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ENZYME ACTIVITIES DURING SUBMERGED FERMENTATION OF RICE MILL FEED BY ASPERGILLUS NIGER, TRICHODERMA VIRIDE AND RHIZOPUS ORYZAE

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ABSTRACT

Rice husk abounds in many rice producing countries. Rice processing residues if not utilized adequately can be a cause for environmental concern with regards to burning excesses. In this study, the submerged fermentation of rice mill feed (RMF) by three indigenous fungi (Aspergillus niger, Trichoderma viride and Rhizopus oryzae was demonstrated in pure and mixed culture for the production of enzymes. Enzymes expressed were protease, phytase, α-amylase, glucoamylase, lignin peroxidase, pectinase and manganese peroxidase. Protease activity was significantly ($p \leq 0.05$) high with *A. niger* (9.00±1.00Iu/ml) than *T. viride* (4.00±0.58Iu/ml) and *R. oryzae.* (6.33±0.33Iu/ml). phytase activity was numerically high in both single and mixed culture submerged fermentation. Lignin peroxidase was significantly ($p \leq 0.05$) higher in mixed culture of all three fungi (48.00±2.31Iu/ml)

The fungi tested and enzymes synthesised have the potential for biodegradation of complex plant material leading to modification, saccharification and nutrient release. This will in turn be utilized beneficially in animal feed formulation and supplementation. Thereby making RMF a valuable agro-industrial by-product from rice production.

Keywords: agro-industrial by-product, enzymes, fungi, rice mill feed, submerged fermentation.

1. INTRODUCTION

Rice mill feed (RMF) often referred to as Rice husk, rice bran or rice offal by rice millers and feed millers in Nigeria is a cheap agro-industrial by-product obtained from one-step milling of

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rice (*Oryza glaberrima*) in West Africa and Nigeria. To farmers in rice growing communities, this waste has no economic value. These communities either have small-scale rice mills or the rice is taken to the city centres for milling. It is made up of bran plus hull and some broken rice. Rice is one of the most consumed food globally. According to an FAO report (FAO, 2017), Global paddy production in 2016 was set to reach 751.9 million tonnes (499.2 million tonnes, milled basis) implying a huge volume of waste after milling. Rice processing residues if not utilized adequately can be a cause for environmental concern with regards to burning excesses. In Nigeria (Ebonyi state) where rice is produced on a large scale, rice husk disposal is a major challenge. Open combustion is commonly carried out which leads to air pollution.

An evaluation of the nutrient composition of rice mill feed as a potential source of feed for monogastric animals indicated an organic matter content of 688gkg^{-1} DM with glutamic acid representing 13% of crude protein and a gross energy concentration of 16.59MJkg^{-1}DM (Ofongo *et al.,* 2008). The Water insoluble NSP were 57.6 % and water soluble NSP were 1.3 % respectively (Ofongo *et al*., 2008). While a one-step milling of rice results in rice mill feed, multiple step milling of rice yields rice bran which can result in the generation of 5 – 8% rice bran (Oliveira *et al*., 2010). Rice bran has been reported to have high concentration of carbohydrates (34 – 49.5%), protein (11 – 14%), lipids (15 – 22%) and crude fibre (of about 11.5%) (Oliveira *et al*., 2010; Kupski *et al*., 2012; Chutmanop *et al*., 2012). With a carbohydrate/protein ratio of 2-3, rice bran provides an excellent medium for enzyme production through solid state fermentation (Chutmanop *et al*., 2012). Compared to rice bran, rice mill feed has a crude protein concentration range of 50 to 60gkg^{-1}DM and crude fibre of 300 – 400gkg^{-1}DM (Ofongo *et al.*, 2008). Its low protein and high concentration of non-starch polysaccharides (NSP) limits its utilization in monogastric animal feeding (Shibuya and Iwasaki, 1985; Saunders, 1986). Monogastric animals do not synthesize NSP-degrading enzymes in their gastrointestinal tract (GIT); however, they overcome this limitation through hindgut fermentation by resident caecal microorganism when a minimal amount of NSP's is contained in their diet. Fungi biodegradation has the benefit of degrading both the soluble and insoluble NSP's present in highly fibrous ingredients. The biodegradation process is mediated through the synthesis of an enzyme complex during growth of the fungi on a substrate. This process involves the formation of single cell proteins which results in increased crude protein concentration of the substrate. Apart from degrading NSP's and increasing the protein content, the sugar level is also increased and hence the energy level of the substrate (Iyayi, 2004; Iyayi and Aderolu, 2004). The net effect is reduced content of NSP's in the substrate and a possible increase in the inclusion and utilization of the substrate in monogastric animal diet. Solid state fermentation (SSF) of RMF can help to depolymerize the NSP's present in it by reducing the cellulose and hemicellulose concentration. Submerged fermentation of RMF also holds potential benefits for enzyme synthesis considering the concentration of its constituents. As a result, the anti-nutritive effect of

