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THE USE OF BANANA PEEL FOR CELLULOLYTIC ENZYMES PRODUCTION BY A LOCAL STRAIN OF *Aspergillus niger*

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ABSTRACT

Banana peel was used as a carbon source to produce cellulases and cellobiase in shake flask cultures by A. *niger*. Peak cellulases and cellobiase activities were obtained on the 6th day of fermentation. The temperature and pH influenced the yield of enzymes. The maximum cellulase activities were recorded at 30°C and pH 5.0. The maximum rate of enzyme activities was obtained when *A. niger* was grown on 1.5% banana peel. Deprivation of ammonium sulphate, peptone or nitrogen source as whole inhibited cellulolytic enzyme formation. The maximum enzyme activities were recorded at 40°C except cellobiase, which had maximum activity at 30°C. The optimum pH of CMCase, FPase and cellobiase was 5.5, 6.5 and 5.5, respectively. The maximum CMCase and cellobiase activities were detected after 1 h, whereas the maximum FPase was detected after 16 h. More than 50% of CMCase, FP-activity and cellobiase retained at 60, 70 and 60°C, respectively.

Keywords: Banana peel, cellulolytic enzymes, *Aspergillus niger*, carboxymethyl cellulase, filter paper-ase, cellobiase.

INTRODUCTION

A. niger, a well-known fungus for the production of cellulolytic enzymes, is used in a many projects on cellulose utilization. The main purpose for investigating the production of cellulases is to turn the production of glucose and single cell protein from cellulosic wastes into an economically feasible process (Youssif, 1996). Work on cellulases production aims at increasing enzyme productivity in order to reduce the price of cellulose utilization processes (Abd-El Naby, 1988).

The range of research topics includes also medium composition and cultured control optimization. However, the search on cheap waste for cellulase production is of a great importance.Agroindustrial and food-processing wastes are available in staggering quantities, which largely become a source of heath hazard. The majority of these wastes contain 30-40% cellulose (Deschamps and Huet, 1985). The use of these wastes for the production of cellulases development a cheaper cellulase for use in many purposes (Abd-ElNaby, 1988).

In this study, therefore, cellulases production by *A. niger* using banana peel was examined. This was for obtaining a cheaper carbon substrate. The optimum temperature and pH for the activity were also established.

MATERIALS AND METHODS

Organism:

A. niger used in this study was obtained from Agric. Microbiol. Dept., Fac. of Agric., Mansoura Univ., Egypt. The culture was propagated and

maintained on Potato Dextrose Agar (PDA) slants at 4°C and subcultured at monthly intervals.

Propagation:

Spore suspension of 7-day old cultures, containing 6.5 x 10^6 spores/1ml in 0.8% NaCl solution, was used as inoculum.

Production of celluloytic enzymes:

A series of 500 ml Erlenmeyer flasks, each containing 100 ml of Chen and Wayman liquid medium, 1991, (pH 5.0), were sterilized at 121°C for 15 min and inoculated with 1 ml of spore suspension. Cultivation was performed on a rotary shaker (180 rev./min). Unless otherwise stated, the cultures were harvested on the 6^{th} day of growth by filtration through glass wool filter and then centrifuged. The clear supernatants were used for enzyme assays. Some factors affecting enzymes production by *Aspergillus niger* such as time course, incubation temperature, pH of culture medium and banana peel concentration and nitrogen source giving up were studied.

Enzyme assays:

Enzyme activities were determined on filtrate samples by measuring the released sugars from substrates. Enzyme activities are expressed as international unit (U), which is defined as micromoles of glucose produced per min under the assay conditions.

CMCase activity:

CMCase activity was done according to the method of Mandels and Weber 1969). 0.5 ml diluted enzyme solution was added to 0.5 ml of 1.0% carboxymethylcellulose (CMC) in 0.05 M citrate-phosphate buffer (pH 4.8). Incubation of the reaction mixture was performed for 30 min at 40°C. The released reducing sugars were determined.

Filter paper activity (FPase):

This was done according to the method of Mandels and Sternberg (1976). To 50 mg (1x5 cm) of Whatman No. 1 filter paper 1.0 ml of 0.05 M citrate phosphate buffer pH 4.8 and 1 ml of diluted culture filtrate were added. The paper strip was coiled by touching the tube to a vibratory mixer. The reaction mixture was incubated at 40°C for 30 min. Released reducing sugars were determined.

