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KINETIC PARAMETERS AND STABILITY OF IMMOBILIZED INVERTASE ENZYME ON SOME DIFFERENT SUPPORTS

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ABSTRACT

The retention activities of invertase enzyme (E.C.3.2.1.26, β-D-fructofuranosidase) from baker's yeast after immobilization on different supports, i.e. sand, chitin, concanavalin A-sepharose and cyanogen bromide-activated sepharose were determined. The efficiency loading capacity of immobilized invertase forms was 72%, 65.2%, 88.8% and 93.3% for the above-mentioned supports, respectively.

The optimum pH values were 4.8, 5.1, and 4.5 for free enzyme, immobilized enzyme with Con A-sepharose and CNBr-activated sepharose, respectively. While, the optimum temperature for immobilized forms was 50°C and 45°C for free form.

The immobilized enzyme forms showed a high stability when compared with its soluble enzyme after storage at 4°C for 21 days.

The free enzyme showed K_m value equalled 4.0 mM which increased after immobilization to 8.0 mM and 14.0 mM for Con A-invertase and CNBr-activated sepharose enzyme complexes.

The bio-conversion percentages of sucrose with free and immobilized invertase on sand, chitin, concanavlin-A sepharose and cyanogen bromide-activated sepharose were 52.9, 53.4, 57.3, 61.2 and 56.2%, respectively. CNBr-activated sepharose invertase complex can be reused 10 times with loss 9.3% of its native activity, but Con A-sepharose invertase complex lost 8.2% after 7 cycles. While, sand-enzyme complex and chitin-enzyme complex lost 29.6% and 25.73% of its initial activity after 5 cycles.

Invertase enzyme (E.C. 3.2.1.26, β-D-fructofuranosidase) catalyses the hydrolysis of sucrose to produce the invert syrup. The industrial use of immobilized enzyme technology improved the development of various immobilization methods either by adsorption, entrapment, cross-linking and covalent binding (Mosbach, 1988).

Immobilized enzymes are of particular importance in this area because they can be readily separated from reaction products and depending on stability, can be reused several times (Lee and Huang, 1995).

Melo and Dsouza (1992) studied the immobilization of invertase by covalently coupled to *Ocimum basilicum* seeds through its carbohydrate moiety. They found that the retention of considerably higher amounts of enzyme activity. Also, the immobilized preparation can be used repeatedly for the hydrolysis of sucrose syrups in a batch process without loss in activity.

Kotwal and Shankar (1997) used macroporous crosslinked polystyrene anion-exchange resin Indian 48-R for the immobilization of crude extracellular invertase from *Sclerotium rolfsii*. The immobilized enzyme retained 70-80% of the activity from soluble enzyme. Immobilization decreased the optimum pH and temperature but improved the heat stability. Conjugated invertase showed a two and sevenfold increase in the K_m and V_{max}, respectively. Moreover, both soluble and immobilized forms showed inhibition at high substrate concentrations. The bound enzyme showed more stability to repeated use and retained 90% of its initial activity after 8 cycles of use in standard conditions.

Vrabel *et al.* (1997) used different supports for the immobilization of invertase from baker's yeast to the purpose of refining the mechanism of inactivation of free enzyme. The immobilization techniques were biospecific adsorption on a concanavalin A/bead cellulose matrix; covalent coupling on an activated bead cellulose matrix and crosslinking of each of these preparations with glutaraldehyde. The biospecifically adsorbed invertase on the concanavalin A-bead cellulose matrix met all criteria for a preparation that should be a convenient model of free enzyme.

Akgol *et al.* (2001) used magnetic polyvinylalcohol (PVAL) microspheres for the immobilization of invertase enzyme by covalent bonding through the amino group and crosslinking with glutaraldehyde. They found that the retained activity of the immobilized invertase was 74%. The K_m value for immobilized invertase (55 mM sucrose) was higher than that of the free enzyme (24 mM sucrose), whereas V_{max} values were smaller for the immobilized invertase. The optimum operational temperature for immobilized form was higher than that of the soluble enzyme. Thermal and storage stabilities were found to increase with immobilized preparations.

Neubert *et al.* (2002) permeabilized cell suspension culture of *Eschscholtzia californica* by Tween 20 or 80, then immobilized by glutaraldehyde. They found that the highest invertase activity was at pH 4.5 and

50°C. The cells had high invertase activity and a good stability. The hydrolysis of the substrate was linear for 5 h reaching 60% conversion.

