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Immobilisation and kinetics of *Penicillium notatum* dextranase on controlled porous glass

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Abstract

Two highly purified enzymic fractions of dextranase (1,6-α-D-glucan 6-glucanohydrolase, EC 3.2.1.11) from *Penicillium notatum* have been immobilised on silanised porous glass modified by glutaraldehyde, carbodiimide and bis-oxirane binding. The marked shifts in the pH and temperature optima as well as the changes in the kinetics (K_m, V_{max}, E_a) of the solid-phase dextranases were observed and discussed. The immobilisation of enzymes on alkylamine glass through the process of glutaraldehyde coupling proved to be the best of the methods studied. The dextranase preparations obtained in this way showed a catalytic activity at wider pH and temperature ranges than those of the free enzymes. They were also characterized by a relatively high affinity to the substrate and good storage stability. The usefulness of the bound dextranase in batch and column hydrolysis of dextran was also established.

Keywords: Dextranase isoforms; Immobilisation; Kinetic properties; Dextran hydrolysis

1. Introduction

Immobilised enzymes have been receiving a great deal of attention [1]. In applied research, the value of solid-phase enzymes lies in their potential as specific, re-usable, non-contaminating catalysts, often of increased stability, for use in industrial processes [2].

Dextranase (α-1,6-glucanohydrolase, EC 3.2.1.11), which hydrolyses the α-1,6-glycosidic linkages in bacterial dextran, is found in higher plants, mammalian tissues, fungi and bacteria [3]. However, the best producers of the dextran depolymerising enzymes are filamentous fungi and those microorganisms which produce extracellular endodextranases are considered the most valuable [4]. Carrier-bound water insoluble dextranase has potential for application in commercial production of clinical dextran in obtaining isomaltose and higher isomaltooligomers and also in continuous removal of dextran from infected diffusive sugar juices [5–8]. The expected qualities which could be most useful in this regard would be the improved thermal stability and the ease of recovery, both factors being of possible importance in the economic viability of such a scheme.

Organic and inorganic supports have been employed in the immobilisation of dextranase [8–14]. Some of these matrices (cellulose derivatives, nylon) are not convenient in large

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scale operations in enzymic reactors because of their low stability and poor resistance to microbial attack. Therefore, the attachment of enzymes to porous glass beads is of commercial interest [15]. Such beads are chemically, mechanically and thermally resistant. They are also resistant to microbial attack and the carrier does not change its configuration over an extensive pH range nor under various solvent conditions and is therefore easier to use in continuous systems. The enzymic preparations thus obtained are inexpensive, easy to manufacture and possess a long working life span. Moreover, the porous glasses can be regenerated by heating or boiling in acid to remove organic materials adsorbed on them.

Recently we have selected a strain of *Penicillium notatum* 1 as a new producer of extracellular dextranase [16]. The enzyme from this fungus was highly purified and two active homogeneous fractions (D_1 and D_2 isoforms), similar in their enzymic and physicochemical properties, were isolated [17]. Both dextranases were typical endoenzymes and isomaltose and isomaltotriose were the principal end products of dextran hydrolysis [18].

Consequently, the present work involves some model experiments on immobilisation of both purified dextranase fractions on porous glass using various covalent methods of enzyme binding to the support. The repeated batch and column hydrolysis of dextran by immobilised enzyme is also described.

2. Materials and methods

2.1. Substrates and chemicals

Dextran (average MW, 110 kDa) was supplied by Polfa Pharmaceutical Works (Kutno, Poland); 3-aminopropyltriethoxysilane (APTES) by Sigma Chemical Co. (St Louis, MO); glutaraldehyde by Merck (Darmstadt, Germany). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 3-mercaptopropyltriethoxysilane (MPTES) were the products of Fluka (Buchs, Switzerland). 3,5-Dinitrosalicylic acid (DNS) was purchased from BDH (Poole, UK). Diglycidyl ether diethylene glycol (bis-oxirane, DEDG-2) was obtained from the Institute of Industrial Chemistry (Warsaw, Poland). Other chemicals were analytical grade reagents purchased locally.

2.2. Enzyme source and assay

Crude (non-purified) dextranase preparation and two highly purified enzyme isoforms (dextranase I, D_1; dextranase II, D_2) were prepared following the methods described in a previous paper [17] and used as a starting material for enzyme immobilisation and dextran hydrolysis.

The standard dextranase assay mixture contained 1 ml of 2% dextran in 0.1 M acetate buffer (pH 5.0) and 0.5 ml of suitably diluted enzyme solution. After 30 min incubation at 50°C, the reducing sugars formed were analysed by DNS method [19]. One unit of dextranase activity was defined as the amount of enzyme that catalysed the liberation of reducing groups equivalent to 1 μmol of isomaltose (measured as maltose) from the substrate per min under the described conditions.

2.3. Immobilisation of dextranase on controlled porous glass (CPG)

Controlled porous glass (Cormay, Lublin) was prepared according to the method described earlier [20]. The specific surface area, S (m^2/g), average pore diameter, D (nm) and average pore volume V (cm^3/g), were 82, 40 and 1.26, respectively. The glass was activated by APTES or MPTES according to the method which permits a high density level of the amino or sulfhydryl groups on the carrier surface [21]. The activated matrices (APTES-CPG, MPTES-CPG) were further modified by bifunctional oxirane (DEDG-2) which finally yielded epoxy-APTES-CPG or epoxy-MPTES-CPG [22,23].

The purified dextranase isoforms (D_1 and D_2) were covalently coupled to APTES-CPG

via amino groups using glutaraldehyde (carrier I) or via carboxyl groups using EDC (carrier II) as described by Lappi et al. [24] and Kaufman and Pierce [25]. Binding of the enzyme by amino groups to epoxy-APTES-CPG (carrier III) or epoxy-MPTES-CPG (carrier IV) was performed following the method given earlier [26,27]. Carrier-bound enzymes were washed copiously with distilled water and kept under water at 4°C until further use. The yield of dextranase immobilisation was calculated from the difference in activities (or protein contents) before and after treatment of the enzyme on the support. Protein was determined using the Lowry method [28].

