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THE USE OF SUGAR BEET INDUSTRIAL BY-PRODUCTS FOR AMYLASES PRODUCTION BY *Aspergillus fumigatus*

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ABSTRACT

The production of amylases utilizing the sugar beet industrial by-products was carried out using *Aspergillus fumigatus*. In this investigation, the time course of α- and β-amylases production, some factors affecting amylases production and some properties of the two obtained enzymes were examined. Obtained data proved that the of sugar beet pulp (SBP) and sugar beet leaves(SBL) showed to be the best C-source for amylases production after the 6th day of fermentation. The proper time for amylases fermentation was found to be 6 days when using sugar beet leaves (SBL), sugar beet pulp + sugar beet leaves (SBP + SBL) or leaves + molasses (SBL + SBM) as carbon source. Data also showed that 33°C and pH 6 were the optimum temperature and initial pH value for the enzymatic production. Regarding the N-source, the presence of peptone (1 gl^{-1}) and $(NH_4)_2 SO_4$ (2 gl^{-1}) showed to be the best source for amylases production. Some properties of *A. fumigatus* amylases were also examined, data showed that 50°C, pH 6 and 60 min., were the optimum temperature, pH and incubation period required for the amylases activities. For thermal stability, α-amylase showed stability after incubation time at 40 and 50°C for 15, 30 and 45 min., while sharp decrease was found at 60°C. β-amylase exhibited little decrease when the enzyme exposed to 40°C for 45 min. High decrease of β-amylase activities were observed at both 50 and 60°C for 45 min. Obtained data can led to maximize the recycling of agro-industrial wastes to get some useful materials such as enzymes and to protect our environment clean.

INTRODUCTION

Nowadays, a great interest has been focused on the enzymatic hydrolysis of starchy materials. Although much researches of this subject have been performed, little detailed work have been done on the sugar industry and microbial enzymes from cellulosic wastes is available so far.

Microbial processes which based on enzymatic saccharification for the production of energy, useful chemicals, single-cell protein and high-cost microbial products, such as antibiotics, and enzymes from lignocellulosics are not yet economically feasible. Amylolytic enzymes are synthesized by a numerous number of microorganisms, though fungi are generally more producers than the most active bacteria. The production of amylase from fungi have been extensively studied and the most active amylases are presently derived from the genera *Trichoderma* and *Aspergillus* (Antisuka *et al.*, 1988 and El-Syiad, 1990). The cost of carbon source has been considered to be the major contribution to amylase production. So, a new approach to reduce the cost of enzyme production is proposed by using the lignocellulosic wastes as a carbon source and as substrate for the saccharification process. Rice, cotton, sugar cane and sugar beet are Egyptian main field crops which generate the highest proportion of locally agricultural and industrial wastes. These wastes can be utilized as carbon

sources for amylases production (Marsden and Grey, 1992). One of these wastes is the sugar industry by-product, either sugar beet pulp, leaves and/or molasses. The chemical composition of these fractions were examined by many workers. Many investigators have also used these wastes to produce many useful materials mainly some hydrolyzed enzymes, sugars and single cell protein. A reduction of cost for enzyme production would improve the economics of saccharification process (Nigam, 1994; Carlsen *et al*., 1996 and Uguru *et al*., 1997). As mentioned by Poonam and Dalel (1995) that the enzymatic hydrolysis of starch has received great attention in the industrial field such as the production of soda crackers, snack foods and pizza. Amylases could be utilized in distilled alcoholic beverage, beer and textile industries, brewing, baking, soft drink canning and milling industries.

From the economic point of view of amylases production, the present work was carried out to use sugar industry by-products such as sugar beet pulp, leaves and/or molasses as low cost carbon source. The examination of some factors affecting amylases production by *Aspergillus fumigatus*, as well as to study some factors affecting the activity of produced enzymes such as incubation time, pH, temperature and thermal stability were also considered.

MATERIALS AND METHODS

Agro-industrial wastes:

The sugar beet pulp (SBP) and sugar beet leaves (SBL) of sugar beet (*Beta vulgaris* L.) and sugar beet molasses (SBM) were obtained and kindly taken from Delta Beet Sugar Company, Belquas, Dakahlia Governorate, Egypt. The prepared wastes used in this study were pulp (SBP), leaves (SBL), pulp + leaves (SBP+SBL), pulp + molasses (SBP+SPM) and leaves + molasses (SBL+SBM). Leaves and pulp were air-dried, milled and sieved to an average size of 250 µm.

