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BIOCHEMICAL STUDIES ON HEMICELLULASE ENZYME AND ITS APPLICATION ON SOME AGRICULTURE WASTES

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ABSTRACT

Peanut pods, midrib and wood powder residues were used throughout the present investigation, as the most abundant wastes. The isolation and determination the quality of resulted hemicellulose from these lignocellulosic materials were studied. Also, the influence of various parameters i.e. pH, temperature, enzyme concentration and isolated hemicellulose concentration on the reaction activity and reaction velocity of hemicellulase enzyme were evaluated.

From these results the cellulose content for the three lignocellulosic materials were found to be 35.71, 36.62 and 38.14% for peanut pods, midrib and wood powder residues, respectively.

On other hand, the crude lignocellulosic materials contained amounts of hemicellulose reached (38.99 %) for peanut pods residue while midrib residue contained (38.31%). However, the hemicellulose from wood powder residue was found to be (33.16%).

The isolated hemicellulosic polymers contained a high percentage of hemicellulose components i.e. 79.45, 78.22 and 76.52 for midrib residue, wood powder residue and peanut pods residue, respectively.

The hemicellulase enzyme showed its maximum activity at pH 4.8 for both xylan and isolated hemicellulose from midrib residue. On the other hand, the maximum reaction activities were found to be at pH equalled to 5.0 for both isolated hemicellulose from peanut pods and wood powder residues, respectively.

Also, the hemicellulase enzyme showed its maximum activity at temperature 55°C for xylan and isolated hemicellulose from peanut pods and wood powder residues. While, the maximum activity was 50°C for isolated hemicellulose from midrib residue.

However, the maximum activity of hemicellulase enzymes by using xylan and isolated hemicellulose were found to be 6.96, 6.56 mM/L/min at enzyme concentration of 0.05 mg/ml, 6.70 mM/L/min at concentration of 0.07 mg/ml and 7.34 mM/L/min at enzyme concentration of 0.10 mg/ml for xylan, wood powder, peanut pods and midrib residues, respectively.

The maximum reaction velocity (V_{max}) for hemicellulase enzyme by using xylan equalled 273.4 mg/ml/min, while the Michael's constant (K_m) was found to be 0.160 g/L. On the other hand, the (V_{max}) valuse for this enzyme with isolated hemicellulose from peanut pods, midrib and wood powder residues were 253.3, 196.7 and 220.0 mg/ml/min, respectively. The K_m values were found to be 0.125, 0.160 and 0.175 g/L for the isolated hemicellulose from abovementioned materials.

Finally, the enzymatic saccharification of isolated hemicellulose from different lignocellulosic wastes under investigation were determined. The maximum value of saccharification process was 86.6% for xylan as standard substrate at concentration of 25 g/L after 48 h under optimum conditions of hemicellulase enzyme. On the other hand, the maximum values of saccharification for isolated hemicellulose from peanut pods, midrib and wood powder residues were 81.0, 81.2 and 79.6%, respectively at substrate concentration of 35 g/L and 45 g/L for incubation period 96 h under optimum conditions of hemicellulase enzymes.

Key words: Hemicellulose – Xylan - Hemicellulase enzyme - Enzymatic saccharification -

INTRODUCTION

Lignocelluloses are the most abundant renewable resource available. The efficient exploitation of this resource requires the use of all of its three major components, i.e., cellulose, hemicellulose, and lignin. Xylans are the major hemicellulose in angiosperms where they account for 20-30% of the dry weight of woody tissue. Xylose is a useful carbon source for producing ethanol or xylitol (Nigam and Singh, 1995; Huitron and Kirchner, 1996).

Hemicellulosic materials are important structural and biomass components in the cell walls of plant material (Sheldon and William, 1996).

Agro-industrial and food processing wastes are available in strategical quantities all over the world, which largely becomes a source of health hazard. The majority of these wastes contain cellulose (30-40%), hemicellulose or xylan (20-40%) and lignin (20-30%). The use of these wastes for the production of strategic chemicals and fuel requires the hydrolysis of all these components (Swaroopa and Krishna, 1996).

Lignocellulosic materials need to different treatments before saccharification by enzymes to give a high yield of sugars (Rivers and Emert, 1988; David *et al.*, 1989; Ramos *et al.*, 1992; Doran *et al.*, 1994 and Neureiter *et al.*, 2002).

The hydrolysis of lignocellulosic materials plays an important role in the conversion of these renewable resources to useful products e.g. foods, syrups, fuels and chemicals. Great deals of interest in the enzymatic hydrolysis of lignocellulosic and extensive studies of its kinetics have been achieved (Ristroph and Humphrey, 1985; Vallander and Eriksson, 1985 and 1991; Shah and Lee, 1992; Saska and Ozer, 1995Giselia *et al.*, 1999; Anders *et al.*, 2003 and Salem, 2004).