2.4. pH-activity profiles, temperature-activity profiles, kinetic constants and activation energy of dextranases

The influence of pH on native dextranases was examined in the standard assay mixture except that 0.1 M phosphate-citrate (McIlvaine) buffers (pH 3.0–8.1) were used instead of acetate buffer. The effect of temperature on dextranase activity was determined in the standard assay mixture except that the reaction temperature was changed gradually from 4 to 70°C and the activity was measured at optimal pH value.

For assays of immobilised enzyme activity, the insoluble enzyme slurry (100 mg wet), dextran (2% aqueous solution, 1 ml) and buffer (0.5 ml) were shaken for 1 h and the released reducing sugars quantified. The relative activity at each pH and temperature was expressed as a percentage of that at the pH and temperature of maximum activity.

The kinetic constants (K_m, V_{max}) for free and immobilised dextranases were determined on an IBM PC employing Wilman 4 (1985) software (M.S.U., MN). The determinations were made at the apparent pH and temperature optima in the assay reaction mixtures and conditions outlined above except that substrate concentrations were changed from 0.2 to 15%.

The activation energy (E_a) was calculated from the Arrhenius equation:

$$E_a = \frac{2.303 \cdot \log \frac{k_2}{k_1} \cdot R \cdot T_1 \cdot T_2}{T_2 - T_1}$$

where E_a is the activation energy (J/mol); k_1, k_2 are the reaction rate constants determined in temperatures T_1 and T_2; R is the gas constant = 8.317/J (mol · K) and T_1, T_2 are the temperature of measurements (K).

2.5. Dextran hydrolysis by immobilised dextranase

2.5.1. Batch hydrolysis

The hydrolysis was conducted in plugged round-bottomed flasks (50 ml) in the presence of 0.01% sodium azide. The reaction mixture (25 ml) in 0.1 M acetate buffer (pH 5.0) contained 4% of dextran and 6.5 units/g substrate of dextranase (crude preparation immobilised on APTES-CPG by glutaraldehyde, carrier I). The flasks were incubated for 20 days at 28°C in a water bath shaker and agitated at 150 rpm. The reaction mixture was replaced every 24 h and the carrier-bound enzyme was washed off with acetate buffer before transferring into the fresh medium. Samples were withdrawn periodically and analysed for reducing power.

2.5.2. Column hydrolysis

The amount of 3 g (wet mass) of immobilised dextranase (7.9 units/g dextran, crude preparation coupled to APTES-CPG using glutaraldehyde, carrier I) was packed under gravity in a column (1.5 × 3.5 cm), equilibrated with 0.1 M acetate buffer (pH 5.0). A solution of dextran (4% in 0.1 M acetate buffer, pH 5.0, 100 ml) was then recycled (by peristaltic pump) through the column at 28°C for 24 h at various flow-rates (0.3–2.0 ml/min). Sodium azide (0.01%) was added to the dextran solutions to prevent the growth of microorganisms. Samples of the column effluent were taken at regular

intervals and the eluted reducing-power was monitored. The efficiency of dextran hydrolysis was studied in the presence and absence of sucrose (20%) in the reaction medium.

The percentage of dextran saccharification based on the total maltose units was calculated using the equation:

$$\text{Saccharification (\%)} = \frac{\text{reducing sugars formed (mg)} \cdot 0.9}{\text{dextran (mg)}} \cdot 100.$$

3. Results and discussion

3.1. Methods of dextranase immobilisation

The physicochemical characteristics of the purified dextranase fractions (D_1 and D_2) isolated from *Penicillium notatum* is shown in Table 1. Both free enzymes showed optimal activity at pH 5.0–5.5 and at 50°C, which was similar to properties of dextranases from other fungi [29]. They also represent an endolytic mode of action, and isomaltose and isomaltotriose were identified as the main final products of dextran hydrolysis.

Dextranase isoforms were immobilised on controlled pore glass using different methods of enzyme binding to the support. The glass was first coated with APTES or MPTES to enrich its surface with amino or sulhydryl groups which are more convenient than -OH groups for the attachment of the enzyme. The chemical activation of initial glass by different silans is depicted in Scheme 1.

The enzyme was covalently coupled to APTES-CPG via amino groups using glutaraldehyde (Scheme 2) or via carboxyl groups using EDC (Scheme 3). The silanised matrices (APTES-CPG, MPTES-CPG) were also modified by bifunctional oxirane (DEDG-2) and dextranase was covalently attached to epoxy-APTES-CPG or epoxy-MPTES-CPG via amino groups as illustrated in Schemes 4 and 5.

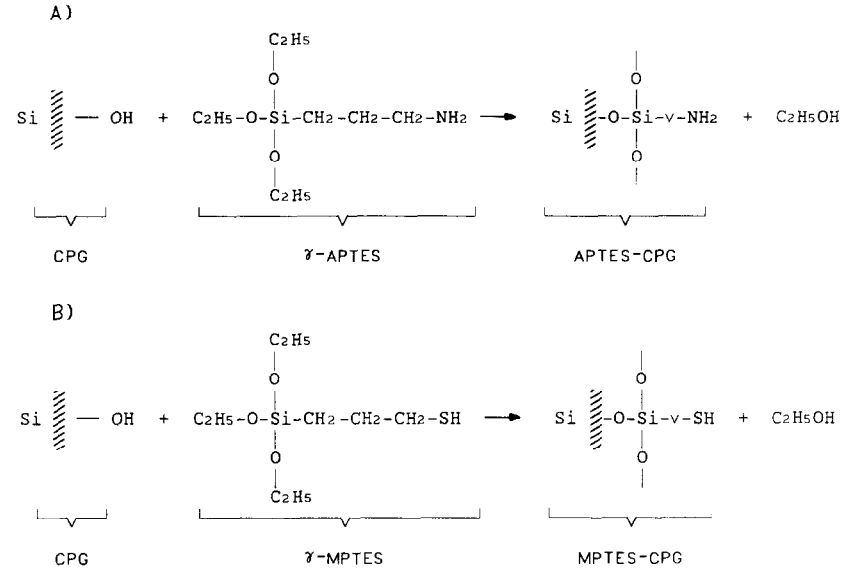
3.2. Properties of immobilised dextranases

Among the properties of the enzyme which might be expected to be changed on immobilisation are the pH and temperature optimum, kinetic constants, and storage stability. These changes have been supposed to arise both from the chemical modification of the enzyme and from physical interference by the carrier. In the latter case, the changes in kinetic behavior could arise from, for example, electrostatic and steric influences or the time required for diffusion of substrates and products. Concentrations of substrates, products and hydrogen ions in the microenvironment of the fixed enzyme would therefore differ from those in the external solution. Thus, the microenvironment in the vicinity of the immobilised enzyme is very different from that of the native enzyme in the solution.

