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INFLUENCE OF CULTIVATION CONDITIONS ON THE PRODUCTION OF *Aspergillus niger* M 2 CELLULASES

Ali, Nadia A. A.

Microbiol. Dept., Soil, Water and Environ. Res. Institute, Agric. Res. Center, Giza, Egypt.

ABSTRACT

Screening of sixteen fungal strains for their cellulases potentiality revealed that *Aspergillus niger* M2 was the most active strain for β–glucosidase, CMCase and FPase production. 1% corn stalk induced greatly *A. niger* cellulases. Barley flour, corn flour and corn stalk were found as the best raw materials, which induced the biosynthesis of all three enzymes examined. Sugar cane molasse (1%) as carbon source had given highest amount of three cellulases which stimulated its production. The presence of corn steep liquor at 0.056% as nitrogen content in the production medium has very necessary for highest biosynthesis of *A. niger* cellulases. 0.310% and 0.155% as phosphorus content of KH_2PO_4 have very necessary for β–glucosidase and CMCase & FPase, respectively. Maximum enzyme productivity was obtained at pH 6.5 and 7.5% of inoculum level to the production medium solution. 35°C and 200 rpm/min was found as the suitable incubation temperature and agitation rate for cellulases biosynthesis. 96 hours was found as the best incubation period for enzyme formation.

Keywords: *Aspergillus niger*, optimizing, cellulase, β-glucosidase, carboxy methyl cellulase (CMCase), filter paper cellulase (FPase)–production, bioconversion, plant raw materials.

INTRODUCTION

In recent years lignocellulosic materials have received a great deal of attention as very promising raw materials for bioconversion to alcohols, other chemicals, single cell protein…etc. Thus the cooperative action of a complex of endoglucanases and exoglucanases is involved in breakdown of such complex carbohydrates. Fungal systems have been the most studied for the production of cellulolytic enzymes for saccharification of cellulosic materials (Malek *et al.*, 1988; Takashima *et al.*, 1998 and Mansour, 2001). Species of *Trichoderma* are considered to be the best cellulases producers through several other fungi also produce cellulase including *Aspergillus wentii* (Srivastava *et al.*, 1987; *A. terreus* (Bastawde, 1992), *Penicillium verruculosum* (Kastel'Yanos *et al.*, 1995), *A. niger* (Selim *et al.*, 2001) and *A. achuleatus* (Mansour, 2001).

Endoglucanases (EC 3.2.1.4), exo-cellobiohydrolases (EC 3.2.1.91) and β–glucosidases (EC 3.2.1.21) are three major types of celulolytic enzymes. Its cellulase system has been widely studied and is considered to be a rational choice for industrial use. These enzymes were act synergistically upon cellulose to produce cellobiose, which is then cleaved by β–1,4-glucosidase to liberate glucose (Sanyal *et al*., 1988; Takashima *et al*., 1998; Romero *et al*., 1999 and Mansour, 2001). The great majority of cellulases used in industry have an acidic pH optimum (4.0 – 5.0). However, nowadays new field of application of these enzymes (such as detergent

production and paper bleaching) are appearing, where cellulases that are active in neutral and alkaline media are required (Abraham & Kurup, 1997; Solov'eva *et al*., 1997 and Mansour, 2001).

In this paper, the production of three kinds of extracellular cellulases by different fungal strains were studied. The optimizing of *Aspergillus niger* M2 cellulases production were also investigated.

MATERIALS AND METHODS

Microorganisms:

Aspergillus achuleatus DSM 63261, and *A. niger* DSM 823 were obtained from Deutsche Sammlung Von Mikroorganismses und Zell Kulturen, Mascheroder weg Ib, D-33, Braunschwerg, Germany.

Aspergillus awamori NRRL 3126, *Fusarium culmorum* NRRL 32188, *Geotrichum candidum* NRRL Y–552 and *Aspergillus foetidus* NRRL 341 were obtained from NRRL ARS culture collection, Northern Regional Research Lab., Agric. Res. Service, Peoria, USA.

Aspergillus niger No. 36, No 26, No. 10, No. 11, No. 12., No. 57, M2, *A. terreus*, *A. wentii* 2001 and *Fusarium* sp. L were obtained from Agric. Microbiol. Dept., Soil, Water and Environ Res. Institute, Agric. Res. Center, Giza, Egypt. These organisms were maintained on PDA medium at 4°C and subcultured monthly.

