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AMYLASE PRODUCTION BY *ASPERGILLUS NIGER* UNDER SUBMERGED FERMENTATION USING *IPOMOEA BATATAS*

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ABSTRACT: Submerged fermentation holds tremendous fungal potentiality in high biomass yield of alpha-amylase. The effect of varying pH, temperature and nitrogen sources of the medium for the productivity of α- amylase from *Aspergillus niger* utilizing *Ipomoea batatas* was investigated. The maximum activity of α-amylase was recorded as 450 U/mg after 7 days of submerged fermentation at pH 7.0 and room temperature 28°C. Among the organic and inorganic nitrogen sources, inorganic sources showed maximum yield in which ammonium nitrate showed highest amylase activity of 475U/mg at the same pH and temperature. The enzyme produced by *Aspergillus niger* can be used in industrial process after characterization.

Keywords: Amylase, *Aspergillus niger, Ipomoea batatas*, Submerged fermentation

INTRODUCTION

Recent discoveries on the use of microorganisms as sources of industrially relavent enzymes have led to an increased in the application of microbial enzymes in various industrial processes. Nowadays, the new potential of using microorganism as biotechnological source of industrially relevant enzymes has stimulated interest in exploration of extracellular enzymatic activities in several microorganisms [Akpan *et al*., 1999; Bilinski and Stewart, 1995; Buzzini and Martini, 2002]. Amylases are hydrolytic enzymes that stand out as a class of enzymes which are of useful applications in the brewing, textile, detergent and pharmaceutical industries [Asghar *et al*., 2000]. A growing new area of application of α-amylase is in the field of laundry and dish washing detergents (Van der Maarel *et al*., 2002).

Amylase of fungal origin was found to be more of stable than the bacterial enzymes on a commercial scale. Many attempts have been made to optimize culture conditions and suitable strains of fungi [Abu *et al*., 2005]. Studies on fungal amylases especially *Aspergillus niger* has been concentrated because of their ubiquitous nature and non fastidious nutritional requirement [Abu *et al*., 2005]. Ikenebomeh Chickundu in the year 1997, reported that *A.niger* shows high amylolytic activity in biomass production. Submerged fermentation holds tremendous potential for the production of enzymes. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source (Canel and Moo, 1980; Tunga and Tunga, 2003). In the present work, the tubers of *Ipomoea batatas* are used for the production of α- amylase. Different process conditions were studied to achieve maximum yield of α-amylase production using various experimental designs.

MATERIALS AND METHODS

SAMPLE COLLECTION

The tubers of *Ipomoea batatas* (Sweet potato) were obtained from the hilly areas of Malappuram and Cannanore Districts of Kerala, India.

ISOLATION OF *ASPERGILLUS NIGER*

Aspergillus species was isolated from the soil and maintained on potato dextrose agar (PDA).

LACTOPHENOL COTTON BLUE STAINING

A loop full of fungal cultures was placed on a clean glass slide, a drop of lactophenol cotton blue stain was mixed with the culture. A clean coverslip was placed over the culture and viewed under the microscope (45X) and the morphology of *Aspergillus niger* was observed and photographed (Plate 1).

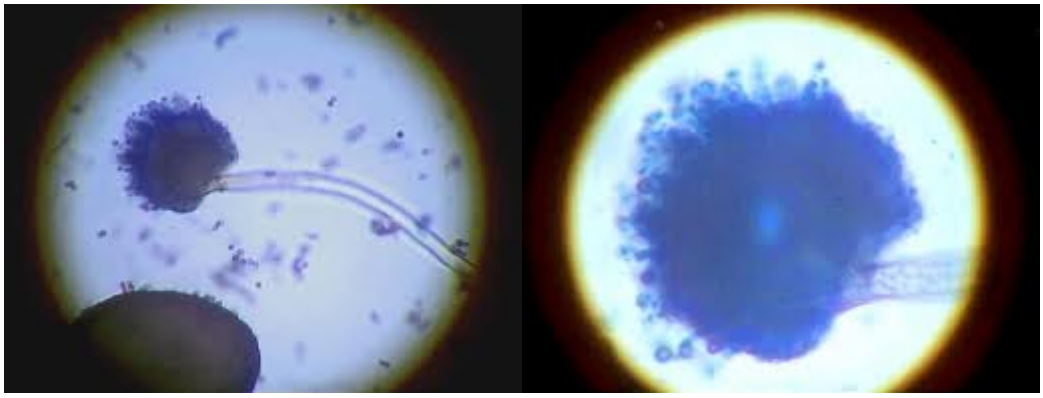

Plate 1: A.niger viewed under microscope after stained with LPCB

SAMPLE PREPARATION

The tubers of the collected sample were washed thoroughly; the skins were removed and chopped into pieces which were then boiled. The boiled sample was grinded and the aqueous extract was obtained by squeezing it using muslin cloth. This extract was used as carbon source in the Czapek dox medium in which the *Aspergillus niger* was inoculated and kept for incubation for 7- days at 3 different temperatures (37°C, 20°C,and 28°C) and 3 different pH (3.0, 5.O, and 7.0).

