PURIFICATION AND CHARACTERISATION OF XYLANASE PRODUCED BY *Trichoderma asperellum*, ISOLATED FROM BANANA PEEL DUMP GROUND IN GBONGAN, AND EDE, OSUN STATE.

BY

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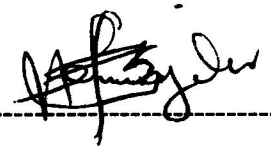
CERTIFICATION

This is to certify that this research work was carried out by OLADAYO, AYOMIDE TOLUWANIMI with matric no MCB/14/2328 under the supervision of Dr H.A. Akinyele.



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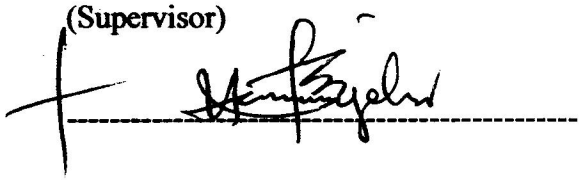

26/3/19

Prof. Bryan Ogeneh — Date

(H O D) ----------

DEDICATION

This project work is dedicated to the Almighty God, the creator of heaven and earth, the Alpha and Omega and to my indispensable parents Mrs OLADAYO, Mr and Mrs BEJIDE and family also to those who helped during my tertiary education, may you all be duly rewarded.

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All glory, honour, and adoration to the Almighty God, The Alpha and the Omega, God who gives understanding to people, The giver of wisdom and the fountain of knowledge, all acknowledgement belong to you and to my precious parents, you will live long to eat the fruit of your labour in Jesus name.

My appreciation also goes to my supervisor Dr H.A Akinyele for his thorough supervision and the father care. I pray God of heaven will prosper you in all your endeavours in life. My appreciation also goes to the Head of the Department, Prof Bryan Ogeneh and all the staff of Microbiology for their contributions both scientifically and morally. Great reward await you all, for God will make you a testimony.

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ABSTRACT.

Xylanase is of great importance especially in the industrial sectors. The demand for this enzyme is so enormous that the search for more of it continues. The aim of this study was to purify the xylanase produced by *Trichoderma asperellum* isolated from banana soils in Gbongan and Ede in Osun State, Nigeria. Xylanase was purified to a saturation of 100% followed by dialysis. The hydrolytic ability of the enzyme against Corn cob, Plantain peel and Banana peel was investigated for 96hrs. Maximum sugar 3.48mg/ml and 3.52mg/ml were produced by A10 and A29 respectively from banana peel. The xylanase from these isolates can be of tremendeous use in industries.

CHAPTER ONE

1.0 INTRODUCTION

Enzymes are distinct biological polymers that catalyze specific chemical reactions, converting substrates into particular products. They are specific in function and speed up reactions by providing alternative pathways with lower activation energy without being consumed. Although enzymes are fundamental elements for cellular and extracellular biochemical processes, they are commercially utilized in a number of food processing industries (Haq *et al.*, 2006).

The manipulation of biotechnological techniques has played an important role in recent advancement, for example, the baking industry Agricultural countries, such as Thailand, produce agro-industrial wastes and by-products in abundance, such as wheat bran, sugar cane bagasse, cassava waste, corn cobs, rice bran and so on, and these form some of the prominent waste materials from the allied food and energy industries. These waste materials, if not handled properly, are sources of environmental pollution as well as, if correctly handled, a potentially valuable add on value to the agro-industries. Thus, producers are increasingly interested to utilize these otherwise waste resources to improve the economic viability of agriculture as well as safeguard the environment. One such increasing drive is to utilize such neglected materials as carbon or nitrogen sources for the bio-production in the production of enzymes that can then be further employed and further processed (Mohammadi 2006; Okafor *et al.*, 2007).

Xylan is the second most abundant biopolymer in the biosphere after cellulose and the major hemicellulosic polysaccharide found in the plant cell wall (Timell 1967). It has a complex structure consisting of α-1,4-linked xylose residues in the backbone to which short side chains of O-acetyl, arabinofuranosyl, D-α- glucuronic and phenolic acid residues are attached. This biopolymer constitutes one third of all renewable organic carbon sources on earth. A considerable amount of xylan is found in solid agricultural and agro-industrial residues, as well as in effluents released during wood processing, but asides representing a significant source of wasted products their inappropriate disposal causes significant damage to the ecosystem (Biely, 1985; Prade, 1996).

Hydrolysis of xylan is an important step towards the degradation of lignocellulosic material in nature for nontoxic recycling, or for further metabolism, such as bio-fermentation, to utilize the trapped carbon and nitrogen source. However, the chemical hydrolysis of lignocelluloses results in hazardous by-products, forcing the use of microbial enzymes that are specific in action for xylan hydrolysis, and so are environmentally friendly options (Biely, 1985). Due to the structural heterogeneity of xylan, complete degradation of this biopolymer requires the synergistic action of different xylanolytic enzymes, such as endo-xylanase, α-xylosidase, α-glucuronidase, α-arabinofuranosidase and esterase. Among these, the most important one is endo-1, 4 xylanase (1, 4-Dxylan xylohydrolase, EC 3.2.1.8), which is also known as xylanase, and which initiates the degradation of xylan into xylose and xylo-oligosaccharides of different sizes (Collins *et al.*, 2006). There are different types of xylanases that differ in their substrate specificities, primary sequences, folds and physicochemical properties (Wong *et al.*, 1988; Collins *et al.*, 2006). These are produced by a number of bacteria and fungi (Kulkarni *et al.*, 1999; Subramaniyan and Prema, 2002).

Filamentous fungi have been reported to be good producers of xylanases of interest from an industrial point of view, due to the extracellular release of the enzymes, their higher yield compared to that produced by yeast and bacteria and also that they produce several auxiliary enzymes that are necessary for debranching of substituted xylans (Haltrich *et al.*, 1996). However, fungal production of xylanases is generally associated with the concurrent production of cellulases (Steiner *et al.*, 1987). Traditionally, the application of xylanases in conjunction with cellulolytic enzymes has been mainly considered for the bioconversion of lignocellulosic materials, especially residues and wastes produced by agriculture and forestry, to produce higher value products, such as ethanol fuel and other chemicals (Biely, 1985; Mandels, 1985). Other potential applications of crude xylanase preparations containing cellulases, glucanases, or pectinases include bread making, fruit juice extraction, beverage preparation, increasing the digestibility of animal feed, converting lignocellulosic substances to feedstock and fiber separation (Beg *et al.*, 2001; Subramaniyan and Prema, 2002), paper and pulp industries (Bajpai, 1997).

However, in the paper and pulp industry, cellulase-free xylanases are required to avoid the adverse effects of damaging the pulp fibers (Haltrich *et al.*, 1996). Moreover, specific xylanases can be used in the pre-bleaching of craft pulps in order to reduce the amount of chlorine required to achieve target brightness (Viikari *et al.*, 1994), and consequently reduce the chloroorganics released in the effluent (Christakopoulos *et al.*, 1996). Cellulase-free

xylanases or those xylanases containing negligible cellulose activity can be obtained by (1) using suitable separation methods, (2) using genetically engineered organisms to produce exclusively xylanase or (3) by applying screening methods and selection of appropriate growth conditions (Balakrishnan *et al.*, 1992).

Characterization of xylanolytic enzymes is important for their biotechnological applications. The cost of the enzyme is one of the main factors that determine the economics of a process and this can be partially achieved by optimizing the fermentation media. Several industrial processes can be carried out using whole cells as sources of enzymes, but the efficiency can be improved by using isolated and purified enzymes. The criteria used to select a particular method for the isolation and purification depends on the intended end use. A high state of purity is generally not required in the food processing, detergent and paper and pulp industries, but it may be necessary to exclude certain contaminating enzymes. Recently, interest in microbial xylanases has markedly increased due to their potential in biotechnological applications and attempts are being made to isolate new strains (Lee-Chiang *et al.*, 2006; Schmeisser *et al.*, 2007).

Xylanase secretion often associates with low or high amount of cellulases. To use xylanase for pulp treatment, it is preferable to use cellulase-free xylanases, since the cellulase may adversely affect the quality of the paper pulp. The most practical approach is the screening for naturally occurring microbial strains capable of secreting cellulase-free xylanases under optimized fermentation conditions. To use xylanase prominently in bleaching process it should be stable at high temperature and alkaline pH (Viikari *et al.*,1990).

Industrial production of enzymes on large scale is associated mainly with substrate. The use of agricultureresidues as low-cost substrates for the production of industrial enzymes is a significant way to reduce production cost. The technique of fermentation using solid state substrate has the great advantage over submerged fermentation due to absence or near absence of aqueous phase that provides natural habitat for growth of microorganisms, economy of the space, simplicity of the media, no complex machinery, equipments and control systems, greater compactness of the fermentation vessel owing to a lower water volume, greater product yields, reduced energy demand, lower capital and recurring expenditures in industry, easier scale-up of processes, lesser volume of solvent needed for product recovery, superior yields, absence of foam build-up, and easier control of contamination due to the low moisture level in the system (Archana *et al.*, 1997). Enzyme

purification is necessary to obtain only one kind of enzyme from complex mixture of protein-enzyme,to determine the active site of the enzyme for substrate binding, Purified enzymes are necessary for therapeutics and medical applications, further to study its kinetics.

1.1 Statement of the problem

During the production of xylanase, other unwanted or impurity enzyme which can affect the use of the enzyme in the industry can be produced with it thereby, the produced enzyme has to be purified. To use xylanase for pulp treatment, it is preferable to use cellulase-free xylanases, since the cellulase may adversely affect the quality of the paper pulp.

1.2 Justification of the study

The various optimized cultured conditions required for the xylanase production coupled with lignocellulosic substrate as carbon source provide a means of producing effective xylanase and the use of this enzyme to replace chemical use in the industry contribute to the reduction in environmental pollution associated with the chemicals, hence, the xylanase produced has to be purified to make it free of other unwanted constituent that can be produced alongside with the enzyme and characterized.

1.3 Aims and objectives of the study.

Aim:

Purification and characterization of xylanase produced by *Trichoderma* specie isolated from banana peel which was obtained from dump ground in gbongan and ede, Osun state.

OBJECTIVES:

To produce xylanase by the isolated xylanolytic fungi.

To purify xylanase produced by *Trichoderma* specie isolated from banana peel obtained from Ikire dump ground.

To determine the characteristics of xylanase produced by *Trichoderma* specie isolated from banana peel obtained from Ikire dump ground.

CHAPTER TWO

2.0 LITERATURE REVIEW.

2.1 Definition of microorganisms.

A microorganism is a living thing that is too small to be seen with the naked eye. They are seen with the aid of a microscope. There are varieties of microbes with different sizes and characteristics. Types of microorganisms include: Bacteria, Viruses, Fungi, Protozoa and Archae. The human body is home to microbes from all of these categories. Microscopic plants are also considered microbes, though they don't generally live on or in the human body. Microbes are tiny form of life and they surround us they are too small to be seen with the naked eye. They are found in the water, soil or in the air. The human body houses millions of these microbes also called microorganisms. Some microbes are beneficial while some are harmful.

