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Research Article

Assessment of Biodegradation Potentials of Some Fungal Species on Cellulosic Medical Waste Materials Generated in Healthcare Facilities within Kaduna Metropolis

***Yunusa. B. A.[1], Ibrahim, H.[1], Abdulkadir, S.[1], Abdulrahman, A. S.[2] and Saidu, A.[2]**

[1]Department of Biological Sciences, Kaduna State University, Kaduna, Nigeria
[2]Department of Applied Biology, Kaduna Polytechnic, Kaduna, Nigeria
****Corresponding Author's email*: bilkeesuyunus@yahoo.com; Phone: +2347066579858**

ABSTRACT
Medical wastes encompass materials produced during patient diagnosis, treatment, immunization, and biomedical research within hospitals. The disposal of these biomedical wastes can pose significant risks, which can lead to heightened environmental pollution, as well as significant public health hazards. This research aimed to assess the biodegradation potential of some fungal species on cellulosic medical waste materials generated from healthcare clinics in Kaduna state. Cellulose medical waste samples were collected from Barau Dikko Teaching Hospital, Alba Clinic, Constitution Road, and Primary Health Care Center, Kabala Costain. A mixture of cow dung, medical wastes and selected individual fungal species was incubated for 14 days at room temperature and the absorbance of each sample was read. An analytical balance was used to assess mass loss after every ten days for days 60, and the extent of biodegradation. Changes in the polymer bond of cellulosic medical waste were determined using Fourier-transform infrared spectroscopy. *Fusarium* sp. showed the highest biodegradation potential of 45.93 %, followed by *Trichoderma s*p. (31.70%); While *Aspergillus* sp. had the least biodegradation potential (27.36%). *Trichoderma* (3280, 1633, 1239, 1033 cm^{-1}), *Fusarium* (3390, 1630, 1239, 1100–1050 cm^{-1}), and *Aspergillus* (3283, 2922, 1640, 1104, 1054 cm^{-1}) all show O–H, C–H, amide, and C–O–C peaks indicating carbohydrates, proteins, and lipids. Therefore, the study confirmed that the isolates play a significant role in the degradation of cellulose medical wastes.

Keywords: Biodegradation; Cellulolytic; Clinics; Fungi; Healthcare; Medical; Waste

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INTRODUCTION

Biomedical waste or bio wastes are those potentially hazardous waste materials, consisting of solids, liquids, sharps, and other laboratory waste. Biomedical wastes differ from other types of hazardous waste, such as industrial wastes, in that it comes from biological sources or is used in the diagnosis, prevention, or treatment of diseases. One of the major sources of biomedical wastes is hospitals and nursing homes. Hospital is one of the complex institutions which are frequented by people. All of them produce waste which is increasing in its amount and type due to advances in scientific knowledge and is creating its impact (Saritha, 2025).

Medical waste encompasses materials produced during patient diagnosis, treatment, immunization, and biomedical research within hospitals. Biomedical wastes are also produced in hospitals, research facilities, health care teaching institutes, clinics, laboratory, blood banks, animal households, and veterinary institutes (Cook *et al.*, 2023). The issue of hospital waste management has gained prominence, especially after the release of the bio medical wastes management and handling. The regulation mandates health care facilities to segregate, sanitize, and appropriately dispose waste in an environmentally-conscientious manner. Hospitals, nursing homes, clinics, dispensaries, animal houses, pathological laboratories, and other facilities must install biological waste treatment facilities as a necessary measure (Hasija *et al.*, 2022). The escalating worry over waste disposal problems in hospitals and other health-care facilities has become more pronounced (Kharat *et al.*, 2024; Janik-Karpinska *et al.*, 2023).

Waste accumulation and storage occur between the point of waste generation and site of waste treatment and disposal. While accumulation refers to the temporary holding of small quantities of waste near the point of generation, storage of waste is characterized by longer holding periods and large waste quantity. Storage areas are usually located near where the waste is treated. Any offsite holding of waste is also considered storage (Saritha, 2025).

The aim of the research was assessment of biodegradation potential of selected fungi on cellulosic medical waste materials generated from healthcare facilities within Kaduna metropolis.