The present investigation aims to study the feasibility of peanut pods, midrib and wood powder residues as the substrates for the enzymatic hydrolysis by using hemicellulase enzymes to produce the soluble reducing sugars (as xylose). Also, the optimum conditions and Kinetic behavior of hemicellulase enzymes were evaluated. Also, the saccharification processes were thoroughly studied to obtain the most suitable conditions for the production of xylose syrup from the different abovementioned materials under this investigation.

MATERIALS AND METHODS

Peanut pods, midrib and wood powder residues (agricultural wastes) were obtained from the farm of Faculty of Agricultural at Moshtohor, Zagazig University, Benha branch, dried and milled then passed through 60 mesh sieve. Xylan, D (+) glucose, D (+) xylose and all other chemicals were used as analytical grade.

Ash content; total lipid; and crude protein of different lignocellulosic wastes under investigation were determined according to the methods described in A.O.A.C. (1995).

Lignin content was carried out by gravimetric method, which described by (Tanaka *et al.*, 1985).

The most commonly used method for xylose determination is the orcinol reagent method as described by (Scheider, 1957). The intensity of green colour developed was measured at 665 nm. Using pye unicam SP6-665 Uv/vis spectrophotometer.

Cellulose and hemicellulose content were estimated gravimetrically after extraction of the lignocllulosic materials under investigation according to the method of Chen and Anderson (1980). Holocellulose was treated with 10% NaOH (100 ml) at 90°C for 3 h and precipitation with HCl at pH 5.0. The precipitated hemicellulose was isolated by centrifugation, then washed with ethanol, dried and weighed.

Hemicellulase enzyme assay was done according to the method of (Ristroph and Humphrey, 1985). The enzyme protein content for the hemicellulase enzyme was determined by the method described by (Bradford, 1976).

The effect of pH on the reaction activity of hemicellulase enzymes were tested at different pH values- i.e. 3.6, 4.0, 4.4, 4.8, 5.0, 5.2, 5.4, 5.6 and pH 6.0 in (0.05) mM acetate buffer using (1%) substrate concentrations of xylan and isolated hemicellulose from different lignocellulasic materials under investigation. Then the test tubes were incubated with enzyme at 50°C for 30 min, followed by measuring resulting of xylose content as mentioned before (Scheider, 1957).

The effect of temperature on the activity of hemicellulase enzymes was determined by the method described by (Dekker, 1983) The enzyme concentration (1.0 ml) was mixed with (4.0ml) of 1% xylan suspension in (0.05 mM) acetate buffer (pH 4.8). Xylan was used as standard compared with the isolated hemicellulose from different lignocellulasic materials then incubated at different temperatures from 35 to 60 °C for 30min. The reaction activity of hemicellulase enzyme was determined by measuring the resulting reducing sugars as xylose (Scheider, 1957).

The enzyme activity of hemicellulases was determined by the method described by (Dekker, 1983) by using different concentrations of enzyme solution. i.e. 0.02, 0.03, 0.05, 0.07, 0.1, 0.15, 0.2, 0.3, 0.4 and 0.5 mg/ml buffer. The reaction mixtures were adjusted to optimum pH in a solution composed of 1.0 ml enzyme solution and 4.0 ml xylan or isolated hemicelliulose from different lignocellulose

materials (1% in 0.05 mM acetate buffer). The reaction was carried out at 50°C for 30 min. then the formed xylose was determined by the method, which described by (Scheider, 1957).

The effect of substrate concentration on the reaction velocity of hemicellulase enzymes was tested by using different concentrations of xylan solution and isolated hemiecllulose, i.e. 0.025, 0.05, 0.10, 0.15, 0.20, 0.30, 0.40 and 0.50% in (0.05) mM acetate buffer, pH 5.0 at 50°C with incubation period of 30 min. The resulting xylose was determined according to the method described above-mentioned.

The enzymatic hydrolysis of isolated hemicellulose from different lignocellulasic wastes under investigation was carried out by the method described by (Saska and Ozer, 1995). Before starting enzyme reaction soaking 1% of isolated hemicellulose from the above-mentioned substrates 50 ml of acetate buffer for 24 h. Samples were taken periodically i.e. 2, 12, 24, 48, 72 and 96 h, heated in boiling water for 10 min, then cooled and centrifuged. The supernatants were analyzed for reducing sugars (as xylose) by the method as described before.

RESULTS AND DISCUSSION

Chemical composition of crude and extracted lignocellulosic residues:

Peanut pods straw, midrib residue and wood powder residues were used throughout the present investigation, as typical examples of the most abundant agricultural residues to evaluate the quality of resulted hemicellulose. The chemical composition of the above-mentioned lignocellulosic materials is tabulated in Table (1).

The obtained results showed that cellulose and isolated hemicellulose after pretreatment by (10% w/v) NaOH and lignin are the main constituents. The results show that the cellulose content for the three lignocellulosic wastes were found to be 35.71, 36.62 and 38.14% for peanut pods, midrib and wood powder residues, respectively. The amount of isolated hemicellulose from wood powder residue contained less amount (76.52%), while; peanut pods its highest content (79.45%). However, the isolated hemicellulose from midrib residue was found to be (78.22%).

