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Effect of industrial tannery effluent on soil fungi and fungal protease/cellulase activity on modified rice husk /modified sawdust medium (MRHM/MSDM)

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The release of unprecedented tannery effluents into agricultural fields constitutes danger to land fill and nutrient cycling as well as organic matter processing. In the present study, tannery effluent discharged soil (polluted landfill), undischarged soil (control) and effluent discharged (waste) were collected from the surrounding areas of tannery industry. The physico-chemical and biological properties and soil protease / cellulose were examined. The study shows that the value obtained from the colour, odour, pH, electrical conductivity and water holding capacity of the polluted soil was black, foul odour, 5.12, 0.72 µMhos cm^{-1} and 0.51 mg/l, respectively. In chemical parameters, organic matter, total nitrogen phosphorus and potassium have the following values: 8.2, 1.2, 0.15 and 0.39% respectively. In all ramifications, the polluted soils showed higher values than the control except that of potassium. The soil protease/ cellulose activities of the selected fungal were observed on different media (modified rice husk, modified saw dust and on control liquid media respectively). The activities were found to be higher on MRHM and MSDM (2.81 and 1.82 IU/ml) at maximum of 45 days than the control liquid media (1.23 IU/ml) at maximum of 15 days; they decreased to 0.312 IU/ml at 30 days. It was found that *Rhizopus nigricans* exhibited the highest cellulase activity (2.81 IU/ml) followed by *Aspergillus niger* (1.82 IU/ml) and the least *Penicillium* species (0.98 UI/ml) respectively.

Key words: Protease/Cellulase, *Rhizopus*, *Aspergillus, Penicillium* solid state/submerge fermentation, tannery waste and contaminated soil.

INTRODUCTION

Soil is an important system of terrestrial ecosystem. There is a direct impact of pollutants ion minerals, organic matter and microbial community of soil (Nagaraju et al., 2007). The discharge of industrial effluent especially

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without treatment may have profound influence on the physico-chemical and biological properties of soils, in relation to soil fertility. A wealth of information on occurrence of changes in properties of soils due to discharge of effluent from other industries is available such as cotton ginning mill (Narasimba et al.,1999), sugar industry (Nagaraju et al., 2007), dairy waste water (David, 2010) and dairy industry (Nizamuddin et al., 2008). Effluents from leather processing, a major industry that produces huge volume of waste water normally discharged to irrigate agricultural lands. This tannery waste water contains a very little amount of proteins except for the sludge waste that has nitrogenous compound from hide and skin of animals. Tannery effluent in the settling reservoir is usually drained to another temporary reservoir, leaving the sludge in the main reservoir for a while, which later becomes a solid waste. The effluent in the temporary reservoir is later released with contaminants into the land fill such as salts and chromium that might affect soil process and crop production (Alvarez-Bernal et al., 2006). During leather processing, the following steps are taken into cognizance: the chemicals use *viz,* lime, sodium sulphate, salt and solvents, which are quite toxic. Thus, they remain one of the worst offenders of the environment (Kamini et al., 2010).

Parkia biglobosa can be found in a belt stretching from Atlantic coast in Senegal to Southern Sudan and Northern Uganda (Thiombiano et al., 2012). The tree currently exists within a wide range of natural communities but is most abundant in anthropic communities where cultivation is semi-permanent (Janick, 2008). Annual production of seeds in Northern Nigeria is estimated at around 200,000t, while the products of the trees are not common in international trade (Ntui et al., 2012).

Yellow-purple or yellow pericarp which contains the seeds is naturally" sweet" and is processed into a valuable carbohydrate food known as "Sikomu and dodawa" among the Yoruba and Hausa people of Nigeria respectively (Olaniji, 2013). The yellow pericarp which is known as waste from *P. biglobosa* is naturally and highly rich in glucose (69%), while the most valuable parts of the locust bean are the seeds themselves which are high in lipid (29%), protein (35%) and carbohydrate (16%) and are good source of fat and calcium for rural dwellers (Gbolagunte et al., 2003; Ntui et al., 2012).

Fermentation processes may be divided into two systems: submerged fermentation (SmF), which is based on microorganisms' cultivation in a liquid medium containing nutrients, and solid state fermentation (SSF), which consists of microbial growth and product formation on solid particles in the absence (or near absence) of water; however, substrate contains sufficient moisture to allow microorganisms' growth and metabolism (Pandey et al., 2008). In recent years, SSF has received more interest from researchers since several studies have demonstrated that this process may lead to higher yields and productivities or better product characteristics than SmF. In addition, due to the utilization of low cost agricultural and agro-industrial residues as substrates, capital and operating costs are lower compared to SmF. The low water volume in SSF has also a large impact on the economy of the process mainly due to smaller fermenter-size, reduced downstream processing, reduced stirring and lower sterilization costs (Pandey et al., 2008).

Fungi and bacteria are the main natural agents that degrade cellulose and their population includes aerobic and anaerobic mesophilic bacteria, filamentous fungi, thermophilic and alkaliphilic bacteria, actinomycetes and certain protozoa (Lederberg, 1992). However, fungi are well known agents that decompose organic matter, in general and cellulosic substrate, in particular (Lynd et al., 2002). Cellulase producing fungi include genera *Trichoderma, Penicillium, Botrytis Neurospora, Aspergillus niger, Aspergillus terreus, Rhizopus stolonifer* and *Aspergilli* (Pothiraj et al., 2006). Fungi are capable of decomposing cellulose, hemicelluloses and lignin in plants by secreting enzymes (Abd-Elzaher and Fadel, 2010).

A variety of microorganisms such as bacteria, fungi, yeast and *Actinomycetes* are known to produce these enzymes (Madan et al., 2002). *Aspergillus*, *Penicillium* and *Rhizopus* are especially useful for producing proteases, as several species of these genera are generally regarded as safe (Madan et al., 2002). *Aspergillus ciavatus* ESI has been recently identified as a producer of extracellular belching stable alkaline protease (Haiji et al., 2008).

