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PRODUCTION OF AMYLASE BY FUNGI THROUGH SUBMERGED FERMENTATION

A. Sattar Qureshi, M. Umar Dahot and A.U.Rehman*

*Institute of Biotechnology and Genetic Engineering and *Dr. M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, Pakistan*

ABSTRACT: Amylase has versatile application in food and industries, which inspired to produce this enzyme by less expensive culture medium using agricultural or forest waste. In the initial stage, amylase production was carried out by different fungal species such as Mucor geophillus, Aspergillus niger, Aspergillus fumigatus, Penicillium lilacinum and mixed culture (A. niger + A. fumigatus) using mineral medium with and without glucose. Highest amount of amylase (34.4 & 1.36 units/ml both) was produced by A. fumigatus when grown at 31 $\pm$ 2 °C with in 48 hours when mineral medium was supplemented with and without glucose respectively in comparison to other fungal species.

Key words: Amylase, Fungi, and Glucose

INTRODUCTION: α-amylase (EC 3.2.1.1, 1, 4-c-D-glucan, glucanohydolases and endo amylase) hydrolyze starch glycogen, and related polysaccharides by randomly cleaving internal α-1, 4-glucosidic linkages (1, 2). It is widely distributed in bacteria, fungi, plants and animals and has a major role in utilization of starch and glycogen (3,4) Alpha amylase has versatile application in food and textile industries, mostly in industrial productions of glucose and high maltose syrup (5). Starch digesting amylases (α-amylase and ß-amylase) are produced from A. niger (6), A. oryzae (7), Rhizopus species (8), Bacillus subtilis (9,10), Bacillus lichenoformis (11), Penicillium expansum (12). Various workers have reported fermentation process using various carbon sources such as rice husk, molasses and pure sugars (glucose and sucrose) for α-amylase production by A.fumigatus, A.niger, M.geophillus and P. lilacinum. In initial stage we have produced α-amylase by fermentation process using pure sugar (glucose) as carbon source for growth of fungi and production of α-amylase.

MATERIAL AND METHODS:

Microorganism: P. lilacinum and M. geophillus were obtained from Research Laboratory department of Chemistry, Shah Abdul Latif University of Khairpur, whereas A. niger and A. fumigatus were isolated and identified in this laboratory. The stock was maintained for A. niger, A. fumigatus and M. geophillus on agar slants, containing (g/l) glucose 20, peptone 10, agar 20 and distilled water, whereas stock culture was maintained for

P. lilacinum on Zpex agar slants, containing (g/l) Zpex agar 4.8 and distilled water. The ingredients were thoroughly mixed and kept in culture tubes sterilized at 15 pounds/cm² for 20 minutes at 121°C. The sterilized slants were inoculated with A. niger, A. fumigatus, M. geophillus and P. lilacinum and incubated at 37°C to obtain luxuriant growth.

Inoculum: A spore suspension was prepared by adding sterilized water to stock culture to get $50x10^6$ spores/ml.

Mineral medium: The mineral medium for fungi containing following reagents /l of solution glucose 10.0g, KH_2PO_4 1.0g, $FeSO_4.7H_2O$ 6.32 mg, $MgSO_4.7H_2O$ 0.25g, $ZnSO_4.7H_2O$ 1.1mg, $MnCl_2.2H_2O$ 3.54mg $CaCl_2.2H_2O$ 46.7mg NH_4NO_3 2.4g.The pH of medium was adjusted at 6.5

Fermentation medium: 100ml of mineral medium with and without sugar supplementation was taken in a 250ml flask. The pH of medium was adjusted 6.5.The flasks were plugged with cotton wool and sterilized at 121°C for 25 minutes at 15 pounds/cm² After cooling at room temperature, the flasks were inoculated with 1.0ml of inoculum containing $50x10^6$ spores/ml. The flasks were incubated at 31$\pm$ 2°C .The flasks were manually shaken few times daily .The culture broth was filtered from mycelium after an interval of 24 hours incubation period, through Whatman No.1 filter paper .The recovered mycelium was dried at 80°C and weighed.

Determination of Protein: Protein content of broth was determined by Lowry et. al., [13] method with bovine serum albumin as standard.

