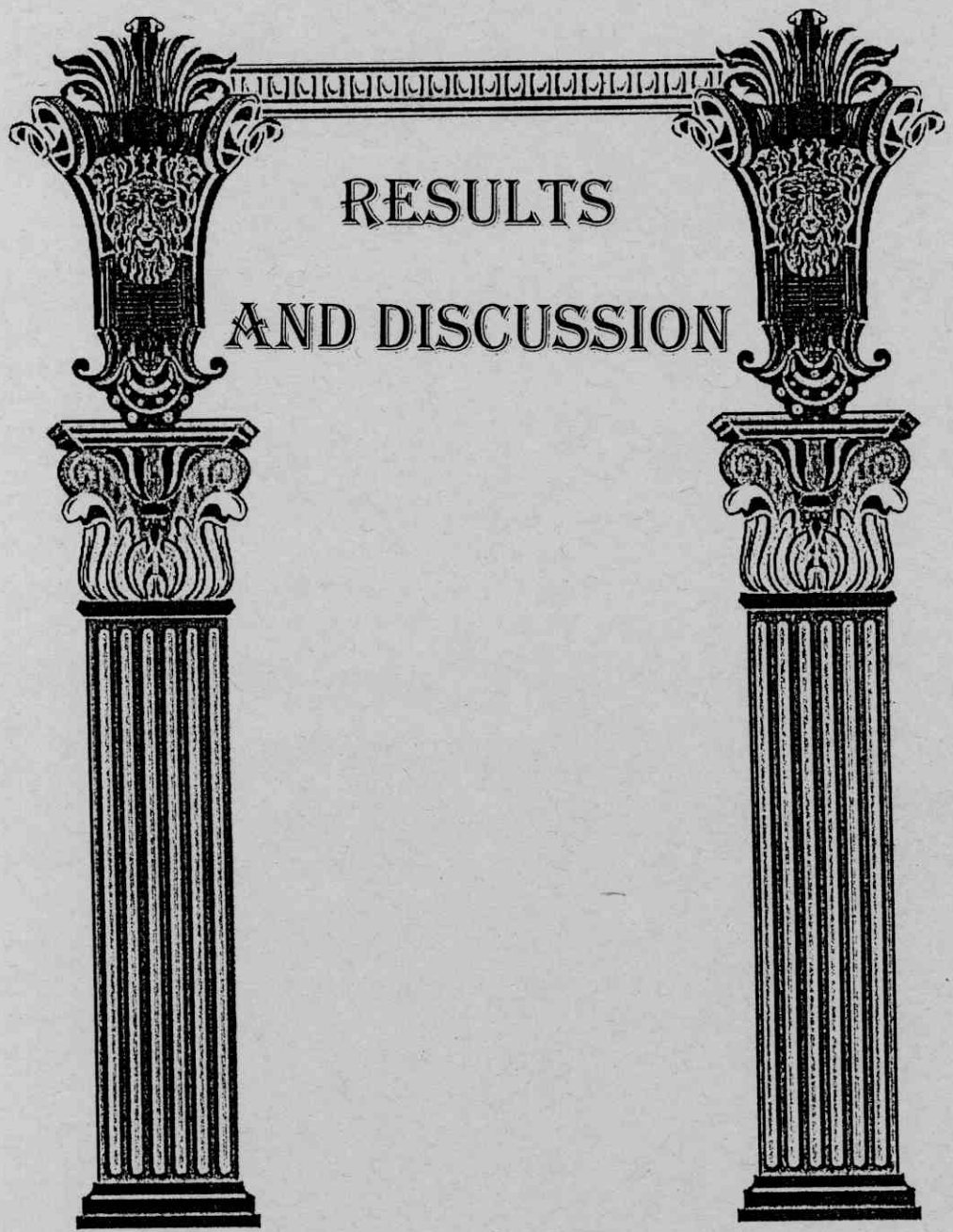

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1. Chemical composition and pretreatment of sugarcane bagasse :

As shown in Table (3), the obtained results showed that cellulose, hemicellulose and lignin are the main components while ash, crude protein and total lipids are occurred in lesser amounts in crude and extracted lignocellulosic residues under investigation.

The data in Table (3) show that, raw sugarcane bagasse contained 43.5% cellulose, 30.4% hemicellulose and 15.3% lignin. On the other hand, ash, crude protein and total lipids were found to be 5.2%, 3.1% and 1.5%) for sugarcane bagasse, respectively.

Bagasse offers numerous advantages in comparison to other crop residues because its low ash content. Also, in comparison to other agricultural residues, bagasse can be considered as a rich solar energy reservoir due its high yields and annual regeneration capacity. **(Ashok *et al.* 2000)**.

From the data presented in Table (3) it can be seen that, cellulose has bean increased to 71.7% and hemicellulose has been decreased to 15.5%. While lignin content was decreased from 15.3 to 5.8%, and ash from 5.2 to 4.2 in bagasse after pretreatment with NaOH. The NaOH has double role, its removes lignin thus increasing the accessibility of the cellulose and also, swells the fibre to make it more permeable to cellulose. It leads to enlargement of the inner surface area of substrate

Table (3): Chemical composition of crude and pretreated sugarcane bagasse.

Source / Components	Sugarcane bagasse	
	Crude %	Pretreated extract %
Cellulose	43.5	71.7
Hemicellulose	30.4	15.5
Lignin	15.3	5.8
Ash	5.2	4.2
Crude protein	3.1	1.5
Total lipids	1.5	0.4

particles, accomplished by partial solubilization and / or degradation of hemicellulose and lignin and the fractionation of the three components and opening of cellulose structure. **Fan and Lee (1983)** noticed the same observation.

Pretreatment of sugarcane bagasse has often been found useful to improve its digestibility and easy access for microbial attack by removing core and noncore lignin fractions, **Doran *et al.* (1994).**

In general, the pretreatment of lignocellulosic materials aimed to obtain high sugar yields and these treatments depend on the nature of substrate and the microbial system employed. Alkali pretreatment solubilized most of the hemicellulose fraction and particularly some depolymerized lignin leaving a residue more effective for enzymatic digestibility, **David *et al.* (1989).**

The previous results indicated that pretreatment with (10%) NaOH at 80°C for 3h was most efficient for the pretreatment of all the lignocellulosic substrates **Foda (1994)**.

These results are in agreements with those reported by **Gabr *et al.* (1991b), Abd El-Malak (1995) and Saad *et al.* (1999).**

4.2. Production of cellulase and hemicellulase enzymes :

Screening tests for cellulolytic and hemicellulolytic activities of *Trichoderma harzianum* and *Aspergillus niger* were achieved by growing them on different media (Media I, II, III and IV) with cellulose, hemicellulose and carboxymethyl

cellulose (CMC), xylan as carbon sources. Fermentation processes were carried out at 30°C for 9 days. The products were analyzed for their protein content.

Data in Table (4) refer to the effect of different media and fungi growth on the production of cellulase and hemicellulase enzymes. The highest values of cellulolytic activities were 178 and 63 μM/L/min in medium III with *Aspergillus niger*, 166 and 22 μM/L/min in medium I with *Trichoderma harzianum*.

On the other hand, the maximum activities of the produced hemicellulase were obtained by using *Aspergillus niger* and *Trichoderma harzianum* with medium III after 9 days (21 and 19 μM/L/min), respectively.

The fungi filtrates of *A. niger* and *T. harzianum* were analyzed for their protein enzymes. The soluble enzyme protein in the filtrate of *A. niger* reached the values of 0.326, 0.188, 0.387 and 0.254 mg protein /ml in media I, II, III and IV, respectively. While the soluble protein enzyme in the filtrate of *T. harzianum* reached the values of 0.373, 0.315, 0.151 and 0.071 mg /ml in media I, II, III and IV, respectively. A similar results was obtained by **Chanal (1988)**.

Table (4): Activities of cellulase and hemicellulase enzymes produced by two fungi and different media.

Enzymes / Type of medium	Fungi	Activity of cellulase (µM/L/min)		Activity of hemicellulase (µM/L/min)
		CMCase	FPase	
Medium I	*Asp.*	158	51	*
	Tri.	166	22	*
Medium II	*Asp.*	75	16	9.0
	Tri.	145	44	5.0
Medium III	*Asp.*	178	63	21
	Tri.	47	11	19
Medium IV	*Asp.*	97	26	20
	Tri.	26	1.0	12

Medium I: Mandel's & Weber 1969.
Medium II : Park et al 2002.
Medium III : Modified of Park medium.
Medium IV : Rice straw only (new medium).
* : Non activity.

4.3. Effect of different parameters on the activity and reaction velocity of cellulase and hemicellulase enzymes :

The major goal of this trial is to study the influence of various parameters such as temperature, pH, enzyme concentration, and substrate concentration on the reaction activity of cellulase and hemicellulase enzymes. The present results illustrated the optimal conditions of cellulase and hemicellulase enzyme activities. Also, such systematic study may help to a great deal in minimizing the enzyme cost in each individual process.

4.3.1. Effect of temperature on the reaction activity of enzymes :

The effect of temperature on the enzyme activity is very complex and interrelated with other variables such as pH, buffer system and substrate concentration. There are two forces acting simultaneously but in opposite direction. 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. The higher of temperature, the more will be inactivation dominant, **Sheldon and William (1996).**

Ten different temperatures, i.e. 34, 38, 42, 46, 50, 54, 58, 62,66 and 70°C were chosen to investigate the optimum temperature of cellulase and hemicellulase enzymes produced by *Aspergillus niger* and *Trichoderma harzianum*. The experiments were carried out at optimum pH for carboxymethyl cellulose

(CMC), extracted cellulose, xylan and isolated hemicellulose from sugarcane bagasse with 1% (w/v) substrate concentration and the incubation period was 30 min.

Table (5) and Fig. (3 and 4) show that the reaction activity of cellulase reached its maximum 164 μM/L/min with *Aspergillus niger* and 175 μM/L/min with *Trichoderma harzianum* at temperature 50°C for carboxymethyl cellulose. On the other hand, the maximum reaction activity of cellulase for extracted cellulose was 92 μM/L/min with *A. niger* and 101 μM/L/min with *T. harzianum* at the same temperature.

Table (6) and Fig. (5 and 6) show that the reaction activity of the produced hemicellulase reached its maximum 19 μM/L/min with *Aspergillus niger* and 19.7 mM/L/min with *Trichoderma harzianum* at temperature 50°C for xylan. On the other hand, the maximum reaction activity for isolated hemicellulose was 18.5 μM/L/min with *A. niger* and 17.8 μM/L/min, with *T. harzianum* at the same temperature. The optimum temperature for cellulose and hemicellulase is in line with those obtained by **Huitron and Kirchner, (1996).**

4.3.2. Effect of pH on the reaction activity of enzymes :

The pH will affect the efficiency of an enzyme and usually there is 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, enzyme concentration, and temperature, which were set constant mostly at optimum.

