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IMMOBILIZATION OF AMYLOGLUCOSIDASE BY USING DIFFERENT SUPPORTS

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ABSTRACT

Amyloglucosidase enzyme from *Aspergillus niger* has been immobilized on different support materials i.e., chicken bone, conçanavalin A-sepharose-6B (con-A-Sepharose), calcium alginate gel and coimmobilized con-A-sepharose within calcium alginate gel. The amount of activity bound relative to an equal amount of free enzyme added were 75.7%, 88.1%, 61.5% and 95.4% for the above mentioned supports, respectively. Bound amyloglucosidase with different supports was the most stable compared with the native enzyme after 35 days.

Kinetic behavior of the immobilized enzyme with different supported were studied. The free enzyme showed K_m value of 1.48 10^{-3} M which was increased after immobilization to 1.85 10^{-3} M, 3.70 10^{-3} M, 1.54 10^{-3} M and 4.14 10^{-3} M for bone, con A-sepharose Ca-alginate gel and combined con A-sepharose-Ca-alginate gel beads.

A decrease in V_{max} values occurred upon enzyme immobilization for all supports, but this largely reflected the amounts of enzyme initially bounded to the supports. The highest amount of reducing sugars was 1165.6 mole glucose/100 ml for free enzyme after incubation time 180 min (3h). While, the immobilized forms reached a maximum amounts of reducing sugars of 434.0, 116.6, 232.5 and 133.0 mole glucose/100 ml for bone, con A-sepharose, Ca-alginate gel and combined con-A-sepharose-Ca-alginate gel beads, respectively after incubation time 150 min. Bound amyloglucosidase on chicken bone gave a high relative activity (95.6%) after three cycles of continuous use at 45°C. But the relative activities were 80% for con A-sepharose after two cycle, 50% for Ca-alginate gel beads after two cycles, this may be due to leaking out of the enzyme from the porous matrix and 70% for coimmobilized enzyme after four cycles.

INTRODUCTION

Amyloglucosidase (E.C.3.2.1.3) enzyme is an exosplitting enzyme that catalyzes the stepwise hydrolysis of -(1, 4) linkages and to a lesser extent -(1, 6) linkages from the nonreducing ends of starch and glycogen. It is one of the most industrially important enzymes which widely used for the production of high-conversion (DE 60-70) and high-dextrose (DE 95) syrups (Norman, 1981).

Amyloglucosidase has been immobilized to create a more controlled reusable enzyme system for commercial purpose. Entrapment in calcium alginate gels is one of the most widely used techniques for immobilizing living cells and enzymes since this technique is suitable for large-scale use according to its simple, cheap and nondenatareing of enzyme proteins (Martinsen *et al.*, 1989).

Qayyum *et al.*, (1985) used calcium alginate for the immobilization of amyloglucosidase by entrapment as concanavalin A-sepharose in order to prevent the leaking out of the enzyme from the porous matrix. They found that Con-A-enzyme complex exhibited marked broadening of pH-activity and temperature-activity and was highly resistant to temperature inactivation even after entrapment in the alginate beads.

Freire and Sant'Annd (1990) immobilized amyloglucosidase enzyme on chitin. They observed a loss of 6% in activity after 20 days at 45°C and pH 4.5.

Schafhauser and Storey (1992) immobilized amyloglucosidase enzyme on granular chicken bone by noncovalent interaction. They found that K_m values for free and immobilized form were 101mg/ml and 95 mg/ml, respectively. While, V_{max} values were 64.3 nmol glucose/ml/min for the soluble enzyme and 26.1 nmol glucose/ml/min for the immobilized enzyme at pH 4.5 (100 mM acetate buffer) for 30 min at 23°C.

The aim of the present work is to immobilize amyloglucosidase enzyme on the granular chicken bone, concanavalin A-sepharose-6B and coimmobilized Con A-sepharose enzyme complex in calcium alginate gels. Also, kinetic properties and stability parameters were studied for free and immobilized amyloglucosidase enzyme. Since these parameters are important from the industrial and economical point of view.

MATERIALS AND METHODS

1. **Amyloglucosidase enzyme** (E.C.3.2.1.3., 1,4- -D-Glucan glucohydrolase) was supplied from NOVO laboratories, INC. Danbury, U.S.A. Amyloglucosidase enzyme was immoblized on different supports, i.e granular chicken bone, concanavalin A-sepharose 6B and Ca-alginate and coimmobilized of Con A-S with ca-alginate according to the methods

described by Schafhauser and Storey (1992), Woodward (1985) and Qayyum *et al.*, (1985), respectively. Soluble starch as substrate, D(+) glucose as standard, Na-alginate, concanavalin A-sepharose 6B and chicken bone were purchased from Sigma chemical Co. (St. Louis, MO).

2. Protein and amyloglucosidase assays:-

Protein content was estimated by the method described by Bradford (1976). Amyloglucosidase activity was determined by using the method described by Attia and Ali (1974). The resulting reducing sugars (as glucose) were estimated by the method described by Nelson's (1944).

