4. RESULTS AND DISCUSSION

The various procedures and reactions attempted in this investigation included the kinetics of four different types of free and immobilized enzymes on celluloses, hemicelluloses extracted and glucose syrup production form the different lignocellulosic sourceses, i.e wheat straw; corn cobs; corn stalks; and sugarcane bagasse. To facilitate the presentation of such massive amount of data in this dissertation, results are divided into several headings including the following titles:-

(1) Pretreatment of agricultural residues.

(2) The chemical composition of crude and extracted lignocellulosic compounds.

(3) Effect of different parameters on the reaction velocity and the activity of the four enzymes, i.e. cellulase complex (cellucalast 1.5 L); Hemicellulase/xylanase (viscozyme 120L, multienzyme); β-gucosidase (Novozym 188); and immobilzed glucose isomerase(sweetzyme type T) enzymes.

(4) Immobilization of β-glucosidase to enhance the saccharification of lignocellulosic materials by the follwing methods :-

(1) Adsorption of enzymes onto affinity materials, coupling on concanavali A-sepharose (Con A-sepharose, CAS) .

(2) Entrapment of enzymes in polyacrylamide gels and calcium alginate gels .

(3) Covalent coupling on cyanogen bromide-activated sepharose .

(4) Immobilization on sand .

(5) Immobilization into membrane matrix by using bovine serum albumin .

(5) Conversion of the pretreatment lignocellulosic materials from the different sourcess to glucose/fructose syrup which include:-

(a) Saccharification of lignocellulosic materails to glucose syrup using cellulase and β-glucosidase enzymes .

(b) Isomerization of saccharified syrup to high-fructose syrup by immobilized glucose isomerase (sweetzyme type T) .

4.1. Pretreatment of agricultural residues:-

The structural ploysaccharides are relatively difficult to hydrolyze to their sugar monomers because of their association with lignin and the crystalline nature of cellulose (Fox, et. al., 1983, 1987).

An ideal pretreatment would accomplished reduction of lignin, with a minimal loss of hemicellulose, concommitant with a reduction of crystallinity and an increase of surface area of cellulose to enzymatic hydrolysis. Therefore a pretreatment of lignocellulosic materials is required to obtain high sugar yields.

4.1.1. Alkali pretreatment of agricultural residues:-

Sodium hydroxide (NaOH) has been most commonly used to increase the susceptibility of lignocellulosic crop residues. The NaOH has double role, its removes the lignin thus increasing the accessibility to the cellulose and also, swells the fiber to make it more permeable to cellulase.

In this study three parameters were examined NaOH concentration, temperature and pretreatment time.

4.1.1.1. Alkali concentration:

The effect of NaOH concentration (2.0-14.0%) on the separation of cellulose, hemicellulose and pentosan from different raw lignocellulosic residues at 80 °C and time 3h are shown in Table (1). Results showed that the amount of the residue was decreased gradually by increasing of NaOH concentration except at 14% NaOH which may be due repolymerization of the products (Chen and Anderson, 1980). On the other hand the level of hemicellulose extraction was increased proportionately with increasing NaOH concentration up to 10% then the rate of increase slowed down. This observation may be due to the loss of significant amount of hemicellulose in the wash stage under high NaOH concentration usage (Fox, et.al, 1989). The amounts of pentosan were sligthy affected under different NaOH pretreatments.

4.1.1.2. Temperature treatments:-

Table (1) showed the results of lignocellulosic pretreatment with 10% NaOH for 3h at different temperatures (40°C - 120°C). The amount of the residue increased gradually by increasing the pretreatment temperature from 40°C to 60°C then began to drop by increasing the temperature. Although the amounts of hemicellulose increased by increasing the pretreatment temperature from 40°C to 120°C. It is note worthy that under moderate condition of temperature the carbohydrate portion of lignocellulose is rendered more biodegradable through various reactions promoting fiber swelling with some solubilization of hemicellulose. At elevated temperature (more than 80°C) more substantial solubilization of constituents results, but this is not necessarily desirable: soluble aromatic specis derived from lignin may caus toxicity in later bioconversion processes. Although undesirable fragmentation reaction at elevated temperatures convert readily biodegradable carbohydrate materials into less degradable species or /and loss the 0-methyl group from the 4-0-methy/ D-glucouronic acid residues of hemicellulose. On the other hand the pentosan compounds were slightly affected by rising the pretreatment temperature from 40 to 120°C (Fox et al.,1989).

4.1.1.3. Time treatment:-

The results for the effect of pretreatment time using NaOH 10% at temperature 80°C are presented in Table (1). The results showed that the pretreatment 3h is sufficient for the isolation of hemicellulose and lignin fractions from the lignocellulosic residues.

The previous results depiced that pretreatment with 10%. NaOH at 80° for 3h was most efficient for the pretreatment of all the lignocllulosic substrates under investigation. These results are slightly differ from those obtained by other workers (Chen and Anderson 1980, Fox, et al., 1984 and 1989).

Table(1): Effect of NaOH concentration, temperature and time on the extraction of cellulose from different soures.

Source	Wheat straw			Corn cobs			Corn stalks			Sugarcane bagasse		
Treatment	Residue %	Hemicell- %	Pentosan %	Residue %	Hemicell- %	Pentosan %	Residue %	Hemicell %	Pentosan %	Residue %	Hemicell %	Pentosan %
A) NaOH %												
2	69.1	5.9	1.8	64.2	24.1	1.0	86.8	17.5	1.6	82.3	11.1	1.6
4	65.4	14.4	1.4	58.2	33.1	1.5	85.6	13.2	2.1	76.3	17.5	1.0
6	60.3	36.2	1.8	57.7	34.5	1.5	79.2	22.8	1.5	73.4	17.4	1.4
8	59.3	28.6	2.0	54.7	38.0	1.4	72.4	27.5	1.2	68.4	22.1	2.6
10	52.8	36.6	1.9	49.9	45.3	1.4	69.4	29.5	1.5	63.1	33.4	1.8
12	49.6	21.8	1.7	33.9	38.8	1.9	63.2	24.9	1.2	53.5	24.6	2.1
14	86.8	10.0	1.8	82.9	12.6	1.2	55.5	22.6	2.0	49.2	19.5	1.6
B)Temperature ^{o}C												
40	78.5	13.3	1.4	72.9	14.5	1.8	80.2	10.6	1.2	84.6	11.1	1.4
60	88.4	9.3	1.7	80.7	13.2	1.8	89.5	4.1	1.8	87.9	8.4	1.3
80	80.6	12.7	1.9	76.9	18.1	1.4	82.8	14.4	1.5	84.0	14.9	1.9
100	65.4	14.4	1.5	58.2	23.1	2.3	85.6	13.2	1.4	76.3	7.5	2.3
120	35.7	19.7	1.6	34.1	36.5	2.5	37.0	13.8	1.9	27.5	11.2	1.8
Time hr												
3	65.4	14.4	1.9	58.2	23.1	1.4	85.6	13.2	1.5	76.3	7.5	1.9
6	73.2	3.9	2.1	75.2	10.6	2.0	87.4	5.9	1.9	85.6	6.8	2.3
12	87.2	4.7	1.8	85.2	11.6	2.0	88.2	6.7	1.8	87.3	3.7	1.9

4.2. The chemical composition of crude and extracted lignocellulosic compounds:-

The preliminary work was carried on the raw and pretreated agricultural residues to evalute the quality of the extracted cellulose. The chemical composition of the lignocellulosic materials (wheat straw, corn cobs, corn stalks, and sugarcane bagasse) are shown in Table (2). The results showed that cellulose, hemicellulose and lignin are the major components while ash and protein occure in lesser amounts in crude and extracts. The pretreatment of the agricultural residues under investigation results in a partial solubilization of the hemicellulose and lignin, therefore their amounts were decreased in the pretreated extract. The results in Table (2) showed that crude sugarcane bagasse contained the highest amount of extracted cellulose (38.5%) while crude corn cobs contained the lowest amount (33.1%). On the other hand, pretreatment of the agricultural residues with 10% NaOH for 3h at 80°C increased the amount of the extracted cellulose between 34.5 and 45.5% for corn stalks and corn cobs, respectively. Although the higher amount of lignin removal was observed in corn cobs (18.3%) than other agricultural residues. Therefore, NaOH pretreatment removes the lignin, thus increasing the accessibility to the cellulose, hence the amount of lignin removal is proportional with cellulose amount in the extract. Lee and Fan (1983) noticed the same observation. Hemicellulose content were reduced by 12.9% and 18.8% after alkali pretreatment for corn stalks and wheat straw respectively. This may be due to the solubility and the hemicellulose and its removal during the wash stage.

Also, NaOH pretreatment extracted some protein compounds and defatted the agricultural residues, therefore the protein content decreased and lipids disappeared in the pretreatment extracts. The obtained results are slightly varied from those obtained byseveral investigators (Chen and Anderson ,1980, Fox, et al., 1987 and 1989) and Tewori, et. al., 1988). This may be due to the enviromental conditions and the difference in cultivar varieties.

4.3. Effect of different parameters on the activity of enzymes:-

One of the major goal of this work is to study the influence of various parameters sush as pH, temperature, enzyme concentration, substrate concentration and salts on the crucial property of each enzyme.

The results of this study are presented in the following pages which made it possible to estimate the optimum conditions for the use of each enzyme in its speific process. Also, such systematic study might help to a great deal in minimizing the enzyme cost in each individual process.

4.3.1. Effect of pH on the enzymes activity:-

The pH will affect the efficiency of an enzyme and usually, there will be an optimum pH value at which activity is at maximum. The optimum pH of each enzyme depends on a number of factors, such as the nature of the buffer, substrate concentration, nature of substrate, presence of activators or inhibitors and etc. To carry out these

Table(2): Chemical composition of crude and pretreated ligno cellulosic materials.

Source	Wheat straw			Corn cobs			Corn stalks			Sugarcane bagasse		
Compononts	crude %	Pretreated extract %	Action of pretr. %	crude %	Pretreated extract %	Action of pretr. %	crude %	Pretreated extract %	Action of pretr. %	crude %	Pretreated extract %	Action of pretr. %
Cellulose	35.9	74.6	+38.7	33.1	78.6	+45.5	36.5	71.0	+34.5	38.5	75.5	+37.0
Hemicellulose	32.9	14.1	-18.8	25.8	10.9	-14.9	27.2	14.3	-12.9	31.5	14.3	-17.2
Lignin	16.6	2.9	-13.7	22.8	4.5	-18.3	20.2	5.6	-2.3	16.9	4.5	-12.4
Ash	7.8	3.4	-4.4	6.0	1.0	-5.0	7.0	4.7	-14.6	4.1	1.9	-2.2
Protein	3.8	1.9	-1.9	5.3	3.2	-2.1	4.2	3.1	-1.1	4.6	2.4	-2.2
Lipids	1.3	---	-1.3	1.4	---	-1.4	1.9	---	-1.9	3.8	---	-3.8
Extractives	1.7	3.1	+1.4	5.6	1.8	-3.8	3.0	1.3	-1.7	1.0	1.4	+0.4

* The values were calculated on the dry weight basis.

experiments, pH values were the only changed parameter while other parameter, e.g. substrate concentration, enzyme concentration and temperature were set constant mostly at optimum.

The reaction activity of the four enzymes under investigation were measured at various pH values. The enzyme activity was plotted aganist the pH and the four characteristic curves were obtained.

The effect of pH on the reaction velocity of cellulase (celluclast 1.5 L) was tested at different pH values and different substrate sources (CMC, wheat straw, corn cobs, corn stalks, and bagasse treatments). Eight solution of these substrate were adjusted at pH values of 3.3- 3.6- 3.9- 4.2- 4.5- 4.8- 5.1 and 5.4.

The obtained results are indicated in Table (3) and Fig. (6a, 6b). The enzyme showed its maximum activities i.e. 3.53; 2.89; 3.01, 2.78, and 3.68 mM/L/min at pH (4.8) with substrate concentration 8.3 g/L of CMC, wheat straw, corn cobs, corn stalks corn stalks and bagasse. while, with substrate concentration 14.3 g/L, the maximum activities were 4.03; 2.93; 2.93; 3.36; 3.40 and 2.71 mM/L/min at the same pH value for the above mentioned substrates. The obtained data confirms the optimum pH for cellulase enzyme celluclast 1.5 L) equals 4.8. This value of pH was in agreement with Lee, et.al., (1982), Caminal, et.al., (1985), Tewori, et.al., (1987) and Novo (1989).

It is worthy to mention that low value of optimum pH (4.8) clearly indicate the importance of such acid media to fit the nature of the catalytic activity of the groups in the active site of the enzyme (Wiseman, 1985). Also, Underkofler et al., (1965) stated that the formation of glucose and disacchorids such as cellobiose at lower pH reduces colour formation in the resulted syrup and aids in preventing microbial contamination during saccharification process .

The effect of p^H on the reaction activity of β-glucosidase was tested at different p^H values. Eleven solution of cellobiose (10mM) were adjusted by pH meter to pH values of 3.6- 3.9- 4.2- 4.5- 4.8- 5.1- 5.4- 5.7 and 6.0 using 50 mM of solution acetate buffer and 50 mM cittrate buffer.

Table (4) and Fig. (7) showed that the maximum activity of B-glucosidase (Novozyme 188) . At pH 4.8 the enzyme exhibit activity equal 51.1 μmol glucose/min/0.1 ml diluted enzyme. But at pH 4.5 the enzyme yield mixmum activity of 55.8 μmol glucose/min/0.1ml enzyme.

Presumably the p^H-rate profile of β-glucosidase in acetate buffer reflects an inhibition of enzyme activity at p^H 4.8 that is related to the concentration of the basic carboxylate anion in the citrate molecule at this p^H. The obtained data are in agreements with Woodword and Wohlpart (1982), Dekker (1986) and Novo (1986). This indicated that the β-glucosidse exhibited imporoved stability in acidic and neutral p^H ranges. Also, the p^H stability of β-glucosidase (Novozyme 188) is stable between p^H 3.6-6.0 in 0.05M acetate buffer.

Table (3): Effect of pH on the reaction Activity of cellulase (celluclast 1.5 L) enzye

pH	Activity mM/ min									
	CMC		Wheat straw		Corn cobs		Corn stalks		Sugarcane bagasse	
	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L
3.3	1.79	2.01	0.13	0.41	0.07	0.04	0.10	0.37	0.44	0.88
3.6	2.03	2.71	0.79	1.02	0.10	0.14	0.20	0.86	1.00	1.78
3.9	2.43	2.64	0.90	1.25	0.60	0.21	0.44	1.32	1.25	2.08
4.2	2.55	3.00	1.37	1.32	1.06	1.11	6.64	1.74	1.69	2.27
4.5	2.66	3.19	1.50	2.36	1.90	2.13	1.67	1.94	2.08	2.59
4.8	3.53	4.03	2.89	2.93	3.01	3.36	2.78	3.40	3.68	2.71
5.1	2.55	3.26	1.97	2.59	2.22	2.33	1.11	2.06	1.71	2.27
5.4	2.43	2.85	1.56	1.76	1.97	1.20	1.02	1.62	1.39	1.62

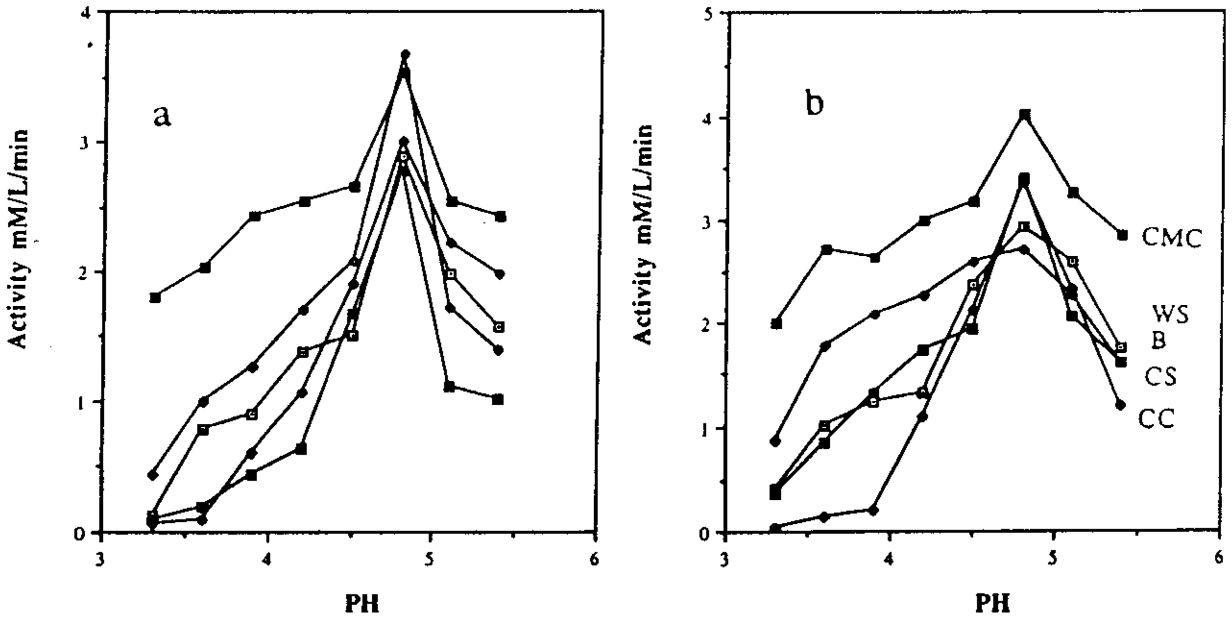
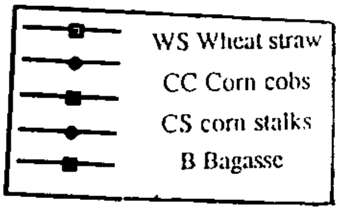


Fig. (6): Effect of pH on the activity of cellulase enzymes.



The effect of p^H on the reaction activity of xylanase/ hemicellulase enzyme complex is shown in Table (5) and Fig (8) . Nine solutions of xylan 1% were adjusted to different pH values (0.05M acetate buffer) stated from p^H 3.6 till 6.0 . The enzyme showed its maximum activity at p^H between 5.0 and 5.4, and the activity amounted to 5.1 μmol xylose/min/0.1 ml enzyme. The value of p^H 5.1 was emphasized by several workers, Dekker (1983), Fournier, et.al., (1985), and Deshpande, et, al., (1986).

The effect of p^H on the reaction activity of immobilized glucose isomerase (sweetzyme type T) is illustrated in Table (6) and shown in Fig (9). Twelve solutions of glucose syrup in 0.05 M Tris-buffer were to p^H values started p^H 6.0 and ended with p^H 9.0. The optimum p^H was 7.5 and the reaction activity reached to 42.44 μmol fructose/min/100 mg immobilized glucose. The value of p^H 7.5 is in agreement with Novo (1992). Mamta and Prabhu (1980) and Foda (1987), they emphasized that the optimum p^H of this enzyme changed between from 7.3 and 8.4, respectively

4.3.2. Effect of Temperature on the activity:-

The effect of temperature on the enzyme activity are very complex and interrelated with other variables such as pH, buffer system and substrate concentration. There are two forces acting simultaneously but in opposite directions. With increasing temperature the activity increase but at a time inactivation is also accelerated, this is due to denaturation of the enzyme protein by heat.

