MICROBIAL DEGRADATION OF POLYURETHANE

BY

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Matric No.: 139595

A Thesis in the Department of Microbiology,
Submitted to the Faculty of Science
in Partial Fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY

of the

UNIVERSITY OF IBADAN

February, 2014

ABSTRACT

Polyurethane is a non-easily degradable synthetic polymer used as packaging material. Its presence and durability in the environment pose global disposal and degradation problem. Polyurethane releases toxic substances with carcinogenic or mutagenic potential during burning in dumpsites. An alternative to burning of waste materials is the use of microorganisms to degrade them. However, little is known about microbial degradation of polyurethane. Therefore, microbial degradation of polyurethane was investigated as an alternative treatment and management method.

Polyurethane samples were collected from a dumpsite in the University of Ibadan and rubbish-bins of two fast foods outlets within Ibadan metropolis. The packs were buried at depths ranging from 15-70 cm in the garden soil of Microbiology Department, University of Ibadan. They were exhumed at predetermined intervals between the dry and rainy seasons of June 2010-June 2012 for isolation of microorganisms. The microbial isolates were screened for ability to utilise polyurethane as nitrogen and/or carbon source using mineral salts medium. Selected polyurethane-utilising microorganisms as carbon and nitrogen sources were characterised and identified using standard microbiological procedures and the advanced bacterial identification software database. Biodegradation study was carried out on sterilised soil in the laboratory and garden soil in the field with the best six polyurethane-utilising bacteria. This was done using complete randomised block design with 4x3x2 factorial experiment for isolates combinations (A: *Pseudomonas alcaligenes* $E_1$4+ *Providencia pseudomallei* $D_2$5, B: *Enterobacter amnigenus* $D_1$2+ *Vibrio* sp. $C_3$2, C: *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1 and D: Consortium of the six bacteria) and biostimulation treatments (cassava peels, potato peels, no peels) at 1 and 3 month periods. Changes in the functional groups of degraded polyurethane samples were determined using Fourier transform infrared spectroscopy. Weight loss of polyurethane samples was monitored by measurement. Data were analysed using ANOVA at $p = 0.05$.

Of the 106 bacterial isolates obtained, 94 utilised polyurethane as carbon, nitrogen or both with highest occurrence (26.0 %) at 70 cm depth. Eighty-seven per cent of the isolates were obtained during the rainy season. Fifteen bacteria isolates that utilised polyurethane as carbon and nitrogen sources were: *Pseudomonas* (4),

Corynebacterium (1), *Providencia* (2), *Enterobacter* (2), *Comamonas* (2), *Micrococcus* (1), *Arthrobacter* (1), *Vibrio* (1) and *Bacillus* (1). Fungi isolates could not utilise polyurethane. Percentage degradation of polyurethane with potato peels, cassava peels and no peels was respectively 91.0, 33.0 and 57.2 % in laboratory and 35.9, 0.0 and 76.3 % in field. Ether peak was removed by B, C and D in the field biostimulated with cassava peels. Carbonyl peak area was reduced by 87.6 % with D biostimulated with potato peels in the laboratory and changes in the functional groups were significant. The highest weight losses were 22.5 and 15.0 % for the field and laboratory studies after one month.

The isolated bacteria degraded the polyurethane by removal of resistant functional groups. Thus they could be used for degradation and management of polyurethane in the Nigeria environment.

Keywords: Polyurethane biodegradation, Biostimulation treatment, Bacteria utilisation, Waste management

Word count: 484

ACKNOWLEDGEMENTS

I thought writing this would be easy, but I realise now that it requires me to take a trip down memory lane for six years. This journey started when I arrived University of Ibadan in September 2007 for my M.Sc. The tricky thing though is that time accumulates debts of gratitude as one traverses different places, spaces and meets different people. Therefore, I should say that the few pages allotted for acknowledging the contributions of many people in this activity can never be enough. Nonetheless, as Ben Okri says ‘‘there are many destinies’’ and that if we fail to keep appointed hour with one destiny, we are bound to fulfil the next. In this spirit therefore, I hope that this activity is a gesture to our shared yet different ‘‘destinies’’, and that this appointed hour is not the only one. We will have other hours to fulfil many appointed ‘‘destinies’’- of acknowledgements. There are many people who have contributed in various ways to my completion of this thesis, the list is almost certainly endless, such is my debt of gratitude.