Fungal strain and cultivation media used:

The fungal strain of *A. fumigatus* used in this investigation was kindly taken from Plant Pathology Dept., Agric. Res. Center, Giza, Egypt. The potato dextrose agar (PDA) medium of Demain and Solomon (1986) was used for maintaining the fungal strain at 5°C till use. The fungal strain was sub-cultured every week during the course of this work. The composition of this medium (gl^{-1}) was potato infusion, 500 ml; glucose, 20 and agar agar, 15. This composition made up to one liter with tap water and adjusted to pH 5.0, then autoclaved at 121°C for 15 minutes. The Chen and Wayman medium (1991) was used for fungal growth and the enzymatic production. The composition of this medium is (gL^{-1}). KH_2PO_4 2.0;$(NH_4)_2SO_4$ 2.0; $MgSO_4.7H_2O$, 0.3; $CaCl_2$, 0.3 and Peptone, 1.0. The wastes of sugar beet (SBP 1%, SBL 1%, SBP 0.5% + SBL 0.5%, SBP 0.5% + SBM 0.5% or SBL 0.5% + SBM 0.5%) were added as a sole carbon source. The initial pH of the medium was adjusted to 5.0 with HCl or NaOH 0.1N solution. The cultural medium was then autoclaved at 121°C for 15 min.

Maintenance of the fungal culture:

The fungal culture was maintained on PDA slants at 5°C till use. Prior to use, the microbial culture was transferred to new PDA slants and re-incubated again at appropriate temperature (28°C) for 72 hr.

Inoculum preparation:

Inoculum was prepared by transferring 12 days old conidia grown on PDA slopes by 5 ml sterile distilled water. Each slant was shaken vigorously for one min. added to 95 ml sterile water. A portion of one ml of this spores suspension, which contains about $10^5 - 10^7$ spores / ml was used for the inoculation of each 100 ml Erlenmeyer flasks each containing 50 ml of the production medium of Chen and Wayman (1991).

Working flasks preparation:

Six groups were prepared, each containing four flasks, three replicates and one as control. Fifty ml of the used cultivation medium was dispensed in 250 ml Erlenmeyer flask, then supplemented with 1% of carbon source and autoclaved at 121°C for 20 min after adjusting the pH to 5.0.

Fermentation procedure and enzymatic activities:

For fermentation process, the autoclaved flasks were then inoculated with standard inoculum size of appropriate dilutions of spores suspension as described above. The incubation was then carried out in a rotary shaker (180 rpm) at 30°C. During 15 days of incubation period, one group of the prepared flasks was taken every 3 days. Some factors affecting the two amylases production were examined. These factors are growth temperature, time course of enzymatic production, initial pH of cultivation media and the omission of nitrogen source from the basal medium was also performed. After that, the fungal mycelium was separated by filtration and the cultural filtrate was taken as enzyme solution. This enzyme solution was used for measuring two enzymes namely α-amylase and β-amylase.

Using these optimum conditions for the enzymatic production, the effect of incubation temperature, pH and reaction time on the enzymatic activities were examined. The inactivation of the temperature and thermal stability was also tested. The optimal pH for amylases was determined using citrate-phosphate buffer (pH, 3.0-5.6) and sodium phosphate buffer (pH 6.0-8.0).

Biochemical analysis :

α-Amylase activity of the cultural centrifugates at different times of cultivation were measured by modified iodine method described by Hernandez and Pirt (1975). A portion of 2.5 ml of 0.4% soluble starch in phosphate buffer pH 7.0 was mixed with 0.5 ml of centrifugate solution and incubated for 15 min at 50°C. The reaction was stopped by adding 1.0 ml of 1.0 N HCl. From each reaction tube, 0.5 ml was taken and mixed with 1.0 ml of 0.2% iodine – 0.4% KI solution and 2 ml distilled water, allowed to stand 15 min at room temperature. The obtained intensities were measured at 620 nm using Spekol II spectrophotometer (PYE Unicam). One unit of α-amylase

activity was defined as the amount of enzyme that hydrolyzed 0.1 mg of soluble starch in 15 min at optimal assay conditions.

β-Amylase activity was measured by Hernandez and Pirt (1975) method. A protein of 2.5 ml of 4.0% soluble starch prepared in phosphate buffer (pH 7.0) was mixed with 0.5 ml of the cultural of filtrate and incubated at 50°C for 15 min. The reaction was stopped by boiling for 10 min. at 100°C. The amount of reducing sugars produced by the crude enzyme was measured by the method of Somogyi (1952) using Spekol II Spectrophotometer at 500 nm. One unit of enzyme activity corresponds to the amount of enzyme that liberated 1 μmol maltose min ml^{-1} at optimal conditions.

