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Bio-oxidation of Nylon-6 by *Aspergillus niger* Isolated from Solid Waste Dumpsites

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Abstract

An Aspergillus niger *strain (AF3) isolated from dumpsites soil sample of Abule-Egba, Lagos, Nigeria, was studied for its potential to degrade a known weight synthetic aliphatic polyamide-6 (generically known as nylon-6) in a submerged culture. The growth activities of the fungus were monitored for three weeks at the room temperature followed by chemical and structural analysis. The fungus reduced the pH of the medium from 6.4 to 4.8 and oxidized the nylon-6 fibre by turning it to yellowish brown. It caused 23.95% loss in weight and reduced the number-average molecular mass of the nylon-6 fibre by 29.77% relative to the control. Structural changes in the molecular functional group of the nylon-6 fibres were ascertained by Fourier transform infrared (FT-IR) spectroscopy. The decrease in the transmittance intensity coupled with increase in the peak area of characteristics functional groups especially of the peaks at 3,304 cm^{-1} corresponding to amide A N-H stretch, 1,641 cm^{-1} and 1,545 cm^{-1} corresponding to amide I and amide II, respectively, as well as disappearance and formation of new peaks confirmed the degradation of the polymer. The discoloration of polymer fibre and the change of the pH by the fungi to create an acidic condition suggested the hydrolytic-oxidation mechanisms through the production of enzymes that initiated the protonation of the hydroxyl group of an intramolecular hydrogen bond followed with the hydrolysis of the carbonyl to produce molecules of lower molecular weight compounds. This study contributes to the possibility of use of the potential in filamentous fungi for large scale biodegradation of recalcitrant nylon-6.*

Keywords: Aspergillus niger, *bio-oxidation, biodegradation, FTIR, nylon-6.*

1. Introduction

Nylon-6 is an aliphatic polyamide, it is a synthetic semi crystalline plastic that is widely used in textile industry as well as an engineering materials for manufacturing of commodity polymers. It has good mechanical properties and high thermal resistance. It is a polymer of high service life because of its stability against many physical and chemical agents. It is considered to be non-biodegradable due to its resistance to microbial and enzymatic attack (Klun *et al*. 2003).

Oxidation of polymers is often seen as a cause of detrimental changes to polymer properties and it's therefore an important factor in the degradation of many polymers (Bernstein *et al*. 2005). The increase in production and environmental accumulation of plastic waste materials is causing increase attention to the study of biodegradation and mineralization of these polymers.

In early studies, the ability of some bacteria isolates including *Pseudomonas, Flavobacterium* and *Alcaligenes* to degrade the nylon-6 monomer and oligomers were reported (Negoro 2000; Baxi and Shah 2000). Tomita *et al*. (2003) isolated a thermophilics bacterium *Geobacillus thermocatenulatus* with ability to degrade some nylons while Sudhakar *et al*. (2007) also reported bacteria mediated

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degradation of nylon-6 and 66 by some marine bacteria. However, few reports that are available on the ability of the fungi to degrade nylon-6 and 66 were only reported in filamentous lignolytic white rot fungi *Bjerkandera adusta* by Klun *et al*. (2003) and *Phanerochaete chrysosporum* by Friedrich *et al*. (2007)

This study therefore reports the oxidative physical and structural changes in nylon-6 caused by an *Aspergillus niger* isolated from a solid waste dumpsite using Fourier Transmittance Infrared (FTIR) spectroscopic analysis.

2. Materials and Methods

2.1 Nylon Fibre and Reagents

Nylon-6 fibre pack of 13 × 13 × 1.5 cm size was purchased from Goodfellow Cambrigde Limited, England, UK. Caprolactam and other analytical reagents were purchased from Zayo-Sigma Chemical Company Limited, Nigeria.

