

## HYDROLYSIS OF SOME AGRICULTURAL CELLULOSIC WASTES BY *Aspergillus awamori* CELLULASES PRODUCED BY SOLID-STATE FERMENTATION

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### ABSTRACT

The use of purified cellulose for bioconversion into cellulases increases the cost of enzyme production. Consequently, there have been attempts to develop a bioprocess to produce such enzymes using different lignocellulosic wastes. The results revealed that: High extracellular cellulases activities were observed after 4 days incubation. Corn stalk (1.5%), sugar cane molasse (1%), corn steep liquor (at 0.056% as nitrogen content) were found as the best inducers for these enzymes biosynthesis. pH 6.5 and 4.5 were found as the favourable pH for  $\beta$ -glucosidase and CMC-ase & FP-ase synthesis, respectively. 35°C, 40 and 35 & 50°C were found as the optimum temperature for these enzymes production, respectively. PH 6.0 and 5.5, 50 and 60°C were found as the pH and temperature optima for  $\beta$ -glucosidase and CMC-ase & FP-ase activities, respectively. These enzymes were completely stable in the pH range between 5.0 – 6.0, outside this pH range, all enzymes lost highest amount of their maximum activities.  $\beta$ -Glucosidase was completely stable up to 50°C. Some deleterious effects were happened to enzyme protein over this temperature degree. CMC-ase and FP-ase were highest stable up to 70°C, above, enzymes activities decreased sharply. This means that these enzymes were thermostable enzymes.  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$  stimulated these enzymes activities. Others such as  $\text{HG}^{+2}$  and  $\text{Fe}^{+2}$  inhibited their activities with much more inhibition. These enzyme mixtures were successful to hydrolyzed untreated and treated cellulosic wastes. Alkali treated materials were hydrolyzed with higher extent ranged between 1.8 to 3.24 times than other untreated ones indicating that these enzymes play an important role for hydrolysis similar materials and recycling such materials to an important biotechnological substances.

**Keywords:** *Aspergillus awamori*, cellulases, solid-state fermentation, bioconversion, hydrolysis, alkali-treated, agricultural cellulosic wastes.

### INTRODUCTION

Cellulolytic enzymes catalyzing the degradation of plant polysaccharides, are important microbial depolymerases for industrial use. Cellulases are used for enzymatic hydrolysis and saccharification of cellulose-containing material and agricultural wastes, in initial purification of urban sewage, in paper production and in preparation of protoplasts for scientific studies. The great majority of cellulases used in industry have an acidic pH optimum (4.0-5.0). However, nowadays new fields of application of these enzymes (such as detergent production, processing of denim, and paper bleaching) are appearing, where cellulases that are active in neutral

and alkaline media are required (Malek *et al.*, 1988; Solov'eva *et al.*, 1997; Takashima *et al.*, 1998; Romero *et al.*, 1999 and Shady *et al.*, 2001).

Fungal systems have been the most studied for the production of cellulolytic enzymes for saccharification of cellulosic materials, the most thoroughly investigated organism being the mesophilic fungus *Trichoderma reesei*. The genera of *Aspergillus*, *Geotrichum*, *Penicillium* and *Neurospora* have shown to be efficient producers of these enzymes on an industrial scale. *Aspergillus awamori* has been used industrially for the production of several enzymes such as glucoamylase,  $\alpha$ -amylase and protease. Another important advantage of *A. awamori* is that it has a long history of safe use for the manufacture of food products destined for human consumption and is regarded as a nontoxic and non pathogenic fungus (Kastel' Yanos *et al.*, 1995; Kvachadze and Yashvili, 1996; Romero *et al.*, 1999 and Lemos *et al.*, 2001).

Therefore, in the present study, evaluated the effect of some agricultural wastes on cellulases production by *Aspergillus awamori* which the experiments were carried out in solid-state fermentation. Some properties of these enzymes were also studied. The bioconversion of some cellulosic materials to fermentable sugars have also been investigated.

## **MATERIALS AND METHODS**

### **Fungal strain:**

*Aspergillus awamori* NRRL 3126 was obtained from NRRL ARS culture collection, Northern Regional Research Lab., Agric. Res. Service, Peoria, USA. The organism was maintained on PDA medium at 4°C and subcultured monthly.