Cellulase is an important extracellular microbial enzyme, which hydrolyzes cellulose. It is also one of the cheapest sources of biomass utilized for pressing in the fruit juice industry and other factories via enzyme bioconversion, which proves to have a high industrial value. A great number of microorganisms, mostly fungi are able to degrade cellulose for their growth and produce a complete set of cellulose for the hydrolysis of cellulose to soluble sugar (Nigam, 2009).

Microbial enzyme has been severally used on various waste materials. However, prohibitive cost limits their extensive utilization especially in developing countries. Because of growing interest, the low cost is easily available and environment friendly. Agricultural residues have been tested as biodegrading recipes by microorganisms as well as metal biosorbent and a number of agro-based plan waste materials such as papaya wood (Saeed et al., 2005) and rice husk (Tarley and Arruda, 2004). Coconut fibres (Espinola et al., 1999) and black grain husk (Saeed and Igbal, 2003) have been

reported as potential degrading recipe by fungi as well as biosorbents for chromium ion removal.

Soil and microbial enzymes occupy a vital role in catalyzing reactions associated with organic matter decomposition and nutrient cycling (Sinsabaugh, 1994). Protaeses participating in the protein catabolism either by degradative or biosynthetic path ways release hormones and pharmacologically active peptides from precursor proteins (Vivian et al., 2008). Proteases are actively involved in carbon recycling and biological transformations of soils fertility (Bolon et al., 2008).

The main objective of this study is to investigate the impact of effluents of leather industry on soil physical properties, biological properties and fungal protease/cellulase activities on the feasibility of rice husk, sawdust in conjunction with conventional media in order to establish an economically viable method for microbial degradation of different substrates by solid state/ submerge fermentation respectively. The abundance of rice husk, sawdust and yellow- purple pericarp in most developing countries as well as its composition and cost effectiveness make them a strong candidate to be used as recipe for removal of many pollutants from aqueous solution using fungi.

MATERIALS AND METHODS

Collection of soil samples with/without effluent discharge

Soil samples with effluent discharge were collected from Mario Jose tannery located at Challawa Industrial Estate, Kano State. Sample without effluent discharge that served as control was collected from adjacent site 3 km away from the leather industry. Soil samples both with and without effluents were used for the determination of physical, biological and fungal properties with respect to their protease/cellulase activities on solid state /submerge medium.

Collection of effluent sample discharge

Effluent samples discharge were collected just 100 m away from Mario Jose tannery. The sample bottles with the caps on were sterilized by autoclaving at a temperature of 121°C for 15 min. These sterilized sample bottles remained capped until the effluent samples were collected at each site. The collected samples were transferred to the laboratory as quickly as possible and kept in the refrigerator in readiness for enumeration of fungal by serial dilution methods after growth on PDA media.

Collection of bio-waste subtrates

Rice husk, sawdust and yellow pericarp from (*P. biglobosa)* were collected from Samaru, Zaria and Sarkin Power, Kaduna and Niger States of Nigeria, respectively.

Physico-chemical properties of the polluted and control land fill were determined in accordance with standard analytical methods (APHA, 2000).

Isolation and identification of fungi from soils and effluent samples

Micro flora such as fungi populations of both soil and effluent samples were enumerated by serial dilution technique. 10 g and 10 ml of each soil and effluent sample respectively were serially diluted and 0.1 ml was gradually spread with a spreader on potato dextrose agar medium for the growth of fungi. Smear of the isolated fungi was prepared in lactophenol cotton blue method. Cultural characteristic such as colure, size of colonies of fungal isolates, size and shape of conidiophores/ fruiting bodies and conidia were measured and recorded. Fungal isolates were identified by matching these characteristics with that of Adawiah (2008).

Cellulase activity determination

The cellulose activity was determined by streaking the identified fungal cultures individually on the carboxy methyl cellulose agar plate and was incubated at 30°C. After 5 days of growth, the zone was identified around the culture by treating the plate with Congo red and NaOH**.**

Inoculum preparation by selected tolerance fungi

Selected tolerance fungi *viz, A. niger, Rhizopus nigrican and Penicillium* sp were maintained by stock culture in PDA agar slants. They were grown at 37°C for 24 h and stored at 4°C for regular sub culturing. 100 ml of inoculums was prepared for each culture using prepared PDA from Irish potato in 250-ml flask. The inoculum was kept in shaker (200 rpm) at 37°C for 24 h in readiness for fermentation processes.

Treatment of agricultural waste (rice husk and sawdust)

Agricultural waste either rice husk or sawdust was procured from Sabon Gari market, Zaria and brought to the laboratory for analysis. The waste was grinded and sieved to obtain a fine texture. Two hundred and fifty grams (250 g) of fine texture was washed and added to 1000 ml of basal media with the following composition (g/L); NH_4SO_4 - 1.0; K_2HPO_4 - 1.0; $MgSO_4.7H_2O$ - 1.0; $CaCO_3$ - 4.0 and $NaNO_3$. They were then mixed and boiled to semi solid slurry after which a prepared solution of 250 g of yellow *P. biglobosa* (Dorowa) was added and boiled to breakdown the complex organic compound to simpler substance as carbon sources. The paste slurry was later spread on a dried sterile tray and oven dried at 40°C for 7 days. The pH of the paste slurry was brought down using *Tamarinda Indica* (Tsamia) from 3.5 to 4.5 and was dried as cake for further analysis.

Biodegradation studies using selected fungal culture for production of protease/cellulase

The degradable capabilities of the three fungi species were selected after testing for their tolerance to Cr SO_4 solution at 4% concentration in the laboratory, while the less tolerance organisms died at 1% concentration (Gbolagunte et al*.,* 2003). Single and combination of these tolerable fungi were studies in modified culture medium *viz, A. niger, R. nigricans* and *Penicilium*

Table 1. Physicochemical properties of contaminated and control land fill of leather industrial area.