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the NSP's can be reduced with a consequent increase in the level of inclusion of RMF in monogastric diets. Solid state fermentation of rice husk has been reported to reduce the fibre fractions of rice husk (Belewu and Banjo, 1999; Belewu and Adenuga, 2005; Belewu *et al*., 2007).

An earlier investigation by Isikhuemhen *et al*. (2014) reported the use of selected white rot fungi (WRF) for the selective delignification of canola biomass via SSF, which resulted in the breakdown of the biomass, sugar release and improved digestibility. According to Isikhuemhen *et al*. (2012), the use of WRF *Lentinus sqarrosulus* Mont. for the degradation of corn stalk also resulted in sugar release under SSF conditions. Such selective degradation involves the selective breakdown of lignin, while hemicelluloses and cellulose are hydrolysed resulting in the saccharification of biomass/ and potential feedstocks. Several authors have reported the use of agricultural residues for the production of ligninolytic enzymes (Mikiashvili *et al.*, 2011; Isikhuemhen *et al*., 2012, 2014).

The aim of this study, was to determine the enzyme activities of selected fungi (*Aspergillus niger, Trichoderma viride* and *Rhizopus oryzae*) during the submerged fermentation of rice mill feed.

2. MATERIALS AND METHODS

The RMF used in this study was purchased from a rice milling plant in Ilorin Kwara state, Nigeria. The mill was a one-step mill, meaning that the husk contained the bran, ground hulls and some broken rice.

2.1 Experimental site and source of fungi

Submerged Fermentation experiment for enzyme activity determination was carried out at the Botany and Microbiology Department of the University of Ibadan. Fungi used were obtained from the culture bank of the Microbial Physiology Laboratory, Department of Botany and Microbiology, University of Ibadan. They were sub-cultured on Potato Dextrose Agar (PDA).

2.2 Preparation of Inoculum

A 1 x 6mm cork borer of 7 days old culture of each fungus (*Aspergillus niger, Trichoderma viride* and *Rhizopus oryzae*) and was dispersed in 15ml sterile deionized water was prepared aseptically. A similar procedure was used to prepare inoculum of mixed culture of *A. niger + T. viride; A. niger + R. oryzae; T. viride + R. oryzae* and *A. niger + T. viride + R. oryzae.* 5ml of each inoculum was used to inoculate the sterile substrate.

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2.3 Determination of Inoculum size and Processing of RMF samples

The inoculum size was determined using a hemocytometer to count the number of spores dispersed in 10ml per 1 x 6 cork borer of 7 days old culture of each fungus. The number of spores for each fungus is as indicated below:

Aspergillus niger: 1.8×10^6

Trichoderma viride: 24.0×10^6

Rhizopus oryzae: 56.0×10^6

The rice husk was in a ground form when collected from the rice mill and so did not require any further processing.

2.4 Determination of enzymes synthesized

A modified version of Mandel and Weber (1969) medium as developed by Hatakka and Pirhonen (1985) was used as the basal medium. Fifty (50) ml of the basal medium and 0.5gm of RMF in 150ml Erlenmeyer flasks were prepared in triplicates for each fungus and their mixed culture. Sterilization was carried out by autoclaving at 121ºC for 15 mins and 15psi. The flask was allowed to cool before inoculating with 3ml of inoculums and incubated for 7 days at 30ºC±2ºC.