MATERIALS AND METHODS

Study Area

The research work was laboratory-based study, which was carried out in microbiology laboratory, Biological Sciences Department, Kaduna State University, Kaduna North which is located between latitudes 10.3764 North and Longitudes 7.7095 East.

Cellulosic medical wastes samples used for this study were collected from Barau Dikko Teaching Hospital, Alba Clinic at Constitution Road, and Primary Health Care Center, Kabala Costain Kaduna North Local Government Area. Cow dung samples used for the isolation of fungi were collected from sites of cow rearing area in Zango, Tudun Wada Kaduna, Kaduna South, and Kabala Costain Kaduna North, Kaduna State, Nigeria.

Sample Collection

The cellulosic medical wastes were collected using a sterilized hand towel and transported to the laboratory in a pre-cleaned polyethylene bag and stored until required (Rose *et al.*, 2022). completely randomized design (CRD) was the sampling method used for collection of fungal substrates (cow dung) in the field. Cow dung (300g) was collected using spatula and transported to the laboratory in a pre-cleaned polyethylene bag and stored at 4^{o}C until required (Glushakova and Kachalkin, 2023).

Source of Fungal Isolates

Three fugal isolates (*Aspergillus, Trichoderma* and *Fusarium* spp.) were collected from the labouratory in the Department of Microbiology, Kaduna State Unibersity, Nigeria. Confirmation of identification was done based on morphological and microscopic characteristics as outlined by Gupta *et al.* (2023).

Screening of Cellulosic Medical Wastes-Degrading Fungi

Preparation of Cellulose Medical Wastes

Cellulosic medical wastes were added to Mineral Salt Medium (MSM) containing the following salts in 1 L distilled water: K_2HPO_4, 1g; KH_2PO_4, 0.2 g; NaCl, 1g; $CaCl_2.2H_2O$, 0.002 g; boric acid, 0.005g; $(NH_4)2SO$, 1g; $MgSO_4.7H_2O$, 0.5g; $CuSO_4.5H_2O$, 0.001g; $ZnSO_4.7H_2O$, 0.001g; $MnSO_4.H_2O$, 0.001g and $FeSO_4.7H_2O$, 0.01g as adopted by Imam *et al.* (2023).

Inoculation of fungal isolates unto Cellulosic Medical Wastes Mixture

Fungal isolates were inoculated onto the mixture and incubated for 14 days under room temperature. then, the absorbance of each sample was read. Fungal isolates which gave highest absorbance were further confirmed by Imam *et al.* (2023).

Biodegradation of Cellulosic Medical Wastes

Cellulose medical wastes were picked up and place onto potato dextrose agar (PDA). fungal cells were added and then incubated for 60 days at room temperature in a stationary position (Imam *et al.*, 2023).

Detection of Biodegradation of Cellulose Medical Wastes

Weight Reduction

Analytical balance was used to assess mass loss after every ten days, and the extent of biodegradation. Percentage weight loss was determined using the formula as adopted by Imam *et al.* (2023) using;

W_T (%) = $wo - wt \div wo \times 100\%$

In this equation, W_T (%) represents the percentage of weight loss after t days of incubation, W0 denotes the initial weight of the waste sample, and WT indicates the weight of the test material following t days of incubation. The weight of the test material was expressed as a percentage by multiplying the difference between the initial weight and the weight after incubation by 100, then dividing by the initial weight (Imam *et al.*, 2023)

Fourier-Transform Infrared Spectroscopy

Fourier- Transform Infrared Spectroscopy (FTIR) analysis was used to determine the changes in the polymer bond of cellulose medical waste after the biodegradation. Fourier transformed infrared (FT-IR) 8400 S Schamdzu spectroscopic were used for the characterization of the analyzed materials. Samples were weight-in at 0.01 g and homogenized with 0.01 g KBr anhydrous by mortar agate. The mixtures were pressed by vacuum hydraulic (Graseby Specac) at 1.2 psi to obtain transparency pellet. Scanned sample passed through infra-red, where its continuing wave by detector that connected to computer and given described of tested sample spectrum. Samples were usually scanned in the absorption area of 600 to 4000 cm-1. The results of analysis consisted of chemical structure, molecular binding form and certain functional groups of tested samples as basic of spectrum type (Imam *et al.*, 2023).