### **Solid-state fermentation and culture condition:**

The fungal strain was cultured on basal nutrient media (Lemos *et al.*, 2001). Fermentations for enzymes biosynthesis were carried out in 500- ml Erlenmeyer flasks, which containing 4 g sugarcane bagasse. The sugarcane bagasse was moistened with 50 ml of an aqueous solution composed of 0.2 g of NaCl, 0.2 g of KH<sub>2</sub>PO<sub>4</sub>, 0.04 g of MgSO<sub>4</sub>.7H<sub>2</sub>O, and 0.5 g of peptone plus 0.5 g of yeast extract as nitrogen source. The pH was adjusted to 4.5 before autoclaving. The cotton -plugged flasks were autoclaved at 121°C for 30 min, allowed to cool to room temperature and inoculated with 1 ml spore suspension ( $1.5 \times 10^6$  spores/ml, approximately). The contents were then mixed thoroughly, and the flasks were incubated at  $28 \pm 2$ °C for 7 days on a rotary shaker (150 rpm), then, mycelia were harvested by filtration and the supernatant were assayed for enzymatic activities.

### **Preparation of fungal spore suspensions:**

Spores appeared on PDA slants were scrapped by using 5 ml sterilized distilled water and dispensed in 50 ml sterilized distilled water containing 8.0 g NaCl / Litre (Hauka *et al.*, 1998).

**Treatment of sugarcane bagasse used for solid-state fermentation:**

Sugarcane bagasse as raw material were milled (1 mesh size = 1.7 mm) and washed thoroughly in distilled water. For alkali-treatment, they soaked in 2 M NaOH for 24 h, steamed for 1 h, repeatedly washed with distilled water until neutral and then oven-dried (Patel and Ray, 1994).

**Enzyme assays:**

$\beta$ - Glucosidase activity was measured according to the method of Saddler (1982). The reaction mixture contained 1 ml culture filtrate and 10 mg salicin in 1 ml 0.05 M acetate buffer (pH 4.8). The reaction was incubated at 50°C for 30 min. The reaction was stopped by the addition of 3 ml of 0.1 N NaOH. One enzyme unit was defined as the amount of enzyme released 1  $\mu$  mole of glucose / min under the above conditions.

Carboxy methyl cellulase (CMC-ase) activity was determined according to the method of Somogyi (1952). The assay mixture of 1.5 ml contained 0.5 ml of 0.05 M citrate buffer (pH 4.8), 0.5 ml of 1% CMC as substrate and 100  $\mu$ L fraction as enzyme source and the rest water. The reaction mixture was incubated at 50°C for 30 min. The reaction was terminated by heating the tubes at 100°C in a boiling water bath for 5 min and then cooled at room temperature. Reducing sugars were determined using glucose as a standard.

Filter paper-ase (FP-ase) activity was determined according to the above method (Somogyi, 1952) except that Whatman No. 1 filter paper (50 mg) were used as substrate instead of CMC. One unit of CMC ase or FP ase activity was identified as the amount of enzyme which released 1  $\mu$  mole / min of reducing sugar measured as glucose under the standard conditions.

**Cellulosic materials:**

Rice straw, wheat straw, maize stalk and cotton stalk were collected from the farm of Fac. of Agric., Mansoura Univ., Mansoura, Egypt. Saw dust was obtained from carpenter workshop. Sugar cane bagasse was obtained from Microbial. Dept., Soil, Water and Environ. Res. Institute, Agric. Res. Center, Giza, Egypt. Wastes were dried at 70°C for 36 hrs., ground in an electric grinder and sieved through a 40 mesh sieve.

**Treatments of cellulosic materials with alkali:**

Sodium hydroxide pretreatment of cellulosic materials were performed with 10% NaOH solution. Ten grams each of cellulosic wastes were mixed with 100 ml each of the NaOH solution separately and allowed to stand at room temperature for 2 hours (Abraham and Kurup, 1997), then washed with distilled water, dried at 40°C and pulverized into a fine powder (El-Azhary, 1991).

## RESULTS AND DISCUSSION

### I- Factors controlling cellulases biosynthesis:

#### 1- Time-course profile:

*Aspergillus awamori* as a filamentous fungi have been widely used to produce industrial enzymes including cellulases because it has a long history of safe use for the manufacture of food products. Thus, results presented in Fig. (1) show the production of these enzymes on sugarcane bagasse as a cellulosic waste and the results revealed that, these enzymes were produced directly upon the cultivation period. The highest levels of  $\beta$ -glucosidase, CMC-ase and FP-ase appeared after 4 days of incubation and decreased thereafter. This means that these enzymes were constitutive in their biosynthesis. These results are similar to those reported by Mansour (2001) and Ali (2001).