3. Determination of optimum binding conditions with chicken bone as support of immobilization:-

3. 1. Effect of pH values on binding conditions:

The effect of pH on the amount of amyloglucosidase which was immobilized to the chicken bone was determined by using different pH values, i.e. 3.0, 4.0, 4.5, 5.0, 5.5 and 6.0 in acetate buffer at concentrations, 50 mM and 100 mM.

3.2. Effect of concentrations of amyloglucosidase on binding conditions:-

The reaction mixture (100 mg bone + 0.5 ml acetate buffer) were incubated at different amounts of amyloglucosidase i.e., 0.1, 0.3, 0.5, 0.8, 1.0 ml concentrated enzyme at 25°C for 30 min. Enzyme assays were performed as described above.

3. 3. Effect of chicken bone amounts on binding conditions:

The immobilization process was achieved at different amounts of bone i.e., 50-75-100-125 and 150 mg.

4. Effect of pH and temperature on free and immobilized amyloglucosidase with different supports:-

The effect of pH on the activity of free and immobilized amyloglucosidase on chicken bone, con A-Sepharose Ca-alginate and Con-A-sepharose entrepped in ca-alginate were tested at different pH values i.e., 3.3, 3.6, 4.2, 4.5, 5.0 and 5.5 acetate buffer (100 mM), 6.1 and 6.8 phosphate buffer (0.1M). While, the effect of temperature on the activity of free and immobilized forms were incubated at different temperatures, i.e., 30, 35, 40, 45, 50, 55, 60 and 65°C for 30 min. The activity of free and immobilized forms were determined by measuring the resulting glucose as described before.

5. Kinetic parameters of free and immobilized amyloglucosidase preparations:-

The kinetic parameters for free and immobilized amyloglucosidase enzyme were determined by using different concentrations of soluble starch as substrate, i.e., 0.05, 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, 1.5, 1.8 and 2.0% in acetate buffer (100 mM, pH 4.5). The enzymatic reactions were incubated for 30 min at

optimum temperature for each enzyme forms. The resulting reducing sugars were determined according to the method described before. K_m and V_{max} values were calculated by using Lineweaver-Burk technique (1954).

6. **Stability and storage half-life of free and immobilized forms** were evaluated according to the method described by Woodward (1985).

RESULTS AND DISCUSSIONS

3.1. Preparations of immobilized amyloglucosidase enzyme on different supports:-

Amyloglucosidase enzyme from *Aspergillus niger* has been immobilized on different support materials i.e., chicken bone, concanavalin A-sepharose (con A-sephorose, CAS), Ca-alginate gel, and coimmobilized (Con A-sepharose entrapped in Ca-alginate). The properties of the different immobilized preparations of amyloglucosidase enzyme are shown in Table (1).

The results showed that amyloglucosidase was bounded with the different supports but with different degrees. These results showed that the highest binding power was found in coimmobilized enzyme form (Con-A-sepharose entrapped in Ca-alginate), since lower amount of enzyme was noticed in washings. This enzyme form was also accompanied with relatively high reaction activity equals to 95.4%. On the other hand, the activiof bound enzyme was 18.5 mole glucose/min. Similar case was also noticed in Con A-sepharose with retention activity amounted to 88.1% and the activity of the resulted complex was found to be 26.3 mole glucose/min. On the other, chicken bone as a support of immobilized enzyme by noncovalent interactions showed a very reasonable results, with retention activity equal to 75.7% free enzyme. Also, the activity of resulted complex was 44.1 μmole glucose/min.

Table (1): Immobilization of amyloglucosidase on different supports:-

Type of support	Enzyme added	Enzyme in supernatant	Bound enzyme		Activity of immobilized enzyme
			Total bound units	Bound enzyme (%)	
Chicken bone (Biobone)	1.725 mg protein/100 mg bone	0.419 mg protein	1.306 mg	75.7	44.1
Con A-sepharose (CAS)	3.45 mg protein/5 ml CAS	0.41 mg protein	3.04 mg	88.1	26.3
Ca-alginate	1.725 mg protein/5 ml Na-alginate	0.665 mg protein	1.06 mg	61.5	25.7
ConA-Sepharose enzyme coimmobilized in Ca-alginate	3.45 mg protein/5 ml Na-alginate	0.16 mg protein	3.29 mg	95.4	18.5

However, Ca-alginate gel beads showed the lowest bounded material which was accompanied with the lowest retention activity i.e., Ca 61.5%, and the activity of gel beads was found to be 25.7 mole glucose/min. The decrement in the activity of immobilized enzyme may be due to the bounded active sites of amyloglucosidase with the support which simply affect the whole body of the enzyme itself. The noticed decrease in the relation activity of the enzyme preparations with Ca-alginate may be due to the linkage of the enzyme with gel. Also, the bound amyloglucosidase has a conformational alteration that affects the active sites. This conclusion is in good agreement with Woodward (1985), Qayyum, *et al.*, (1985) and pieters *et al.*, (1992) reportes.

