# Effect of Various Process Parameters of Fungal Amylase from *Aspergillus Spp.*

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***Abstract:*** **- Filamentous fungi have been widely used to produce hydrolytic enzymes for industrial applications. Fungal enzymes amylase were produced using fungus *Aspergillus spp*. under solid state fermentation. Screening of isolate no. S2 from soil was done to produce amylase. Various cultural conditions such as incubation time, temperature, pH, carbon source and nitrogen source were optimized. The temperature varied from 10 to 60 °C , pH from 3 to 11, and incubation period from 0 to 192 hrs. The optimum temperature of amylase was observed at 30°C, optimum pH was observed at 6.0 and maximum incubation period of amylase was seen at 72 hrs. The carbon source as sucrose and nitrogen source as urea were found optimum for maximum production of amylase from isolate no. S2.**

***Key words*: Amylase, *Aspergillus spp*, solid state fermentation**

## I. INTRODUCTION

Amylase are enzymes which hydrolyse the starch molecules in to polymers as they consist of glucose units. Amylase hydrolyses carbohydrates into smaller units and converts them into even smaller units such as glucose. A number of digestive enzymes including amylase are required to produce fructose in large quantities (Archana *et al*, 2011).

Amyalse is classified as α-amyalse, β-amylase, and γ-amyalse. The α-amylases are calcium metalloenzymes, and is not functioning if calcium is absent. α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or , glucose and "limit dextrin" from amylopectin. . β-amylase is also produced from bacteria, fungi and plants. From the non-reducing end, β-amylase catalyzes the hydrolysis of the second -1,4 glycosidic bond. γ-amylase will cleave (1-6) glycosidic linkages and most efficient in acidic environments and has an optimum pH of 3. Alpha amylase is ubiquitous in distribution, with plants , bacteria and fungi being the major sources. Most of the microbial alpha amylases belong to the family 13 glyosyl hydrolases, and they contribute numerous common properties.

Amylase is derived from several fungi, yeasts, bacteria and actinomycetes. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. Fungal sources are confined to terrestrial isolates, mostly to *Aspergillus* species and to only one species of *Penicillium, P. brunneum* (Pandey *et al*., 2000; Haska and Ohta., 1994).

There are two fermentation techniques: submerged fermentation and solid state fermentation. Solid state fermentation (SSF) is better for the production of enzymes. SSF utilizes solid substrates, like bran, bagasse, paddy straw, other agricultural waste and paper pulp (Subramaniyam and Vimala., 2012). Filamentous fungi are suitable microorganisms for solid state fermentation, mainly because their morphology which allows them to colonize and penetrate the solid substrate (Rahardjo, 2005).

Starch degrading amylolytic enzymes have great importance in biotechnological application ranging from food, fermentation, textile and paper industries. Amylases are enzymes which hydrolyze the starch molecules in to polymers consists of glucose units (Takata et al., 1992). Amylase also widely use in industry for various applications, like chapatti industry, building product industries, chocolate industry, sanitary waste treatment,retardation of staling in baking industry, reduction of haze formation in juices. (Van der Maarel et al., 2002).

## II. MATERIALS AND METHODS

*Media*

Potato Dextrose Agar Medium

*Chemicals*

Starch , Iodine, NaCl, KCl, $CaCl_2$, $Na_2HPO_4$, $MgSO_4$, $FeSO_4$, Glucose, $NH_4Cl$, DNSA.

*Method*

*Isolation of Fungi from Soil Sample*

Soil samples were collected in a sterile polyethylene container from the garden of KBS NATRAJ College , Dairy farms, Plastic industry and Dump sites in outer region of Vapi, Gujarat – 396195, India. The soil samples were then suspended in sterile water and appropriate dilutions were plated by spread plate technique in the basal medium plate containing (/100ml) NaCl 0.08g; KCl 0.08g; $CaCl_2$ 0.01g; $Na_2HPO_4$ 0.2g; $MgSO_4$ 0.2g; $FeSO_4$0.01g; Glucose 0.8g; $NH_4Cl_2$ 0.2g; Agar 3.2g; pH-6.2 . The plates were incubated at 30°C for 3-7 days. After incubation period the basal agar plates were observed for colonies showing zone of clearance against dark blue background after addition of gram's iodine purified by streaking on basal agar plate (kumar and Duhan, 2011).

