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Hydrolytic Degradation of Nylon-6 by *Pseudomonas aeruginosa*HE858284 Isolated from Solid Waste Dumpsites in Lagos State, Nigeria

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Abstract
Polyamide-6 (also known as nylon-6) is one of the biodegradable-resistant synthetic polymers used in the manufacturing of commodity plastic materials. The environmental effects of the persistence of this material in landfill pose a global problem of disposal system. Knowledge of the microbial pattern of interaction with this plastic will provide the biological resources and scientific basis for the development of sustainable disposal and treatment method.

Pseudomonas aeruginosa (HE858284), one of the bacterial isolates from Solid Waste dumpsites that were screened for their ability to utilize ε-caprolactam (the monomer of the nylon-6) as a sole source of carbon and nitrogen for growth and identified base on 16SrDNA gene sequences was tested for nylon-6 degradation. Intermediate products in the culture medium were monitored using High Performance Liquid Chromatography (HPLC) while biodegradation of the nylon-6 was monitored using Fourier Transmittance Infrared Spectroscopy (FTIR), average number molecular mass (M_n) and weight loss. An un-inoculated experiment served as the control. Data obtained were analysed using ANOVA.

The HPLC analysis of the culture supernatant revealed the presence of 6-aminohexanoic acid, ε-caprolactam and some un-identified oligomers as the degradation products of the nylon-6 fibre. The changes observed in the FTIR spectra of different functional group confirmed the effect of microbial degradation of the nylon-6 fibre. The degradation potential of 12.82 % reduction in M_n and 5.23% weight loss within 90 days was recorded in this *Pseudomonas aeruginosa* (HE858284).These changes were found to be significant at $p = 0.05$.

This ability in the bacteria isolate from solid waste dumpsites will serves as baseline information for the bio-treatment of the nylon-6 polymer.

Key words: Nylon-6, Ɛ-Caprolactam , Solid waste dumpsite, Microbial degradation, 16SrRNA.

1. Introduction

Polyamides, generically designated as Nylon is the first synthetic polymer to have been successfully used for commercial purposes and ever since it first came on the market, nylon's many uses have greatly influenced most facets of human lives, and remained the strongest of all man-made fibres in common use (Meplestor, 1997). It is a thermoplastic semi-crystalline synthetic polymer that contains chain of recurring amide groups bonded with hydrogen bonding.While natural polymers are readily broken down by microbes, synthetic polymers are more resistant because the enzymes required to break down these synthetic polymers are not found in nature. Another reason is that these synthetic polymeric materials are mostly insoluble, hydrophobic and lack points of hydrolytic attack by extracellular enzymes produced by the microorganisms other than the terminus of the carbon chain (Palmisano and Pettigrew, 1992).

Polyamides might seem to be susceptible to microbial attacks due to their structural similarity to protein. However, this has been refuted in many tests and it is well known that these compounds especially polyamides-6 are generally resistant to microbial degradation when the number average molecular weight is greater than 1000 (Andreoni *et al*., 1993).

Nylon-6 was generally regarded as a xenobiotic polymer (Oppermann *et al*., 1998). Its reporteddegradation by a thermophilic bacterium*Bacillus pallidus* (Tomita *et al.,* 2003) and marine strains of *Bacillus cereus, Bacillus sphaericus, Vibrio furnisii,* and *Brevundimonas vesicularis* (Sudhakar *et al*, 2007) brighten the hope of the prospects for microbial degradation of polymer. However, the degree of microbial degradation has been shown to be lower in the larger molecule (Prijambada *et al.,* 1995).

After Nylon products are used and are no longer wanted they are thrown in the garbage and this causes environmental problems because Nylons has an exceptionally slow decay rate, which is responsible for a buildup of Nylon products in landfills worldwide (Smith 2009). Therefore, to contribute to the ways of ameliorating the

challenges of accumulation and non-degradability of nylon products in our environment, this study look at the ability and pattern of degradation of nylon-6 by some Ɛ-Caprolactam utilizing bacteria isolates from solid waste dumpsites. The aim of the study is to provide us with the knowledge of biological resources and possible scientific basis required for development of a suitable disposal system that will ensure a sustainable treatment of this material.