First of all, I acknowledge the generous intellectual support of my supervisor, Prof. Obasola E. Fagade throughout my postgraduate studies for his inestimable contributions. He has been magnanimous as a mentor, often times going beyond the call of duty. Sir, I am deeply grateful to your dedicated mentorship, my prayer is that the heart of God will open to you and generations of your children. I am also grateful to all the staff of the Department of Microbiology, University of Ibadan namely; Prof. S.A. Odunfa, Prof. A.I. Sanni, Prof. A.A. Onilude, Drs. A.A. Ogunjobi (PG, Coordinator), O.O. Adelowo, S.T. Ogunbanwo, Sherifah Wakil, for all the seminars and informal sessions which allowed my mind to wander into rich and diverse intellectual landscapes; and also in a special way, the members of Environmental Microbiology and Biotechnology Unit for giving me all the necessary support during this period of study. Thanks go to the family of Dr and Mrs K.S. Chukwuka, for the warmth, generosity and kindness; they always created that much needed sense of belonging- a home away from home. Thank you Uncle and God bless you.

I also appreciate the academic encouragement received from Prof. J.B.I. Aderiye, Prof. A.C. Odebode, Prof. A.E. Ayodele, Drs. A.M. Salaam, A.T.P. Ajuwape and Dr. Clementina Adenipekun.

This thesis would not have been completed without the financial support of my epitome of love and kindness, Dr. John Obiaghanwa Okoye, who made sure that finance, was never a stumbling block but always tried to bring out the best in me, Dr. Jay, God will open to you and your family doors of favours beyond your imagination. Without his support, I would not have had the peace of mind required for sustained academic activity, thanks and may God bless you, Sir.

Worthy of note is equally the encouragement and support from Dr. George & Mrs. Ugonma Uzonwanne, Mr & Mrs E. S. K Uzoma, Chief (Engr.) Obi R. Ekezie, my friend Dr. Ijeawele P. Adiewere, and my Spiritual Director Rev. Fr. Cyriacus A. Oguh. May God bless you and I am indeed grateful to you. I acknowledge the efforts and kind contributions of Mr A. Adebayo of Redeemer's University, Lagos who analysed the PUR samples using FTIR and Dr. A.I. Oladosu of Chemistry Department, U.I for helping with the interpretation of my FTIR spectra to the success of this project work, may God bless them all. I also appreciate my colleagues and friends in the Department of Microbiology as a whole.

To my beloved parents Sir Michael & Lady Priscilla Okolie, I appreciate your contributions towards my academic pursuit and life generally. Thank you for your love, care and understanding and my lovely siblings- Ikenna, Chizoba, Kelechi, Tochukwu, Arinzechukwu, Chibuike, Chukwuebuka and Anyaehiechukwu, may God Almighty bless you and give you long lives to reap good fruits of your labour. To my beloved God-given husband Chima, I say, 'B' you are one in a trillion, thanks for your love and understanding. May God bless you.

Finally and most importantly, my heartfelt gratitude goes to Almighty God, the giver of divine wisdom and understanding; my Lord and personal Saviour Jesus Christ and to the Seat of Wisdom- the Blessed Virgin Mary, my powerful intercessor, for unfailing love and favours all through the course of the program. I AM INDEED GRATEFUL!

Blessing I. Okolie

CERTIFICATION

I certify that this work was carried out by Blessing Ifeoma OKOLIE in the Department of Microbiology, University of Ibadan

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DEDICATION

This research project is dedicated to my beloved parents, **Sir Michael & Lady Priscilla Okolie** and my lovely siblings for their love and selfless sacrifices.

In a very special way, it is dedicated to **Dr. John Obiaghanwa Okoye- an epitome of love and kindness** and his lovely family for always being there for me.

