# DEGRADATION OF POLYAMIDE-6 BY MICROBIAL ISOLATES FROM SOLID WASTE DUMPSITES IN LAGOS STATE, NIGERIA

BY

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## ABSTRACT

Polyamide-6 (also known as nylon-6) is one of the biodegradation-resistant synthetic polymers used in the manufacturing of commodity plastic materials. The environmental effects of the persistence of this material in landfill and on surface water bodies pose global problems that endanger public health. Knowledge of the microbial distribution and pattern of their interaction with these plastics will provide the biological resources and scientific basis for the development of sustainable disposal and treatment method. Therefore, microbial degradation of polyamide-6 and its monomers were investigated.

Soil samples were randomly collected at five spots to a depth of 15cm and mixed to form composites from each of the three selected dumpsites at Olusosun, Abule-Egba and Isheri-Igando in Lagos state. Microbiological analysis was carried out on the samples on bimonthly intervals over a period of eight months. The fungal and bacterial isolates 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 on a synthetic solid medium and were phenotypically characterized. The 16SrRNA gene sequences were used for the molecular typing of the isolates. The isolates with the best growth within 120hrs in ε-caprolactam medium were 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 Transform 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.

A total of 64 bacteria and 22 fungi were isolated from the soil samples. Sixteen species of the bacterial isolates made up of the genera *Pseudomonas* (5), *Alcaligenes* (3), *Corynebacterium* (2), *Leucobacter* (2), *Bordetella* (1), *Proteus* (1), *Providencia* (1) and *Lysinibacillus* (1) were capable of utilising ε-caprolactam as the sole source of carbon and nitrogen up to a minimum inhibitory concentration of 20 $gL^{-1}$. The fungi were identified as five species of *Aspergillus* and a species of *Penicillium*. *Proteus vulgaris* utilized 97.2%, *Bordetella petrii* 92.5%, *Pseudomonas aeruginosa* (NTS1) 90.5% and *Alcaligenes faecalis* (2ABA2) 82.3% of 10 $gL^{-1}$ ε-caprolactam within 120hrs compared to the control experiment. The HPLC analysis of the culture supernatant revealedthe 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 groups confirmed the effect of microbial degradation of the nylon-6 fibre. *Aspergillus*

*niger* was the most efficient of the fungi isolates in degrading the polyamide-6. It achieved 29.77 % reduction in polyamide-6 $M_n$ and 23.95 % weight loss. Moreover, *P. aeruginosa* (NTS1) degraded Polyamide-6 with 12.82 % reduction in $M_n$ and 5.23% weight loss. These changes were found to be significant at $p = 0.05$.

*Aspergillus niger* and *Pseudomonas aeruginosa* degraded nylon-6 fibre and this serves as baseline information for the bio-treatment of the nylon polymer.

**Keywords**: Nylon-6, Ɛ-Caprolactam , Solid waste dumpsite, Microbial degradation, 16SrRNA.
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Above all the people that have been mentioned is **God Almighty Allah** for sparing my Life, granting me good health, protecting me and providing my sustenance. If not for **His** grace, this achievement will have remained a fiction. *Alhamdulillah Rabil Alamin*.

Hassan A. Sanuth
October, 2012

## CERTIFICATION

I certify that this work was carried out by Mr. Hassan Adeyemi Sanuth, under my supervision in Department of Microbiology, University of Ibadan.

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## DEDICATION

This Study is dedicated to:

The Memory of my late father, Alhaji (Chief) S. I. Sanuth.

My Mother, Alhaja I. A. Sanuth and my wife, Mrs. Lateefat M. Sanuth.

And to my children, Mutmainat Adedamola, Fadlul'azeem Adekambi, Ikram Aderinsola and Ziyad Adelekan for their endurance throughout the period of this study.

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## ABBREVIATIONS

| | |
|---|---|
| BLAST | Basic Local Alignment Search Tool |
| CFU | Colony Forming Unit |
| Conc. | Concentration |
| DNA | Deoxy ribonucleic acid |
| FTIR | Fourier Transmission Infra-Red Spectroscopy |
| HPLC | High Performance Liquid Chromatography |
| MEGA | Molecular Evolutionary Genetics Analysis |
| MIC | Minimum Inhibitory Concentration |
| NA | Nutrient Agar |
| NCBI | National Centre for Biotechnology Information |
| OD | Optical Density |
| PCR | Polymerase Chain Reaction |
| PDA | Potato Dextrose Agar |
| PEG | Polyethylene Glycol |
| ppm | Part Per Million |
| TVC | Total Bacteria Viable Colony Counts |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Polyamides (Nylons)

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, its many uses have greatly influenced most facets of human lives, and it remained the strongest of all man-made fibres in common use (Meplestor, 1997). Nylon is a thermoplastic semi-crystalline synthetic polymer that contains chains of recurring amide groups bonded by hydrogen bonding. Nylon was invented in 1935 by Wallace Carothers at DuPont's research facility in the DuPont Experimental Station, West Virginia, USA. This began the explosion of completely synthetic materials that imitate natural materials in the fabric market as nylon was created entirely from petrochemicals. Previous artificial fabrics such as rayon and acetate were plant-based (Boyd and Philips, 1993). Nylon is a silky material and was first used commercially in manufacturing of a toothbrush with nylon bristles. Thereafter, it was used for making women stockings; and many other products have been made from it. (Schwartz and Goodman, 1982).

Nylon is one of the most important classes of synthetic polymeric materials, with wide application in the fields of fibres and plastics, because of their thermoplastic and silky nature. The properties of polyamides when fabricated into manufactured articles are considerably affected by the amount of crystallinity present. Unlike other polymer classes, the degree of crystallinity of the polyamides can vary by as much as 40%, depending on how the fabrication was carried out. Nylon fibres are therefore used in many applications, including fabrics, bridal veils, carpets, musical strings, and rope. Solid nylon is used for hair combs and mechanical parts such as gears and other low- to medium-stress components previously casted in metal while engineering-grade nylon is processed by extrusion, casting, and injection molding (Schwartz and Goodman, 1982). In general, the polyamides are usually referred to as condensation polymers and they may be synthesized in one of two ways, either by the reaction of dicarboxylic acids with diamines, or from specific amino acids or their cyclic derivatives - the lactams. These two approaches result in two types of polyamides: Nylon6/6 (Polyhexamethylene adipamine) and Nylon-6 (Polycaprolactam). Nylon-6 is

the most common commercial grade of molded nylon. It is available in glass-filled variants which increase structural and impact strength and rigidity.

### 1.2 Health and Environmental Hazards

Nylon is not yet considered an hazardous chemical, but Malloy and Grubb (2008) reported three main effects that can be caused by nylon. These were:-

a. Irritation of mucous membranes in the nose and throat, the eye and skin,
b. Respiratory hazard arising from dust produced during secondary operations, such as grinding, sanding or sawing and
c. Gastrointestinal discomfort resulting from ingestion or inhalation.

The authors also reported that the most common products of Nylon's thermal breakdown included carbon monoxide, carbon dioxide, ammonia, aliphatic amines, ketones, nitriles and hydrogen cyanide. However, some of these products may differ, depending on various factors such as temperature, time of exposure and environmental factors. After nylon products are used and are no longer wanted they are thrown in the garbage and this causes a problem because nylon has an exceptionally slow decay rate, which is responsible for a buildup of nylon products in landfills worldwide (Smith 2009). Its accumulation and non-degradability in landfills renders it to be of environmental concern. The disposal of this polymer which is being produced in large quantities, is posing serious environmental problem, in that the rate of their accumulation in the environment is higher while the natural microbial mediated degradation forces are not effective (Thompson *et al*., 2009). These polymeric materials are not easily degradable because they are insoluble, hydrophobic and lack points of hydrolytic attack by extracellular enzymes produced by the microorganisms other than the terminus of the carbon chains (Palmisano and Pettigrew, 1992). The degradation of these polymers have been reported to require several tough chemical and physical processes because natural forces were not very effective due to slow rate of degradation compared to their accumulation in the environment (Sudhakar *et al*., 2007).

### 1.3 Biodegradation of Nylon

Several attempts have been made by Fukumura (1966), Shama and Wase (1981), Negoro *et al*. (1994), Prijambada *et al*. (1995) and Negoro (2000) to degrade nylon materials using microorganisms. Both the cyclic and the linear monomers of nylon-6

(caprolactam and 6-aminohexanoic acid) as well as the oligomer, consisting of a small number of monomeric units of nylon-6, have been found to be metabolized by numerous microorganisms. These include the bacteria species of the genera *Pseudomonas, Flavobacterium, Achromobacter* and *Corynebacterium* (Fukumura, 1966; Negoro *et al*., 1994; Prijambada *et al*., 1995; Negoro, 2000) and the fungal genera *Absidia, Aspergillus, Byssochlamis, Penicillium, Rhodotorula and Trichosporon* (Shama and Wase, 1981). With increased polymerization to over 100 monomers, the material became more recalcitrant (Prijambada *et al.*, 1995). Nylon-6 was generally regarded as a xenobiotic polymer (Oppermann *et al*., 1998). Its degradation 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) have been reported. These reports brighten the hope of the prospects of the polymer's microbial degradation. On the other hand, fungal degradation of the polymer by both physical attachment and enzymatic attack with a manganese peroxidase by lignolytic fungi-*Phanearochaete chrysosporium* and *Bjerkandera adusta* (Klun *et al*., 2003 and Friedrich *et al*., 2007) were the only reported cases of nylon 6 degradation by fungi. The degree of microbial degradation has been shown to be lower in the larger molecule (Prijambada *et al.*, 1995).

### 1.4 Project Justification

The concern over environmental and health impacts of solid and effluent wastes disposal continue to be one of the greatest global challenges. The management of Municipal solid waste (MSW) has been classified as one of the challenges facing Environmental Protection Agencies in developing countries of the world (Ogwueleka, 2009). The ever increasing volumes of plastic debris, industrial waste effluents, sludge and other solid waste materials that constitute MSW accumulating in waste dumpsites creates problem of degradation; especially the synthetic polymeric plastic materials that were produced and introduced into the environment for the past few decades. Many of these plastics are not biodegradable and persist almost indefinitely in the environment (Kawai, 1995; Alexander, 1999; Shimao, 2001; Madigan *et al.*, 2003). The volume of solid waste being generated continues to increase at a faster rate than the ability of the environmental management agencies. Therefore, technical resources are needed to parallel this growth (Ogwueleka, 2009).

In Nigeria, Oyeniyi (2011) in his study of Waste Management in contemporary Nigeria found that solid waste management has overwhelmed the Nigerian government and Environmental hazards of varying magnitude dangerously threaten human and animal lives in most urban centers in Nigeria. In Lagos state, polymeric plastic materials constitutes over 15% of the total solid waste generated (Salami *et al.*, 2011). Solid waste management has emerged as one of the greatest challenges facing state and local government environmental protection agencies in Nigeria. Lagos State being an industrialised megacity with a population of about 18 millon, designated four dumpsites in Gbagada, Olusosun, Abule-Egba, and Solous to meet the disposal of solid wastes generated as a result of rapid urbanization. At these dumpsites, scavengers sort out items for reuse and recycling. The essence of this treatment is to reduce the volume of waste being ultimately landfilled. In most cases the retrieved items become raw materials for other processes. These, to a large extent, enhanced environmental aesthetics in major parts of the state. However, there is the need for more research into the development of new methods that will bring about effective and environmentally safe processes for the rapid degradation of the recalcitrant polymeric plastic materials in the waste accumulated.

Therefore, the aim of this research is to identify bacterial and fungal isolates associated with the degradation of nylon-6 polymer in dumpsites and laboratory condition, study their degradation products and provide information on possible development and application of biological methods to compliment its management

## 1.5 Research Objectives

The objectives of the study, therefore, were to

a. Isolate and screen for nylon-6 monomer (Caprolactam) degrading microorganisms (fungi and bacteria) from solid waste dumpsites.
b. Study the optimum physico-chemical conditions for the growth of the microorganisms.
c. Study the potential of the isolates to utilize nylon-6 fibre in broth culture.
d. Analyze the degradation ability of the isolates using physical and chemical parameters.
e. Characterise the potential degrading bacteria isolates biochemically and molecularly.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 History and Synthesis of Polyamides

Polyamides also known as Nylon are synthetic semi-crystalline thermoplastic polymers containing recurring amides links (R-CONH-R) in the main chain of the polymer (Kohan, 1973). Polyamide was developed by Wallace Carothers and other scientists at Du Pont in 1935 and became the first commercially produced synthetic fibres in 1938 (Mark and Whiby, 1940). It was first used in the production of a nylon-bristled toothbrush in 1938, followed by women's stockings in 1940. There are two common methods of making nylon for fiber applications. In one approach, molecules with an acid (COOH) group on each end are reacted with molecules containing amine ($NH_2$) groups on each end. The resulting nylon is named on the basis of the number of carbon atoms separating the two acid groups and the two amines. These are formed into monomers of intermediate molecular weight, which are then reacted to form long polymer chains. This reaction is refered to as polycondensation reaction of diacids and diamines (Kroschwitz and Howe-Grant, 1996). An example of this polyamide is Polyamide-6,6. The numerical suffix specifies the numbers of carbons donated by the monomers; the diamine first and the diacid second. In the second approach on the other hand, a compound which has an acid at one end and an amine at the other is polymerized to form a chain with repeating units of $(-NH-[CH_2]_n-CO-)_x$ and the product is named based on the number of carbon per unit of the chain. In this equation, if n=5, then nylon 6 is the assigned name. An example of this is polyamides-6 which is produced by ring opening polymerization of ε-caprolactam (Schwartz and Goodman, 1982). Structurally, the chemical elements of polyamides are carbon, hydrogen, nitrogen, and oxygen.

Polyamide (Nylon) was intended to be a synthetic replacement for silk and was substituted for it in many different products after silk became scarce during World War II. It replaced silk in military applications such as parachutes and flak vests, and was used in many types of vehicle tires (Salmawi *et al.*, 1993). Nylon fibres are used in

many applications including fabrics, bridal veils, carpets, musical strings and rope. Solid nylon is used in hair combs and for mechanical parts such as machine screws, gears and other low- to medium-stress components previously casted in metal. Nylon 6 is the most common commercial grade of molded nylon and is available in variants structures which increase impact strength, rigidity and lubricity (Nagata and Kiyotsukuri, 1992).

## 2.2 General Properties of Polyamides

Polyamides are semi-crystalline polymers with both crystalline and amorphous phases. Crystallinity results from the polar amide groups, which undergo hydrogen bonding between the carbonyl and amine groups in adjacent sections of the polyamide chains. The regular alignment of the amides groups allows a high degree of hydrogen bonding hence, the more random the amorphous region the lower the degree of hydrogen bonding. These linear aliphatic polyamides are able to crystallize mostly because of strong intermolecular hydrogen bonds through the amide groups (Dasgupta *et al* 1996), and because of Vander Walls forces between the methylene chains. Nylon 6 had adjacent chains that are anti parallel and the hydrogen bonding is between adjacent chains within the same sheet (bisecting the $CH_2$ angles). The crystalline structure contribute to the hardness, strength, chemical resistance, creep resistance and temperature stability of the nylon. It also contributes to the impact resistance and high elongation. This crystalline nature of the material is responsible for its wear resistance, chemical resistance and thermal resistance. These properties have made nylon the strongest of all man made fibres in common use (Meplestor, 1997). Polyamides are insoluble in common organic solvents at room temperature. However, they are soluble in formic acid, phenols, mineral acids and fluorinated alcohols such as 2,2,2-trifluoroethanol (Tuzar, 1991).

Polyamides generally have high melting points which are a function of the strong hydrogen bonding between the chains. They are hygroscopic as water bind to the hydrogen bonds of the polar amide groups. Water absorption is greater in the amorphous regions due to greater availability of amides groups. Moisture strongly affects properties of polyamides such as the glass transition temperature ($T_g$) and act as a plasticiser increasing its flexibility and toughness. Polyamides are reported to be degraded by hydrolysis at elevated temperatures (Kroschwitz and Howe-Grant, 1996).

## 2.3 General Applications of Polyamides

Polyamides are significantly commercial polymeric materials representing approximately 4% of the total world consumption of principal polymers. Polyamides-6 accounts for approximately 54% of polyamides produced while Polyamides-6,6 accounts for approximately 36% (Kroschwitz and Howe-Grant, 1996). Almost 75% of polyamides are used as fibres while about 15% are used as engineering plastics. Fibre uses include carpet, textiles, home furnishings and industrial applications (Meplestor, 1997). Engineering uses vary from wheel covers, plastic handles, radiator end tanks, fuel hoses, hair dryers, lawnmowers, gears and bearings (Dasgupta *et al.*,1996). The nylon fibre has outstanding durability and excellent physical properties. Its toughness makes it a major fibre of choice in carpets; including needle punched and floor-covering products (Meplestor, 1997).

In certain applications, the performance of nylon fibre is hard to beat. However, because of its higher cost, it is used in specialized applications where its performance can justify the increased cost. It is used as a blending fibre in some cases, because it conveys excellent tear strength. It is used in moderate quantities, because it is more expensive than polyester, polypropylene, or rayon (Raghavendra, 2004). The most widely used nylon products in the textile industry are a form of nylon 6, the others are mainly used in tubing extrusion, injection molding, and coatings of metal objects (Dasgupta *et al.*, 1996).

Versatility is an outstanding characteristic of Nylon in the textile industry. It can be made strong enough to with-stand the strength of tire cords, fine enough for sheer, high fashion hosiery and light enough for parachute cloth and backpacker's tents. Nylon is used both alone and in blends with other fibres, where its chief contributions are strength and abrasion resistance. Nylon washes easily, dries quickly, needs little pressing, and holds its shape well since it neither shrinks nor stretches (Meplestor, 1997).

Some particular applications are as follows:

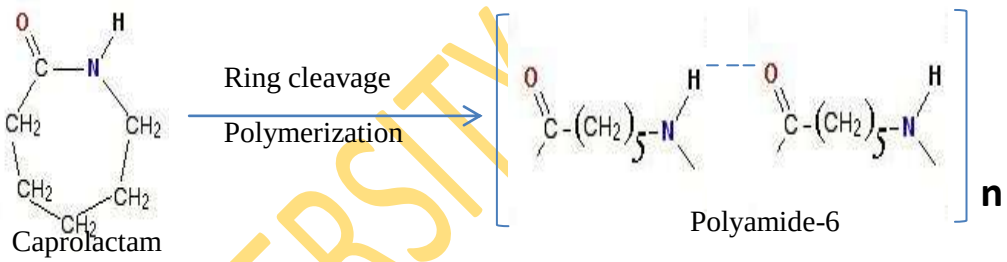
- It is mostly found in garment interlinings and wipes where it supplies strength and resilience.
- In Ni/H and Ni/Cd batteries, nylon fibres are used as Nonwovens separators.

- Nylon fibres are used for the manufacture of split table-pie fibres. These fibres find application in high performance wipes, synthetic suede, heat insulators and battery separators.
- Nonwovens developed from nylon are found in automotive products, athletic wear and conveyor belts.

### 2.4 Polyamides-6 (Nylon-6)

Nylon-6 or polycaprolactam is a polymer developed by Paul Schlack at IG Farben to reproduce the properties of nylon 6,6 without violating the patent on its production. Unlike most other nylons, nylon 6 is not a condensation polymer, but instead is formed by ring-opening polymerization. This makes it special in the comparison between condensation and addition polymers. Its competition with nylon 6,6 and the example it set have also shaped the economics of the synthetic fibre industry. It was given the trademark Perlon in the year 1952 (Galanty and Bujtas, 1992).

#### 2.4.1 Synthesis of Nylon-6

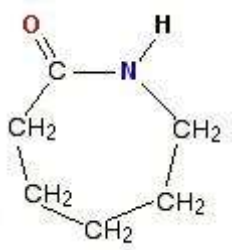



Nylon-6 begins as pure caprolactam. As caprolactam has 6 carbon atoms, it got the name Nylon-6. When caprolactam is heated at about 533 Kelvin in an inert atmosphere of nitrogen for about 4-5 hours, the ring breaks and undergoes polymerization. Then the molten mass is passed through spinnerets to form fibres of Nylon-6 (Galanty and Bujtas, 1992). During polymerization, the peptide bond within each caprolactam molecule is broken, with the active groups on each side re-forming two new bonds as the monomer becomes part of the polymer backbone. Nylon-6 therefore resembles natural polypeptides more closely such that hydrolysis of caprolactam would result in the production of amino acid as end products. Oligomers are formed by ring cleavage polymerization of caprolactam, resulting in less than 100 monomeric units of 6-aminohexanoate (Ahx). These can be linear or cyclic oligomers (Prijambada et al., 1995).

### 2.4.2 Properties of Nylon-6

Nylon-6 according to Bjarnason *et al*. (2004) is semi crystalline polyamide possessing high tensile strength. Nylon-6 is 100% elastic under 8% of extension. It is highly resistant to abrasion, chemicals like acids, alkalis, etc. Nylon-6 are wrinkle proof with tenacity-elongation at break ranges of 18% -45% from 8.8g/d- 4.3 g/d. Tensile strength of nylon-6 is higher than that of wool, silk, or cotton. It has specific gravity of 1.14 and melting point of 263$^{o}$C. Nylon-6 is permanently set by heat and steam and its extremely chemically stable to mildew or bacterial effects but degraded by light as natural fibres

### 2.5 Caprolactam, the Monomeric unit of Nylon-6




Caprolactam is an organic compound consisting of carbon, nitrogen, oxygen and hydrogen with the formula $(CH_2)_5C(O)NH$. It is the monomeric precursor used for the manufacture of nylon-6 (Baxi and Shah, 2000). Mostly, caprolactam is synthesized from cyclohexanone via an oximation process using hydroxylammonium sulfate followed by catalytic Beckmann rearrangement process step. Caprolactam is a stable white crystalline solid at 20°C. At that temperature the vapour pressure is low (<0.1 kPa). It is highly soluble in water (4560 g/l at 20°C). Caprolactam has an unpleasant pungent odour and has been reported to have toxic effect on human, animals, plants and microorganisms.(Shama and Wase, 1981). It has been shown to be mutagenic and capable of inducing chromosomonal damage (Sheldon, 1989; Vogel, 1989). Although caprolactam is readily biodegradable, its spillage in water causes damage to the ecosystem due to rapid degradation resulting in extensive oxygen consumption.

Caprolactam is intended to be used by industrial manufacturing companies as a chemically reacted intermediate for other products. It is the raw material for Nylon-6 which is produced almost exclusively by hydrolytic polymerization of caprolactam (Prijambada *et al*., 1995).

## 2.6: Disposal and Management of Polyamides and other Synthetic Polymers

Synthetic polymeric materials constitute a significant percentage of the solid waste generated worldwide. The variability of these materials and sources is one of the main difficulties of solid waste management. Polyamides and other synthethic polymers are mostly used as packaging materials because they are non-toxic but the problem of their disposal is becoming more and more severe (Jayasekara *et al*., 2005). Also, because majority of these synthetic polymers are non-polar they are found to be chemically inert. This creates potential environmental problems during disposal of the polymers. Preferred options for disposal of polyamides are (1) recycling, (2) incineration with energy recovery, and (3) landfill (Edinburg Plastics, Inc., 2011).

### 2.6.1 Recycling of Polyamides

With the daily increase in strength of the Green Movement across the developed countries, fibre manufacturers are adapting and become more creative, developing ways to save and improve the environment. Besides being environment friendly and reducing $CO_2$ emissions, polyamides and other polymeric plastics recycling can be an important factor of increased profitability for compounders and processors (Brueggemann Chemical, 2011). In Nigeria, stable demand for recycled polyamides has not been pronounced and there is little or no recycling infrastructure for it. Recycled polyamide is a synthetic fibre based on polymer waste recovery. The waste material can either come from post-consumer stream such as fishing nets, carpets and used apparel or from post-industrial stream i.e. waste occurring during production such as fibre waste during yarn production, or fabric waste from the cutting rooms.

There are two main recycling methods:

**Mechanical**: the raw material is cleaned, cut and re-melted to be spun into yarn.

**Chemical:** the raw material is cleaned, cut, de-polymerized to the base-molecule and then re-polymerized with the help of chemical additives. It can then be processed into textiles. Because of technical limitations, recycled polyamide is not always 100% recycled; yarns are available from 50 to 100% recycled content (Textile Exchange, 2011). Recycling of household waste polymeric plastics is difficult because it contains mixture of different plastics. Recycling and reuse inevitably leads to a reduction in solid waste but have limited value in solving waste management problems because

direct recycling is applicable to waste of plastics processing facilities only (Shah *et al*., 2008).

### 2.6.2 Disposal of Polyamides by Incineration

The high fuel value of polymeric materials makes incineration very desirable for material that cannot be recycled, but the incinerator must be capable of scrubbing out acidic combustion products. Incineration of polymeric plastics seems to be simple and straight forward but also not free of problems (Shah *et al*, 2008). Polyamides and many polymers like Polyvinyl chloride (PVC), Polyurethane and other halogen and nitrogen containing polymers can form corrosive and toxic substances upon burning and can cause health hazards or pollute the environment (Tsuji *et al*., 2006). Torben Lenau (2003) reported that incineration of polyamides mainly produces water and carbon dioxide with heating value equivalent to 1/2 kg of oil.

### 2.6.3 Landfill disposal of polymers

Millions of tonnes of polymeric plastic waste including polyamides are buried in landfill sites around the world each year. In Nigeria and many other African cities, the management of urban solid waste is one of the most immediate and serious environmental problems facing governments. The monthly total mean mass of waste collected from selected study area in four local governments in Lagos state for a period of twenty-four months were 658.44 tonnes, 653.39 tonnes, 538.28 tonnes and 2151.62 tonnes respectively for Ikoyi, Ebute-Metta, Gbagada and Mushin (Oyelola *et al*., 2011). The synthetic polymeric materials are reported to be approximately over 15% (w/v) of the Municipal solid waste generated in Lagos state (LAWMA, 2012). Disposal of polyamides by landfill causes long-term problems as non-polar polymers are non-biodegradable and take many years to break down (Smith 2009). In Nigeria there is no properly designed landfill that has underlay to prevent leachates arising from municipal wastes from getting to the ground water. There are mostly designated dumpsites as operated in Lagos and some other states of the country.

## 2.7 Degradation of Polymeric materials

### 2.7.1 Auto Oxidation Degradation

Polymeric materials that are exposed to outdoor abiotic conditions (i.e. weather, ageing and burying) can undergo transformations (mechanical, light, thermal, and chemical)

which contribute more or less to the resistance of the polymeric materials to biodegradation. In most cases, these abiotic parameters contribute to weaken the polymeric structure, and in this way favour undesirable alterations (Helbling *et al*., 2006; Ipekoglu *et al*., 2007). Sometimes, these abiotic parameters are useful either as a synergistic factor, or to initiate the biodegradation process (Jakubowicz , 2003).

As with most polymers, nylon are susceptible to molecular weight degradation when expose to elevated temperatures, light, acid, and alkali. The first reaction to take place in the oxidative degradation of a nylon will be abstraction of a hydrogen from the polymer by some initiating species. This will be followed by reaction of the thus-formed alkyl radical with oxygen to form a peroxy radical. This radical can then itself abstract hydrogen to form hydroperoxide. The hydroperoxide is easily split into an alkoxy radical and a hydroxyl radical, both of which can abstract further hydrogen from the polymer. The result is thus a cascade of reactions, known as an autoxidation cycle, or chain reaction. As the autoxidation cycle proceeds, however, the number of chain-scission reactions increases, and begins to predominate. The nylon molecular-weight loss then accelerates, resulting in catastrophic loss of structural integrity once the molecular weight has reduced by 30%–40% (Nakamura *et al*., 2006).

In the absence of oxygen, and to some extent in conjunction with the above reactions, direct chain breaking via non-radical routes also takes place. In this case, the reaction proceeds by hydrogen transfer via cyclic intermediates. These reactions may involve any of the possible hydrogen on the polymer chain, and the result is splitting off from the polymer of cyclic species such as monomers, dimers, trimers, etc.

### 2.7.2 Photo Degradation

In abiotic degradation of polymers, the action of light radiation is one of the most important parameters. The Norrish reactions express photo degradation that transforms the polymers by photo ionisation and chain scission. Photo degradation can result from this Norrish reaction, and/or cross linking reactions, or oxidative processes (Nakamura *et al.,* 2006). Norrish reactions have been recently described during photo degradation of Polylactic acid (PLA) and Polycaprolactone (PCL) (Tsuji *et al*., 2006). In the case of photo oxidation of nylons, the reaction will depend on the wavelength of light being absorbed, which in turn will depend on the presence of chromophoric species. At low wavelengths, this can be the amide group itself, while impurities, such as coloured species created during manufacture or processing, can account for energy absorption at

longer wavelengths. Chain-scission reactions here occur again by a mixture of radical and non radical routes, although the specific pathways involved may differ somewhat from those occurring in thermal processes (Nakamura *et al*., 2006).

### 2.7.3 Thermal Degradation

Thermal degradation of thermoplastic polymers occurs at the melting temperature when the polymer is transformed from solid to liquid (Ojumu *et al*., 2004). Generally, the environmental temperature is lower than the melting point of thermoplastic polymers. However, some thermoplastic polymers such as Polycaprolactone (PCL) has melting temperature of 60 $^{o}$ C which is close to environmental temperature. This is the case for the thermopile stage of composting. Otherwise, temperature may influence the organisation of the macromolecular framework. Biodegradable polymers such as PCL, PBA (polybutylene adipate) or cellulose are semi crystalline polymers; they possess amorphous and crystalline regions (Wyart, 2007). Structural changes take place at their glass transition temperature (Tg), the mobility and the volume of the polymeric chains are therefore modified. Above Tg (rubbery state), the disorganisation of the chains facilitate the accessibility to chemical and biological degradations (Iovino *et al*., 2008). Under Tg (glassy state), the formation of spherulites may take place, generating inter spherulitic cracks and the brittleness of the thermoplastics polymers (El-Hadi *et al*., 2002). Detailed studies on the thermal degradation of nylon 6 show that such reactions result in the formation of caprolactam, with the reaction taking place in two ways: either from the chain ends (rapid) or within the polymer chain (slow). Under normal circumstances, non-oxidative thermal breakdown of nylons occurs predominantly via these non-radical routes, and thus is not susceptible to suppression by use of standard stabilizing additives (Iovino *et al*., 2008).

### 2.7.4 Chemical Degradation

Chemical transformation is the other most important parameter in abiotic degradation. Atmospheric pollutants and agrochemicals may interact with polymers changing the macromolecule properties (Briassoulis, 2005). Oxygen is the most powerful of the chemicals provoking the degradation of materials. The atmospheric form of oxygen (i.e. $O_2$ or $O_3$) attacks covalent bonds producing free radicals. The oxidative degradation depends on the polymer structure (e.g. unsaturated links and branched chains) (Duval, 2004). These oxidations can be concomitant or synergic to light

degradation to produce free radicals. Like the products of Norrish reactions, peroxyl radicals resulting from the oxidative degradation can lead to cross linking reactions and/or chain scissions. Hydrolysis is another way by which polymers can undergo chemical degradation (Muller *et al*., 1998; Tsuji and Ikada, 2000; Yi-Heng *et al*., 2004). To be split by $H_2O$, the polymer must contain hydrolysable covalent bonds as it occurs in groups like ester, ether, anhydride, amide, carbamide (urea), ester amide (urethane) and so forth. Hydrolysis is dependent on parameters as water activity, temperature, pH and time. The design of materials with controlled life span needs the choice of specific monomers to obtain a copolymer with the wanted hydrophilic characteristics (Yew *et al.*, 2006). Well organised molecular frameworks (crystalline domains) prevent the diffusion of $O_2$ and $H_2O$, limiting in this way the chemical degradation. Oxidative and hydrolytic degradations on a given material are more easily performed within disorganised molecular regions (amorphous domains).

### 2.8 Stages of Biodegradation in Polymers

Biodegradation according to American Society for Testing and Materials (ASTM) is the process by which organic substances are broken down into smaller compounds using the enzymes produced by living microbial organisms. The microbial organisms transform the substance through metabolic or enzymatic processes. Although biodegradation processes vary greatly, the final product of the complete degradation is most often carbon dioxide and/or methane (Dommergues and Mangenot, 1972). Organic material can be degraded **aerobically**, with oxygen, or **anaerobically**, without oxygen. At present, the complexity of biodegradation is better understood and cannot be easily summarised (Grima, 2002). The biodegradation of polymeric materials includes several stages and can be defined as any change in the polymer at molecular level that alters the properties of the polymer (Belal, 2003). Biodegradation of polymeric material therefore, can stop at any of the following stages

a. The combined action of microbial communities, other decomposer organisms or/and abiotic factors fragment the biodegradable materials into tiny fractions. This step is called **biodeterioration** (Eggins and Oxley, 2001; Walsh, 2001).
b. Microorganisms secrete catalytic agents (i.e. enzymes and free radicals) able to cleave polymeric molecules reducing progressively their molecular weight. This process generates oligomers, dimers and monomers. This step is called **depolymerisation**.

c. Some molecules are recognized by receptors of microbial cells and can go across the plasmic membrane. The other molecules stay in the extracellular surroundings and can be the object of different modifications. In the cytoplasm, transported molecules integrate the microbial metabolism to produce energy, new biomass, storage vesicles and numerous primary and secondary metabolites. This step is called **assimilation**.

d. Concomitantly, some simple and complex metabolites may be excreted and reach the extracellular surroundings (e.g. organic acids, aldehydes, terpens, antibiotics, etc.). Simple molecules as $CO_2$, $N_2$, $CH_4$, $H_2O$ and different salts from intracellular metabolites that are completely oxidized are released in the environment. This stage is called **mineralisation**.

