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OPTIMIZATION OF FERMENTATION MEDIUM FOR α-AMYLASE PRODUCTION BY FREE AND IMMOBILIZED *Aspergillus niger* ON PALM TREE FIBERS

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ABSTRACT

Various strains of *Aspergillus niger* were screened for α- amylase synthesis. Strain 3 proved to be the highest producer (1.82 U. ml^{-1}). Production of α- amylase by *Aspergillus niger 3* in batch shaking system was optimized. The highest α- amylase production (5.63 U. ml^{-1}) at 30 ºC was achieved when *Aspergillus niger 3* was allowed to grow aerobically in a buffered medium (pH 7.0) containing 2 % dextrin and 1.6 % tryptone. The enzyme was found to be highly inducible by dextrin, while the presence of glucose or fructose repressed its synthesis. Palm tree fiber (PTF) was evaluated for the first time as a novel fungal cells supporting material. Conidiospores were firmly attached onto the rough surfaces of the PTF as proved by SEM photomicrographs. The immobilized preparations were used in batch system for α- amylase production with maximum sp. activity of 0.49 U. $ml^{-1}.mg^{-1}$. Such sp. activity was improved to 0.8 U. $ml^{-1}.mg^{-1}$ by modifying fermentation broth to contain 0.5 % dextrin and 2.4 % tryptone.

INTRODUCTION

Alpha amylases (E.C. 3.2.1.1. 1, 4-α-D-glucan glucohydrolase) are starch- degrading enzymes that catalyze the hydrolysis of internal α-1, 4-D-glycosidic bonds in polysaccharides with the retention of α-anomeric configuration in the products. Most of the α-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activities (Ramachandran *et al.,* 2004).

α-amylases are widely used enzymes with wide spectrum of applications such as: starch saccaharification (Sims and Chryan, 1992), medicinal and analytical chemistry (Kandra, 2003), paper industry (Sigoillot *et al* ., 2004), baking industry (Kim *et al* ., 2006), brewing, detergent and textile industries (Hernandez *et al* ., 2006).

α-amylases are produced by animals, plants and microorganisms. However, industrial production of such enzymes utilized microbial sources due to economical purposes (Djekrif-Dakhmouche *et al*., 2006). α -amylase are synthesized by several molds such as: *Aspergillus terreus* (Shambe and Ejembi, 1987), *Calvatia gigantean* (Kekos and Macris, 1987) *Mucor sp*. (Mohapatra *et al*., 1998), *Aspergillus flavus* (El-Safey and Ammar, 2004) *Aspergillus oryzae,* (Rahardjo *et al*., 2005) , *Thermomyces lanuginosus* (Kunamneni *et al.*, 2005) *Aspergillus niger* (Djekrif-Dakhmouche *et al*., 2006)*, Leucoagaricus gongylophorus* (Silva *et al*., 2006) and *Aspergillus awamori* (Prakasham *et al*., 2007)*,* as well as by several bacterial strains such as *Clostridium sp*. (Hyun and Zeikus, 1985), *Bacillus amyloliquefacience* (Argirakos *et al*., 1992), *Bacillus licheniformis* (Ivanova *et al*., 1995) and *Bacillus subtilis* (Ben Messaoud *et al*., 2004) .

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However, commercially, α-amylase has been produced by *Apergillus niger* (fungal α-amylase) and Bacillus *subtilis , B.licheniformis* and *B. amyloliquefacience* (bacterial α-amylase). Fungal α-amylase is un thermostable and its end product is maltose, whereas bacterial α- amylase characterized by its thermostability and dextrin is the end product of its action (Gerrard *et al*., 1997).

α- amylases from molds are generally produced by solid state fermentation (Rahardjo *et al*., 2005), submerged culturing (Hernandez *et al*., 2006) and the more recent immobilized cells system (Prakasham *et at*., 2007). The use of viable immobilized cells offers several advantages over the use of free cells, such as: the re-use of biocatalysts, relative ease of product separation, prevention of cells washout, reduced risk of contamination and increased operational stability (Konsoula and Liakopulou-Kyriakides, 2006).

