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Research Article

Extracelullar Cellulase Production by Solid State Bioprocesses of *Artemisia annua* L. Agro Waste

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Abstract

Agrowaste residues are made up of cellulosic and hemicellulosic materials. These cellulosic wastes are ideal for the growth of microorganisms which in turn convert the wastes to organic manures. Biodegradation potential of two fungal species isolated from soils of *Artemisia annua* plantation was investigated. The two fungal strains were selected from a preliminary test carried out on fungal isolates using Carboxymethylcellulose agar plates. Congo red test was performed and *Aspergillus niger* and *Trichoderma viride* showed highest hydrolytic zones and were used for the biodegradation of the *A. annua* agrowaste. Biodegradation of the agrowaste lasted for 7 and 14 d. The percentage weight loss for pulverized agrowaste obtained for *T. viride* was 27 and 21% after the 7th and 14th d of degradation while for *A. niger* it was 32 and 24%. The percentage weight loss for non-pulverized agrowaste obtained for *T. viride* was 34 and 22% after 7th and 14th d degradation process while the figures obtained for *A. niger* was 36 and 30% respectively. The enzyme produced and its activity was determined under different process parameters including effects of different concentration of Carboxymethylcellulose powder, incubation period, temperature and pH. The pulverization of the *A. annua* waste into powder gave better results as it helped in high conversion yields obtained as against the non-pulverized waste material.

Keywords: Biodegradation, solid state processes, *Artemisia annua*, agrowaste, carboxymethylcellulose.

Introduction

Cellulase enzyme consisted mainly of endoglucanases which degrade the inner regions of cellulose to disrupt the polymer chains, cellobiohydrolases which degrade the cellulose ends releasing cellulose oligomers and cellobiose and β-glucosidases which turn the latter molecule to glucose (Bhat, 2000; Sukuruman *et al*., 2009; Fang *et al*., 2010). High cost production of these enzymes has hindered the industrial application of cellulose bioconversion. As a result, the conversion of cellulosic biomass to fermentable sugars using cellulases derived from cellulolytic organisms has been suggested as a feasible process and offers potential to reduce the use of fossil fuels and also reduce environmental pollution (Lynd *et al*., 1999). There is however a need for continuous search for organisms with production of cellulase enzymes in high amounts and to optimize their enzyme production parameters. Industrial application of cellulases in the food, textile, laundry, baking, brewing, pulp and paper industries and genetic engineering has been reported (Ojumu *et al.,* 2003; Gilna and Khaleel, 2011). Thambirajah *et al*. (2005) reported that agricultural wastes contain a high proportion of cellulosic matter which is easily decomposed by a combination of physical, chemical and biological processes.

The bunch consists of 70% moisture and 30% solid; of which holocellulose accounts for 65.5%, lignin 21.2%, ash 3.5%, hot water-soluble substances 5.6% and alcohol-benzene soluble 4.2%. Filamentous fungi, particularly *Aspergillus* and *Trichoderma* species, are common soil inhabiting fungi well known as efficient producers of these cellulases (Gupta and Madamwar, 1994; Peij *et al*., 1998; Singh *et al*., 2009). Solid state fermentation (SSF) has been used effectively and has proven more efficient in bioconversion processes of cellulosic biomass. In solid substrate cultivation, microbial biomass and product formation takes place on the surface of solid substrates. Solid state fermentation (SSF) is the growth of microorganisms on moist solid materials in absence or near absence of free flowing water (Canal and MooYoung, 1980; Satyanarayana, 1994) and is suitable for the growth of fungal species that grow well at water activities that are relatively low. The present work therefore mainly focused on the optimization of different cultural conditions needed for rapid growing fungi *Aspergillus niger* and *Trichoderma viride* isolated from *Artemisia annua* plantation soil in order to enhance the extracellular endoglucanase synthesis under solid state fermentation. This optimized fermentation conditions would be helpful in utilizing these organisms in large scale bioprocess systems.