Table (1): Chemical composition of crude and pretreated different lignocellulasic wastes (g/100 g on dry weight basis).

Components	Peanut pods residue		Midrib residue		Wood powder residue	
	Crude %	Isolated hemicelluose%	Crude %	Isolated hemicelluose%	Crude %	Isolated hemicelluose%
Moisture	8.28	7.90	8.40	8.32	7.85	7.43
Crude protein	3.26	2.80	2.21	2.09	2.80	2.48
Ash	4.32	6.27	5.81	7.59	5.57	6.32
Ether extract	1.33	-	1.27	-	1.32	-
Lignin	16.39	11.48	15.78	12.10	19.01	14.68
Cellulose	35.71	-	36.62	-	38.14	-
Hemicellulose	38.99	79.45	38.31	78.22	33.16	76.52

From the obtained results, it could be concluded that the pretreatment of lignocellulosic materials with NaOH decreased the lignin from 16.39 to 11.48% for peanut pods residue, 19.01 to 14.68% for wood powder residue and 15.78 to 12.10% for midrib residue. Also, this pretreatment causes a decrease in extracted some protein compounds while the ash content was increased. Therefore NaOH pretreatment of lignocellulosic residues under investigation removes some lignin, thus increasing the accessibility to the hemicellulose, hence the amount of lignin removal is proportional with hemicellulose amount in the extract. These results are in agreement with those reported by (Ramos et al., 1992 and Alves *et al.*, 2002).

Effect of different parameters on the activity and reaction velocity of hemicellulase enzyme:

The reaction activity of hemicellulase enzyme under investigation was measured at different pH values with (0.05 mM) acetate buffer and different substrates i.e. xylan as standard substrate and isolated hemicellulose from peanut pods, midrib and wood powder residues.

Nine solutions of xylan and isolated hemicellulose from different abovementioned lignocellulosic wastes were adjusted using pH meter model RB glass electrode designed by Sergeant-Weish to pH values of 3.6, 4.0, 4.4, 4.8, 5.0, 5.2, 5.4, 5.6 and 6.0.

The obtained results are illustrated in Table (2) and Fig. (1). The enzyme showed its maximum activity at pH 4.8 for both xylan and isolated hemicellulose from midrib residue were 11.45 and 9.44 mM/L/min, respectively. On the other hand, the maximum reaction activities were found to be 9.56 and 7.38 mM/L/min for both isolated hemicellulose from peanut pods residue and wood powder residue at pH 5.0, respectively. The obtained results are in a good agreement with that reported by (Giselia *et al.*, 1999). Also, the same trend was observed with obtained D-xylose (Table, 1), since the maximum amount of D-xylose was found at pH 4.8 and 5.0.

The effect of temperature on the reaction activity of hemicellulase enzyme was tested at six different temperatures, i.e. 35, 40, 45, 50, 55 and 60°C. The reaction mixtures of the experiments were carried out at optimum pH for xylan and isolated hemicellulose from peanut pods, midrib and wood powder residues with 1% (w/v) substrate concentration and incubation period for 30 min.

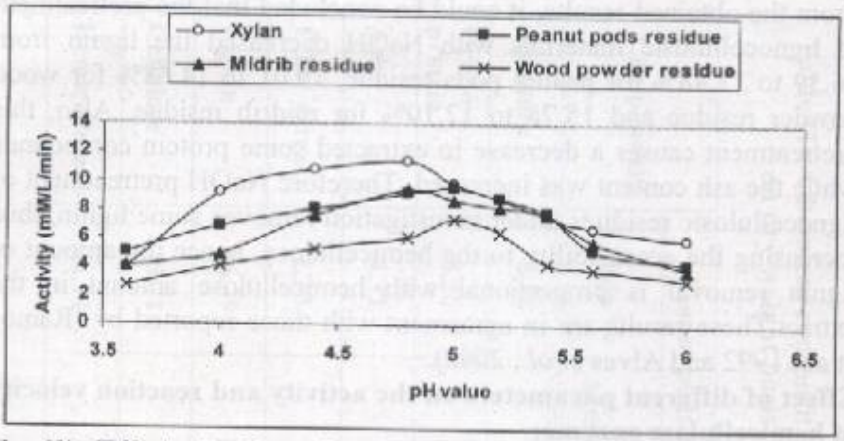


Fig. (1): Effect of pH on the activity of hemicellulase enzyme with xylan and isolated hemicellulose from different wastes

Data in Table (3) and Fig. (2) shows that the reaction activity reached to its maximum 8.89 mM/L/min, 6.83 mM/L/min and 6.56 mM/L/min at temperature 55°C for xylan and isolated hemicellulose from peanut pods and wood powder residues, respectively. On the other hand, the maximum reaction activity of isolated hemicellulose from midrib residue was 7.78 mM/L/min at temperature 50°C. These results indicated that the maximum reaction activity of hemicellulase enzyme with xylan polysaccharide as standard substrate is higher when compared with isolated hemicellulose from different lignocellulosic materials. Such results may be due to that xylan polysaccharide is considered as homoglycan polysaccharide while the other hemicellulosic of lignocellulosic wastes are heteroglycan, which may hinder the action of hemicellulase enzyme, and hence, less amount of obtained D-xylose. Such values for optimum temperature and maximum activity are in agreement with those reported by Ristroph and Humphrey (1985), Gilbert *et al.* (1992) and Salem (2004).