Statistical analysis:

The experimental data were statistically analyzed using the analysis of variance by the Factorial Design method. This has been done in order to a certain whether the experimental observation of tested parameters were real and the differences between them are significant (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Time course of amylases production by *Aspergillus fumigatus*:

For the fungus *Aspergillus fumigatus*, the production of α-amylase was in its peak at the 6th day of the fermentation in case of using the pulp of sugar beet. The same finding was found in case of using leaves (SBL), pulp + leaves (SBP+SBL) and leaves + molasses (SBL+SBM) as carbon sources, since the peak of α-amylase was found after the 6th day of the fermentation. For β-amylase, the peak of the production was found also at the 6th day of the fermentation in case of all tested wastes as can be seen in Table (1). The waste contained pulp + leaves (SBP+SBL) showed to be the best to produce β-amylase being 0.529 U/ml of the cultural filtrate. This value is followed by 0.467 U/ml when using the pulp (SBP) of sugar beet waste alone as a carbon source to grow *Aspergillus fumigatus*. These results are in agreement with those obtained by Domingues and Peralta (1993), as well as Selim (2001).

Table 1. Effect of sugar beet wastes and time course on amylases production by *Aspergillus fumigatus* grown at 30°C (reaction mixture was done at 40°C for 15 min using citrate phosphate buffer pH 4.8)

Waste Used	3 days		6 days		9 days		12 days		15 days	
	α- U/ml	β- U/ml	α- U/ml	β- U/ml	α- U/ml	β- U/ml	α- U/ml	β- U/ml	α- U/ml	β- U/ml
SBP	2.082	0.163	3.999	0.467	2.556	0.320	1.980	0.211	1.336	0.091
SBL	2.716	0.086	3.980	0.313	2.946	0.221	2.483	0.139	1.799	0.084
P + L	2.878	0.115	5.317	0.529	3.443	0.350	2.871	0.150	1.771	0.095
P + M	2.899	0.043	3.617	0.408	3.387	0.319	1.682	0.258	1.336	0.086
L + M	2.551	0.122	4.371	0.384	3.312	0.372	2.136	0.229	1.834	0.078

α-amylase: U/ml cultural filtrate. β-amylase: U/ml cultural filtrate.
P + L = SBP + SBL P + M = SBP + SBM L + M = SBL + SBM

They found that both α- and β-amylases production by *Aspergillus fumigatus* increased with time proceeded to the 6th day then start to decrease. In addition, Uguru *et al.* (1997) found that the optimum production of amylases was at the end of the 4th day of incubation. Carlsen *et al.* (1996) noticed that alpha-amylase production appears closely coupled to the growth of *Aspergillus oryzae*.

The obtained data are also subjected to the statistics. Analyzed results showed high significance between all tested factors. These factors were waste material, incubation period (in day), and values of examined enzymes (Table 2). In addition, the coefficient of variation is calculated for the tested fungus to be 2.91%. Furthermore, the fungal growth was also followed during the fermentation periods by measuring the final pH values of the cultural medium. Obtained data showed little changes of the media pH especially in the first period of incubation, after that, the value of pH seems to be steady.

Table 2: Values of the analysis of variance of data obtained by *Aspergillus fumigatus* for amylases fermentation

Tested items	Source of variance	Degree of freedom	Mean square an Significance
Time course	Factor A	4	0.016**
	Factor B	4	0.091**
	AB	16	0.002**
	Factor C	1	0.080**
	AC	4	0.007**
	BC	4	0.004**
	ABC	16	0.003**
	Error	150	0.0000125
	Total	199	

A: Waste used. B: Incubation temperature. C: Examined enzymes.

Factors affecting amylases production:

Effect of incubation temperature:

All physico-chemical processes responsible for the functional activity of cells, to a greater or lesser extent are dependent on temperature. This is because the microbial growth and consequently the product formation are the results of a complex series of chemical reactions which are greatly affected by the temperature. To determine the optimum temperature for the enzyme production, shake flasks experiments were realized at different temperatures, 24, 27, 30, 33 and 36°C. Both α-amylase and β-amylase were examined and obtained data are plotted in Fig. (1). Data showed that 33°C was the most favourable for α-amylase and β-amylase being 6.70 and 0.584 U/ml of *A. fumigatus* cultural filtrate. This activity was 81.79% at 30°C, and 80.59% at 36°C for α-amylase. Also, the same activity was recorded with β-amylase under the same conditions, since 92.29 and 79.79% of amylase activities were recorded at 30 and 36°C, respectively. Furthermore, 24 and 27°C recorded the lowest values of the enzymatic activity represent in percentages form being 49.4 & 65.67% for α-amylase and 24.48 & 44.52% for β-amylase, respectively. These data are in accordance with those obtained by Selim (2001), who found that the optimum temperature for α- and β-amylases

production by *Aspergillus fumigatus* was 33°C. Data show that the amylase production decreased at temperature higher or lower than optimum temperature (33°C). Topiwala and Sinclair (1971) noticed that increasing of cultivation temperature may appreciably change the physical properties of the cultural medium and hence indirectly affect the cell metabolism. Obtained results are also subjected to the statistical analysis and obtained values showed high significance between the two tested factors and their effect (Table 5). The value of the coefficient of variation was found to be 0.46%.

