International Journal of
Agriculture and Biosciences
www.ijagbio.com P-ISSN: 2305-6622 E-ISSN: 2306-3599 editor@ijagbio.com

RESEARCH ARTICLE

# The Effect of Some Organic Nitrogen Sources on the Production of Amylase from *Aspersigllus niger* U3 Using Rice Bran

*[1]Damisa D, HB [2]Sharif and RH [2]Otitolaiye

[1]Department of Microbiology, Federal University of Technology, Minna, PMB 65 Bosso Road, Minna Nigeria
[2]Department of Applied Science, Kaduna Polytechnic, PMB 2021, Kaduna, Nigeria

**ARTICLE INFO**

Received: April 01, 2013
Revised: April 07, 2013
Accepted: April 14, 2013

**Key words:**
Amylase
Nitrogen
Replacement
Submerged

***Corresponding Address:**
Damisa D
damisaduro@yahoo.com

**ABSTRACT**

Fermentation media formulation from some organic nutrient sources in partial or full replacement of nitrogen requirements is receiving attention as a means of backward integration. Submerged Culture fermentation was used to study the effect of nitrogen replacement of amylase fermentation medium using organic sources. *Aspergillus niger* isolated from the soil was used as the chemical factory while rice bran was used as the carbon source. The amylase activity of the nitrogen sources were low although. Cotton seed gave an amylase activity of 1.282 IU/ml which was the highest recorded for this work. Peptone had amylase activity of 0.962 IU/ml whereas Blood and Ground-nut cake (Kuli-Kuli) had an amylase activity of 0.713IU/ml and 0.512IU/ml respectively. Rice bran for this work appears to be a good carbon source and a good replacement for starch as the quality of enzyme produced compared well with starch substrate. Amylase production level was 33.1% higher in activity when peptone was replaced with cotton seed. Cotton seed may be used in full replacement of Peptone.

**Cite This Article as:** Damisa D, HB Sharif and RH Otitolaiye, 2013. The effect of some organic nitrogen sources on the production of amylase from *Aspersigllus niger* u3 using rice bran. Inter J Agri Biosci, 2(2): 87-89. www.ijagbio.com

## INTRODUCTION

Microbial enzymes are becoming increasingly important for their technical and economic advantages. Amylases have important applications in diverse industries such as baking, brewing detergent, medicine, textile, paper and pharmaceuticals. Extracellular amylases have been found in various species of fungi (Pandey *et al*., 2000) and bacteria (Srivastave and Baruah, 1986; Tanaka *et al*., 1996). Among the microorganisms that have amylolytic activity, *Aspersgillus niger* is one of the well known producers. Different culture conditions greatly affect the production of amylase. The optimization of the fermentation medium therefore plays an important role in enzyme production (Bakri *et al*., 2003). Amylase production by *Aspersgillus niger* has been found maximum when cassava starch was used as substrate compared to sugar cane bagasse or rice straw (Haq *et al*., 2002; Yao – xing *et al.,* 2008). It therefore holds that certain wastes can be converted to products of economic value for example the rice bran. The organisms need essential elements such as nitrogen, carbon, phosphorus and sulphur for growth and subsequent amylase production. The concentrations of these elements have a profound effect on the yield of amylase. The industrial uses of amylases have increased in recent time and glucoamylase is the most produced around the world.

## MATERIALS AND METHOD

### Isolation and identification of *Aspergillus niger* strain from soil

The *Aspergillus* culture was isolated from soil by serial dilution method of Clark *et al.* (1988), 1g soil sample was dissolved in 100ml sterilized distilled water. the starch digesting, α -amylase was screened according to method described by Bergmann *et al.* (1988) and Akpan *et al*. (1999) *Aspergillus niger* colonies producing large clear zone were picked and purified on PDA. Identification was based on cell and colony morphology characteristics as described by Rasper and Fennel (1965). The young colonies of *Aspergillus niger* were aseptically picked up and transferred to PDA slants and incubated at room temperature for 5-6 days for maximum growth.

### Growth medium

The medium contained (%w/v): Rice Bran (2%); $KH_2PO_4$ (0.2%), $(NH4)_2\ SO_4$ (0.14%); $CaCl_2$ (0.03%); $MgSO_4.7H_2O$ (0.03%), Urea (0.03%), Trace element

solution contain in 500ml (2.5g$FeSO_4$, 1.0g $CoCl_2$, 1.76g $ZnSO_4$ 0.98g $MnSO_4$). 0.2% peptone was added and thereafter in the other flasks it was replaced by 0.2% cotton seed, 0.2% of blood meal and 0.2% of groundnut cake ("Kuli-Kuli") to test for the effect of the organic nitrogen source on the enzyme production. The pH of the medium was adjusted to 7.2 with 0.1M HCl, medium was sterilized by autoclaving at 121 $^0$C for 15 30min.