Media and culture conditions:

The fungal strains were cultured on basal nutrient medium (Kvachadze and Yashvili, 1996), which had the following composition (%): 1 microcrystalline cellulose, 0.13 $(NH_4)_2SO_4$, 0.68 KH_2PO_4, 0.05 $MgSO_4$. $7H_2O$, 0.02 $CaCl_2$, 0.15 peptone and 1.5 corn-steep liquor. The pH was adjusted to 4.5 before autoclaving. One ml of spore suspensions containing approximately 1 x 10^6 spores were used to inoculate 500 ml Erlenmeyer flasks containing 100 ml of the above liquid medium. Flasks were incubated at 30°C $\pm$ 1 for 4 days, at which time, maximum yield of enzyme was produced on a rotary shaker (200 rpm). Mycelia were harvested by filtration and the supernatant 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 corn stalk:

Corn stalk as raw material was milled (1 mesh size = 1.7 mm) and washed thoroughly in distilled water. For alkali-treatment, it 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:

β-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 inoculated 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 μ mole of glucose / min under the above conditions.

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 μL 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.

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 CMCase or FPase activity was identified as the amount of enzyme which released 1 μ mole / min of reducing sugar measured as glucose under the standard conditions.

RESULTS AND DISCUSSION

Screening of some fungal strains for their cellulases activity:

Data on the comparative cellulases activity of some fungal strains are presented in Table (1). The screening of the sixteen fungal strains for cellulose degrading enzymes potentiality revealed that *Aspergillus niger* M 2 was the most active β-glucosidase, CMC ase and FP ase producers. Their enzymes activities were 0.8, 0.16 and 0.17 U/ml/min., respectively. *Aspergillus awamori* NRRL 3126 was found in the second order for these cellulases biosynthesis. Other strains tested, *e.g*., *Aspergillus wentii* 2001, *A. niger* NO. 12 and *Fusarium* sp. were very poor for or have not CMCase and FPase potentiality. Therefore, *Aspergillus niger* M 2 was used for further experiments to optimizing cellulases production.

Effect of corn stalk concentration on *Aspergillus niger* M 2 cellulases production

The results on the effects of corn stalk concentrations as plant raw materials on β-glucosidase, CMC ase and FP ase are shown in Table (2). Corn stalk was added as the sole carbon source at concentrations ranging from 1% to 5% (w/v). The results acheived show that corn stalk concentration greatly affected on *Aspergillus niger* M 2 cellulases production. Increasing corn stalk concentration above 1% resulting sharp decreasing of all cellulases examined. At low concentration (1%) of this plant raw materials used as a waste, cellulases activities induced greatly. Also, this result revealed that, higher concentration of the substrate repressed the biosynthesis of *A. niger* M 2 cellulases. Maximum enzyme activity reached 0.85, 0.19 and 0.18 Units /ml/min, for β-glucosidase, CMC ase and FP ase,

respectively. Optimum carbon source concentration for cellulases production by other fungi varied between 0.5% to 1% (w/v) depending on composition of the medium (Mandels and Weber, 1969), and that higher cellulosic material concentrations were inhibitory. Thus 1% appears to be the most suitable for maximum enzymes production. El-Sawah *et al* (1989) reported that 1% of barley straw was the most inducers for *Aspergills terreus* cellulases production. These results are similar to those obtained by Mansour (2001).

Table (1): Enzyme activities of some fungal strains during submerged of corn stalk (cultivation for 4 days).

Fungal culture	β-Glucosidase	CMCase	FPase
	U/ml		
Aspergillus niger DST 823	0.21	0.03	0.00
Aspergillus niger No. 36	0.35	0.10	0.10
Aspergillus niger No. 26	0.40	0.08	0.06
Aspergillus niger No. 57	0.33	0.04	0.00
Aspergillus niger M2	0.80	0.16	0.17
Aspergillus terreus	0.50	0.10	0.05
Aspergillus awamori NRRL 3126	0.60	0.16	0.15
Aspergillus achuleatus DST 63261	0.45	0.15	0.10
Fusarium culmorum NRRL 3288	0.34	0.00	0.00
Aspergillus wentii 2001	0.25	0.07	0.03
Aspergillus niger No. 10	0.22	0.00	0.00
Aspergillus niger No. 11	0.41	0.10	0.08
Aspergillus niger No. 12	0.65	0.01	0.00
Fusarium sp.	0.31	0.00	0.04
Geotrichum candidum NRRL Y-552	0.55	0.06	0.02
Aspergillus foetidus NRRL 341	0.21	0.00	0.00

Table (2): Effect of corn stalk concentration on cellulases biosynthesis during the growth of *Aspergillus niger* M 2.