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CHAPTER ONE

INTRODUCTION

1.1 PLASTICS

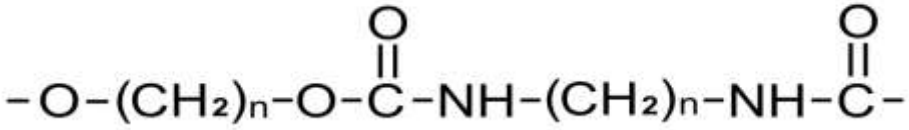
Plastics are polymers used in a wide variety of industries and commerce. They were developed as strong, lightweight, durable and bio-inert materials and have been applied to a wide range of commodities. They are used in almost all the fields ranging from sophisticated articles such as prosthetic hips and knee joints to disposable food utensils. Approximately 30% of the plastics are used worldwide for packaging applications such as food, pharmaceuticals, cosmetics, detergents and chemicals (Shah, 2007). Plastics replaced many natural materials such as metals, woods and gravel. Due to the growth in residues that pollute the environment, the use of synthetic polymers has posed great concern. Cangemi *et al*. (2008) opined that new uses of plastics are on the increase because new and numerous applications are frequently emerging. The enormous production and utilization of polymers led to their accumulation in the environment after use. Plastics are not easily degraded by microorganisms, and thus have become a serious source of pollution affecting both flora and fauna (Arutchelvi *et al*., 2008).

As the use of plastics increases so does the amount being dumped into landfills and the rate of depletion of landfills is being greatly impacted by the presence of these substances (Rowe and Howard, 2002). Biodegradation and mineralization of plastic wastes have been given an increasing attention because of its xenobiotic origin and recalcitrant nature. Orhan and Buyukgungor (2000) reported that plastics biodegradation is problematic and accumulates in the environment at a rate of 25 million tons per year.

The potential for the biodegradation of synthetic polymeric materials, or the lack thereof, has been studied from the 1960's. The majority of the early research on biodegradation of synthetic polymer dealt with issues of biodeterioration as an inherent negative property, rather than the development of materials with relatively short lifetimes. However, as social and political concerns grow about the management of municipal solid waste, the hazards of plastic litter to wildlife, and plastic film mulching in agriculture, interest in the development of biodegradable synthetic polymers emerged (Pettigrew and Johnson, 1996).

1.2 POLYURETHANES

Polyurethanes (PUR) are synthetic polymers developed by Ottor Bayer in 1938 at the beginning of the World War II as a substitute for rubber (Oceguera-Cervantes *et al*., 2007). Polyurethanes are formed by the synthesis of three basic components: the condensation of a polyol and a poly- or di-isocyanate and an extender using various low-molecular-weight prepolymer blocks (Urgun-Demirtas *et al*., 2007). PUR is classified roughly into two types; polyester PUR and polyether PUR, depending on the type of polyols from which they were synthesized (Morton and Surman, 1994). The terminal hydroxyl group allows for alternating blocks, called "segments", to be inserted into the PUR chain (Howard, 2002). Blocks providing rigid crystalline phase and containing isocyanate and the chain extender are referred to as "hard segments" (Fried, 1995); while those yielding generally either noncrystalline or amorphous rubbery phase and containing polyester/polyether are called "soft segments" (Young and Lovell, 1994; Urgun-Demirtas *et al*., 2007). The general formula for PUR is linear and represented by



where n is the number of repetitions.

Depending on the chemical structures of the polyisocyanates and polyols, PUR can adopt various forms ranging from flexible to rigid and from low density to solid elastomer (Howard, 2012). This chemical composition of PUR precludes them from being classified as pure plastics and hence are called mixed polymer. The urethane group, which is the basis of this class of mixed polymer, represents a small part of the macromolecule and some PUR products do not contain a urethane group. Despite the lack of this base unit, all PUR are based on the composition of polyisocyanates. The polyisocyanate polyaddition is distinct from polymerization and polycondensation for the production of synthetic polymers and this feature explains their versatility (Howard, 2012).

