PRODUCTION OF B-1,4-MANNOSIDASE ENZYME BY *ASPERGILLUS ORYZAE* PT4 OBTAINED FROM DECAYING POTATO PEELS

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Abstract: Refined and complex mannan-containing substrates were investigated for their ability to induce β-1,4-mannosidase enzyme production in *Aspergillus oryzae* PT4 in a solid substrate fermentation condition, while optimizing the fermentation process using the best complex carbon source and One Factor at Time (OFAT) approach. Various carbon, nitrogen sources and some environmental factors were varied and enzyme titre assayed. Regulation of enzyme production in this organism was also studied in the presence of 0.2g of easily utilizable sugars per gram of the complex carbon source. Copra meal was the best carbon source after Locust Bean Gum. Addition of nitrogen source while using copra meal reduced the amount of enzyme produced by this isolate. The pH of 5.0, moisture content of 100% v/v, inoculum size of 8% v/v, temperature of 30 ºC and incubation time of 10 days were the best fermentation condition for the production of β-1,4-mannosidase by *Aspergillus oryzae* PT4. Mannosidase enzyme production in this isolate was highly inductive. Using the above set of conditions, enzyme titre was optimized from 39.24±0.19 to 87.55±3.80 U/gds.

Keywords: *Aspergillus oryzae*, Optimization, Mannolyitc enzymes, β-1,4-mannosidase, Solid State Fermentation and Agro-waste biomasses.

Introduction

Mannolytic enzymes or mannan-degrading enzymes are enzymes that are able to hydrolyze mannopyranosyl linkages namely pure mannan, glucomannan, galactomannan and galactoglucomannan (Sundu and Dingle, 2003). At least 2 groups of mannolytic enzymes are prominent, based on their activity. Some are exo-acting β-mannanases while others are endo-acting β-mannanases. A complete breakdown of mannan polysaccharides into D-mannose and other constituent sugars is done by the synergistic action of β-mannanases, β-mannosidases and accessory enzymes such as β-galactosidases, α-L-arabinofuranosidases, β-glucosidases and esterases (Nascimento *et al*., 2014).

β-Mannosidase otherwise called β-D-mannoside mannohydrolase (EC 3.2.1.25) is an exoglycosidase mainly known for the breaking down of terminal β-linked mannosides in various mannan containing substrates. It is very essential for the complete hydrolysis of mannan oligosaccharides to mannose and because of this it has been found useful in many industrial applications such drilling of oil and gas, coffee extraction, pulp and paper industries as bleach-enhancing agent (Moreira and Filho, 2008). In medical science, β-mannosidases are used for the synthesis of oligosaccharides (Nashiru *et al.*, 2001). B-mannosidases have been either extracted or produced from mammals, plants, fungi (*Aspergillus*, *Sclerotium rolfsii*, *Trichoderma reesei*), bacteria (*Cellulomonas fimi*, *Thermobifida fusca*, *Thermotoga neapolitana*, *Streptomyces* and *Bacillus*) (Fliedrová *et al*., 2012).

Several works have been done on the production and characterization of mannosidases from bacteria and fungi; genes of enzymes have been cloned and overexpressed in bacteria and fungi. Agricultural wastes are abundant in our environment and can be used as feed stocks in the production of lignocellulosic enzymes, including mannosidases. Multiple fermentation techniques such as solid-state fermentation, submerged fermentation and co-culturing have been developed for the production of these enzymes (Huang *et al.*, 2023). However, there still need to optimize the fermentation processes for yield promotion. The current study therefore is aimed at producing and optimizing β-1,4-mannosidase production in *Aspergillus oryzea* PT4 obtained decaying potato peels.

Materials and Methods

The Isolate

Aspergillus oryzae PT4 previously identified molecularly with accession number (KR871216) was obtained from the Department of Microbiology, Akwa Ibom State University. It was sub-cultured and preserved on Potato Dextrose Agar Slant at 4 ºC in a refrigerator.

