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IMMOBILIZATION AND EVALUATION OF THE PRODUCED CELLULASE ENZYME FROM DIFFERENT FUNGI

BY

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ABSTRACT

Cellulase enzyme produced from different fungi has been immobilized on two different supports, i.e. gel fertilizer and chitin. The immobilized enzyme with chitin as a support had retention activity lower than that of gel fertilizer. The retention activities of immobilized enzyme on chitin were found to be 89%, 60% and 72% for *T. reesei*, *T. harzianum* and *A. niger*, respectively. While, these values were 97, 95 and 96% for the above-mentioned enzymes which were immobilized on gel fertilizer as a support. The optimum temperature for immobilized enzyme on chitin was established at 55°C for *T. reesei*, *T. harzianum* and *A. niger*. But the optimum temperature of immobilized enzyme on gel fertilizer was 60°C for *T. reesei*, and *A. niger* and 55°C for *T. harzianum*. On the other hand, the optimum pH in case of gel fertilizer support was 5.0 for *T. reesei*, and *A. niger* and 4.8 for *T. harzianum*. While, the optimum pH for immobilized enzyme on chitin was 4.8 for either *T. reesei*, or *A. niger*. But *T. harzianum* showed that the maximum activity at pH 4.6. Kinetic behavior of the immobilized enzyme with different supports was studied. Michaelis constant (K_m) of immobilized cellulase enzyme from *T. reesei* were determined to be 0.42 and 0.22 g/100 ml CMC in case of gel fertilizer and chitin support, respectively. On the other hand, K_m values of immobilized cellulase enzyme produced from *T. harzianum* were calculated to be 0.18 and 0.2 g/100 ml CMC for gel-cellulase complex and chitin-cellulase complex, respectively. While, K_m of immobilized cellulase enzyme produced from *A. niger* was 0.2 g/100 ml in case of gel fertilizer and 0.52 g/100 ml for chitin-cellulase enzyme complex.

The stability of immobilized cellulase enzymes forms were found to be more stable in comparison with native form. The immobilized cellulase enzyme on gel fertilizer from different studied sources of fungi (*T. reesei*, *T. harzianum* and *A. niger*) lost 40% of its original activity after 4 cycles. While, the immobilized enzyme on chitin lost 70% of its activity after 4 times.

INTRODUCTION

Enzymes are being used increasingly as catalysts for biochemical conversion, but they are expensive and have limited stability. Consequently, chemists attempted to immobilize enzymes by different techniques for decreasing enzyme expenses and increasing enzyme stability, (Woodward, 1985).

Roy, *et al.*, (1984) immobilized cellulolytic and hemicellulolytic enzymes from *Macrophomina phaseolina* within acrylamide polymer. They found that K_m values of free and immobilized enzyme preparations were 3.02 and 4.76 mg/L for cellulase enzyme and 2.38 and 3.92 mg/L for hemicellulase enzyme, respectively. The maximum productivities of soluble sugar obtained from filter paper and cotton as substrate using immobilized cellulase enzyme were 409.32 and 67.56 mg reducing sugar/g substrate/mg enzyme protein bound to matrix. Also, the immobilized enzyme preparations retained the original activity of different cellulolytic and hemicellulolytic enzymes up to 25-29 times.

Garcia, *et al.*, (1989) used iron oxide (Fe_3O_4, magnetite) with a particle size of 325 mesh as the solid support for immobilization of cellulase enzyme. The highest specific activity of the immobilized enzyme was 5.9 mM. glucose/g bound protein/h and retaining enzymatic activity was 128%. The optimum pH was 5.5 compared to 4.0 of the free enzyme. The half-life of the immobilized cellulase enzyme (IMC) was extended to 272 h compared to 0.77 h of the free enzyme.

Park and Kajiuchi (1995) modified cellulase enzyme with amphilic copolymers made of α-allyl-ω-methoxy polyoxyalkylene (POA) and maleic acid anhydride (MAA) to improve the cellulose hydrolytic reactivity and cellulase separation. The maximum degree of modification (DM) of 55%, the modified cellulase activity retained more than 80% of the unmodified native cellulase activity. The modified cellulase has greater stability against temperature, pH, organic solvents, and demonstrated greater conversion of substrate than native cellulase enzyme.

The aim of the present work is to study the immobilization by covalent attachment of the produced cellulase enzyme from different fungi on gel fertilizer and chitin as an inexpensive supports. Also, kinetic behavior of immobilized cellulase forms and recycling were evaluated, since these parameters are very important for the industrial process.

MATERIALS AND METHODS

1. **Cellulolytic enzyme complex** i.e. Endo-1,4-ß-glucanase (E.C. 3.2.1.4.) and Exo-1,4-ß-glucosidase (E.C. 3.2.1.74.) were produced from different fungi (*T. reesei, T. harzianum* and *A. niger*).

2. **Isolation of cellulase from fermentation liquor**:- The enzyme was isolated from fermentation liquar according to the method described by Tabossum. *et al.*, (1990).
3. **Immobilization of the produced cellulase enzyme:** Immobilization of enzyme was carried out by using gel fertilizer material as a solid support. This material was obtained from Agriculture research Center, Giza, Egypt under the commercial name "Barbary plant". The solid support was purified by washing with distilled water and methanol (1: 5), then rewashed by distilled water and air dried at 50°C. The immobilization of cellulase enzyme on the activated gel fertilizer solid support was achieved by the adsorption of the free enzyme (0.187 mg enzyme protein) on 300 mg of the support for 1 hr with intermittent mixing, followed by standing for 24 h at 4°C. The immobilized enzyme form was washed with distilled water to remove the unattached enzyme protein. The amount of the immobilized form was measured. Also, the enzyme activity and kinetic parameters were determined and compared with that free form.

   Another attempt for immobilization of the produced cellulase enzyme was carried on chitin (chitosan polysaccharide) according to the method described by Synowiecki *et al.*, (1981). Water-soluble carboxymethyl cellulose (CMC) as substrate, D(+) glucose as standard. Chitin was purchased from Sigma chemical Co.
4. **The enzyme activity activity** was expressed as the resultant reducing sugars (as glucose) by the method described by Nelson's (1944) which was modified by Somogyi (1952).
5. **Protein content** was estimated by the method described by Bradford (1976).
6. **Evaluation of immobilized cellulase:**

   The different parameters which affect the enzyme activity were determined in order to evaluate immobilized , cellulase enzyme (e.g. concentration of substrate, pH and temperature.

   The effect of substrate concentration on the reaction activity of immobilized cellulase enzyme forms were tested by using carboxy-methylcellulose at different concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 20%.

   The enzyme activity of immobilized cellulase enzyme forms were tested at different pH values i.e 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5.
   The activity of immobilized cellulase enzyme forms were tested at different temperatures i.e. 30, 35, 40, 45, 50, 55 and 60°C.

   The effect of enzyme concentration on the reaction activity of immobilized cellulase enzyme forms were tested at different concentrations of immobilized cellulase enzyme between 0.378 mg enzyme proteins till 2.232 mg enzyme proteins by using (1% w/v) of carboxymethylcellulose (CMC) .