The term ‘‘biodegradation” indicates the predominance of biological activity in this phenomenon. Hence, low level of degradation of polymer can influence properties such as elasticity and permeability, whereas high level degradation can result in loss of material and the formation of sizeable cracks in the fibre samples (Niels and Peter, 2001). However, in nature, biotic and abiotic factors act synergistically to decompose organic matter. Several studies about biodegradation of some polymers show that the abiotic degradation precedes microbial assimilation (Kister *et al.*, 2000; Proikakis *et al.*, 2006). Consequently, the abiotic degradation must not be neglected.

Biodegradation can be a key feature of synthetic polymers within the frame of sustainable development. Polymers are widely used and our daily life could not be imagined without them. The different stages of biodegradation (biodeterioration, biofragmentation and assimilation) have to be observed when environmental conditions change.

### 2.8.1 Biodeterioration

Deterioration is a superficial degradation that modifies mechanical, physical and chemical properties of a given material. Abiotic effects provoking deterioration are described above. This section focuses on the biological aspects of deterioration. The biodeterioration is mainly the result of the activity of microorganisms growing on the surface or/and inside a given material (Hueck, 2001; Walsh, 2001). Microorganisms act by mechanical, chemical and/or enzymatic means (Gu, 2003). Microbial development depends on the constitution and the properties of polymer materials. The

specific environmental conditions (e.g. humidity, weather and atmospheric pollutants) are also important parameters (Lugauskas *et al*., 2003). Microorganisms involved in biodeterioration are very diverse and belong to bacteria, protozoa, algae, fungi and lichenaceae groups (Wallström *et al*., 2005). They can form consortia with a structured organisation called biofilms (Gu, 2003). This microbial mat, which works in synergy, provokes serious damages on different materials (Flemming, 1998). The development of different microbial species, in a specific order, increases the biodeterioration facilitating in this way the production of simple molecules. All these substances act as carbon and nitrogen sources, as well as growth factors for microorganisms (Crispim and Gaylarde, 2005). Studies show that atmospheric pollutants are potential sources of nutrients for some microorganisms (Zanardini *et al*., 2000; Nuhoglu *et al*., 2006). Mitchell and Gu (2000) reported the deposition of sulphur dioxide, aliphatic and aromatic hydrocarbons from urban air on several polymer materials. These adsorbed pollutants may also favour material colonisation by other microbial species. Organic dyes are also potential nutrients for these microorganisms (Tharanathan, 2003; Faÿ *et al*., 2007).

### 2.8.2 Biofragmentation

Fragmentation is a lytic phenomenon necessary for the subsequent event called assimilation. A polymer is a molecule with a high molecular weight, unable to cross the cell wall and/or cytoplasmic membrane. It is indispensable to cleave several bonds to obtain a mixture of oligomers and/or monomers. The energy to accomplish scissions may be of different origins: thermal, light, mechanical, chemical and/or biological. Microorganisms use different modi operandi to cleave polymers. They secrete specific enzymes or generate free radicals. Biofragmentation is mainly concerned by enzymes that belong to oxidoreductases and hydrolases. Cellulases, amylases and cutinases are hydrolases readily synthesised by soil microorganisms to hydrolyse naturally abundant polymers (e.g. cellulose, starch and cutin). These polymers are, in some industrial composites, co-extruded with polyesters to increase the biodegradability (Chandra and Rustgi, 1998). Some enzymes with an activity of depolymerisation of (co)polyesters have been identified (Muller, 2006). They are lipases and esterases when they attack specifically carboxylic linkages and they are endopeptidases if the cleaved bond is an amide group.

A polymer is considered as fragmented when low molecular weight molecules are found within the media. The most used analytical technique to separate oligomers with different molecular weight is the size exclusion chromatography (SEC) (Marqués-Calvo *et al*., 2006). HPLC and GC are usually used to identify monomers and oligomers in a liquid (Gattin *et al*., 2002; Araujo et al., 2004) or in a gaseous phase (Witt *et al*., 2001). After purification, intermediates molecules can be identified by MS (Witt *et al*., 2001). Monomer structures may be determined by NMR (Marten et al., 2005), functional chemical changes are easily detected by FTIR (Nagai *et al*., 2005; Kim *et al*., 2006).

### 2.8.3 Assimilation

Assimilation is the unique event in which there is a real integration of atoms from fragments of polymeric materials inside microbial cells. This integration brings to microorganisms the necessary sources of energy, electrons and elements (i.e. carbon, nitrogen, oxygen, phosphorus, sulphur and so forth) for the formation of the cell structure. Assimilation allows microorganisms to grow and to reproduce while consuming nutrient substrate (e.g. polymeric materials) from the environment. Naturally, assimilated molecules may be the result of previous biodeterioration and/or biofragmentation. Monomers surrounding the microbial cells must go through the cellular membranes to be assimilated. Some monomers are easily brought inside the cell due to specific membrane carriers. Other molecules to which membranes are impermeable are not assimilated, but they can undergo biotransformation reactions giving products that can be assimilated or not. Inside cells, transported molecules are oxidised through catabolic pathways resulting in the production of adenosine triphosphate (ATP) and constitutive elements of cells structure. Depending on the microbial abilities to grow in aerobic or anaerobic conditions, there exist three essential catabolic pathways to produce the energy to maintain cellular activity, structure and reproduction: aerobic respiration, anaerobic respiration and fermentation. Assimilation is generally estimated by standardised respirometric methods (Krzan *et al*., 2006). It consists of measuring the consumption of oxygen or the evolution of carbon dioxide (Pagga, 1997). This is generally measured with GC. But in some cases, FTIR is preferred (Itavaara and Vikman, 1995; Lefaux *et al*., 2004). Few biodegradability tests using complex media such as the use of a radiolabelled polymer to perform $^{14}CO_2$ respirometry (Reid *et al*., 2001; Rasmussen *et al*., 2004). However,

this hazardous and expensive test requires particular lab room, specific equipment, training and is time consuming.

Biodegradation of xenobiotic compounds has been recognised as a useful way to eliminate environmental pollutants. However, the efficiency of removal is highly dependent on the specific enzymes that can catalyze the desire degradation reactions (Negoro, 2000). In addition, from the environmental point of view, microbial degradation of synthetic compound provided a good system for studying how microorganism acquire the ability to degrade such compounds. The identification of microorganisms that can degrade a synthetic polymeric material and the characterization of the enzymatic mechanism that are employed by the microorganisms are fundamental to the understanding of polymer biodegradation. While detailed studies of some naturally occuring polymers and their derivatives, such as cellullose and starch are available the processes of biodegradation of most synthetic polymers especially polyamides-6 are not generally understood (Deguchi *et al.,* 1998). Polyamide (PA) fibres have high crystallinity and low moisture regain. They exhibit excellent physical properties of strength, flexibility, toughness, stiffness, and wear resistance. Beside these properties, they also demonstrate low friction coefficient, low creep and good chemical resistance (Vertommen *et al,*. 2005; Jia *et al*., 2006). However, hydrophobicity makes it difficult for microorganism to utilize the product. Studies have reported the isolation of some bacteria strains with ability to degrade polyamides through the production of enzymes essential for degradation of the compound. These include *Pseudomonas aeruginosa* (Kulkarni and Kanekar 1998), *Arthrobacter* sp. strain KI72 (formerly called a *Flavobacterium* sp.) Negoro (2002) and *Agromyces sp* KY5R, from activated sludge of sewage disposal plant (Yasuhira *et al.* 2007). But little is known of the level of the biodegradation ability. Friedrich *et al*., (2007) reported the inability of fifty-eight fungi isolated from a nylon producing factory to degrade the recalcitrant synthetic polyamide-6 except the wood degrading fungi *Bjerkandera adusta* collected from a culture center after incubation for several weeks and *Phanerochaete chrysosporium* (Klun *et al*., 2003)

**2.8.4 Enzymatic Degradation**:

In cases where microorganisms are known to produce enzymes that show activity on a specific type of polymer, purified enzyme preparation can be used to study the effect

of variations in the polymer substrate. Partial hydrolysis of Polyamide fibres and oligomers has been demonstrated with proteases (Kakudo *et al.*, 1993; Fischer-Colbrie *et al*., 2004). Biochemical studies on the biodegradation of nylon-6, 6 by a lignin-degrading fungus (Deguchi *et al*., 1998; Nomura *et al*., 2001) revealed the presence of peroxidase enzyme. The characteristics of the purified protein such as molecular weight, absorption spectrum, and requirements for 2, 6 dimethoxyphenol oxidation were found to be identical to those of manganese peroxidase. Moreover, Klun *et al*., (2003) also reported oxidases from the lignolytic fungi *Bjerkandera adusta* have been used to depolymerise polyamides. The enzymatic modification of synthetic materials has immense potential both in the functionalization of bulk materials, such as polyamide, polyacrylonitrile or polyester, and in the production of polymers for special applications, such as medical devices and electronics (Deguchi *et al*., 1998; Tauber *et al.*, 2000; Yoon *et al*., 2002; Silva *et al*., 2005).

**2.9 Biodegradation of Nylon Oligomer and Monomers**

The presence of caprolactam as unreacted monomer of nylon-6 and linear oligomer that fail to polymerize as well as the cyclic oligomer that undergo head to tail condensation in nylon-6 by-product contribute to the increase in the industrial waste material entering the environment. Owing to their polluting and toxic nature, their removals from waste streams become necessary. Due to the need for biodegradation of these environmental pollutants, two bacterial strains, *Flavobacterium* sp. KI72 (Kinoshita *et al.*, 1977) and *Pseudomonas* sp. NK87 (Kanagawa *et al.*, 1989) that can degrade 6-aminohexanoate-cyclic dimer, were isolated from nylon-6 factories waste. These two bacteria strains almost completely utilized 1% of the cyclic dimer within 24hours. *Pseudomonas aeruginosa* strain, MCM B-407 was also isolated from activated sludge to treat waste from a factory producing nylon-6 (Kulkarni and Kanekar, 1998). This organism was able to remove ε-caprolactam with simultaneous reduction in chemical oxygen demand (COD). The degradation of caprolactam in wastewater was found to be optimal over a wide range of pH from 5.0 to 9.0, at a temperature of 30$^{o}$C. Biochemical studies of the degradation ability of the bacteria strains revealed that three enzymes produced by *Flavobacterium* sp. K172 are responsible for the degradation of the oligomers. The enzymes are 6-aminohexanoate-cyclic-dimer hydrolase (EI, EC 3.5.2.12) (Kinoshita *et al.*, 1977), 6-aminohexanoate-dimer hydrolase (EII, EC 3.5.1.46) (Kinoshita *et al*., 1981) and endotype 6-

aminohexanoate-oligomer hydrolase (EIII) (Negoro *et al*., 1992; Kakudo *et al*., 1993). Restriction analyses and electron microscopy revealed that the Enzymes observed in the nylon oligomer degradation activities of *Flavobacterium* sp. K172 were encoded on plasmid (Negoro *et al*., 1992). In *Pseudomonas* sp.NK87, the enzymes were also plasmid meditated but with divergent amino acid sequence except for EI enzyme that showed 99% homology to the sequence of that from the *Flavobacterium* sp. Holloway and Morgan, (1986) reported from the conjugation experiment that the ability to utilize the caprolactam was detected in the plasmids of several *Pseudomonas* strains studied. However, very little is actually reported about the biodegradation of higher molecular weight polyamides.

**2.10 Biodegradation of Nylon-6**

Polyamide-6 is considered non-degradable due to its resistance to microbial enzymatic attack. Although, its amide linkages are comparable to peptide bonds in protein but the two differ in that the alkyl segment of polyamide-6 makes it less polar than proteins and there is an absence of various side chains found in proteins (Klun *et al*., 2003). Also, its strong cohesive intermolecular force caused by hydrogen bonds between the molecular chains of the nylon makes it less degradable compared to polyesters (Tomita *et al.*, 2003). Polyamide-6 degradation by fungi was first reported in the lignolytic fungus, *Phanerochaete chrysosporum* by Klun *et al*. (2003) while Fredrich *et al.* (2007) also documented the ability of *Bjerkandera adjusta* to degrade this recalcitrant synthetic polymer out of the fifty-eight (58) fungi isolates tested on the polymer. The degradation of the polymer was observed through the decrease in the average molecular mass as well as physical damages to the fibre under a scanning electron microscope (SEM). Continuous attempts to degrade this polymer using micro-organisms lead to the discovery by Tomita *et al.* (2003) of a thermophilic bacterium (*Bacillus pallidus*) that was reported to grow on nylon-6 and nylon-12 thereby resulting in marked decrease in the molecular weight of the polymers. Sudhakar *et al*., (2007) also reprted four (4) marine bacteria *Bacillus cereus, Bacillus sphericus, Vibro furnisii and Brevundimonas vescularis* that degraded nylon-6 and nylon-66 in mineral salt medium under submerged enrichment conditions with the polymer as the sole carbon source. Available literature showed that several attempts have been made to degrade these polyamides using microorganisms, but the degree of microbial

degradation has been shown to be lower the larger the molecule (Friedrich *et al*, 2007). Therefore, considering the wide spread use of polyamide-6 and the diversity of microorganisms in nature and the lack of report on studies on the polyamide-6 degrading potential of microorganisms from landfill or municipal solid waste dumpsites that serve as the final depository of these materials necessitated this study. Municipal waste dumpsites are known to be a source of great variety of microorganisms, it is believe that the potentials of microorganisms to degrade this polymer has not been exhaustibly investigated. Study of the microbial isolates from these sites may lead to the discovery of potentially active microbial strains that could be exploited in developing the biotechnology for waste polymer degradation.

# CHAPTER THREE
# MATERIALS AND METHODS

## 3.1 Sterilization of Media, Solutions and Apparatus

Media and solutions used in this study were sterilized by autoclaving at 121$^{o}$C, 15 psi pressure for 15 minutes. The glassware and other apparatus were sterilized in an oven at 160$^{o}$C for two hours. After sterilization, the media and solutions were cooled to room temperature and then stored under refrigeration for their subsequent use.

## 3.2 Sampling Dumpsites

Solid waste dumpsites in Lagos namely, Abule-Egba, Isheri-Igando, Olusosun, and Nichemtex Solid waste collection point in Ikorodu Lagos state were chosen as sites for sampling.

**Abule-Egba** dumpsite is located along Lagos- Abeokuta Express way at Oke-Odo, Lagos state. It is geographically located at 6$^{o}$ 38' 19" N 3$^{o}$ 18' 11" E and site occupies about 10.2 hectares of land in the Western part of Lagos in Alimosho Local Government Area. The dumpsite receives waste from the densely populated residential area and two major Markets. The residual life span is approximately 8 years (LAWMA).

**Isheri-Igando** dumpsite also known as "Solous" dumpsite is situated at Igando along Isheri-Igando road in Alimosho Local Government Area of Lagos State, Nigeria It is geographyically located at 6$^{o}$ 34' 20" N 3$^{o}$ 15' 13" E. Solous dumpsites according to LAWMA started operations in the year 1996 and is sub-divided into three (3) sections namely, Solous I (closed), Solous II and III (existing). The sampled site for this study was Solous I which covered an area of about 3.0 hectares. The new dumpsites i.e.

Soluos II is on 7.8 hectares of land with average life span of 5 years while Soluos III is a new site with approximately 5 hectares of land with average life span of 5 years. Each site receives an average of about 2,250 m$^{3}$ of waste per day.

**Olusosun** dumpsite is located beside Lagos- Ibadan Express way at Ojota in Kosofe Local Government Area of Lagos State, Nigeria at the geographical position of 6$^{o}$ 35' 57" **N**, 3$^{o}$ 22' 57" **E**. It is the largest dumpsite in Lagos State with about 18 metres deep

and covers an area of close to 42.7 hectres of land (LAWMA). This dumpsite receives Municipal solid wastes from various residential and industrial areas ranging from Ikosi Ketu, Oregun industrial estates, the commercial area of Kudirat Abiola way, and Ojota. It also receives waste from designated waste loading stations as far as Mushin, Ebute-Meta and Ikoyi. This dumpsite according to Lagos State Waste Management Authority (LAWMA) is said to be the repository of more than 50% of about 9,000 metric tonnes of solid waste generated in the Lagos Metropolis on a daily basis and said to have a residual life span of 20 years.

**Nichemtex** is a textile manufacturing company geographically located at $6^{o}$ 34' 36" **N**, $3^{o}$ 29' 20" **E** in Ikorodu area of Lagos state. The company operates a solid waste collection point, from which LAWMA collects the solid waste for onward transfer to other dumpsites. Being a textile manufacturing company, waste generated is expected to contain polyamides because of its acclaimed wide use in the textile industry.

In all the existing dumpsites in the state, the waste streams were made up of domestic, market, commercial, industrial and institutional origins. The areas around the dump are surrounded mostly by residential houses while scavengers are found in active sorting of recycle able wastes especially the polymeric plastics and metals.

### 3.3 Sample Collection

Soil samples were collected by bimonthly in April, June, August and October 2009. These samples were randomly collected at five spots to a depth of 15cm using a cleaned soil auger and mixed to form a composite sample for each of the dumpsites. The soil samples were collected in clean polyethylene bags. They were labelled according to collection sites as AB- Abule Egba dumpsite samples, IS- Isheri dumpsite samples, OR- Olusosun dumpsite samples and NTS- Nichemtex Textile dumpsite samples. The samples were thereafter transported to Environmental Microbiology laboratory, University of Ibadan in ice chests and kept in the refrigerator at $4^{o}$C prior to microbiological analysis. Soil samples were also collected from the backyard dumpsite of Botany Department, University of Ibadan and also from a buried cloth samples in Ibadan to serve as negative and positive control samples.

### 3.4 Estimation of Total Viable Bacteria Counts (TVC).

One gram each of the thoroughly mixed composite soil samples was suspended in 9 ml of sterile saline peptone water (Olutiola *et al*., 2000) and serially diluted tenfold in the

same medium. 1 ml each of appropriate dilutions was inoculated into sterile Petri plates containing already prepared cool Nutrient agar by pour plate method. Inoculated plates in duplicates were incubated at 30$^{o}$C overnight in an inverted position and distinct bacteria colonies were counted after 48 hrs.

### 3.5 Isolation of Bacteria

Morphologically distinct bacteria colonies from the 48 hrs incubated culture were subcultured by streaking on fresh nutrient agar plates until pure bacteria colonies were obtained. Pure culture of each bacteria strain was stored on nutrient agar slants in a refrigerator at 4$^{o}$C for further studies.

### 3.6 Screening for Caprolactam utilizing ability of the Bacteria isolates

The method described by Baxi and Shah (2002) was employed. Caprolactam utilizing abilities of the bacteria isolates were qualitatively tested by streak inoculation of a 24 hrs old culture on already prepared sterilized Basal medium for caprolactam (as shown in Appendix I). Plates were inoculated in duplicates and incubated at 30$^{o}$C for 48 hrs. Bacterial isolates growing as morphologically distinct colonies on the medium were presumed to have the ability to utilize the caprolactam as source of both carbon and energy and were subjected to further confirmation in broth culture.

### 3.7 Determination of the bacteria ability to utilize Caprolactam in broth Culture

#### 3.7.1 Growth studies for bacteria isolates

Sixteen bacterial isolates that grew on the solid basal medium within 48hrs were selected for growth confirmation in broth of same medium. Isolates grown in peptone water for 18 to 24 hrs was used as inoculum. 1ml each of the broth were inoculated into 100 ml of the 1% (w/v) of caprolactam basal medium in 150 ml conical flasks and incubated at 35$^{o}$C on the G24 Environmental incubator shaker (New Brunswick Scientific Co.Inc., Edison USA) at 180 rpm for 120 hrs. Culture samples were aseptically withdrawn every 24 hrs and bacterial growth determined turbidimentrically by measuring the optical density at wavelength of 600nm using UV –Visible Spectrophotometer (Perkin Elmer Lambda 25).

### 3.7.2 Changes in pH of culture medium during bacteria growth

The changes in pH of the caprolactam basal medium were determined every 24 hrs using Hanna Laboratory pH meter (HANNA Instruments HI 2210) at every 24hrs. The pH meter was calibrated using standard buffers 4, 7 and 9. The glass tip electrode was inserted into the culture broth in such a way that the glass bulb of the electrode was totally covered by the broth. pH reading were taken when the digital indicator became stable.

### 3.7.3 Changes in concentration of caprolactam in the growth medium.

The change in concentration of caprolactam in the growth medium was determined after 96 hrs of the experiment. Sample cultures were centrifuged at 10,000 rpm at 4$^{o}$C for 10 minutes to harvest the bacteria cells and the cell free supernatants. 1 ml of the cell free supernatant was injected into High Performance Liquid Chromatography (HPLC) (Cecil – Adept System 4 (Analytical) with a UV-Visible detector at 200 nm. The flow rate of the mobile phase was 1ml min$^{-1}$ in TSK-GEL ODS-80TM 4.6mm x7.5cm column (Sigma-Aldrich). The HPLC was standardised with known concentrations of caprolactam. Methanol: water 40:60(v/v) was used as the mobile phase. The result was read in duplicates and the mean concentration was used in calculation of percentage reduction in caprolactam.

$$\% \text{ Reduction} = \frac{\text{Concentration in abiotic control} - \text{Concentration in biotic culture}}{\text{Concentration in abiotic control}} \times 100$$

### 3.7.4 Estimation of Amino acid produced using Ninhydrin Reaction method.

6-aminohexanoic acid was also estimated as an intermediate product in the cell free supernatant of the culture after centrifugation using ninhydrin reaction method (Friedman, 2004). 1ml of ninhydrin solution (0.35g in 100ml of ethanol) was added to 5ml of the culture supernatant in caped test tubes, heated at temperature between 80$^{o}$C-100$^{o}$C for 5minutes and cooled to room temperature. The absorbance was read at 570nm using spectrophotometer. Absorbances of previously known concentrations of 6-aminohexanoic acid were used to construct a standard curve for the estimation of the 6-aminohexanoic acid content of the supernatant. This was calculated using the Beer-Lamberts law of absorptivity.

### 3.8 Determination of Minimum Inhibitory Concentration (MIC) of Caprolactam on different bacteria isolates

The MIC of capolactam on the bacterial isolates was determined using the method described by Baxi and Shah (2002). Pure culture of each bacteria isolate was grown in Nutrient broth and incubated on rotatory shaker four 24 hrs. The bacteria cells were harvested by centrifugation at 10,000 rpm at 4$^{o}$C for 10 minutes, and cells suspended in saline solution. 5 ml each of the suspended isolates were inoculated into 50 ml of the sterilized basal medium and incubated at 30$^{o}$C for 24 hours on rotatory shaker. The broth was observed for bacterial growth visually by turbidity and/or pellet of microorganisms in the bottom of the conical flask. The last flask in the concentration series that does not demonstrate growth were considered as the minimum inhibitory concentration (MIC) of the caprolactam for the bacteria isolate.

### 3.9 Effect of Temperature on Growth of the Bacterial Isolates in Caprolactam broth

This was carried out to determine the optimal temperature for the growth of the bacteria isolates. 10 ml test tubes containing 5ml of basal medium were inoculated with 0.1ml of bacterial cells previously harvested from 24 hrs culture of each bacterial isolates and incubated separately at temperatures 27°C, 30°C, 35°C and 40°C, respectively for 24 hrs. The growth of the isolates was turbidimetrically measured at 600 nm at 0 and 24 hrs. The data obtained were used to plot a graph to determine the optimum growth temperature of the respective bacterial isolates.

### 3.10 Effect of pH on growth of the Bacterial Isolates in Caprolactam broth

To determine the optimal pH for the growth of the isolate, the pH value of the basal medium of caprolactam was adjusted to 6.0, 6.4, 7.0, 7.4, 8.0 and 8.5 using 20% HCl and 0.1N NaOH with the aid of Hanna pH meter. It was inoculated with 0.1ml bacterial cells harvested from 24 hrs culture of the bacterial isolates and incubated at 30$^{o}$C on shaker for 24 hrs. The growth of the microorganisms was turbidimetrically determined at 600 nm at 0 and 24 hrs. The data obtained were used to plot a graph to determine the optimum growth pH of the respective bacterial isolates.

### 3.11 Biochemical Characterization of Bacteria Isolates

#### 3.11.1 Gram's reaction

The bacteria cells were cultured overnight on nutrient agar plates. Each bacterial isolate was smeared in a drop of water on a cleaned and grease free glass slide and air dried. The prepared slides were Gram stained as described by Olutiola *et al*. (2000). The stained slides were examined under light microscope with oil immersion objectives. Gram positive bacteria cells were characterised by purple colouration while Gram negative cells were characterized by pink colouration.

#### 3.11.2 Oxidase Test

The modified method of Kovacs (1956) cited by Persley (1980) was used to detect the presence of oxidase. Filter paper strips were soaked in freshly prepared oxidase reagent (1% tetramethyl p-phenylene diamine hydrochloride). A loopful of bacteria growth from 24 hours culture on nutrient agar plate was smeared on the soaked filter paper with a sterile platinum wire loop. The result was read within 10 seconds. A deep purple colour showed a positive result while absence of the colouration indicates a negative result.

#### 3.11.3 Catalase Test

The ability of the isolates to decompose Hydrogen peroxide ($H_2O_2$) by the production of catalase enzyme was tested as described by Olutiola *et al.* (2000). 18 hours culture of the test organism was picked with a sterile wire loop and emulsified in 3% hydrogen peroxide ($H_2O_{2)}$ on grease free cleaned glass slides. The production of effervescence indicates a positive reaction for catalase while the absence of effervescence indicates a negative reaction.

#### 3.11.4 Motility Test

The hanging-drop method described by Olutiola *et al.* (2000) was used. Vaseline was placed round the edge of the hollow in a clean cavity slide and a loopful of bacteria culture was dropped carefully on the coverslip placed on the bench. The cavity slide was inverted over the coverslip in such a way that the drop was at the center of the cavity and the cavity slide was pressed down gently to firmly keep the cover slip in the position with the Vaseline. The cavity slide was inverted quickly and smoothly with

the drop still hanging. This preparation was examined under the light microscope for vigorously moving organism.

### 3.11.5 Gelatin Hydrolyses Test.

The ability of the isolates to hydrolyse gelatin was tested in broth culture as described by Olutiola *et al.* (2000). Nutrient broth supplemented with 15% gelatin was prepared, dispensed into test tubes and sterilized in autoclave at 121$^{o}$C for 15minutes. The tubes were later stab inoculated with a 24 hrs old culture of the test organisms and were incubated at 30$^{o}$C for 7 days. The culture was thereafter transferred into a refrigerator for 15minutes to solidify any unhydrolysed gelatin. Liquifaction of the gelatin confirmed positive test for the organism.

### 3.11.6 Indole Test

The bacteria isolates were grown for 5 days on a shaker incubator in broth containing 1.0% tryptone and 0.5% yeast extract according to the modified method as cited by Olutiola *et al*. (2000). To 1ml of the broth culture was added Kovac's reagent. The development of a cherry red colour that faded away after 15 minutes indicated a positive reaction while absence of a colour change indicated a negative result.

### 3. 11. 7 Urease Test

The method of Fahy and Hayward (1983) was used to detect the presence of urease. The basal medium contained in g/l $NH_4H_2PO_4$ 0.5; $K_2HPO_4$ 0.5; $MgSO_4.7H_2O$ 0.2; NaCl 5; glucose 10; yeast extract (Difco) 10; Cresol red 0.016; agar 15; autoclaved in a flask. A 20% solution of urea was filter-sterilised and added to the sterile basal medium to give a final concentration of 2% urea. The medium was dispensed in 10ml quantities to 25 ml screw-capped bottles and bottles slanted so as to give slope with a butt of approximately 2.5 cm depth and a slant of 4.0cm length. The slanted agar was inoculated with each isolate and incubated at 30$^{o}$C. Production of a red-violet colour on the slant within 3days indicated a positive reaction.

### 3.11.8 Citrate Utilization Test.

Koser Citrate medium was prepared by dissolving 24 g in 1000 ml distilled water and dispensed in 5 ml quantities into test tubes. The tubes were sterilized at 121$^{o}$C for 15 minutes in an autoclave and cooled in slant position. The slopes were then inoculated

with 24 hrs old culture of the test organisms and incubated at 30$^{o}$C for 48 hrs. Change in the medium colour from green to blue indicated a positive result while absence of colour changes indicated a negative result.

### 3.11.9 Methyl Red Test

Glucose phosphate broth was prepared as described by Cheesebrough (1984) by dissolving in g/l peptone, 5; glucose, 5 and di-potassium hydrogen phosphate ($K_2HPO_4$) 5; in distilled water. Few drop of methyl red reagent was added and 5ml each of the medium was dispensed into tubes and sterilized at 115$^{o}$C for 10 minutes in an autoclave. The tubes were then inoculated with loopful of each bacteria isolates and incubated at 35$^{o}$C for 48hours. Colour change from yellow to red indicated positive result.

### 3.11.10 Hydrogen Sulphide Test

The ability of the bacteria isolates to decompose organic sulphur or reduce inorganic sulphur to produce hydrogen sulphide was tested using the method described by Olutiola *et al.* (2000). Thiosulphate broth was prepared by adding 0.01% sodium thiosulphate into nutrient broth. The broth was dispensed into a screw cap bottles and sterilized at 121$^{o}$C for 15 minutes with lead acetate soaked paper strip.. The bottles were inoculated and screwed with the soaked filter paper in its mouth. The bottles were incubated at 35$^{o}$C for 5days with un-inoculated bottle as control. Dark colouration of the lead acetate paper strip indicated positive result.

### 3.11.11 Nitrate Reduction Test

Nitrate peptone broth was prepared by adding 0.1% of Potassium nitrate into peptone water. Ten millilitres each of the broth was then dispensed into screw capped bottles and sterilised in an autoclave at 121$^{o}$C for 15 minutes with inverted Durham tubes. The medium was inoculated with the loopful 24 hrs old culture of the test organisms and incubated at 35$^{o}$C for 5 days. After which 0.5 ml of Griess-liosvay reagent (1% sulphanilic acid in 5N acetic acid and 0.6% dimethy naphthyl napthylamine) was added to determine the reduction of nitrate to nitrite.The presence of gas in the inverted tubes indicated formation of nitrogen gas signifying complete reduction of nitrate while change of medium to red or brown showed incomplete reduction.

### 3.11.12 Sugar fermentation Tests.

The ability of the bacteria isolates to ferment various sugars was carried out as described by Olutiola *et al.* (2000) using 1% appropriate sugar in a basal medium containing peptone 1% and NaCl 0.1% with the addition of phenol red as indicator. Ten millilitres each of the broth was dispensed into test tube with inverted Durham tubes and sterilised at 115$^{o}$C for 10 minutes in an autoclave. Each tube was inoculated with a loop full of the isolate and incubated at 35$^{o}$C for 48 hours. Acid production was shown by a change in colour of the medium from red to yellow and gas production indicated production of $CO_2$ from fermentation.

## 3.12 Molecular Characterization of Bacteria Isolates

### 3.12.1 Extraction of Total Genomic DNA

Total genomic DNA of the bacteria isolates were extracted at the National Centre for Cell Sciences (NCCS), Pune, India using the method described by Ausubel *et al.* (1987). Each bacterial strain was inoculated into 5ml Nutrient broth (Difco) for 72 hrs at 27$^{o}$C. In an eppendorf tube, 1.5 ml of the culture was spinned in a microcentrifuge for 5 min to obtain compact pellets. The supernatant was discarded and the pellet resuspended in 567 µl TE buffer unto which was added 30 µl of 10 % SDS and 3 µl of 20 mg/ml proteinase k. It was mixed thoroughly and incubated 37$^{o}$C for 1 hr. This was followed with the addition of 100 µl of 5M NaCl and mixed thoroughly. Then 80 µl of CTAB/NaCl solution was added mixed and incubated for 10 minutes at 65$^{o}$C. Thereafter, equal volume of 0.8 ml of chloroform/isoamyl alcohol was added, mixed and spin in a microcentrifuge for 5 minutes. The aqueous, viscous supernatant was thereafter removed into a fresh microcentrifuge tube where equal volume of phenol/chloroform/isoamyl alcohol was added and spinned for 5 minutes. The aqueous supernatant was also transferred into fresh tube and the DNA precipitated by adding 0.6 ml of isopropanol. The precipitate was thereafter washed with 70% ethanol, dried and resuspended 100 µl of TE buffer.

### 3.12.2 Process of PCR Amplification

The PCR assay was performed using Applied Biosystems Thermocycler, model 9800 with 1.5 µl of DNA extract in a total volume of 25 µl. The PCR master mix contained 2.5 µl of 10X PCR reaction buffer (with 1.5 M $MgCl_2$), 2.5 µl of 2 mM dNTPs, 1.25 µl

of 10 pm/µl of each oligonucleotide primers 8F and 1392R, 0.2 µl of 3U/ µl Taq DNA polymerase and 15.76 µl of ultra pure PCR water. Initially denaturation was accomplished at 94°C for 3 min. Thirty-two cycles of amplification consisted of denaturation at 94°C for 30sec, annealing at 55°C for 30sec and extension at 72°C for 90 min. A final extension phase at 72°C for 10 min was performed. The PCR product was purified by PEG-NaCl method. The sample was mixed with 0.6 times volume of PEG-NaCl,20% [PEG(MW6000) and 2.5 M NaCl] and incubated for 20 min at 37°C. The precipitate was collected by centrifugation at 3,800 rpm for 20 min. The pellet was washed with 70% ethanol, air dried and dissolved in 12 µl sterile distilled water.