β-1,4-Mannosidase Assay

β-Mannosidase activity was assayed using *p*-nitrophenyl-β-D-mannoside as substrates. The reaction mixture included: 250 µL of the substrate (1mM) was added to 500 µL of 50 mM citrate buffer (pH 5.0). To start the reaction, 250 µL of enzyme preparation was added to the mixture. This setup was then incubated at 50ºC for 10 minutes. To stop the reaction, 2.0 mL of 1M Na_2CO_3 solution was added to the mixture. The absorbance of *p*-nitrophenol released was measured using spectrophotometer at 405 nm. One unit of enzyme activity is defined as the amount of enzyme

producing 1 µmol *p*-nitrophenol per minute under the assay condition (Norita *et al.* 2010).

Inoculum preparation

One millitre (1 mL) of the spore suspension in a culture slant was standardized to spore concentration of $1x10^6$ spores/mL using the Neubeur counting chamber (Sae-lee, 2007).

Production of β-1,4-Mannosidase enzymes by Aspergillus oryzae PT4 using different carbon sources.

Both complex and refined carbon sources were used in submerged and solid state fermentation to determine the inductive ability of these substrates on the isolate for β-1,4-Mannosidase production. Copra meal, palm kernel cake and soy bean meal (agro-waste) were used in solid state fermentation experiment while birchwood xylan, locust bean gum, carboxymethyl cellulose, glucose, xylose and arabinose (refined carbons) were used submerged fermentation.

In a solid state culture setup, the dried powered substrates were wetted with ML medium 50 % (v/w) moisture concentration (Santiago *et al.*, 2007). The contents of the flasks were then autoclaved at 121°C for 15min. On cooling, standardized fungal spores (4% v/w of $1x10^6$ spores/mL) of *Aspergillus oryzae* PT4 were inoculated into the set up and then incubated at 30°C for 72 hours in a stationary mode. Fifty millilitres (50 mL) of the sodium citrate buffer (pH 5.0) were used to extract the produced enzyme as supernatant after centrifugation at 10,000 rpm for 10 mins. The presence of β-1,4-Mannosidase activity was assayed as previously outlined.

In submerged fermentation setup, the refined carbon sources (1% w/v) were supplemented into ML1 medium. The setup was sterilized, cooled, inoculated with standardized fungal spores (4% v/v) and incubated at 30°C for 7days. The entire broth was then centrifuged and the supernatant assayed for enzyme activity.

Effect of nitrogen sources on the production of β-1,4-Mannosidase by Aspergillus oryzae PT4

The best complex carbon source supporting the production of the enzyme was supplemented with various nitrogen sources at 4% w/w in solid culture fermentation (Darah and Omar 2010). They were Urea, Peptone, Yeast Extract, Casein, Tryptone, Soy bean Meal, KNO_3 and NH_4NO_3. Moisture content was kept at 50% (v/w), pH at 5.0 and incubation was done at 30°C for 7 days.

Test of environmental factors on the production of β-1,4-Mannosidase by Aspergillus oryzae PT4

In these series of experiments, the best carbon and nitrogen sources were used while other factors such as initial pH, moisture content, inoculum size, temperature, incubation time were varied in a bit to optimize the production conditions of β-1,4-Mannosidase by the *Aspergillus oryzae* PT4. The experimental ranges of these factors were pH (3.0 to 7.0); moisture content (50 to 150%v/w); inoculum size (1 to 10% v/w); temperature (27 to 40 °C) and incubation time (3 to 14 days). These factors were carried out in a stepwise manner by keeping the best factors already determined while varying the next.

Regulation of β-1,4-Mannosidase production by Aspergillus oryzae PT4

Easily utilizable sugars such as glucose, galactose, arabinose and xylose were supplemented at different instances in a culture set up containing copral meal as the complex carbon substrate at 0.2g/g. after incubation, the enzyme produced were harvested and assayed. The results obtained were compared to the concentration of enzyme gotten in the absence of simple sugars (Mabrouk and El Ahwany, 2008).

Results and Discussion

Optimization of the production conditions of for the production β-1,4-mannosidase by Aspergillus oryzae PT4.

