funiculosum* 258 dextranase immobilized on chitosan by covalent binding appears to be more stable than *P. aculeatum* dextranase immobilized on bentonite [15].

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Table 6
Immobilization of *P. funiculosum* dexhanase by covalent binding

| Carrier | Added enzyme (U g carrier$^{-1}$) A | Unbound enzyme (U g carrier$^{-1}$) B | Immobilized enzyme (U g carrier$^{-1}$) I | Immobilization yield (%) = (I/A−B) × 100 |
|---|---|---|---|---|
| AS-alumina | 918 | 490.40 | 260.45 | 60.86 |
| Chitin | 918 | 170.63 | 175.96 | 23.53 |
| Chitosan | 918 | 177.15 | 599.87 | 80.93 |
| HydroxyapaOte | 918 | 405.73 | 273.67 | 53.38 |
| CNBr-Sepharose-CL4B | 918 | 507.12 | 149.14 | 36.26 |

due to the gradual decrease of matrix porosity, and hence development of diffusional resistance for both the substrate and products. Similar results were reported for other immobilized enzymes by entrapment in polyacrylamide [16,17].

From the previous studies *P. funiculosum* 258 dextranase was immobilized on various carriers by different methods of immobilization including: physical adsorption, ionic binding, covalent binding and entrapment. Of these immobilized enzymes, that prepared by covalent binding on chitosan (through a spacer group, GA) showed the highest immobilized activity (599.8 U g$^{-1}$ carrier) and the highest immobilization yield (80.93%). This preparation was therefore used throughout this study.

### 3.8. Properties of chitosan immobilized dextranase

The enzyme immobilized on chitosan retained about 63% of the specific activity exhibited by the free enzyme (90 U mg$^{-1}$ protein). This value is favourably comparable to that reported by Ramesh and Singh [18] for immobilized bacterial dextranase on zirconia-coated alkylamine glass (62%). The decrease of specific activity after immobilization is a common phenomenon and may be attributed to diffusional limitation [19] (i.e. resistance of the substrate to diffuse into the matrix and resistance of the products to diffuse out). In addition, the fixation process of the enzyme on the immobilization carrier may impair or prevent the proper conformational changes required for catalysis [20]. In general, the retained specific activity of immobilized *P. fimiculosum* 258 dextranase on chitosan (63%) was higher than those reported for other immobilized dextranases from *P. funiculosum* (30%, Sugiura and Ito [21]) and *Brevibacterium fuscum* (30%, Sugiura and Ito [22]).

The optimal pH of the immobilized dextranase was shifted to a higher value (6.0) from pH 5.5, which was the optimal for the free enzyme (Table 8). These effects may be dependent on the ionic environment around the active site of the enzyme. A shift of the optimal pH of the immobilized dextranase has already been reported [18].

The optimal reaction temperature was shifted from 60°C for the free dextranase to 80°C for the immobilized enzyme (Table 8). This higher value of the optimal reaction temperature for the immobilized enzyme indicated that the applied immobilization procedure (covalent binding) contributed to greater stability. Using Arrhenius plots, the calculated values of the activation energy were 2.14 and 6.68 kcal mol$^{-1}$ for the free and immobilized dextranase, respectively (Table 8). The higher value of the activation energy of the immobilized form indicated that the applied immobilization procedure introduced conformational changes in the structure of the enzyme molecule which impeded the enzyme catalyzed reaction. Increasing the activation energy of the immobilized enzymes were similarly reported [23].

The rate of heat inactivation of the soluble and immobilized enzyme were investigated. In general, the immobilized enzyme was more thermostable than the free enzyme. For example, the calculated half-life for

Table 7
Immobilization of *P. funiculosum* dextranase by entrapment in polyacrylamide*

| Cross-linking concentration (%) | Added enzyme (U 10 ml gel$^{-1}$) A | Unbound enzyme (U 10 ml gel$^{-1}$) B | Immobilized enzyme (U 10 ml gel$^{-1}$) I | Immobilization yield (%) = (I/A−B) × 100 |
|---|---|---|---|---|
| 2 | 918 | 154.28 | 359.45 | 47.00 |
| 3 | 918 | 135.36 | 161.26 | 20.54 |
| 4 | 918 | 124.90 | 132.52 | 16.70 |
| 5 | 918 | 111.03 | 97.07 | 12.02 |

* Final gel concentration = 7%.

