4. RESULTS AND DISCUSSION

4.1. Suitable conditions for the production and evaluation of the β-galactosidase from *Kluyveromyces marxianus*:

Lactase, the enzyme that hydrolysis lactose to glucose and galactose, is of potentially great importance of the dairy industry. In the caurse of estabilishing a screening program to find the best microbial sources of the enzyme production, it became apparent that a rapid and sensitive. There is considerable interest, from both nutritional and technological view points, in the enzymic hydrolysis of lactose in milk, **Park *et al.* (1979)**.

Lactase enzyme (β-D-galactosidase, E.C.3.2.1.23) was produced and extraced from *Kluyveromyces marxianus* yeast.

Several factors is importance in the study of enzyme reactions were investigated in establishing conditions for the production of lactase enzyme. It shoud be pointed out that there many factors that affect the specific properties of β-galactosidase enzyme during preparation and storage. When these factors was studied must be keeping the other factors constant.

4.1.1. Effect of incubation period on the production of β-galactosidase enzyme:

The effect of incubation period on the enzyme activity level, protein content and specific activity were investigated during 1-15 days at 30°C. The obtained results are illustrated in Table (1) and Fig. (1). Data indicated that the supernatant β-galactosidase activity increased continuously as the incubation

Table (2): Effect of different incubation temperatures on the production of β-galactosidase enzyme from *Kluyveromyces marxianus*.

Incubation temperature (°C)	β-galactosidase activity (unit/ml conc.)	Enzyme-protein content (mg/ml conc.)	Specific activity (unit/mg)
25	3.96	0.90	4.40
30	5.36	0.95	5.64
35	4.36	0.91	4.79
40	3.66	0.87	4.22

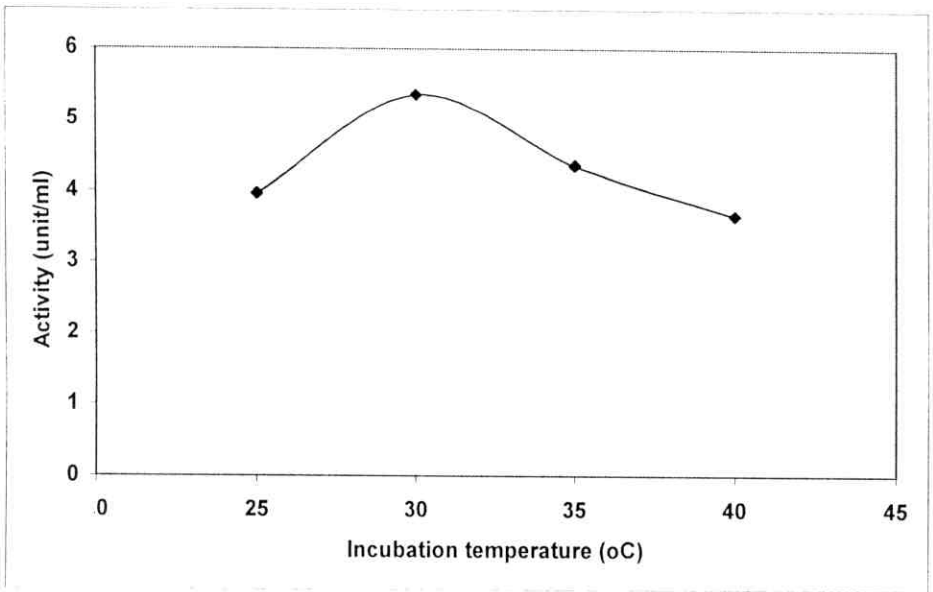


Fig. (2): Effect of different incubation temperature on the production of β-galactosidase enzyme from *Kluyveromyces marxianus*.

4.1.3.1. Effect of temperature on the reaction activity of the produced β-galactosidase:

The effect of temperature on the enzymatic rate of lactase activity was investigated. Data in Table (3) showed that with increasing temperature the activity will be increase but at a time inactivation is also accelerated, this is due to denaturation of the enzyme protein by heat. Temperatures studied were 35, 40, 45, 50, 55, 60, 65 and 75°C. The lactose solution (20%) and buffer components of the reaction mixtures in test tubes were incubation period of 20 min at pH 6.4 (0.1 M) phosphate buffer. The rate enzymatic hydrolysis of lactose by the produced β-galactosidase was measured and shown in Table (3) and Fig. (3).

Results indicated that the decline in enzymatic activity at 60°C, may be due to heat inactivation of the enzyme protein denaturation, **Stano *et al.* (1996)**. The maximum reaction activity of the produced β-galactosidase was 1456.9 mg glucose/ml (72.85 mg/ml/min) at 55°C. On the other hand, the amount of activity was decreased and equaled 30.63 mg/ml/min at 70°C. Such results are higher with that reported by **Chen and Tsen (1991)** and similar to be obtained by **Rogalski and Lobarzewski (1995) and Poor *et al.* (1997),** they reported that the optimum temperature between 50-55°C for the native β-galactosidase produced from *Penicillium notaum*.

Table (3): Effect of temperature on the reaction activity of the produced β-galactosidase enzyme.

Temperature (°C)	Glucose concentration (mg/ml)	Activity (mg/ml/min)
35	390.7	19.54
40	582.8	29.14
45	887.4	44.37
50	1145.6	57.28
55	1456.9	72.85
60	1210.6	60.53
65	922.4	46.12
70	712.6	30.63

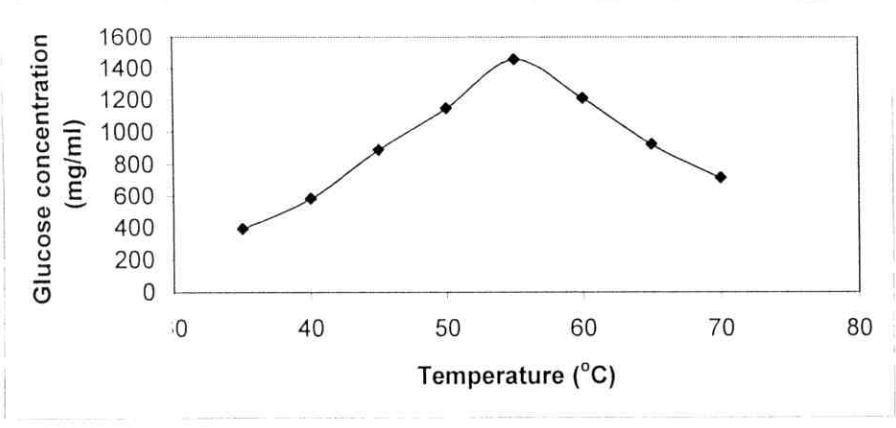


Fig. (3): Effect of temperature on the reaction activity of the produced β-galactosidase enzyme.