Application of immobilized fungal cells for the production of various products have been reported (Bazaraa, 1990; Bazaraa *et al*., 1998; Sankpal *et al*., 1999; Angelova *et al*., 2000; Demirel *et al*., 2005; Skowronek and Fiedurek, 2006; Nighojkar *et al*., 2006; Ganguly *et al*., 2007). Several carriers for cells were used for the immobilization process: calcium alginate (Kuek, 1991), Wood shavings (Hamdy, 2001), non woven materials (Sankpal and Kulkarni, 2002; Papagianni and Mattey, 2004), *k*-carrageenan (Ellaiah *et al*., 2004) and polyurethane (Mandal and Banerjee, 2005). Generally, lower productivity with the immobilized systems than with conventional systems was observed and this probably due to some limitation in mass transfer (substrate, product and oxygen) (Bazaraa, 1990). He also reported for the first time the use of loofa sponge as a novel supporting material for fungal cells and indicated higher productivities for the immobilized reactor system than the conventional fermentation system.

Screening, evaluation of new mold strains producing α- amylase as well as the development of new, effective immobilization methods are necessary to improve the efficiency of mass transfer and system productivity. Therefore, in this study different *Aspergillus niger* strains were tested for their α- amylase production abilities, the production of α- amylase from the selected strain was optimized and palm tree fiber (PTF) were evaluated for the first time as a novel carrier in fungal cells immobilization.

MATERIALS AND METHODS

Fungal strains and conidiospore suspensions

The code numbers and sources of *Aspergillus niger* strains used in this study are listed in Table 1. All strains were maintained on malt extract agar at 4 ºC (Merck, Darmstadt, Germany) and transferred monthly. Spore suspensions were prepared as described by (Khattab and Bazaraa, 2005), microscopically counted (7.8×10^7 spores $.ml^{-1}$), stored at 4 ºC and utilized as stock inoculum.

Table (1): Code numbers and sources of used *Aspergillus niger*

Strains code	Sources
Aspergillus niger 3	Department of Biotechnology, NRC Cairo
Aspergillus niger 1(EMCC-104)	Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Cairo
Aspergillus niger 2 (NRRL-326) *Aspergillus niger* 5 (NRRL-2322) *Aspergillus niger* 7 (NRRL-599) *Aspergillus niger* 8 (NRRL-3)	Northern Regional Research Laboratories, Peoria, IL, USA
Aspergillus niger 4 *Aspergillus niger* 9 *Aspergillus niger* 10	Department of Microbiology, Faculty of Agriculture, Cairo University, Giza.
Aspergillus niger 6 (FSM-3)	Food Science Department, Faculty of Agriculture, Cairo University, Giza.

Media

Screening medium

Starch agar (Difco Co., Detroit, MI, USA) with added triton x-100 (0.1%) as growth limiting factor was utilized to screen the different test organisms for their amylase activity.

Fermentation medium

The fermentation medium (FM) described by (Yabuki *et al*., 1977) was used for α-amylase production. This medium contains (gL^{-1}): peptone, 20; starch, 30; KH_2PO_4, 5; and Mg $SO_4.7H_2O$, 2.5. The pH of the medium was adjusted to 7.0 using NaOH and HCl prior to sterilization (15 min at 121 ºC).

Fermentation and α-amylase production

Erlenmeyer flasks (250 ml) containing 100 ml of the FM were inoculated with 1% of the stock inoculum. Growth was aerobically carried out at 30 ºC for the desired time under shaking condition (100 rpm). The formed mycelia were harvested by filtration on Whatman no.1 (Whatman Ltd., Maidstone, England) and α-amylase activity was determined in the filtrate.