2. Experimental

2.1 Nylon Fibre and Reagents

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

2.2 Bacterial Isolate and Inoculum

The Isolation and characterization of the *Pseudomonas aeruginosa* (HE858284) was reported in Sanuth *et al.* (2013). The inoculum was prepared by culturing the bacteria isolate in Nutrient broth on an incubator shaker G24 Environmental incubator shaker (New Brunswick scientific Co.Inc.Edeison USA) at 180 rpm for 48 hrs; cells were harvested by centrifugation at 7,000 rpm for 10 minutes, washed with and suspended in normal saline solution.

2.3 Biodegradation of Nylon-6 Fibre by the Bacteria Isolate

The bacteria isolate was tested for its ability to degrade nylon-6fibre in submerged culture using the synthetic medium as described by Baxi and Shah, (2002). Previously weighed strips of the nylon-6 fibre of 25 mm x 25 mm x 1 mm diameter were added as the sole source of carbon and nitrogen in 100 ml of the basal medium at pH 7.2 in 250 ml conical flasks. Cultures were sterilized in autoclave at 121^{o}C for 15 minutes and thereafter inoculated with1ml of the harvested cells of the bacteria isolate suspended in normal saline solution. The experiment was incubated in triplicates at 35^{o}C on the G24 Environmental incubator shaker (New Brunswick scientific Co.Inc.Edeison USA) at 180 rpm for 3 months.

2.4 Biodegradation Analysis

2.4.1 Determination of Nylon-6 fibre Weight Loss

The weight loss of the previously weighed nylon-6 fibre was determined after it was recovered from the culture broth and washed with distilled water to remove any biofilm layer and dried in the oven at 60^{o}C overnight. Both the biotic and abiotic treated fibre was reweighed using a digital analytical balance (A&D Model GR 200; capacity 210/0.0001g).

The percentage weight loss was calculated using the formula as follows:

$$\% \text{ Weight loss} = \frac{W1 - W2}{W1} \times 100$$

Where, W1 = weight of nylon-6 fibre before the experiment and W2 = weight of nylon fibre after the experiment.

2.4.2 Determination of changes in Number Average Molecular Mass (M_n) of the Nylon-6 Fibre

The relative viscosity of the nylon-6 fibre was measured using Ubbelholde Viscometer at room temperature. 1% (w/v) of the nylon-6 fibre in 5M solution of H_2SO_4 was ran through the Viscometer after the previous run of the solvent without the nylon-6 fibre. The time it takes the liquid to travel through the calibrated marks measuring bulb was measure for each of the samples in replicates and the relative viscosity (η_{rel}) was calculated as

$$\eta_{rel} = \eta / \eta_o$$

Where η_{rel} is the relative viscosity of the fibre sample, η is the viscosity of the solution and η_o is the viscosity of the solvent.

The number average molecular mass (M_n) was computed from the relative viscosity using the equation $M_n = 11500(\eta_{rel}-1)$ (Ciaperoni and Mula, 2001).

2.4.3 High Performance Liquid Chromatography (HPLC).

At the termination of the experiment, the bacteria cells were harvested by centrifugation at 10,000 rpm under 4^{o}C for 10 minutes. The cell free supernatants was analysed for degradation products using High Performance Liquid Chromatography (HPLC) at the Multidisciplinary Central Research Laboratory (MCRL) of University of Ibadan, Nigeria. The standard was made up of Caprolactam and 6-aminohaxanoic acid (50:50 v/v). The mobile phase

consisted of methanol: water using concentration of 60:40 (v/v). The analyses were performed on Cecil-Adept System 4 (Analytical); UV-Visible detector; CE 4900 Power stream software at 200 nm and the flow rate of the mobile phase was 1 mlmin-1 in TSK-GEL ODS-80TM 4.6 mm × 7.5 cm column.(Sigma-Aidrich)

2.4.4 Spectroscopic Analysis of structural changes in Nylon-6 fibres.
Fourier transform infrared (FTIR) spectrophotometer was used to assess the changes in the structure of nylon-6 fibre that can be attributed to the effect of the microorganism at the termination of the experiment. The nylon-6 fibre was recovered from the culture broth and washed to remove any external component. The washed fibre was then dissolved in 2,2,2 trifluro ethanol (Sigma-Aldrich Co), poured into a glass Petri dish and allowed to dry overnight in the fume chamber. The fibre membrane obtained was analysed at the Analytical Laboratory of the Redeemer University, Nigeria (RUN) using Fourier Transform Infrared Spectrometer (FTIR) Schimatzu IR-4800S at room temperature in transmission mode. The changes in the functional groups of the biodegraded and undegraded nylon-6 fibre were compared in the produced spectra with special interest on the amide and carbonyl region.