The effect of enzyme concentration on the reaction activity of hemicellulase enzyme was tested with different concentrations i.e. 0.02, 0.03, 0.05, 0.07, 0.10, 0.15 and 0.20 mg/ml. The reaction mixtures were carried out at optimum temperature and optimum pH for each isolated hemicellulose from different lignocellulosic materials at 1% (w/v) substrate concentration.

Table (2): Effect of pH on the activity of hemicellulase enzyme with xylan and isolated hemicellulose from different wastes

pH value	Xylan		Peanut pods residue		Midrib residue		Wood powder residue	
	Obtained of D-xylose (mM/L)	Activity mM/L/min	Obtained of D-xylose (mM/L)	Activity mM/L/min	Obtained of D-xylose (mM/L)	Activity mM/L/min	Obtained of D-xylose (mM/L)	Activity mM/L/min
3.6	116.67	3.89	150.00	5.00	123.33	4.11	80.00	2.67
4.0	277.80	9.26	206.67	6.89	146.10	4.87	123.23	4.11
4.4	324.60	10.82	243.33	8.11	228.60	7.62	156.67	5.22
4.8	343.50	11.45	276.67	9.20	283.33	9.44	183.34	6.11
5.0	294.90	9.83	286.67	9.56	263.33	8.78	221.53	7.38
5.2	266.67	8.89	266.67	8.89	246.67	8.22	196.56	6.55
5.4	226.67	7.56	236.67	7.89	231.54	7.72	130.00	4.33
5.6	201.53	6.72	156.67	5.22	176.67	5.89	116.67	3.89
6.0	180.00	6.00	130.00	4.33	116.34	3.88	96.45	3.22

Xylan acts as standard substrate.

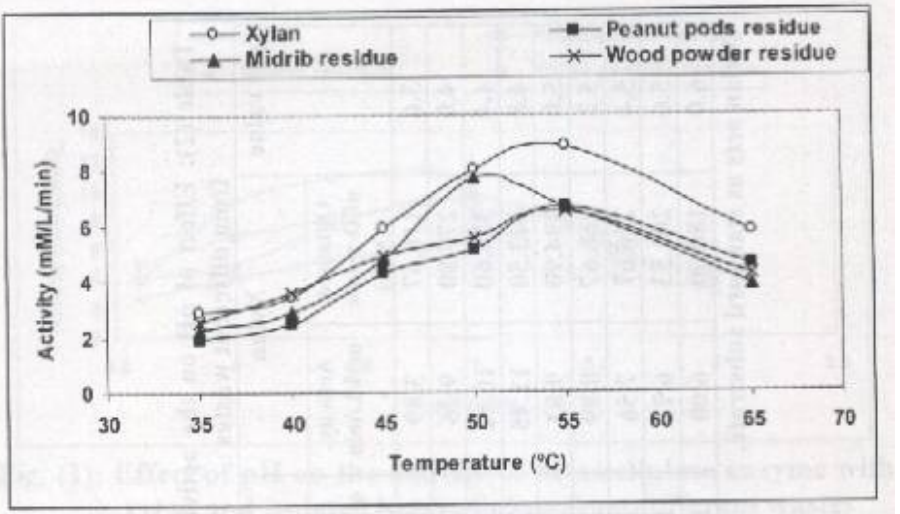


Fig. (2): Effect of temperature on the activity of hemicellulase enzyme with xylan and isolated hemicellulose from different wastes

The obtained results are shown in Table (4) and Fig. (3). From these data indicate that the activity of hemicellulase enzyme was increased by increasing the enzyme concentration until reached to its maximum reaction velocity at the optimum enzyme concentrations. The maximum activity of hemicellase enzymes by using xylan as standard substrate was found to be 6.96 mM/L/min at enzyme concentration of 0.05 mg/ml. On the other hand, the maximum activity of hemicillulase enzyme by using isolated hemicellulose from peanut pods residue gave reaction activity 6.70 mM/L/min at concentration of 0.07 mg/ml. The maximum reaction activity with isolated hemicellulose from midrib and wood powder residues were 7.34 and 6.56 mM/L/min at enzyme concentration of 0.05 and 0.10 mg/ml, respectively.

The above results indicated that increasing enzyme concentration more than the optimum values led to a decrease in the overall reaction activity. The differentiations in optimum enzyme concentration may be due to the nature of isolated hemicellulose from different lignocellulosic materials and the inhibition effect of the product (as D-xylose), which effect in opposite direction of the reaction. Such explanation was introduced by (Dekker, 1983 and Gibert *et al.*, 1992).