Dry weight of the mold mycelia

After filtration, mycelia were washed twice with distilled water and dried at 70 ºC under vacuum to constant weight (Ramachandran *et al.*, 2004) .

Enzyme assay

α -amylase activity was assayed by determining the reducing sugars liberated from starch using dinitosalicylic acid method (Miller, 1959).To one ml enzyme source, 3 ml soluble starch (1%, w/v) containing 0.1 M sodium acetate buffer (pH 4.5) and 10 mM Ca^{++} ion were added. The mixture was incubated at 30 ºC for 30 min in a shaker water bath and the formed reducing sugars were then measured. One unit of α -amylase activity was defined as the amount of enzyme that produces 1 µmole of maltose per min under the conditions described above. Also, specific activity was calculated as units of enzyme per mg formed biomass.

Strain selection

The test organisms were screened for their abilities to hydrolyze starch on the basis of clearing zones produced on plates of starch agar. Cultures was point inoculated on the center of starch agar plates (containing 0.1% triton x-100), and incubated at 30 ºC for up to 7 days. After incubation, for the desired time plates were flooded with iodine solution and the formed clearing zones were then measured in millimeter (Mishra *et al.*, 2004).

Factors regulating α -amylase production

Carbon source. Growth was carried out as previously described utilizing one of the following carbon sources (2%): glucose, fructose, sucrose, lactose, maltose, dextrin and starch. Biomass, α -amylase activity and pH were periodically determined.

Carbon source concentration. Five levels of dextrin (0.5, 1.0, 2.0, 3.0, and 4.0%) were tested in the FM. Biomass, α -amylase activity and pH were determined at time intervals.

Nitrogen source. Organic (peptone, tryptone, yeast extract and urea containing 12, 12.7, 9.8 and 46.6 % nitrogen, respectively) as well as inorganic (ammonium nitrate, ammonium sulfate and ammonium chloride) sources were evaluated for their effect on α –amylase synthesis. The medium utilized in this experiment was as described earlier except it contained (gL^{-1}): dextrin, 2; and different amounts of nitrogen sources containing equal amounts of nitrogen (0.2%).

Nitrogen source concentration. The effect of five levels (0.8, 1.6, 2.4, 4.0 and 5.6%) of tryptone was examined on both growth and α –amylase activity in the FM containing 2% dextrin.

Effect of pH. Growth was carried out in the FM (containing 2%dextrin and 1.6%tryptone) at different initial pH levels (5 to 8) and monitored as well as α –amylase activity at intervals. In parallel, another experiment was conducted under the same conditions except with the removal of $K H_2 PO_4$ (unbuffered system) from the medium. pH was adjusted prior to sterilization utilizing NaOH and HCl.

Immobilization on PTF

The palm tree fibers (PTF) were soaked in distilled water (30 ºC, 24 h), sliced into squares (1.0×1.0 cm) and sterilized (121ºC, 20 min) in water. After sterilization, PTF squares were soaked in sterile FM (containing 2% dextrin, 1.6% tryptone, pH 7.0) till used. Various PTF weights were separately added to 250 ml Erlenmeyer flasks containing 50 ml FM, inoculated with the prepared spore suspension (2% inoculum) and incubated at 28 ºC under shaking (100 rpm) for 6 days. Clear fermentation broth samples were daily withdrawn for the determination of α –amylase activity, biomass and pH.

Scanning electron microscopy (SEM)

The SEM was used to examine the surface and interior of the PTF before and after fungal cell immobilization according to the method described by (Bazaraa and Hamdy, 1989). The PTF was fixed by immersion in 2.5% glutaraldehyde-sodium phosphate buffer (0.13 M, pH 7.2) for 1h. The specimens were washed in three changes of buffer and then post fixed in 1% osmium tetroxide for 2 h, washed again with buffer, and dehydrated using

ascending series of ethanol level (20, 30, 40, 50, 60, 70, 80, 90 and 100%, v/v) for 15 min at each step. The specimens were dried (30 min) at critical point in an auto Samdri-815 critical point dryer (Tousimis Research Corporation, Rockville, ML, USA) using liquid carbon dioxide. The specimens were then mounted on aluminum stubs, coated with 25 mm of gold in a Hummer Sputter Coater (Alexandria, VA, USA) and examined using the Jeol SEM- 5200 (JEOL, Japan).

