*The Chittagong Univ. J. B. Sci.,Vol. 3(1 &2):pp.11-20 , 2008.*

# FACTORS RESPONSIBLE FOR PRODUCTION OF AMYLASES FROM *ASPERGILLUS FUMIGATUS* FRESENIUS

MUNIRA AKHTER, MD. TOWHID HOSSAIN AND M. N. ANWAR*
Department of Microbiology, University of Chittagong, Chittagong- 4331, Bangladesh.

## ABSTRACT

*Aspergillus fumigatus* Fresenius showed maximum amylase production at 27°C with an initial culture pH of the medium 6.0 after 72 h. of incubation. One per cent soluble starch and 0.15% ammonium nitrate in the medium supported the highest amylase activity. During enzyme-substrate reaction maximum enzyme activity was observed at 50°C and pH 4.0 with 2% starch.

**Key words:** Factors, Amylases, *Aspergillus fumigatus*

## INTRODUCTION

Amylases represent a group of enzymes of great importance to the food industry and other needs of life. They were also one of the first to be produced industrially by microorganisms (Reed 1975). Amylases have most widely been reported to occur in microorganisms, although they are also found in plants and animals. Two major classes of amylases have been identified in microorganisms, namely α-amylase and glucoamylase. They have immense potential for commercial production. In addition, β-amylase, which is generally of plant origin, has also been reported from a few microbial sources (Pandey *et al.* 2000). Since starch is the only natural substrate to be hydrolyzed by amylases, it is desirable to recover potential microbial isolates producing high level of amylase active on raw starch (Pandey *et al.* 2000). Fungal amylase activity is influenced to a great extent by pH (Abu-Zeid 1997, Filipov *et al.* 1982, Kachelkina and Oreshchenko 1980, Okolo *et al.* 1995, Olasupo *et al.* 1996, Rahman *et al.*1993), temperature (Abu-Zeid 1997, Okolo *et al.* 1995, Olasupo *et al.* 1996, Srivastava and Baruah 1986), incubation period (Bormiss *et al.* 1981, Marzan *et al.* 2001, Rahman *et al.*1993), various carbon and nitrogen sources (Guo *et al.* 1988, Lachmund and Ruttkowski 1990,Mahmoud 1993, Okolo *et al.* 1995) and also substrate concentration. Considering the above, the present work has been under taken to investigate the influence of some factors on the production and activity of amylases from the fungal isolate *Aspergillus fumigatus*.

* Corresponding author.

## MATERIALS AND METHODS

*Organism*

*Aspergillus fumigatus* Fresenicus was obtained from the Department of Microbiology, University of Chittagong, Bangladesh.

*Culture Medium*

*Aspergillus fumigatus* was cultured in defined salt medium (Almieda *et al.* 1997) containing 0.5% $KH_2PO_4$, 0.67% $K_2HPO_4$, 0.1% $(NH_4)_2SO_4$, 0.01% $MgSO_4.7H_2O$ and 4% soluble starch.

*Assay of Enzyme Activity*

Assay system for amylase activity was carried out by measuring the amount of reducing sugar following Nelson's modification of Somogyi method (Nelson 1944). The reaction mixture contained 5 ml of 1% soluble starch prepared in 0.2 M citrate phosphate buffer (pH 6.0), 1 ml of 0.2 M citrate phosphate buffer and 1 ml of enzyme solution. The reaction mixture was incubated at 37˚C for 1 h. Then 1 ml of alkaline copper reagent was added to 1 ml of reaction mixture, and the mixture was boiled for 20 min, cooled and 1 ml of arsenomolybdate colour reagent was added. After 10 min the reaction mixture was diluted with 22 ml of distilled water and the absorbance was measured at 550 nm. Enzyme activity was expressed as the amount of glucose released per ml of enzyme extract per unit time (Mohadevan and Sridhar 1982). [One unit (U) enzyme activity is the amount of enzyme required to release 1µmol glucose equivalent in 1 min under the assay condition].

*Protein Estimation*

Protein was determined by the method of Lowry *et al.* (1951).

*Biomass yield*

Biomass was determined by dry weight basis (mg/g substrate).