Table (5): Effect of temperature on the reaction activity of cellulase enzyme with CMC and extracted cellulose from sugarcane bagasse.

Substrate / Temperature (°C)	Activities (µM/L/min)			
	CMC		Extracted cellulose	
	Asp.	*Tri.*	*Asp.*	*Tri.*
34	71	68	28	31
38	100	93	31	49
42	119	117	50	55
46	142	155	65	80
50	164	175	92	101
54	140	158	76	87
58	131	157	61	74
62	130	130	46	62
66	99	120	34	46
70	86	94	29	44

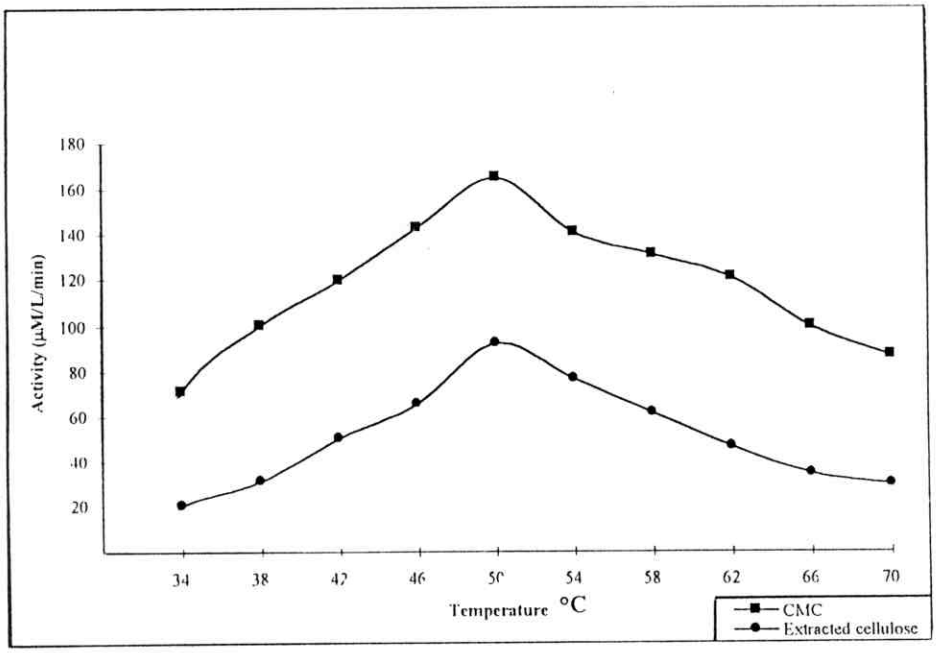

Fig (3) : Effect of temperature on the reaction activity of cellulase by *Aspergillus niger*.

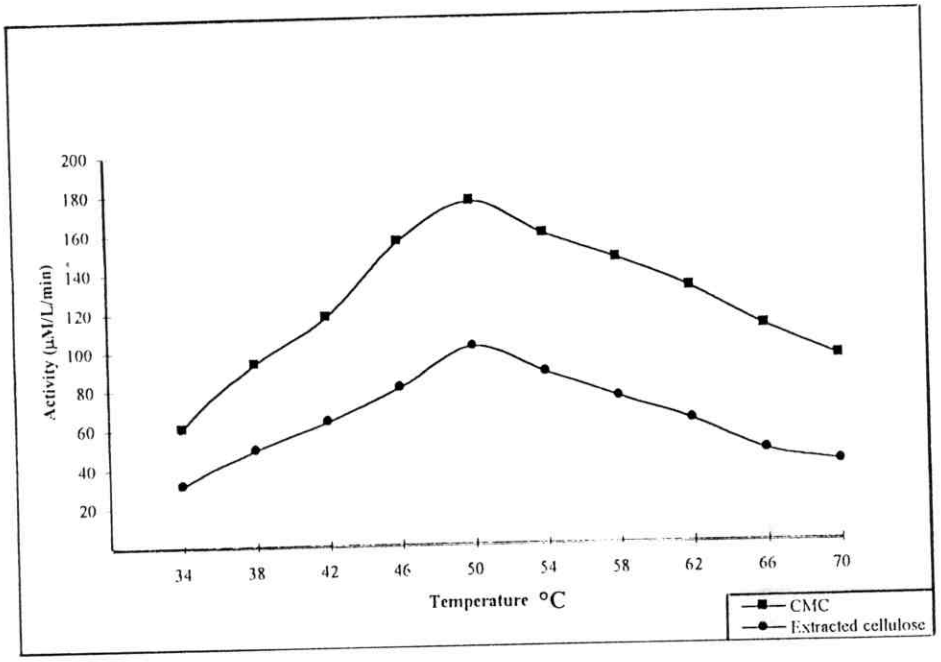

Fig (4): Effect of temperature on the reaction activity of cellulase by *Trichoderma harzianum*.

Table (6): Effect of temperature on the reaction activity of hemicellulase enzyme with xylan and isolated hemicellulose from sugarcane bagasse.

Substrate / Temperature (°C)	Activities (µM/L/min)			
	xylan		Isolated hemicellulose	
	Asp.	*Tri.*	*Asp.*	*Tri.*
34	7	9.9	5.4	5.9
38	8	12.7	6.4	8.4
42	9	16.1	8.3	11.4
46	14	17.8	12.6	12.9
50	19	19.7	18.5	17.8
54	18	17.7	17.1	16
58	16.3	16.5	13.9	13.5
62	14.6	15	13.6	11.8
66	13.3	12.1	12	11
70	8.8	10.9	8.6	8.2

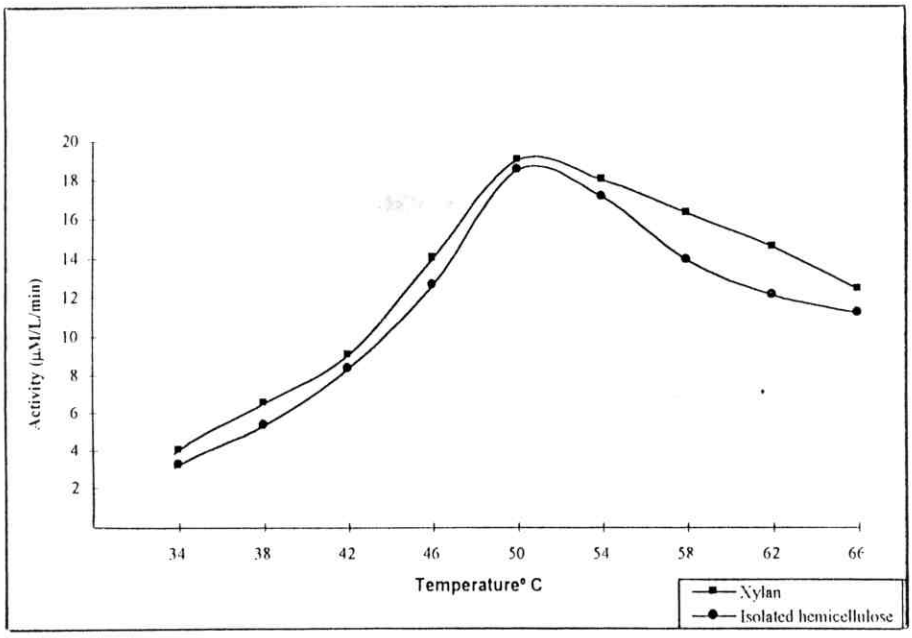

Fig (5) : Effect of temperature on the reaction activity of hemicellulase enzyme by *Aspergillus niger*.

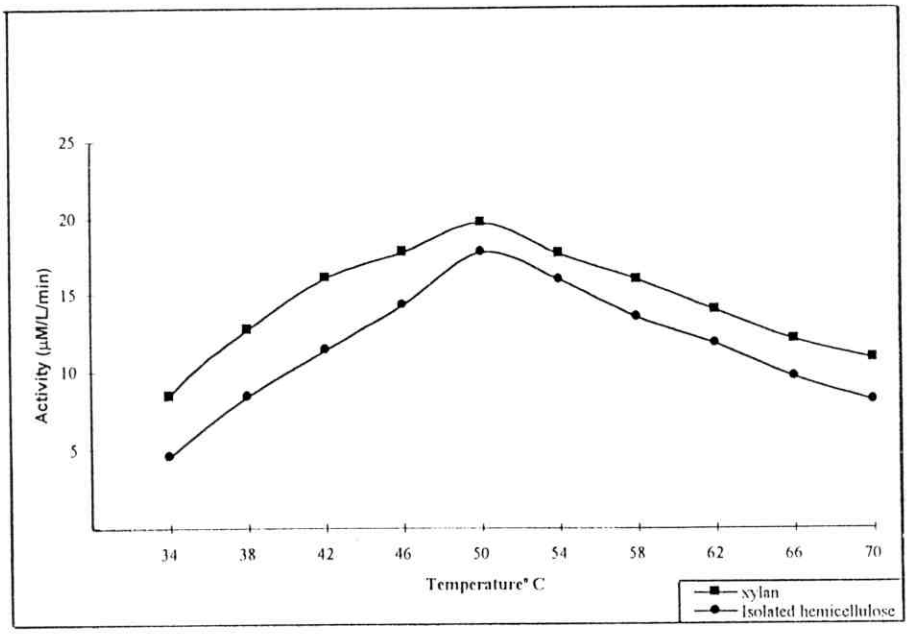

Fig (6): Effect of temperature on the reaction activity of hemicellulase enzyme by *Trichoderma harzianum*.

The effect of pH on the reaction activity of cellulase and hemicellulase enzymes were tested at different pH values with (0.05 mM) acetate buffer on different substrates of carboxymethyl cellulose (CMC), extracted cellulose, xylan and isolated hemicellulose from sugarcane bagasse.

Nine solutions of these substrates were adjusted to pH values 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, 5.0 and 5.2 with 0.05 μM acetate buffer. The obtained resulted are illustrated in Table (7 and 8) and Fig. (7-10).

From the obtained results it can be noticed that the maximum activity of cellulase enzyme on carboxymethyl cellulose (CMC) was 161 μM/L/min with *A. niger* and 171μM/L/min with *T. harzianum* at pH 4.8 which is more acidic than that of treated lignocellulosic materials under investigated.