4.1.3.2. Effect of pH on the reaction activity of crude β-galactosidase enzyme:

The optimum pH for the enzyme preparation used in these experiments was as certained by using (0.1 M) phosphate buffer of various pH levels in the assay procedure.

Eight solutions of lactose (20%) were adjusted to different pH values started from 5.8 till 7.2. The reaction activity of the produced β-galactosidase was carried out at the optimum temperature occurred before (55°C) for 20 min incubation period.

The obtained data are tabulated in Table (4). The reaction activity is plotted against the pH values and the curve was obtained and was shown in Fig. (4). From the above-mentioned results in Table (4) and Fig. (4), it can be observed that the maximum activity of the produced β-galactosidase was 73.68 mg/ml/min at pH 6.2. These results are in agreement with that reported by **Arora and Sinha (1989)** and **Ladero *et al.* (2000)**.

4.1.3.3. Effect of enzyme concentration on the reaction activity of the produced β-galactosidase enzyme:

The effect of different enzyme concentrations on the enzyme activity of the produced β-galactosidase from *K. marxianus* was evaluated. Different amounts i.e. 0.5. 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml were tested in (0.1 M) phosphate buffer at optimum pH (6.2) and at 55°C for 20 min incubation period. The obtained results are shown in Table (5) and illustrated in Fig. (5).

Table (4): Effect of pH value on the reaction activity of the produced β-galactosidase enzyme.

pH	Glucose concentration (mg/ml)	Activity (mg/ml/min)
5.8	721.8	36.09
6.0	1215.2	60.76
6.2	1473.5	73.68
6.4	850.99	42.22
6.6	473.2	23.66
6.8	370.8	18.54
7.0	237.7	11.89
7.2	194	9.7

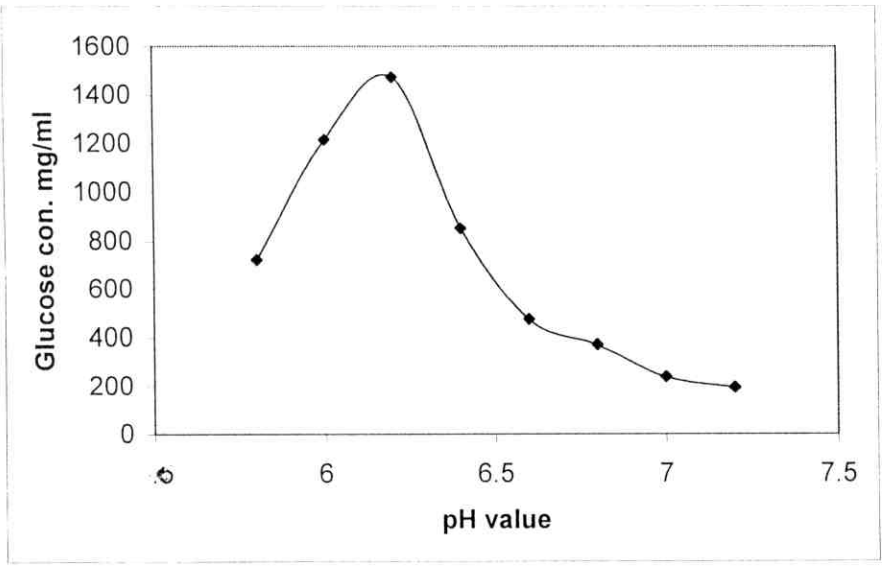


Fig. (4): Effect of pH value on the reaction activity of the produced β-galactosidase enzyme.

Table (5): Effect of enzyme concentration on the reaction activity of the produced β-galactosidase enzyme.

Enzyme amounts (ml)	Glucose concentration (mg/ml)	Activity (mg/ml/min)
0.5	245.0	12.25
1.0	387.4	19.37
1.5	500.0	25.00
2.0	751.7	37.59
2.5	814.5	40.25
3.0	1185.4	59.27
3.5	1473.0	73.65
4.0	1652.4	82.62
4.5	1545.6	77.28
5.0	1292.4	64.62

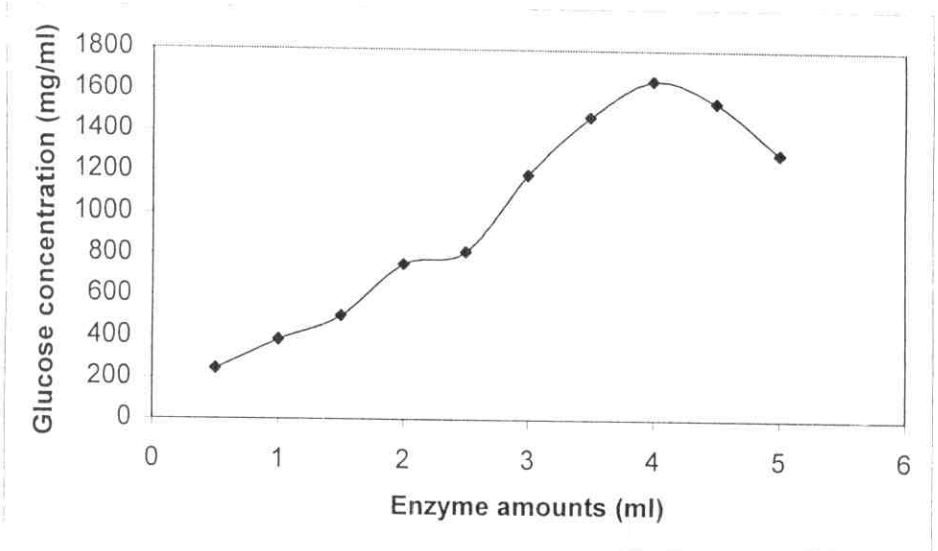


Fig. (5): Effect of enzyme concentration on the reaction activity of the produced β-galactosidase enzyme.

The obtained results indicated that the activity of the crude preparation β-galactosidase enzyme reached its maximum 82.62 mg/ml/min at enzyme amounted 4.0 ml. From these results, it could be stated that increasing enzyme concentration to extra high levels lead to a significant decrease in the overall reaction activity. This observation may be attributed to the rate of the reverse reaction (inhibition effect) of the product which might produced in opposite distraction leading to general decrease in the final product, **Illanes *et al.* (1998)**. These results are in agreement with that reported by **Sufang *et al.* (1999)** and **Albayrak and Yang (2002)**.