Table (4): Effect of pH on the reaction activity of β-glucosidase (Novozym 188) enzyme.

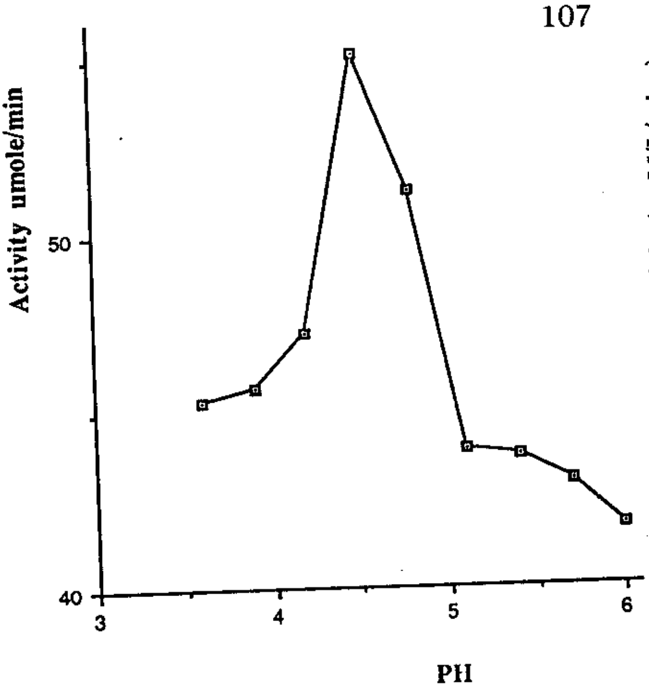
pH	Glucose content mM/L	activity μmol glucose /min
3.6	161.0	44.5
3.9	164.5	45.7
4.2	170.4	47.2
4.5	215.3	55.8
4.8	198.4	51.1
5.1	184.3	50.2
5.4	157.9	43.9
5.7	154.8	43.0
6.0	150.0	41.7

Table(5): Effect of pH on the reaction activity of xylanase/ Hemicllulase enzyme (viscozyme 12oL).

pH	Xylose content mM/L	Activity mM/L/min
3.6	90.0	3.0
3.9	96.7	3.2
4.2	101.7	3.4
4.5	115.7	3.9
4.8	143.3	4.8
5.1	150.0	5.0
5.4	153.0	5.1
5.7	125.0	4.2
6.0	105.0	3.5

Table(6): Effect of pH on the activity of immobilized Glucose isomerase (Sweetzyne type T) enzyme.

pH	Activity μ mol fructose/min/100 g Enzyme
6.0	19.63
6.5	22.96
7.0	24.07
7.1	26.50
7.3	39.81
7.5	42.44
7.8	38.89
8.0	37.04
8.2	34.26
8.4	31.48
8.6	22.22
9.0	19.26



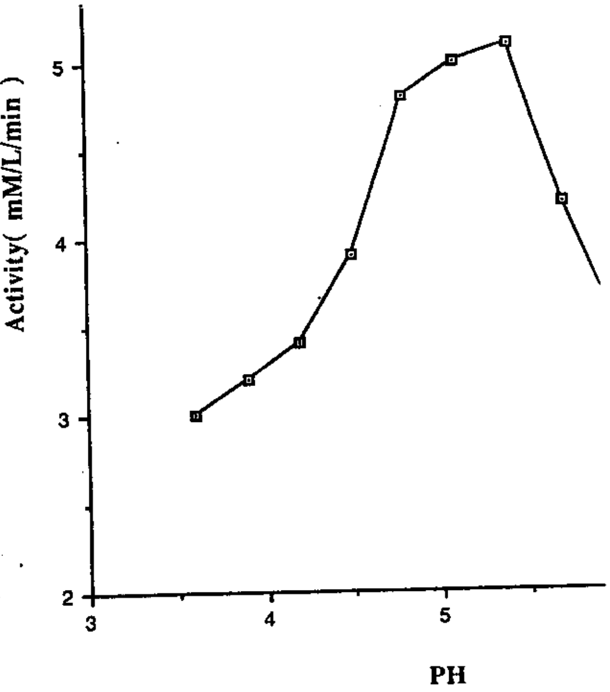


Fig. (7): Effect of pH on the activity of β-glucosidase

Fig. (8): Effect of pH on the activity of hemicellulas

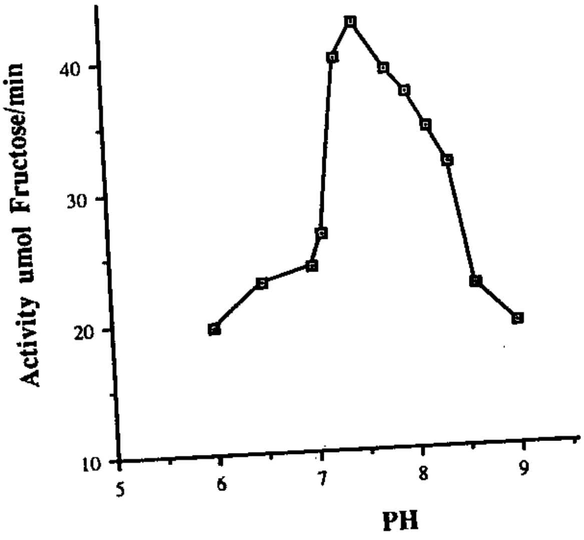


Fig. (9): Effect of pH on the activity of immobilized glucose isomerase enzyme

The higher of temperature, the more will inactivation dominate (Mattenheimer,1976).

The optimum region of temperature of any enzyme occured when the two forementioned factors of increase initial rate and decreased active life of the enzyme are balanced to produce the most producet in a resonable time. It is not easily to determine an exact value for the optimum tempreature because it is a some what vague concept, and will depend on the lenght of time over which the measurments are made. Most enzymes often have optimum temperature in the range 45-53°C while the enzyme from the bacteria that live in volcanic hot springs may have optima at 80 °C (Yudkin and Offord, 1980).

Eight different temperatures, i.e, 35- 40- 45- 50- 55- 60- 65 and 70 °C were chosen to investigate the optimum of cellulase (celluclast 1.5 L). The experiments were carried out at p^H 4.8 for CMC and pretreatments lignocellulosic, wheat straw; corn stalks, and sugarcane bagasse with two concentrations of substrate (8.3 g/L and 24.3 g/L) and reaction period of each experiments was 1 h. Tables (7) and Fig (10a, 10b) show that the effect of temperature on the activity of cellulase. The obtained results indicated that the optimum temperature was 50 °C for both CMC, and pretreatments lignocellulosic materials. The maximum activity showed values of 4.20; 2.89; 3.01; 2.78; and mM/1/min with 8.3 g/L as substrate, respectively. With 14.3 g/L of substrate the enzyme showed a maximum activity of 3.82; 3.13; 3.36; 3.40; and 2.71 mM/L/min, respectively. Such values of

Table(7): Effect of temperature on the reaction activity of cellulase (celluclast 1.5 L)

Temperature	Activity mM/L min									
	CMC		Wheat straw		Corn cobs		Corn stalks		Sugarcane bagasse	
°C	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L
35	0.69	0.95	1.11	1.18	1.04	1.18	1.04	1.18	1.04	1.18
40	1.22	1.33	2.22	1.62	2.00	1.76	2.34	2.36	2.69	2.18
45	1.97	2.31	2.36	2.43	2.08	2.50	2.71	2.82	2.85	2.43
50	4.2	3.82	2.89	3.13	2.01	3.36	2.78	3.40	3.68	2.71
55	1.93	2.09	2.13	2.41	2.18	2.18	2.73	2.55	2.78	2.31
60	1.75	1.12	1.34	1.81	1.44	1.62	2.22	2.08	2.08	2.13
65	1.60	0.68	1.30	1.71	1.16	1.20	1.30	1.57	1.25	1.16
70	1.57	0.58	1.11	1.46	0.58	1.00	1.09	0.97	1.04	1.02

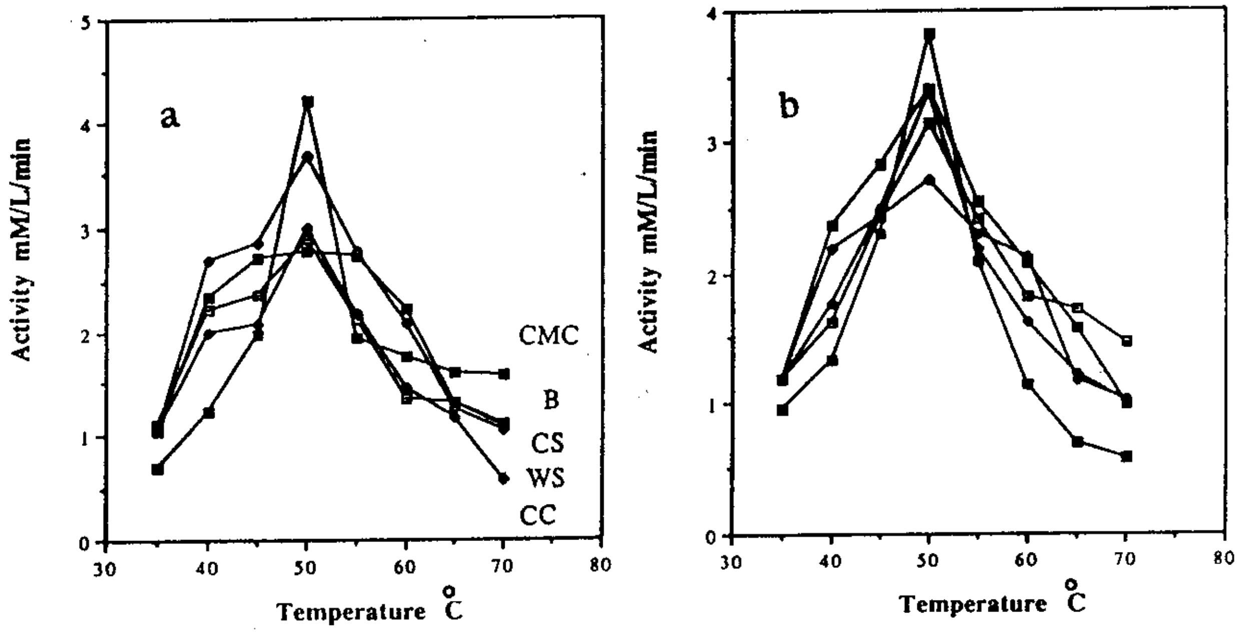


Fig. (10): Effect of temperature on the activity of cellulase enzymes.

WS Wheat straw
CC Corn cobs
CS corn stalks
B Bagasse

optimum temperature for cellulcast 1.5 L are in agreement with several works in the conversion of cellulose to glucose by cellulase enzyme (Vallander and Eiksson,1985 & 1991 and Tjerneld, et. al., 1985).

The effect of temperature on the reaction activity of β-glucosidase was tested at different temperature starated from 30°C and ended with 70°C, results are tabulated in Table (8) and Fig.(11) . The obtained results showed that at first increase in temperature was accompanied with a rapid increase in the reaction activity of the enzyme till reaching its optimum temperature, then began gradually decrease slowly. The optimum temperature was 50°C with reaction activity of 55.83 μmol glucose/min/0.1ml enzyme. The optimum temperature for β-glucosidase enzyme is in agreement with those reported by Woodward and Wohlpart (1982), Grous, et. al., (1985) Woodward (1985), Breuil, et. al., (1986), and Dekker (1986).

The effect of temperature on the reaction activity of xylanase/hemicellulase (viscozyme 120 L) are shown in Table (9) and Fig. (12). The experments were carried out at different temperatures between 40 and 70 °C, pH 4.8 (50 mM acetate buffer). enzyme concentration (20% (v/v), diluted 1:5, 0.1 ml used) and xylan concentration 1% (w/v; 0.8 ml used) for 30 min reaction time. The optimum temperature for this enzyme under the above conditions is 50°C with reaction activity is 4.3 mM/L/min/0.1 ml diluted enzyme. This value of optimum temperature were empherature were

Table(8): Effect of temperature on the activity of β-glucosidase (Novozym/88) enzyme.

Temperature °C	Glucose content mM/L	Activity μmol glucose/min
30	151.85	41.97
35	163.85	42.60
40	175.72	48.81
45	180.00	50.0
50	213.22	55.83
55	168.52	46.81
60	141.84	39.40
65	72.43	20.12
70	58.21	16.17

Table (9): Effect of temperature on the reaction activity of Hemicellulase (viscozyme 120L).

Temperature °C	Xylose content mM/L	Activity mM/L/nin
40	61.7	2.1
45	91.7	3.1
50	128.3	4.3
55	116.7	3.9
60	90.0	3.0
65	65.8	2.2
70	62.5	2.1

emphasized by several workers before (Dekker, 1983 and Deshpande, et.al.,1986).

Table (10) and Fig (13) showed the effect of temperature on the activity of immobilized isomerase (sweetzyme type T). Resultes showed the optimum temperature was 60°C with glucose syrup 6.0 mM and p^H 7.5 (0.05 M Tris-buffer) for 60 min. The maximum activity at the optimum temperature for this enzyme was 35.56 μmol fructose/ min/ 100mg immobilized enzyme. These resultes are in was agreements with Foda (1987) and Novo (1992) .

4.3.3. Effect of enzyme concentration on the activity of the enzymes:-

The effect of enzyme concentration on the reaction activity of cellulase enzyme (celluclast 1.5L) was tested with different concentrations between 50 μ L/100ml and ended 400 uL/100mol enzyme (acetate buffer, pH 4.8, 50 mM). The obtained results are shown in Tables (11) and Figs. (14a,14b).

These results indicate that the activity of cellulase enzyme reached its maximum i.e. 4.31 mM/L/min and 4.44 mM/L/min with the two different concentration (8.3 and 14.3 g/L) of CMC at enzyme concentration equal. to 200 μL/100 ml buffer. While the maximum activities of the treated lignocellulosic substrates (8.3 g/L) i.e wheat straw, corn cobs, corn stalks and sugarcane bagasse were 3.61, 2.92, 3.01 and 3.01mM/L/min at concentration 350, 300 and 250 μL/100ml buffer of cellulase enzyme. On the other hand, the maximum activities

Table (10): Effect of temperature on the activity of immobilized isomerase (Sweetzyme type T) enzyme.

Temperature's °C	Activity μmol fructose/ min /100 g Enzyme	
	30 min	60 min
40	8.89	9.87
45	12.69	16.49
50	15.84	23.70
55	18.71	30.19
60	35.56	32.41
65	34.06	27.41
70	30.67	20.74
75	23.71	16.67
80	14.34	10.36

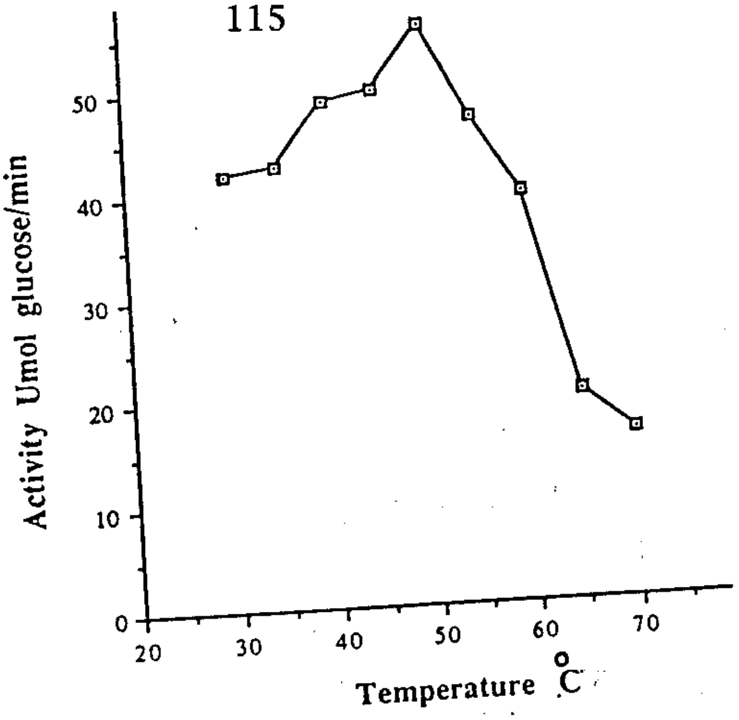


Fig. (11): Effect of temperature on the activity of β-glucosidase enzyme.

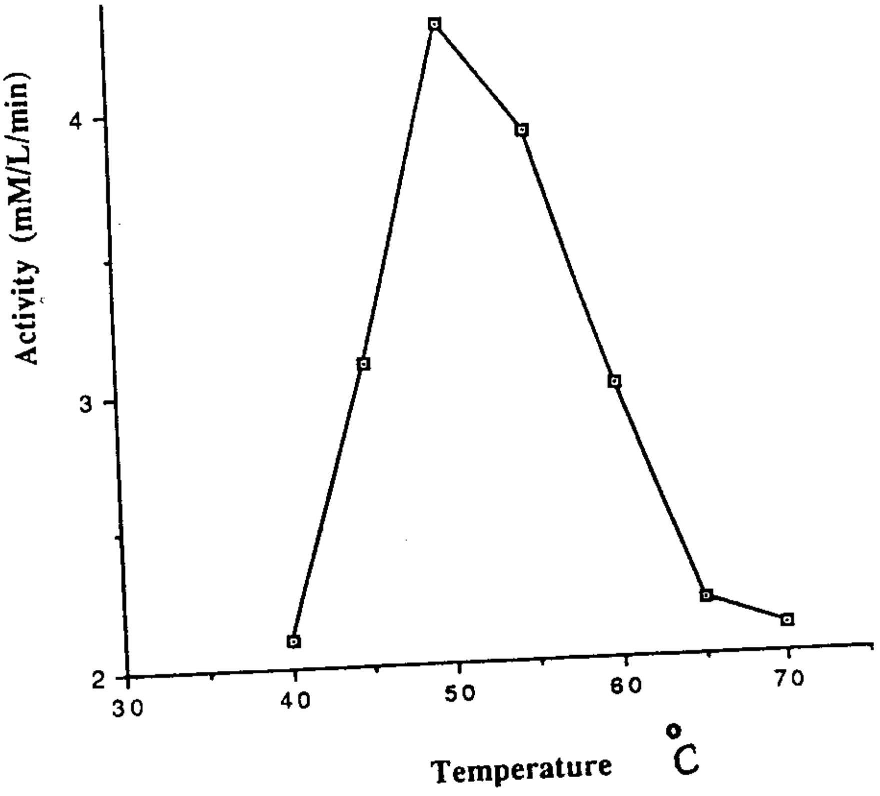


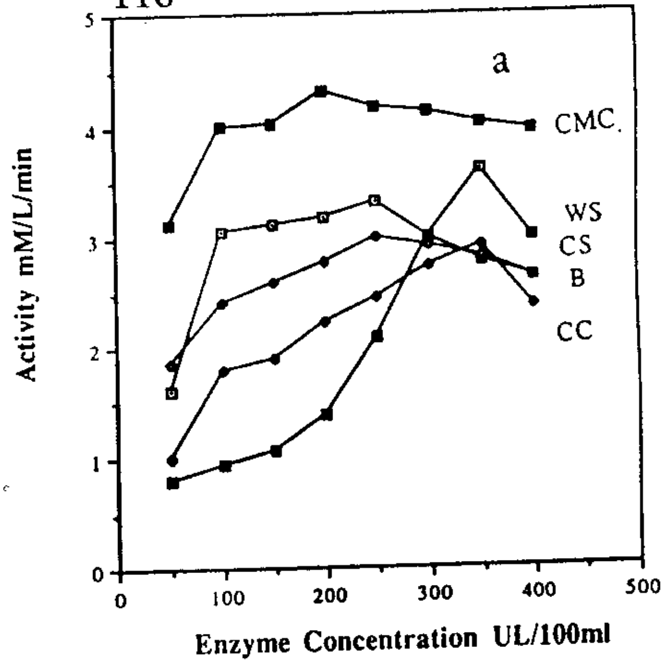
Fig. (12): Effect of temperature on the activity of hemicellulase enzymes.

with the other concentration (14.3 g/L) with the above mentioned substrates were 2.45, 4.60, 3.70 and 3.24 mM/L/min at enzyme concentration of 250, 200, 200, 350 μL/100ml buffer. From the above results, it could be concluded that, the maximum activities of cellulase enzyme with different ligndcellulosic materials were obtained by using enzyme concentration ranged between 200-350 μL/100ml buffer. Also, the obtained data indicate that increasing enzyme concentration beyond these values lead to a decrease in the overall reaction activity. This observation may be attributed to the inhibition effect of the product (glucose and cellobiose) wich proceed in opposite direction leading to a decrease in the final product (Yudkin and Offord 1980). Such expanation was introduced by Tewari, et. al., (1988); Vallander and Eriksson (1991), where they observed that the inhibition of cellulase enzyme was proportional to the concentration of the products glucose and cellobiose.