3 RESULTS AND DISCUSSIONS

3.1 Microbial Growth

The ability of the *Pseudomonas aeruginosa* HE858284 to grow in the nylon-6 fibre medium over the period of 3 months in the absence of alternative source of carbon and nitrogen with the corresponding changes in the pH of the culture medium is shown in Figure 1. The spectrophotometric readings of the bacteria growth at 600 nm showed the increase in the optical density of the culture from 0.468 on day zero to 1.138 on 90th day of the culture incubation. Also, pH of the medium changed from initial 7.0 gradually to 8.5 as at the 90th day. This observation indicated the ability of the isolates to produce enzymes which acted on the fibres to make available to the microorganisms the inherent nutrient requirement for growth in the nylon-6 fibre. Changes in the pH of culture medium towards alkaline pH values were also recorded. Similar observation was reported for *Baccillus cereus* in a nylon-6 culture medium in the study of Sudhakar *et al*., (2007). This change in pH may be due to the possibility of production of amine or ammonia as part of the hydrolytic products of the nylon-6 fibre degradation.

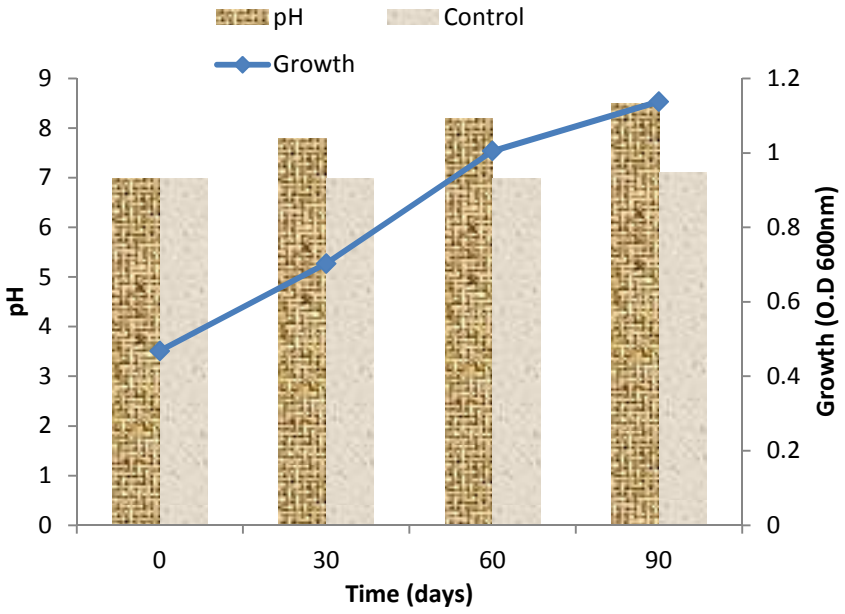


Fig. 1: Growth of the *Pseudomonas aeruginosa* and pH changes in the nylon-6 medium. Line graph shows the growth at optical density (OD) readings recorded at 600nm and Bar graph for pH changes over the 90 days incubation.

3.2 Weight Loss and Changes in Number average molecular mass (M_n)

The weight loss of the submerged nylon-6 fibre sample over a period of 3 months as shown in Table 1 indicated a mean average weight loss of 0.0315 g in the fibre treated with *Pseudomonas aeruginosa* representing a weight loss of 5.23% when compared to a similar weight loss of 0.0305 g recorded in the un-inoculated control fibre over same period of incubation. This observation suggested the possible production of extracelluar enzymes that possess specificity for the nylon-6 and or its components by the microbial isolate. The exocellular activity of such enzymes 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.