4.1.3.4. Effect of substrate concentration on the reaction activity of the produced β-galactosidase enzyme:

Substrate concentration is one of the most important factors which affects on the efficiency and velocity of the enzyme reaction. The rate of the enzymatic reaction of lactase was studied as a function of the lactose concentration. During the course of these experiments the reaction mixture consistes of 1.0 ml of crude enzyme in several concentrations of lactose in (0.1 M) sodium phosphate buffer at the optimum pH and temperature which estimated before. The reaction was carried out at different concentrations i.e. 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 5.0 g/L of lactose as a substrate. The obtained results are tabulated in Table (6) and Fig. (6a, b). The rate of the most enzyme reactions increase up to a certain point with increasing concentrations of the substrate. By plotting the obtained D-glucose against the concentration of the substrate, the curve

was obtained, Fig. (6a). From this curve indicate an increase in the activity with increasing substrate concentration until a maximum rate (V_{max}) is reached and further increase in substrate concentration dose not show any positive effects and the reaction rate of enzyme depends on the time necessary for the enzyme to act on the substrate.

Results showed that the reaction velocity to convert lactose to glucose was increased up to 3.5 g/L and maximum reaction velocity (V_{max}) of 1120.6 mg glucose/ml. The Michaels constant of β-galactosidase enzyme can be obtained by the half point of the experiment curve and clearly shown in Fig. (6a) and is equal 1.30 g/L when using lactose as a substrate. The obtained K_m acquired by **Lineweaver and Burkplots (1954)**, Fig. (6b) was equally to that obtained by experimentally curve. These values are slightly higher than that reported by **Rejikumar and Surekha (2001)** and **Ladero *et al.* (2001)**.

Table (6): Effect of substrate concentration on the reaction velocity of the produced β-galactosidase enzyme.

Substrate concentration [S] (g/L)	1/[S]	Glucose concentration (mg/ml)	Reaction velocity [V]	1/[V] $x10^{-3}$
0.5	2.0	324.5	311.28	3.21
1.0	1.0	443.7	487.22	2.05
1.5	0.67	632.4	600.32	1.67
2.0	0.50	781.4	679.15	1.47
2.5	0.40	887.4	737.24	1.36
3.0	0.33	976.8	781.81	1.28
3.5	0.29	1120.6	817.10	1.22
4.0	0.25	821.4	845.74	1.18
5.0	0.20	596.5	889.37	1.12

V_{max} = 1120.6
K_m = 1.30 g/L

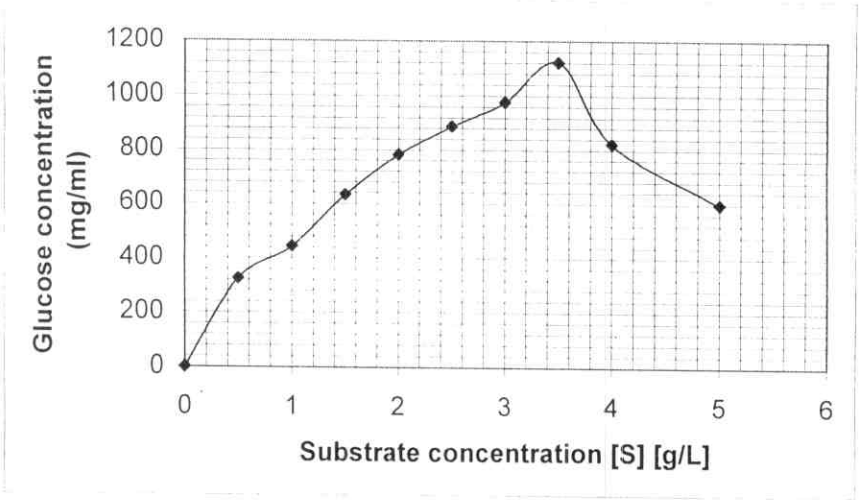


Fig. (6a): Effect of substrate concentration on the reaction velocity of the produced β-galactosidase enzyme.

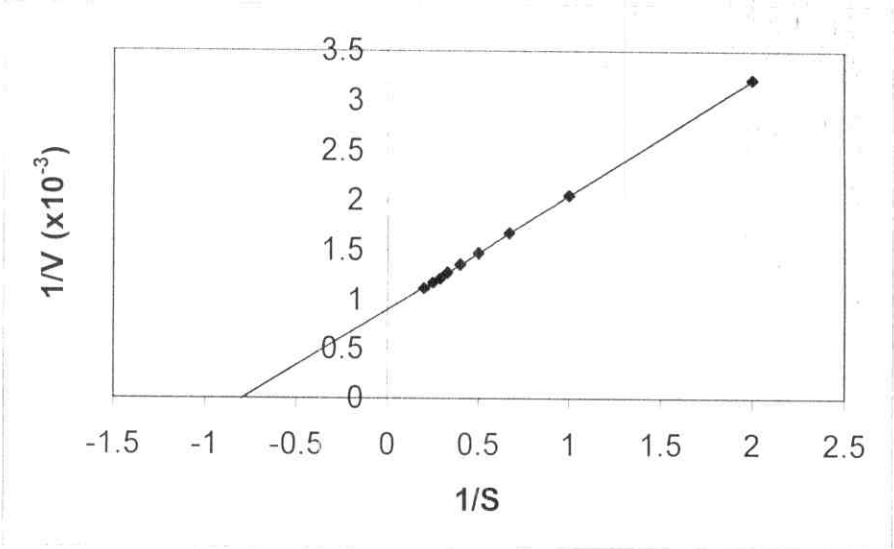


Fig. (6b): Lineweaver-Burk plots for the produced β-galactosidase enzyme.

4.2.: Effect of different methods and supports on the immobilization of the produced *K. marxianus* β-galactosidase enzyme:

The present part of this study deals with attempts for immobilization of the produced β-galactosidase enzyme by using various immobilization techniques and different support materials in order to find the optimal immobilization method and support for this enzyme.

A number of techniques have been used for β-galactosidase enzyme immobilization including covalent binding, adsorption and matrix entrapment. This enzyme has been immobilized on different supports i.e. sodium alginate, sand, chitin and con A-sepharose with different attachment to insoluble materials i.e. entrapment within Ca-alginate, adsorption onto a solid supports like sand and chitin and covalent coupling with con A-sepharose.

4.2.1. Effect of Na-alginate concentration on entrapment of the produced β-galactosidase enzyme:

Different concentrations of Na-alginate were used to evaluate the effect of the immobilization process on the activity and rentation activity after entrapped within Ca-alginate gel beads.

The obtained results are illustrated in Table (7). From these results it can be seen that the retention activity of immobilized enzyme increased progressively with the increase of Na-alginate. Rentention activity and effectiveness factor of immobilized β-galactosidase were 58.40 and 82.45% at a concentration (2%) sodium alginate. While, at 4% (w/v) sodium

Table (7): Effect of Na-alginate concentration on the activity of immobilized β-galactosidase enzyme.