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Materials and methods

Collection and processing of soil samples: Soil samples were collected from 1-10 cm depth of three different locations of *Artemisia annua* plantation. The samples were placed in separate labeled polythene bags and were carried to the laboratory where further experimental works were carried out. All the samples brought to the laboratory for isolation were processed within 3-5 h after collection. The ecological parameters of the soil samples including pH, percentage moisture content and temperature were determined using methods of Ogbonna and Pugh (1982). About 10 g of each of soil samples were suspended in sterile distilled water in the ratio of 1:5. The pH of the soils samples were assessed using digital pH meter and were recorded in triplicates. For the percentage moisture content, a weight of 30 g of soil from each soil sample was dried to a constant weight in hot air oven set at 110°C. The percentage moisture content was calculated by computing the loss in weight on drying as a fraction of initial weight of samples and multiplied by 100 and then recorded. This experiment was done in triplicates. The temperature of the soil samples were determined at the site using thermocouple and recorded in triplicates.

Isolation and identification of fungi from the soil samples: Isolation of fungal species was done using pour plate method of Okoko and Ogbomo (2010). Serial dilutions of the soil samples were made. One mL of 10^{-4} dilution was plated out on sterile petri plates containing Potato Dextrose Agar (PDA) media. The plates were incubated at 25°C for 5 d to isolate fungal colonies. The colonies showing significant fungal growth were selected and placed on fresh Petri plates containing PDA medium. Series of sub-cultures were made to obtain pure cultures of the isolated fungi and were maintained at 4°C. The cultures were identified based on microscopic and macroscopic characteristics. Lactophenol cotton blue staining was performed for microscopic observation. A drop of lactophenol cotton blue stain was placed on clean grease free glass slide, a small tuft of the fungus, was transferred unto the stain using a flamed, cooled needle and was teased. A thin cellotape was placed above it and the slide was observed under low and high power objectives of the microscope. Identification of each of the fungal isolates was done using identification scheme of Samson *et al.* (1984), Nagamani *et al*., (2006), Dubey and Maheshwari (2007). Percentage frequency of occurrence was determined using the formula:

$$\%FO = \frac{\text{Number of positive samples}}{\text{Total number of samples}} \times \frac{100}{1}$$

Primary screening for cellulolytic activity: The fungal isolates were grown on basal salt solution as originally defined by Mandels *et al.* (1974) with slight modification (KH_4PO_4, 1.4; NH_4NO_3, 1.0; KCl, 0.5; $MgSO_4.7H_2O$, 0.1; $FeSO_4.7H_2O$, 0.01; H_2O, 1000 L) supplemented with 1% Carboxymethylcellulose (CMC) powder. Holes were bored at the center of the plates using sterile 5 mm cork borer. Spore suspension of each pure fungus was introduced in the holes. A set of three petri plates were used for each fungus as replicates. The plates were incubated at 25±2°C for 5 d. After the incubation period, plates were flooded with Congo red solution (0.1%) for 5 minutes and then decolorized with 1m Nacl solution for 15 minutes. Clear zones were observed and the diameter of the zones were measured (mm) in replicates and recorded.

Biodegradation of A. annua agro waste using Solid State Fermentation (SSF): This experiment was performed using modified method of Kaur and Satyanarayana (2004). A portion of the agrowaste residue was pulverized (p) while the other portion was non-pulverized (n). This experiment was done using sixteen (16) 500 mL conical flasks, eight flasks for each fungal species and each agrowaste (pulverized and non-pulverized). One control was set up for each of the agrowaste residues. Forty (40) grams of pulverized *A. annua* waste was introduced into eight (8) of the conical flasks and were labeled appropriately. Another forty (40) g of non- pulverized *A. annua* agro waste was poured into the remaining eight (8) conical flasks. A volume of 100 mL of sterile distilled water was added to already prepared 100 mL basal medium and poured into the flasks in the ratio of 1:5. The flasks were plugged with cotton wool and autoclaved. The flasks were allowed to cool and were inoculated with 5 mL of spore suspension of actively growing *Trichoderma viride* and *Aspergillus niger*. The contents of the flasks were mixed thoroughly to ensure uniform distribution of the inoculum. The two flasks set aside as controls were not inoculated. The whole flasks were incubated at 25°C for 14 d. The initial pH of the mixture was taken and pH was also read at 24 h interval for 5 d using digital pH meter. The contents of the flasks were moistened throughout the biodegradation period of experiment to avoid dryness. A portion of each of the samples was removed every 24 h interval for 5 d, centrifuged at 10,000 rpm for 10 min and the supernatant was used to measure for growth and enzyme activity. The optical density was measured at 540 nm using Jenway spectrophotometer and the results were recorded. This method was used for the detection of protein within cell free extract with the help of Biuret reagent with Bovine Serum Albumin (BSA) as standard (Lee, 1992). The results were recorded in replicates. At the end of the 7^{th} and 14^{th} d of incubation, a set of 4 flasks each for both pulverized and non-pulverized agrowaste residues for the two fungal species were removed and the experiment terminated. The enzyme was extracted by the addition of sterile distilled water.