The optimum activity of this enzyme on extracted cellulose from sugarcane bagasse was found to be at pH 4.8 and the maximum reaction activites were 93 μM/L/min with *A. niger* and 97 μM/L/min with *T. harzianum* for the above-mentioned treated lignocellulosic material. Such relative low values of optimum pH clearly indicate the importance of such acid media to fit the nature of the catalytic activity of the groups in the activity side of enzyme. These results are in agreement with those reported by **Abd El-Malak (1995), Huitron and Kirchner (1996).**

Table (7): Effect of pH on the reaction activity of cellulase enzyme with CMC and extracted cellulose from sugarcane bagasse.

Substrate / pH	Activities (µM/L/min)			
	CMC		Extracted cellulose	
	Asp.	*Tri.*	*Asp.*	*Tri.*
3.6	80	84	35	33
3.8	100	93	45	41
4.0	119	101	54	50
4.2	128	121	76	56
4.4	139	143	78	67
4.6	154	164	85	79
4.8	161	171	93	97
5.0	152	155	80	83
5.2	138	135	66	56
5.4	123	127	57	37

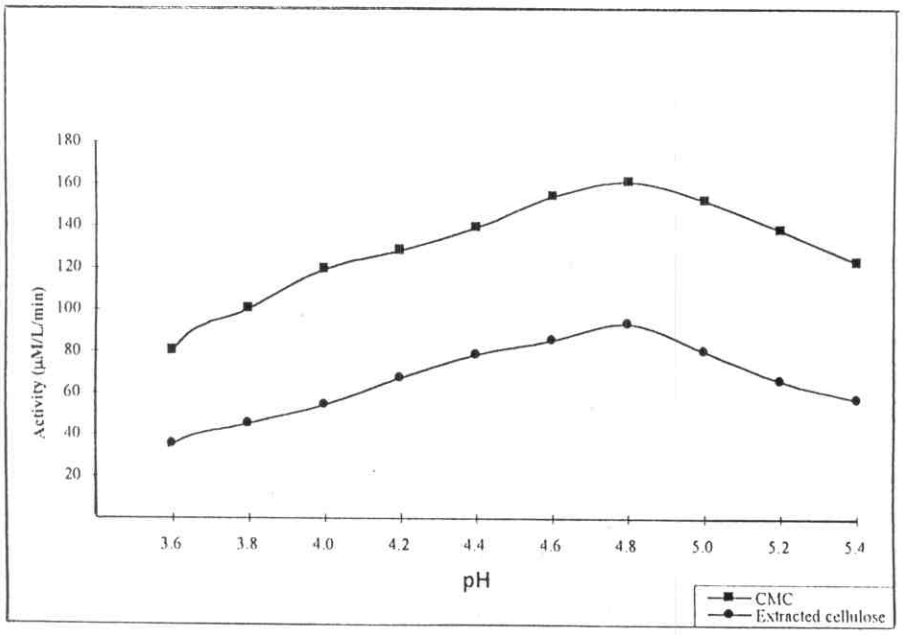

Fig (7) : Effect of pH on the reaction activity of cellulase by *Aspergillus niger.*

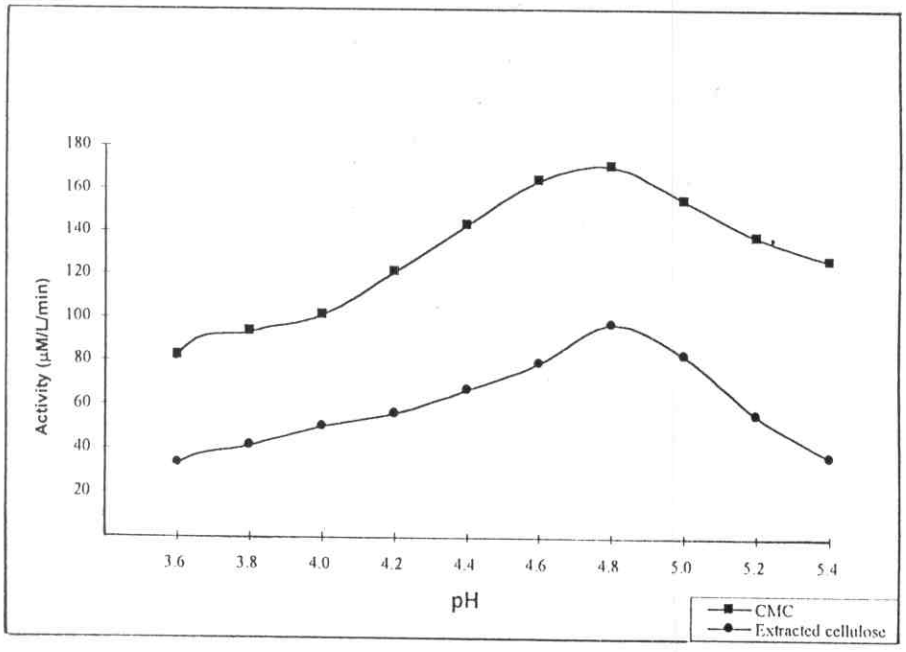

Fig (8): Effect of pH on the reaction activity of cellulase by *Trichoderma harzianum.*

Table (8): Effect of pH on the activity of hemicellulase enzyme with xylan and isolated hemicellulose from sugarcane bagasse.

Substrate / pH	Activities (µM/L/min)			
	Xylan		Isolated hemicellulose	
	Asp.	*Tri.*	*Asp.*	*Tri.*
3.6	8.7	10.8	6.1	7.0
3.8	11.1	14.1	8.6	10.1
4.0	12.4	15.6	10.5	11.6
4.2	13.5	16.7	11.5	12.5
4.4	14.6	17.2	13.2	13.1
4.6	15.6	17.8	14.3	14.0
4.8	16.9	18.5	15.4	14.4
5.0	18.3	19.8	16.0	15.5
5.2	17.7	18.4	15.4	14.2
5.4	16.5	17.4	14.1	13.4

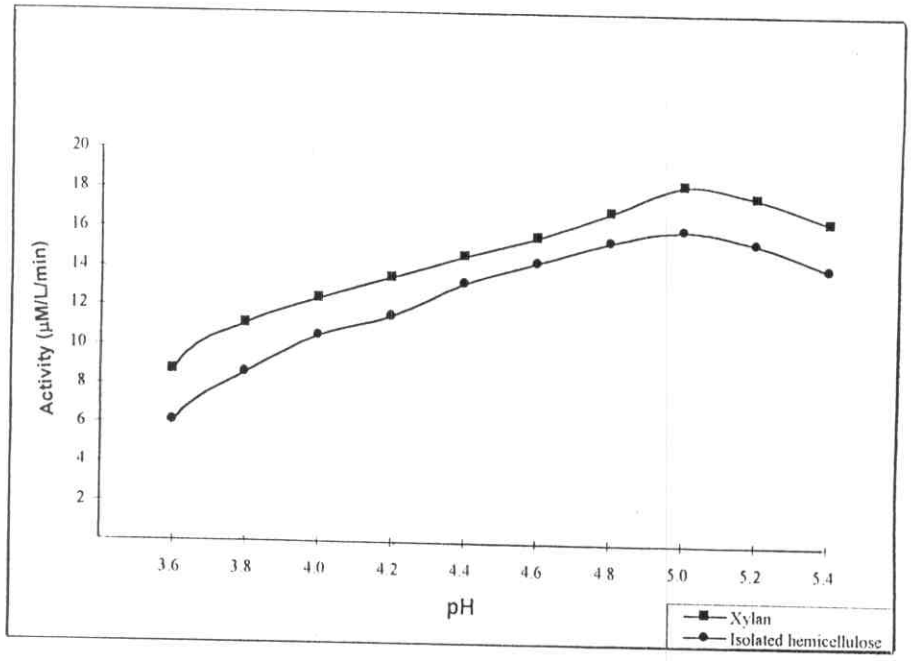

Fig (9) : Effect of pH on the reaction activity of hemicellulase by *Aspergillus niger*.

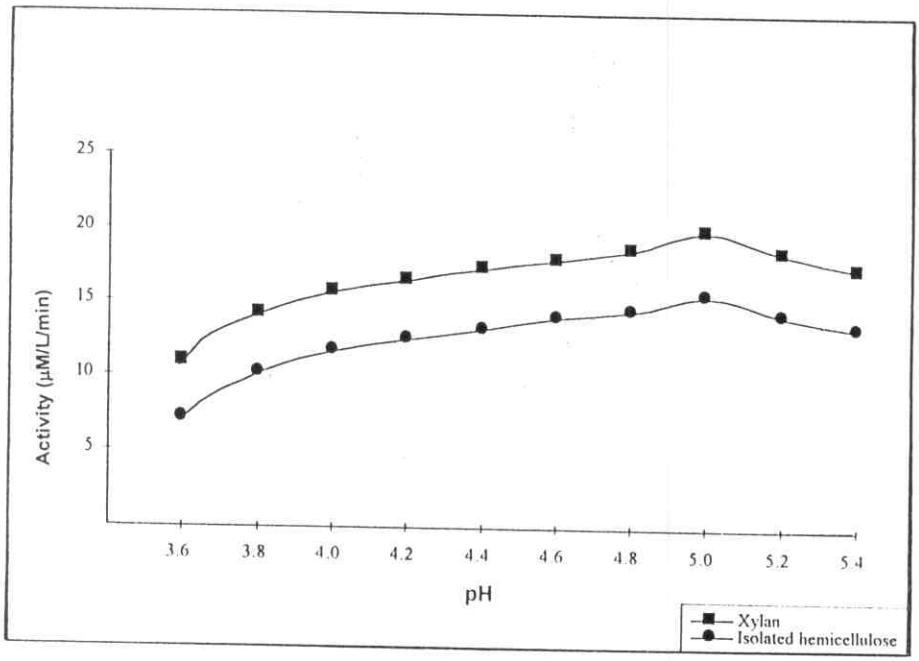

Fig (10): Effect of pH on the reaction activity of hemicellulase by *Trichoderma harzianum*.

From the above-mentioned results in Table (8) and Fig (9 and 10), it can be observed that the maximum activity of hemicellulase enzyme on xylan was 18.3 μM/L/min with *A. niger* and 14.8 μM/L/min with *T. harzianum* at pH 5.0.

On the other hand, the optimum activity of this enzyme with isolated hemicellulose from sugarcane bagasse was found to be at pH 5.0 and the maximum reaction activities were 16.0 μM/L/min with *A. niger* and 15.5 μM/L/min with *T. harzianum* for the above-mentioned treated lignocellulosic material. Such relative low values of optimum pH clearly indicate the importance of such acid media to fit the nature of the catalytic activity of the groups in the activity side of enzyme. These results are in agreement with those reported by **Saad *et al.* (1999).**

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

The effect of enzyme concentration on the enzyme activity was tested with different concentrations i.e 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000 and 3000 μL/100 ml buffer solution. The obtained results are shown in Tables (9 and 10) and Fig. (11-14).