Properties	Contaminated soil	Control soil
Colour	Black	Brown
Odour	Foul	Normal
pH	5.12	6.90
Electric conductivity μ (Mhoscm^{-3})	0.72	1.27
Water holding capacity (M/g^{+1}) of soil	0.51	0.3
(Texture)		
Sand(g)	56	73
Silt(g)	24	21
Clay(g)	16	8
Organic matter (%)	8.2	4.32
Total nitrogen (%)	1.21	0.15
Phosphorus (%)	0.15	0.01
Potassium (%)	0.39	1.04

sp of all the three fungi. Twenty gram of the modified rice husk medium was added to different volumes (50, 65, 75, 85 and 100 ml) of distilled water in 250-ml conical flask. The pH was adjusted to 5.2 and sterilized in an autoclave at 121°C for 15 min. The solution was allowed to cool. The inoculum of fungi cell suspension was prepared by adding 5 ml of sterile distilled water to freshly grown fungal mycelia in slant agar bottle. 4 ml of spore suspension containing *A. niger* was then inoculated into the conical flask containing sterile MRHM and deionized water. The conical flasks were incubated for 0, 15, 30 and 45 days at 32°C. This procedure was carried out using potato dextrose medium (PDM) as the growth substrate on *R. nigricans* and *Penicillium* sp as inoculants. All the samples were filtered at specific interval using dampened cheese cloth and were centrifuged at 600 rpm for 15 min. The clear supernatant was used as a source of extracellular enzyme**.**

Protease assay

Protease activity was determined according to the modified Anson's method. 1.0 ml of the culture broth was taken in a 100 ml flask and 1.0 ml of pH 7.0 phosphate buffer added to it. 1 ml of the substrate (2% Hammersten's casein pH 7.0) was added to buffer enzyme solution and incubated at 37°C for 10 min in a water bath. At the end of 10 min, 10.0 ml of 5NTCA (trichloroaceticacid) was added to stop the reaction. The precipitated casein was then filtered off and 5.0 ml of the filtrate was taken in a test tube. To this, 10.0 ml of 0.5 N NaOH solution and then 3.0 ml of the folin ciocalteu reagent (one ml diluted with 2 ml of distilled water) were added. Final readings were taken in a spectrophotometer at 750 nm. Blanks of the samples were prepared by adding the TCA before the substrate. The effect of various factors like inoculums size, carbon source, nitrogen sources, pH and temperature on the production of protease was studied.

RESULTS AND DISCUSSION

Physico-chemical properties of contaminated and control land fill like those of pH in Table 1, showed considerable increased from 5.12 to 6.90. Soil texture in terms of gram of sand, silt and clay was 56, 24, and 16 in the contaminated soils and 73, 21, 8 in the control soil, respectively. Higher water holding capacity was observed in contaminated soil than control; values were found to be 0.53 and 0.3 mg/l respectively. The electrical conductivity of both contaminated and control soil was 0.72 and 1.24 μMhos cm^{-1} respectively. Increased water holding capacity and decreased electrical conductivity in contaminated soil may be due to the accumulation of organic wastes such as amino acid residues and alkalis in tannery industries (Alvare-Bernal et al., 2006). The parameters like organic matter, total nitrogen, phosphorus and potassium all in percentage were higher in all ramifications from contaminated land fill than the control soil except potassium content. The values of above properties of contaminated soil sample were 8.2, 1.21, 0.15 and 0.39% and that of the control soil were 4.32, 0.15,0.01 and 1.04% respectively. The microbial populations of soil samples and tannery effluent discharges are shown in Tables 2, 3 and 4. The fungal populations were relatively higher in control land fill by about 2 times than those of tannery waste polluted landfill and tannery waste effluent. The control soil sample contains the fungal population with 20.0 x 10^3 colony forming units (CFU/g) of the soil recorded in respect to soil with effluent discharges as against the tannery waste polluted landfill. The fungal population had 20.0 x 10^3 CFU/g being the highest from control soil followed by tannery waste polluted landfill of 9.7 x 10^3 CFU/g; the least recorded was 4.8 x 10^3 CFU/ml by effluent waste.

The morphological and microscopic characteristics of

Table 2. Microbial population in control soil.

Isolate Code	Macroscopic characteristics			Microscopic characteristics				Probable organism
	Colour of special hyphae	Colour of subtrate hyphae	Shape of hyphae	Nature of hyphae	Presence of special structure	Appearance of Sporangiophore	Characteristics of spire head	
B01	Black	Brown	Oval	Non – septate	Round columns present	Long erect non-separate	Multinucleated vesicle	*Aspergillus niger*
B02	Brown	Black	Globose	Long non septate	Rhizoid stolon columnal	Long sporran guim	Large and round at the Apex	*Rhizopus nigricans*
B03	Whitish yellow	Brownish green	Oval	Septate	Foot cell giving rice to conidiophores	Long erect non separate conidiophores	Vesicles small into multinucleated	*Aspergillus flavus*
B04	Grayish green	Grayish blue	Globose	Septate	Foot cell present	Long erect non separate conidiophores	Radiating sterigma	*Aspergillus fumigates*
B05	Microscopic present pseudomy chamy clono arthrosphire	Blasphire celium pure present	-	Septate	No foot cell	Microscopic white Cream	Microscopic white to Cream	*Candida* sp
B06	Green/blue greenish	Greenish	Globose	Septate	Foot cell give rise to conidiophores	Erect and non separate conidiophores	Finger-like sterigma	*Penicillum* sp
B07	Green/blue green	Greenish	Globose	Septate	Foot cell giving rise to conidiophores	Short erect non separate conidiophore broom like	Brown like sterigma	*Penicillium* sp
B08	Upper white to permanent light brownish under amber	Light brown under amber	No conidiosphore	Septate	Foot cell present	Non present	Roundish oval pear shape	*Trichophyton schoenleinii*
C01	Gray white	PMX Varieties	Globose	Septate	Foot cell not seen	Short erect non-separate conidiosphore	Multinucleated conidia	*Cephalosporium* sp
C02	Cotton white	White and gray	-	Septate with dichotomous	Initially yeast like then metamophosis into mycellum	Separate arthrospore	White	*Geotrichum* sp
C03	Wooly greenish colour	Velvety	-	Septate	No foot cell	Separation of nitrosphine within the cell wall	Elongated rounded thin wall at the end	*Coccidiodes immitis*
C04	White to brownish with reverse	Brown reverse	Oval short conspicuous Centro mere	Septate	Foot cell not seen	Conidiosphore are short or oval	Round oval conidia	*Paraccocidiodes Brasilensis*

(TOTAL CFU/g): $20.0x10^3$.