The effect of enzyme concentrations on the reaction activity oc cellobiase β-glucosidase) enzyme are shown in Table (12) and Fig.(15). Seven proceeded enzyme concentrations started with 25 to 200 uL/ml. With increasing of enzyme concentration the activity was increased and reached its maximum i.e. 83.0/μmol glucose/min at enzyme concentration of 200 u L/ml. However, under such high concentration of enzyme, the reaction rate is dependendent on substrate concentration. Such results are in agreement with that obtained by Dekker (1986).

Table (11): Effect of enzyme concentration on the reaction activity of cellulase (celluclast 1.5 L) enzyme

Enzyme concentration	Reaction Activity mM/L/ min									
	CMC		Wheat straw		Corn cobs		Corn stalks		Sugarcane bagasse	
µ/100 ml	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L	8.3 g/L	14.3 g/L
50	3.13	3.61	1.62	1.80	1.00	0.60	0.79	0.83	1.85	1.71
100	4.00	3.82	3.06	1.85	1.79	1.71	0.93	1.90	2.41	2.22
150	4.03	4.17	3.13	2.13	1.90	2.41	1.06	2.78	2.59	2.41
200	4.31	4.44	3.19	2.22	2.22	4.60	1.39	3.70	2.78	2.78
250	4.17	4.03	3.00	2.45	2.45	4.21	2.08	3.38	3.01	2.87
300	4.12	3.82	3.33	2.04	2.73	4.03	3.01	3.24	2.92	3.01
350	4.02	3.73	3.61	1.99	2.92	4.07	2.78	2.92	2.82	3.24
400	3.96	2.50	2.99	1.94	2.36	2.64	2.64	2.82	2.64	2.69



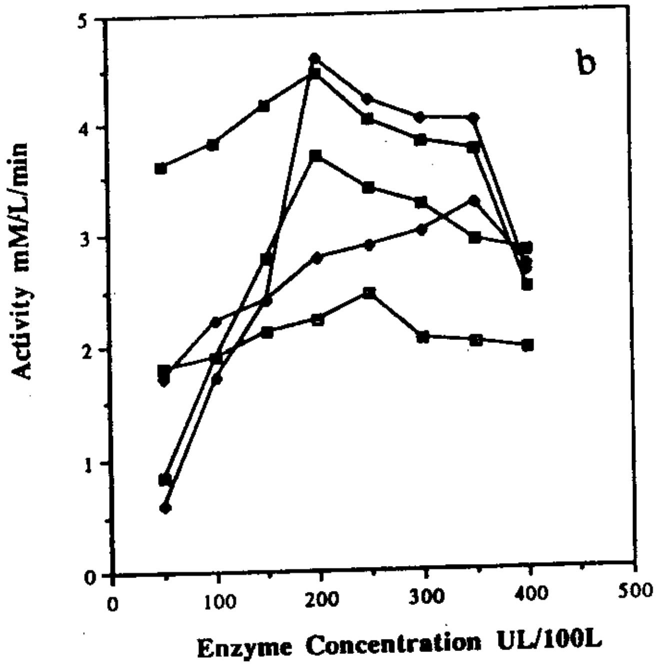


Fig. (14): Effect of enzyme concentration on the activity of cellulase enzymes

WS Wheat straw
CC Corn cobs
CS corn stalks
B Bagasse

Table (13) and Fig.(16) illustrate the effect of different concentrations (dilutions with 50 mM sodium acetate buffer) 1:5, 1:10, 1:20, and 1:50) of xylanase/ hemicellulase (viscozyme 120L) using different volumes with xylan concentration 1% and buffer in a final vilume of 1:0 ml, then incubated at 50°C for 30 min and pH.

The maxmum reaction activities were 3.6; 3.0 2.9, 3.7, and 3.9 mM/L/min with 0.1 ml; 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml of the enzyme dilutions 1:5, however, with 1:50 dilutions the maximum activity were 2.7; 1.6; 1.1; 1.8; and 2.2 mM/L/min. An increase in the amount of xylanase enzyme occured increasing in the activity with different dilutions. The xylanase enzymes exhibited a nonlinear relationship between enzyme activity and enzyme concentration although the enzyme solution was diluted 50-fold nonlinaearity was observed. This observation suggested that the enzyme solution contained compounds of inhibitory nature towards xylanase. Some of these inhibitors are thought to be polyphenolic and probably arise from lignin-heteroxylan linkage groups in the hetero-xylan preparations used growth substrate in fermentation (Dekker,1983).

Attempts to achieve a linear relationship between the xylanase activity and its concentration by adsorption of these inhibitory compoundsby using polyvinylpyrollidone (pup) and partially successful adsorption was achieved. Linearity relationship being observed when 0.02-0.2 ml of enzyme used (Maloney, et.al., 1985).

Table(12): Effect of enzyme concentration on the activity of β-glucosidase (Novozum 188) enzyme

Enzyme concentration µL	Glucose content mM/L	activity µmol glucose/min
25	139.3	38.7
50	141.8	39.4
75	144.0	40.0
100	154.8	43.0
125	191.9	53.3
150	230.4	64.0
200	298.8	83.0

Table(13): Effect of enzyme concentration on the reaction activity of xylanase/Hemicellulase (viscozyme 120 L) enzyme.

Enzyme Concentration	Volume of enzyme			
	0.1 mL		0.5 mL	
	Xylose content mM/L	Activity mM/L min	Xylose content mM/L/min	Activity mM/L /min
1:5	106.7	3.6	116.7	3.9
1:10	101.7	3.4	108.3	3.6
1:20	93.3	3.1	80.0	2.7
1:50	80.0	2.7	66.7	2.2

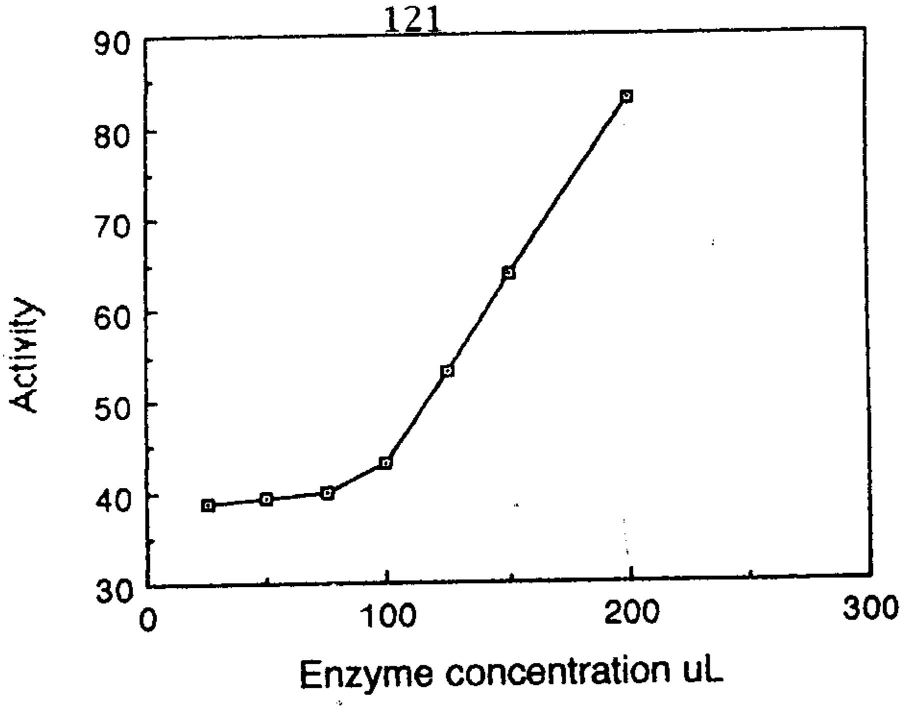


Fig. (15): Effect of enzyme concentration on the activity of β-glucosidase enzyme

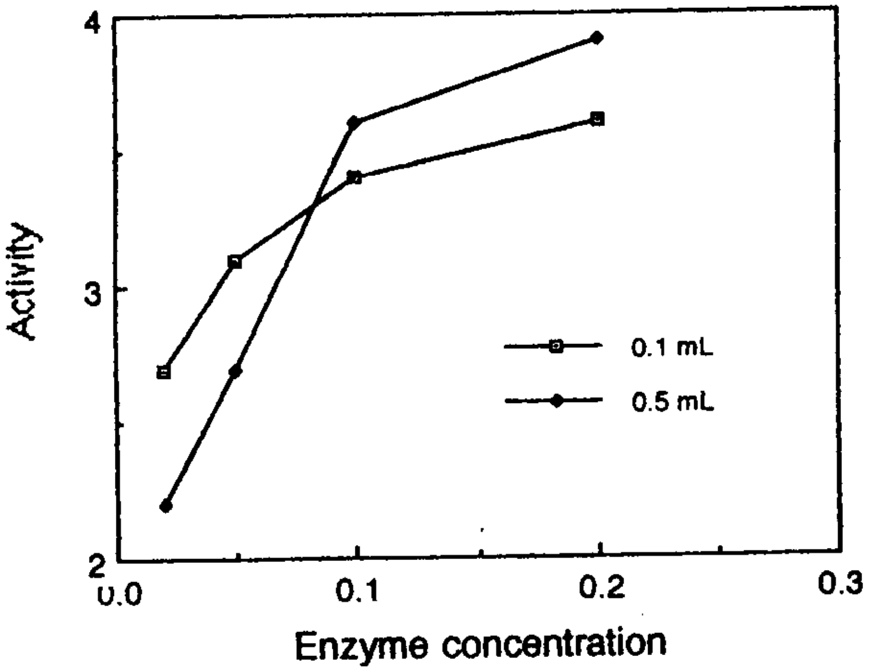


Fig. (16): Effect of enzyme concentration on the reaction activity of Xylanase/Hemicellulase enzyme.

The effect of different sweetzyme type-T concentrations i.e. 50 - 100- 150- 200- 250- 300- 350- 400- 450 and 500 mgIGIs dry solid of enzyme/5ml reaction mixture was tested with substrate concentration of 6 mM (2.0 ml), Tris-buffer, pH 7.5 (1.0 ml), $MgSO_4 . 7H_2O$. 0.01M (1.0ml) and $CoCl_2$ (0.003M (1.0ml) at 60^oC for 30 and 60 min.

The obtained results (Table,14, Fig.17) indicate that as the enzyme concentration increases, the activity of immobilized glucose isomerase increases until reached its maximum i.e. 50.37 and 60.74 u mol fructose/min at 30 and 60 min, respectively. The obtained results are in agreement with that obtained by Foda (1987) with immobilized glucose isomerase (sweetzyme type Q).

4.3.4. Effect of substrate concentration:-

Substrate concentration is one of the most important factors which affects the velocity of the enzyme reaction (Dekker,1986 and Szczodrak,1988).

During the course of these experiments the amount of each enzyme in the individal experiments, pH, temperature, and other factors were constant.

4.3.4.1. Effect of substrate concentration on the activity of cellulase enzymes:-

The effect of substrate concentration on the reaction velocity of these enzymes was throughly examined using carboxymethyl cellulose (CMC, as standard) and cellulose pretreated from the different

Table(14): Effect of Enzyme concentration on the activity of immobilized Glucose isomerase (Sweetzyme type T) enzyme.

Enzyme concentration mg	Activity μmol fructose	
	30 min	60 min
50	10.00	13.06
100	13.33	18.61
150	16.94	19.94
200	20.00	23.70
250	22.96	27.41
300	24.67	27.78
350	25.19	28.15
400	44.45	51.15
450	45.93	55.66
500	50.37	60.74

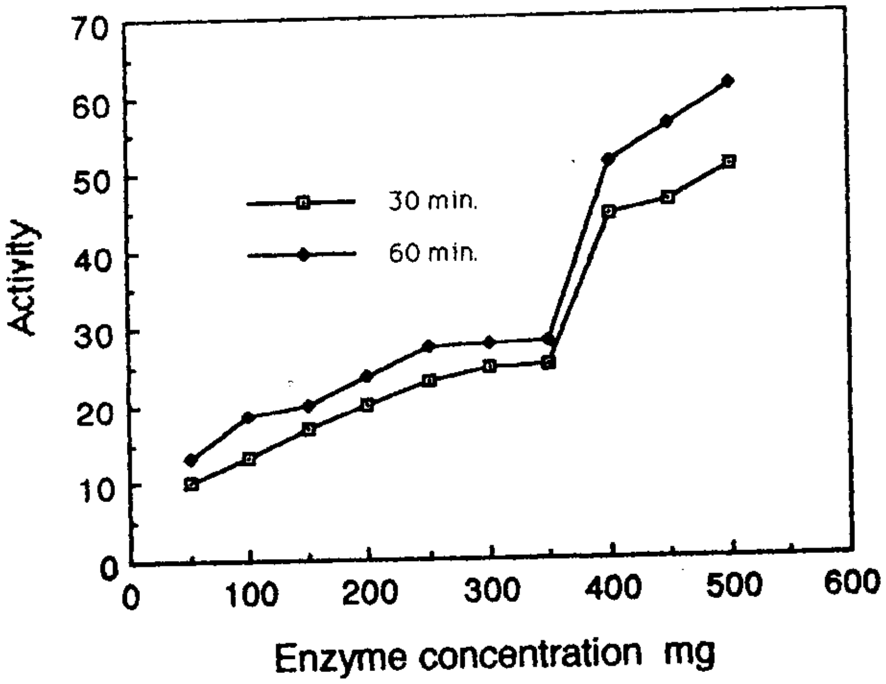


Fig. (17): Effect of enzyme concentration on the activity of immobilized glucose isomerase.

lignocellulosic materials (wheat straw, corn cobs, corn stalks, and sugarcane bagasse). Cellulase complex (celluclast 1.5L) enzyme reaction was carried out under constant temperature 50 ^{o}C in 0.05M acetate buffer pH 4.8. Different substrate concentrations in the range between 2.3 and 20.3 g/L were investigated using a constant enzyme concentration.

The effect of substrate concentration on the reaction activity of cellulase (celluclast 1.5 L) enzyme at different periods i.e 60 and 120 min for carboxmethylcellulose wheat straw, corn cobs, corn stalks and sugarcane bagasse as substrates are illustrated in Tables (15, a, b, c, d, e). By plotting the obtained reducing sugars (as glucose) against the concentration of the substrates, the Figures (18, a, b, c, d, e) were obtained.

From these results, the enzyme activity increased with the increasing of substrate concentration until its maximum (Vmax). Afterwards, the maximum velocity of cellulase enzyme reaction was decreased with the increasing of different substrate concentration.

An explanation for such enzymic behaviour was introduced by Caminal, et. al.,(1985), Dekker (1986), Converse, et. al., (1988) and Fredrik, et. al., (1988). At low substrate concentration, the enzyme is surround only by a small number of substrate molecules and the chances that a molecule to hit the active centre of the enzyme are fewer than with high substrate concentration. With increasing substrate concentration, the peroid of the time necessary for a new substrate molecule to hit a free bond decreases constantly until the enzyme is surrounded by many substrate molecules to fill a bond immeditely after having released the product as follows.

E + S <-----------> ES <---------> E + P

E: Enzyme, S:Substrate and p: product. Once the product is formed, and released, the free active center can bind another substrate molecule. The maximum reaction velocity (Vmax) for cellulase enzyme for 60 min by using of CMC and pretreatment lignocellulosic materials i.e, wheat straw, corn cobs, corn stalks, and bagasse were determined to be 252.3; 201.4; 208.3; 204.2; and 229.2 mM/L, respectively. It is clear that the maximum reaction velocity was lower in lignocellulosic materials than CMC due to many factors such as the preteratment process, and to the different structure of them in which lignocellulosic materials contained hemicellulose which lead to reduce the maximum reaction velocity (Wald et. al., 1984, Szczodrak, 1988).

The Michaelis constant (K_m) is one of the most useful and important parameter in enzyme kinetics. K_m of cellulose enzyme was experimentally obtained from Figures (18a, 18b, 18c, 18d, 18e) by taking the substrate concentration at the half point of V_{max}. The Michaelis constant of cellualse (celluclast 1.5 L) were 4.4 g/L; 3.0 g/L, 3.0 g/L; 2.6 g/L and 2.5 g/L for CMC, wheat straw, corn cobs, corn stalks and bagasse for 1h incubation period. While these values were 2.6 g/L, 4.2 g/L; 3.29 g/L; 3.0 g/L and 3.0 g/L for 2h incubation period. The obtained different values of K_m may be due to the fransformation of cellulose into a form highly resistant to enzymatic attack and product inhibition i.e. cellobiose and glucose (Lee and Fan, 1983; Holtzapple, et. al., 1984a).

It is important to mention that K_m constant was one more determined by Lineweaver and Burk Plotts (1954) . The obtained K_m values were almost equal to that obtained firstly by experimental curve.

4.3.4.2. Effect of substrate concentration on the reaction velocity of β-glucosidase (Novozym 188) enzyme:-

The effect of substrate concentration on the reaction velocity of β-glucosidase (cellobiase, Novozym 188) is shown in Table (26) and Fig. (25). The reaction was carried out at constant temperature at 50°C with incubation period 30 min in 0.05 M acetate buffer pH 4.8 with concentration of cellobiose between 0.68 and 10.0 mM as a substrate concentration in all experiments.

The obtained results illustrated that the Vmax for activity cellobiase enzyme was 55.0 μmol glucose/min(201.0 mM/L). Michaelis constant was obtained by taking the substrate concentration at the half point of Vmax which was found to be 0.4 mM. On the basis of Lineweaver-Burk plotts, the km and Vmax of β-ghucosidase enzyme was found to be 0.45 mM and 201.0mM/L, respectively. Generally, the obtained data is conscident with that reported by woodward and wohlpart (1982) and Dekker (1986).