Determination of R. Sugar: Reducing sugar content of broth was determined by Miller method [14] with glucose as standard.

Determination of Sugar: Total sugar content of broth was determined by Montgomery method [15] with glucose as standard.

Determination of α-Amylase Aactivity: α-Amylase activity was determined by Fischer and Stein [16] method 1.0ml of culture broth was added to 1.0ml of 1% soluble starch and kept in water bath at 37°C for 15 minutes .The reaction was terminates by addition of 2.0ml DNS (3,5-dinitro- salicylic acid) reagent. Colour developed due to the reducing sugar by heating the reactant in boiling water bath for 5minutes and was cooled at tap water. The activity was determined by Spectro-photometr at 540nm.

RESULT AND DISCUSSION:

The table 1 shows the results of amylase synthesis when medium was used without sugar by A. fumigatus. The maximum production of α-amylase (1.36 units/ml) was achieved at 168 hours and then decreased with the increase of time period. The concentration of total sugar and reducing sugar was absent at the initial period of growth but after 48 hours the concentration increases with the increase of growth period up to 192 hours and then decreased. Fluctuation was noted in the final pH in the culture broth throughout study period.

Table- 1: A. fumigatus was grown on mineral medium; without sugar at 31± 2°C and pH 6.5

Time	Final pH	Mycelial weight (g/50ml)	Total Sugar (mg/ml)	Reducing Sugar (mg/ml)	Amylase Activity (units/ml)	Total Protein (mg/ml)
24	6.30	0.005	-	-	-	0.00013
48	6.21	0.01	-	-	-	0.00013
72	6.26	0.01	0.045	0.039	-	0.017
96	6.12	0.01	0.067	0.059	0.32	0.012
120	6.22	0.01	0.093	0.08	0.84	0.013
144	6.35	0.03	0.098	0.08	0.69	0.014
168	5.94	0.03	0.12	0.10	1.36	0.007
192	5.89	0.03	0.15	0.12	0.93	0.019
216	5.64	0.02	0.19	0.14	0.64	0.018
240	6.51	0.02	0.2	0.18	0.18	0.01

The table 2 shows the results of amylase synthesis when medium was used without sugar by A. niger .The maximum production of α-amylase (0.91 units/ml) was achieved at 168 hours and then decreased with the increase of time period. The concentration of total sugar and reducing sugar was absent at the initial period of growth but after 48 hours the concentration increases with the increase of growth period. Fluctuation was noted in the final pH at the culture broth throughout study period.

Table 2: A. niger was grown on mineral medium; without sugar at 31± 2°C and pH 6.5

Time	Final PH	Mycelial weight (g/50ml)	Total Sugar (mg/ml)	Reducing Sugar (mg/ml)	Amylase Activity (units/ml)	Total Protein (mg/ml)
24	6.23	0.01	-	-	-	0.005
48	6.21	0.01	-	-	-	0.015
72	6.11	0.01	0.08	0.04	-	0.015
96	6.25	0.01	0.091	0.062	-	0.1
120	6.22	0.01	0.11	0.09	0.52	0.12
144	6.32	0.03	0.13	0.103	0.69	0.14
168	6.17	0.04	0.154	0.11	0.91	0.11
192	5.70	0.03	0.167	0.16	0.64	0.01
216	6.05	0.02	0.18	0.162	0.19	0.004
240	6.50	0.02	0.21	0.169	0.11	0.05

The table 3 shows the results of amylase synthesis when medium was used without sugar by M. geophillus. The maximum production of α-amylase (0.77 units/ml) was achieved at 192 hours and then decreased with the increase of time period. The concentration of total sugar and reducing sugar increases with the increase of growth period. Fluctuation was noted in the final pH at the culture broth throughout study period.

