
Effect of different salts of metals on production of fungal alpha amylase by solid state fermentation utilizing agricultural wastes

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Enzymes are among the most important products obtained for human needs through microbial sources. Molds play an important role in producing starch degrading enzymes. Alpha amylase is one of the most important starch hydrolyzing enzyme, which have large applications in food, pharmaceutical, fermentation & chemical industries, can be obtained from molds. *Aspergillus* spp. have a long history on producing those enzymes. Solid state fermentation is gaining its popularity day by day in scientific world in production of industrially important enzymes due to its simplicity over conventional submerged method. Considering this facts alpha amylase was produced from *Aspergillus oryzae* under solid state fermentation. House hold agro-wastes, which are considered as one of the major pollutants due to unfavorable gas production via natural fermentation beside creating disposal problem, were used as medium in our present study. Investigations were carried out to evaluate the effect of different chemical compounds of various metals such as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, KH_2PO_4 and $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ etc. on production of enzyme alpha amylase obtained from *Aspergillus oryzae* by solid state fermentation utilizing agricultural wastes.

Key words : Alpha amylase, solid state fermentation, *Aspergillus oryzae*, agricultural wastes, metal salts

INTRODUCTION

Solid state fermentation (SSF) holds tremendous potential for the production of enzyme. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme sources as reported by Tengerdy (1998). In addition to the conventional applications in food and fermentation industries, microbial enzymes have attained significant role in biotransformation involving organic solvent media, mainly for bioactive compounds. This system offers numerous advantages over submerged fermentation (SmF) system, including high volumetric productivity, relatively higher concentration of the products, less effluent generation, requirement for simple fermentation equipment, etc as reported by Tengerdy (1998), Pandey (1991, 1992, 1994) and

Doelle *et al.* (1992). Agro-industrial residues are generally considered the best substrate for SSF processes, and use of SSF for production of enzyme is no exception to that. Sugarcane bagasses, wheat bran, rice bran, banana waste, tea wastes, etc have been used as substrate material in SSF by Mitra *et al.* (1994), Selvakumar *et al.* (1994, 1998), Babu and Satyanarayan (1994), Nigam and Singh (1994), Pandey and Radhakrishen (1993), Pandey *et al.* (1995) and Tengerdy (1996).

The processing of fruits and vegetable leads to large amount of organic residues which are a kind of agricultural wastes. This wastes are one of the cause of environmental pollution. In general most of this agricultural wastes is used as cattle feed or converted to biogas or compost. However, greater environmental and economic benefit could result

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from the conversion of this by-products of higher value. Bio-conversion of this wastes not only reduces disposal problem but also environmental pollution along with production of value added products as reported by Bose *et al.* (2004).

Enzymes are among the most important products obtained for human needs through microbial sources as reported by Pandey *et al.* (1999). Pandey *et al.* (2000) also quoted that "microbial amylases could be potentially useful in the pharmaceutical and fine-chemical industries if enzymes with suitable properties could be prepared". Alpha-amylases (1,4-alpha-D-glucan glucanhydrolase, EC 3.2.1.1) is a widely distributed secretory enzyme which is one of the most popular and important form of industrial amylases as stated by Gupta *et al.* (2003). Comparative studies have been made on alpha-amylase production using different substrates by DeAlmeida *et al.* (1997), Shankaranand *et al.* (1992) and Shah *et al.* (1991). *Thermomyces lanuginose* a thermophilic fungus has been reported to be an efficient producer of alpha-amylase by Jensen and Olsen (1992) and Amesén *et al.* (1998). Studies on acid stable alpha amylase also have been carried out using *A. kawachii* IFO 4308 by Sudo *et al.* (1994). Other microorganisms like *Saccharomyces copis capsularia*, *B. coagulans* etc have also been reported as good alpha-amylase producers by Soni *et al.* (1996) and Babu and Satyanarayan (1995) simultaneously.

Metal salts play very important role in metabolic activity of microorganisms. It can initiate or inhibit the production rate of various enzymes and organic acids required in our day to day life. Metal salts act as the source for metal ions (eg. cation/anion) and regulate the metabolic activity of the organism. Various metal salts such as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ etc. have been used in evaluating the production of metabolites and enzymes from various fungal sources by Shankaranand and Lonsane (1992) and Nandakumar *et al.* (1999) & plants sources by Oboh (2005) and Rao *et al.* (2005).