EXTRACTION OF AMYLASE FROM THE FERMENTATION MEDIUM

After incubation, the fermentation medium was harvested by centrifugation at 10,000 rpm for 10 minutes at 4°C. The supernatant was collected and subjected for estimate the amylase activity.

EFFECT OF TEMPERATURE

To study the effect of temperature on amylase production, the submerged fermentation was carried out at different temperatures (20°C, 37°C, 28°C).

EFFECT OF pH

The fermentation medium was prepared by varying the p values (3.0, 5.0, and 7.0) for the production of amylase.

EFFECT OF NITROGEN SOURCES

The effect of nitrogen source on enzyme production was studied by replacing the nitrogen source in Czapek dox medium, pH -7.0 with organic nitrogen sources such as peptone, casein, yeast extract and inorganic nitrogen sources such as NH_4NO3, KNO3 and incubated at room temperature 28ºC for 7 days.

ENZYME ASSAY

Amylase activity was estimated by the analysis of reducing sugar released during hydrolysis of 1% (w/v) starch in 0.1 M sodium citrate buffer by the Dinitrosalicylic acid (DNS) method (Miller, 1959). One unit of amylase activity was defined as the amount of enzyme that releases 1mMol of reducing sugar as glucose per minute under assay condition.

(VI) PROTEIN ESTIMATION

Protein content of the enzyme extracts was estimated by the method of Lowry. *et. al* (1951), using bovine serum albumin as the standard.

RESULT AND DISCUSSION

Different fermentation parameters were optimized for α- amylase production by conducting series of experiments and the results are discussed as under.

Effect of temperature

Duplicate flask containing 30gm of fresh boiled tuber of *Ipomoea batatas* was autoclaved, inoculated and incubated at different temperature of 37ºC, 20°C and room temperature (28°C) for seven days. The maximum activity of α- amylase was noted in the enzyme extracts incubated at room temperature (28ºC) of a pH-7 in *Ipomoea batatas* (450 U/mg) (shown in Table-1 & Fig-1). It is reported that best enzyme production in *A.niger* at room temperature both in Submerged Fermentation and Solid State Fermentation [Varalakshmi *et al.,* 2009] and reported 30°C be the best for enzyme production by *Penicillium fellutanum* [Kathiresan & Manivannan, 2006].

Table 1. The effect of pH-3 at different temperature on fungal enzyme production.

CONCENTRATION (µg)	pH	TEMPERATURE	AMYLASE ACTIVITY U/mg
50	3.0	20ºC	93.75
100	3.0	20ºC	131.25
50	3.0	28ºC	187.5
100	3.0	28ºC	300
50	3.0	37ºC	75
100	3.0	37ºC	150

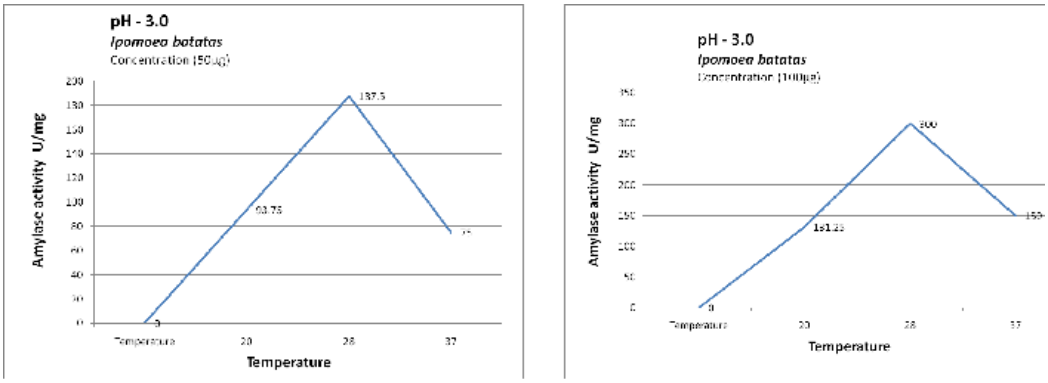

Fig. 1. The effect of pH-3 on amylase production at different temperatures.

