IMMOBILIZTION OF α-AMYLASE AND ITS APPLICATION FOR THE PRODUCTION OF GLUCOSE SYRUP

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ABSTRACT

Alpha-amylase has been immobilized on five different supports, Ca-alginate gel beads, sand, chitin, concanvalin A-sepharose and cyanogen bromide-activated sepharose. The activity of native α-amylase enzyme equalled 96.0 μmM glucose/L/min. The highest retention activity was achieved by cyanogen bromide-activated sepharose and amounted to 91.7%, but this value was 90.4% with Con A-sepharose. On the other hand, Ca-alginate, sand and chitin complexes gave the lowest retention activity i.e. 78.4, 71.2 and 72.8%, respectively.

The optimum pH values of free and immobilized alpha-amylase with Ca-alginate gel beads were found to be 6.2 and 7.0. However, maximum activities for free and immobilized forms were obtained at the same temperature, 50°C.

The maximum reaction velocity (V_{max}) and K_m values were 2036.6 μmM glucose/L and 0.55 g/100 ml buffer for free form. However, the V_{max} and K_m values equalled to 1579 μmM glucose/L and 0.75 g/100 ml buffer in the case of Ca-alginate gel beads immobilized form.

The relative activities of bounded α-amylase were 66.59, 76.55, 74.15, 92.14 and 93.62% for Ca-alginate, sand, chitin, Con A-sepharose and CNBr-activated sepharose after incubation period 96 days, respectively.

Reusability of immobilized α-amylase enzyme forms was assayed after 7 times use. The highest relative activity (92.96%), however it lost 7.04% of its initial activity after 7 cycles with enzyme-CNBr-activated sepharose complex. But, Con A-sepharose enzyme complex lost about 9.84% of its activity after 6 times. On the other hand, Ca-alginate enzyme gel beads decreased (30.0%) of its original activity after 5 times use.

The enzymatic hydrolysis of soluble starch for the production of glucose syrup were found to be 1172.28, 2256.5, 1670.50, 1621.51 and 2262.18 μmM glucose/L by using free and immobilized α-amylase on Ca-alginate, sand, chitin and CNBr-sepharose, respectively after 120 min. While, Con A-sepharose yielded 2210.05 μmM glucose/L after 150 min.

Keywords: Alpha-amylase, immobilized form, Reusability.

INTRODUCTION

Enzymes immobilization on insoluble supports have been extensively developed in the industrial applications of enzymes. Advantages are the possibility of using continuous processes with insoluble enzymes, facilitation of enzyme recovery, and in some cases immobilization of an enzyme will result in an enhanced stability (Lamb and Stuckey, 1999).

Noda *et al.* (2001) studied the immobilization of alpha-amylase onto chitosan beads for the production of maltose. They found that the immobilized alpha-amylase exhibited an activity of 142 U/g carrier. The optimum temperature of the immobilized alpha-amylase increased by 20°C, and thermostability was improved by about 10°C compared to free enzyme.

Dey *et al.* (2003) studied the immobilization of alpha-amylase by entrapment in calcium alginate gel beads. They found that the optimum pH and temperature were 4.9 and 57°C, respectively, but the apparent activity was 25.6 U/g of beads. The immobilized enzyme showed a high operational stability by retaining almost 85% of the initial activity after seventh use.

Lim *et al.* (2003) studied the immobilization of barley alpha-amylase on silica particles using a covalent binding. They found that the optimal conditions for the hydrolysis were pH 4.5, 40°C, calcium ion concentration (0.002 M) and immobilized enzyme loading of 30 mg/ml. At these conditions, the immobilized enzyme was able to hydrolyze wheat starch particles at concentrations as high as 100 mg/ml with a final conversion of 90% after 24 h of operation.

Bayramoglu *et al.* (2004) immobilized alpha-amylase onto poly (2-hydroxyethyl methacrylate) membranes. The optimum pH value of alpha-amylase was not affected by the immobilization reaction. Michaelis constant (K_m) of alpha-amylase was significantly larger (ca. 2.3 times) upon immobilization, whereas V_{max} was smaller for immobilized alpha-amylase. In a 120 h continuous operation at 35°C, only 4% of immobilized alpha-amylase activity was lost.

The aim of this investigation was to study the attempts of immobilization for alpha-amylase which the most popular enzyme used for industrial purposes in the starch solubilization process. Different supports were used in techniques of immobilization i.e. Ca-alginate gel beads, sand, chitin, cyanogen bromide activated-sepharose (CNBr-Sepharose) and concanavalin A-sepharose (Con A-Sepharose). Also, the optimal conditions and kinetic behaviour of free and immobilized enzyme were carried out. Besides that, the liquefaction process was thoroughly studied to obtain the most suitable conditions for glucose syrup production from soluble starch.

MATERIALS AND METHODS

1. Alpha-amylase enzyme: (E.C.3.2.1.1. α →1, 4 glucan-4-gluconohydrolase)

It was supplied from Novo Industria A/S Co. Denmark.

2. Supports for the immobilization techniques:

Alginic acid, sodium salt, conconavalin A-sepharose and cyanogen bromide-activated sepharose (CNBr-Sepharose) were obtained from Sigma Chemical Co. (ST Louis, MO 63178 USA). While, chitin was purchased from Alderich Chem. Co. Sand was obtained from Sina desert, Egypt.

The commercial substrates used in this study were D(+) glucose, soluble starch, glutaraldehyde, 3- Aminopropyltriethoxysilane (APTES), Na-alginate, sodium metaperiodate, cyanoborohydride and ethylenediamine were obtained from Sigma Chemical Co. Also, all other reagents were purchased from El-Gomharya Chemical Co., Cairo, Egypt.

