ASSESSMENT OF BIODEGRADATION POTENTIALS OF *FUSARIUM OXYSPORUM* AND *ASPERGILLUS NIGER* ON BIOMEDICAL WASTE AND WASTE MANAGEMENT IN URBAN KANO, NIGERIA

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ABSTRACT

The aim of this study is to assess the potentials of fungi in biodegrading biomedical waste. In this study, biodegradation of blood-stained cotton wool wastes by two species of fungi, *Fusarium oxysporum* and *Aspergillus niger* was assessed under ranges of temperature conditions. Pure cultures of the test fungi were inoculated on 25 g of test material and maintained under temperature ranges of 15-25°C, 25-35°C and 35-45°C for 60 days respectively. Degradation process of test material was represented by the weight loss and transformation of materials. A weight loss of 89.74 %, 52.08 % and 35.92 % were recorded under temperature ranges of 25-35°C, 15-25°C and 35-45°C by *Fusarium oxysporum* respectively. However, the loss of weight from *Aspergillus niger* under the same temperature ranges were 64.00 %, 13.40 % and 21.36 % respectively. *Fusarium oxysporum* was more effective at temperatures of 25-35°C, which is similar to the environmental temperature condition of the study area. There is significant difference in the weight loss of biomedical waste treated with the fungal species ($p< 0.05$), however no significant difference was observed on the effect of temperature condition in the biodegradation process ($p=0.44$). The products of the biodegraded test material included water, alcohols, alkyls, cellulose biomass among others, as confirmed by Fourier Transform Infrared Spectroscopy (FTIR). These findings suggest that, fungal organisms employed in the study can be utilized as excellent biodegraders of healthcare wastes which are cost effective and environmentally safe.

Keywords: *Aspergillus niger*, Biodegradation, Biomedical waste, *Fusarium oxysporum*, FTIR, Temperature ranges.
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INTRODUCTION

Biomedical waste is described by USA Environmental Protection Agency [1], under its Medical Waste Tracking Act (MWTA) as any solid generated from diagnosis, treatment or immunization of humans and animals and through the process of conducting research. These kind of wastes present serious and widespread consequences among population, particularly in countries with huge urban population and little capacity to handle epidemics as in the sub Saharan region. The World Health Organization (WHO) estimated that about 8 to 16 million new cases of Hepatitis B Virus (HBV); 2-5 million cases of Hepatitis C virus (HCV) and 80,000 to 160,000 cases of Human Immune Deficiency virus (HIV) are reported annually, due to exposure caused by indiscriminate disposal of biomedical waste [2].

In Nigeria, waste in general is poorly managed. Waste materials are often disposed on streets, open places, drains, ditches, to mention but few. Medical waste materials are particularly treated in similar manner; thus, increase public exposure to disease pathogens as reported by Alagoz & Kocasoy; Abah & Ohimain [3, 4]. It is apparent from this description that, biomedical waste posed serious health risk to a population, therefore effective but efficient disposal of this kind of waste is very necessary. However, the technologies for safe and effective disposal of biomedical waste are lacking in Nigeria for obvious reasons. This study is prompted by the desire to explore the potentials of *Fusarium oxysporum* and *Aspergillus niger* for biodegradation of contaminated cotton wool widely disposed in the environment, as no literature is available on the potentials of these two fungal organisms, hence; the first study of its kind. Adoption of conventional procedures within limited resources in our situation was employed. The study aimed at assessing the potentials of *Fusarium oxysporum* and *Aspergillus niger* to biodegrade contaminated cotton wool generated from medical practice through isolation of the fungal specie from cow dung, laboratory production of pure cultures, measurement of weight reduction and determination of byproducts of biodegradation using FTIR.

MATERIALS AND METHODS

Research design

The study was carried out at Multipurpose Laboratory of the Department of Biological Sciences, Bayero University, Kano. The Sampling method adopted for the study was completely randomized design (CRD) Three (3) treatments and one control for each fungal organism were used as the experimental set up. The experiment was replicated three times. Laboratory safety procedures using live specimen was maintained during the experimentation based on the guidelines of Cheesbrough [5].