Table 1. The Weight loss

	EXPERIMENTS	INITIAL WEIGHT	FINAL WEIGHT	WEIGHT LOSS
Pseudomonas aeruginosa	A	0.9468	0.6906	0.2562
	B	0.9226	0.6743	0.2483
	C	0.9247	0.6722	0.2525
	Mean Average	**0.9314**	**0.6790**	**0.2523**
Control	A	0.9426	0.9074	0.0352
	B	0.943	0.9134	0.0296
	C	0.9424	0.9009	0.0415
	Mean Average	**0.9427**	**0.9072**	**0.0354**

Also, the reduction in the viscosity number average molecular mass of the nylon-6 fibres treated with this bacteria isolate (Table 2) showed reduction in the average number molecuar weight (M_n) from 3795.75 $gmol^{-1}$ to 3419.94 $gmol^{-1}$, representing a reduction of 12.82 % when compared to the un-inoculated control treatment. Statistical analysis of the changes using Duncan[a,b] Multiple Range Test, showed the changes to be significant at 95% confidence. Molecular weight reduction as reported by Deguchi *et al*. (1998) may probably be due to the hydrolysis or oxidative chain scission occuring in the polymer.

Table 2. The mean Changes in Viscosity Average Molecular Mass in the degraded nylon-6 fibre

Treatment	**Average number molecular mass (M_n) ($gmol^{-1}$)**
Control treatment	3795.75[a]
Pseudomonas aeruginosa	3419.94[b]
%	12.82%

a. Uses Harmonic mean sample sizes

b. Alpha = 0.05

3.3 HPLC Analysis

The HPLC chromatograms of the standards (Cyclic and linear monomers), the supernatant of the un-inoculated control experiment and that of the culture treated with *Pseudomonas aeruginosa* is as shown in the Figure 2 (a –c). Figure 2a shows the peaks of the standards. The 6-aminohexanoic acid resolved between 0.58-1.01 minutes retention time (Rt.) while the Caprolactam resolved between 1.35 -1.41minutes retention time (Rt.).

The chromatogram of the un-inoculated control (figure 2b) showed the presence of the linear monomer (6-aminohexanoic acid) at retention time of 1.00 minute with a peak area of 4165.0 mAs but the cyclic monomer (Caprolactam) was not seen in the control experiment. The chromatogram of that from the *Pseudomonas aeruginosa* treated sample on the other hand (figure 2c) showed the presence of the 6-aminohexanoic acid at retention time of 1.01 minutes with peak area of 4882.3 mAs, also the presence of one other oligomer at retention time of 1.10 and Caprolactam at retention time of 1.35 minutes with peak

area of 373 mAs and 82 mAs respectively. The presence of the 6-aminohexanioc acid (linear monomer) in both the treated and the un-treated control experiment suggested the possibility of partial chemical hydrolysis due to abiotic activities such as the temperature effect of autoclaving and reactions of the inorganic chemicals of the minimal salt medium. This Similar observation of slight increase in linear monomer in the abiotic control was reported in the study of Friedrich *et al*., (2007) and was accredited to be the monomer impurities in the nylon fibres.

However, the increases in the peak areas of the linear monomer of the bacteria treatment compared to that of the un-treated control experiment, coupled with formation of caprolactam and some other unidentified oligomers corroborated the degradation potentials of the bacteria. This is also in agreement with the earlier reports of Baxi and Shah (2000) and Friedrich *et al*. (2007).