Table (3): Effect of temperature on the activity of hemicellulase enzyme with xylan and isolated hemicellulose from different wastes

Temperature (°C)	Xylan		Peanut pods residue		Midrib residue		Wood powder residue	
	Obtained of D-xylose (mM/L)	Activity mM/L/min	Obtained of D-xylose (mM/L)	Activity mM/L/min	Obtained of D-xylose (mM/L)	Activity mM/L/min	Obtained of D-xylose (mM/L)	Activity mM/L/min
35	86.67	2.89	56.67	1.89	66.6	2.22	76.67	2.56
40	103.33	3.44	73.33	2.44	86.67	2.89	106.67	3.56
45	176.67	5.89	130.00	4.33	144.35	4.81	148.52	4.95
50	240.00	8.00	153.33	5.11	233.33	7.78	166.67	5.56
55	266.67	8.89	205.00	6.83	215.00	7.17	196.67	6.56
65	173.33	5.78	136.67	4.56	116.67	3.89	126.45	4.22

Xylan acts as standard substrate.

Table (4): Effect of enzyme concentration on the activity of hemicellulase enzyme with xylan and isolated hemicellulose from different wastes

Enzyme concentration	Xylan		Peanut pods residue		Midrib residue		Wood powder residue	
	Obtained of D-xylose (mM/L)	Activity mM/L/min	Obtained of D-xylose (mM/L))	Activity mM/L/min	Obtained of D-xylose (mM/L)	Activity mM/L/min	Obtained of D-xylose (mM/L)	Activity mM/L/min
0.02	124	4.13	79.64	2.65	96.50	3.22	66.40	2.21
0.03	150.00	5.00	139.45	4.65	136.20	4.54	124.20	4.14
0.05	208.67	6.96	189.65	5.69	210.45	7.02	138.55	4.62
0.07	166.67	5.56	201.10	6.70	220.15	7.34	165.55	5.52
0.10	155.32	5.18	174.35	5.81	198.20	6.61	196.80	6.56
0.15	138.20	4.61	155.56	5.18	165.25	5.51	165.25	5.51
0.20	83.33	2.78	138.20	4.61	124.00	4.13	125.20	4.17

Xylan acts as standard substrate.

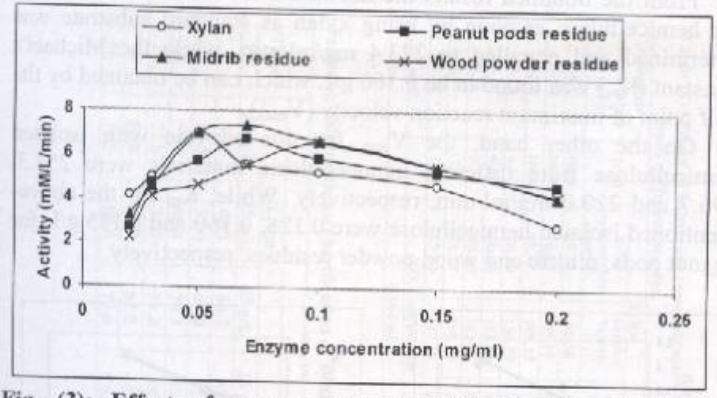


Fig. (3): Effect of enzyme concentration on the activity of hemicellulase enzyme with xylan and isolated hemicellulose from different wastes

The effect of different substrate concentrations were tested at 0.025, 0.05, 0.10, 0.15, 0.20, 0.30, 0.40 and 0.50 g/L using (0.05 mM) acetate buffer solution at optimum pH and temperature which estimated before were mentioned in Table (5) and Fig. (4a & b). The rate of the most enzyme reaction were increased up to a certain point with increasing concentration of substrate till it reached its maximum velocity (V_{max}).

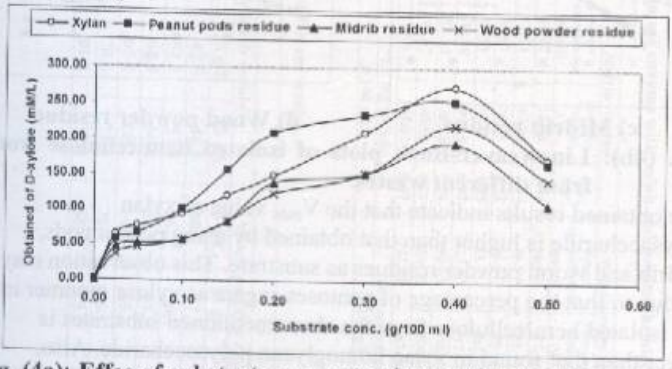
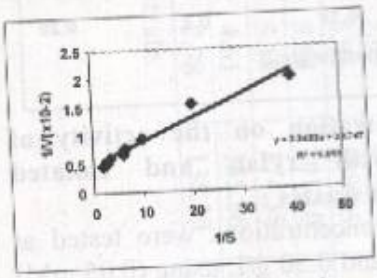