The present investigation was carried out to elucidate the effect of immobilization on the stability and kinetic parameters of invertase enzyme. Various supports have been used to immobilize invertase i.e. sand, chitin, concanavalin A-sephorase (Con-A-S) and coupling with cyanogen bromide-activated sepharase. Since these parameters are vey important for the industrial process from the economical point of view.

MATERIALS AND METHODS

1. Invertase enzyme (E.C.3.2.1.26, β-D-fructofuranosidase):

Invertase enzyme (grade VII from bakers yeast) was purchased from Sigma Chemical Co.. The obtained enzyme was immobilized on different supports i.e. sand, chitin, concavalin A-sepharose and cyanogen bromide-activated sepharose 4 B according to the methods described by Brotherton *et al.* (1976), Synowicki *et al.* (1981), Vrabel *et al.* (1997) and Shahid *et al.* (1996), respectively.

Sucrose, D(+) glucose, as standards and the buffer components (sodium acetate and acetic acid) were obtained from Sigma Chemical Co. (St. Louis, MO). Con-A-Sepharose, cyanogen bromide-activated sepharose, chitin and glutaraldehyde (25%) were supplied from the products of Koch-Light (Colubrook, Bucks, England). Other chemicals and reagents were analytical grade.

2. Enzyme assays:

The activity of free and insoluble enzyme preparations was determined by the procedure described by Vrabel *et al.* (1997). Protein concentrations were estimated according to the method of Bradford (1976). The resulting reducing sugars (as glucose) were determined by the method described by Somogyi (1952). Values shown in the Figures and Tables represent the average of at least two experiments performed in duplicate.

Stability of free and immobilized invertase was evaluated according to the method described by Wiseman (1985).

3. Kinetic parameters of free and immobilized invertase preparations:

The different parameters which affect on the enzyme activity were determined in order to evaluate insoluble enzyme preparations i.e. pH, temperature and substrate concentration. The enzymatic reactions were incubated for 20 min at optimum pH and temperature value for each enzyme forms. The resultant reducing sugars were measured according to the method described before.

4. Effect of incubation periods on the enzymatic hydrolysis of sucrose:

The enzymatic hydrolysis of sucrose (0.146 mole) with free and immobilized invertase on different supports were determined under the optimum conditions of each preparations enzyme forms. The reaction mixtures were incubated from 15 to 240 min and the resulting reducing sugars were measured by the method described before.

5. Effect of recycles on the activity of immobilized invertase:

The relative activity of each preparations of immobilized invertase forms was assayed under standard conditions after 10 cycles with repeated washing.

RESULTS AND DISCUSSION

In the present work, the attempts for immobilization of invertase enzyme (β-D-fructofuranosidase, E.C. 3.2.1.26) will be discussed. Various immobilization techniques and different supports, i.e. sand, chitin, concanavalin A-sepharose and CNBr-activated sepharose were used in order to find the optimal convenient method and support for this enzyme under investigation.

1. Effects of immobilization on the invertase enzyme:

The activities of immobilized invertase on the above-mentioned support materials were determined. The quantity of the enzyme bounded to each support besides that, the retention activity after immobilization has to be considered in comparing such supporting materials. The data for the efficiency of immobilization were illustrated in Table (1). The highest efficiency loading capacity of immobilized form was found with cyanogen bromide-activated sepharose (CNBr-activated sepharose), since the support was bounded with large amount of enzyme added and the enzyme coupling was strong enough to prevent dissociation. The retention activity was found to be 93.3% of its native activity for CNBr-activated sepharose form. This result may be due to that CNBr-activated complexes are porous and active sites of enzymes are quite accessible to the substrates leading to the relatively high effectiveness factor values. Such explanation was introduced by Vrabel *et al.* (1997). The lowest yield of immobilization (65.2%) was observed for the adsorption of invertase on alkylamine chitin. On the other hand, the biospecific adsorption of invertase on concanavalin A-sepharose (Con A-S) gave a yield with retention activity of 88.8%. Also, the activated sand as a solid support yield a retention activity equalled 72.0%. The efficiency steadily decreased with increase of the enzyme load. The decrement in the efficiency at higher enzyme load can be correlated to overcrowding of the enzyme on the matrix as there was a decrease in the specific activity of the bound enzyme (Kotwal and Shankar, 1997). While, the decrease in the retention activity of immobilized invertase on sand and chitin might be attributed to weakness bounded because the attachment of enzyme on activated solid support depended on the display area of surface size of the support and types of attachment bonds or cause diffusion limitations which markedly decrease the observed enzyme activity. Such conclusions was explained by Melo and Dsouza (1992).