3. 2. Optimal binding conditions of amyloglucosidase immobilization onto chicken bone:-

3. 2. 1. Effect of pH and ionic strength:

Table (2) show the effect of pH and ionic strength on the amount of amyloglucosidase immobilized on chicken bone. From these results the amount of enzyme bounded increased progressively with the increase of pH value it reached a maximum amounts at pH 5.0 at different concentration of acetate buffer i.e., 50 mM and 10 mM.

3. 2. 2. Effect of enzyme concentration:

Table (3) show the effect of different concentrations of amyloglucosidase (0.1-1.0 ml, concentrated dialysis enzyme) on the amount of enzyme immobilized to the bone. The amounts of amyloglucosidase on chicken bone adsorbed increased up to 0.8 ml concentrated enzyme (1.38 mg protein/100 mg bone). The activity of adsorbed enzyme onto bone under the above condition was found to be equalled 71.4 μmole glucose/min (relative activity 100%).

3. 2. 3. Effect of amount bone:-

Table (4) show the effect of amount bone on amyloglucosidase immobilized onto bone. It is observed that increasing the amount of chicken bone (with a constant amount of enzyme) will increase the amount of enzyme adsorbed onto bone till reaching a maximum rate then, further increase in amount bone does not effect. This occured when the surface area of bone is saturated with enzyme. The maximum amount of bone reached 125 mg bone/0.8 ml concentrated enzyme. It showed a maximum activity of 67.2 mole glucose/min/125 mg bone-enzyme complex.

3.3. Stability of free and immobilized amyloglucosidase on different supports:-

The stabilities of different preparations of immobilized forms were assayed at optimum temperature and optimum pH for each preparations and results are shown in Table (5) and Fig. (1). The relative activity of each immobilized forms were estimated at different periods (1-35 days) comparing with free enzyme. From these results, chicken bone was most stable comparing with the free enzyme and other supports, i.e., relative activity was 93.4% after 35

days. Other supports for immobilization amyloglucosidase enzyme showed lower stability. The relative activities obtained after 35 days were 90.2%, 86.4%, 89.9% and 87.4% for Con A-sepharose, Ca-alginate gel beads, coimmobilized form and free enzyme, respectively.

Table (2): Effect of pH and ionic strength on the amount of amyloglucosidase immobilized onto bone.

PH	Relative Activity (%)	
	50 mM acetate	100 mM acetate
3.0	(76.5%)	(71.1%)
4.0	(80.2%)	(78.3%)
4.5	(86.0%)	(86.0%)
5.0	(100.0%)	(100.0%)
5.5	(64.2%)	(56.5%)
6.0	(61.7%)	(47.8%)

The standard amount of enzyme (5 ml, 0.863 mg protein) was added to bone at each pH.

Table (3): Effect of amounts of amyloglucosidase immobilized onto bone.

Amounts of enzyme (ml)	Protein enzyme content	Activity	Relative Activity (%)
0.1	0.173	48.3	(67.6%)
0.3	0.518	53.6	(75.1%)
0.5	0.863	63.0	(88.2%)
0.8	1.380	71.4	(100%)
1.0	1.898	52.5	(73.5%)

Table (4): Effect of amount of bone on amyloglucosidase immobilized onto Bone:

bone:Amount of bone (mg)	mg protein in washing	Activity	Relative activity (%)
50	0.120	44.1	(65.6%)
75	0.121	56.7	(84.4%)
100	0.112	58.8	(87.5%)
125	0.089	67.2	(100%)
150	0.094	52.5	(78.1%)

3. 4. Effect of pH on the reaction activity of immobilized amyloglucosidase on different supports comparing with its free enzyme:-

The obtained results are illustrated in Table (6) and Fig. (2). The maximum activity was at pH 4.5 for free enzyme and immobilized amyloglucosidase on Ca-alginate and con A-sepharose entrapped in Ca-