2.2 Types of microorganisms.

Bacteria: They are single-cell organisms, some require oxygen for survival while others do not. The aerobes require the use of oxygen while the anaerobes do not require oxygen. Some bacteria loves heat (Thermophiles) while some prefer a cold environment (Psychrophiles).

Most bacteria are not harmful to humans. Many of them either live on or inside our body and helps to stay healthy. For instance, lactic acid bacteria in the bowel supports digestion, other bacteria helps the immune system by fighting germs. There are a lot of bacteria that helps in production of certain food through fermentation e.g Youghurt, sauerkraut or cheese, less than 1% of all bacteria are responsible for causing diseases.Bacteria are ubiquitous as they can be found everywhere. Infact, bacteria are regarded as the most widely distributed of all organisms. Bacteria have been described as friends as well as foes of man. This is a description that could be said to be appropriate for this group of microorganisms. This is because bacteria are beneficial to mankind. At the same time, they are harmful to mankind. The beneficial roles of bacteria are seen in agriculture, industry, medicine and many others miscellaneous activities.

Viruses: Viruses are non-cellular entities that consist of a nucleic acid core (DNA or RNA) and surrounded by a protein coat. Although viruses are classified as microorganisms they are not considered living organisms. Viruses cannot reproduce outside a host cell and cannot metabolize on their own. Viruses often infect prokaryotic and eukaryotic cells causing diseases. Viruses are obligate parasites which are not capable of independence existence outside living organisms. Viruses are very deadly as they are very harmful they are capable of causing diseases of man which include influenza, yellow fever, measles smallpox and AIDS. Viruses are also responsible for diseases of animals and plants. They are also beneficial as they are used in biological control of certain insects and bacteria, they are used in advanced studies in genetics, particularly in understanding heredity.

Fungi: Fungi are any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, Fungi, which is separate from the other eukaryotic life kingdoms of plants and animals. A characteristic that places fungi in a different kingdom from plants, bacteria, and some protists is chitin in their cell walls. Similar to animals, fungi are heterotrophs; they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Fungi do not photosynthesize. Growth is their means of mobility, except for spores (a few of which are flagellated), which may travel through the air or water. Fungi are the principal decomposers in ecological systems. These and other differences place fungi in a single group of related organisms, named the Eumycota (true fungi or Eumycetes), which share a common ancestor (form a monophyletic group), an interpretation that is also strongly supported by molecular phylogenetics.

Protozoa: Protozoa are defined as a diverse group of unicellular eukaryotic organisms. Historically, protozoa were defined as single-celled animals or organisms with animal-like behaviors, such as motility and predation. The group was regarded as the zoological counterpart to the "protophyta, which were considered to be plant-like, as they are capable of photosynthesis. Protozoa, as traditionally defined, are mainly microscopic organisms, ranging in size from 10 to 52 micrometers. Some, however, are significantly larger. Among the largest are the deep-sea–dwelling xenophyophores, single-celled foraminifera whose shells can reach 20 cm in diameter. Free-living forms are restricted to moist environments, such as soils, mosses and aquatic habitats, although many form resting cysts which enable them to

survive drying. Many protozoan species are symbionts, some are parasites, and some are predators of bacteria, algae and other protists. Organisms traditionally classified as protozoa are abundant in aqueous environments and soil, occupying a range of trophic levels. The group includes flagellates (which move with the help of whip-like structures called flagella), ciliates (which move by using hair-like structures called cilia) and amoebae (which move by the use of foot-like structures called pseudopodia).

Archae: The archae constitute a domain and kingdom of single-celled microorganisms. These microbes (archaea; singular archaeon) are prokaryotes, meaning they have no cell nucleus or any other membrane-bound organelles in their cell. Archaeal cells have unique properties separating them from the other two domains of life, Bacteria and Eukaryota. The Archaea are further divided into multiple recognized phyla. Classification is difficult because the majority have not been isolated in the laboratory and have only been detected by analysis of their nucleic acids in samples from their environment. Archaea and bacteria are generally similar in size and shape, although a few archaea have very strange shapes, such as the flat and square-shaped cells of *Haloquadratum walsbyi*. Despite this morphological similarity to bacteria, archaea possess genes and several metabolic pathways that are more closely related to those of eukaryotes, notably the enzymes involved in transcription and translation. Other aspects of archaeal biochemistry are unique, such as their reliance on ether lipids in their cell membranes, including archaeols.

2.3 Enzymes

An enzyme is a naturally occurring protein that catalyzes chemical reactions in biological systems. Enzymes promote the breakdown of complex feed molecules into smaller chemical fractions such as glucose or amino acids that are digestible by the ruminant animal. As an example, the enzyme cellulase initiates the breakdown of cellulose (fiber) into sugars. There are numerous enzymes of industrial interest which include pectinase enzyme, xylanase, lipase, lactase, cellulose, amylase, proteases, glucose and invertase.

2.3.1 **Pectinase**

Plants, filamentous fungi, bacteria and yeasts produce the pectinase enzymes group with wide use in the food and beverages industries. The enzyme is employed in the food industries for

fruit ripening, viscosity clarification and reduction of fruit juices, preliminary treatment of grape juice for wine industries, extraction of tomato pulp, tea and chocolate fermentation (Almeida *et al.*, 2005), vegetal wastes treatment, fiber degumming in the textile and paper industries (Sorensen *et al.*, 2004;Lima, *et al.*, 2000), animal nutrition, protein enrichment of baby food and oil extraction. The main application of the above mentioned enzyme group lies within the juice processing industry during the extraction, clarification and concentration stages (Martin, 2007). The enzymes are also used to reduce excessive bitterness in citrus peel, restore flavor lost during drying and improve the stableness of processed peaches and pickles. Pectinase and glucosidase infusion enhances the scent and volatile substances of fruits and vegetables, increases the amount of antioxidants in extra virgin olive oil and reduces rancidity. The advantages of pectinase in juices include, for example, the clarification of juices, concentrated products, pulps and purees; a decrease in total time in their extraction; improvement in the production of juices and stable concentrated products and reduction in waste pulp; decrease of production costs; and the possibility of processing different types of fruit (Uenojo and Pastore 2007). For instance, in the production of passion fruit juice, the enzymes are added prior to filtration when the plant structure's enzymatic hydrolysis occurs. This results in the degradation of suspended solids and in viscosity decrease, speeding up the entire process (Paula *et al.*, 2004). Several species of microorganisms such as Bacillus, Erwinia, Kluyveromyces, Aspergillus, Rhizopus, Trichoderma, Pseudomonas, Penicillium and Fusarium are good producers of pectinases (De Gregorio, *et al.*, 2002). Among the microorganisms which synthesize pectinolytic enzymes, fungi, especially filamentous fungi, such as Aspergillus niger and Aspergillus carbonarius and Lentinus edodes, are preferred in industries since approximately 90% of produced enzymes may be secreted into the culture medium. In fact, several studies have been undertaken to isolate, select, produce and characterize these specific enzymes so that pectinolytic enzymes could be employed not only in food processing but also in industrial ones. High resolution techniques such as crystallography and nuclear resonance have been used for a better understanding of regulatory secretion mechanisms of these enzymes and their catalytic activity. The biotechnological importance of microorganisms and their enzymes triggers a great interest toward the understanding of gene regulation and expression of extracellular enzymes.

2.3.2 Lipases

Lipolytic enzymes such as lipases and esterases are an important group of enzymes associated with the metabolism of lipid degradation. Lipase-producing microorganisms such as *Penicillium restrictum* may be found in soil and various oil residues. The industries Novozymes, Amano and Gist Brocades already employ microbial lipases. Several microorganisms, such as *Candida rugosa*, *Candida antarctica*, *Pseudomonas alcaligenes*, *Pseudomonas mendocina* and *Burkholderia cepacia*, are lipase producers (Jaeger and Reetz, 1998). Other research works have also included *Geotrichum sp.* (Burkert *et al.*, 2004), *Geotrichum candidum*, *Pseudomonas cepacia*, *Bacillus stearothermophilus*, *Burkholderia cepacia* (Bradoo *et al.*, 2002), *Candida lipolytica* (Tan *etal.*, 2003) *Bacillus coagulans*, *Bacillus coagulans* (Kumar *et al.*, 2005), *Pseudomonas aeruginosa*, *Clostridium thermocellum*, *Yarrowia lipolytica* (Dominguez *et al.*, 2003) and *Yarrowia lipolytic*. The fungi of the genera *Rhizopus*, *Geotrichum*, *Rhizomucor*, *Aspergillus*, *Candida* and *Penicillium* have been reported to be producers of several commercially used lipases. The industrial demand for new lipase sources with different enzymatic characteristics and produced at low costs has motivated the isolation and selection of new lipolytic microorganisms. However, the production process may modify their gene expression and change their phenotypes, including growth, production of secondary metabolites and enzymes. Posterior to primary selection, the production of the enzyme should be evaluated during the growth of the promising strain in fermentation, in liquid medium and / or in the solid state. However, it is evident that each system will result in different proteins featuring specific characteristics with regard to reactions' catalysis and, consequently, to the products produced (Asther *et al.*, 2002).

2.3.3 Cellulases

Cellulases are enzymes that break the glucosidic bonds of cellulose microfibrils, releasing oligosaccharides, cellobiose and glucose. These hydrolytic enzymes are not only used in food, drug, cosmetics, detergent and textile industries, but also in wood pulp and paper industry, in waste management and in the medical-pharmaceutical industry (Bhat and Bhat, 1997). In the food industry, cellulases are employed in the extraction of components from green tea, soy protein, essential oils, aromatic products and sweet potato starch. Coupled to hemicellulases and pectinases they are used in the extraction and clarification of fruit juices. After fruit crushing, the enzymes are used to increase liquefaction through the degradation of

the solid phase. The above enzymes are also employed in the production process of orange vinegar and agar and in the extraction and clarification of citrus fruit juices. Cellulases supplement pectinases in juice and wine industries as extraction, clarification and filtration aids, with an increase in yield, flavor and the durability of filters and finishers (Pretel, 1997). Cellulase is produced by a vast and diverse fungus population, such as the genera *Trichoderma*, *Chaetomium*, *Penicillium*, *Aspergillus*, *Fusarium* and *Phoma*; aerobic bacteria, such as *Acidothermus*, *Bacillus*, *Celvibrio*, *pseudonoma*, *Staphylococcus*, *Streptomyces* and *Xanthomonas*; and anaerobic bacteria, such as *Acetovibrio, Bacteroides, Butyrivibrio, Caldocellum, Clostridium, Erwinia, Eubacterium*, Pseudonocardia, *Ruminococcus* and *Thermoanaerobacter*. *Aspergillus* filamentous fungi stand out as major producers of cellulolytic enzymes. It is worth underscoring the filamentous fungus *Aspergillus niger*, a fermenting microorganism, which has been to produce of cellulolytic enzymes, organic acids and other products with high added value by solid-state fermentation processes. (Castro and Pereira Jr. 2010).