Table 3: M. geophillus was grown on mineral medium; without sugar at 31± 2°C and pH 6.5

Time	Final pH	Mycelial weight (g/50ml)	Total Sugar (mg/ml)	Reducing Sugar (mg/ml)	Amylase Activity (units/ml)	Total Protein (mg/ml)
24	6.27	0.01	0.05	0.045	-	0.0014
48	6.25	0.01	0.051	0.046	-	0.00013
72	6.27	0.01	0.069	0.061	-	0.004
96	6.25	0.01	0.071	0.069	-	0.004
120	6.21	0.01	0.093	0.08	0.38	0.025
144	6.04	0.03	0.099	0.085	0.24	0.029
168	6.18	0.03	0.12	0.10	0.63	0.004
192	5.73	0.05	0.154	0.14	0.77	0.002
216	6.13	0.10	0.164	0.151	0.26	0.002
240	6.53	0.02	0.187	0.166	0.21	0.002

The table 4 shows the results of amylase synthesis when medium was used without sugar by mixed culture (A. niger and A. fumigatus). The maximum production of α-amylase (1.14 units/ml) was achieved at 168 hours and then decreased with the increase of time period. The concentration of total sugar and reducing sugar increases with the increase of growth period. Fluctuation was noted in the final pH at the culture broth throughout study period.

Table 4: Mixed culture (A. fumigatus and A. niger) was grown on mineral medium with out sugar at 31± 2°C and pH 6.5

Time	Final pH	Mycelial weight (g/50ml)	Total Sugar (mg/ml)	Reducing Sugar (mg/ml)	Amylase Activity (units/ml)	Total Protein (mg/ml)
24	6.26	0.005	-	-	-	0.005
48	6.15	0.01	0.058	0.050	-	0.008
72	6.17	0.01	0.069	0.061	-	0.03
96	6.19	0.01	0.093	0.082	-	0.03
120	6.17	0.01	0.105	0.095	0.50	0.005
144	6.32	0.03	0.11	0.098	0.48	0.004
168	6.22	0.03	0.113	0.10	1.14	0.03
192	6.28	0.06	0.12	0.10	0.87	0.031
216	6.41	0.03	0.12	0.10	0.42	0.004
240	6.41	0.02	0.135	0.12	0.40	0.001

The table 5 shows the results of amylase synthesis when medium was used without sugar by P. lilacinum. The maximum production of α-amylase (1.03 units/ml) was achieved at 240 hours and with the increase of time period amylase production increases. The concentration of total sugar and reducing sugar increases with the increase of growth period. Fluctuation was noted in the final pH at initial stage whereas after 120 hours decreases with increase of growth period.

Table 5: P. lilacinum was grown on mineral medium without sugar at 31 ± 2°C & pH 6.5

Time	Final pH	Biomass weight (g)	Total Sugar (mg/ml)	Reducing Sugar (mg/ml)	Amylase Activity (units/ml)	Total Protein (mg/ml)
24	6.28	0.005	-	-	-	0.00013
48	6.23	0.01	-	-	-	0.00014
72	6.25	0.01	0.04	0.031	-	0.0112
96	6.22	0.01	0.061	0.053	-	0.0114
120	6.33	0.01	0.09	0.079	0.33	0.013
144	6.10	0.01	0.11	0.087	0.71	0.014
168	5.71	0.02	0.11	0.09	0.760	0.009
192	5.65	0.03	0.125	0.099	0.91	0.019
216	5.20	0.02	0.14	0.12	0.99	0.018
240	5.21	0.03	0.152	0.126	1.03	0.01

The table 6 shows the results of amylase synthesis when medium was used with sugar by P. lilacinum. The maximum production of α-amylase (25.2 units/ml) was achieved at 24 hours and with the increase of time period amylase production decreases. The concentration of total sugar and reducing sugar decreases with the increase of growth period after 48 hours. Fluctuation was noted in the final pH at culture broth throughout growth period.

Table 6: P.lilacinum was grown on mineral medium with sugar at 31 ± 2°C and pH 6.5

Time	Final pH	Mycelial weight (g/50ml)	Total Sugar (mg/ml)	Reducing Sugar (mg/ml)	Amylase Activity (units/ml)	Total Protein (mg/ml)
24	5.85	0.21	14.2	10.6	25.2	0.17
48	4.12	0.2	50	46.0	18.2	0.13
72	3.61	0.34	21.5	18.4	15	0.21
96	4.21	0.39	18.6	14.8	7.1	0.28
120	4.01	0.46	7.1	4.05	2.28	0.19
144	3.24	0.11	2.3	1.28	1.86	0.23
168	2.09	0.38	0.85	0.68	0.89	0.17
192	3.63	0.17	0.75	0.29	0.89	0.23
216	4.5	0.08	0.72	0.24	1.24	0.23
240	5.7	0.08	0.48	0.2	1.20	0.29