Na-alginate concentration (% w/v)	Activity of β-galactosidase enzyme					Effectiveness factor (D/C) 100 %	Retention activity (D/A) 100 %
	Enzyme added (A)	Enzyme protein (mg/ml)	In washing (B)	Entrapped within Ca-alginate			
				Theoretical (A-B) = C	Actual complex (D)		
2	397.35	0.77	115.89	281.46	232.05	82.45	58.40
4	397.35	0.77	139.07	259.28	266.48	103.17	67.06

alginate, the retention activity and effectiveness factor were 67.06 and 103.17% of the original enzyme activity. The reduction in enzyme activation may be due to the hindered conformational changes in the immobilized enzyme which occurred during immobilization process, **Ates and Mehmetoglu (1997)**. Also, it is inapplicable to most enzymes, since the pore size of gel is so large that enzymes leak out from the support, **Husain *et al.* (1985)**. These results are in agreement with those reported by **Abd El-Aleem and Foda (1998)**

4.2.2. Retention activity of preparation β-galactosidase enzyme on other different supports:

Also, the produced β-galactosidase enzyme has been immobilized on other different support materials i.e. sand, chitin and concanavalin A-spherose by using different methods of the immobilization process. The properties of the different immobilized preparations β-galactosidase are of major importance. The quantity of the enzyme bounded to each support besides the retention activity after immobilization has to be considered in comparing such suppoting materials. The obtained data were tabulated in Table (8). This enzyme has been bounded with all the evaluated different supports but with different retention activities. The highest efficiency loading capacity of immobilized enzyme form was found with a support of con A-sepharose, since the lowest enzyme loss was noticed in washing, which simply means that the support was bounded with the large amount of enzyme added. The retention activity was found to be 85.0%. On the other hand, other two supports showed

Table (8): Retention activity of preparations β-galactosidase enzyme on different supports.

Supports	Activity of β-galactosidase enzyme					Effectiveness factor (D/C) 100 %	Retention activity (D/A) 100 %
	Enzyme added (A)	Enzyme protein (mg/ml)	In washing (B)	Adsorben in supports			
				Theoretical (A-B) = C	Actual complex (D)		
Sand	397.35	3.85	92.71	304.64	298.01	97.82	75.00
Chitin	397.25	3.85	66.22	331.13	281.45	84.99	70.83
Con A-sepherose	397.25	3.85	16.56	380.79	377.48	99.13	85.00

the lowest bounded material which was accompanied with the lowest retention activity. The retention activities were amounted to 75.0 and 70.83% with sand and chitin as a supports, respectively.

Pore size restrictions may grossly alter the available surface area for binding of the enzyme or cause diffusion limitation which markedly decrease the observed enzyme activity. Both the sand and chitin compositions can have significant effects on the number of active bonding site, microenvironment, pH and surface charge. These effect will be cause changes in the activity and performance of the bound enzymes, **Brotherton *et al.* (1976)**.

The decrement in the retention activity of the immobilized β-galactosidase enzyme on sand and chitin might be attributed to the bounded active sites of this enzyme with the support which simply affect the whole body of the enzyme itself. Besides, that the adsorption of this enzyme on surface area of each support was weakness bounded because the enzyme attachment on a solid support depended on the display area of support surface size and the types of bonds of attachment i.e. Hydrogen bonding and Van der Waals, **Foda (1999)** and **Illanes *et al.* (2000)**.

4.2.3. Evaluation of immobilized β-galactosidase enzyme within Ca-alginate gel beads:

The optimum factors influence the immobilized enzyme reaction e.g. pH, temperature and substrate concentration were determined for the immobilized β-galactosidase within Ca-alginate. Also, stability, reuse of immobilized preparations on other different supports were evaluated.

4.2.3.1. Effect of pH on the activity of immobilized form:

The effect of pH on the reaction activity of entrapped β-galactosidase enzyme within calcium alginate gel beads is shown in Table (9) and Fig. (7). Different pH values (5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0 and 7.2) using (0.1 M) phosphate buffer were tested versus the reaction enzyme activity. As shown in Table (9). The immobilized form showed optimum pH was 6.4 revealed that the maximum amount of glucose concentration was 215.2 mg/ml. Also, the enzyme activity amounted to 10.76 mg/ml/min. When compared with the native β-galactosidase enzyme, the optimum pH value was found to be 6.2. The differentiation in optimum pH values of soluble and immobilized form may be due to the conformation changes in the immobilized enzyme and diffusion limitation of matrix gel, **Becerra *et al.* (2001)**. These pH values of immobilized form are accordance with those reported by **Sridhar and Dutta (1991) and Illanes *et al.* (2000)**.

4.2.3.2. Effect of temperature on the activity of immobilized enzyme within Ca-alginate gel beads:

The effect of different temperatures on the activity of immobilized enzyme gel beads are presented in Table (10) and Fig. (8). The enzyme activity increased with increasing temperature and reach its maximum value at 50°C and tend to decrease at higher temperature. The higher decrement in the activity of immobilized enzyme on calcium alginate gel beads may be due to the partially denaturation of protein enzyme at higher temperatures, **Illanes *et al.* (2000)**.

Table (9): Effect of pH value on the reaction activity of immobilized β-galactosidase within Ca-alginate.

pH	Glucose concentration (mg/ml)	Activity (mg/ml/min)
5.8	119.2	5.96
6.0	145.6	7.28
6.2	184.4	9.22
6.4	215.2	10.76
6.6	196.3	9.82
6.8	174.6	8.73
7.0	129.1	6.46
7.2	112.0	5.60

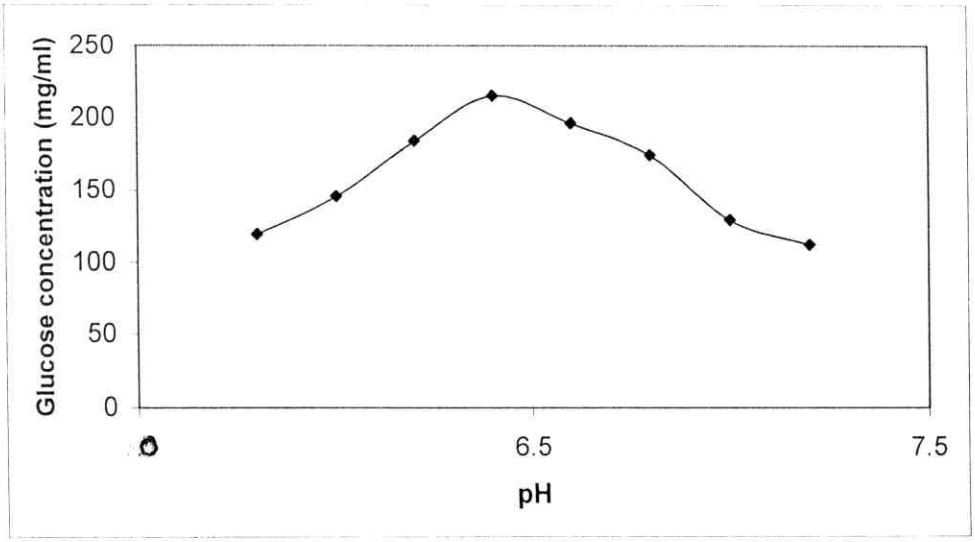


Fig. (7): Effect of pH value on the reaction activity of immobilized β-galactosidase in Ca-alginate.