2.3.4 Amylases

Amylases started to be produced during the last century due to their great industrial importance. In fact, they are the most important industrial enzymes with high biotechnological relevance. Their use ranges from textiles, beer, liquor, bakery, infant feeding cereals, starch liquefaction-saccharification and animal feed industries to the chemical and pharmaceutical ones. Currently, large quantities of microbial amylases are commercially available and are almost entirely applied in starch hydrolysis in the starch-processing industries. The species *Aspergillus* and *Rhizopus* are highly important among the filamentous fungus for the production of amylases. In the production of amyloglucosidase, the species *Aspergillus niger, A. oryzae, A. awamori, Fusarum oxysporum, Humicola insolens, Mucor pusillus, Trichoderma viride* . Species Are producing amylase. *Aspergillus niger, A. fumigatus, A. saitri, A. terreus, A. foetidus foetidus, Rhizopus, R. delemar* (Pandey *et al.*, 2005), with special emphasis on the species of the genera *Aspergillus sp., Rhizopus sp. and Endomyces sp* (Soccol *et al.*, 2003). In fact, filamentous fungi and the enzymes produced thereby have been used in food and in the food-processing industries for decades. In fact, their GRAS (Generally Recognized as Safe) status is acknowledged by the U.S. Food and Drug Administration in the case of some species such as *Aspergillus niger* and *Aspergillus oryzae*. The food industry use amylases for the conversion of starch into dextrins. The latter are employed in clinical formulas as stabilizers and thickeners; in the conversion of starch

into maltose, in confectioneries and in the manufacture of soft drinks, beer, jellies and ice cream; in the conversion of starch into glucose with applications in the soft-drinks industry, bakery, brewery and as a subsidy for ethanol production and other bio-products; in the conversion of glucose into fructose, used in soft drinks, jams and yoghurts (Aquino *et al.*, 2003, Nguyen *et al.*, 2002). Amylases provide better bread color, volume and texture in the baking industry. The use of these enzymes in bread production retards its aging process and maintains fresh bread for a longer period. Whereas fungal amylase provides greater fermentation potential, amyloglucosidase improves flavor and taste and a better bread crust color. Amylases are the most used enzymes in bread baking. Amylases have an important role in carbon cycling contained in starch by hydrolyzing the starch molecule in several products such as dextrins and glucose. Dextrins are mainly applied in clinical formulas and in material for enzymatic saccharification. Whereas maltose is used in confectioneries and in soft drinks, beer, jam and ice cream industries, glucose is employed as a sweetener in fermentations for the production of ethanol and other bioproducts. The above amylases break the glycosidic bonds in the amylose and amylopectin chains. Thus, amylases have an important role in commercial enzymes. They are mainly applied in food, drugs, textiles and paper industries and in detergent formulas. Results from strains tested for the potential production of amylases, kept at 4°C during 10 days, indicated that the wild and mutant strains still removed the nutrients required from the medium by using the available substrate. This fact showed that cooling maintained intact the amylase's activities or that a stressful condition for the fungus caused its degradation and thus consumed more compounds than normal (Smith *et al.*, 2010). The best enzyme activity of microbial enzymes occurs in the same conditions that produce the microorganisms' maximum growth. Most studies on the production of amylases were undertaken from mesophilic fungi between 25 and 37°C. Best yields for amylase were achieved between 30 and 37°C for *Aspergillus sp.*30°C for A. niger in the production of amyloglucosidase 30°C in the production of amylase by A. Oryza(Tunga,R., Tunga B.S, 2003) 55°C by thermophile fungus *Thermomonospora*, and 50°C by *T. lanuginosus* in the production of amylase. However, no reports exist whether increase in enzyme activity after growth of fungus in ideal conditions and kept refrigerated at 4°C for 10 days has ever been tested.

2.4 XYLANASE

Xylanases are hydrolases depolymerizing the plant cell component xylan and are the second most abundant polysaccharide. Xylanases are produced by fungi, bacteria, yeast, marine algae, protozoans, snails, crustaceans, insect, seeds, etc., but the principal commercial source is filamentous fungi. Genes encoding xylanases have been cloned in homologous and heterologous hosts with the objectives of overproducing the enzyme and altering its properties to suit commercial applications. There has been much industrial interest in xylanases, as a supplement in animal feed, for the manufacture of bread, food and drinks, textiles, bleaching of cellulose pulp, and xylitol production. Use of xylanases could greatly improe the overall economics of processing lignocellulosic materials for the generation of liquid fuels and chemicals. Recently, cellulase-free xylanases have received great attention in the development of environmental friendly technologies in the paper and pulp industry. In microorganisms that produce xylanases, low molecular mass fragments of xylan and their positional isomers play a key role in regulating its biosynthesis. Xylanase and cellulase production appear to be regulated separately, although the pleiotropy of mutations, which causes the elimination of both genes, suggests some linkage in the synthesis of the two enzymes. Future work on understanding the functional significance of xylanase multiplicity, the mechanisms of xylanase pre-bleaching, and the structural confirmation of xylanases could lead to improved or alternative applications of xylanases. Xylanase production has been reported in a wide spectrum of microorganisms, including bacteria, actinomycetes, yeasts and filamentous fungi. In the past few years, xylanases have drawn significant attention of the scientific fraternity due to their widespread biotechnological applications such as: (i) in pretreatment of lignocellulosic waste to simple sugars; (ii) production of bio-butenol; (iii) animal feed processing; (iv) improvement of bread quality; (v) biobleaching of fabrics; (vi) pulp bleaching; (vii) silage production; and (viii) treatment of organic waste. The main bottleneck in commercial applications of xylanase-based enzymatic processes is the bulk production of xylanases at an economically viable rate. Therefore, it is exigent to work on cost-effective strategies for large-scale production of xylanases by microbes.

2.5 Application of Microbial Xylanase

Interest in microbial xylanases has increased markedly because of their wide range of potential biotechnological applications in pulp and paper industry, production of xylo-

oligosaccharides, texture improvement of bakery products, textile industry, nutritional improvement of pig and poultry feed, fruit softening and clarification of juices and wines, bioconversion of lignocellulosic material and agro-wastes to fuels and chemical feed stocks, production of pharmaceutically active polysaccharides for use as antimicrobial agents or antioxidants, detergents, extraction of coffee, plant oils and pigments, and degumming of plant fibers such as flax, hemp and jute (Wong *et al.,* 1998).

2.5.1Pulp biobleaching in pulp and paper industry

The main application of xylanases is in the bleaching of pulp. The conversion of wood into paper involves pulping (often kraft pulping) and bleaching. Kraft process involves pre-treatment of wood shavings with a combination of NaOH and sodium sulphide at 165°C in a digester. During this process, about 90-95% of the hemicellulose and lignin are dissolved and partially degraded. The deposited lignin imparts a dark color to the pulp (Damiano *et al.,* 2003). This is followed by washing and pre-bleaching of the brown mass to remove minor impurities and a part of the remaining lignin. Subsequently, chemical bleaching is carried out which may use ozone, chlorine, chlorine dioxide, hydrogen peroxide and sodium hydroxide. The main advantage of the Kraft process is the possibility of recovering the chemical products from the black liquor. On the other hand, the disadvantages are the high initial costs, the strong smell of gases emitted by the process, low yield (40–50%) and the high cost of bleaching (Damiano *et al.,* 2003). The use of chlorine based bleaching results in the production of organochlorine compounds which are discharged in the effluent. These compounds are highly toxic, mutagenic, persistent and harmful to biological systems (Bajpai and Bajpai, 1999). Environmental regulations have restricted the use of chlorine compounds in bleaching processes in the paper and cellulose industries, especially in Western Europe and North America. Special attention has been given to using xylanase in pre-bleaching, which would lower the amount of chlorine compounds used by up to 30%, so that a 15–20% reduction in organo-chlorines in the effluents could be achieved (Bajpai, 2004). The utilization of xylanases could lead to the replacement of 5–7 kg of chlorine dioxide per ton of Kraft pulp thereby reducing environmental pollution (Polizeli *et al.,* 2005).

2.5.2 Fruit juice clarification

Fruit juices obtained by simple extraction are cloudy, viscous and turbid (Uhlig, 1998). Visual perception of turbidity in fruit juices is the result of finely suspended particles in it. The cloudy juices have low yield, less acceptability and are difficult to pasteurize and concentrate (Rai *et al.*, 2003). Except for citrus juices, most industrially processed fruit based beverages, including both juices and wines, are clarified during processing in order to avoid undesirable turbidity, haze, and sediments in the final products (Pozeli *et al.*, 2005). Enzymes are used to obtain optimal juice clarity, yield and a quality product that ensures consumer appeal. Plant cell wall degrading enzymes such as xylanase, hemicellulases and cellulases are used industrially to improve theclarification and yield of fruit juice and to enhance the quality of product (Abdullah *et al.*, 2014).

2.5.3 Textile industry

The xylanolytic complex can be used in the textile industry to process plant fibres, such as of flax, hemp, jute, sisal and bast, cotton and jute. For this purpose, the xylanase should be free of cellulolytic enzymes. (Garg *et al.*, 2012), reported that enzymatic treatment of jute resulted in release of more reducing sugar, weight loss, increase the whiteness and brightness of fabric and decrease the yellowness of the fibric compared to conventional process.

2.5.4 Baking industry

Xylanases are also used in wheat flour for improving dough handling and quality of baked products (Garg *et al.*, 2001). Enzymatic hydrolysis of non-starch polysaccharides leads to the improvement of Rheological properties of dough, bread specific volume and crumb firmness. Xylanases break down the hemicellulose in wheat-flour, helping in the redistribution of water, increase the bread volumes, delay crumb formation, improve resistance to fermentation, leaving the dough softer and easier to knead. For application of xylanase in the baking industry, it must be active at temperatures below 35°C (Juturu and Wu, 2011).

The enzyme can even substitute the addition of different emulsifiers and other chemical additives used in bread production. Xylanase improves the bread quality with an increase in specific bread volume. Xylanases are used as additives in the baking industry to increase the elasticity of the gluten network. Elasticity improves handling and stability of the

dough. Several xylanases from bacterial and fungal sources are used in baking industries (Pariza and Johnson, 2001).

2.5.5 Animal feed

Xylanases are used in animal feed industry along with others enzymes to break down arabinoxylans present in the feed, reducing the viscosity of the raw material. The arabinoxylan found in the cell walls of grains have an anti-nutrient effect on poultry. Xylanase is added to feed containing maize and sorghum (both of which are low viscosity foods) so as to improve the digestion of nutrients in the initial part of the digestive tract, resulting in a better use of energy (Polizeli *et al.*, 2005). Endoxylanase and cellulose treatment of forages produces better quality silage that improves the subsequent rate of plant cell wall digestion by ruminants. As a result of endo-1,4-β-xylanase treatment, there is increased nutritive sugar and that is useful for digestion in cow and other ruminants. Thermo-stable xylanase would be beneficial in animal feeds if added to the feeds before the pelleting process (typically carried out at 70–95 °C). In addition, for this latter application the enzyme must be highly active at the temperature (approximately 40 °C) and pH (approximately pH 4.8) of the digestive tract (Collin *et al.*, 2006).

2.5.6 Xylooligosaccharide and xylitol production

Xylanases may be used for the production of xylooligosaccharides, which act as probiotics. Xylooligosaccharides containing 2–6 xylose units linked via β-(1–4)-xylosidic linkages can be generated from abundantly available xylan containing agro-residues. The production of value-added saccharides from lignocellulosic materials would be an interesting and emerging alternative because these raw materials are renewable and do not compete with food crops and are also less expensive than the conventional agricultural feed stocks.(Anand *et al.*, 2013) There are many applications of xylanases which can be exploited for industrial use in bioconversion of lignocellulosic waste to value added products.

