Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.46, 2016
www.iiste.org

# Cellulase Production by Fungi Isolated from Odo-Aremu Dumpsite in Ado-Ekiti, Nigeria

Benjamin A. Oso Abdallah A. Muhammad Pius A Okiki*
Department of Biological Sciences, AfeBabalola University, AdoEkiti, Nigeria

**Abstract**
The study was aimed at using waste-soil fungi for production of cellulase enzyme. Fungi were isolated from soil samples obtained from Odo-Aremu dumping sites in Ado-Ekiti, Nigeria. Three fungal species namely *Aspergillus niger, Aspergillus flavus*and *Trichoderma spp.* were isolated. The fungal isolates were assayed for their ability to produce cellulase enzyme as well as variable conditions that suited the production of the enzyme. Temperature, days of incubation and pH of assay medium were all significant in the enzyme production. Varied chaff concentrations however had no significant influence on enzyme production. All three isolates showed cellulase synthesis best 8 days after incubation at 37$^{o}$C and enzyme activity was assayed to be best at 45$^{0}$C. Enzyme synthesis was also assayed to be best at oatmeal chaff concentration of 3% and enzyme activity was best between pH 4-7.
**Keywords:** Cellulase, Oatmeal chaff, dumpsite, enzyme activity, *Aspergillus, Trichoderma*

## 1.0. Introduction

The term soil refers to the outer loose material of the earth crust. It may be regarded as a three-phase system composed of solids, liquids and gases, dispersed to form a heterogeneous matrix (Davies and Williams, 1999). On the whole, the soil is composed of five major components, these include: Mineral matter, Water, Organic matter, Air and living Organisms. The various components of the soil environment constantly change and the quantities of these constituents are not the same in all soil but vary with locality. Living portion of the soil body composes of small animals and microorganisms; but it is generally considered that microorganisms play the most important role in the release of nutrient and carbon dioxide for plant growth (Davies and Williams, 1999).

The bacteria are usually the most abundant group. Soil bacteria can be rod, (bacilli) cocci (spherical) spirilla (spirals), of these bacilli are more numerous than the others. They are one of the major groups of soil bacteria population and are very widely distributed. Fungi are microscopic cells that usually grow as long threads or strands called hyphae. Hyphae interact with soil particles, roots, and rocks forming a filamentous body that promotes foraging for food. These networks release enzymes into the soil and break down complex molecules that the filaments then reabsorb. Fungi act like natural recycling bins, reabsorbing nutrients in the soil (James, 2011).

The number and type of microorganism present in a particular soil would be greatly influenced by geographical location, soil temperature, soil type, soil pH, organic matter contents, cultivation, aeration and moisture content (Davies and Williams, 1999).

Many microorganisms including fungi and bacteria had been found to degrade cellulose and other plant cell wall fibers. In nature, mixtures of hydrolytic enzymes collectively known as cellulases perform degradation of cellulosic biomass. The cellulases include endo-acting (endoglucanases, EGs) and exo-acting (cellobiohydrolases, CBH) enzymes, which act in a synergistic manner in biomass-degrading microbes (Dashtban *et al.*, 2009). The cellobiose and cello- dextran products of exoglucanases and CBH are inhibitory to their activity. Thus, efficient cellulose hydrolysis requires the presence of β-glucosidases to cleave the final glycosidic bonds of cellobiose-producing glucose (Maki *et al.*, 2009). Cellulolytic fungi can use cellulose as a primary carbon source. Pure, crystalline cellulose, such as SolkaFloc, Avicel, and cotton are good cellulose inducers, but expensive (Ahamed, 2008).

Although a large number of microorganisms are capable of degrading cellulose, only a few of these microorganisms produce significant quantities of cell-free enzymes capable of completely hydrolysing crystalline cellulose in vitro. Fungi are the main cellulase-producing microorganisms (Sundaramoorthi, 2011). Fungi of the genera *Trichoderma* and *Aspergillus* are thought to be cellulase producers, and crude enzymes produced by these fungi are commercially available for agricultural use. Fungi of the genus *Trichoderma* produce relatively large quantities of endo-ß-glucanase and exo-ß-glucanase, but only low levels of ß-glucosidase, while those of the genus *Aspergillus* produce relatively large quantities of endo-ß-glucanase and ß-glucosidase with low levels of exo-ß- glucanase production (Sundaramoorthi, 2011).