Table(15,a):Effect of substrate concentration on the reaction velocity of cellulase (celluclats 1.5 L) enzyme.

Substrate conc. [S] g/L	1/S X 10^{-2}	60 min obtained RS mm/L	Reaction velocity (v)	1/v X 10^{-3}	120 min obtained RS mm/L	Reaction velocity (v)	1/v X 10^{-3}
2.3	4.35	52.78	104.75	9.55	56.3	88.3	11.3
5.3	1.90	163.89	177.29	5.64	235.4	137.5	7.3
8.3	1.20	252.30	219.40	4.56	283.6	162.6	6.2
11.3	0.88	250.0	246.90	4.05	251.2	177.6	6.6
14.3	0.70	250.17	266.28	3.76	246.5	188.1	5.3
17.3	0.58	250.00	280.66	3.56	245.8	145.4	5.1
20.3	0.49	237.33	291.76	3.43	241.7	200.9	5.0

* Substrate: CMC carboxymethyl cellulose

Table(15,b): Effect of wheat straw extracted concentrations on the reaction velocity of cellulase (celluclast 1.5 L) enzyme at different periods..

substrate concentration (S) g/L	1/[S] X10-1	06 min. obtained reducing sugar mM/L	Reaction velocity (v)	1/v X10^{-3}	120 min. obtained reducing sugar mM/L	Reaction velocity (v)	1/v X10^{-3}
2.3	4.35	125.00	104.17	9.60	159.72	135.00	7.41
5.3	1.90	152.78	139.18	7.10	187.50	180.38	5.54
8.3	1.20	173.61	153.48	6.52	194.44	198.91	5.03
11.3	0.88	145.83	161.24	6.20	222.22	208.97	4.79
14.3	0.70	187.50	166.12	6.02	243.06	215.30	4.64
17.3	0.58	180.56	169.47	5.90	229.17	219.64	4.55
20.3	0.49	201.39	171.92	5.82	187.50	222.80	4.49

Table(15,c): Effect of corn cobs extracted concentrations on the velocity of cellulase (Celluclast 1.5 L) enzmye at different periods.

		06 min.			120 min.		
substrate concentra -tion (S) g/L	1/[S] X10-1	obtained reducing sugar mM/L	Reaction velocity (v)	1/v X10-3	obtained reducing sugar mM/L	Reaction velocity (v)	1/v X10-3
2.3	4.35	131.94	89.91	11.12	166.67	104.20	9.60
5.3	1.90	168.89	134.06	7.46	180.56	148.94	6.71
8.3	1.20	180.56	155.17	6.44	222.22	169.05	5.92
11.3	0.88	208.33	167.55	5.97	194.44	180.47	5.54
14.3	0.70	201.39	175.69	5.69	215.28	187.85	5.32
17.3	0.58	215.28	181.44	5.51	166.67	192.99	5.18
20.3	0.49	180.56	185.72	5.38	173.61	196.79	5.08

* Substrat: corn cobs extracted.

Table(15,d): Effect of corn stalks extracted concentrations on the velocity of cellulase (Celluclast 1.5 L) enzmye at different periods.

		06 min.			120 min.		
substrate concentra -tion (S) g/L	1/S X10-1	obtained reducing sugar mM/L	Reaction velocity (v)	1/V X10-3	obtained reducing sugar mM/L	Reaction velocity (v)	1/V X10-3
2.3	4.35	141.67	120.12	8.33	166.67	104.15	9.60
5.3	1.90	183.33	156.62	6.38	162.50	145.26	6.88
8.3	1.20	166.67	171.03	5.85	187.50	163.10	6.13
11.3	0.88	170.83	178.73	5.60	208.33	173.07	5.78
14.3	0.70	204.17	183.54	5.45	154.17	179.44	5.57
17.3	0.58	166.67	186.81	5.35	154.17	183.86	5.44
20.3	0.49	170.83	189.19	5.29	170.83	187.10	5.34

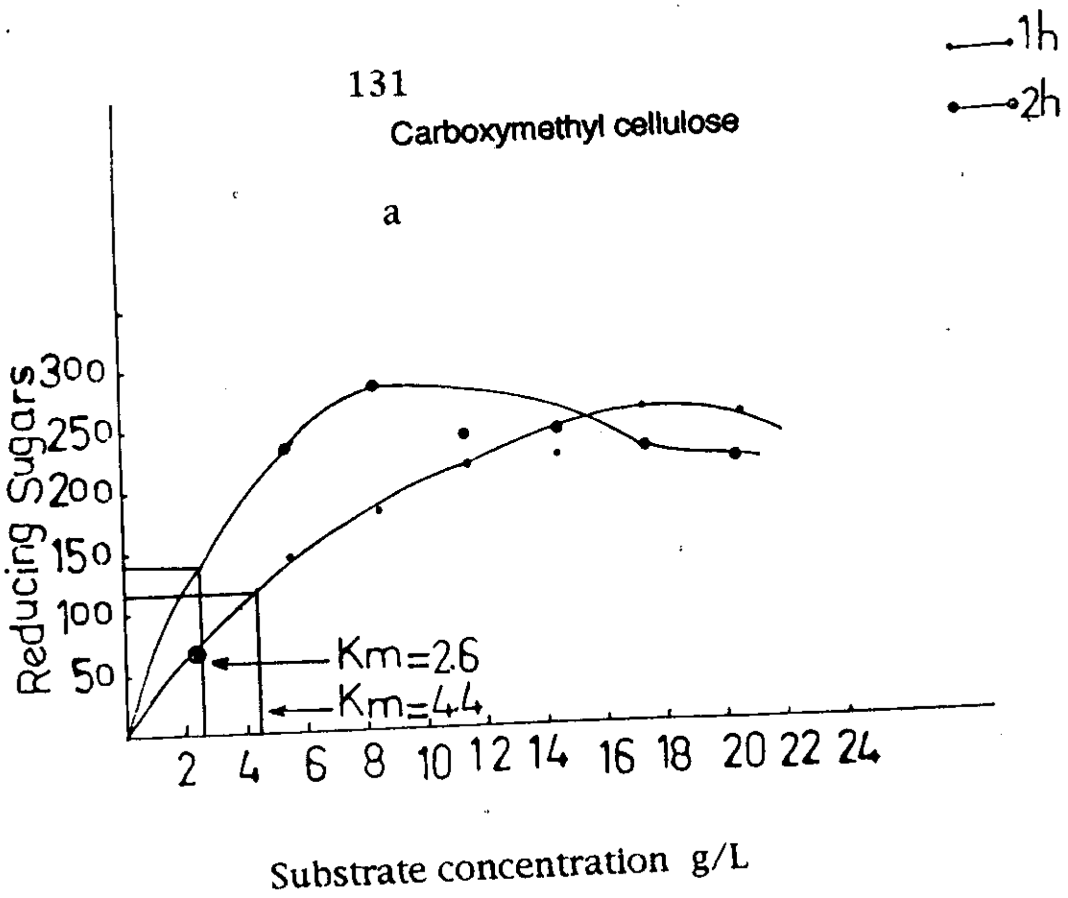
* Substrate: corn stalks extracted.

Table(15,e): Effect of sugarcane bagasse extracted concentrations on the velocity of cellulase (Celluclast 1.5 L) enzmye at different periods.

		06 min.			120 min.		
substrate concentra -tion (S) g/L	1/S X10-1	obtained reducing sugar mM/L	Reaction velocity (v)	1/V X10^{-3}	obtained reducing sugar mM/L	Reaction velocity (v)	1/V X10^{-3}
2.3	4.35	183.33	147.22	6.79	127.50	75.41	13.26
5.3	1.90	204.17	181.46	5.51	133.33	116.48	8.59
8.3	1.20	220.83	193.96	5.16	137.50	137.19	7.29
11.3	0.88	191.67	200.43	4.99	154.17	149.67	6.68
14.3	0.70	162.50	204.39	4.89	162.50	158.01	6.33
17.3	0.58	229.17	207.07	4.83	200.00	163.98	6.10
20.3	0.49	166.67	208.99	4.78	145.83	168.46	5.94

* Substrate: Sugarcane bagasse extracted.

Carboxymethyl cellulose



wheat straw

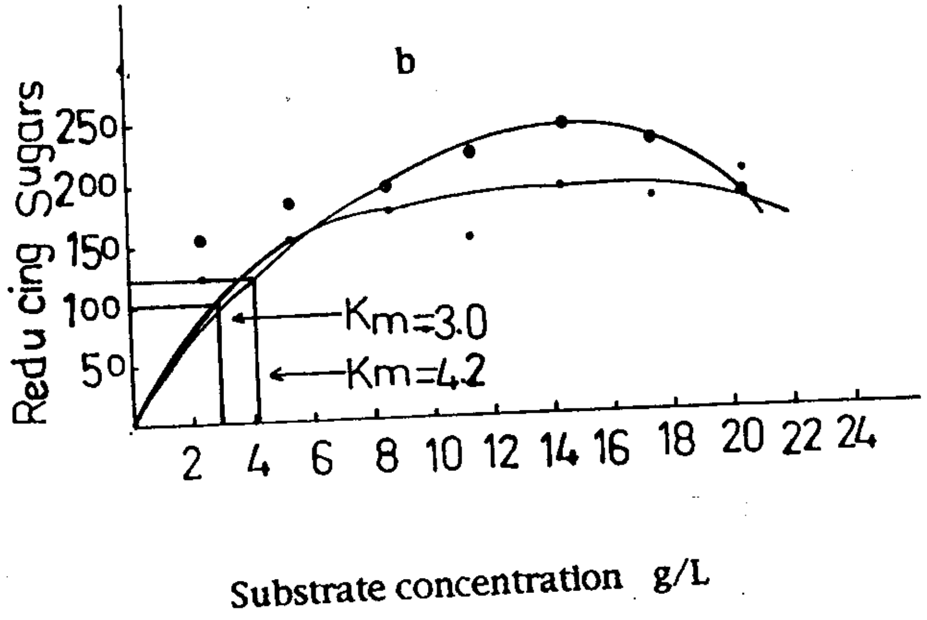


Fig. (١٨): Relationship between substrate concentration and obtained D-glucose of cellulase enzyme.

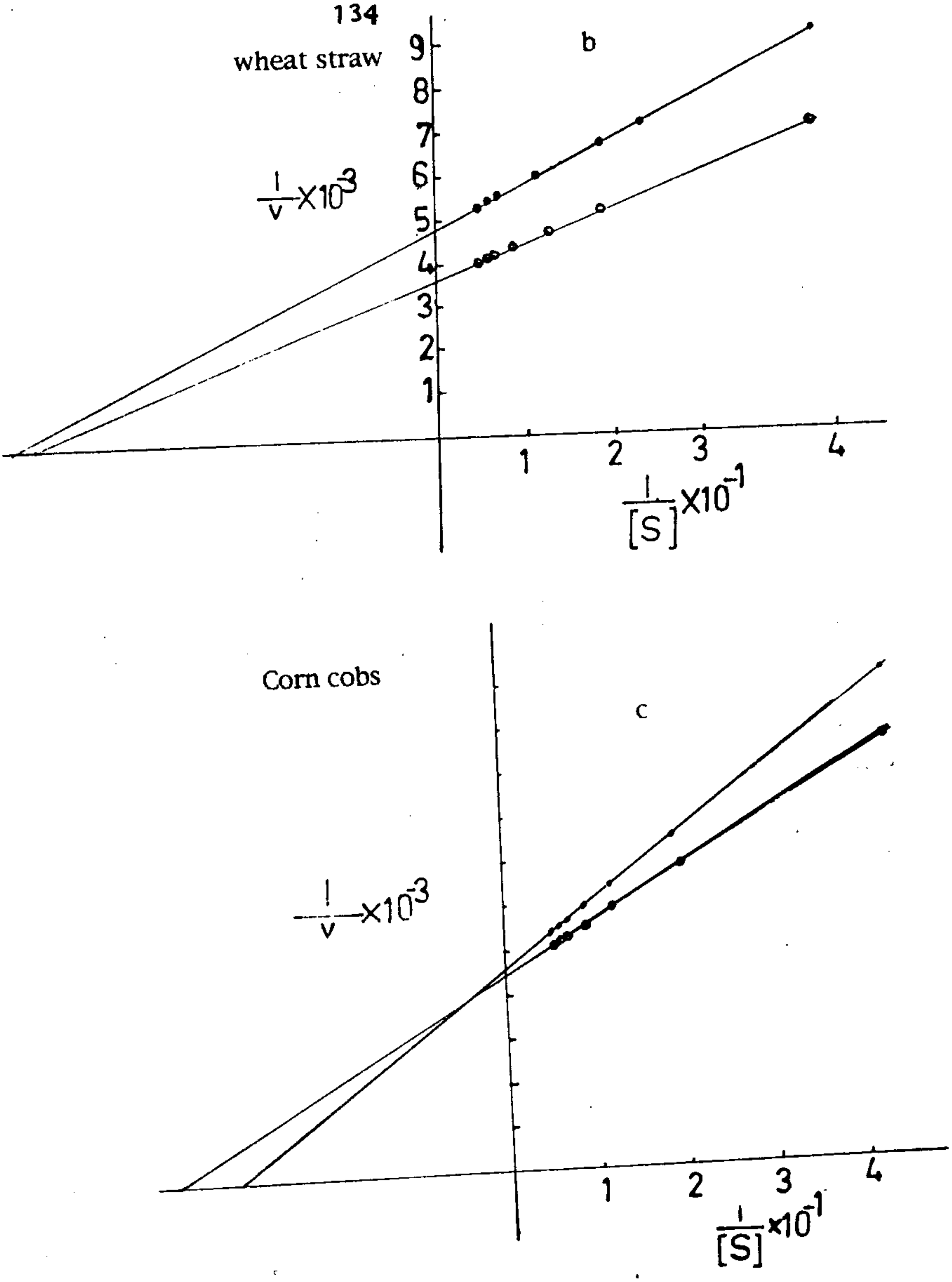
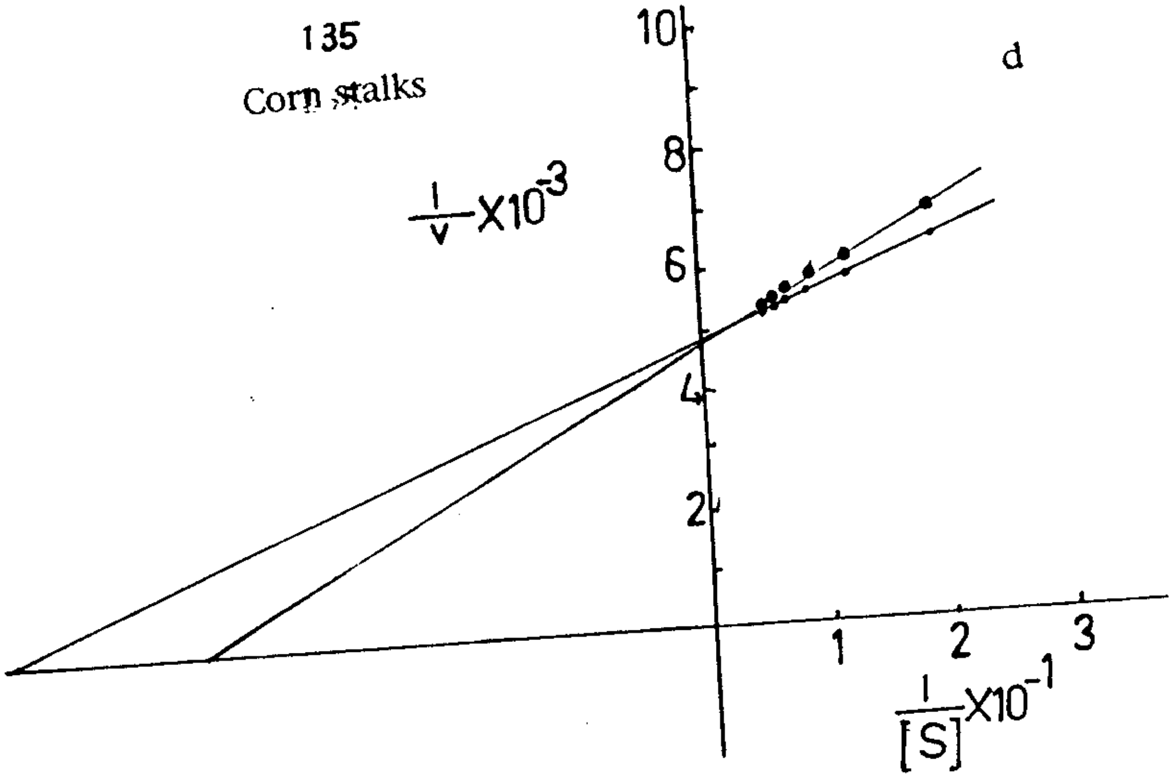


Fig. (14): Lineweaver Burk plotts of cellulase enzymes .

Corn stalks



Sugarcane bagasse

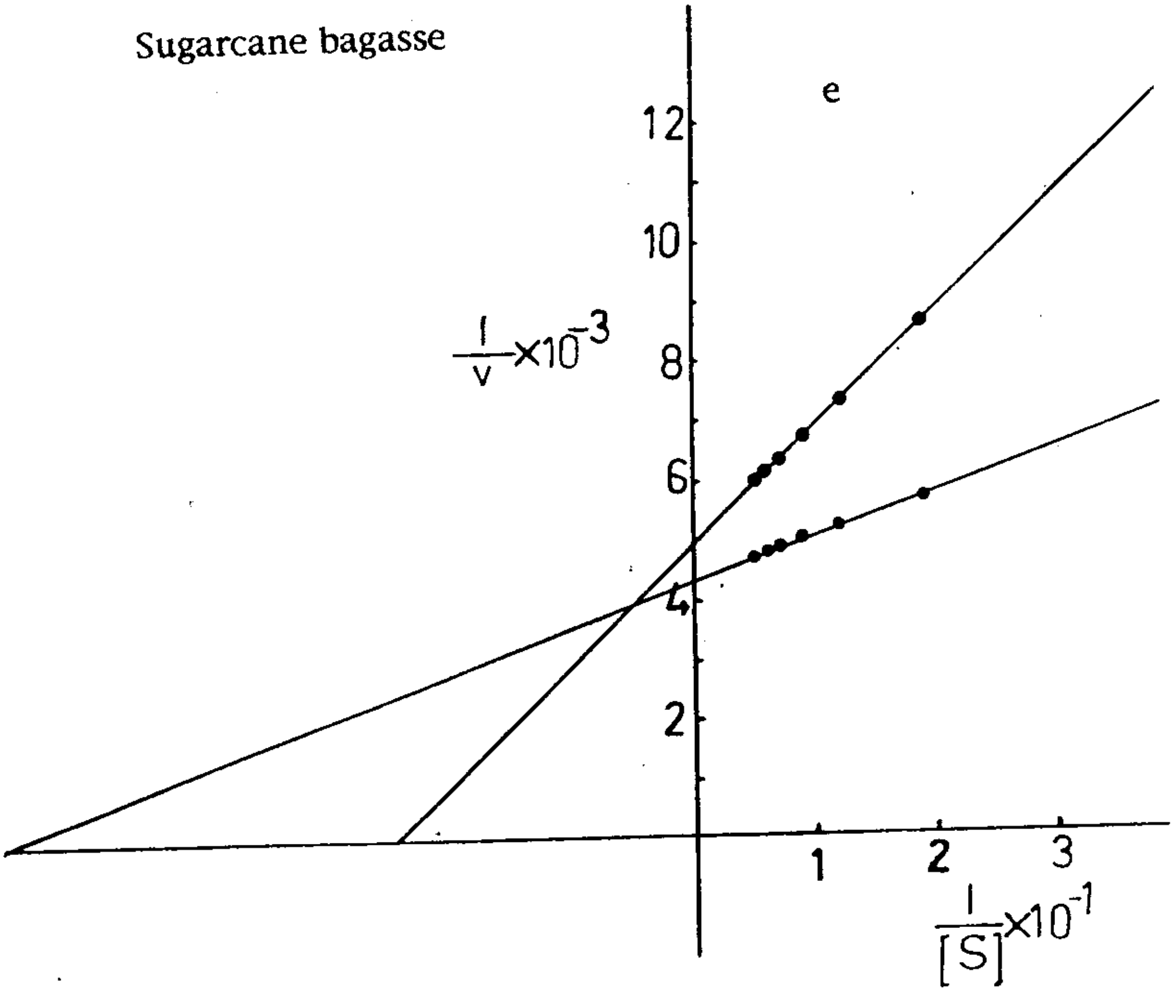


Fig. (19): Lineweaver Burk plotts of cellulase enzymes.