Cellobiase activity:

The assay was performed by a modification of the method described by Berghem and Patterson (1974) as follows: to 1.0 ml of 0.4% cellobiose dissolved in 0.05 M citrate-phosphate buffer (pH 4.8), 0.5 ml diluted culture filtrate was added. The reaction mixture was incubated at 40°C for 15 min. The reaction was stopped by heating the reaction mixture in a boiling water bath. The glucose released was determined.

Determination of reducing power:

The amount of reducing power was determined by the method of Nelson (1944) and Somogyi (1952) with glucose as a standard.

RESULTS AND DISCUSSION

The first part of this work deals with the production of cellulases and cellobiase by *A. niger* using banana peel as the sole carbon source in the basal culture medium

Effect of time-course:

The production of cellulolytic enzymes from the used fungus as a function of incubation time was determined in order to determine suitable sampling intervals for further experiments. The time-course of enzyme production by *A. niger* is shown in Fig. (1). The results showed that the enzymes were maximized in their productivity on the sixth day of incubation, there after the enzyme activities reduced. In subsequent experiments cultures were sampled after 6 days. Maximum enzyme production by *A. niger* was reported after about 7 days by Abd-El Naby (1988). Kang *et al.* (1999) found that the maximum yield of β-glucosidase by *A. niger* KK2 mutant grown on rice straw was obtained after 7 days of incubation.

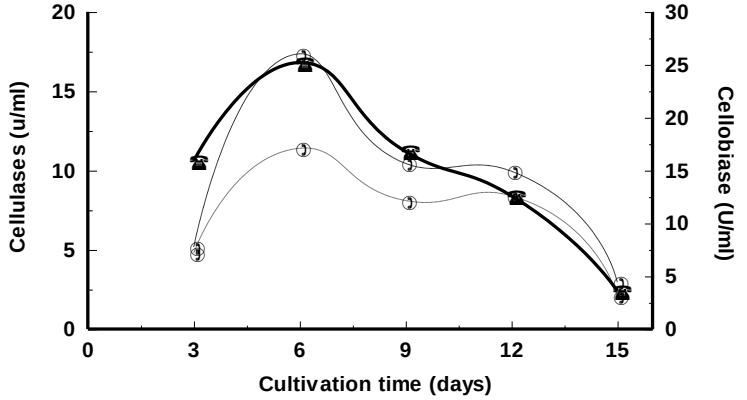

Fig. (1): Effect of time course on the production of CMCase (—), FPase (….) and cellobiase (- - -) *A. niger* in liquid medium (pH 5.0 at 30°C with 1% banana peel).

Effect of incubation temperature:

As shown in Fig. (2), the rate of cellulases production was influenced by the cultivation temperature. The production of celluloytic enzymes after 6 days in basal medium (pH 5.0) increased as the temperature of incubation increased up to 30°C. The maximum enzyme production attained at 30°C. The yields of enzymes were 16.4, 11.1 and 26.3 (U/ml) for CMCase, FP-ase and cellobiase, respectively. Many investigators reported that optimum temperature for cellulolytic enzyme biosynthesis are between 27-30°C (Sternberg, 1976; Allen and Andreotti, 1982; Cauchon and Leduy, 1985; Webb *et al.* 1986, Doppelbauer *et al.*, 1987 and Youssif, 1996).

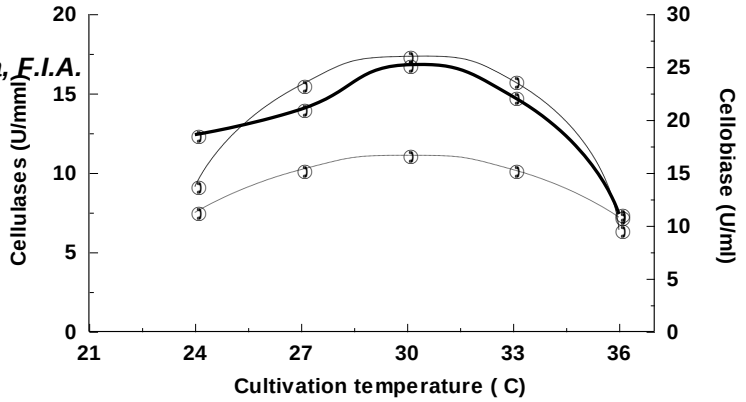

Fig. (2): Effect of cultivation temperature on the production of CMCase (—), FPase (….) and cellobiase (- - -) by *A. niger* in liquid medium (pH 5.0 with 1% banana peel for 6 days).