Due to polyurethanes' range of properties, they are diverse group of synthetic polymers that are widely used as raw materials in various industrial applications including medical; for production of catheters, cardiac valves; automotive; for car seats, brake pads; and furniture as insulating foams (flexible and rigid foams), adhesive constructional materials, paddings and packaging (Howard, 2002; Urgun-Demirtas *et al*., 2007). Being such a versatile polymer, PUR production has increased, but this has brought with it the problem of its safe disposal. Each year, more than 5 million tons of shredder residue containing different plastics and PUR foams is generated in the United States and Canada (Oceguera-Cervantes *et al*., 2007).

1.2.1 Applications of Polyurethanes

Polyurethanes are present in many aspects of modern life. They represent a class of polymers that have found a widespread use in the medical, automotive and industrial fields (Howard, 2002; 2012).

Polyurethanes are replacing older polymers for various reasons; they have increased tensile strength and melting points making them more durable (Bayer, 1947). In the medical field, PUR elastomers are being used in place of other elastomers due to their higher elasticity and toughness, and resistance to tear, oxidation and humidity (Dombrow, 1957; Saunders and Frisch, 1964; Ulrich, 1983). Their resistance to degradation by water, oils, and solvents make them excellent for the replacement of plastics (Saunders and Frisch, 1964). As coatings, they exhibit excellent adhesion to

many substances, abrasion resistance, electrical properties and weather resistance for industrial purposes (Saunders and Frisch, 1964; Urbanski *et al*., 1977; Fried, 1995). PUR has lower density and greater flexibility and thus auto manufacturers are replacing latex rubber in car seats and interior padding with PUR foam (Ulrich, 1983). The United States government is phasing out chlorinated rubber in marine and aircraft and coatings because they contain environmentally hazardous volatile organic compounds and replacing them with PUR (Hegedus *et al*., 1989; Reisch, 1990).

1.3 Biodegradation of Polyurethanes

The susceptibility of the PUR to microbial degradation is highly dependent on the chemical structure of its constituents (Darby and Kaplan, 1968). The burden of plastic waste in the environment can be reduced by exploiting the biodegradability of plastics such as polyurethane through microorganisms in the environment which show great potential for PUR degradation (Cosgrove *et al*., 2010). Polyester PUR are considered to be comparatively susceptible to microbial attack (Morton and Surman, 1994), whereas polyether PUR are relatively more resistant to this kind of microbial attack (Darby and Kaplan, 1968). This difference has been suggested to be due to the PUR biodegradation mechanism which involves exo-type depolymerization in the ether PUR but endo-type depolymerization in the ester PUR (Nakajima-Kambe *et al.*, 1999).

Both fungi such as *Aspergillus fumigatus, Chaetomium globosum, Gliocladium roseum* and *penicillium citrinum* (Pathirana and Seal, 1984) and bacteria *Corynebacterium* sp. and *Enterobacter agglomerans* (Kay *et al.*, 1991) have been isolated from the surface of soil-buried polyester PUR and tested in vitro for PUR degrading ability. More fungi have been isolated in comparison with bacteria. *Gliocladium roseum* was isolated from polyester PUR buried for 28 days in soil (Pathirana and Seal, 1984), whilst a number of isolates from the genera *Aspergillus*, *Emericella*, *Fusarium*, *Penicillium*, *Trichoderma* and *Gliocladium* were recovered from the surface of polyester PUR foam buried in soil for 28 days (Bentham *et al.*, 1987) and *Nectria gliocladioides, Penicillium ochrochloron* and *Geomyces pannorum* were isolated from PUR buried in soil for 44 days by Barrat *et al*. (2003)

Few bacteria can degrade polyester PUR as sole carbon source but recently a number of bacteria from the genera *Pseudomonas*, *Comamonas* and *Bacillus* have been identified that can degrade colloidal polyester PUR in vitro (Howard and Blake, 1998; Allen *et al.*, 1999; Ruiz *et al.*, 1999; Rowe and Howard, 2002). The development of new strategies based on the utilization of biopolymers and the discovery of microorganisms (fungi and bacteria) able to utilize PUR as a source of carbon and nitrogen is leading the move to a greener chemical industry (Darby and Kaplan, 1968; Nakajima-Kambe *et al.*, 1995; 1999).