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The enzyme extract was filtered through cheese cloth and was centrifuged at 10000 rpm for 10 min at 4°C. The supernatant obtained was kept and used for enzyme assay using Carboxymethylcellulose (CMC) as cellulose inducer. The content of the flasks containing the biodegraded *A. annua* waste samples were dried separately to constant weight using dry air oven. The cellulose degradation by each fungal species was expressed as percentage weight loss. The loss in weight was determined using the formula:

$$\% \text{ loss in weight} = \frac{w - w_1}{w_1} \times \frac{100}{1}$$

where,
w = initial weight and w_1 = final weight

Effect of different concentrations of Carboxymethylcellulose (CMC) on enzyme activity: The experiment was done using the modified methods of Omojasola *et al*. (2008). Effects of different concentrations of the substrate (CMC) ranging from 0.5-3% on enzyme production and activity were investigated. The reaction mixture containing 0.5 mL of enzyme source was taken in a test tube, 0.5 mL of citrate buffer (pH 4.8) was added, and 0.5 mL of different concentration (0.5, 1, 2 and 3%) of CMC was added and incubated at 30°C for 30 minutes. The reaction was stopped by the addition of 1 mL of DNSA solution. The mixture was boiled for 10 minutes and cooled in water for colour stabilization and was read at 540 nm. Enzyme activity expressed in international units (IU) is defined as micromoles (µmol) of glucose released per min per mL of culture filtrate.

Effects of different incubation temperature on enzyme production and activity: For optimum temperature for cellulase production, the reaction mixture was incubated at different temperatures of 25- 50°C for 30 minutes. One (1) mL of crude enzyme for each isolates was collected separately and 1 mL of citrate buffer (pH 4.8) was added to the crude enzyme. One (1) mL of 1% CMC was also added and incubated. DNSA reagent was added to stop the reaction and was heated for 10 minutes. After cooling, the mixture was read at 540 nm using spectrophotometer as described by Jeffries (1996).

Effects of different pH on enzyme production and activity: The optimum pH for enzyme production was determined by incubating 1 mL crude enzyme, 1 mL 1% CMC in 1 mL 0.05M citrate buffer at different pH (3-9) for 30 minutes at 50°C. Reducing sugars thus released were estimated by the Dinitrosalicyclic acid reagent method at 540 nm using spectrophotometer as described by Jeffries (1996).

Statistical methods: The analyses on the ecological parameters of the soil samples, plate assay for cellulose enzyme activity and changes in pH of the *A. annua* agrowaste during biodegradation process were performed using three replicates. All data obtained were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Duncan multiple range test was used to compare the means at P≤0.05.

Results

Ecological parameter of the soil samples: The moisture content, pH and temperature of soil samples were determined and results as shown in Table 1 indicated that the soils collected from location A showed moisture content of 28.25±0.14, pH value of 7.20±1.30 and temperature of 24.23±0.54°C. Soil collected from location B showed moisture content, pH and temperature values as 28.55±0.11, 6.42±0.60 and 24.35±0.67 respectively. Samples collected from location C showed moisture content, pH value and temperature values as 28.35±0.17, 6.41±0.20 and 24.16±0.62 respectively.

Table1: Ecological parameter of the soil samples.

Soil samples	Moisture content	pH	Temp (°C)
Location A	28.25±0.14	7.20±1.30	24.23±0.54
Location B	28.55±0.11	6.42±0.60	24.35±0.67
Location C	28.35±0.17	6.41±0.20	24.16±0.62

Fungi isolated from the experimental soil samples: The fungal isolates were identified as *Aspergillus niger, A. tamarii, Curvularia lunata, Fusarium oxysporum, Rhizopus stolonifer* and *Trichoderma viride*. *Aspergillus* species was found to be the most dominant fungal isolates. *Aspergillus niger* and *T. viride* had the highest frequency of occurrence of 100% from the various habitats, *R. stolonifer* had 50% frequency of occurrence having been isolated from two of the soil samples. *Aspergillus tamarii, C. lunata* and *F. oxysporum* had frequency of occurrence of 33.3%. The details of the results are presented in Table 2.