Sample collection
Samples for biodegradation test were collected in accordance with standards recommended by ICRC and WHO [6, 7]. Contaminated cotton wool was collected from Out Patient Department (OPD) of Bayero University, Kano Clinic and Accident and Emergency Surgical Theater of Murtala Muhammad Specialist Hospital, both within Kano metropolis. Samples of fungal organisms were collected from cow dung adopted from Lakshimi *et al.* [8]. Samples were collected using a wooden spatula and placed in sterile polythene bag, and transported immediately to the laboratory for isolation

Isolation of fungal organisms
Serial dilution procedure was employed for isolation of fungal organisms by dissolving 1 g of cow dung in sterile test tube containing 10 ml of distilled water and mixed properly at room temperature. This solution was immediately diluted up to 10^{-6}. Inoculation was carried out using pour plate method; six petri dishes were labeled according to the power of dilution; 1 ml of cow dung solution from each test tube was added into the labeled petri dishes respectively. 20 ml of molten agar was then subsequently added to the plates, the plates were allowed to solidify and incubated at room temperature (28˚C) for seven (7) days as described by Altayyar *et al.* [9].

Identification of fungal organisms
Initial cultures and subsequent isolates were observed under the microscope. Diagnostic features were noted and matched with notable diagnostic keys of Raper & Fennel; Nelson *et al.* [10, 11] for the identification of *Aspergillus* and *Fusarium* respectively.

Biodegradation of cotton wool by *Fusarium oxysporum* and *Aspergillus niger*
The procedure described by Dill Macky [12] was employed for preparing spore suspension. Spore suspension of fungal species were prepared by adding 10ml of distilled water to the culture plates. The spores were then carefully removed and preserved in sterile capped bottles for subsequent use in the biodegradation of blood-stained cotton wool. The test organisms were tested on the waste material under 3 conditions; (15-25˚C, 25-35˚C and 40-50˚C), and in the presence of ultraviolet light and oxygen. Cotton wool waste (25 g) was placed on solid agar plate and inoculated with spore suspension of the test fungi. Control plates were also set up, but without inoculum. The samples were incubated aerobically for 60 days [13].

Measuring the degree of biodegradation
The degree of biodegradation was determined by the level of weight loss over a period of time. The equation below explains how the fungal spore degraded the contaminated cotton wool over a period of 60 days.

$$W_T(\%) = w_o - w_t \div w_o \times 100\% \quad \ldots\ldots 1 \text{ [14].}$$

Where $W_T(\%)$, is the percentage weight loss after t days of incubation,

w_o is the initial weight of the waste sample material

w_t is the weight of the test material after (t) days of incubation

Fourier Transform Infrared (FTIR) spectroscopic analysis
The degraded cotton material was further subjected to FTIR analysis to identify the substances formed from the degradation activities of the fungal organism.

RESULTS AND DISCUSSION

The results of the study showed that both fungal organisms were able to biodegrade the cotton wool waste, however *Fusarium oxysporum* was more effective than *Aspergillus niger* at all temperature conditions. Similarly, the temperature condition that favors the biodegradation process at the highest rate was room temperature. Figure 1 shows the weight reduction in cotton wool waste treated with *Aspergillus niger* at different temperature conditions over a period of sixty days. From the study, the weight of the test material was reduced from 25 g to 21.65 g at low (15-25 °C) temperature, from 25 g to 4.95 g at indoor (25-35°C) temperature, and from 25 g to 19.40 g at high (35-45°C) temperature.

Figure 2 shows the weight reduction in cotton wool waste treated by *Fusarium oxysporum.* From the study, the weight of the test material was reduced from 25 g to 11.98 g at low temperature, from 25 g to 2.56 g at indoor temperature, and from 25 g to 16.02 g at high temperature. It is apparent from the results that the activities of the fungal organisms were most effective in degrading the cellulosic cotton material at room temperature. Temperatures regarded as low (15-25°C) and high (> 35°C) temperatures were considered in this experiment because higher temperatures in most cases hastens microorganism activities in general. However, *Aspergillus niger* and *Fusarium oxysporum* performed remarkably under room temperature (25-35 °C) in the study area. The study also showed that, as cotton wool degrades the weight of the test material decreases, this is largely due to the action of the fungi that stick to the surface of the test material and release extracellular enzymes which penetrates and breakdown the structure of cellulose biomass into new simpler biomass, gaseous molecules, mineral salts other products including water molecules. This corresponds with the findings of Khubaib & Mujahid [15].