Effect of pH

Aspergillus niger was inoculated in *Ipomoea batatas* was incubated at room temperature 28ºC for 7 days. The enzyme was extracted and the amylase produced at different pH was recorded. The maximum yield of amylase was in pH-7 and the amylase production was 450 U/mg in *Ipomoea batatas* (shown in Table 1,2,3 & Figure -1,2, 3). In contrary to our results Varalakshmi *et al*., (2009) reported the maximum enzyme activity of 75 U /mg of protein at pH- 9.5. Others have reported acidic pH optima for amylases from *A.niger* [Hernandes *et al*., 2006; Mitieri et al., 2006]. Different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth [Lehninger, 1982].

Effect of Nitrogen source

Addition of organic sources such as peptone, casein and yeast extract to the medium resulted in a considerable increase in the production of alpha amylase compared to the control (53U/mg in *Ipomoea batatas*). Media supplemented with peptone showed maximum amylase activity compared to casein and yeast extract (Fig.4). Suganthi. *et al*., in the year 2011 reported that nitrogen source increases the yield of alpha amylase produced in ground nut oil cake medium. Similarly the supplementation with inorganic nitrogen sources to amylase production by *Aspergillus niger* is done with success increase in the yield of enzyme in submerged fermentation [Pandey, 2005]. Among the inorganic nitrogen sources ammonium nitrate showed highest amylase activity (see Fig.4). Our results are in agreement found that the nitrogen supplementation enhances the production of the organism and have increased in the biomass cropped [Anupama and Ravindra, 2001]. Previous findings have shown that peptone, sodium nitrate and casein hydrolysate are good nitrogen supplements for amylase production in *A.fumigatus* [Got *et al*.,1998] *A.niger* [Pandey *et al*.,1994] and *A.oryzae* [Pederson and Neilson,2000].

Table 2. The effect of pH-5 at different temperature on fungal enzyme production.

50	5.0	20ºC	131.25
100	5.0	20ºC	206.25
50	5.0	28ºC	225
100	5.0	28ºC	375
50	5.0	37ºC	168.75
100	5.0	37ºC	318.75

Table 3. The effect of pH-7 at different temperature on fungal enzyme production.

50	7.0	20°C	168.75
100	7.0	20°C	262.5
50	7.0	28°C	318.75
100	7.0	28°C	450
50	7.0	37°C	225
100	7.0	37°C	356.25

Effect of Nitrogen sources on *Ipomoea batatas*

Table 4. The effect of different nitrogen sources at temperature 28°C, pH- 7 on fungal amylase production after 7 days incubation.

NITROGEN SOURCE	CONCENTRATION (µg)	pH	TEMPERATURE	AMYLASE ACTIVITY(U/mg)
INORGANIC NITROGEN SOURCES				
NH_4NO_3	50	7.0	28	277.5
NH_4NO_3	100	7.0	28	475
KNO_3	50	7.0	28	225
KNO_3	100	7.0	28	375
ORGANIC NITROGEN SOURCES				
Peptone	50	7.0	28	101.25
Peptone	100	7.0	28	236.25
Caesin	50	7.0	28	60
Caesin	100	7.0	28	112.5
Yeast extract	50	7.0	28	60
Yeast extract	100	7.0	28	135

Figures indicating the effect of different parameters on fungal enzyme production

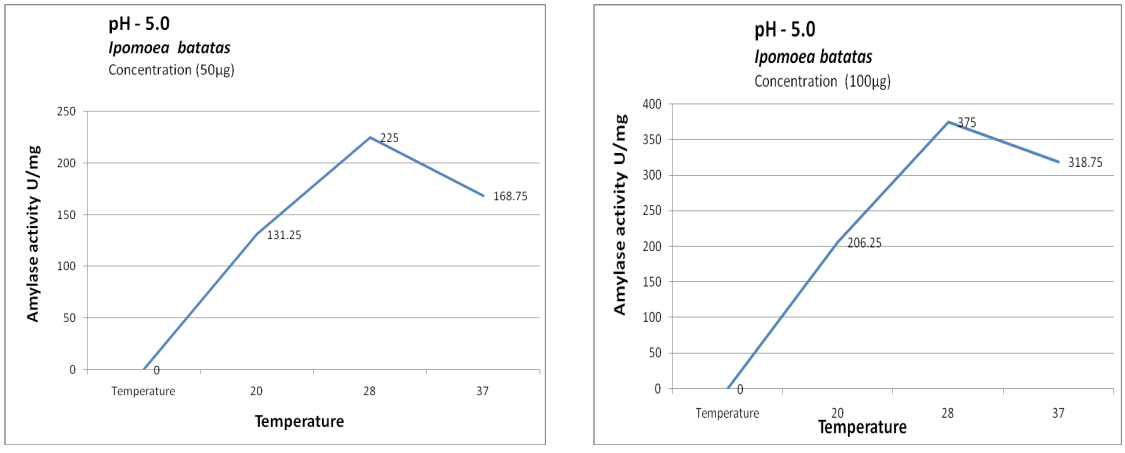

Fig. 2. The effect of pH-5 on amylase production at different temperatures.