Table 2. Fungi isolated from the soil samples.

Isolates	A	B	C	Total	%FO
Aspergillus niger	+	+	+	3	100
A. tamarii	-	-	+	1	33.3
Culvularia lunata	+	-	-	1	33.3
Fusarium oxysporum	-	+	-	1	33.3
Rhizopus stolonifer	-	+	+	2	50
Trichoderma viride	+	+	+	3	100
Total	3	4	4	11	

Primary screening for cellulolytic activity: The primary screening carried out on the fungal isolates indicated that there was hydrolysis of cellulose by the tested fungal species. The rate of hydrolysis differed from organism to organism. *Aspergillus niger* and *Trichoderma viride* recorded the highest hydrolytic zone diameters of 73.50±0.60 and 70.75±0.48 mm.

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Table 3: Screening of cellulose activity.

Fungal isolates	Zone of clearing (mm)
Aspergillus niger	73.50±0.60
Trichoderma viride	70.75±0.48
Rhizopus stolonifer	66.00±0.55
Aspergillus tamarii	51.25±0.52
Culvularia lunata	45.75±1.14
Fusarium oxysporum	0.00±0.00

Table 4. Changes in pH of *A. annua* agrowaste during the biodegradation process.

	Days				
Fungal species	1	2	3	4	5
*Aspergillus niger (n)**	3.30±0.02	4.33±0.85	4.94±1.30	4.84±0.96	4.73±0.54
*A. niger (p)**	3.41±0.59	6.20±1.01	5.96±1.46	5.94±0.55	5.83±0.77
Trichoderma viride (n)	3.44±0.61	6.03±0.98	5.93±1.39	5.84±0.60	5.73±0.82
T. viride (p)	3.31±0.03	5.84±1.10	5.83±1.28	5.67±0.89	5.65±0.84

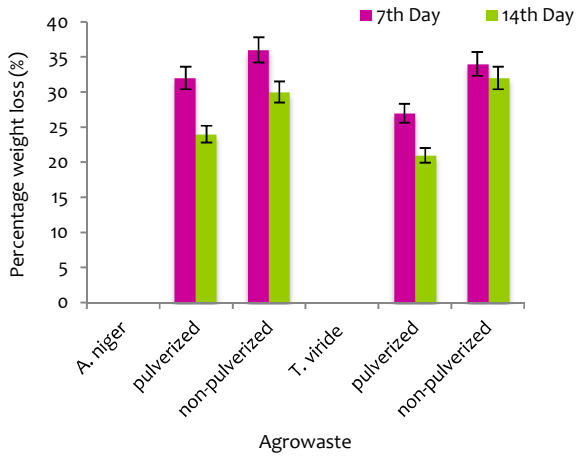
*n = non-pulverized A. annua agrowaste*p = pulverized A. annua agrowaste; Values are means of three determinations ±SE.*

Rhizopus stolonifer, *A. tamarii* and *Curvularia lunata* recorded zone diameters of 66.00±0.55, 51.25±0.52 and 45.75±1.14 mm respectively. *Fusarium oxysporum* had no zone of hydrolysis showing that the species is non-cellulolytic and could not produce the hydrolytic enzyme. *Aspergillus niger* and *T. viride* having produced the highest zones of clearance were selected for further experimentation. The details of the results are presented in Table 3.

Biodegradation of A. annua agrowaste: The relative efficiency of the test fungi to degrade *A. annua* agrowaste was studied *in vivo*. The results indicated that the two test fungi, *Aspergillus niger* and *Trichoderma viride* degraded *A. annua* agrowaste. *Trichoderma viride* was observed as the best in degrading the cellulose of *A. annua* process waste which was evident in the weight loss observed after the biodegradation process. The percentage weight loss for pulverized agrowaste obtained for *T. viride* was 27 and 21% after the 7^{th} and 14^{th} d of degradation while for *A. niger*, it was 32 and 24%. The percentage weight loss for non-pulverized agrowaste obtained for *T. viride* was 34 and 22% after 7^{th} and 14^{th} d degradation process. The values obtained for *A. niger* was 36 and 30% respectively. The details of the results are presented in Figure 1.