Fig. 1A–B shows the activity of free and immobilised dextranase isoforms on modified glasses (carriers I–IV) at different pH values. The native dextranase fractions exhibited their optima at pH 5.0 (D_1) and 5.5 (D_2). The pH-of-activity profiles for the insolubilised enzymes were altered appreciably by immobilisation, although not in identical ways. Except for dex-

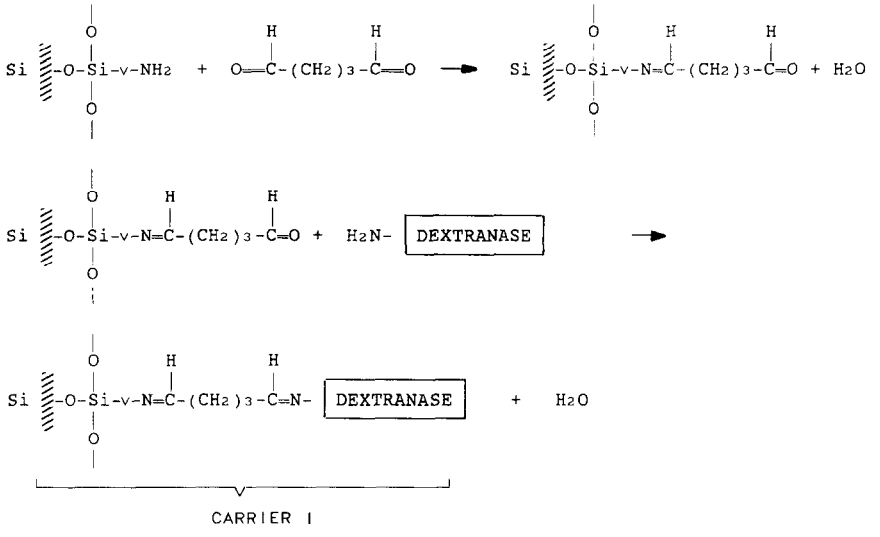
Table 1
Characteristics of the purified dextranase fractions from *P. notatum*

Enzyme isoform	Molecular mass (kDa)	Isoelectric point (pI)	Optimal pH	Optimal temperature (°C)	Kinetic constants		Main hydrolysis products
					K_m (mM)	V_{max}	
D_1	55.8	4.90	5.00	50.0	0.46	0.22	isomaltose, isomaltotriose
D_2	50.1	4.75	5.50	50.0	1.69	3.10	isomaltose, isomaltotriose

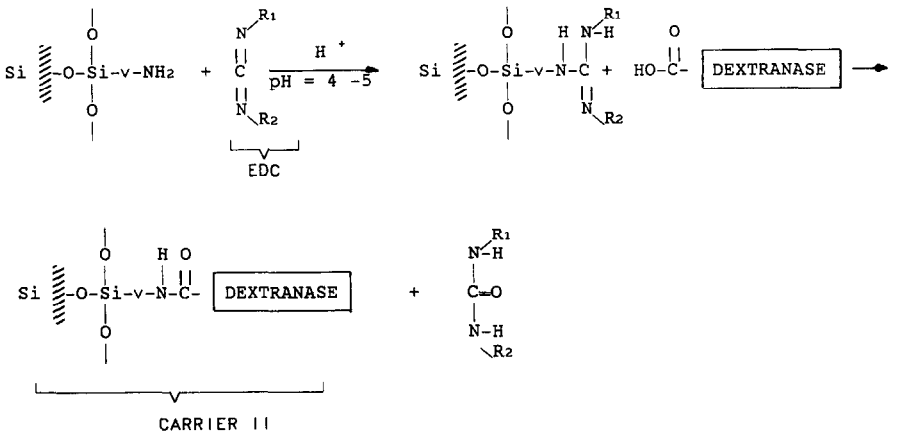


Scheme 1. Silanisation of CPG by APTES (A) and MPTES (B).

tranase D_1 coupled to carrier IV (epoxy-MPTES-CPG), the pH optima of all other immobilised preparations were shifted towards the acidic region. The largest displacements of optimal activity (by 1.0–2.0 pH units) were observed in the case of dextranase D_1 immobilised on alkylamine derivative of porous glass (carrier I) and carrier III (epoxy-APTES-CPG) as well as for fraction D_2 covalently attached to carrier I and IV. The pH optima of other carrier-bound enzymes were displaced only to a small extent (0.5 pH unit) to the acidic side in comparison with the free dextranase isoforms. Among the enzymic preparations studied, the purified dex-



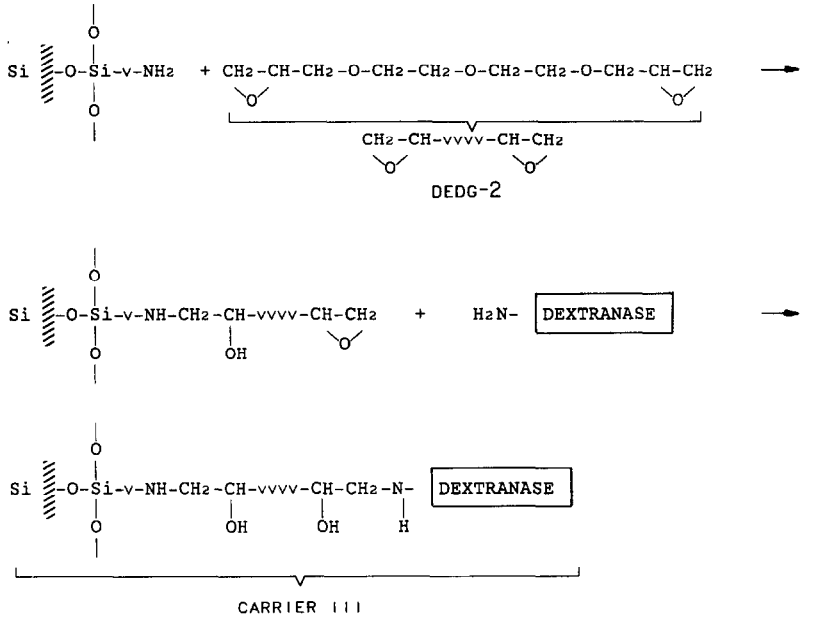
Scheme 2. Binding of dextranase to APTES-CPG by glutaraldehyde (carrier I).