2.6 Xylanase producing microorganisms

Microorganisms have been regarded as a good source of useful enzymes because they multiply at extremely high rates and synthesize biologically active products that can be controlled by humans. In recent years, there has been a phenomenal increase in the use of enzymes as industrial catalysts. These enzymes offer advantages over the use of conventional chemical catalysts for numerous reasons: they exhibit high catalytic activity and a high

degree of substrate specificity, they can be produced in large amounts, they are highly biodegradable, they pose no threat to the environment and they are economically viable. Xylanases derived from microorganisms have many potential applications in the food, feed, and paper pulp industries (Collins *et al.*, 2005). Complete xylanolytic enzyme systems,which including all of these activities, have been found to be widespread among fungi (Sunna *et al.*, 1997 and Belancic,1995), actinomycetes (Eligir, 1994) and bacteria, and some of the most important xylanolytic enzyme producers include *Aspergillus, Trichoderma, Streptomyces, Phanerochaetes, Chytridiomycetes, Ruminococcus, Fibrobacteres, Clostridia* and *Bacillus* (kulkari *et al.*, 1999).

The ecological niches of these microorganisms are diverse and widespread and typically include environments where plant materials accumulate and deteriorate, as well as in the rumen of ruminants. Although there have been many reports on microbial xylanases since the 1960s, the prime focus has been on plant pathology related studies (lebeda *et al.*,2001). Only during the 1980's did the use of xylanases for biobleaching begin to be tested (Viikari *et al.*,1994). Since 1982, several microorganisms including fungi and bacteria, have been reported to readily hydrolyze xylans by synthesizing1,4-β-D endoxylanases (E.C. 3.2.18) and β-xylosidases (EC.3.2.1.37). The production of xylanases must be improved by finding more potent fungal or bacterial strains or by inducing mutant strains to excrete greater amounts of the enzymes. Moreover, the level of microbial enzyme production is influenced by a variety of nutritional and physiological factors, such as the supply of carbon and nitrogen, physical circumstances and chemical conditions (Nagar *et al.*, 2010).

2.6.1 Fungi

Filamentous fungi are particularly interesting producers of xylanases and other xylan-degrading enzymes because they excrete the enzymes into the medium and their enzyme level are much higher than those of yeast and bacteria. In addition to xylanases, fungi produce several auxiliary enzymes required for the degradation of substituted xylan (Polizeli *et al.*, 2005).The fungal genera *Trichoderma*, *Aspergillus*, *Fusarium*, and *Pichia* are considered great producers of xylanases (Adsul *et al.*, 2005). White-rot fungi have also been shown to produce extracellular xylanases that act on a wide range of hemicellulosic materials, are useful as food sources (Buswell, 1994) and produce metabolites of interest to the pharmaceutical, cosmetic, and food industries (Quinnge *et al.*, 2004). White-rot basidiomycetes normally secrete large amounts of these enzymes to degradelignocellulosic materials. For example, *Phanerochaete chrysosporium* produces high levels of α-

glucuronidase (Castaniers *et al.,* 1995), and *Coriolus versicolor* produces a complex xylanolytic combination of enzymes (Abd El-Nasser *et al.*,1997). Xylanase is also produced by *Cuninghamella subvermispora* when growing on plant cell-wall polysaccharides or on wood chips (Souza-Cruz *et al.*, 2004).

Fungal xylanases are generally associated with celluloses (Steiner *et al.*, 1987). On cellulose these strains produce both cellulase and xylanase, which may be due to traces of hemicellulose present in the cellulosic substrates (Gilbert *et al.*, 1993); however, selective production of xylanase may be possible using only xylan as the carbon source. The mechanisms that govern the formation of extracellular enzymes with regards to the carbon sources present in the medium are influenced by the availability of precursors for protein synthesis. Therefore, in some fungi, growing the cells on xylan uncontaminated by cellulose under a lower nitrogen/carbon ratio may be a possible strategy for producing xylanolytic systems free of cellulases [Beily, 1994]. Another major problem associated with fungi is the reduced xylanase yield in fermenter studies. Agitation is normally used to maintain the medium homogeneity, but the shearing forces in the fermenter can disrupt the fragile fungal biomass, leading to the reported low productivity. Higher rates of agitation may also lead to hyphal disruption, further decreasing the xylanase activity [Subramaniyan *et al.*, 2000].

2.6.2 Bacteria

Xylanases have been reported in *Bacillus*, *Streptomyces* and other bacterial genera that do not have any role related to plant pathogenicity (Estebar *et al.*, 1982) The extreme thermophile *Rhodothermus marinus* has been reported to produce α-L-arabinofuranosidase (Gomes *et al.,* 2000), and two different polypeptides with α-arabinofuranosidase activity from *Bacillus polymyxa* were characterized at the gene level for the production of α-arabinofuranosidases (Morales *et al.,*1985). Bacteria, just like many other industrial enzymes, have fascinated researchers due to their alkaline-thermostable xylanase-producing trait (Subramaniyan *et al.*, 2002). The optimum pH of bacterial xylanasesare, in general, slightly higher than the optimal pH of fungal xylanases (khasin *et al.,*1985), which is a suitable characteristic in most industrial applications, especially the paper and pulp industries.

Noteworthy producers of high levels of xylanase activity at an alkaline pH and high temperature are *Bacillus* spp. (Subramaniyan *et al.*, 2002). When considering only temperature, a handful of xylanases that's how optimum activity at higher temperatures have been reported from various microorganisms. These include *Geobacillus thermoleovorans*, *Streptomyces sp*, *Bacillus firmus*, *Actinomadura sp* and *Saccharopolyspora pathunthaniensis*,

all of which produce xylanases that show activity between 65 and 90°C (Verma *et al.*, 2012). One xylanase, reported from *Thermotoga sp* (Yoon *et al.*, 2004), has been shown to exhibit a temperature optima between 100 and 105 °C.

2.7 Production of xylanases under solid state fermentation and submerged fermentation

Microbial xylanase may be produced in submerged (SmF) or solid substrate fermentation (SSF). SmF involves growth of the desired microorganism as a suspension in liquid medium in which various nutrients are either dissolved or suspended as particulate solids. It is the preferred method for the production of most of the commercial enzymes, principally because sterilization and process control are easier in these systems. About 90% of the total xylanase are produced worldwide by most of the xylanase using submerged fermentation techniques (Polizeli *et al.,* 2005). On the other hand, SSF is the growth of microorganisms on moist substrates in the absence or near absence of free-flowing water. The solid substrates act as source of carbon, nitrogen, minerals and growth factors and have the capacity to absorb water in order to provide natural habitat and growth requirements of microbes. Enzyme production in SSF is usually much higher than that of submerged fermentation (Haltrich *et al.*, 1996). Enzyme production in SSF offer several economical and practical advantages over submerged cultivation such as need of simpler equipment, a simple fermentation medium, requirement of less energy, higher product yield, reduced waste water output, lower capital and operational costs, low catabolic repression, and does not require a rigorous control of fermentation parameters (Pandey *et al.*, 1999). Bacterial systems are being increasingly investigated for the production of enzymes and metabolites through SSF.

Xylanase production in SSF is of interest in many countries with abundant biomass and agro-residues. Culture conditions for xylanase production in SmF or SSF may differ for various bacterial strains/species. Each bacterial strain may have a different set of optimal conditions for maximum enzyme yield. Therefore, optimization of culture conditions is essential for obtaining maximum enzyme yield from the desired bacterial strain. Optimum concentration of inoculum is necessary for maintaining balance between proliferating biomass and available nutrients to produce maximum enzyme level. Inoculum size required for SSF is generally much larger than that required for SmF. Cultivation period for the

enzyme production is the time taken by the microorganism for utilizing the available nutrients from the culture medium for the synthesis of desired product. The period of incubation depends upon the type of fermentation, growth of microorganism and its growth pattern. Generally, the incubation period required by a microorganism for xylanase production in SSF is longer as compared to SmF. Further, the duration of incubation period also varies according to the microorganism being smaller for bacterial cultures as compared to fungi because the growth rate of the former is faster than the latter. (Corrol and Ortega, 2006)

Incubation temperature and pH are the most important physical variables affecting the microbial growth and enzyme production. Each bacterial strain results in maximum enzyme yield at its unique optimum growth temperature and pH of the production medium. The pH of the production medium may change during fermentation due to metabolic activities of the microorganism. Different types of microorganisms, species and genus require unique cultivation temperature and pH for enzyme production. The optimum pH for xylan hydrolysis is around five for most fungal xylanases and they are normally stable between pH values of two and nine. The pH optima of bacterial xylanases are generally slightly higher than the pH optima of fungal xylanases. Alkalophilic *Bacillus* species and actinomycetes produce xylanases with high activity at alkaline pH value (Biely, 1985). Most of the fungal xylanases tolerate temperatures below 50°C (Subramaniyan and Prema, 2002). As compared to fungi, bacteria are good source of alkaline and thermo stable xylanases (Kumar *et al.*, 2013).Carbon and nitrogen source are the main components of the production medium required for the growth of microorganism. The choice of substrate is important for the enzyme production. Xylanases are usually inducible enzymes secreted in media containing pure xylan or xylan-rich residues (Balakrishnan *et al.*, 1997).

Various lignocellulosic materials, such as wheat bran, wheat straw, corn cobs, sugarcane bagasse, cassava bagasse, barley bran rice straw,and saw dust have been reported to act as good inducers of xylanase synthesis (Beg *et al.*, 2000). Nitrogen source can be provided in either inorganic (KNO3,ammonium chloride, ammonium di-hydrogen phosphate etc) or organic form (peptone, yeast extract, beef extract etc.), the later being more effective in stimulating xylanase production in most fermentation processes. Agitation and aeration are generally used to meet the oxygen demand and uniform mixing of nutrients during fermentation process.(Dhillon 2000). Although most xylanase manufacturers produce these enzymes using submerged fermentation (SmF) techniques (nearly for 90% of the total xylanase sales worldwide) (Polizeli *et al.*,2005) the enzyme productivity via solid-

state fermentation (SSF) is normally much higher than that of submerged fermentation (Agnihotri *et al.*,2010). The growing interest in using solid-state fermentation (SSF) techniques to produce awide variety of enzymes, including xylanases from fungal origins, is primarily due to the economic and engineering advantages of this process (Pandey *et al.*,1999).

The advantages of SSF processes over SmF include a low cultivation cost for the fermentation, lower risk of contamination (Beg *et al.*, 2001), improved enzyme stability, mimicking the natural habit of the fungus, production of enzymes with higher specific activities, generation of a protein-enriched by-product, and easier downstream processing of the enzymes produced (Considine *et al.*,1989). SSF conditions are especially suitable for the growth of fungi, as these organisms are able to grow at relatively low water activities, contrary to most bacteria and yeast, which will not proliferate under these culture conditions (Corral *et al.*, 2006).