**Inoculum and fermentation**

The *Aspergillus niger* stored on PDA slant was Inoculated on fresh PDA plates by point inoculation method and incubated at room temperature for one week to obtain confluent growth. 0.1% Tween 80 was used to wash off the conidia from the surface of the PDA plates into a sterile conical flask. This was used as the inoculum stock for the fermentation. Twenty milliliter quantities (20ml) of the inoculum were transferred from the stock culture into 250ml flask containing 200ml of fermentation medium. The flasks were incubated for 192hours at 28±2$^0$C on a rotatory shaker set at 150rpm. At the end of each day, 10ml quantities of the culture filtrate were centrifuged at 4500rpm for 10min to obtain a clear supernatant. The clear supernatant (crude enzyme) was used for estimation of α - amylase; the enzyme activity was expressed in number of units. One unit of enzyme was defined at the amount of enzyme (protein) in milligram required for hydrolysis of starch to produce a millimolar of reducing sugar (glucose) in 1 hour under the assay conditions. The specific activity was defined as number of units per gram protein.

**Enzyme assay**

Enzyme activity was determined by Dinitrosalicylic Acid (DNS) method described by Miller, (1959) The reaction mixture contained 2ml crude solution of enzyme, 1.0ml of distilled water. The reaction mixture was incubated at 37 $^0$C for 30 min and the reaction was terminated by addition of 3ml of DNS solution. After stopping the reaction the tubes were placed in boiling water bath for 5 min, cooled and absorbance was determined at 540 nm. The amount of glucose produced was calculated by referring to the standard plot using glucose as the reducing sugar.

## RESULTS

The α - amylase production potential of the mold was observed by the zone of starch hydrolysis around the *Aspergillus niger* in the petridishes.

The result of the effect of organic nitrogen sources: "Kuli-kuli", peptone, cotton seed and blood is presented in Figure 1. The amylase activity of the nitrogen sources was low the first day (Day1). The activity rose thereafter for all the nitrogen sources used. Cotton seed gave an amylase activity of 1.282 IU/ml which was the highest recorded for this work. It peaked off at day 4 (Figure 1). Peptone had amylase activity of 0.962 IU/ml at day 4 too whereas Blood and "Kuli-Kuli" peaked their activity at day 5 and 6 with amylase activity of 0.713IU/ml and 0.512IU/ml, respectively.

The result suggest that though peptone is the typical organic nitrogen source often used in the production of amylase using *Aspergillus niger* it can be replace wholly by cotton seed. Bertolin *et al.* (2003) have been able to use wheat bran in their work as full replacement for starch in the production of glucoamylase using *Aspergillus awamori.* Urea has been replaced as a suitable nitrogen source for glucoamylase synthesis in submerged fermentation*;* however the use of cotton seed compared favourably with the Urea.

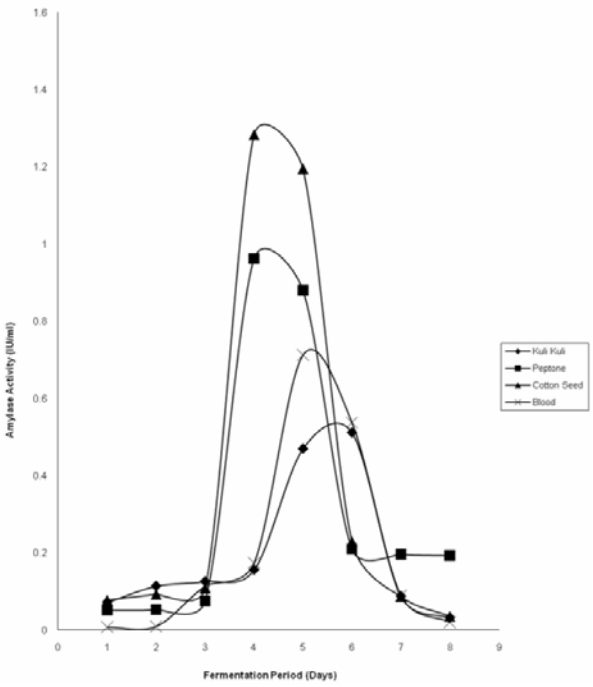


**Fig. 1:** Effect of Different Organic Nitrogen Sources Amylase Production by *Aspergillus niger*

## DISCUSSION

The presence of α - amylase producing *Aspergillus niger* from the soil agrees with earlier reports of Rehana and Nand (1989). Adebiyi and Akinyanju in (1998) found that the soil is known to be a repository of amylase producing microorganisms. *Aspergillus niger* isolate used for the work produced zones that are very sharp and contrast with the blue-black background as described by (Akpan *et al*., 1999a). A number of reports exist regarding the influences of various environmental conditions like effect of pH value and temperature optimum, incubation period, carbon sources, nitrogen sources and metal ion on the production of thermostable α-amylase by *Aspergillus niger* (Okolo *et al*., 2006; Nagamine *et al.,* 2003; Francis *et al.,* 2002; Carlsen and Nielson, 2001; Pedersen and Nielson, 2000).