Stability of free and immobilized of the produced cellulase enzyme were evaluated according to the method described by Woodward (1985). While, reusing of immobilized enzyme forms were estimated according to the method described by Garcia, *et al.*, (1989).

RESULTS AND DISCUSSION

The effect of the immobilization process on the activity and stability of each immobilized enzyme was studied. Generally, the immobilized enzymes showed less specific activity than the native enzyme.

Nowadays, various immobilization technique and support materials are used in order to find the optimal immobilization technique and support for the produced cellulase enzyme.

1. Preparations of immobilized cellulase enzyme produced from different fungi:-

The cellulase enzyme produced from different fungi has been immobilized on two different support materials i.e. gel fertilizer and chitin polymer. Such systematic study may be helpful in finding the most convenient support which has the highest stability and proper enzyme activity. The quantity of the enzyme bounded to each support besides the retention activity after immobilization comparing with the two supports were investigated. The results in Table (1) illustrate that the gel fertilizer is more effective for immobilization of the produced enzymes from different sources since no enzymatic protein was noticed in washings, which simply means that the support was bounded with all the enzyme added. The latter phenomenon was also accompanied with relatively high retention activity which amounted to 97% for *T. reesei* , 95% for *T. harzianum* and 96 % for *A. niger*.

On the other hand, the immobilized enzyme with chitin support had retention activity lower than that of gel fertilizer. The retention activities were found to be 89, 60 and 72% of the orginal activity for *T. reesei*, *T. harzianum* and *A. niger*, respectively. The noticed decrement in the retention activity of the enzyme preparation with chitin support may be attribute to that the active sites of this enzymes were partially bounded with the support which simply after the whole body of the enzyme itself and/or the bound enzyme had a conformational alteration that affect the active sites (Saad.,1992).

2. Factors affecting the activity and reaction velocity of immobilized enzyme from different sources:

The optimum factors influence the immobilized enzyme reaction and stability were determined for both gel fertilizer and chitin i.e. temperature, pH, enzyme concentration and substrate concentration.

Table (1): Preparations of immobilized cellulase enzyme produced from fungi

Enzyme Source	Cellulase enzyme units								
	Gel fertilizer					Chitin			
	Native protein enzyme	Protein after washing Gel. F.	Bound maximum (A)	Activity of complex (B)	Retention activity B/A %	Protein after washing Gel. F.	Bound maximum (A)	Activity of complex (B)	Retention activity B/A %
T. reesei	54.5	-	54.5	53.0	97.0	22.0	32.5	29.0	89.0
T. harzianum	40.6	0.4	40.2	38.2	95.0	9.0	31.6	18.9	60.0
A. niger	51.3	-	51.3	49.3	96.0	18.0	33.3	24.0	72.0

2.1. Effect of temperature on the activity of immobilized cellulase enzyme:

The effect of temperature on initial rate of cellulose hydrolyzed by the immobilized preparations of the produced cellulase was measured as shown in Figs (1 a , b).

Different temperatures between 35, 40, 45, 55, 60 and 65°C were examined to investigate the optimum temperature of the immobilized enzyme on gel fertilizer and chitin. The immobilized enzyme on gel fertilizer upport, yielded the maximum activity 81.5 and 44.9 μmM/L/min for *T. reesei.* and *A. niger* at temperature equalle 60°C. While, the maximum activity of immobilized enzyme produced from *T. harzianum* was found to be 28.6 μmM/L/min at 55°C. On the other hand, the maximum activities of immobilized cellulase enzyme on chitin were 30.1, 20.9, 29.9 μmM/L/min was established at 55°C for *T. reesei, T. harzianum*, and *A. niger.*

Such results are in agreement with that reported by Garcia, *et al.*, (1989).

2.2. Effect of pH on the activity of the immobilized cellulase enzyme preparations:-

The effect of pH on the activity of the immobilized enzyme on gel fertilizer and chitin are shown in Figs. (2 a , b). Different pH values i.e 4.2; 4.4; 4.6; 4.8; 5.0; 5.2 using (0.1 M) acetate buffer were established versus the enzyme activity. The optimum pH in case of gel fertilizer support revealed that the maximum activities of immobilization with 1 % (CMC) as substrate were 82.3; 48.5 μmM/L/min for *T. reesei*, and *A. niger* at pH 5.0, while, the maximum activity of immobilized enzyme with gel fertilizer i.e. 37.2 μmM/L/min was found to be at pH 4.8 for *T. harzianum.*

The maximum activity was at pH 4.8 for immobilized enzyme on chitin for *T. reesei,* which gave values 32.1 μmM/L/min and 30.7 μmM/L/min for *A. niger.* On the other hand, *T. harzianum* showed that the maximum activity i.e. 26.4 μmM/L/min of immobilized cellulase enzyme at pH 4.6. The obtained data are in agreement with those reported by Roy *et al.*,(1984) .

The explanation for such phenomenon that the enzyme is attached to a negatively charged matrix. It seems that the low values of optimum pH (4.6 - 4.8) in the case of chitin support was shifted clearly which indicated the importance of such acid media (0.1 M) acetate buffer to fit the nature of the active site of cellulase enzyme. Therefore, cellulase enzyme bound on the matrix attracting a thin "film" of positive hydrogen ions thereby creating a microenvironment for the bound enzyme, that has a highest hydrogen ion concentration in the surrounding solution where the pH is actually measured i.e. higher pH values, (Wiseman, 1985).