2.2 Organism and Culture

The fungus strain (AF3) was one of the five fungi isolated in our laboratory from Soil samples from a major designated Solid waste dumpsites (Abule Egba Dumpsite) in Lagos state, on Potato Dextrose Agar (PDA) and identified based on it colony morphology and the microscopic observation of it hyphae and conidiophores compared with the Compendium of soil fungi (Domsch *et al*. 1980)

2.3 Biodegradation of Nylon-6 Fibre

The ability to degrade nylon-6 fibre in submerged culture of synthetic medium using the method described by Friedrich *et al*. (2007) was determined. 100 ml of the medium at pH 6.4 dispensed into 250 ml conical flasks with previously weighed pieces of 2.5 cm × 2.5 cm of the1mm thick nylon-6 fibre. Five millimetres of suspended fungi spores scraped from 7 days old plate culture of the fungi isolates on PDA was used as inoculums. The experiments together with the uninoculated control were set up in triplicate and incubated in stationery position for 3months followed by monthly monitoring analysis.

2.4 Experimental Analysis

2.4.1 Mycelia Growth Weight and Nylon-6 Fibre Weight Loss

Mycelia growth (Dry mycelia weight), and the pH of the culture were analysed at monthly intervals by harvesting the mycelia mat with a dried and previously weighed filter paper, dried in the oven at 60^{o}C to a constant weight using digital Analytical Balance (A&D Model GR 200) while the recovered nylon-6 fibre was thoroughly washed with distilled water, dried and reweighed. The mycelia weight and the fibre weight loss were determined by difference from the initial weight. The pH of the filtrates was also determined using JENWAY model 3520 pH meter.

2.4.2 Number-Average Molecular Mass

The relative viscosity of the recovered nylon-6 fibres were determined by dissolving 1% of the nylon in 4M H_2SO_4 and measured with Ubelholde viscometer. The number-average molecular mass (M_n) were calculated from the equation $M_n = 11{,}500(\eta_{rel}-1)$, (Ciaperoni and Mula 2001), where η_{rel} is the relative viscosity of the fibre sample.

2.4.4 FT-IR Analysis

The recovered nylon-6 fibres were dissolved in 2,2,2 trifluro-ethanol, poured into a glass Petri dish and allowed to dry overnight in a fume chamber. The membrane formed was analysed using Fourier Transform Infrared Spectrometer (FT-IR) (Schimatzu IR-470) at room temperature in transmission mode. The changes in the functional groups of the treated and untreated nylon-6 fibre were compared.

3. Results and Discussion

3.1 Fibre Discoloration and pH Changes

Figure 1 shows the yellowish brown discoloration effect of the oxidation on the nylon-6 fibre. This observation has been reported to be a common phenomenon in the mechanism of aliphatic polyamide oxidation especially during thermal oxidation (Li and Hu 1998).

Discoloration of polyamide during oxidation is said to be caused by formation of α-ketoamide groups chromophores due to oxidation of the methylene group adjacent to the carbonyl of the amide (Li and Hu 1998). However, the medium was not discoloured but only the nylon-6 fibre as observed in this study.

The ability of this fungal isolate to change the pH to value of the culture medium from 6.4 to 4.8 may have contributed to the degradation effect on the nylon-6 fibre. This may conform to the observations of Dam and Ogilby (2001) that reported the pH dependence degradation of polyamide in chlorinated water and observed that degradation was most pronounced at pH value less than 5.

3.2 Microbial Growth on Nylon-6 Fibre Medium

The ability of the fungi isolates to grow in the broth culture containing the nylon-6 fibre as the sole nitrogen source (Fig. 2) and the corresponding changes in the pH of the growth medium (Fig. 3) indicated the activity of the fungal isolates on the substrate. Although, Friedrich *et al*. (2007) reported the inability of the *Aspergillus species* isolated in their studies to degrade the nylon-6 fibre but their report was however silent on the growth of the *Aspergillus* isolates in the submerged culture. Therefore the ability of this fungal isolate to grow and change the pH of the culture may result in their degradation effect on the nylon-6 fibre.