Table (1): Retention activities of preparations immobilized invertase enzyme on different supports:

Supports	Activity of invertase enzyme (μ mole/L/min.)					Effective-ness factor (D/C) x 100%	Retention activity (D/A) x 100%
	Added (A)	Protein mg/ml	In wash-ing (B)	Adsorption or coupling with supports			
				Theoretical (A-B) = C	Actual D		
Sand	297.2	7.90	75.00	222.2	213.90	96.3	72.0
Chitin	297.2	7.90	41.67	255.5	193.75	75.8	65.2
Con A-sepharose	297.2	7.90	25.00	272.2	263.90	97.0	88.8
CNBr-activated sepharose	372.2	11.85	22.00	350.2	348.80	99.6	93.3

2. Evaluation of free and immobilized invertase preparations with different supports:

There are several factors influence on the enzyme activity and reaction velocity of free and immobilized invertase on Con A-sepharose and CNBr-activated sepharose were measured.

The pH activity profiles of soluble and immobilized forms has been shown in Table (2) and Fig. (1). The optimum pH values of the soluble and immobilized preparations on Con A-sepharose and CNBr-activated sepharose were 4.8, 5.1 and 4.5, respectively. This shift can be correlated to the polycationic nature of the matrix. These results for optimum pH are slightly differentiations with those reported by Melo and Dsouza (1992) and Kotwal and Shankar (1997).

Table (2): Effect of pH value on the activity of free and immobilized invertase enzyme with different supports.

pH value	Free enzyme		Con A-sepharose invertase complex		CNBr-activated sepharose invertase complex	
	R.S.* content μmole/L	Activity μmole/L/min	R.S.* content μmole/L	Activity μmole/L/min	R.S.* content μ mole/L	Activity μmole/L/min
3.6	1527.78	76.39	1388.89	69.44	1166.67	58.33
4.0	1666.67	83.33	1500.00	75.00	1444.44	72.22
4.2	1722.22	86.11	1611.11	80.56	1722.22	86.11
4.5	1944.44	97.22	1694.44	84.72	2138.89	106.94
4.8	2055.56	102.78	1805.56	90.28	2083.33	104.17
5.1	1638.89	81.94	1861.11	93.06	1952.25	97.61
5.3	1472.22	73.61	1416.67	70.83	1750.00	87.50
5.6	1361.11	68.06	1305.56	65.28	1390.00	69.50

R.S.* = Reducing sugars (as glucose).

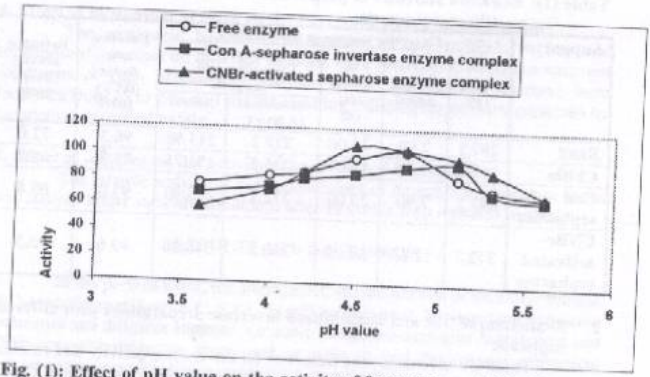

Fig. (1): Effect of pH value on the activity of free and immobilized invertase enzyme.

The effect of temperature on the enzyme activity of invertase (free and immobilized forms) was studied by carrying out the enzyme assay at the desired temperature. The Con A-invertase and CNBr-activated sepharose invertase complexes exhibited a retain greater activity at higher temperature (50°C) compared with free enzyme (45°C) as shown in Table (3) and Fig. (2).

Table (3): Effect of temperature on the activity of free and immobilized invertase enzyme with different supports.