The obtained results indicate that, the activity of cellulase enzyme reached its maximum 157.6 μM/L/min with *A. niger* and 106.1 μM/L/min with *T. harzianum* at concentration 1200 μL/100 ml buffer of enzyme solution with carboxymethyl cellulose (CMC) as substrate. While, the maximum activity of

Table (9) : Effect of enzyme concentration on the reaction activity of the produced cellulase with CMC and extracted cellulose from sugarcane bagasse.

Substrate / Enzyme conc. (µl / 100 ml)	Activities (µM/L/min)			
	CMC		Extracted cellulose	
	Asp.	*Tri.*	*Asp.*	*Tri.*
100	15.0	9.5	6.8	4.4
200	25.7	18.8	10.4	9.4
400	44.6	29.7	21.5	13.9
600	83.2	45.3	31.3	18.9
800	117.0	76.1	48.8	25.2
1000	155.2	94.1	64.7	30.5
1200	157.6	106.1	67.4	35.0
1400	149.3	101.4	52.5	32.1
1600	138.6	93.2	43.5	25.8
1800	129.0	82.4	36.0	20.3
2000	110.5	71.6	29.2	16.4
3000	70.8	32.9	13.7	7.2

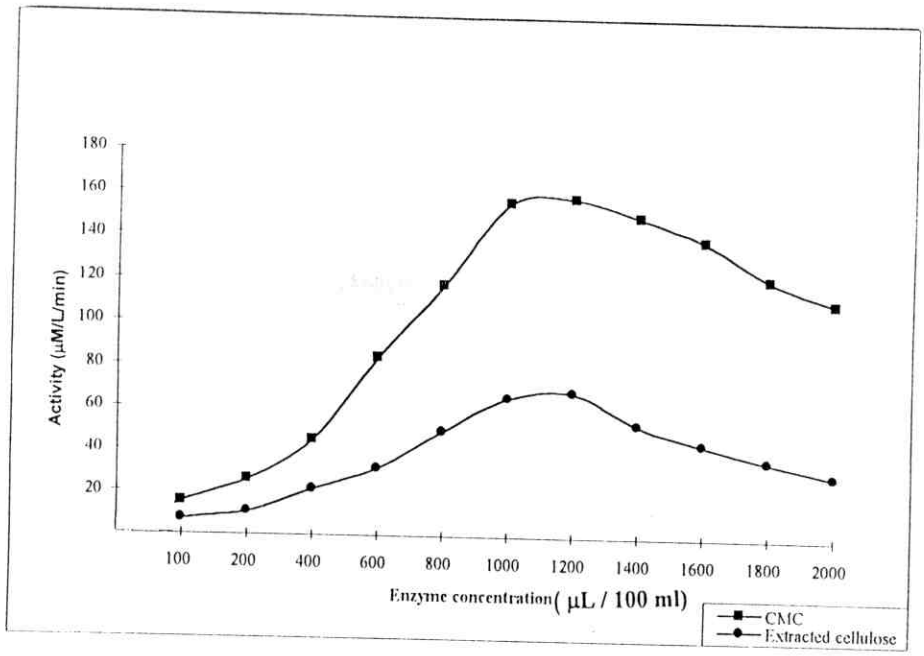

Fig (11) : Effect of enzyme concentration on the reaction activity of cellulase by *Aspergillus niger.*

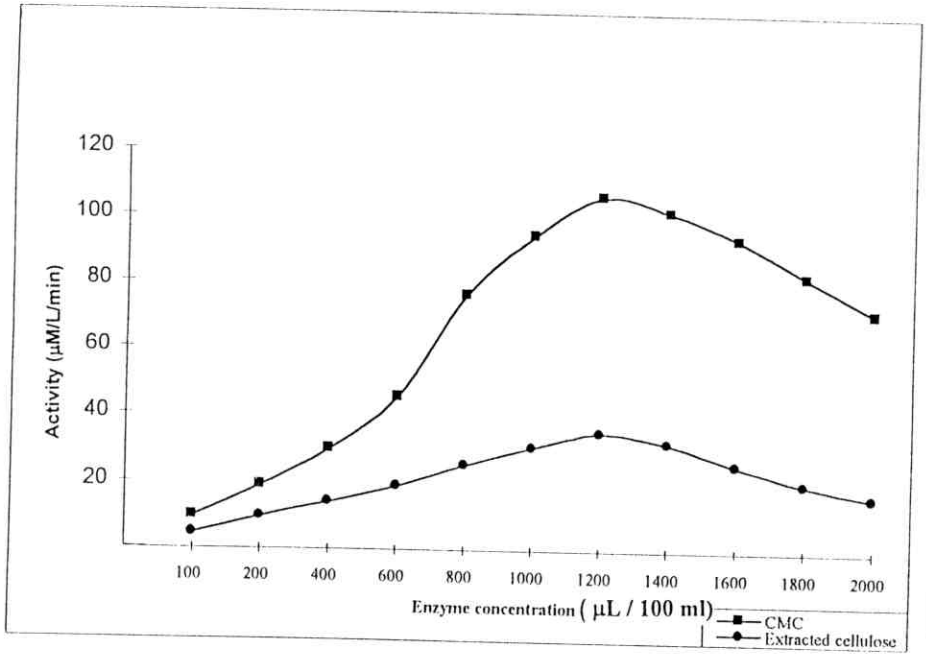

Fig (12): Effect of enzyme concentration on the reaction activity of cellulase by *Trichoderma harzianum.*

Table (10) : Effect of enzyme concentration on the reaction activity of hemicellulase with xylan and isolated hemicellulose from sugarcane bagasse.

Substrate / Enzyme conc. (µl / 100 ml)	Activities (µM/L/min)			
	Xylan		Isolated hemicellulose	
	Asp.	*Tri.*	*Asp.*	*Tri.*
100	5.3	9.3	4.1	7.2
200	9.4	11.6	7.1	9.8
400	11.1	13.8	9.1	11.4
600	13.8	15.3	11.4	13.3
800	16.6	16.4	14.5	14.6
1000	18.6	17.2	17.3	15.5
1200	18.3	16.7	16.1	15.1
1400	16.1	14.8	14.2	13.2
1600	14.2	12.6	12.5	11.0
1800	12.7	10.5	11.1	9.2
2000	8.8	7.9	6.9	5.4
3000	6.2	5.6	5.4	4.2

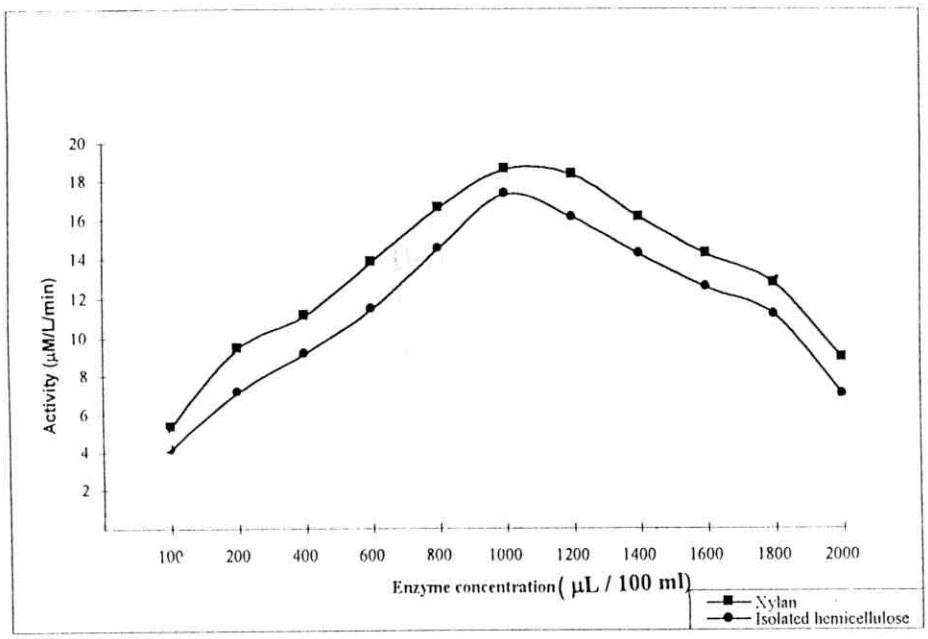

Fig (13) : Effect of enzyme concentration on the reaction activity of hemicellulase by *Aspergillus niger*.

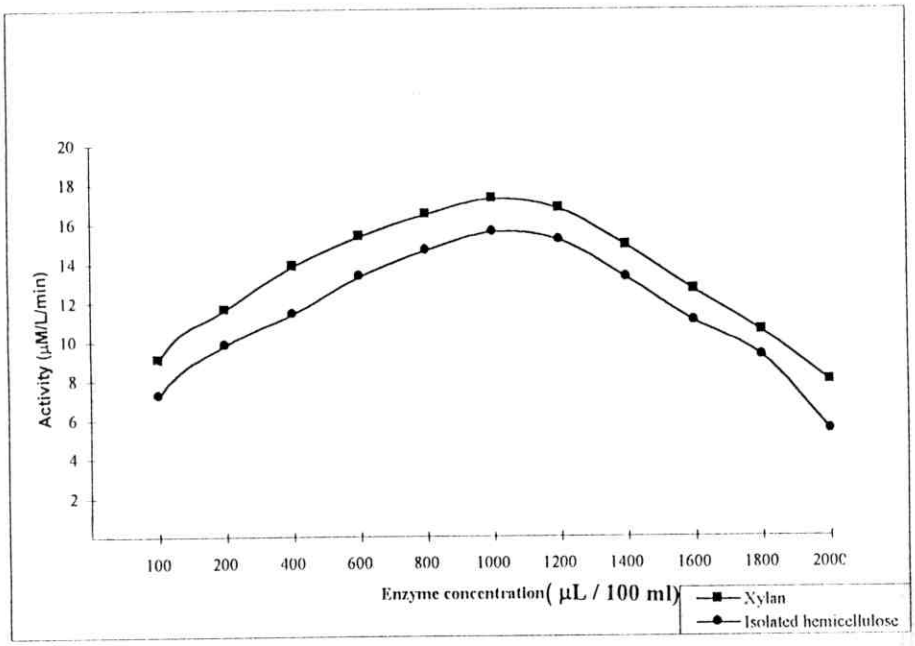

Fig (14): Effect of enzyme concentration on the reaction activity of hemicellulase by *Trichoderma harzianum*.

this enzyme on extracted cellulose was at enzyme concentration equaled 1200 µL/100 ml buffer, which gave reaction activity of 67.4 with *A. niger* and 35.0 µM/L/min with *T. harzianum*.