The table 7 shows the results of amylase synthesis when medium was used with sugar by A. niger. The maximum production of α-amylase (25.6 units/ml) was achieved at 24 hours and with the increase of time period amylase production decreases. The concentration of total sugar and reducing sugar decreases with the increase of growth period after 48 hours. Fluctuation was noted in the final pH at culture broth throughout growth period.

Table 7: A. niger was grown on mineral medium with sugar at 31 ± 2°C and pH 6.5

Time	Final pH	Mycelial weight (g/50ml)	Total sugar (mg/ml)	Reducing sugar (mg/ml)	Amylase activity (Units/ml)	Total Protein (mg/ml)
24	3.88	0.05	41.2	37	25.6	0.18
48	2.14	0.13	31.52	25.2	20.61	0.0475
72	1.78	0.695	33.25	26	7.7	0.1275
96	1.71	0.395	17.3	12.7	8.6	0.205
120	1.955	0.375	5.4	2.9	12.6	0.1525
144	2.16	0.665	2.1	1.5	4.85	0.17
168	1.82	0.475	1.5	0.98	7.3	0.155
192	1.85	0.23	0.7	0.575	1.74	0.1025
216	1.76	0.215	0.55	0.33	1.26	0.1175
240	2.02	0.3	0.31	0.24	0.89	0.1725

The table 8 shows the results of amylase synthesis when medium was used with sugar by M. geophillus. The maximum production of α-amylase (17.4 units/ml) was achieved at 48 hours and with the increase of time period amylase production decreases. The concentration of total sugar and reducing sugar decreases with the increase of growth period. Fluctuation was noted in the final pH at culture broth throughout growth period.

Table 8: M.geophillus was grown on mineral medium with sugar at 31 ± 2°C and pH 6.5

Time	Final pH	Mycelial weight (g/50ml)	Total sugar (mg/ml)	Reducing sugar (mg/ml)	Amylase activity (Units/ml)	Total protein (mg/ml)
24	2.895	0.06	15.485	14.525	16	0.155
48	2.455	0.105	14.8	14.025	17.4	0.055
72	1.775	0.81	7.2	6.625	8.05	0.0975
96	1.78	0.35	3.1	2.9	5.5	0.12
120	2.625	0.355	2.6	2.1	4.85	0.0775
144	2.17	0.345	1.31	1.085	1.31	0.12
168	1.85	0.425	0.72	0.625	3.5	0.0775
192	1.735	0.305	0.55	0.46	4.4	0.0975
216	1.86	0.255	0.45	0.39	1.16	0.045
240	2.095	0.35	0.315	0.29	0.76	0.055

The table 9 shows the results of amylase synthesis when medium was used with sugar by A. fumigatus. The maximum production of α-amylase (34.4 units/ml) was achieved at 48 hours and with the increase of time period amylase production decreases. The concentration of total sugar and reducing sugar decreases with the increase of growth period after 48 hours. Fluctuation was noted in the final pH at culture broth throughout growth period.

Table 9: A. fumigatus was grown on mineral medium with sugar at 31 ± 2°C and pH 6.5

Time	Final pH	Mycelial weight (g/50ml)	Total sugar (mg/ml)	Reducing sugar (mg/ml)	Amylase activity (Units/ml)	Total protein mg/ml
24	4.57	0.052	13.735	12.485	19.8	0.13
48	2.52	0.11	19.225	18.8	34.4	0.09
72	2.13	0.285	12.8	10.5	5.6	0.1025
96	4.695	0.325	4.4	3.15	11.1	0.225
120	1.735	0.25	1.2	0.98	15.55	0.09
144	2.28	0.26	1.2	0.86	1.52	0.2075
168	1.78	0.345	1.1	0.81	4.2	0.2
192	3.62	0.28	1.1	0.75	1.36	0.1
216	4.405	0.295	0.96	0.72	2.44	0.0775
240	4.475	0.27	0.89	0.71	1.28	0.0675

The table 10 shows the results of amylase synthesis when medium was used with sugar by Mixed culture (A. niger and A. fumigatus). The maximum production of α-amylase (26.3 units/ml) was achieved at 48 hours and with the increase of time period amylase production decreases. The concentration of total sugar and reducing sugar decreases with the increase of growth period. Fluctuation was noted in the final pH at culture broth throughout growth period.