Effect of different carbon sources on the production of β-1,4-mannosidase

Locust bean gum was the best inducer of mannosidase with a recorded activity of 44.46±0.24 U/gds. This was followed by copra meal with enzyme activity of 39.24±0.19 U/gds. Production in the presence of soybean meal, xylan and xylose were not significantly different with individual activities of 30.95±0.19, 33.67±0.35 and 31.22±0.10 U/gds respectively. Substrates with least inductive effect for mannosidase production were arabinose and glucose with enzyme titre of 20.07±1.02 and 17.79±0.70 U/gds respectively (Table 1).

Table 1: Production of β-1,4-mannosidase by *Aspergillus oryzae* PT4 using different carbon sources in a solid state fermentation at 50% moisture level.

	Enzyme/ Enzyme Activity (U/gds)
Substrates	Mannosidase
Soy-Bean meal	*****30.95±0.19$^{dc\neq}$
Copra meal	39.24±0.19^{b}
PKC	28.80±2.46^{de}
Xylan	33.67±0.35^{c}
CMC	26.82±0.62^{e}
LBG	$^{+}$**44.46±0.24^{a}**
Xylose	31.22±0.10^{dc}
Arabinose	20.07±1.02^{f}
Glucose	17.79±0.70^{f}

*******Data are presented as Mean±SE of results in triplicates.*

$^{\neq}$ Values with same superscript are not significantly different (P<0.05) using Duncan's Multiple Range Test

$^{+}$Values in bold font are higher than other values on the same column

Copra meal, the cheap carbon source, has been reported by some other authors to be a good inducer of mannolytic enzymes (Youssef *et al.*, 2006). The report of Antia *et al.* (2023) showed that copra meal is high in mannose and amino acids, informing the possible reason for its inductive ability on mannosidase production by *Aspergillus oryzae* PT4.

Effect of different nitrogen sources on production of β-1,4-mannosidase by Aspergillus oryzae PT4.

Aspergillus oryzae PT4 did not need additional nitrogen to produce mannosidase as the recorded activities of was highest in the absence of additional nitrogen sources. Enzyme activities of 62.04±2.38 U/gds was recorded for this enzyme (Table 2). This is similar to the observation of Marouk and El Ahwany (2008) that omitting nitrogen source from the growth medium of *Bacillus amyloliquefaciens* when potato peel was the sole carbon source resulted in the production of high quantities of mannanase. From the above results it can be concluded that some lignocellulosic material can serve both as carbon and nitrogen sources (Gomes *et al.*, 2007). Thus, further addition of nutrients in form of nitrogen may lead to excess nutrient availability.

Table 2: Effect of different nitrogen sources on the production of β-1,4-mannosidase by *Aspergillus oryzae* PT4 using the best supporting complex carbon source in a solid state fermentation at 50% moisture level.

	Enzyme/Enzyme Activity (U/gds)
Nitrogen Sources	Mannosidase
Peptone	*47.17±0.23$^{b\neq}$
Yeast Extract	16.07±0.38^{f}
Urea	18.83±0.04^{e}
KNO_3	15.58±0.18^{f}
Casein	30.82±0.36^{c}
Soybean Meal	24.07±0.17^{d}
Tryptone	20.67±0.11^{e}
Ammonium Sulphate	13.62±0.07^{f}
Control	$^{+}$**62.04±2.38^{a}**

***Data are presented as Mean±SE of results in triplicates.*

$^{\neq}$ Values with same superscript are not significantly different (P<0.05) using Duncan's Multiple Range Test

$^{+}$ Values in bold font are higher than other values on the same column

Effect of pH on the production of β-1,4-mannosidase by Aspergillus oryzae PT4.

The influence of initial pH of the culture medium on β-1,4-mannosidase production was investigated within the pH range of 3.0 to 7.0 (Table 3). Production of mannosidase from *A. oryzae* PT4 at pH 3.0, 4.0, 6.0, and 7.0 were not significantly different. Activities of 39.99±0.38, 34.92±0.77, 36.85±1.59 and 42.25±4.83 U/gds were recorded at those pH levels respectively. Maximum production of mannosidase was at pH 5.0 (60.52±1.42 U/gds).