Statistical analysis

Results were subjected to one way analysis of variance (ANOVA) (Rao and Blane, 1985)**,** and results were the average of 3 experiments.

RESULTS AND DISCUSSION

Screening of different *Aspergillus niger* strains for their α –amylase activity

The maximum α –amylase activity was obtained after 7 days in clearing zone method utilizing starch agar plates and 4 days only when activity (U. ml^{-1}) was determined in FM broth (data not shown). Data (Table 2) indicate that in both cases, *Aspergillus niger* 3 exhibited the highest activity (10 mm and 1.8 U. ml^{-1}) and *Aspergillus niger* 8 was the lowest with almost 8.0% of the total activity of that obtained with strain 3. Therefore, strain 3 was selected as strain of choice for further experiments. High correlation (r = 0.97) between the two used methods was noted. Therefore, the clear zone qualitative detection method can give an indication about the approximate α –amylase activity.

Table (2): Screening of various *Aspergillus niger* strains for α -amylase activity*.

Strains	α-amylase activity	
	Clear zone ±SD (mm)	(U.ml^{-1})±SD
Aspergillus niger 1(EMCC-104)	** 7^{d} ± 0.14	0.77^{e} ± 0.00
Aspergillus ngier 2 (NRRL-326)	9^{b} ± 0.00	1.19^{b} ± 0.01
Aspergillus niger 3	10^{a} ± 0.07	1.82^{a} ± 0.02
Aspergillus niger 4	7^{d} ± 0.21	0.77^{e} ± 0.01
Aspergillus niger 5 (NRRL-2322)	8^{c} ± 0.07	1.16^{c} ± 0.05
Aspergillus niger 6 (FSM-3)	6^{e} ± 0.21	0.65^{f} ± 0.05
Aspergillus niger 7 (NRRL-599)	8^{c} ± 0.14	1.10^{d} ± 0.07
Aspergillus niger 8 (NRRL-3)	4^{g} ± 0.14	0.15^{g} ± 0.01
Aspergillus niger 9	6^{f} ± 0.14	0.70^{f} ± 0.04
Aspergillus niger 10	7^{d} ± 0.21	0.83^{e} ± 0.01

*** The cultivation time was 7 days and 4 days in clearing zone method and FM broth , respectively.**

**** Means followed by different superscripts within columns are significantly different at the 5% level.**

Effect of carbon source

The test organism was able to grow in all tested carbon sources and the highest biomass (11.7 mg.ml^{-1}) was achieved in medium containing maltose**.** Sucrose followed by glucose, starch, dextrin then fructose supported the growth descendingly and 10.0, 9.4, 9.0, 6.5 and 5.4 mg biomass.ml^{-1}were obtained, respectively. Data also show that the weakest growth was obtained with lactose (2.6 mg.ml^{-1}). Data also reveal that the lowest pH value (3.0) was obtained with the highest growth, while the highest pH (4.6) was obtained with the lowest recorded biomass. Different carbon sources were tested in order to evaluate their effect on both of growth and α –amylase production by *Aspergillus niger* 3. Results (Table 3) indicate that the dextrin highly induced the enzyme synthesis (2.8 U.ml^{-1}). While sucrose, lactose, starch and maltose partially induced the synthesis and 67.9, 53.6, 53.6 and 39.3 % of the activity obtained with dextrin were noted, respectively. On the other hand, the presence of glucose or fructose as fast consuming sugar in the FM resulted in catabolite repression and the enzyme therefore was not synthesized. It is of interest to report that the highest sp. activity (0.58 U.mg^{-1}) was obtained with lactose and this was due to the low biomass resulted from the use of such carbon source. Similarly, Silva *et al*. (2006) reported the induction of α –amylase of *Leucoagaricus gongylophorus* by starch and maltose as with *Aspergillus sp*. Also, Baks *et al*., (2006) indicated that starch has the largest influence on α –amylase synthesis while glucose has the lowest.