4.3.4.3. Effect of substrate concentration on the reaction velocity of hemicellulase complex enzyme.

The effect of xylan concentration on the reaction velocity of xylanase (hemicellulase complex, viscozyme 120 L) was carried out by using standard xylan polysaccharide. And then it was applied on different isolated hemicelluloses of ligncellulosic materials i.e. wheat straw, corn cobs, corn stalks and sugarcane bagasse. The obtained results are shown in Table (16,17, 18, 19, 20) and Figs. (20a, 20b, 21a, 21b, 21c, 21d). The results illustrate that V_{max} of hemicellulase mixture by using concentrations of xylan substrate ranged 10 to 60 g/L were 43.3 and 93.3mM/L for 30 and 60 min. The obtained K_m values were calculated to be 11.0 and 12.3 g/L. K_m values which were aquired by Lineweaver and Burk plotts, Fig. (20b, 21a,21b, 21c, 21d) were equally to that obtained by experimentally curve. These values are slightly higher than that obtained by Dekker (1983).

V_{max} of hemicellulase complex with wheat straw, corn cobs, corn stalks and sugarcane bagasse were 146.7, 100.0, 108.3 and 123.3 mm/L after 30 min incubation period. While these values were 150, 125, 123.3 and 140 mm/L for 60 min. From these results, it has been observed that V_{max} values increased with increasing the of reaction period. Michaelis constant (K_m) values were obtained by expermentally curves, and also, by Linweaver and Burk plotts. The obtained results for both methods were in good harmony and these results are partially in agreement with that reported by Dekker (1983).

The differences of Vmax between the above mentioned substrates may be due to that these lignocellulosic materials contained an unalike hemicellulose content i.e. 36.6, 26.60, 29.50 and 33.40%, respectively for wheat straw, corn cobs, corn stalks and sugarcane bagasse. It is clearly that these values of hemicellulose content of substrates are proportaional with the obtained Vmax values of hemicellulase (viscozyme 120 L) enzyme.

4.3.4.4. Effect of substrate concentration on the reaction velocity of immobilized glucose isomerase enzyme

The effect of substrate concentration on the reaction velocity of immobilized glucose isomerase, IGIs (sweetzyme, type T) is shown in Table (21) and Fig. (22a, 22b). The reaction was applied on glucose syrup containing different concentrations of glucose i.e. 1-2-3-4-5-6 and 7 mM.

The results showed that the reaction velocity to convert glucose to fructose was increased up to 0.6 mM of D-glucose and maximum reaction velocity (Vmax) of 750.0 μmol fructose/100 mg immobilized glucose isomerase. At higher concentration of glucose the conversion process was inhibited and the reaction slow down until no further change in velocity was observed (zero order reaction). On plotting (v) as fructose content in μmol against [s] which is the substrate concentration in mM, Michaleis constant (K_m) and Vmax of immobilized glucose isomerase (sweetzyme, type T) were determined to be 2.3 mM fructose and 750.0 μmol fructose/100 mg immobilized

Table (16): Effect of xylan concetration on the reaction activity of hemicellulase enzyme

		30 min.			60 min.		
substrate concentra -tion (S) g/L	1/[S] X10-1	obtained D-xylose sugar mM/L	Reaction velocity (v)	1/v $X10^{-2}$	obtained D-xylose sugar mM/L	Reaction velocity (v)	1/v $X10^{-2}$
10	1.00	30.0	20.6	4.9	43.3	41.8	2.4
15	0.67	33.3	25.0	4.0	60.0	51.3	1.9
20	0.50	41.3	27.9	3.6	80.0	57.8	1.7
40	0.25	42.7	34.0	2.9	93.3	71.4	1.4
60	0.17	43.3	36.6	2.7	86.7	77.4	1.3

Table (17):Effect of hemicellulose concentration from wheat straw on the reaction activity of hemicellulase enzyme.

substrate concentra -tion (S) g/L	1/[S] X10-1	obtained D-xylose sugar mM/L	Reaction velocity (v)	1/v $X10^{-3}$	obtained D-xylose sugar mM/L	Reaction velocity (v)	1/v $X10^{-3}$
5	2.0	76.6	81.7	12.2	91.6	93.8	10.7
10	1.0	93.3	105.0	9.5	136.3	115.4	8.7
20	0.5	126.7	122.5	8.2	143.3	130.4	7.7
25	0.4	146.7	126.7	7.9	150.0	133.9	7.5

Table (18): Effect of hemicellulose concentration from corn cobs on the reaction activity of hemicellulase enzyme

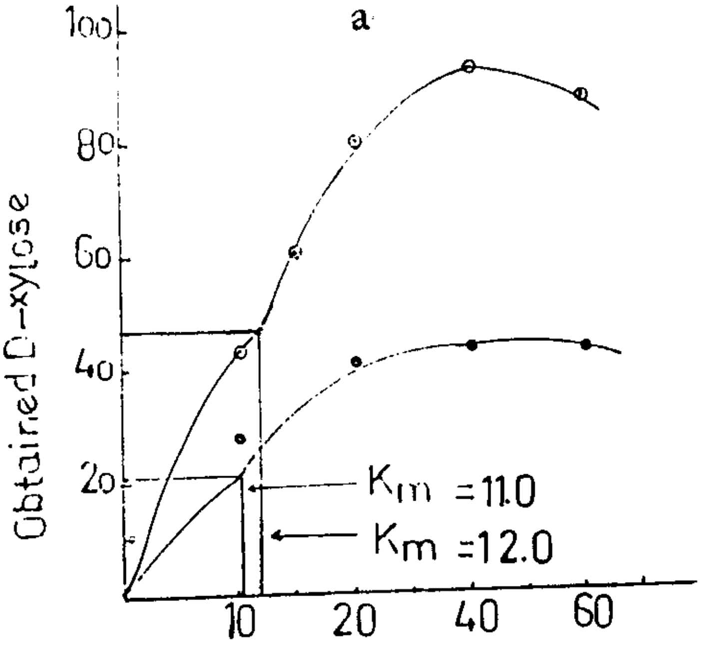
		06 min.			120 min.		
substrate concentra -tion (S) g/L	1/[S] X10-1	obtained D-xylosesugar mM/L	Reaction velocity (v)	1/v $X10^{-3}$	obtained D-xylose sugar mM/L	Reaction velocity (v)	1/v $X10^{-3}$
5	2.0	34.9	40.0	25.0	42.0	50.0	20.0
10	1.0	61.6	57.1	17.5	80.0	71.4	14.0
20	0.5	91.7	72.7	13.8	105.2	90.9	11.0
25	0.4	100.0	76.9	13.3	125.0	96.2	10.4

Table (19) Effect of hemicellulose concentration from corn stalks on the reaction activity of hemicellulase enzyme

substrate concentra -tion (S) g/L	1/[S] X10-1	obtained D-xylose sugar mM/L	Reaction velocity (v)	1/v $X10^{-3}$	obtained D-xylose sugar mM/L	Reaction velocity (v)	1/v $X10^{-3}$
5	2.0	46.7	45.0	22.2	55.0	55.9	17.9
10	1.0	68.3	63.5	15.7	93.3	76.9	13.0
20	0.5	88.3	80.0	12.5	105.0	94.6	10.6
25	0.4	108.3	84.4	11.8	123.3	99.2	10.0

Table (20): Effect of hemicellulose concentration from sugarcane bagasse on the reaction activity of hemicellulase enzyme

substrate concentra -tion (S) g/L	1/[S] X10-1	obtained D-xylose sugar mM/L	Reaction velocity (v)	1/v $X10^{-3}$	obtained D-xylose sugar mM/L	Reaction velocity (v)	1/v $X10^{-3}$
5	2.0	41.7	49.2	20.3	63.3	66.7	15.0
10	1.0	73.3	73.3	14.2	98.3	90.3	11.1
20	0.5	91.7	89.5	11.1	130.0	109.8	9.1
25	0.4	123.3	94.6	10.6	140.0	114.8	8.7



Substrate concentration g/L

Fig. (20): Relationship between substrate concentration and D-xylose content of hemicellulase.

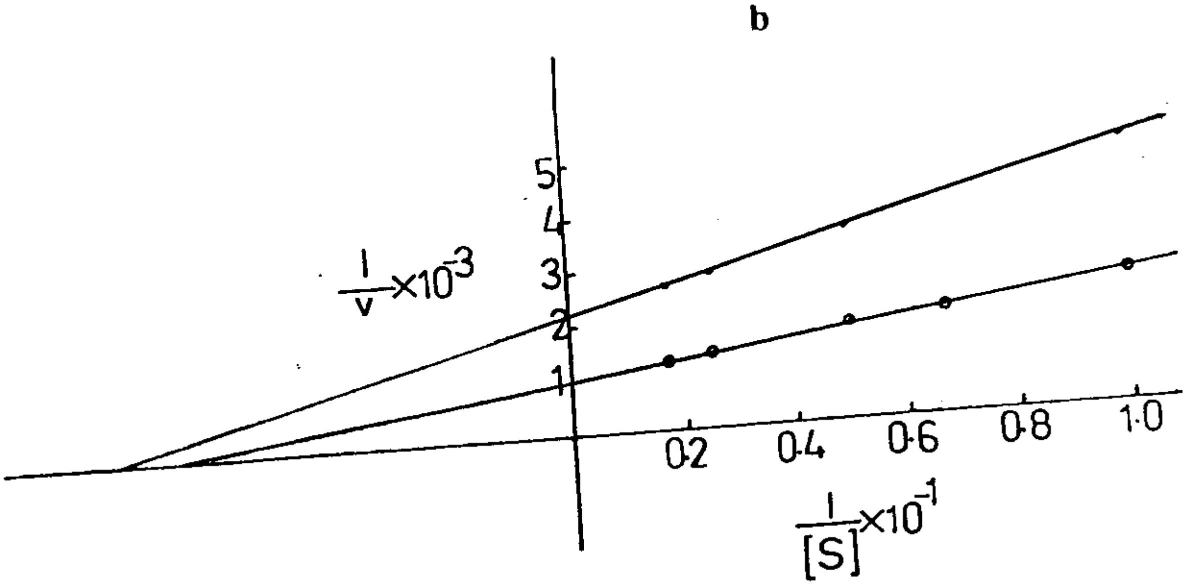


Fig. (20): Lineweaver Burk plotts of hemicellulase enzyme.

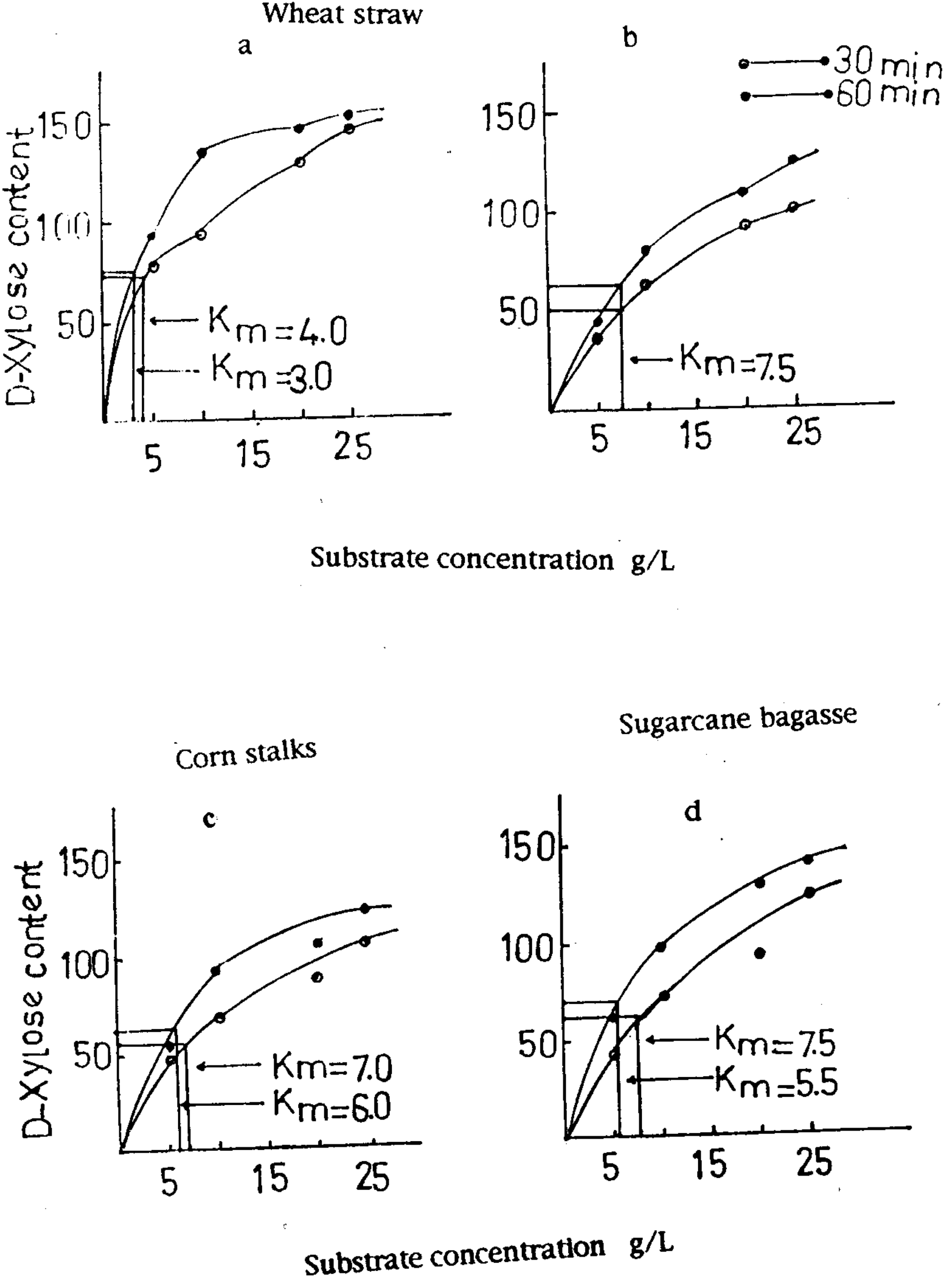


Fig. (21): Relationship between substrate concentration and D-xylose content of hemicellulase enzyme.

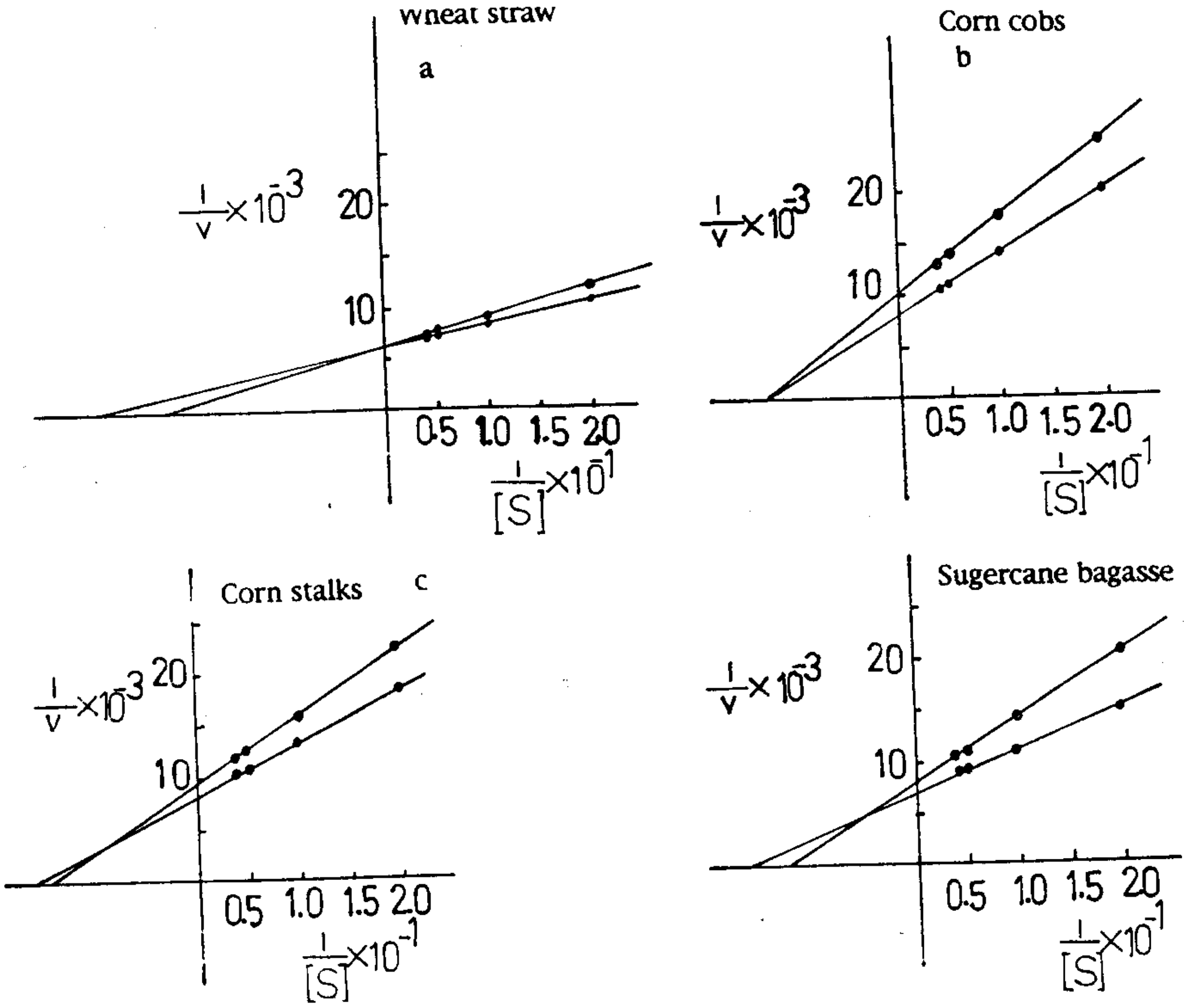


Fig. (29): Lineweaver Burk plotts of hemicellulase enzymes.

enzyme . On the other hand, Foda (1987) found that the Km of isomerase enzyme (sweetzyme type Q) was 0.028 mM/L with a maximum activity of 16.8 μ mol/min/500mg immobilized enzyme and substrate concentration equal 0.8 mM/L of glucose. Also the K_m constant was determined by lineweaver and Burk plot. The obtained value was in agreement with that obtained by experimental curve.