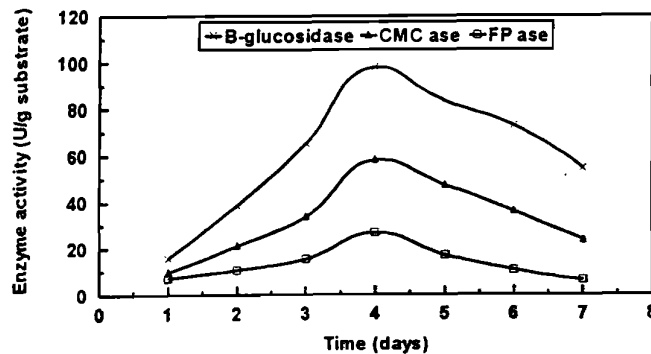


Fig. (1): Time-course profile of the biosynthesis of *Aspergillus awamori* cellulases.

#### 2- Effect of some cellulosic wastes:

The use of purified cellulose as a substrate or/and carbon and energy source for bioconversion into cellulases increases the cost of enzyme production. Consequently, there have been attempts to develop a bioprocess to produce such enzyme using different lignocellulosic wastes. The results presented in Table (1) show that high level of extracellular cellulases activities were observed on cultivation of *A. awamori* on milled cellulosic wastes. Generally,  $\beta$ -glucosidase, CMC-ase and FP-ase were produced with much amount with the use of corn stalk, which induced or stimulated these enzymes biosynthesis. This, also, indicated that these enzymes, were constitutive enzymes and induced greatly with cellulosic materials. Wheat bran also induced or stimulated the highest enzymes biosynthesis, but found in the second order. These results may be attributed to the over hydrolysis of these substances lead to higher amount of soluble and simple sugars that induce more synthesis of these enzymes. Magazine and news paper were found as repressed the biosynthesis of these enzymes, this may be due to its

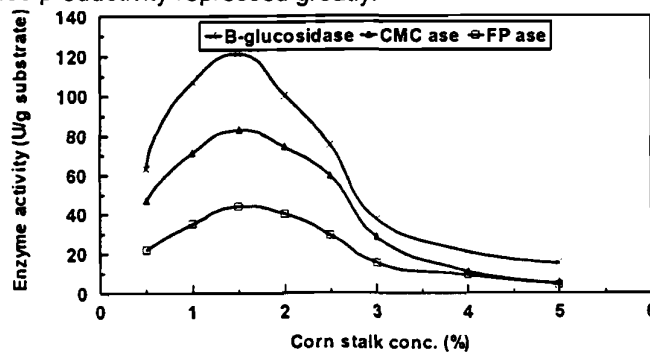
contains of some ingredients such as printed ink inhibited the enzyme synthesis. Fadel and Foda (1993) found that alkali-treated corn cobs were most suitable for cellulases biosynthesis. These results are in agreement with those obtained by Mansour (2001) and Ali (2001).

**Table (1): The biosynthesis of cellulases during the fermentation of some cellulosic wastes.**

| Cellulosic wastes | Enzyme activity (U/g substrate) |         |        |
|-------------------|---------------------------------|---------|--------|
|                   | $\beta$ -Glucosidase            | CMC-ase | FP-ase |
| Corn stalk        | 105                             | 67      | 33     |
| Wheat bran        | 103                             | 63      | 31     |
| Rice bran         | 86                              | 53      | 25     |
| Sugarcane bagasse | 98                              | 60      | 27     |
| Rice straw        | 65                              | 43      | 21     |
| Wheat straw       | 97                              | 57      | 29     |
| Banana waste      | 62                              | 32      | 17     |
| Filter paper      | 90                              | 57      | 25     |
| Magazine paper    | 45                              | 51      | 13     |
| Citrus peel       | 90                              | 37      | 19     |
| News paper        | 45                              | 41      | 11     |
| Corn flour        | 100                             | 59      | 23     |
| Barley flour      | 102                             | 61      | 21     |
| CMC               | 85                              | 37      | 16     |

**3- Effect of corn stalk concentrations:**

Results on the effect of corn stalk concentration as the best inducers on cellulases production were presented in Fig. (2). The results achieved show that, cellulases production were affected greatly with corn stalk concentration, which the increasing of its concentration up to 1.5% resulting higher increasing of these enzymes biosynthesis. Above this concentration, enzymes productivity decreased sharply, which repressed greatly the synthesis of these enzymes. Mansour (2001) and Ali (2001) found that 1% of corn stalk induced cellulases production and above this concentration, enzymes productivity repressed greatly.