Table (10): Effect of temperature on the reaction activity of immobilized β-galactosidase within Ca-alginate.

Temperature (°C)	Glucose concentration (mg/ml)	Activity (mg/ml/min)
35	274.8	13.74
40	314.5	15.73
45	450.3	22.52
50	496.6	24.83
55	432.3	21.62
60	397.3	19.87
65	261.6	13.08
70	146.4	7.32

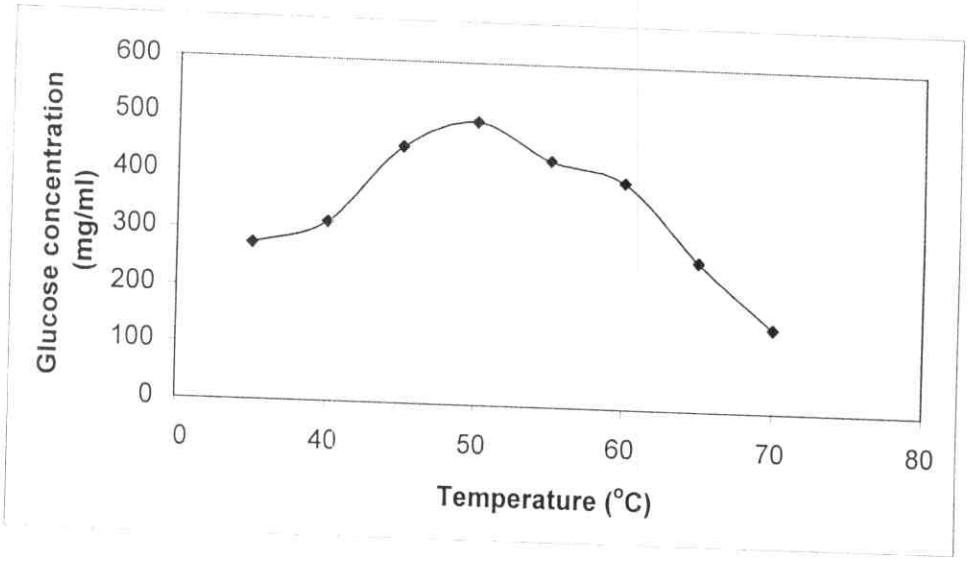


Fig. (8): Effect of temperature on the reaction activity of immobilized β-galactosidase in Ca-alginate.

The immobilized enzyme had optimum temperature of 50°C with maximum activity of 24.83 mg glucose/ml/min/ 100 mg wet beads, while the native form had a optimum of 55°C. Such values for optimum temperature are in good agreement with that obtained by **Pizzichini *et al.* (1991) and Yang *et al.* (1993)** which they noticed that the optimum temperature was 57°C. On the other hand, **Berger *et al.* (1995)** reported that the immobilized enzyme showed increased temperature optima comparing with the free enzyme, optimum temperature for activity towards ONPG was 70°C.

4.2.3.3. Effect of substrate concentration on the reaction velocity of immobilized enzyme with Ca-alginate gel beads:

The rate of the reaction velocity of immobilized enzyme with different substrate concentrations is shown in Table (11) and Figs. (9a and 9b).

The reaction was carried out at different concentrations, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 5.0 g/L of lactose as substrate. From these results, it is clear that the maximum reaction velocity (V_{max}) was found to be 734.84 mg glucose/ml at a substrate concentration of 4.0 g/L. These decrease in V_{max} largely reflect the percentage of enzyme initially immobilized on Ca-alginate. Also, this observation can be attributed to steric effect. On the other hand, Michaeles-Menten constant (K_m) for immobilized enzyme was determined and shown in Fig. (9a and 9b). The value of K_m was found to be 1.5 g/L for immobilized form and compared with native β-galactosidase (K_m = 1.3 g/L).

Table (11): Effect of substrate concentration on the reaction velocity of immobilized β-galactosidase within Ca-alginate.

Substrate concentration [S] (g/L)	1/[S]	Glucose concentration (mg/ml)	Reaction velocity [V]	1/[V] $x10^{-3}$
0.5	2.0	274.8	252.60	3.96
1.0	1.0	351.0	404.16	2.47
1.5	0.67	474.8	505.20	1.98
2.0	0.50	565.0	577.37	1.73
2.5	0.40	656.6	631.50	1.58
3.0	0.33	807.4	673.60	1.48
3.5	0.29	945.6	707.28	1.41
4.0	0.25	1010.4	734.84	1.36
5.0	0.20	796.8	777.23	1.29

V_{max} = 1010.4
K_m = 1.50 g/L

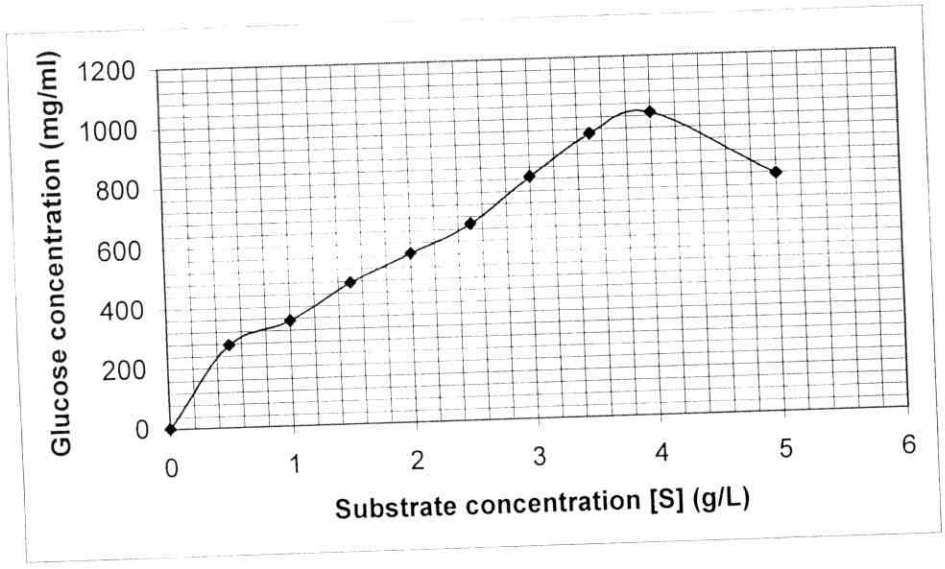


Fig. (9a): Effect of substrate concentration on the reaction velocity of immobilized β-galactosidase in Ca-alginate.