*Preparation of Inoculum*

The culture of the PDA slant was first incubated at 30°C for 24 h. The activated spores were removed and suspended in Tween-80 (0.01%,v/v) and 2mL of spores suspension ($2\times10^6$ per mL) was used for inoculation in SSF flasks.

*Enzyme production*

Enzyme production was carried out under solid state fermentation (SSF). For SSF, 5 grams of rice straw was weighed into 250 mL Erlenmeyer flasks and were autoclaved at 121°C for 15 minutes at 15 psi pressure. After cooling the rice straw was moistened with 20 ml sterile basal medium which had the following composition (100 ml) NaCl 0.08g; KCl 0.08g; $CaCl_2$ 0.01g; $Na_2HPO_4$ 0.2g; $MgSO_4$ 0.2g; $FeSO_4$ 0.01g; Glucose 0.8g; $NH_4Cl_2$ 0.2g; pH-6.2. The fermentation flask were inoculated with 2 ml of spore suspension ($2\times10^6$ spores per mL). The contents were mixed thoroughly and incubated for 7 days at 30°C under static condition.

*Extraction of enzymes* (Vardhini *et al*., 2013)

In SSF the enzyme was extracted using 20 mL of distilled water. The contents of the flask were crushed with the help of a glass rod and were shaken for 30 minutes to harvest the enzymes from the fungal cells . The whole contents were then filtered through a four layered cheese cloth. The filtrate obtained was centrifuged at 5000 rpm for 20 minutes at 4°C. The clear brown coloured supernatant was as crude enzyme .

*Enzyme assays and protein determination*

Enzyme assay was carried out based on the dinitro salicylic acid method (Millern, 2010). 1.0 mL of crude enzyme and 1mL of 1% soluble starch in phosphate buffer ( pH 7 ) was added in test tube. The test tubes were covered and incubate at 30°C for 10 minutes (Burhan et al., 2003). Then 1.0 mL DNS reagent was added in each test tube to stop the reaction and kept in boiling water bath for 10 minutes. After boiling cool the test tubes at room temperature, final volume was made to 10 mL with distilled water. The absorbance was read at 540 nm by spectrophotometer. The amylase activity was determined by using a calibration curve for glucose. One unit of enzyme activity was defined as the amount of enzyme that released 1 μmol of glucose per minute.

*Optimization of Amylase Production*

The present work involves optimization of different parameters governing amylase production. The effects of various nitrogen sources, carbon source, incubation temperature, etc on amylase production were examined by one factor at a time method.

*Effect of Time Period*

Effect of time period was performed with 2 ml of spore suspension ($2\times10^6$ spores per mL) of selected fungal strain. The broth was incubated for different time period (24h, 48h,72h, 96h,120h, 144h, 168h. ) at 30°C for 3 days. The crude enzyme was extracted after 3 days, centrifuged and the cell free supernatant was used as the source of crude amylase enzyme.s

*Effect of Carbon source*

Effect of carbon source on amylase production was studied by taking different carbon sources i.e. fructose, maltose, lactose, sucrose, starch, mannitol was determined by replacing glucose in the production medium used for moistening of solid substrate. The cell free supernatant was used as the source of crude amylase enzyme. Similarly, To study the effect of different concentrations of the best carbon source, the concentration of selected carbon source was varied from 1-6g/100ml.

*Effect of Nitrogen source*

Effect of different nitrogen sources including yeast extract, peptone, beef extract, and their combinations, peptone , urea was determined by replacing ammonium sulphate in the production medium used for moistening of solid substrate. A control is represented with ammonium sulfate use as nitrogen source was also performed. The cell free supernatant was used as the source of crude amylase enzyme. Similarly, to study the effect of different concentrations of the selected nitrogen source, the concentration of nitrogen source was varied from 1-6 g/100ml.