**Fig. (2): Effect of corn stalk concentration on *A. awamori* cellulases biosynthesis.**

**4- Effect of various carbon sources:**

Data presented in Table (2) show that the addition of simple sugar and/or polysaccharides in the production media greatly affected the biosynthesis of these enzymes, which induced its production with much more. The highest enzymes synthesis were obtained with the addition of sugarcane molasses and glucose syrup to the fermentation media. Other materials also induced and stimulated the biosynthesis of these enzymes, but with lowest induction than those obtained with sugarcane molasses and glucose syrup. Mansour (2001) and Ali (2001) reported similar results.

**Table (2): The biosynthesis of *A. awamori* cellulases during the fermentation of some carbon sources.**

| Carbon sources          | Enzyme activity (U/g substrate) |         |        |
|-------------------------|---------------------------------|---------|--------|
|                         | $\beta$ -Glucosidase            | CMC-ase | FP-ase |
| Glucose                 | 188                             | 157     | 31     |
| Galactose               | 155                             | 105     | 25     |
| Lactose                 | 199                             | 67      | 29     |
| Manitol                 | 205                             | 73      | 23     |
| Sorbitol                | 163                             | 45      | 27     |
| Xylose                  | 167                             | 39      | 5      |
| Sucrose                 | 169                             | 28      | 15     |
| Fructose                | 175                             | 85      | 37     |
| Arabinose               | 177                             | 77      | 41     |
| Glycerol                | 195                             | 115     | 58     |
| Vinasse                 | 168                             | 98      | 47     |
| Soluble starch          | 205                             | 167     | 93     |
| Sugarcane molasse       | 235                             | 176     | 97     |
| Beet molasse            | 177                             | 154     | 87     |
| Glucose syrup           | 223                             | 167     | 89     |
| Control (without sugar) | 121                             | 83      | 44     |

These carbon sources (1%) were added to the production media containing 1% corn stalk.

**5- Effect of different nitrogen sources:**

To investigate the effect of different organic and inorganic nitrogen sources on cellulases production, data presented in Table (3) show that, generally organic nitrogen sources induced the biosynthesis of these enzymes, but inorganic ones repressed its biosynthesis. Highest enzymes productivity were observed with the use of corn steep liquor (CSL) in the fermentation media. Thus, it was chosen as the most suitable nitrogen source used for highest cellulases production, peptone +  $(\text{NH}_4)_2 \text{SO}_4$  were highest induced the enzymes secretion, but found in the second order. These observation were similar to those obtained by Mansour (2001) and Ali (2001).

**6- Effect of different concentrations of CSL:**

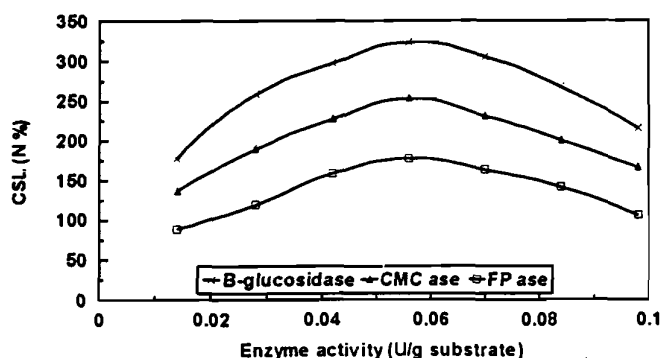
Results presented in Fig. (3) shows that increasing of CSL up to 0.056% as nitrogen content in the production media increased the

biosynthesis of these enzymes, thereafter, enzymes productivity decreased sharply. These results may be due to its contains of growth substances such as minerals and vitamins and other ingredients induced the biosynthesis of these enzymes. These means that the presented of CSL in the production media was very necessary for over production of cellulases. Mansour (2001) and Ali (2001) reported similar results.

**Table (3): Effect of different nitrogen sources on the biosynthesis of cellulases.**

| Nitrogen sources  | Enzyme activity (U/g substrate) |         |        |
|---|---------------------------------|---------|--------|
|   | $\beta$ -Glucosidase            | CMC ase | FP ase |
| KNO <sub>3</sub>  | 137                             | 66      | 43     |
| NaNO <sub>3</sub>   | 125                             | 39      | 47     |
| NH <sub>4</sub> NO <sub>3</sub>                           | 193                             | 97      | 62     |
| (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>          | 117                             | 65      | 55     |
| Peptone   | 199                             | 167     | 93     |
| Yeast extract   | 187                             | 155     | 67     |
| Peptone + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 250                             | 185     | 115    |
| Corn steep liquor   | 258                             | 189     | 117    |
| Control (peptone + yeast extract)                         | 235                             | 176     | 97     |

These nitrogen sources were added to the production media at the same level of N content.