4.3.5. Effect of Mg^{++} and Co^{++} ions on the activity of immobilized glucose isomerase (sweetzyme typr T) enzyme

Frequenty the interaction between enzyme and substrate is mediated by some small ions or molecules. There is often a degree of specifity shown for these ions which is oftenly due to the carefully tailored size of the binding site, or the need for an electric field of a paraticular intesity. An enzyme that requires one of these ions is glucose isomerase which require Mg^{++} and Co^{++} ions to acquire complete function. Many investigators have emphasized the importance of these ions especilly Mg^{++} in activity of glucose isomerase, Mamat and Prabhu (1980), Benaiges, et.al., (1986), and Novo (1992). On the other hand, the required concentrations of Mg^{++} to carrey out the enzymatic reaction have to be carefully since excess of this ions often inhibit the reaction (Mattenheimer, 1976).

Different concentration of Mg^{++} and Co^{++} ions, ranged between $2.5X10^{-3}$ to $100X10^{-3}$ M for Mg^{++} ions and from $2.5 X10^{-4}$ to $100 X 10^{-4}$ M for the other metal, were used and the reaction activity was

Table(21): Effect of substrate concentration on the reaction velocity of immobilized Glucose isomerase (Sweetzyme **T**ype T) enzyme.

Substrate conc.mM [S]	1/[s]	60 min		
		Fructose content mM/L	Reaction velosity v	1/v $X10^{-2}$
1	1.00	222.2	227.3	0.44
2	0.50	305.6	348.8	0.29
3	0.33	444.4	424.5	0.24
4	0.25	555.6	476.2	0.21
5	0.20	666.7	513.7	0.19
6	0.17	750.0	542.2	0.18
7	0.14	694.4	564.5	0.18

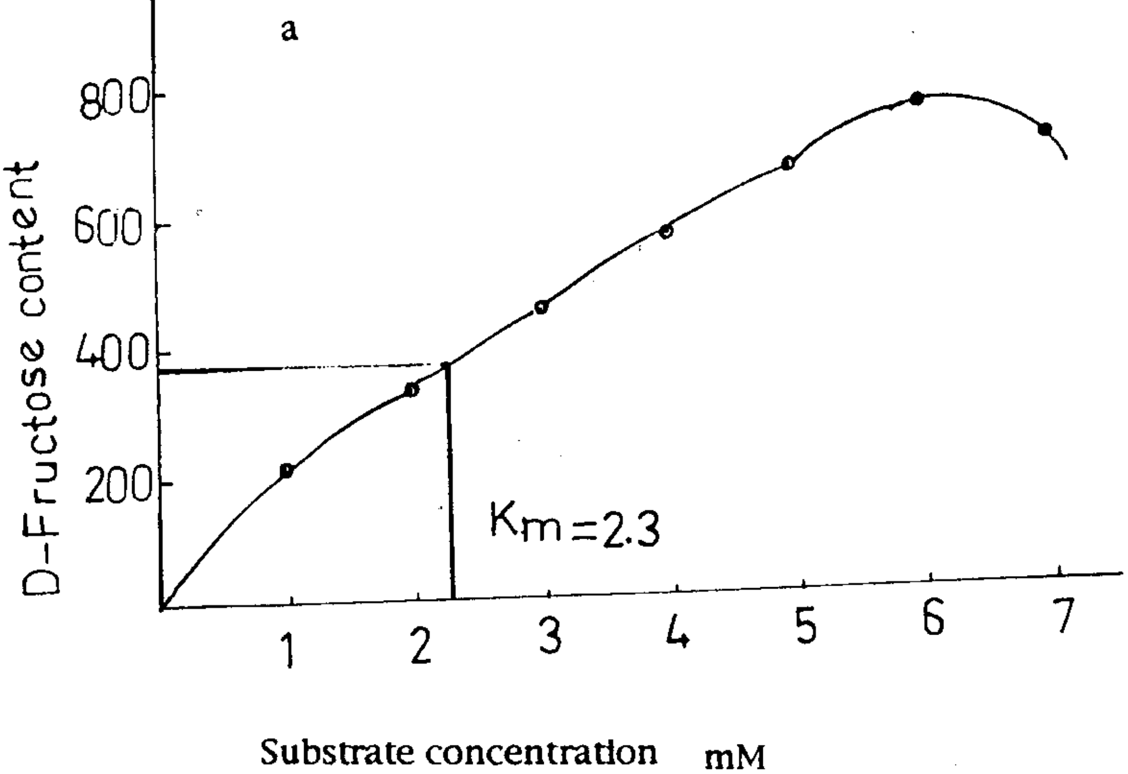


Fig. (22): Relationship between substrate concentration and fructose content of glucose isomerase.

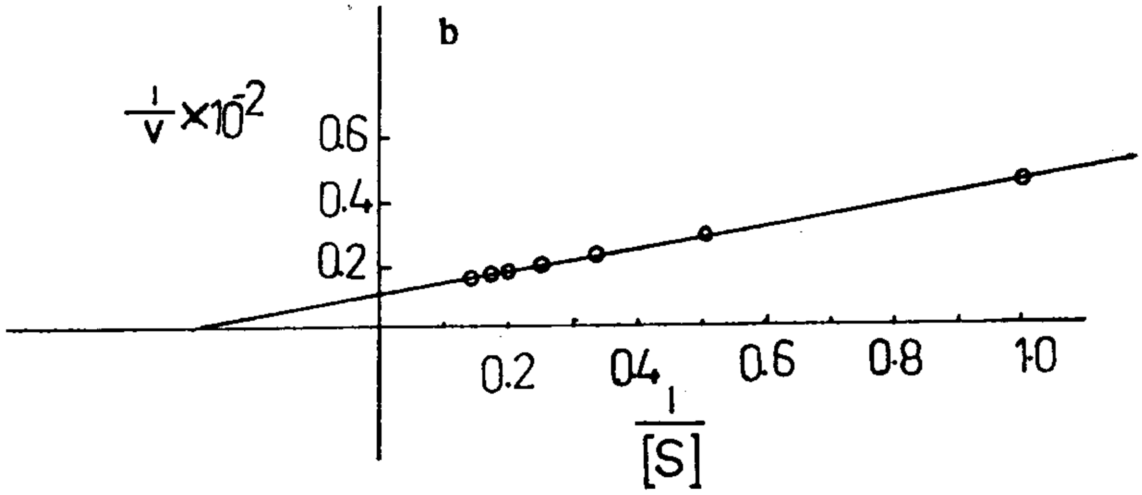


Fig. (22): Lineweaver Burk plotts of glucose isomerase enzyme.

estimated at optimum conditions for 60 min. The obtained results are recorded in Table (22) and shown inFig (23).

The results illustrate that both Mg^{++} and Co^{++} ion act as important activators for conversion reaction. But, it has to be mentioned that at concentration higher than ($10X10^{-3}M$) of Mg^{++} ions and ($30.0X10^{-4}M$) of Co^{++} ions, the reaction activities began to fall down. In other words, it has been observed that the increment of activity for immobilized isomerase enzyme reached its maximum (191.48% and 157.41%) by using ($10X10^{-3}M$) Mg^{++} and ($30X10^{-4}M$) Co^{++} ions, respectively. On the other hand, these values were decreased with the increasing of metal ions until 65.74% and 25.93% by using ($100X10^{-3}$ M) Mg^{++} and $100 X 10^{-4}M$) Co^{++} ions.

This phenomenon might be attributed to the autoinhibition effect induced by excess of metal ions. Such explanation was previously reported by (Sproull, et. al., 1976 and Novo,1992).

Also, it has been found that when the reaction was done in the presence of both Mg^{++} ($10.0 X 10^{-3}M$) and Co^{++} ($30.0 X 10^{-4}M$), the increment reached 167.54%. This result indicated that the increment of enzyme activity in the presence of Mg^{++} ($10X10^{-3}M$) alone as mentioned before was higher than that obtained in the presence of both of them(Mg^{++} Co^{++} ions). On the other hand, the mixture of Mg^{++} ions with the Co^{++} ions lead to increase in the activity by 10.13% than that obtained in the presence of Co^{++} alone.

Such results may be due to the competition between the two differnt ions i.e. Mg^{++} plus Co^{++} ions in the reaction media which mightbe favoured for Mg^{++} ions (Bell, 1977). Such explanation was introduced by Saad, et. al., (1991) during their studies on the effect of Mn^{++} ions in the presence of Mg^{++} ions on the activity of immobilized glucose isomerase (sweetzyme type-Q) enzyme.

4.4. Stabilization of enzymes:

Enzymes having a nutural marked tolerance to high temeratures are becoming increasing improtant in a wide range of industrial processes. Scientifically, it is interesting to understanding the mechanism and factors underlying protien stabilisation at elevated temperatures. In addition to prolonged half-lives at high temperatures, many thermostable enzymes also show a strong resistance to chemical denaturation by urea, sodium dodecyl sulphate or organic solvents. Also some enzymes possess unusually high affinities for their, compared with their mesophilic counterparts, possibly as a result of substrate lability at the high growth temperature of the parent thermophilic specie (Atkinson,1976).

All stability experiments were carried out under the conditions contemplated for the enzymatic conversion of cellulose to glucose (50°C, pH 4.8) and enzyme concentration diluted 1:100 (3.1 mg protein/ml) in the presence of 0.01% sodium azide as a preservative.

Table(22): Effect of Mg++ and Co++ ions concentration on the reaction activity of immobilized glucose isomerase (sweetzyme type-T) enzyme.

Metal concentration M		Activity µmol fructose/min		Percentage increment of reaction activity	
Mg^{++} $X10^{-3}$ M	Co^{++} X 10^{-4} M	Mg^{++}	Co^{++}	Mg^{++}	Co^{++}
0.0	0.0	16.20	16.20	-----	------
2.5	2.5	34.26	18.50	111.48	14.20
5.0	5.0	35.14	29.60	116.91	82.72
10.0	10.0	47.22	34.70	191.48	114.20
15.0	20.0	43.52	40.70	168.64	151.23
25.0	30.0	41.67	41.70	157.22	157.41
30.0	40.0	39.81	39.8	145.74	145.70
40.0	50.0	37.96	32.4	134.32	100.00
70.0	60.0	32.41	28.2	100.06	74.07
80.0	70.0	30.09	27.8	85.74	71.60
100.0	100.0	26.85	20.4	65.74	25.93
$10X10^{-3}M+30X10^{-4}M$		43.34		167.54	

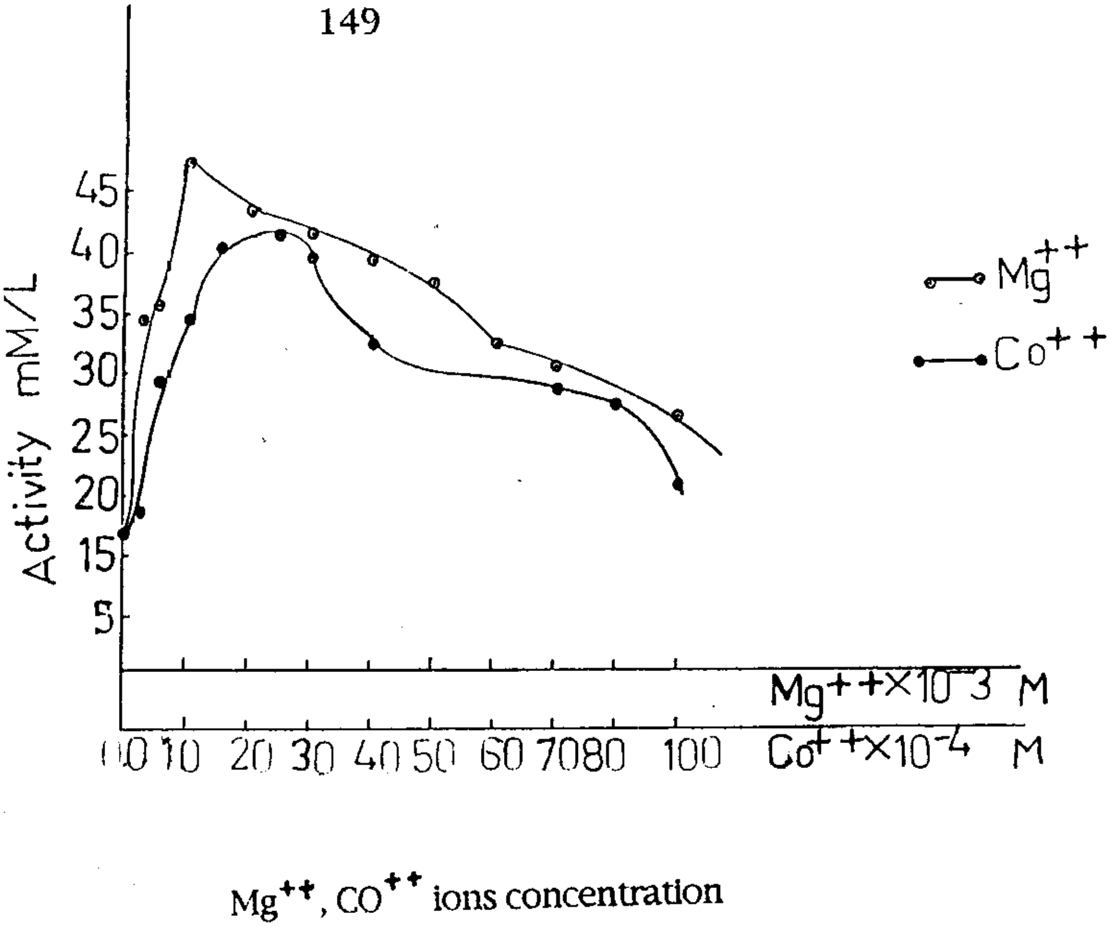


Fig. (23): Effect of Mg++ and Co++ ions concentration on the activity of isomerase enzyme.

4.4.1.Stability of cellulase enzymes:-

The stability of cellulase mixture enzymes i.e. endo-β-1,4 glucanase (Cx) and cellobiohydrolase were studied by using CMC (1%) as a substrate for the first enzyme and filter paper (FP) for the latter ones. The obtained results were illustrated in Fig (24a). From these results, on the basis of relative activity, it could be observed that endo-B-1,4 glucanase lost 25.8% while cellobiohydrolase enzyme was decreased by 19.7%, after 96 h. The obtained results are in agreement with that reported by Reese and Mandels (1980).

4.4.2.Stability of β-glucosidose enzyme:-

Fig (24c) reflect the activity of β-glucosidase (Novozym 188) from Aspergillus niger. The results indicated that a gradual decrese in its relative activity had been occurred until reached 90.6%, compared with orginal activity, after 120 h. The obtained data is conscident with that reported by Matteau and Saddler (1982).

4.4.3. Stability of xylanase/hemicellulase (viscozyme 120L) enzyme:-

The stability of xylanase (viscozyme 120L) was carried out with two different concentrations of viscozyme (1:5 and 1:10 dilution) using 0.05 M ocetate buffer sdution at 50 °C, pH 5 and incubation period reached 96 h. Fig (24c) exhibit the results of enzyme stability. These results show that the relative activities were decreased as the time increased. The loss of relative activity of this enzyme reached 18.9% in case of enzyme diluted 1:5 and 22.2% for enzyme diluted 1:10 at the end of incubation period. The obtained data is in agreement with that reported by Lilly, (1976).

4.4.4.Stability of glucose isomerase (sweetzyme type T):-

Fig (24d) illustrated the stability of immobilized glucose isomerase (Sweetzyme type T). The reaction was achieved using a mixture of glucose syrup (0.6mM) in Tris-buffer pH 7.5, containing 100 mg immobilized glucose iosmerase and $MgSO_4. 7H_2O$ ($3.0X10^{-3}$), then incubated at a constant temperature (60^oC). The results show that enzyme is stable after 240 h and a loss 1.3% of the relative activity was Occurred. This result is very important from the economical point of view, since the glucose isomerase enzyme has become the first commercial insolubilised enzyme. In addition, the stability of enzyme during the isomenization process acts as a significant factor in the production of high fructose syrup with a high percentage of fructose (Fullboox and Godfrey,1976 and Novo,1992).

4.5. Immobilization of ß-D-glucosiduse (Novozyme 188) enzyme:

The most common methods about immobilized enzymes (insolubilized) are matrix-bound, carrier-bound, support-bound, entrapped and microencapsulated. The effect of this immobilization process on the activity and stability of each immobilized enzyme is of major importance. In a number of cases the stability was found to be increased. Such phenomenon was accomplished by either covalent multiple-point attachment of enzymes or by embedding the enzyme in

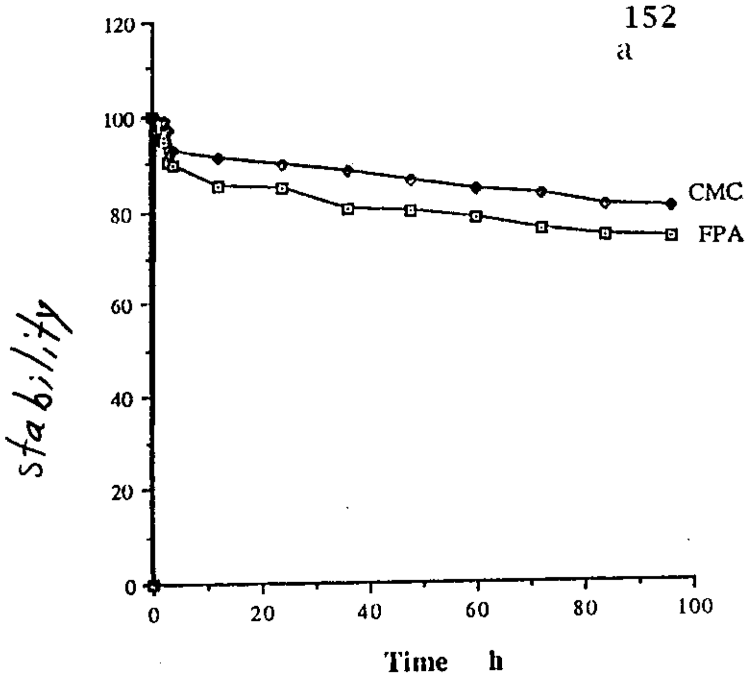


Fig. (24a): Stability of cellulase enzymes

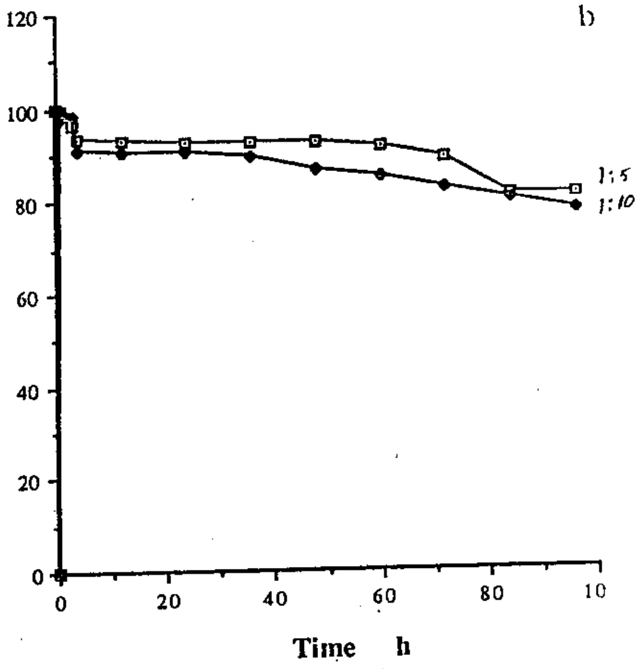


Fig. (24b): Stability of hemicellulase enzymes

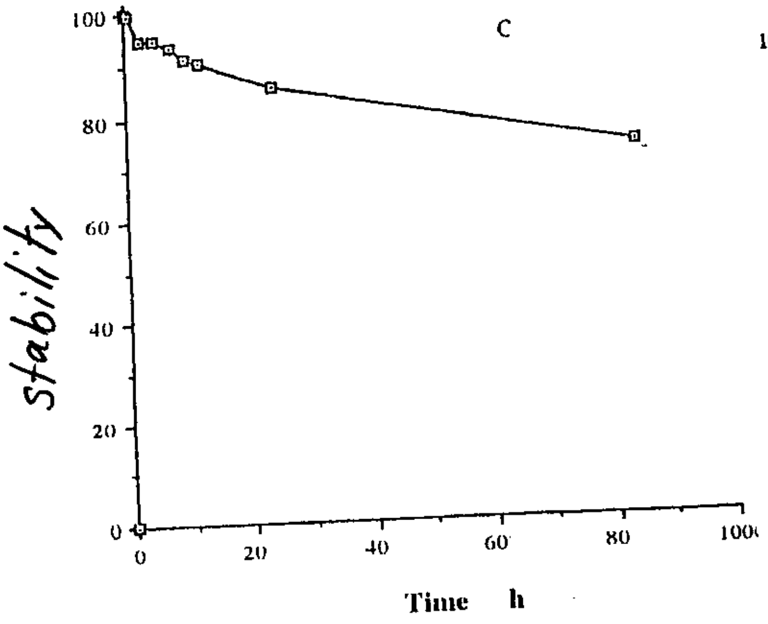


Fig. (24c): Stability of β-glucosidase enzyme.