Table 2
Effect of different carbon sources on the production of extracellular dextranase by *P. funiculosum* 258

| Carbon source | Dry weight of mycelium (mg 20 ml culture $^{-1}$) | Protein content of culture filtrate (mg ml$^{-1}$) | Dextranase activity (U ml$^{-1}$) |
|---|---|---|---|
| *Dextran (%, w/v)* | | | |
| 0.5 | 33.3 | 0.34 | 2.4 |
| 1.0 | 67.0 | 0.42 | 12.6 |
| 1.5 | 77.2 | 0.52 | 13.0 |
| 2.0 | 95.4 | 0.53 | 14.1 |
| 2.5 | 136.3 | 0.58 | 27.3 |
| 3.0 | 225.7 | 0.62 | 30.7 |
| 3.5 | 230.4 | 0.64 | 41.8 |
| 4.0 | 233.0 | 0.67 | 36.5 |
| Glucose (1.0%) | 72.4 | 0.4 | 0.08 |
| Lactose (1.0%) | 57 6 | 0.45 | 0.07 |
| Dextrin (1.0%) | 69.5 | 0.26 | 0.14 |
| Starch (1.0%) | 87.3 | 0.16 | 0.14 |

The effect of substitution of dexuran (MW, 260 000) in the basal medium by other carbon sources (glucose, lactose, dextrin or starch), on an equal carbon basis, was investigated. The aforementioned carbon sources led to low (with dextrin or starch) or negligible (with glucose or lactose) dextranase production (Table 2). These results are similar to those reported by other investigators [4,13]. Maximal enzyme yield (41.8 U ml$^{-1}$) was obtained at a dextran concentration of 3.5% (w/v). This value is about 2.3-fold that obtained at 1% (w/v) dextran concentration in the basal medium.

On an equivalent nitrogen basis, the nitrogen source in the basal medium (1.0% (w/v) $NaNO_3$ and 0 2% (w/v) yeast extract) containing 3.5% (w/v) dextran was substituted by different nitrogen sources. These included organic (yeast extract, peptone, tryptone, casein hydrolyzate, and urea) and inorganic ($NaNO_3$ and $(NH_4)_2SO_4$) nitrogen sources. The results (Table 3) showed that the combination of $NaNO_3$ (1.0%, w/v,) and yeast extract (0.2%, w/v) was the most effective for enzyme production (41.8 U ml$^{-1}$).

As previously elucidated, the dextranase production medium contained both $MgSO_4$ and $K_2HPO_4$. The effect of their concentrations were studied and the results indicated that maximal enzyme activity was detected at 0.06% (w/v) $MgSO_4$ and 0.4% (w/v) $K_2HPO_4$ concentrations. Other authors have used similar concentrations for $MgSO_4$ and $K_2HPO_4$ for maximal fungal dextranase production [4,13].

From the preceding results, the optimized culture medium consisted of (g l$^{-1}$): dextran (MW 260 000), 35.0; $NaNO_3$, 10; $K_2HPO_4$, 4.0; yeast extract, 2.0; $MgSO_4 \cdot 7H_2O$, 0.6; KCl, 0.2; $FeSO_4 \cdot 7H_2O$, 0.001. The initial pH was adjusted at 8.0. The optimum culture medium of *P. funiculosum* yielded 41.8 U ml$^{-1}$ dextranase activity which affected a 3.1-fold increase of dextranase activity compared to the original medium (Table 1).

Table 3
Effect of different nitrogen sources on the production of extracellulardextranase by *P. funiculosum* 258

| Nitrogen source* | Dry weight of mycelium (mg 20 ml culture$^{-1}$) | Protein content of culture filtrate (mg ml$^{-1}$) | Dextranase activity (U ml$^{-1}$) |
|---|---|---|---|
| $NaNO_3$ + Yeast (control) | 112.70 | 0.73 | 41.80 |
| $NaNO_3$ | 274.70 | 0.52 | 1.20 |
| Yeast extract | 358.50 | 2.11 | 27.50 |
| Ammonium sulphate | 100.60 | 0.42 | 0.31 |
| Peptone | 155.2 | 1.87 | 26.61 |
| Tryptone | 198.50 | 1.90 | 20.81 |
| Casein hydrolyzate | 250.60 | 1.96 | 14.60 |
| Urea | 241.5 | 0.70 | 00.97 |

* On an equivalent nitrogen basis.

### 2.2. Carriers for enzyme immobilization

Chitin, colloidal chitin, chitosan, hydroxyapatite, and sepharose Cl-4B were obtained from Sigma Chemicals Co., USA. Acrylamide, bisacrylamide and alumina were from BDH Chemicals Ltd., Poole, England. Amberlite IR 120, Dowex 50W were from Fluka, Switzerland. DEAE-cellulose DE-52 and ecteola cellulose were from Whatman Ltd., Springfield, Mill, Maidstone, Kent, England. All other chemicals used were of analytical grade.