Change in pH of the A. annua agrowaste during biodegradation process: The progress of the agrowaste degradation was monitored by observing the changes in pH of the mineral media. The results indicated that there was a gradual increase in pH of the medium up to 5 d of incubation. The initial pH of the medium on the 1^{st} d of incubation was very low for the two test fungi but as hydrolysis and or degradation of the cellulose progressed, the pH of the medium increased from highly acidic to less acidic. The details of the results are presented in Table 4.

Figure 1. Percentage weight losses of *A. annua* agrowaste by test fungi.



Protein assay on the test fungi for pulverized and non-pulverized A. annua agrowaste during degradation process: The results of protein estimation showed that protein was produced by the test fungi into medium. *Aspergillus niger* had the highest protein production of 18.81 U/mL on the 2^{nd} d and the lowest of 0.61 U/mL on the 5^{th} d. *Trichoderma viride* had the highest on the 2^{nd} d producing 14.4 IU/mL and the lowest production of 1.2 on the 5^{th} d. The protein assay for the test fungi showed that the organisms produced protein during the biodegradation process of the *A. annua* agrowaste. For the pulverized samples, *A. niger* (p) and *T. viride* (p) produced the highest amount of protein 272.8 IU/mL and 243.3 IU/mL respectively.

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Effect of different concentrations of CMC during the biodegradation process: The different concentrations of CMC used produced different effects on the culture filtrate during the biodegradation process. Enzyme production was higher with higher concentrations of CMC. This was observed in the two test fungi for both the pulverized and non-pulverized *A. annua* agrowaste. *Trichoderma viride* produced more enzymes for both pulverized and non-pulverized agrowaste than *Aspergillus niger*, indicating that the former is a better hydrolyser of CMC. For non-pulverized, *T. viride* and *A. niger* produced 241.3 IU/mL and 179.81 U/mL respectively at 3% and 270.5 IU/mL and 264.1 IU/mL respectively for pulverized. The results are presented in Figure 3.

Effect of different temperatures for cellulase production during degradation process: Different incubation temperatures also affected enzyme production in the test fungi. The results showed that for *A. niger*, the highest enzyme production was recorded at 50°C for both pulverized and non-pulverized and the lowest enzyme production was recorded at 25°C. *Trichoderma viride* had its highest enzyme production at 30°C and the lowest at 50°C for non-pulverized. For the pulverized the highest enzyme production was at 30°C and the lowest at 25°C. The details of the results are presented in Figure 4.

Effect of different pH on cellulase production: The results of the effects of different pH on enzyme production during the *A. annua* agrowaste degradation showed that for the non-pulverized agrowaste, *A. niger* had its peak enzyme production at pH 3 and its lowest at pH 9. For the pulverized agrowaste, *A. niger* had its highest and lowest enzyme production at pH 5 and pH 9 respectively. For *T. viride*, its highest and lowest enzyme production was at pH 3 and pH 7 respectively for the non-pulverized agrowaste and its highest and lowest enzyme production was recorded at pH 5 and pH 9 respectively for the pulverized agrowaste. The details of the results are presented in Figure 5.

Discussion

The screening of the various soil samples yielded a total of six fungi with *Aspergillus niger* and *Trichoderma viride* having the highest frequency of occurrence of 100%. The isolated fungi are ubiquitous in nature and have been isolated severally from soil and other natural habitats (Abdel-Hazef, 1994; Guatam *et al*., 2010; Guatam *et al* 2010; Rahna *et al*., 2012; Sivakumaran, 2014). The pH and percentage moisture content of the soil samples were within the ranges that support microbial growth in culture. The soils collected from location A showed moisture content of 28.25±0.14 pH value of 7.20±1.30 and temperature of 24.23±0.54.

Figure 3. Effect of different concentrations of CMC on cellulase production during the degradation of *A. annua* agrowaste.

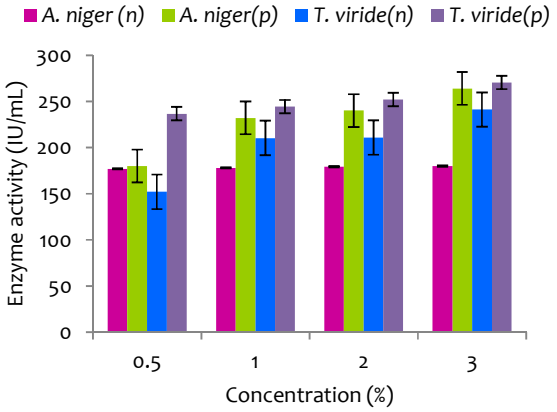


Figure 4. Effect of different temperatures on cellulase production during degradation process.