Scheme 3. Binding of enzyme to APTES-CPG using carbodiimide (carrier II).

tranase fractions immobilised on carrier I showed the most widely spread pH profiles. Enzyme D_1 preserved 35% of its optimal activity even at pH 8.0 while D_2 form was much less active in the alkaline region.

Chemical modification of an enzyme often results in an altered pH-of-activity profile [30] and immobilisation frequently has the same effect, especially if the insoluble support is polyionic [31]. The marked shifts to lower pH optima of water insoluble dextranases were observed in the present investigations. This observation suggests that the silanised porous glass modified by glutaraldehyde, EDC or bis-oxirane, behaves as a ionic medium. Such displacements of pH optima on immobilisation have been recorded previously for various immobilised enzymes [20,32]. According to Trevan [33] this



Scheme 4. Schematic representation of enzyme coupling to epoxy-APTES-CPG by DEDG-2 (carrier III).

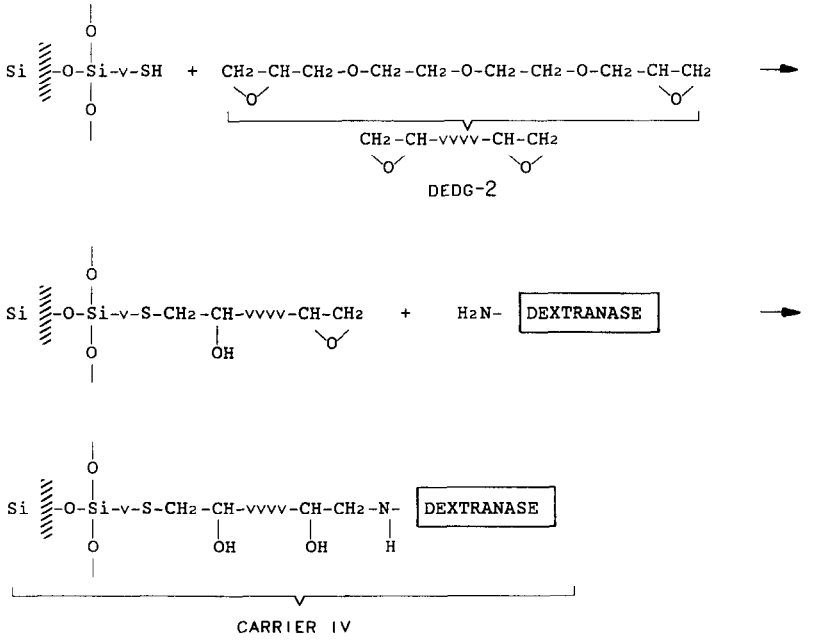
phenomenon may be caused by partitioning of protons which are affected by ionised groups in the matrix. It is also possible to relate such a shift to the effect of the electrostatic potential of the polyelectrolyte carrier on the local concentration of hydrogen ions [34].

There is a number of reports which indicated that immobilisation of an enzyme often results in an altered temperature-of-activity and thermostability profiles compared with those obtained for soluble catalyst [35,36].

Examining the influence of the incubation temperature on the activity of both the native and carrier-bound forms of *P. notatum* dextranase, significant differences can be observed (Fig. 2A–B). Both free dextranase fractions (D_1 and D_2) were optimally active at 50°C. The same temperature optima reached on immobilisation dextranase D_1 coupled to carrier II (MPTES-CPG modified by EDC) and fraction D_2 attached to carrier IV (epoxy-MPTES-CPG). In these cases, however, the solid-phase preparations showed a marked broadening of the temperature profiles related to those of the soluble forms. The temperature rise, for example, to 70°C led to rapid inactivation of native enzymes while the immobilised forms preserved from 51% (D_1, carrier II) to 58.5% (D_2, carrier IV) of their optimal activity in these conditions.

For other immobilised preparations, large shifts in temperature optima were noted. The D_1 isoform of dextranase bound to carrier IV and carrier III (epoxy-APTES-CPG) exhibited its temperature optimum at lower values (35 and 40°C, respectively) than the free enzyme. Contrary to the native form, the same enzyme attached to alkylamine glass using glutaraldehyde (carrier I) possessed a widely spread temperature-of-activity profile with a maximum at 70°C. In the case of fraction D_2, immobilised on carrier I and carrier II, the relatively high temperature optimum (60°C) was obtained while the same enzymic preparation immobilised on carrier III reached its maximal activity at only 35°C. The immobilised dextranase preparations which achieve the higher optimal temperature for dextranolysis can be highly useful in sugar refining where the temperature of the diffusive juice range from 55 to 60°C [37].

Table 2 gives a comparative data on the kinetic constants and activation energy of both soluble and immobilised dextranase fractions. It



Scheme 5. Schematic representation of dextranase attachment to epoxy-MPTES-CPG using DEDG-2 (carrier IV).

may be seen from this that there occurred an alternation in the kinetics of the bound dextranase as expected for enzymes upon immobilisation [38]. The apparent Michaelis constant, K_m, was appreciably higher for carrier-bound dextranase isoforms (except dextranase D_1 immobilised on carrier III) than for native enzymes indicating a significant decrease in the affinity of the bound dextranases for their substrate i.e. dextran. The catalytic activity, expressed by V_{max}, was lower for dextranase D_2 immobilised on all carriers used but higher for enzyme D_1 in comparison with free enzymes. The activation energy (E_a) of the immobilised dextranase preparations exhibited a decline (except the enzyme D_1 immobilised on carrier III)