To keep costs down, it is therefore important to use a less expensive substrate. Many cellulosic materials such as wood, wastepaper, wheat straw, corncob, wheat bran, and fruit pomace have been studied as potential substrates for the production of cellulase. In this study however, analysis of cellulase production on oatmeal-chaff is investigated.

The aim of this work was to study the potential of using waste-soil fungi for the production of cellulase

 

enzyme as a means of local sourcing of cellulolytic fungi and cost-effective processes for enzyme production.

## 2.0. Materials And Methods

### 2.1. *Soil sample collection*

Soil samples were obtained from Odo-Aremu dumping site, Ado-Ekiti, Nigeria. Soil samples were collected about 10-15cm below the surface of the dump-soil and packed in polythene bag. Soil samples were then transported to the Microbiology laboratory, AfeBabalola University, Ado-Ekiti.

### 2.2. *Isolation of Fungi*

Using soil suspension method also, 1 g of soil sample was taken and mixed with 100ml of sterile distilled water. Serial dilution was then done up to $10^{-5}$ (Williams *et al.,* 1972). A volume of 1ml of each solution was then inoculated onto prepared Potato dextrose agar (PDA) platesand PDA plates were incubated at room temperature of 28$^{o}$C for 3-5 days. Emerging fungi were then sub-cultured onto fresh potato dextrose agar plates.

### 2.3. *Screening of fungal isolates for cellulase production*

For enzyme synthesis, oatmeal chaff medium was used throughout. Oatmeal chaff was obtained by running distilled water over Quaker oats on a muslin cloth. This washed off most of the starch, leaving the chaff, which was then dried, in an oven at 85$^{0}$C for 48 hours. The oatmeal chaff medium was prepared by suspending 20g of the dry chaff in a litre of distilled water together with 5g-yeast extract. This was dispensed into 150-ml conical flasks in 30-ml amounts per flask, care being taken to shake the medium well before dispensing into each flask. The flasks were then autoclaved for 15 minutes at 121$^{0}$C (Oso, 1978).

To obtain the inoculums, the fungi were grown for 3 days at 45$^{0}$C in Petri dishes on starch-yeast-extract-agar (SYEA) with the following composition: soluble starch, 10g; yeast extract, 2g; $KH_2PO_4$, 1g; $MgSO_4.7H_2O$, 0.5g; agar, 20g; distilled water, 1 litre. Each flask of the chaff medium was inoculated with one disk (5mm diameter) of agar and mycelium obtained by using a sterile cork borer. Un-inoculated flaks of the chaff medium served as control (Oso, 1978).

### 2.4. *Enzyme Assay*

After growth had been allowed to proceed for the required length of time at the required temperature, the cultures were filtered and cellulolytic activity of the filtrates was determined. The assay medium was 0.55% carboxymethyl cellulose (CMC) in 0.55M acetate buffer (pH 5.5) and 9ml of this were incubated with 1 ml of the fungus filtrate for 1 hour at 45$^{o}$C. Filtrates of the un-inoculated control were also obtained and similarly assayed, to estimate the amount of reducing sugars released, 1ml of dinitrosalicylic acid (DNSA) reagent was added to 1ml of the filtrate-CMC reaction mixture and the transmittance was determined at 540nm using an SP 600 Spectrophotometer. Transmittance was set at 100% with the filtrate-CMC reaction mixture of the un-inoculated control. Dinitrosalicylic acid reagent was prepared by combining 1.0g DNSA with 20ml 2N NaOH and 20g potassium sodium tartrate in 100ml distilled water. The transmittance of standard aqueous solutions of D-glucose of various concentrations (0-10mg per ml) was determined and used to construct a graph of % transmittance as related to mg of glucose per ml. the amount of reducing sugar produced by 1ml of fungus filtrate from the CMC assay medium was calculated from this graph. Cellulolytic activity of the filtrates was then expressed in terms of the amount of total reducing sugars (RS) per ml. three replicate determinations were carried out in each case and the mean of the three values was taken (Oso, 1978).