### 3.12.3 DNA Sequencing

The sample was sequenced using a 96-well Applied Biosystems sequencing plate according to the manufacturer's instructions. The thermocycling for the sequencing reactions began with an initial denaturation at 94°C for 2 min, followed by 35 cycles of PCR consisted of denaturation at 94°C for 10 s, followed by annealing at 50°C for 10 s, and extension at 60°C for 4 min using primers 704F and 907R. The samples were purified using standard protocols described by Applied Biosystems, Foster City, USA. To this, 10 µl of Hi-Di formamide was added and vortexed briefly. The DNA was denatured by incubating at 95°C for 3 min, kept on ice for 5-10 min, and was sequenced in a 3730 DNA analyzer (Applied Biosystems) following the manufacturer's instructions. The obtained sequences of bacterial 16S rDNA (using 704F and 907R primers) were analysed using Sequence Scanner (Applied Biosystems) software and the 16S rDNA sequence contigs were generated using Chromas Pro.

### 3.12.4 Identification of the Bacteria Isolates

The 16S rDNA sequences were used for a BLAST (Basic Local Alignment Search Tool) search at the GenBank database hosted at the National Centre for Biotechnology Information (NCBI) (www.ncbi.nim.nih.gov) to confirm the identity of the isolates.

### 3.12.5 Phylogenetic Analyses

The 16S rDNA gene sequences were aligned using the Molecular Evolutionery Genetics Analysis (MEGA) software version 5. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 2.14781234 was determined. The percentage of replicate trees in which the associated

taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method Tamura *et al*. (2011) and were in the units of the number of base substitutions per site. The analysis involved 15 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5 (Tamura *et al*., (2011).

### 3.13 Total Fungal Counts and Isolation of Pure Fungal Culture

One millilitre of appropriate dilutions of the composite soil sample was inoculated into sterile Petri plates unto which was added an already prepared Potato Dextrose Agar to make pour plates. Inoculated plates in duplicates were incubated at 27$^{o}$C and morphologically distinct fungal colonies were counted after 96 hrs. Pure fungal cultures were obtained by picking mycelia from distinct fungal colonies and sub culturing onto freshly prepared potato dextrose agar plates using inoculating needle until pure fungal colonies were obtained. Pure fungal strains cultures were stored on potato dextrose agar slants in a refrigerator at 4$^{o}$C for further studies.

### 3.14 Screening for Caprolactam utilizing ability of the Fungi Isolates

The ability of the fungal isolates to utilise caprolactam was tested on glucose-mineral medium (Deguchi *et al*., 1997) as modified by Friedrich *et al.* (2007), using spread plate method. Spores were scraped from 7 days old fungal cultures and suspended in sterile distilled water. 0.5 ml of the spore suspension was inoculated onto already prepared basal medium. Inoculated plates in duplicates were incubated at 27oC for 7 days. Those fungi that grew with morphologically distinct mycelia colonies on the basal medium were selected for ability to use the caprolactam as nitrogen source for growth.

### 3.15 Identification of the fungi isolates

This was done using standard technique as described by James and Natalie (2001). Pure culture of the fungi isolates were inoculated onto Potato Dextrose Agar and incubated for 7 days. A drop of lacto-phenol cotton blue stain was placed on a clean glass slide with the aid of a needle and a small portion of the mycelium from the fungal

cultures was removed and placed in the drop of lacto-phenol cotton blue stain and the mycelium was spread very well on the slide with the aid of the needle. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objective lenses respectively under a light microscope. The fungi were identified morphologically and microscopically based on spore colour, shape, surface ornamentation and wall structures of the hyphae and conidiophores in accordance with information in Compendium of soil fungi (Domsch *et al.*, 1980).

### 3.16 Effect of Caprolactam on growth of the different fungal isolates

The fungal isolates were grown on potato dextrose agar (LabM, IDG diagnostics, UK) for 5 days. Plugs (5 mm) of the fungal cultures were bored on growing mycelia colonies and picked with sterilized inoculating needles into 5 mm diameter well bored on freshly prepared modified glucose-mineral medium (Friedrich *et al*, 2007) of varied caprolactam concentration using a sterilized 5mm cork borer. Inoculated plates in duplicates were incubated at 27$^{o}$C for 7 days and the radial mycelia growth of the fungi in the varied concentration series was monitored.

### 3.17 Confirmation of Caprolactam utilizing ability of the Fungi Isolates in broth Culture

#### 3.17.1 Studies of the fungi growth in broth culture

The mycelia weight and changes in medium pH was used to monitor the ability of the fungi isolates to utilize caprolactam as a nitrogen source for growth. The fungi isolates were inoculated into the caprolactam basal broth medium with addition of 1% glucose and incubated in static position for 15 days. Dry mycelia weight were determined after harvesting the mycelia mass by filtration using a previously weighed Whatman Filter Paper followed by drying in the oven at 60$^{o}$C to constant weight. The mycelia weight was calculated as the difference between the weight of the filter paper ($W_0$) and the final weight of the residue and the filter paper ($W_1$). i.e.

$$\text{Mycelia Weight} = W_1 - W_0.$$

The pH of the culture was measured at 5 days interval using a pH meter (HANNA Instruments HI 2210) to confirm the activity of the fungi isolates.

### 3.17.2 Determination of changes in concentration of caprolactam in the growth medium

Changes in concentration of caprolactam and of 6-aminohexanoic acid produced as an intermediate product were determined in the final filtrate using HPLC and Spectrophotometry ninhydrin method as described before in section 3.7.3.

## 3.18 Biodegradation of Nylon-6 fibre Experiments

### 3.18.1 Biodegradation of Nylon-6 Fibre by Bacteria Isolates

Four (4) bacteria isolates that possessed the ability to grow on caprolactam were tested for their ability to degrade nylon-6 fibre in submerged culture. The basal medium as described by Baxi and Shah, (2002) was used. Strips of nylon-6 fibre were purchased from Goodfellow Cambrigde Limited, United Kingdom. Previously weighed strips of the nylon-6 fibre (25 mm x 25 mm x 1 mm) diameter were added as the sole source of carbon and nitrogen in the basal medium. 100 ml of the medium at pH 7.2 were dispensed into 250 ml conical flasks and inoculated after sterilization.

The inocula were prepared by culturing the selected isolates in Nutrient broth on an incubator shaker for 48 hrs; cells were harvested by centrifugation at 7,000 rpm for 10 minutes, washed with and suspended in normal saline solution. The mediumt was inoculated with 1ml of the harvested cells in triplicates and incubated at 35oC on the G24 Environmental incubator shaker (New Brunswick scientific Co.Inc.Edeison USA) at 180 rpm. The experiments were monitored for 3 months and analysed monthly for the following.

#### 3.18.1.1 Determination of the bacteria growth in the Nylon-6 culture broth

The culture broth was sampled monthly and analysed for growth turbidimetrically. 5ml of the culture broth was filled into the designated cuvette and the optical density was read at wavelength of 600nm using UV –Visible Spectrophotometer (Perkin Elmer Lambda 25).

#### 3.18.1.2 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 inoculated and un-

inoculated treated nylon were 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-6 fibre after the experiment.

### 3.18.1.3 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 in an Ubbelholde viscometer at room temperature. 1% (w/v) of the nylon-6 fibre in 5 M 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 (ηrel) was calculated as

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

Where $\eta_{rel}$ is the relative viscosity of the fibre sample, η is the viscosity of the solution and $\eta_o$ is the viscosity of the solvent.

The number average molecular mass (Mn) was computed from the relative viscosity using the equation Mn= 11500(ηrel-1) (Ciaperoni and Mula, 2001).

### 3.18.1.4 High Performance Liquid Chromatography (HPLC) Analysis

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, University of Ibadan, Nigeria. A mixture of caprolactam and 6-aminohaxenoic acid was used as a standard (50:50v/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) with 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)

### 3.18.1.5 Fourier Transform Infrared (FTIR) 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 microorganisms at the termination of the experiment. This test was used to examine the differences in the chemical component of biodegraded nylon-6 fibre compared to undegraded nylon-6.

The nylon-6 fibre was recovered from the culture broth and was 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's University, Ogun State, 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 degraded and undegraded nylon-6 fibre were compared in the spectra obtained.

### 3.18.2 Biodegradation of Nylon-6 Fibre by Fungi Isolates

The Six (6) fungi isolates that had the ability to utilise Caprolactam at Concentration not less than 10 $gl^{-1}$ were tested for their ability to degrade nylon-6 fibre in submerged culture using the synthetic medium described by Friedrich *et al*., (2007). Strips of nylon-6 fibre were added as the sole source of nitrogen in the basal medium. 100 ml of the medium in 250 ml conical flasks at pH 6.4 were inoculated in duplicates with 5 ml of suspended fungi spores scraped from 7 day old plate culture of the fungi isolates previously grown on PDA and incubated in stationary position for 3 months, while the following analyses were carried out monthly.

### 3.18.2.1 Determination of Mycelia Weight

The mycelia mats of the fungal isolates were harvested by filtration using a previously weighed filter paper after which the filter paper with the mycelia mat were oven dried at 60$^{o}$C until constant weight. The weight of the mycelia was determined by subtracting the weight of the filter paper from the total weight of filter paper plus mycelia mat. The mean mycelia dry weight of the cultures was then determined from

the duplicates. The change in pH value of the culture medium was measured using Hanna Laboratory pH meter as describe above.

### 3.18.2.2 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 filtrate residue and the percentage weight loss was calculated as described above.

### 3.18.2.3 Determination of changes in Number Average Molecular Mass ($M_n$)

This was carried out using the same methods as described above.

### 3.18.2.4 Fourier Transform Infrared (FTIR) Spectroscopic Analysis of structural changes in the Nylon-6 fibres.

This was carried out using the same methods as described above.

# CHAPTER FOUR

# RESULTS

## 4.1 Description of Sampled Dumpsites

The map of Lagos state indicating the sampled dumpsites in their respective Local Government Area (LGA) of the state is shown in Figure 4.1.

**Olusosun** dumpsite is located beside Lagos- Ibadan Express way at Ojota in Kosofe local government area of Lagos State. **Abule-Egba** dumpsite is located in Oke-odo the Western part of Lagos in the Northern part of Alimosho Local Government area of the state. **Isheri-Igando** dumpsite is situated at Igando along Isheri-Igando road in Southern part of Alimosho Local Government Area of Lagos State,

**Nichemtex** is a company located along Igbogbo -Bayeku road in Ikorodu Local Government area. Lagos state presently operates on 20 Local Government and 37 Local Development Area. In order to meet the exigency of solid waste disposal, illegal dumpsites are being operated in some of these Local governments especially those that are a bit far from the metropolis. Among these are Anthony Village dumpsite in Shomolu LGA, Isolo / Oke-Afa dumpsites in Oshodi-Isolo LGA and Itire dumpsite in Surulere LGA. All have been officially closed since 1992 but are still un-officially, serving some area of the Mainland. Among the earlier officially established but now closed dumpsites is Pelewura dumpsite located at Adeniji Adele in the central Lagos Island Local Government. According to LAWMA it was closed in 2008 and with a loading station for transfer to Olusosun dumpsite. Pelewura waste loading centre serve as loading station for waste generated from the commercial and residential areas of Lagos Island, Ikoyi, and Victoria Island. However, in order to meet the rapid urbanization and the status of Mega city, LAWMA has also designated some satellite sites comprising of Owutu in Ikorodu LGA, Sangotedo in Eti–Osa LGA and Temu in Epe LGA dumpsites. These sites are serving as back–up for the three major designated dumpsites in the state.

## 4.2 Sample labelling and Codes

Soil samples were taken at areas close to polyamides manufactured products waste found in the dumpsites as shown in Plate 4.1. The samples were labelled according to collection sites and the isolated organisms labelled according to the samples as shown in Table 4.1

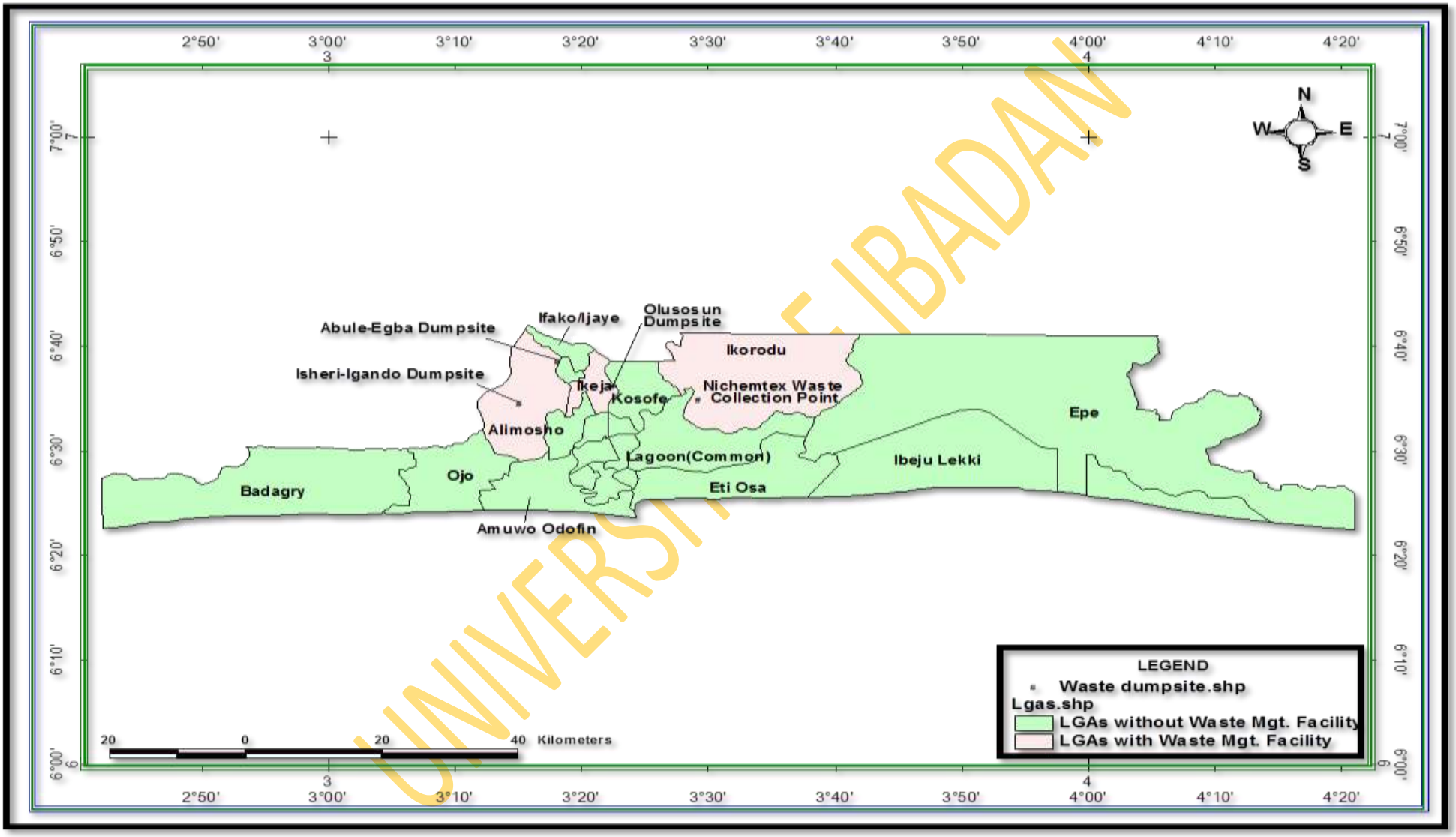



**Figure 4.1.** Map of Lagos state indicating the location of the sampled dumpsites

a. Tyre collection centre at Olusosun dumpsite, Ojota, Lagos.

b. Insulating material waste at Solus dumpsites, Isheri-Igando, Lagos.

c. Textiles waste materials at Abule-Egba dumpsites, Abule-Egba, Lagos.

**Plate 4.1(a-c).** Different waste types from sampled dumpsites.

**Table 4.1.** Sample Codes and Sites sampled for isolation of Microorganisms

| Codes | Interpretations | Date of sampling |
|---|---|---|
| ABA | Abule-Egba first sampling | April 2009 |
| ABB | Abule-Egba second sampling | June 2009 |
| ABC | Abule-Egba third sampling | August 2009 |
| 2ABA | Abule Egba fourth samples | October 2009 |
| ISA | Isheri first sampling | April 2009 |
| ISB | Isheri second sampling | June 2009 |
| ISC | Isheri third sampling | August 2009 |
| ORA | Olusosun first sampling | April 2009 |
| ORB | Olusosun second sampling | June 2009 |
| ORC | Olusosun third sampling | August 2009 |
| ORD | Olusosun fourth sampling | October 2009 |
| NTS | Nichemtex samples | October 2009 |
| BO | Botany department soil sample | August 2009 |
| FBC | Buried cloth samples | August 2009 |

Bacteria isolates obtained were named numerically according to the samples (Table 4.3).

### 4.3 Total Viable Bacteria Colony Counts (TVC)

The mean value of the total viable bacteria colony counts in the soil samples collected from each of the dumpsite and number of pure isolates obtained are shown in Table 4.2. Soil samples collected from Abule-Egba dumpsite had the highest total viable bacteria counts of 2.3 x $10^7$ cfu/g, followed by that of Olusosun dumpsite with 2.0 x $10^7$ cfu/g, Isheri-Igando dumpsite with 1.80 x $10^7$ cfu/g and the least of 1.2 x $10^7$ cfu/g was recorded in soil sample from Nichemtex waste collection points. The number of pure culture isolated from each of the dumpsite was 23, 21, 14 and 6 for Olusosun, Abule-Egba, Isheri/Igando and Nichemtex respectively (Table 4.2).

### 4.4 Screening for Caprolactam utilizing ability of the Bacteria isolates

The result of the screening for the ability of bacteria isolates to utilize caprolactam in a solid basal medium is shown in Table 4.3. Sixteen of the isolates grew on the basal medium within 48 hrs of incubation, nine others grew on the medium within 96 hrs while twenty two other isolates were found to have grown after the eleventh day of incubation. However, seventeen of the sixty four isolated bacteria did not grow in the basal medium. The result showed ubiquitous presence of caprolactam utilizing bacteria in dumpsites, positive and negative control sites as shown in the growth of isolates from these environments within 48 hrs (Table 4.3)

### 4.5 Biochemical Characterization of Bacteria Isolates

Table 4.4 showed the result of the biochemical and physiological tests carried out on the sixteen (16) bacteria isolates. Five of the isolates were Gram positive rods while the remaining eleven isolates were Gram negative rods.

The Gram positive rods consisted of two strains of *Corynebacterium ammoniagenes*, two strains of *Leucobacter aridicollis* and a strain of *Lysinibacillus sphearicus*. Both strains of *Corynebacterium* had yellowish colonies and were positive for catalase, urease, hydrogen sulphide and indole productions. Both utilised citrate, hydrolysed gelatin and reduced nitrate with gas production. Sugar fermentation tests were negative for Lactose, maltose, xylose and Galactose except for *C. ammoniagenes* (FBC2) that is weekly positive for glucose.

**Table 4.2.** Total viable bacteria colony counts (TVC) and number of isolated pure culture in the soil samples taken from the dumpsites.

| **Sampling Sites** | **Mean of TVC ($cfug^{-1}$) x $10^7$** | **No of pure bacteria isolates** |
|---|---|---|
| Abule-Egba | 2.38 ± 15 | 21 |
| Isheri-Igando | 1.80 ± 14 | 14 |
| Olusosun | 2.02 ± 22 | 23 |
| Nichemtex | 1.23 ± 10 | 6 |
| Botany Department | 1.86 ± 15 | 3 |
| Buried Cloth | 1.36 ± 10 | 3 |

Values represent a mean of four (4) samplings.

**Table 4.3.** Qualitative screening of growth patterns of caprolactam utilizing bacteria isolates obtained from the sampled dumpsites in Lagos State and University of Ibadan Botany Department backyard dumpsite.

| Dumpsites | ISOLATES CODES | GRADE |
|---|---|---|
| Abule-Egba | ABA1 | + |
| | ABA2 | ++ |
| | ABA3 | + |
| | ABA4 | ++ |
| | ABA5 | - |
| | ABA6 | - |
| | ABB1 | + |
| | ABB2 | - |
| | ABB3 | + |
| | ABC1 | + |
| | ABC2 | + |
| | ABC3 | +++ |
| | ABC4 | - |
| | ABC5 | + |
| | 2ABA2 | +++ |
| | 2ABA3 | + |
| | 2ABA4 | +++ |
| Isheri-Igando | ISA1 | + |
| | ISA2 | ++ |
| | ISA3 | ++ |
| | ISB1 | ++ |
| | ISB2 | ++ |
| | ISC1 | +++ |
| | ISC2 | ++ |
| | ISC3 | + |
| | ISC4 | ++ |
| | ISC5 | + |
| | ISC6 | +++ |
| | ISC7 | +++ |
| Olusosun | ORA1 | - |
| | ORA2 | - |
| | ORA3 | - |
| | ORA4 | + |
| | ORA5 | - |
| | ORA6 | - |

**KEY**:

+++ = Growth within 48hrs

++ = Growth within 96hrs

+ = Growth within 11days

- = No Growth

| | | |
|---|---|---|
| | ORA7 | - |
| | ORA8 | - |
| | ORB1 | - |
| | ORB2 | + |
| | ORB3 | + |
| | ORB4 | - |
| | ORB5 | +++ |
| | ORC1 | +++ |
| | ORC2 | - |
| | ORC3 | + |
| | ORC4 | - |
| | ORC5 | + |
| | ORC6 | - |
| | ORD1 | + |
| | ORD2 | + |
| | ORD3 | + |
| | ORD4 | + |
| Nichemtex | NTS1 | +++ |
| | NTS2 | +++ |
| | NTS3 | ++ |
| | NTS4 | - |
| | NTS5 | + |
| | NTS6 | +++ |
| University of Ibadan Botany | FBC1 | +++ |
| department backyard dumpsite | FBC2 | +++ |
| (negative Controls) | FBC3 | +++ |
| | BO1 | + |
| Buried cloth Ibadan (positve | BO2 | +++ |
| control) | BO3 | +++ |

The isolated *Leucobacter aridicollis* strains (BO2) had a cream coloured colony with smooth edge and was negative for indole production while strain (BO3) showed a whitish coloured colony and produced indole. Both strains are non-motile and negative for urease, nitrate, methyl red and gelatin hydrolysis. They are positive for catalase, oxidase, citrate and sulphide production. Sugar fermentation tests was only positive for galactose, other sugars tested were not fermented by the two strains The isolated *Lysinibacillus sphearicus* was a whitish colony with flat surface, it was positive for oxidase, catalase, citrate, and gelatin hydrolysis but negative for nitrate, urease, methyl red, indole and sulphide production. A sugar fermentation test was only positive for glucose.

The isolated Gram negative isolates belong to the genera *Pseudomonas, Alcaligenes, Providencia* and *Proteus.* Five strains of *Pseudomonas aeruginosa* were isolated in this study with abilities to utilize caprolactam. They had transparent colonies with varied shapes but mostly with pigmentation that diffused through the medium and are motile. Isolated strains are positive for catalase, oxidase, citrate, methyl red, hydrogen sulphide and gelatin hydrolysis but negative for urease and indole production. Sugar fermentation tests were positive for glucose and xylose only. Three species of *Alcaligenes* were isolated with two of them confirmed as *A. faecalis* while one cannot be identified to species level. They had large waxy circular colonies and motile. All the isolated strains of this genus were positive for oxidase, catalase, citrate, nitrate and gelatin hydrolysis but negative for indole, urease and methyl red. Sugar fermentation tests were positive for glucose and galactose in the two *Alcaligenes faecalis* strains (2ABA2) and (ORC1) but *Alcaligenes species* (ORB5) did not ferment galactose but only glucose. The isolated *Providencia vermicola* (FBC1) is gram negative rod with circular mucoidal colony. It was positive for catalase, urease, citrate and indole production but negative for oxidase, nitrate, methyl red, hydrogen sulphide and gelatin hydrolysis. This strain fermented glucose, lactose, maltose and xylose with gas production but did not ferment sucrose and galactose. The isolated *Proteus vulgaris* strain had a swarming colony with lobate edges. It was positive for oxidase, catalase, urease, nitrate, hydrogen sulphide production and gelatin hydrolysis but negative for indole production. It fermented all the sugar tested except lactose (Table 4.4).

### 4.6 Molecular Characterization and Phylogenetic Analyses of Bacteria isolates

The result of the 16S rDNA sequences of the sixteen bacteria isolates is shown in Table 4.5. Basic Local Alignment Search Tool (BLAST) analysis of the sequences in the GenBank (www.ncbi.nim.nih.gov) showed that seven of the isolates belong to the group of Gamma (γ) Proteobacteria and of the genera *Pseudomonas*, *Proteus* and *Providencia* while four of the isolates belong to the group Actinobacteria in the genera *Corynebacterium* and *Leucobacter*. Similarly, four other isolates were of the group Beta (β) Proteobacteria belonging to the genera *Alcaligenes* and *Bordetella* while the last one belong to the group Firmicutes in the genus *Lysinibacillus.*

Figure 4.2 showed the Phylogenetic tree of the isolates constructed with Molecular Evolution Genetics Analysis (MEGA) version 5. The phylogenetic analysis of the isolates using the neighbour joining method based on Maximum Composite likelihood revealed that the isolates clustered into four grouping pattern of close phylogenetic resemblance.

*Corynebacterium ammoniagenes* (FBC3 showed 96 % 16S rDNA homology with *Corynebacterium ammoniagenes* (X82056) in NCBI Genbank These isolates clustered with 99 % nucleotides alignment with *Leucobacter aridicollis* (BO2). *Leucobacter aridicollis* (BO2) shared 97 % 16S rDNA homology with *L. aridicollis* (AJ781047) in the Genbank while *L. aridicollis* (BO3) shared 93 % nucleotide homology with same bacteria. However, both *Leucobacter aridicollis* (BO2) and (BO3) did not cluster together.

*Alcaligenes faecalis* (2ABA2) and *A. faecalis* (ORC1) clustered together on the phylogenetic tree showing 90 % similarity with each other while both isolates shared 97 % 16s rDNA sequence similarity with *A. faecalis* in NCBI Genbank nucleotides sequence database with accession number EF0111115. These two isolates also showed 76 % similarity with *Alcaligenes* species (ORB5) which shared 95% 16S rDNA homology with an uncultured *Alcaligenes* species with accession number EU272864 in NCBI Genbank nucleotides database. Also in this group was the *Bordetella petrii* (2ABA4) that shared 98 % 16S rDNA nucleotides homology with *B. petrii* EU082174 in the Genbank database but clustered with 65 % similarity with *A. faecalis* (ORB5).

**Table 4.4.** Biochemical characteristics of caprolactam utilizing bacteria isolates from solid waste dumpsites.

| | Isolates Codes | Shape | Gram | Reaction | Oxidase | Catalase | Urease | Nitrate | Citrate | Indole | Methyl red | $H_2S$ | Gelatin | Motility | Glucose | Lactose | Sucrose | Maltose | Xylose | Galactose | Isolates Identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ABC3 | Short rod | - | + | + | - | + | + | - | + | + | + | + | + | - | - | - | + | - | | *Pseudomonas aeruginosa* |
| 2 | 2ABA2 | Rod | - | + | + | - | + | + | - | - | + | + | + | + | - | - | - | - | + | | *Alcaligenes faecalis* |
| 3 | 2ABA4 | Rod | - | + | + | + | - | + | - | - | - | - | + | + | - | - | - | + | - | | *Bordetella petrii* |
| 4 | BO2 | Rod | + | + | + | - | - | + | - | - | + | - | - | - | - | + | - | - | + | | *Leucobacter aridicollis* |
| 5 | BO3 | Rod | + | + | + | - | - | + | + | - | + | - | - | - | - | - | - | + | + | | *L. aridicollis* |
| 6 | FBC1 | Rod | - | - | + | + | - | + | + | + | - | - | + | +G | +G | - | +G | +G | - | | *Providencia vermicola* |
| 7 | FBC2 | Rod | + | - | + | + | +G | + | + | - | + | + | - | + | + | + | + | + | - | | *Corynebacterium ammoniagenes* |
| 8 | FBC3 | Rod | + | - | + | + | + | + | + | - | + | + | - | + | + | + | + | + | - | | *C. ammoniagenes* |
| 9 | ISC1 | Rod | + | - | + | - | - | + | - | - | - | + | - | + | - | - | - | - | - | | *Lysinibacillus sphaericus* |
| 10 | ISC6 | Short rod | - | + | + | - | + | + | - | + | + | + | + | + | - | - | - | + | - | | *P. aeruginosa* |
| 11 | ISC7 | Short rod | - | + | + | - | + | + | - | + | + | + | + | + | - | - | - | + | - | | *P. aeruginosa* |
| 12 | NTS1 | Short rod | - | + | + | - | + | + | - | + | + | + | + | + | - | - | +G | + | - | | *P. aeruginosa* |
| 13 | NTS2 | Rod | - | + | + | + | +G | + | - | - | + | + | + | + | - | - | - | + | + | | *Proteus vulgaris* |
| 14 | NTS6 | Short rod | - | + | + | - | + | + | - | + | + | + | + | + | - | - | - | + | - | | *P. aeruginosa* |
| 15 | ORB5 | Rod | - | + | + | - | + | + | - | - | + | + | + | + | - | - | - | - | - | | *Alcaligenes species* |
| 16 | ORC1 | Rod | - | + | + | + | + | + | - | - | + | + | + | + | - | - | - | - | + | | *A. faecalis* |

*Pseudomonas aeruginosa* (ISC7) shares 99 % 16S rDNA identity with *Pseudomonas aeruginosa* (AY486366) in the NCBI Genbank database. *P. aeruginosa* (NTS1) on the other hand shares 99 % identity with *P.aeruginosa* with accession number (HM224410) in the Genbank database while *P. aeruginosa* (ABC3) shares 99 % identity with *P. aeruginosa* FJ374125. All the three aforementioned isolates aligned with each other with 97 % relationship on the phylogenetic tree but did not cluster with the other two *Pseudomonas aeruginosa* strains (ISC6) and (NTS6). However, *Pseudomonas aeruginosa* (ISC6) and (NTS6) shared 97 % and 96 % 16S rDNA nucleotides sequence identities with *P. aeruginosa* HQ457019 and *P. aeruginosa* DQ300346 respectively in the Genbank database. Also in the same phylogenetic group are *Providencia vermicola* (FBC1) identified based on 98 % 16S rDNA nucleotides sequence identity with *P. vermicola* HM582228 in the Genbank database and *Proteus vulgaris* (NTS2) that shared 100 % 16S rDNA nucleotides sequence identity with *P. vulgaris* FJ799903 in the database. *Providencia vermicola* (FBC1) showed 40 % alignment with *Pseudomonas aeruginosa* (NTS1), *P. aeruginosa* (ABC3) and *P. aeruginosa* (ISC7) and also 30 % alignment with *C. ammoniagenes* (FBC3) and *L. aridicollis* (BO2) isolates in the group. *Proteus vulgaris* (NTS2) on the other hand aligned with 54 % to *Pseudomonas aeruginosa* (NTS6) and *Pseudomonas aeruginosa* (ISC6).

*Lysinibacillus sphearicus* is the only member of the isolates belonging to the group Firmicutes and it shared 98 % 16S rDNA nucleotide sequence homology with *Lysinibacillus sphaericus* in the NCBI Genbank with accession number HM771260. It clustered with 94 % alignment to the actinobacteria group.