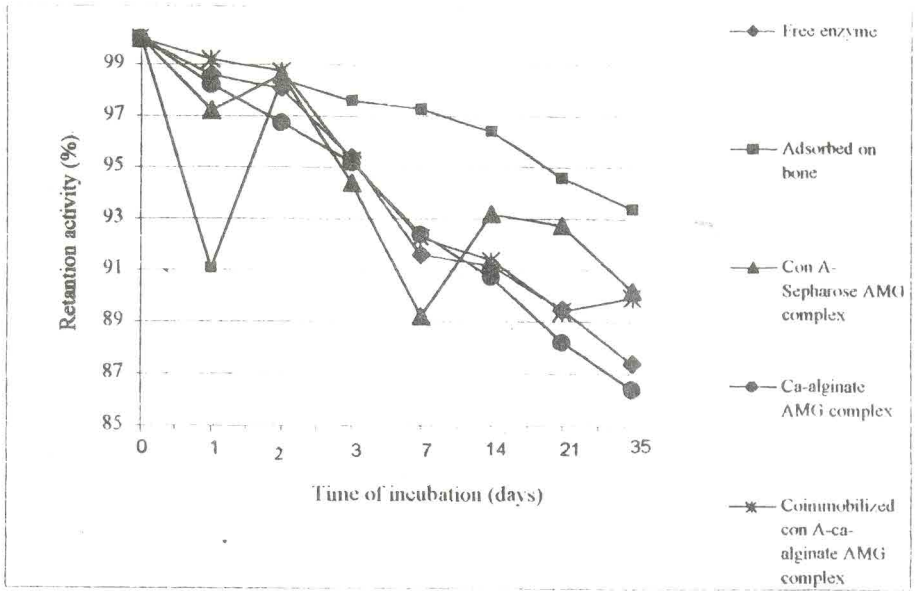
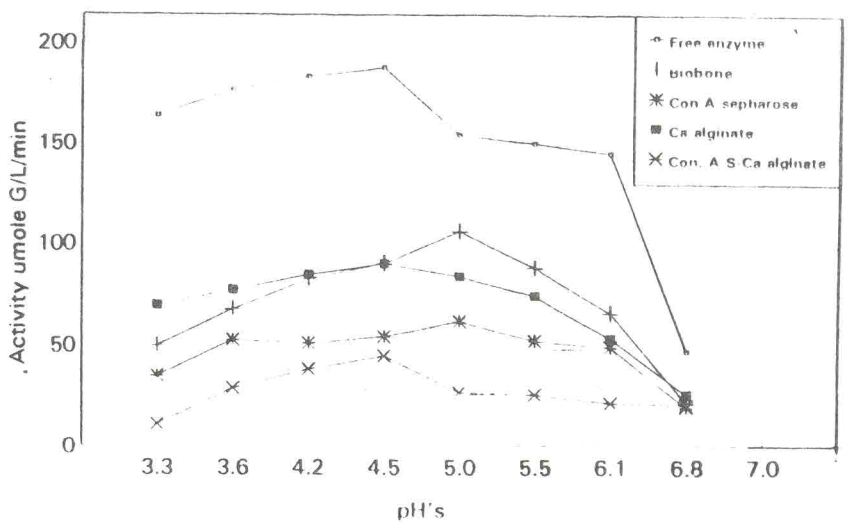


Fig (1): Stability of free and immobilized amyloglucosidase on different supports at 5°C

Fig(2): Effect of pH on the activity of free and immobilized amyloglucosidase enzyme ondifferent supports



alginate. While, the immobilized enzyme on chicken bone and con-A- sepharose as supports exhibited some broadening in the pH activity.

The maximum activities were 105.0 mole glucose/min/125 mg bone-enzyme complex and 60.9 mole glucose/min/100 ml suspension of con A-sepharose-enzyme complex, respectively. The obtained results are in good agreement with those obtained by Schafhauser and Storey (1992) and Qayyum, *et al.*, (1985). The differentiation in optimum pH values of free and immobilized enzyme on bone and con A-sepharose as supports may be due to the conformation changes in the immobilized enzyme upon immobilization process.

Table (5): Stability of free and immobilized amyloglucosidase on different supports at 5°C.

Type of support	Activity or Relative Activity (%) µmole G/L/min							
	Time of incubation (days)							
	0	1	2	3	7	14	21	35
Free enzyme	105.0	103.5	103.0	100.2	96.2	95.8	94.0	91.8
	100%	98.6%	98.1%	95.4%	91.6%	91.2%	89.5%	87.4%
Adsorbed	46.2	45.8	45.5	45.1	45.0	44.5	43.7	43.2
on bone	100%	91.1%	98.4%	97.6%	97.3%	96.4%	94.6%	93.4%
Con A-Sepharose	26.3	25.6	25.9	25.1	23.5	24.5	24.4	23.7
AMG complex	100%	97.2%	98.6%	95.4%	89.2%	93.2%	92.7%	90.2%
Ca-alginate AMG	51.5	50.6	49.8	49.0	47.6	46.7	45.4	44.5
Complex	100%	98.2%	96.7%	95.2%	92.4%	90.7%	88.2%	86.4%
Coimmobilized	17.9	17.8	17.7	17.1	16.5	16.4	16.0	16.1
Con A-ca-alginate AMG complex	100%	99.2%	98.7%	95.3%	92.3%	91.4%	89.4%	89.9%

Table (6): Effect of pH on the activity of free and immobilized amyloglucosidase enzyme on different supports:-

PH'S	Activity or / Relative activity (%) µmole G/L/min				
	Free enzyme	Biobone	Con A-sepharose	Ca-alginate	lized (Con A-S-Ca-alginate)
3.3	163.8	49.4	34.7	69.3	10.5
3.6	176.4	67.2	42.0	76.7	28.4
4.2	182.7	81.9	50.4	84.0	37.8
4.5	186.9	89.3	53.6	89.3	44.1
5.0	153.3	105.0	60.9	82.9	26.3
5.5	149.1	87.2	51.5	73.5	25.2
6.1	144.0	65.1	48.3	52.5	21.0
6.8	46.2	21.0	18.9	25.2	20.0

3. 5. Effect of temperature:

Table (7) and Fig. (3) show the effect of temperature on the reaction activity of free and immobilized enzyme forms. The free enzyme gave a maximum activity equalled 231.0 mole glucose/min/0.1 ml diluted enzyme (1:50) at 55°C. On the other hand, the immobilized enzymeon chicken bone, Con A-sepharose and coimmobilized form yielded the maximum activity at 45°C and the maximum activities were 123.9 mole glucose/min/125 bone, 70.4 mole glucose/min/100 l suspension of immobilized form and 87.2 mole glucose/min/100 mg wet beads of con A-sepharose entrapped in Ca-alginate, respectively. The optimum temperature in the case of Ca-alginate support was 50°C, and the reaction activity equals to 89.3 mole glucose/min/100 mg wet beads of immobilized enzyme. Such results are in agreement with that reported by Qayyum, *et al.*, (1985) and Woodward (1985).

Table (7): Effect of temperature on the activity of free and immobilized amyloglucosidase enzyme on different supports:-

Temp. (°C)	Activity or /Relative activity (%) μmole G/L/min				
	Free enzyme	Biobone	Con A-sepharose	Ca-alginate	Coimmobilized (Con A-S-Ca-alginate)
30	70.2	56.3	32.6	21.3	18.2
35	87.3	78.7	47.5	36.2	38.7
40	118.7	92.4	53.6	47.3	49.4
45	145.3	123.9	70.4	78.8	87.2
50	186.9	115.6	60.9	89.3	70.4
55	231.0	105.0	47.3	67.3	43.4
60	115.5	81.9	36.6	32.1	30.2
65	86.6	78.8	29.1	18.7	17.6

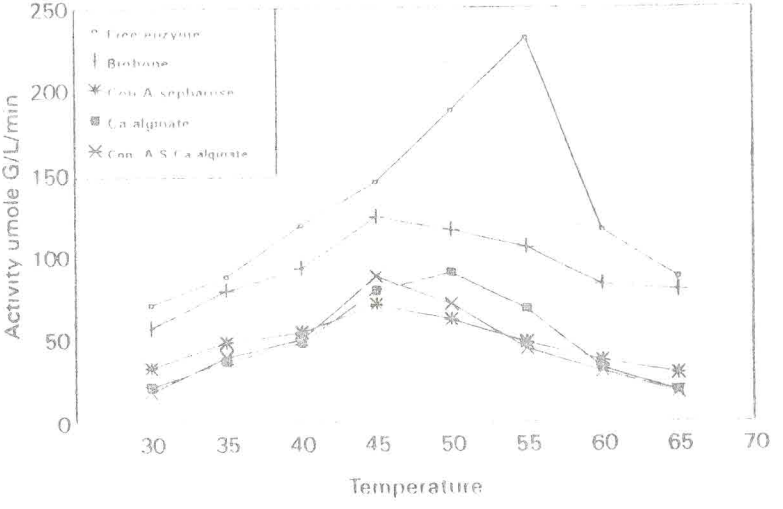
3. 6. Effect of substrate concentration:

The effect of substrate concentration on the reaction activity and velocity of free and immobilized amyloglucosidase on different supports are shown in Table (8) and Fig. (4a, 4b). These results indicated that the reaction velocity was increased progressively with the increase of the substrate until it reached a maximum reaction velocity (V_{max}) for free enzyme 851.9 mM/100 ml at a substrate concentration of 11.11 M 10^{-3} (1.8% w/v) potato starch. While, the maximum reaction velocity of immobilized forms were 543.2 mM/100 ml, 126.5 mM/100 ml, and 342.6 mM/100 ml for immobilized enzyme with chicken bone, con A-sepharose and Ca-alginate gel, respectively at a substrate concentration of 9.2 M 10^{-3} (1.5% w/v). However, the maximum velocity of coimmobilized enzyme was 163.5 mM/100 ml at the same substrate concentration of soluble form. These decreases in V_{max} largely reflect the percentage of enzyme initially immobilized on different supports. Also, this observation can be attributed to steric effect (Handa, *et al.*, 1982).

Table (8): Effect of substrate concentration on the reaction velocity of free and immobilized amyloglucosidase enzyme on different supports.