3. Alpha-amylase enzyme assay:

The enzyme activity of alpha-amylase was measured according to the method described by Yoo *et al.* (1987). The resulted reducing sugars (as glucose) were estimated by using the method described by Somogyi (1952).

4. Immobilization methods for α-amylase enzyme:

Alpha-amylase was entrapped with calcium alginate according to the method described by Dey *et al.* (2003). Also, this enzyme was immobilized on sand according to the method of Brotherton *et al.* (1976). Another attempts for immobilization of α-amylase enzyme on chitin was carried out according to the method of Flov and Hayashida (1983). Also, immobilization of α-amylase enzyme on concanavalin A-sepharose by adsorption was done according to the method of Woodward (1985). However, the immobilization of enzyme on cyanogen bromide activated sepharose (CNBr-sepharose) was achieved according to the method of Mosbach (1988).

5. Evaluation of free and immobilized α-amylase enzyme:

The activity of alpha-amylase enzyme was tested on different pH values in (0.1 M) acetate and phosphate buffers, using 2.5 ml soluble of starch solution (1%) as substrate in presence (0.5 ml) of sodium chloride (10 g/L) and 0.5 ml diluted enzyme. The incubation period was 20 min at 50°C and the resulted glucose was carried out according to the method mentioned by Somogyi (1952).

Also, the activity tested at different temperatures using 2.5 ml of soluble starch solution (1%) in phosphate buffer (0.1 M, pH 6.2). The resulted glucose was carried out as mentioned before.

The effect of substrate concentration on reaction velocity of free and immobilized alpha-amylase within Ca-alginate was teseted by using different concentrations of soluble starch solutions, i.e., 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0% in (0.1 M) phosphate buffer, pH (6.2) at 50°C for 20 min. The produced glucose was carried out as mentioned before.

6. Stability of immobilized α-amylase enzyme:

The relative activity (%) of immobilized α-amylase on different supports, i.e. Ca-alginate, sand, chitin, CNBr-activated sepharose and Con A-sepharose were evaluated according to the method described by Woodward (1985).

7. Reusability of immobilized enzyme:

The relative activity (%) of each preparation on different above-mentioned supports was assayed under standard conditions after 7 cycles with repeated washing. The immobilized enzymes forms were resuspended in fresh substrate and the procedure was repeated.

8. Enzymatic hydrolysis of soluble starch by using free and immobilized enzyme on different supports:

The enzymatic hydrolysis of soluble starch with free and immobilized α-amylase enzyme forms on different supports was determined under the optimum conditions for each preparation enzyme form. The reaction mixture was incubated at different periods from 15 to 240 min and the resulting reducing sugars were estimated by the method described before.

RESULTS AND DISCUSSION

1. Immobilization of alpha-amylase on different supports:

Alpha-amylase enzyme have been immobilized on five different support materials i.e. Ca-alginate gel beads, sand, chitin, concanavalin A-sepharose and cyanogen bromide-activated sepharose. The quantity of the enzyme bounded to each support besides the reaction activity after immobilization has to be considered in comparing such supporting materials.

Alpha-amylase was bounded with the above-mentioned different supports as shown in Table (1). From these results the highest retention activity was found in cyanogen bromide-activited sepharose which simply means that the support was bounded with higher amount of enzyme added. The latter phenomenon was also accompanied with retention activity which amounted to 91.7%.

Similar results were also noticed in concanvalin A-sepharose but with less activity of the resulted complex and the retention activity in this case was 90.4%. However, Ca-alginate enzyme-complex retained 78.4% of its original activity with 4.0% Na-aglinate. On the other hand, sand and chitin

had been the lowest bounded materials which were accompanied with the lowest retention activity, 71.2% and 72.8%, respectively.

Table (1): Retention activities of immobilized α-amylase enzyme on different supports.

Supports	Activity of α-amylase enzyme					Effectiveness factor (D/C)100 (%)	Retention activity (D/A)100 (%)
	Added (A)	Protein mg/ml	In washing (B)	Immobilized forms: Theoretical (A-B) = C	Immobilized forms: Actual complex (D)		
Ca-alginate	125	2.02	28.0	97.0	98.0***	101.03	78.4
Sand	125	2.02	32.0	93.0	89.0**	95.7	71.2
Chitin	125	2.02	29.0	96.0	91.0**	94.8	72.8
CNBr-sepharose	125	2.02	18.7	106.3	114.6*	107.8	91.7
Con A-sepharose	125	2.02	15.0	110.0	113.0*	102.7	90.4

** Activity refers to the amount contained in 0.1 ml suspension of CNBr-sepharose and Con A-sepharose-enzyme complex.*

*** Activity refers to the amount contained in 1.0 g enzyme complex with sand or chitin.*

**** Activity refers to the amount contained in 1.0 ml Ca-alginate gel-enzyme complex.*

The decrement in the activity of the immobilized alpha-amylase might be attributed to the bounded active sites of this enzyme itself and/or the bound alpha-amylase has a conformational change in the immobilized enzyme, which has been occurred during immobilization process (Woodward, 1985). However, the highest retention activity of alpha-amylase enzyme on both Con A-sepharose and cyanogen bromide-activated separose may be due to the glycoprotein nature of this enzyme which made the attachment between the enzyme and substrate more strong (Wiseman, 1985).