The above results indicated that increasing enzyme concentration beyond these values led to a decrease in the overall reaction activity. This observation may be attributed to the inhibition effect of the product glucose which produce in opposite direction. These results are in agreement with those reported by **Zaki (1998) and Saad *et al.* (1999).**

From the data presented in Table (10) it is clear that, the activity of hemicellulase enzyme reached its maximum 18.6 µM/L/min with *A. niger* and 17.2 µM/L/min with *T. harzianum* at concentration 1000 µL/100 ml buffer of enzyme solution with xylan as substrate. While, the maximum activity of this enzyme on isolated hemicellulose was at enzyme concentration equaled 1000 µL/100 ml buffer, which gave reaction activity of 17.3 with *A. niger* and 15.5 µM/L/min with *T. harzianum*. These results are in agreement with those reported by **Ristroph and Humphery (1985) and Saad *et al.* (1999).**

4.3.4. Effect of substrate concentration on the reaction velocity of enzymes :

Substrate concentration is one of the most important factors, which affects the reaction velocity and activity of the enzyme reaction. During the course of these experiments the amount of enzyme, pH and temperature in the individual experiments being constant.

The effect of different substrate concentrations on the reaction velocity of cellulose and hemicelulase enzymes were tested using carboxymethyl cellulose (CMC) and xylan as standards beside to extracted cellulose and isolated hemicellulose from sugarcane bagasse were used.

The obtained results in the before-mentioned factors for cellulase enzyme are shown in Tables (11 and 12) and Fig. (15 and 17). The rate of the most enzyme reaction increase up to a certain point with increasing concentration of substrate till it reached its maximum velovity (V_{max}).

By plotting the obtained reducing sugars against the substrate concentrations, four curves were obtained, Fig. (16 and 18) for carboxymethyl cellulose (CMC) and extracted cellulose from sugarcane bagasse. These curves indicate that any increase of the substrate concentration was accompanied with the increment of activity until reached its maximum, beyond this concentration any further increase in substrate concentration does not show any positive effect and the reaction rate of enzyme depends on the time necessary for the enzyme to act on the substrate.

The maximum reaction velocity (V_{max}) of cellulase enzyme were 158.0 μM/L/min with *A. niger* and 168.2 μM/L/min with *T. harzianum* for CMC and 46.9 μM/L/min with *A. niger* and 44.8 μM/L/min with *T. harzianum* for extracted cellulose at substrate concentration 10g/L.

However if (v) plotted against [s], the Michaelis constant (K_m) of cellulase enzyme can be obtained by the half point of the experimental curve as shown in Fig. (16 and 18). K_m of cellulase enzyme were 2.0 and 5.2 g/L with *A niger* and *T. harzianum* for CMC respectively, while 5.5 and 6.6 g/L with *A niger* and *T. harzianum* for extracted cellulose respectively.

The differentiation in the obtained values of K_m may be due to the transformation of cellulose into a form highly resistant to enzymatic attack and product inhibition, **Lee *et al.* (1992)** and **Medve *et al.* (1998).** Also, it is clear that the maximum reaction velocity (V_{max}) was lower and K_m values was higher in pretreated lignocellulosic residues than carboxymethyl cellulose, this observation may be due to many factors such as pretreatment process and impurities substrate.

It is important to mention that K_m constant was once more determined by **Lineweaver and Burk (1954)**. The obtained K_m was almost equal to that obtained firstly by experimental curves as shown in Figs. (16 and 18).

Table (11): Effect of substrate concentration on the reaction velocity of cellulase enzyme produced from *Aspergillus niger*.

[S] (g/L)	1/[S]	CMC			Extracted cellulose		
		Obtained of D-glucose (µ M/L/min)	Reaction velocity (v)	1/v X10^{-3}	Obtained of D-glucose (µ M/L/min)	Reaction velocity (v)	1/v X10^{-3}
2	**0.500**	79.9	79	12.66	12.7	12.5	80
4	**0.250**	122.2	105.3	9.45	19.9	19.7	50.8
6	**0.167**	125.4	118.5	8.44	26.9	24.5	40.7
8	**0.125**	140.6	126.4	7.91	33.7	27.8	35.9
10	**0.100**	158.0	131.7	7.59	46.9	30.3	33.0
12	**0.083**	155.2	135.4	7.39	44.2	32.2	31.1
14	**0.071**	154.9	138.3	7.23	43.1	33.7	29.7
16	**0.0625**	154.4	140.4	7.12	41.9	34.9	28.6
18	**0.0556**	147.6	142.2	7.03	41.1	35.9	27.9
20	**0.050**	146.1	143.6	6.96	39.9	36.8	27.2

V_{max1} = 158.0 **V_{max2} = 46.9**

K_{m1} = 2 **K_{m2} = 5**

Calc. K_{m1} = 2.0 **Calc. K_{m2} = 5**

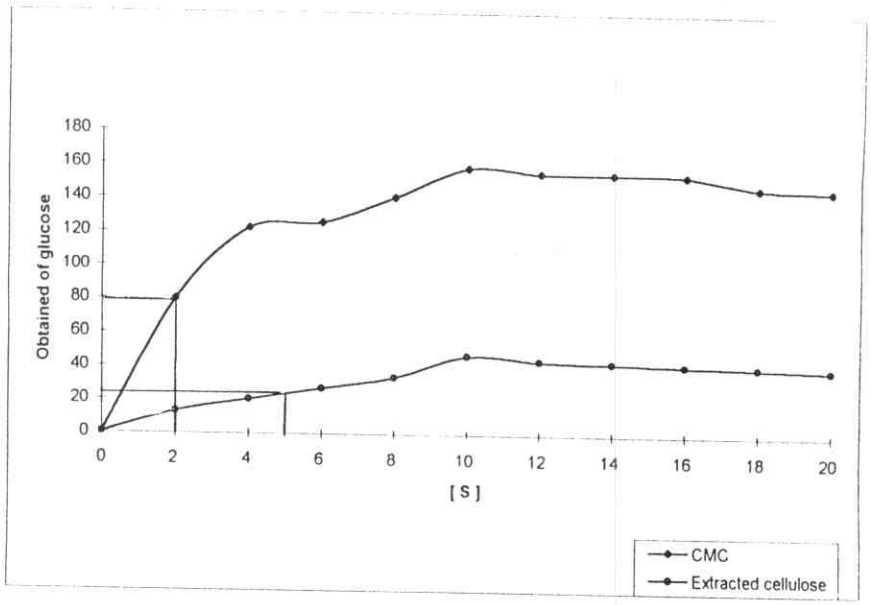

Fig (15) : Effect of substrate concentration on the reaction velocity on cellulase enzyme by *Aspergillus niger*.

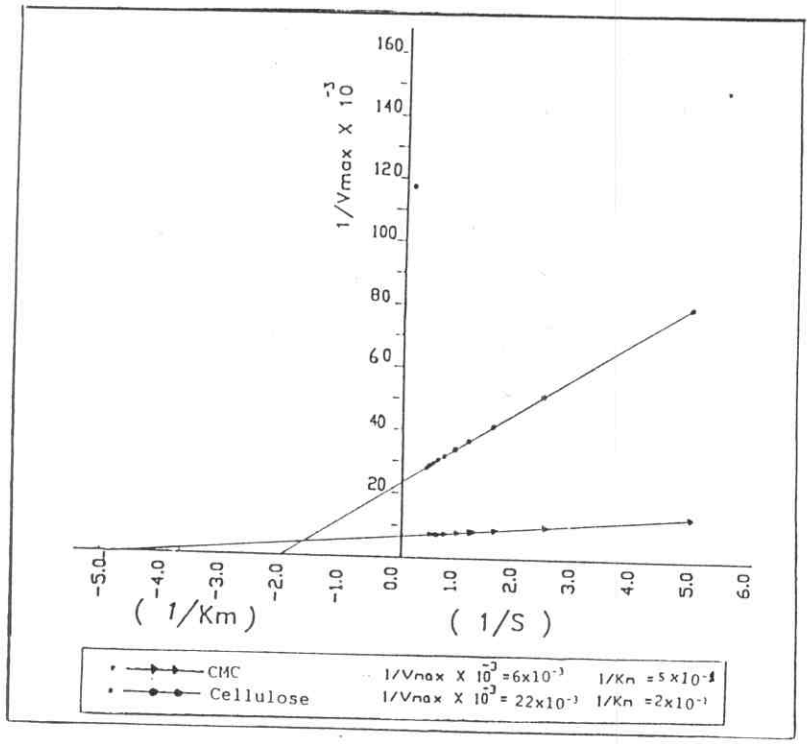

Fig (16): Lineweaver-Burk plots of cellulase enzyme by *Aspergillus niger*

Table (12): Effect of substrate concentration on the reaction velocity of cellulase enzyme produced from *Trichoderma harzianum*.

[S] (g/L)	1/[S]	CMC			Extracted cellulose		
		Obtained of D-glucose (μ M/L/min)	Reaction velocity (v)	1/v X10^{-3}	Obtained of D-glucose (μ M/L/min)	Reaction velocity (v)	1/v X10^{-3}
2	**0.500**	65.8	62.3	16.10	10.1	10.5	95.2
4	**0.250**	93.9	90.9	11.00	12.8	17.1	58.5
6	**0.167**	131.6	107.4	9.31	22.6	21.5	46.5
8	**0.125**	155.7	118.0	8.47	33.6	24.7	40.4
10	**0.100**	167.4	125.5	7.97	44.8	27.2	36.8
12	**0.083**	168.2	131.1	7.63	43.2	29.1	34.4
14	**0.071**	163.4	135.3	7.40	42.2	30.6	32.7
16	**0.0625**	157.2	138.7	7.21	38.9	31.9	31.3
18	**0.0556**	155.5	141.5	7.07	37.8	32.9	30.7
20	**0.050**	155.2	143.8	6.95	29.8	33.8	29.6