Substrate concentration mM [S]			Free enzyme			Immobilized enzyme on chicken bone			Immobilized enzyme on concanavalin A-sepharose			Immobilized enzyme on Ca-alginate gels			Coimmobilized enzyme conA-sepharose in Ca-alginate gel		
(%W/V)	[S] $M\times10^{-3}$	1/[S]	Obtained R.S. µmole glucose	Reaction velocity (V)	$1/V\times10^{-3}$	Obtained R.S. µmole glucose	Reaction velocity (V)	$1/V\times10^{-3}$	Obtained R.S. µmole glucose	Reaction velocity (V)	$1/V\times10^{-2}$	Obtained R.S. µmole glucose	Reaction velocity (V)	$1/V\times10^{-3}$	Obtained R.S. µmole glucose	Reaction velocity (V)	$1/V\times10^{-2}$
0.05	0.31	3.23	287.0	147.5	6.78	166.7	78.0	12.82	12.3	9.8	10.22	77.2	57.4	17.42	9.3	11.4	8.77
0.10	0.61	1.64	327.2	248.6	4.02	203.7	129.4	7.73	20.3	17.9	5.59	138.9	97.2	10.29	21.6	21.0	4.76
0.30	1.84	0.54	444.4	472.1	2.12	213.0	270.9	3.69	30.9	42.0	2.38	194.4	186.5	5.36	27.8	50.3	2.00
0.50	3.07	0.33	574.1	574.8	1.74	253.1	338.9	2.95	49.4	57.4	1.74	246.9	228.2	4.38	52.5	69.6	1.44
0.70	4.29	0.23	604.9	633.4	1.58	325.9	379.5	2.64	67.9	67.9	1.47	277.8	252.1	3.97	61.7	83.2	1.20
0.90	5.52	0.18	666.7	671.8	1.49	388.9	406.8	2.46	74.7	75.7	1.32	287.0	267.9	3.73	89.5	93.4	1.07
1.10	6.75	0.15	703.7	707.3	1.41	413.6	426.3	2.35	88.3	81.7	1.22	2.99.4	279.0	3.58	111.1	101.3	1.00
1.30	7.98	0.12	787.0	718.6	1.39	481.5	441.0	2.27	100.0	86.4	1.16	314.8	287.2	3.48	135.8	107.7	0.93
1.50	9.20	0.11	805.6	733.8	1.36	543.2	452.3	2.21	126.5	90.2	1.11	342.6	293.5	3.41	151.2	112.8	0.89
1.80	11.11	0.09	851.9	751.8	1.33	524.7	465.7	2.15	89.5	94.9	1.05	333.3	300.9	3.32	163.5	119.11	0.84
2.00	12.35	0.08	833.3	760.7	1.31	456.8	472.4	2.12	52.5	97.3	1.03	259.3	304.6	3.28	129.6	122.5	0.82

Fig(3): Effect of temperature on the activity of free and immobilized amyloglucosidase enzyme ondifferent supports



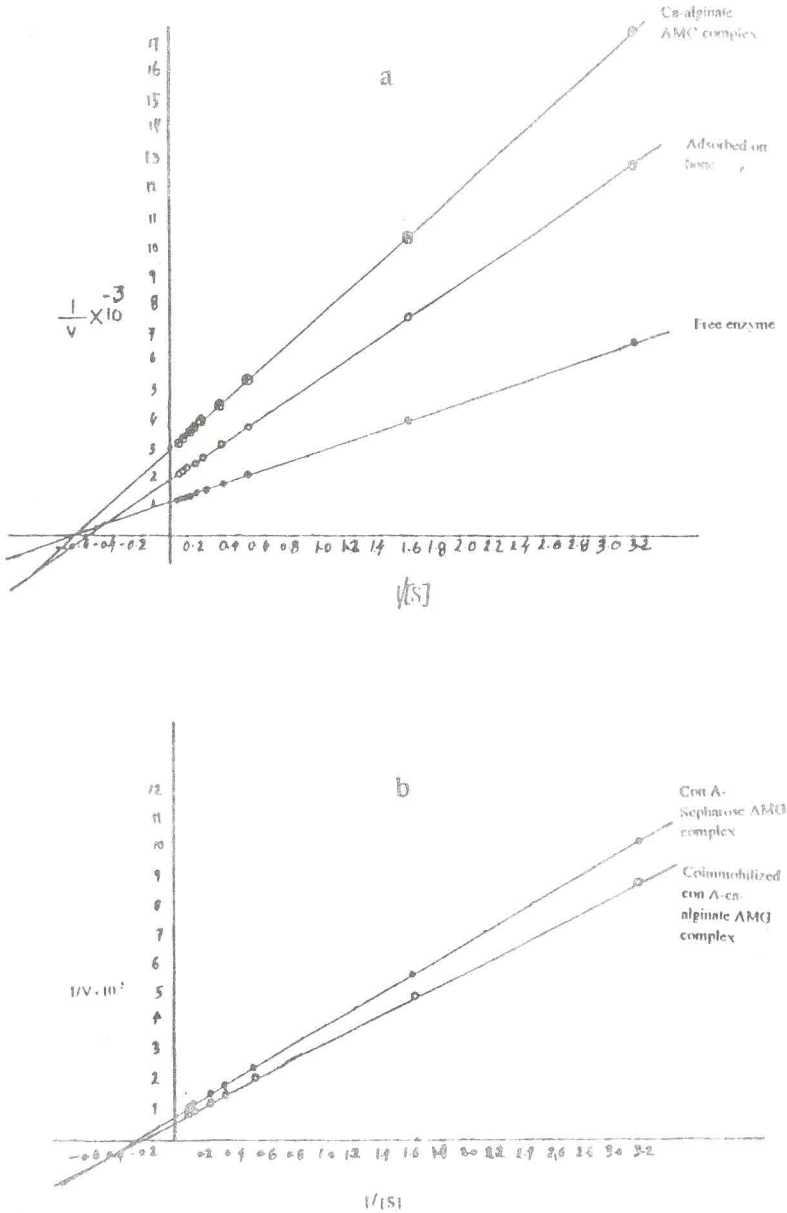


Fig. (4a, 4b): Lineweaver-Burk plots of immobilized amyloglucosidase enzyme on different supports.