**Table 4.5.** Phylogenetic Identities of the bacterial isolates using NCBI online database

| Isolates Code | Length of the nucleotide sequences | Accession number of nearest Homology | % similarity | Name of the organism | Phylogenetic group |
|---|---|---|---|---|---|
| ORB5 | 1214 | EU272864.1 | 95 | *Alcaligenes sp.* | β -Proteobacteria |
| ORC1 | 1198 | EF0111115.1 | 97 | *Alcaligenes faecalis* | ,, |
| 2ABA2 | 1328 | EF0111115.1 | 97 | *A. faecalis* | ,, |
| 2ABA4 | 1294 | EU082174.1 | 98 | *Bordetella petrii* | , |
| NTS2 | 1382 | FJ799903.2 | 100 | *Proteus vulgaris* | γ –Proteobacteria |
| NTS1 | 1361 | HM224410.1 | 99 | *Pseudomonas aeruginosa* | ,, |
| NTS6 | 703 | DQ300346.1 | 96 | *P. aeruginosa* | ,, |
| ABC3 | 1357 | FJ374125.1 | 99 | *P. aeruginosa* | ,, |
| ISC6 | 370 | DQ300346.1 | 97 | *P. aeruginosa* | ,, |
| ISC7 | 1331 | AY486366.1 | 99 | *P. aeruginosa* | ,, |
| FBC1 | 1369 | HM58281.1 | 98 | *Providencia vermicola* | ,, |
| FBC2 | 1158 | X82056.1 | 96 | *Corynebacterium ammoniagenes* | Actinobacteria |
| FBC3 | 1158 | X82056.1 | 96 | *C. ammoniagenes* | ,, |
| BO2 | 1301 | AJ781047.1 | 97 | *Leucobacter aridicollis* | ,, |
| BO3 | 673 | AJ781047.1 | 93 | *L. aridicollis* | ,, |
| ISC1 | 1310 | HM771260.1 | 98 | *Lysinibacillus sphaericus* | Firmicutes |

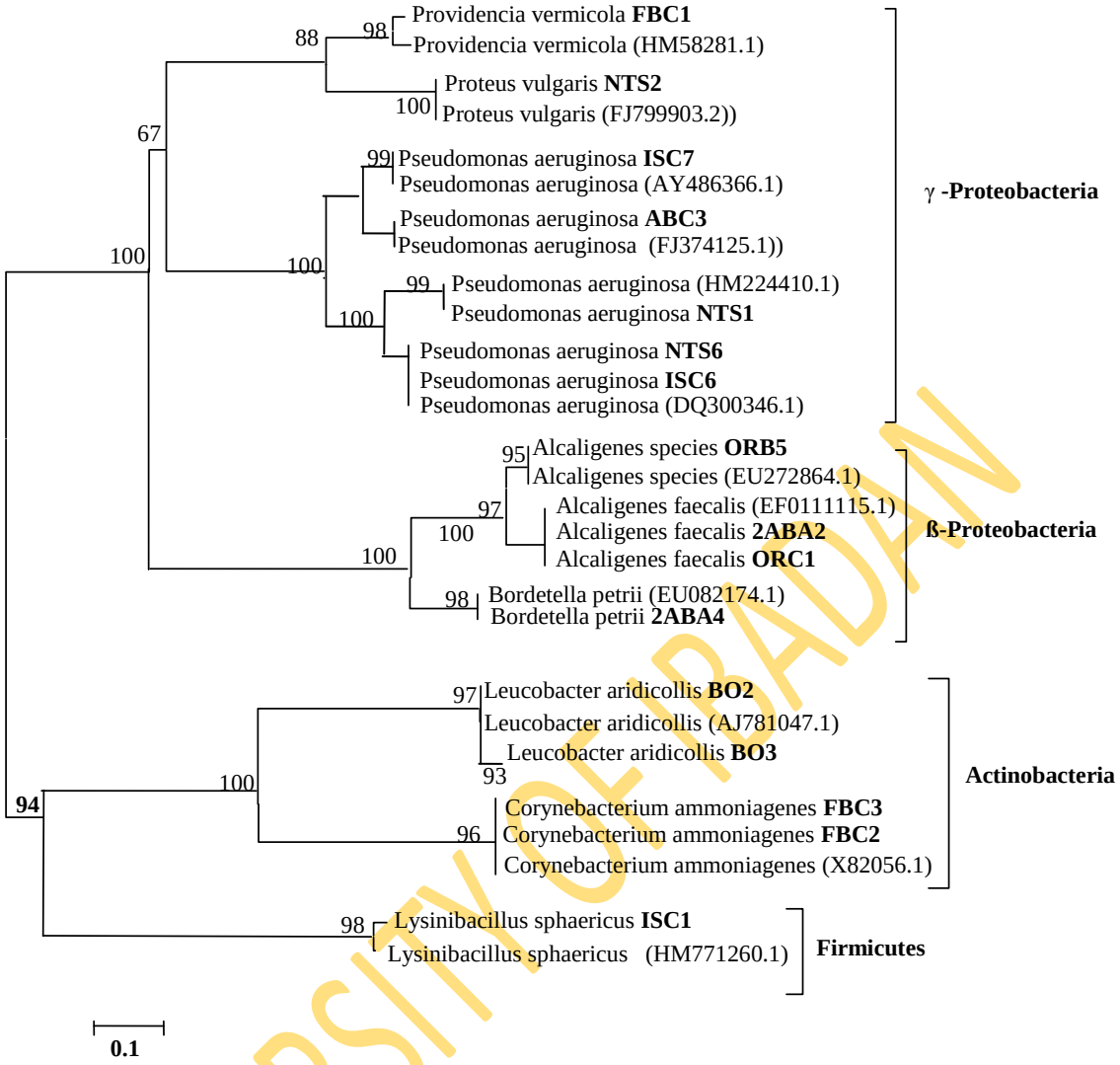



**Figure 4.2** Phylogenetic relationship of the bacteria isolates used in the study of degradation of nylon-6.

## 4.7 Confirmation of Caprolactam utilizing ability of the Bacteria Isolates in broth Culture medium

### 4.7.1 Growth studies for bacteria isolates

Figure 4.3 (a-c) shows the growth pattern of the selected sixteen bacterial isolates that grew on the solid basal medium within 48 hrs when inoculated into the broth of caprolactam basal medium. All the sixteen bacteria isolates grew on the basal medium. *Lecucobacter aridicollis* (BO3) showed an exponential growth phase within 48 hrs, *Corynebacterium ammoniagenes* (FBC3), *Pseudomonas aeruginosa* (NTS1), *P. aeruginosa* (NTS6) and *Alcaligenes faecalis* (ORC1) showed exponential growth phase up to 96 hrs while the eleven other isolates grew exponentially within 72 hrs. *Pseudomonas aeruginosa* (NTS1) and *P. aeruginosa* ( NTS6) showed relatively stationery growth phases between 48 and 96 hrs while similar growth phase was observed in *Pseudomonas aeruginosa* (ABC3) between 72 and 96hrs. Generally, the pH of the growth medium ranged from 6.9 to 8.3 (Table4.6).

Also, Table 4.7 shows the percentage reduction in caprolactam concentration by each bacteria isolate in the broth basal medium using High Performance Liquid Chromatography (HPLC). *Proteus vulgaris* (NTS2) reduced the concentration by 97.2 %, followed by *Bordetella petrii* (2ABA4) with 92.5 %, *P. aeruginosa* (NTS1) with 90.5 %, and *Alcaligenes faecalis* (2ABA2) with 82.3 % respectively. The least reduction of 53.8 % was by *Corynebacterium ammoniagenes* (FBC3).

Estimation of 6-aminohexanoic acid produced as intermediate product of the caprolactam degradation by the bacterial isolates is shown in Figure 4.4. *Bordetella petrii* (2ABA4) produced the highest concentration of 1269.30 ppm followed by *Proteus vulgaris* (NTS1) with 870ppm, *Alcaligenes faecalis* (2ABA2) and *P. aeruginosa* (NTS1) produced 848.60 ppm and 790.40 ppm respectively. The least value of 440.20 ppm was produced by *Corynebacterium ammoniagenes* (FBC2) which is 321 % higher when compared with the un-inoculated control.

## 4.8 Minimum Inhibitory Concentration (MIC).

Table 4.8 shows the minimum inhibitory concentration of the caprolactam on the sixteen different bacteria isolates that were able to grow on the solid basal medium of caprolacatam within 48 hours out of the sixty four isolated bacteria (Table 4.5). Thirteen of the isolates grew well in the presence of caprolactam up to 15 $gL^{-1}$ except *Bordetella petrii* (2ABA4), *Alcaligenes faecalis* (2ABA2) and *Proteus vugaris* (NTS2) that grew well at concentration of 20 $gL^{-1}$ but none of the isolates grew at concentrations of 25 $gL^{-1}$and above

### 4.9 Effect of Temperature on Growth of the Bacterial Isolates

The optimum growth temperature of 35$^{o}$C was observed in all the bacteria isolates except *Providencia vermicola* (FBC1) and *Proteus vulgaris* (NTS2) that had their highest growth at 30$^{o}$C (Figure 4.5a-c).

### 4.10 Effect of pH on growth of the Bacterial Isolates

The optimal growth pH for the isolates was found to be 7.5 except the *Proteus vulgaris* (NTS2) and *P. aeruginosa* (ABC3) that had their optimum growth at pH 7.0. (Figure 4.6a-c)

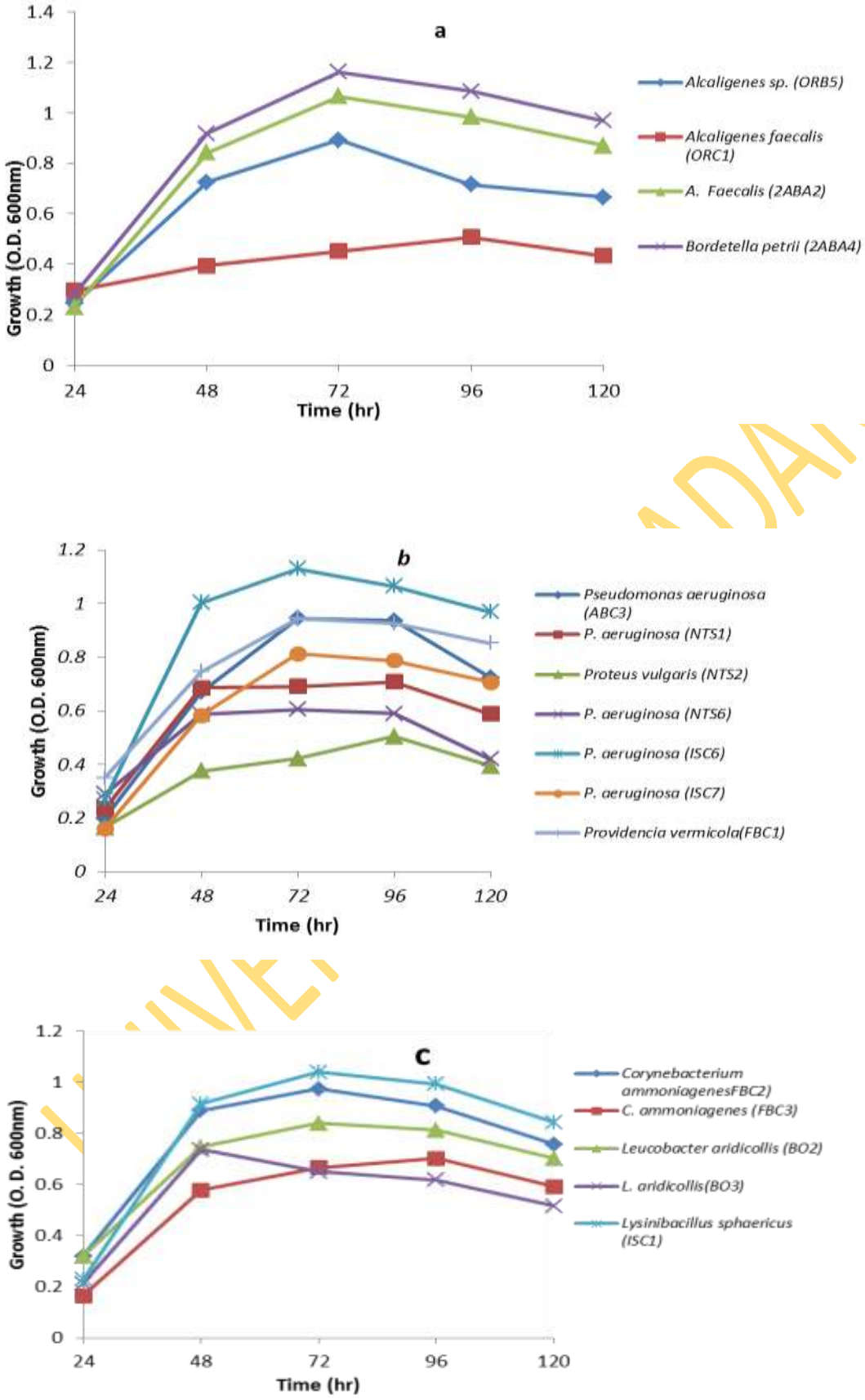



**Figure 4.3** (a-c). The growth pattern of the sixteen selected bacteria isolates in broth of the basal medium of caprolactam.

**Table 4.6.** Changes in pH during growth of bacteria in Caprolactam basal broth

| **Bacteria isolates** | **Time of incubation (hours).** | | | | | |
|---|---|---|---|---|---|---|
| | **0** | **24** | **48** | **72** | **96** | **120** |
| *Bordetella petrii(2ABA4)* | 6.9 | 7.7 | 8.0 | 8.2 | 8.2 | 7.9 |
| *Pseudomonas aeruginosa(ABC3)* | 6.9 | 7.6 | 7.7 | 8.2 | 8.1 | 8.3 |
| *Alcaligenes faecalis(2ABA2)* | 6.9 | 7.7 | 7.9 | 8.4 | 8.3 | 8.4 |
| *P. aeruginosa(ISC7)* | 6.9 | 7.7 | 7.8 | 8.0 | 8.1 | 8.3 |
| *P. aeruginosa (ISC6)* | 6.9 | 7.7 | 7.8 | 8.0 | 8.1 | 8.3 |
| *Lysinibacillus sphaericus (ISC1)* | 6.9 | 7.5 | 7.8 | 8.2 | 8.1 | 8.3 |
| *Alcaligenes species (ORB5)* | 6.9 | 7.7 | 8.0 | 8.3 | 8.2 | 8.3 |
| *A. faecalis (ORC1)* | 6.9 | 7.6 | 8.0 | 8.2 | 8.1 | 8.2 |
| *P. aeruginosa(NTS1)* | 6.9 | 7.4 | 7.7 | 8.1 | 8.1 | 8.3 |
| *P. aeruginosa(NTS6)* | 6.9 | 7.7 | 7.8 | 8.3 | 8.1 | 8.3 |
| *Proteus vulgaris(NTS2)* | 6.9 | 7.6 | 7.7 | 8.0 | 8.1 | 8.3 |
| *Leucobacter aridicollis(BO2)* | 6.9 | 7.6 | 8.1 | 8.2 | 8.1 | 8.2 |
| *Leucobacter aridicollis (BO3)* | 6.9 | 7.6 | 7.7 | 8.2 | 8.2 | 8.3 |
| *Corynebacterium ammoniagenes(FBC3)* | 6.9 | 7.4 | 7.8 | 8.1 | 8.0 | 8.2 |
| *Providencia vermicola (FBC1)* | 6.9 | 7.5 | 7.9 | 8.2 | 7.9 | 8.2 |
| *Corynebacterium ammoniagenes(FBC2)* | 6.9 | 7.1 | 7.9 | 8.1 | 8.0 | 8.2 |
| Control treatment | 6.9 | 6.9 | 6.9 | 6.9 | 6.8 | 6.9 |

**Table 4.7.** Percentage reduction in caprolactam concentration by the bacteria isolates after 120 hours of incubation.

| Bacteria isolates | % Reduction of caprolactam |
|---|---|
| *Proteus vulgaris(NTS2)* | **97.2** |
| *Bordetella petrii(2ABA4)* | **92.5** |
| *P. aeruginosa(NTS1)* | **90.5** |
| *Alcaligenes faecalis(2ABA2)* | **82.3** |
| *P.aeruginosa(ISC7)* | **71.0** |
| *Lysinibacillus sphaericus (ISC1)* | **70.9** |
| *P.aeruginosa (ISC6)* | **69.9** |
| *Corynebacterium ammoniagenes(FBC2)* | **68.6** |
| *Pseudomonas aeruginosa(ABC3)* | **66.8** |
| *Providencia vermicola (FBC1)* | **63.0** |
| *P. aeruginosa(NTS6)* | **61.0** |
| *Alcaligenes species (ORB5)* | **60.8** |
| *Alcaligenes faecalis (ORC1)* | **68.9** |
| *Leucobacter aridicollis (BO3)* | **59.9** |
| *Leucobacter aridicollis(BO2)* | **59.4** |
| *Corynebacterium ammoniagenes(FBC3)* | **53.8** |

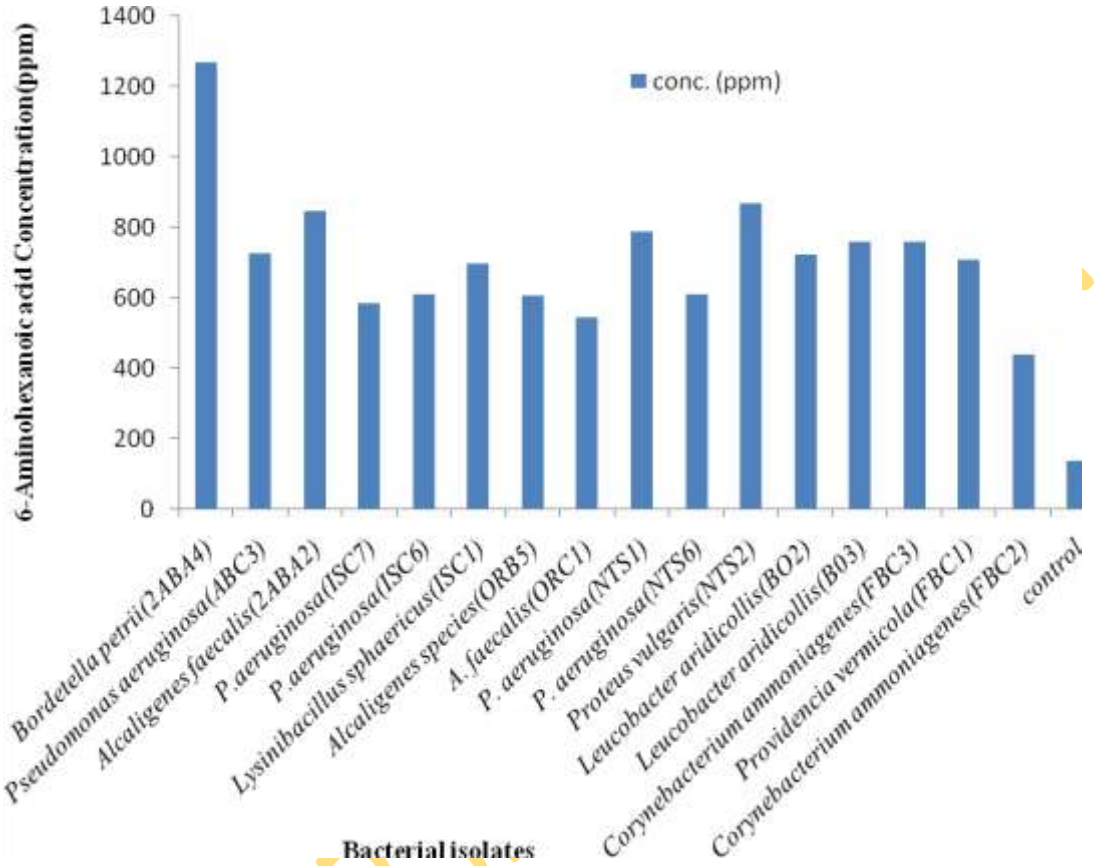



**Figure 4.4.** Estimated concentration of 6-aminohexanoic acid produced by each isolate in the culture after 96 hrs.

**Table 4.8.** Minimum Inhibitory Concentration (MIC) of Caprolactam on different bacteria isolates

| **Isolates Code** | **Isolates Identity** | **Inhibitory Caprolactam Concentration $gL^{-1}$)** |
|---|---|---|
| 2ABA4 | *Bordetella petrii* | 25 |
| ABC3 | *Pseudomonas aeruginosa* | 20 |
| 2ABA2 | *Alcaligenes faecalis* | 25 |
| ISC7 | *P. aeruginosa* | 20 |
| ISC6 | *P. aeruginosa* | 20 |
| ISC1 | *Lysinibacillus sphearicus* | 20 |
| ORB5 | *A. species* | 20 |
| ORC1 | *A. faecalis* | 20 |
| NTS1 | *P. aeruginosa* | 25 |
| NTS6 | *P. aeruginosa* | 20 |
| NTS2 | *Proteus vulgaris* | 25 |
| BO2 | *Leucobacter aridicollis* | 20 |
| BO3 | *Leucobacter aridicollis* | 20 |
| FBC3 | *Corynebacterium ammoniagenes* | 20 |
| FBC1 | *Providencia vermivola* | 20 |
| FBC2 | *C. ammoniagenes* | 20 |

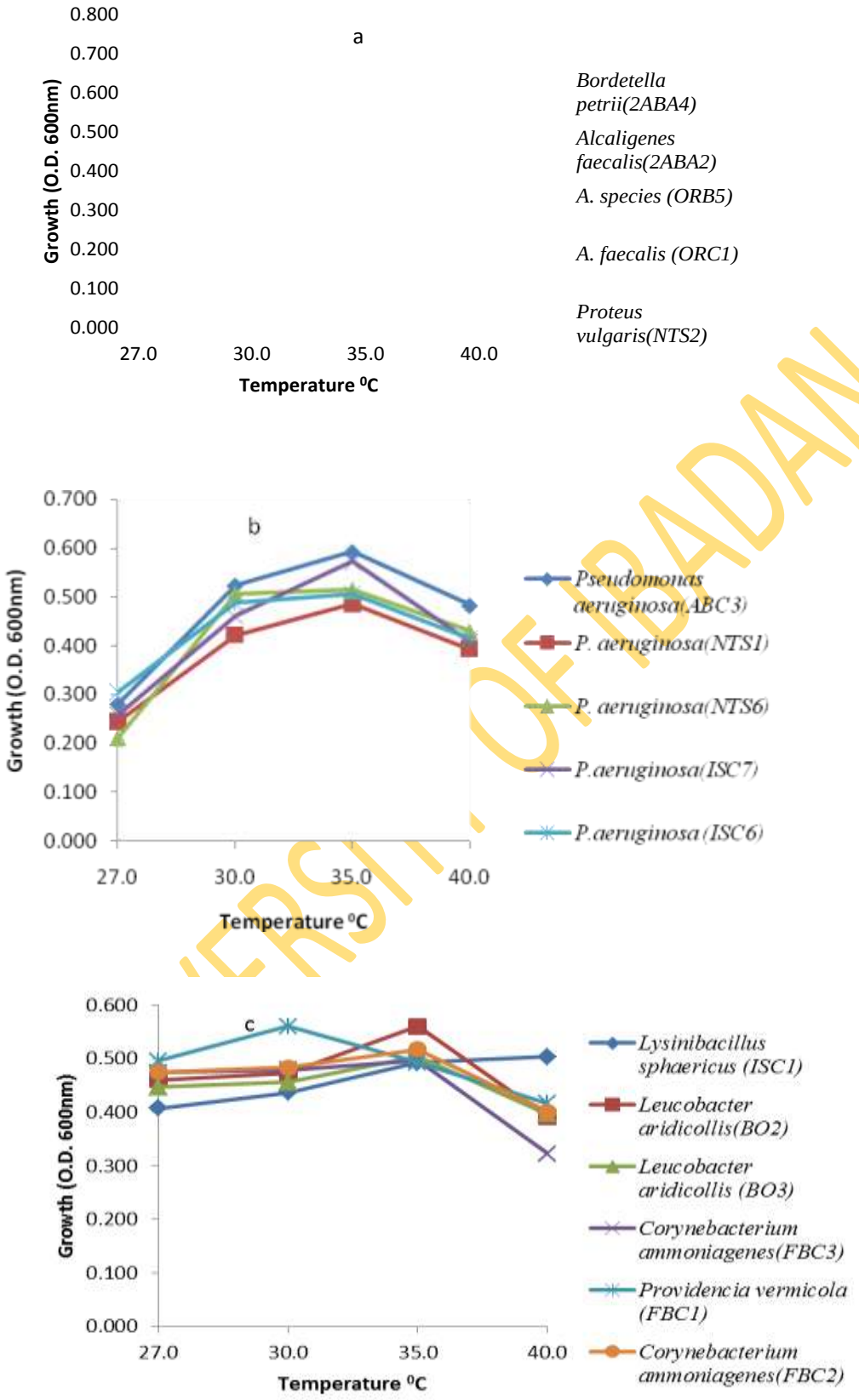



**Figure 4.5 (a-c).** Optimal temperature for growth of the bacteria isolates in Caprolactam

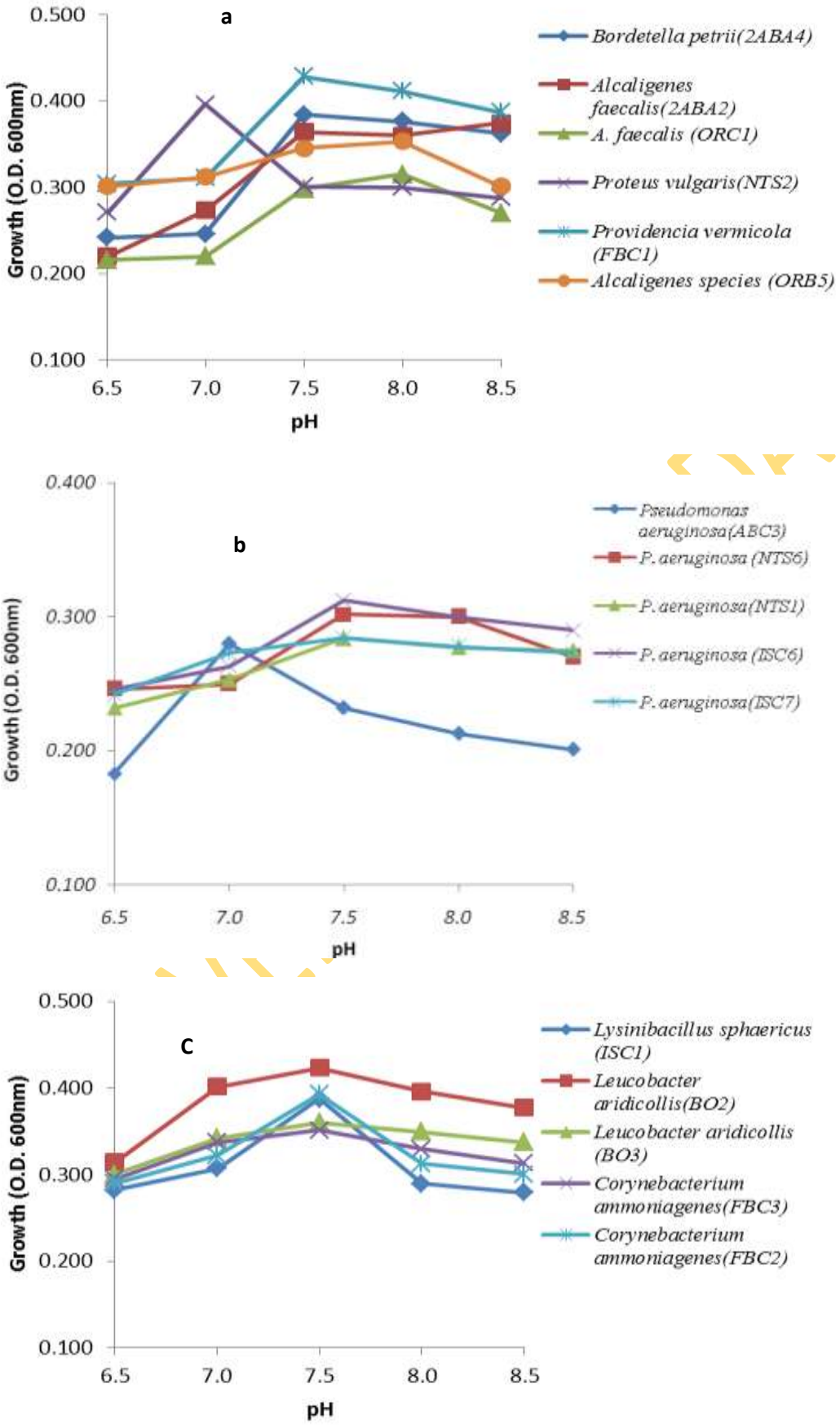



**Figure 4.6 (a-c).** Optimal pH for growth of the bacteria isolates in Caprolactam

### 4.11 Total Fungal Colony Counts of collected soil samples

The mean value of total fungi counts from the soil samples collected from the various dumpsites and the number of the pure fungi isolates obtained are shown in Table 4.9. Soil sample collected from Olusosun dumpsite had the highest fungi counts of 2.3 x $10^6$ cfu/g, followed by 2 x $10^6$ cfu/g in soil samples from Abule-Egba dumpsite and 1.7 x $10^6$ cfu/g in soil sample from Isheri-Igando dumpsites while the least count of 9 x $10^6$ cfu/g was recorded in the soil sample from Nichemtex waste collection point. A total of twenty two fungi were isolated from the soil samples collected from the dumpsites. This was made up of Seven (7) fungal isolates each from Abule-Egba and Olusosun while five (5) and three (3) isolates were isolated from samples collected from Nichemtex and Isheri/ Igando respectively.

### 4.12 Caprolactam utilizing ability of the Fungi Isolates

Out of the twenty two (22) fungi isolates that were screened on caprolactam basal medium agar supplemented with 1% glucose, only six (6) fungal isolates grew and sporulated within 15 days on the medium others did not grow (Table 4.10)

### 4.13 Identification of the fungi isolates

The six fungi isolates that grew and sporulated in the basal medium of the caprolactam were identified as five different species of the genus *Aspergillus* and a species of the genus *Penicillium* (Table 4.11).

### 4.14 Effect of Caprolactam on growth of the different fungal isolates

Table 4.12 show the growth of the six fungi isolates at varied concentrations of 5 to 30 $gL^{-1}$ of caprolactam. The mycelia growth of the isolates reduced gradually with the increase in concentration of caprolacatam. The highest myelia growth at 30 $gL^{-1}$ of caprolactam was observed in *Aspergillus terreus* (ORAF1) with growth diameter of 30.0 mm, followed by *A. niger* (AF3) with mycelia growth of 23.0 mm at same concentration. The least mycelia growth diameter of 10.0 mm was recorded in *Penicilium citrinum* (NTSF3) at caprolactam concentration of 30 $gL^{-1}$.

**Table 4.9.** Total fungal colony counts and number of isolated pure culture in the soil samples from each of the dumpsites.

| **Sampling Sites** | **Mean of Fungal Counts (cfug$^{-1}$) X10$^{5}$** | **Number of pure fungi isolates** |
|---|---|---|
| Abule-Egba | 20 ± 3 | 7 |
| Isheri-Igando | 17 ± 1 | 3 |
| Olusosun | 23 ± 2 | 7 |
| Nichemtex | 9 ± 1 | 5 |

Mean values represent a mean of four (4) samplings.

**Table 4.10. Growth of the fungi isolates in basal medium of caprolactam**

| ISOLATES | REMARKS |
|---|---|
| AF1 | No growth |
| AF2 | Growth with sporulation |
| AF3 | Growth with sporulation |
| AF4 | No growth |
| AF5 | No growth |
| AF6 | No growth |
| AF7 | No growth |
| OF1 | Growth with sporulation |
| OF2 | Growth with sporulation |
| OF3 | No growth |
| OF4 | No growth |
| OF5 | Growth with sporulation |
| OF6 | No growth |
| OF7 | No growth |
| IF1 | No growth |
| IF2 | No growth |
| IF3 | No growth |
| NF1 | Growth with sporulation |
| NF2 | No growth |
| NF3 | No growth |
| BF1 | No growth |
| BF2 | No growth |

**KEY**

AF – Fungi isolated from Abule-Egba soil sample

OF – Fungi isolated from Olusosun soil sample

IF – Fungi isolated from Isheri soil sample

NF – Fungi isolated from Nichemtex soil sample

BF – Fungi isolated from the buried cloth in Ibadan

**Table 4.11**. Identification of Caprolactam utilizing fungi isolated from the Lagos dumpsites

| Fungi Codes | Characteristics | Tentative Identification |
|---|---|---|
| NF3 | Dark green colour with pale yellow reverse on plate. Septate hyphae with direct attachment long conidiophore. Conidia are spherical | *Penicillium citrinum* |
| AF2 | Lime green colour with a cream reverse. Wooly texture. Conidia are globose. One sterigma. | *Aspergillus flavus* |
| AF3 | Black and wooly. Reverse is white. Conidiophores are long terminating in a globose vesicle. Primary sterigma as twice the secondary sterigma. | *A. Niger* |
| OF1 | Light brown with velvety texture, crowded irregular size sterigma, round conidia. | *A. Terreus* |
| OF2 | Blue-green colour with light orange reverse, primary and secondary sterigma of equal colour, globose conidia. | *A. versicolor* |
| OF5 | Dirty green colour with wooly texture, flask shape vesicle, Conidia are coarse and globose. | *A. parasiticus* |

**Table 4.12.** Fungal growth diameter (mm) at varied concentration of caprolactam ($gL^{-1}$)

| | **Caprolactam concentrations ($gL^{-1}$)** | | | | | |
|---|---|---|---|---|---|---|
| **Isolates** | **5** | **10** | **15** | **20** | **25** | **30** |
| *Penicillium citrinum* (NF1) | 25 | 23 | 23 | 11 | 10 | 10 |
| *Aspergillus flavus* (AF2) | 45 | 45 | 41 | 23 | 17 | 16 |
| *A. niger* (AF3) | 56 | 54 | 52 | 40 | 28 | 23 |
| *A. terreus* (OF1) | 65 | 65 | 62 | 42 | 30 | 30 |
| *A. versicolor* (OF2) | 54 | 32 | 32 | 30 | 23 | 20 |
| *A. parasiticus* (OF5) | 42 | 32 | 28 | 25 | 15 | 10 |

## 4.15 Confirmation of Caprolactam utilizing ability of the Fungi Isolates in broth Culture medium

### 4.15.1 Growth studies of fungi isolates

Figure 4.7 showed that all the isolates grew within the first 5 days in the basal medium. The highest mycelia weight of 0.13 g for *Aspergillus niger* (AF3) followed by *A. versicolor* (OF2) with 0.10 g and the least mycelia weight of 0.04 g for *A. flavus* (AF2). However, there was decrease of between 25 to 80 % in the mycelia weight of the isolates between $10^{th}$ and $15^{th}$ days except in *Penicillium citrinum* (NF3) and *Aspergillus terreus* (OF1) with mycelia weight increases of 0.02 g and 0.01 g on $10^{th}$ day representing 33.3 % and 20.0 % compared to their respective mycelia weight at the 5th day. The pH of all the cultures (Fig. 4.8) decreased with the fungal growth. The highest decrease in pH value occurred in the culture medium of *A. parasiticus* from 6.4 to 4.8 while the least change in pH value recorded was 5.9 for *A. niger* at the $15^{th}$ day.

### 4.15.2 Changes in concentration of caprolactam in the growth medium

Caprolactam utilization by the fungi isolates was determined as a percentage reduction in value obtained compared to the un-inoculated control using HPLC peak area estimation (Table 4.13). 86.5 % reduction in peak area was obtained for *Aspergillus terreus*, followed by 75.48 % and 73.35 % respectively for *A. niger* and *Penicillium citrinum*. While the least reduction of 59.29 % was recorded for *A. parasiticus*.

The spectrophotometric estimation of the 6 aminohexanoic acids produced in the fungi culture filtrates (Table 4.13) indicated that 5356 ppm of the compound was present in the culture of *Penicillium citrinum* at the $15^{th}$ day of incubation followed by 2964 ppm and 1156 ppm *for Aspergillus terreus* and *A parasiticus* respectively. It was observed that the amino acids estimated in the fungi culture filtrates did not correspond proportionally with the percentage reduction in caprolactam for each of the isolates. This observation suggested the possibility of amino acid assimilation by the microorganisms for growth.