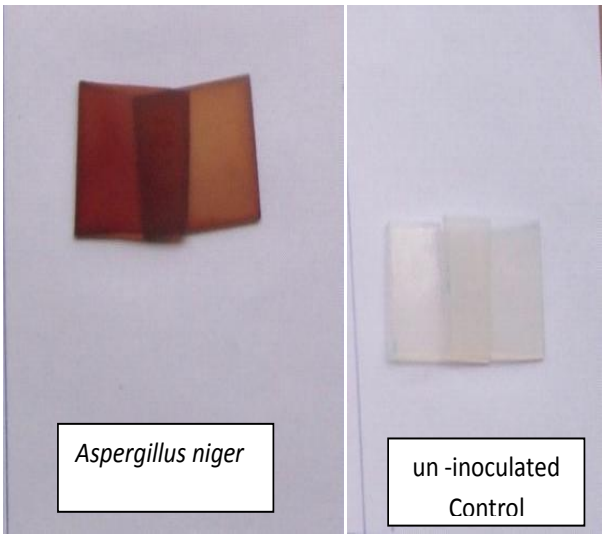

Fig. 1. Sample of the oxidised nylon-6 fibre and the uninoculated control.

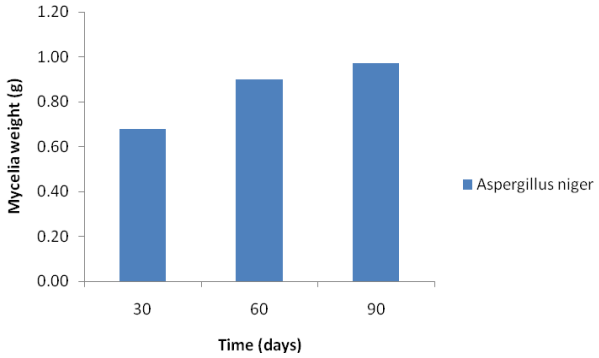

Fig. 2. The growth of the *Aspergillus niger* strain in submerged culture.

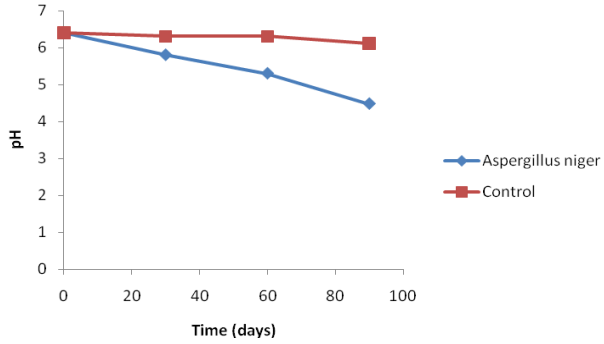

Fig. 3. pH changes of the culture medium.

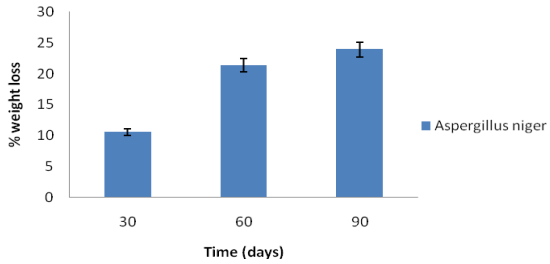

Fig. 4. Percentage loss in weight of the *Aspergillus niger* treated nylon-6 fibre.

3.3 Weight Loss and Changes in Number-Average Molecular Mass (M_n)

There was gradual loss in weight of the treated fibres over the 90 days of the experiment that resulted in 23.95% weight loss compared to the uninoculated control (Fig. 4).

The number average molecular mass decreases with time over the 90 days of the experiment. The treated nylon fibre decreased from 4,436.88 $gmol^{-1}$ to 2,748.45 $gmol^{-1}$, i.e. 29.77% reduction relative to the uninoculated control (Fig. 5). Similar observation was recorded in lignin degrading fungus *Bjerkandera adusta*. This may be due to the possibility of extracelluar enzymes production

by the microbial isolates which may act on the nylon-6 fibres so as to make available inherent nutrient requirement of the microorganisms from the fibres. The exocellular activity of such enymes according to Wales and Sagar (1988) will remove successive monomer units from the chain ends of the polymer fibre resulting in a disproportionate weight loss with relative effect on the tensile strength. Also, molecular weight reduction accordibg to Deguchi *et al*. (1997) is probably caused by hydrolysis or oxidative chain scission.