fungal cultures isolated from soil samples with/without tannery industry effluents are listed in Table 2 on the basis of a comparison of these characteristics with those recorded by Adawiah (2008). Twelve isolates identified *viz, A. niger*, *Aspergillus flavus*, *R. nigricans*, *Aspergillus fumigatus*, *Candida* sp, *Geotrichum* sp, *Penicillium notatum*, *Penicillium expansium*, *Coccidiodes immitis, Trichophyton schoenleinii, Paraccocidiodes bransilensis* and *Cephalosporum* sp from the control land fill. The former two samples of tannery waste polluted landfill and tannery effluent waste had five each as seen in Table 2, Plate 2 and Plate 3 except in Plate 1 that shows only the pollutants in polluted lands respectively. The dry modified rice husk medium recipe and the growth of *Rhizopus nigrican* from tannery effluent/chrome buffing dust tolerance species are shown in Plate 4 and 5 both are significantly presented in readiness for biosorption studies in-situ. Abundance and activities of micro flora in soil strata are controlled by the availability of water, nutrient, pH, concentration of metal ions, and hydrodynamic communication with the ground

Table 3. Microbial population in tannery wastes dumpsite (polluted landfill).

Organisms	Colony forming unit (CFU/g)
Aspergillus niger	3.0×10^3
Rhizopus nigricans	1.20×10^3
Aspergillus flavus	2.0×10^3
Aspergillus fumigatus	2.20×10^3
Penicillium sp	1.30×10^3

(TOTALCFU/g)= 9.7×10^3.

Table 4. Microbial population in tannery wastes effluent.

Organisms	Colony forming unit (CFU/ml)
Aspergillus niger	1.20×10^3
Rhizopus nigricans	1.0×10^3
Aspergillus flavus	1.2×10^3
Aspergillus fumigatus	1.4×10^3

(TOTAL CFU/ml)=4.8×10^3.

Plate 1. Dumpsite for chromium sulphate salts and chrome buffing dust (CBD).

Plate 2. Mixed culture of fungi isolated from control landfill.

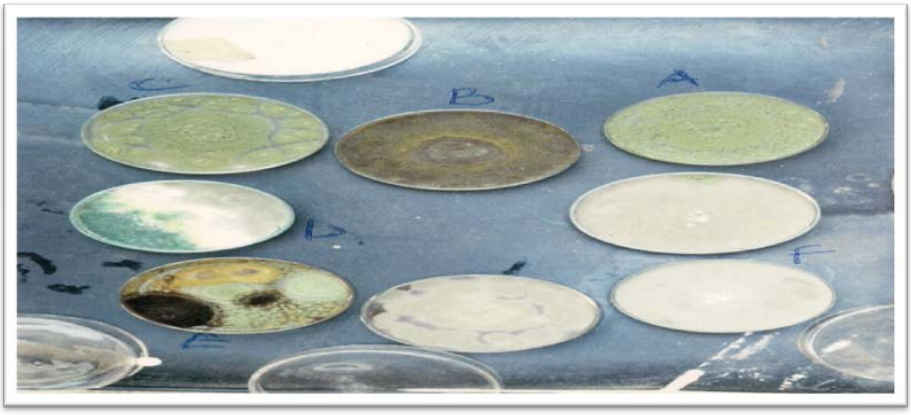


Plate 3. Mixed culture from tannery effluents /chrome buffing dust. A-*Aspergillus fumigatus,* B-Old culture of *Aspergillus niger,* C-*Aspergillus fumigatus,* D-*Penicillium* sp, E-*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigates*.

Plate 4. Dry modified rice husk medium.

Plate 5. *Rhizopus nigricans* from tannery effluents /chrome buffing dust.

Table 5. Protease/cellulose activity of fungi isolates as indicated by their relative clearance zone after their tolerance level of $CrOSO_4$ at 4% concentration were tested.

Name of the organisms	Zone of clearance
Aspergillus niger	++++ (Abundance growth)
Rhizopus nigricans	+++ (Moderate growth)
Penicillium sp	+++ (Moderate growth)

surface and so on. Environmental stresses brought about by the contamination could be a reason for the reduction in microbial species but increasing the population of few serving species. The soil samples collected from polluted sites were mostly affected by waste water irrigation due to the presence of heavy metal which affects the population densities of fungi. The differences between the sampled sites regarding their richness on microbial isolates appear to be closely linked to the degree of heavy metal pollution. Generally, pollution of soil and water by heavy metals may lead to a decrease in microbial diversity. This is due to the extinction of species sensitive to the stress imposed, and enhanced growth of other resistant species. The sources of pollutant as well as long period of exposure are also the important factors regulating stress and fungal adaptation. In determining the tolerance of the fungi isolated and characterized from land fill and waste water, three common and dominant chromium sulphate solution tolerance fungi isolated belonged to the genera of *A. niger, R. nigricans* and *Penicillium* sp. They were spotted for tolerance of chromium sulphate concentration of 4.0% as well as their clearance zone test so as to dictate their cellulolytic activity on solid media as seen in Table 5. On the basis of these tolerance and clearance zone, they were tested for protease /cellulase activities. The isolates were purified through repeated streaking onto casein agar plates. Among the isolates, the organisms were selected based on highest zone formation (casein hydrolysis) on casein agar. The potential strains were maintained on agar slants and stored at 4°C in readiness for protease/ cellulose activity determination. Prepared broth culture of the three selected fungi (*R. nigricans, A. niger* and *Penicillium* sp) and their cleared zone with more than (++) on solid agar using state fermentation on locally available modified rice husk medium (MRHM) modified sawdust (MSDM) and control medium that is, potato dextrose medium (PDM). The culture organisms were incubated for 0, 15, 30 and 45 days at 32°C. There was a gradual increase in protease/ cellulose activity in the solid state fermentation up to 45 days while in the submerge, it shows rapid growth of protease/cellulose activity at maximum of 15 days and later got depleted beyond. *R. nigricans* isolate exhibited higher maximum protease/cellulose activity (2.8 IU/ml) on modified rice husk followed by *A. niger* (1.82 IU/ml) on saw dust and least was observed on *Penicillum* sp respectively in comparison to control (PDM) soluble as seen in Figures 1, 2 and 3 respectively. The possible explanation to these selected fungal serving as promising protease/cellulase producing organisms for further studies. In tanneries, such isolates may be useful for dehairing and bating processes during tanning operations and also for hydrolysis of proteinaceous waste in the discharged tannery effluent (Sivaprakasam et al., 2011). Soil is an important sub - system of the terrestrial ecosystem. The direct discharge of industrial effluents, especially without treatment, to soil may alter its biological properties. The discharge of effluent from tannery industry containing hazardous heavy metals affects microbial population in soil.