### 2.5. *Effect of temperature on cellulase synthesis*

Several flasks of the 2% oatmeal chaff medium were inoculated at 25, 37, and 45$^{0}$C. At 48-hour intervals, three flasks were removed from each temperature, the contents were filtered and cellulase activity of the filtrate was determined at 45$^{0}$C. This was continued for 12 days (Oso, 1978).

### 2.6. *Effect of temperature on enzyme activity*

Flasks of the 2% oatmeal chaff medium were inoculated and incubated at 45$^{0}$C for 5 days. These were subsequently filtered and the cellulase activity of the filtrate was determined at 20, 30, 45, and 50$^{0}$C (Oso, 1978).

### 2.7. *Effect of oatmeal chaff concentration on enzyme synthesis*

Oatmeal chaff medium was prepared as previously described, buy with various concentrations of chaff: 0, 0.25, 1, 2, 3, and 4%. The pH at each concentration was adjusted to 6.8 and each medium was dispensed in 30 ml amounts in 150 ml of flasks. After sterilization the flasks were inoculated and incubated at 45$^{0}$C for 5 days. The cellulase activity of the filtrates was then determined at 45$^{0}$C. The pH of the filtrates was also measured (Oso, 1978).

 

2.8. *Effect of pH on enzyme activity*
Some flasks of the 2% chaff medium were inoculated and incubated at 45$^{o}$C for 5 days, and for the enzyme assay 0.55% CMC was prepared as previously described. This CMC was divided into nine portions and the pH was adjusted to 2, 4, 6, 7, and 9 respectively using appropriate buffer solutions. Cellulase activity was determined by incubating 1 ml of the fungus filtrate with 9ml of these solutions for 1 hour at 45$^{0}$C (Oso, 1978).

**3.0. Results**
Three fungal species namely *Aspergillus niger, Aspergillus flavus* and*Trichoderma sp.* were isolated from the soil samples. All the isolated fungi produced cellulase enzyme. The fungai showed cellulase synthesis best on the 8$^{th}$ day of incubation at 37$^{o}$C (Figs.1-3) and enzyme activity was best at 45$^{o}$C (Fig. 6). Enzyme synthesis was best with oatmeal chaff concentration of 3% (Fig. 4) and also at pH 4-6 (Fig. 5).

Temperature significantly influenced enzyme production: *A. niger* (F=10.174, P=0.002); *A. flavus*(F=11.698, P=0.001); *Trichoderma sp.* (F=4.555, P=0.028) (Figs. 1-3). Oatmeal chaff concentration has no significant influence on enzyme production by the fungi (F=1.147, P=0.350) (Fig. 4). The effect of pH on enzyme production was very significant (F=5.726, P=0.006) (Fig. 5).

**4.0. Discussion**
The dumpsite in this study is characterised with large heaps of fresh and decaying refuse dump. Results from this study reveal the presence of microorganisms known to be associated with waste biodegradation and their active roles in the decaying of the refuse dump through production of enzymes and antibiotics. Studies were carried out on cellulase production capabilities of microorganisms associated with dumpsite. The fungi were tested for their abilities to produce cellulase.

The fungal species isolated from the dump included *Aspergillus niger, Aspergillus flavus, Trichoderma sp.* The fungal species isolated were found to be capable of growth at 45$^{o}$C and found to be capable of producing cellulase, which is involved in the decaying of organic matter present in the dump. The fungal isolates showed cellulase synthesis best on the 8$^{th}$ day of incubation at 37$^{o}$C and enzyme activity was best at 45$^{o}$C. Enzyme synthesis was found to be best with oatmeal chaff concentration of 3% and also at pH 4-6.

Cellulolytic fungi are potentially less expensive alternative of producing cellulase using lignocellulosic waste as substrate. This alternative would provide profitable means of disposing of lignocellulosic waste as well as provide cheaper methods for the production of cellulase enzyme by industries. Further research should therefore be made on the production of cellulase enzyme by cellulolytic fungi using oatmeal chaff as well as other lignocellulosic waste as substrates.