Data Analysis

The data collected from the series of investigations and experiments was systematically organized. The variations in the responses of the variables to different times, and weight were statistically analyzed using ANOVA, facilitated by the Statistical Package for the Social Sciences (SPSS) software, version 16.0.

RESULTS

Table 1 shows the degree of biodegradation of cotton wool by *Aspergillus* Species, as indicated by weight loss under room temperature conditions. The weight of the treated cellulosic waste materials decreased modestly from 46.15g to 34.10g after 60 days period. The degree of biodegradation of cotton wool by *Fusarium* Species as indicated by weight loss under room temperature conditions. The treated cellulosic waste exhibited an average weight reduction from 41.57g to 24.30g after 60 days. At room temperature, a significant weight loss was recorded, decreasing from 25g at day zero to 2.56g after 60 days. Ultimately, *Fusarium* Species. demonstrated the most substantial weight reduction across all experimental conditions, where the weight decrease (Table 3).

The degree of biodegradation of cotton wool by *Trichoderma* Species as indicated by weight loss under room temperature conditions. The treated cellulosic waste exhibited an average weight reduction from 42.26g to 30.41g after 60 days (Table 1).

The characteristics of intramolecular bonding in cellulosic fibers, both prior to and following biodegradation. The vibrational peaks observed in the ranges of 3283 cm-1 and 1500-1200 cm-1, which correspond to O-H stretching and C-O stretching, exhibit an increase from the untreated to the treated waste as cotton wool undergoes degradation. In comparison to the untreated waste, the intensity of the vibrational stretching in the 3600-3200 cm-1 range becomes more pronounced, indicating a transformation in the –OH functional groups of cellulose to those of water molecules. This observation provides evidence of biodegradation and the emergence of new molecular structures. Additionally, new bands were identified in the treated samples at 1372, 1316, and 1160 cm-1, which are associated with –OH bending, -C-H deformation, and C-O stretching, respectively. The peaks at 1640 cm-1, linked to C-O methane hydrogen bonding and C-H bending of cellulose in the virgin sample, were absent in the biodegraded waste, further confirming the degradation process. Moreover, the absorption peak at 2922 cm-1, related to –C-H stretching in the virgin sample, also vanished in the biodegraded material. These findings indicate a modification in the carboxyl groups of the cellulosic waste following microbial treatment (Figure 1).

Table 1: Reduction in Weight of Waste Materials Due to Biodegradation by Some Fungi

Fungal species	Initial weight (g)	Weight after 10 days	Weight after 20 days	Weight after 30 days	Weight after 40 days	Weight after 50 days	Weight after 60 days	Average weight reduction	Percentage reduction (%)
Aspergillus spp.	43.6±2.41	41.7±2.63	39.6±2.57	37.6±2.48[b]	35.6±2.37[b]	33.5±2.43[b]	31.7±2.31[c]	11.9±0.12[b]	27.4±1.28[b]
Fusarium spp.	40.6±1.00	37.4±1.41	34.6±1.07	32.1±1.39[a]	29.1±1.62[a]	26.1±1.71[a]	23.01±1.48[a]	17.6±0.49[c]	43.3±2.29[c]
Trichoderma spp.	40.3±1.98	38.3±1.97	36.4±2.10	34.4±1.99[ab]	32.2±1.95[ab]	30.4±2.04[b]	28.3±2.13[b]	11.9±0.15[b]	29.9±1.83[b]
Control	38.8±1.00	37.5±1.11	36.7±0.09	35.6±1.08[ab]	34.6±1.29[b]	33.7±1.23[b]	32.6±0.98[c]	6.19±0.11[a]	15.9±0.43[a]
p value	0.051	0.074	0.054	0.037	0.011	0.004	0.001	<0.001	<0.001