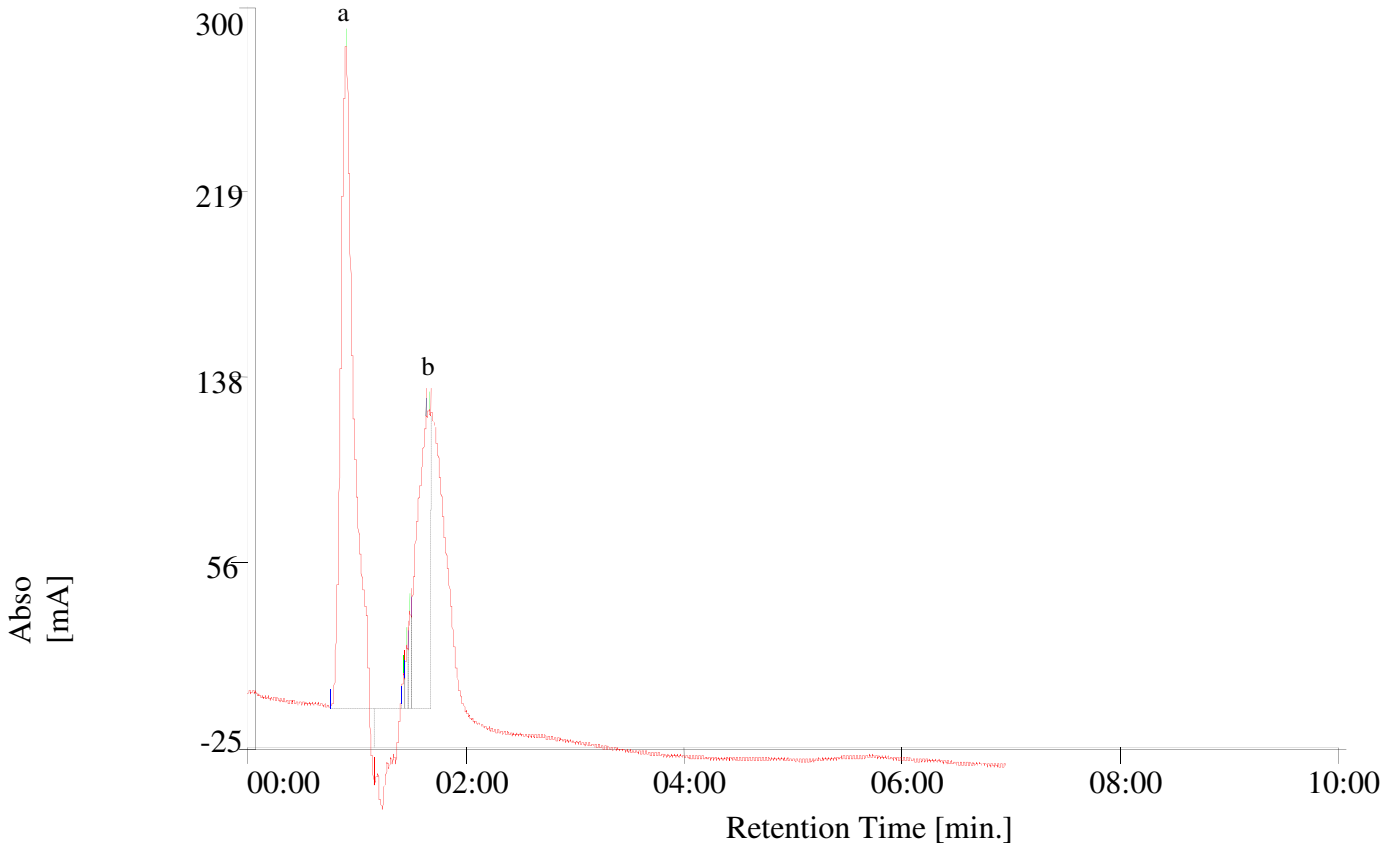


Figure 2a. Chromatogram of the standards: (a) 6-aminohexanoic acid with retention time between 0.58- 1.01 minutes and (b) Caprolactam with retention time between 1.35 - 1.41minutes