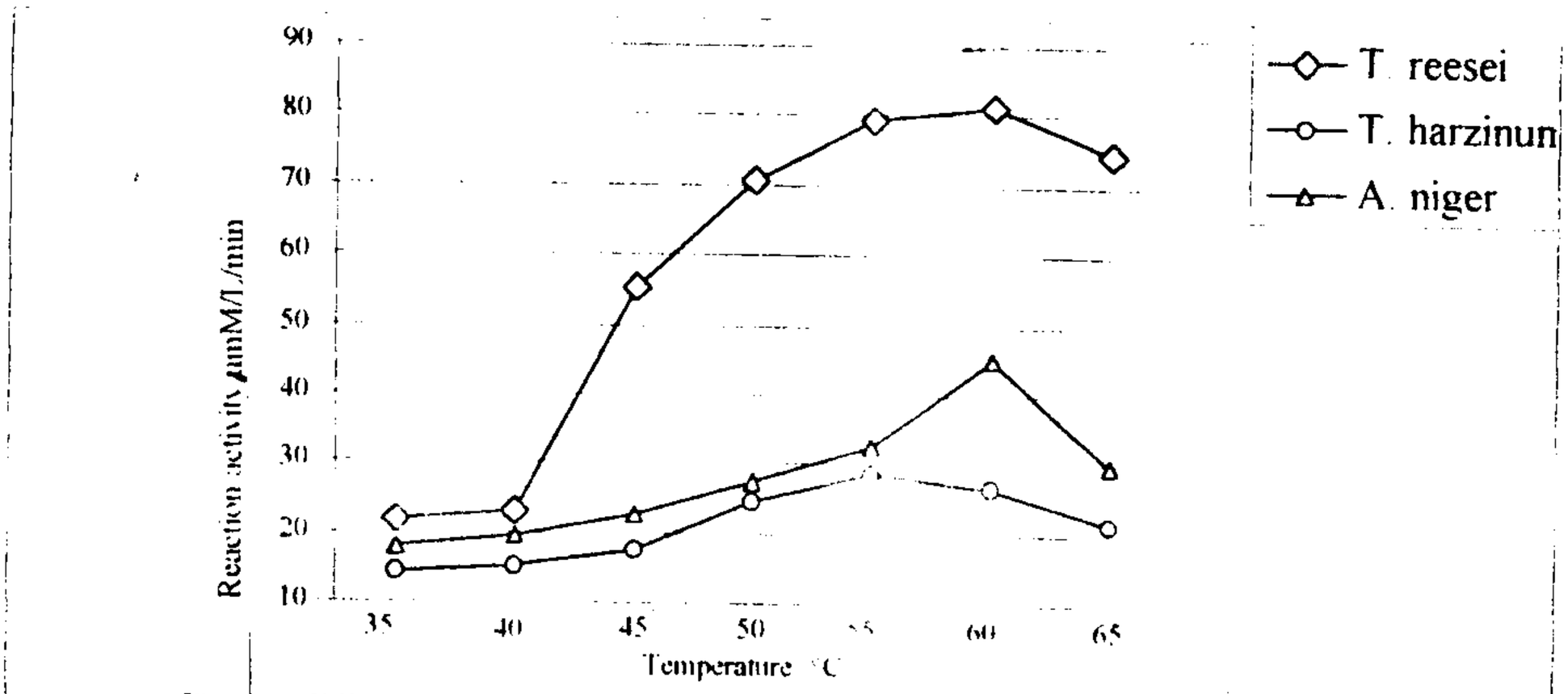

Fig (1 a): Effect of temperature on the activity of immobilized enzyme on gel fertilizer