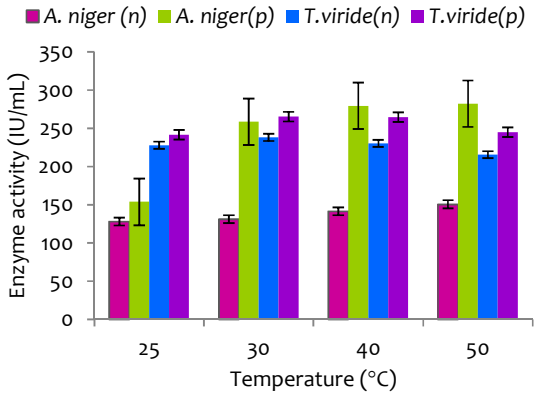
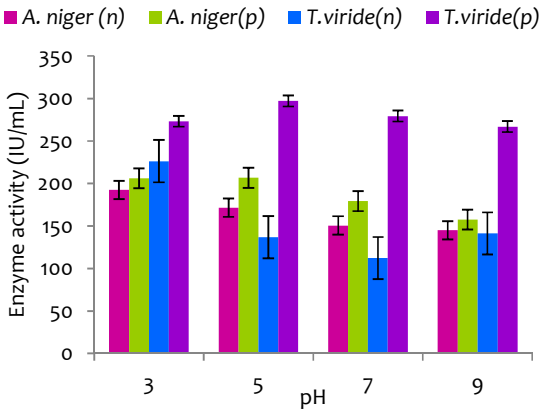


Figure 5. Effect of different pHs on cellulase production during degradation process.



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Soil collected from location B showed moisture content, pH and temperature values as 28.55±0.11, 6.42±0.60 and 24.35±0.67 respectively. Samples collected from location C showed moisture content, pH value and temperature values as 28.35±0.17, 6.41±0.20 and 24.16±0.62 respectively (Table 1). The isolated fungi from different soil samples were further screened for cellulose hydrolysis using cellulose agar plates. The fungal species that recorded the highest zones of clearing were *Aspergillus niger* and *Trichoderma viride* with 73.50 and 70.75 mm respectively and were selected for further work. These findings coincide with that of Guatam *et al*. (2012) in which *T. viride* recorded a very wide hydrolytic zone together with other fungal isolates (Table 3). The biodegradation of *A. annua* waste was done using Solid State Fermentation (SSF) and the results indicated enzyme production and enzyme activity. Cellulases are enzymes which hydrolyse the β-1,4-glycosidic linkage of cellulose and are synthesized by microorganisms during their growth on cellulosic materials. The biodegradation of cellulosic raw materials is now a global problem of current biotechnology due to food, energy and chemicals demand for them that are on the increase (Lee and koo, 2001). In plant substrates both cellulose and hemicelluloses are present. Cellulase systems are capable of degrading hemicelluloses as well as cellulose (Lynd *et al*., 2002).

In this study, cellulosic waste made up of *A. annua* waste materials were subjected to biodegradation using spore suspension of cellulose producing fungi, *Aspergillus niger* and *Trichoderma viride.* The experiment was conducted for 14 d and the results indicated degradation of the wastes which was determined as the percentage weight loss in triplicates. The average percentage weight loss for pulverized *A. annua* agrowaste obtained for *T. viride* was 27 and 21% after the 7^{th} and 14^{th} d of degradation while for *A. niger* it was 32 and 24%. The percentage weight loss for non-pulverized *A. annua* agro waste obtained for *T. viride* was 34 and 22% after 7^{th} and 14^{th} d degradation process. The figure obtained for *A. niger* was 36 and 30% respectively (Figure 2). A similar work by Kadarmoidheen *et al*. (2012) reported similar trend where *Trichoderma* sp., *Aspergillus* sp. and *Fusarium oxysporum* reduced the cellulose and hemicellulose contents of paddy straw by degradation processes. It was observed during the degradation process of the *A. annua* wastes that there was a shift in pH (Table 4). The initial pH was high acidity which was between 3.30 and 3.44 for the pulverized and non-pulverized *A. annua* agrowastes which gradually increased as the incubation period increased, though the pH was still within the acidic range (between 4 and 6) throughout the 5 d period the pH was taken. Fungi generally prefer acidic environment for their metabolic activities and enzymatic hydrolysis of celluloses are done mostly in acidic pH.