**REFERENCES:**
Ahamed, A., and Vermette, P.(2008). Enhanced enzyme production from mixed cultures of Trichodermareesei RUT-C30 and *Aspergillus niger* LMA grown as fed batch in a stirred tank bioreactor. *Biochemical Engineering* 42: 41-46.

Davies, C. and Williams, B. (1999). Genus Bacillus. In: *Bergeys Manual of Systematic Bacteriology* [Sneath, P.H. Ed] Williams and Wikins Company Baltimore.

Dashtban, M., Schraft, H., Qin, W. (2009).Fungal bioconversion of lignocellulosic residues; opportunities & perspectives.*International Journal of Biological Science* 5: 578–595.

James, J.H. (2011). *The Role of Soil* Fungus.*Agriculture and Natural Resources.*Ohio State University Extension SAG-14-11.

Maki, M., Leung, K.T., Qin, W. (2009).The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass.*International Journal of Biological Science* 5: 500–516.

Oso B.A. (1978). The Production of Cellulase by *Talaromycesemersonii.Mycologia,* LXX: 577-585.

Sundaramoorthi, C., Vengadesh, P.K., Gupta, S., Karthick, K., Tamilselvi, N. (2011).Production and characterization of antibiotics from soil-isolated actinomycetes.*International Journal of Pharmaceutics* 2011; 2(4): 114-118.

 