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The choice of the substrate is of great importance for the selection of the fermentation process and the successful production of xylanases. In this context, purified xylans can be excellent substrates because the low molecular weight compounds derived from them are the best xylanase inducers. The use of such substrates has led to increased yields of xylanase production and a selective induction of xylanases, with concomitantly low cellulase activity in a number of microorganisms. However, for large-scale processes other alternatives have to be considered due to the cost of such substrates. Some lignocellulolytic substrates such as barley husk, corn cobs, hay, wheat bran or straw have been compared in relation to pure substrates, and many have performed significantly better than isolated xylans (or celluloses) with respect to theyields of xylanase in large-scale production processes. Solid-state fermentation processes arepractical for complex substrates, including agricultural, forestry and food processing residues and wastes, which are used as inducing carbon sources for the production of xylanases (Corral *et al.*, 2006).

The use of abundantly available and cost-effective agricultural residues, such as wheat bran, corn cobs, rice bran, rice husks, and other similar substrates, to achieve higher xylanase yields via SSF allows the reduction of the overall manufacturing cost of biobleached paper. This has facilitated the use of this environmentally friendly technology in the paper industry (Beg *et al.*, 2007).

2.8 Cloning and expression of xylanases

To meet specific industrial needs, an ideal xylanase should have specific properties, such asstability over a wide range of pH values and temperatures, high specific activity, and strong resistance to metal cations and chemicals (Qui *et al.*, 2010). Other specifications include cost-effectiveness,eco-friendliness, and ease of use (Taibi *et al.*, 2010). Therefore, most of the reported xylanases do not possess all of the characteristics required by industry (Verma *et al.*, 2012). Native enzymes are not sufficient to meet the demand, due to low yields and incompatibility of the standard industrial fermentation processes (Ahmed *et al.*,2009). Therefore, molecular approaches must be implemented to design xylanases with the required characteristics (Verma *et al.*, 2012). Heterologous expression is the main tool for the production of xylanases at the industrial level (Ahmed *et al.*,2009). Protein engineering (alteration or modification of existing proteins) by recombinant DNA technology could be useful in improving the specific characteristics of existing xylanases (Verma *et al.*, 2012). Genetic engineering and recombinant DNA technology allow the large-scale expression of xylanases in homologous or heterologous protein-expression hosts. As industrial applications require cheaper enzymes, the elevation of expression levels and efficient secretion of xylanases are vital for ensuring the viability of the process (Juturu *et al.*, 2011).

An increasing number of publications have described numerous xylanases from several sources and the cloning, sequencing, mutagenesis and crystallographic analysis of these enzymes (Kulkarni *et al.*, 1999). The available amino acid sequence data, X-ray crystallographic data, molecular dynamics and computational design of xylanases provide information that authenticates the relationship between the structure and function of xylanases. All of these methods aid in the design of xylanases that are required in industrial processes, such as improvement of the stability of xylanases at higher temperatures and alkaline pHs (Verma *et al.*, 2012). To attempt these processes for commercial purposes, genes encoding several xylanases have been cloned in homologous and heterologous hosts (kulkarni *et al.*,1999, perez j *et al.*, 2002). Recombinant xylanases have shown equivalent or better properties than the native enzymes, and the xylanase genes from anaerobic microorganisms have also been expressed successfully in hosts that can be employed in the fermentation industry (Ahmed *et al.*, 2009).

2.9 Occurrence of Xylanases (EC 3.2.1.8)

Xylanases are produced mainly by microorganisms and take part in the breakdown of plant cell walls, along with other enzymes that hydrolyze polysaccharides. Microbial sources of xylanases include bacteria (Beg *et al.*, 2001; Sunna and Antranikian, 1997) actinomycetes

(Kumar *et al.*, 2013) and fungi (Polizeli *et al.*, 2005;Sunna and Antranikian, 1997). Xylanases can also be found in marine algae, protozoans, crustaceans, insects, snails and seeds of land plants (Sunna and Antranikian, 1997; Kuhad *et al.*, 1997).

Bacterial xylanases mainly belong to the genus *Bacillus*, C*ellulosimicrobium Chromohalobacter, Geobacillus, Paenibacillus, Flavobacterium* and *Arthrobacter.* Among the bacteria, *Bacilli*are the most important xylanolytic enzyme producers, which are known to produce elevated levels of xylanase exhibiting activity at alkaline pH and high temperature (Beg *et al.*, 2001 ; Mamo *et al.*, 2006; Sunna and Antrakian, 1997).

2.10 Lignocellulose

Lignocelluloseis the name given to the material present in the cell wall of higher terrestrialplants, made up of micro fibriles of cellulose embedded in an amorphous matrix of hemicellulose and lignin (Martínez *et al.*, 2009). These three types of polymers are strongly bonded to one another and represent more than 90% of the vegetable cell's dry weight. The quantity of each polymer varies according to the species, harvest season and, also, throughout different parts of the same plant. In general, softwoods (gymnosperms such as pine and cerus) have higher lignin content than hardwoods (angiosperm such as eucalyptus and oak).

Hemicellulose content, however, is higher in gramineous plants. In average, lignocellulose consists of 45% of cellulose, 30% of hemicellulose and 25% of lignin (Glazer and Nikaido, 2007). Lignocellulosic materials also include agricultural residues such as straws, stalks, cobs, bagasses,sawdust, groundnut shells. Agro-industrial Wastes as substrates for microbial enzymes production. Lignocellulose is the world's main source of renewable organic matter and the chemical properties of its components make it a materials of great biotechnological value. Therefore,lignocellulosic materials have received growing attention due to the potential of conversion of this material into many high added value products such as chemical compounds, fermentation substrates, feedstock andbiofuels (Demirbsas, 2008).

2.11 Cellulose

Cellulose is the most abundant organic compound on Earth and the main constituent of plant cell walls. It consists of linear chains of approximately 8,000 to 12,000 residues of D-glucoselinked by α-1,4 bonds (Timell, 1967). Cellulose chains exhibit a flat structure, stabilized by internal hydrogen bonds. All alternate glucose residues inthe same cellulose chain are rotated at 180°. One glucose residue is the monomeric unit ofcellulose and the dimer, cellobiose, is the chain's repetitive structural unit (pozeli *et al.*, 2005).

Cellulose chain is polarized, once there is a non- reducing group at one of its end, At the opposite end there is a reducing group. Parallel cellulose chains interact, through hydrogen bonds and vander-waals forces,resulting in microfibriles, which are very extensive and crystalline aggregates (Glazer and Nikaido, 2007). The microfibriles are made up of approximately 30-36 glucan chains, exhibit a 2-10 nm diameter and are cross-linked by other components of the cell wall, such as the xiloglucans (Arantes and Saddler, 2010).

The cellulose microfibriles networks are called macrofibrils, which are organized in lamellas to form the fibrous structure of the many layers of plant cell wall (Glazer and Nikaido, 2007). In cellulose fibers, crystaline and amorphous regions alternate. The crystaline regions arevery cohesive, with rigid structure, formed by the parallel configuration of linear chains,which results in the formation of intermolecular hydrogen bonds, contributing to cellulose insolubility and low reactivity, at same time making its less reactive, water entrance difficult.(Glazer & Nikaido, 2007).

2.12 Hemicellulose

Hemicellulose is the second group of most abundant polyssacharide in plant cell wall different fromcellulose, it is made up of non-crystalline hetero-polyssacharides (Aspinall, 1959). They are also defined as polyssacharides present in plant cell wall and intercellularly (in the middle lamella), extracted from higher terrestrial plant tissues through alkaline treatment or as certain carbohydrates of cereal endosperms, which are non-starch polyssacharides that are described as cereal gum or pentosans (Timell, 1967; Wilkie, 1979).

Based on the chemical properties of its components, including only cell wall polyssacharides non covalently bonded to cellulose made up by α-(1,4)-linked pyranosyl residues that have the *O*-4 in the equatorial region. In a general way, the hemicellulose fraction makes up 15 to 35% of plant biomass, representing a great renewable source of biopolymers which may contain pentoses (α-D-xylose, α-Larabinose), hexoses (α-D-mannose, α-D-glucose, α-D-galactose) and/or uronic acids (α-D-glucuronic, α-D-4-O-methylgalacturonic and α-D-galacturonic acids). Other sugars such as α- L-rhamnose and α-L-fucose may also be present in small amounts and the hydroxyl groups of sugars can be partially substituted with acetyl groups (Gírio *et al.*, 2010). Therefore, hemicllulose classification depends on the type of monomer constituent and these may be called xyloglucans, xylans (xyloglycans), mannans (mannoglycans) and α-(1→3,1→4)-glucans (mixed-linkage α-glucans). Galactans, arabinans and arabinogalactans are also present.

2.13 Lignin

Lignin is a complex organic polymer that form important structural materials in the tissue of plant0s. Aproximately 20% of the total carbon fixed by photosynthesis in land ecosystems is incorporated into lignin. It is a complex and recalcitrant aromatic polymer, without defined repetitive units (Hammel and Cullen, 2008), its precursors are three *p*-hydroxycinnamyl alcohols or monolignols (*p*-coumaryl, coniferyl and sinapyl) and their recently reported acylated forms).

The association of lignin and hemicelluloses occurs through covalent linkages such as benzyl ester bonds with the carboxyl group of 4-*O*-methyl-d-glucuronic acid in xylan andmore stable ether.

2.14 Chemical structure and distribution of xylan (the substrate for xylanase)

Xylan is the substrate for xylanase. It a major component of hemicelluloses present in plant cell walls and is the second most abundant polysaccharide next to cellulose in nature, accounting for approximately one-third of all renewable organic carbon on earth (Biely, 1985; Prade, 1995). The term hemicellulose refers to a group of non-cellulosic polysaccharides which include xylan,xyloglucan (a heteropolymer of D-xylose and D-glucose), glucomannan (a heteropolymer of D-mannose and D-glucose), galactoglucomannan (a heteropolymer of D-galactose, D-glucose and D-mannose), arabinogalactans (a heteropolymer of D-galactose and arabinose) (Shallom and Shoham, 2003).