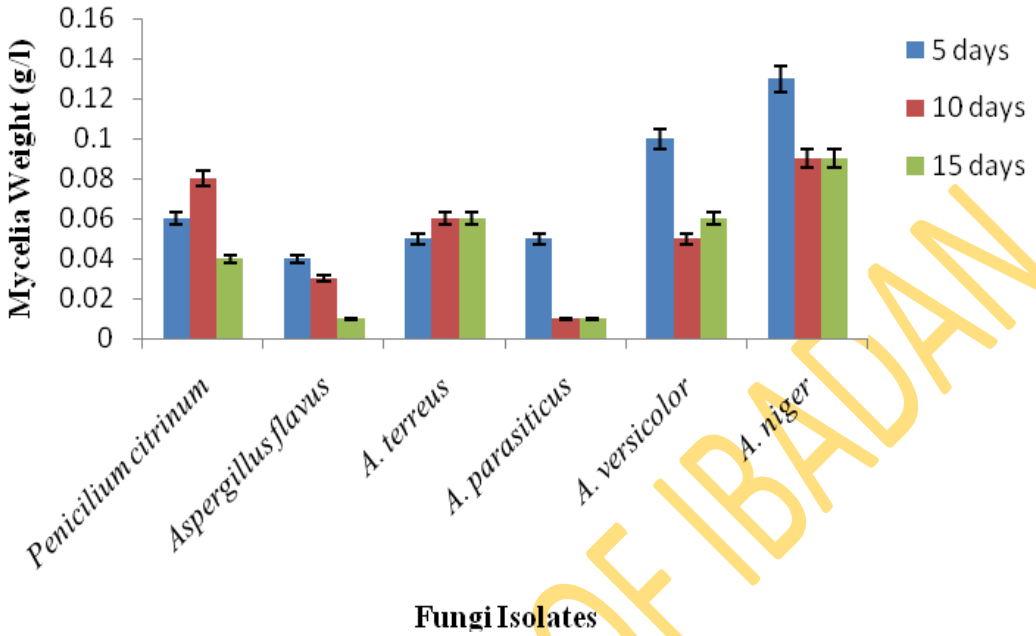



**Figure 4.7.** Changes in Mycelia Weight of fungi isolates in basal medium of Caprolactam over 15 days.

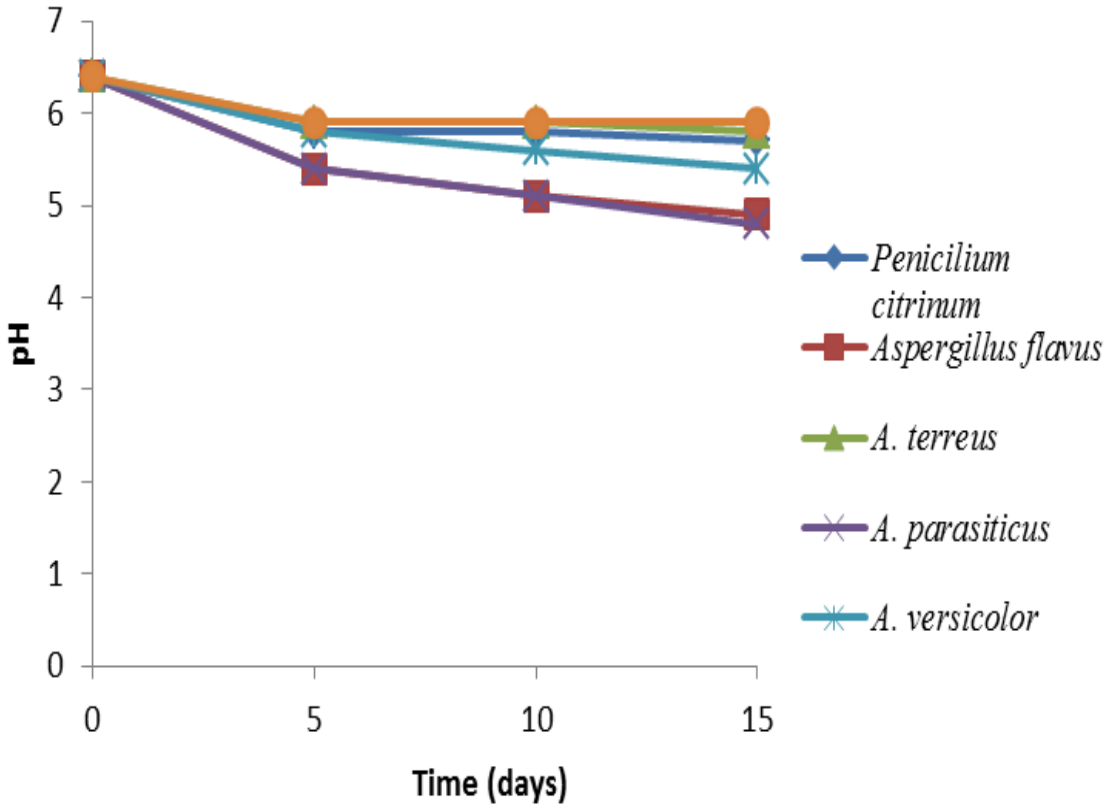



**Figure 4.8.** Changes in pH of the caprolactam basal medium during growth of the fungi isolates over 15 days.

**Table 4.13**. Percentage reduction in caprolactam concentration and corresponding amino acid produced by the fungi isolates after fifteen (15) days.

| Isolates | % Reduction in caprolactam concenttration | 6-aminohexanoic acid concentration (ppm) |
|---|---|---|
| *Penicilium citrinum* | 73.35 | 5356 |
| *Aspergillus flavus* | 69.09 | 440 |
| *A. niger* | 75.48 | 670 |
| *A. terreus* | 86.5 | 2964 |
| *A. versicolor* | 60 | 480 |
| *A. parasiticus* | 59.29 | 1156 |

### 4.16 Biodegradation of Nylon-6

### 4.16.1 Biodegradation of Nylon-6 Fibre by Bacteria Isolates

#### 4.16.1.1 Growth Changes

Figure 4.9 shows the growth of a isolates over the period of 3 months in the broth medium containing nylon-6 as the only source of carbon and nitrogen. The growth recorded indicated the ability of all the isolates to utilize the fibre with the maximum growth of 1.861 absorbance recorded for *Bordetella petrii* followed by 1.628 absorbance recorded for culture inoculated with *Alcaligenes faecalis* after 90days of incubation. The lowest growth of 1.138 absorbance was recorded in culture inoculated with *Proteus vulgaris* at optical density of 600 nm.

#### 4.16.1.2 Weight Loss

The weight loss of the submerged nylon-6 fibre sample over a period of 3 months as shown in Fig. 4.10 indicated a decrease over the period of incubation. Highest weight loss of 5.23 % was recorded in nylon fibre treated with *Pseudomonas aeruginosa* followed by 5.03 % weight loss in the nylon fibre treated with consortium of all the bacterial isolates. The least weight loss of 3.41 % after 90days of degradation was recorded in nylon-6 fibre treated with *Bordetella petrii*.

#### 4.16.1.3 Changes in Number average molecular mass ($M_n$)

Figure 4.11 shows the viscosity number average molecular mass as calculated from the relative viscosity. The number average molecular mass (Mn) decreases over the period of incubation. The highest decrease of 12.82 % was recorded in nylon-6 fibre treated with *Pseudomonas aeruginosa* that decreased from 3795.75 $gmol^{-1}$ to 3364.49 $gmol^{-1}$ after 90 days. The least performance was recorded in nylon-6 treated with *Bordetella petrii.* It decreased from3795.75 $gmol^{-1}$ to 3601.58 $gmol^{-1}$. Statistical analysis of the changes showed the changes to be significant at 95% confidence (Table 4.14).

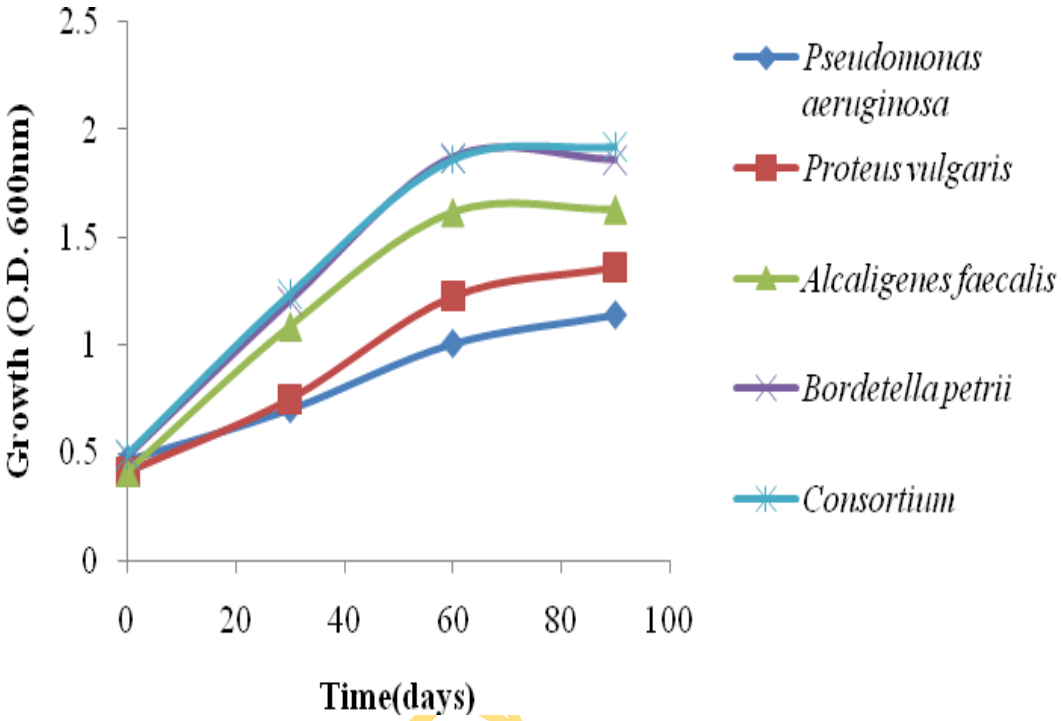



**Figure 4.9.** Changes in growth of bacteria isolates in nylon-6 basal medium.

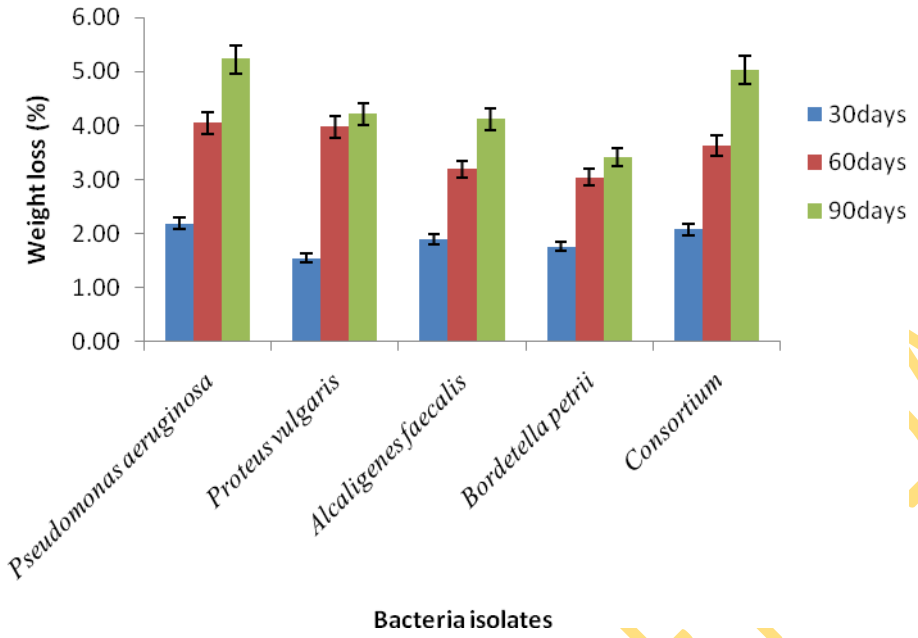
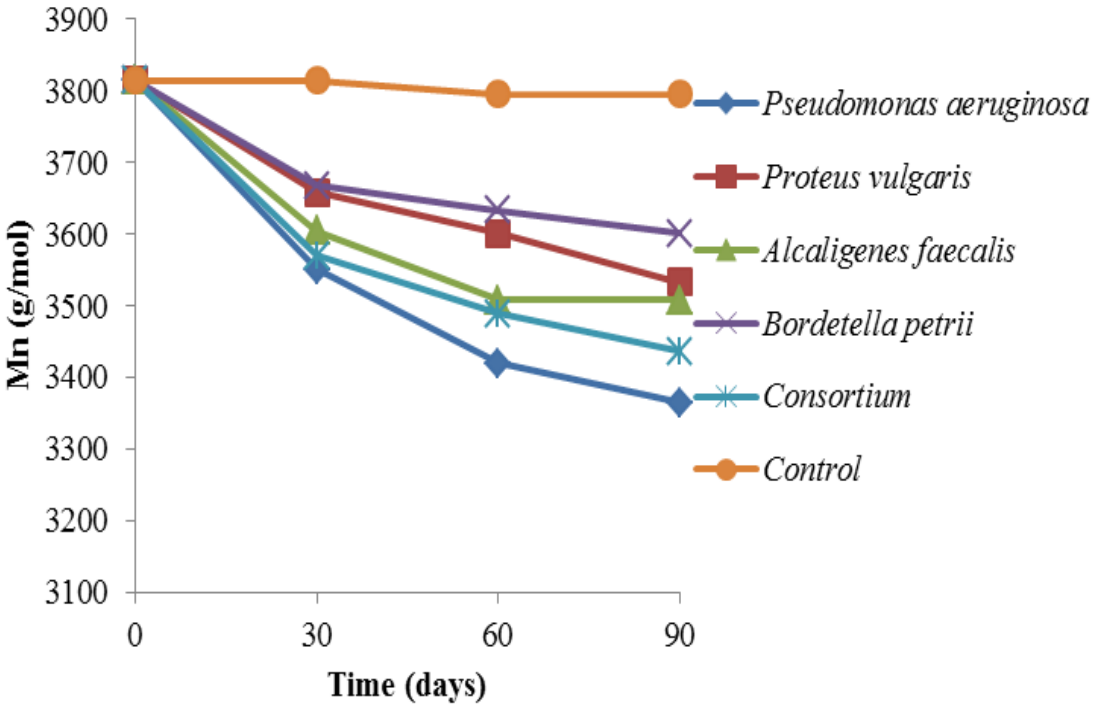



**Figure 4.10.** Percentage weight loss of nylon-6 fibre treated with bacteria isolates.




**4.11.** Changes in number average molecular mass(Mn) of nylon-6 fibre treated with the bacteria isolates over 90 days period using the viscosity technique.



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**Table 4.14**. Statistical Analysis of Changes in Weight loss and Viscosity average Molecular mass using Duncan[a,b] Multiple Range Test.

| **Treatment** | **Weight loss** | **Average number molecular mass ($M_n$) ($gmol^{-1}$)** |
|---|---|---|
| Control treatment | 0.0305[a] | 3795.75[a] |
| *Bordetella petrii* | 0.0310[ab] | 3601.58[b] |
| *Proteus vugaris* | 0.0312[b] | 3532.68[bc] |
| *Alcaligenes faecalis* | 0.0313[b] | 3507.63[bc] |
| *Pseudomonas aeruginosa* | 0.0315[b] | 3419.94[c] |
| Consortium of isolates | 0.0314[b] | 3476.31[bc] |

a. Uses Harmonic mean sample sizes
b. Alpha = 0.05

#### 4.16.1.4 Results of the HPLC Analysis

Figure 4.12(a –g) show the HPLC chromatogram of the nylon-6 treatment supernatants after three months incubation in submerge bacteria cultures. The two monomers used as the standards (6-aminohexanoic acid and caprolactam) were mixed together after many independent runs to produce a composite standard. Figure 4.12a shows the peaks of the monomers in the standards. The 6-aminohexanoic acid was eluted between 0.58-1.01 minutes retention time (Rt.) while the Caprolactam was eluted between 1.35 -1.41 minutes retention time (Rt.).

Figure 4.12b showed the presence of the linear monomer (6-aminohexanoic acid) in the uninoculated control at retention time of 1.00 minute with a peak area of 4165.0 mAs. The cyclic monomer (caprolactam) was not seen in the control experiment.

The chromatographic analysis of the supernatant of the nylon-6 treated with *Pseudomonas aeruginosa* (Figure 4.12c) showed the presence of the 6-aminohexanoic acid at retention time of 1.01 minutes with peak area of 4882.3 mAs, the presence of one other product at retention time of 1.10 and caprolactam at retention time of 1.35 minutes with peak area of 373 mAs and 82 mAs.

Figure 4.12d shows the analysis of the supernatant of the nylon-6 treated with *Proteus vulgaris*. The presence of 6-aminohexanoic acid was detected at the retention time of 1.01 minutes with peak area of 5001.9 mAs. And caprolactam at retention time of 1.35 minutes with peak area of 126.4 mAs.

The analysis of the supernatant of the *Alcaligenes faecalis* treated culture (figure 4.12e) showed the presence of 6-aminohexanoic acid at 0.59 minutes with peak area of 4294.8 mAs and four other products at retention times of 1.11, 1.17, 1.19 and caprolactam at retention time of 1.38 minutes with peak areas of 306.8, 84.5, 85.2 and 9.0 mAs respectively.

Figure 4.12f, shows chromatogram of the supernatant of the culture treated with *Bordetella petrii*. It indicated the presence of 6-aminohexanoic acid at retention time of 1.01 minutes with peak area of 4447.1 mAs, and the presence of one other product at retention time of 1.10 and caprolactam at retention time of 1.37 minutes with peak area of 568.8 and 54.4.

Analysis of the supernatants of the nylon-6 treated with the consortium of the isolates revealed the presence of 6-aminohexanoic acid at retention time of 1.01minutes with peak

area of 4498.2 mAs, two other peaks at 1.25, 1.29 and caprolacatm at retention time of 1.36 minutes with peak areas of -1.7, -0.1 and 18.6 mAs respectively(4.12g).

**4.12.1.5 FTIR Analysis**:

The analysis of the FTIR spectra of the degraded samples is shown in Figure 4.13(a-e). Figure 4.13a shows the analysis for the nylon-6 fibre treated with *Pseudomonas aeruginosa*. It revealed the replacement of the peak at 1464 $cm^{-1}$ wavelength corresponding to the asymmetric C-H bending vibrations of methyl-alkyl group with two other peaks ( 1473 and 1458 $cm^{-1}$) of methyl-alkyl group with peak area of 6.37 and 4.112 $cm^2$ 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 %.

Figure 4.13b shows the spectral analysis of the nylon-6 fibre treated with *Proteus vulgaris*, indicating the replacement of the peak at 1464 $cm^{-1}$ wavelength corresponding to the asymmetric C-H bending vibrations of methyl-alkyl group with peak area 11.44 $cm^2$ with two other peaks (1473 and 1458 $cm^{-1}$) corresponding to methyl-alkyl functional group but at higher absorbance of 9.281 and 14.165 $cm^2$ peak areas respectively. The O-H stretching vibrations of intramolecular hydrogen bond at 3649, 3853 and 3904 $cm^{-1}$ wavelength disappeared in this treated sample but with increase in absorbance of similar band at 3736 $cm^{-1}$ from peak area of 1.552 to 2.215 $cm^2$. The calculated cumulative decrease in peak area compared to the control was -26.86%.

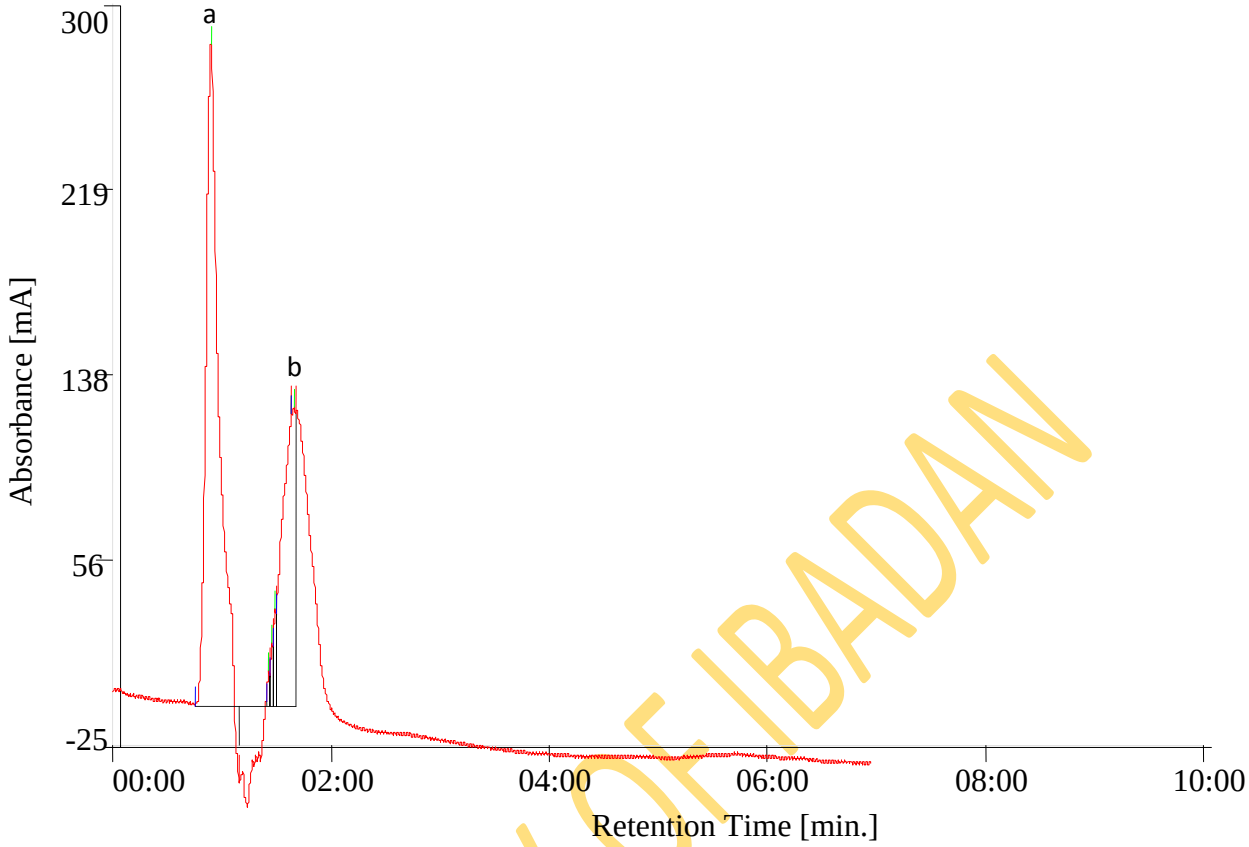



**Figure 4.12a.** Chromatogram of the standards: (a) 6-aminohexanoic acid with retention time between 0.58- 1.01 minutes and (b) Caprolactam with retention time between 1.35 - 1.41minutes

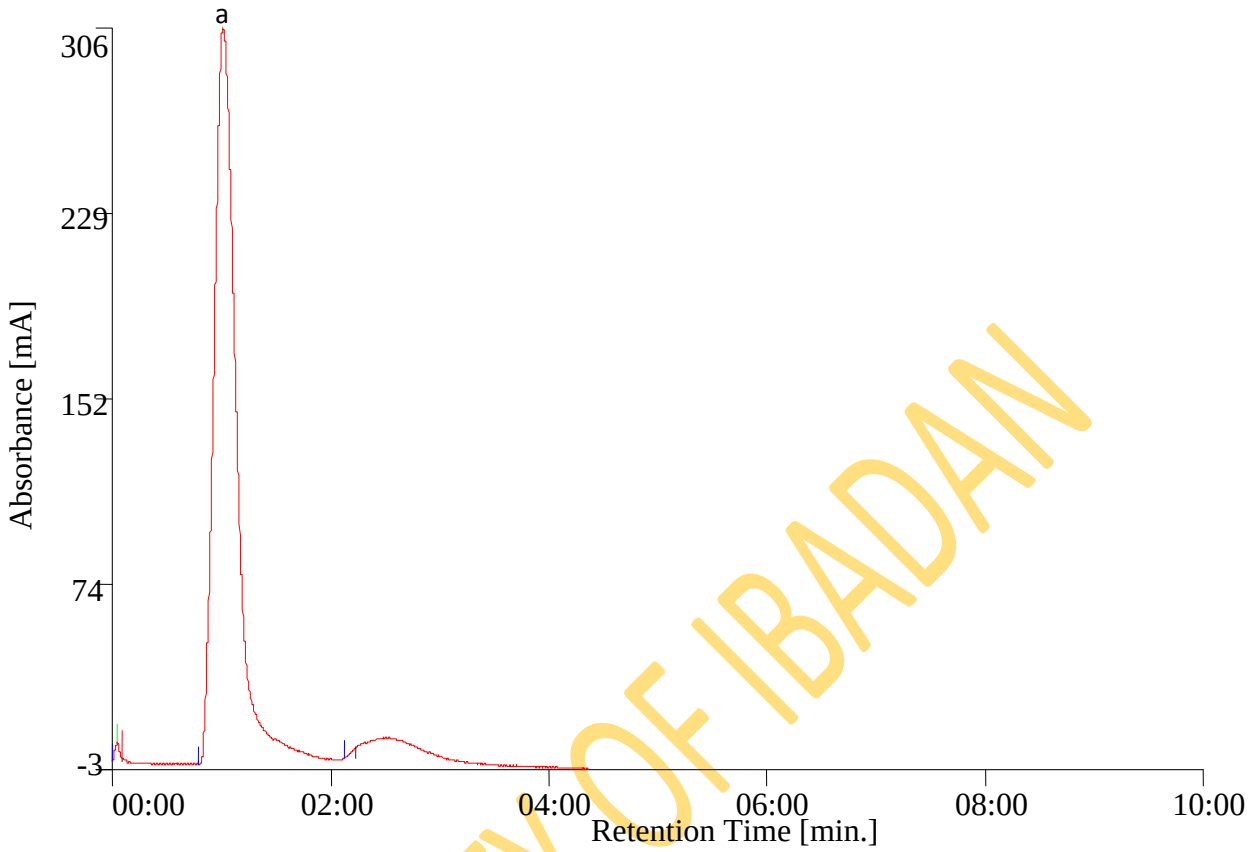



**Figure 4.12b.** The Chromatogram of un-inoculated control treatment of nylon-6 showning the presence of: (a) 6-aminohexanoic acid.

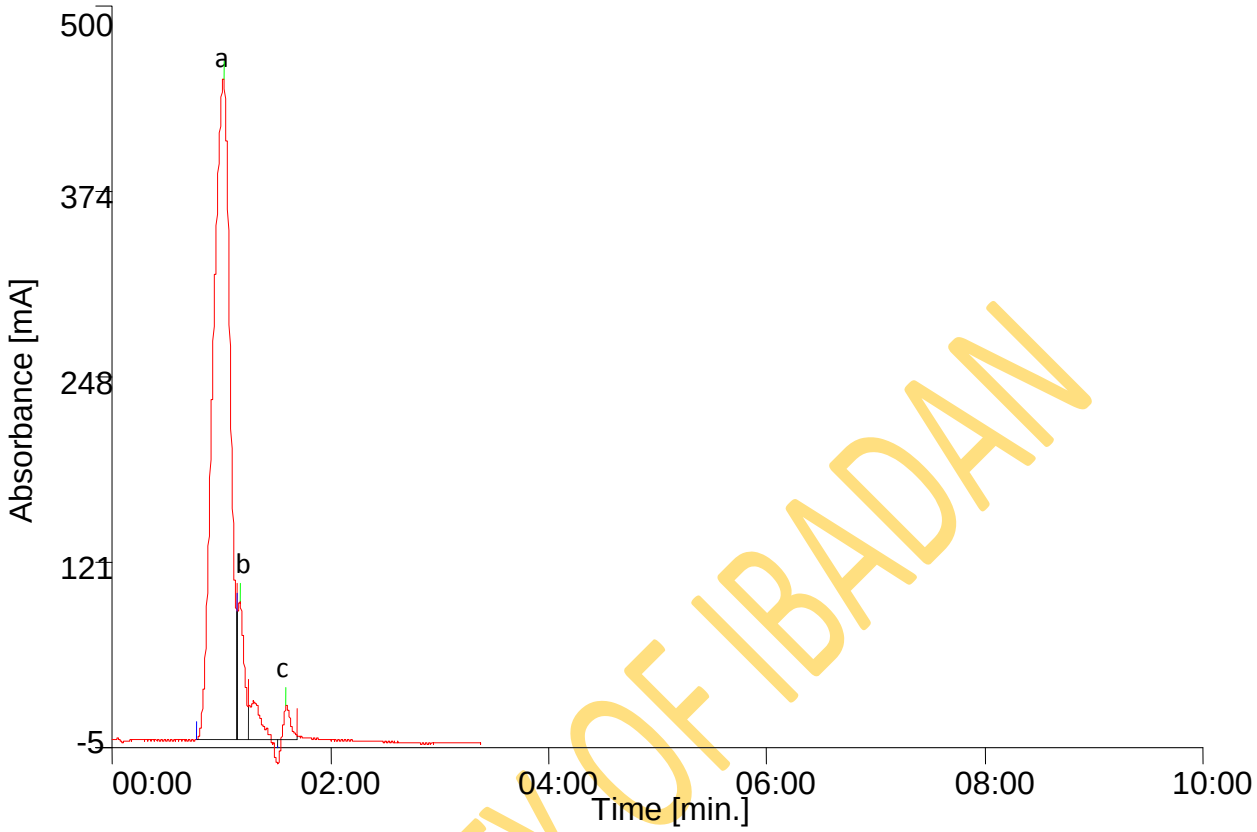



**Figure 4.12c.** The Chromatogram of the *Pseudomonas aeruginosa* (NTS1) treated nylon-6 supernatant, showing: (a) 6-aminohexanoic acid (b) Un-identified Oligomer (c) Caprolactam.

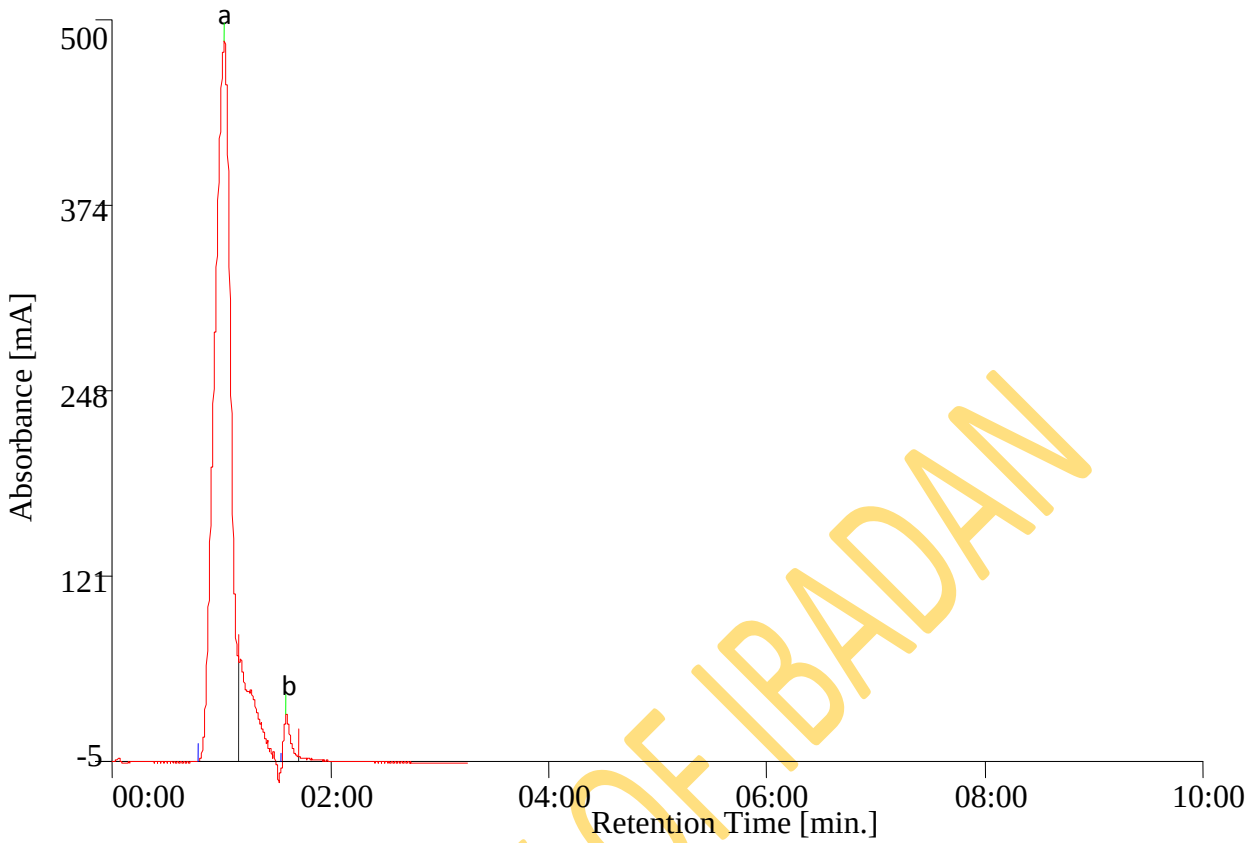



**Figure 4.12d.** The Chromatogram of the *Proteus vulgaris* (NTS 2) treated nylon-6 supernatant, showing: (a) 6-aminohexanoic acid (b) Caprolactam.