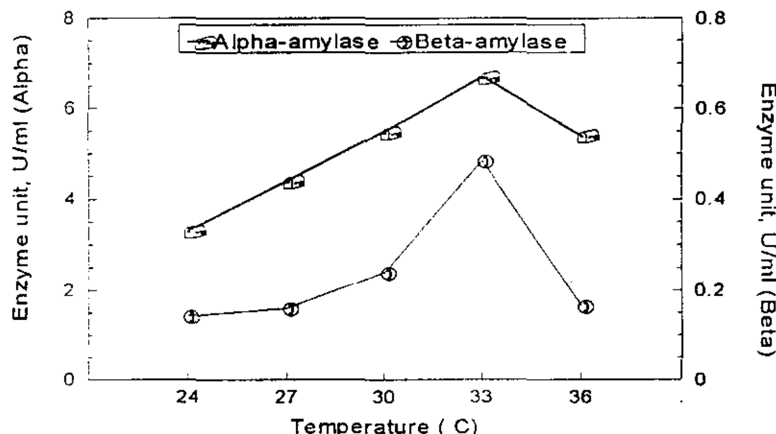

Fig. 1. Amylases activities produced by *Aspergillus fumigatus* at different incubation temperatures

Effect of the initial pH:

The initial pH of the growth medium was adjusted at various levels with 0.1 N HCl or 0.1 N NaOH solution prior to sterilize the medium containing flasks. Listed data in Table (3) showed that increasing of the initial pH resulted to a gradual increase in amylase activities of both α- or β-amylase up to pH 6.0. The α-amylase units at pH 6 were equal to 6.82 U/ml of the cultural filtrate of *A. fumigatus* after the 6th day. The percentages of activities were represent 1.03, 45.89 and 68.48% at pH 5, 4 and 3, respectively. At pH 7, the value of the enzymatic unit was 4.63, which is lower than that of pH 6 by about 32.12%. The decrease ratio reached to 59.38% at pH 8, while 62.61% decrease was found when the initial pH was 9.0.

The same trend of these results was found in case of β-amylase. General increase in the enzymatic activity was found with the increase of pH value from 3 to 6. Again the cultural medium with pH 6 gave the highest value of β-amylase activity being 0.595 U/ml of the cultural filtrate of *A. fumigatus*. Then the value of β-amylase decreased again up to pH 9. Similar results were obtained by Magmoud (1993) who found that optimal amylase yield was obtained at pH 6.0. The obtained results are in disagreement with those obtained by Selim (2000), who found that the maximum production of both α- and β-amylases by *Aspergillus fumigatus* was observed at pH 5.0.

Data of the statistical analysis showed high significance between the tested factors and its interaction as can be seen in Table (5). The value of the coefficient of variation was also calculated to be 0.6%. These data proved that both enzymes are considered to be neutral enzymes. The increase percent of the enzymatic activities was also calculated and obtained results are listed in the same Table.

Table 3: Effect of the initial pH-values on the production of amylases by *A. fumigatus* grown at 33°C after 6 days of incubation

pH	α-amylase		β-amylase	
	U/ml	%	U/ml	%
3	2.15	31.52	0.146	24.54
4	3.69	54.11	0.244	41.01
5	6.75	98.97	0.580	97.48
6	6.82	100.0	0.595	100.0
7	4.63	67.88	0.383	64.37
8	2.77	40.62	0.235	39.49
9	2.55	37.39	0.230	38.66

Effect of N-source:

In order to examine the N-source elemination on the production of amylases, four forms of the cultural medium were used. These forms are the complete medium (Chen and Wayman, 1991) (M_1), complete medium without $(NH_4)_2SO_4$ (M_2), (M_1) without peptone (M_3) and (M_1) without peptone and $(NH_4)_2SO_4$ (M_4). From the data recorded in Table (4), it could be noticed that the medium M_1 was the best in case of pH 5, 6 and 7 for α-amylase production. The value of α-amylase at pH 6 was higher than that at pH 5 by about 0.12 U/ml of the cultural filtrate. On the other hand, when the initial pH of the medium M_2 was 7.0 gave value of α-amylase lower than that at pH 6 by about 1.84 U/ml of the cultural filtrate.