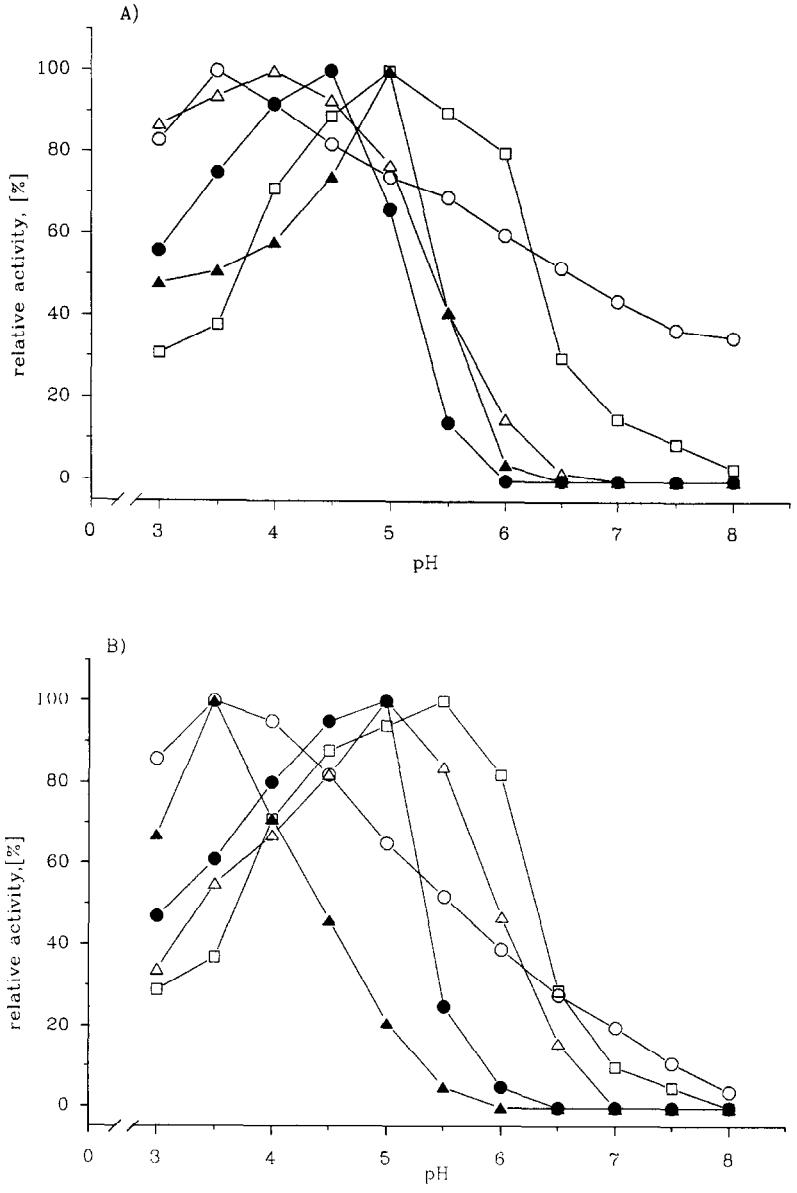


Fig. 1. pH-activity profiles for native and immobilised on modified carriers dextranase isoforms. Experimental details are as described in Section 2. (A) D_1 isoform; (B) D_2 isoform. (– □ –) native enzyme; enzyme immobilised on (– ○ –) carrier I; (– ● –) carrier II; (– △ –) carrier III; (– ▲ –) carrier IV.

as compared to the soluble enzyme isoforms (Table 2) indicating an increase in stability of the enzymes upon immobilisation [39,40]. The lowest E_a value was established for dextranase D_1 coupled to carrier I (APTES-CPG modified by glutaraldehyde).

The changes in the kinetics of immobilised enzymes are controlled mainly by four factors i.e. (1) change in enzyme conformation, (2) steric effects, (3) microenvironmental and (4) bulk and diffusional effects [38]. The higher K_m values for the solid-phase enzymes may be a result of a number of effects. The migration of substrate from the solution to the microenvironment of an immobilised enzyme can be a major factor in the rate of the enzyme reaction. A