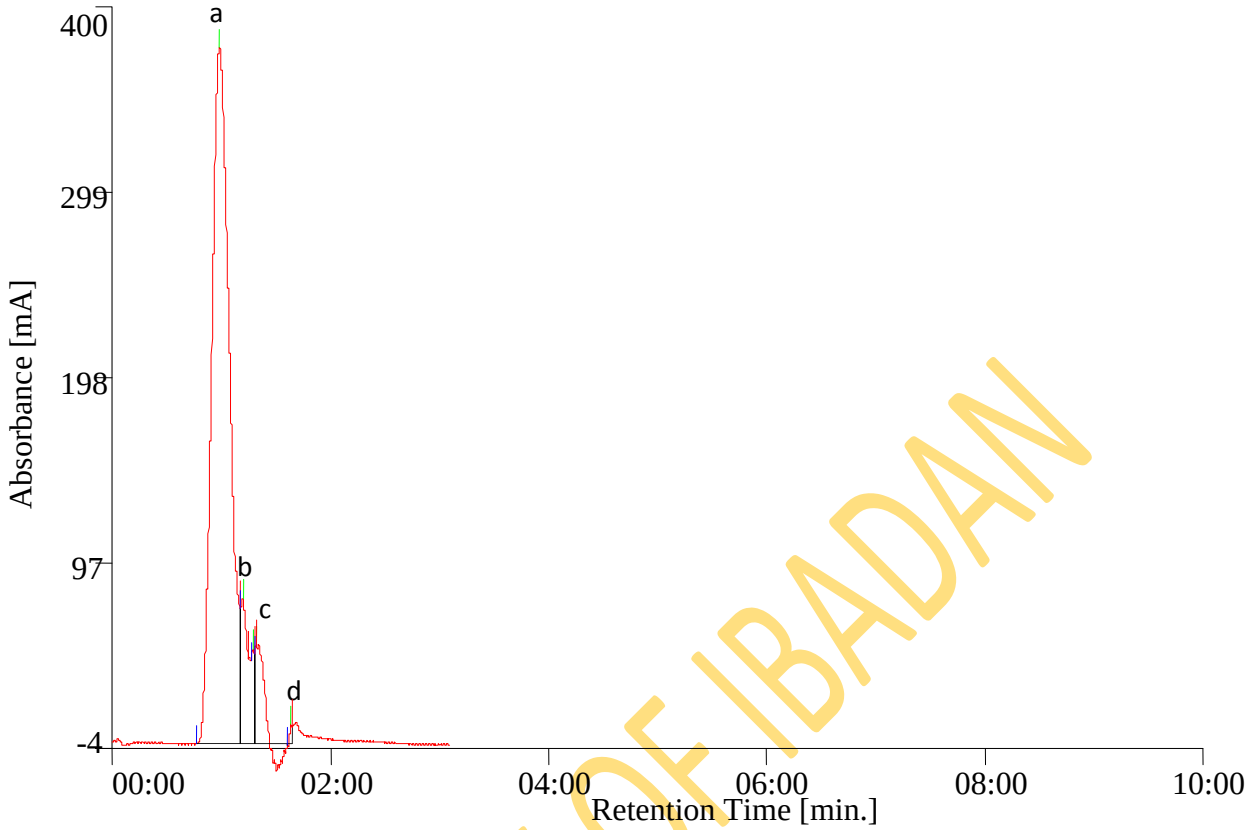



**Figure 4.12e.** The Chromatogram of the *Alcaligenes faecalis* (2ABA2) treated nylon-6 supernatant showing: (a) 6-aminohexanoic acid (b) Un-identified Oligomer (c) Un-identified oligomer (d) Caprolactam.

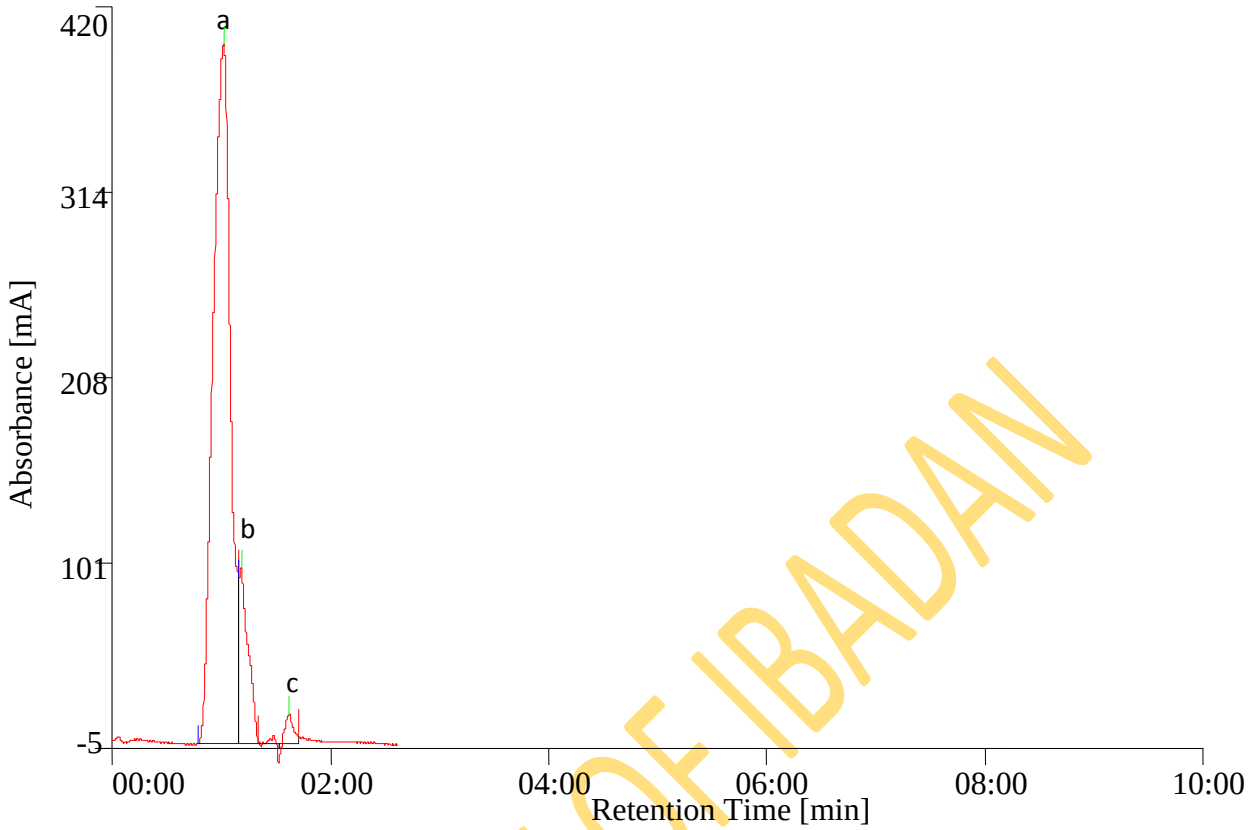



**Figure 4.12f.** The Chromatogram of the *Bordetella petrii* (2ABA4) treated nylon-6 supernatant showing: (a) 6-aminohexanoic acid (b) Un-identified Oligomer (c) Caprolactam.

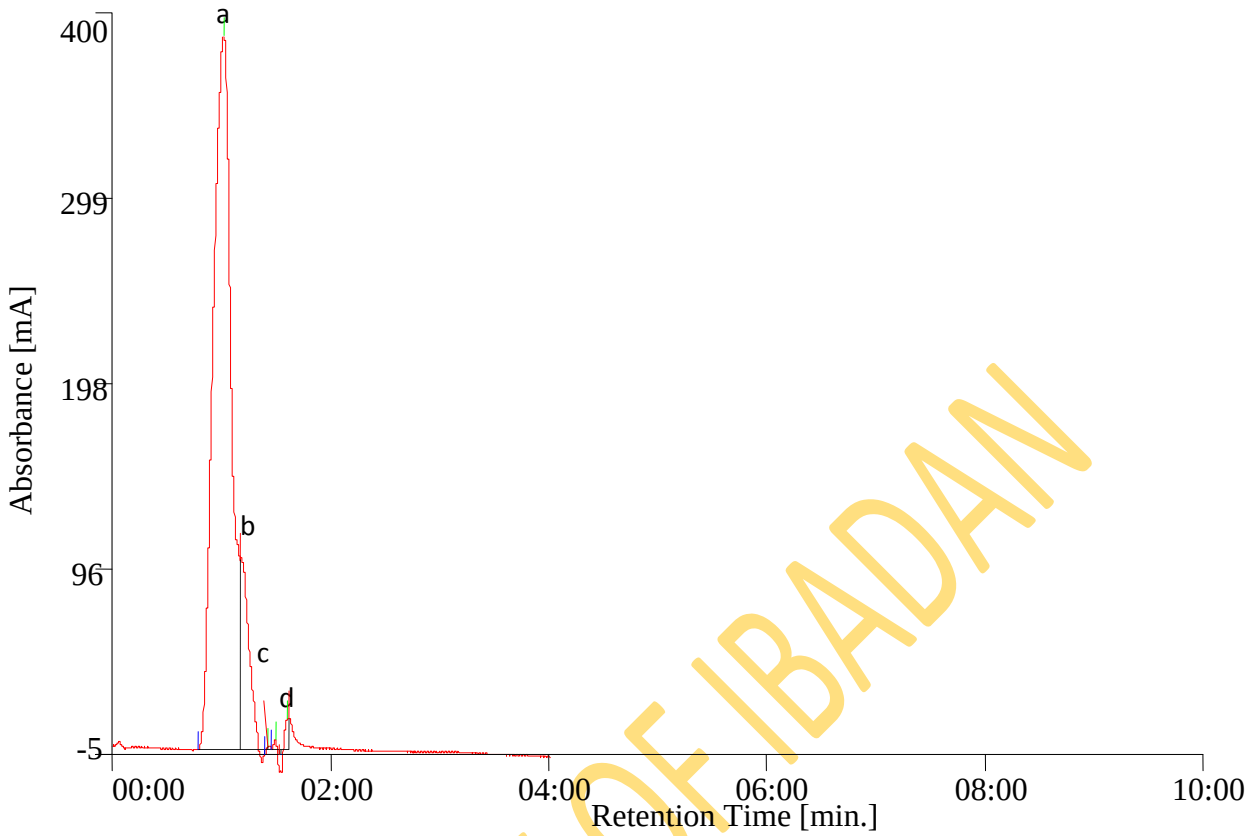



**Figure 4.12g.** The Chromatogram of the supernatant of nylon-6 treated with consortium of the bacteria isolates, showing: (a) 6-aminohexanoic acid (b) Un-identified Oligomer (c) Un-identified oligomer (d) Caprolactam.

The spectral analysis of the nylon treated with *Alcaligenes faecalis* (figure 4.13c) showed the disappearance of the O-H stretching vibrations of intramolecular hydrogen bond at 3649 $cm^{-1}$ wavelength and the peak at 1869$cm^{-1}$ corresponding to anhydride of carbonyl of the amide. New peaks formed include 669 and 833 $cm^{-1}$ corresponding to the C-H bend of alkyne group and C-H disubstituted out of plane bend, 1242, 1340 and 1437 $cm^{-1}$ corresponding to C-N stretching vibration of primary amine, C-H bend of methyne (CH) and asymmetric C-H bend of methyl (CH3) functional groups respectively. Also formed are peaks at 2681 and 2748 $cm^{-1}$ of C-H stretching vibrations of methyl functional group. The percentage cumulative change in the peak area of the treated nylon fibre compared to the un-inoculated control was -49.91 %.

The analysis of the *Bordetella petrii* treated nylon fibre (Figure 4.13d) showed the formation of only one new peak corresponding to C-H stretching vibrations of methyl functional group at 2748 $cm^{-1}$ and the disappearance of peaks at 1869 $cm^{-1}$ of the anhydride of carbonyl of the amides and 3649 $cm^{-1}$ corresponding to the O-H stretching vibrations of intramolecular hydrogen bond. The absorbance of many peaks in the treated nylon fibre increases compared to corresponding peaks in the un inoculated control resulting in cumulative change of -41.32 %.

Figure 4.13e shows the spectra analysis of the nylon-6 treated with the consortium of the bacteria isolates. It also revealed the disappearance of peaks at 1869 $cm^{-1}$ of the anhydride of carbonyl of the amides and 3649 $cm^{-1}$ corresponding to the O-H stretching vibrations of intramolecular hydrogen bond only. But with the formation of new peaks which include 1242 $cm^{-1}$ corresponding to C-N stretching vibration of primary amine and 2748 $cm^{-1}$ corresponding to C-H stretching vibrations of methyl functional group. The percentage cumulative change in the peak area of the treated nylon fibre compared to the un-inoculated control was -22.04 %.

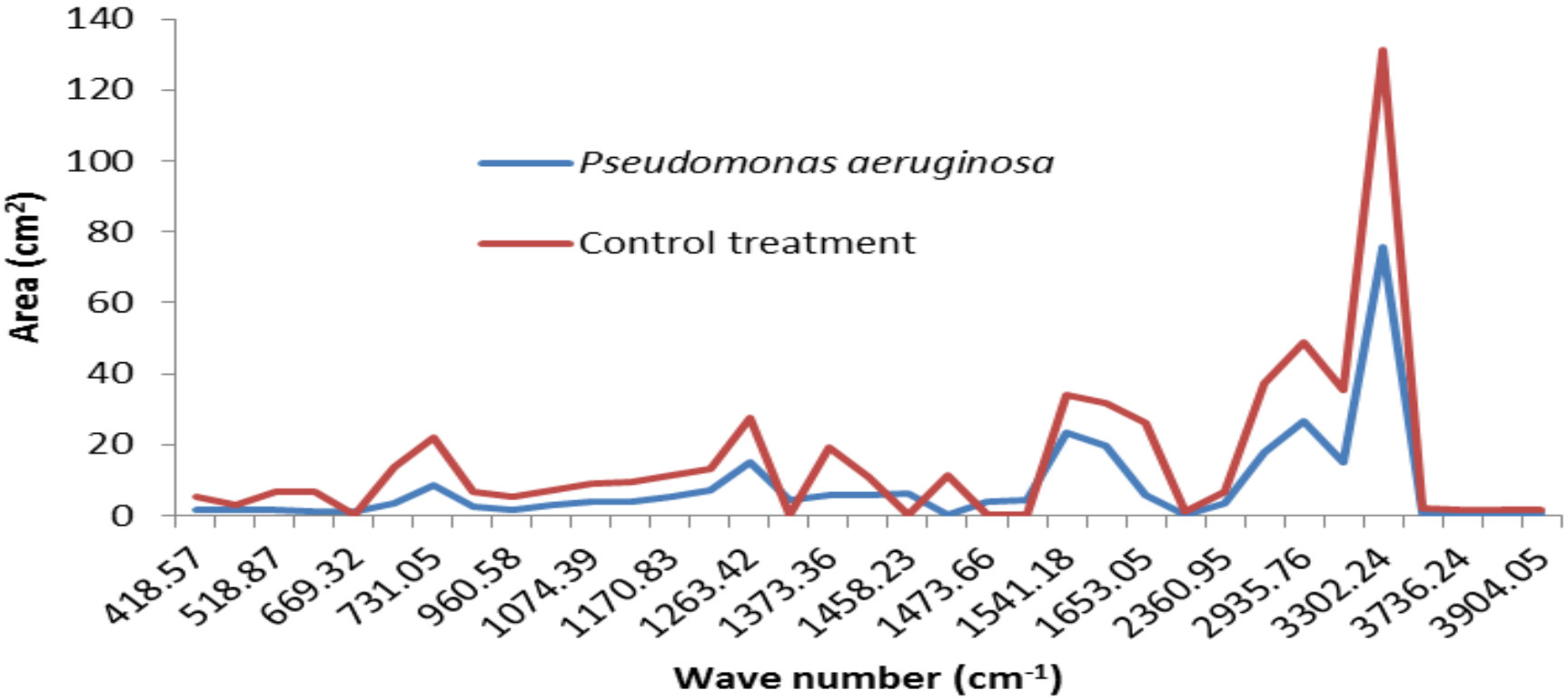



**Figure 4.13a.** FTIR Spectra analysis of nylon-6 fibres treated with *Pseudomonas aeruginosa* (NTS1) after 90 days of biodegradation.

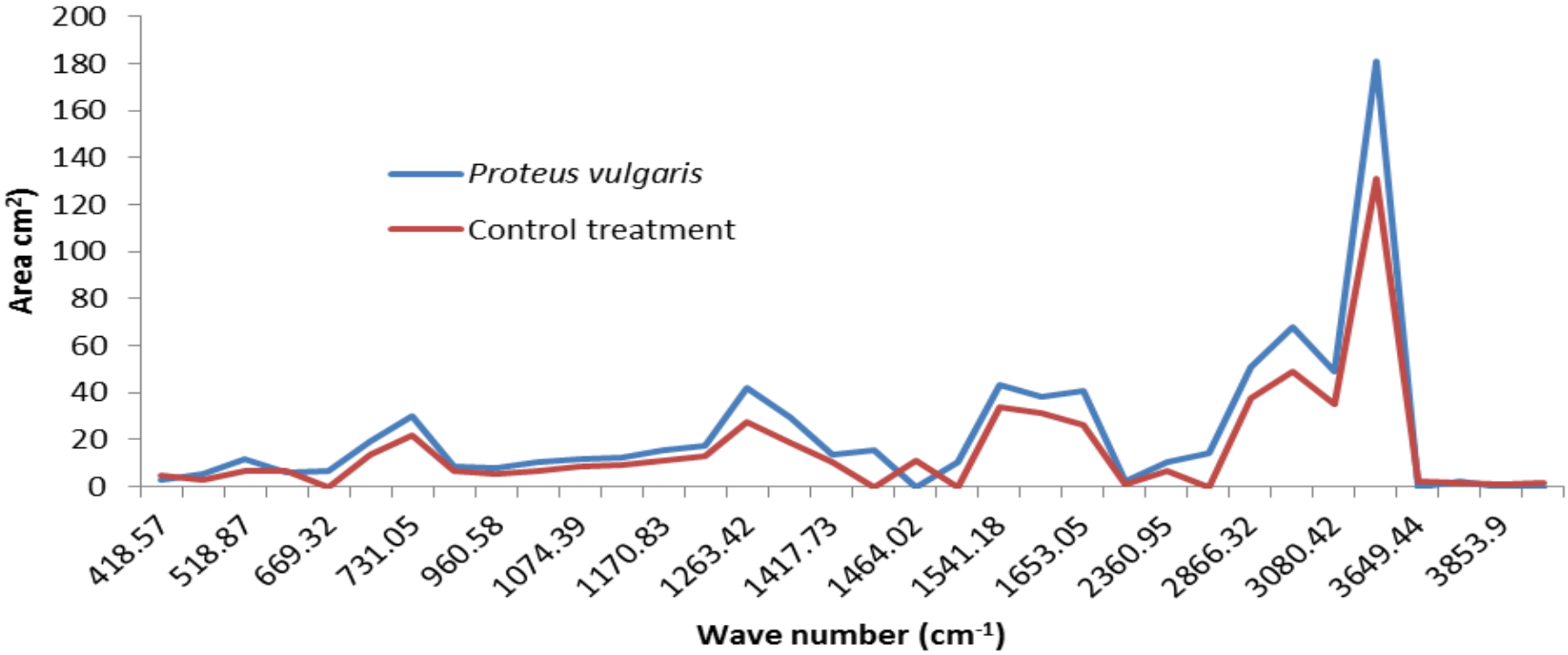



**Figure 4.13b.** FTIR Spectra analysis of nylon-6 fibres treated with *Proteus vulgaris* (NTS2) after 90 days of biodegradation

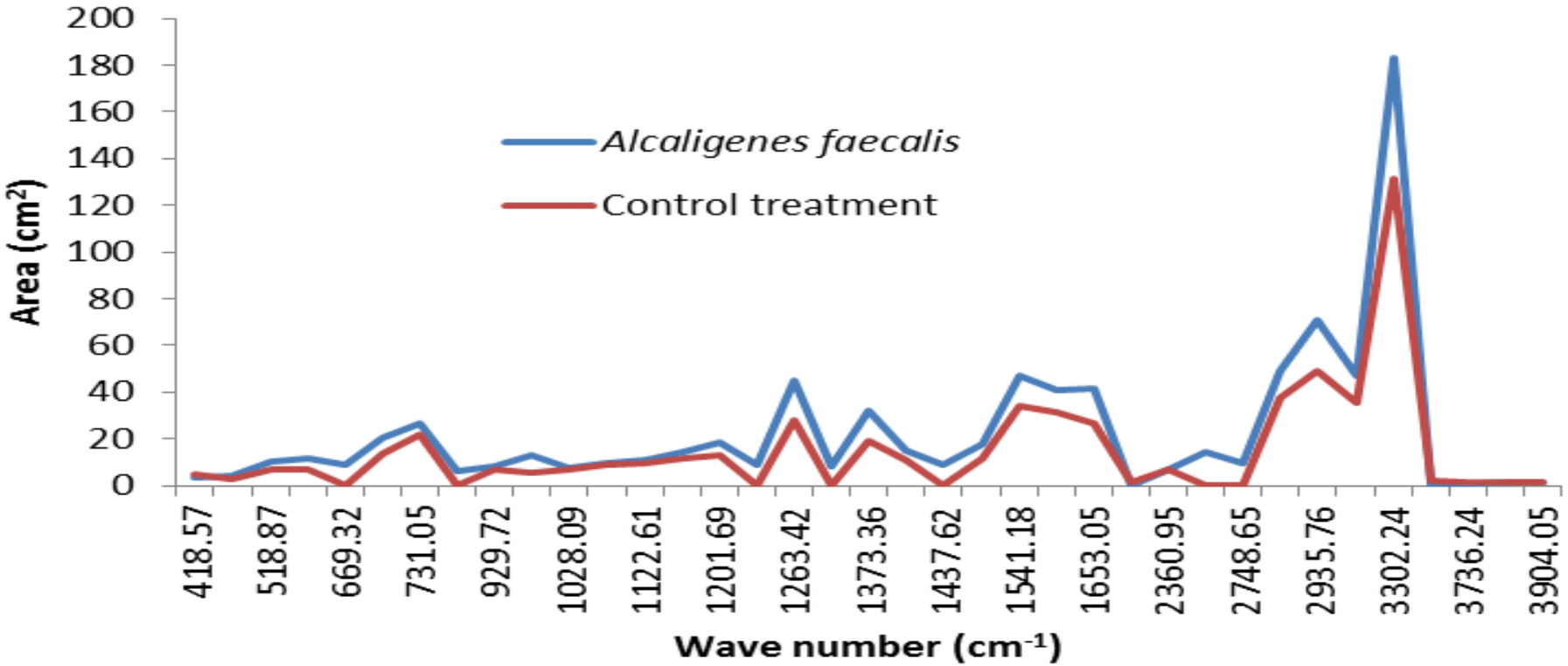



**Figure 4.13c.** FTIR Spectra analysis of nylon-6 fibres treated with *Alcaligenes faecalis* (2ABA2) after 90 days of biodegradation

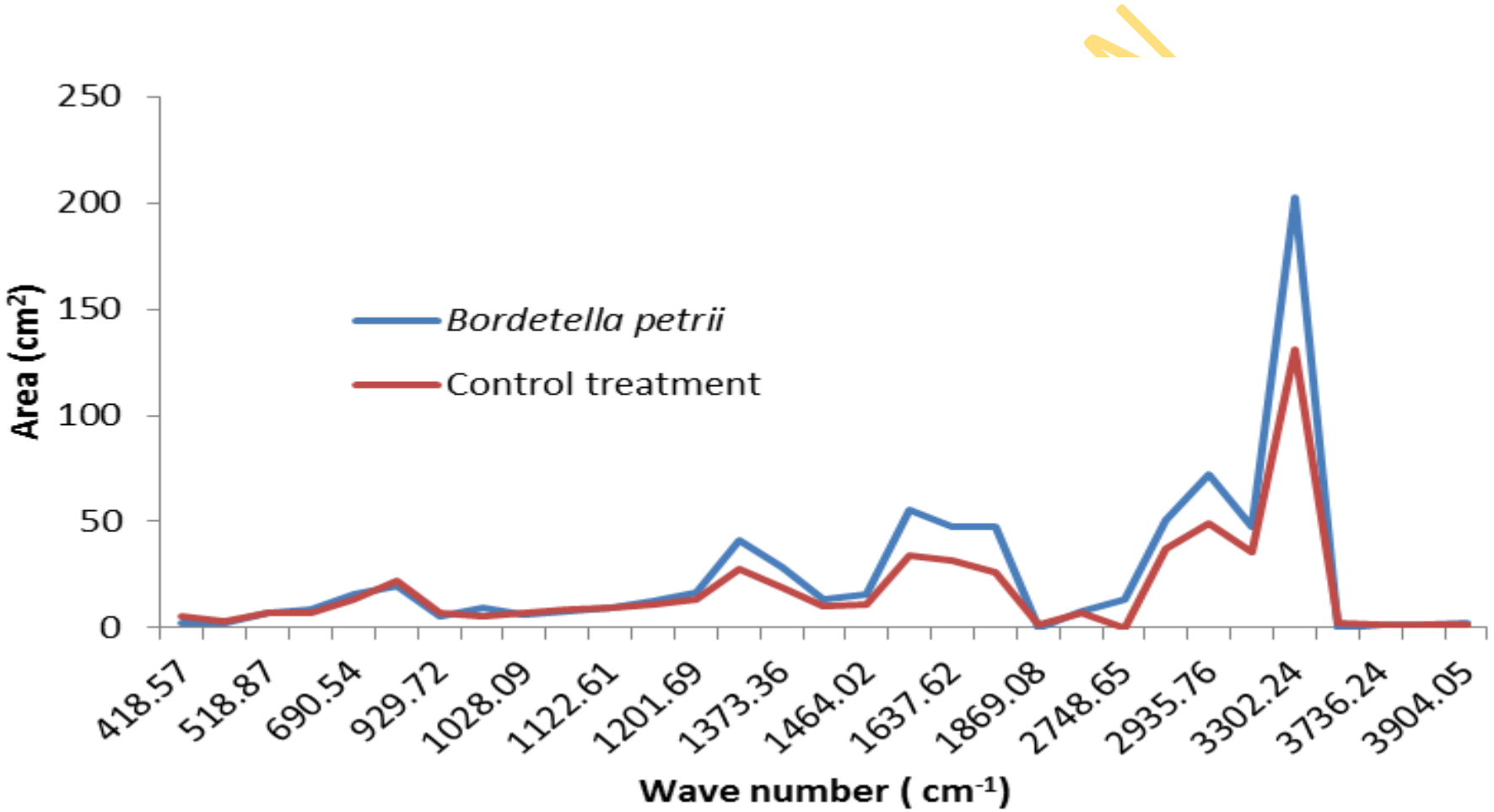



**Figure 4.13.** FTIR Spectra analysis of nylon-6 fibres treated with *Bordetella petrii* (2ABA4) after 90 days of biodegradation

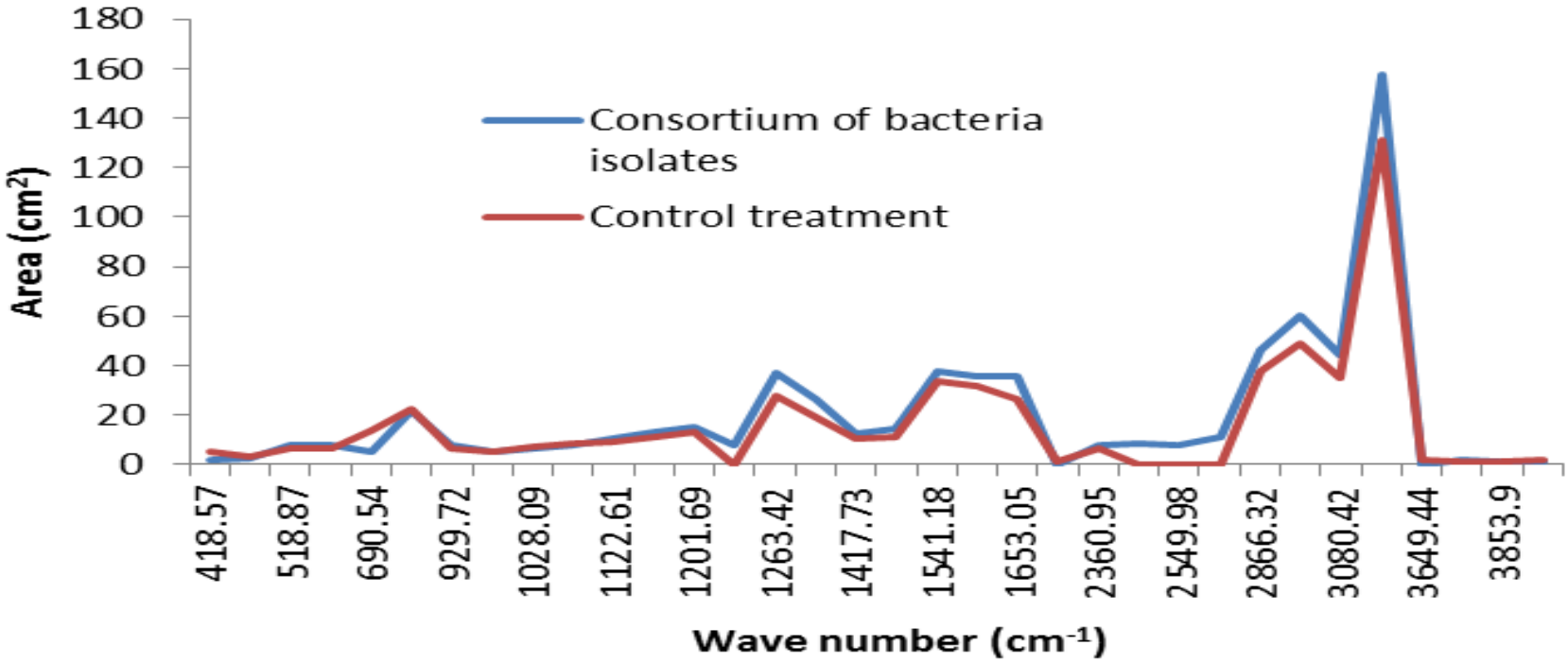



**Figure 4.13e**. FTIR Spectra analysis of nylon-6 fibres treated with the consortium of bacteria isolates after 90 days of biodegradation**.**

### 4.16.2 Biodegradation of Nylon-6 Fibre by Fungi Isolates

#### 4.16.2.1 Coloration of fibre

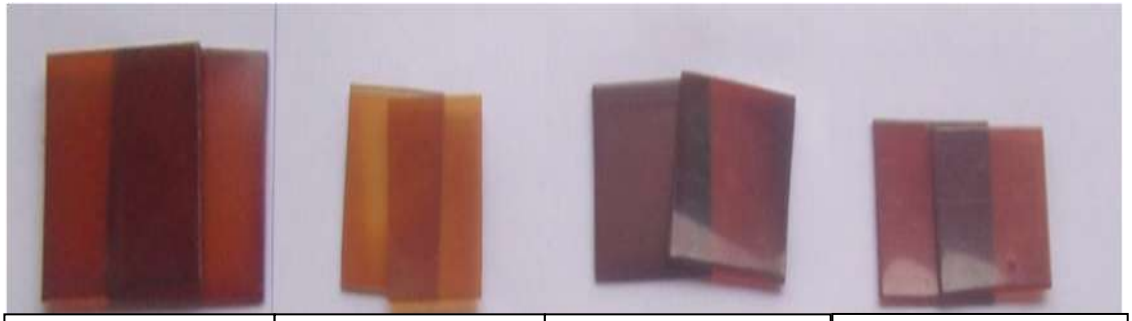
Changes in the colouration of the culture medium and the fibre with days of degradation were observed. The colour of the medium and the Nylon-6 fibre changed from colourless to brown in all the liquid cultures except in the culture of *Aspergillus niger* (AF3) where the fibre alone changed to brown but the medium remained unchanged (Plate 4.2).

#### 4.16.2.2 Mycelia weight

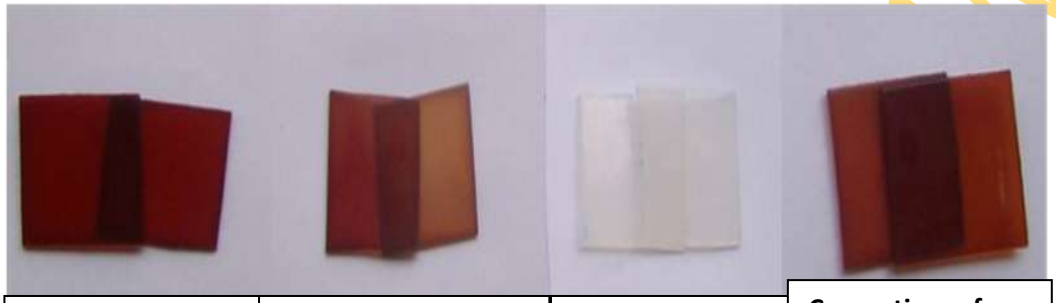
Fungi growth was monitored in the submerged culture using the mycelia weight. The continuous increase in mycelia weight was recorded for the fungi over the 90days of incubation (Figure 4.14). *Aspergillus niger* showed the highest mycelia weight of 0.68 g after 30 days, 0.90 g after 60 days and 0.97 g after 90 days. Next to it was the consortium of which 0.56 g was recorded after 30 days, 077 g after 60 days and 0.95 g after the 90 days. The least growth of 0.13 g after 30 days, 0.62 g after 60 days and 0.72 g after 90 days was recorded in the culture with *Penicilium centrinum*. Corresponding pH changes was recorded as shown in Figure 4.14. The pH decreases generally from 6.4 to the lowest of 4.19 in culture inoculated with *A. parasiticus*.