The optimum pH 5.0 for this enzyme production is similar to that reported by Chantorn *et al.* (2013); Ufot *et al.* (2023) amongst other researchers. Several works on fungal enzyme production have reported pH range of 4.0 and 6.0 as the optimum pH range for their production and this is a reflection of their optimal growth pH.

Table 3: Effect of initial pH on the production of β-1,4-mannosidase by *Aspergillus oryzae* PT4 using the best carbon and nitrogen sources in a solid-state fermentation at 50% moisture level.

	Enzyme/Enzyme Activity (U/gds)
pH	Mannosidase
3	*39.99±0.38$^{b\neq}$
4	34.92±0.77^{b}
5	$^{+}$**60.52±1.42^{a}**
6	36.85±1.59^{b}
7	42.25±4.83^{b}

***Data are presented as Mean±SE of results in triplicates.*

$^{\neq}$ Values with same superscript are not significantly different (P<0.05) using Duncan's Multiple Range Test

$^{+}$ Values in bold font are higher than other values on the same column

Effect of different moisture levels** on the production of **β-1,4-mannosidase by Aspergillus oryzae PT4

Table 4 shows the effect of moisture content on the production of β-1,4-mannosidase by the fungal isolate. Mannosidase production by *A. oryzae* PT4 gradually increased as the moisture level increased. After it peaked at 100% (v/w) moisture, a gradual decrease in production was also observed. Mannosidase production was 85.46+082 U/gds at 100% (v/w). Lowest production of mannosidase was at 50% and 150% (v/w) moisture levels.

Moisture concentration of above 100% has been reported by Onilude *et al.* (2012a) for mannosidase production by a bacterium. Depending on the substrate used as well as the organism, moisture demand could be as low as 50 % (v/w) and as high as 150 % (v/w). This variation could be as a result of difference in the rate of water absorption and availability exhibited by different substrates (Pandey, 2003).

Table 4: Effect of moisture content on the production of β-1,4-mannosidase by *Aspergillus oryzae* PT4 in solid state fermentation incubated at 30^{o}C for 7days

	Enzyme/Enzyme Activity (U/gds)
Moisture (%, v/w)	Mannosidase
50	*48.01±2.14$^{d\neq}$
75	60.54±3.35^{b}
100	$^{+}$**85.46±0.82^{a}**
125	59.08±1.88^{bc}
150	52.03±2.96^{cd}

*****Data are presented as Mean±SE of results in triplicates.*
ǂ Values with same superscript are not significantly different (P<0.05) using Duncan's Multiple Range Test
+ Values in bold font are higher than other values on the same column

Effect of different inoculum sizes on the production of β-1,4-mannosidase by Aspergillus oryzae PT4

Mannosidase production at 4, 8 and 10% (v/w) inoculum sizes were 73.58±8.45 U/gds, 60.62±6.80 U/gds and 56.17±7.38 U/gds. These values were not significantly different and they were reached after a sharp rise in enzymes production from 6.36±0.10 U/gds at 1% (v/w) inoculum size to 34.69±6.59 U/gds at 2% (v/w) inoculum size (Table 5). In solid state fermentation, inoculum size must be evenly distributed and in sufficient numbers for the organism to grow and produce their metabolites effectively. Fungal spores grow by initially getting attached to the outer surface of the substrates particles slowly. It then begins to multiply and with the development of hyphae, it penetrates the substrates. Hence, a suitable inoculum size is needed if the highest enzyme production must be achieved (Ibrahim *et al.*, 2012; Antia *et al.*, 2019).

Table 5: Effect of inoculum size (% v/w) on production of β-1,4-mannosidase by *Aspergillus oryzae* PT4 in solid state fermentation incubated at 30ºC for 7days using the best moisture level of 100% (v/w).