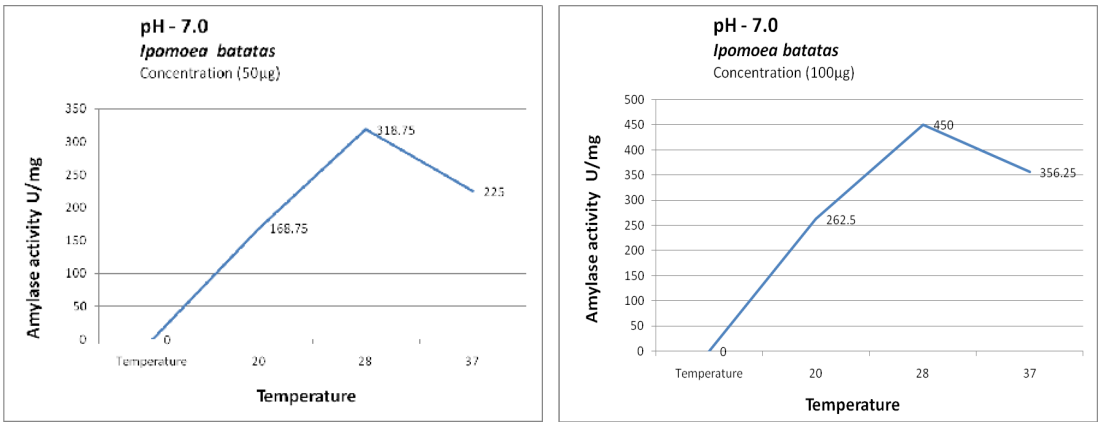

Fig. 3. The effect of pH-7 on amylase production at different temperatures.

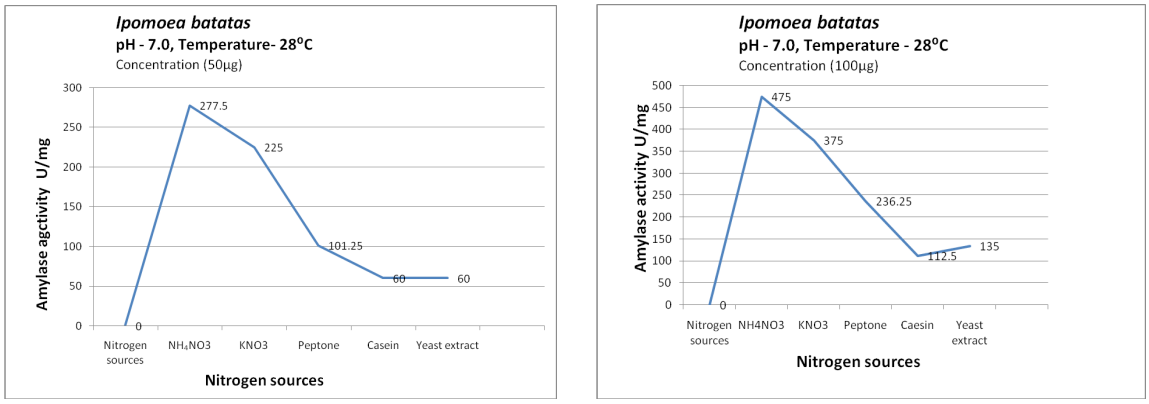

Fig. 4. Effect of different nitrogen sources on fungal amylase production at pH- 7.0 and temperature 28^{O}C.

CONCLUSION

Higher yield of α-amylase production from *A.niger* was possible by Submerged Fermentation. The maximum production with 450U/mg of α- amylase was observed in tuber of *Ipomoea batatas* at room temperature (28^{0}C) with a pH of 7.0.

From the results the media supplemented with ammonium nitrate gave more biomass yields with 475U/mg amylase activity. This highlights the importance of nitrogen sources to increase biomass yield. Apparently the preferred nitrogen source for amylase production by *A.niger* was ammonium nitrate.In conclusion the amylase activity was higher at a pH 7.0 in 28^{o}C when *Ipomoea batatas* was used as a substrate.