The study also showed that at high temperature, biodegradation has occurred. This indicates that high temperature not more than 50˚C also favors biodegradation, as any temperature above

that can denature the enzymes. This finding correlates with that of Sivakumaran [16], where the maximum temperature tolerated for biodegradation of cellulose by fungi was 50˚C, and any temperature above 50˚C results in decline in biodegradation process. Test materials treated under low temperature showed the least change in weight or rate of biodegradation. This was largely due to inactive state of enzymes at low temperature.

Plate I (a & b): Cotton wool wastes before and after degradation by *Aspergillus niger.*

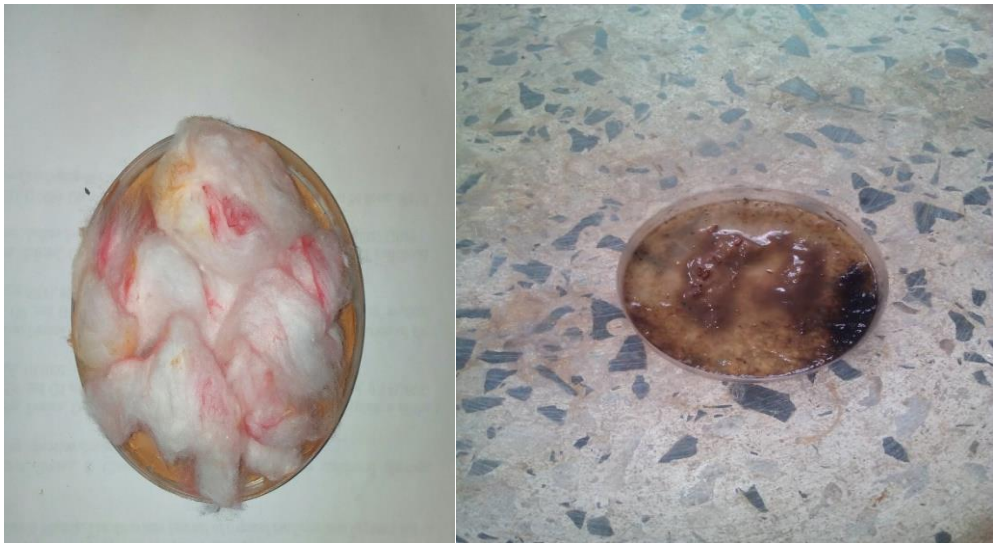

Plate II (a & b): Cotton wool wastes before and after biodegradation by *Fusarium oxysporum.*