Inoculum size (%, v/w)	Enzyme/Enzyme Activity (U/gds) Mannosidase
1	*6.36±0.10^{cǂ}
2	34.69±6.59^{b}
4	$^{+}$**73.58±8.45^{a}**
8	**60.62±6.80^{a}**
10	**56.17±7.38^{a}**

***Data are presented as Mean±SE of results in triplicates. 1 ml of the inoculums contained about 1x10^{6} spores*
ǂ Values with same superscript are not significantly different (P<0.05) using Duncan's Multiple Range Test
+ Values in bold font are higher than other values on the same column

Effect of different incubation temperature on the production of β-1,4-mannosidase by Aspergillus oryzae PT4

The effect of incubation temperature on β-1,4-mannosidase production by the fungal isolate was examined (Table 6). Mannosidase production at 27ºC was 79.92+0.80 U/gds. Production increased to 87.33±0.36 U/gds at 30ºC and 87.18+0.49 U/gds at 45ºC. The two (2) values were not significantly different. Production dropped by 20 units at 40ºC with a record activity of 56.22±0.81 U/gds.

Temperature of 30ºC has been reported widely by many authors as being optimal for enzyme synthesis by fungal and some bacterial isolates (Onilude *et al.*, 2012a; Rashid *et al.*, 2012). It is possible that the optimum temperature for enzyme production by any microorganism reflects best growth temperature of the producing organism in solid state fermentation experiments (Antia *et al*., 2019). According to Rashid *et al.* (2012), the optimum temperature for enzyme production in solid state fermentation also are affected by factors like moisture content, airflow and oxygen level.

Table 6: Effect of incubation temperature on production of **β-1,4-mannosidase by *Aspergillus oryzae* PT4** in solid state fermentation incubated for 7days using the best moisture levels and inoculum size.

Temperature (ºC)	Enzyme/Enzyme Activity (U/gds) Mannosidase
27	*****79.92±0.80^{bǂ}
30	$^{+}$**87.33±0.36^{a}**
35	**87.18±0.49^{a}**
40	56.22±0.81^{c}

***Data are presented as Mean±SE of results in triplicates.*
ǂ Values with same superscript are not significantly different (P<0.05) using Duncan's Multiple Range Test
+ Values in bold font are higher than other values on the same column

Effect of incubation time on the production of β-1,4-mannosidase by Aspergillus oryzae PT4

Table 7 shows the production of β-1,4-mannosidase between 4 to 18 days of cultivation. Samples were taken at 3 and 4 days intervals and assayed for the quantity of enzymes produced. The quantity of enzymes measured rose gradually from 36.71±0.05 U/gds at day 4 to 75.39±0.05 U/gds at day 7 and peaked at 87.55±3.80 U/gds at day 10. After which a sharp drop in activity was observed at day 14 (16.81±0.81 U/gds) and 18 (18.50+0.08 U/gds); both values were not different significantly. The optimum time of 10 days recorded for mannosidase of *A. oryzae* PT4 could be due to the fact that mannosidase act on the oligomers of mannan polysaccharides produced after the actions of mannanase, cellulase and xylanase on the intact polymeric chain of the mannan biomass (Onilude *et al*., 2012b).

Table 7: Effect of incubation time (days) on the production of β-1,4-mannosidase by *Aspergillus oryzae* PT4 in solid state fermentation incubated at 30ºC for 7days using the best inoculums, moisture and initial pH levels.

Incubation Time (days)	Enzyme/Enzyme Activity (U/gds) Mannosidase
4	*****36.71±0.05^{cǂ}
7	75.39±0.05^{b}
10	$^{+}$**87.55±3.80^{a}**
14	16.81±0.18^{d}
18	18.50±0.08^{d}

*****Data are presented as Mean±SE of results in triplicates.
ǂ Values with same superscript are not significantly different (P<0.05) using Duncan's Multiple Range Test
+ Values in bold font are higher than other values on the same column

Repressive and Inductive effects of simple sugars on β-1,4-mannosidase Production by Aspergillus oryzae PT4

There was a severe inhibitory effect associated with the addition of simpler and easily utilizable carbohydrates on the production of mannosidase by the fungal isolate. Galactose had the highest repressive effect on mannonidase followed by xylose with activities of 18.33±0.18 U/gds and 28.34±0.06 U/gds respectively (Table 8).