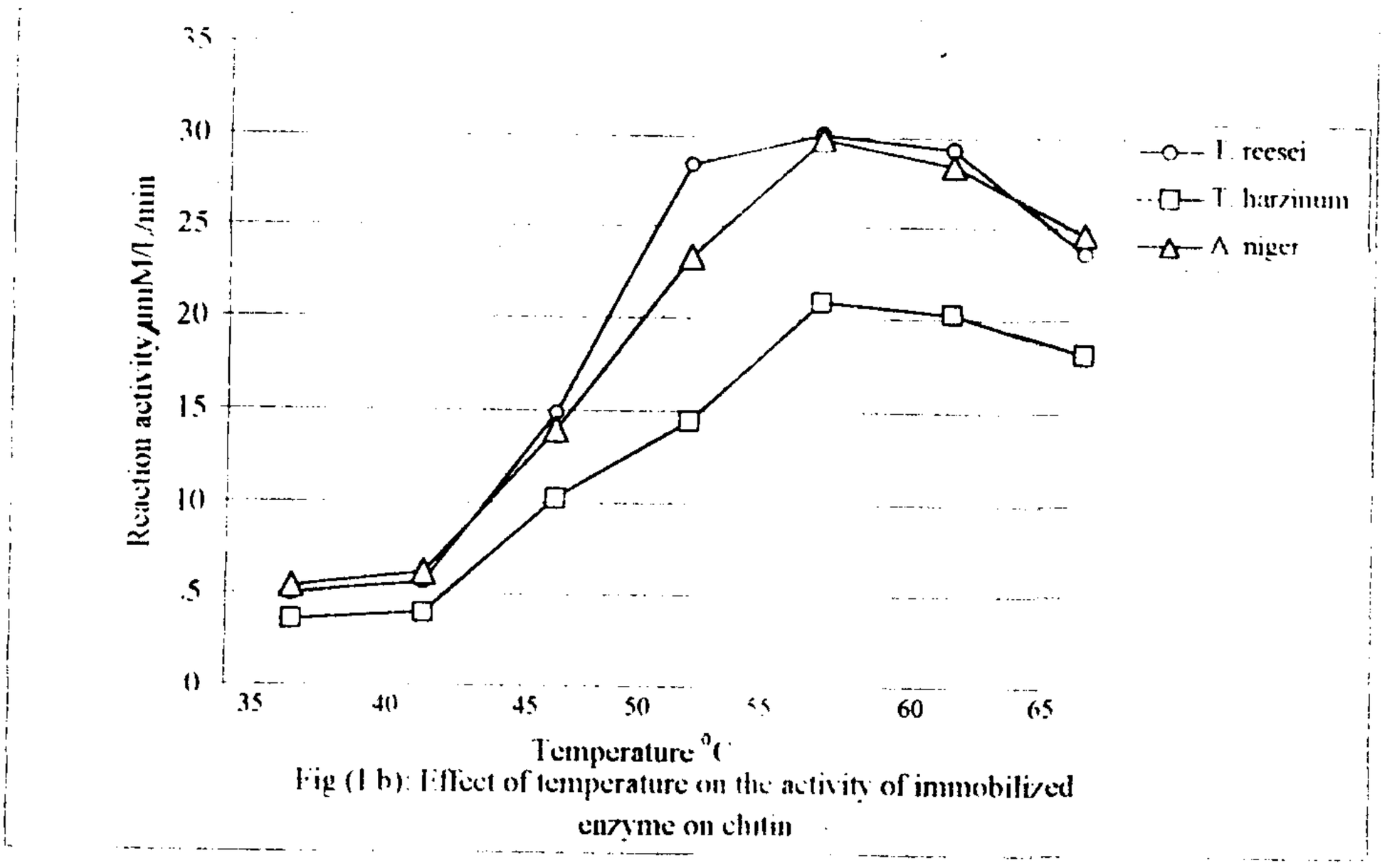

Fig (1 b): Effect of temperature on the activity of immobilized enzyme on chitin

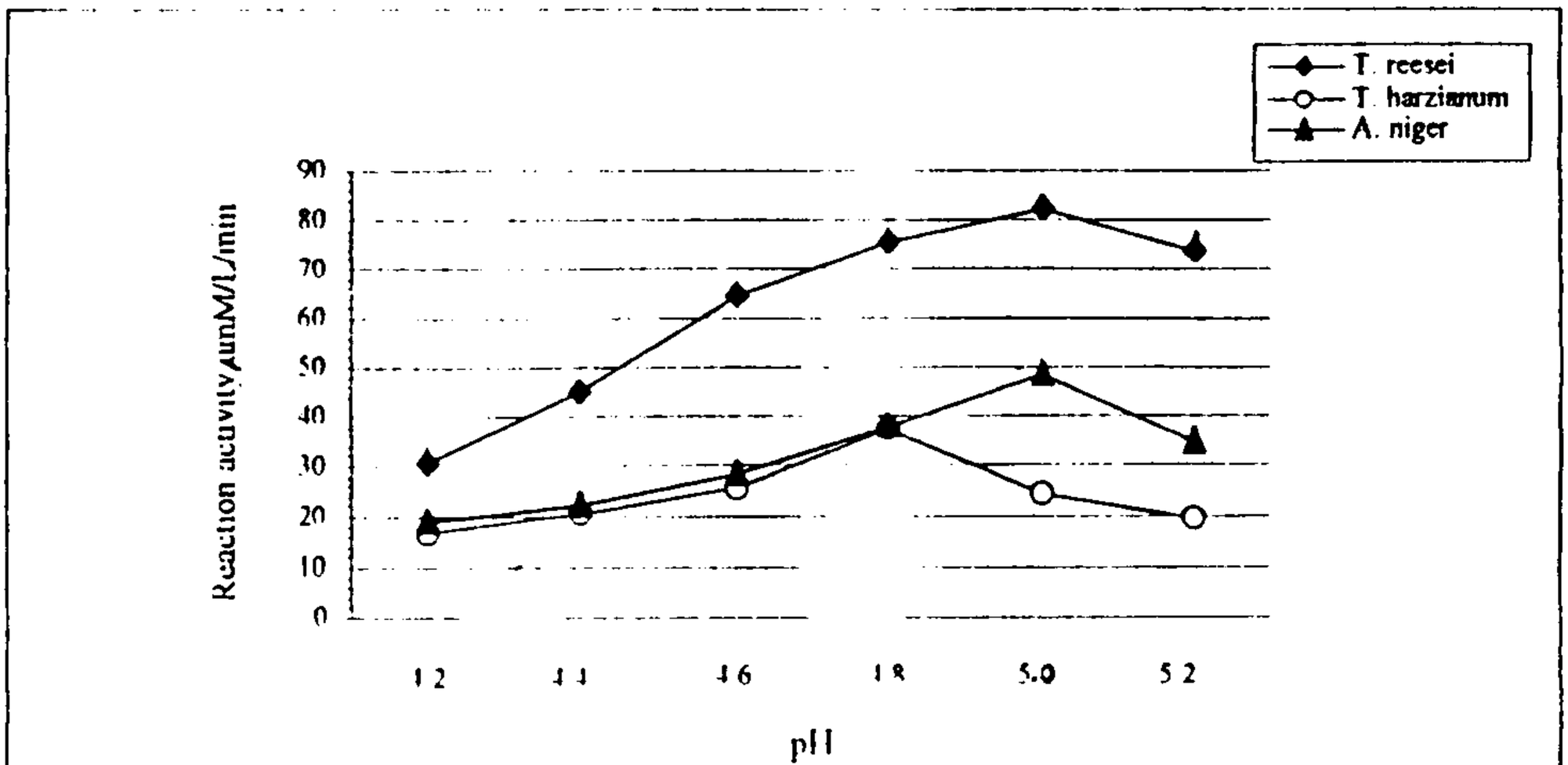

Fig (2 a) Effect of pH on the activity of immobilized enzyme on gel fertilizer

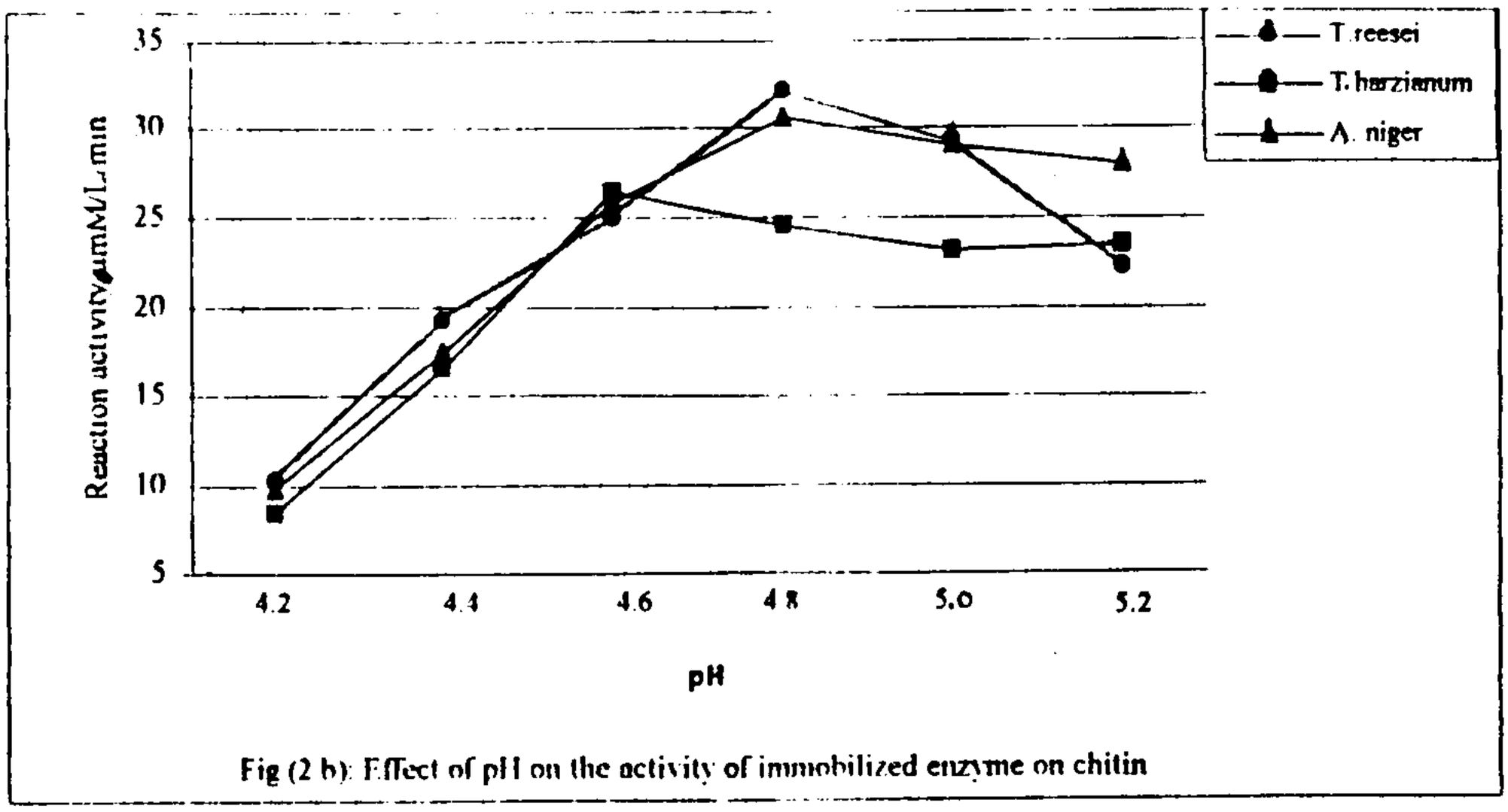

Fig (2 b): Effect of pH on the activity of immobilized enzyme on chitin

2.3. Effect of immobilized enzyme concentration on the reaction activity of cellulase enzyme:

The effect of immobilized enzyme concentration (enzyme proteins) on the reaction activity of cellulase enzyme production from different fungi was tested with different enzyme proteins between 0.378 mg enzyme proteins till 2.232 mg enzyme proteins. The obtained results shown in Fig (3) which indicated that the activity of immobilized cellulase on gel fertilizer as support with CMC as substrate were 88.4, 28.6 and 81.2 μmM/L/min with 1.87 mg/ml protein enzymes with *T. reesei*, *T. harzianum*, and *A. niger*, respectively.