The noticed decrement in the retention activity of the enzyme preparations with sand and chitin may be due to the desorption of the enzyme from the support as they effect such linkages. Also, changes in experimental conditions such as pH, ionic strength, temperature and type of solvent can cause desorption. Besides, that the enzyme may be conformationally different when fixed on a support, alternatively it may be attached to the solid carrier in a way that would render certain parts of the enzyme molecule less accessible to substrate. Such effects due to the diffusion limitation or perturbation of catalytic pathway of the enzyme reaction would reflect events arising from the fact that enzyme-substrate

interactions occur in a different micro environmental effects when an enzyme is immobilized on a solid support (Mosbach, 1988 and Lim *et al.*, 2003).

2. Evaluation of immobilized enzyme:

The effect of pH on the reaction activity of free and immobilized α-amylase within Ca-alginate was investigated in a batch system at 50°C and the results are presented in Table (2) and Fig. (1). Optimal conversion of soluble starch was obtained at pH 6.2 and the maximum activity was found to be 84.6 µmM glucose/L/min for free α-amylase form. However, the immobilized enzyme within Ca-alginate gel beads exhibits the maximum activity at pH 7.0 and the activity amounted to 63.9 µmM glucose/L/min. This difference is possibly due to the interactions between the enzyme and the polymeric matrix. Similar observations upon immobilization of α-amylase have been reported by Dey *et al.* (2003).

The effect of temperature on the reaction activity of free and immobilized α-amylase was evaluated. Table (3) and Fig. (2) show that the maximum activity for free and immobilized form were obtained at the same temperature, 50°C, which could be considered a balance between the increase of initial activity and destruction of the enzyme at high temperature. The maximum rates of reaction for free and immobilized form were 55.6 and 22.5 µmM glucose/L/min, respectively. Such observations are similar with that reported by Dey *et al.* (2003).

Table (2): Effect of pH on the reaction activity of free and immobilized α-amylase enzyme within Ca-alginate.

pH	Free enzyme		Immobilized form	
	Obtained of D-glucose (µmM/L)	Activity (µmM/L/min)	Obtained of D-glucose (µmM/L)	Activity (µmM/L/min)
4.4	973.0	48.7	166.8	8.3
4.6	1067.5	53.4	305.8	15.3
4.8	1195.4	59.8	417.0	20.9
5.0	1351.1	67.6	444.8	22.2
5.2	1467.8	73.4	511.5	25.6
5.5	1512.3	75.6	533.8	26.7
5.7	1634.9	81.7	589.4	29.5
6.0	1651.3	82.6	611.6	30.6
6.2	1692.4	84.6	722.8	36.1
6.5	1501.2	75.1	778.4	38.9
6.7	1340.0	67.0	945.2	47.3
7.0	1289.9	64.5	1278.8	63.9
7.2	1028.6	51.4	611.6	30.6
7.5	784.0	39.2	500.4	25.0

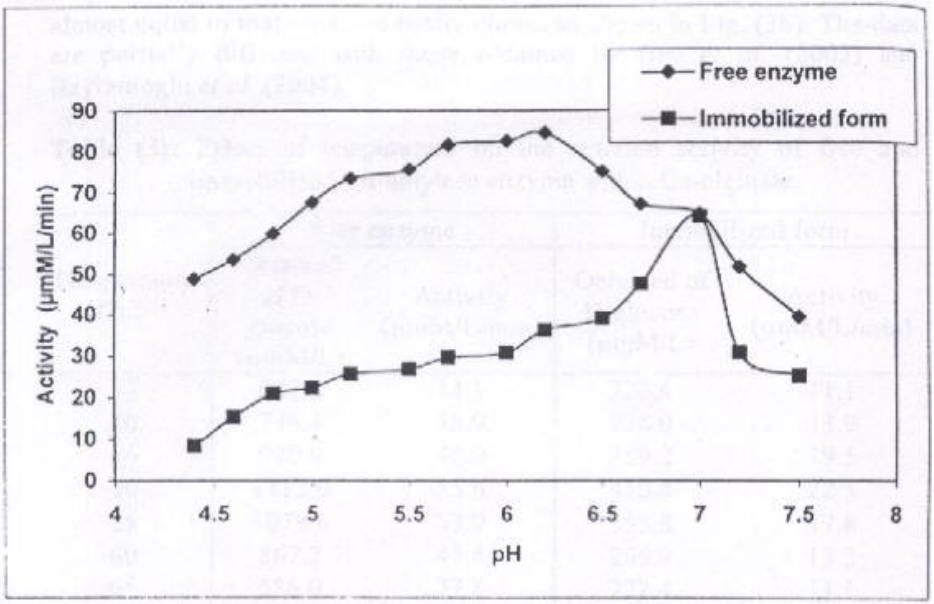

Fig. (1): Effect of pH on the reaction activity of free and immobilized α-amylase enzyme within Ca-alginate.