*Saccharification*

For saccharification the enzyme preparation (crude) was adjusted to pH 4.0 and sodium azide (0.2%) was added to inhibit the microbial growth. Hydrolysis was carried out under stationary condition in 25 ml screw cap test tubes at 50˚C with substrate (starch) concentration of 7.5% (w/v) for 24 h. and 48 h. of intervals (Begum *et al.* 1993). The sugar content in the hydrolysate was measured by Nelson's modification of Somogyi method (Nelson 1944).

Per cent of saccharification was calculated as follows:

$$\text{Saccharification \%} = \frac{\text{mg of reducing sugar/ml}}{\text{mg of substrate /ml}} \times 100$$

*Effect of Culture Conditions on Amylase Production*

The fungal isolate was grown at various pH (4, 5, 6, 7, 8 and 8.5) and temperature (10ºC, 27ºC, 37ºC and 45ºC) for various incubation periods (48, 72, 96, 120 and 144 h.) using different nitrogen sources ($NH_4NO_3$, $NH_4Cl$, $(NH_4)_2HPO_4$, glutamine, glycine and peptone) for the optimization of growth conditions and to investigate the effects of these factors on amylase production. To determine optimum percent of carbon and nitrogen sources, the study was also carried out with 1 to 5% carbon and 0.05 to 0.30% nitrogen sources keeping other experimental conditions at optimum.

*Effect of Incubation Time on Amylase Activity*

To determine the effect of incubation time on enzyme activity the enzyme substrate mixture was incubated at different incubation time (15, 30, 45, 60, 75 and 90 min).

Effect of pH and Temperature on Amylase Activity

The effect of pH on amylase activity was determined by incubating the reaction mixture at pH 3.0 to 8.0 using citrate phosphate buffer (adjusted with 1% $Na_2HPO_4$.7H2O solution). The optimum temperature for enzyme activity was also determined by assaying the enzyme activity at various temperatures from 35 to 55ºC.

## RESULTS AND DISCUSSION

*Effect of incubation period on amylase production*

When the fungus was incubated at different incubation period the isolate was found to produce maximum amylase at 72 h. of incubation period (Table 1). Though 72 h. of incubation showed the highest amylase activity but the highest biomass yield was recorded at 96 h. of incubation period. The colour of the supernatant was sand gold. The pH of the supernatant ranged from 5.02 to 6.72. The maximum protein and the saccharification rate were recorded at 96 and 120 h. of incubation period, respectively. Production of maximum amylase after 72 h. of incubation period by fungi was reported by many workers (Cherry *et al.* 2004, Gargova and Nauchni 1979, Mahmud 2006, Rahman *et al*. 1993).

TABLE 1: EFFECTS OF INCUBATION PERIOD ON PRODUCTION OF AMYLASES BY *A. FUMIGATUS.*

| Incubation Period (In hrs) | Biomass yield (mg/g) | Reducing sugar (µg/ml) | Protein (µg/ml) | Saccharification % | Amylase activity (U/ml) |
|---|---|---|---|---|---|
| 48 | 290 | 307 | 243 | 0.76 | 370 |
| 72 | 439 | 333 | 490 | 0.83 | 378* |
| 96 | 525* | 370 | 611* | 0.92 | 365 |
| 120 | 375 | 489* | 557 | 1.22* | 287 |
| 144 | 323 | 478 | 522 | 1.19 | 295 |

*Maximum yield

*Effect of temperature and pH on amylase production*

The effect of pH and temperature on the enzyme production was conducted. The optimal temperature for enzyme production was found at 27$^{o}$C (Table 2). The highest protein, reducing sugar production and saccharification rate were recorded at 37°C. The pH of the culture filtrates varied from 5.97 to 6.55. Similar observation was made by many researchers (Fabiana *et al.* 1999, Mahmud 2006, Rahman *et al*. 1993).