Effect of initial pH value:

To optimize the production of cellulolytic enzymes further, the effect of initial pH was investigated and the results are shown in Fig. (3). The pH greatly affected the enzyme activity of culture filtrates. Enzyme activities increased by the increase of the pH of the culture medium from 3.0 to 5.0. A pH range 5.0 to 6.0 seemed to be optima for cellulolytic enzymes production. Outside this range, enzyme biosynthesis was repressed, which was lowered to some extent. The enzyme productivity was maximized at pH 5.0, indicating that the *A. niger* protease was produced on acidic medium and the increase of acidity or alkalinity of the medium by which organism was surrounded, profoundly affected enzyme formation. These results are in agreement with those reported for cellulase and cellobiase by Mandels and Andreotti (1978).

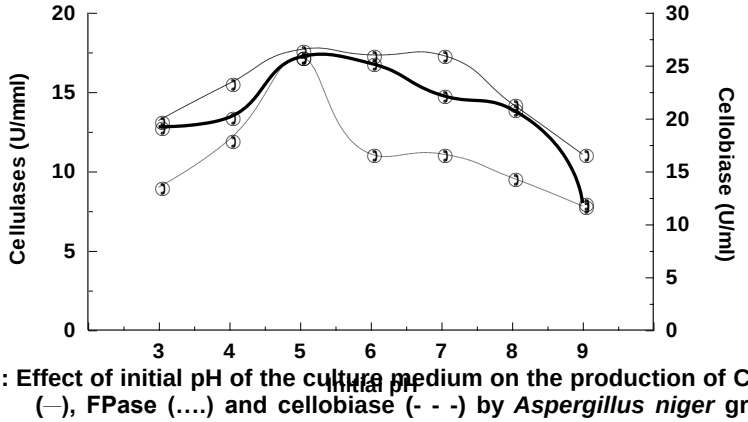

Fig. (3): Effect of initial pH of the culture medium on the production of CMCase (—), FPase (….) and cellobiase (- - -) by *Aspergillus niger* grown in liquid medium with 1% banana peel for 6 days at 30°C.

Effect of banana peel concentration:

The effect of banana peel at various concentrations was investigated for production of cellulolytic enzymes by used fungus. The optimal banana peel concentration for enzyme production was 1.5% (Fig. 4). Increasing the concentrations of banana peel discouraged enzyme production. Youssif (1996) found that 1.5% beet pulp achieved the maximum values of both

CMCase and FPase by *Trichoderma viride* and further increase in concentration caused gradual repression of enzyme biosynthesis. Selim (2000) found that the maximum yield of amylases was obtained when banana peel, at a concentration of 0.5% was added as a carbon source into the basal medium employed for enzyme production.

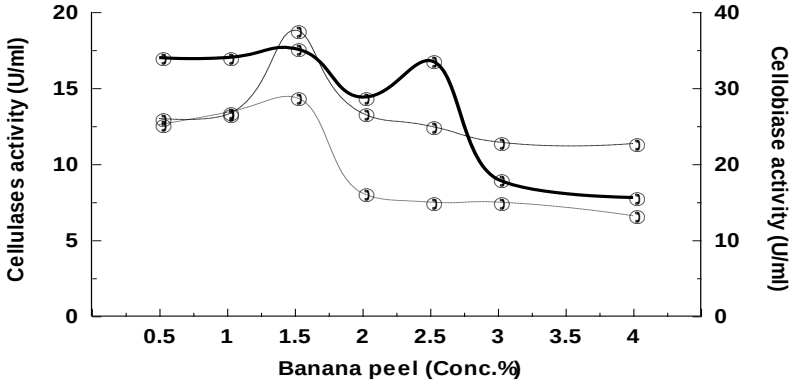

Fig. (4): Effect of banana peel concentration on the production of CMCase (—), FPase (….) and cellobiase (- - -) *A. niger* grown in liquid medium with 1% banana peel for 6 days at 30°C.