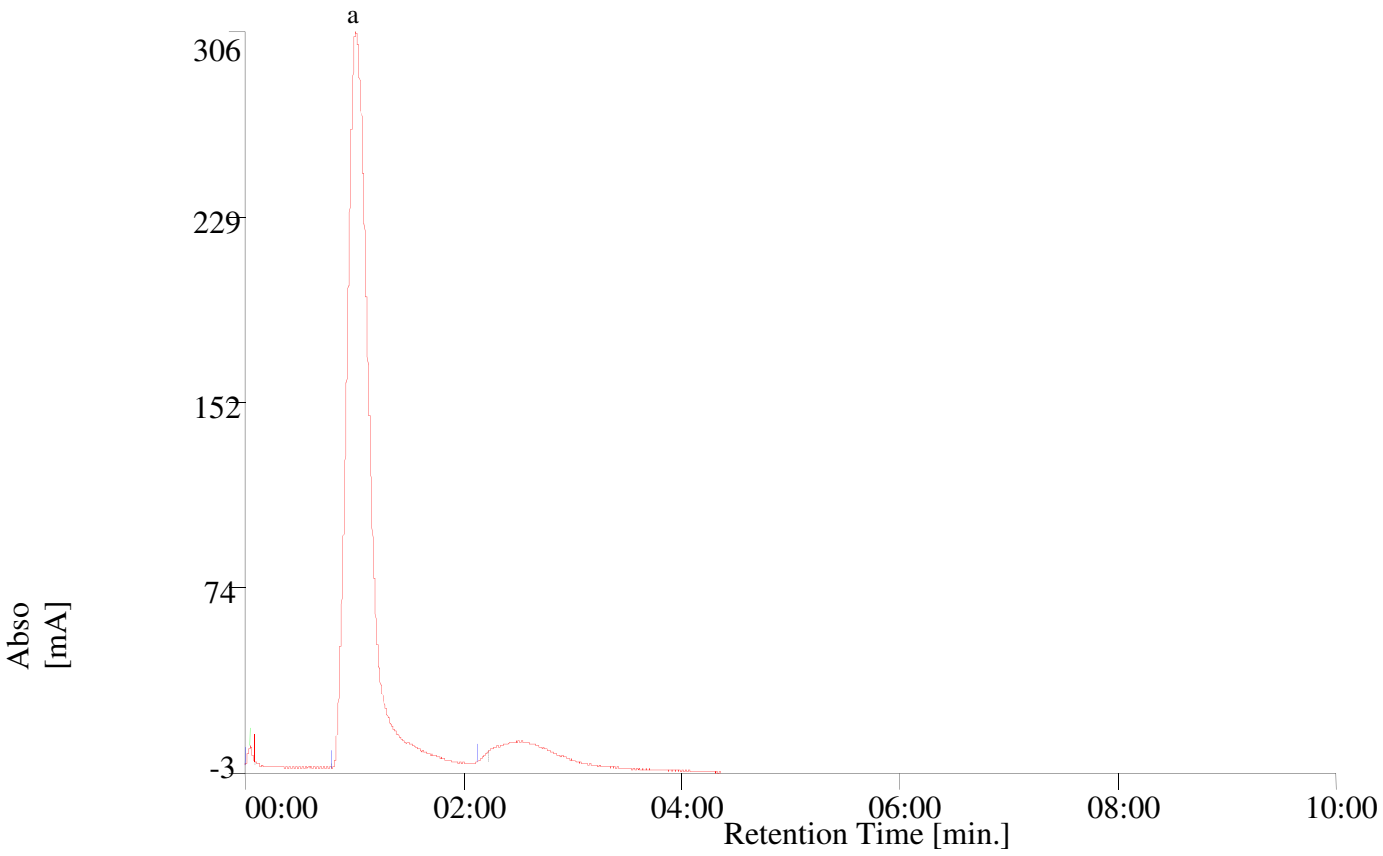


Figure 2b. The Chromatogram of un-inoculated control treatment of nylon-6 showning the presence of: (a) 6-aminohexanoic acid.