TABLE 2: EFFECTS OF INCUBATION TEMPERATURE ON PRODUCTION OF AMYLASES BY *A. FUMIGATUS.*

| Incubation temperature ($^{o}$C) | Biomass yield (mg/g) | Reducing sugar (µg/ml) | Protein (µg/ml) | Saccharification % | Amylase activity (U/ml) |
|---|---|---|---|---|---|
| 10 | 00 | 306 | 290 | 0.76 | 239 |
| 27 | 211 | 430 | 738 | 1.07 | 370* |
| 37 | 414* | 466* | 788* | 1.16* | 353 |
| 45 | 343 | 401 | 753 | 1.00 | 264 |

The optimum pH for enzyme production was 6.0 (Table 3). Maximum protein formation, biomass yield and saccharification rate were recorded at pH 8.0, 7.0 and 6.0, respectively. The results are in agreement with the findings of other investigators who observed maximum enzyme activity, protein and biomass formations at pH 6.0 by *Aspergillus* spp. (Abu *et al.* 2005, Abu-Zeid 1997, Fabiana *et al.* 1999, Okolo *et al.*1995) and other fungi (Mahmoud 1993) and bacteria (Freer 1993).

TABLE 3: EFFECTS OF pH OF MEDIUM ON PRODUCTION OF AMYLASES BY *A. FUMIGATUS.*

| Medium pH | Biomass yield (mg/ml) | Reducing sugar (µg/ml) | Protein (µg/ml) | Saccharification % | Amylase activity (U/ml) |
|---|---|---|---|---|---|
| 4.0 | 231 | 341 | 570 | 0.85 | 337 |
| 5.0 | 308 | 338 | 720 | 0.84 | 350 |
| 6.0 | 434 | 399* | 770 | 0.99* | 481* |
| 7.0 | 529* | 354 | 529 | 0.88 | 338 |
| 8.0 | 280 | 368 | 771* | 0.92 | 334 |
| 8.5 | 240 | 385 | 760 | 0.96 | 326 |

*Effect of nitrogen sources on amylase production*

In the present study the fungal isolate exhibited the highest amylases formation and biomass yield in $NH_4NO_3$ containing medium (Table 4). The highest reducing sugar and saccharification rate were recorded with $(NH_4)_2HPO_4$. The culture filtrate exhibited highest level of soluble protein when grown in the medium containing $NH_4Cl$ as nitrogen source. Effects of organic and inorganic nitrogen sources on amylase production have been reported by other workers (Cherry *et al.* 2004, Lachmund and Ruttkowski 1990, Mahmoud 1993, Tryavsogolova *et al.* 1986, Xiangzhang *et al.* 1988). It was also reported that amylase production was induced by inorganic nitrogen (Hizukuri and Susumu 1983).

TABLE 4: EFFECTS OF DIFFERENT NITROGEN SOURCES ON PRODUCTION OF AMYLASES BY *A. FUMIGATUS.*

| Nitrogen sources | Biomass Yield (mg/g) | Reducing sugar (µg/ml) | Protein (µg/ml) | Saccharification % | Amylase activity (U/ml) |
|---|---|---|---|---|---|
| $NH_4NO_3$ | 248* | 400 | 520 | 1.00 | 405* |
| $NH_4Cl$ | 162 | 377 | 721* | 0.94 | 349 |
| $(NH_4)_2HPO_4$ | 81 | 406* | 370 | 1.01* | 381 |
| Glycine | 224 | 384 | 529 | 0.96 | 357 |
| Glutamine | 127 | 346 | 671 | 0.86 | 364 |
| Peptone | 40 | 365 | 360 | 0.91 | 319 |

*Effect of starch and nitrogen concentrations on amylase production*

The effect of starch (1-5%) and $NH_4NO_3$ (0.05-0.30%) concentrations was studied under otherwise identical culture conditions and the results are

summarized in Table 5. Maximum biomass and protein yields were recorded with 5% starch and 0.30% $NH_4NO_3$. The table 6 shows the maximum amylase production that recorded in the culture supernatant with 1% starch and 0.15% $NH_4NO_3$ in the basal medium. It was observed that the increase of the concentration of $NH_4NO_3$ gradually decreased the synthesis of amylase formation.

The effect of concentration of nitrogen sources on microbial amylase production has been reported by other workers (Bormiss *et al.* 1981, Hizukuri and Susumu 1983, Tryasogolova *et al.* 1986, Xianzhang *et al.* 1988).