V_{max1} = 168.2 **V_{max2} = 43.2**

K_{m1} = 3.5 **K_{m2} = 6**

Calc. K_{m1} = 3.3 **Calc. K_{m2} =5.8**

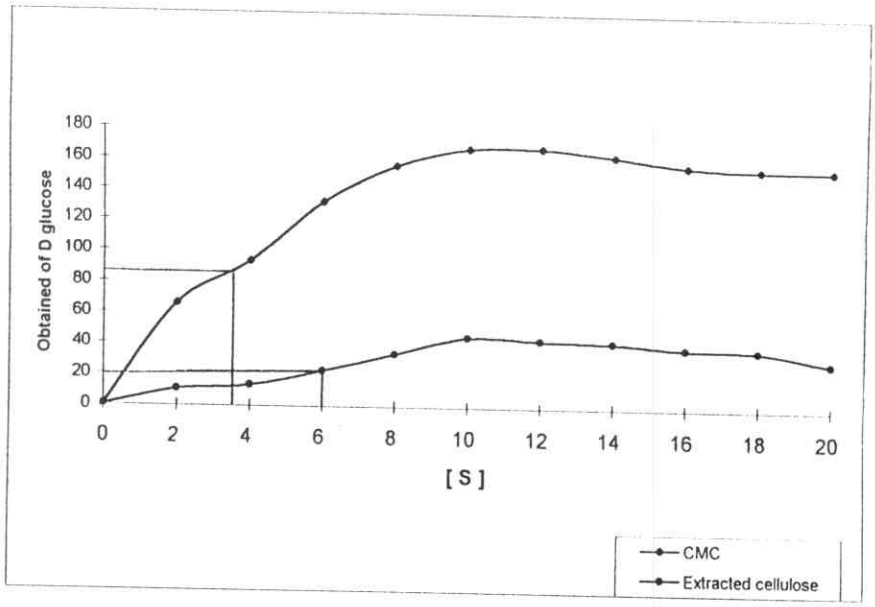

Fig (17) : Effect of substrate concentration on the reaction velocity of cellulase enzyme by *Trichoderma harzianum.*

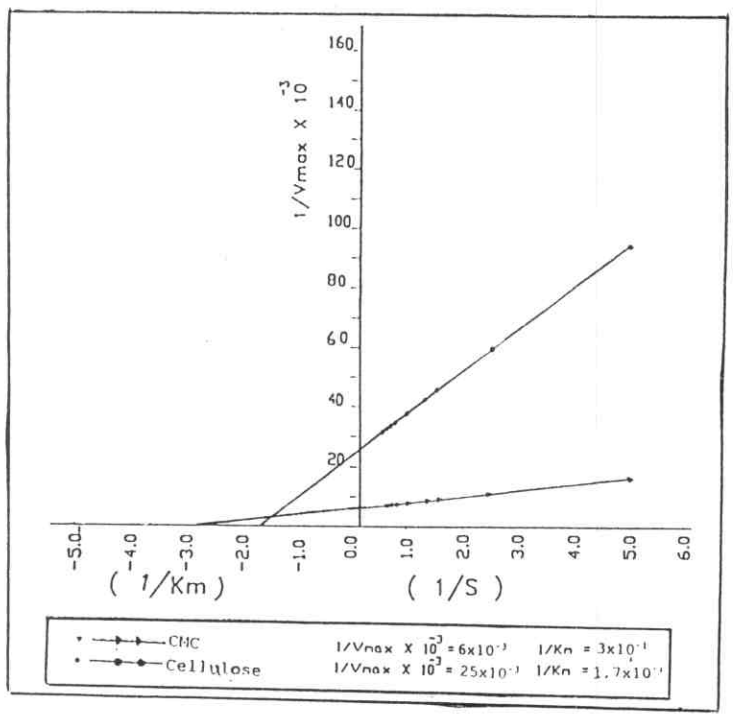

Fig (18): Lineweaver-Burk plots of cellulase enzyme by *Trichoderma harzianum.*

The obtained results in the mentioned factors for hemicellulase enzyme are shown in Table (13 and 14) and Fig. (19 and 21).

The maximum reaction velocity (V_{max}) of the produced hemicellulase enzyme were 25.3 μM/L/min with *A. niger* and 24.4 μM/L/min with *T. harzianum* for xylan and 20.9 μM/L/min with *A. niger* and 20.0 μM/L/min with *T. harzianum* for isolated hemicellulose at substrate concentration 12 g / L.

However if (v) plotted against [s], the Michaelis constant (K_m) of hemicellulase enzyme can be obtained by the half point of the experimental curve as shown in Fig. (19 and 21). K_m of hemicellulase enzyme were 4.3 and 3.4 g/L with *A niger* and *T. harzianum* for xylan, respectively while the K_m values were 4.6 and 6.5 g/L with *A niger* and *T. harzianum* for isolated hemicellulose, respectively.

It is important to mention that, K_m constant was once more determined by **Lineweaver and Burk (1954)** The obtained K_m was almost equaled to that obtained firstly by experimental curves as shown in Figs. (20 and 22).

Table (13) : Effect of substrate concentration on the reaction velocity of hemicellulase enzyme produced from *Aspergillus niger*.

[S] (g/L)	1/[S]	Xylan			Isolated hemicellulose		
		Obtained of D-xylose (μ M/L/min)	Reaction velocity (v)	1/v X10^{-3}	Obtained of D-xylose (μ M/L/min)	Reaction velocity (v)	1/v X10^{-3}
2	**0.500**	8.2	7.9	125.5	6.9	6.3	157.9
4	**0.250**	11.0	12.1	82.7	9.1	9.7	102.9
6	**0.167**	15.1	14.6	68.4	12.4	11.8	84.5
8	**0.125**	17.0	16.3	61.3	15.2	13.3	75.4
10	**0.100**	18.1	17.6	56.9	16.9	14.3	69.8
12	**0.083**	25.3	18.5	54.1	20.9	15.1	66.1
14	**0.071**	24.6	19.2	52.1	19.3	15.7	63.6
16	**0.0625**	24.4	19.8	50.6	16.9	16.2	61.6
18	**0.0556**	23.2	20.3	49.4	16.8	16.7	60.1
20	**0.050**	22.2	20.7	48.4	15.8	16.9	56.9

V_{max1} = 25.3 **V_{max2} = 20.9**

K_{m1} = 5 **K_{m2} = 4.6**

Calc. K_{m1} = 4.7 **Calc. K_{m2} =4.1**

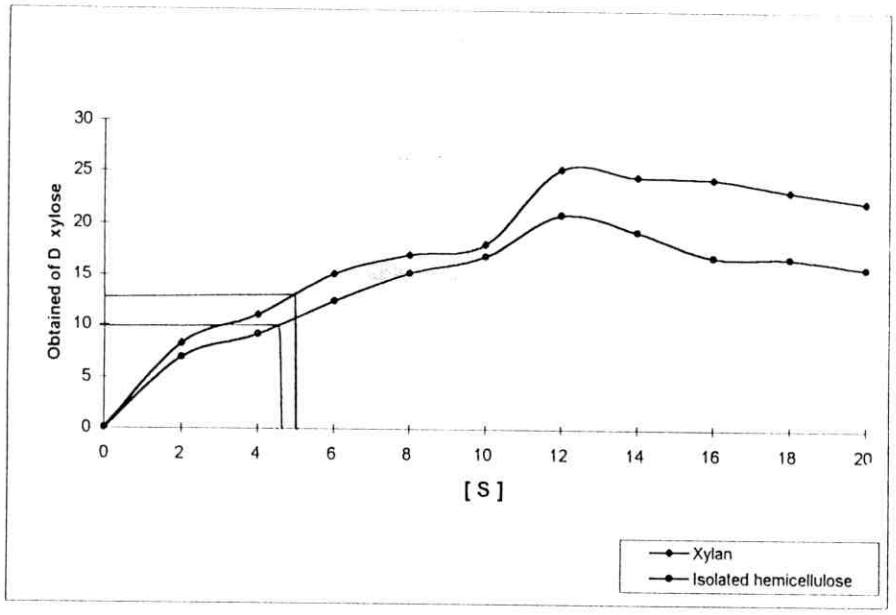

Fig (19) : Effect of substrate concentration on the reaction velocity of hemicellulase enzyme by *Aspergillus niger.*

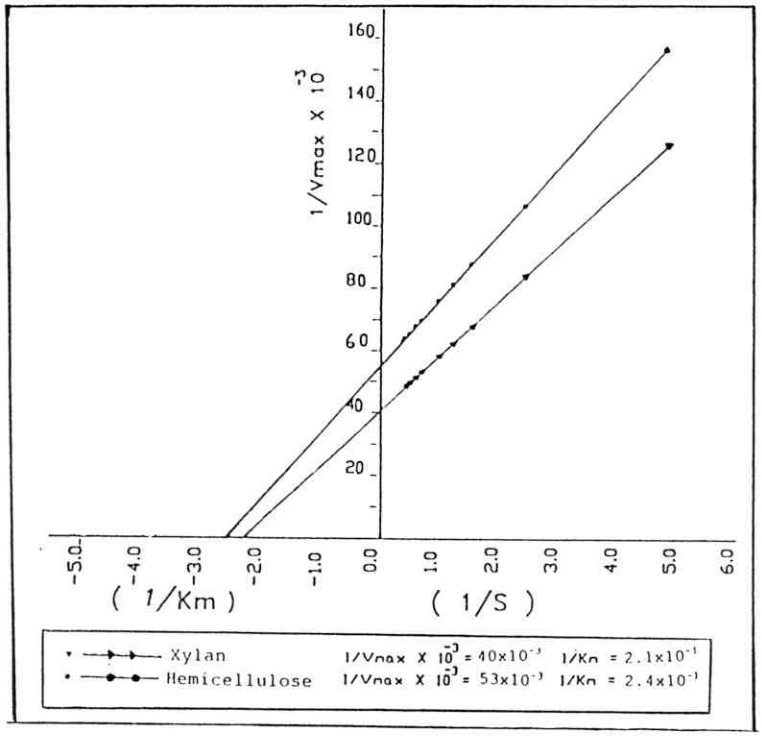

Fig (20): Lineweaver-Burk plots of hemicellulase enzyme by *Aspergillus niger.*

Table (14): Effect of substrate concentration on the reaction velocity of hemicellulase enzyme produced from *Trichoderma harzianum*.