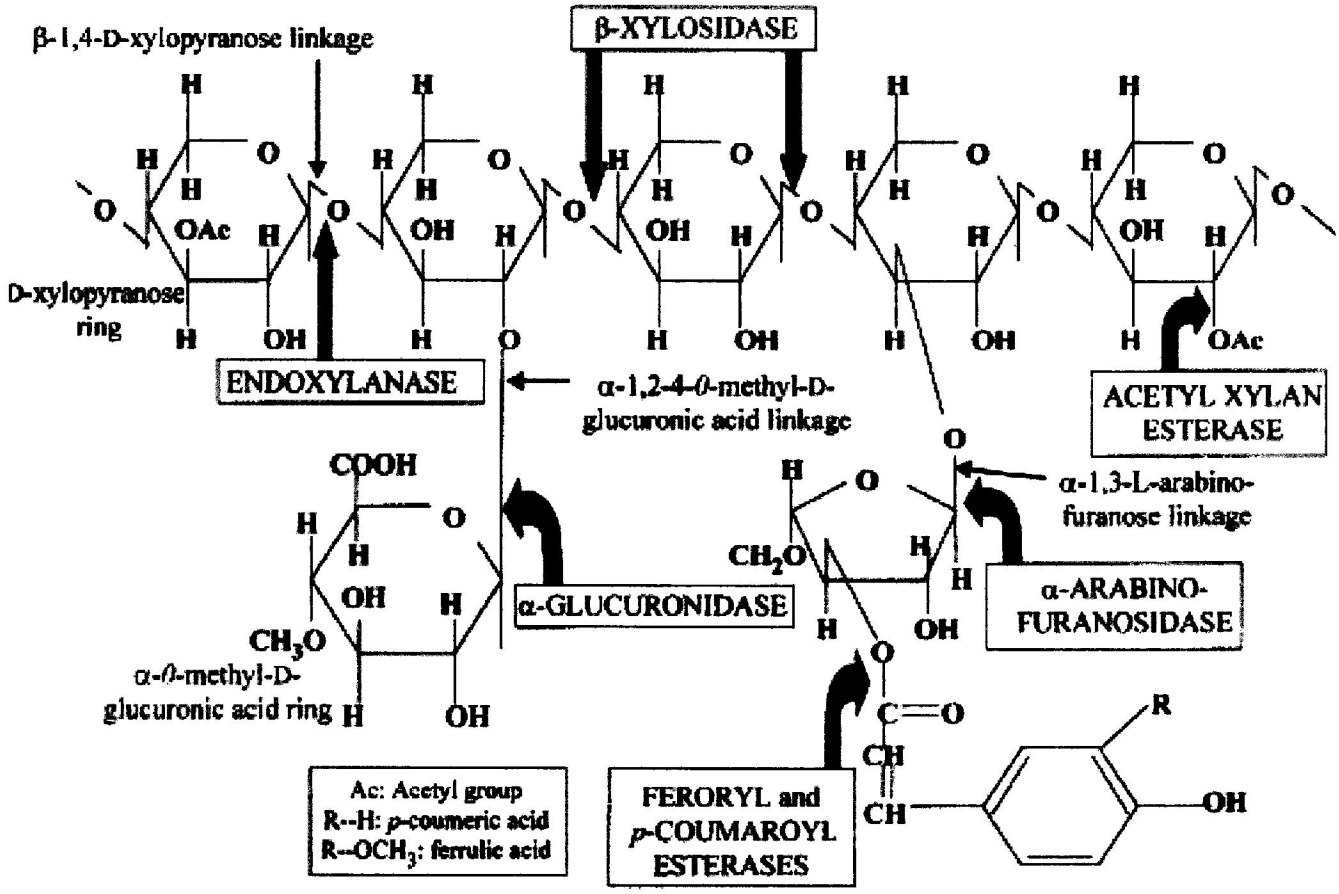
In plant cell wall, hemicelluloses occur in association with cellulose and lignin constituting lignocellulose which on an average contains 35-45% cellulose, 20-30% hemicellulose and 8-15% lignin. β-1,4-xylans are mainly found in secondary walls, the major component of mature cell walls in wood tissue.

A variety of xylanolytic enzymes are produced by microbes. Owing to the heterogeneity and complex chemical nature of xylan, its complete hydrolysis to its constituent sugars requires the concerted action of several enzymes including endo-1,4-β-xylanase(1,4-β-D-xylan xylanohydrolase; EC 3.2.1.8), β-D-xylosidase (1,4-β-D-xylan xylohydrolase; EC 3.2.1.37), α-L-arabinofuranosidase (α-L-Arabinofuranosidase; EC 3.2.1.55), acetylxylan esterase (EC 3.2.1.6), α-D-glucuronidase (α-glucosiduronase; EC 3.2.1.139) ,feruloyl esterase (EC 3.1.1.73) and p-coumaroyl esterase (Beg *et al.*, 2001; Biely, 1985; Kuhad *et al.*, 1997).Complete xylanolytic enzyme systems, including all of the above activities have been found to be quite widespread among fungi (Belancic *et al.*, 1995; Sunna and Antranikian, 1997), actinomycetes (Elegir *et al.*, 1994) and bacteria (Sunna and Antranikian, 1997).

Among xylanolytic enzymes, endoxylanase and β-xylosidase are the key enzymes responsible for xylan hydrolysis. Xylan does not form tightly packed structures and is thus more accessible to hydrolytic enzymes. Consequently, the specific activity of xylanases is 2-3 orders of magnitude greater than for cellulase hydrolysis of crystalline cellulose(Gilbert and Hazlewood, 1993).

Endoxylanase (EC 3.2.1.8) catalyzes cleavage of the internal β-1,4-glycosidic bonds in the xylan backbone producing xylobiose, xylotriose and a small fraction of xylooligosaccharides with higher degree of polymerization. β-xylosidase acts on these xylooligomers releasing xylose. Xylan is not attacked randomly, but the bonds selected for hydrolysis depend on the chain length, degree of branching and the presence of substituents in the substrate molecule (Li et al., 2000). Although many xylanases are knownto release xylose during the hydrolysis of xylan or xylooligosaccharides, xylobiase activity has only been reported in β-xylosidases(Wong et al., 1988).

β-D-Xylosidases (EC 3.2.1.37) catalyzes the hydrolysis of small xylooligosaccharides and xylobiose releasing xylose from the non-reducing terminus. These can be classified according to their relative affinities for xylobiose and larger xylooligosaccharides.



Chemical structure of plant xylan showing the sites of cleavage by different xylanolytic enzyme (Beg *et al.*, 2001).

2.15 Production of Xylanase

Xylanase is produced by diverse genera and species of bacteria, actinomycetes and fungi.while several bacterial species secrete high levels of extra-cellular xylanase, filamentousfungi secrete high amounts of extra-cellular proteins where xylanase secretion oftenaccompanies cellulolytic enzyme (Polizeli *et al.*, 2005). Microbial endo-1,4-β-xylanases (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) are the preferred catalysts for xylan hydrolysis due totheir high specificity, mild reaction conditions, negligible substrate loss, and side productgeneration. However, cost of the enzymatic hydrolysis of a biomass is one of the main factors limiting the economic feasibility of this process. Therefore, the production of xylanase

must beimproved by finding more potent fungal or bacterial strains or by inducing mutant strains that can secrete greater amounts of the enzyme (Dekker *et al.*, 1976).

2.16 Xylanase Distribution among Microorganisms

Xylanases, the xylan hydrolyzing enzymes, are ubiquitous and diverse by nature(Collins *et al.*, 2005). A number of different sources including bacteria (Gilbert *et a*l., 1993;Sunna *et al.*, 1997) fungi (Sunna *et al.*,1997 ;Kuhad *et al.*, 1998).Actinomycetes (Ball *et al.*, 1988) and yeast(Hrmova *et al.*, 1984;. Liu *et al.*, 1998) have been reported to produce xylanases.

Various bacterial and fungal species such as (*Thermomonospora* sp, *Bacillu s*sp, *Melanocarpus albomyces*, *Chaetomium thermophilum*, *Nonomuraea flexuosa*, *Streptomyces* sp., *Dictyoglomus* sp., *Thermotogales* sp., *Thermoactinomyces thalophilus, Thermoascus aurantiacus, Fusarium proliferatum, Clostridium abusonum* and *Arthrobacter* are reported to produce alkalo-thermophilic xylanases (Garg *et al.*, 2011; Khandeparkar *et al.*, 2005).Bacteria just like many industrial enzymes fascinated the researchers for alkalinethermostable endo-1, 4-β-xylanase producing trait. *Bacillus* SSP-34 produced higher levelsof endo-1, 4-β-xylanase activity under optimum nitrogen condition. *Bacillus* SSP-34 produced endo-1, 4-β-xylanase with activity of 506 IU/ml in the optimized medium (Subramaniyan *etal.*,2006). Earlier, (Rattoet *al.* 1992), reported endo-1,4-β-xylanase with an activity of 400 IU/ml from *Bacillus circulans*. It had optimum activity at pH 7 and 40% of the activity was retained at pH9.2. Fungi produce endo-1, 4-β-xylanases which tolerate temperatures up to 50°C. In general, with rare exceptions, fungi are reported to produce endo-1,4-β-xylanases and have an initialcultivation pH lesser than pH 7.0. The pH optima of bacterial endo-1,4-β-xylanases are in general slightly higher than the pH optima of fungal endo-1,4-β-xylanases. In most of the industrial applications, especially paper and pulp industries, the low pH required for the optimal growth and activity of endo-1, 4-β-xylanase necessitates additional steps in the subsequent stages which make fungal endo-1,4-β-xylanases less suitable.(Beg *et al.*, 2001).

2.17 Xylanases: Classification and Characteristics

Endoxylanase (endo-1,4-β-xylanase, EC 3.2.1.8), β-xylosidase (xylan 1,4-β-xylosidase, EC 3.2.1.37), α-glucuronidase (α-glucosiduronase, EC 3.2.1.139), α-arabinofuranosidase (α-L-arabinofuranosidase, EC 3.2.1.55) and acetylxylan esterase (EC 3.1.1.72) constitute a typical xylan-degrading enzyme system (Choi *et al.*, 2000). Endo-β-1,4-xylanases bring about the

catalytic hydrolysis of the rachis of xylan-producing xylooligosaccharides, which in turn can be converted to xylose by β-xylosidase (Zhang *et al.*, 2007).Depending on the amino acid sequence homologies and hydrophobic cluster analysis, xylanases can be classified chiefly into two families of glycosyl hydrolases (GH): (i) family F or GH10; and (ii) family G or GH11 (Jeffries, 1996; Zhou *et al.*, 2009). However, other glycosyl hydrolase families, 5, 7, 8 and 43, have also been reported to have different catalytic divisions with a proven endo-1,4-β-xylanase activity (Collins *et al.*, 2006). Xylanases belonging to family G are of low molecular mass with isoelectric point (pI) values of 8–9.5 as compared with those in family F which are of high molecular mass with lower pI values 6.5-7.0 (Buchert *et al.*, 1995). The favourable pH for xylan hydrolysis has been reported to be around 5 pH for most fungal xylanases (Yu *et al.*, 1987), whereas pH optima of bacterial xylanases is slightly higher than that fungal xylanase

Endo-1,4-β-xylanases

Endo-1,4-β-xylanase (1,4-bβ-D-xylan xylanohydrolase; EC 3.2.1.8) results in the cleavageof the glycosidic bonds in the xylan rachis and retards the degree of polymerization ofstarch (Li *et al.*, 2000). Endoxylanases can be demarcated based on the final productsreleased from the hydrolysis of the xylan rachis (e.g. xylose, xylobiose and xylotriose,arabinose). The optimum activity of endoxylanases occurs at temperatures between 40°C and 80°C, and between pH 4.0 and 6.5. Endoxylanases from bacteria and fungi are single subunit proteins with molecular weights from 8.5 kDa to 85 kDa and pI values between 4.0 and 10.3 and the majority of them are glycosylated (Polizeli *et al.*, 2005).

β –Xylosidases

β -D-Xylosidases (1,4-D-xylan xylohydrolase; EC3.2.1.37) have been classified on the basis of their relative affinities for xylobiose and high molecular weight xylooligosaccharides. β-D-Xylosidases may be monomeric, dimeric or tetrameric with molecular weights ranging from 26 kDa to 360 kDa. They are secreted by different types of bacteria and fungi (Corral and Villasenor-Ortega, 2006). Purified β-xylosidases generally do not hydrolyse xylan, their best substrate is xylobiose and their affinity for xylooligosaccharides is inversely proportional to its degree of polymerization. They are, however, capable of cleaving synthetic substrates such as p-nitrophenyl- and o-nitrophenyl-β- D-xylopyranoside (Polizeli *et al.*, 2005). The optimum temperature for the enzyme activity can differ from 40°C to 80°C, but the majority

of β-xylosidases give optimum activity at 60°C. The thermo-stability of β-xylosidases greatly differs in microorganisms (Rizzatti *et al.*, 2001).