**Fig. (3): Effect of corn steep liquor concentrations on cellulases production.**

#### 7- Effect of initial pH:

It is well known that, the initial pH value of the fermentation medium has a great effect on the growth of the organism, on the permeability of the cell membrane as well as on the biosynthesis and stability of the enzyme (Fadel and Abd-ElKader, 1994). Accordingly, results in Fig. (4) shows the effect of initial pH of the fermentation medium on the level of biosynthesis of cellulases. The maximum productivity of  $\beta$ -glucosidase was attained at pH 6.5. But the highest level of CMC-ase and FP-ase were observed at pH 4.5,

thereafter enzymes productivity decreased sharply. Therefore, it can be observed that the differences in pH were more disadvantageous for these enzymes synthesis. These results are in agreement with those obtained by Fadel (1994); Fadel & Abd-El-Kader 91994) and Ali (2001).

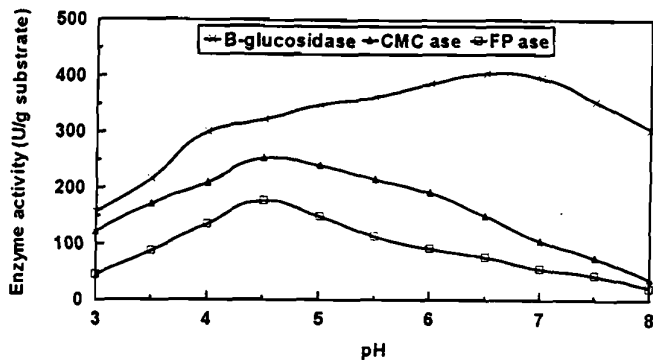


Fig. (4): Effect of initial pH on enzymes production.

**8- Effect of incubation temperature on enzymes production:**

Results presented in Fig. (5) show that  $\beta$ -glucosidase biosynthesis reached its maximum at 35°C. But, CMC-ase productivity reached its maximum at 40°C. While, FP-ase biosynthesis shows higher level of its yield at 35°C and 50°C, this means that, two fractions of this enzyme were found. Above or below these temperatures optima, enzymes biosynthesis decreased sharply. These results are similar to those obtained by Kvachadze & Yashvili (1996), Mansour (2001) and Ali (2001).

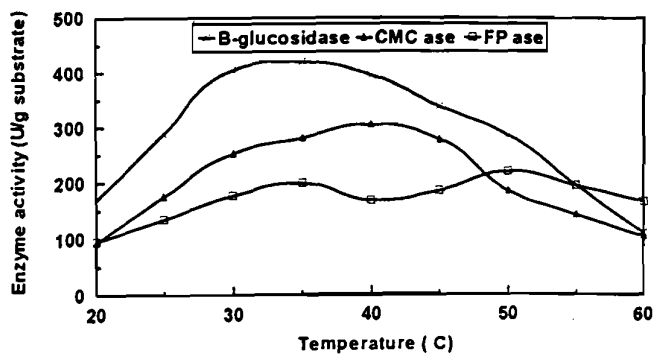


Fig. (5): Effect of incubation temperature on enzyme production.



## II. Enzyme properties:

### 1- PH and temperature optima:

The data illustrated in Fig. (6) suggest that the optimum pH for  $\beta$ -glucosidase isolated from *A. awamori* was found to be 6.0, but, the optimum pH for CMC-ase and FP-ase activities were found to be 5.5. The temperature optimum for maximal  $\beta$ -glucosidase activity was 50°C, whereas, CMC-ase and FP-ase were maximized at 60°C (Fig. 7). These results means that these enzymes had acidic in their nature and thermostable enzymes. These results are in agreements with those obtained by Solov'eva *et al.* (1997); Riou *et al.* (1998); Abd-El-Naby *et al.* (1999) and Shady *et al.* (2001).