Temperature (°C)	Free enzyme		Con A-sepharose invertase complex		CNBr-activated sepharose invertase complex	
	R.S.* content µ mole/L	Activity µ mole/L/min	R.S.* content µ mole/L	Activity µ mole/L/min	R.S.* content µ mole/L	Activity µ mole/L/min
30	985.00	49.25	429.00	21.45	647.00	32.35
35	1027.78	51.39	555.56	27.78	708.34	35.42
40	1388.89	69.44	833.33	41.67	805.56	40.28
45	2083.33	104.17	1166.67	58.33	888.89	44.44
50	1944.44	97.22	1750.00	87.50	1266.67	63.33
55	1777.78	88.89	1500.00	75.00	1013.89	50.70
60	1527.78	76.39	1388.89	69.44	833.34	41.67
65	1250.00	62.50	1290.60	64.53	513.89	25.70
70	972.22	48.61	1190.20	59.54	416.67	20.83

R.S.* = Reducing sugars (as glucose).

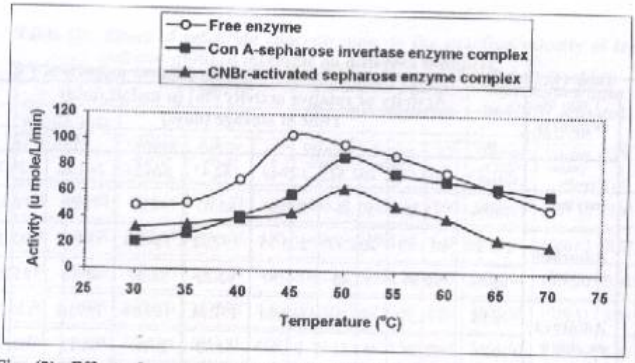

Fig. (2): Effect of temperature on the activity of free and immobilized of invertase enzyme.

The superior temperature stability of the bound enzyme indicates the rigidity of the enzyme structure in the bound form. These data are in agreement with those reported by Akgol *et al.* (2001) and Neubert *et al.* (2002).

However, immobilization caused an increase in the stability of immobilized invertase enzyme, compared to its soluble counterpart. The obtained results are shown in Table (4) and illustrated in Fig (3). The bound enzyme with sand, chitin, Con A-sepharose and CNBr-activied sepharose showed a high stability if compared to its soluble enzyme. The retention activities of immobilized forms were 85.25, 80.65, 88.12 and 91.15%, respectively with above-mentioned supports at 4°C for 21 days storage period. The increment stability of immobilized forms if compared with free form may be due to changes in the conformation that would lead these preparations were more useful towards the inactivation behavior of free enzyme. Such explanation was introduced by Vrable *et al.* (1997).

3. Kinetics of free and immobilized invertase with Con A-sepharose and CNBr-activated sepharose:

The rate of the reaction velocity of free and prepared immobilized invertase enzyme on Con A-sepharose and CNBr-activated sepharose was studied at different substrate concentrations as described before. The data are shown in Table (5) and Fig (4a & b). From these results, the maximum reaction velocity (V_{max}) was found to be 2500.00 µmM glucose/L and Michales constant (K_m) was 4.0 mM for free form. On the other hand, these values of V_{max} and K_m were 2277.8 µmM glucose/L, 8.0 mM for Con A-invertase complex and 2666.7 µmM glucose/L and 14.0 mM for CNBr-activated sepharose enzyme complex. It is clear that the K_m values for immobilized forms were higher than that obtained for free form, which simply means a less affinity of the enzyme matrix to the substrate had been occurred.

Table (4): Stability of free and immobilized invertase on different supports at 4°C.

Type of supports	Activity or relative activity (%) (μ mole/L/min) Time of storage (days)							
	0	1	2	3	4	7	14	21
Free enzyme	221.43	208.67	202.27	197.62	182.43	174.13	164.28	159.76
	100%	94.25	91.36	89.26	82.40	78.65	74.20	72.16
Adsorbed on sand	213.90	213.89	208.33	205.56	197.22	184.40	184.17	182.35
	100%	99.99	97.40	96.10	92.20	86.21	86.10	85.25
Adsorbed on chitin	193.8	191.70	187.53	185.65	177.78	169.40	163.08	156.30
	100%	98.91	97.84	95.79	91.73	87.41	84.15	80.65
Con A-sepharose enzyme complex	263.90	263.33	261.11	258.25	245.22	236.67	235.53	232.55
	100%	99.78	99.16	97.86	92.92	89.68	89.25	88.12
CNBr-activated enzyme complex	186.10	185.00	183.33	182.75	177.78	172.25	171.40	169.63
	100%	99.41	98.51	98.20	95.53	92.56	92.10	91.15