*Effect of pH*

Effect of initial pH of the medium on amylase production was studied by adjusting the pH of the production medium used for moistening of solid substrate in the range of 4 to 9 using 1 N NaOH and 1N HCL after sterilization . The cell free supernatant was used as the source of crude amylase enzyme.

*Effect of Temperature*

Effect of temperature on cellulase production was studied by incubating the production medium used for moistening of solid substrate with 2 ml of spore suspension ($2\times10^6$ spores per mL) different temperatures including 20°C, 25°C ,30°C ,35°C and 37°C for 3 days under static conditions . The cell free supernatant was used as the source of crude amylase enzyme.

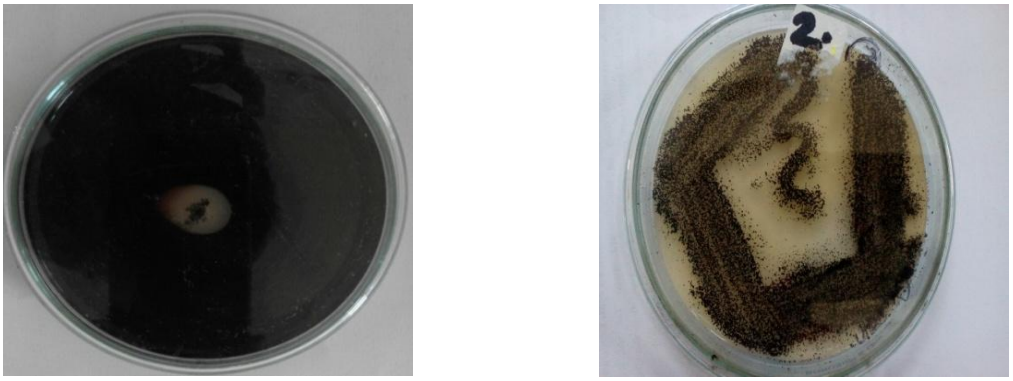
## III. RESULT AND DISCUSSION

*Isolation and Screening of Amylase producing fungi*

Screening of fungal isolates for amylase production was done by starch hydrolysis on starch agar plate. Various environmental samples were plated by spread plate technique on starch agar plates and incubated at 30 $^{O}$ C for 5-6 days. A total of 13 fungal cultures were selected for the production of amylase under solid state fermenatation using rice straw as solid substrate. The results observed shows that isolate S2 shows maximum amylase activity 1.05 U/gm of dry substrate at 72 hr of incubation under static condition..

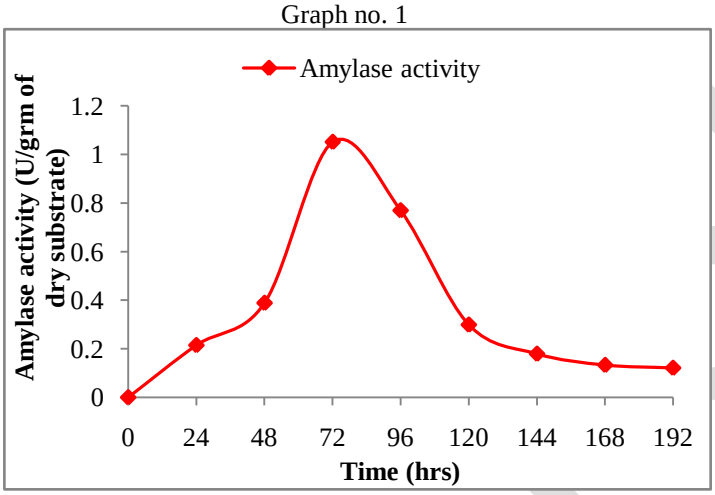
Fig-1 Screening on amylase production (a) zone of clearance of amylase on starch agar plate. (b) Isolation of *Aspergillus* sp.