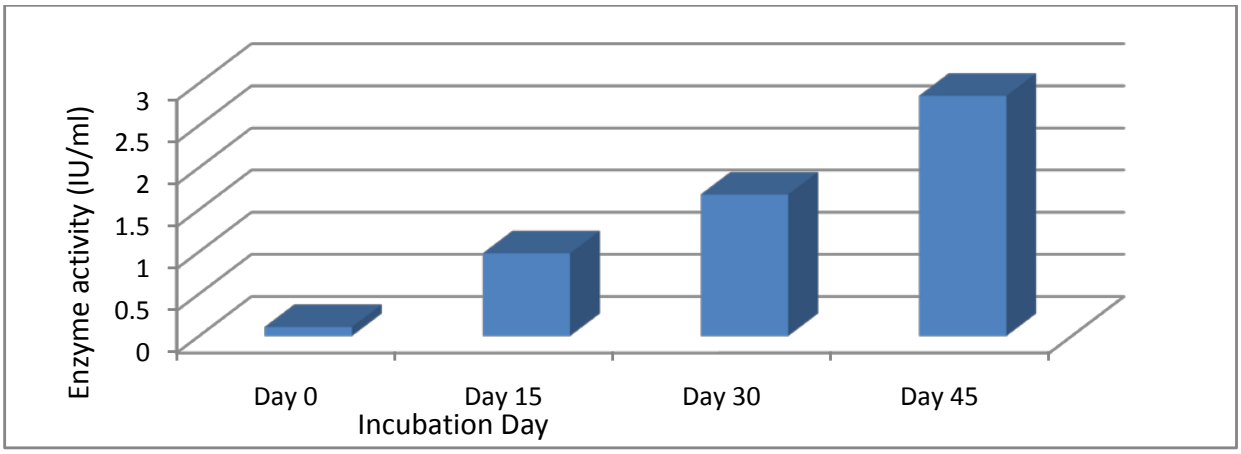
The fungal cultures isolated in the present investigation need to be further studied in depth for their proteolytic/cellulolytic potential to be exploited for conversion of proteose/cellulose waste materials into value-added and useful products.

Conclusion

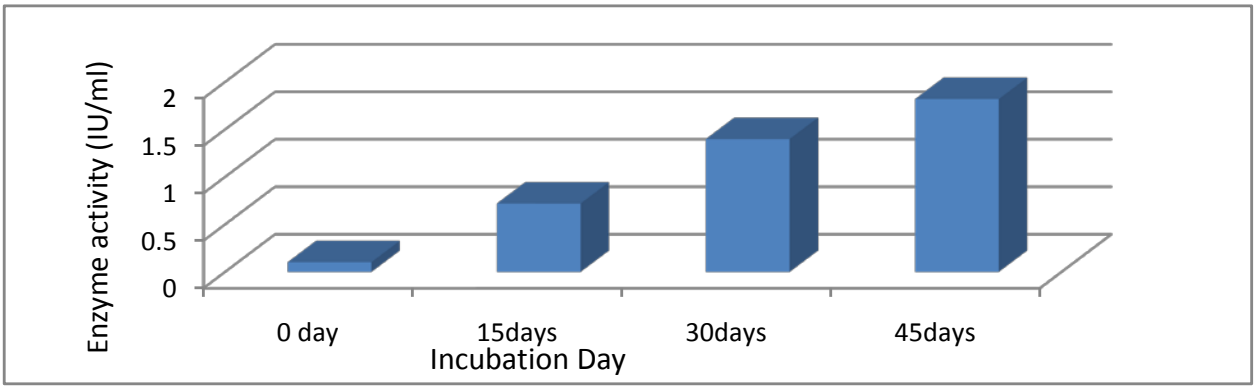
Strains of pectinolytic cellulolytic fungi including *R. nigricans* and *A. niger* have been isolated from control and polluted landfill in Challawa Industrial Estate, Kano State of Nigeria. Rice husk waste and sawdust waste have been identified as a low-cost substrate for pectinase/cellulase production in comparison with potato dextrose medium as submerge fermentation by the strains *R. nigricans* and *A. niger*. In other words, higher levels of pectinase activity were obtained by SSF compared to SmF. The use of modified rice husk for pectinase production will not only reduce the production costs of enzyme but also help decrease pollution load due to the agro-industrial waste. Further studies are to be conducted in depth for their cellulose waste material to be converted to value added and useful product.

Conflict of Interests

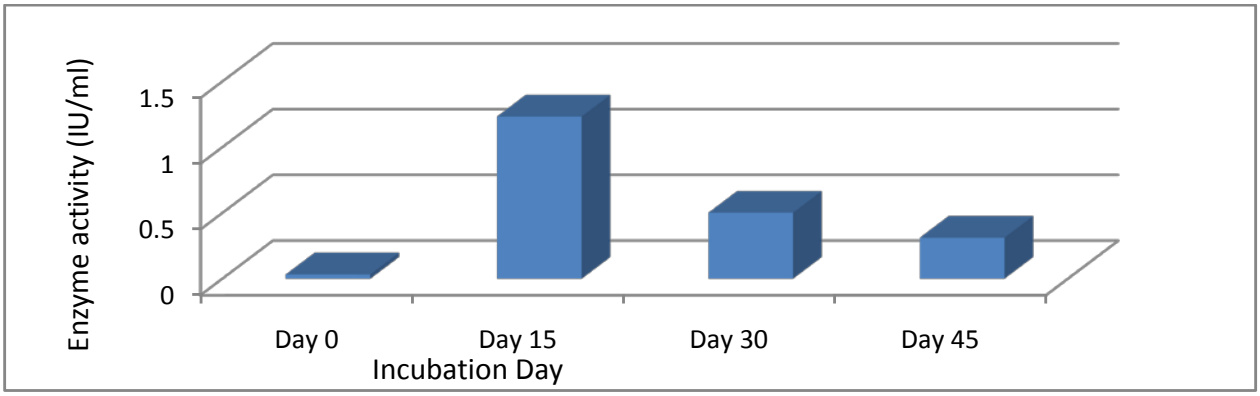
The author(s) have not declared any conflict of interests.