The effect of substrate concentration on the reaction velocity of free and immobilized α-amylase within Ca-alginate gel beads were estimated and the results were illustrated in Table (4) and Fig. (3a & b). By plotting the obtained glucose against the substrate concentrations, two curves were obtained, Fig. (3a & b) for free and immobilized form. The obtained results indicated that any increase of the substrate concentration was accompanied with the increment of activity until reached its maximum, beyond this concentration. Any further increase in substrate concentration does not show any positive effect and the reaction rate of enzyme depends on the time necessary for the enzyme to act on the substrate. The maximum reaction velocity (V_{max}) of free α-amylase was 2036.6 µmM glucose/L. Michaelis constant (K_m) of this enzyme can also be obtained by the half point of the experimented curve as shown in Fig. (3a). K_m was found to be 0.55 g/100 ml buffer in the case of free α-amylase. However, the V_{max} and K_m values equalled to 1579 µmM glucose/L and 0.75 g/100 ml buffer in the case of immobilized α-amylase enzyme. The obtained data indicate that apparent K_m value of immobilized enzyme was found to be 1.3 times higher than that of the free enzyme, but the differences in these values may be due to the decrement in the affinity between the substrate and the immobilized enzyme which yield an increase in K_m, also may be the result of the conformational changes and diffusion limitations. It is important to mention that K_m constant was once more determined by Lineweaver and Burk method (1954). The obtained K_m was

almost equal to that obtained firstly curves as shown in Fig. (3b). The data are partially different with those obtained by Dey *et al.* (2003) and Bayramoglu *et al.* (2004).

Table (3): Effect of temperature on the reaction activity of free and immobilized α-amylase enzyme within Ca-alginate.

Temperature (°C)	Free enzyme		Immobilized form	
	Obtained of D-glucose (μmM/L)	Activity (μmM/L/min)	Obtained of D-glucose (μmM/L)	Activity (μmM/L/min)
35	682.8	34.1	222.4	11.1
40	778.4	38.9	278.0	13.9
45	920.0	46.0	389.2	19.5
50	1112.0	55.6	450.4	22.5
55	1078.6	53.9	355.8	17.8
60	867.2	43.4	266.9	13.3
65	556.0	27.8	222.4	11.1
70	250.2	12.5	177.9	8.9

Table (4): Effect of substrate concentration on the reaction velocity of free and immobilized α-amylase within Ca-alginate gel.

Substrate concentration (% w/v)		Free enzyme			Immobilized form		
	1/[S]	Obtained D-glucose (μmM/L)	Reation velocity [V]	1/[V] ($x10^{-4}$)	Obtained D-glucose (μmM/L)	Reation velocity [V]	1/[V] ($x10^{-4}$)
0.5	2.00	1015.6	966.2	10.3	617.4	631.6	15.8
1.0	1.00	1556.8	1309.0	7.6	973.0	902.3	11.1
1.5	0.67	1751.4	1484.6	6.7	1167.6	1052.7	9.5
2.0	0.50	1879.2	1591.4	6.3	1223.2	1148.4	8.7
2.5	0.40	2029.4	1663.1	6.0	1501.2	1214.6	8.2
3.0	0.33	1951.4	1714.6	5.8	1579.0	1263.2	7.9
3.5	0.29	1712.4	1753.5	5.7	1565.2	1300.4	7.7
4.0	0.25	1556.8	1783.7	5.6	1490.2	1329.7	7.5
4.5	0.22	1506.7	1800.0	5.5	1278.8	1353.4	7.4
5.0	0.20	1390.0	1827.9	5.4	1062.0	1373.0	7.3

Free form

V_{max} = 2036.6 μmM/L

K_m = 0.55 g/100 ml

$$\frac{1}{V} = 4.91 \times 10^{-4} + \frac{0.55}{2036.6} \times \frac{1}{S}$$

Immobilized form

V_{max} = 1579.0 μmM/L

K_m = 0.75 g/100 ml

$$\frac{1}{V} = 6.33 \times 10^{-4} + \frac{0.75}{1579} \times \frac{1}{S}$$

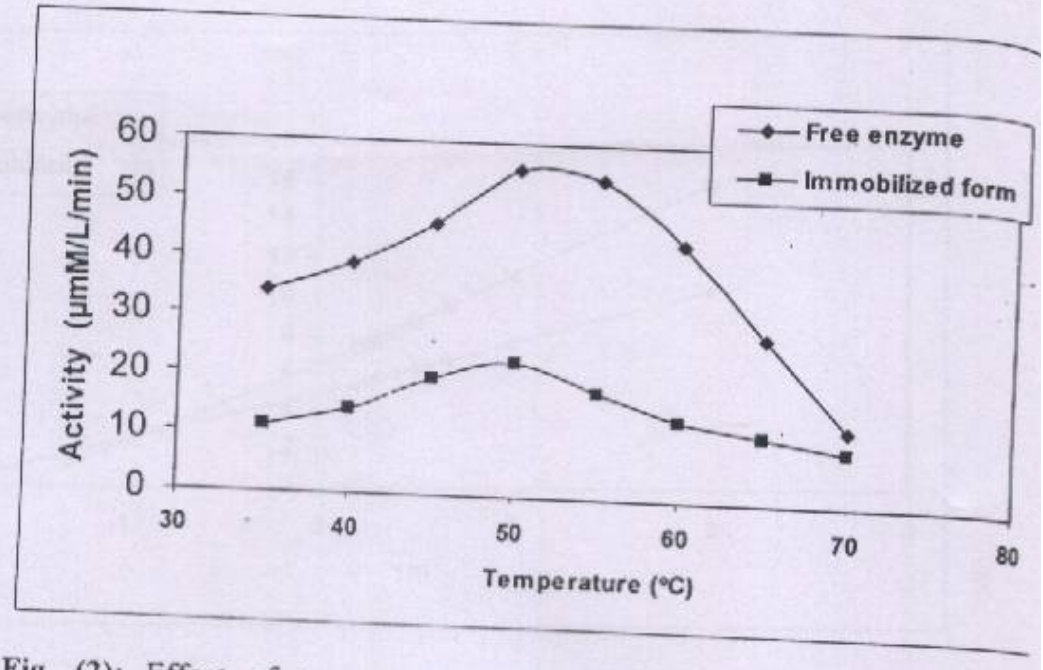

Fig. (2): Effect of temperature on the reaction activity of free and immobilized α-amylase enzyme within Ca-alginate.