On the other hand, the maximum activities of immobilized enzyme on chitin reached its maximum i.e. 44.7, 37.7 and 42.4 μmM/L/min at enzyme concentration equal 1.87 mg/ml enzyme protein with the above-mentioned fungi.

2.4. Effect of substrate concentration on the reaction velocity of immobilized cellulase enzyme with different support:

The effect of substrate concentration on the reaction velocity of immobilized cellulase enzyme production from different fungi is shown in Tables (2, 3, and 4).

The results showed that the reaction velocity to convert CMC to reducing sugars was increased up to 1.8 % and maximum reaction velocity (V_{max}) of 190.2 μmM/L/min with gel fertilizer as a support.

While, the maximum reaction velocity was 56.6 μmM/L/min at the same substrate concentration (CMC) for chitin-cellulase enzyme complex as shown in Table (2). Michaelis constant (k_m) of immobilized cellulase enzyme produced from *T. reesei* were calculated to be 0.42 and 0.22 g/100 ml of carboxymethyl cellulose as substrate (CMC) with gel fertilizer and chitin, respectively. Such behavior certainly leads to a decrease in the affinity between the substrate and the immobilized enzyme form which gave an increase in k_m value . (Saad, 1992). The decrease in reaction velocity of chitin cellulase enzyme complex may be due to its conformation when immobilized on the support. Alternatively it may be attached to the solid carrier in a way that would hinder certain parts of enzyme molecule less accessible to substrate, (Goldstein, 1976).

On the other hand, the effect of substrate concentration on the reaction velocity of immobilized cellulase enzyme produced from *T. harzianum* was determined as shown in Table (3). The results illustrated that (V_{max}) of gel-cellulase enzyme complex and chitin cellulase enzyme complex were 137.3 and 56.5 μmM/L/min, respectively. The obtained K_m values were calculated by Lineweaver and Burk plots and shown in Figs (4, a, b, c). These values are slightly higher than that obtained by Jain and Wilkins (1987).

Table (2): Effect of substrate concentration on the reaction velocity of immobilized enzyme by T. reesei.

Substrate Conc.S%	1/S	Native enzyme			Immobilized on gel fertilizer			Immobilized on chitin		
		Obtained reducing sugars µmM/L/ min.	Reaction velocity (v)	(1/v) x10^{-3}	Obtained reducing sugars µmM/L/ min.	Reaction velocity (v)	(1/v) x10^{-3}	Obtained reducing sugars µmM/L/ min.	Reaction velocity (v)	(1/v) x10^{-3}
0.2	5.00	75.20	68.60	14.60	65.50	61.40	16.30	26.30	27.30	36.60
0.4	2.50	99.30	100.10	9.90	69.80	92.80	10.80	30.20	37.00	27.00
0.6	1.67	111.30	118.20	8.50	110.70	111.80	8.90	32.70	41.90	23.90
0.8	1.25	125.40	130.00	7.70	130.40	124.70	8.02	35.40	44.90	22.30
1.0	1.00	130.50	138.20	7.20	134.80	133.90	7.50	38.00	46.90	21.31
1.2	0.83	142.20	144.30	6.90	145.50	140.80	7.10	38.90	48.40	20.70
1.4	0.71	155.10	149.00	6.70	167.20	146.30	6.80	43.60	49.50	20.20
1.6	0.63	183.20	152.70	6.50	183.60	150.70	6.60	45.00	50.40	19.80
1.8	0.56	185.20	155.80	6.40	190.20	154.20	6.50	56.60	51.00	19.60
2.0	0.50	1798.40	154.90	6.50	190.00	157.20	6.30	57.30	51.60	19.40

Table (3): Effect of substrate concentration on the reaction velocity of immobilized enzyme by T. harzianum.

Substrate Conc. S%	1/S	Native enzyme			Immobilized on gel fertilizer			Immobilized on chitin		
		Obtained reducing sugars µmM/L/ min.	Reaction velocity (v)	(1/v) x10^{-3}	Obtained reducing sugars µmM/L/ min.	Reaction velocity (v)	(1/v) x10^{-3}	Obtained reducing sugars µmM/L/ min.	Reaction velocity (v)	(1/v) x10^{-3}
0.2	5.00	72.20	68.00	14.70	70.60	72.30	13.80	25.20	28.25	35.40
0.4	2.50	77.30	89.90	11.00	77.00	94.70	10.60	26.40	37.70	26.50
0.6	1.67	82.50	100.80	9.90	85.20	105.70	9.50	35.40	42.30	23.60
0.8	1.25	94.90	107.20	9.30	92.70	112.10	8.90	38.90	45.20	22.10
1.0	1.00	97.60	111.50	8.90	100.50	116.40	8.60	40.70	47.00	21.30
1.2	0.83	112.00	114.60	8.70	115.00	119.40	8.40	45.20	48.40	20.70
1.4	0.71	120.30	116.80	8.50	127.00	112.60	8.20	48.00	49.40	20.20
1.6	0.63	122.80	118.60	8.40	129.00	123.40	8.10	50.70	50.20	19.90
1.8	0.56	132.70	120.00	8.30	137.30	124.80	8.00	56.50	50.90	19.80
2.0	0.50	131.40	121.20	8.30	137.00	125.90	7.90	56.00	51.40	19.50

Table (4): Effect of substrate concentration on the reaction velocity of immobilized enzyme by A. niger.

Substrate Conc. S%	1/S	Native enzyme			Immobilized on gel fertilizer			Immobilized on chitin		
		Obtained reducing sugars umM/L/ min.	Reaction velocity (v)	$(1/v) \times 10^{-3}$	Obtained reducing sugars umM/L/ min.	Reaction velocity (v)	$(1/v) \times 10^{-3}$	Obtained reducing sugars umM/L/ min.	Reaction velocity (v)	$(1/v) \times 10^{-3}$
0.2	5.00	69.40	69.70	14.30	55.00	57.40	17.40	64.20	63.30	15.80
0.4	2.50	78.80	92.10	10.80	77.00	79.50	12.60	75.30	86.50	11.60
0.6	1.67	85.20	103.20	9.70	78.90	91.20	11.00	80.20	98.50	10.20
0.8	1.25	92.70	109.80	9.10	84.40	98.40	10.20	86.90	105.80	9.50
1.0	1.00	97.30	114.20	8.80	89.90	103.40	9.70	90.00	110.70	9.00
1.2	0.83	102.90	117.30	8.50	96.20	107.00	9.30	95.20	114.30	8.70
1.4	0.71	112.30	120.00	8.30	105.70	109.60	9.10	115.70	116.90	8.60
1.6	0.63	122.30	121.50	8.20	118.30	111.70	9.00	125.30	119.00	8.40
1.8	0.56	135.90	122.90	8.10	129.20	113.40	8.80	136.20	120.70	8.30
2.0	0.50	144.00	124.10	8.00	128.70	115.00	8.60	134.10	122.00	8.10