#### 4.16.2.3 Nylon-6 fibre weight loss

The changes in the weight of nylon-6 fibres treated with the fungi isolates compared with the un-inoculated control treatment was expressed as percentage loss in weight (Figure 4.15). There was a gradual loss in weight of the treated fibres over the 90 days of the incubation with the fungi. The highest loss of 23.95 % was obtained in fibre treated with *A.niger* over the period followed by 22.40 % in consortium while the least of 15.10 % was obtained in the fibre treated with *A. flavus* over the same period.



| *Aspergillus terreus* treatment | *A. versicolor* treatment | *A. parasiticus* treatment | *Penicilium citrinum* treatment |
|---|---|---|---|



| *A. flavus* treatment | *A. niger* treatment | Control treatment | Consortium of fungi treatment |
|---|---|---|---|

**Plate 4.2** Plate of nylon-6 fibres treated with different fungi isolates, (consortium represents all fungi isolates together)

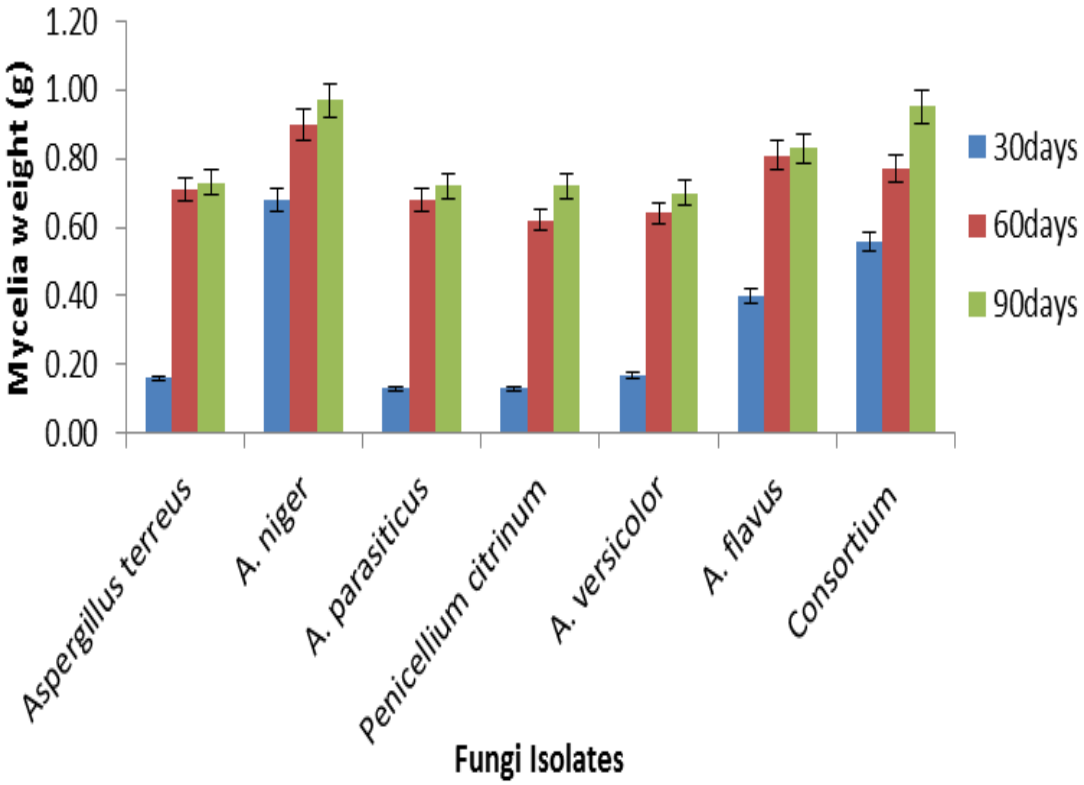



**Figure 4.14.** Mycelia Weight of the fungi isolates in Nylon-6 submerged broth culture within 90 days.

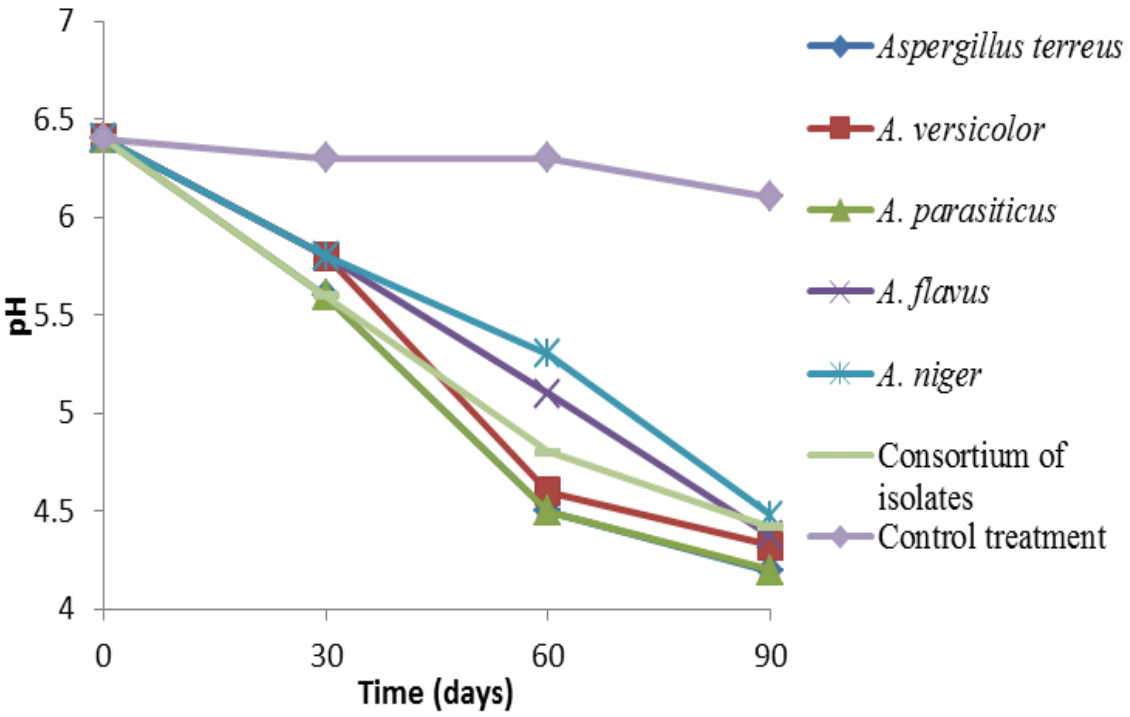



**Figure 4.15.** Changes in the pH of the nylon-6 culture medium during the growth of fungi isolates over 90 days incubation period.

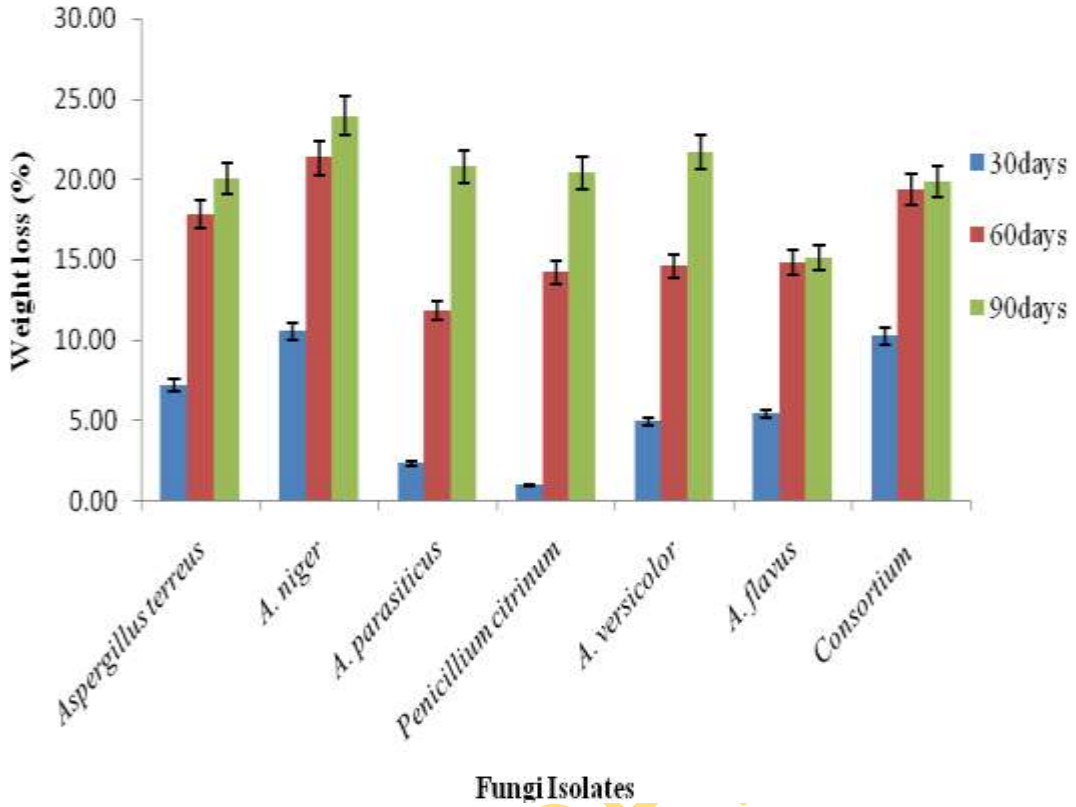



**Figure 4.16.** Percentage weight loss in nylon-6 fibre during the incubation period

### 4.16.2.4 Changes in Number Average Molecular Mass ($M_n$)

Figure 4.17 shows the number average molecular mass determined using viscosity technique of the nylon-6 fibres exposed to the fungi isolates and the control treatment without inoculation. The number average molecular mass decreases with time over the 90 days period incubation. The highest reduction was observed in the nylon fibre treated with the consortium of fungal isolates, decreasing from 4436.88 $gmol^{-1}$ to 2548.17 $gmol^{-1}$after 90 days constituting 34.88 % decrease in number average molecular mass relative to the control. This was followed by *A. niger* which decreased from 4436.88 $gmol^{-1}$ to 2748.45 $gmol^{-1}$ (29.77 % reduction), *A. terreus* decreased to 3043.19 $gmol^{-1}$ (22.23 %), *A. flavus* decreased to 3274.05 $gmol^{-1}$ (16.33%) and *Penicilium citrinum* which decreased to 3278.37 $gmol^{-1}$ (16.22%). The least reduction was recorded in *A. parasiticus* which decreased to 3794.25$gmol^{-1}$ representing 3.04% compared to the control over the 90 days period of degradation.

### 4.16.2.5 FTIR Analysis:

Figure 4.18 (a-g) shows the analysis of Fourier Transform Infra Red (FTIR) spectra of the fungi treated nylon-6 fibres. The analysis of the spectra of the nylon-6 fibre treated with *Aspergillus terreus* (Fig. 4.18a) shared the disappearance of peaks at 596 and 690 $cm^{-1}$ corresponding to C-H out of plane bend vibrations of amides as well as the peaks at 929 and 1026 $cm^{-1}$ corresponding to crystalline alkyl group. However, new peaks were formed at 405, 445, 640 and 877 $cm^{-1}$ respectively corresponding to C-H out of bending vibration of substituted amide groups. Using the peak area values of the spectra, there were general decreases in the absorbance of the peaks resulting in total percentage decrease of about 67.02 % compared to the uninoculated control. Also in the nylon fibre treated with *A. versicolor* (Fig. 4.18b), similar appearance of peaks at 428, 619, 781 and 877 $cm^{-1}$ corresponding C-H out of plane bending vibration of substituted amide groups were observed with the appearance of another new peak at 3450 $cm^{-1}$ corresponding to the free N-H stretching of primary amine. The percentage total decrease in peak area was calculated to be 26.77 % compared to the untreated control. In the fibre treated with *A. parasiticus* (Fig. 4.18c), new peaks are found to be formed at 418 and 451 $cm^{-1}$ corresponding to the C-H out of plane bend vibration and a peak at 3414 $cm^{-1}$ corresponding to free N-H stretching vibrations of amine while the peak at 3506.7 $cm^{-1}$ corresponding to hydroxyl (OH) stretching vibrations of intramolecular hydrogen bond disappeared.

Figure 4.18d showed the spectra analysis of the nylon fibre treated with *Penicillium citrinum*, it revealed the formation of new peaks at 451, 781 and 877 $cm^{-1}$ wavelength corresponding to C-H out of bending vibration of substituted amide groups and a peak at 3414 $cm^{-1}$ corresponding to free N-H stretching vibrations of amine while the peak at 3506.7 $cm^{-1}$ corresponding to hydroxyl (OH) stretching vibrations of intramolecular hydrogen bond disappeared. However, rather than general decrease in the absorbance there were increases in the absorbance of the peaks at 1371 and 1462 $cm^{-1}$ corresponding to C-H bending vibrations of methylene alkyl group and at 2864 $cm^{-1}$ corresponding to the symmetric stretching vibrations of alkyl group.

The spectra analysis of the nylon fibre treated with *A. flavus* (Fig. 4.18e) showed the formation of new peaks at 405 and 451 $cm^{-1}$ corresponding to the C-H out of bend vibrations of amides and at 3450 $cm^{-1}$ corresponding to N-H stretching vibration of primary amine respectively. Although there were increases in absorbance of most peaks, there were decreases in these peaks at wavelength of 1371, and 1462 $cm^{-1}$ corresponding to C-H bending vibrations of methylene alkyl group and 3304 $cm^{-1}$ corresponding to N-H stretching of secondary amine. The Peak at 3506.7 $cm^{-1}$ corresponding to O-H of intramolecular hydrogen bond also disappeared in this spectrum.

Figure 4.18f showed the spectra analysis of the nylon-6 fibre treated with *A. niger*. There were general increase in the absorbance of most peaks with the exception of peaks at 1170 and 1201 $cm^{-1}$ corresponding to C-N stretch of tertiary amine. There were formations of new peaks at 418 and 588 $cm^{-1}$ corresponding to the C-H out of plane bend vibrations of amides as well as peaks at 3414 and 3471 $cm^{-1}$ corresponding to N-H stretching vibrations of primary amine. Disappearance of the peak due to O-H of intramolecular hydrogen bond (3506.7 $cm^{-1}$) and the peaks corresponding to the out of bending vibrations of the amides was also shown in the spectra.

The spectra of the nylon-6 fibre treated with the consortium of the fungi isolates is shown in Figure 4.18g. There are new peaks corresponding to out of plane bend vibrations of amides (960 and 428 $cm^{-1}$) with 3471 and 1475 $cm^{-1}$ corresponding to N-H stretching vibration of amine as well as disappearance of the 3506.7 $cm^{-1}$ corresponding to the O-H of intramolecular hydrogen bond and 974 $cm^{-1}$ corresponding to C-H out of bend vibrations of amides.

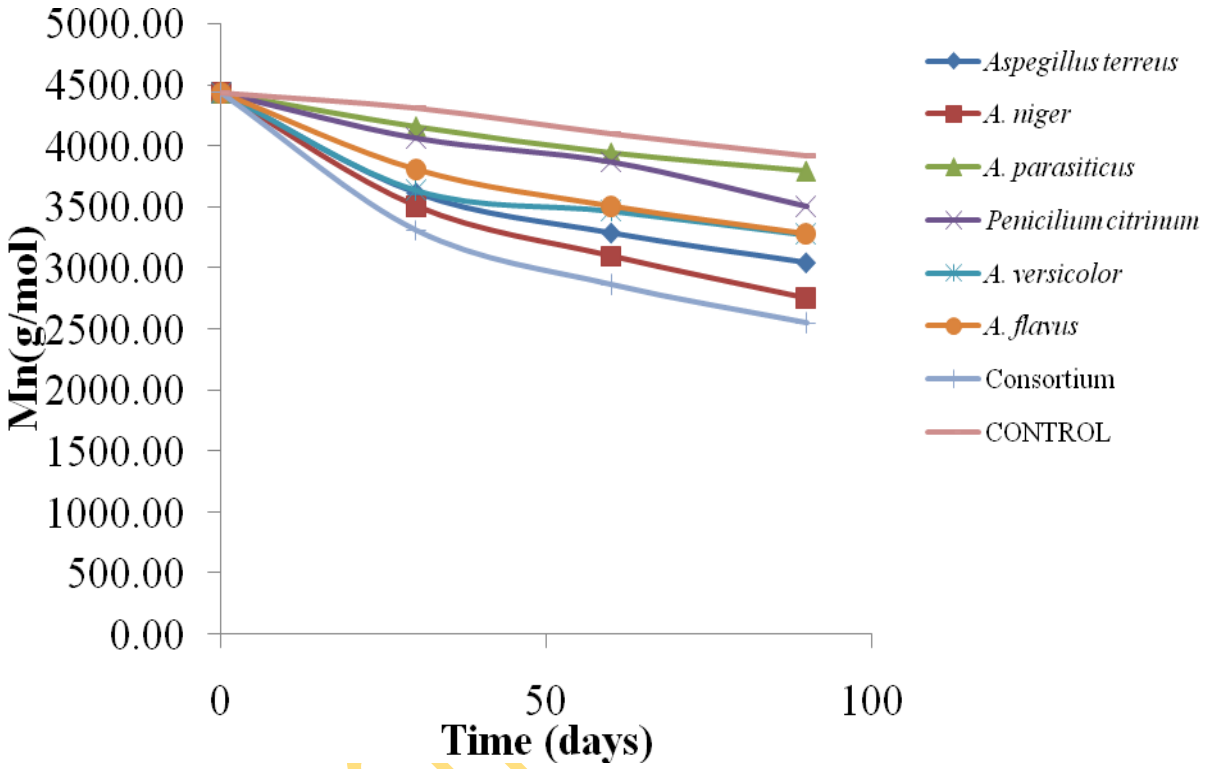



**Figure 4.17**. Changes in number average molecular mass ($M_n$) of nylon-6 fibres treated with fungi isolates over 90 days period using the viscosity technque.

**Table 4.15. Statistical Analysis of Changes in Weight loss and Viscosity average Molecular mass using Duncan[a,b] Multiple Range Test.**

| Treatment | Weight loss | Average number molecular mass ($M_n$) ($gmol^{-1}$) |
|---|---|---|
| Control treatment | $0.0165^{c}$ | $4436.88^{f}$ |
| *Aspegillus parasiticus* | $0.0189^{b}$ | $3794.25^{cf}$ |
| *Penicillium citrinum* | $0.0190^{ab}$ | $3278.37^{e}$ |
| *A. flavus* | $0.0188^{b}$ | $3274.05^{d}$ |
| *A. versicolor* | $0.0193^{ab}$ | $3463.59^{cd}$ |
| *A. terreus* | $0.0196^{ab}$ | $30403.19^{c}$ |
| *A. niger* | $0.0205^{a}$ | $2748.45^{b}$ |
| Consortium of isolates | $0.0199^{ab}$ | $2548.17^{a}$ |

a. Uses Harmonic mean sample sizes

b. Alpha = 0.05

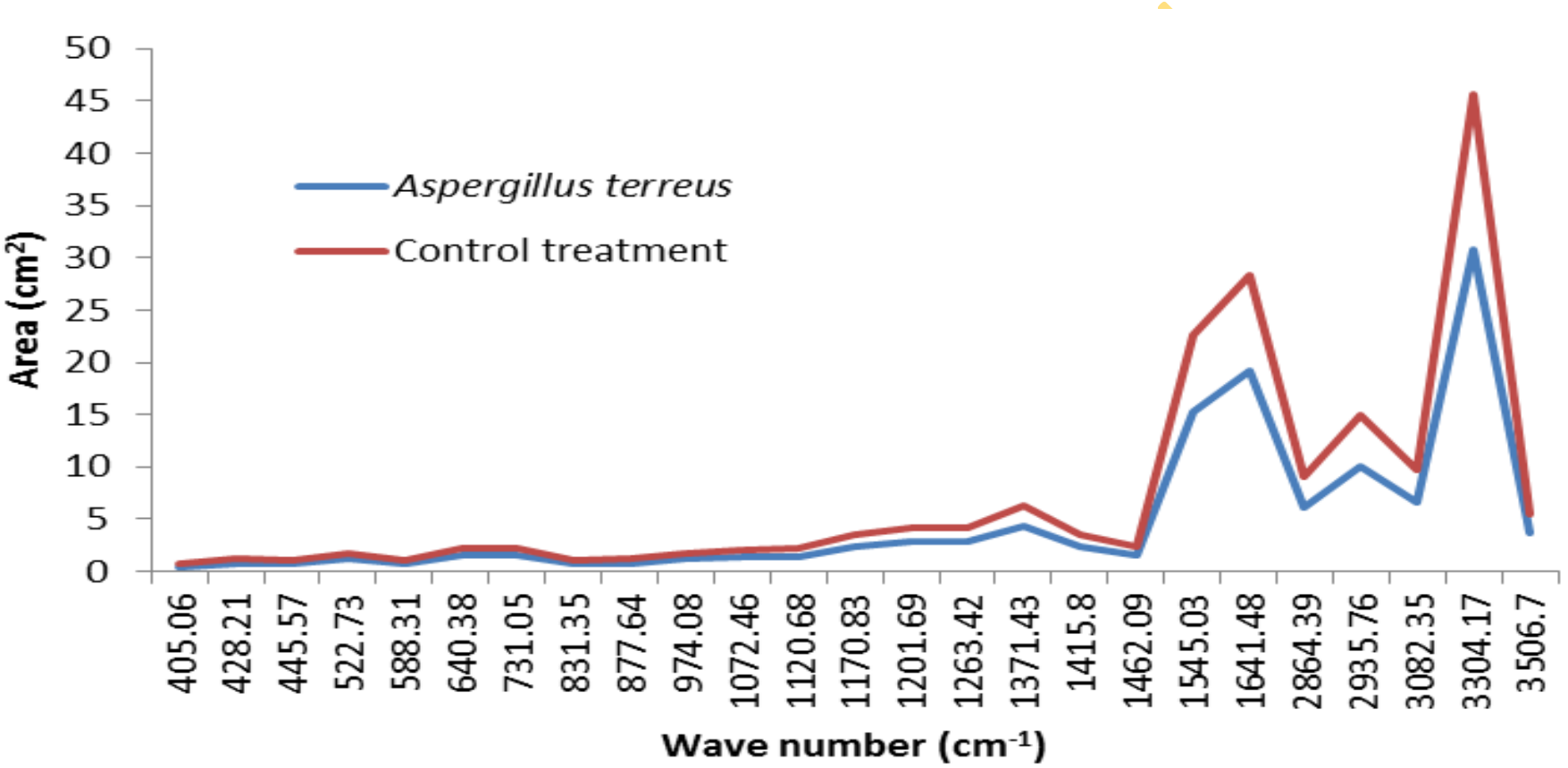



**Figure 4.18a.** FTIR Spectra analysis of nylon-6 fibres treated with *Aspergillus terreus* (OF1) after 90 days of biodegradation.

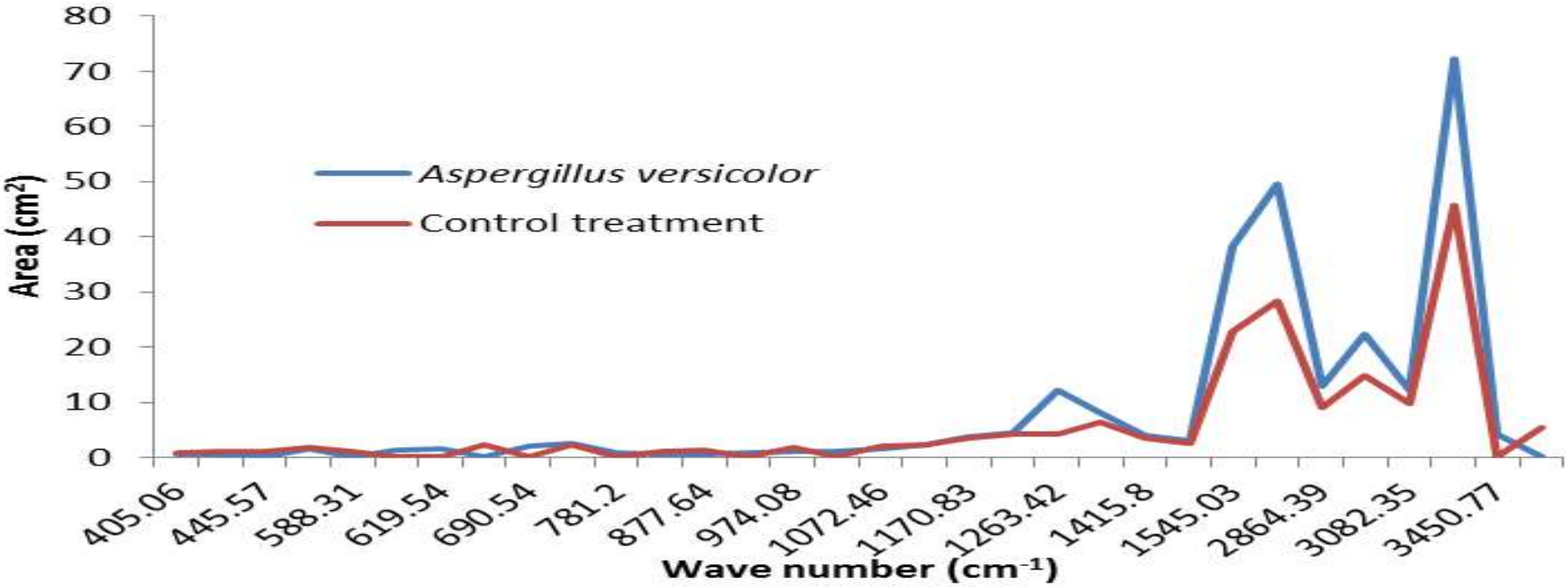



**Figure 4.18b**. FTIR Spectra analysis of nylon-6 fibres treated with *Aspergillus versicolor* (OF2) after 90 days of biodegradation

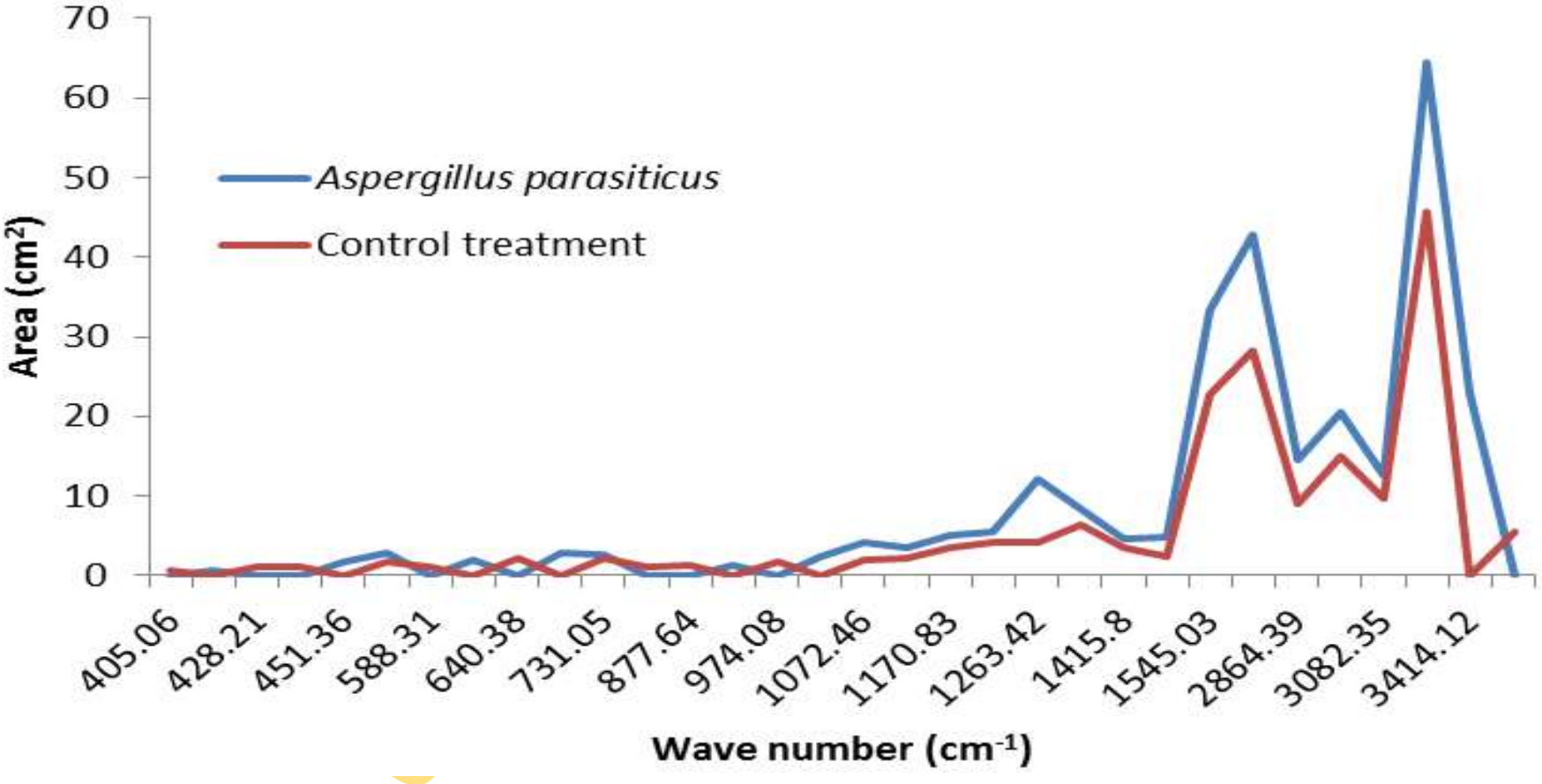



**Figure 4.18c.** FTIR Spectra analysis of nylon-6 fibres treated with *Aspergillus parasiticus* (OF5) after 90 days of biodegradation

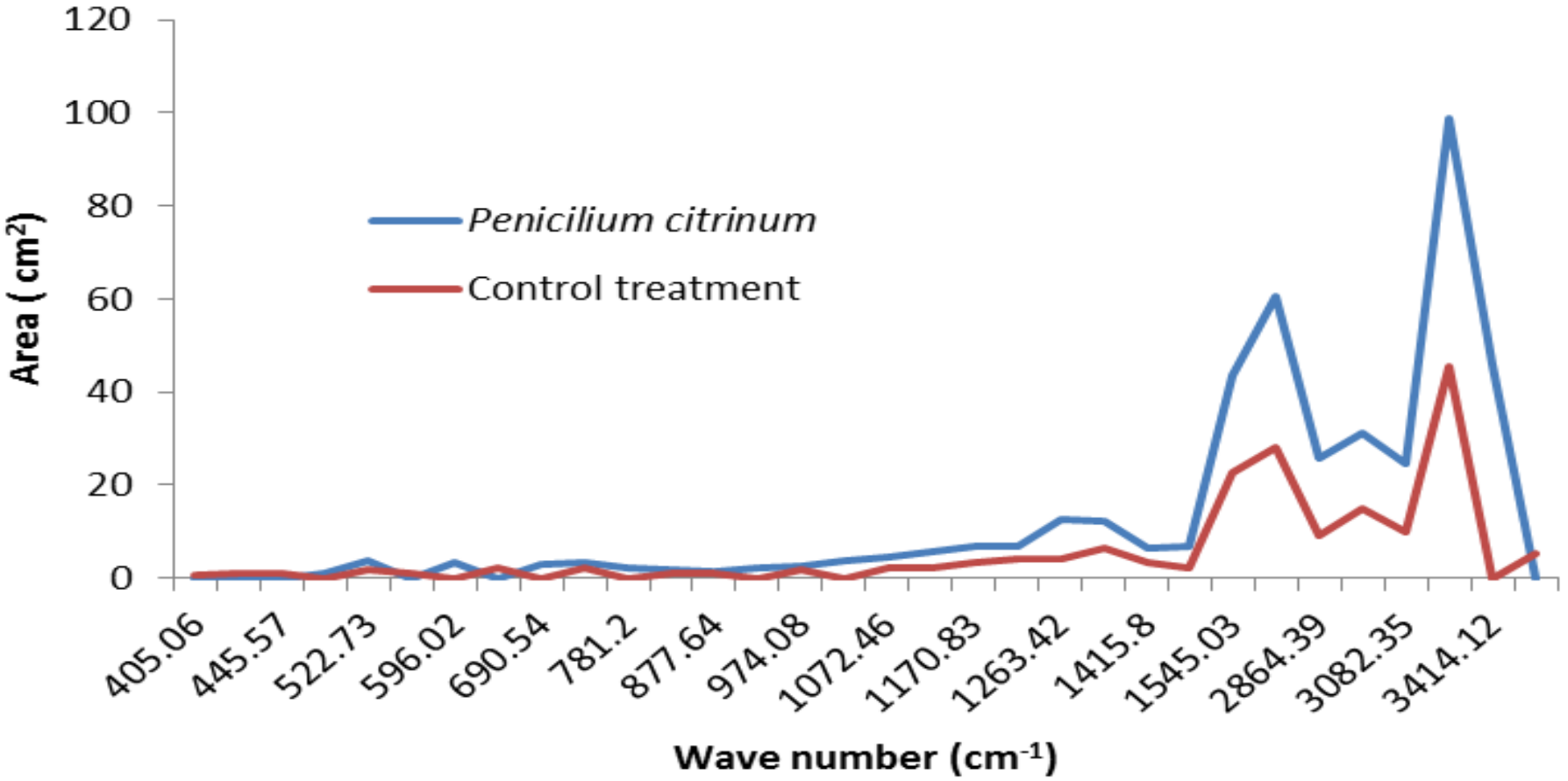



**Figure 4.18d**. FTIR Spectra analysis of nylon-6 fibres treated with *Penicillium citrinum* (NF3) after 90 days of biodegradation