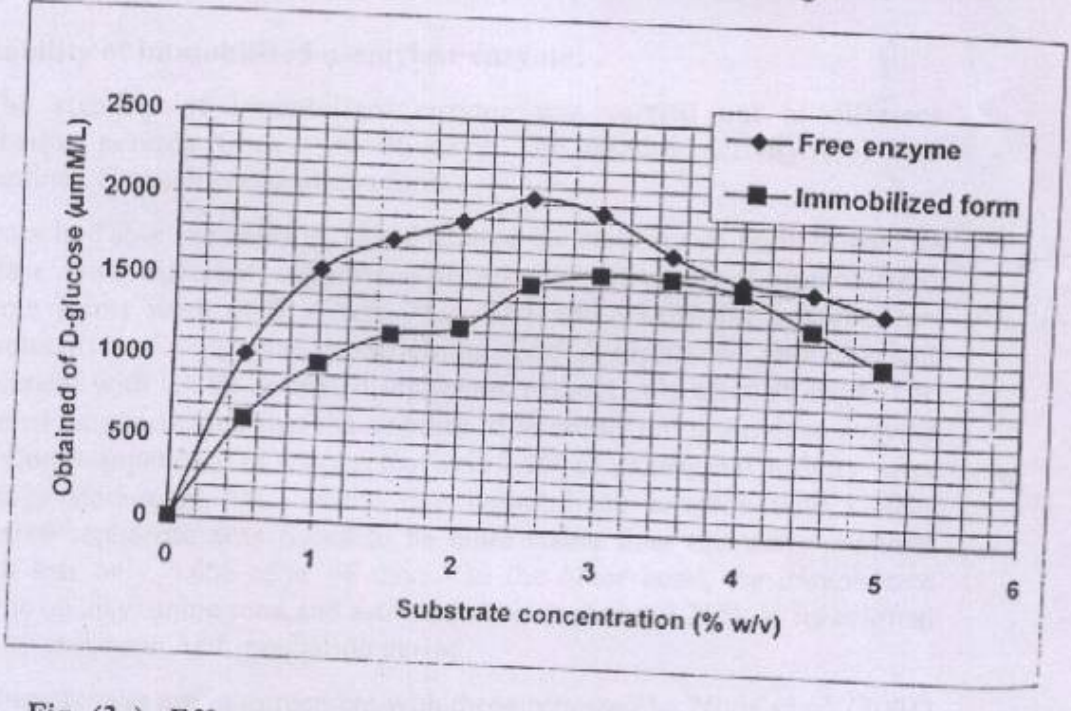

Fig. (3a): Effect of substrate concentration on the reaction velocity of free and immobilized α-amylase within Ca-alginate.

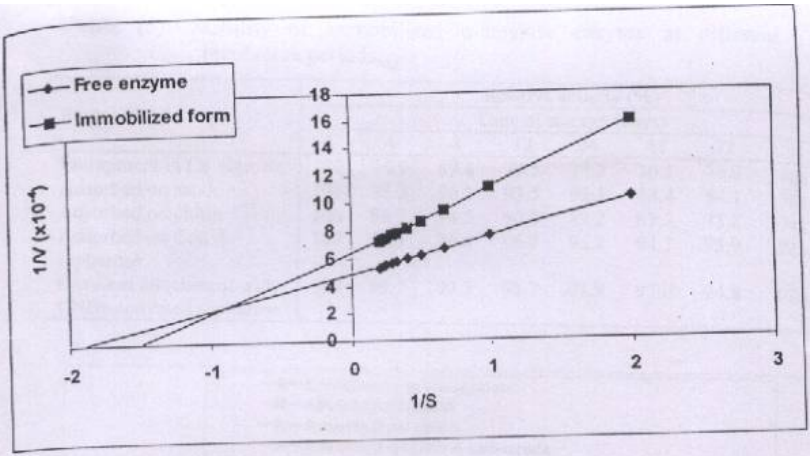

Fig. (3b): Lineweaver-Burk plots for the free and immobilized α-amylase within Ca-alginate

3. Stability of immobilized α-amylase enzyme:

The stability of immobilized enzyme was carried out at different incubation periods from 4 to 96 days. The relative activity (%) was determined for each preparations form.

Data in Table (5) and Fig. (4) illustrated the stability of immobilized α-amylase with different supports. The relative activities of immobilized enzyme forms were 66.6 , 76.6, 74.2, 92.1 and 93.6% for adsorbed on entrapment in Ca-alginate, sand, chitin, Con A-sepharose and covalent attachment with CNBr-activated sepharose, respectively after 96 days. The obtained data indicated that the stability of immobilized α-amylase enzyme with Con A-sepahrose as support lost only 7.9% of its original activity after 96 days storage at 4°C. While, the immobilized enzyme with CNBr-activated sepharose was found to be more stable than the other supports which lost only 6.4% after 96 days. On the other hand, the immobilized enzyme on alkylamine sand and activated chitin lost about 25% of its original activity at the same of incubation period.

These results are in agreement with those repeated by Noda *et al.* (2001)

Table (5): Stability of immobilized α-amylase enzyme at different incubation periods.