Effect of $(NH_4)_2SO_4$ or/and peptone giving up:

As shown in Fig. (5), both ammonium sulphate and peptone was required to support cellulolytic enzymes formation by *A. niger.* Deprivation of ammonium sulphate or peptone resulted in suppression of celluloytic enzymes synthesis. These results are in agreement with those for *A. wentii* (Srivastava *et al.*, 1987) and *A. niger* (Abd-El-Naby, 1988). They reported the suitability of ammonium sulphate as the inorganic N and proteose peptone as the organic N source for the production of active cellulase and cellobiase by *Aspergillus* sp.

Some properties of cellulase activity:

Temperature optima:

To determine the optimum temperature of enzyme activity, different temperatures were tested to determine enzyme activity. Data illustrated in Fig. (6) assured that 40°C was the optimum temperatures for CMCase and FP-activity. On the other hand, 30°C was the optimum temperatures for cellobiase activity. Increasing or decreasing the temperature outside the range 30-50°C resulted in progressive drop in the activity. Kanamoto *et al.* (1979) found that the optimum temperature for two carboxymethyl cellulose hydrolyzing enzymes (F-I and F-V) of *A. aculeatus* were 45 and 55°C, respectively. Kang *et al.* (1999) found that the optimal temperature for enzyme activity of *A. niger* mutant was 60-70°C.

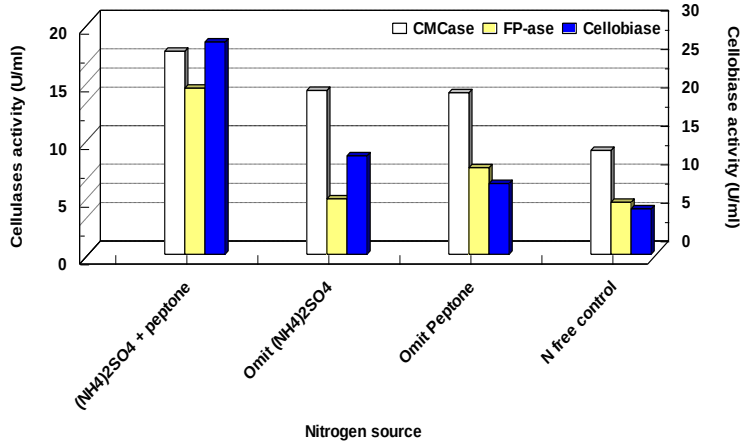

Fig. (5): Effect of $(NH_4)_2SO_4$ or/and peptone giving up on the production of CMCase (—), FPase (….) and cellobiase (- - -) *A. niger* grown in liquid med

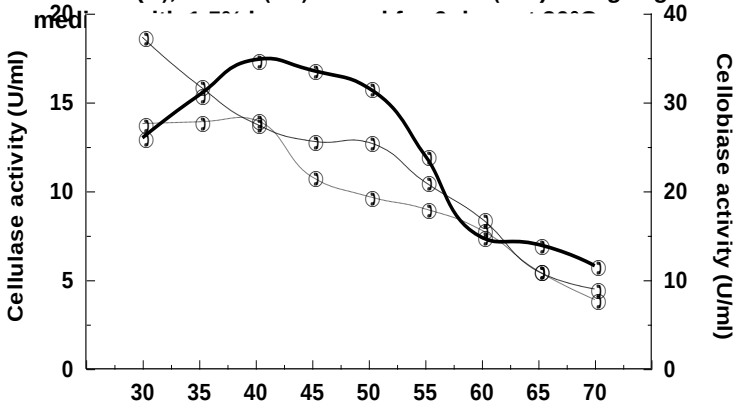

Fig. (6): Effect of reaction temperature on the activity of CMCase (—), FPase (….) and cellobiase (- - -) of *A. niger* (reaction was done at pH 4.6 for 15 min, 30 min and 60 min for cellobiase, CMCase and FPase, respectively).

Optimum pH:

The results (Fig. 7) indicated that CMCase, FP-activity and cellobiase showed their pH optima at 5.5, 6.5 and 5.5, respectively. Kang *et al.* (1999) found that the optimum pH for β-glucosidase by A*. niger* was 4.8. Kanamoto *et al.* (1979) found that the optimum pH of two carboxymethyl cellulose hydrolyzing enzymes (F-I and F-V) of *A. aculeatus* were 4.0-4.5 and 5.0, respectively. Kang *et al.* (1999) found that the optimal pH for enzyme activity of *A. niger* mutant was 4.8.