Arabinofuranosidases

There are two types of α-arabinofuranosidases based on their mode of action: exo-α-L-arabinofuranosidase (EC 3.2.1.55), degrading p-nitrophenyl-α-L-arabinofuranosidase and sand endo-1,5-α-L-arabinase (EC 3.2.1.99) hydrolysing linear arabinans (De Vries *et al.*, 2000). Subsequently after release of the arabinose, there is no degradation in the xylan backbone as there will be production of xylooligosaccharides.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sub-culturing of fungal isolates

Already characterized fungi isolate A10 and A29 was sub-cultured on potato dextrose agar to test the viability of the isolate before further analysis. Further sub-culturing was carried out until a pure isolate was observed.

3.2 Collection of lignocellulosic substrate

The lignocellulosic substrates, corn cob, plantain peel, and banana peel were used for the experiment. The substrate gotten was washed, dried and grinded into finer particles to pass through the international standard sieve.

3.3 Basal media preparation

The basal medium was prepared according to Mandel and Sternburg specifications i.e 1litre of distilled water.

3.4 Enzyme production.

About 5grams of substrate was added into conical flask and sterilized at 121°C for 15minutes and 45ml of basal medium was added and then autoclaved. 1ml of the inoculum was added to the mixture and then incubated at 28°C for 6days.

3.5 Enzyme extraction.

After incubation,the crude enzyme was extracted by adding 60ml of 50mM sodium citrate buffer(pH 5.3) and mixed thoroughly and shaked to allow to stand for an hour. It was filtered through Whatmans filter paper. Extract obtained was centrifuged at 8000rpm for 15minutes using a centrifuge.

3.6 Enzyme purification.

Ammonium sulphate precipitation.

Crude enzyme filtrate obtained by growing the fungi at established optimal enzyme production conditions for the isolates were subjected to ammonium sulphate precipitation within limits of 0 to 100% saturation (Dixon and webb,1971). One hundred millilitres (100ml) of crude enzyme filtrate was sequentially treated with solid ammonium sulphate to 0-20, 20-60, 60-80, and 80-100% saturation by slowly adding 11.4g, 26.2g, 14.3g and 15.7g of the salt into the solution till it dissolves respectively. The mixture of each batch of saturation was slowly and continuously stirred until the salt completely dissolved in the medium. After 24hours at 4°C, the precipitate was recovered by centrifugation at 1500g for 15minutes using 4°C. The supernatants were then treated to the next batch of saturation until the required level of saturation was reached. The precipitate of each batch of saturation was then pooled together and re-suspended to initial volume of the crude enzyme filtrate with the sodium citrate buffer.

Dialysis.

Preparation of dialysis tubing.

The dialysis tube were cut into smaller pieces and boiled in de-ionised water for 30minutes and later 30minutes in 1litre of solution containing 0.2M Na2HCO3, 0.01M EDTA and 2 litre of distilled water. After thorough rinsing twice in de-ionised water, the tubes were again boiled in 0.01M EDTA for 10minutes. Thereafter, the tubes were thoroughly rinsed in changes of de-ionised water and 25% ethanol. The tubes were washed with de-ionised water and tied one end with a clean twine rope before used (www.Bioprotocol.com).

Dialysis procedure.

Ten millilitres of the rede-ionised precipitated protein of the isolates culture filtrate in the sodium citrate buffer was poured in the dialysis tube which was then tied at the other end and dialysis against 1litre of the same buffer in a beaker. The dialysis was allowed to take place for 24hours against several changes of the buffer.

3.7 Pretreatment of lignocellulosic substrate

The substrates were alkaline treated by autoclaving the washed and dried substrate at 121°C for 30mintues with 0.25M of NaOH (20ml/g substrate). The substrate was recovered by filtration through whatmans filter paper were thoroughly washed with deionized water and neutralized with 0.25M of HCL. The substrate were finally washed with many changes of deionized water and dried at 65°C in the oven to constant weight (Singh et al. 1988).

3.8 Hydrolysis of substrate

A suspension of substrate (10g/ml) was prepared by adding 100ml of 50M sodium citrate buffer to 1g of the substrate. 15ml each of the substrate suspension was sterilized at 121°C for 20minutes in 250 ml conical flask. 5ml of partially purified enzyme was added to the substrate. Hydrolysis was performed at 50°C for 5days and samples were withdrawn at 24hrs, 48hrs, 72hrs and 144hrs for analysis of reducing sugar produced. The resultant filtrate following filtration was assayed for total releasing sugar using DNSA method and a colour change was observed (Miller 1959), The released sugar is expressed as equivalent to xylose.

CHAPTER FOUR.

4.0 RESULTS

4.1 Viability testing of fungal isolates (A10 and A29).

The already characterized fungal isolates *Trichoderma asperellum* A10 and A29 were sub-cultured on potato dextrose agar to test its viability. After five days, the growth was observed and further sub-culturing was carried out on potato dextrose agar(PDA) until a pure isolate was observed.

4.2 Enzyme production.

After the treatment of the lignocellulosic wastes they were added into the basal medium. Three different lignocellulosic waste were used for the enzyme production which are the banana peel, plantain peel and corn cob. The substrates were used in enzyme production using two different fungal isolates A10 and A29. The enzyme production was carried out in duplicates. The fungal spores were injected into the medium and then incubated for 6days for maximum enzyme production. After which, enzyme extraction was carried out using sodium citrate buffer. The solution was filtered using whatsman filter paper.

4.3 Enzyme purification.

Enzyme purification was carried out by two procedures which include the ammonium sulphate precipitation and the dialysis tubing process which includes the use of dialysis tube for purification. The crude enzyme produced was subjected to ammonium sulphate precipitation within limits 0-100%. The solid ammonium sulphate was completely dissolved in the solution. Precipitates was recovered from the solution after 24hrs. The supernatant were then used for the next batch of the saturation until the required level of saturation was reached. Precipitates of each batch of saturation were pooled together and then re-suspended

to initial volume of each enzyme after which the dialysis tubes were treated and prepared then the dialysis process was carried out on the enzyme.

4.4 Hydrolysis of the substrate.

Hydrolysis was carried out using 15ml of the substrate suspension and 5ml of the purified enzyme. DNSA method was used to test for colour change. Hydrolysis was carried out for four days and samples were withdrawn at interval and the absorbance was read on the spectrophotometer at 540nm.

4.5 Effect of Xylanase of *Trichoderma asperellum* strains on the hydrolysis of corn cob.

Enzyme activity of corn cob at different hours using the Xylanase produced by the two isolates(A10 and A29) were measured and shown at figure1. The highest enzyme activity was recorded for isolates A10 at 96hrs (2.84mg/ml) with enzyme activity of 2.04mg/ml for A29 at 96hrs. There was a rapid increase in enzyme activity from day1 to day 4.

4.6 Effect of Xylanase of *Trichoderma asperellum* strains on the hydrolysis of Banana peel.

Enzyme activity of banana peel at different hours of hydrolysis using the enzyme produced by two fungal isolates (A10 and A29) were measured and represented in figure2. The highest enzyme activity was recorded after 96hrs for both enzymes. For banana peel enzyme activity of 3.48mg/ml was recorded for isolate A10 and enzyme activity of 3.52mg/ml was recorded for isolates A29.

4.7 Effect of Xylanase of *Trichoderma asperellum* strains on the hydrolysis of plantain peel.

Enzyme activity of plantain peel was measured at differemt hours for isolates A10 and A29. They are represented on figure3. The highest enzyme activity was recorded at 96hrs.Enzyme activity of 3.6mg/ml was recorded for isolate A10 and 2.68mg/ml was recorded for A29.

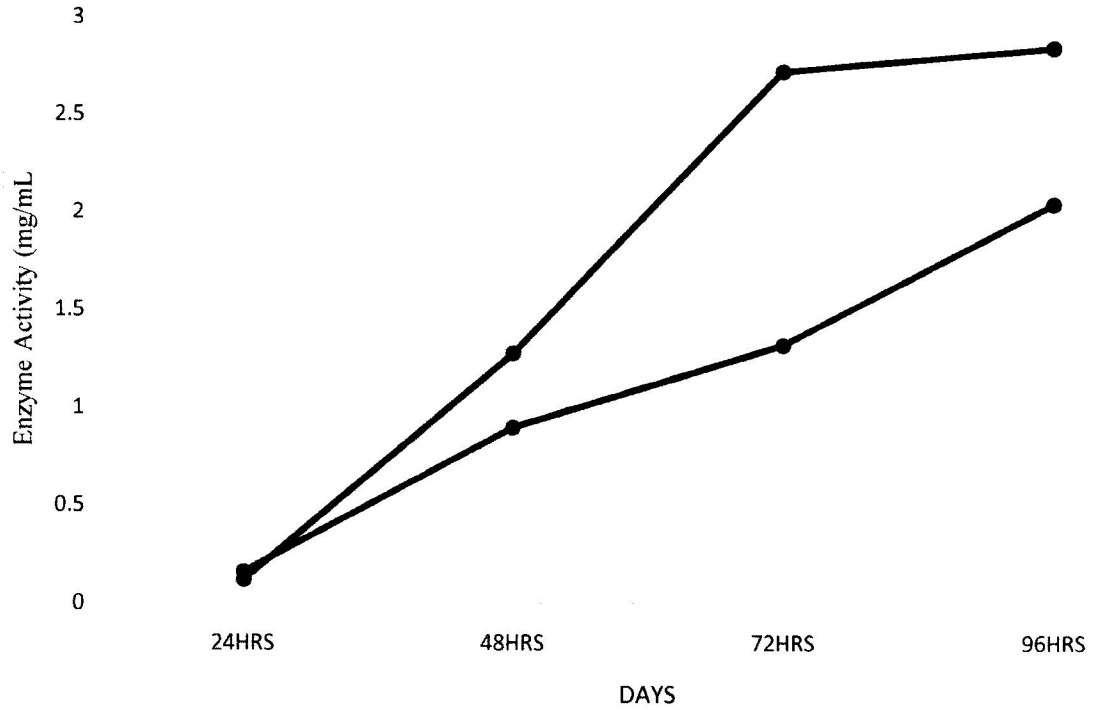


Figure 1: Effect of xylanase of *Trichoderma asperellum* strains on the hydrolysis of corn cob