Immobilized forms	Relative activity (%) Time of storage (days)							
	0	4	8	12	24	48	72	96
Entrapment in Ca-alginate	100	94.9	89.8	79.5	74.2	70.1	70.0	66.6
Adsorbed on sand	100	98.3	96.3	93.5	93.1	88.4	84.1	76.6
Adsorbed on chitin	100	96.2	94.5	90.8	89.2	87.3	75.8	74.2
Adsorbed on Con A-sepharose	100	99.1	98.4	96.7	95.2	94.3	93.9	92.1
Covalent attachment with CNBr-activated sepharose	100	99.7	99.5	98.2	97.9	97.0	94.8	93.6

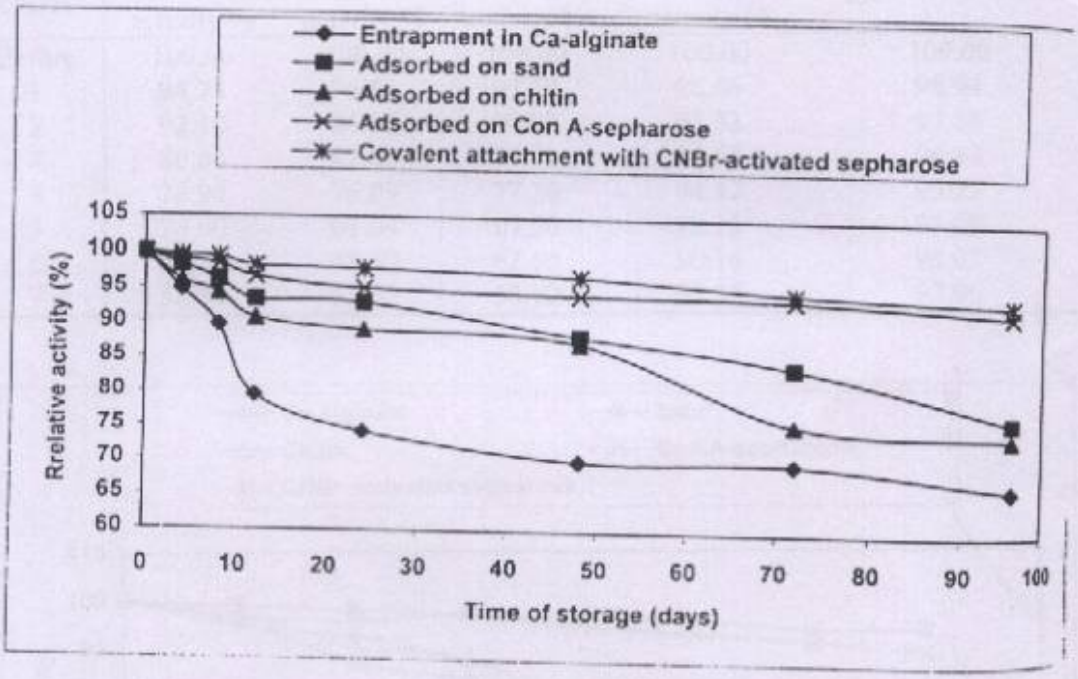

Fig. (4): Stability of immobilized α-amylase enzyme at different periods.

4. Reusability of immobilized α-amylase enzyme:

The relative activity (%) of immobilized enzyme forms on different supports is assayed after 7 times recycles with repeated washing and the obtained results are shown in Table (6) and Fig. (5). The reusability of immobilized α-amylase with Ca-alginate gave lowest relative activity (70.0%), its loss reached approximately 30.0% of its original activity after 5 times use. This observation may be attributed to the linkage of the immobilized enzyme from with the gel molecules. While, the decreament of immobilized enzyme activities on alkylamine sand and activated chitin were 23.91% and 18.62% of its original activity after 4 times use, this may be due to desorption of enzyme from surface area of these supports.

However, CNBr-activated sepharose enzyme complex exhibited the highest relative activity (92.96%) therefore its lose reached 7.04% of its initial activity after 7 recycles. But, the immobilized enzyme with Con A-sepharose showed a relative activity (90.16%) and lost only 9.84% of its original activity after 6 times use. Therefore, it could be concluded that the reusability values of immobilized α-amylase with the above-mentioned supports are similar with those reported by Dey *et al.* (2003) and Bayramoglu *et al.* (2004).

Table (6): Reuse of immobilized α-amylase enzyme.

Number of cycles	Ca-alginate	Sand	Chitin	Con A-sepharose	CNBr-activated sepharose
	Relative activity %	Relative activity %	Relative activity %	Relative activity %	Relative activity %
Before	100.00	100.00	100.00	100.00	100.00
1	94.73	95.65	97.50	96.66	98.94
2	92.10	86.95	90.00	95.33	97.88
3	86.84	82.60	87.50	96.66	96.82
4	78.94	76.09	77.50	94.12	95.73
5	70.00	63.04	67.50	92.15	95.09
6	60.52	58.69	62.50	90.16	93.03
7	52.84	55.65	50.40	88.33	92.96