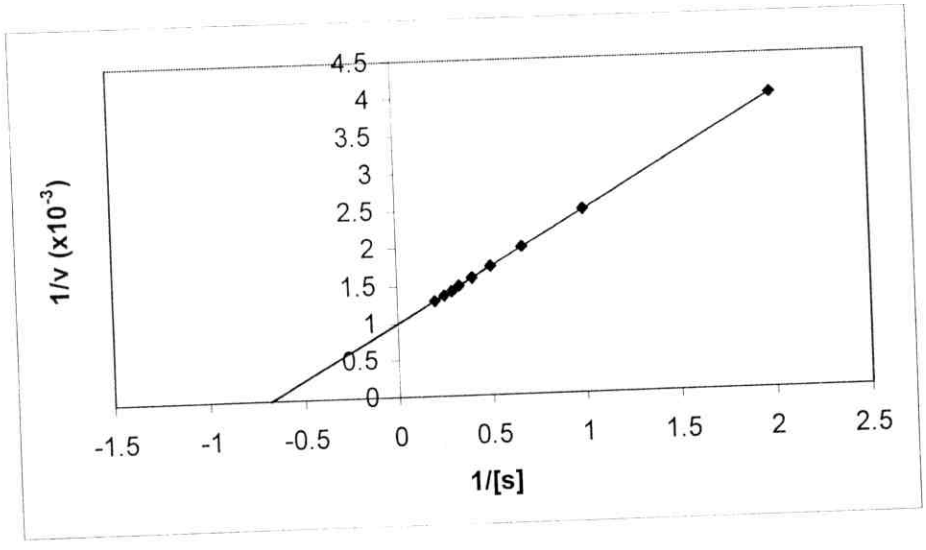


Fig. (9b): Lineweaver-Burk plots for the immobilized β-galactosidase in Ca-alginate.

It is clear that the K_m value for immobilized form was increased which simply means a less affinity between the immobilized enzyme and the substrate. This may be due to the chemical nature of support material and diffusional resistances to the translocation of substrate and/or product, **Woodward and Capps (1992)**. Also, K_m value was determined by **Lineweaver and Burk (1954)** technique. The obtained values using 1/S against 1/v were almost equal to the results obtained in the experimental curve. These results are partially differ in agreement with that reported by **Hernaiz and Crout (2000)** they noticed that the value of K_m for the immobilized enzyme on Eupergit C (epoxy-activated acrylic beads (14.2 mM) was approximately 3-fold higher than that of the free enzyme (5.0 mM) for the same substrate. Such behaviour certainly leads to a decrease in the affinity between the substrate and the immobilized enzyme which gave an increase in K_m value may be due to the differ conformationally, alternatively it may attached to the solid carrier in a way that would render certain parts of the enzyme molecule less accessible to substrate, **Goldstein (1976).**

4.2.3.4. Stability of the immobilized β-galactosidase on different supports:

The stability of the immobilized β-galactosidase on Ca-alginate gel beads, sand, chitin and concanavalin A-sepharose were studied by using (20%) lactose as a substrate with different time of storage at 4°C. The obtained results were illustrated in Table (12) and Fig. (10). Data indicated that a gradual decrease in its relative activity had been occurred. On the basis of relative

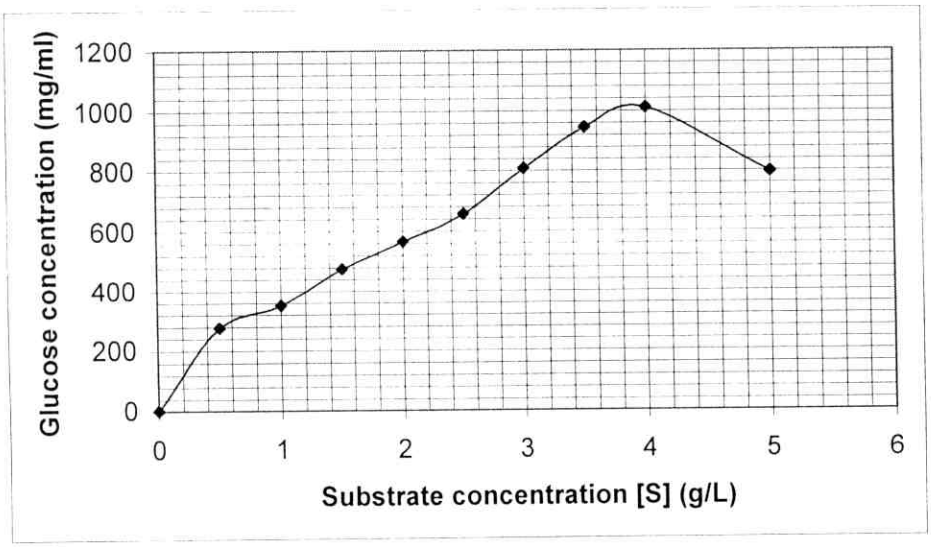
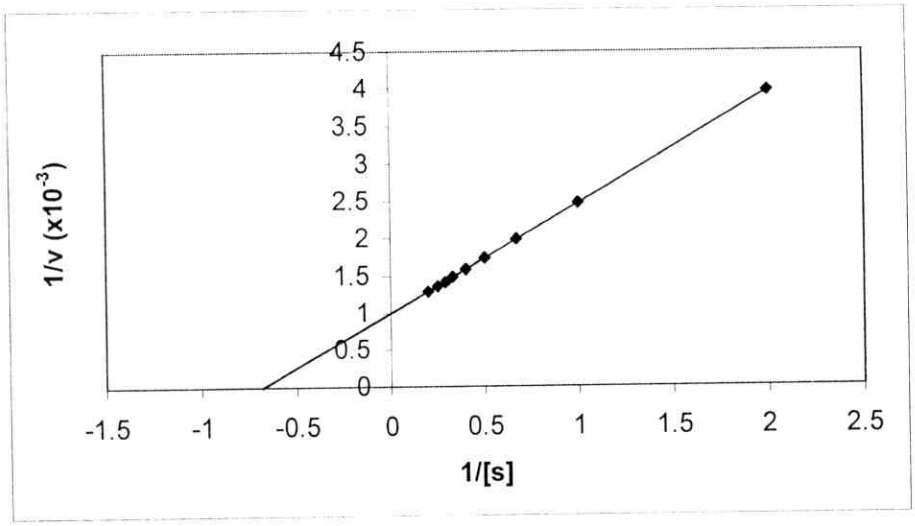


Fig. (9a): Effect of substrate concentration on the reaction velocity of immobilized β-galactosidase in Ca-alginate.