Table (3): Effect of different carbon sources on growth of *Aspergillus niger* 3, pH and α-amylase activity*.

Carbon source	Culture pH	Biomass (mg. ml^{-1})	α –amylase (U . ml^{-1}) ±SD	Sp. activity (U .mg^{-1})
Glucose	3.6	9.4	0.0 ± 0.00	0.00
Fructose	3.7	5.4	0.0 ± 0.00	0.00
Sucrose	3.7	10.0	**1.9^{b} ± 0.01	0.19
Maltose	3.0	11.7	1.1^{d} ± 0.07	0.09
Lactose	4.8	2.6	1.5^{c} ± 0.01	0.58
Dextrin	3.6	6.5	2.8^{a} ± 0.04	0.43
Starch	3.6	9.0	1.5^{c} ± 0.01	0.17

*** The cultivation time was 3 days in FM (100 ml) containing different carbon sources (2%).**

****Means followed by different superscripts within columns are significantly different at the 5% level.**

Effect of dextrin concentration

Different dextrin concentrations (up to 4 %, w/v) were tested in order to determine their effect on growth, change in pH level and α –amylase production (Table 4). Results indicate that the enzyme activity increased with the increase in dextrin concentrations reaching its maximum activity (2.80 U.ml^{-1}) at 2 % and persisted thereafter. Therefore, dextrin concentration of 2 % was used for further experiments. Also, maximum sp. activity was obtained at 2 % dextrin and decreased after that due to the increase in biomass. Data

reveal positive correlation between biomass and dextrin concentration and the lowest pH value (3.4) was obtained at 4 % dextrin

Table (4): Effect of dextrin concentrations on growth of *Aspergillus niger* 3, pH and α-amylase activity*.

Dextrin (%)	Culture pH	Biomass ($mg.ml^{-1}$)	α –amylase ($U.ml^{-1}$) ±SD	Sp. activity ($U.mg^{-1}$)
0.0	4.6	3.4	0.0 ± 0.00	0.00
0.5	3.9	4.3	**1.4^{c} ± 0.01	0.33
1.0	3.5	5.6	2.2^{b} ± 0.02	0.40
2.0	3.5	6.5	2.8^{a} ± 0.03	0.43
3.0	3.5	6.9	2.8^{a} ± 0.05	0.41
4.0	3.4	12.6	3.0^{a}± 0.02	0.23

*** The cultivation time was 3 days in FM (100 ml) containing different Concentrations of dextrin.**

****Means followed by different superscripts within columns are significantly different at the 5% level.**

Effect of nitrogen source

The results of α –amylase production, changes in culture pH and biomass as affected by different nitrogen sources are described in Table 5. In general, inorganic nitrogen sources appeared to be not suitable for α –amylase production. This was probably due to the lack of some essential growth factors in such sources (Bazaraa and AL-Dagal, 1999). Results show that tryptone was the best nitrogen source used for α –amylase production (5.6 $U.ml^{-1}$, considered as 100%) followed by yeast extract and peptone, where the α–amylase activities were 43.3 and 24.9% of that obtained with tryptone, in succession. The inorganic nitrogen sources, ammonium sulfate and ammonium nitrate exerted negative effect on α –amylase synthesis, where the activities were 21.4 and 17.9% of that obtained with tryptone, respectively (Table 5). On the other hand, ammonium chloride completely prevented α –amylase synthesis with moderate growth obtained. Literature indicated that organic nitrogen sources are in general better than inorganic sources in supporting growth as well as α –amylase synthesis, in this respect. McMahon *et al*. (1999) reported that, Yeast extract was the best tested organic source for the production of α -amylase by *Streptomyces sp.* Moreover Pedersen and Nielsen. (2000) reported the increase in α -amylase productivity (110 to 156%) of *Aspergillus oryzae* when yeast extract used as an additional nitrogen source. In addition, Hayashida *et al*. (1988) and Cheng *et al*. (1989) reported that organic nitrogen sources (beef extract, peptone and com steep liquor) supported maximum α - amylase production by tested bacterial strains. On the other hand, Morkeberg *et al*. (1995) differently reported that the use of $(NH_4)_2SO_4$ as inorganic nitrogen source maximized α –amylase synthesis by *Aspergillus oryzae*.