The basal medium used contained per litre KH_2PO_4 (2.0gm); $(NH_4)_2SO_4$ (2.1gm); $MgSO_4.7H_2O$ (0.3gm); $CaCl_2.6H_2O$ (0.3gm); $MgSO_4.H_2O$ (1.56mg); $ZnSO_4.7H_2O$ (1.4mg); $CoCl_2.6H_2O$ (2.66mg); yeast extract (0.5gm) all regulated to a pH of 5.0. The inoculum used was prepared with a 1× 6 cork borer of each fungus in (mono and mixed culture) from 7 days old culture plates dispersed in sterile deionized water. During the period of incubation, the flasks were placed on a rotary shaker (80rpm) for one hour daily. At the end of the incubation period, the contents of each flask were filtered through Whatman number 1 filter paper. The filtrate without prior purification was used to determine the following enzymes: Phytase activity was assayed according to the method of Mohan *et al*. (2004); Protease activity according to the method of McDonald and Chen (1965); Pectinase activity according to the method of Miller 1959; Manganese peroxidase activity according to the method of Glenn and Gold (1985) and Lignin peroxidase was assayed according to the method of Tien and Kirk (1988). Enzyme activity was taken as international unit per ml (Iu/ml) of crude enzyme filtrate

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2.5 Experimental design and statistical analysis

Completely randomized design was used for the experiment having seven treatments and three replicates per treatment. Data obtained were subjected to one-way Analysis of Variance (ANOVA) and significant means separated by Duncan Multiple Range Test using SPSS version 17 (SPSS Inc, Chicago, USA).

3. RESULTS AND DISCUSSION

The enzyme activities of *Aspergillus niger, Trichoderma viride* and *Rhizopus oryzae* during submerged fermentation of rice husk is presented in Table 1 (pure culture of each individual fungus).

Table 1: Enzyme activity (Iu/ml) in pure fungus culture fermentation

Enzymes	*A. niger*	*T. viride*	*R. oryzae*
Protease	9.00 ± 1.00^{a}	4.00 ± 0.58^{b}	6.33 ± 0.33^{b}
Phytase	25.33 ± 2.40^{a}	21.33 ± 4.67^{a}	16.00 ± 2.00^{a}
Pectinase	25.67 ± 0.88^{ab}	18.67 ± 1.45^{b}	27.00 ± 3.46^{a}
Lignin peroxidase	21.33 ± 0.88^{a}	18.67 ± 1.45^{a}	22.33 ± 2.19^{a}
Manganese peroxidase	10.67 ± 2.03^{a}	6.33 ± 0.67^{b}	13.00 ± 0.00^{a}

^{abc}: means along the same row are significantly different ($p\leq0.05$). Each data is expressed as mean ± standard error (n=3)

Enzyme activity in single culture fungi fermentation of rice mill feed

Protease activity was significantly highest ($p\leq0.05$) with *Aspergillus niger* (9.00±1.00 Iu/ml) than *T. viride* (4.00±0.58 Iu/ml) and *R. oryzae* (6.33±0.33 Iu/ml) which are not significantly different from each other ($p\geq0.05$). Phytase activity was numerically high across the 3 treatments. Values recorded ranged from 25.33±2.40 Iu/ml in *A. niger* to 16.00±2.00 in *R oryzae*. Pectinase activity was significantly ($p\leq0.05$) higher with *R. oryzae* (27.00±3.46 Iu/ml) than *T. viride* (18.67±1.45 Iu/ml). A value of 25.67±0.88 Iu/ml was recorded with *A. niger* but not significantly ($p\geq0.05$) higher than that recorded for *T. viride*. Manganese peroxidase activity was significantly higher ($p\leq0.05$) in *A. niger* (10.67±2.03 Iu/ml) and *R. oryzae* (13.00±0.00 Iu/ml) than *T. viride* (6.33±0.67 Iu/ml).