[S] (g/L)	1/[S]	Xylan			Isolated hemicellulose		
		Obtained of D-xylose (µ M/L/min)	Reaction velocity (v)	1/v X10^{-3}	Obtained of D-xylose (µ M/L/min)	Reaction velocity (v)	1/v X10^{-3}
2	**0.500**	6.7	6.8	147.5	5.8	4.7	215.1
4	**0.250**	9.5	10.6	94.3	8.0	7.6	132.5
6	**0.167**	12.9	13.1	76.5	11.1	9.5	105.0
8	**0.125**	16.7	14.8	67.6	12.2	11.0	91.2
10	**0.100**	18.3	16.1	62.3	15.5	12.1	83.0
12	**0.083**	24.4	17.0	58.8	20.0	12.9	77.5
14	**0.071**	22.3	17.8	56.2	18.6	13.6	73.6
16	**0.0625**	21.8	18.4	54.3	16.6	14.2	70.6
18	**0.0556**	18.6	18.9	52.8	16.3	14.7	68.4
20	**0.050**	17.8	19.4	51.6	13.8	15.0	66.5

V_{max1} = 24.4 **V_{max2} = 20.0**

K_{m1} = 5.6 **K_{m2} = 5.4**

Calc. K_{m1} = 5 **Calc. K_{m2} =5.2**

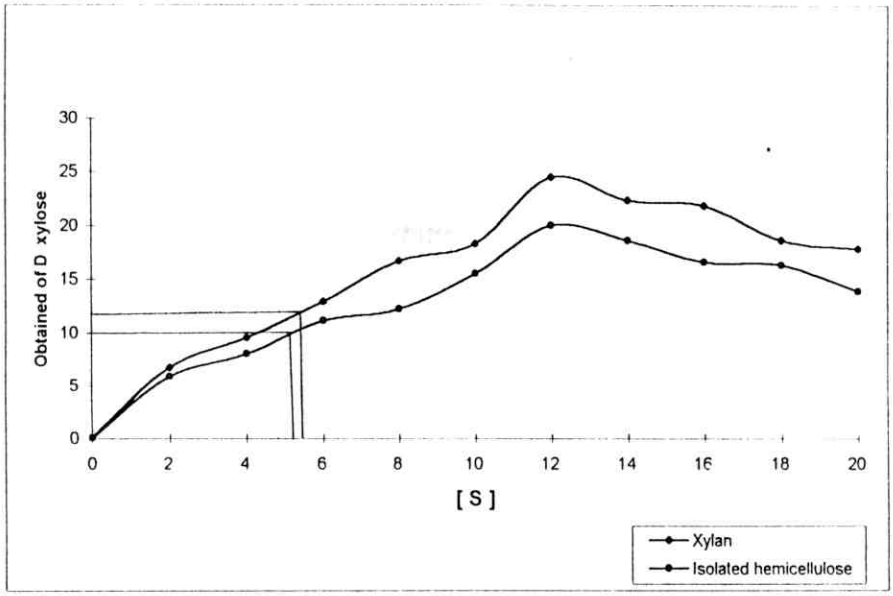

Fig (21) : Effect of substrate concentration on the reaction velocity of hemicellulase enzyme by *Trichoderma harzianum.*

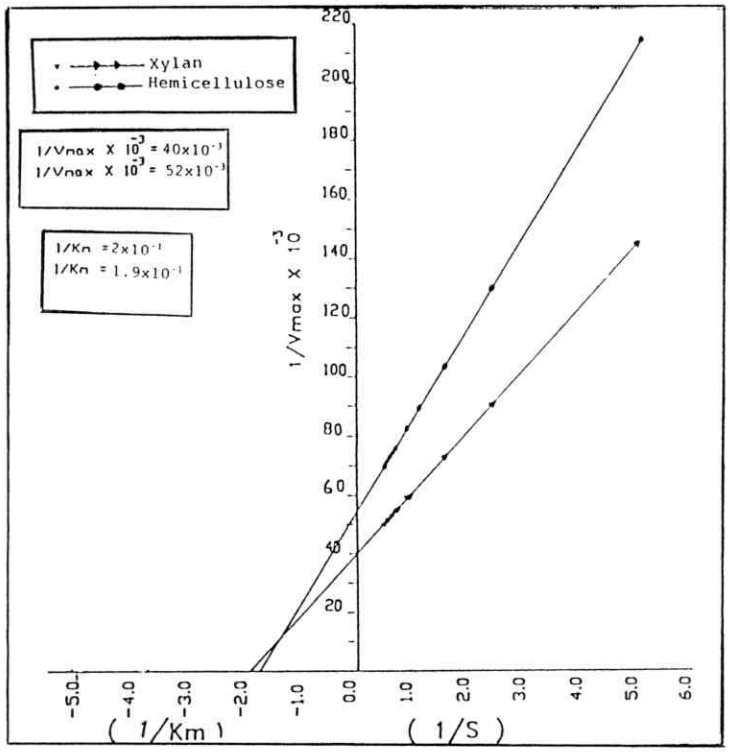

Fig (22): Lineweaver-Burk plots of hemicellulase enzyme by *Trichoderma harzianum.*

4.4. Stability of the produced cellulase and hemicellulase enzymes :

Data in Table (15) and Fig (23 a and b) illustrated the stability of cellulase enzyme which was produced from *A. niger* and *T. harzianum*. These results show that the relative activities were decreased as the time increased. The loss of relative activity of cellulase enzyme from *A. niger* reached 11.6 % while, the loss percentage of relative activity with the other once was 11.9 % after 96 hr.

The stability of the produced hemicellulase enzyme was carried out at different incubation periods from 1 to 96 hr.

Table (16) and Fig (24 a and b) exhibit the results of enzyme stability. The results indicated that a gradual decrease in its relative activity had been occurred until reached 91.1% and 95.4% compared with the original activity after 96 hr. for *A. niger* and *T. Harzianum*, respectively.

Table (15) : Stability of the produced cellulase enzyme.

Time (hr)	Relative activity %	
	Asp.	*Tri.*
0	100	100
1	98.7	84.4
2	97.6	84.0
4	92.8	86.1
12	94.2	86.6
20	89.7	88.4
24	89.4	85.3
38	88.6	81.1
48	89.4	82.9
60	89.7	78.5
72	88.6	78.0
84	88.9	77.5
96	88.4	77.2

Table (16) : Stability of the produced hemicellulase enzyme.

Time (hr)	Relative activity %	
	Asp.	*Tri.*
0	100	100
1	98.5	99.2
2	98.8	99.5
4	98.0	97.7
12	98.2	97.3
20	96.2	96.7
24	95.4	95.9
38	96.0	95.4
48	95.7	95.1
60	94.3	95.5
72	92.1	95.0
84	91.3	96.1
96	91.1	95.4

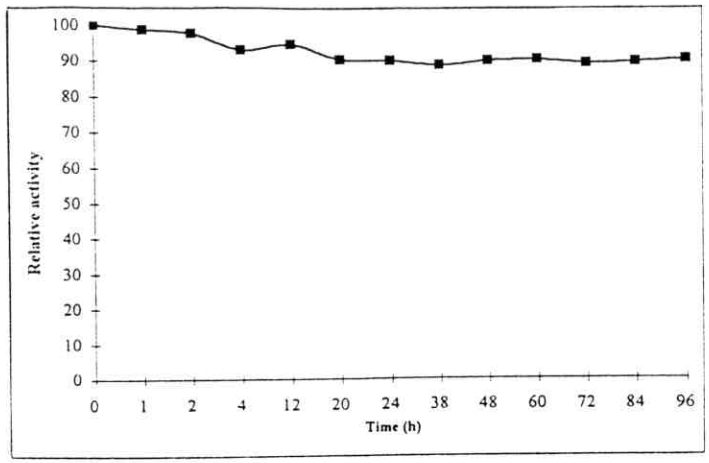

Fig (23 a) : Stability of cellulase by *Aspergillus niger.*

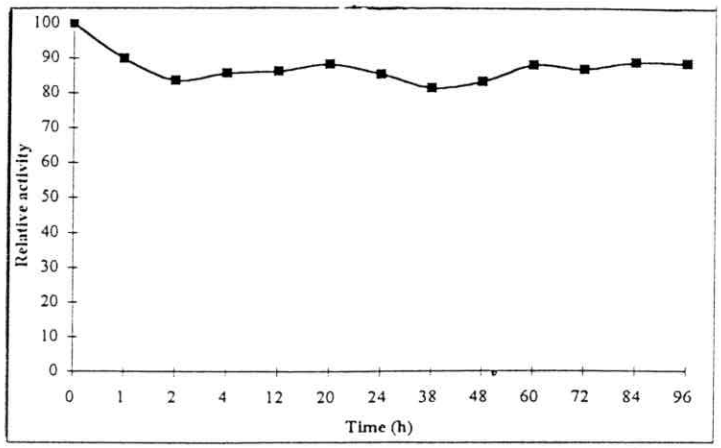

Fig (23 b) : Stability of cellulase by *Trichoderma harzianum.*

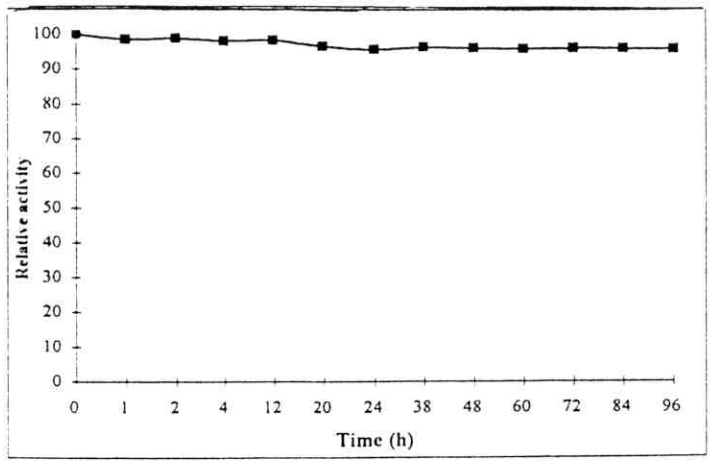

Fig (24 a) : Stability of hemicellulase by *Aspergillus niger*

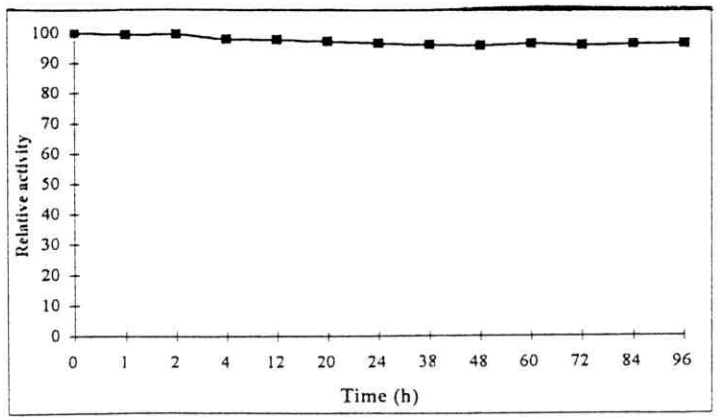

Fig (24 b) : Stability of hemicellulase by *Trichoderma harzianum.*

4.5. Enzymatic saccharficiation of extracted cellulose and isolated hemicellulose from sugarcane bagasse :

The aim of this process is to hydrolyze the extracted cellulose and isolated hemicellulose, from sugarcane bagasse to reducing sugars containing a high percent of D-glucose and D-xylose.