Culture filtrate of (*Rhizopus nigricans*) on modified rice husk.

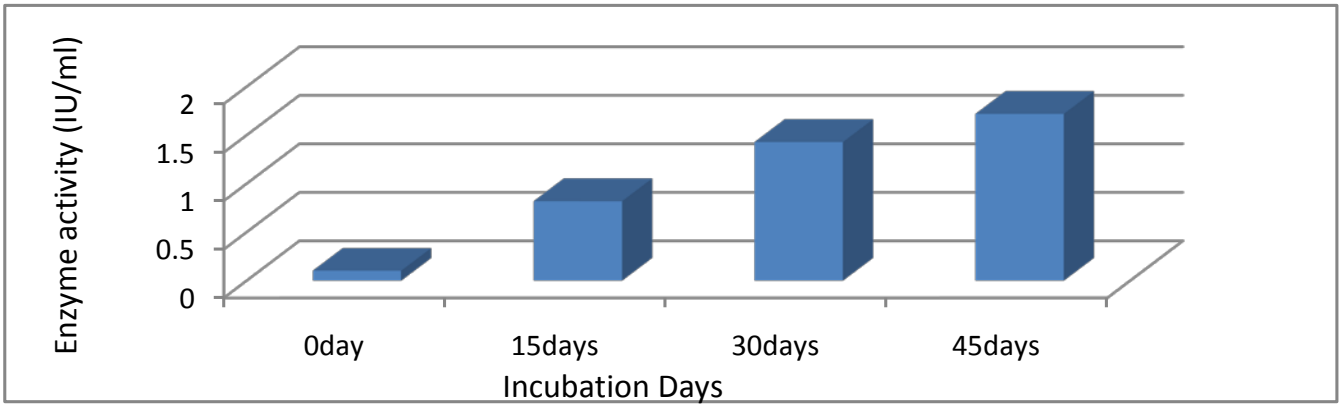


Culture filtrate of (*Rhizopus nigricans*) on modified saw dust.

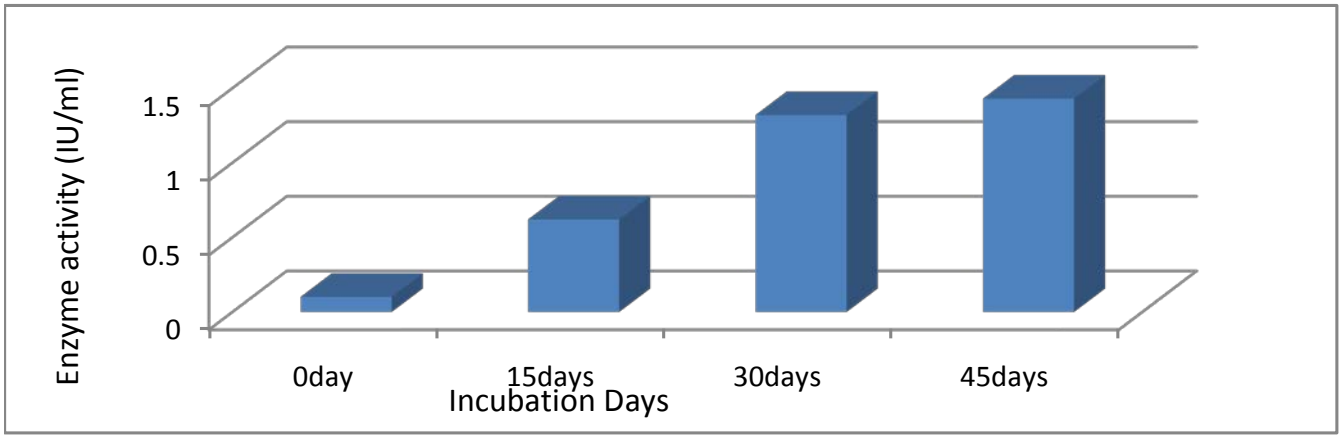


Culture filtrate of (*Rhizopus nigricans*) on Potato dextrose medium.

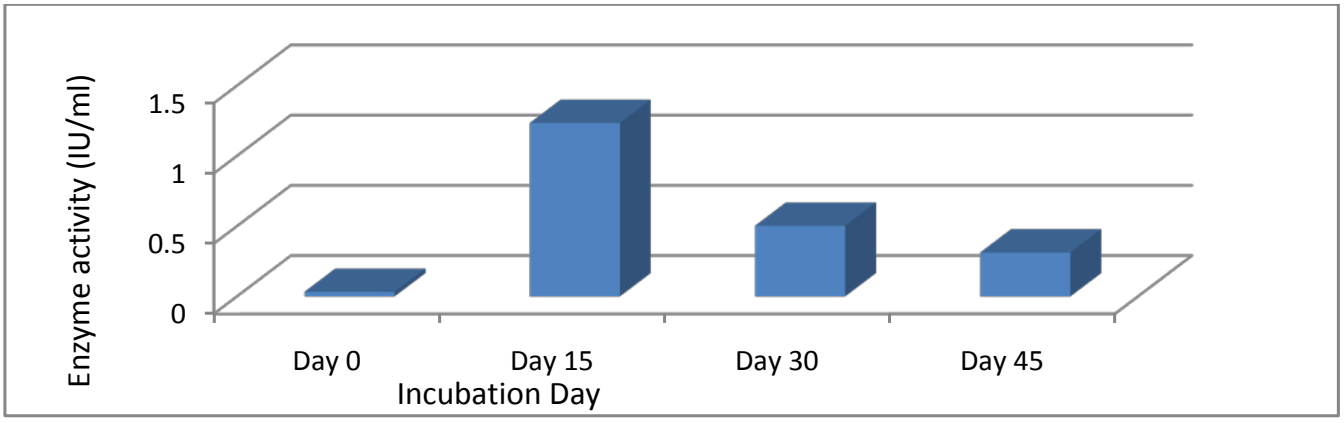
Figure 1. Enzyme estimation from culture filtrates of *Rhizopus nigricans*. Reveals that amount of protease/ cellulose was increase with increase in incubation time up to 45 days on both modified rice husk and sawdust (2.8 IU/ml) and (1.82 IU/ml) respectively in comparison with the control medium which shows an increase of the activity at maximum of 1.23 IU/ml for 15 days and decrease to 0.312 IU/ml at 30 days.