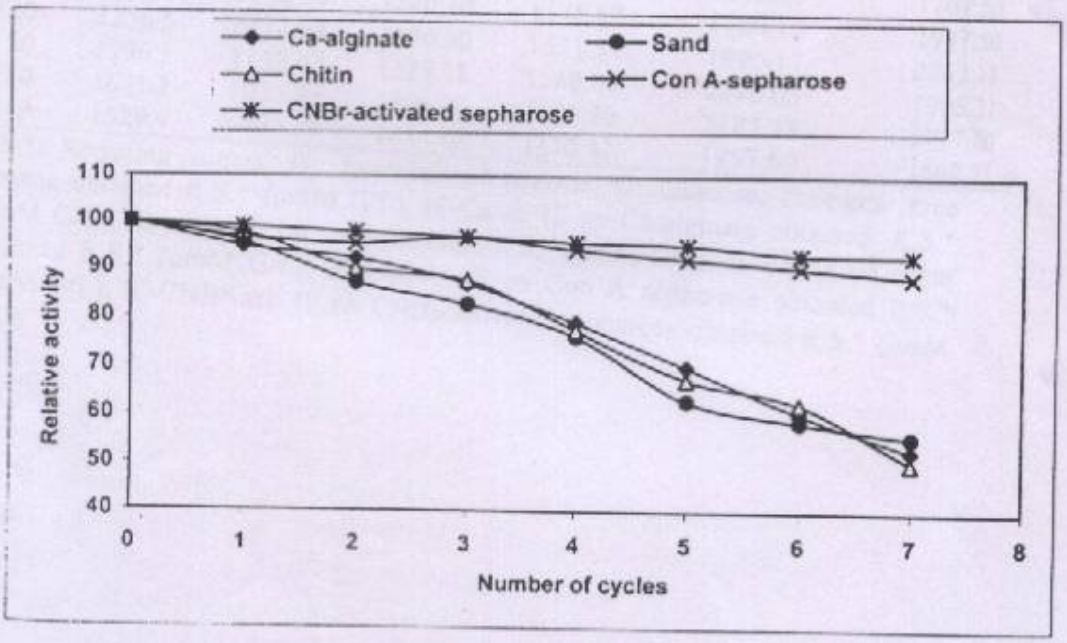

Fig. (5): Reuse of immobilized α-amylase enzyme.

5. Enzymatic hydrolysis of soluble starch with free and immobilized α-amylase enzyme:

The effect of time for continuous hydrolysis of soluble starch by using free and immobilized α-amylase forms on different supports is shown in Table (7) and Fig. (6). These results indicated that the reducing sugars (as glucose) increase with increasing incubation time till reached its maximum 2256.5 µmM glucose/L for free form after 120 min. However, the obtained results of maximum reducing sugars were 1172.28, 1670.50, 1621.51 and 2262.18 µmM glucose/L for immobilized α-amylase on Ca-alginate, sand, chitin and CNBr-sepharose, respectively after incubation period 120 min. On the other hand, the immobilized form with Con A-sepharose gave its maximum reducing sugars, 2210.05 µmM glucose/L after continuous hydrolysis for 150 min. These results are partially differentiation from those reported by Lim *et al.* (2003) and Bayramoglu *et al.* (2004).

Table (7): Enzymatic hydrolysis of starch with free and immobilized α-amylase enzyme on different supports.

Time (min)	Free-enz	IE-Ca-al	IE-sand	IE-ch	IE-Con A	IE-CNBr-act
15	1655.7	997.55	815.95	925.15	1232.50	1125.15
30	1775.1	1117.51	925.56	1022.55	1440.62	1130.25
60	1980.2	1130.40	1040.60	1365.58	1650.25	1267.50
90	2212.4	1142.50	1270.16	1576.57	1780.15	1997.50
120	2256.5	1172.28	1670.50	1621.51	1990.17	2262.18
150	1996.5	1137.22	1325.15	1560.00	2210.05	1995.22
180	1671.2	1035.15	1206.20	1375.50	2185.18	1777.60
240	1529.9	905.18	1155.10	1175.17	1957.60	1662.55

R.S.*: Reducing sugars, IE: Immobilized enzyme, G: Glucose, Free-enz: Free enzyme obtained R.S.* (µmM G/L), IE-Ca-al: IE on Ca-alginate obtained R.S.* (µmM G/L), IE-sand: IE on sand obtained R.S.* (µmM G/L), IE-ch: IE on chitin obtained R.S.* (µmM G/L), IE-Con-A: IE on Con A sepharose obtained R.S.* (µmM G/L), IE-CNBr-act: IE on CNBr-activated sepharose obtained R.S.* (µmM G/L).