Also, K_m for free and immobilized forms were determined and shown in Fig. (4a, 4b). The values of K_m were 1.48 10^{-3} M for soluble enzyme and 1.85 10^{-3}M, 3.70 10^{-3}M, 1.54 10^{-3}M and 4.14 10^{-3}M for immobilized enzyme on chicken bone, con A-sepharose, Ca-alginate and coimmobilized con A-sepharose-enzyme complex in Ca-alginate, respectively. It is clear that the K_m values for immobilized forms were increased which simply means a less affinity between the immobilized enzyme and the support. This may be due to the chemical nature of support materials and diffusional resistances to the translocation of substrate and/or product.

Consequently, an increase in K_m value on immobilization means that a higher substrate concentration is required to achieve the same of reaction observed with the free enzyme (Woodward, 1985). Immobilized enzyme on chicken bone as support showed low value of k_m (1.85 10^{-3} M), and also high V_{max} (542.2 mM glucose/100 ml) compared with other reaction activities of immobilized forms on other supports. Also, K_m value determined by Lineweaver and Burk (1954) technique, the obtained values using 1/(S) againts 1/v were almost equal to the results obtained in the experimental curve.

3. 7. Effect of time on the hydrolysis:

The effect of incubation time for continuous hydrolysis of potato starch using soluble and immobilized forms on different supports are shown in Table (9). From these results the obtained reducing sugars (as glucose) increase with increasing incubation time till reaching a maximum 1165.6 mole glucose/100 ml for soluble form after 180 min (3h). However, the obtained reducing sugars were 434.0, 116.6, 232.5 and 133.9 mole glucose/100 ml for immobilized enzyme on chicken bone, CAS, Ca-alginate, and coimmobilized, respectively after incubation time of 150 min.

Table (9): Effect of time on the hydrolysis.

Time (min)	Reducing sugars μmole G/100 ml				
	Free enzyme	Bone-Enzyme complex	CAS-enzyme complex	Ca-alginate enzyme complex	Coimmobilized
15	70.1	54.6	27.5	48.4	29.1
30	140.2	69.4	31.6	67.0	39.3
60	275.3	73.2	41.5	70.1	41.5
90	545.6	143.2	49.6	102.3	63.9
120	771.5	226.0	89.3	139.5	119.0
150	942.5	434.0	116.6	232.5	133.9
180	1165.6	434.0	80.6	199.0	121.5
240	1023.0	224.2	79.4	148.8	94.2

3. 8. Reuse of immobilized amyloglucosidase enzyme:-

The activity or relative activity (%) of the immobilized enzyme forms after 5 times with repeated washing is shown in Table (10). The immobilized

enzyme on bone loss 65.8% of its activity after 5 times, this may be due to readsorbed of enzyme from surface area of bone as support. While, the decreament of immobilized enzyme on con A-separe was 52.1% after 5 times. However, ca-alginate enzyme complex exhibited the lowest relative activity therefor it losses 80.0% of its activity after 5 cycle. This observation may be attributed to the linkage of the enzyme with gel. Therefore, it could be concluded that the immobilized enzyme on chicken bone can be use 3 times with only loss 4.4% of its relative activity.

Table (10): Reuse of immobilized amyloglucosidase with different supports:-

No. of cycle	BioBone *		Con-A-Sepharose*		Ca-alginate**		Colmmobilized** CoA-Ca-alginate complex	
	Activity µmole G/L/min	Relative activity (%)	Activity µmole G/L/min	Relative activity (%)	Activity µmole G/L/min	Relative activity (%)	Activity µmole G/L/min	Relative activity (%)
Before	46.2	100%	26.3	100%	51.5	100%	17.9	100%
1	45.8	99.1	26.3	100	37.38	73.4	18.0	100.6
2	45.2	98.7	21.0	79.8	25.2	48.9	17.0	95.2
3	44.1	95.6	16.8	63.9	22.4	43.5	12.6	70.4
4	18.9	40.9	11.6	44.1	12.6	24.5	12.6	70.4
5	15.8	34.2	12.6	47.9	10.3	20.0	8.4	46.9

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

** Activity refers to the amount contained in 100 mg wet beads of ca-alginate and Con A-sepharose Ca-alginate enzyme complex.