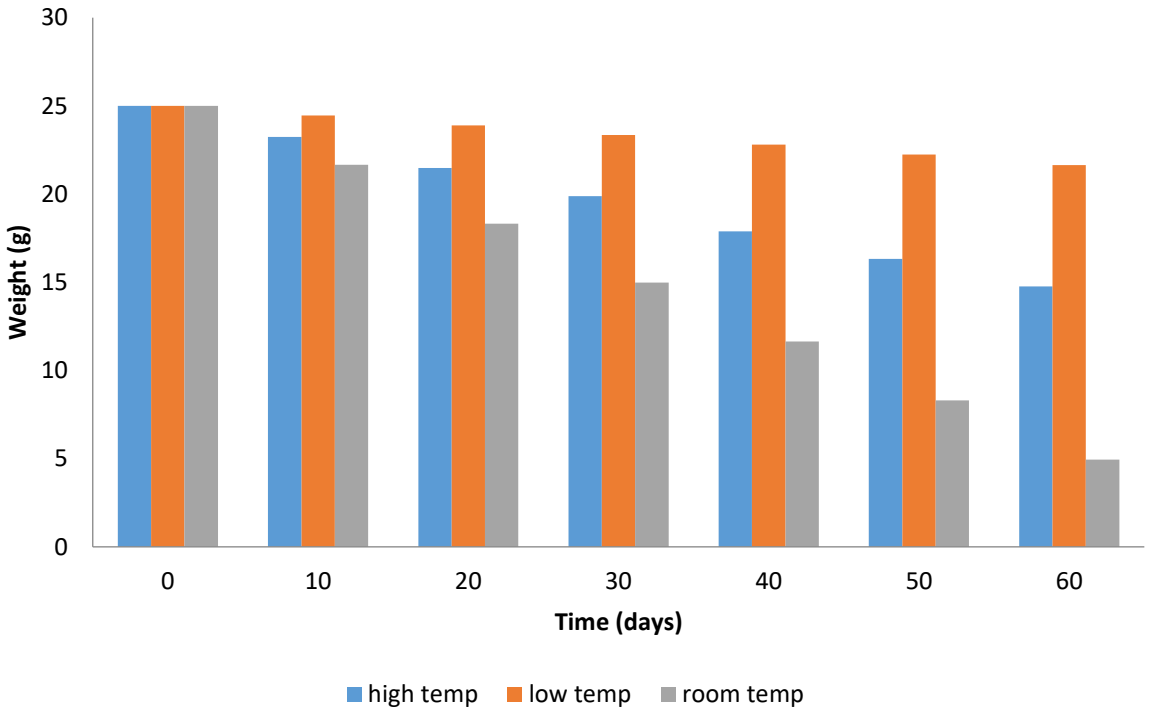

Figure 1: Weight reduction in cellulosic medical waste treated with *Aspergillus niger* under different temperature ranges, over a period of sixty days.

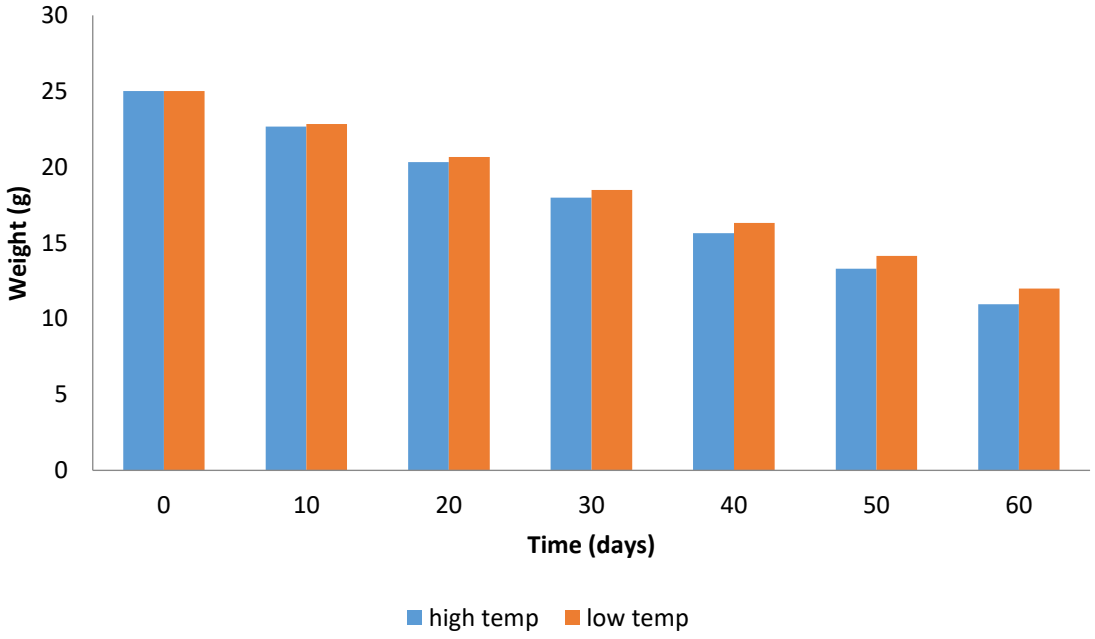

Figure 2: Weight reduction in cellulosic medical waste treated with *Fusarium oxysporum* under different temperature conditions over a period of sixty days.

Table 1: Biodegradation levels in grams and percentage (%) of cellulosic waste materials by fungal organisms under different temperature conditions over a period of sixty (60) days.