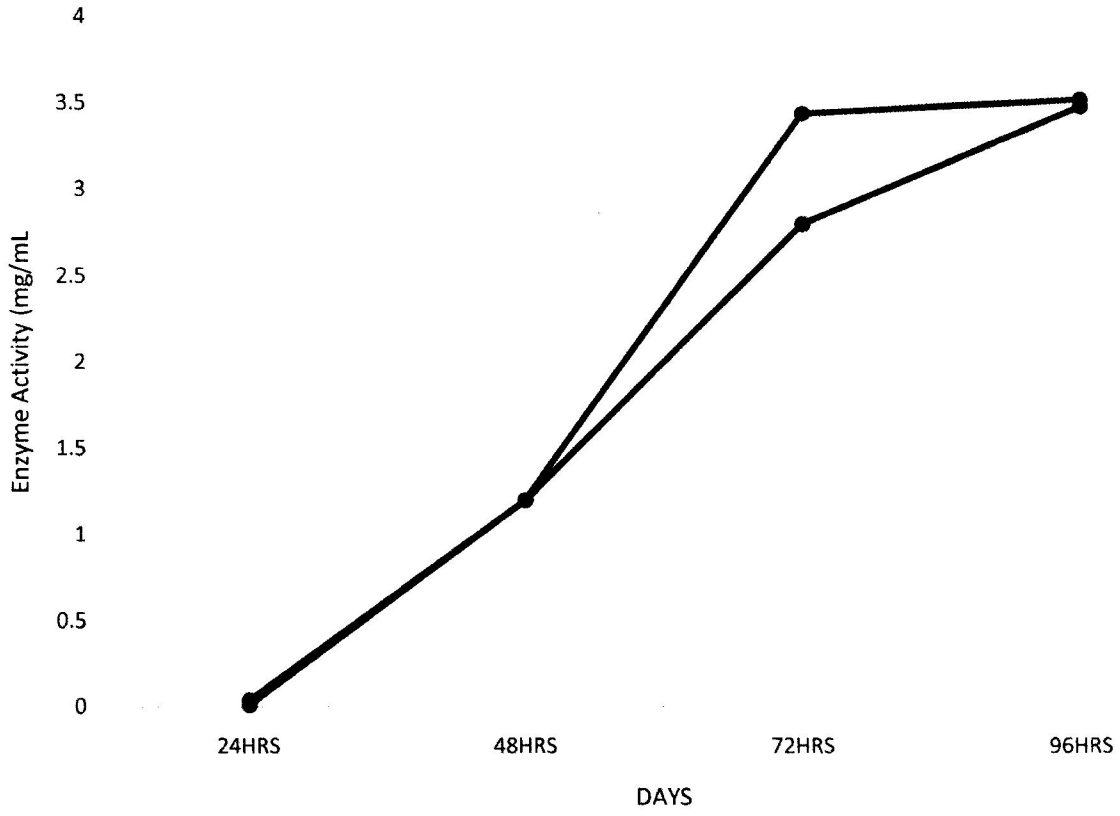


Figure 2: Effect of xylanase of *Trichoderma asperellum* strains on the hydrolysis of Banana peel

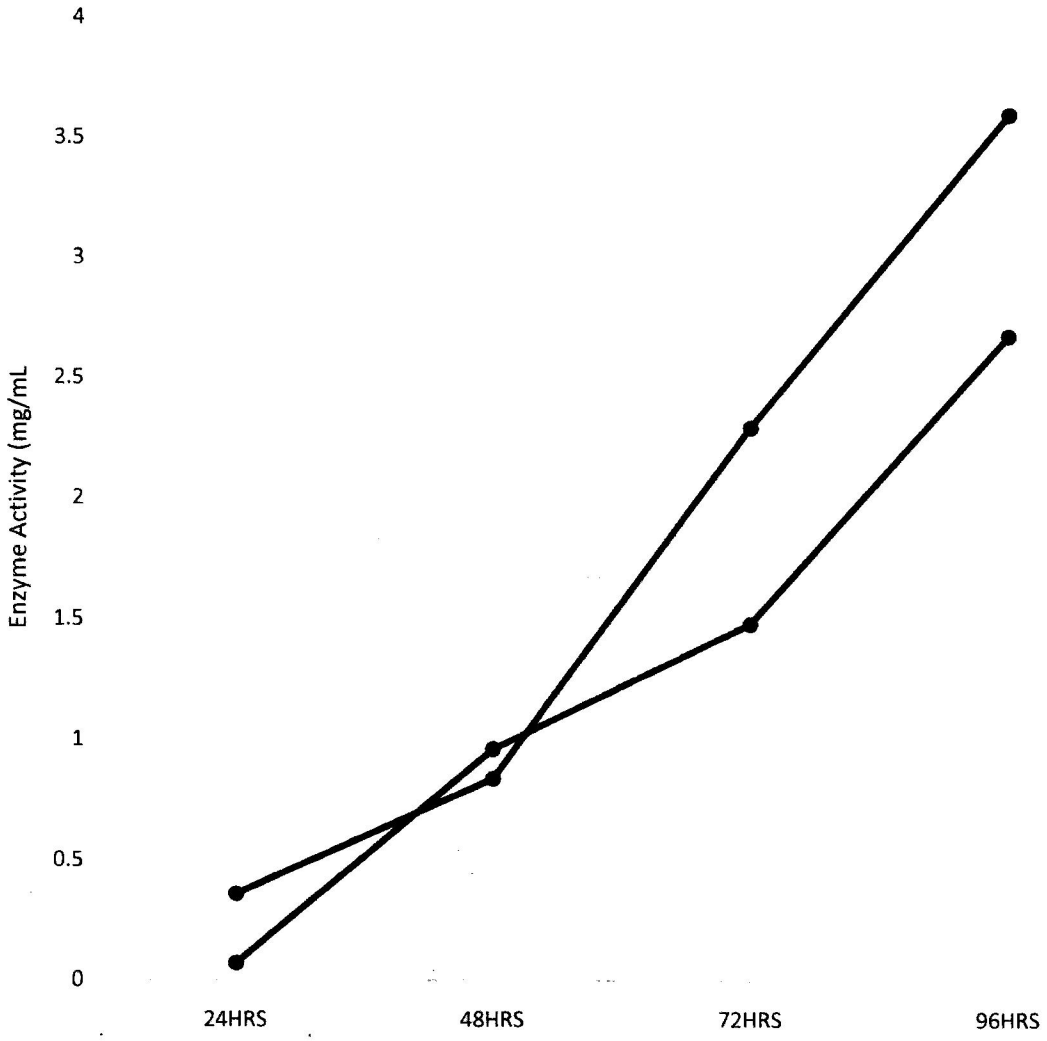


Figure 3 Effect of xylanase of *Trichoderma asperellum* strains on the hydrolysis of Plantain peel

CHAPTER FIVE.

5.0 DISCUSSION.

Trichoderma species have been reported to be a very good producer of enzyme (Bailey *et al.*, 1992). Carbon is highly important in the production of enzymes, xylanase inclusive. The interest in the use of lignocellulosic waste for enzyme production is due to the low cost and ease production of xylanases and also the use of this wastes helps in keeping the environment clean and also in recycling. The ability of the isolates to produce xylanase is in conformity with of Ajijolakewu *at al.*, (2015). Ajijolakewu *et al.*, (2015) had earlier reported the production of xylan degrading potential of *Trichoderma species.*

During the enzyme production process, there are some unwanted impurities produced alongside with the enzyme. This impurities affect the use of the enzyme in the industry. Enzyme purification is necessary to obtain only one kind of enzyme from complex mixture of protein-enzyme, to determine the active site of the enzyme for substrate binding, Purified enzymes are necessary for therapeutics and medical applications, further to study its kinetics. The agro-industrial wastes such as corn cob, banana peel, and plantain peel are good substrates for the induction of xylanolytic enzyme, (Ravindran *et al.*, 2018). All the lignocellulosic wastes used were able to elicit xylanase production to different degrees.

The purified enzyme was applied to for the degradation of the pre-treated substrates(Corn cob, Banana peel and Plantain peel). For the corn cob, the purified enzyme was applied for the degradation of the pre-treated corn cob at different interval of time. There was increase in the amount of sugar generated from 24hrs to 96hrs for the two isolates. The increament in the amount of sugar for A10 after 72hrs was gradual compared with what was obtained between 24 to 72hrs, The initial reluctance of the action of the action of the enzyme before 24hrs might be due to the fact that the enzyme is just being used to the environment. The decrease

in the activity of the enzyme as the time increases might also be due to the fact that the sugar produced was inhibiting the activity of the enzyme due to product catabolic repression (Nwodo *et al.*, 2007). At 96hrs, the amount of sugar increament was not as rapid as that of 24hrs to 72hrs. The reason for this might be as a result of the enzyme being far away from the substrate. For A29, there was also an increament from 24 to 96 hours. There was also an initial reluctance before 24hrs. which might be due to the fact that the enzyme is just getting used to the environment (Crispen *et al.*, 1996). The enzyme is trying to adjust to the conditions of the medium. There was a rapid increament in the amount of sugar at 48hrs. After 72hrs, the increament became gradual. It was not as rapid as before. At 96hrs, there was a noticeable increase in the amount of sugar generated. These imbalances can be as a result of the strains used in the production of the enzyme. (Desai *et al.*, 2016) reported corn cob as a good substrate for the production of xylanase by A.niger DX-23.

Banana peel gave a very high enzymatic activity for the two strains (A10 and A29). This can be because the isolates were gotten from banana peel dump ground so it is used to the system (Crispen *et al.*, 1996). The initial reluctance at 24hrs is always due to the introduction of the enzyme to the substrate. For banana peel, there was an increament in enzyme activity for the two isolates. The high enzymatic activity can also be as a result of high hemicelluloses present in banana peel. For A10, there was an initial increase in enzymatic activity from 24 to 48hrs, the growth was rapid after which there was a great increase in amount of sugar from 48 to 72hrs from 72hrs to 96hrs, there was a little increase this might be as a result of the sugar production inhibiting the enzymatic activity of the enzyme due to product catabolic repression (Nwodo *et al.*, 2007). For A29, there was initial reluctance at 24hrs to 48hrs, the growth was rapid at 48hrs to 72hrs. At 72hrs to 96hrs, the amount of sugar was not as rapid as other hours. This might be because the the produced sugar is trying to cause a catabolic repression but the amount of sugar produced is not sufficient to cause the catabolic

repression. When it is compared with A10, At 48hrs to 72hrs higher sugar were released and it was capable of causing the decrease in enzyme activity.

For plantain peel, the isolate A10 at 24hrs to 48hrs there was a little generation of sugar but from 48hrs to 96hrs there was a noticeable increament in the enzymatic activity this can be that it took a longer hour for the enzyme to acclamatize with the environment (Crispen *et al.*, 1996). and once it got acclamatized, it started generating sugar at a very rapid rate till the 96hours. While for the isolate A29, there was also a rapid generation of sugar from 24hours to 48 hours. From 48 to 72 hours, there was a little reduction in the rate of production of sugar which might be that the enzyme wasn't getting in much contact with the substrate but at 72 to 96hours the sugar generation increased and the production became more rapid (Crispen *et al.*, 1996).

5.2 CONCLUSION

From this work, it can be deduced that agro industrial wastes are good substrates for the induction of xylanlolytic enzyme may be due to the presence of high level of cellulose and hemicellulose in them. The use of this enzyme to replace chemicals used in different industrial sectors will contribute to the reduction in environmental pollution constitute by the chemicals.

5.3 RECOMMENDATION

Further work can be done to purify the enzyme to homogeneiety.

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APPENDIX.

Preparation of media and reagents.

1. Potato Dextrose Agar

2% PDA — 2.0g

0.5% Agar agar— 0.5g

Distilled water— 100ml

2. 1% DNSA

DNSA — 1g

20% Potassium sodium tartarate — 20g

3. 2M NaOH

NaOH — 8g

Distilled water — 100ml