Table 4: Effect of N-source elemination under different initial pH's on amylases production by *A. fumigatus* grown on M_n at 33°C for 6 days

Initial pH	Media used	α-amylase		β-amylase	
		U/ml	%	U/ml	%
5	M_1	6.73	100.0	0.583	100.0
	M_2	5.28	78.45	0.385	66.04
	M_3	4.43	65.82	0.087	14.92
	M_4	3.09	45.91	0.052	8.92
6	M_1	6.85	100.0	0.594	100.0
	M_2	5.94	86.72	0.417	86.72
	M_3	4.62	67.45	0.097	16.33
	M_4	4.37	63.80	0.084	14.14
7	M_1	4.69	100.0	0.374	100.0
	M_2	4.10	87.42	0.278	74.30
	M_3	3.70	78.89	0.048	22.46
	M_4	3.05	65.03	0.039	10.43

M_1 = Complete medium. M_2: Complete without $(NH_4)_2SO_4$
M_3: Complete without peptone. M_4: Complete without (Peptone + $(NH_4)_2SO_4$).

The complete medium (M_1) showed to be the best for β-amylase production. The highest value of enzyme (0.594 U/ml) was detected when the initial pH was 6.0, but it was 0.583 U/ml at pH 5.0. The lowest value (0.039 U/ml) of β-amylase was observed with pH 7.0 when using complete medium without peptone and ammonium sulphate. Data revealed that the deprivation of ammonium sulphate and/or peptone resulted in suppression of β-amylase synthesis and they are required to support amylases production by *A. fumigatus*. Ram Krishna *et al.* (1990) examined various nitrogenous compounds such as $NaNO_3$ and KNO_3. These sources were the best for amylase production at 45°C by *M. thermophila* D14. Data of the statistical analysis showed high significance between the tested factors and their interaction as can be seen in Table (5).

Table 5: Values of the analysis of variance of data obtained by *A. fumigatus* for amylases optimization

Tested items	Source of variance	Degree of freedom	Mean square and significance
Growth temperature	Factor A (Temp.)	4	0.044**
	Factor B (Enz.)	1	1.391**
	AB	4	0.007**
	Error	20	0.0001
	Total	29	
Initial pH of growth media	Factor A (pH)	6	0.067**
	Factor B (Enz.)	1	0.875**
	AB	6	0.029**
	Error	28	0.0001
	Total	41	
Nitrogen Sources in growth media	Factor A (pH)	2	0.059**
	Factor B (Med.)	3	0.149**
	AB	6	0.005**
	Factor C (Enz.)	1	2.791**
	AC	2	0.008**
	BC	3	0.023**
	ABC	6	0.077**
	Error	48	0.0001
	Total	71	

Some properties of amylases produced by *A. fumigatus*:

Extra-cellular enzymes are biosynthesized and excreted into the surrounding environments in order to breakdown the high molecular weight substances to make their cellular uptake possible for formation of new living materials of growth and reproduction. From this fact, it could be logically to suppose that the optimal temperature and pH of enzyme action are likely the same as those of microbial growth by which these enzymes were given and their production conditions, too.

Effect of temperature on amylases activities:

Results in Table (6) showed the effect of temperature on amylases activities produced by *Aspergillus fumigatus* after 15 min. The activities of amylases either α-amylase or β-amylase are increased with the increasing of

the temperature of the reaction mixture. The optimum temperature was 50°C for both tested enzymes, since the activities were 7.512 and 0.650 U/ml of the cultural filtrate for α-amylase and β-amylase, respectively. This result is disagreement with that obtained by Selim (2001), who found that the optimum temperature for amylases activities of *Aspergillus fumigatus* was 60°C. Ram Krishna *et* al. (1990) found that α-amylase produced by *Myceliophthora* thermophila D14 (ATCC 48 104) was active in a broad temperature range (50-60°C) and displayed activity optimum at 60°C and pH 5.6. On the other hand, this result is in agreement with that obtained by Bheila and Altossar (1984) and Ali *et al.* (1990), who found that the optimum temperature for amylases activities of *Aspergillus spp.* was 50°C. The same results were also found by Domingues and Peralta (1993) and Selim (2001) using *Aspergillus oryzae* and *Aspergillus fumigatus*, respectively.

Table 6: Effect of reaction mixture temperature on the activity of amylases produced by *A. fumigatus*

Temp. (°C)	α-Amylase		β-Amylase	
	U/ml	Fold	U/ml	Fold
30	2.360	0.31	0.189	0.29
35	3.580	0.48	0.260	0.40
40	6.750	0.90	0.597	0.91
45	7.287	0.97	0.550	0.85
50	7.512	1.00	0.650	1.00
55	7.219	0.96	0.613	0.94
60	7.024	0.94	0.478	0.74
65	5.526	0.73	0.432	0.66
70	4.551	0.61	0.315	0.48

Reaction mixture was done using acetate buffer, pH 4.8 for 15 min.