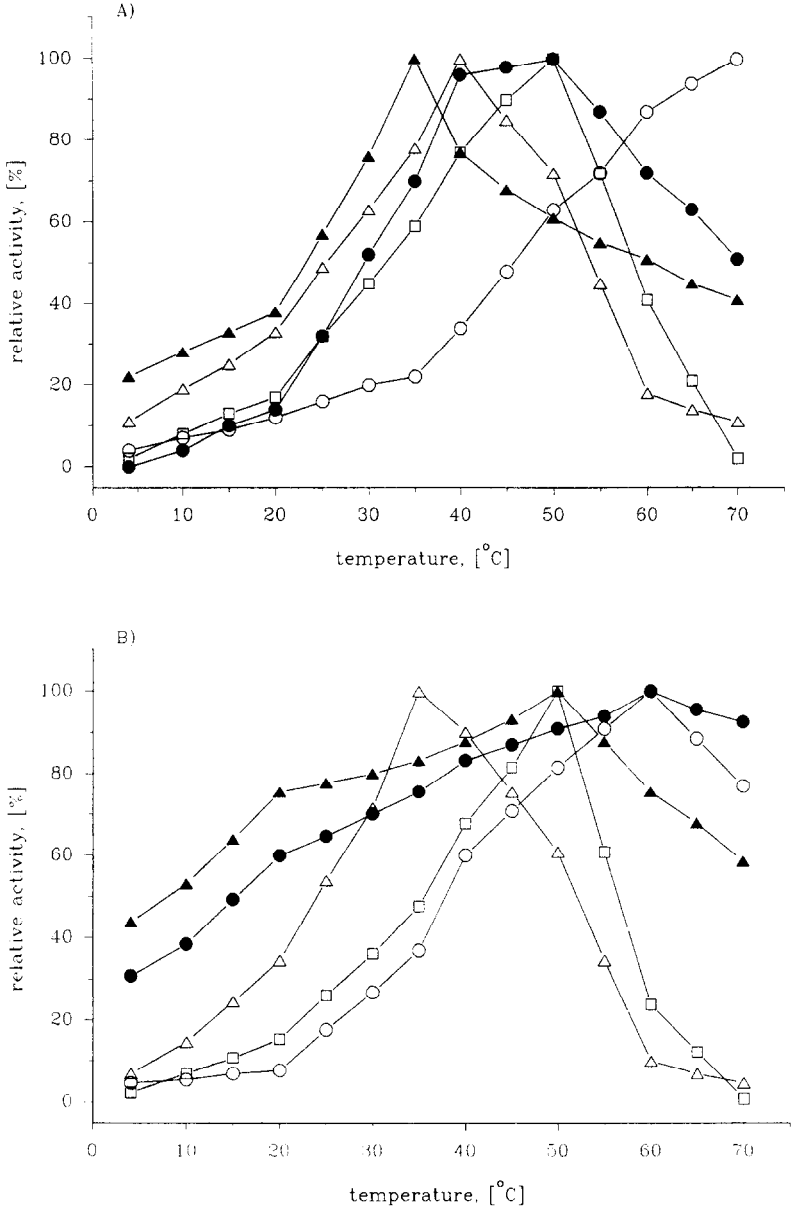


Fig. 2. Temperature-activity profiles for native and immobilised on modified carriers dextranase isoforms. Experimental details are as described in Section 2. (A) D_1 isoform; (B) D_2 isoform. (– □ –) native enzyme; enzyme immobilised on (– ○ –) carrier I; (– ● –) carrier II; (– △ –) carrier III; (– ▲ –) carrier IV.

Table 2
Kinetic constants (K_m, V_{max}) and activation energy (E_a) of the native and immobilised on modified carriers dextranase isoforms

Enzyme form	Fraction					
	D_1			D_2		
	K_m (mM)	V_{max}	E_a (kJ/mol)	K_m (mM)	V_{max}	E_a (kJ/mol)
Native	0.46 ± 0.01	0.22 ± 0.02	21.6 ± 1.01	1.69 ± 0.08	3.10 ± 0.23	36.7 ± 2.29
Immobilised (carrier I)	9.80 ± 0.16	1.04 ± 0.07	6.39 ± 0.21	6.31 ± 0.26	0.84 ± 0.05	20.1 ± 1.21
Immobilised (carrier II)	42.9 ± 0.20	4.41 ± 0.17	17.1 ± 0.89	2.45 ± 0.15	0.18 ± 0.01	30.2 ± 2.02
Immobilised (carrier III)	0.37 ± 0.01	0.23 ± 0.02	22.2 ± 0.89	19.1 ± 0.31	2.03 ± 0.06	18.1 ± 0.90
Immobilised (carrier IV)	49.3 ± 0.17	4.89 ± 0.31	13.1 ± 0.58	13.2 ± 0.33	1.46 ± 0.08	14.7 ± 0.89

diffusion film which covers the surface of an insoluble particle was proposed [41] and within which the substrate concentration is lower than in the solution. The rate at which substrate passes over the insoluble particle affects the thickness of the diffusion film, which in turn determines the concentration of substrate in the vicinity of the enzyme and hence the rate of reaction.

A further reason for diffusion may be the adsorption of dextran on glass or its association with the reagents coating the glass. Dextrans are adsorbed by Celite coated with diazotised 1,3-diaminobenzene [42]. Linear dextrans, however, were adsorbed to a lesser degree (1.2 g/g of matrix) than branched dextrans (7.9 g/g of matrix). An equation that correlates apparent K_m values of immobilised enzymes and the corresponding value of the free enzyme in terms of both diffusional and electrical effects has been derived by Hornby et al. [43]. According to the derived equation, like charges on substrate and carrier would increase K_m, whereas unlike charges would decrease K_m. A diffusional limiting layer around the fixed enzyme would always impede entrance of substrate, so that the apparent K_m value is raised.

Table 3 shows the effect of storage on the activity of crude dextranase coupled to the two modified carriers on which the purified form of enzyme achieved the maximal affinity to the substrate (see Table 2). It should be noted that the crude enzymic preparation was a mixture of both dextranase isoforms (D_1 and D_2). The alkylamine conjugated dextranase (carrier I) showed relatively good stability if stored at 28°C and preserved about 63% of its original activity even after two weeks while the enzyme attached to carrier III (epoxy-APTES-CPG) was labile and it lost over 90% of its initial activity in the same period of time.

Taking into account the discussed properties of immobilised dextranases, it appears that the most convenient and useful enzymes for dextran hydrolysis in the recycle process are the both purified isoforms of dextranase immobilised on carrier I. This conclusion results from the following aspects: (i) the chosen enzymic preparations possessed widely spread pH-of-activity profiles (see Fig. 1A–B), (ii) their optimal temperature ranged from 60 to 70°C (see Fig. 2A–B), (iii) they showed relatively high affinity to the substrate and good storage stability (see Tables 2 and 3). Thus, the dextranase bound to the alkylamine porous glass (carrier I) was used in the further studies. However, for the efficient saccharification of dextran, non-purified dextranase preparation containing both dextranase isoforms was applied.

Table 3
The time stability of immobilised dextranase [a] at 28°C

Time (days)	Activity retained on storage (% of original)	
	carrier I	carrier III
0	100.0	100.0
1	99.0	90.0
14	63.2	9.8
28	38.5	5.5
180	0.3	0.0

[a] Crude (non-purified) enzyme preparation was used.

Table 4
Conversion of dextran to sugar syrup on recycling a solution of dextran through a column of immobilised dextranase for 24 h [a]

Time (h)	Flow rate (ml/min)	Residence time [b] (min)	% saccharification		% saccharification/residence time (min^{-1})	
			without sucrose	with sucrose	without sucrose	with sucrose
1	2.0	3.09	8.40	7.40	2.70	2.40
3	2.0	3.09	33.2	25.4	10.7	8.20
6	2.0	3.09	41.4	39.4	13.4	12.7
12	2.0	3.09	44.2	42.8	14.3	13.8
24	2.0	3.09	57.4	55.0	18.6	17.8
1	0.7	8.85	11.5	4.10	1.30	0.47
3	0.7	8.85	34.5	22.4	3.90	2.53
6	0.7	8.85	42.6	37.7	4.81	4.26
12	0.7	8.85	45.3	44.3	5.11	5.01
24	0.7	8.85	62.3	60.3	7.04	6.82
1	0.3	20.6	6.90	4.50	0.33	0.22
3	0.3	20.6	14.9	21.9	0.72	1.06
6	0.3	20.6	44.2	39.6	2.15	1.29
12	0.3	20.6	48.8	47.0	2.37	2.28
24	0.3	20.6	65.0	62.4	3.15	3.03

[a] Column hydrolysis was performed by coupled to carrier I dextranase (non-purified preparation) at various flow-rates in the presence and absence of sucrose (20%). Other experimental details are as described in Section 2.
[b] The residence time is the time for which the liquid is flowing through the column, i.e. residence time = packed column volume (ml)/flow rate (ml/min) = 6.185 ml/flow rate.