The alpha amylase productivity of the *Aspergillus niger* on rice bran using different organic nitrogen sources indicated that all the sources have potential for α-amylase production. Increase in the incubation period resulted to decrease in the production of α-amylase by culture of *Aspergillus niger*. It may be due to the fact that after maximum production of α-amylase enzyme (maximum

incubation time), the production of other by product and depletion of the nutrients. These byproducts inhibited the growth of fungi and hence enzyme formation (Duochaun *et al*., 1997). Rice bran for this work appears to be a good carbon source and a good replacement for starch as the quality of enzyme produced compared well with the work done by Okolo *et al*. (2006) using starch and peptone.

The present increase in amylase production when peptone was replace with cotton seed was 33.1%. This is a very good increase and suggests that this nitrogen source is a better replacement then the rest, that is, Blood and "Kuli-kuli"

## REFERENCES


Adebiyi CAB and G Akinyaanju, 1998. Thermophilic amylase producers from the soil Nigeria, Journal of Science and Technology, 11: 30-38.

Akpan I, MO Bankole, AM Adesemowo and GO Lantunde-Date, 1999b. Production of alpha amylase by *Aspergillus niger* in a cheap solid medium using rice bran and agricultural material, Tropical Science, 39: 77-79.

Bakri Y, M Al-jazairi and G Al-kayat. 2008. Xylanase production by a newly isolated *Aspergillus niger* 557 in submerged culture. Polish Journal Microbiology, 57: 249-251.

Bertolin TE, JAV Costa and GD Pasquali, 2003. Glucoamylase production in batch and fed-batch solid state fermentation: effect of maltose and starch addition, Journal Microbiology Biotechnology, 11: 13-16.

Bergmann FW, J Abeand and S Hizukuri, 1988. Selection of microorganism which produce raw-starch degrading amylase, Applied Microbiology and Biotechnology 27: 443-446.

Carlsen M and J Nielson 2001. Influence of carbon sources on alpha amylase production by *Aspergillus oryzae*, Applied Microbiology and Biotechnology, 57: 346-349.

Clark H, E Gaeldrich, PW Kabler and CM Huff, 1988, Applied Microbiology. 1st Edn. International Book Company, New York, 53.

Duochaum L, Y Yijun, P Youliang, S Chongyao, Z Peijin and H Yicum, 1997. Purification and properties of thermostable alpha amylase from thermophilic fungus thermomyces lanuginosus. Acta. Microbiology, 37: 107-117.

.

Francis F, A Sabu, KM Nampoothiri, S Szakaas and A Pandey, 2002. Synthesis of alpha amylase by oryzae in solid state fermentation, Journal of Basic Microbiology 42: 3206-3206.

Haq I, A Khan, WA Butt, S Ali and MA Qadeer, 2002. Effect of nitrogen and carbon sources on xylanase production by mutant strain of *Aspergillus niger* GCBMX-45, Online Journal of Biological Sciences, 2: 143-144.

Miller G. C. (1959) use of Dinitrosalicylic Acid Reagent for the determination of Reducing sugar. *Analytical Chemistry* 31: 426-428.

Nagamine K, K Murashima, T Kata, H Shimoi and K Ito, 2003. Mode of alpha amylase producing the Schochu Koji mold *Aspergillus kawachii*, Biotechnology and Biochemistry 67: 21191-21202.

Okolo BN, Ezeogu and CN Mba, 2006. Production of raw starch digesting amylase by grown on native starch sources, Journal of Science Food and Agriculture, 69: 109-115.

Pandey A, P Selvakumar, and I Ashakumary, 1996. Performance of a column bioreactor for glucoamylase synthesis by *Aspergillus niger* in SSF. Process Biochemistry 31: 43-46.

Pedersen H and J Nielsen, 2000. The influence of nitrogen sources on the α - amylase productivity of *Aspergillus oryzae* in continous cultures. Applied Microbiology and Biotechnology, 53: 278-281.

Raana K, M Tasneem, I Haquand and H Mukhtar, 2004. Effect of additional carbon and nitrogen on the production of xylanase by a mutant strain of *Aspergillus niger* GCBCX-20. International Journal of Biology and Biotechnology, 1: 529-533.

Rasper KB and DJ Fennel, 1965. The Genus Asperillus. 1st Edn. Williams and Wilkins, Baltimore, USA.

Rehana FV and K Nand, 1989. Preliminary studies on the production of thermostable α-amylase by a mesophilic strain of *Bacillus licheniformis*, Chemical Microbiology Technology, 12: 8-13.

Srivastava RAK and JN Baruah, 1986. Culture condition for production of thermostable amylase by bacillus stearothermophilus Applied and Environmental Microbiology, 51: 179-184.

Tanaa TE, Y Ishimoto and MT Shimomura, 1996. Purification and some properties of raw starch binding amylase of clostridium butyricum T-7 isolated from mesophilic methane sludge, Agricultural and Biology Chemistry, 51: 399-405.