Table 8: Repressive and Inductive effects of simple sugars (0.2 g/g) on the production of β-1,4-mannosidase by *Aspergillus oryzae* PT4 in a solid-state fermentation.

	Enzyme/Enzyme Activity (U/gds)
Sugars (0.2 g/g)	Mannosidase
control	+***92.26±0.09**[a,f]
Glucose	35.31±0.37[b]
Galactose	18.33±0.18[d]
Arabinose	35.82±0.15[b]
Xylose	28.34±0.06[c]

***Data are presented as Mean±SE of results in triplicates.*
[f] Values with same superscript are not significantly different (P<0.05) using Duncan's Multiple Range Test
[+] Values in bold font are higher than other values on the same column

The presence of easily utilizable sugars are known to delay inducible enzyme production like mannosidase. Many mannan utilizing enzymes are largely inducible (Singh *et al*., 2003). Mabrouk and El-Ahwany (2008) reported inhibition of mannan-degrading enzymes in *Sclerotium rolsfii* and *Bacillus amyloliquefaciens* in the presence of easily utilizable glucose monomers.

Conclusion

This study presented the optimization of β-1,4-mannosidase production by *Aspergillus oryzae* PT4 in a solid-state fermentation using the OFAT approach. Copra meal without additional nitrogen source supported the production of this enzyme more than the other carbon sources tried out. This is good as it signifies reduction in the overhead cost during a commercial production of this enzyme using the *Aspergillus oryzae* PT4. The production of β-1,4-mannosidase in this organism is inductive and need the following conditions for enhanced enzyme production: pH of 5.0, moisture content of 100% v/v, inoculum size of 8% v/v, temperature of 30 ^{o}C and incubation time of 10 days.

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Conflict of Interest

There is no conflict of interest.

References

Anita, U. E., Stephen, N. U., Onilude, A. A., and Ibanga, I. A. (2019). Studies of the Nutritional, Environmental Effects and Repressive Nature of Simple Sugars on the Production of endo-β-mannanase by Aspergillus flavus PT7 on Solid State Fermentation. *Journal of Advances in Biology & Biotechnology*, **21**(4), 1-12.

Antia, U. E., Stephen, N. U., Onilude, A. A., Udo, I M. and Amande T. J. (2023). Bioconvertibility of mannan-containing polysaccharides to bioethanol: a comparative study of palm kernel cake and copra meal feedstocks. *Biomass Conversion and Biorefinery* **13**, 5175–5186.

Chantorn, S. T., Buengsrisawat, K., Pokeseam, A., Sombat, T., Dangpram, P., Jantawon, K., Nitisinprasert, S. 2013. Optimization of extracellular mannanase production from Penicillium oxalicum KUB-SN2-1 and application for hydrolysis property. *Songklanakarin Journal of Science and Technology*, **35**(1):17-22

Darah, S. I. and Omar, I. 2010. Utilization Of Palm Kernel Cake For The Production Of Mannanase by an indigenous filamentous fungus, *Aspergillus niger* USM F4 Under Solid Substrate Fermentation. *The Internet Journal of Microbiology* **9**(1)

Fliedrová, B., Gerstorferová, D., Křen, V., and Weignerová, L. (2012). Production of Aspergillus niger β-mannosidase in Pichia pastoris. Protein Expression and Purification, **85**(2), 159–164.

Gomes, J., Terler, K., Kratzer, R., Kainz, E. and Steiner, W. 2007. Production of thermostable ß-mannosidase by a strain of *Thermoascus aurantiacus*. Isolation, partial purification and characterization of the enzymes. *Enzyme and Microbial Technology*, **40**: 969-975.

Ibrahim, D., Puspitaloka, H., Rahim, R. A. and Hong, L. S. 2012. Characterization of Solid State Fermentation Culture Conditions for Growth and Mananase Production by *Aspergillus niger* USM F4 on Rice Husk in Tray System. *British Biotechnology Journal*, **2**(3)133-145.