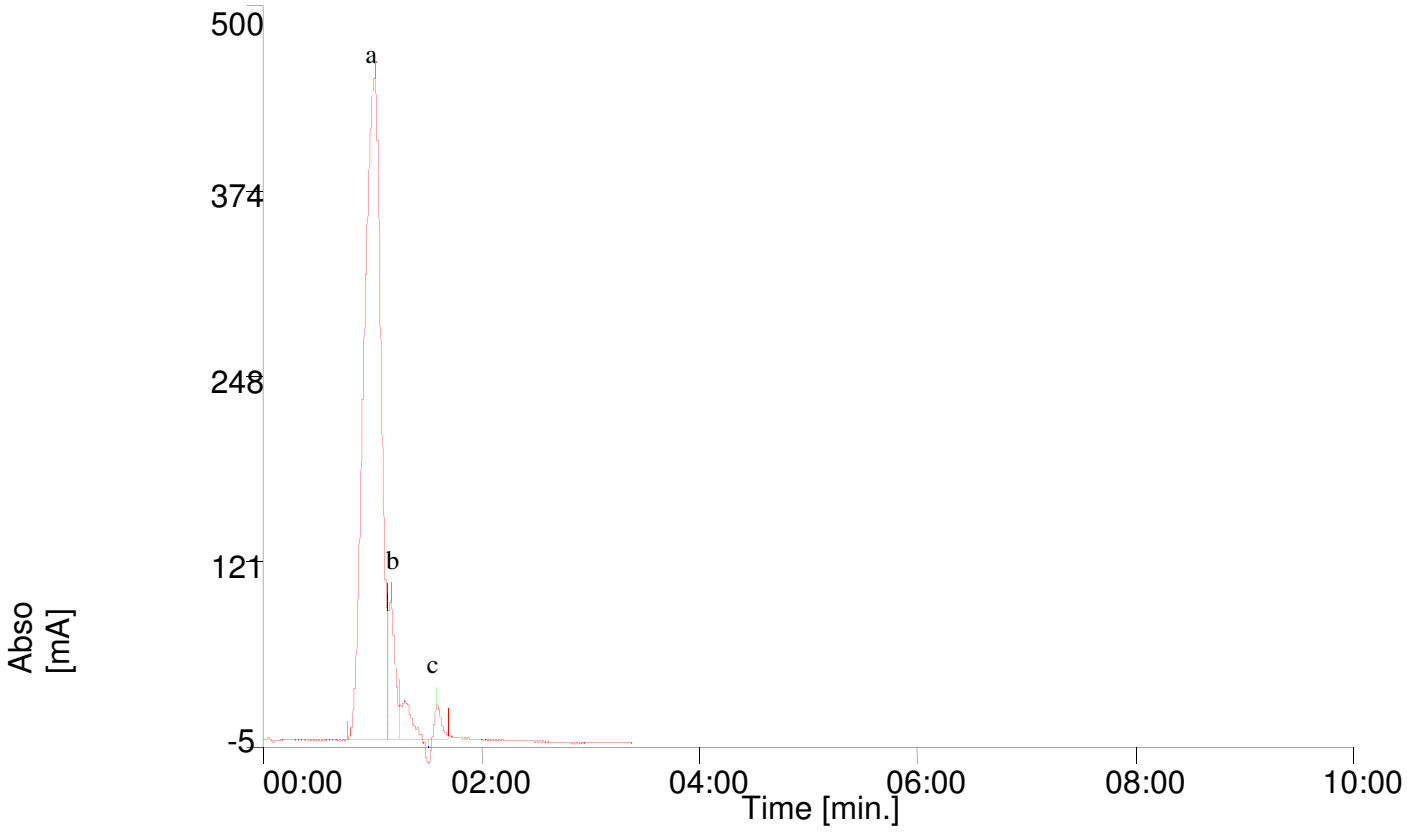
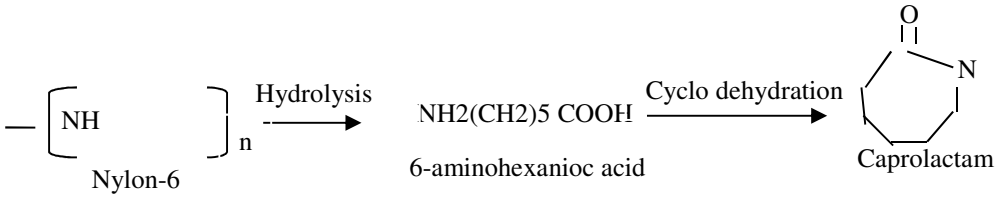


Figure 2c. The Chromatogram of the *Pseudomonas aeruginosa* (NTS1) treated nylon-6 supernatant, showing: (a) 6-aminohexanoic acid (b) Un-identified Oligomer (c) Caprolactam.

The suggested hydrolysis based on the HPLC analysis of the degradation products's supernatant can be represented as shown



The major detected degradation products are 6-aminohexanoic acid and caprolactam but there were traces of some oligomers that could not be identified in the course of this study.