1.4 Statement of problem and Justification

The low cost and ease of manufacture have increased global plastic demand more than 150-fold, with the production of 1.5 million tons in 1950 and 245 million tons as of 2006 (Plastics Europe, 2008). Tremendous increases in the manufacture and consumption of plastics including polyurethanes over recent decades have led to numerous ecological and economic concerns. The persistence of synthetic polymers introduced into the environment by industries, influence of western world and urbanization poses a major threat to natural ecological systems. Despite recognition of the persistent pollution problems posed by PURs, global production is still increasing, with the largest increases expected in developing nations. The sheer volume of plastics produced each year presents a problem for waste disposal systems

In Nigeria, the indiscriminate disposal of plastics in the environment coupled with inadequate waste management techniques have led to various forms of PURs constituting environmental nuisance in the ecosystem. With more and more plastics being utilized, environmental problems caused by their non-biodegradable characteristics have raised appreciable ecological concerns about the increase in production and accumulation of plastic wastes. As non-degradable plastics build up in the environment and cause increasing problems for disposal, it is therefore becoming more important that the biodegradability of these polymers be thoroughly understood. Ecological problems connected with burial of industrial wastes claim special attention to the processes of processing wastes and recycling of used plastic goods.

The scale of this problem and the recalcitrance of some polymers to degradation necessitated the investigation into effective methods for biodegradation of PUR. Microorganisms have been found to be able to degrade many pollutants that cause waste management problems. A basic understanding of the biological processes leading to biochemical degradation will advance the development of new bioremediation techniques.

Since environmental pollution by PUR wastes has become a serious issue and cause waste management problems, an understanding of how these PUR can undergo biodegradation may aid in the development of strategies to exploit these processes for waste management. The current research focuses on biodegradation of Polyurethane, which is a base material widely used in many industries and employed in everyday applications. This will assist in combating the waste pollution problems caused by this type of plastic wastes and enhance bioremediation of the contaminated environment.

Therefore, there is need to study the role and pattern of microbial degradation of PUR polymer as this will provide information necessary for the development and application of biological resources as agents for sustainable treatment of PUR polymer waste.

The objectives of this study therefore were to;

- Isolate, screen and identify polyurethane degrading bacteria from PUR samples buried in soil over a period of two years
- Select PUR utilizing bacteria strains for bioaugmentation and biostimulation processes in laboratory
- Carry out *ex situ* and *in situ* PUR degradation in soil by the selected bacterial isolates with and without stimulation and natural soil flora.
- Determine and evaluate the structural changes that occur in the experimentally degraded PUR samples above using Fourier Transform Infra-Red (FTIR) analysis of recovered PUR samples.

CHAPTER TWO
LITERATURE REVIEW

2.1 Plastics and Polyurethanes

Plastics are the products of the 20th century. They are largely synthetic materials made from crude oil, an extremely inexpensive but non-renewable resource. In their original forms, plastics were mimicking and replacing natural products such as lacguer, shellac, amber, horns, husks and tortoise shell (APME, 1999). Many plastics are both physically and chemically robust and cause waste management problems (Bouwer, 1992). The polyurethanes are a diverse group of synthetic polymers that are used in a variety of industrial applications, including furniture, insulating foams, adhesive constructional materials, fibers, paddings, paint, synthetic leather and rubber goods (Shah *et al*., 2008).

2.2 Raw Materials of Polyurethanes

The raw materials used in the synthesis of PUR are classified into polyisocyanates, polyols, catalysts and auxiliary materials (Table 2.1). Some of the auxiliary materials used are chain-extension agents (e.g. short-chain diols), crosslinking agents (e.g. short-chain polyols with three or more hydroxyl groups) that react with isocyanate groups, addition agents for PUR manufacture process and improvement agents (e.g. silicone compounds used as antifoams and aromatic esters as flexibilizers) (Nakajima-Kambe *et al*., 1999).