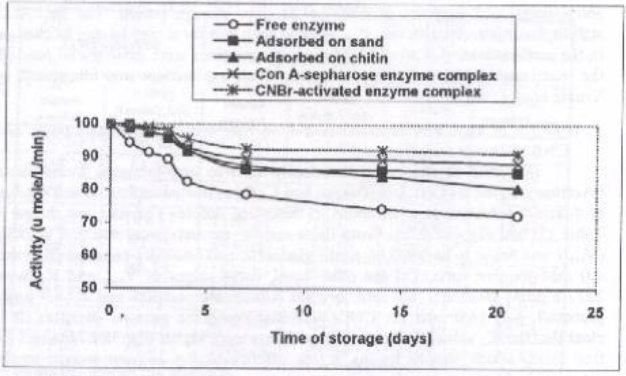

Fig. (3): Stability of free and immobilized invertase at 4°C.

Table (5): Effect of substrate concentration on the reaction velocity of free and immobilized invertase on different supports.

Substrate concentrate mM [S]	1/[S]	Free enzyme			Immobilized form on Con A-sepharose			Immobilized form on CNBr-activated sepharose		
		Obtained of R.S.* µmM/L	Reaction velocity (v)	1/V x 10^{-4}	Obtained of R.S.* µmM/L	Reaction velocity (v)	1/V x 10^{-4}	Obtained of R.S.* µmM/L	Reaction velocity (v)	1/V x 10^{-4}
10	0.100	1944.44	1785.71	5.60	1333.33	1265.44	7.90	1166.67	1111.13	9.00
20	0.050	2166.67	2083.33	4.80	1638.89	1591.29	6.28	1416.67	1568.65	6.37
30	0.033	2305.56	2205.89	4.53	1861.11	1758.79	5.69	1694.44	1818.20	5.50
40	0.025	2500.00	2272.73	4.40	2055.56	1856.50	5.39	1805.56	1975.33	5.06
50	0.020	2138.89	2314.81	4.32	2194.44	1920.52	5.21	2305.56	2083.36	4.80
60	0.017	1972.22	2343.75	4.27	2277.78	1965.71	5.09	2472.22	2162.19	4.62
70	0.014	1833.33	2364.86	4.23	2222.22	1999.31	5.00	2666.67	2222.25	4.50
80	0.013	1555.56	2380.95	4.20	2027.78	2025.77	4.94	2333.33	2269.53	4.41
90	0.011	1305.56	2393.62	4.18	1527.78	2045.94	4.89	2166.67	2307.72	4.33

R.S.* = Reducing sugars (as glucose).

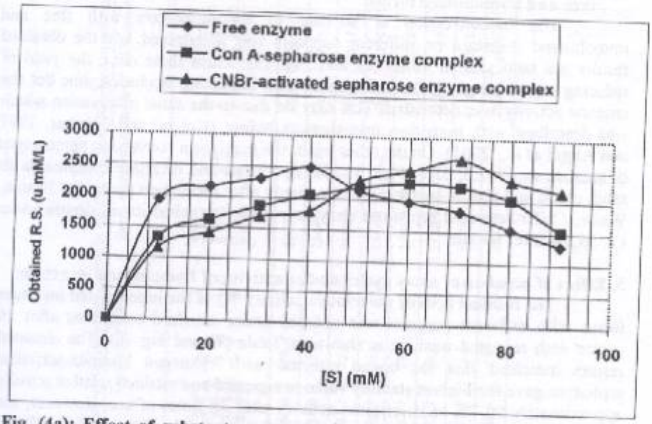

Fig. (4a): Effect of substrate concentration on the reaction velocity of free and immobilized invertase.