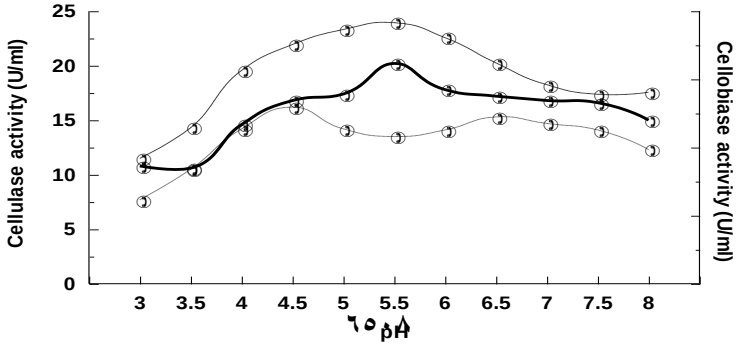

Fig. (7): Effect of pH value on the activity of CMCase (—), FPase (….) and cellobiase (- - -) of *A. niger* (reaction was done at 30, 40 and 40°C for cellobiase, CMCase and FPase, respectively).

Effect of incubation time on enzyme activity:

Incubation time of enzyme was varied from 15 to 120 min, with keeping the other operational conditions (pH and temperature) constant. The results illustrated in Fig. (8) indicated that the activities increased gradually with the increase of time. In the early time of celulolysis, cellulose hydrolysis was very high up to 60-75 min, after that hydrolysis continued in a very slow rate. Therefore, 60 min was chosen as the best reaction time of CMCase and cellobiase activities. The optimum reaction time for FPase activity was 16 h.

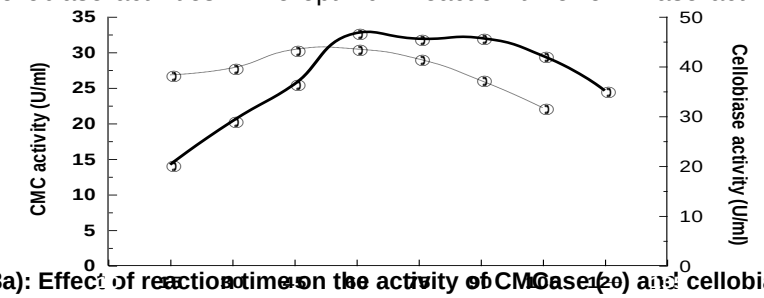

Fig. (8a): Effect of reaction time on the activity of CMCase (—) and cellobiase (- - -) of *A. niger*.

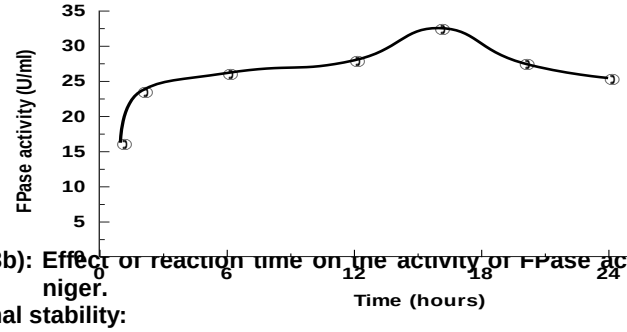

Fig. (8b): Effect of reaction time on the activity of FPase activity of Aspergillus niger.

Thermal stability:

Thermal inactivation on CMCase, FPase and cellobiase activities was investigated by measuring the residual activities after incubating the culture filtrate at 40, 50, 60 and 70°C for 15, 30 and 45 min. It is clear from Fig. (9) that the enzyme was stable at 40 and 50°C for 45 min, and lost less than of 50% of activity after 45 min at 00°C. Also, the results show clearly that enzyme lost about 50% from maximum activity at 70°C after 45 min. These results show that this enzyme was a thermostable and suitable to use in some industrial processings exposed to high temperature for 45 minutes. Abd-El Naby (1988) reported similar results on cellulases and cellobiase of *Aspergillus niger* and *Trichoderma viride.*