Data showed also that the maximum value of the enzymatic activity was found at 50°C, which are significantly different from the optimum growth temperature (33°C). At 60°C, the amylase activities decreased to 7.024 U/ml then decreased to 5.526 at 65°C and lastly to 4.551 U/ml at 70°C. This thought to be accounted for by the denaturing effect of heat on the enzymatic operation. The sharp decline of enzymatic stability and the presence of break at about 55-60°C suggest the possibility that conformational changes of the enzyme protein occurred around the temperature mentioned above. The same results were found by Uchino (1982). Amylase obtained by Uguru *et al.* (1997) showed its optimum activity at 70°C and was stable at the temperature range of 30-65°C.

Obtained results are also subjected to the statistical analysis using the factorial randomized design. Analyzed results showed high significant differences between the tested factors as shown in Table (7). The coefficient of variation is also calculated to be 0.52%.

In addition, both α- and β-amylases showed activities in a wide range of temperature between 40 and 60°C with maximum value at 50°C. De Mot and Verachtert (1985) found that α-amylase maintained at 50°C for 1 hr lost 8.1% of its original activity, but β-amylase lost 38.9% of its original activity. Results obtained here are in accordance with those obtained by Domingues

and Peralta (1993). Amylase obtained by Uguru *et al.* (1997) retained more than 5% of its activity at 80°C for 30 min.

Effect of pH on amylases activity :

Since enzymes are proteins containing many ionizable groups, the effect of pH on enzymes is due to changes in the different states of ionization of the components of the system. The enzymatic reaction mixture subsequently may undergo such changes, either the free enzyme, substrate, and the (E-S) complex, as explained by Bailey and Ollis (1986). Eleven pH levels were used (citrate-phosphate buffer pH 3.0-5.6 and sodium phosphate buffer pH 6.0-8.0) to examine their effect on the amylases activity, which produced by *Aspergillus fumigatus*. The activity of α-amylase was gradually increased with increasing the pH value up to pH 6.0 being 8.068 U/ml of the cultural filtrate. This value was decreased at pH 8. The activity of α-amylase at pH 6 is much higher than that at pH 3.0 by 3.4 times, while this ratio decreased to 1.35 at pH 8.0. In case of β-amylase, both pH of 5.6 and 6.0 gave the highest values of the enzymatic activities being 0.635 and 0.695 U/ml of the cultural filtrate (Fig. 2).

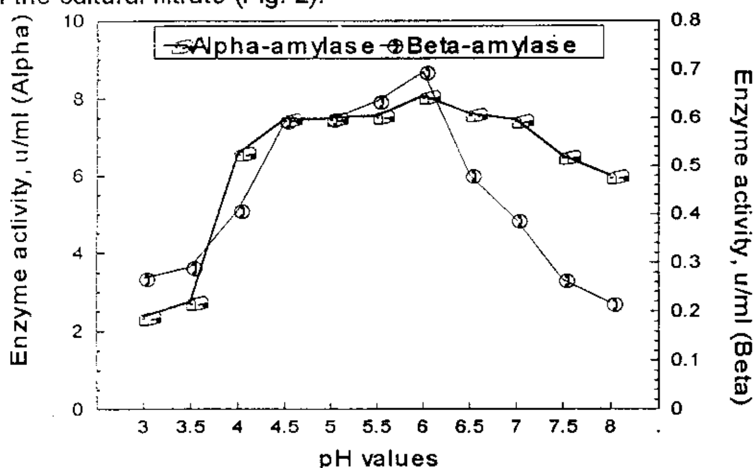

Fig. 2. Effect of pH values of the enzyme reaction mixture on amylases activities

Obtained data also showed that pH 6 was the optimum for both two tested enzymes of α- and β-amylases. These data indicated that α-amylase obtained by *Aspergillus fumigatus* is neutral enzyme. Selim (2001) obtained acidic amylases from *Aspergillus fumigatus* using some agricultural wastes since the optimum pH value was 4.6. Neutral amylases were found by *Aspergillus fumigatus*, since the optimum pH was 6.0 as reported by Domingues and Peralta (1993). Pikke *et al.* (1995) observed the stability of α-amylase obtained by *A. oryzae* at pH 6.0. Amylase obtained by Uguru *et al.* (1997) using *A. niger* showed optimum pH at 5.5 and was stable over the range of 4.0-7.5. The optimum pH for amylase activity was 5-6 as reported by Ram-Krishna *et al.* (1990).

Obtained results are also statistically analyzed using the factorial randomized design. Analyzed results showed high significance between the tested factors as shown in Table (7). Additionally, the coefficient of variation is also calculated to be 5.8%.