Studies have been done to evaluate the effect of various metal salts e.g. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and

$\text{KH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$ with different concentrations (which were selected randomly) on the production of fungal alpha amylase utilizing agricultural wastes as the fermentation medium.

MATERIALS AND METHODS

Microorganism

Aspergillus oryzae was maintained on Czapek Dox agar medium consisting of Glucose, 5%, NaNO_3 , 0.2%, KCl, 0.05%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05%, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001%, KH_2PO_4 , 0.1%, Agar, 3% with a pH of 5 and stored under refrigerated condition at 4°C.

Utilization of agricultural wastes for SSF

House hold agro-wastes (i.e. vegetable and fruit wastes) were used as substrate in this study. These agro-wastes were dried at 60°C in Tray drier (ICT, India) for 4 h and made to powder in a Mixer grinder (jx 5, Bajaj Electronics Ltd, India). These agro-waste powders used as medium for SSF throughout the study.

Utilization of metal salts

Production of enzyme alpha amylase by *Aspergillus* sp. varied with the addition of different metal salts with different concentration. Metal salt solutions were prepared in double distilled water & the concentrations were maintained at ppm level. The metal salts used were as follows :

- (i) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (as a source of Fe^{2+})
- (ii) $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (as a source of Cu^{2+})
- (iii) $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (as a source of Mn^{2+})
- (iv) $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (as a source of Zn^{2+})
- (v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (as a source of Mg^{2+})
- (vi) $\text{KH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$ (as a source of PO_4^-)

Production of alpha amylase by SSF

Production of alpha amylase by *Aspergillus oryzae* was carried out using 20 g agro-waste material & 20 ml of metal salts solutions with desired concentration in 100 ml conical flask. A set of flasks for each metal salts were taken and plugged with cotton wool. The fermentation was carried out

under stationary condition at 30°C. Alpha amylase secreted into the spent medium was monitored at regular interval of time. After 6 day of incubation, flasks were removed and enzyme was extracted with double distilled water by shaking for 4 h at 30°C. The ratio of waste to water was 1:2.5 w/v. Solid were removed by filtration followed by centrifugation at 10,000 rpm (C-24, REMI, India) for 20 min. Clear supernatant was used for alpha amylase activity measurement.

Enzyme assay

Alpha amylase activity was determined at 30°C by mixing 2.5 ml of diluted (adequate dilution with distilled water) enzyme solution with 0.5 ml of 1.1% soluble starch dissolved in 0.05 M Imidazole-HCl buffer, pH 7.0. This reaction mixture was incubated for 1 h. After incubation the reaction was stopped by adding 1 ml of dinitrosalicylic acid (DNS) solution. The mixture prepared was heated in boiling water bath for 5 min. Absorbance at 540 nm wavelength (Spectrophotometer, U-2000, Hitachi, Japan) was measured after cooling the DNS-sample mixture at room temperature.

Activity of enzyme produced was measured in Units (IU). One (1) unit of alpha amylase activity is defined as the amount of enzyme that releases 1 μm (micromole) of reducing sugar per minute from soluble starch at pH 7.0 and 30°C.

RESULTS AND DISCUSSION

Activity of different enzymes depends on presence of different metal ions. Thus, investigations were carried out to study the effect of metal ions for production of alpha amylase enzyme. From the result it was clear that maximum activity of enzyme obtained was 24% at 0.1 ppm concentration of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ with a control showing 17% activity (Fig. 1), 21% at 1 ppm concentration of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ with a control showing 19% activity (Fig. 2), 48% at 1 ppm concentration of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ with a control showing 6% activity (Fig. 3), 28% at 10 ppm concentration of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ with a control showing 3% activity (Fig. 4), 40% at 1 ppm concentration of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ with a control showing 7% activity (Fig. 5) and 29% at 10 ppm

concentration of $\text{KH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$ with a control showing 6% activity (Fig. 6).

A comparison between the metal salts also indicate the effectiveness of the $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ for production of alpha amylase when compared with other metal salts used (i.e. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{KH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$) (Fig. 7).