Fungal specie	Initial weight (g)	Final weight(g) at LT (15-25°C)	Average % loss at LT	Final weight(g) at RT (25-35°C)	Average % loss at RT	Final weight(g) at HT (35-45°C)	Average % loss at HT
Aspergillus niger	25.00	21.65	13.40	4.95	80.00	19.40	40.96
Fusarium oxysporum	25.00	11.98	52.00	2.56	89.76	16.02	56.00

Key:
LT: Low temperature
RT: Room temperature
HT: High temperature

FTIR of waste materials treated with *Aspergillus niger.*

The nature of intra molecular bonding of the cellulosic fibers before and after biodegradation is illustrated in Figure 3(I & II). A key interaction is measured in the region of the major functional group, at exactly 3335 cm^{-1} which results from the hydrogen bonding interaction between $3OH_5$ adjacent to the beta-glycosidic bond of the cellulose structure, this shows evidence of hydrolysis, thus showing degradation of cotton material. The vibrational peaks around 1500-1200cm^{-1} attributed to C-O stretch increases from the virgin to the treated waste as cotton wool degrades. According to Khubaib *et al.* [17], the increase in the spectral band shows that the main functional group has been attacked by microorganisms, degraded and converted into a new biomass. Similarly, new bands appeared in treated sample at 1372, 1316 and 1160cm^{-1} attributed to –OH bending, -C-H deformation and C-O stretch. These corresponds to the findings of Yang *et al.* [18] which showed that, as time passes, the structure of cotton collapses, absorption of water increases and new products were formed. The peak at wavelength of 1205cm^{-1} ,1428cm^{-1} and 2899cm^{-1} attributed to C-O methane hydrogen bonding, C-H bending of cellulose and –C-H stretch present in virgin sample disappeared in the biodegraded waste material, which showed changes in carboxyl group of cellulosic waste after microbial treatment, thus, confirming degradation.

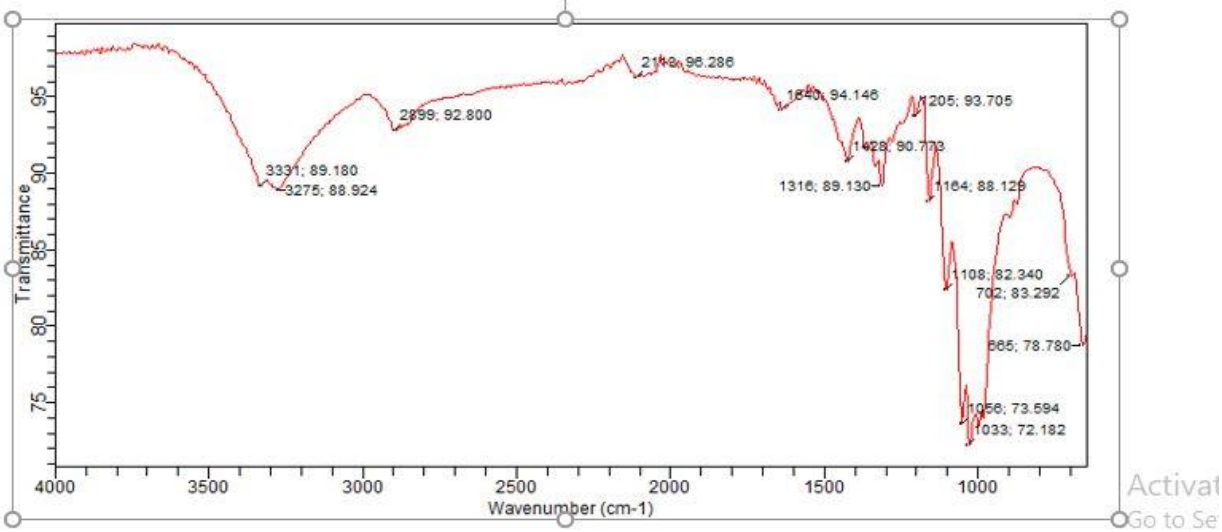

Figure 3 (I): FTIR Spectra of cellulosic waste before degradation.