Table (5): Effect of nitrogen sources on growth of *Aspergillus niger* 3, pH and α-amylase activity*.

Nitrogen sources	Culture pH	Biomass (mg.ml^{-1})	α –amylase (U.ml^{-1}) ±SD	Sp. activity (U.mg^{-1})
Peptone	3.6	8.5	**1.4^{c} ± 0.00	0.16
Tryptone	3.0	8.4	5.6^{a} ± 0.02	0.67
Yeast extract	2.7	8.2	2.4^{b} ± 0.01	0.30
Urea	3.5	6.6	1.3^{d} ± 0.04	0.22
NH_4NO_3	2.0	5.4	1.0^{f} ± 0.02	0.19
$(NH_4)_2SO_4$	2.4	7.9	1.2^{e} ± 0.01	0.15
NH_4Cl	2.0	6.1	0.0 ± 0.00	0.00

***The cultivation time was 5 days in FM (100 m l) containing (2%) dextrin and different nitrogen sources (0.2% N_2).**

****Means followed by different superscripts within columns are significantly different at the 5% level.**

Effect of tryptone concentration

The production of α –amylase and biomass were influenced by tryptone concentration (Table 6). The highest α –amylase production (5.6 U.ml^{-1}) was achieved at 1.6% tryptone and the activity significantly decreased at higher tryptone concentration reaching no activity at 5.6%. On the other hand, a positive relation between biomass and tryptone concentration was observed up to tryptone concentration of 2.4% after which a constant biomass was obtained.

Table (6): Effect of tryptone concentrations on growth of *Aspergillus niger* 3, pH and α-amylase activity*.

Tryptone (%)	Culture pH	Biomass (mg.ml^{-1})	α –amylase (U .ml^{-})±SD	Sp. activity (U.mg^{-1})
0.0	2.8	0.1	0.0 ± 0.00	0.00
0.8	2.8	7.9	**0.7^{b}± 0.00	0.08
1.6	3.0	8.4	5.6^{a} ± 0.02	0.67
2.4	2.8	8.9	0.7^{b} ± 0.03	0.08
4.0	3.8	9.0	0.6^{c} ± 0.01	0.07
5.6	3.9	9.1	0.0 ± 0.00	0.00

***The cultivation time was 5 days in FM (100 ml) containing (2%) dextrin and different concentrations of tryptone as nitrogen source.**

****Means followed by different superscripts within columns are significantly different at the 5% level.**

Effect of pH on enzyme production

Results (Table 7) indicate that *Aspergillus niger* 3 grew well in buffered (contained KH_2PO_4) as well as unbuffered medium. Data reveal the maximum biomass 8.4 and 7.6 mg. ml^{-1} at pH 7.0 (buffered) and 8 (unbuffered), respectively. It is of interest to report that after 5 days of fermentation, no α –amylase activity was detected in the unbuffered media at all tested pH levels and reversely the activity was obvious in the buffered medium with maximum activity (5.63 U.ml^{-1}) at pH 7.0. Similar pH changing pattern was noted during mold growth in both medium (data not shown) therefore, it could be concluded that the presence of phosphate ions is essentially for enzyme synthesis and not as buffering agent. Ueno *et al*.