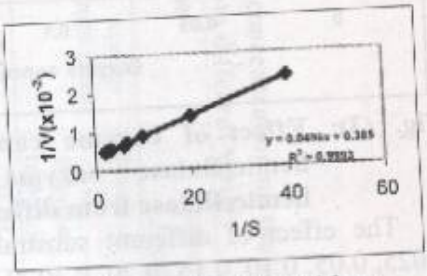
Fig. (4a): Effec of substrate concentration on the reaction velocity of hemicellulase enzyme with xylan and isolated hemicellulose from different wastes

From the obtained results the maximum reaction velocity (V_{max}) for hemicellulase enzyme by using xylan as standard substrate was determined and equalled to 273.4 mg/ml/min, while the Michael's constant (K_m) was found to be 0.160 g/L which can be obtained by the half point of maximum reaction velocity (V_{max}).

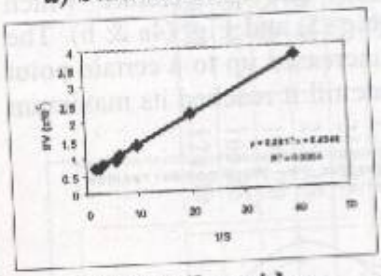
On the other hand, the V_{max} for this enzyme with isolated hemicellulose from different lignocellulosic materials were 253.3, 196.7 and 220.0 mg/ml/min, respectively. While, K_m for the above-mentioned isolated hemicellulose were 0.125, 0.160 and 0.175 g/L for peanut pods, midrib and wood powder residues, respectively.



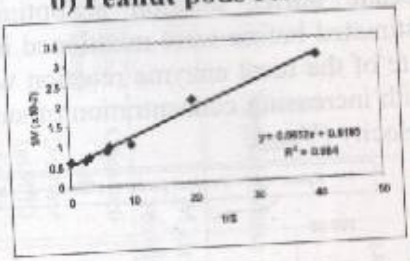
a) Standard substrate



b) Peanut pods residue



c) Midrib residue



d) Wood powder residue

Fig. (4b): Lineweaver-Burk plots of isolated hemicellulose fro from different wastes.

The obtained results indicate that the V_{max} value of xylan polysaccharide is higher than that obtained by using peanut pods, midrib and wood powder residues as substrate. This observation ma be due to that the percentage of pentoses sugars as xylose monmer i the isolated hemicellulose from the abovementioned substrates is lower than that found in xylan homoglycan polysaccharide. Also, Lineweaver and Burk plots (1954) were carried out and are illustrat in Fig. (4b). There are alignment between the two techniques.

Table (5): Effect of substrate concentration on the reaction velocity of hemicellulase enzyme with xylan and isolated hemicellulose from different wastes.

Substrate concentration [S] (g/L)	1/[S]	Xylan			Peanut pods residue			Midrib residue			Wood powder residue		
		Xylose concentration (mg/ml)	Reaction Velocity (V)	1/[V] X10^{-2}	Xylose concentration (mg/ml)	Reaction Velocity (V)	1/[V] X10^{-2}	Xylose concentration (mg/ml)	Reaction Velocity (V)	1/[V] X10^{-2}	Xylose concentration (mg/ml)	Reaction Velocity (V)	1/[V] X10^{-2}
0.025	40.00	63.33	50.58	1.98	56.67	42.17	2.37	46.67	26.58	3.76	36.67	27.50	3.64
0.05	20.00	76.67	65.10	1.54	66.67	72.29	1.38	51.21	46.83	2.14	46.59	49.04	2.04
0.10	10.00	93.33	105.15	0.95	101.22	112.44	0.89	56.67	75.65	1.32	56.67	80.00	1.025
0.15	6.67	120.00	132.29	0.76	155.32	138.00	0.72	83.33	95.18	1.05	83.33	101.54	0.98
0.20	6.00	150.00	151.89	0.66	208.67	155.69	0.64	138.20	109.28	0.92	123.33	117.33	0.85
0.30	3.33	210.00	163.96	0.61	233.33	178.59	0.56	153.33	128.28	0.78	152.65	138.95	0.72
0.40	2.5	273.37	195.29	0.51	253.33	192.76	0.52	196.67	140.50	0.71	220.00	153.04	0.65
0.50	2.00	173.33	207.12	0.48	166.67	202.40	0.49	110.0	149.02	0.67	143.33	162.96	0.61
V_{max} =		273.4 mg/ml/min			253.3 mg/ml/min			196.7 mg/ml/min			220.0 mg/ml/min		
K_m =		0.160 g/L			0.125 g/L			0.160 g/L			0.175 g/L		

Table (6): Enzymatic saccharification of xylan and isolated hemicellulose from different wastes.