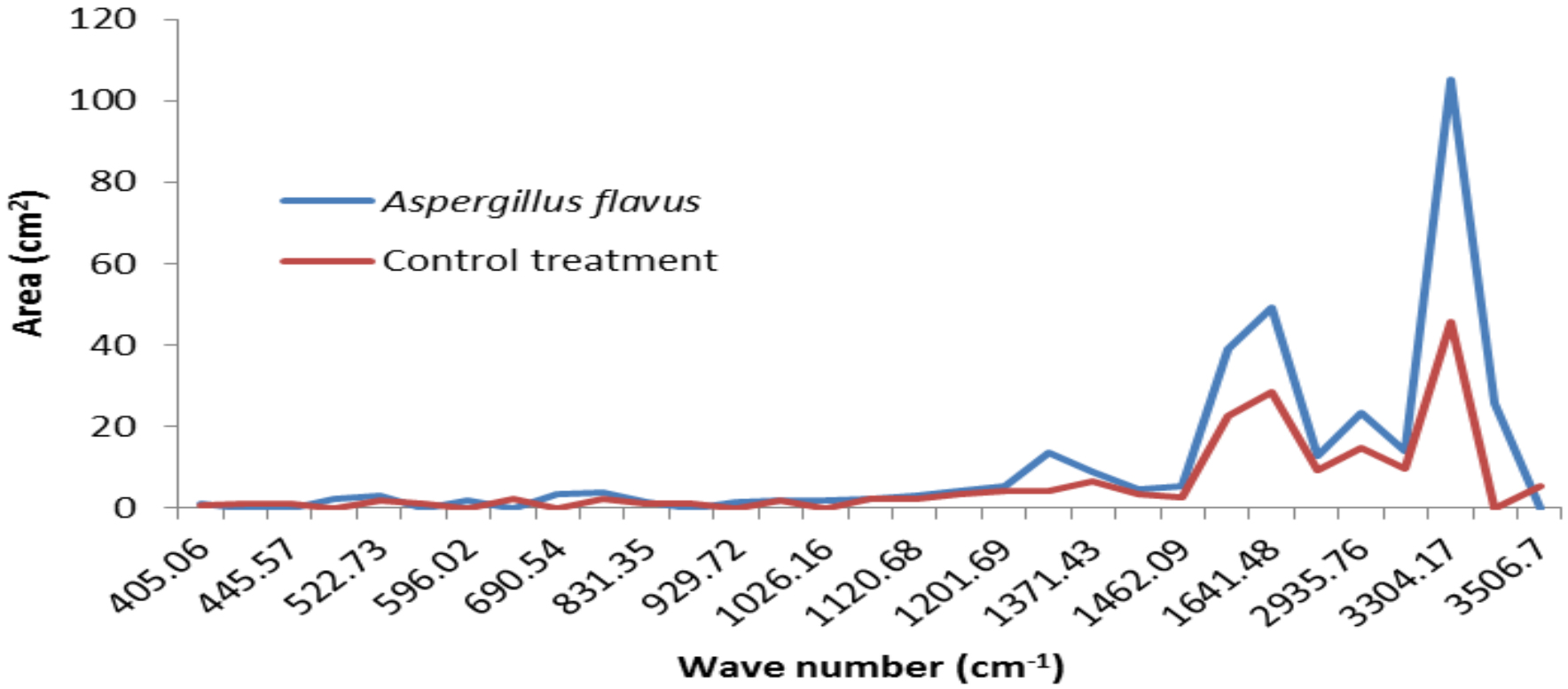



**Figure 4.18e.** FTIR Spectra analysis of nylon-6 fibres treated with *Aspergillus flavus* (AF2) after 90 days of biodegradation

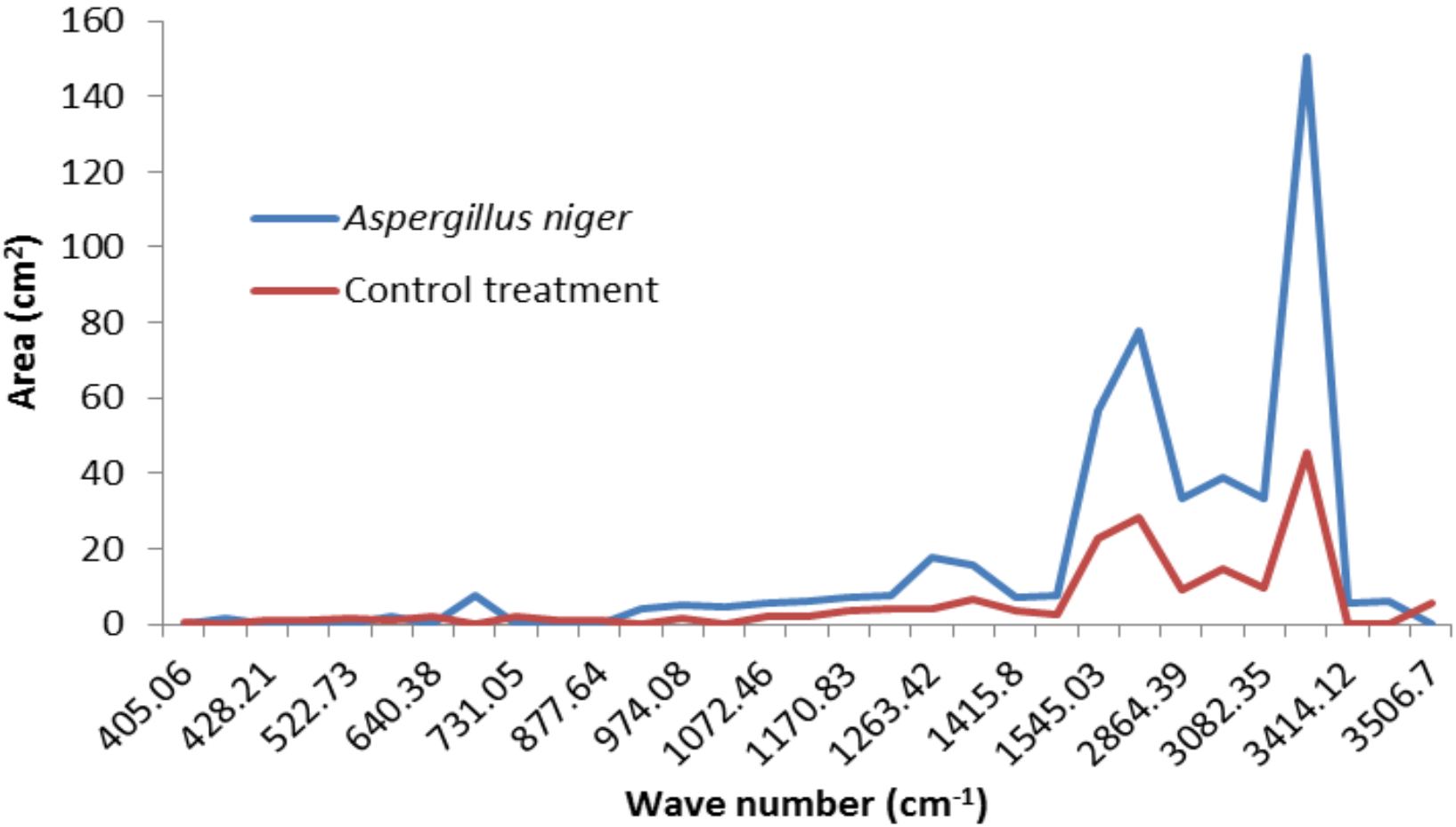



**Figure 4.18f.** FTIR Spectra analysis of nylon-6 fibres treated with *Aspergillus niger* (AF3) after 90 days of biodegradation

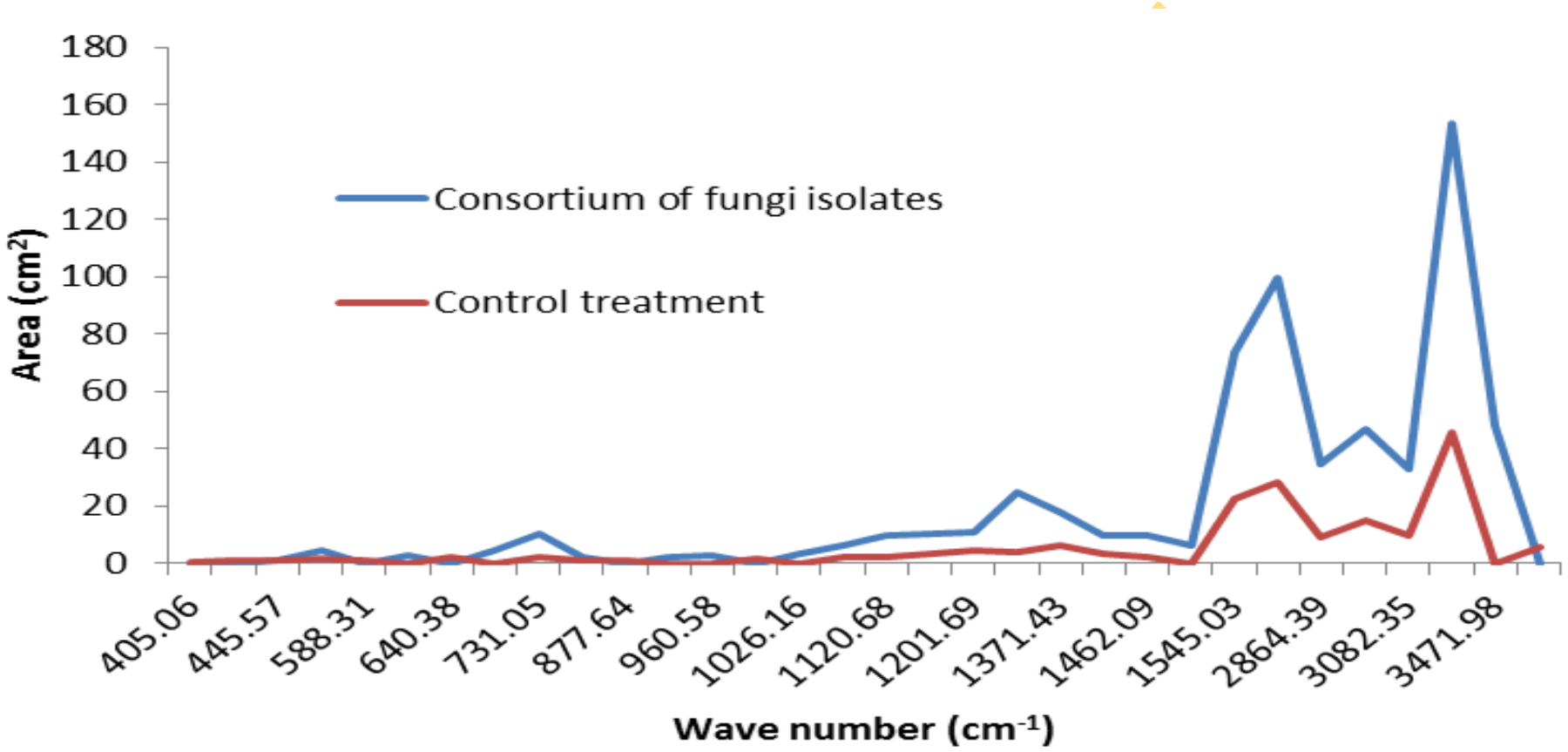



**Figure 4.18g**. FTIR Spectra analysis of nylon-6 fibres treated with the consortium of fungi isolates after 90 days of biodegradation

# CHAPTER FIVE

# DISCUSSION

## 5.1 Microbial Population of soil samples from the Dumpsites

Sixteen heterotrophic bacteria isolates capable of utilizing ε-caprolactam out of the sixty four bacterial isolates from the dumpsites were identified as member of the genera *Alcaligenes* (3), *Bordetella*, *Proteus*, *Pseudomonas* (5), *Providencia*, *Corynebacterium* (2), *Leucobacter* (2) and *Lysinibacillus*. All these bacteria genera have been reported in studies involving solid waste dumpsites biodegradation ( Obire *et al*., 2002; Aliyu, 2010; Ajuzie *et al.*, 2010). High occurrence of *Aspergillus* and *Penicillium* species in these dumpsites are similar to the observation of Obire *et al*. (2002) that reported 25.3 % and 12.6 % of *Aspergillus* and *Penicillium* respectively out of the total viable fungal population of Waste Dumpsites in Port harcourt, Nigeria. The distribution of microorganisms may also be a subject of the waste composition of the dumpsites. However, the isolation of caprolactam utilizing microorganisms from dumpsites, positive and negative control sites as in this study showed that caprolactam utilizing bacteria are not restricted to dumpsites. This is similar to the observation of the presence of hydrocarbon utilizing bacteria in soils of petroleum contaminated soil as well as pristine soils (Fagade, 1990). Although, the presence of Hydrocarbon utilizing bacteria were used as index of petroleum contamination of such sites but this could not be established in this study for the presence of caprolactam utilizing potential in the isolates.

## 5.2 ε-caprolactam utilization potential of the Bacteria Isolates.

The ability of the sixteen of the sixty four bacteria isolated in this study to utilize ε-caprolactam as growth substrates agreed with the observations of earlier studies. For instance, Fukumura (1966) reported the ability of *Achromobacter cycloclastes* W2 and *Corynebacterium aurantiacun* B2 to split ε-caprolactam and produced the corresponding amino acid. Also, Kulkarni and Kaneker (1998) reported the isolation of *Pseudomonas aeruginosa* MCM B-407 from activated sludge and *P. putida* MCM B-408 from cow dung. Both isolates was utilized in remediation of ε-caprolactam from nylon-6 waste water. In a similar study, Baxi and Shah (2000) isolated *Alcaligenes*

*faecalis, Arthrobacter citrus, Bacillus sphaericus and Rhodococcus rhodochrous* from different soil samples of a nylon-6 manufacturing site and these isolates were able to degrade caprolactam in solution. More recently, Wang and Lee (2007) isolated ε-caprolactam denitrifying bacteria including *Hyphomicrobium* species, *Methylosinus pucelena* and *Magnetospirillum* species from waste water treatment system of chemical manufacturing company . Caprolactam degradation in a *Pseudomonas* strain had been reported to be plasmid dependence. Boronin *et al.* (1984) characterized naturally occurring conjugative plasmids that is found to be responsible for caprolactam degradation in several *Pseudomonas* strains capable of growing on caprolactam as a sole source of carbon and nitrogen. Their study found that the ability to grow on caprolactam and aminocaproic acid as sole sources of carbon or nitrogen and adipic acid as a sole source of carbon could be transferred in interspecies crosses. And that all transconjugants harboured corresponding large plasmid DNAs. It was suggested that the discovered plasmids possessed the genetic material controlling several consecutive reactions of caprolactam catabolism. In an ealier study, Negoro *et al*. (1980) demonstrated the transformation of the plasmid DNA coding for nylon oligomer-degrading enzymes from a wild *Flavobacterium* strain to a curred strain of *Flavobacterium* species K172. A similar plasmid was reported to be responsible for this ability in *P. putida* strain BS836 (Esikova *et al*., 1992).

Although, most of the bacteria isolated in this study may have been found to be associated with environmental samples and biodegradation or biodeterioration (Kulkarni and Kanekar, 1998; Baxi and Shah, 2002), there was little or no report on the biodegrading potential in *Proteus vulgaris.* This organism is known to be a pathogen of human and warm blooded animals. On the other hand, *Bordetella petrii* which was ascribed to be the only environmental species out of the eight member species of the genus of *Bordetella* has also been reported to be an opportunistic pathogen (Gross *et al.*, 2008), it has been isolated in a clinical patient (Norman *et al*., 2005*).*

The expanded metabolic range as observed in these bacteria genera may be due to physiological adaptation as a result of alteration in enzyme substrate specificity as reported by Clarke and Slater, (1986) or mutation resulting in alteration of uptake specificity (Negoro 2000).

### 5.3 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of 20 $gL^{-1}$ of caprolactam observed for the isolates from this study is higher when compared to those reported by previous studies. For example, Baxi and Shah (2002) reported 15 $gL^{-1}$ of caprolactam as the upper growth limit for the *Alcaligenes faecalis* isolated from nylon-6 manufacturing plant waste. While previous reports on *Pseudomonas aeruginosa* MCM 407 and *P. putida* MCM 408 isolated from activated sludge and Cow dung respectively reported upper range of 1365 $mgL^{-1}$ (Kulkarni and kaneker, 1998). Similarly, 1631 $mgL^{-1}$ was reported as the highest caprolactam tolerance concentration in *Paracocus versutus* MDC-3 (Wang and Lee, 2007).

The percentage of the caprolactam utilized by the isolates from this study ranges between 97.2 % and 53.8 % in the least performing bacteria isolates at 10 $gL^{-1}$ concentration within 5 days, this is similar to the observation of Baxi and Shah (2002) that reported 95-97 % removal by *Alcaligenes faecalis*. This may be due to the ability of the bacteria to utilize the caprolactam substrate as physiological nutrient for growth. Therefore, the bacteria strains isolated in this study displayed better caprolactam utilization potential compared to those of earlier reports and will be expected to play better role in remediation of caprolactam polluted ecosystems.

### 5.4 Optimal Growth Conditions of pH and Temperature in Caprolactam Medium.

The growth of these bacteria isolates in the ranges of pH 7.0 to 8.5 is similar to the observation reported by Kulkarni and Kanekar (1998) in his study with *Pseudomonas aeruginosa* MCM B-407 that the isolate performed best in the pH range of 5.0 to 9.0 after 48 hrs of incubation. This indicated the ability of the isolates to thrive under pH fluctuation. In this study, majority of the isolates had their optimal growth at pH 7.5. The optimal temperature ranges for these isolates fall within the mesophilic ranges of 30 – 35$^{o}$C. This may be a reflection of the isolates environmental source. Kurian *et al.*, (2003) reported a temperature range of 17 to 39 $^{o}$C for various studied Solid waste dumpsites in India.

The distribution of microbial isolates to a large extend depend on the conditions of pH and temperature. The ability of the bacteria isolates to adapt more quickly to varied pH and temperature may be responsible for the relative high distribution in the studied soil

samples. More so, that bacterium survives better in alkaline environment relative to fungi.

### 5.5 Caprolactam utilizing ability of the Fungi Isolates

This study showed the utilization of ε-caprolactam by the fungi isolates. The caprolactam utilization potential (Table 4.13) is 86.5 % by *Aspergillus. terreus*, 75.5 % by *A. niger*, 69.1 % by *A. flavus*, 60 % by *A. versicolor*, 59.3 % by *A. parasiticus*, and 73.5 % by *Penicilium citrinum* within the 15 days of incubation. Similar utilization potential was reported by Shama and Wase (1981) in fungi genera including *Aspergillus, Absidia, Penicillium, Rhodotorula* and *Trichosporon*. However, the bacteria isolates showed utilization potential ranges between 97.2 % by *Proteus vulgaris* to the least utilization of 53.8 % by *Corynebacterium ammoniagenes* within 5 days (Table 4.5). This observation may be due to the ability of the bacteria isolates to utilize the caprolactam as the sole source of its both carbon and nitrogen compared to the fungal isolates that require a co-metabolic source of carbon in other to grow in the caprolactam medium. Hence the supplementation of glucose in the fungi cultured medium.

### 5.6 6-Aminohexanoic Acid as degradation products of Caprolactam

The detection of amino acid in the culture supernatant of the bacteria isolates (Fig. 4.3) and in the filtrates of the fungal isolates (Table 4.13) suggested the breakdown of the caprolactam to the corresponding substrate unit (i.e. 6 - aminohexanoic acid) and related amino acid required for the microbial growth. This obseravation agreed with that of Karlsson and Albersion (1998) that reported the detection of 6-aminohexanoic acid as the main degradation products of caproamide. Also, Kulkarni and Kanekar, (1997) reported simultaneous detection of caprolactam and aminocaporic acid using high performance liquid chromatography. However, while 5356 ppm of 6-aminohexanoic acid was recorded in the filtrate from *Penicillium* species the highest concentration in filtrates of the *Aspergillus* species was 2964 ppm in filtrate from *A. terreus* (Table 4.13) This observation may be due to rapid assimilation of the amino acid for mycelia growth by the *Aspergillus* species compare to that of the *Penicilium citrinum*.

### 5.7 Molecular Characterization and Phylogenetic Analyses

Analysis of the 16SrDNA nucleotide sequence is often used to identify and classify organisms while phylogenetic relationships between closely related species are usually defined based on the highly conserve regions of their aligned sequences. In this study, the the diversity of the caprolactam degrading bacteria isolates (Table 4.8) were discovered to be of three phylogenetic divisions, the Proteobacteria (γ and β), Actinobacteria and Firmicutes). These bacteria isolates are made up 43.75 % γ-proteobacteria, 25 % β-proteobacteria, 25 % actinobacteria and 6.25 % firmicutes. This observation could not be said to be the representation of the bacteria distribution in the selected study dumpsites but of the caprolactam degrading isolates. Hence, this may possibly be responsible for the lower percentage of 6.25 % of firmicutes compare to the percentage occurrence reported by some earlier workers such as Ajuzie *et al.* (2010) that reported 83.33 % aerobic spore bearers (mostly firmicutes) and Obire *et al*. (2002) that reported 21% *Staphylococcus*, followed by 16.8 % *Streptococcus* and then 15.2 % *Bacillus* in their studies of microbial population of dumpsites. Although, it will be pertinent to note that microbial population of any municipal waste dumps will depend on the source and composition of the component waste materials. However this study showed proteobacteria (68.5 %) and actinobacteria ( 25 %) as the dominant caprolactam degrading groups in the studied dumpsites.

The phylogenetic tree (Fig.4.6) revealed the close evolutionary relationship based on the alignment and the conserved regions of their 16S rDNA. This study showed the maximum similarity and divergence between the bacteria isolates. In the γ-proteobacteria,the genera *Providencia* and *Proteus* cluster together with 88 % alignment while their alignment with *Pseudomonas* was 67 %. *Lysinibacillus sphaericus* (ISC1) which belong to the firmicutes serves as the out-group in the phylogenetic tree and it showed 94 % alingnment to both the *Leucobacter* and *Corynebcaterium* species that belong to the group actinobacteria. Although 16SrDNA sequence analysis is a rapid method of identifying unknown bacteria base on easy amplification of the conserved region, the degree of divergence may be better explained at the level of ATP binding protein (abc) gene sequence analyses (Dewangan *et al.*, 2009).

### 5.8 Coloration of Nylon-6 Fibre

Coloration of the polymer was observed in all the fungi treated fibre (plate 4.2). A dark brown coloration was observed in the nylon-6 samples treated with *Aspergillus parasiticus* and *Penicillium citrinum*. The coloration in the nylon-6 fibre treated with *A. versicolor* was orange brown while samples treated with other *Aspergillus* species showed moderate brown colouration. But the control treatment that was also subjected to same conditions with the treated samples except inoculation with fungi isolates remains unchanged. This coloration may be due to oxidation of the nylon-6 fibre by the activities of the extracellular enzymes produced by these fungi isolates. Similar phenomenon was reported to occur during the thermal oxidation of aliphatic polyamide (Rongfu and Xingzhu, 1998). Although there is difference of opinion on the coloration mechanism of the polyamide in the course of the thermo-oxidative degradation however, Rongfu and Xingzhu (1998), in their hydrolysis of the oxidized nylon-6 showed that the coloration was due to the formation of an α-ketoamide group chromophore which was produced by oxidation of the methylene group adjacent to carbonyl of the amide group in the polyamide. Bio-oxidation may therefore be suggested as a possible mechanism of the degradation of nylon-6 fibre as observed in these fungi isolates.

### 5.9 Microbial growth on Nylon-6 fibre medium

The ability of the fungi isolates to grow in the broth culture containing nylon-6 fibre as the sole source of nitrogen is evident in the mycelia weight of the fungi isolates. *Aspergillus niger* showed 0.97g mycelia weight in 90 days (Fig. 4.14). The corresponding changes in the pH of the growth medium from 6.4 to as low as 4.13 in culture treated with *Aspergillus parasiticus* (Fig. 4.15) further indicated the potential of the fungal isolates to produce enzymes with specificity for the nylon-6 fibre and or its components. Although, Friedrich *et al.*, (2007) reported the inability of the *Aspergillus* species isolated in their study to degrade nylon-6 fibre but their report was silent on the growth of the *Aspergillus* isolates in submerged culture. However, Niels and Peter (2001) reported the pH dependence degradation of polyamide in chlorinated water and observed that degradation was most pronounced at pH value less than 5. Therefore the ability of these fungal isolates to adjust the pH to value less than 5 may have contributed to the degradation effect on the nylon-6 fibre.

The ability of the bacteria isolates to grow in the nylon-6 fibre medium over the period of 3 months (Fig. 4.9) to attain as much as 1.861 absorbance growth density in *Bordetella petrii* from the initial inoculum density of 0.486 absorbance and the least growth density of 1.13 absorbance from initial inoculum density of 0.468 in *Proteus vugaris* in the absence of alternative source of carbon and nitrogen indicated the ability of the bacteria isolates to utilize the nylon-6 fibre for growth. Although contrary to the observation of the changes of pH in the fungal culture medium, the pH of the bacteria inoculated nylon culture medium changed towards alkaline pH values. Similar observation was reported for *Baccillus cereus* in a nylon-6 culture medium in the study of Sudhakar *et al*., (2007). This observation may be due to the possibility of production of amine or ammonia as part of the hydrolysis products as it may lead to changes in the pH.

### 5.10 Changes in Viscosity Number Average Molecular Mass ($M_n$) and Weight Loss

The reduction in the viscosity number average molecular mass with corresponding loss in weight of the nylon-6 fibres treated with bacteria isolates (Table 4.14) showed the highest weight loss in the fibre treated with *Pseudomonas aeruginosa* of 0.0315 g 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. On the other hand the degree of reduction is more pronounced in the nylon-6 fibres treated with the fungal isolates (Table 4.15). *Aspergillus niger* treated nylon-6 fibres showed a weight loss of 0.0205 g and while the molecular weight reduced to 2748.88 $gmol^{-1}$ from the initial 4436.88 $gmol^{-1}$. This represented 29.77 % when compared to the un-inoculated nylon-6 control treatment. This observation showed that the fungi isolates performed better than the bacteria isolates. This may be due to co-metabolism effect resulting from the glucose supplemet of the culture treated fungi. This potential in the microbial isolates suggested the production of extracelluar enzymes by the fungi and bacteria isolates that possess specificity for the nylon-6 and or its components which acted on the fibres to make available to the microorganisms the inherent nutrient requirement in the the nylon fibres. 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.

Also, molecular weight reduction according to Deguchi *et al*. (1998) is probably caused by hydrolysis or oxidative chain scission.

### 5.11 FTIR Analysis of the Nylon-6 Fibres

FTIR analysis of the treated nylon-6 fibres revealed the changes in chemical functional groups of the fibres. This observed changes which include the disappearance of some peaks corresponding to certain functional groups and formation of some peaks corresponding to some new functional groups, according to Sudhakar *et al*. (2007) this observation may be due to the process of hydrolysis and oxidation.

In the FTIR analysis of the fungal treated nylon-6 fibres, general changes in the intensity of peaks between 500 – 1400cm$^{-1}$ was observed in fibres treated with respective isolates. Absorptions in this region according to Rusu *et al.* (2009) are attributed to amorphous methylene sequences responsible for the crystalline form of polyamide-6. Characteristic peaks in this region including peaks at 596 and 690 corresponding to C-H out of plane bend as well as 929 and 1026 corresponding to crystalline alkyl group were found to disappeared in the treated fibres. This observation coupled with the changes in the intensity of peaks in the amorphous region is an indication of decrease in the extent of crystallinity of the nylon fibre upon degradation. Also the disappearance of peaks such as 3506cm$^{-1}$ corresponding to the hydroxyl (O-H) indicated breakage of the intramolecular hydrogen bonding.

Formation of new peaks such as 3450-3471 cm$^{-1}$ and 1475 cm$^{-1}$ corresponding to the free aliphatic primary amine as well as increase in some peaks area especially of peaks at 3304 cm$^{-1}$ corresponding to amide A N-H stretch, 1641 cm$^{-1}$ and 1545 cm$^{-1}$ corresponding to amide I and amide II respectively as observed in the case of nylon-6 fibre treated with *Aspergillus niger* may be due to the cleavage in the intramolecular hydrogen bond as well as cleavage in carbon to carbon (C-C) and or carbon to nitrogen (C-N) bonds thereby resulting in formation of corresponding amines, carboxylate, 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). According to Deguchi *et al*. (1997), similar degradation occurred through hydrolysis and oxidation by white rot fungi and reported that methylene group adjacent to the nitrogen atom in the polymer was probably attacked by the peroxidase enzyme and subsequently the reaction proceeds through autoxidation.

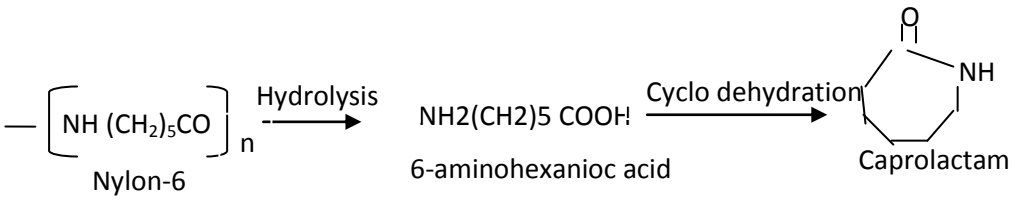
Also, in the analysis of the bacteria treated nylon-6 fibres, similar observations were recorded. There were breakdown and formation of peaks but worthy of note is formation of hydroxyl groups corresponding to tertiary alcohol at 1364 $cm^{-1}$, hetero-oxy compound at 1508 $cm^{-1}$ and out of plane 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).

**5.12: HPLC Analysis**

The HPLC Analysis of the bacteria culture supernatants using 6-aminohexanoic acid (the linear monomer of nylon-6) and Caprolactam (the cyclic monomer) as monitoring standards revealed the presence of the nylon-6 linear monomer in both the experimental and the uninoculated control cultures. However, the cyclic monomer (caprolacatm) or traces of oligomers were not found in the control treatment. This suggested the possibility of partial chemical hydrolysis due to abiotic activities such as the temperature effect of autoclaving and reaction of inorganic chemicals of the minimal salt medium. 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.

In the bacteria inoculated cultures, there were increases in the peak areas of the linear monomer compared to the abiotic control in the supernatants of all the analysed bacteria treated nylon-6 fibres. This observation coupled with formation of caprolactam and some other unidentified oligomers corroborated the degradation claims. This is in agreement with the earlier reports of Baxi and Shah (2000) and Friedrich *et al*. (2007).

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.

# CHAPTER SIX

## SUMMARY AND CONCLUSIONS

This study investigated the possibility of isolating from solid waste dumpsites microorganisms with ability to metabolize the recalcitrant nylon-6 plastic and its precursors based on the high adaptive potential of microbes to their environmental conditions.

Sixty four bacteria and twenty two fungi isolates were obtained from soil samples from three major accredited solid waste dumpsites and a waste collection point of a textile industry in Lagos state, Nigeria. Sixteen bacteria and six fungi isolates with best growth on caprolactam medium were selected and studied for their caprolactam and nylon-6 degrading potential in laboratory cultures. These selected microbial isolates from solid waste dumpsites were able to utilise caprolactam as a source of carbon and energy for growth thereby resulting in the removal of the substrate from solution. Caprolactam tolerable concentration was found to be up to 20 $gL^{-1}$ in these isolates.
The bacteria isolates were molecularly characterised based on their 16SrDNA nucleotide sequence as member of the genera *Alcaligenes* (3), *Bordetella*, *Proteus, Pseudomonas* (5), *Providencia*, *Corynebacterium* (2), *Leucobacter* (2) and *Lysinibacillus*. The fungi isolates were characterized based on their cultural and microscopical characteristics as five species of *Aspergillus* and a species of *Penicillium*.

*Proteus vulgaris* and *Bordetella petrii* isolated in this study showed the best caprolactam utilization potential with ability to remove 97.2 % and 92.5 % of 10 $gL^{-1}$ within 120 hrs while similar potential of 86.5% and 75.48% were recorded in *Aspergillus tereus* and *A. niger* respectively.

Biodegradation effects of the isolates were detected by the oxidative discoloration of the polymer as observed in the fungi treated fibres and the decrease in weight and viscosity average number molecular mass ($M_n$) observed in the treated fibres.

This biodegradation study showed *A. niger* to be the best fungi isolate with highest reduction of 29.77 % in the $M_n$ as well as 23.95 % loss in weight while the best bacterial isolate was found to be *Pseudomonas aeruginosa* (NTS1) with 12.82 % reduction in $M_n$ and 5.23 % weight loss. This may due to the versatile ability of fungi to attach to materials under the presence of the least minimal moisture condition.

The degradation effect was further confirmed with the changes in the intensities of different functional group components of the polymer as well as the formation of new functional groups as revealed by the FTIR spectroscopic analysis.

HPLC analysis of the degradation products of the bacteria treated fibres revealed the production of both the linear and cyclic monomers of nylon (6-aminohexanoic acid and caprolactam) and some un-identified oligomers that suggested the mechanisms employed by the organisms to likely be of enzymatic hydrolysis.

Therefore, the results of this study demonstrated the following:

1. The possibility of utilization of these isolates for caprolactam remediation in industrial effluents. And that adequate knowledge of their physiology and kinetics could make them useful in the design of suitable bioreactor for textile and other chemical industry.
2. The potential of the isolates to partially degrade the recalcitrant nylon-6 polymer. It gives the hope of possible degradation in waste dumpsites.
3. This study also reported the Caprolactam utilization in *Proteus vulgaris* which is known to be an obligate pathogen, suggesting the production of an enzyme specific for its utilization
4. Serve as a baseline research into the potential of the isolates from solid waste dumpsites in nylon-6 degradation especially of lower filamentous fungi due to their abundance, easy ability to colonize materials and high production of extracellular enzymes.

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