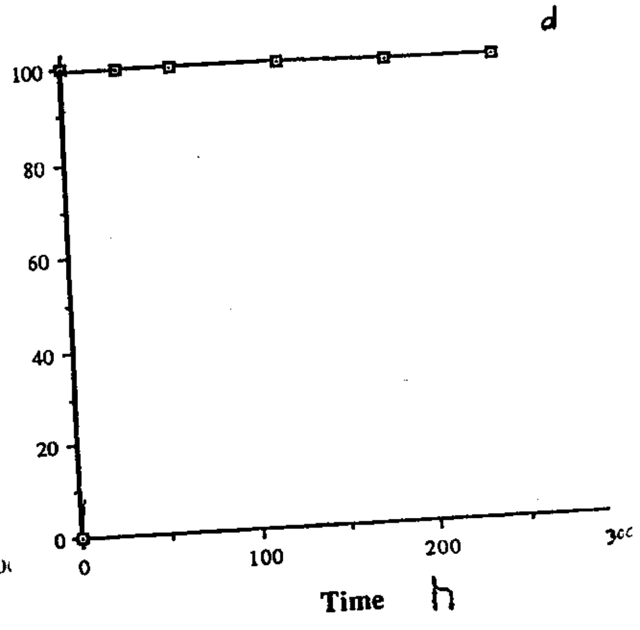


Fig. (24d): Stability of immobilized glucose isomerase a stabilizing and protecting microenviroment, i.e. enzymes bound to matrix (Mosbach,1976). With regard to activity, immobilized enzymes generally showed less specific activity than the native enzyme. However, recently more sophisticated methods being developed, for minimizing the losse in activity.

Nowadays, various immobilization technique and support materials are used in order to find the optimal immobilization technique and support for one particular enzyme. The enzyme β-D-glucosidase from Aspergillus niger has been immobilized through its carbohydrate moiety on 6 different support materials. Concanavalin A-sepharose (con A-sepharose, CAS), cyanogen bromide activated sepharose (CNBr- sepharose), calcium-Alginate gel, polyacrylamide gel (entrapped), and form a membrane with bovine serum albumn (by cross-linkage) were used. Such a systematic study might be helpful in finding the most convenient support which has the highest stability and proper enzyme activity after its attachment with support. The properties of the different immobilized preparations of β-D-glucosidase 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 supporting materials.

As shown in Table (23) β-D-glucosidase was bounded with all the tested different supports but with different degrees, such property is designed as protein loading capacity. The highest loading capacity was found in con A-sepharose, since no enzyme 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 amounted to 98.0%.

Similar case was also noticed in bovine serum albumin but with loss activity of the resulted complex and consequently the retention activity in this case was 95.2%. On the other polyacrylamide gel showed the lowest bounded material which was accompanied with the lowest retention activity, i.e. Ca 57.0%. However, two supports showed a very reasonable results, CNBr-sepharose and sand. The first showed a retention activity of about 88.0% and Ca 80.0% for the second respectively. The decrement in the activity of the immobilized β-D-glucosidase enzyme might be attributed to the bounded active sites of this enzyme with the support which simply affect the whole body of the enzyme itself and/or the bound β-D-glucosidase has a conformational alteration that affects the active sites (Saad et.al., 1991). The high adsorption phenomenon of β-D-glucosidase enzyme on both con A-sepharose and CNBr-sepharose indicated the glucoprotein nature of this enzyme (Woodward and Wohlport ,1982). The noticed decrease in the retention activity of the enzyme preparations with calcium alginate (Table, 23) might be attributed to the liankage of the enzyme with gel.

Tanaka, et al., (1984) stated that immobilization using calcium alginate gel is inapplicable to enzymes. But the pore size of the gel formed is so large that enzyme leak out from the support even enzyme molecular weights exceeds $3X10^5$.

Table(23): Preparations of immobilized β-D-Glucosidase enzyme.

Type of support	β-D-glucosidase Units*				
	Added	In Washings	Bound maximum A	Activity of compex B	Retention activity B/A%
Concanavalin A-separose	55.0	----	55.0	54.0	98.0
CNBr-Activated sepharose	84.0	27.0	57.0	50.0	87.7
Polyacrylamide gel	27.0	12.0	15.0	8.5	56.7
Calcium alginate	55.0	23.5	31.5	23.9	74.6
Sand	84.0	16.0	68.0	54.0	79.4
Bovine serum Albumin	84.0	----	84.0	80.0	95.2

* Units refers to the amount contained in 0.1 ml suspension of immobilized enzyme

Table(24): Properties of native and immobilized β-D-glucosidase preparations at 50 °C and pH (4.5 - 4.8).

Type of support	pH maximum stability	Km mM	Vmax mM/L/min
Native enzyme	3.3-5.4	0.45	201.0
Concanavalin A-sepharose	3.3-5.4	0.40	192.3
CNBr-Activated sepharose	3.3-5.7	0.55	181.1
Polyacrylamide gel	3.3-5.4	0.35	68.0
Calcium alginate	3.3-5.4	1.80	161.3
Sand	3.3-5.1	----	----
Bovin serum Albumin	3.3-5.1	----	----

4.5.1. Kinetic studies of the immobilized enzyme with different supports:

Kinetic studies of β-D-glucosidase enzyme immobilized on different supports are introduced in Table (24). The maximum velocity (Vmax) and Michaelis-Menten constant (K_m) of the immobilized β-D-glucosidase on different supports were determined at 50 °C and at pH (4.5-4.8), and compared with native enzyme (unimmobilized using cellobiose as a substrate. However substrate concentration is one of the most important factors which affects the velocity of the enzyme reaction. The rate of most enzyme reactions increased up to a certain point with increasing concentration of substrate. The native enzyme showed K_m value of 0.45 mM which was decreased after immobilization to 0.40 mM and 0.35 mM for concanavalin A-sepharose and polyacrylamide gel, respectively .

Such phenomenom simply means that the affinity between the immobilized enzyme and substrate was high enough and concequently Km decreased. However, the decrease in the K_m of an enzyme on immobilization can have practical advantages since the reaction will be faster at lower concentration of substrate (Woodward,1985).

On the contrary, the K_m values for the two other supports calicum alginate and CNBr-sepharose were increased which simply means a less affinity between the immobilized enzyme and the support. However, the increment in K_m value was two-fold in the case of calcium alginate compared with that of CNBr-sepharose,i.e. 1.80 and

0.90 mM, respectively. Consequently, an increase in Km value on immobilization means that a higher substrate concentration is required to achiere the same of reaction observed with the free enzyme.

In spite of the low value of Km obtained with the immobilized enzyme on polyacrylamide gel support (0.35 mM) its maximum velocity (Vmax) was also very low 68.0 mM/L compared with other reaction velocities. Con A-separose and CNBr-sepharose showed the highest Vmax 192.3 and 181.1 mM/L respectively.

The pH stabilities of the immobilized β-D-glucosidase enzyme on different supports were estimated with regard to pH degrees. The immobilized enzyme on CNBr-sepharase showed more stability at higher pH range 3.3-5.7. However, native enzyme and most other immobilized enzymes showed a pH stability ranged from 3.3-5.4.

4.5.2. Stability of native and immobilized enzymes:

The stabilities of different preparations of immobilized enzyme were assayed at 50^{o}C, pH4.8 and showen in Table (25) . The relative activity of each immobilized technique were determined at different periods of incubation and all compared with native enzyme. The decay in activity can be correlated with the time of incubation. CNBr-sepharose support after enzyme incubation was most stable one compared with the native enzyme, relative activity was 96.7% after 120 h.

Table(25): Stability of native and immobilized β-D-glucosidase enzyme.

Type of Support	Relative activity (%)									
	Time of incubation (h)									
	0	1	2	3	4	24	48	72	96	120
Native enzyme	100	98.7	98.1	96.9	95.8	94.7	94.7	93.6	91.2	90.6
concanavalin A-sepharose	100	98.2	97.4	95.4	94.1	92.6	91.9	90.7	88.3	86.5
CNBr- Activated sepharose	100	99.4	98.8	98.9	98.7	98.4	98.3	98.0	97.7	96.7
Polyacrylamide gel	100	97.8	96.7	95.6	94.4	87.8	86.7	85.4	83.3	77.8
Calcium alginate	100	95.3	92.4	89.6	83.3	71.8	68.4	63.2	60.4	58.2
Sand	100	99.1	98.0	97.9	97.8	95.2	95.4	94.8	90.3	86.3
Bovine serum	100	99.1	99.5	98.4	98.7	98.2	97.4	97.2	96.0	94.6

Calcium alginate gel polyacrylamide as immobilization materials showed the lowest enzyme stability. The relative activities obtained after 120h were 58.2% and 77.8% respectively. However, such decrement in those values could be explained on the hypothes introduced by Tanaka, et.al. (1984). He stated that the rapid decrease in the relative activity of the immobilized enzyme owing to mass transfer on the surface of the gel. He concluded that such phenomenon could artificially controlled by coating the surface of the gel with polymers having branching structures. Such polymers will bind electrostatically to carboxylate ions in alginate gel to shrink the pore size of the gel support and form an enzyme-gel bridge leading to an expand capacity of the gel. Sand as a solid support and very cheap showed a very resonable results, since it retained about 86.3% of the total reative activity after 120 h.

After complete screening of the support materials as mentioned before, two main supports were choosen to continue this study. These choice was carried owing to its inexpensive prices and obtained criterion in the last 3 Table (23, 24, 25).

CNBr-sepharose and sand showed a reasonable retention activity, Km values, Vmax values and rather good stability. Therefore different preparations of β-D-glucosidase immobilized by CNBr-separose and sand were used in a continous study. It is very important to identify the circumestances for each immobilized enzyme with specific support. The optimum factors influence the immobilized enzyme reaction and stability were determined for both CNBr-sepharose and sand, i.e.

substrate concentration, pH, temperature, incubation and enzyme concentration.

4.5.3.Kinetic behaviour of native and immobilized enzyme:-

The kinetic behaviour of the native and immobilized β-glucosidase enzyme on CNBr-sepharose was studied as shown in Table (26) and Fig.(25a,25b). Different substrate concentrations were used from 0.68 mM till 10.00 mM. of cellobiose. The immobilized enzyme on the 2 supports was compared with its native enzyme to study the effect of immobilization on different parametess.

In general the obtained glucose after hydrolysis of cellobiose by the native enzyme was in higher quantities when compared with that obtained from the immobilized one. when the substrate concentration was very low, 0.68 mM cellobiose, the difference of the resulted glucose content from native enzyme and the immobilized was very high, 161.3 mM/L and 101.5 mM/L respectively.

This simply means that the hydrolysis reaction at lower concentration for the immobilized enzyme resembles only 58.9% of the native one. When the substrate concentration was raised till 5.40 mM, the difference between both native enzyme and immobilized enzyme was decreased, 194.0 mM/L and 181.1 mM/L. Such data might be indicate that the most suitable substrate concentration for the immobilized enzyme is about 5% as shown in Table (26). Also, it can be noticed from the same Table (26) regarding the reaction velocity criteria that it goes almost paralell with the obtained glucose for both native and immobilized enzymes. On the other hand, the Michaelis constant (K_m) values for both native and immobilized were calculated. K_m value of the native enzyme was 0.45 mM while that of the immobilized enzyme was little higher 0.55 mM. However, K_m values of both native and immobilized enzymes were emphasized by determing Lineweave and Burk plot (1954) as shown in Fig. (25b). The obtained values using 1/[S] againist 1/v were almost equal to obtained in the calculation of K_m values, as shown in Fig.(25a) by half way of the experimental curve. The Michaelis constant (K_m) is one of the most useful and important paramenter in understanding the kinetic behaviour of enzymes. Handa, et. al. (1982). Consequently, K_m for native enzyme was lower than that of the immobilized enzyme which might be attributed to the binding of CNBr-separose with β-D-glucosidase.

Such behaviour certainly leads to a decrease in the affinity between the substrate and the immobilized enzyme which gave an increase in K_m (Saad,1992). A detailed explanation for the decrease in reaction velocity and the quantity of evolued glucose when compared with the native enzyme were introduced by Goldstein (1976). He stated that the enzyme may be conformationally different when on the support, alternatively it may attached to the solid carrier in a way that would render certain parts of the enzyme molecule less accessible to substrate.

Also, the chemical nature of the support material may cause electrostatic or hydrophobic interactions between the matrix

Table(26): Effect of substrate concentration on the reaction velocity of native and immobilized β-glucosidose with CNBr-sepharose.

		Native enzyme			Immobilized enzyme		
substrate concentr-ation (S) mM	1/[S]	Glucose content mM/L	Reaction velocity (v)	1/v X10^{-2}	Glucose content mM/L	Reaction velocity (v)	1/v X10^{-2}
0.68	1.48	161.3	116.4	0.86	101.5	77.9	1.28
1.35	0.74	166.0	145.5	0.69	120.1	108.7	0.92
2.70	0.37	174.0	166.3	0.60	123.8	135.8	0.74
5.40	0.19	194.0	179.1	0.56	181.1	155.2	0.64
10.0	0.10	201.0	192.3	0.52	165.9	166.1	0.60

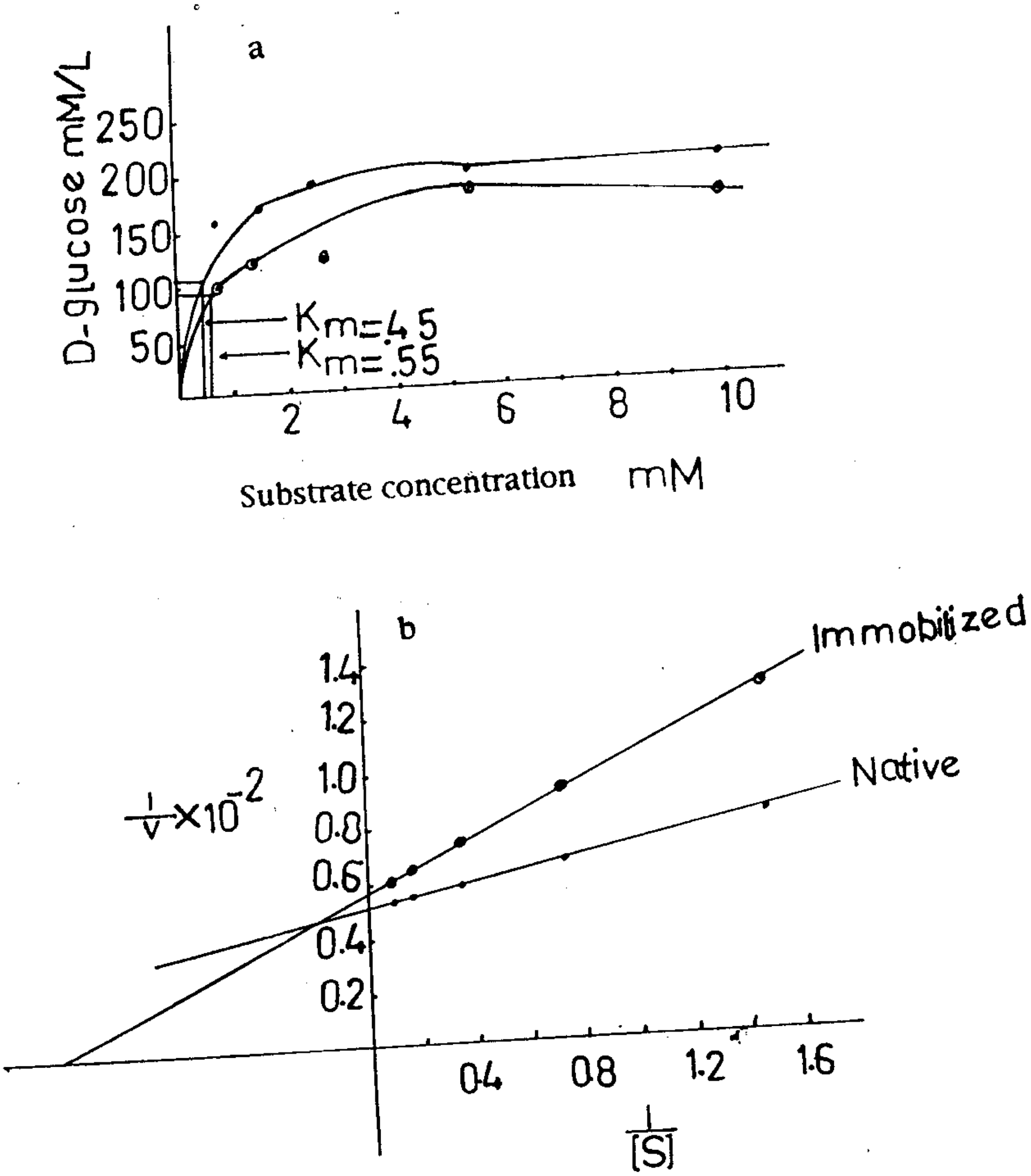


Fig. (25): Relationship between substrate concentration and D-glucose content on native and immobilized β–glucosidase enzyme.