**(a)** **(b)**



*Effect of Incubation Period on Amylase production*

Graph no. 1

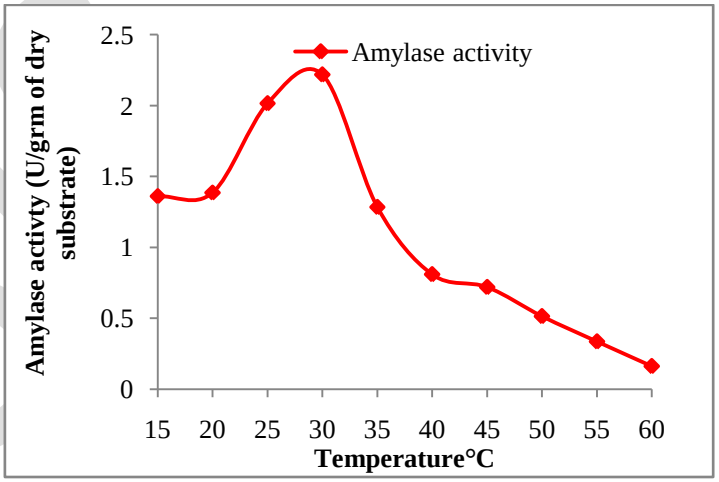



The above graph No. 1 shows that fungal amylase from selected isolate was produced by using solid-sate fermentation at different time intervals ranging from 24, 48, 72, 96, 120, 144, 168 and 194 hrs incubation. It was found that the maximum yield of amylase activity was observed at 72 hrs of incubation (1.05 U/gm of dry substrate).

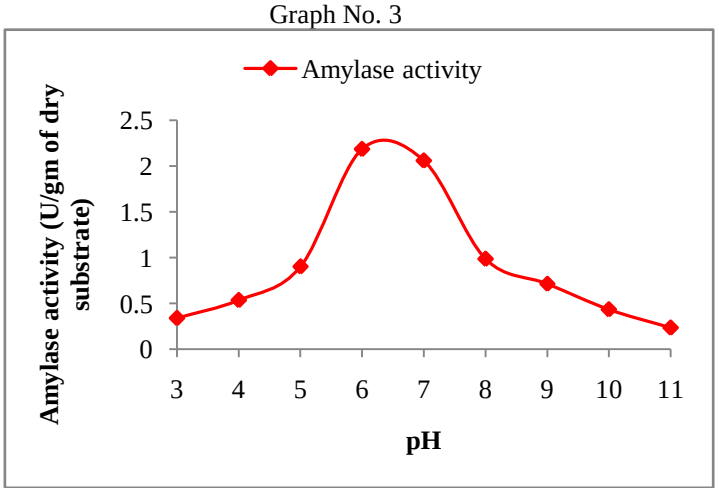
Similarly, Chimata *et al* (2010) reported the production of extracellular amylases by solid state fermentation (SSF) employing a laboratory isolate Aspergillus sp. MK07 and found that amylase production (164 U/g) was highest on 3rd day of incubation period at 30°C. The result of our study varied with the above study.

*Effect of Temperature and pH on Amylase production*

Graph No. 2




Graph No. 3



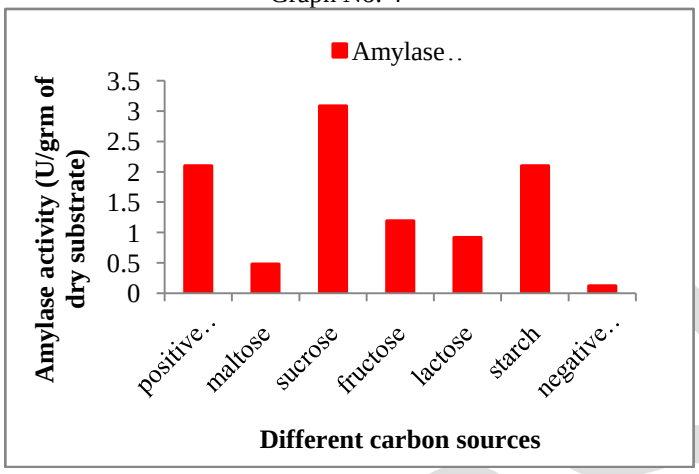

In order to investigate the role of temperature on amylase enzyme production, different incubation temperature were considered like 15°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C,