Saccharification processes were applied at different concentrations of extracted cellulose of 40,80, 120 and 300 g/L using (0.05 mM) acetate buffer solution. These processes were carried out by utilization cellulase enzyme mixture at concentration of 1200 µL / 100 ml enzyme solution. The experiments were achieved in a shaking incubator at optimum temperature and pH for different periods ranged between 2hrand 96hr. From the data presented in Table (17) and Fig (25 a and b) it can be concluded that, the maximum values of saccharification process for cellulose from sugarcane bagasse at substrate concentration 250 g/L after 96 hr under optimum conditions of cellulose enzyme were 61 and 68% by using *A. niger* and *T. harzianum*, respectively.

On the other hand, the enzymatic sacharification of isolated hemicellulose was achieved at different concentrations of hemicellulose i.e. 40,80,120 and 250 g/l for different periods 2,12,24,48,72 and 96 hr.

Data in Table (18) and Fig (26 a and b) indicated that the maximum values of saccharification process for isolated hemicellulose from sugarcane bagasse at substrate concentration of 250 g/l after 96 hr under optimum conditions of

hemicellulase enzyme were 31.2 and 27% when using *A. niger* and *T. harzianum*, respectively.

In despite of the maximum percentages of saccharification processes with both extracted cellulose and isolated hemicellulose were achieved by using the substrate concentration of 250 g/l after 96 hr., but it can be considered that the best values of saccharification processes were achieved with the substrate concentration of 120 g/l after 72 hr. because of using less amounts of substrates with low time and the final results are so close. These results are in agreement with those previously obtained by **Okeke and Obi (1995) and Huitron and Kirchner (1996).**

Table (17) : Enzymatic saccharification of different concentrations of extracted cellulose:

Substrate / Time (hr)	% Saccharification							
	40 g / l		80 g / l		120 g / l		250 g / l	
	Asp.	*Tri.*	*Asp.*	*Tri.*	*Asp.*	*Tri.*	*Asp.*	*Tri.*
2	21.7	23.8	25.2	27.7	30.6	33.5	31.2	34.4
12	24.7	26.3	27.6	31.3	32.9	40.5	33.1	41.2
24	28.8	30.2	31.4	37.6	40.5	50.1	39.1	49.8
48	32.1	37.7	44.0	47.9	57.0	62.9	57.2	61.4
72	34.8	38.4	47.6	49.5	59.6	65.1	60.8	67.5
96	34.6	38.5	47.9	50.1	60.1	66.0	61.5	68.0

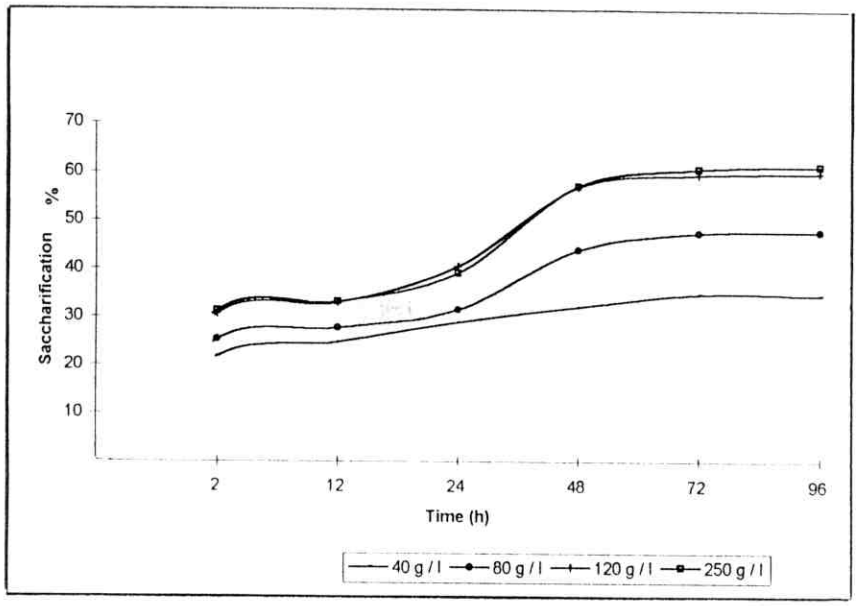

Fig (25 a) : Effect of time and substrate concentration on the enzymatic saccharification process of extracted cellulose using *Aspergillus niger*.

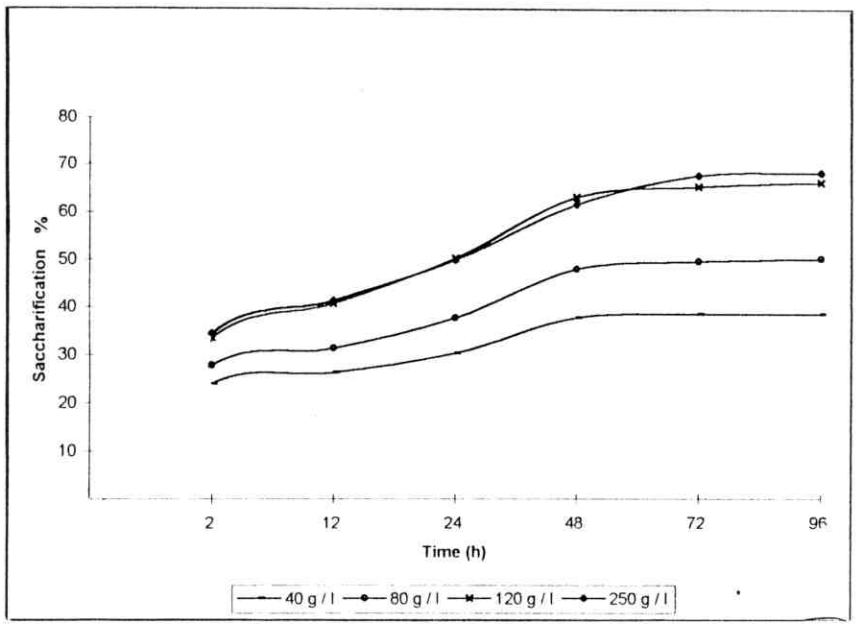

Fig (25 b) : Effect of time and substrate concentration on the enzymatic saccharification process of extracted cellulose using *Trichoderma harzianum*.

Table (18): Enzymatic saccharification of different concentrations of isolated hemicellulose:

Substrate / Time (hr)	% Saccharification							
	40 g / l		80 g / l		120 g / l		250 g / l	
	Asp.	*Tri.*	*Asp.*	*Tri.*	*Asp.*	*Tri.*	*Asp.*	*Tri.*
2	10.0	8.8	12.4	11.2	14.4	12.4	13.6	12.5
12	13.2	10.0	15.6	13.6	18.1	15.2	17.2	15.0
24	16.8	13.6	21.0	18.0	22.8	19.3	21.2	20.1
48	21.2	18.4	25.2	22.8	29.2	24.5	28.4	25.1
72	24.4	20.8	28.0	25.6	31.6	26.8	30.8	26.3
96	25.6	21.3	29.2	26.0	32.0	27.6	31.2	27.0

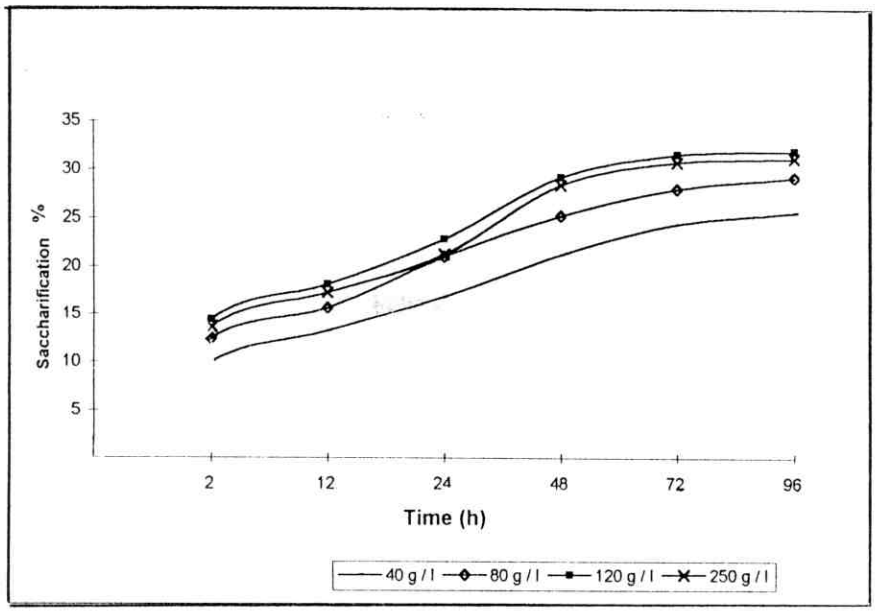

Fig (26 a) : Effect of time and substrate concentration on the enzymatic saccharification process of isolated hemicellulose using *Aspergillus niger*.

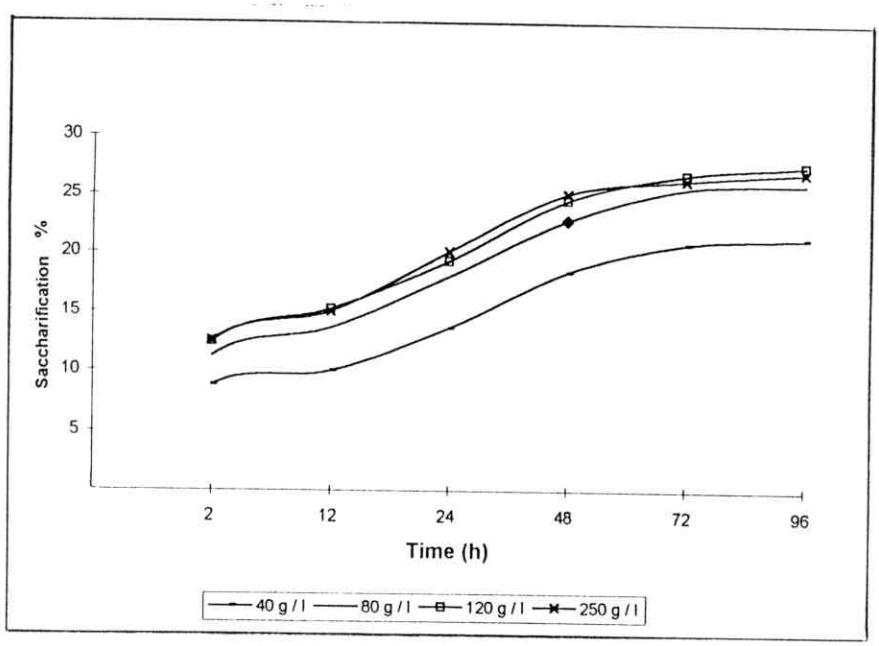

Fig (26 b) : Effect of time and substrate concentration on the enzymatic saccharification process of isolated hemicellulose using *Trichoderma harzianum*.