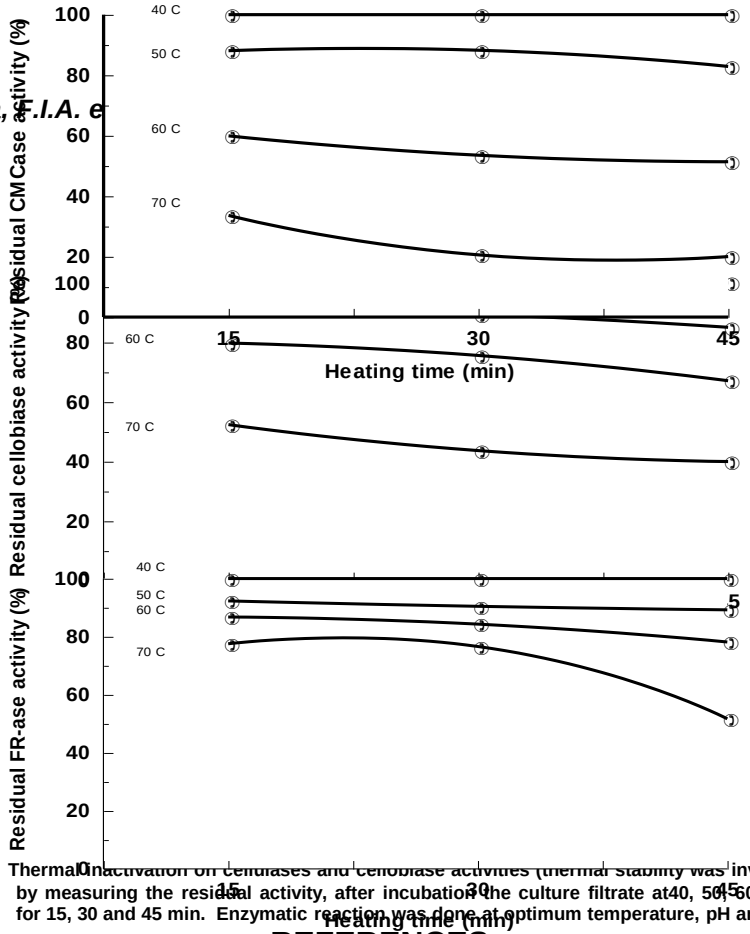

Fig. (9): Thermal inactivation on cellulases and cellobiase activities (thermal stability was investigated by measuring the residual activity, after incubation the culture filtrate at40, 50, 60 and 70C for 15, 30 and 45 min. Enzymatic reaction was done at optimum temperature, pH and time).

REFERENCES

Abd-El Naby, M. A. (1988). Biochemical studies on fungal cellulases. Ph. D. Thesis, Fac. of Sci. Mmansoura Univ., Mmansoura, Egypt.

Allen, A. H. and Andreott, R. E. (1982). Cellulase production in continuous and fed-batch cutulre by *Trichoderma reesei* MCG80 [Lecture]. Biotechnol. Bioeng. 12: 451-459.

Berghem, L. E. R. and Pettersson, L. G. (1974). The mechanism of enzymatic cellulose degradation: Isolation and some properties of a β-glucosidase from *Trichoderma koningii.* Eur. J. Biochemm. 46: 295-305.

Cauchon, N. and Leduy, A. (1985). Novel process for the production of cellulolytic enzymes. Biotechnol. Bioeng. 27: 456-462.

Chen and Wayman (1991). Cellulose production induced by carbon sources derived from waste newspaper. Process Biochemmistry 26: 93-100.

Deschamps, F. and Huet, M. C. (1985). Xylanase production in solid-state fermentation: A study of its properties. Appl. Microbiol. Biotech. 22: 177-180.

Doppelbauer, R.; Esterbauer, H.; Steiner, W.; Lafferty, R. M. and Steinmuller, H. (1987). The use of lignocellulosic wastes for production of cellulase by *Trichoderma reesei* . Appl. Microbiol. Biotechnol. 26: 485-494.

Kanamoto, J.; Sakamoto, R.; Arai, M. and Murao, S. (1979). Enzymatic properties of two carboxymethyl cellulose hydrolyzing enzymes from *Aspergillus aculeatus.* J. Ferment. Technol. 57(3): 163-168.

Kang, S. W.; Ko, E. H.; Lee, S. S. and Kim, S. W. (1999). Over production of β-glucosidase by *Aspergillus niger* mutant from lignocellulosic biomass. Biochem. Lett. 21: 647-650.