Corn stalk conc. (%)	β-Glucosidase	CMC ase	FP ase
	U/ml		
1.0 (Control)	0.85	0.19	0.18
2.0	0.80	0.16	0.15
3.0	0.60	0.10	0.13
4.0	0.12	0.05	0.06
5.0	0.05	0.00	0.02

Effect of various cellulosic materials on cellulases biosynthesis:

The use of an available cellulosic wastes and by-products as carbon source in the growth medium would reduce the costs of enzyme production. Table (3) shows that corn stalk, barley flour and corn flour were the most suitable materials used for induction and biosynthesis of β-glucosidase, CMC ase as well as FP ase. But, the production of these enzymes was different with the difference of the substrate. The results obtained indicated

that delignification of cellulosic wastes by alkali increases their suitability for cellulases biosynthesis by the fungus used. These results may be attributed to much substrate lead to higher amount of soluble products that induce more biosynthesis of the enzymes (Rao *et al*., 1983 and Acebal *et al*., 1986). Fadel and Foda (1993) found that alkali-treated corn cobs were most suitable for cellulase biosynthesis. Mansour (2001) reported similar results and observation.

Table (3): Biosynthesis of enzymes during the growth of *A. niger* M 2 on various cellulosic materials.

Added materials	β-Glucosidase	CMC ase	FP ase
	U/ml		
Corn stalk* (Control)	0.90	0.20	0.17
Wheat bran	0.90	0.15	0.10
Rice bran	0.46	0.15	0.03
Bagasse	0.64	0.12	0.11
Rice straw	0.63	0.10	0.10
Banana waste	0.62	0.14	0.14
CMC	0.40	0.06	0.06
Filter paper	0.40	0.10	0.10
News paper	0.45	0.20	0.09
Magazine paper	0.45	0.20	0.10
Barley flour	1.10	0.19	0.16
Corn flour	1.00	0.20	0.15
Citrus peel	0.90	0.15	0.00
Sun flower calthde meal	0.75	0.07	0.09

*** These carbon sources were added at the same concentration 1%.**

Induction of cellulases by various carbon sources:

Table (4) shows the level of β-glucosidase, CMC ase and FP ase activities produced in the culture filtrate of *A. niger* in the presence of different carbohydrates used as sole carbon sources at 1% final concentrations. The highest level of the three enzymes activities were obtained using sugar cane molasse as carbon source. Soluble starch also induced the enzyme production, which present in the second order for these enzyme biosynthesis.

Other carbon sources such as lactose, glycerol, mannitol and vinasse were also induced the biosynthesis of all three enzymes. Fadel and Foda (1993) reported that cellobiose was found as the best inducers for cellulase and β-glucosidase production. These results are harmony with those obtained by Mansour (2001).

Effect of medium composition on enzyme biosynthesis:

From results given in Table (5), it could be noticed that cellulases biosynthesis were affected greatly with the presence or absent of any of the ingredients of the production medium. The absence of corn steep liquor repressed greatly these enzymes biosynthesis, which at this treatment, enzyme activity reduced sharply. The absence of any other ingredient also

reduced these enzymes secretion. But, the present of all ingredients induced the biosynthesis of all cellulases examined. These means that the present of carbon, nitrogen, phosphate and other sources were played an important role in cellulases production. Mansour and Saber (2001) found similar observation and results.

Table (4): Biosynthesis of enzymes during the growth of *A. niger* M 2 on various carbon sources.