3.3. Dextran hydrolysis by immobilised dextranase

One of the most important reasons for the preparation of solid-phase enzymes is that they afford simple methods for separating the enzymes from reaction products, for example, by filtration or centrifugation. Alternatively, a column packed with a suitable solid matrix to which an enzyme has been attached may be used with the reaction solution flowing through it, where the recycle of the substrate is an additional advantage of such a system. Both above-mentioned processes (batch and column) have been used for dextran hydrolysis by immobilised dextranase in relation to stability, reusability and flow characteristics.

Fig. 3 illustrates the hydrolytic activity of immobilised dextranase (measured as the efficiency of dextran saccharification) in the batch hydrolysis system. The bound enzyme could be used repeatedly for four 24 h cycles without any appreciable loss in sugar yield which reached average value of 68.5% after every 24 h of enzymic hydrolysis. A little decrease in dextran saccharification (by only 8% in relation to the

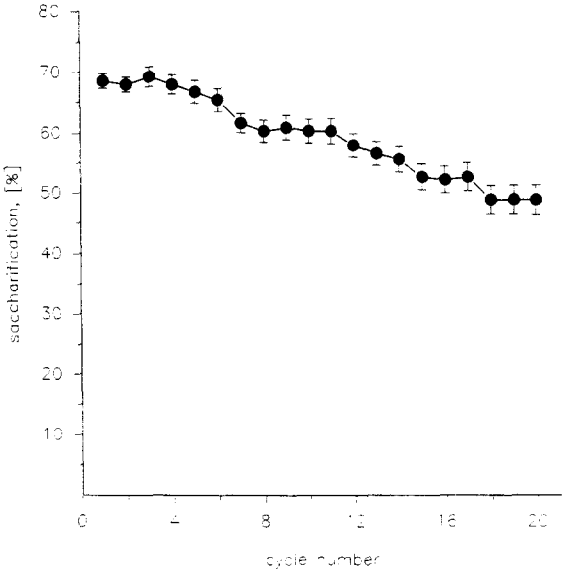


Fig. 3. Repeated batch hydrolysis of dextran using immobilised on alkylamine glass (carrier I) *P. notatum* dextranase. The hydrolysis was performed in shaken flasks in 24 h cycles.

first cycle) was recorded from the 5th up to the 11th cycle. Later the hydrolysis efficiency declined distinctly and in the twentieth cycle it achieved a value of about 49%.

In order to evaluate the usefulness of the immobilised enzyme in a recycling system, the immobilised dextranase was packed in a column and used for the conversion of dextran into sugar syrup at different recirculation flow rates of substrate solution. In sugar refining, the hydrolysis of dextran in infected diffusive juices takes place at a high content of sucrose that can affect the efficiency of dextran hydrolysis. Therefore, the effect of sucrose concentration (20%) on the yield of dextran hydrolysis was additionally studied. The results are summarised in Table 4.

After 1 h of hydrolysis the degree of dextran saccharification was between 6.9 and 11.5%, depending on the recirculation rate, while after 24 h the sugar yield increased up to 65%, when the residence time of substrate in the column was the longest (20.6 min) and its recirculation velocity was the smallest (0.3 ml/min). The effect of the substrate flow rate on saccharification process was mainly on the control of mass transfer of the dextran through the carrier-bound enzyme similarly as in the heterogeneous reaction between the cross-linked dextran (insoluble substrate) and a soluble dextranase described by Suga et al. [44]. It should be also stressed that the addition of sucrose into the reaction medium did not affect distinctly the ratio (% saccharification/residence time, nor the total yield of sugar obtained during the hydrolysis. Besides, the crude enzyme preparation contained practically no invertase activity which could decrease sucrose content in sugar juices [16,17]. A relatively constant ratio (% saccharification/residence time) obtained in the successive cycles of hydrolysis (Fig. 4) gives an indication that the packed column was catalytically active over the whole of hydrolysis period. Thus, the readiness with which reasonable flow rates were obtained was encouraging and suggests that further applications might be practicable.

To sum up, the immobilisation of dextranase from *P. notatum* on alkylamine porous glass through the process of glutaraldehyde coupling is the best of the methods studied. The alkylamine conjugated dextranase showed catalytic activities at a wider pH and temperature range

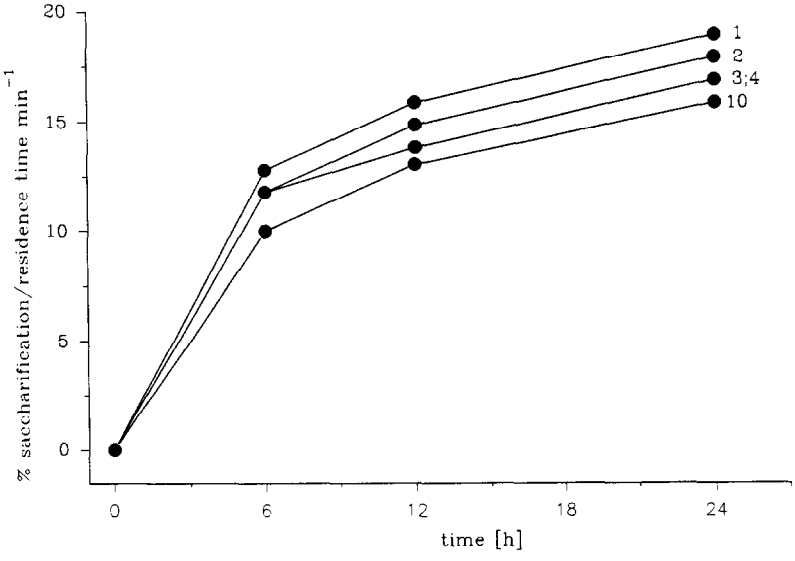


Fig. 4. Effect of time on the ratio (% saccharification/residence time) on passage of a solution of dextran through a column of immobilised dextranase. Hydrolysis was performed in 24 h cycles at a flow rate of 2 ml/min using dextranase immobilised on alkylamine glass (carrier I). The numbers in the particular curves represent the successive cycle of hydrolysis.

than those of the free enzyme. It was also characterized by the relatively high affinity to the substrate and good storage stability. These properties and the fact that the material can be packed into the column show that the porous glass holds potential possibilities as a matrix for dextranase immobilisation. The production of matrix with various degrees of controlled porosity could therefore offer the basis for a reactor-separator with the immobilised enzyme.