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4.2.3.4. Stability of the immobilized β-galactosidase on different supports:

The stability of the immobilized β-galactosidase on Ca-alginate gel beads, sand, chitin and concanavalin A-sepharose were studied by using (20%) lactose as a substrate with different time of storage at 4°C. The obtained results were illustrated in Table (12) and Fig. (10). Data indicated that a gradual decrease in its relative activity had been occurred. On the basis of relative

Table (12): Stability of the immobilized β-galactosidase with different supports at different times.

Immobilized β-galactosidase form		Activity and relative activity (%)							
		Time of storage (hrs)							
		0	4	8	12	24	48	72	96
Ca-alginate enzyme complex		241.7	231.8	228.5	225.5	198.7	182.2	158.9	135.8
	%	100.0	95.9	94.5	93.3	82.2	75.4	65.9	56.2
Adsorbed on sand		397.3	367.5	347.7	317.8	298.1	278.1	258.3	228.5
	%	100.0	92.5	87.5	80.0	75.0	70.0	65.0	57.5
Adsorbed on chitin		281.5	248.3	238.4	228.1	218.5	198.7	181.6	168.7
	%	100.0	88.2	84.7	81.0	77.6	70.5	64.5	60.0
Con A-sepharose enzyme complex		629.1	628.2	618.4	606.6	589.1	586.5	583.1	582.4
	%	100.0	99.9	98.3	96.4	93.6	93.2	92.7	92.6

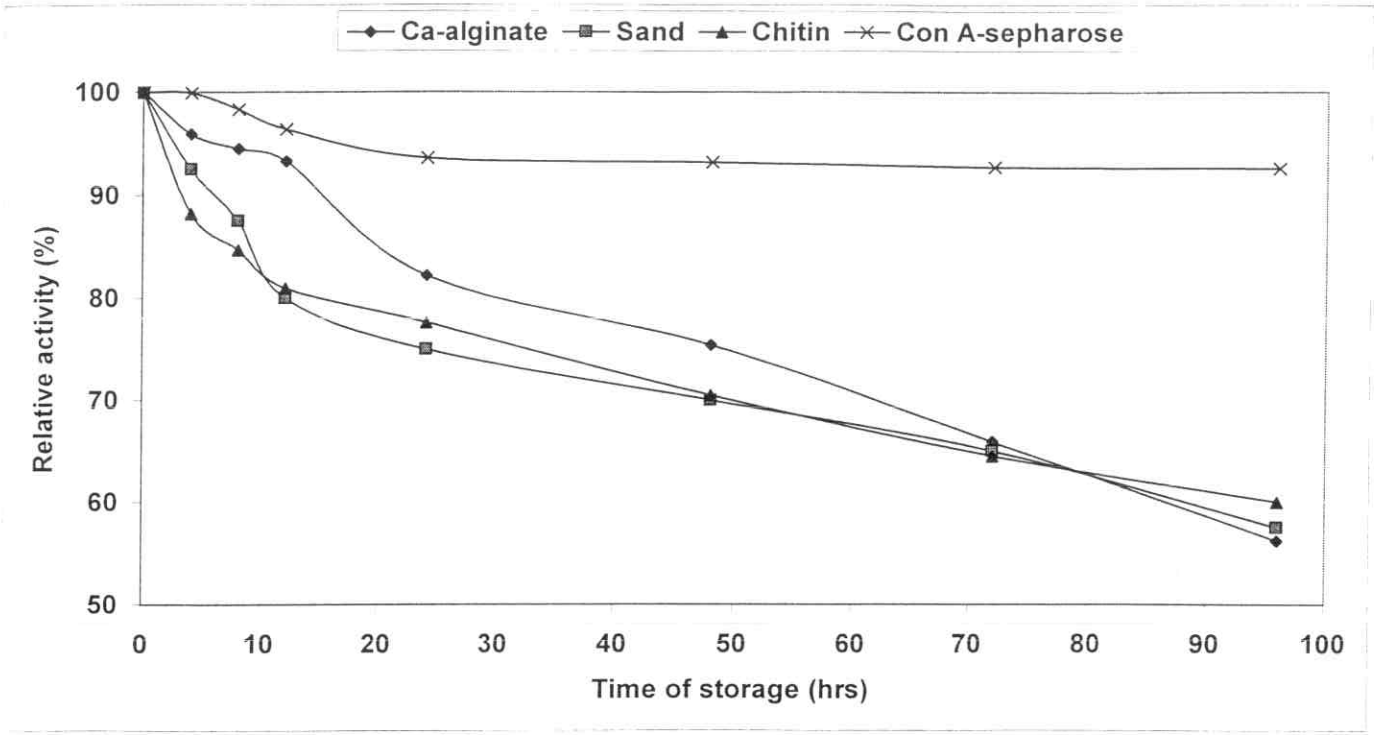

Fig. (10): Stability of the immobilized β-galactosidase with different supports

activity, it could be observed that the immobilized β-galactosidase on Ca-alginate, sand and chitin lost 43.8, 42.5 and 40.0% after 96 h, respectively. On the other hand, the loss of relative activity of immobilized enzyme on con A-sepharose complex reached 7.4%, compared with original activity. The obtained data is concident with that reported by **Hernaiz and Crout (2000)** and **Ladero *et al.* (2001)**.

4.2.3.5. Effect of incubation periods on the enzymatic hydrolysis of lactose:

The rate of enzymatic hydrolysis of lactose at different incubation periods by using soluble and immobilized forms on different supports were studied. The obtained results are tabulated in Table (13) and Figs. (11). From these results it is clear that the obtained glucose increase with increasing incubation time till reached a maximum amounted to 1321.2 mg glucose/ml for native enzyme after 150 min. On the other hand, the high levels of enzymatic hydrolysis of lactose (20%) were reached after 120, 60, 150 and 150 min for immobilized forms on Ca-alginate, sand, chitin and con A-sepharose, respectively.

However, the obtained glucose were 410.6, 678.1, 437.1 and 1125.8 mg glucose/ml for immobilized forms on different supports after the above-mentioned incubation periods. It is obviously clear from Table (13) that immobilized enzyme on different supports are less more efficient in hydrolyzing lactose as compared with the soluble enzyme. The reduction of lactose hydrolysis with immobilized forms may be due to inhibition of

Table (13): Effect of time on the enzymatic hydrolysis of lactose by using free and immobilized β-galactosidase with different supports.