Enzyme activity in mixed culture fungi fermentation of rice husk

Enzyme activities of mixed culture of *Aspergillus niger, Trichoderma viride* and *Rhizopus oryzae* during solid-state fermentation of RMF is presented in Table 2. In mixed culture, the lower protease activity was recorded in *A. niger* + *T. viride* and *T. viride* + *R. oryzae*, whereas the highest protease activity was recorded in the mixed culture of all three fungi (21.00±1.53Iu/ml), which is not significantly different ($p \geq 0.05$) from that of *A. niger* + *R. oryzae* mixed culture. Using rice bran as substrate, Chutmanop *et al*. (2008) reported high levels of protease activity (1200U/g dry solids) expressed by *Aspergillus oryzae.* Similarly,

Sumantha *et al*. (2006) reported protease activity of 22 – 214 U/g of dry solid. Results obtained in this study is not unusual because most commercial proteases are produced mostly from the genera *Aspergillus, Rhizopus, Fusarium* and *Penicillin* according to earlier reports (Chutmanop *et al*., 2008; Ali and Vidhale, (2013).

Table 2: Enzyme activity (Iu/ml) in mixed fungus culture fermentation

Enzymes	*A. niger* + *R. oryzae*	*A. niger* + *T. viride*	*T. viride* + *R. oryzae*	*A. niger* + *T. viride*+ *R. oryzae*
Protease	17.33±0.88^{a}	9.00±1.15^{b}	12.67±1.45^{b}	21.00±1.53^{a}
Phytase	31.33±8.09^{a}	28.33±2.33^{a}	28.00±1.00^{a}	29.67±8.09^{a}
Pectinase	38.00±2.08^{b}	31.33±1.45^{b}	36.00±2.52^{b}	51.00±1.73^{a}
Lignin peroxidase	38.00±2.08^{b}	28.33±1.45^{c}	35.67±1.20^{b}	48.00±2.31^{a}
Manganese peroxidase	15.33±3.18^{a}	16.33±0.88^{a}	11.67±0.67^{a}	15.33±0.33^{a}

^{abc}: means along the same row are significantly different ($p \leq 0.05$). Each data is expressed as mean ± standard error (n=3)

Phytase activity was numerically higher in mixed culture fermentation than in single culture fermentation but was not significantly different ($p \geq 0.05$) among the three fungi in pure cultures and in mixed cultures. Values recorded ranged between 31.33±8.09 Iu/ml to 28.00±1.00 Iu/ml. Bhargav *et al.* (2008) reported phytase production using various agro-processing wastes by bacteria (*Bacillus subtilis, B. amyloliquefaciens, E. coli*) fungi (*Aspergillus oryzae, A. fumigates, A. ficcum, A. flavipas)* yeast (*Hansenula polymorpha, Schwannioyces caselii, S. occidenta*lis) under SSF. The high concentration of phosphorous (Ofongo *et al.*, 2008) in RMF could have been a probable factor with regards to phytase activity thereby implicating availability of substrate as a factor to consider in enzyme synthesis from agro-industrial by-products. Pectinase

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activity followed a similar trend with the highest ($p \leq 0.05$) value of 51.00±1.73 Iu/ml recorded in a mixed culture of *A. niger* + *T. viride* + *R. oryzae*. Similarly, pectinase activity recorded in this study was comparable among the three fungi in pure cultures, but under mixed culture of the three fungi, the highest pectinase activity recorded (51.00±1.73iu/ml) was significantly higher ($p \leq 0.05$) than the mixed culture of any two of the fungi studied. Microbial production of pectinolytic enzymes has been reported to be mainly from filamentous fungi, yeasts and non-filamentous bacteria (Jacob, 2009). Lignin peroxidase activity was highest in *R. oryzae* (22.33±2.19 Iu/ml) but was not significantly higher ($p \geq 0.05$) than activity of lignin peroxidase (48.00±2.31Iu/ml) expressed when all the three fungi were present in mixed culture of *A. niger* + *R. oryzae* and *T. viride* + *R. oryzae*. Thus, exhibiting synergistic enzyme production. Manganese peroxidase activities was significantly lower ($p \leq 0.05$) than Lignin peroxidase in all cases. In pure culture, the highest Manganese peroxidase activity was expressed by *R. oryzae* (13.00±0.00 Iu/ml), which was not significantly different ($p \geq 0.05$) from that expressed by *A. niger but* was significantly different ($p \leq 0.05$) from the concentration produced by *T. viride*. Under mixed culture fermentation, the enzyme activity by all the three fungi combined and combination of any two of the fungi yielded Manganese peroxidase in order of 15Iu/ml that were not significantly different ($p \geq 0.05$) across all treatments. Mikiashvili *et al.* (2011) reported a peroxidase activity of 2.33 – 22.55U/l by different strains of *Grifola frondosa* cultivated on oak saw dust. Isikhuemhen *et al.* (2012) reported Manganese peroxidase activity of 2.32 – 15.40U/l and lignin peroxidase activity of 2.17 – 27.42U/l by *Lentinus squarrosulus* under SSF on corn stalk substrate. Isikhuemhen *et al.* (2014) further reported Manganese peroxidase (0.23 – 50.36U/l) and lignin peroxidase (0.45 – 65.35U/l) activity by different WRF during SSF of Canola oil processing agro-wastes. This is indicative that fungi may be suitable in synthesis of lignin peroxidase and manganese peroxidase as recorded in the results obtained from the current study and that reported by other authors.