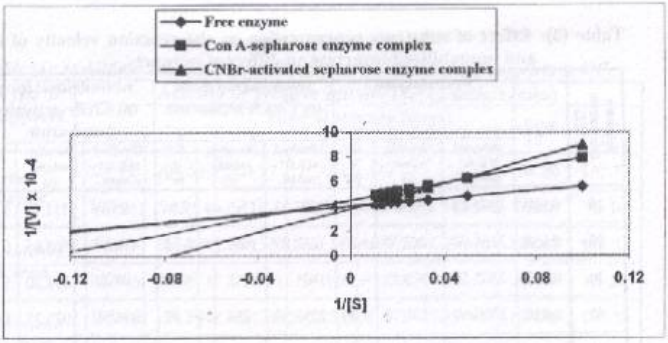

Fig. (4b): Lineweaver-Burk plots of free and immobilized invertase.

These observations may be due to the chemical nature of support materials and diffusional resistances to the translocation of substrates and products (Woodward, 1985). These values are in agreement with those reported by Akgol *et al.* (2001).

4. Effect of incubation periods on the enzymatic hydrolysis of sucrose with free and immobilized forms:

The bioconversion of sucrose to its monomers with free and immobilized invertase on different supports was determined and the obtained results are tabulated in Table (6) and Fig. (5). From these data, the yield of reducing sugars (as glucose) was increased with increasing incubation time but the enzyme activity was decreased. This may be due to the same observation which was described with numerous investigators before (Kotwal and Shankar, 1997 and Akgol *et al.*, 2001). On the other hand, the maximum conversion percentages of sucrose were 52.9, 53.4, 57.3, 61.2% for soluble and invertase complexs with sand, chitin and Con A-sepharose, respectively after incubation period for 30 min. While, CNBr-activated sepharose enzyme complex reached its maximum value i.e. 56.2% after 60 min.

5. Effect of number of assay cycles on the activity of immobilized invertase:

The residual activity (as relative activity %) of the immobilized invertase forms with different supports was assayed under standard conditions after 10 cycles with repeated washing is shown in Table (7) and Fig. (6). The obtained results indicated that the bound enzyme with cyanogen bromide-activated sepharose gave the highest stability value to repeated and retained relative activity approximately 90.7% of its original activity after 10 times of use. However, the immobilized invertase on sand and chitin showed the lowest retention relative activities i.e. 41.81 and 37.49% after 10 cycles. But, Con A-sepharose invertase complex lost only 19•61% of its relative activity after 10 cycles of restability.

Table (6): Effect of incubation periods on the enzymatic hydrolysis of sucrose with free and immobilized invertase forms.

Time of incubation (min)	Free enzyme			Sand-enzyme complex			Chitin-enzyme complex			Con A-Sepharose enzyme complex			CNBr-activated sepharose enzyme complex		
	Obtained of D-glucose µ mole/L	Activity µ mole/L/min	Conversion (%)	Obtained of D-glucose µ mole/L	Activity µ mole/L/min	Conversion (%)	Obtained of D-glucose µ mole/L	Activity µ mole/L/min	Conversion (%)	Obtained of D-glucose µ mole/L	Activity µ mole/L/min	Conversion (%)	Obtained of D-glucose µ mole/L	Activity µ mole/L/min	Conversion (%)
15	2555.6	170.4	51.2	2272.2	151.5	45.5	2192.2	146.1	43.9	2833.3	180.9	56.7	2183.3	145.6	43.7
30	2638.9	87.9	52.9	2666.7	88.9	53.4	2861.1	95.4	57.3	3055.6	101.9	61.2	2305.6	76.9	46.2
60	2583.3	43.1	51.7	2500.0	41.7	50.0	2611.1	43.5	52.3	2500.0	41.7	50.0	2805.6	46.8	56.2
90	2388.9	26.5	47.8	2444.4	27.2	48.9	2500.0	27.8	50.0	2333.3	25.9	46.7	2361.1	26.2	47.3
120	2333.3	19.4	46.7	2250.0	18.8	45.1	2388.9	19.9	47.8	2277.8	19.0	45.6	2252.2	18.8	45.1
150	2222.2	14.8	44.5	2138.9	14.3	42.8	2138.9	14.3	42.9	225.0	15.0	45.1	2181.1	14.5	43.7
180	2027.8	11.3	40.6	2055.6	11.2	41.2	2083.3	11.6	41.7	2111.1	11.7	42.3	1994.4	11.1	39.9
240	1750.0	7.9	35.0	2027.8	8.5	40.6	2000.0	8.3	40.1	1944.4	8.1	38.9	1684.6	7.0	33.7