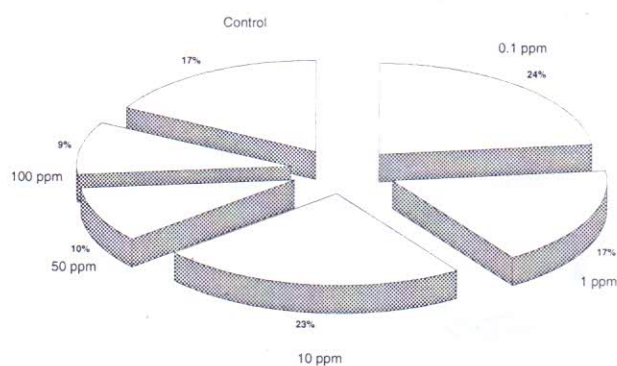


Fig. 1 : Effect of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ on production of Fungal Alpha amylase.

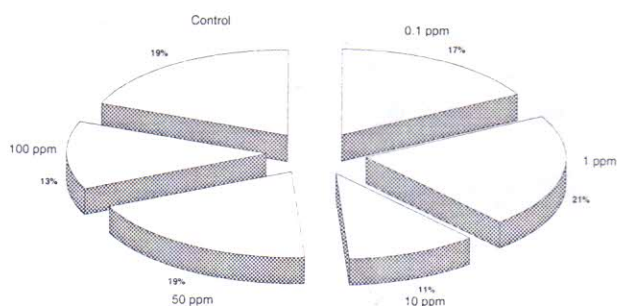


Fig. 2 : Effect of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ on production of Fungal Alpha amylase.

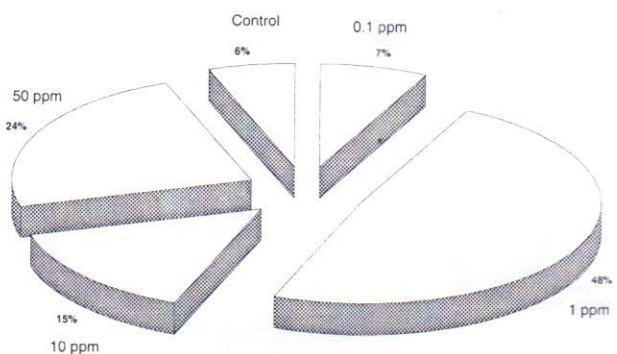


Fig. 3 : Effect of $\text{MnCl}_2 \cdot 7\text{H}_2\text{O}$ on production of Fungal Alpha amylase.

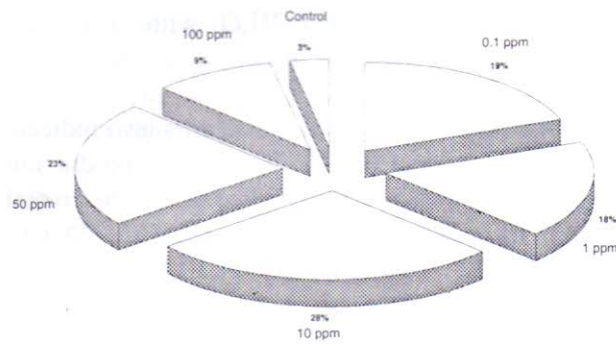


Fig. 4 : Effect of $ZnSO_4 \cdot 7H_2O$ on production of Fungal Alpha amylase.

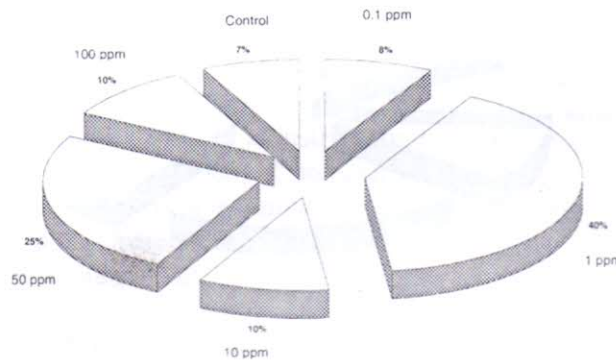


Fig. 5 : Effect of $MgSO_4 \cdot 7H_2O$ on production of Fungal Alpha amylase.