Culture filtrate of (*Aspergillus niger*) on modified rice husk medium

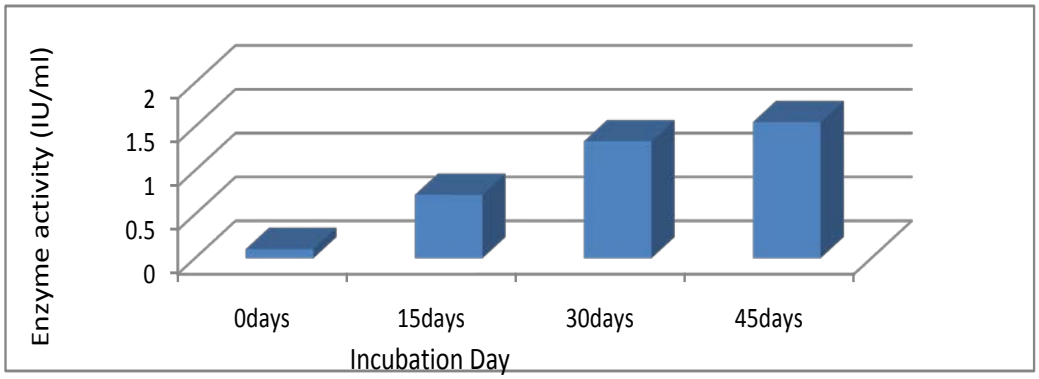


Culture filtrate of (*Aspergillus niger*) on modified saw dust medium

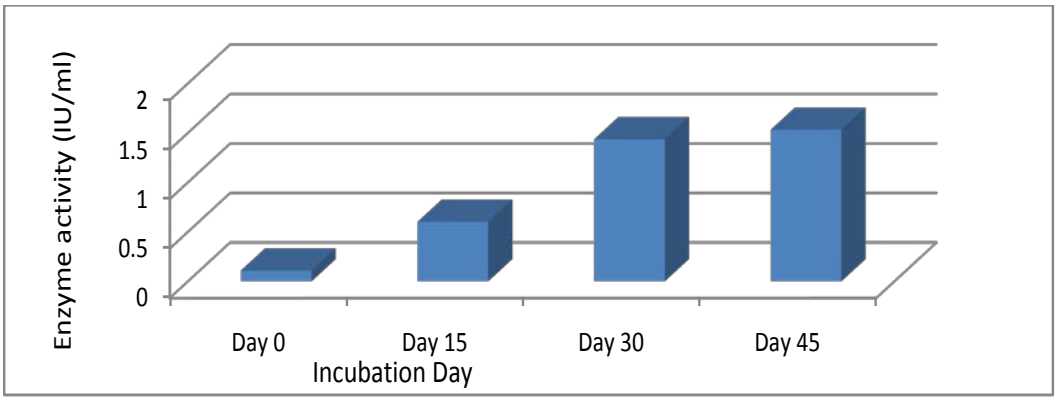


Culture filtrate of (*Aspergillus niger*) in Potato Dextrose Medium

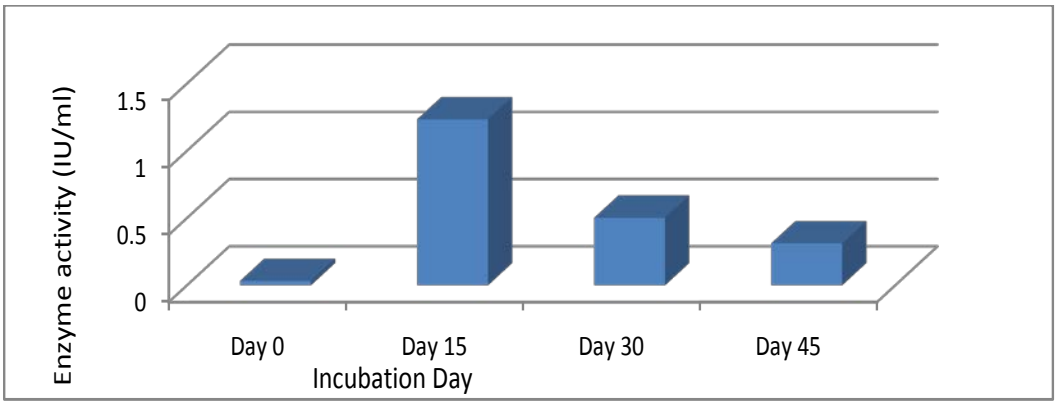
Figure 2. Enzyme estimation from culture filtrates of *Aspergillus niger*. Reveals that the amount of cellulase product was increase with increase in incubation time up to 45 days on both modified rice husk and sawdust (1.72 IU/ml) and(1.43 IU/ml) respectively in comparison with the control medium which shows an increase of the activity at maximum of 1.23 IU/ml for 15 days and decrease to 0.24 IU/ml at 30 days.



Culture filtrate of (*Penicillium* sp) on modified rice husk medium



Culture filtrate of (*Penicillium* sp) on modified saw dust medium



Culture filtrate of (*Penicillium* sp) on potato dextrose medium

Figure 3. Enzyme estimation from culture filtrates of *penicillium* sp. Reveals that amount of protease/ cellulose was increase with increase in incubation time up to 45 days on both modified rice husk and sawdust (1.55IU/ml) and(1.34IU/ml) respectively in comparison with the control

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REFERENCES

Abd-Elzaher FH, Fadel M (2010). Production of Bioethanol via Enzymatic Saccharification of Rice Straw by Cellulase Produced by Trichoderma Reesei under Solid State Fermentation. New York Sci. 3:72-78.