(immobilized enzyme + support) and other low molecular weight species. The latter are present in the reaction medium leading to a certain decrease of the immobilized enzyme activity towards the substrate itself. A third effect would arise from diffusional resistances to the translocation of substrate and/or product.

4.5.4. Effect of pH on the activity of the immobilized β-glucosidase preparations:

The pH activity curve of native and immobilized enzyme on CNBr-Sepharose and sand is shown in Table (27) and Fig.(26). Different pH's values ranged from 3.3 to 6.0 using 0.05 M acetate buffer were established versus the enzymes activity which measured as μmol glucose/min/unit of enzyme. The maximum activity was at pH 4.5 for both the native β-glucosidase and the immobilized enzyme on CNBr-sepharose. Consequently, pH 4.5 could be considered the optimum pH for native and CNBr-sepharose immobilized enzyme. The β-glucosidase enzyme immobilized on sand as support exhibited some broadening in the pH activity. It showed a maximum activity (45.7 μmol glucose/min/1.0 g sand-enzyme complex) at optimum pH 4.8.

The optemium pH in the case of sand support was shifted towards the alkaline solution when compared with the other enzyme immobilized on CNBr- sepherose or with native one, pH4.5 and pH4.8 respectively. However, Katchalski et. al., (1971) introduced an explanation for such phenomena when the enzyme is attached to a negatively charged matrix. It seems that the low values of optimum pH (4.5-4.8) clearly indicated the importance of such acid media

Table(27): Effect of pH on the activity of β-glucosidase preparations.

pH'S	Activity µmol glucose/min/ enzyme unit		
	Native enzyme	CNBr-separose*	Sand**
3.3	40.1	26.2	23.1
3.6	45.3	32.0	25.2
3.9	46.1	36.4	26.1
4.2	47.2	44.9	37.7
4.5	55.1	46.1	42.3
4.8	51.2	36.1	45.7
5.1	43.9	33.1	39.2
5.4	43.7	31.0	28.0
5.7	43.0	23.2	21.4
6.0	41.7	20.1	19.6

* Activity refers to the amount contained in 0.1 ml suspenion of CNBr-sepharose enzyme complex.

** Activity refers to the amount contained in 1.0 g of sand-enzyme complex.

(acetate buffer, 0.05 M) to fit the nature of the active site of the β-D-glucossidase enzyme.The latter which found on the matrix attracting a then "film" of positive hydrogen ions, thereby creating a microinvioment for the bound enzyme, that has a highest hydrogen ion concentration than the concentration in the surrounding solution where the pH is actually measured (i.e. higher pH values).

4.5.5.Effect of temperature on the activity of immobilized β-D-glucosidase preparations:

The effect of temperature on the initial rate of cellobiase hydrolysis catalysed by the native and immobilized preparations of β-D-glucosidase was measured and shown in Table (28) and Fig.(27). Nine different temperatures, i.e. 30-35-40-45-50-55-60-65 and 70°C were used to investigate the optimum temperature of the native and immobilized enzyme on CNBr-separose and sand.

The experiment were carried out at pH 4.5 for native and immobilized enzyme on CNBr-sepharose, pH 4.8 for immobilized enzyme on sand and the reaction period of each experiments was 30 min. The maximum activity of both the native enzyme (55.8 μmol glucose/min/0.1ml enzyme) and the immobilized enzyme on CNBr-sepharose (48.9 μmol glucose/min/0.1ml suspension of matrix enzyme) was established at 50°C. While, the immobilized enzyme on sand yielded the maximum activity (46.2 μmol glucose/min/1.0 g immobilized enzyme on sand) at temparture equals to 55°C. Such results are in agreement with that reported by Sundstrom, et.al.,(1981), Woodward and Wohlpart (1982) and Woodward (1985).

However, raising the temperature more than 55°C was found to lead to a serious decrease in the activity due to the denaturation of the enzyme protein.

4.6. Enzymatic saccharification of treated cellulosic materials:

Saccharification processes include the stages of conversion of extracted cellulose from different pretreated lignocellulosic materials into mixture of mono- and oligomers containing a high percent of D-glucose. These processes were carried out by using the mixture of enzumes i.e. cellulase (celluclast 1.5 L) and β-glucosidase (Novozym 188) enzymes under optimal conditions (0.93 FPU, 0.46 CMCase units cellulase and 55.8 units for β–glucosidase).

The saccharification process was applied on different extracted cellulose from lignocellulosic materials i.e. wheat straw, corn cobs, corn stalks and sugarcane bagasse, at concentrations: 10, 20, 25 and 50 g/L by utilizing 0.05M acetate buffer solution and pH 4.8. The experiments were achieved in a shaking incubator at 50 °C and 200 rpm. Also, enzymatic saccharification were accomplished for different periods ranged between 1 to 96 h. Since this point is very important from the economical stand point of view, because any decreasing of the time required to saccharification process, simply means great saving in the final expenses of product. The obtained results of saccharification processess for different treated lignocellulosic materials are shown in Table (29), and Fig.(28a, 28b, 28c, 28d).

Table(28): Effect of Temperatur on the activity of β-glucosidase preparations.

Temperature	Activity [μ mol glucose/min enzyme unit		
oC	Native enzyme	CNBr-separose*	Sand **
30	41.9	41.2	34.3
35	42.6	42.0	36.3
40	48.8	43.3	40.2
45	49.8	44.7	41.8
50	55.8	48.9	45.3
55	46.8	45.6	46.2
60	39.4	43.9	44.2
65	20.1	39.3	37.4
70	16.2	33.4	28.2

* Activity refers to the amount contained in 0.1 ml suspenion of CNBr-sepharose enzyme complex.

** Activity refers to the amount contained in 1.0 g of sand-enzyme complex.

From these results, it can be easily show that the highest values of saccharification process were 70.0, 72.0, 79.0, 72.0 and 75.0% for extracted cellulose of wheat straw at concentrations: 10,20,25 and 50/L, respectively. The period of reaction was 96h except the latter one was incubated for 48h only. Also, the obtained data indicate that lignocellulosic corn cobs material gave the highest saccharification process values: 82.0, 86.0, 88.0 and 89% at the abovementioned concentrations and periods 48,48, 72 and 96h, respectively. On the other hand, these values equalled to 71.0, 72.0, 81.0 and 80% in case of utilizing the extracted cellulose of corn stalks at periods 96, 96, 36, and 96h. Finally, the latter one i.e. extracted cellulose of sugarcane bagasse achieved highest values equivalent to 73.0, 76.0,77.0 and 79.0% after incubation time for 72h for all the abovementioned concentrations.

In general, from the above results it might be concluded that the maximum values of saccharification process were 79.0, 89.0, 81.0 and 79.0%, respectively for extracted cellulose of wheat straw, corn cobs, corn stalks and sugarcane bagasse at concentrations 25,50, 50 and 50 g/L with incubation periods 96,96, 36 and 72h. Also, the obtained data is conscident with that reported by Szczodrak (1988) and Ooshima, et. al. (1991). However, it has to be mentioned in this aspect that the extracted cellulosic materials from corn cobs achieved maximum saccharification values higher than acquired by other substances. Such result might be attributed to that the lignocellulosic materials of corn cobs contained a higher content of cellulose i.e. 78.6% and less amount of hemicellulose i.e. 10.9% than that found in extracted lignocellulosic materials of wheat straw, corn stalks and sugarcane bagasse as show in Table (2).

Also, it is important to metion that Gusakov, et.al. (1992) reported that the most negative factors in hydrolysis of lignocellulosic materials by cellulase enzymes, was the inhibition of these enzymes by cellobiose and glucose sugars. Also, they found that a significant effect was produced by enzyme inactivation, to a lesser extent, by nonspecific binding of cellulose to lignin, leading to a decrease in the efficiency of hydrolysis process. Such explanation can be introduced to interpret why the saccharification process did not reach its completeness with different lignocellulosic materials under investigation. And also, how to a large extent, the obtained results are reasonable for achievment the saccharification process under the abovementioned optimum conditions, respectively.

4.7. Monomerization and isomerization of saccharified lignocellulosic materials:

The aim of this process is firsty to increase the percemt of D-glucose of saccharified cellulosic materials by the conversion of cellobisoe disaccharide to D-glucose monomer.

Secondly, the resulted syrup from saccharification was enzymatically isomerized by glucose isomerase enzyme (sweetzyme type T). The resulted mixture of monomers and oligomers syrup, which was produced from enzymatic saccharification of different cellulosic materials i.e. wheat straw, corn cobs, corn stalks and

Table(29): Enzymatic saccharification of pretreatment of different extracted cellulose.

Substrate	Wheat straw				Corn cobs				Corn stalks				Sugarcane bagasse			
con.	10 g/L	20 g/L	25 g/L	50 g/L	10 g/L	20 g/L	25 g/L	50 g/L	10 g/L	20 g/L	25 g/L	50 g/L	10 g/L	20 g/L	25 g/L	50 g/L
Time (hr)																
1	38.0	45.0	54.0	61.0	53.0	55.0	57.0	60.0	45.0	47.0	54.0	55.0	50.0	58.0	59.0	59.0
2	41.0	47.0	59.0	62.0	57.0	63.0	62.0	65.0	47.0	50.0	57.0	62.0	53.0	59.0	66.0	63.0
12	45.0	50.0	65.0	66.0	60.0	67.0	67.0	65.0	48.0	59.0	67.0	68.0	57.0	60.0	67.0	72.0
24	47.0	51.0	67.0	70.0	65.0	75.0	80.0	77.0	50.0	64.0	75.0	71.0	60.0	63.0	70.0	72.0
36	50.0	60.0	71.0	71.0	68.0	83.0	84.0	80.0	54.0	68.0	81.0	73.0	66.0	72.0	74.0	75.0
48	58.0	63.0	74.0	75.0	82.0	86.0	87.0	82.0	57.0	70.0	71.0	76.0	68.0	74.0	76.0	77.0
72	63.0	67.0	76.0	73.0	79.0	83.0	88.0	86.0	63.0	72.0	73.0	79.0	73.0	76.0	77.0	79.0
96	70.0	72.0	79.0	72.0	77.0	81.0	86.0	89.0	71.0	72.0	78.0	80.0	72.0	75.0	75.0	74.0

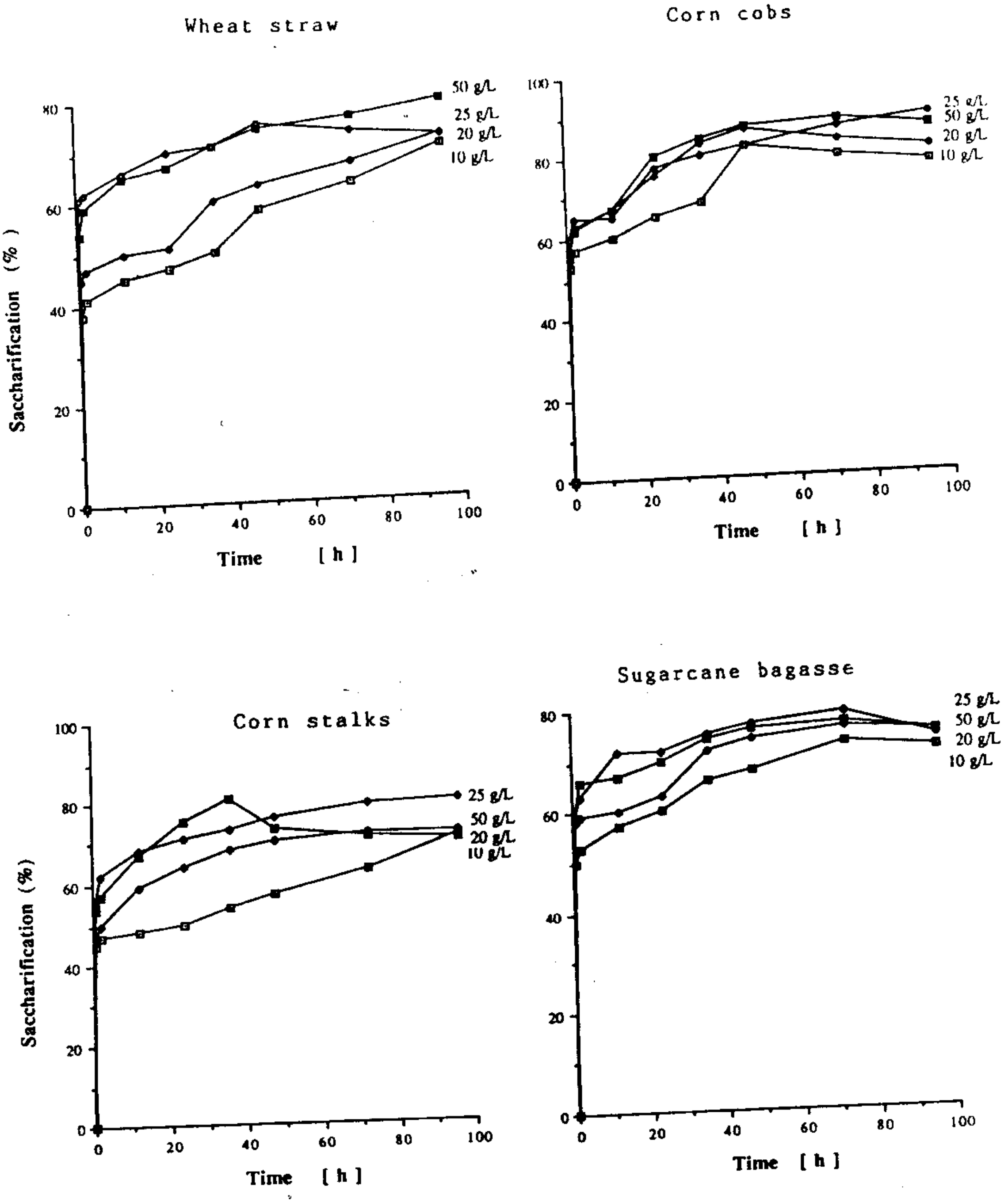


Fig.(28): Enzymatic saccharification of lignocellulosic materials.

sugarcane bagasse were used as substrate for β-glucosidase enzyme was used in two immobilized forms i.e. cyanogen bromide-activated sepharose (CNBr-Sepharose) and sand complexes.

It is important to carry out the purification of resultant glucose syrup before the accomplishment of isomerization process since, some impurities affect the stability and activity of glucose isomerase enzyme . Purification was carried out with activated carbon powder, followed by ion exchange chromatography cation resin (Amberlite IR 120-A) and anion resin (Dowex 1X8-400) to eliminate impurities, e.g. peptides, amino acids and ions of salts which inhibit or inactivate the immobilized glucose isomerase (Sweetzyme type T) enzyme.

The purified glucose syrup preparations were transferred quantitatively to a column containing a mixture of $MgSO_4.7H_2O$ ($10X10^{-3}$ M) and glucose isomerase enzyme (sweet zyme type T). The flow rate was controlled at 0.1 ml/min. using a proportionating pump for recycling the isomerization process for 12,24,48,72, and 96 hrs. The other process paramters i.e. temperature, pH, enzyme concentration ..... etc. were adjusted at the optimum conditions according to the abovementioned obtained results. The results are shown in Tables (30, 31, 32, 33) .

From these results, it has been observed that the conversion values of glucose to its isomer i.e. fructose increased as the reaction time increased until reached its maximum i.e. 78.7, 70.0 and 72.3% after 96h, in case of completement of monomerization process by

Table(30): Wheat straw conversion to fructose through immobilized β-glucosidase followed by glucose isomerase.

Time h	Glucose content mM/L		Fructose content mM/L		Conversion (%)	
	Sand	CNBr-sepharose	Sand	CNBr-sepharose	Sand	CNBr-sepharose
0	72.8	75.5	0.00	0.00	0.00	0.00
12	17.3	16.8	25.0	18.1	34.3	24.0
24	12.5	11.1	31.5	33.4	43.3	44.2
48	8.4	6.6	41.7	45.9	57.3	60.8
72	4.3	3.6	40.8	57.0	56.0	75.5
96	2.5	1.8	39.8	54.5	54.7	72.2

Table(31): Corn cobs conversion to fructose through immobilized β-glucosidase followed by glucose isomerase.

Time h	Glucose content mM/L		Fructose content		Conversion (%)	
	Sand	CNBr-sepharose	Sand	CNBr-sepharose	Sand	CNBr-sepharose
0	89.6	98.8	0.00	00.0	00.0	00.0
12	54.4	68.9	18.5	14.5	20.6	15.0
24	53.5	34.5	27.8	43.8	31.0	44.5
48	25.9	19.2	38.5	60.1	43.0	62.8
72	11.9	13.5	41.7	72.3	46.5	73.2
96	4.9	11.0	51.0	77.8	57.9	78.7

Table(32): Corn stalks conversion to fructose through immobilized β-glucosidase followed by glucose isomerase.

Time h	Glucose content mM/L		Fructose content mM/L		Conversion (%)	
	Sand	CNBr-sepharose	Sand	CNBr-sepharose	Sand	CNBr-sepharose
0	88.4	93.2	0.00	0.00	0.0	0.0
12	61.9	45.1	7.70	11.50	8.7	12.3
24	38.2	22.0	25.20	29.27	28.5	31.4
48	25.1	17.8	32.00	33.00	36.4	35.4
72	18.1	9.7	53.00	48.70	60.2	52.2
96	11.9	3.6	54.96	64.90	62.2	70.0

Table(33): Sugarcane bagasse conversion to fructose through immobilized β-glucosidase followed by glucose isomerase.

Time	Glucose content mM/L		Fructose content mM/L		Conversion (%)	
	Sand	CNBr-sepharose	Sand	CNBr-sepharose	Sand	CNBr-sepharose
0	71.2	76.4	0.00	0.0	0.0	0.0
12	58.2	64.3	12.3	11.4	17.3	15.0
24	38.4	34.2	30.4	33.2	42.7	43.5
48	20.6	21.1	39.5	43.6	55.5	57.1
72	16.1	13.6	44.6	54.0	62.6	70.7
96	13.7	11.4	46.2	55.2	64.9	72.3

using CNBr-sepharose-β-glucosidase complex with saccharified extracted cellulose followed by isomeration process of corn cobs, corn stalks and sugarcane bagasse. while these values were 57.9, 62.2 and 64.9% by using sand-β-glucosidase complex for achievement the monomerization on the abovementioned substances. On the other hand, saccharified extracted cellulose of wheat straw a chieved it highest values i.e. 75.5 and 56.0% after 72 h, then the reaction rate began fell down. This point is very important from the economical point of view since decreasing the time required to isomerization process by almost 25%, simply means great saving in final expencies of the process. However, such point need to more investigated to come to conclusive results.

Generally, from the abovementioned results, it could be concluded that the maximum values of conversion process which performed by using CNBr-sepharose-β-glucosidase complex for monomerization, followed by isomerization process were higher than the obtained values of conversion in case of achievement of monomerization by using sand-β-glucosidase complex. Such results might be attributed to the differences in the stability of two forms of immobilized enzyme under the same conditions. It has been found that CNBr-sepharose-β-glucosidase preparation more stable than sand-β-glucosidase complex, Table (28). Therefore, the amount of glucose sugar which was produced during the monomerization process by CNBr-sepharose enzyme complex would be higher than that obtained by the other immobilized form. The isomerization values in case of