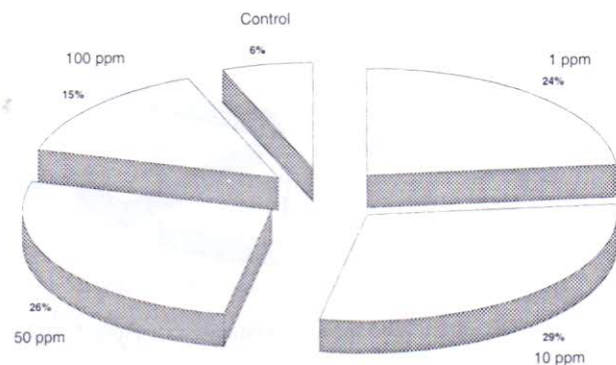


Fig. 6 : Effect of $KH_2PO_4 \cdot 7H_2O$ on production of Fungal Alpha amylase.

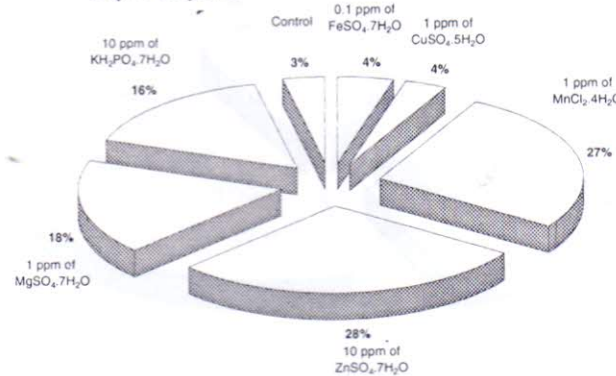


Fig. 7 : Comparison of Effect of Various Metal salts on production of Fungal Alpha amylase.

Except some concentrations of $FeSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ all other metal salts used in our experiment gave an increased activity of enzyme alpha amylase when compared with control. The maximum activity of alpha amylase was obtained when $ZnSO_4 \cdot 7H_2O$ solution was used in medium at a concentration of 10 ppm. Thus, it is clear that Zn^{2+} ion helps the production of alpha amylase when compared to other metal ions used in our present study from *Aspergillus oryzae* utilizing agricultural wastes by solid state fermentation.

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REFERENCES

- Amesen, S.; Eriksen, S. H.; Olsen, J. and Jensen, B. 1998. Increased production of α -amylase from *Thermomyces lanuginosus* by the addition of Tween 80. *Enz Microb Technol.* **23** : 249-252.
- Babu, K. R. and Satyanarayana, T. 1994. Production of bacterial enzyme by solid state fermentation. *J Sci Ind Res.* **55** : 464-467.
- Babu, K. R. and Satyanarayana, T. 1995. α -Amylase Production by thermophilic *Bacillus coagulans* in Solid State Fermentation. *Process Biochem.* **30** : 305-309.
- Bose, D.; Ghosh, U. and Gangopadhyay, H. 2004. Production of amyloglucosidase enzyme from agricultural wastes by solid state fermentation using *Aspergillus oryzae*. Biohorizon 2004, 6th National Symposium on Biochemical Engineering and Biotechnology, IIT Delhi. BIB-09.
- DeAlmeida, S. E. M.; Mizuta, K and Giglio, R. 1997. *Pycnoporus sanguineus* : a novel source of [alpha]-amylase. *Mycol Res.* **101** : 188-190.
- Doelle, H. W.; Mitchell, D. A. and Rolz, C. E. (eds.). 1992. *Solid State Fermentation*, Elsevier, London.
- Gupta, R.; Gigras, P.; Mohapatra, H.; Goswami, V. K. and Chauhan, B. 2003. Microbial ' α '-amylases : a biotechnological perspective. *Process Biochem.* **38** : 1599-1616.
- Jensen, B. and Olsen, J. 1992. Physicochemical properties of a purified alpha-amylase from the thermophilic fungus *Thermomyces lanuginosus*. *J Enz Microb Technol.* **14** : 112-116.
- Mitra, P.; Chakraverty, R. and Chandra, A. L. 1994. Production of proteolytic enzymes by solid state fermentation. *J Sci Ind Res.* **55** : 439-442.
- Nandakumar, M. P.; Thakur, M. S. Raghavarao, K. S. M. S. and Ghildyal, N. P. 1999. Studies on catabolite