Time (h)	Saccharification (%)											
	Xylan			Peanut pods residue			Midrib residue			Wood powder residue		
	25 g/L	35 g/L	45 g/L	25 g/L	35 g/L	45 g/L	25 g/L	35 g/L	45 g/L	25 g/L	35 g/L	45 g/L
1	43.5	46.1	64.7	39.1	42.1	45.3	32.9	42.5	46.8	40.5	47.9	48.1
2	55.3	57.0	66.0	41.2	44.0	49.8	48.6	52.1	57.0	56.3	58.7	59.6
12	59.3	60.1	70.1	47.9	50.1	61.4	61.2	63.3	60.8	62.9	61.6	64.3
24	72.8	77.7	79.8	59.6	64.7	67.2	68.0	65.4	68.2	64.2	65.4	68.0
48	86.6	84.9	82.2	68.4	72.8	70.4	73.9	70.6	71.9	70.4	66.1	72.5
96	79.5	80.6	73.9	75.5	81.0	72.6	77.2	81.2	79.0	68.6	75.7	79.6

However, the differentiation in the obtained values of (K_m) may be due to transformation of isolated hemicellulose into a form highly resistant to enzymatic attack, and differ in the main units of structure, which lead to reduce the affinity between the active sites of enzyme and substrate concentration.

These results are differ partially with those observed by (Ristroph and Humphery, 1985) they found that the apparent K_m values and V_{max} of xylanases enzymes from *Thermomonspora* sp. were 1.54 g/L and 0.066 µmol /ml/min at xylan concentrations between 0.5 to 4.0 g/L. However, Palop *et al.* (1991) found that the values of K_m and V_{max} were 1.05 g/L and 0.118 mol/ml/min of xylan as substrate concentration when evaluated of xylanase enzyme from *Clostridium celerecrescens*.

Enzymatic saccharification of xylan and isolated hemicellulose from lignocellulosic wastes:

Saccharification processes were carried out for xylan as a standard substrate and isolated hemicellulose from peanut pods, midrib and wood powder residues at different concentrations of 25, 35 and 45 g/L using (0.05 mM) acetate buffer solution. These processes were applied by hemicellulase enzyme mixture under optimum conditions. The experiments were achieved in a shaking water bath at optimum pH and temperature for different periods i.e., 1, 2, 12, 24, 48 and 96 h. The obtained results are illustrated in Table (6).

From these data, it can be concluded that the maximum value of saccharification process for xylan at substrate concentration 25 g/L after 48 h under optimum conditions of hemicellulase enzymes was 86.6%.

On the other hand, the enzymatic saccharification of isolated hemicellulose from peanut pods, midrib and wood powder residues was determined. The maximum values of saccharification process were 81.0, 81.2 and 79.6% for isolated hemicellulose from peanut pods, midrib and wood powder residues, respectively at substrate concentration of 35 g/L and 45 g/L after 96 h under optimum conditions of hemicellulase enzymes. Such results might be attributed to that the isolated hemicellulose and hence its content of pentose monomers from peanut pods and midrib residues were higher than that found in wood powder residue as shown in Table (1). These results are in agreement with those previously obtained by (Ramos *et al.*, 1992 and Soderstrom, 2003).

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دراسات كيميائية حيوية على إنزيم الهيمسليلوليز وتطبيقاته على بعض المخلفات الزراعية

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في الآونة الأخيرة أصبحت المخلفات الزراعية من المواضيع الرئيسية للدراسة نظرا لما تسببه من مشاكل التلوث البيئي علاوة علي إمكانية الحصول منها على مصادر للغذاء والطاقة بطرق غير تقليدية .

وفي هذا البحث تمت دراسة المواد اللجنوسليوليزية lignocellulosic الخاصة بمخلفات قرون الفول السوداني وجريد النخل ونشارة الخشب لإلقاء الضوء علي ملامحها الكيميائية وكذلك دراسة تأثير بعض معاملات الاستخلاص علي نشاط إنزيمات الهيمسليلوليز hemicellulase بهدف استخدامها في تحليل هذه المركبات بالإضافة إلي ذلك أجريت عمليات التقييم للحصول علي الظروف المثلي لعمل هذا الإنزيم . وأيضاً أجريت عمليات التسكر باستخدام هذا الإنزيم لتحويل الهيمسليلوز hemicellulose إلي شراب الزيلوز الذي له دور هام في الأغراض الصناعية.

أوضحت النتائج المتحصل عليها أن نسبة السليلوز الخام في مخلفات قرون الفول السوداني وجريد النخل ونشارة الخشب هي ٣٥,٧١، ٣٦,٦٢، ٣٨,١٤%على التوالي، بينما كانت نسبة الهيمسليلوز الخام تساوي ٣٨,٩٩، ٣٨,٣١، ٣٣,١٦% على التوالي. كما أظهرت النتائج أن نسبة الهيمسليلوز بعد الفصل للمخلفات تحت الدراسة هي على التوالي ٧٩,٤٥، ٧٨,٢٢، ٧٦,٥٢% (على أساس الوزن الجاف). كما انخفضت نسبة اللجنين من ١٦,٣٩% إلى ١١,٤٨ لمخلف قرون الفول السوداني، من ١٥,٧٨% إلى ١٢,١٠% لمخلف جريد النخل، بينما انخفضت النسبة من ١٩,٠١% إلى ١٤,٦٨% في مخلف نشارة الخشب وذلك بعد المعالجة الكيميائية بالصودا الكاوية لهذه المخلفات الخام.