Table 10: Mixed culture (A. fumigatus and A. niger) was grown on mineral medium with sugar at 31 ± 2°C and pH 6.5

Time	Final pH	Mycelial weight (gr/50ml)	Total sugar (mg/ml)	Reducing sugar (mg/ml)	Amylase activity (units/ml)	Total protein mg/ml
24	2.9	0.045	47.2	45.9	22.8	0.185
48	2.405	0.115	35.45	34	26.3	0.0775
72	1.77	0.385	12.4	11.1	5.9	0.0875
96	1.695	0.36	6.56	5.75	5.35	0.195
120	4.175	0.355	2.1	1.89	15.1	0.0775
144	2.18	0.26	1.085	0.91	2.27	0.2175
168	1.895	0.425	1.06	0.89	3.8	0.0925
192	3.14	0.24	0.96	0.76	0.88	0.1075
216	4.425	0.235	0.785	0.69	0.56	0.12
240	6.05	0.22	0.58	0.48	0.455	0.195

In this study, four microorganism such as, Mucor geophillus, Aspergillus niger, Aspergillus fumigatus, Penicillium lilacinum and mixed culture (A. niger + A. fumigatus) were grown on medium with and without glucose. Among these microorganisms A. fumigatus produces highest amount of amylase. It is concluded that the yield of α-amylase varies from carbon to carbon source and microorganism to microorganism but media composition and pH of culture medium also play an important role in enzyme production [16-18].

Work is under progress for the use of different carbon sources.

REFERENCES:

1. Gilles, C., T. P. Astler, G.M.Mourn, C. Cambillau and F. Payan, Eur .J. Biochem. **238**: 561 (1996)
2. Witt,W. and J.Sauter, J. Phytochem. **441:** 365 (1996)
3. Hagenimana, V., L.P.Vezina and R.E.Simardd, J. Agri. Food Chem. **40**: 1777 (1992).
4. Swamy, M .V. and G. Seenayya, Process Biochemistry **31**: 157 (1995)
5. Fogarty, W. M. and E. J. Bourke, J. Chem. Tech. Bioechnol. **33B:** 145 (1983).
6. Aoki, K., M. Aral and Y. Minda, Agri. Biol. Chem. **35**: 1913, (1970).
7. Qureshi,E.I. and M.A.Malik, Pak. J. Sci. Ind. Res. **11**: 377 (1968).
8. Windish, W. M. and N. S. Mhatre, Adv. Apl. Microbiol., **7**: 273 (1965).
9. Yamane, K. and B. Maruo, J. Bacteriol. **120**: 792 (1974).
10. Qadeer, M. A., J. I. Anjum and R. Akhtar, Pak. J. Sci. Ind. Res. **23**: 25 (1980).
11. Dobreva, E., E. Emanuilova, P. Kosturkova and M. Beschkov, Acta Biotechnol. **5:** 187 (1985).
12. Belloc,A., J. Florent, D. Mancy, and J. Varrier, U.S. Patent 3, 906, 113 (1975).
13. References, T. S., R. montgomrey; Biochem. Biophys. Acta **48:** 591 (1961)
14. Lowry,O. H., N. J.Rosrbroug, A. L. Farr and R. J. Randadll, J. Biol. Chem. **193:** 265 (1951)
15. Miller; J. L., Anal. Chem. **31**: 426 (1959).
16. Takasaki, Y., Agric. Biol. Chem., 40: 1523 (1976)
17. Macris, B.J., M. Paspaliari and D. Kekos., Biotechnol. Lett., 4: 785 (1982)
18. Shoemarker, S.P., J.C.Raymond and R. Burner., Basic Life Sci., 18: 89 (1981)