Carbon sources	β-Glucosidase	CMC ase	FP ase
	U/ml		
Corn stalk (Control)	0.90	0.20	0.17
Glucose	1.97	0.30	0.08
Galactose	1.00	0.25	0.05
Lactose	2.00	0.15	0.08
Mannitol	2.10	0.16	0.06
Sorbitol	1.50	0.11	0.07
Xylose	1.70	0.10	0.00
Sucrose	1.50	0.07	0.05
Fructose	1.60	0.20	0.09
Arabinose	1.70	0.18	0.10
Soluble starch	2.90	1.90	0.26
Glycerol	2.00	1.90	0.12
Vinasse	1.70	0.50	0.14
Sugar cane molasse	3.50	1.90	0.28
Beet molasse	2.10	0.60	0.06
Glucose syrup	1.10	0.05	0.10

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

Table (5): Influence of medium composition on the biosynthesis of enzymes during the growth of *A. niger* M 2.

Ingredients						β-Glucosidase	CMC ase	FP ase
$(NH_4)_2SO_4$	KH_2PO_4	$MgSO_4.7H_2O$	$CaCl_2.2H_2O$	Peptone	Corn steep liquor	U/ml		
+	+	+	+	+	+	3.5	1.90	0.28
-	+	+	+	+	+	3.3	0.91	0.27
+	-	+	+	+	+	1.6	0.50	0.05
+	+	-	+	+	+	3.3	0.80	0.19
+	+	+	-	+	+	3.6	0.85	0.18
+	+	+	+	-	+	3.7	0.90	0.16
+	+	+	+	+	-	2.1	0.30	0.07

Effect of corn steep liquor (CSL) concentrations on cellulases production:

The medium was supplemented with corn steep liquor (CSL) at various concentrations ranged from 0.014% to 0.098% as nitrogen content. Results presented in Table (6) shows that increasing of CSL up to 0.056% as

nitrogen content increased the secretion of all cellulases tested with much more amount. In other words, CSL up to 0.056% stimulated and induced greatly the biosynthesis of these enzymes. These results may be due to its contains of growth factors such as minerals and vitamins and other ingredients. Above or below of this concentration, cellulases reduced greatly, which these concentrations repressed the enzyme formation. This means that the present of CSL in the production media was very necessary for cellulases production. Mansour and Saber (2001) and Mansour (2001) reported that the present of CSL supported cellulases production.

Table (6): Effect of corn steep liquor concentrations on the biosynthesis of enzymes production by *A. niger* M 2.

Corn steep liquor concentration		β-Glucosidase	CMC ase	FP ase
N %	Ml %	U/ml		
0.014	0.044	2.1	0.10	0.05
0.028*	0.088	3.5	0.90	0.18
0.042	0.132	4.9	1.50	1.00
0.056	0.176	5.1	1.70	1.09
0.070	0.220	5.0	1.70	0.70
0.084	0.264	4.3	1.30	0.40
0.98	0.308	3.6	1.00	0.25

*** Control.**

Effect of phosphorus sources on enzyme productions

Effect of various sources of phosphorus on the biosynthesis of cellulases were investigated. These results are presented in Table (7) which revealed that extracellular cellulases were accumulated at the highest rates in the media containing KH_2P_4. $(NH_4)HPO_4$ also stimulated these enzymes production, but was found in the second order. Rock phosphate and $Ca_3(PO_4)_2$ were repressed with much more the biosynthesis of β-glucosidase and completely repressed the biosynthesis of CMC ase and FP ase. This means that the form of phosphorus was very necessary for enzyme production which it was a core for metabolism processes in any living organisms. Similar results were also observed by Kvachadze & Yashvili (1996) and Mansour (2001).

Table (7): Effect of phosphorus source on the biosynthesis of enzymes during the growth of *A. niger* M 2.

Phosphorus source	β-Glucosidase	CMC ase	FP ase
	U/ml		
KH_2PO_4 (Control)	5.2	1.90	1.10
K_2HPO_4	2.3	0.90	0.90
NaH_2PO_4	3.5	1.00	0.80
$(NH_4)HPO_4$	4.0	1.00	0.90
$Ca_3(PO_4)_2$	0.5	0.00	0.00
Rock phosphate	0.4	0.00	0.00

Effect of KH_2PO_4 concentrations on enzymes production:

The kind of phosphorus form affected greatly the enzymes biosynthesis. Also, the rate of cellulases biosynthesis directly depended on the level of this compound on the production media (Table 8). The most favorable concentration of KH_2PO_4 which stimulated the enzymes formation was 0.310% for β-glucosidase and 0.155% for CMMCase and FPase, respectively. Thereafter these concentrations, enzyme production decreased sharply. Mansour (2001) found that 0.2325% as phosphorus content induced *Aspergillus achuleatus* DSM 63261 cellulases production.