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REFERENCES

1. Abu EA, Ado SA, James DB. 2005. Raw starch degrading amylase production of mixed culture of *Aspergillus niger and Saccharomyces cerevisae* grown on Sorghum pomace, *Afr. J. Biotechnol*. 4; 785-790.
2. Akpan I, Bankjole Mo, Adesermowo Am and Lantunde – Data 1999. Production of α- amylase by *Aspergillus niger* in a cheap solid medium using rice bran and agricultural material. *Trop. Sci*; 39; 77-79.*Braz. Arc. Biol. Tech* 2001; 44: 79-88.
3. Anupama and Ravindra P, Studies on production of single cell protein by *A.niger* in solid state fermentation of rice bran.
4. Asghar, M, M.J. Asad and M. Arshad.2000. Alpha- amylase from *Aspergillus niger* in waste bread medium. *Proc.*2nd *Int.Symp, Technol. For Environ. Monit and Agro- Appil*. October 18-20, Tekirdag, Turkey.
5. Bilinski and Stewart.1995. Production and Characterization of α- amylase from *Aspergillus niger*.18: 551-556.
6. Buzuhi and Martini. 2002. Extracellular enzymatic activity profiles in yeast and yeast like strains isolated from tropical environments. *Journal of Applied Microbiology*. 93: 1020-1025.
7. Canel and Moo Young.1980. Production of α- amylase with *Aspergillus orizae* on spent brewing grain by solid substrate fermentation. Applied Biochemistry and Biotechnology. 102: 453-461
8. Fogarty WM and Kelley C T. 1980.Amylase, amyloglucosidase and related glucanases. *In; Microbial Enzymes and bioconversions*, Rose, A.H (Ed). Academic Press, London. 115-170.
9. Goto CE, Barbosa EP, Kistner L-do-CL, Gandra RF, Arrias VL, Peralta RM. 1998- Production of amylase by *Aspergillus fumigatus*. *Revista de Microbiologia* 29, 99-103.
10. Hanes, B. and S.Stedt. 1988. *Milling and Baking technology*, pp: 415-70. John Wiley and Sons USA.
11. Hernandez MS, Marilu RR, Nelson PG and Renato PR.2006. Amylase production by *Aspergillus niger* in submerged cultivation on two wastes from food industries. *J. Food Eng*. 73: 93-100.
12. Kathiresan K and Manivannan S. 2006.Amylase production by *Penicillium fellutanum* isolated from Mangrove rhizosphere soil. *African Journal of Biotechnology*. 5: 829-832.
13. Kaur P, Grewal HS, Kocher GS. 2003- Production of α- amylase by Aspergillus niger using wheat bran in submerged and solid state fermentation. Indian Journal of Microbiology 3, 143- 145.
14. 14. Lowry O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin- Phenol reagents. J. Biol. Chem. 48: 17-25.
15. Miller G.L. 1959. Use of Dinitrosalicylic acid reagent for determination of reducing sugar, Anal. Chem. 31, 426-429.
16. Nigam.P, and Singh D.1995. Enzymes and microbial enzymes involved in starch processing enzymes Microb. Technol.17: 770-778.
17. Pandey A, Webb C, Soccol CR,LArroche C.2005. Rice bran as a substrate for proteolytic enzyme production. Enzyme technology, New Delhi: Asiatech Publishers, Inc.197.

18. Pandey A, Selvakumar P and Ashakumary L.1994. Glucose production by A.niger on rice bran is improved by addition of nitrogen sources.World J.Microbial. Biotechnol. 10:348-349.
19. Pederson H and Neilson J.2000. The influence of nitrogen sources on alpha amylases productivity of A.oryzae in continuous cultures. Appl.Microbiol.Biotechnol. 53: 278-281.
20. Tunga R and Tunga B S.2003. Extracellular amylase production by Aspergillus orizae under solid state fermentation. International Centre for Biochemistry, Olaka University, Japan. 12.
21. Van der Maarel, M.J.E.C., B.Van der Veen, J.C.M. Vitdehaag, H. Leemhuis and L. Dijkhuizen. 2002. Properties and application of starch converting enzymes of α-amylase family.J. Biotechnol. 49: 137- 55.
22. Varalakshmi KN, Kumudini BS, Nandini BN, Solomon J, Suhas R, Mahesh B and Kavitha AP.2009.Production and characterization of alpha amylase from Aspergillus niger JGI 24 isolated in Bangalore. Polish Journal of Microbiology. 58: 29-36