Moreira, L. R. S. and Filho, E. X. F. 2008. An overview of mannan structure and mannan-degrading enzymes system. *Applied Microbiology Biotechnology*, **79**: 165-178.

Nascimento, A. S., Muniz, C., Aparício, J. R., Golubev, R. and Polikarpov, I. (2014). Insights into the structure and function of fungal β-mannosidases from glycoside hydrolase family 2 based on multiple crystal structures of the *Trichoderma harzianum* enzyme. *The FEBS*

Journal, *281*(18), 4165-4178. https://doi.org/10.1111/febs.12894

Norita, M. S., Rosfariza, M., and Ariff, A. B. (2010). Evaluation of the activities of Concentrated crude mannan-degrading enzymes produced by *Aspergillus niger*. *Malaysian Journal of Microbiology*, **6**(2):171-180.

Nashiru, O., Zechel, D. L., Stoll, D., Mohammadzadeh, T., Warren, R.A.J. and Withers S.G., (2001) β-Mannosynthase: synthesis of β-mannosides with a mutant β-mannosidase. *Angewandte Chemie International Edition*. **40**:417–420.

Onilude, A. A., Fadahunsi, I. F., Garuba, E. O., and Antia, U. E. (2012a). Production of alkaline β-mannosidase by Bacillus sp. 3A in Solid State Fermentation using different Agro Wastes. *Researcher*, **4**(1), 48-54.

Onilude, A. A., Fadahunsi, I. F., Antia, U. E., Garuba, E. O., and Ja'afaru, M. I. (2012b). Characterization of crude alkaline β-mannosidase produced by Bacillus sp. 3A isolated from degraded palm kernel cake. *AU Journal of Technology*, **15**(3).

Pandey, A. 2003. Solid-state fermentation. *Biochemical Engineering Journal*, **13**: 81–84.

Rashid, S A., Ibrahim, D. and Omar, I. C. 2012. Mannanase production by *Aspergillus niger* USM F4 via solid substrate fermentation in a shallow tray using palm kernel cake as a substrate. *Malaysian Journal of Microbiology*, **8**(4): 273-279.

Santiago, S. D. N., Gonzalez, C. R., Almendarez, B. G., Fernandez, F. J., Jurado, A. T. and Ochoa, S. H. 2007. Physiology, morphological, and mannanase production studies on *Aspergillus niger* UAM-GS mutants. *Electronic Journal of Biotechnology*, **9**: 1.

Sae-Lee, N. 2007. The production of fungal mannanase, cellulose and xylanase using palm kernel meal as a substrate. *Walailak Journal of Science and Technology*, **4**: 67-82.

Singh, C., Ahuja, N., Batish, M., Capalash, N., Sharma, P. 2003. Biobleaching of wheat straw-rich-soda-pulp with alkalophilic laccase from γ-*Proteobacterium* JB: Optimization of process parameters using response surface methodology. *Bioresource Technology*, **99**:7472-7479.

Sundu, B. and Dingle, J. 2003. Use of enzymes to improve the nutritional value of palm kernel meal and copra meal. *Queen land poultry and Sciences symposium* **11**: (14) 1-15.

Mabrouk, M.E.M. and El Ahway, A.M.D. 2008. Production of ß-mannanase by *Bacillus amylolequifaciens* 10A1 cultured on potato peels. *African Journal of Biotechnology* **7**(8): 1123-1128.

Ufot, E. A., and Antia, U. E. Udomessien, C. K. and Umoh, V. J., (2022). Enzymatic Activities of Halotolerant and Halophilic Fungi Isolated from Iko River Estuary, South-South Nigeria. *Journal of Advances in Biology & Biotechnology*, *25*(8), 12-27.

Youssef, A. S., El-Naggar, M. Y., El-Assar, S. A. and Beltagy, E. A. 2006. Optimization of Cultural Conditions for ß-mannanase Production by a Local *Aspergillus niger* Isolate. *International Journal of Agriculture and Biology*, **8**(4):539-545.