Adawiah BI (2008). Isolation, Characterization and Identification of Microorganism from Soil Contaminated with Pesticides. A Thesis Submitted to Faculty of Chemical and Natural Resources Engineering, University Malasiya, Pahang, pp. 18-19.

Alvarez-Bernal DSM, Olalde-Portugal JT, Contreras-Remos N, Trujillo-Tapia V (2006). Effect of tanneries waste water on chemical and biological soil characteristics. Appl. Soil Ecol. 33:267-277. http://dx.doi.org/10.1016/j.apsoil.2005.10.007

APHA-AWWA-WEF (2000). Standard methods for the examination of water and waste water, 20th Edn. American Public Health Association, American Water Works Association. Water Federation, Washington DC.

Bolon NST, Rowarth M, Delaluzmora D, Curtin D (2008). Biological transformation and bioavailability of nutrient elements in acid soils as affected by liming Develop. Soil Sci. 32:413-446.

David shyambabu S (2010). Protease and protease and phaspatase activity of soil contaminated with dairy waste water Asian J. Microbiol. Biotechnol. Environ. Sci. 26:711-714.

Espinola A, Adamiam R, Gome LMB (1999). An innovation technology: Natural coconut fibre as adsorptive medium in industrial waste water clean up waste treat. Clean Technol. Proc. 3:2057-2066.

Gbolagunte GO, Okonkwo EM, Okizie NO, Silas DE (2003). Biotechnological detoxification of chrome from tannery waste: Preliminary studies. Nig. J. Biotechnol. 12(1):9-17.

Haiji M, Rebai A, Gharsallah N, Nasri M (2008). Optimazation of alkaline protease production by Aspergillus clavatus ESI in Mirabilis jalapa tuber powder using statistical experimental design. J. Appl. Microbiol. Biotechnol. 79:915-923. http://dx.doi.org/10.1007/s00253-008-1508-0

Janick KJ (2008). Parkia biglobosa: African Locust Bean. The encyclopedia of fruit and Nuts, Willingford U.K CABI North American Office, pp. 395-400.

Kamini NR, Hemachander C, Sandana–Mala JG, Puvanakrishnan R (2010). Microbial enzyme technology as an alternative to conventional chemicals in leather Industry. Curr. Sci. (Bangalore) 77(1):80-86.

Lederberg J (1992). Cellulases. In: Encyclopaedia of Microbiology (Vol. 1; A-C). Academic Press, Inc.

Lynd LR, Weimer PJ, Pretorius IS (2002). Microbial cellulose utilization: fundamentals and biotechnolgy. Microbiol. Mol. Biol. Res. 66:506-577. http://dx.doi.org/10.1128/MMBR.66.3.506-577.2002

Madan M, Dhillon S, Singh R (2002). Production of alkaline protease by a UV mutant of Bacillus polymyxa. Indian J. Microbiol. 42:155-159.

Nagaraju LG, Narasimba B, Rangaswami V (2007). Impact of effluents of sugarcane industry on soil physicochemical and biological properties. J. Ind. Pollut. Contam. 23:73-76.

Narasimba GA, Sridevi A, Venkata SR, Rajasekhar B (1999). Effects of cotton gaining mill. Effluent on soil enzymatic activities and nitrogen mineralization in soil. J. Chem. Pharm. Res 3:126-137.

Nigam PS (2009). Production of bioactive secondary metabolites. In: Nigam PS, Pandey A, editors. Biotechnology for agro-industrial residues utilization. First ed. Netherlands: Springer, pp. 129-145. http://dx.doi.org/10.1007/978-1-4020-9942-7_7

Nizamuddin SA, Sridevi A, Narasimba G (2008). Impact of dairy factory effluents on soil enzyme activities. Eco. Environ. Conserv. 14:89-94.

Ntui VO, Uyuh EA, Urua IS, Ogbuv, Okpako EC (2012). Regeneration of Parkia biglobosa Benth: An important tree species. Afr. J. Microbiol. Biotechnol. Res. 2(1):167-177.

Olaniji A (2013). Locust Bean Products Non-wood News No.10 Retrieved http://www.Fao.org/docrep/005/y464e/y4640e02ht#paia 1309276.

Pandey PK, Verma Y, Choubey S, Pandey M, Chandrasekhar K (2008). Biosorptive removal cadmium from contaminated groundwater and industrial effluents. Bioresour. Technol. 99:4420-4427. http://dx.doi.org/10.1016/j.biortech.2007.08.026

Pothiraj C, Balaji P, Eyini M (2006). Enhanced production of cellulases by various fungal cultures in solid state fermentation of cassava waste. Afr. J. Biotechnol. 5:1882-1885.

Saeed A, Iqbal M (2003). Bioremoval of cadmium from aqueopus solution by black gram husk (Cicer arientinum). Water Res. 37:3472-3480. http://dx.doi.org/10.1016/S0043-1354(03)00175-1

Saeed A, Iqbal M, Akhtar MW (2005). Biosorption of heavy metals using rice milling by product. Characterisation and application for the removal of metals from aqueous effluents. Chemosphere 54:987-995.

Sivaprakasam S, Dhandapani B, Mahadevan S (2011). Optimization studies on production of a salt- tolerant protease from Pseudomonas aeruginosa strain BC1 and its application on tannery saline waste water treatment. Brazil. J. Microbiol. 42:1506-1515.

Sinsabaugh RL (1994). Enzymatic analysis of analysis of microbial pattern and process. Biol. Fertil. Soils 17:69-74. http://dx.doi.org/10.1007/BF00418675

Tarley CRT, Arruda MA (2004). Study on biosorption of Cr (VI) by Mucor hiemalis. Biochem Eng. J. 23:185-192.

Thiombiano DN, Lamien N, Dibong DS, Boussim IJ, Belen B (2012). The role of woody species in managing food shortage in Burkina Faso. Sechrasse, 23(2):86-93.

Vivian HL, Funkelstein D, Steven Bark J, Shinrong H (2008). Protease for processing proneuro peptides into peptide Neuro transmitters and hormones. Annu. Rev. Pharmacol. Toxicol. 348:393-423.