Mandels, M. and Andretti, R. E. (197\8). Problems and challenges in the cellulose to cellulase fermentation. J. Process Biochem. 3(5): 6-11.

Mandels, M. and Sternberg, D. (1976). Recent advances in cellulase technology. J. Ferment. Technol. 54(1): 267-286.

Mandels, M. and Weber, J. (1969). The production of cellulases. Adv. Chem. Series. (Hajny and Reese, E. T., eds), 95: 391. Amer. Chem. Washington, D. C.

Neish, A. C. (1952). " Analytical methods for bacterial fermentation ", Repor no., 46-8-3, National Research Council of Canada, Second Revision, November, p. 34.

Nelson, N. A. (1944). A photometric adaptation absorption of the Somogyi method for the determination of glucose. *J. Biol. Chem*. 153: 375-380.

Selim, A. E. I. (2000). Production and some properties of α- and β-amylases from *Aspergillus fumigatus* grown on some agricultural wastes. 5th Internat. Conf. On Chemistry and its Role in Development. Mans. Sci. Bull. (A Chem.) 27(1): 57-73.

Somogyi, M. (1952). Notes on sugar determination. *J. Biol. Chem*. 195: 19-23.

Srivastava, S. K., Gopalkrishnan, K. S. and Ramachandran, K. B. (1987). The production of β-glucosidase in shake-flasks by *Aspergillus wentii*. J. Ferment. Technol. 65(1): 95-99.

Sternberg, D. (1976). Production of cellulase by *Trichoderma* sp. Biotechnol. And Bioeng. Symp. No. 6, 35-53.

Webb, C; Fukuda, H. and Atkinson, B. (1986). The production of cellulase in a spouted bed fermentor using cells immobilized in biomass support particles. Biotechnol. And Bioeng. 28: 41-50.

Youssif, Nariman O. A. (1996). Utilization of some industrial and agricultural wastes of sugar crops " production of *Trichoderma reesei* celluloytic enzymes by fermentation of sugar crops agroindustrial by-products. Ph. D. Thesis, Fac .of Agric, Tanta Univ, Egypt.

إستخدام قشر الموز لإنتاج إنزيمات السليوليز من عزلة محلية من فطر الأسبرجلس نيجر

فتحى إسماعيل على حوقه ، عبد الله العوضى إبراهيم سليم ، سامية محمد مرسى بيومى
قسم الميكروبيولوجى – كلية الزراعة – جامعة المنصورة – المنصورة – مصر .

تم إستخدام قشر الموز كمصدر وحيد للكربون لإنتاج الإنزيمات المحللة للسليوليز من فطر الأسبرجلس نيجر فى مزرعة مهتزة . وقد وجد أقصى إنتاج للإنزيمات فى بيئة الزرع كان بعد ٦ أيام ، وأن أنسب درجة حرارة هى ٣٠°م ، وأفضل pH للإنتاج ٥,٥٠ ، وعند دراسة تأثير تركيزات المخلف المضافة إلى بيئة بيئة الزرع تبين أن أمثل تركيز لتخليق الإنزيمات موضع البحث هو ١,٥% من قشر الموز ، وقد أدى

حذف أى من سلفات الأمونيوم أو البيتون أو كليهما من بيئة الزرع المستخدمة فى الإنتاج إلى تثبيط تخليق الإنزيمات .

وقد وجد أن أقصى نشاط لإنزيات السليوليز كان عند٤٠°م ، بإستثناء إنزيم السلوبيوز فقد وجد أقصى نشاط له عند٣٠°م ، وعند دراسة تأثير درجات الـ pH على نشاط الإنزيمات وجد أن أمثل درجة pH لنشاط للإنزيمات المحللة للكربوكسى ميثيل سليوليز وورق الترشيح والسلوبيوز هى٥,٥٠، ٦,٥ ، ٥,٥٠ ، على الترتيب ، وعند دراسة أنسب وقت لتحضين الإنزيم إتضح أنه فى حالة الإنزيم المحلل للكربوكسى ميثيل سليلوز والإنزيم المحلل للسلوبيوز كان أنسب فترة للتحضين بعد ساعة بينما فى حالة الإنزيم المحلل لورق الترشيح فقد كان أنسب وقت للتحضين بعد ١٦ ساعة ، كما لوحظ ثبات الإنزيمات بدرجة عالية على درجة حرارة ٤٠- -٥٠°م .