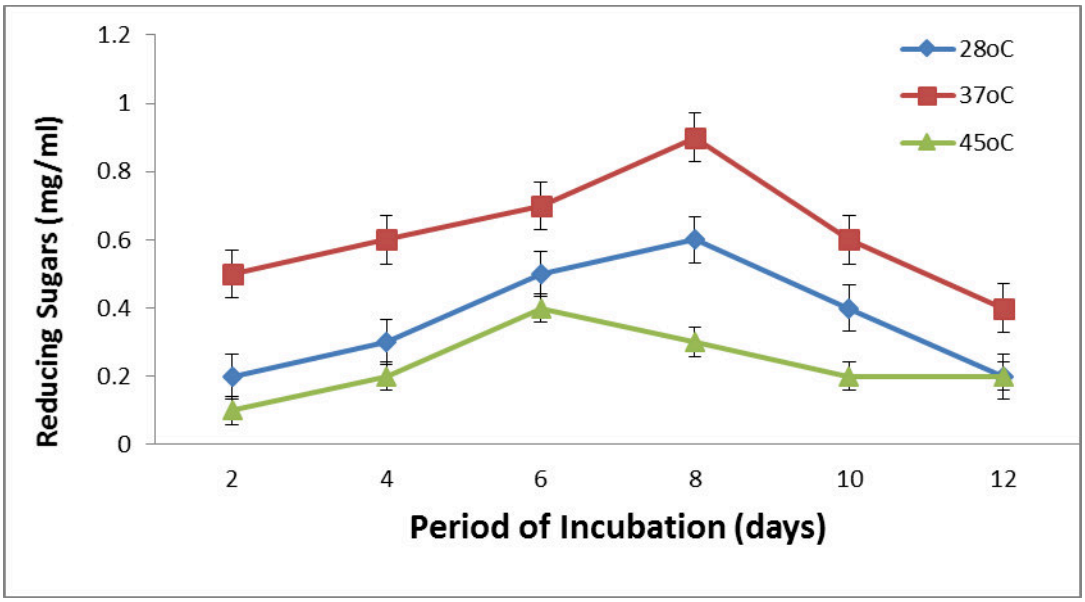


**Figure 1:** Effect of temperature on cellulase synthesis by *Aspergillus niger* (F=10.174, P=0.002).

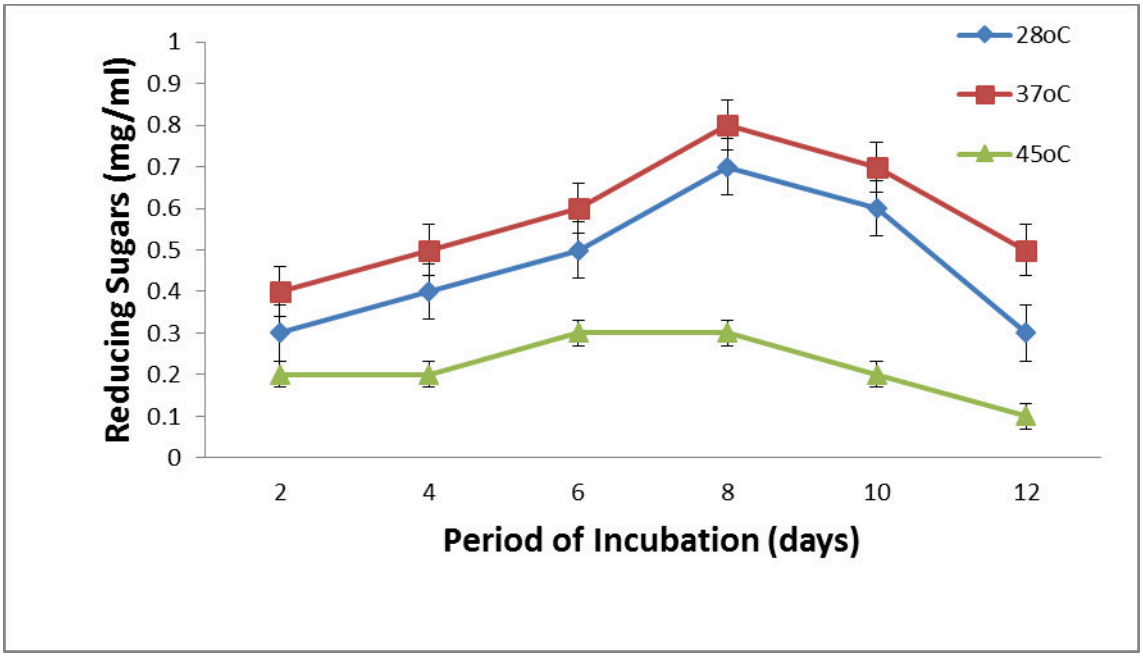


**Figure 2:** Effect of temperature on cellulase synthesis by *Aspergillus flavus* (F=11.698, P=0.001).

 