تأثير الظروف المختلفة علي درجة نشاط إنزيم الهيمسليلوليز:

تم دراسة تأثير درجة الحموضة (pH) علي درجة نشاط الإنزيم وكان أفضل معدل للتحليل الإنزيمي عند درجة حموضة ٤,٨ بالنسبة لكل من الزيلان كمادة قياسية والهيمسليلوز المفصول من مخلف جريد النخل وكانت درجة النشاط الإنزيمي هي علي التوالي١١,٤٥، ٩,٤٤ ميكروملليمول زيلوز/لتر/دقيقة. أما بالنسبة للهيمسليلوز المفصول من مخلفات قرون الفول السوداني ونشارة الخشب فكان أفضل معدل للتحليل عند درجة حموضة٥,٠ حيث كان النشاط الإنزيمي لهما علي التوالي يساوي ٩,٥٦، ٧,٣٨ ملليمول زيلوز/لتر/دقيقة.

أوضحت النتائج المتحصل عليها أن الحرارة المثلي لإنزيم الهيمسليلوليز هي٥٥ م° لتحليل الزيلان كمادة قياسية وكذلك بالنسبة لكل من الهيمسليلوز الناتج من مخلفات قرون الفول السوداني ونشارة الخشب حيث كان أعلي معدل للتفاعل الإنزيمي يساوي ٨,٨٩، ٦,٨٣، ٦,٥٦ ملليمول زيلوز/لتر/دقيقة علي التوالي. بينما كانت درجة الحرارة المثلي

لأعلي معدل تحلل إنزيمي للهيميسليلوز المفصول من مخلف جريد النخل هي ٥٠°م ودرجة النشاط تساوي ٧,٧٨ ملليمول زيلوز/لتر/دقيقة.

تم دراسة تأثير تركيز الإنزيم علي درجة نشاط إنزيم الهيميسليلوليز وعملية التحلل الإنزيمي لكل من الزيلان كمادة قياسية والهيميسليلوز المفصول من كل من مخلفات قرون الفول السوداني وجريد النخل ونشارة الخشب حيث وجد أن أعلي معدل للتحلل بالنسبة للزيلان كان ٦,٩٦ ملليمول زيلوز/لتر/دقيقة عند تركيز إنزيمي ٥ جم/١٠٠ مللي محلول منظم ، أما بالنسبة لكل من الهمسيليلوز المفصول من المخلفات السابقة فكان أعلي معدل هو ٦,٧٠ ، ٧,٣٤ ، ٦,٥٦ ملليمول زيلوز/لتر/دقيقة عند تركيزات ٧جم/١٠٠مللي لكل من قرون الفول السوداني وجريد النخل ، ١٠جم/١٠٠ مللي مع نشارة الخشب.

تم دراسة تأثير تركيز المادة المتفاعلة علي نشاط الإنزيم وأوضحت النتائج أن أعلي معدل للتفاعل بالنسبة للزيلان كانت ٢٧٣,٣٧ملليجرام زيلوز/لتر/دقيقة عند تركيز ٠,٤ جم/لتر بينما كانت٢٥٣,٣٣، ١٩٦,٦٧، ٢٢٠ ملليجرام زيلوز/لتر/دقيقة عند نفس التركيز السابق لكل من مخلفات قرون الفول السوداني وجريد النخل ونشارة الخشب علي التوالي، بينما قيم ثابت ميكالس منتن فكانت ٠,١٦، ٠,١٢٥، ٠,١٦، ٠,١٧٥ جم/لتر لكل من الزيلان والهيميسليلوز المفصول من المخلفات تحت الدراسة علي التوالي.

عملية التسكر Saccharification:

تم إجراء عملية التسكر تحت الظروف المثلي السابقة لنشاط إنزيم الهيميسليلوليز ولمدة ١، ٢، ١٢، ٢٤، ٤٨ ، ٩٦ ساعة لكل من الزيلان والهيميسليلوز المفصول من المخلفات تحت الدراسة. أظهرت النتائج أن أعلي معدل للتسكر (٨٦,٦%) عند تركيز ٢٥ جم/لتر من الزيلان كمادة متفاعلة بعد ٤٨ ساعة. بينما كانت هذه المعدلات للتسكر مع الهيميسليلوز المفصول من مخلف قرون الفول السوداني وجريد النخل ونشارة الخشب فكانت ٨١,٠، ٨١,٢ ، ٧٩,٦% تحت الظروف المثلي للإنزيم وعند تركيزات تساوي ٣٥جم/لتر، ٤٥جم/ لتر ولمدة ٩٦ ساعة.

ونظراً لأهمية هذه الدراسة فإنها ستلقي مزيداً من الضوء عليها في الدراسات المستقبلية حيث ان هذه الدراسة مهمة من الناحية التكنولوجية والصناعية.