Table (8): Effect of KH_2PO_4 concentrations on the biosynthesis of enzymes during the growth of *A. niger* M 2.

Phosphorus conc. (%)	β-Glucosidase	CMC ase	FP ase
	U/ml		
0.0775	3.9	0.70	0.50
0.155 (Control)	5.2	1.90	1.10
0.2325	6.0	1.00	0.90
0.310	6.3	1.00	0.50
0.3875	5.4	0.50	0.10

Effect of initial pH:

The pH of the production medium was greatly affected the enzyme formation. The accumulation of cellulases had a pH optimum of 6.5 (Table 9). The activity of three enzymes cellulases decreased thereafter. Similar results were reported by Kvachadze and Yashvili (1996). While, Mansour (2001) reported that pH 4.0 was the optimum pH for *Aspergillus achuleatus* cellulases production.

Table (9): Effect of initial pH on the biosynthesis of enzymes during ther growth of *A. niger* M 2.

pH		β-Glucosidase	CMC ase	FP ase
Initial	Final	U/ml		
4.0	3.1	3.1	1.0	0.50
4.5*	3.3	5.4	1.9	1.10
5.0	3.4	5.5	1.9	1.30
5.5	3.1	5.6	2.1	1.30
6.0	3.3	6.5	2.2	1.60
6.5	3.7	7.8	3.9	2.00
7.0	6.5	4.2	2.5	1.10
7.5	7.0	3.5	1.5	0.05

*** Control.**

Effect of inoculum size:

In this experiment, inoculum size was varied from 2.5 to 15% of the cultural solution of production medium. Data in Table (10) show that maximum yield of enzymes was obtained at 7.5% of inoculum level to the medium solution. Increasing or decreasing this inoculum level decreased the

three enzymes production. At the lowest volume of inoculum, the production of enzymes were decreased with the decreasing of cell, which responsible of enzyme secretion. At the higher volume of inoculum, lowest amount of enzyme was observed, this may be due to decreasing or absent of one of the nutritional factors from the production media. Mansour & Saber (2001) and Mansour (2001) reported similar results.

Table (10): Effect of inoculum size on the biosynthesis of enzymes during the growth of *A. niger* M 2.

Inoculum size		β-Glucosidase	CMC ase	FP ase
Ml %	D.W %			
2.5	0.075	4.1	2.1	1.0
5.0*	0.150	7.8	3.9	2.0
7.5	0.225	8.9	4.5	2.2
10.0	0.300	6.3	3.6	1.6
12.5	0.375	4.3	3.0	1.0
15.0	0.450	2.7	2.5	0.5

*** Control.**

Effect of agitation rate:

The effect of agitation rate on cellulase production by *Aspergillus niger* M 2 is present in Table (11), which the agitation rate ranged from 100 to 250 rpm/min. These results show that the agitation rate affected greatly on cellulases production. Enzyme formation increased greatly up to 200 rpm/min, thereafter, enzyme synthesis decreased sharply. These results mean that submerged fermentation increased the biosynthesis of these enzymes with much more than static culture up to 200 rpm. These results are similar to those obtained by Mansour and Saber (2001) and Mansour (2001).

Table (11): Effect of agitation rate on the biosynthesis of enzymes during the growth of *A. niger* M 2.

Agitation rate (rpm)	β-Glucosidase	CMC ase	FP ase
	U/ml		
100	1.0	1.0	0.0
125	3.5	2.1	0.9
150	4.7	3.5	1.1
175	5.3	4.0	1.7
200	9.0	4.5	2.2
225	7.4	3.0	1.8
250	6.2	1.5	0.9

Effect of incubation temperature:

Results presented in Table (12) show that the highest cellulases activities (β-glucosidase, CMC ase and FP ase) were observed at 35°C. Above or below this temperature, the biosynthesis of these enzymes were decreased sharply. These results are similar to those obtained by Kvachadze and Yashvili (1996) and Mansour (2001).

Table (12): Effect of incubation temperature on the biosynthesis of enzymes during the growth of *A. niger* M 2.