(1987) stated that phosphate is very important in regulation both of growth as well as α -amylase synthesis by *Aspergillus oryzae*. They also reported a significant increase in α –amylase production when ≥ 0.2 M phosphate levels were used. Similar findings were noticed with *Bacillus amyloliquefaciens*, where low levels of phosphate ≤ 0.1 M resulted in sharp decrease in both of cell density and α -amylase production. On the other hand, high phosphate concentrations ≥ 0.4 M inhibited enzyme production (Hillier *et al*., 1997).

Table (7): Effect of different pH on growth of *Aspergillus niger* 3, and α-amylase activity*.

pH	Biomass (mg. ml^{-1})		α -amylase ($U.ml^{-1}$) ±SD	
	Unbuffered	buffered	Unbuffered	buffered
5	7.5	NT	0.00	**NT
6	5.4	7.3	0.00	†0.80^{c}±0.05
7	5.9	8.4	0.00	5.63^{a}±0.01
8	7.6	6.7	0.00	1.20^{b}±0.01

***The cultivation time was 5 days in FM (100 ml) containing 2% dextrin, 1.6% tryptone and without or with $K H_2 PO_4$ 0.5% at different pH levels. **Not tested.**

†Means followed by different superscripts within columns are significantly different at the 5% level.

Immobilization

The SEM of PTF prior to immobilization (Fig. 1) showed two distinctive areas (A and B). The first (A) was network of fibers with different direction and thickness with large intraspaces between fibers (Fig. 1 a), while, the area B was almost solid and consisted of very compact channels covered with very rough surfaces (Fig. 1 b). Therefore, only the areas which consisted of fibers network (A) were used in immobilization since mass transfer problems will be obviated due to the large intraspaces between fibers. After inoculation with conidiospores suspension (Fig. 2 a), the round shape conidiospores (2-4 µm in diameter) were firmly attached to the surfaces of the network. Spores germination on PTF was detected within the first 10 h (data not shown) followed by rapid growth with hypha branching as well as septa formation. The mycelia covered the entire PTF surfaces with starting sporiolation after 24 h (Fig.2 b). Results indicated that PTF had large, rough surfaces aiding the attachment of spores and hypha. Also, it exhibited large internal spaces which facilitate nutrient mass transfer, product secretion as well as air movement. Also, the PTF is very light, durable and very cheap (palm tree waste). These results were very close to these reported by Bazaraa *et al*. (1998) who used loofa sponge as a novel carrier in compactin synthesis.

Fig. (1): Scanning electron micrographs for the surface of PTF prior to immobilization.
(a) The network fibers and the various directional changes and thickness. Bar 10 µm.
(b) The compact channels with rough surfaces. Bar 500 µm.

Fig. (2): Scanning electron micrographs for the surface of PTF after immobilization.
(a) Exactly after inoculation with conidiospores. Bar 10 µm.
(b) After 24h of *Aspergillus niger* . Noted the complete coverage of the PTF surface with mold mycelia. Bar 100 µm.

Immobilized batch system

PTF squares (1.0×1.0 cm) were used in batch system at different concentrations for α –amylase production and the results were summarized in Table 8. Data reveal that by increasing the concentration of PTF from 0.25 to 2.0 g. 50 ml^{-1}, the biomass increased from 7.1 to 15.9 g and the specific activity increased up to 0.49 $U.ml^{-1}.mg^{-1}$ at PTF concentration of 1.0 g and decreased after that. At PTF concentration 1.5 and 2.0 g a complete blockage occured after 2 days of fermentation due to the extra growth of the mold. Also, at concentration of 0.25, 0.5 and 1.0 g the blockage occured but after 4, 4 and 3 days of fermentation, respectively. The maximum specific activity (0.49 $U.ml^{-1}.mg^{-1}$) was lower than that obtained with free cells (0.72 $U.ml^{-1}.mg^{-1}$). Therefore, a medium modification is a must to reduce the formed biomass and consequently increase the specific activity. The modification was by limiting carbon source to 0.5% and increase tryptone concentration to 2.4 %. Results in Table 8 indicate 90.3 and 88.2 % reduction in biomass at PTF concentration of 0.5 and 1.0 g, respectively. On the other hand, the specific activity increased up to 0.8 ($U.ml^{-1}.mg^{-1}$) at 0.5 g PTF. Such results will be of great impact on the continuous production of α –amylase since it will delay the blockage of the bioreactors by mold biomass and therefore, could be used for long operation time.