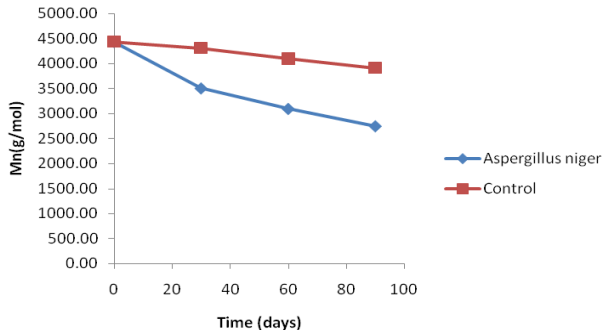

Fig. 5. Changes in number-average molecular mass (M_n) of the nylon-6 fibre with days of incubation.

3.4 FT-IR Analysis

Figures 6 and 7 show FTIR spectra of the fungus treated nylon-6 fibres and the uninoculated control.

The peak at wavelength number 3,506.7 cm^{-1} corresponding to hydroxyl (O-H) functional group of the hydrogen bonds was found to have disappeared in the treated fibres when compared with the control. This indicated the breakage of the intracellular hydrogen bonding while the formation of new peak at wavelength 3,471.98 cm^{-1} corresponding to free N-H stretch of the aliphatic primary amine (John 2000) was observed in the spectral of the treated nylon-6 fibres.

Furthermore, there were increases in the peak area at 3,304.17 cm^{-1} which corresponding to the N-H stretch of amide A, peaks 2,935.76 cm^{-1} and 2,864.39 cm^{-1} corresponding to asymmetric and symmetric CH_2 stretch of methyl group and increases in the peaks area at 1,641.48 cm^{-1} and 1,545.03 cm^{-1} corresponding to carbonyl of the amide I and amide II.