Control = cellulosic medical wastes without fungal treatment

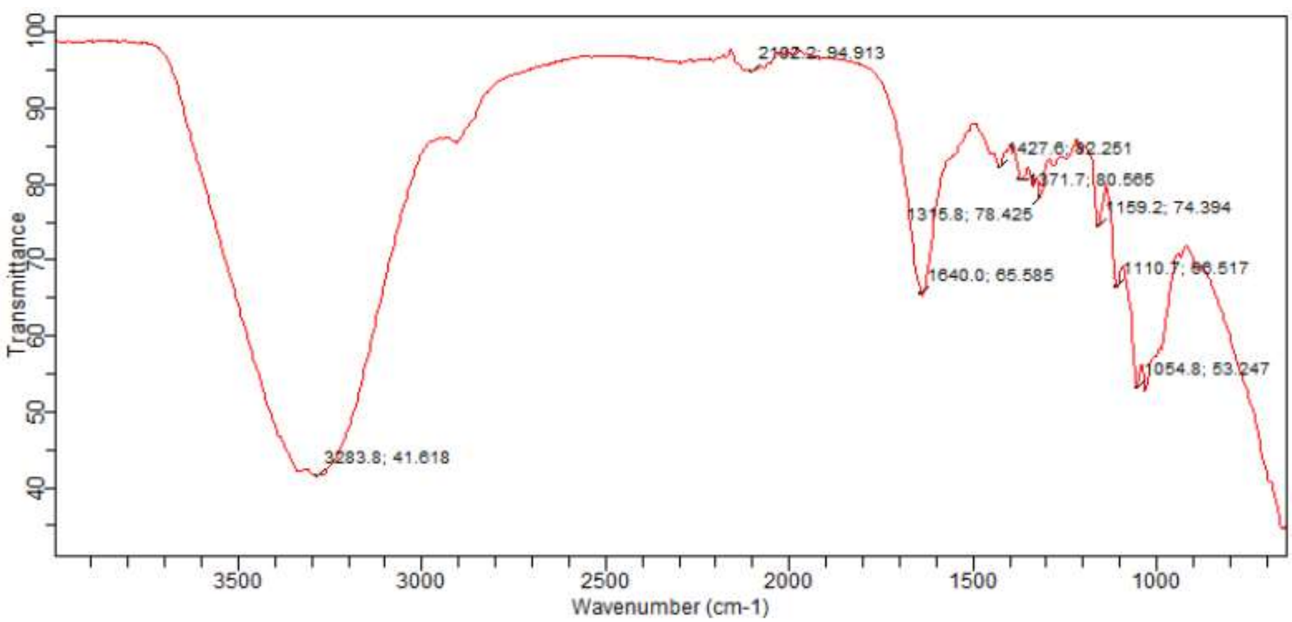

Figure 1: FTIR of Waste Materials Treated with *Aspergillus* Species

The intra-molecular bonding characteristics of cotton wool, both prior to and following degradation. The absorption peaks observed in the ranges of 3190-3280 cm-1, 1100-1300 cm-1, and 1400-1600 cm-1, which correspond to the –OH stretching, C-O stretching, and C-H bending respectively, exhibit a decline as the cotton wool undergoes degradation. Conversely, the absorption peak at 1205 cm-1, associated with –C-O stretching, shows an increase following microbial treatment. Additionally, new absorption bands emerged in the biodegraded material that were absent in the original sample, specifically in the ranges of 1547, 1376, 1160, and 1112 cm-1, which are attributed to C=C stretching, –OH bending, and C-O stretching of the alcohol group. This indicates that cellulose has been decomposed, resulting in the formation of alcohol and other byproducts. Furthermore, the absorption peaks at 2899 cm-1 and 2113 cm-1, linked to C-H stretching and –C---H stretching in the virgin sample, were no longer present in the biodegraded sample. These findings suggest a reduction in the carboxyl groups of cellulose, which is likely due to the cleavage of various bonds and the generation of new products as a result of fungal biodegradation (Figure 2).