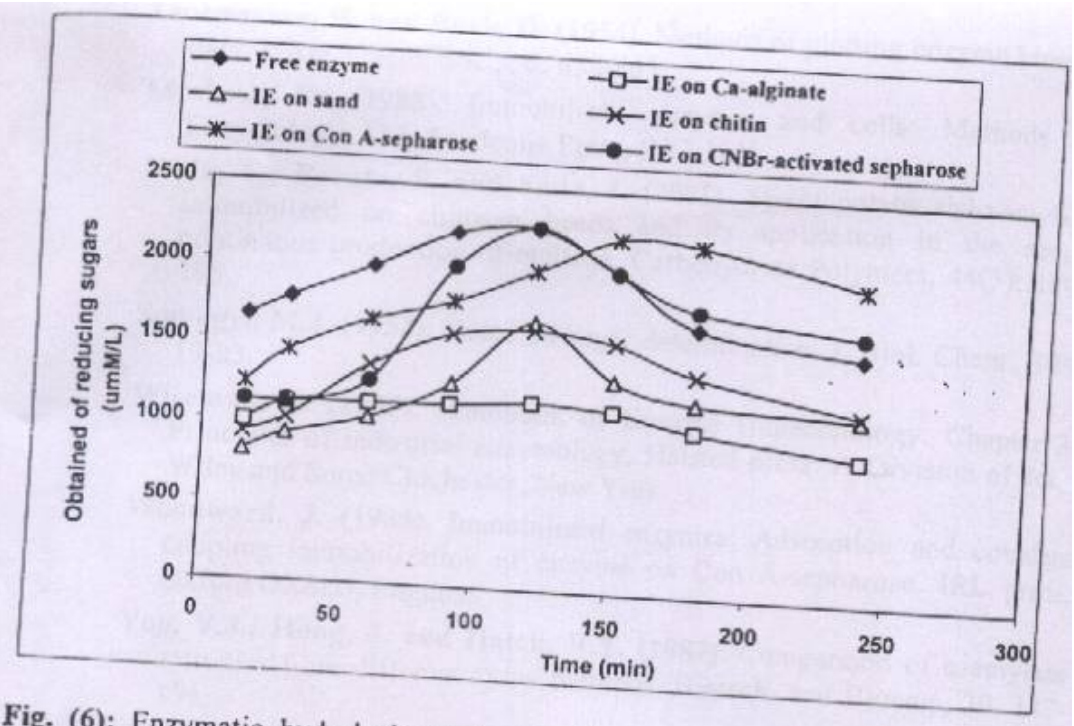

Fig. (6): Enzymatic hydrolysis of starch with free and immobilized α-amylase enzyme on different supports.

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تحميل إنزيم ألفا-أميليز واستخدامه فى إنتاج شراب الجلوكوز

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الملخص العربى

أمكن فى هذا البحث تحميل إنزيم ألفا أميليز لاستخدامه أكثر من مرة لانتاج شراب الجلوكوز من النشا الذائب فى النظام المستمر. وكذلك دراسة الظروف المختلفة التى تؤثر على درجة النشاط للأنزيم فى صورته الحرة والغير ذائبة حيث تم تحميل هذه الأنزيم على خمس دعامات ذات طبيعة تركيبية مختلفة باستخدام وسائل متنوعة وهى ألجينات الكالسيوم والرمل والكيتين والكونيكانافلين-أ-سيفاروز والسيانوجين بروميد المنشط سيفاروز.

أظهرت النتائج المتحصل عليها أن نسب الاسترجاع لمعقدات إنزيم ألفا أميليز مع كل من

أظهرت الدراسة أن درجة الـ pH المثلى لأنزيم ألفا أميليز المحمل كانت ٧,٠ بينما كانت للأنزيم الحر ٦,٢. أما درجة الحرارة المثلى وصلت ٥٥°م مع الفا أميليز المحمل والحر.

أوضحت نتائج تأثير تركيز المادة المتفاعلة للأنزيم الحر أن السرعة القصوى لنشاط الإنزيم (٢٠٣٦,٦) ميكروملليمول جلوكوز/لتر أما ثابت ميكاليس-منتن فكان ٠,٥٥ جرام/١٠٠ مللى. بينما كانت السرعة القصوى لنشاط إنزيم ألفا أميليز المحمل على كالسيوم ألجينات هى ١٥٧٩ ميكروملليمول جلوكوز/لتر بينما ثابت ميكاليس-منتن يساوى ٠,٧٥ جرام/١٠٠ مللى محلول منظم.

أجريت أيضا دراسة درجة ثبات الأنزيم المحمل على الدعامات المختلفة تحت الدراسة حيث كانت درجة النشاط لمعقدات أنزيم ألفا أميليز بعد تخزين ٩٦ يوم هى ٦٦,٥٩، ٧٦,٥٥، ٧٤,١٥، ٩٢,١٤، ٩٣,٦٢% مع دعامات جل الكالسيوم-ألجينات، الرمل، الكيتين، والكونيكانافلين-أ-سيفاروز، السيانوجين بروميد على التوالى.

أظهرت نتائج معقدات الأنزيم مع الدعامات المختلفة أنه يمكن استخدامها أكثر من مرة. حيث أمكن استخدام معقد إنزيم ألفا أميليز مع ألجينات الكالسيوم ٥ مرات مع فقد حوالى ٣٠% من درجة نشاطه بينما أمكن إعادة استخدام معقد الإنزيم مع الرمل والكيتين ٤ مرات مع فقد ٢٣,٩١، ١٨,٦٢% على التوالى. كما أظهرت نتائج معقدات الإنزيم مع السيانوجين بروميد أعلى معدل لدرجة النشاط حيث استرجع المعقد ٩٢,٩٦% من درجة نشاطه بعد استخدامه ٧ مرات مع نسبة فقد ٧,٠٤% ولكن الأنزيم المحمل على الكونيكانافلين-أ-سيفاروز فقد حوالى ٩,٨٤% من درجة نشاطه بعد إستخدام ٦ مرات.

أوضحت النتائج أن أعلى مستويات لمعدل التحلل الأنزيمى لانتاج شراب الجلوكوز لمحلول النشا الذائب (٥%) هى ١١٧٢,٢٨، ٢٢٥٦,٥، ١٦٧٠,٥، ١٦٢١,٥١، ٢٢٦٢,١٨، ٢٢١٠,٠٥ ميكروملليمول جلوكوز/لتر لأنزيم الألفا أميليز الحر والمحمل على كل من جل الكالسيوم-ألجينات، الرمل، والكيتين، السيانوجين بروميد مع أوقات للتفاعل ١٢٠ دقيقة، ١٥٠ دقيقة مع الكونيكانافلين-أ-سيفاروز على التوالى.

أوضحت هذه الدراسة أن الدعامات الطبيعية المستخدمة الرخيصة الثمن ذات كفاءة عالية فى تحميل الأنزيمات ويمكن تطبيقها فى المجالات الصناعية على نطاق واسع.