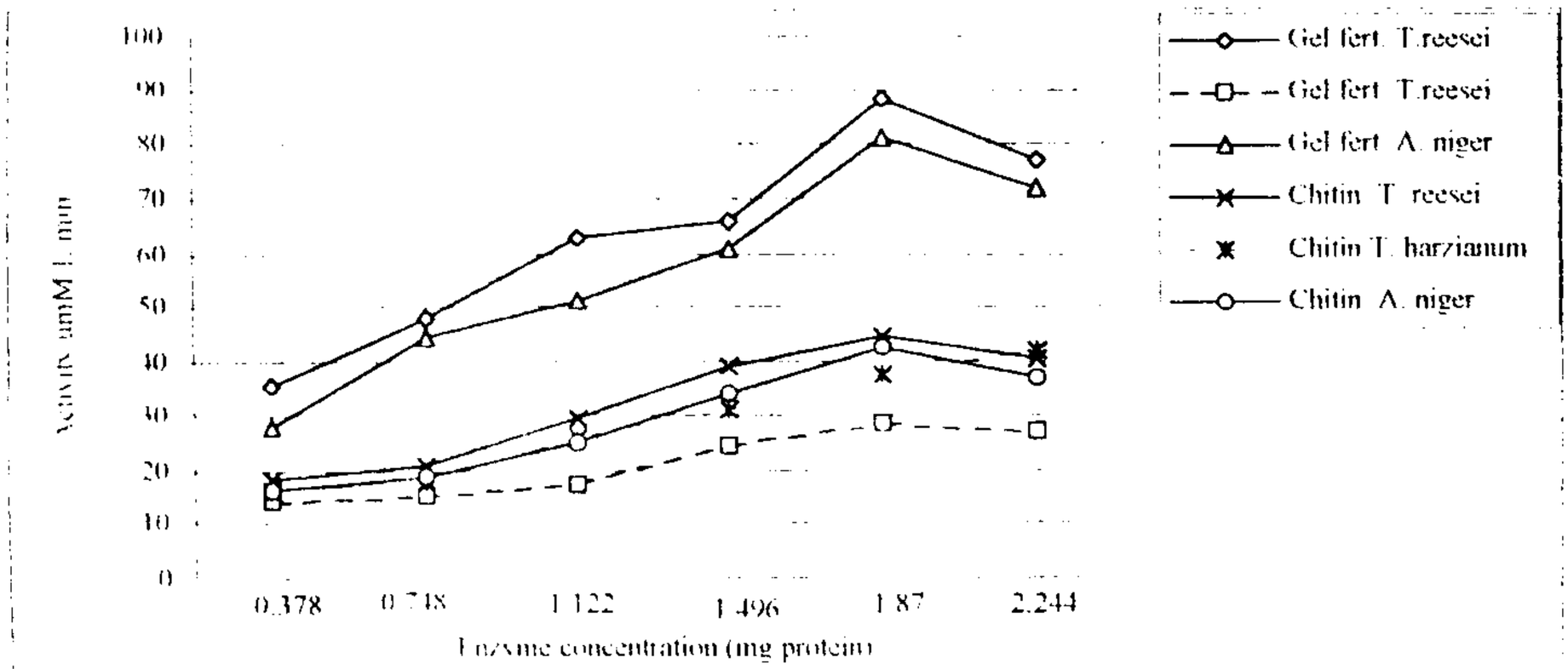

Fig (3): Effect of immobilized enzyme concentration on the activity of cellulase enzyme

Table (4) showed that, V_{max} and K_m of immobilized cellulase enzyme produced from *A. niger* were 129.2 μmM/L/min, 0.2 g/100 ml for CMC in case of gel fertilizer as a support and 136.2 μmM/L/min, 0.52 g/100 ml for chitin-cellulase enzyme complex, respectively. These results are partially in agreement with that reported by Roy, *et al.*, (1984).

3. Stability of free and immobilized cellulase enzyme:-

The stability at 5°C of free and immobilized cellulase enzyme produced from different sources was studied by using (1 %) CMC as a substrate at different periods of time (4, 8,12, 24 and 48h). The obtained results are illustrated in Table (5). From these results, on the basis of relative activity, it could be observed that free enzyme from different sources lost 4 %, 4.4 % and 4.3 % of its original activity for *T. reesei*, *T. harzianum* and *A. niger* at 5°C after 48 h, respectively. The immobilized forms on gel fertilizer were more stable if compared with free form after 48h. The loss of the relative activities reached 3.1, 4.2 % and 2.8% in gel fertilizer while, the loss of activities reached 14.9 %, 17.4 and 15.0 % with chitin-cellulase complex for *T. reesei*, *T. harzianum* and *A. niger* at 5°C after 48h. The obtained results are in agreement with that reported by Garcia, *et al.*, (1989).

4: Reuse of immobilized enzymes:

The relative activity of the immobilized enzymes after reusing at 50°C for up to 4 times with repeated washings is shown in Table (6). The immobilized cellulase enzyme on gel fertilizer from different sources of fungi (*T. reesei*, *T. harzianum*, and *A. niger*) lost 40 % of its original activity after 4 times. The noticed decrement in the