TABLE 5: EFFECTS OF DIFFERENT CONCENTRATIONS OF STARCH AND NITROGEN SOURCES ON THE PRODUCTION OF REDUCING SUGAR, PROTEIN AND SACCHARIFICATION RATE (%) BY *A. FUMIGATUS.*

| *% $NH_4NO_3$* **as nitrogen source** | **% Starch as carbon source** | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1.0** | | | **2.0** | | | **3.0** | | | **4.0** | | | **5.0** | | |
| | A | B | C | A | B | C | A | B | C | A | B | C | A | B | C |
| 0.05 | 330 | 478 | 3.3 | 402 | 622 | 2.01 | 337 | 594 | 1.12 | 433 | 946 | 1.08 | 451* | 919 | 0.90 |
| 0.10 | 381 | 763 | 3.81* | 303 | 498 | 1.51 | 274 | 566 | 0.91 | 439 | 980 | 1.09 | 379 | 898 | 0.75 |
| 0.15 | 375 | 457 | 3.75 | 279 | 572 | 1.39 | 244 | 539 | 0.81 | 233 | 991 | 0.58 | 301 | 865 | 0.60 |
| 0.20 | 377 | 477 | 3.77 | 223 | 658 | 1.11 | 275 | 572 | 0.91 | 206 | 830 | 0.51 | 343 | 924 | 0.69 |
| 0.25 | 330 | 436 | 3.3 | 179 | 541 | 0.89 | 271 | 460 | 0.90 | 187 | 898 | 0.46 | 278 | 992 | 0.55 |
| 0.30 | 254 | 454 | 2.54 | 252 | 514 | 1.26 | 250 | 590 | 0.83 | 240 | 867 | 0.60 | 336 | 997* | 0.67 |

Note: A = Reducing sugar (µg/ml), B = Protein (µg/ml), C = Saccharification rate (%).
*Maximum yield.

TABLE 6: EFFECT OF DIFFERENT CONCENTRATIONS OF STARCH AND NITROGEN SOURCES ON PRODUCTION OF AMYLASE BY *A. FUMIGATUS.*

| **% $NH_4NO_3$ as nitrogen source** | **% Starch as carbon source** | | | | |
|---|---|---|---|---|---|
| | **1.0** | **2.0** | **3.0** | **4.0** | **5.0** |
| | Amylase activity (U/ml) | | | | |
| 0.05 | 189 | 148 | 195 | 208 | 78 |
| 0.10 | 179 | 177 | 156 | 76 | 159 |
| 0.15 | 456* | 174 | 227 | 229 | 223 |
| 0.20 | 221 | 220 | 204 | 211 | 187 |
| 0.25 | 192 | 199 | 126 | 225 | 278 |
| 0.30 | 217 | 178 | 193 | 219 | 178 |

*Effect of incubation time, temperature and pH on amylase activity*

Incubation time, temperature and pH are most important factors which markedly influence enzyme activity. The maximum amylase activities of *Aspergillus fumigatus* were recorded at 50°C for 30 min at pH 4.0, after which the activity decreased (Fig 1)). Similar amylase activities at acidic pH and high temperature were reported by many workers (Akhter *et al.* 1999, Campos and Felix 1995, Dey *et al.* 2002, Glymph and Stutzenberger 1977, Teodoro and Martin 2000).

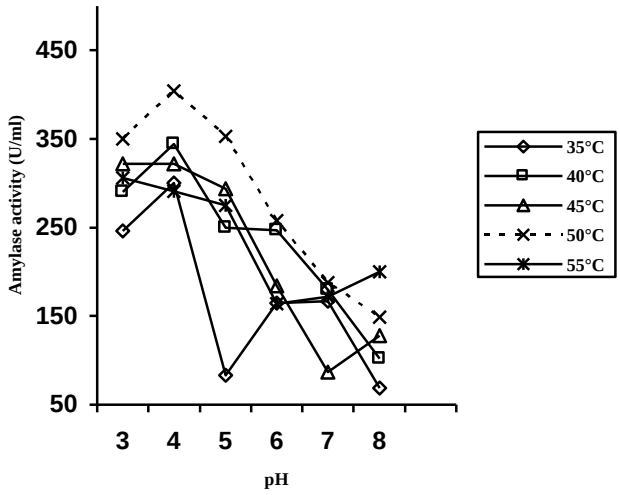


FIG 1: EFFECT OF pH AND TEMPERATURE ON AMYLASE ACTIVITY BY *A. FUMIGATUS.*

In the present observation, high temperature (50°C) and low pH (4.0) for maximum enzyme activity indicate a good commercial probability of the amylase enzymes produced by the fungal isolate *Aspergillus fumigatus.*