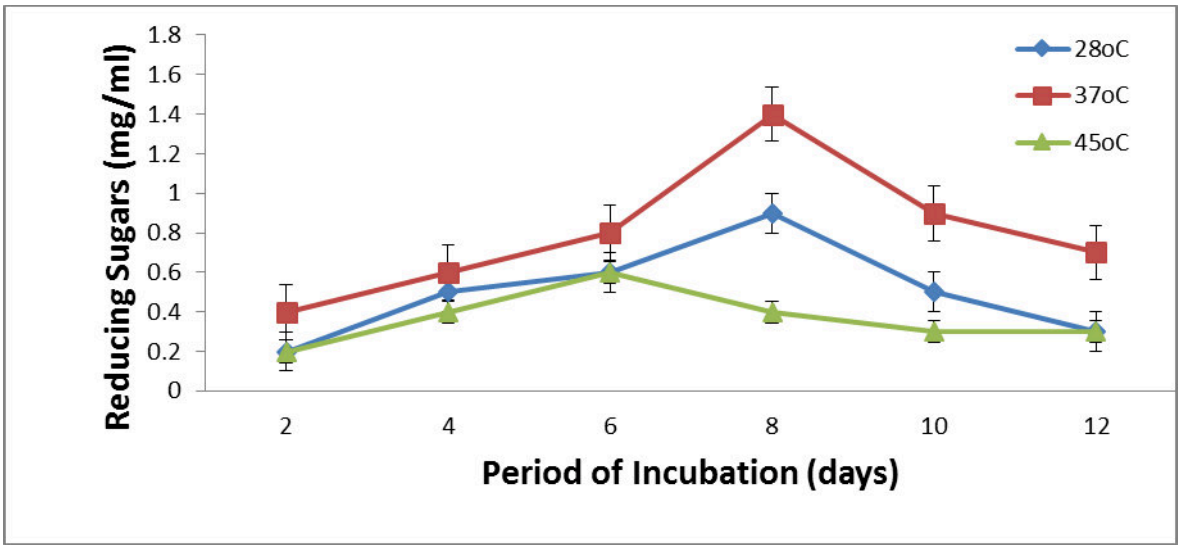


**Figure 3:** Effect of temperature on cellulase synthesis by *Trichoderma sp*. (F=4.585, P=0.028).

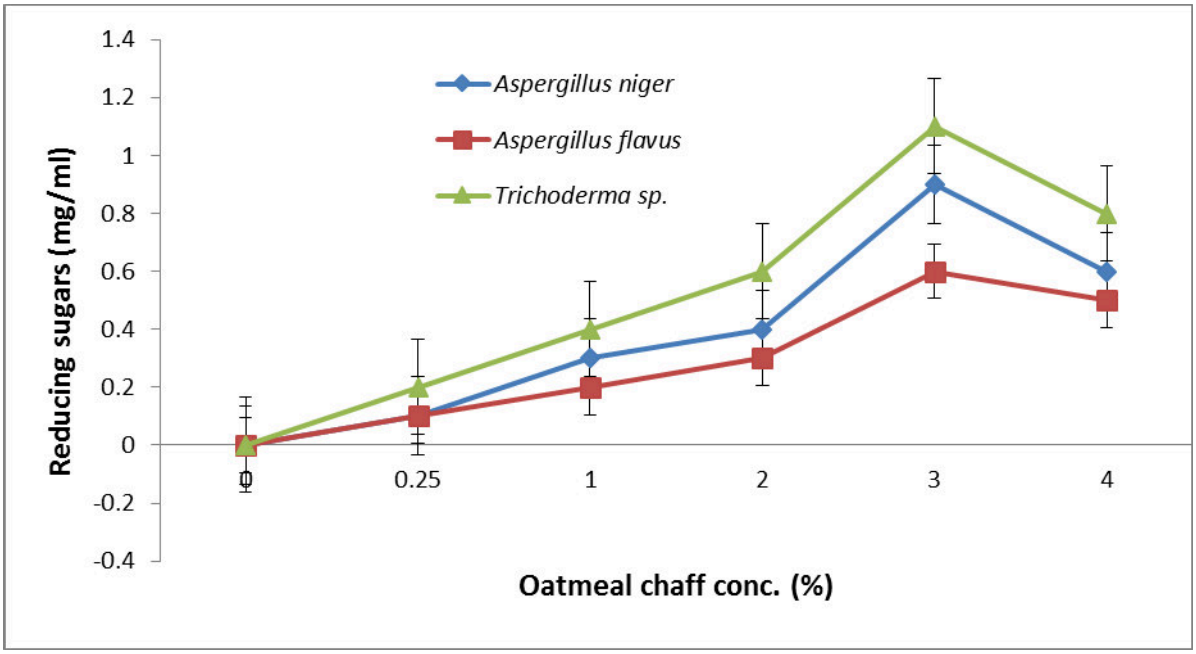


**Figure 4:**The Effects of oatmeal chaff concentration on enzymatic activities of fungal filtrates from a CMC substrate (F=1.147, P=0.350).

 