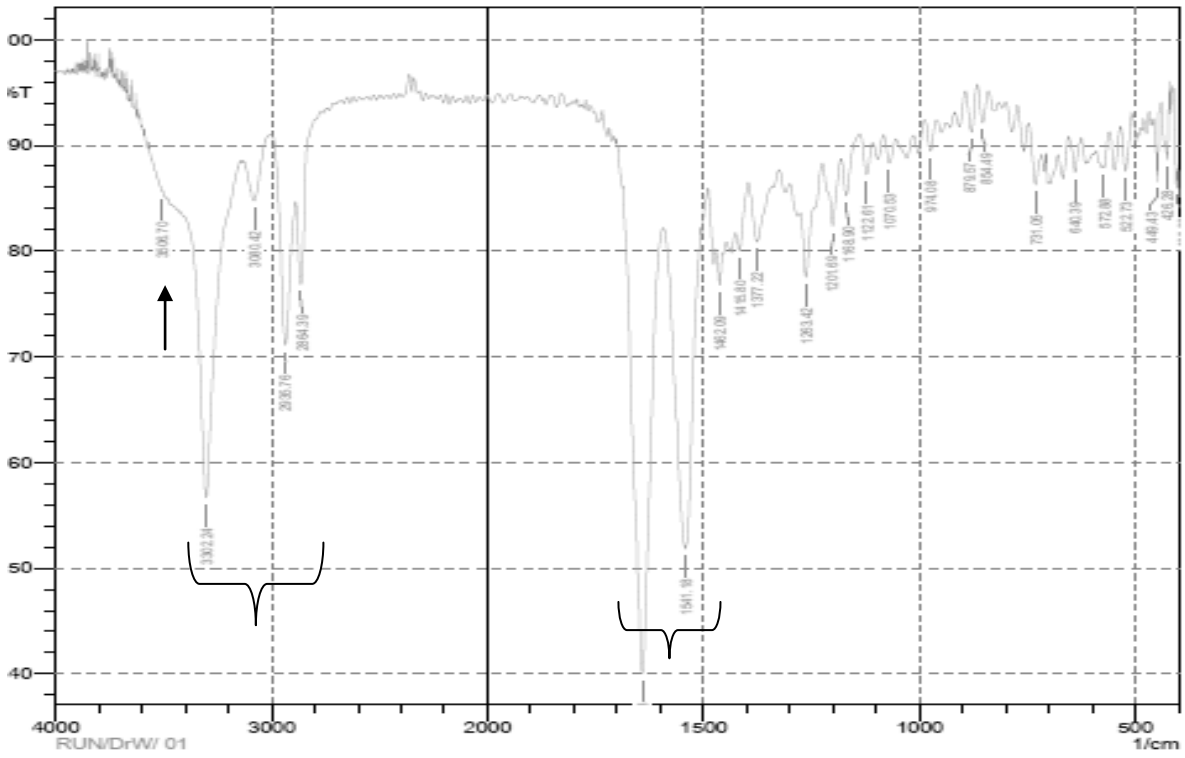

Fig. 6. FT-IR spectra of the uninoculated control.

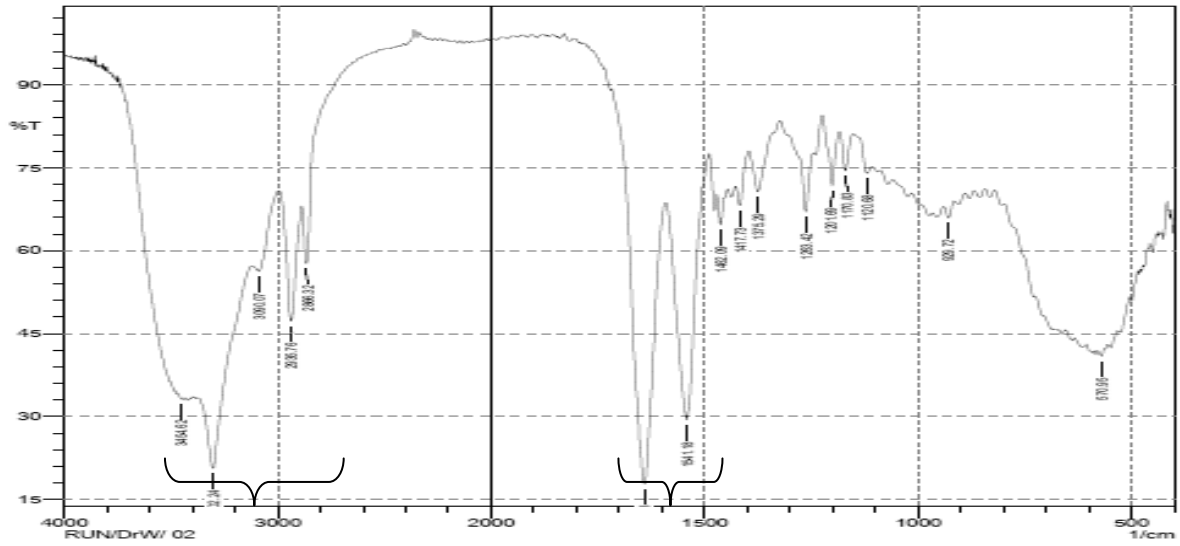

Fig. 7. FT-IR spectra of the *Aspergillus niger* treated nylon-6 fibre.

The increases in these intensities may be due to the cleavages in the intramolecular hydroxyl (O-H), carbon to carbon (C-C) and or carbon to nitrogen (C-N) bonds thereby resulting in formation of corresponding amines, amides, methyl and other degradation products. Similar observations were reported in earlier studies (Deguchi *et al*. 1997; Sudhakar *et al*. 2007; and Ibrahim *et al*. 2009). Deguchi *et al*. (1997) reported oxidative degradation by a white rot fungus IZU-54. The methylene group adjacent to the nitrogen atom in the polymer was said to be probably attacked by the peroxidase enzyme produced by the fungus while subsequently the degradation proceeds through autoxidation.

Conclusion

In conclusion, since biodegradation of polymeric materials can be defined to include any change in molecular level that alters the properties of the polymer, this oxidative ability of the fungus is considered a positive step in biodegradation of nylon-6.

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