4. FTIR Analysis:

The analysis of the FTIR spectra of the degraded samples is shown in Figure 3. The analysis for the nylon-6 fibre treated with *Pseudomonas aeruginosa* reavealed the replacement of the peak at 1464 cm-1 wavelength corresponding to the asymmetric C-H bending vibrations of methyl-alkyl group of a peak area 11.44 cm2 with two other peaks (1473 and 1458 cm-1) corresponding to same functional group and of 6.37 and 4.112 cm2 peak areas respectively. Also shown in the spectra was the formations of O-H bend of phenol or a tertiary alcohol at 1364 cm-1 with corresponding associated band of O-H out of plane bend at 669 cm-1. Presence of another peak at 1508 cm-1 corresponding to simple hetero-oxy compound was also detected. The percentage cumulative decrease in the absorbance of the nylon fibre when compared to the un-inoculated control was calculated to be 48.65%. Similar changes corresponding to disappearance of some

certain functional groups and formation of some new functional groups were reported by Sudhakar *et al.* (2007), this observations may be due to the processes of hydrolysis and oxidation. Also worthy of note is formation of hydroxyl groups corresponding to tertiary alcohol at 1364 cm^{-1}, hetero-oxy compound at 1508 cm^{-1} and out of plane hydroxyl group at 669 cm^{-1} as revealed in the *Pseudomonas aeruginosa* treated nylon-6 fibre. This may be due to alkaline hydrolysis as the bacteria isolates changed the pH of the culture medium towards alkaline. The degradation mechanism was likely to be through electrophilic attack catalysed by an alkaline contrary to the acidic protonation of the hydroxyl end-group of the intramolecular hydrogen bond. The alkaline attacked the hydroxyl end group of the carbonyl to form a lactam ring which is further hydrolysed by random alkaline attack on the carbon of the carbonyl group. Thus new molecules of low molecular weight were produced (Lucas *et al.,* 2008).

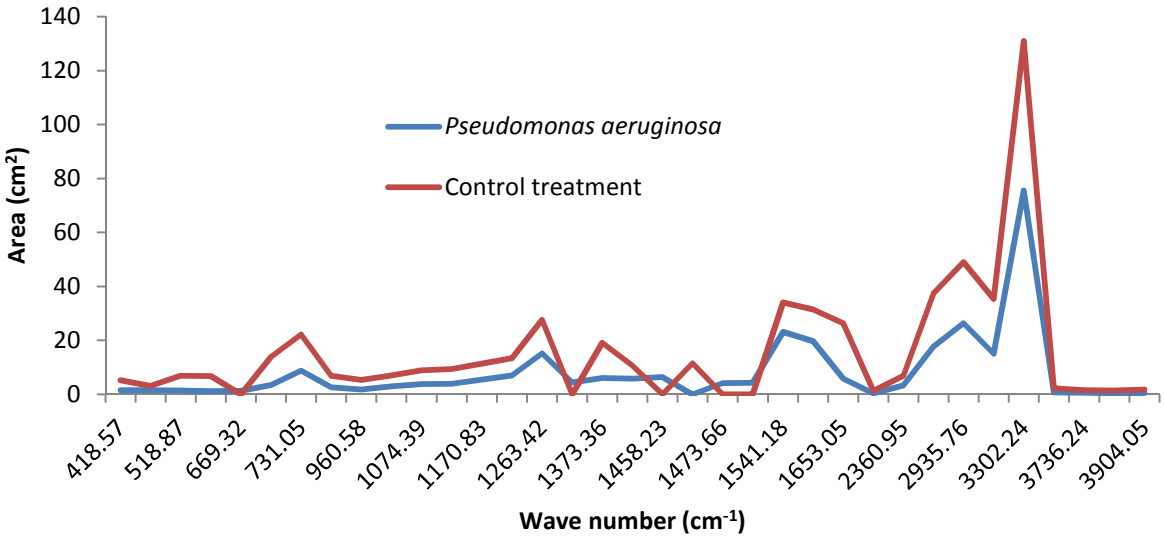


Figure 3. FTIR Spectra analysis of nylon-6 fibres treated with *Pseudomonas aeruginosa* (NTS1) after 90 days of biodegradation.

Conclusion

In conclusion, this study confirmed the potential of this strain of *Pseudomonas aeruginosa* to partially degrade the recalcitrant nylon-6 polymer. It gives the hope of possible utilization of these isolates in the development and application of biological methods to compliment its management.

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