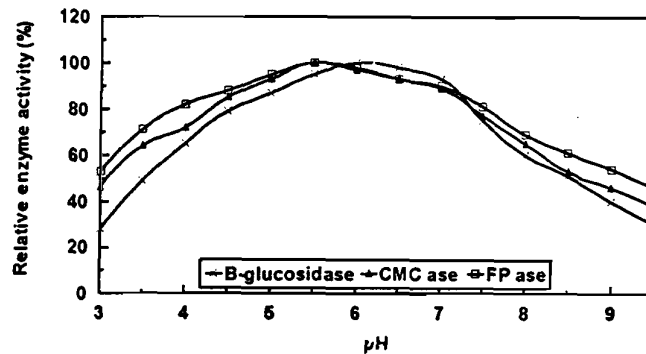


Fig. (6): pH optima of *A. awamori* cellulases.

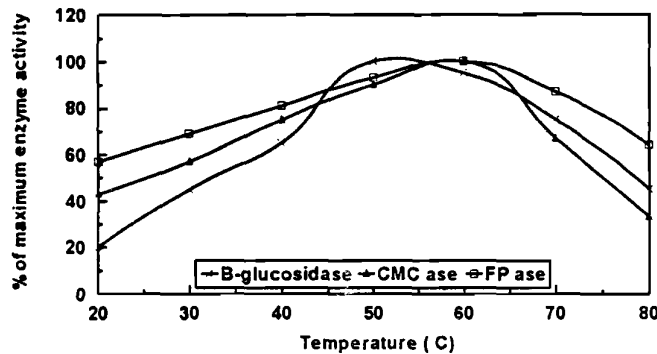


Fig. (7): Temperature optima of *A. awamori* cellulases.

### 2- PH-stability of cellulases:

The enzymatic activities showed favourable pH stabilities in acidic region (Fig. 8). Cellobiase ( $\beta$ -glucosidase) activity was completely stable at pH 5.0-6.0 and lost only 15 and 13% from its maximum activity at pH 4.0 and 7.0, respectively. Deleterious effects were observed with highly extent at

alkaline side. This means that this enzyme was acidic in their nature. CMC-ase activity was completely stable at pH 6.0-7.0. An increment of lost activity was observed outside this pH range. FP-ase activity was highly or completely stable at PH 5-6, over or below this pH range, lost of its activity was increased gradually. Therefore, these enzymes played a vital role in acidic industrial processes manufactured on cellulosic wastes. Similar results were reported by Hayashi *et al.* (1993); solov'eva *et al.* (1997); Riou *et al.* (1998) and Shady *et al.* (2001).

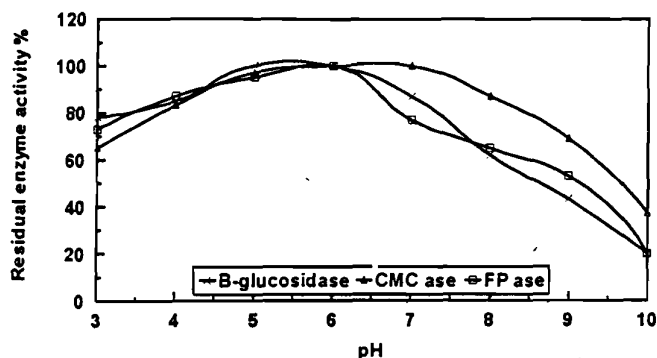


Fig. (8): pH stability of *A. awamori* cellulases.

### 3- Thermal stability of cellulases:

The thermostability of *A. awamori* cellulases showed highly stability or full activities of these enzymes at temperature up to 50, 60 and 70°C for  $\beta$ -glucosidase, CMC-ase and FP-ase, respectively (Fig. 9). But, their activities were almost inactivated at temperature above, which, they lost 60, 37 and 31% of their activities, respectively, when preincubation were performed at 90°C. These means that these enzymes were thermostable and successful in biotechnological process requires high temperature. Similar observations were reported by Kundu *et al.* (1988); Riou *et al.* (1998); Peshin & Mathur (1999) and Shady *et al.* (2001).

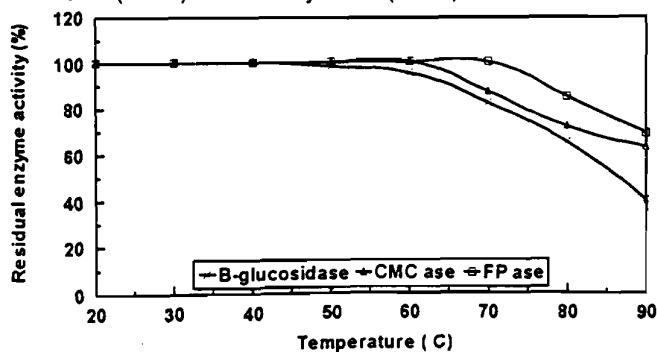


Fig. (9): Thermal stability of *A. awamori* cellulases.