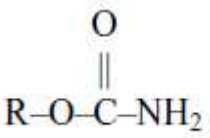
As polyols, polyether and polyester polyols are generally used. PUR synthesized from polyester polyol is termed polyester PUR, and that synthesized from polyether polyol is termed polyether PUR. Although most PUR used in the past is polyether PUR, polyester PUR has become the focus of attention at present because of its biodegradability and therefore its advantages from the viewpoint of waste treatment,

but not within our country Nigeria where polyether PUR is currently in use as seen in the PUR samples used in this study. This is due to its cost effectiveness as observed by Howard (2012; 2002) who is of the opinion that polyether derivatives are inexpensive to produce as prepolymers, which can lower the overall cost of polymer production; and also in Medical field, due to its show of resistance to macromolecular oxidation, hydrolysis and calcification (Marchant, 1992).

Polyisocyanates include aromatic and aliphatic compounds. Among these, tolylene diisocyanate and diphenylmethane diisocyanate are the most commonly used. Since an isocyanate group generates carbon dioxide on reacting with water, foam-type PUR is synthesized by the addition of a small quantity of water during the synthetic process (Nakajima-Kambe *et al*., 1999).

2.3 Physical and chemical properties of Polyurethanes

Polyurethane is a polymer in which the repeating unit contains a urethane moiety. Urethanes are derivatives of carbamic acids which exist only in the form of their esters (Dombrow, 1957). This structure can be represented by the following, generalized amide-ester of carbonic acid:



Variations in the R group and substitutions of the amide hydrogen produce multiple urethanes. Although all PUR contain repeating urethane groups, other moieties such as urea, ester, ether and aromatic maybe included (Saunders and Frisch, 1964). The addition of these functional groups may result in fewer urethane moieties in the polymer than functional groups. The urethane linkage results most readily through the reaction of an isocyanate, –N=C=O, with an alcohol, –OH (Dombrow, 1957; Kaplan *et al*., 1968). The hydrogen atom of the hydroxyl group is transferred to the nitrogen atom of the isocyananate and the major advantage of PUR is that the chain is not composed exclusively of carbon atoms but rather of heteroatoms, oxygen, carbon and nitrogen (Bayer, 1947).

Table 2.1: Raw materials used in synthesis of PUR

Material	Chemical name
Polyisocyanate	2, 4-Tolylene diisocyanate (2,4-TDI)
	2, 4- TDI/2, 6-TDI (80/20 mixture)
	4-4´- Diphenylmethane diisocyanate
	1, 3- Xylylene diisocyanate
	Hexamethylene diisocyanate
	1, 5-Naphthalene diisocyanate
Polyol	
Polyester-type	Poly (butylene adipate)
	Poly (ethylene butylene adipate)
	Poly (ethylene adipate)
	Polycaprolactone
	Poly (propylene adipate)
	Poly (ethylene propylene adipate)
Polyether-type	
	Poly (oxytetramethylene) glycol
	Poly (oxypropylene) glycol
	Poly (oxypropylene)-poly (oxoethylene) glycol
Chain extension or Crosslinking agent	
	I, 4-Butanediol
	Ethylene glycol
	1, 3-Butanediol
	2, 2-Dimethyl-1, 3-propanediol
	Trimethylolpropane
	Glycerol
	1, 2, 6-Hexanetriol

Source: Nakajima-Kambe *et al*., 1999

The synthesis of PUR and polyurethane-urea is represented in Figure 2.1. Diisocyanates are employed in PUR production reactions because they will react with any compound containing active hydrogen (Dombrow, 1957). For industrial applications, a polyhydroxyl compound can be used. Similarly, polyfunctional nitrogen compounds can be used at the amide linkages. By changing and varying the polyhydroxyl and polyfunctional nitrogen compounds, different PUR can be synthesized (Dombrow, 1957). Polyester or polyether resins containing hydroxyl groups are used to produce polyester- or polyether-PUR, respectively (Urbanski *et al*., 1977).

Variations in the number of substitutions and the spacing between and within branch chains produce PUR ranging from linear to branched and flexible to rigid. Linear PUR are used for the manufacture of fibers and molding (Urbanski *et al*., 1977). Flexible PUR are used in the production of binding agents and coatings (Saunders and Frisch, 1964). Flexible and rigid foamed plastics, which make up the majority of PUR produced, can be found in various forms in the industries (Fried, 1995). Using low molecular mass prepolymers, various block copolymers can be produced; the terminal hydroxyl group allows for alternating blocks, called segments, to be inserted into the PUR chain. Variation in these segments results in varying degrees of tensile strength and elasticity. Blocks providing rigid crystalline phase and containing the chain extender are referred to as hard segments (Fried, 1995). Those yielding an amorphous rubbery phase and containing the polyester/polyether are called soft segments. Commercially, these block polymers are known as segmented PUR (Young and Lovell, 1994).