The FTIR graphs illustrating the biodegradation of cotton wool by *Trichoderma* Species. The absorption peaks observed in the ranges of 3287-3390 cm-1, 1630 cm-1, and 1300-1000 cm-1, which correspond to the –OH stretch, -C=-C- stretch, and –C-O stretch respectively, diminish as the treated waste material undergoes degradation. Following degradation, the treated waste material exhibited new peaks in the regions of 1369 cm-1 and 937 cm-1, which were absent in the virgin sample. These peaks are attributed to –OH bending and C-H bending, indicating the formation of new molecular bonds and consequently, new products. Furthermore, the absorption peak at 2899 cm-1, associated with –C-H bending in the untreated waste material, was completely eliminated after fungal degradation, providing evidence of the molecular bonds present in cellulose (Figure 3).

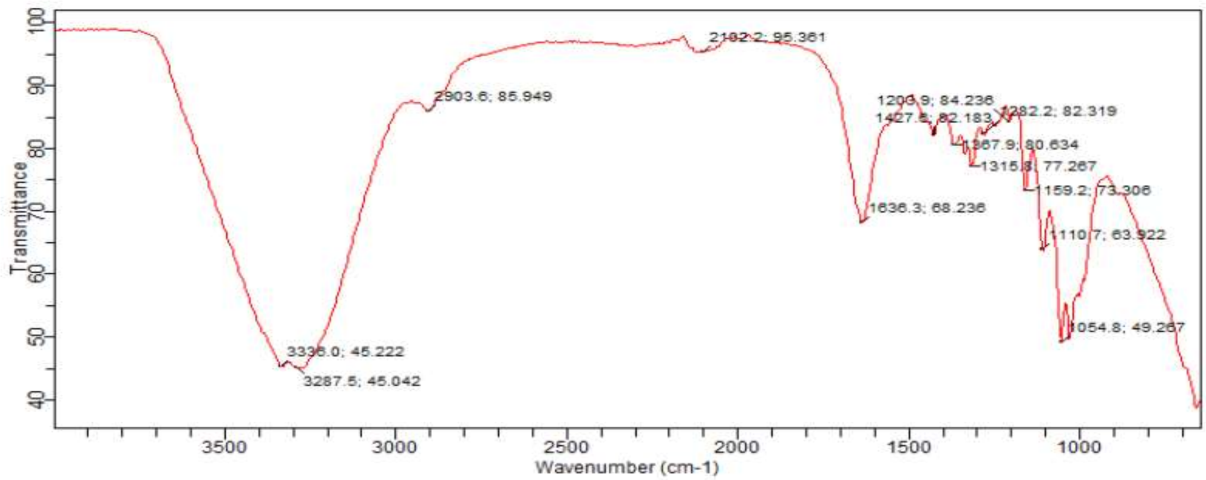

Figure 2: FTIR of Waste Materials Treated with *Fusarium* Species

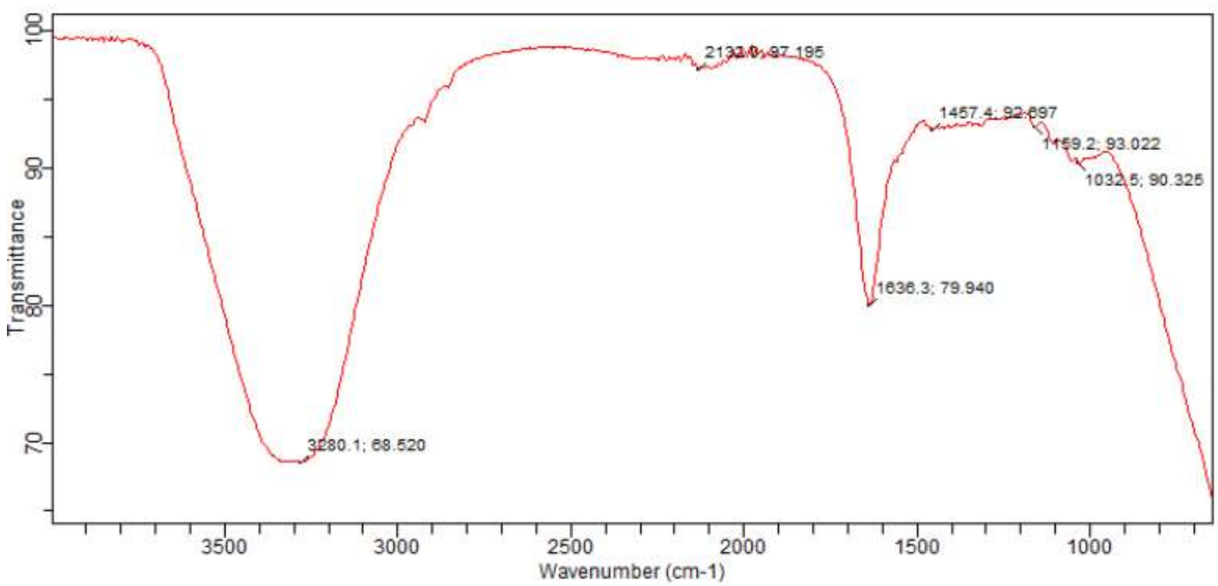

Figure 3: FTIR of Waste Materials Treated with *Trichoderma* Species

DISCUSSION

Generally, fungi exhibit better sporulation and activity at room temperature, with increased growth and metabolic activity at elevated temperatures. As with other enzyme-mediated processes, fungal biodegradation is regulated by cellulolytic enzymes, which are sensitive to temperature, and typically, these enzymes become denatured at elevated temperatures. This result aligns with the research conducted and Gao *et al.* (2022) who identified the optimal temperature for the biodegradation of cellulose by *Aspergillus Specie*. Temperatures exceeding this threshold resulted in a decreased biodegradation rate (Yi *et al.,*2022).

The outcomes of cotton wool biodegradation by *Fusarium* Species*,* and *Trichoderma* Species over a duration of 60 days indicated that *Fusarium* Specie exhibited a similar degradation pattern to that of *Aspergillus* Species. In contrast, *Trichoderma* Species demonstrated a more rapid biodegradation rate. This observation may be attributed to the enzymes produced by these fungi. These results confirm the cellulolytic capabilities of *Aspergillus* Species *Fusarium* Species *and Trichoderma* Species*,* in degrading cotton wool, aligning with the findings of Selim *et al.* (2021).