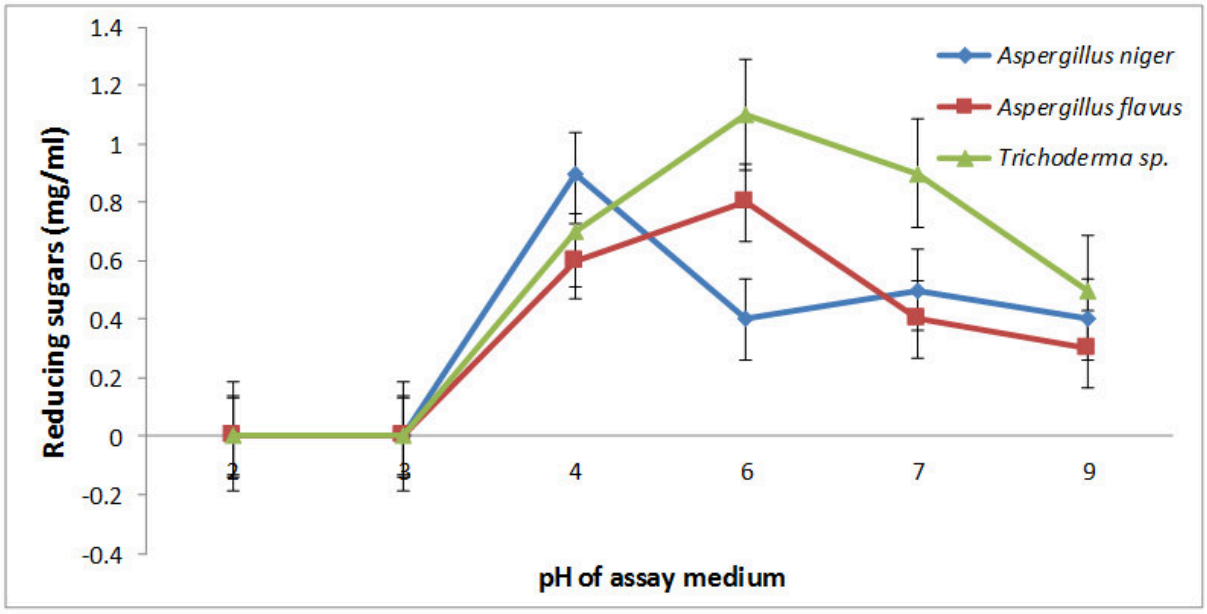


**Figure 5:**The Effects of pH on enzymatic activities of fungal filtrates from a CMC substrate (F=5.726, P=0.006).

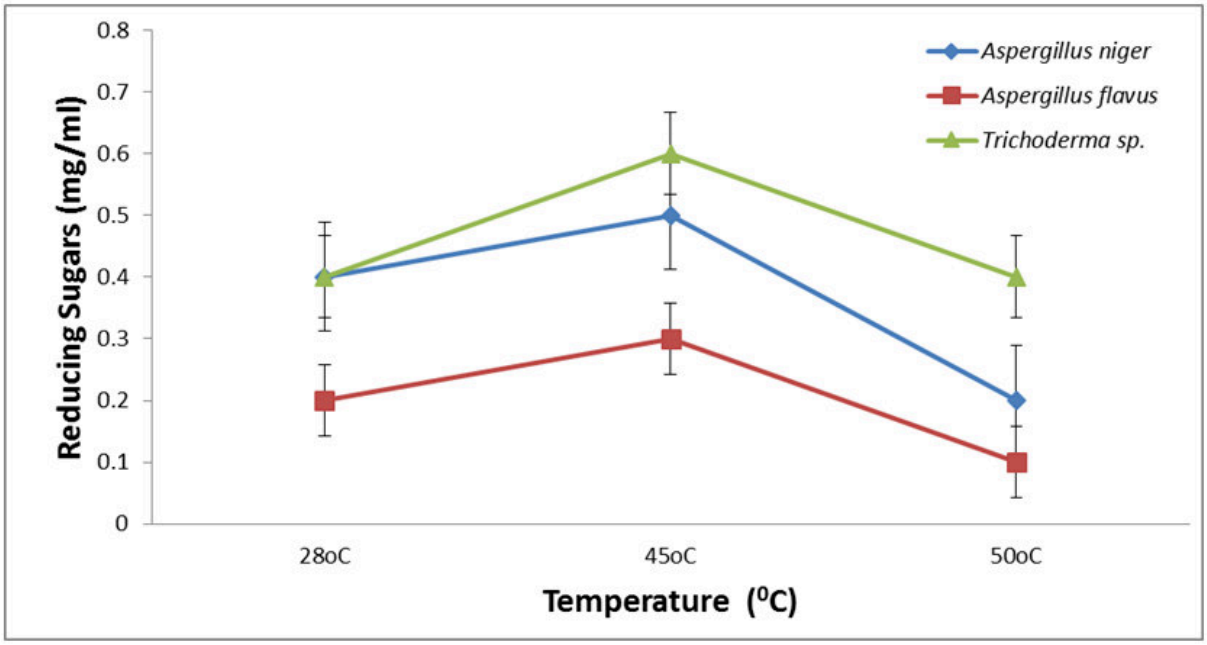


**Figure 6:** The Effects of temperature on enzymatic activities of fungal filtrates from a CMC substrate (F=2.000, P=0.193)