Table 7: Values of the analysis of variance of data obtained for properties of amylases produced by *Aspergillus fumigatus*

Tested Items	Source of variance	Degree of freedom	Mean square and significance
Temperature	Factor A	8	0.156**
	Factor B	1	0.1326**
	AB	8	0.058**
	Error	38	0.000
	Total	53	
pH	Factor A	10	0.077**
	Factor B	2	3.770**
	AB	20	3.036**
	Error	44	0.001
	Total	76	

Effect of incubation time on amylases activity:

In addition, to determine the optimum time of the reaction mixture of both α-amylase and β-amylases, the activities were measured at different incubation periods from 10 to 70 min with 10 min increment. Obtained results are graphed in Fig. (3) showed that the optimum incubation time for both α- and β-amylases was 50 min. Similar results were obtained by Selim (2001) where 50 min were the optimum time for α- and β-amylases activities.

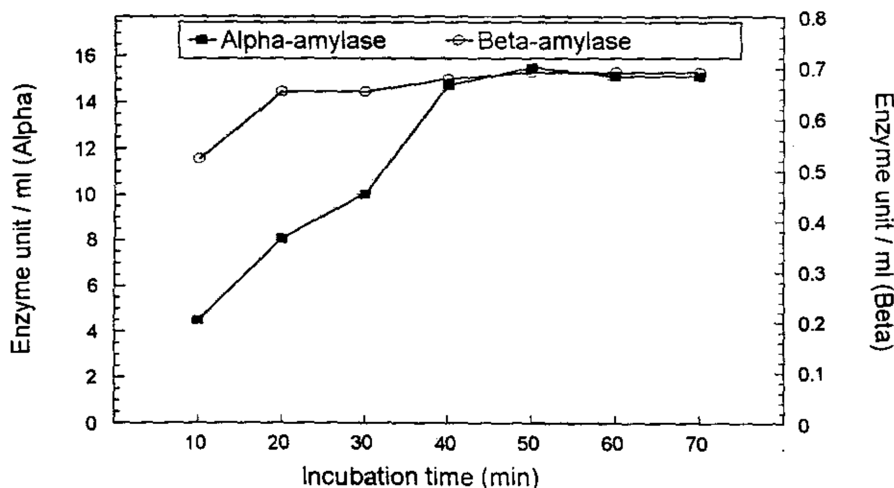

Fig. 3: Effect of the incubation time of the reaction mixtures on the amylases activities produced by *Aspergillus fumigatus* The incubation was done at 50°C and pH 6.0.

Thermal stability of amylases activity:

The effect of thermal stability on both α-amylase and β-amylase was measured after incubating the cultural filtrate at 40, 50 and 60°C for 15, 30 and 45 min. Afterwards, the cultural filtrate was immersed in an ice bath and then the residual activities were measured. The effect can be seen in Figs. (4 and 5) fo α-amylase and β-amylase, respectively. Plotted data clearly show that α-amylase was stable after incubation at 40 and 50°C for 15, 30 and 45 min. High decreases of β-amylase activities were found at both 50 and 60°C for 45 min as shown in Fig. (5). Carlsen *et al.* (1996) stated that stability of α-amylase from *A. oryzae* was found to be very high in the pH range 5-7. More than 95% of the enzymatic activity remains after 14 days at room temperature at pH 6.0. Ram-Krishna *et al.* (1990) found that amylase obtained from *M. thermophila* D14 was active in a broad temperature range, 50-60°C and displayed activity optima at 60°C.

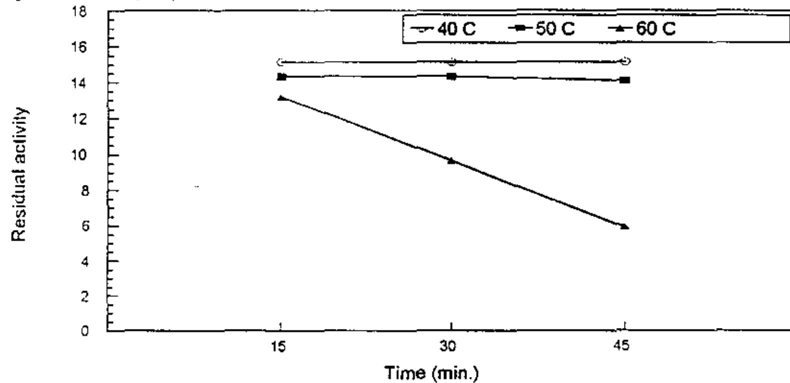

Fig. 4. Effect of temperature on the α-amylase produced by *Aspergillus fumigatus* (crude enzyme was exposed to 40, 50 or 60°C for 15, 30 or 45 min. and residual activity was measured)