The conditions of the plates before and after the biodegradation indicated notable physical alterations on the surfaces of the waste materials, including the shrinkage or absorption of the solid agar medium utilized. This indicates that the fungi not only degraded the cotton wool but also consumed the solid agar during the process. A similar phenomenon of discoloration following microbial treatment was reported by (Freeman *et al.* (2024).

Fourier Transform Infrared (FTIR) spectroscopy employed to verify the biodegradation of cellulose by examining the alterations in the spectra that represent the functional groups of cellulose after degradation. Key indicators of biodegradation include changes in the intensity of the spectral bands, shifts in their positions, and the emergence or disappearance of bands in both untreated and degraded cotton wool as noted by (Smith *et al.* (2021).

The intramolecular bonding of cellulose fibers degraded by *Aspergillus* Species correspond to the primary functional groups of cellulose, namely the hydroxyl (-OH) and C-O stretching, exhibit an increase in the biodegraded material. The enhancement of these spectral bands indicates that the primary functional groups have been targeted by microorganisms, leading to their degradation and transformation into new biomass (Kassem *et al.*, 2023). The overall results demonstrated that as cotton undergoes degradation by *Aspergillus* Species, the carboxyl groups in cellulose are disrupted, resulting in the collapse of the cellulose structure and the emergence of new products (Subramanian *et al.*, 2022).

CONCLUSION

Aspergillus species, *Fusarium oxysporum* and *Trichoderma* species were isolated and identified from cow dung. Confirmation of identification using molecular techniques showed *Fusarium* has 99.0% similarly index with *Aspergillus*. *Fusarium* showed the highest biodegradation potentials with the mean of 43.34% in 60 days followed by *Trichoderma* with 30.07% while *Aspergillus* species showed the least with 27.38%. FTIR of waste materials shows that isolates provided further validation of the degradation process by indicating the presence of functional groups associated with the potential breakdown products, which primarily included alcohols, alkenes, alkyls, alkynes, carboxylic acids, and methyl groups.

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