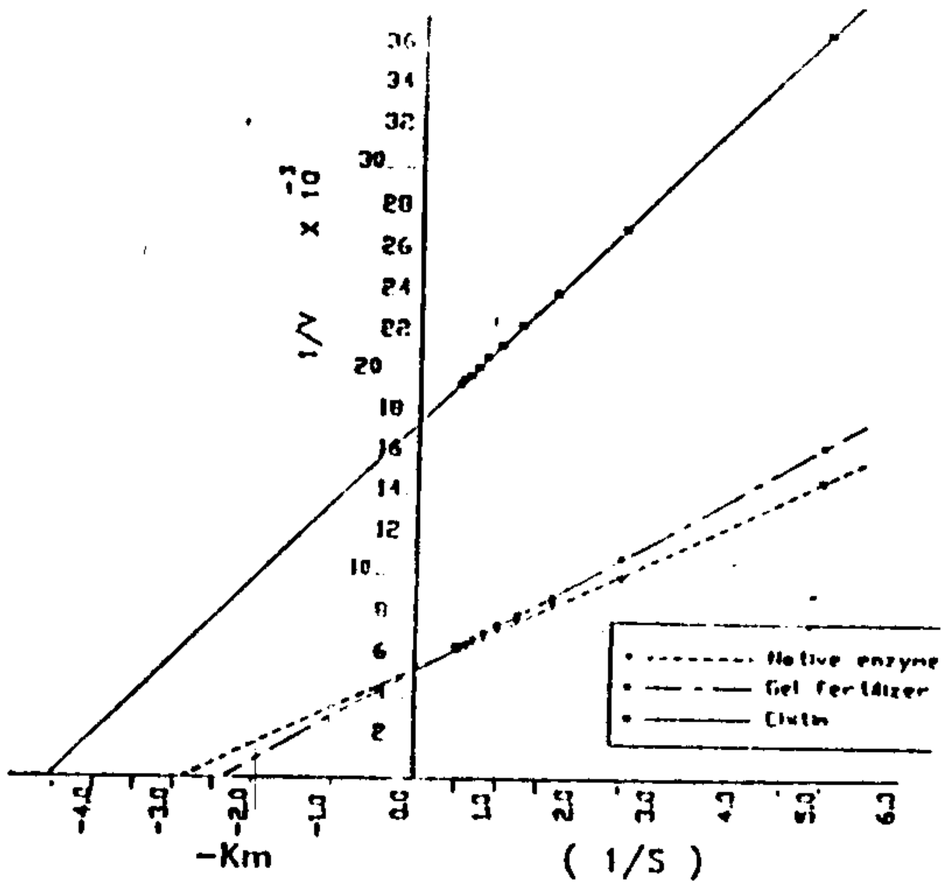

Fig 4,a) Lineweaver Burk plotts of cellulose enzymes by T. reesei

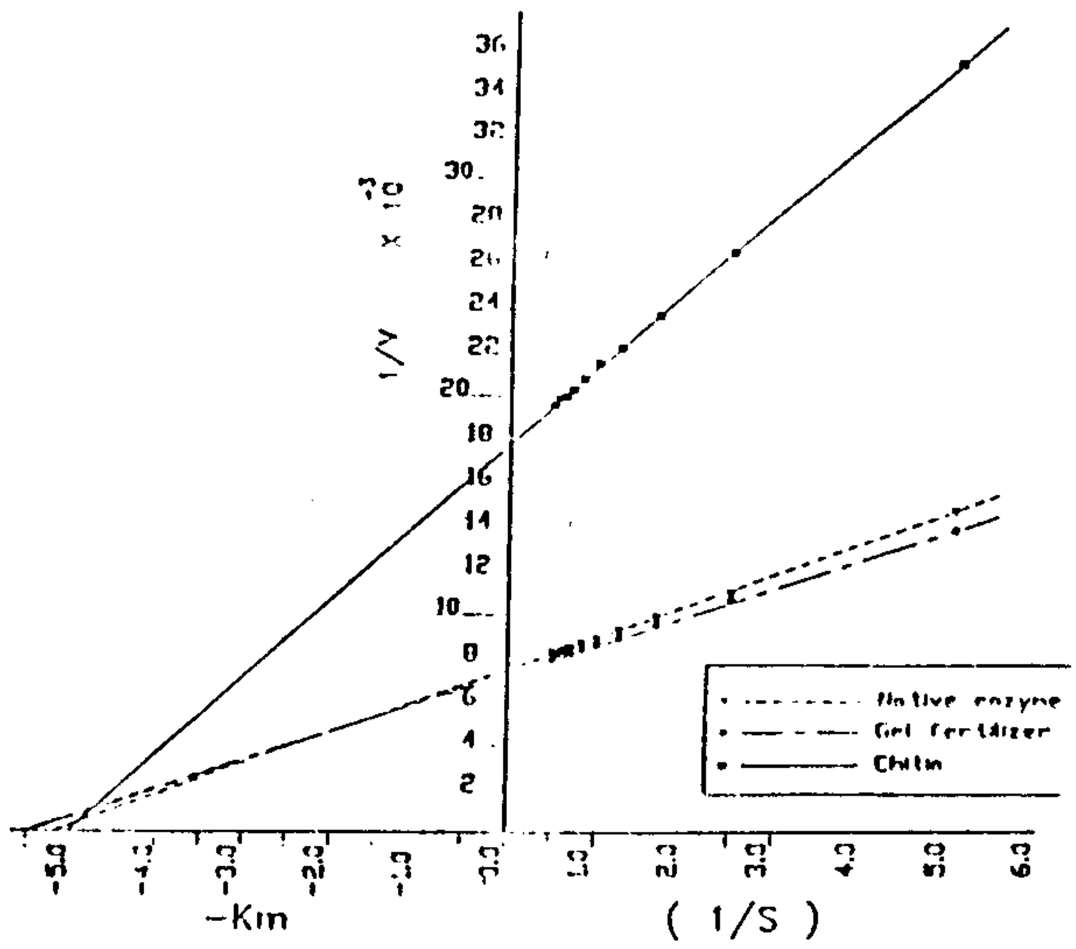

Fig 4,b) Lineweaver Burk plotts of cellulose enzymes by T. Harzianum

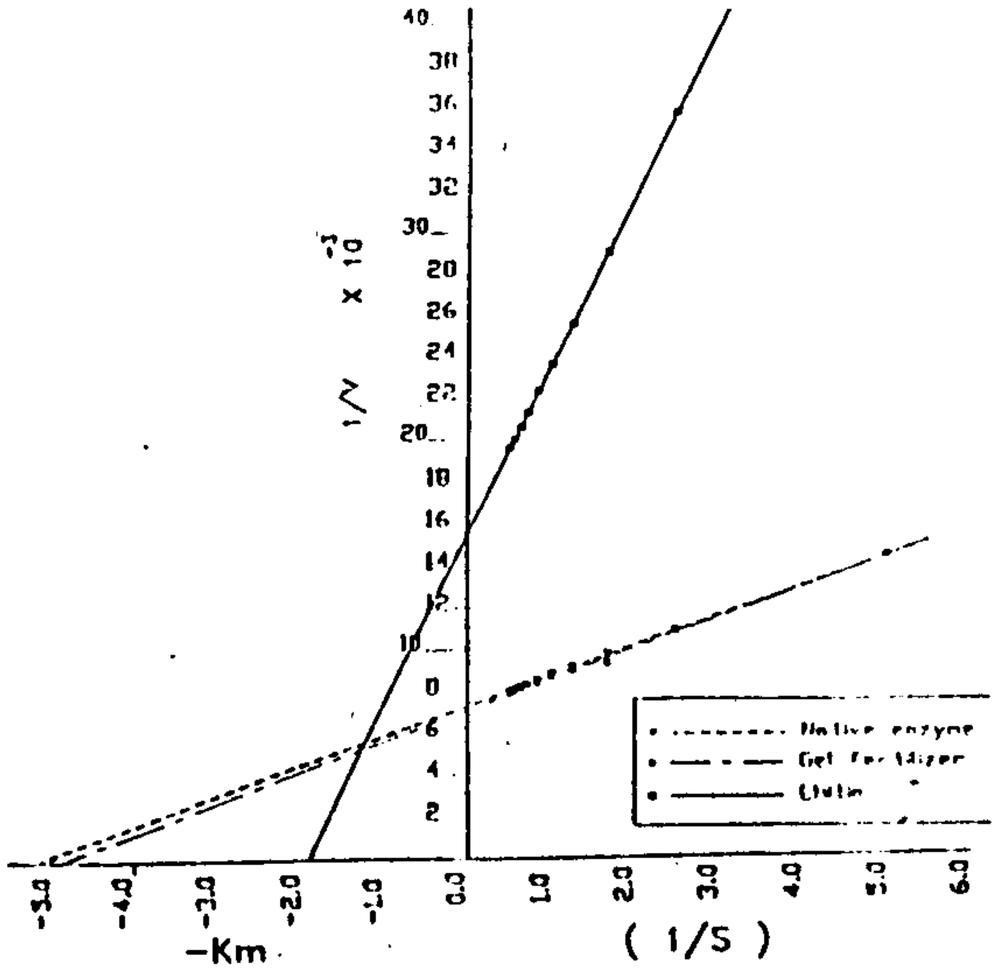

Fig 4 c) Lineweaver Burk plotts of cellulose enzymes by A. niger

Table (5): Stability of free and immobilized cellulase enzyme by gel fertilizer and chitin