Time of incubation (min)	Free enzyme (mg/ml)	Enzymatic hydrolysis of lactose with immobilized β-galactosidase on different supports (mg glucose/ml)			
		Ca-alginate*	Sand**	Chitin***	Con A-sepharose****
15	1198.7	281.5	354.3	294.7	652.3
30	1225.2	294.7	390.7	314.6	950.3
60	1278.1	370.9	678.1	354.3	990.3
90	1291.4	397.4	658.3	390.7	1009.9
120	108.0	410.6	635.1	423.8	1046.4
150	1321.2	321.2	477.5	437.1	1125.8
180	976.8	271.5	447.7	255.0	837.8
240	837.8	248.3	327.8	225.2	776.8

* Rate of hydrolysis refers to the amount contained in 25 beads.
** Rate of hydrolysis refers to the amount contained in 0.1 g of sand-enzyme complex wet.
*** Rate of hydrolysis refers to the amount contained in 0.1 ml of chitin-enzyme complex wet
**** Rate of hydrolysis refers to the amount contained in suspension of con A-sepharose.

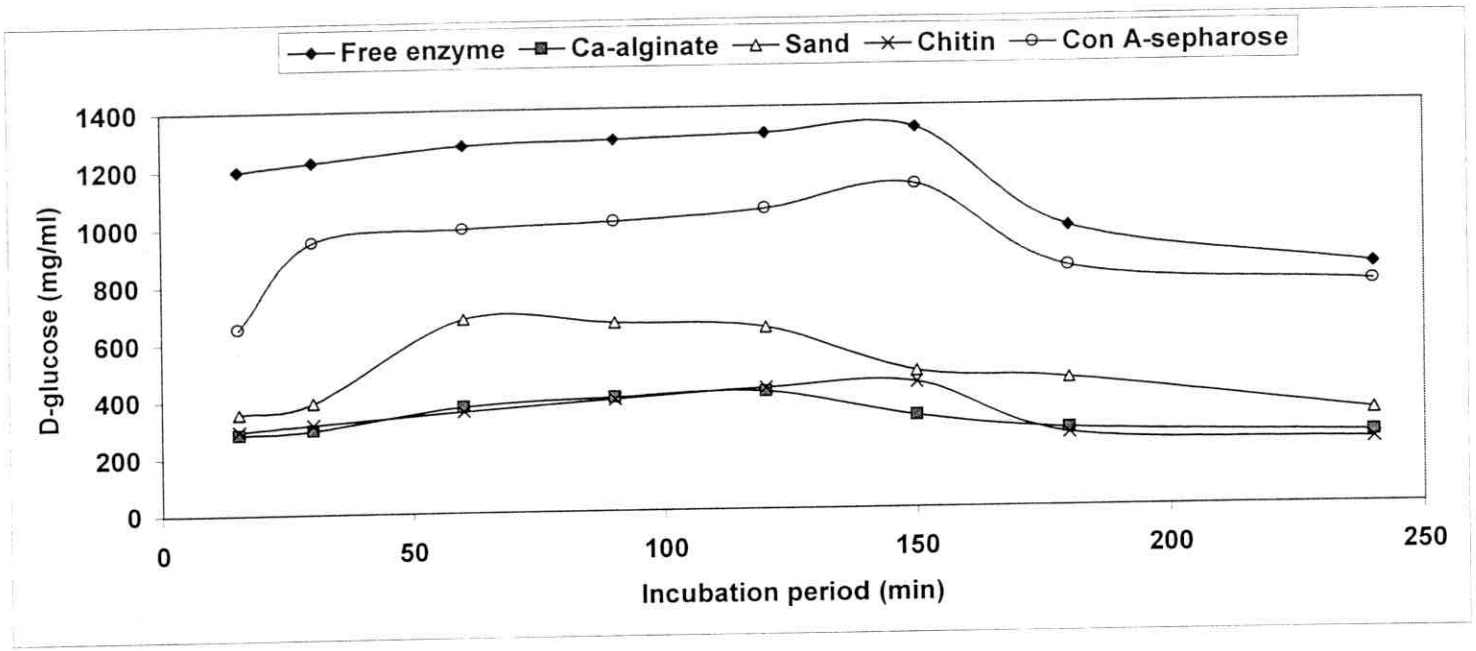

Fig. (11): Effect of time on the enzymatic hydrolysis of lactose by using free and immobilized β-galactosidase with different supports.

the product which accumulated in the gel during the continuous hydrolysis as explained by **Nakhapetyan *et al.* (1991)**.

4.2.3.6. Reuse of immobilized β-galactosidase enzyme on different supports:

The activity or relative activity (%) of the immobilized β-galactosidase enzyme on different supports, e.g. sand, chitin, Ca-alginate gel beads and Con A-sepharose after 6 times with repeated washing were evaluated. The obtained data are tabulated in Table (14).

From these results the decreament of relative activity of all supports were found to be after 6 times. The immobilized enzyme on each sand and chitin were lost about 43.26 and 44.94% of its activity after 6 times, this may be due to readsorbed of immobilized β-galactosidase enzyme on the above-mentioned supports from surface area of these supports and physical loss of weakly bound enzyme from the support or a more rapid denaturation of one component of this enzyme, Illanes *et al.* (1998). On the other hand, the immobilized form on Ca-alginate lost about 42.90% of its original activity after 6 times. The noticed decreament in the relative activity of β-galactosidase preparation with Ca-alginate might be attributed to the linkage out of the enzyme from the gel beads. While, Con A-sepharose enzyme complex exhibited the highest relative activity therefore it losses 24.33% of initial activity after 6 cycle. Therefore, it could be concluded that the immobilized enzyme on sand can be use 3 times with only 15% loss of its relative activity when compared with the immobilized form on Con A-sepharose.

But at the same number of cycles the Ca-alginate enzyme complex retained 81.41%, it can be used sand for industrial application and Ca-alginate for food industry.

Table (14): Reuse of immobilized lactase enzyme on different supports.

Number of cycles	Sand		Chitin		Ca-alginate gel beads		Con A-sepharose	
	Activity mg/ml/min.	Relative activity (%)	Activity mg/ml/min.	Relative activity (%)	Activity mg/ml/min.	Relative activity (%)	Activity mg/ml/min.	Relative activity (%)
fresh	711.92	100.00	692.05	100.00	516.55	100.00	1029.80	100.00
1	658.94	92.56	639.07	92.34	480.01	92.93	990.07	96.14
2	946.36	90.79	602.65	84.65	450.33	87.80	937.15	91.03
3	605.23	85.01	566.22	81.82	420.53	81.41	880.08	85.46
4	500.58	70.31	506.62	73.21	384.10	74.36	851.26	82.67
5	463.97	65.72	428.84	61.97	344.37	66.67	811.47	78.80
6	403.97	56.74	381.06	55.06	294.97	57.10	779.20	75.67