**4- Effect of some activators or inhibitors:**

The effects of some metal ions and EDTA (activators or inhibitors) on the activities of *A. awamori*  $\beta$ -glucosidase, CMC-ase and FP-ase were presented in Table (4). Among these materials tested,  $Hg^{+2}$ ,  $Fe^{+2}$ , EDTA had been found to be strongly inhibitory to these enzymes, which showed significant inactivation. Also, these enzymes were also found to be inhibited by other ions such as  $Cu^{+2}$  and KCl, but with a slight extent. In contrast,  $Ca^{+2}$  and  $Mg^{+2}$  were found as activators for these enzymes, which their activities were stimulated and increased with a considerable degree. These results indicated that this enzymes is a metallo-activated ones and these specific cations could play a role in these enzymes functions. Similar observation were reported by Kundu *et al.* (1988); Riou *et al.* (1998); Abdel-Naby *et al.* (1999) and Shady *et al.* (2001).

**Table (4): Effect of some activators and inhibitors on enzyme activities.**

| Activators and inhibitors | Enzyme activity (U/g substrate) |         |        |
|---------------------------|---------------------------------|---------|--------|
|                           | $\beta$ -Glucosidase            | CMC ase | FP ase |
| None                      | 100                             | 100     | 100    |
| CaCl <sub>2</sub>         | 117                             | 108     | 105    |
| MgCl <sub>2</sub>         | 119                             | 135     | 107    |
| MnCl <sub>2</sub>         | 105                             | 88      | 61     |
| CuCl <sub>2</sub>         | 87                              | 82      | 67     |
| NaCl                      | 95                              | 105     | 96     |
| KCl                       | 97                              | 99      | 93     |
| FeCl <sub>2</sub>         | 47                              | 23      | 57     |
| HgCl <sub>2</sub>         | 33                              | 16      | 26     |
| PbCl <sub>2</sub>         | 52                              | 57      | 60     |
| EDTA                      | 49                              | 53      | 42     |

**Enzymatic hydrolysis of some agricultural cellulosic materials:**

Utilization of *A. awamori* cellulases for hydrolysis of some agricultural cellulosic wastes was shown in Table (5). The results easily indicated that these enzymes were able to degrade all these materials (such in alkali treated form or/and untreated one), but with different extent as well as with time of hydrolysis. Wheat straw, cotton stalk and rice straw were found as the most degraded materials. These means that, highly affinity between these enzymes and these materials were presented and these materials were more readily for enzymatic hydrolysis than any of other wastes. But saw dust was found as the lowest hydrolysis one After 24 hours of hydrolysis, the bioconversion of untreated materials reached 28, 32, 17, 25, 3.9 and 37% of rice straw, wheat straw, sugarcane bagasse, maize stalks, saw dust and cotton stalks, respectively. But, the degree of hydrolysis of these alkali treated substances, were reached to 70, 82, 55, 45, 9.5 and 76% of these materials, respectively. These means that, the hydrolysis of these alkali treated cellulosic wastes reached 2.50, 2.56, 3.24, 1.8, 2.44 and 2.05 times of untreated ones.

Table (5): Enzymatic hydrolysis of some agricultural cellulosic wastes.

| Agricultural wastes | % Conversion               |     |     |     |     |                |     |     |     |     |
|---------------------|----------------------------|-----|-----|-----|-----|----------------|-----|-----|-----|-----|
|                     | Untreated                  |     |     |     |     | Alkali treated |     |     |     |     |
|                     | Time of hydrolysis (hours) |     |     |     |     |                |     |     |     |     |
|                     | 1                          | 2   | 5   | 10  | 24  | 1              | 2   | 5   | 10  | 24  |
| Rice straw          | 6                          | 9   | 14  | 21  | 28  | 11             | 18  | 29  | 48  | 70  |
| Wheat straw         | 8                          | 12  | 17  | 24  | 32  | 14             | 23  | 35  | 59  | 82  |
| Sugarcane bagasse   | 4                          | 6   | 9   | 12  | 17  | 5              | 12  | 23  | 34  | 55  |
| Maize stalks        | 5                          | 7   | 11  | 19  | 25  | 4              | 10  | 15  | 24  | 45  |
| Saw dust            | 0.8                        | 1.2 | 1.9 | 2.7 | 3.9 | 2.0            | 3.5 | 6.5 | 8.2 | 9.5 |
| Cotton stalks       | 8                          | 12  | 19  | 26  | 37  | 11             | 17  | 33  | 47  | 76  |

Therefore, these enzymes play an important role in practical saccharification of cellulosic wastes. Also, the results indicated that the addition of such enzymes to animals food stuffs were very necessary in order to improve its digestion and raised its feed value. Therefore, the direct application of culture filtrate as enzymes sources for hydrolysis of cellulosic wastes also shows great potential for future development in countries like Egypt. Similar observations were reported by El-Azhary (1991); Fadel & Foda (1993) and Bhat (2000).