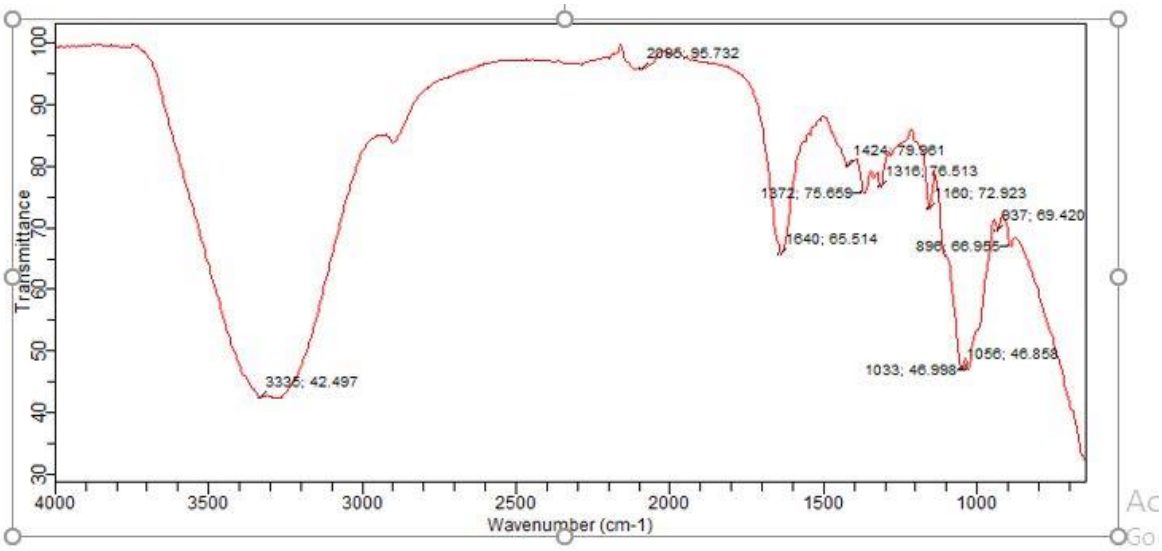

Figure 3 (II): FTIR spectra of Cellulosic Waste after degradation by *Aspergillus niger*.

FTIR of waste materials treated with *Fusarium oxysporum.*

The intra molecular bonding of cotton wool before and after degradation is illustrated in Figure 4(I & II). A key interaction is measured in the 3275 cm^{-1} regions from the OH bonding interaction of the cellulose structure as in *Aspergillus niger*. Next to this region is the C-H bonding region which is much narrower between 3000-2800 cm^{-1} and corresponds to the C-H moieties. In the mid –IR wavenumber range, vibrations from other heteroatoms like nitrogen and double bonds at exactly 2899 cm^{-1} present in the virgin sample are absent in the biodegraded cellulose. This shows that fungi have attacked and broken the structure of cellulose. The most complex region in the spectra of cellulose FTIR is the finger region from 1500-660 cm^{-1} which contains signals from different sp^3 single bonds vibrational nodes. Finally, between 1100 and 600 cm^{-1} is the region of multiple bands, corresponding to heavy atom bending and rotation [19]. The results confirmed biodegradation of cotton wool and corresponds to the findings of Yang *et al.* and Hulleman *et al.* [18, 20].