- repression in solid state fermentation for biosynthesis of fungal amylases. *Letters in Applied Microbiology*. 29 : 380-384.
- Nigam, P. and Sing, D. 1994. Processing of agricultural wastes in solid state fermentation for cellulolytic enzymes production. *J Sci Ind Res*. 55 : 457-463.
- Oboh, G. 2005. Isolation and characterization of amylases from fermented cassava (*Manihot esculenta* Crantz) wastewater. *African Journal of Biotechnology*. 4(10) : 1117-1123.
- Pandey, A. 1991. Aspects of fermenter design for solid-state fermentations. *Process Biochem*. 26 : 355-361.
- Pandey, A. 1992. Recent process developments in solid-state fermentation. *Process Biochem*. 27 : 109-117.
- Pandey, A. 1994. *Solid State Fermentation*. Pandey, A editor. Wiley Eastern Publishers, New Delhi. 3-10.
- Pandey, A. and Radhakrishnan, S. 1993. The production of glucoamylase by *Aspergillus niger* NCIM 1245 . *Process Biochem*. 28 : 305-309.
- Pandey, A.; Ashakumary, L. and Selvakumar, P. 1995. Copra waste — A novel substrate for solid-state fermentation. *Biores Technol*. 51 : 217-220.
- Pandey, A.; Selvakumar, P.; Carios, R. S. and Nigam, P. 1999. Solid state fermentation for production of industrial enzymes. *Current Science*. 77(1) : 149-162.
- Pandey, A.; Nigam, P.; Carlos, R. S.; Vanete, T. S.; Sing, D. and Radjiskumar, M. 2000. Advance in microbial amylases (Review). *Biotechnol Appl Biochem*. 31 : 135-152.
- Rao, M. Srinivasa; Reddy, N. S; Rao, G. Venkateswara and Rao, Sambasiva. 2005. Studies on the extraction and characterization of thermostable α -amylase from pericarp of *Borassus indica*. *African Journal of Biotechnology*. 4(3) : 289-291.
- Selvakumar, P.; Ashakumary, L. and Pandey, A. 1994. Microbial synthesis of starch saccharifying enzyme in solid culture. *J Sci Ind Res*. 55 : 443-449.
- Selvakumar, P.; Ashakumary, L.; and Pandey, A. 1998. Biosynthesis of glucoamylase from *Aspergillus niger* by solid-state fermentation using tea waste as the basis of a solid substrate. *Biores Technol*. 65 : 83-85.
- Shah, N. K.; Ramamurthy, V. and Kothari, R. M. 1991. Comparative profiles of fungal alpha amylase production by submerged and surface fermentation. *Biotechnol Lett*. 13 : 361-364.
- Shankaranand, V. S. and Lonsane, B. K. 1992. Ability of *Aspergillus niger* to tolerate metal ions and minerals in solid-state fermentation system for the production of citric acid. *Process Biochem*. 29 : 29-37.
- Shankaranand, V. S.; Ramesh, M. V. and Lonsane, B. K. 1992. Idiosyncrasies of solid-state fermentation systems in the biosynthesis of metabolites by some bacterial and fungal cultures. *Process Biochem*. 27 : 33-36.
- Soni, S. K.; Bath, K. S. and Soni, R. 1996. Production of amylases by *Saccharomycopsis capsularis* in solid state fermentation. *Indian J Microbiol*. 36 : 157-159.
- Sudo, S.; Ishikawa, T. and Sato, T. 1994. Comparison of acid-stable-amylase production by *Aspergillus kawachii* in solid-state and submerged cultures . *J Ferment Bioeng*. 77 : 483-489.
- Tengerdy, R. P. 1996. Cellulase production by solid substrate fermentation. *J Sci Ind Res*. 55 : 313-316.
- Tengerdy, R. P. 1998. *Advances in Biotechnology* (ed. Pandey, A.), Educational Publishers and Distributors, New Delhi. pp. 13-16.

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