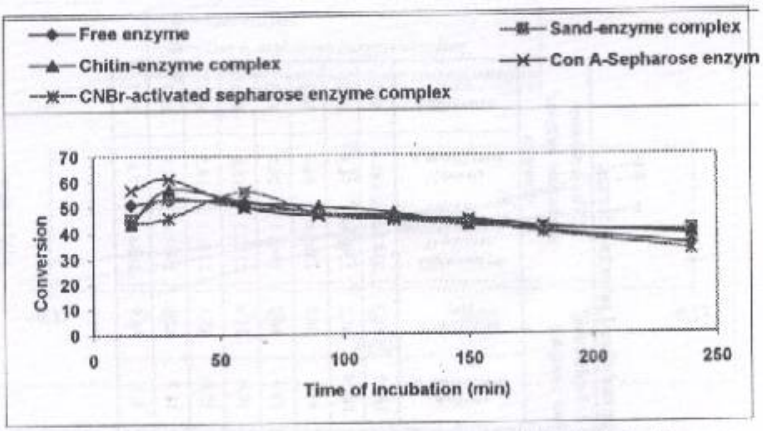

Fig. (5): Effect of incubation periods on the enzymatic hydrolysis of sucrose with free and immobilized invertase forms

Table (7): Reuse of immobilized invertase on different supports.

No of cycles	Sand		Chitin		Con A-sepharose		CNBr-sepharose	
	Activity µmole/L/min	Relative activity (%)	Activity µmole/L/min	Relative activity (%)	Activity µmole/L/min	Relative activity (%)	Activity µmole/L/min	Relative activity (%)
Before	213.90	100	193.80	100	263.90	100	372.20	100
1	212.44	99.32	193.33	99.21	263.61	99.89	371.16	99.72
2	206.11	96.36	187.89	96.95	263.35	99.79	366.32	98.42
3	192.22	89.86	186.36	96.16	261.66	99.15	365.91	98.31
4	178.33	83.37	161.86	83.52	258.28	97.87	364.38	97.90
5	150.56	70.39	143.94	74.27	249.86	94.68	358.09	96.21
6	136.67	63.89	127.73	65.91	243.69	92.34	354.52	95.25
7	125.56	58.70	101.28	52.26	242.29	91.81	350.50	94.17
8	122.78	57.40	94.71	48.87	231.89	87.87	346.89	93.20
9	120.00	56.10	80.93	41.76	216.16	81.91	343.39	92.26
10	89.44	41.81	72.65	37.49	212.15	80.39	337.59	90.70

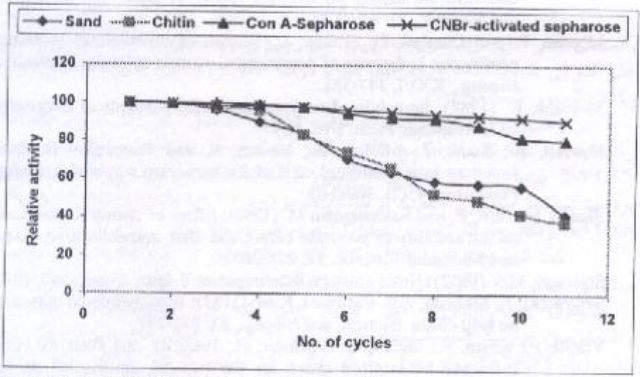

Fig. (6): Reuse of immobilized invertase on different supports.

The obtained data of Con-A sepharose, CNBr-activated sepharose enzyme are closed with those reported by Kotwal and Shankar (1997) who found that the immobilized invertase retained about 90% of its activity after 8 cycles.

From the obtained results it can be concluded that the activity and stability of invertase immobilize on nature supports like sand and chitin were found to be very satisfactory and this makes it possible to consider their industrial use in the production of invert sugar syrups, despite the low cost incidence in traditional hydrolysis using enzyme in solution.