50°C, 55and 60°C and the effect of pH on the activity of the amylase was studied at pH 3.0 to 11.0 .

The above graph No. (2) and (3) shows that the optimum pH was 6.0 and the optimum incubation temperature was 30°C which gave the highest enzyme productivity ( 2.18 U/gm of dry substrate) and (2.21 U/gm of dry substrate) respectively.Similarly, Kathiresan and Manivannan (2006) , reported 30°C to be the best for enzyme production by *Penicillium fellutanum* and (Ramesh and Lonsane, 1991) reported that fungal amylase could be denatured at high pH value. The results of our study varied with the above study.

*Effect of Carbon Source on Amylase production*

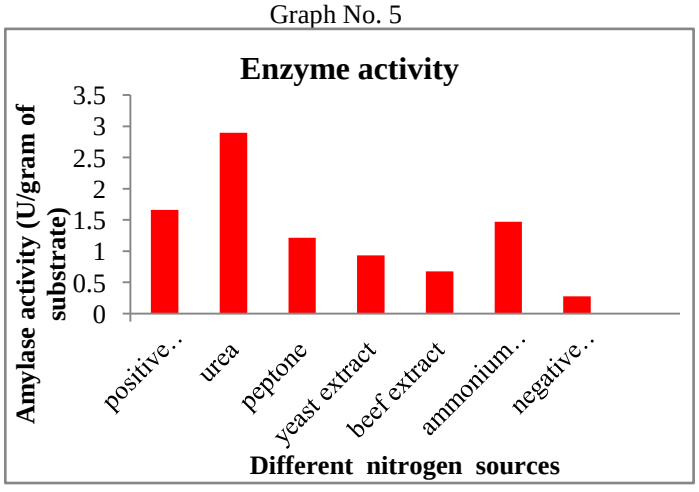
Graph No. 4




The above Graph No. 4 shows that the effect of carbon sources on growth and enzyme production by the isolate was determined by growing the test organism in SSF medium . The highest amylase activity was shown with sucrose and the activity was found to be 3.08 U/gm of dry substrate .In contrast, maximum enzyme production by glucose, has been reported by Nandakumar et al. (1999) for A. Niger CFTRI 1105 and Alva et. al (2007) for aspergillus sp. JGi 12.

*Effect of Nitrogen Sources on Amylase Production*

Graph No. 5




The above graph No. 5 shows the appropriate nitrogen source for amylase production by the isolate, the test organism was cultivated in SSF medium with either of different nitrogen sources. The highest amylase activity showed by urea was found to be ( 4.67 U/gm of dry substrate). Many workers reported that urea as an organic nitrogen source produces maximum amylase (Hamilton et al., 1999). $NH_4CL$ and $(NH_4)_2SO_4$ showed inhibitory effect on enzyme production . It might be due to the pH changes associated with their use in the medium (Ashokkumar, 2001).

## IV. CONCLUSION

Higher yield of amylase production from *Aspergillus spp.* was possible by solid state fermentation. Various parameters viz. Incubation time, temperature, pH, carbon and nitrogen source were studied to optimize the conditions to carry out SSF using rice straw by *Aspergillus* sp. The enzyme has a great potential for starch industry because of its amylolytic potential and environmental friendly approach.