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تحميل إنزيم الأميلوجلوكوسيديز بواسطة إستخدام دعامات مختلفة

فرحات فوده على فوده

قسم الكيمياء الحيوية الزراعية-كلية الزراعة بمشتهر-جامعة الزقازيق-فرع بنها

يهدف هذا البحث إلى دراسة تحميل إنزيم الأميلوجلوكوسيديز بإستخدام دعامات مختلفة هى مسحوق عظم الدجاج ، الكونيكانافالين ا-سيفاروز ، الكالسيوم الجينيت جل وتغليف معقد الإنزيم بالكونيكانافالين ا-سيفاروزبالكالسيوم الجينيت جل لتحويله من الصورة الذائبة إلي الصورة الغير ذائبة بغرض إمكانية إستخدامة فى النظام المستمر وكذلك دراسة الظروف المثلى والسلوك الحركى للإنزيم المحمل مقارنة مع حالته الذائبة وكذلك إستخدامة عديد من المرات حيث أن هذه النقطة ذات أهمية صناعية وإقتصادية نظراً لأهمية هذا الإنزيم فى عملية التسكر للنشا وإنتاج شراب الفراكتوز.

أوضحت الدراسة أن قوة ربط الدعامات المختلفة لإنزيم الأميلوجلوكوسيديز هى ٧و٧٥٪ ، ١و٨٨٪ ، ٥و٦١٪ ، ٤و٩٥٪ من كمية الإنزيم المضافة للدعامات مسحوق عظم الدجاج ، الكونيكانافالين ا-سيفاروز ، الكالسيوم الجينيت جل وإعادة تحميل معقد الإنزيم بالكونيكانافالين ا-سيفاروزبالكالسيوم الجينيت جل على التوالى.

كذلك أوضحت الدراسة أن الظروف المثلى لتحميل هذا الإنزيم على مسحوق عظم الدجاج هى على درجة حموضة مثلى (pH) تساوى ٥ بإستخدام ٥٠ ملليمول خلات صوديوم، أقصى كمية أنزيم ذائب يمكنها أن ترتبط مع مسحوق العظم هى ٨ ر٠ملى إنزيم ذائب (٣٨ر١ ملليجرام بروتين) على أنسب كمية من العظم وتساوى ١٢٥ ملليجرام مسحوق العظم. كما أن الإنزيم المرتبط بالدعامات المختلفة أوضح درجة ثبات عالية لمدة ٣٥ يوم تحضين على درجة ٥٥م مقارنة للإنزيم الحر.

وقد أوضحت النتائج أن درجة الحموضة المثلى pH للإنزيم المحمل على كل

من مسحوق العظم و الكونيكانافالين ا-سيفاروز هى ٥ بينما الإنزيم الحر والمحمل فى الكالسيوم الجينيت والمعاد تحميله فكانت ٥ر٤ وقد أوضحت الدراسة أيضا أن درجة الحرارة المثلى للإنزيم الحر ومعقد الأنزيم مع كل من مسحوق العظم ، الكونيكانافالين ا-سيفاروز والمعاد تحميله تساوى ٥٥ م وكانت ٥٠ م لمعقد الإنزيم مع الكالسيوم الجينيت جل.

أظهرت الدراسة الحركية لهذا الإنزيم أن ثابت ميكاليس للإنزيم الحر يساوى ٤٨ر١ ١٠ $^{-٣}$مولر بينما لمعقد الإنزيم مع الدعامات المختلفة فكان ٨٥ر١ ١٠ $^{-٣}$ مولر، ٧٠ر١ ١٠ $^{-٣}$مولر، ٥٤ر١ ١٠ $^{-٣}$مولر، ١٤ر٤ ١٠ $^{-٣}$مولر لكل من مسحوق عظم الدجاج، الكونيكانافالين ا-سيفاروز، الكالسيوم الجينيت جل وإعادة تحميل الكونيكانافالين ا-سيفاروز بالكالسيوم الجينيت جل على التوالى. وقد يعزى زيادة ثابت ميكالس للإنزيم المحمل على الدعامات المختلفة عن الإنزيم الحر إلى إنخفاض جاذبية المادة المتفاعلة بالإنزيم وكذلك التأثيرات الناتجة عن إنخفاض تركيز المادة المتفاعلة نتيجة لوجود طبقة بين الإنزيم المحمل ومحلول التفاعل.

كما أوضحت الدراسة أن السرعة القصوى للإنزيم الحر تساوى ٩ر٨٥١ ميكرومول جلوكوز بينما للإنزيمات المحملة فكانت ٢ر٥٤٣ ميكرومول جلوكوز، ٥ر١٢٦ ميكرومول جلوكوز، ٦ر٣٤٢ ميكرومول جلوكوز و ٥ر١٦٣ ميكرومول جلوكوز على التوالى.

كما أوضحت الدراسة أن أعلى كمية من السكريات المختزلة أمكن الحصول عليها بعد مدة ٣ ساعات لعمل الإنزيم المستمر. أما بالنسبة لإستخدام الإنزيم المحمل عديد من المرات فقد أظهرت الدراسة أن الأنزيم المحمل على مسحوق عظم الدجاج أمكن إستخدامة ٣ مرات مع فقد ٦ر٤٪ من درجة نشاطه على٤٥ م بينما المعقدات الأخرى أظهرت فقد ٣٦-٥٧٪ من درجة النشاط بعد إستخدامها ٣ مرات.