### 2.3. Culture medium and cultivation

The basal growth medium for enzyme production had the following composition (g $l^{-1}$): dextran (MW, 260 000), 10.0; $NaNO_3$, 10; $K_2HPO_4$, 4.0; yeast extract, 2.0; $MgSO_4 \cdot 7H_2O$; 0.2; KCl, 0.2; $FeSO_4 \cdot 7H_2O$, 0.001. The initial pH was 6.0. The same culture medium was used for inoculum preparation for all the fermentation experiments. Cultivation was in 100 ml Erlenmeyer flasks each containing 20 ml of sterile medium. The flasks were incubated at 30°C under static conditions for different incubation periods (i.e. 7, 10, and 14 days). In some cases, flasks were shaken in a rotary shaker at 180 rpm under the same conditions used for static cultures. The culture broth from each flask was filtered off and the filtrates were centrifuged in a refrigerated centrifuge ($4500 \times g$) for 15 min. The clear culture filtrates were taken for enzyme assays.

### 2.4. Detection of ochratoxins

This was carried out according to the method of Reddy et al. [5].

### 2.5. Assay for dextranase activity

Dextranase activity was determined according to the method of Tsuru et al. [6]. A total of 4.0 ml of 1.0% (w/v) dextran (MW, 260 000, grade A, BDH), in 0.05 M acetate buffer (pH 5.5) was added to the enzyme solution or a weighed sample of the immobilized enzyme. The reaction mixture was incubated at 40°C for 10 min in a shaking water bath. The reducing sugars released was determined as isomaltose by the method of Somogyi [7]. One enzyme activity unit (U) was defined as the amount of enzyme that liberate one micromole of isomaltose per min reaction under the assay conditions.

### 2.6. Immobilization methods

The culture filtrate obtained from the optimized medium was precipitated with methanol. The fraction precipitated at 96% methanol (specific activity 90 U $mg^{-1}$ protein) showed the highest dextranase activity. This partially purified enzyme was used for preparation of the immobilized enzyme.

#### 2.6.1. Immobilization by physical adsorption

Tannin-chitosan and Tannin-sepharose were prepared by the method reported by Sakai et al. [8]. A total of 1 g of each carrier (chitin, chitosan, colloidal chitin, tannin-chitosan and tannin-sepharose) was incubated with the enzyme solution (918.36 U of *P. funiculosum* dextranase in 2 ml of 0.05 M acetate buffer, pH 5.5) at 4°C for 12 h. The unbound enzyme was removed from the carriers by washing with 0.05 M acetate buffer (pH 5.5) until no activity or soluble protein were detected.

#### 2.6.2. Immobilization by ionic binding

A total of 1 g of cation exchanger (equilibrated with 0.05 M acetate buffer, pH 5.5) or anion exchanger (equilibrated with 0.05 M phosphate buffer, pH 7.5) was incubated with 1 ml of the enzyme solution (895.4 U of *P funiculosum* dextranase) in the same buffer at 4°C for 12 h. The unbound enzyme was removed by washing with the same buffer until no activity or protein was detected.

#### 2.6.3. Immobilization by covalent binding

A total of 1 gram chitosan was shaken in 25 ml of 0.01 M HCl containing 2.5%, (v/v) glutaraldehyde (GA) for 2 h at 30°C. The solubilized chitosan was precipitated by the addition of 1 ml of 0.1 M NaOH. The precipitates were collected by filtration and washed with distilled water to remove the excess GA. The wet chitosan was mixed with 2.5 ml of the enzyme solution containing 918.0 U of dextranase. After being shaken for 1 h at 4°C, the unbound enzyme was removed by washing with distilled water.

Aminoalkylsilane-alumina (AS-alumina) was prepared by the method of Weetall [9]. In separate experiments, 1 g of chitin, hydroxyapatite, and AS-alumina was treated with 5 ml of 2.5%, (v/v) GA for 2 h at 30°C. The carriers were collected by filtration and washed with distilled water to remove the excess GA. Each of the wet carrier was shaken with 2.5 ml of the enzyme solution (918.0 U dextranase) for 2 h at 30°C and the unbound enzyme was removed as described above.

Cyanogen bromide activated sepharose (CNBr-sepharose)-CL 4B was prepared by the method of March et al. [10]. A total of 0.3 g was incubated with 1 ml of the enzyme solution (containing 275.4 U dextranase) for 12 h at 4°C. The unbound enzyme was removed as described above.

#### 2.6.4. Immobilization by entrapment

Polyacrylamide gel was prepared according to the method of Roy et al. [11]. The polymerization mixture