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## REFERENCES

[1]. Al-delaimy KSD, Microbial enzymes and Biotechnology. Philadelphia University –Jordan. (2002) 340 page.
[2]. Ashokkumar B, Kayalvizhi N and Gunasekaran P. Optimization of media for fructofuranosidase production by Aspergillus niger in submerged and solid state fermentation. Process. Biochem (2001) 37: 331-338.
[3]. Chimata MK , Sasidhar P and .Challa S. 2010. Production of extracellular amylase from agricultural residues by a newly isolated Aspergillus species in solid state fermentation. Afri J Biotechnol (2010) 9(32):5162-5169.
[4]. Ellaiah P, K. Adinarayana, Y. Bhavani, P. Padmaja and B. Srinivasula, Optimization on process parameters for amylase production under SSF by a newly isolated Aspergillus sp. Process Biochemistry (2003) 38: 615-620.
[5]. Hamilton LH, Kelly CT and Fogarty, WM. Production and properties of the raw starch-digesting α- amylase of Bacillus species IMD 435. Process Biochem.(1999) 35(1– 2):27–31.
[6]. Haska R and Ohta Y, Starch/Starke (1994) 46: 480-485.
[7]. Kathiresan K, Manivannan S. -Amylase production by Penicillium fellutanum isolated from mangrove rhizosphere soil. Afr. J. Biotechnol. 5 (2006) 829-832.
[8]. Khan JA and Yadav SK, Production of alpha amylases by Aspergillus niger using cheaper substances employing solid state fermentation .International J. Plant,Animal Environ. sci.(2011) 1(3):100-108.
[9]. Kumar A and Duhan JS , Production and Characterization of Amylase Enzyme Isolated from Aspergillus niger MTCC-104 Employing Solid State Fermentation, Int. J. Pharma and Bio Sciences,(2011) 2(3): 250-258.
[10]. Nandakumar MP, MS Thakur, KSMS, Raghavarao and NP Ghildyal, Studies on catabolite repression in solid-state fermentation for biosynthesis of fungal amylases. Lett. Appl. Microbiol (1999) 29: 380-384.

[11]. Pandey A, Nigam P, Soccol CR, Soccol, VT, Singh D, Mohan R, Advances in microbial amylases. Biotechnol. Appl. Biochem, (2000), 31: 135–152.
[12]. Rahardjo Y.S.P. Weber F.J. Haemers S, Tramper J. Rinzema A. Aerial mycelia of Aspergillus oryzae accelerate -amylase production in a model solid-state fermentation system. Enzyme Microb. Technol. 36 (2005) 900–902.
[13]. Ramesh MV, Lonsane BK, Regulation of a-amylase production in Bacilus Iicheniformis M27 by enzyme end-products in submerged fermentation and its overcoming ln solid state fermentation system. Biotechnol Len. (1991) ;13:355-360.
[14]. Shailima Vardhini Rd, Reddi Naik B, Neelima M And Ramesh B, Screening And Production Of A-Amylase From Aspergillus Niger Using Zero Value Material For Solid State Fermentation, International Journal of Pharmacy and Pharmaceutical Sciences, 2013 ;5(1): 55-60
[15]. Subramaniyam R and Vimala R, Solid state and submerged fermentation for the production of bioactive substances: A comparative study. Int J Sci Nature.(2012) ;3(3):480-486.
[16]. Takata H, Kuriki T, Okada S, Takesada Y, Iizuka M and Imanaka T, Action of neopullulanase. Neopullulanase catalyzes both hydrolysis and transglycosylation at alpha-(1, 4) and alpha-(1, 6) glucosidic linkages. J. Biol. Chem. (1992) 267, 18447–18452.
[17]. Tanyildizi MS, Ozer D, and Elibol, M. Production of bacterial α-amylase by B.amyloliquefaciens under solid substrate fermentation. Biochem. Eng. J. 37 (2007) 294– 297.
[18]. Van der Maarel, Van der Veen, M. J. E. C., Utdehaag, B., Leenhuis, H. and Dijkhuizen L, Properties and applications of starch converting enzymes of the alpha amylase family. J. Biotechnol (2002) 94: 137-155.