## REFERENCES

ABU, E.A., ADO, S.A. AND JAMES, D.B. 2005. Raw starch degrading amylase production by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae* grown on sorghum pomace. *African J. Biotechnol.* **4**(8):785-790.

ABU-ZEID, A. M. 1997. Production, purification and characterization of an extracellular alpha-amylase enzyme isolated from *Aspergillus flavus*. *Microbios.* **89**(358): 55-56.

AKHTER, S., HOSSAIN, M.T., RAHMAN, M.S., MANCHUR, M.A. AND ANWAR, M.N. 1999. Isolation of amylase producing fungi and their amylase activity. *The Chittagong Univ. J. Science.* **23**(2):107-113.

ALMEIDA, E.M. D.S., MIZUTA, K. and GIGILO, J.R. 1997. *Pycnoporus sanguineus*: a novel source of α-amylase. *Mycol, Res*. **101**(2): 188-190.

BEGUM, A. A. CHOWDHURY, N. AND SARDAR, A. H. 1993. Production of cellulose by fungal isolates in solid state fermentation and saccharification of rice bran and sugarcane bagasse. *Bangladesh J. Microbiol.* **10**(1):21-27.

BORMISS, R., SCHMIDT, D., RUFTOFF, F. E., HEINZ, T. A. L., STEFFEN, P. WOLFGANY, H. AND URSULA, G. 1981. Enzyme mixture for saccharification of grain mash. *Ger (East) D.D* **152**: 142.

CAMPOS, L. AND FELIX, C.R. 1995. Purification and Characterization of Flucoamylase from *Humicola grisea*. *Appl. Environ. Microbiol.* **61**(6):2436-2438.

CHERRY, H.M., HOSSAIN, M.T. AND ANWAR, M.N. 2004. Extracellular glucoamylase from the isolate *Aspergillus fumigatus*. *Pak. J. Biol. Sci.* **7**(11): 1988-1992.

DEY, G., NAGPAL, V. AND BANERJEE, R. 2002.Immobilization of alpha-amylase from *Bacillus circulans* GRS313 on Coconut fiber. *Applied. Biochemistry and Biotechnology*. **103**(1-3): 303-314.

FABIANA G. M., FRANCIELI A.D.L., SOPHIA R.F.P., VERIDIANA L., CRISTINA G.M D. S. AND ROSANE M. P.1999. The production of amylases by *Aspergillus tamarii*. *Rev. Microbiol*. **30**(2).Sao Paulo.

FILIPOV, S.A., AVAKYANTS, S.P. AND BARTENEV, S.P. 1982. Conditions for the immobilization of cells of microscopic fungi and their use for enzymatic hydrolysis of starch. *Ferment. Spirt. Prom-st*. **2**:30-31.

FREER S.N. 1993. Purification and characterization of the extracellular alpha-amylase from Streptococcus bovis JB1. *Appl. Environ. Microbiol*. **59**(5):1398-1402.

GARGOVA, S. AND NAUCHNI, T. 1979. Study of some condition for the biosynthesis of amylolytic enzymes by *Aspergillus sp. 16/132, Vkusova prom-st. Plovdiv*, **26**(1): 319-330.

GLYMPH, J.L. AND STUTZENBERGER, F.J. 1977. Production, purification and characterization of alpha-amylase from *Thermomonospora curvata. Appl. Environ. Microbiol.* **34**(4): 391-397.

GUO, Y.,HAUNG, L., ZHAO, M., CHEN, H., PENG, X., LUO, G AND LI, L. 1988.Production of glucoamylase by immobilized cells. Conditions for batch fermentation and semicontineuous fermentation. *Shipin Yu Fajiao Gongye* **2**: 28-31.