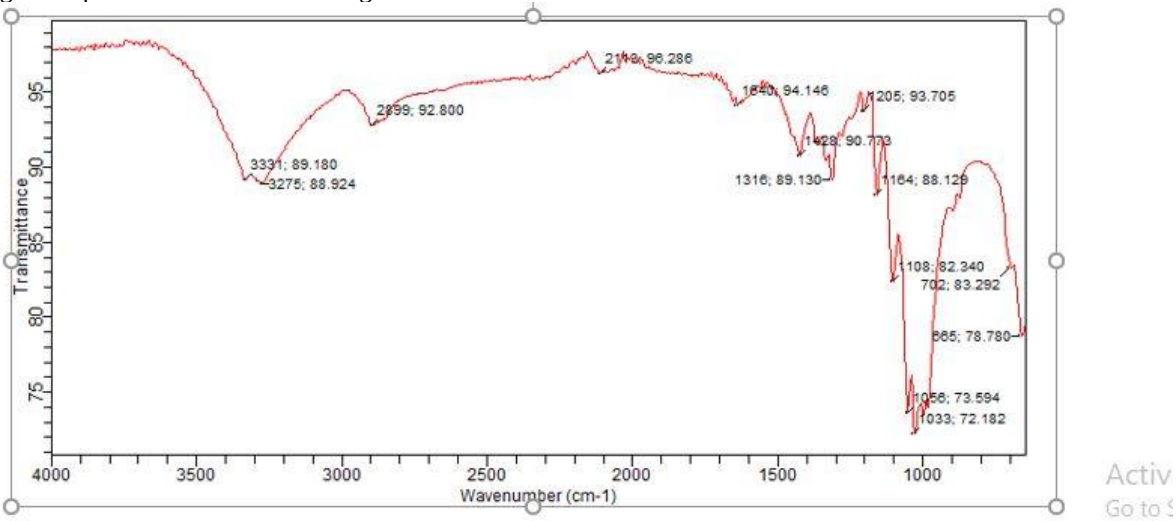

Figure 4(I): FTIR Spectra of cellulosic waste before degradation by *Fusarium oxysporum*

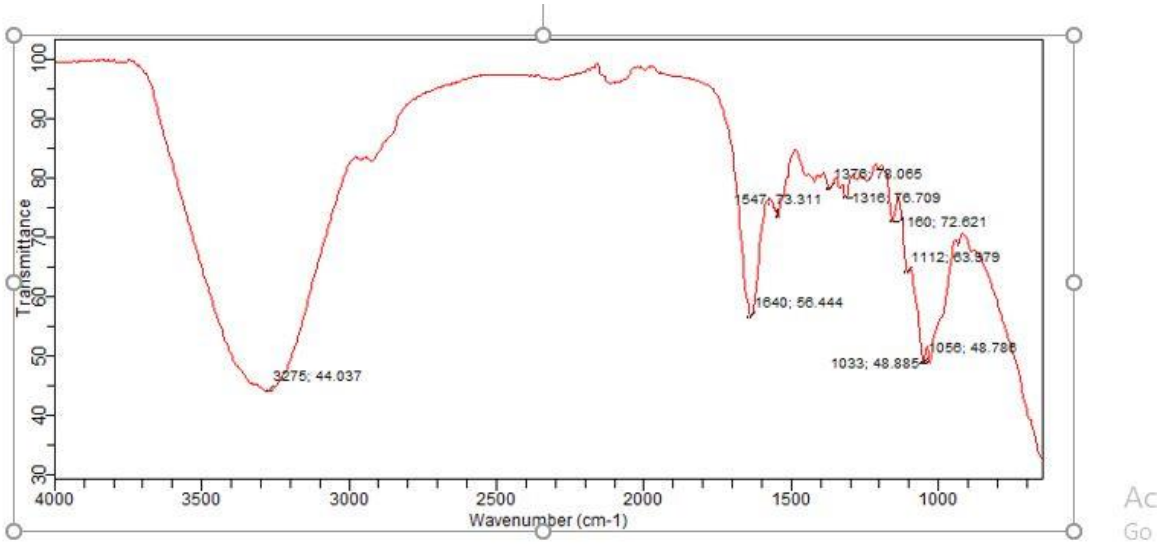

Figure 4(II): FTIR Spectra of cellulosic waste after degradation by *Fusarium oxysporum.*

CONCLUSION

The study shows that *Aspergillus niger* and *Fusarium oxysporum* were efficient agents of biodegradation of cellulosic hospital wastes, however *F. oxysporum* was more effective. Similarly, room temperature (25-35°C) was found to be more effective than low (15-25°C) and high (35-45°C) temperatures. FTIR spectra before and after degradation further confirmed the degradation process based on the functional groups of the products of the breakdown process such as; alykyls, carboxylic acids, alcohols and water. This showed that fungal organisms can be utilized as environmentally safe and cost-effective biological agents of biodegradation of hospital wastes, especially in developing countries like Nigeria, taking into consideration the financial status of the country coupled with less priority given to the health sector.

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Table 2: Analysis of variance (ANOVA) for fungal organisms and temperature conditions.

Source of variation	SS	Df	MS	F	P-value	F-crit
Fungal organism	324.56628	2	162.283	10.852	0.00526	4.4589
Temperature conditions	61.8728	4	15.468	1.034	0.4460	3.837
Error	119.625	8	14.953			
Total	506.065	14				