This further buttress the impact of substrate availability on products of fermentation either solid state or submerged fermentation processes. In addition, inoculum size in mixed culture fermentation is another likely factor responsible for values recorded in the current study. Earlier reports suggest that high inoculum level resulted in higher enzyme activity recorded (Zambare, 2010 and Kunamueni *et al.,* 2005). High concentrations of spores were attributed for increased enzyme production because of higher substrate specificities coupled with substrate availability.

4. CONCLUSION

While some are Agro-processing wastes are returned to farms and ploughed back into the soil for nutrient enhancement, many are disposed or abandoned in the environment causing pollution and other nuisance. In this study, the submerged fermentation of RMF was demonstrated using three

indigenous fungi (*Aspergillus niger, Trichoderma viride* and *Rhizopus oryzae*) both in pure and mixed culture for the production of enzymes. Oxidative, hydrolytic and other types of biomass degradation enzymes were expressed. The enzymes have the potential of biodegradation of complex plant material leading to the modification, saccharification and nutrient enrichment of the substrate, which could be utilized beneficially in animal feed supplementation.

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REFERENCES

1. Ali, S.S. and Vidhale, N.N. 2013. Protease production by *Fusarium oxysporium* in solid-state fermentation using rice bran. *American Journal of Microbiological Research,* 1(3): 45 – 47.
2. Bhargav, S., Panda, B.P., Ali, M., and Javed, S. 2008. Solid-state fermentation: an overview. *Chem. Biochem. Eng*. 22(1): 49 – 70.
3. Belewu, M.A. and Adenuga, O. S., 2005. Effect of treating rice husk with *Aspergillus niger* on the feed intake and digestibility of WAD goat's. *J. Raw Material Res.* 2. (1): 19 - 25.
4. Belewu, M.A. and Banjo, N. O., 1999. Bio delignification of rice husk and sorghum Stover by edible mushroom (*Pleurotus sajor caju*). *Tropical Journal of Animal Science*. 1 (37): 137 - 142.
5. Belewu, M. A., Ayinde, O.E. and Morakonjo, A.O., 2007. Biochemical changes of some waste agricultural residues after solid state fermentation. *Global J. of Agricultural Science* 13 (2): 161 - 164.
6. Chutmanop, J., Chuichulchrmm S., Chist, Y. and Srinophakun, P. 2008. Protease production by Aspergillus oryzae in solid-state fermentation using agro-industrial substrates. *Journal of Chemical Technology and Biotechnology*, 83: 1012 – 1018.
7. FAO 2017. Rice monitor: Production, international trade, rice utilization and domestic prices. Volume XX Issue No. 1
8. Glenn, J.K. and Gold, M.H. (1985). Purification and characterization of an extracellular Mn (II)-dependent peroxidase from the lignin degrading basidiomycete *Phanerochaete chrysosporium. Arch. Biochem. Biophys.* 242,329-341.
9. Hatakka, A. I. and Pirhonen, T. I, 1985. Cultivation of wood rotting fungi on agricultural lignocellulosic materials for production of crude protein. *Agricultural wastes* 12, 81 - 97.