Temperature (°C)	β-Glucosidase	CMC ase	FP ase
	U/ml		
20	2.1	1.1	0.8
25	4.6	2.1	1.7
30*	9.0	4.5	2.2
35	10.2	5.2	2.6
40	7.1	3.0	1.5
45	4.2	1.6	0.7
50	2.0	0.6	0.0

***Control.**

Effect of incubation period:

Table (13) shows the level of β-glucosidase, CMC ase and FP ase activities released in culture medium of *Aspergillus niger* M 2 during 168 hours incubation. The highest levels of these enzymes were after 96 hours. Then, the enzymes formation decreased sharply. Also, the results show that these enzymes were present in the culture medium after 24 hours incubation Cellulases were obtained after 12-14 days incubation in culture of *Penicillium funiculosum* (Kanthan *et al*., 1985), while, Fadel and Abd-ElKader (1994) reported that 9 days were the suitable incubation period for the highest FPase and β-glucosidase production, but it was after 8 days incubation for CMC ase. These results are in agreement with those obtained by Mansour (2001).

Table (13): Time-course profile of the biosynthesis of enzymes during the growth of *A. niger* M 2.

Time (hrs)	Final pH	β-Glucosidase	CMC ase	FP ase
		U/ml		
24	6.5	1.5	0.9	0.6
48	6.5	3.0	2.1	1.0
72	6.0	6.5	3.2	1.2
96*	5.1	10.5	5.3	2.6
120	5.1	10.	4.7	1.7
144	5.0	7.5	3.6	1.0
168	5.0	4.5	3.0	0.2

*** Control.**

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العوامل المؤثرة على إنتاج إنزيمات السيليوليز من فطر الأسبرجلس نيجر م2

نادية عبد الهادى عوض على

قسم الميكروبيولوجى-معهد الأراضى والمياه والبيئة-مركز البحوث الزراعية-الجيزة-مصر

نظراً للعديد من الإستخدامات البيوتكنولوجية الجديدة التى تستخدم فيها الإنزيمات القائمة على تحليل المواد السيليلوزية فقد هدف هذا البحث لتوفير الظروف البيئية والغذائية المثلى التى تؤدى إلى إنتاج هذه الإنزيمات بكميات عالية ، وقد أوضحت الدراسة النتائج التالية:

1. بدراسة قدرة 16 سلالة فطرية لإختبار كفاءتها على إنتاج إنزيمات السيليلوليز تبين أن فطر الأسبرجلس نيجر هو أفضلها قدرة على تخليق إنزيمات البيتاجلوكوسيديز والكربوكسى ميثيل سليوليز وإنزيم الفلتر بيبر .
2. توافر سيقان الذرة المطحون بنسبة 1% فى بيئة النمو كان لها حث جيد على إنتاج الإنزيم .
3. تواجد دقيق الشعير أو دقيق الذرة أو سيقان الذرة المطحون فى بيئة النمو أدى إلى زيادة إنتاجية هذه الإنزيمات بدرجة عالية .
4. إضافة مولاس قصب السكر (بنسبة 1%) إلى بيئة الإنتاج أدى إلى الحصول على إنتاجية عالية من الإنزيمات موضع الدراسة .
5. تواجد منقوع الذرة بنسبة 0.056% كنيتروجين عنصرى فى بيئة النمو أدى إلى الحصول على كمية عالية من هذه الإنزيمات .
6. توافر فوسفات البوتاسيوم ثنائية الهيدروجين بنسبة 0.31% و 0.155% كنسبة فوسفور فى بيئة النمو كان له حث جيد لإنتاج إنزيمات البيتاجلوكوسيديز والكاربوكسى ميثيل سليلوليز وإنزيم الفلتر بيبر على الترتيب .
7. كانت درجة pH 6.5 ، 7.5% كنسبة لقاح من حجم بيئة النمو و°35م كدرجة حرارة تحضين هى الظروف المثلى لإنتاج هذه الإنزيمات .
8. 200 لفة/دقيقة كمعدل تقليب و 96 ساعة كوقت تحضين دعمت إنتاج الإنزيمات بدرجة عالية .

ولذلك لابد من توافر هذه الظروف البيئية والغذائية حتى يتسنى الحصول على أعلى معدل من هذه الإنزيمات الهامة فى تحليل المواد السليلوزية من فطر الأسبرجلس نيجر م2 .