HIZUKURI AND SUSUMU 1983. *Aspergillus* strains and their use to produce D-glucose from starch. *Eur. Pat. Appl. Up*. 134, 546.

LACHMUND, A. AND RUTTKOWSKI, E. 1990. Investigations on the expression and regulation of the alpha-amylase gene from Aspergillus oryzae. *DECHEMA Biotechnol. Conf* **4**:399-402.

KACHELKINA, A.I. AND ORESHCHENKO, L.I. 1980. Saparating alpha-amylase from a culture medium**.** *U.S.S.R.* 668:348.

LOWRY, O.H., ROSEBROUGH, N. J., FARR, A. F., AND RANDALL, R. J. 1951. Protein Measurement with the Folin-Phenol Reagent. *J. Biol. Chem.* **193**:265-275.

MAHADEVAN A.AND SRIDHAR R. 1982. *Methods in physiological plant pathology.* Siva Kami Pub. Madras**.** PP.316

MAHMOUD, A. L. E. 1993. Different factors affecting growth and amylase production by fungi inhabiting poultry feeds. *J. Basic Microbiol.* **33**(3): 187-192.

MAHMUD, N. 2006. Isolation, purification and characterization of amylase from *Listeria denitrificans* and *Aspergillus versicolor*. *M.S. thesis* pp. 106-171.

MARZAN, L.W., M.A. MANCHUR, M.T. HOSSAIN AND M.N. ANWAR 2001.Production of protease and alpha-amylase by *Fusarium poae*. *Bangladesh J. Microbiol.* **18**:127-134.

NELSON, N. 1944. A Photometric adaptation of the Somogy method for the determination of glucose**.** *J. Biol. Chem* **153**: 375-380.

OKOLO, B.N. EZEOGU, LEWIS M. I., AND CHARLES N. 1995. Production of raw starch digesting amylase by *Aspergillus niger* grown on native starch sources. *J. Sci. Food Agric.* **69**(1): 109-115.

OLASUPO, N.A., TENIOLA, O.D., OKASUN, R., OMAWAYE, A., *OLATROPE, S.O. AND SCOTT E.M.B. 1996. Studies on an amylolytic strain of Saccharomyces* cerevisiae isolated from yam tuber. *J. Basic Microbiol.* **36**: 288-293.

PANDEY, A., NIGAM, P. SOCCOL, C. R., SOCCOL, V. T. SINGH, D. AND MOHAN, R.2000. Review advances in microbial amylases. *Biotechnol.Appl. Biochem.* **31**:135-152.

RAHMAN, A.K.K.M.S., M.F. RAHMAN, P.K. NATH, M.M. HOQ, AND M. HOSSAIN. 1993. Glucoamylase activity of some selected strains of *A. niger* and *A. oryzae*. *Bangladesh J. Microbiol.* **10**(2): 107-110.

REED, G. 1975. *Enzyme in food processing (2nd edition).* Academic press, New York 10005 U.S.A.

TEODORO C.E.D.S. AND MARTIN, M.L.L. 2000. Culture conditions for the production of thermostable amylase by *Bacillus* sp. *Braz J. Microbiol*. **3**(1): 4.

SRIVASTAVA, R. A. K. and BARUAH, J. N., 1989. Culture condition for production of thermostable amylase by *Bacillus stearothermophilus Appl. Environ. Microb*. **51**: 179-184.

TRYAVSOGOLOVA, T.V., KAZAKOV, A.V., BURTSEVA, E.I., DVADTSALOVA, E.A. AND USTINNIKOV, B.A. 1986.Improved fermentation conditions for producer of alpha-amylase. *Ferment. Spirt. Prom-st* **5**:36-37.

XIANGZHANG, X., CHUANXIAN, G., WENHAIR, Q., WEIWEI, X., YUNLIN, Z., CHEN, X.Y. AND JIANWEI, R.W. 1988. Studies on the technology of production of alpha-amylase by a mutant B.S.796. *Shengwn Gongeneng Xuebao.* **4**(2):119-126.

Manuscript received on 11.10.07; Accepted on 26.6. 08

*The Chittagong University Journal of Biological Sciences*, Vol. 3( 1 & 2): *pp.*11-20, 2008.