Table (8): Effect of PTF concentrations on growth and α-amylase synthesis by *Aspergillus niger* 3*.

PTF (g. 50 ml^{-1})	Growth ($mg.ml^{-1}$)	α-amylase activity ($U.ml^{-1}$)	Sp. activity ($U.ml^{-1}$ mg^{-1})
0.25	7.1(0.16)	0.71(0.48)	0.10(0.30)
0.50	7.2(0.70)	2.22(0.56)	0.30(0.80)
1.00	7.6(0.90)	3.70(0.47)	0.49(0.50)
1.50	11.4(1.40)	1.40(0.16)	0.10(0.11)
2.00	15.9(2.30)	1.11(0.48)	0.07(0.21)

* **The cultivation time was 3 days in FM (100 ml) containing 2 % dextrin and 1.6 % tryptone.**

Means between parenthesis were obtained for the modified medium (0.5% dextrin and 2.4% tryptone)

CONCLUSIONS

The α –amylase production by *Aspergillus niger* 3 was optimized. This enzyme proved to be highly induced by dextrin. Under the optimized conditions, the productivity increased 3.0 folds. The PTF as a novel carrier for fungal cells seemed to be very suitable in immobilization processes. However, more research is needed for the application of the immobilized preparations in both continuous and consecutive batch cycles.

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الظروف المثلى لانتاج انزيم الالفا اميليز بواسطة خلايا *Aspergillus niger* الحرة و المقيدة على ليف النخيل.

نجوى محمد الشيمى, وائل احمد بازرعه- رضا محجوب ابو العلا
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تم اختبار العديد من سلالات *Aspergillus niger* من حيث قدرتها على انتاج الالفا اميليز . و أظهرت السلاله *Aspergillus niger 3* أعلى انتاج للانزيم (1.82وحدة انزيم. مل$^{-1}$). تم اختبار الظروف المثلى باستخدام المزارع المهتزة بنظام الدفعات لأنتاج انزيم الالفا اميليز بواسطة سلالة *3 Aspergillus niger* و تم الحصول على أعلى أنتاج من الانزيم (5.63 وحدة انزيم. مل$^{-1}$) عند تنمية الفطر فى بيئة تحتوى على 2٪ دكسترين, 1.6٪ تربتون و درجة حرارة 30م^{o} عند درجة آس هيدروجينى 7.0 كما أظهرت النتائج ان الدكسترين عمل كمحفزا لأنتاج الالفا اميليز بعكس الجلوكوز و الفركتوز الذى كان وجودهما مثبطا لأنتاج الانزيم. و تم تقيم ليف النخيل كمادة دعامية جديدة لتحميل الفطريات حيث أظهرت صور الميكروسكوب الالكترونى الماسح التصاق الجراثيم الفطرية على الاسطح الخشنة لألياف النخيل. و تم أنتاج انزيم الالفا اميليز عن طريق خلايا الفطر المقيدة على ليف النخيل باستخدام نظام الدفعات و كان اعلى نشاط نوعى 0.49وحدة انزيم. مل$^{-1}$. ملجم$^{-1}$ و ازداد الى 0.8 وحدة انزيم. مل$^{-1}$. ملجم$^{-1}$ عند استخدام بيئة معدلة تحتوى على0.5 ٪ دكسترين, 2.4٪ تربتون.