The results are in line with that of Milila *et al*. (2005) in his study on hydrolyses of various cellulosic materials by *Aspergillus candidus* were the maximum enzyme activity was observed at pH 5 for rice husk and pH 3 and 4 for guinea corn stalk and saw dust respectively. The increase in pH could be as a result of ammonification process where ammonium is produced in to the medium. In line with this, protein estimation revealed that proteins were released into the medium during the degradation process. *Aspergillus niger* had the highest protein production of 18.8 IU/mL on the 2^{nd} d and the lowest of 0.6 IU/mL on the 5^{th} d. *Trichoderma viride* had the highest on the 2^{nd} d producing 14.4 IU/mL and the lowest production of 1.2 on the 5^{th} d. The protein assay carried out also showed that large quantities of proteins were produced by the test fungi during the degradation process. For the pulverized samples, *A. niger* (p) and *T. viride* (p) produced the highest amount of protein 272.8IU/ml and 243.3 IU/mL respectively. Kadarmoidheen *et al*. (2012) reported that maximum yields of single cell protein and cellulase, under solid-state fermentation were obtained when the culture was incubated at pH 5.5 for 18 days at 30°C with 12% substrate. The different concentrations of CMC used produced different effects on the culture filtrate during the biodegradation process. Enzyme production was higher with higher concentrations of CMC. This was observed in the two test fungi for both the pulverized and non-pulverized *A. annua* agrowaste as shown in Figure 3. *Trichoderma viride* produced more enzymes for both pulverized and non-pulverized *A. annua* agrowaste than *Aspergillus niger,* indicating that the former is a better hydrolyser of CMC. For non-pulverized agrowaste, *T. viride* and *A. niger* produced 241.3 IU/mL and 179.8 IU/mL respectively at 3% and 270.5 IU/mL and 264.1 IU/mL respectively for pulverized agrowaste. Baig (2005) has reported that glucose and fructose represses the production and activity of cellulase while lactose, avicel and CMC induced the production of cellulase by *Trichoderma* spp.

Different incubation temperatures also had effects on enzyme production in the test fungi. The results showed that for *A. niger*, the highest enzyme production was recorded at 50°C for both pulverized and non-pulverized and the lowest enzyme production was recorded at 25°C. *Trichoderma viride* had its highest enzyme production at 30°C and the lowest at 50°C for non-pulverized agrowaste. For the pulverized agrowaste, the highest and lowest enzyme production was at 30°C and 25°C respectively and an optimum temperature of 50°C (Figure 4). This result is similar to findings by Oyeleke *et al.* (2010). During the biodegradation of *A. annua* agrowastes, it was observed that the microbial metabolism took place at lower pH as shown in Figure 5.

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The effects of different pH on enzyme production during the agrowaste degradation showed that for the non-pulverized agrowaste, *A. niger* had its peak enzyme production was at pH 3 and its lowest at pH 9. For the pulverized agrowaste, *A. niger* had its highest and lowest enzyme production was at pH 5 and pH 9 respectively. For *T. viride*, its highest and lowest enzyme production was at pH 3 and pH 7 respectively for the non-pulverized agrowaste and its highest and lowest enzyme production was recorded at pH 5 and pH 9 respectively for the pulverized agrowaste. Ogura *et al.* (2006) in their experiments carried out to optimize the culture conditions for the enzyme production using temperature and pH. *Bacillus subtilis* had an optimum pH of 5. Other *Bacillus* species according to previous studies by them had optimum pH range of 5-6.5.

Conclusion

The biodegradation of *Artemisia annua* agro-wastes under Solid State Fermentation method was studied using *Aspergillus niger* and *Trichoderma viride*. The results showed that the selected test fungi had different cellulose degrading ability for the cellulose substrate used in the study. Their ability to degrade cellulosic waste makes them applicable in waste management. Treated cellulases from fungal isolates can be used in industries such as food processing industries, brewery, pulp and paper industries and in making detergent. Further work is being done to characterize the cellulases as well as the proteins produced by the test fungi.

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