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إستخدام بعض المخلفات الزراعية للإنتاج العالى لإنزيمات السليوليز من فطر الأسبرجلس أوامورى بطريقة الزرع الصلب  
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و عفاف هاتم محمود رمضان  
قسم الميكروبيولوجيا - معهد الأراضى والمياه والبيئة - مركز البحوث الزراعية - الجيزة -  
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- نظراً لتراكم العديد من المخلفات السليولوزية مما يشكل خطر كبير على البيئة فقد إتجهت الأبحاث الحديثة نحو إستغلال هذه المخلفات وإعادة تدويرها وإستخدامها فى إنتاج إنزيمات السليوليز الهامة صناعياً جنباً إلى جنب نحو تحويل هذه المخلفات إلى سماد عضوى ولذلك فقد هدفت هذه الدراسة إلى إستغلال بعض هذه المخلفات فى إنتاج إنزيمات السليوليز من فطر الأسبرجلس أوامورى الأمن غذائياً ، وإستغلال هذه الإنزيمات فى تحليل بعض المواد السليولوزية والخشبية كجانب تطبيقى لهذه الدراسة ، وقد أوضحت الدراسة للنتائج التالية:
1. تم الحصول على أعلى كمية من هذه الإنزيمات بعد 4 أيام تحضين .
  2. توافر سيقان الأذرة المطحون بنسبة 1,0% فى بيئة الإنتاج ومولاس قصب السكر 1% ومنقوع الأذرة بنسبة 0,056% كنسبة نيتروجين فى بيئة التخمر أدى إلى إنتاج أعلى كمية من هذه الإنزيمات
  3. كانت درجة pH 6,0 ، 4,5 ، هى المثالية لإنتاج إنزيم البيتا جلو كوسيديز وإنزيم الكاربوكسى ميثيل سليوليز وإنزيم المحلل لورق الترشيح ، على الترتيب ، فى حين كانت درجة 3,0 م و 4,0 م و 5,0 م هى المثالية لإنتاج هذه الإنزيمات ، على الترتيب .
  4. كانت درجة pH 6 ، 5,0 و 5,0 م و 6,0 م هى المثالية لنشاط البيتا جلو كوسيديز وإنزيمات CMC و FP ، على الترتيب .
  5. أظهرت هذه الإنزيمات ثبات شبه كامل فى المدى من درجات الـ pH من 5 - 6 وخارج هذا المدى ظهرت التأثيرات الضارة لبروتينات هذه الإنزيمات .
  6. أظهر إنزيم البيتا جلو كوسيديز ثبات شبه كامل حتى 5,0 م فى حين تحملت بروتينات إنزيمات الـ CMC والـ FP حتى 7,0 م ثم بدأ تتأخر نشاط هذه الإنزيمات نظراً للتأثيرات الضارة للحرارة العالية على بروتينات هذه الإنزيمات مما يعنى أن هذه الإنزيمات ثابتة تجاه درجات الحرارة .
  7. كان لتوافر بعض أيونات المعادن مثل الكالسيوم والمغنسيوم تأثير حتى جيد على نشاط هذه الإنزيمات فى حين كان للبعض الآخر مثل الزنك والحديد تأثير تثبيطى لهذه الإنزيمات .
  8. نجحت هذه الإنزيمات فى تحليل العديد من المخلفات السليولوزية سواء المعاملة بالقوى أو الغير معاملة وإن وصلت نسبة تحلل هذه المخلفات المعاملة إلى معدل كبير تراوح بين 1,8 : 3,24 مرة قدر تحلل المخلفات الغير معاملة .
- وفى النهاية فقد أوضحت هذه الدراسة أن فطر الأسبرجلس أوامورى نجح فى إنتاج إنزيمات السليوليز بكفاءة عالية على المخلفات السليولوزية وأن هذه الإنزيمات تلعب دوراً مهماً فى تحليل المخلفات الزراعية السليولوزية والخشبية وإعادة تدويرها إلى مواد هامة بيوتكنولوجياً.