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References

[1] W. Gerhartz, Enzymes in Industry: Production and Applications, VCH Verlagsgesellschaft, Weinheim, 1990.
[2] L.W. Powell, Immobilized biocatalyst technology, in: W.M. Fogarty, C.T. Kelly (Eds.), Microbial Enzymes and Biotechnology, Elsevier Applied Science, London, New York, 1990.
[3] R.L. Sidebotham, Adv. Carbohydr. Chem. Biochem. 30 (1974) 371.
[4] A. Hattori, K. Ishbashi, Agric. Biol. Chem. 45 (1981) 2347.
[5] D.F. Day, D. Dim, Ann. N.Y. Acad. Sci. 672 (Ezyme Engineering XI) (1992) 573.
[6] M. Sugiura, A. Ito, Chem. Pharm. Bull. 22 (1974) 1593.
[7] M. Ulitzsch, C. Scholz, German (East) Patent DD 255,952 (Cl.C12P19/08) and DD 255,953 (Cl.C12P19/08) (1988).
[8] Madhu, K.A. Prabhu, Enzyme Microb. Technol. 7 (1985) 279.
[9] J.F. Kennedy, I.M. Kay, Carbohydr. Res. 56 (1977) 211.
[10] J.F. Kennedy, I.M. Kay, Carbohydr. Res. 59 (1977) 553.
[11] C.J. Gray, C.M. Livingstone, Biotechnol. Bioeng. 19 (1977) 349.
[12] M. Kobayashi, S. Yanagihara, T. Kitae, E. Ichishima, Agric. Biol. Chem. 53 (1989) 2211.
[13] N.W.H. Cheetham, G.N. Richards, Carbohydr. Res. 30 (1973) 99.
[14] V. Ramesh, C. Singh, Biochem. Biophys. Res. Commun. 97 (1980) 779.
[15] H.H. Weetall, Science 166 (1969) 616.
[16] J. Szczodrak, M. Pleszczyńska, J. Fiedurek, J. Ind. Microbiol. 13 (1994) 315.
[17] M. Pleszczyńska, J. Rogalski, J. Szczodrak, J. Fiedurek, Mycol. Res. 100 (1996) 681.
[18] M. Pleszczyńska, J. Szczodrak, J. Rogalski, J. Fiedurek, Mycol. Res. 101 (1997) 69.
[19] G.L. Miller, Anal. Chem. 31 (1959) 426.
[20] J. Rogalski, J. Szczodrak, A. Dawidowicz, Z. Ilczuk, A. Leonowicz, Enzyme Microb. Technol. 7 (1985) 395.
[21] A. Dawidowicz, J. Rogalski, Polish Patent No. 152959 (1991).
[22] L. Sundberg, J. Porath, J. Chromatogr. 90 (1974) 87.
[23] J. Rogalski, A. Dawidowicz, Polish Patent No. 149845 (1990).
[24] D.A. Lappi, F.E. Stolzenbach, N.O. Kaplan, M.D. Kamen, Biochem. Biophys. Res. Commun. 69 (1976) 878.
[25] B.T. Kaufman, J.V. Pierce, Biochem. Biophys. Res. Commun. 44 (1971) 608.
[26] M. Landt, S.C. Boltz, L.G. Butler, Biochemistry 17 (1978) 915.
[27] W.H. Scouten, Synthetic methods, in: P.J. Elving, J.D. Winefordner (Eds.), Affinity Chromatography: Bioselective Adsorption on Inert Matrices, John Wiley and Sons, New York, Chichester, Brisbane, Toronto, 1980, pp. 42–84.
[28] O.H. Lowry, N.J. Rosebrough, A.L. Faar, R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[29] O.N. Zinchenko, V.I. Shishlo, O.V. Krivosheeva, A.G. Lobanok, Prikl. Biokhim. Mikrobiol. 29 (1993) 851.
[30] G.E. Means, R.E. Feeney, Chemical Modification of Proteins, Holden-Day, San Francisco, 1971.
[31] L. Goldstein, Y. Levin, E. Katchalski, Biochemistry 3 (1964) 1913.
[32] J. Rogalski, A. Dawidowicz, A. Leonowicz, J. Mol. Catalysis 93 (1994) 233.
[33] M.D. Trevan, Immobilized Enzymes: An Introduction and Applications in Biotechnology, John Wiley and Sons, Chichester, New York, Brisbane, Toronto, 1980.
[34] R. Axén, P.-Å. Myrin, J.-C. Janson, Biopolymers 9 (1970) 401.
[35] H. Maeda, H. Suzuki, A. Yamauchi, A. Sakimae, Biotechnol. Bioeng. 16 (1974) 1517.
[36] H. Maeda, H. Suzuki, A. Yamauchi, A. Sakimae, Biotechnol. Bioeng. 17 (1975) 119.
[37] E. Stoppok, K. Buchholz, Zuckerindustrie 119 (1994) 476.
[38] W.R. Vieth, K. Venkatasubramanian, Chem. Technol. 4 (1974) 47.
[39] G.J. Melrose, Rev. Pure. Appl. Chem. 21 (1971) 83.
[40] L. Goldstein, Kinetic behavior of immobilized enzyme systems, in: S.P. Colowick, N.O. Kaplan, K. Mosbach (Eds.), Methods in Enzymology, vol. 44, Immobilized Enzymes, Akademic Press, New York, San Francisco, London, 1976, p. 397.
[41] M.D. Lilly, G. Kay, A.K. Sharp, R.J.H. Wilson, Biochem. J. 107 (1968) 5P.
[42] J.F. Kennedy, S.A. Barker, C.A. White, Carbohydr. Res. 38 (1974) 13.
[43] W.E. Hornby, M.D. Lilly, E.M. Crook, Biochem. J. 107 (1968) 669.
[44] K. Suga, G. van Dedem, M. Moo-Young, Biotechnol. Bioeng. 17 (1975) 185.