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حركية وثبات إنزيم الأنفرتيز المحمل على بعض الدعامات المختلفة

فرحات فودة على فودة

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يهدف هذا البحث إلى دراسة تأثير التحميل باستخدام الطرق المختلفة بغرض تحويل إنزيم الأنفرتيز من الصورة الذائبة إلى الصورة غير الذائبة حيث تم استخدام دعامات ذات طبيعية تركيبية مختلفة وهى الرمل والكيتين والكونيكاناڤلين-أ-سيفاروز والسيانوجين بروميد المنشط سيفاروز على الخواص الوظيفية لنشاط الأنزيم ودرجة الثبات له. وكذلك دراسة الظروف المثلى والسلوك الحركى للأنزيم المحمل مقارنة مع الأنزيم الحر وأيضا إمكانية استخدام الأنزيم المحمل أكثر من مرة حيث أن هذه النقطة هامة من الناحية الإقتصادية والصناعية نظرا لأهمية هذا الأنزيم فى إنتاج السكر المحول من السكروز.

وأوضحت النتائج أن نسبة درجة النشاط المرتجعة لإنزيم الإنفرتيز المحمل هى ٧٢,٠%، ٦٥,٢%، ٨٨,٨%، ٩٣,٣% من كمية الأنزيم المضافة للدعامات الرمل والكيتين والكونيكاناڤلين-أ-سيفاروز والسيانوجين بروميد المنشط سيفاروز على التوالى.

وقد أوضحت النتائج أن درجة الحموضة المثلى pH لإنزيم الإنفرتيز الحر والمحمل على كل من الكونيكانافلين-أ-سيفاروز والسيانوجين المنشط سيفاروز هى على التوالى ٤,٨، ٥,١، ٤,٥، بينما كانت درجة الحرارة المثلى للأنزيم الذائب ٤٥°م وكانت للصور المحملة ٥٠°م.

وأظهرت النتائج أن درجة ثبات الأنزيم المحمل على الدعامات المختلفة زادت بنسب متفاوتة مقارنة مع الأنزيم الحر حيث أعطى نسبة درجة نشاط مرتجعة ٧٢,١٦% بينما كل من الرمل والكيتين والكونيكانافلين-أ-سيفاروز والسيانوجين بروميد المنشط سيفاروز أعطت درجة نشاط مرتجع ٨٥,٢٥%، ٨٠,٦٥%، ٨٨,١٢%، ٩١,١٥% وذلك بعد فترة تخزين لمدة ٢١ يوم على درجة ٤°م.

كما أوضحت الدراسة الحركية لإنزيم الإنفرتيز أن السرعة القصوى وثابت ميكائيس-منتن لهذا الأنزيم فى صورته الذائبة تساوى ٢٥٠٠ ميكرومول جلوكوز/لتر، ٤ ملليمول بينما الصورة غير الذائبة على كل من الكونيكانافلين-أ-سيفاروز والسيانوجين بروميد المنشط سيفاروز تساوى (٢٢٧٧,٨ ميكرومول جلوكوز/لتر، ٨ ملليمول)، (٢٦٦٦,٧ ميكرومول جلوكوز/لتر، ١٤ ملليمول) على التوالى. وقد يعزى إرتفاع قيمة ثابت ميكائيس-منتن للأنزيم المحمل إلى إنخفاض تركيز المادة المتفاعلة فى وسط التفاعل نتيجة لحدوث تثبيط جزئى نتيجة ارتباط الأنزيم بالدعامات المختلفة.

كما أوضحت النتائج أن أعلى نسبة تحويل السكروز إلى السكر المحول باستخدام الأنزيم الحر والأنزيم المحمل على الدعامات المختلفة كانت ٥٢,٩%، ٥٣,٤%، ٥٧,٣%، ٦١,٢% بعد فترة تحضين ٣٠ دقيقة، ٥٦,٢% لمدة ٦٠ دقيقة على التوالى.

أما بالنسبة لاستخدام الأنزيم المحمل عديد من المرات فقد أظهرت النتائج أن معقد الأنزيم مع السيانوجين بروميد المنشط سيفاروز أمكن استخدامه ١٠ مرات مع فقد ٩,٣% من درجة نشاطه بينما معقد الأنزيم مع الكونيانافلين-أ-سيفاروز فقد ٨,٢% من نشاطه بعد ٧ مرات بينما كانت نسبة الفقد لمعقد الأنزيم مع كل من الرمل والكيتين تساوى ٢٩,٦%، ٢٥,٧٣% من درجة النشاط بعد الاستخدام خمس مرات.