Relative activity (%).									
Time of incubation (h)	T. reesei			T. harzianum			A. niger		
	Free enzyme	Immobilized on Gel f.	Immobilized on Chitin	Free enzyme	Immobilized on Gel f.	Immobilized on Chitin	Free enzyme	Immobilized on Gel f.	Immobilized on Chitin
0	100	100	100	100	100	100	100	100	100
4	97.4	97.8	87.2	96.8	96.9	84.6	97.0	98.2	86.3
8	97.0	97.6	86.0	96.6	96.7	84.0	96.8	98.0	86.0
12	96.5	97.4	86.3	96.2	96.4	83.7	96.4	97.8	85.7
24	96.5	97.0	85.7	95.9	96.0	83.0	96.0	97.5	85.3
48	96.0	96.9	85.1	95.6	95.8	82.6	95.7	97.2	85.0

relative activity of enzyme preparations with gel fertilizer might be attributed to the linkage of the covalnt bond between the enzymatic protein with gel fertilizer. While, the immobilized enzyme on chitin lost 70 % of its activity after 4 times. This decay may be result of physical loss of weakly bound enzyme from the support or a more rapid denaturation of one component of the enzyme (Garcia *et al.*, 1989).

Table (6): Reusing of immobilized enzymes.

NO. of Recycle	Relative activity %					
	Immobilized enzyme on gel fertilizer			Immobilized enzyme on chitin		
	T. reesei	*T. harzianum*	*A. niger*	*T. reesei*	*T. harzianum*	*A. niger*
0	100	100	100	100	100	100
1	93.6	91.9	94.9	85.9	82.6	83.4
2	85.1	83.4	85.0	66.0	64.7	66.4
3	71.1	71.5	70.9	48.3	47.6	48.0
4	62.3	59.2	60.3	38.5	37.0	38.5

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تحميل وتقييم إنزيم السليوليز المنتج من فطريات مختلفة

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** مركز البحوث الزراعية– قسم الميكروبيولوجى

يهدف هذا البحث إلى دراسة تحميل إنزيم السليوليز لتحويله من الصورة الذائبة إلى الصورة الغير ذائبة وكذلك دراسة الظروف المثلى والسلوك الحركى للإنزيم المحمل على دعامتين ذات طبيعة تركيبية مختلفة وهما جيل المخصب (بربرى بلانت) والكيتين وذلك لإمكانية إستخدامه فى النظام المستمر لتفادى عملية التثبيط الناتجة من إستخدام نظام الوجبه. وكذلك إستخدامه عديد من المرات حيث أن هذه النقطة ذات أهميه تطبيقيه فى تحويل المواد السليلوزية إلى سكريات أحادية (جلوكوز) والذى يدخل فى العديد من الصناعات الغذائية علاوة على إمكانية تحويله إلى كحول أثيلى وبذلك يمكن لحد ما تقليل التلوث البيئى.

أوضحت الدراسة أن قوة ربط الكتين كدعامة لإنزيم السليوليز المنتج من فطريات تراى ريزيا، تراى هيرزينيم والأسبرجلاس نيجر هى ٨٩%، ٦٠%، ٧٢% من كمية الإنزيم المضافة للدعامه على التوالى بينما كانت هذه القيم تساوى ٩٧%، ٩٥%، ٩٦% للإنزيمات سابقة الذكر والتى تم تحميلها على جيل المخصب.

وقد أوضحت الدراسة أن درجة الحرارة المثلى للإنزيم المحمل على الكتين والمنتج من فطريات*T. reesei ' T. harzianum' A. niger* هى ٥٥°م بينما الحرارة المثلى كانت ٦٠°م للإنزيم المحمل على جيل المخصب (بربرى بلانت) لفطريات *T. reesei ، A. niger* بينما كانت ٥٥°م لفطر *T. harzianum* . كما تم دراسة درجة الحموضة (pH) على درجة نشاط الإنزيم المحمل بجيل المخصب فكانت ٥ للإنزيم المنتج من فطريات *T. reesei* و*A. niger* بينما كانت ٨ر٤ لفطر *T. harzianum* .

وأظهرت دراسة السلوك الحركى لإنزيم المحمل أن معدل التفاعل إزداد مع الصور المختلفة المحمله حتى وصل إلى نقطة معينه مع زيادة تركيز المادة المتفاعلة. أظهرت النتائج أيضا أن ثابت ميكاليس للإنزيم المحمل على جيل المخصب والكتين والمنتج من فطر *T. reesei* يساوى ٤٢ر. ، ٢٢ر. جرام/١٠٠ مللى مادة متفاعلة بينما مع فطر *T. harzianum* يساوى ١٨ر. ، ٢٠ر. جرام/١٠٠ مللى مادة متفاعلة لمعقد الإنزيم مع جيل المخصب والكتين. وأيضا عند تحميل الإنزيم الناتج من فطر *A. niger* مع جيل المخصب فإن ثابت ميكالس يساوى ٢ر. بينما مع الكتين يساوى ٥٢ر. وقد يعزى هذه الإختلافات فى قيم K_m للإنزيم المنتج من الفطريات المختلفة والمحملة على دعامات مختلفة إلى حدوث بعض التغيرات فى طبيعة الأنزيم المحمل (Conformationally) بعد الإرتباط مما يؤثر على جاذبية الأنزيم للمادة المتفاعلة ومن ثم تأثيره على ثابت ميكاليس K_m.

كما أوضحت الدراسة أن إنزيم السليوليز المحمل على الدعامات المختلفة أوضح درجة ثبات عالية لمدة ٤٨ ساعة تحضين على درجة ٥٥م.

أما بالنسبة لإستخدام الأنزيم المحمل إعديد من المرات فقد أظهرت الدراسة أن الأنزيم المنتج من الفطريات سابقة الذكر والمحملة مع جيل المخصب أمكن إستخدامه ٤ مرات مع فقد ٤٠% من درجة نشاطه بينما معقد الكتين – إنزيم فقد ٧٠% من درجة النشاط بعد إستخدام ٤ مرات.