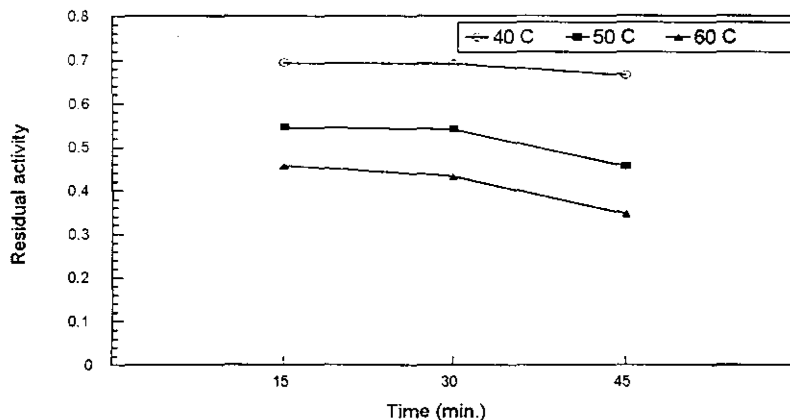

Fig. 5. Effect of temperature on the β-amylase produced by *Aspergillus fumigatus* (crude enzyme was exposed to 40, 50 or 60°C for 15, 30 or 45 min. and residual activity was measured)

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إستخدام مخلفات صناعة سكر البنجر لإنتاج إنزيمات الأميليز بواسطة فطر أسبرجلس فيوميجانس

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تم دراسة إنتاج إنزيمات الأميليز ألفا وبيتا بإستخدام المخلفات الناتجة من صناعة سكر البنجر بواسطة فطر أسبرجلس فيوميجاتس. في هذا البحث تم دراسة تاثير الوقت اللازم لإنتاج كلا من الإنزيمين تحت الدراسة وكذلك تاثير مصدر الكربون المناسب متمثلا فى المخلفات الصناعية وكذلك دراسة تاثير مصدر النيتروجين. كما تم دراسة درجة الحرارة المناسبة للنمو وكذلك رقم حموضة الوسط الغذائى المستخدم للإنتاج . وقد بينت النتائج المتحصل عليها أن مخلفات لب البنجر والاوراق كان أفضل مصدر كربون لإنتاج ألفا أميليز بواسطة فطر أسبرجلس فيوميجاتس وذلك بعد ستة أيام من عملية التخمير. كذلك بينت النتائج أن كل المخلفات المستخدمة مثل أوراق بنجر السكر ، اللب ، المولاس ومخلوط الأوراق مع المولاس ومخلوط اللب مع الأوراق أعطى أيضا أفضل إنتاجية للإنزيمات تحت الدراسة بعد ٦ أيام من عملية التخمير. بالنسبة لإنزيم بيتا أميليز كان ايضا مخلوط اللب وأوراق بنجر السكر أحسن مصدر كربون. أوضحت النتائج أيضا أن درجة الحرارة المثلى لنمو الفطر لإنتاج ألفا وبيتا أميليز هى ٣٣ °م وأن رقم الحموضة المناسب هو ٦ بالنسبة لمصدر النيتروجين أوضحت النتائج أن أفضل مصدر نتروجين يتمثل فى ١ جم ببتون + ٢ جم كبريتات أمونيوم لكل لتر بيئة غذائية وهى البيئة الكاملة.

ولقد تم دراسة صفات الإنزيمات الناتجة والتى بينت نتائجها أن ٥٠ °م هى المثلى لنشاط الإنزيم المنتج وأن رقم حموضة مخلوط التفاعل الإنزيمى هو ٦ وأن فترة التفاعل الإنزيمى هى ٥٠ دقيقة لكل من ألفا وبيتا أميليز. وعند دراسة الثبات الحرارى أوضحت النتائج أن إنزيم ألفا أميليز كان ثابتا على ٤٠ ، ٥٠ °م لمدة ١٥ ، ٣٠ ، ٤٥ دقيقة ، بينما كان هناك إنخفاض ملحوظ عند ٦٠ °م. بالنسبة للبيتا أميليز أوضح إنخفاض ضئيل عند تعرض الإنزيم على ٤٠ °م لمدة ٤٥ دقيقة. كذلك وجد إنخفاض واضح للبيتا اميليز عند تعرضه على ٥٠ و ٦٠ °م لمدة ٤٥ دقيقة. ومن النتائج المتحصل عليها يمكن القول بضرورة تعظيم إستغلال المخلفات الصناعية لإنتاج مواد مفيدة مثل الإنزيمات التى تستخدم فى مجال الصناعة وكذلك للمساهمة فى حماية البيئة المحيطة بنا من التلوث بهذه المخلفات فى حالة تركها بدون إستغلال أو تدوير.