10. Isikhuemhen, O.S., Mikiashvili, N. A., Senwo, Z. N. and Ohimain, E. I. 2014. Biodegradation and Sugar Release from Canola Plant Biomass by Selected White Rot Fungi. *Advances in Biological Chemistry*. 4: 395-406
11. Isikhuemhen, S. O., Mikiashvili, N. A., Adenipekun, C. O., Ohimain, E. I. and Shahbazi, G. 2012. The tropical white rot fungus, *Lentinus squarrosulus* Mont.: Lignocellulolytic enzymes activities and sugar release from cornstalks under solid state fermentation. *World Journal of Microbiology and Biotechnology*. 28 (5): 1961-1966
12. Iyayi, E.A. and Aderolu, Z.A.2004. Enhancement of the feeding value of some agro - industrial by - products for laying hens after their solid-state fermentation with *Trichoderma viride*. *African Journal of Biotechnology* vol 3 (3) pp 182 - 185.
13. Iyayi, E.A. 2004. Changes in the cellulose, sugar and crude protein contents of agro - industrial by - products fermented with *Aspergillus niger, Aspergillus flavus* and *Penicillium sp. African Journal of Biotechnology.* Vol3: (3), pg. 186 - 188.
14. Jacob, N. 2009. Pectinolytic enzymes. In: Biotechnology for Agro – industrial residues utilization. **Singh-Nee Nigam**, Poonam, **Pandey**, Ashok (Eds.). ISBN 978-1-4020-9942-7 Springer link.
15. Kunamneni, A., Permaul, K. and Singh, S. 2005. Amylase production in solid state fermentation by the thermophilic fungus *Thermomyces lanuginosus*. *Journal of Bioscience and Bioengineering,* 100:168-171.
16. Mandel, M. and Weber, J. 1969. The production of cellulases in: Cellulases and their applications (Gound, R. F. Ed) Advanced Chemical Series 95, Amer. Chem. Soc. Washington D. C.
17. Mcdonald, C.E. and Chen, L.L., 1965. Lowry modification of the Folin reagent for determination of proteinases activity. *Ann. Biochem*. 10: 175.
18. Mikiashvili, N. A., Isikhuemhen, O.I, Ohimain, E. I. 2011. Lignin degradation, ligninolytic enzymes activities and exopolysaccharide production by *Grifola frondosa* strains cultivated on oak sawdust. *Brazilian Journal of Microbiology*. 42: 1101-1108.
19. Miller, L.G. 1959. Use of dinitro salicylic acid reagent for the determination of reducing sugars. *Analytical Chemistry* 31, 426 – 428.
20. Mohan, S., Anthony, M. V. and Kasinathan, P. 2004. Submerged fermentation of phytase by *Aspergillus niger* and *Aspergillus fumigatus*. *Asian Journ. Microbiol. Biotech. And Environ. Sci.* Vol. 6 (1): 107 – 111.
21. Ofongo, S.T., Kehraus, S., Iyayi, E.A. and Südekum, K. H. 2008: Rice Mill Feed: an Agro-industrial By-product with Potential for Rural Development. In: Proceedings of Tropentag (Conference on International Research on Food Security, Natural Resources Management and Rural Development) University of Hohenheim, Stuttgart-Germany. 7th - 9th October, 2008. Pp 439

ISSN: 2455-6939

Volume:04, Issue:06 "November-December 2018"

22. Oliveira, M.D.S. Feddern, V., Kupski L., Cipolatti, E.P., Badiale-Furlong, E. and de Souza-Soares, L.A. 2010. Physico-chemical characterization of fermented rice bran biomass. CyTA-*Journal of Food*, 8(3): 229 – 236.
23. Saunders, R. M. 1986. Rice bran: Composition and potential food uses. *Food Reviews International* 1: 465 – 495.
24. Shibuya, N. and Iwasaki, T. 1985. Structural features of rice bran hemicellulose. *Phytochemistry* 24: 285 - 289.
25. Sumantha, A., Deepa, P., Sandhya, C., Szakacs, G., Socc,i C.R., Pandey, A. 2006. Rice bran as a substrate for proteolytic enzyme production. *Brazilian Archives of Biology and Technology*, 49(5): 843 – 851.
26. Tien, M., and T. K. Kirk. 1988. Lignin peroxidase of *Phanerochaete chrysosporium*. *Methods Enzymol*. 161:238–249.
27. Zambare, V. 2010. Solid state fermentation of *Aspergillus oryzae* for Glucoamylase production on agro residues. *International Journal of Life Science*, 4: 16 – 25.