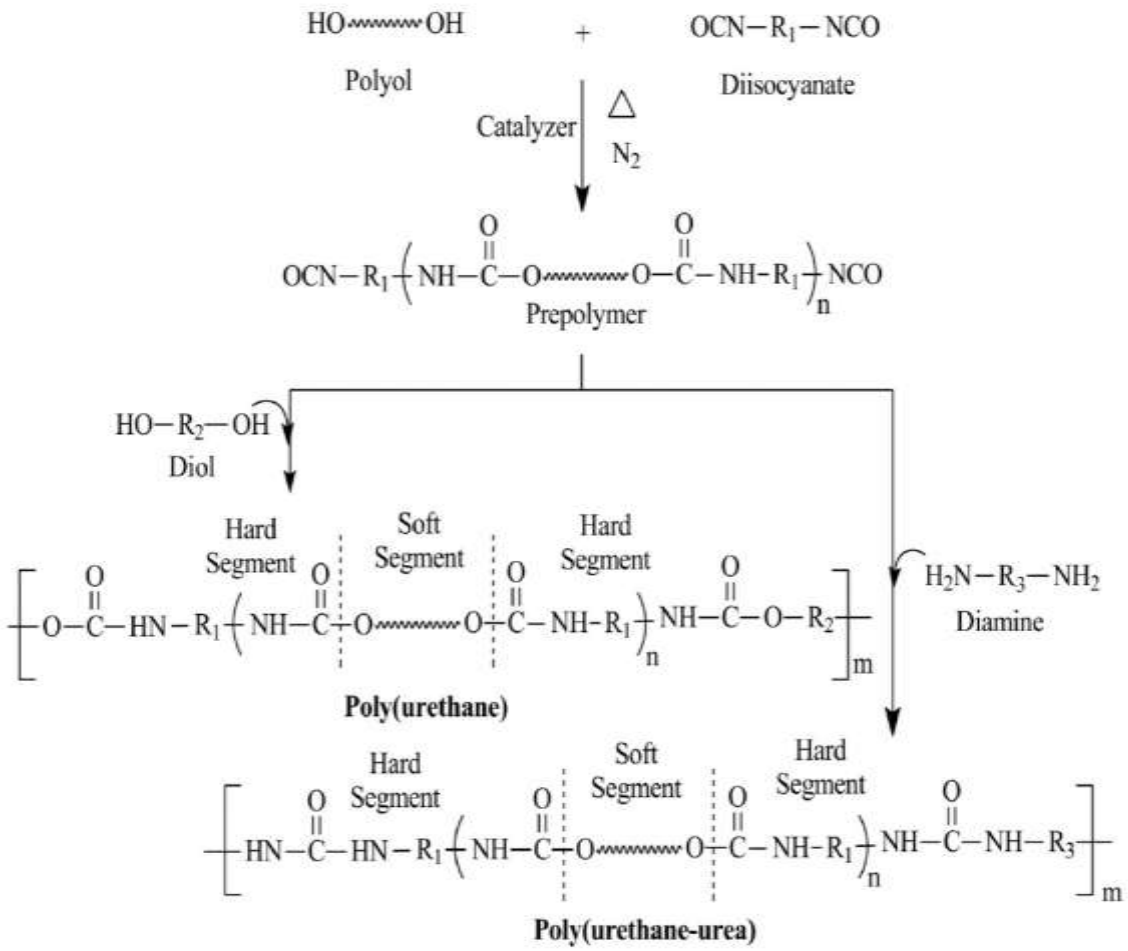


Figure 2.1: Synthesis of PUR and polyurethane-urea

Source: Juan *et al*., 2013.

2.4 Disposal of plastics and hazards

The plastic packaging materials are strong, lightweight, easily processable, energy efficient and inert in nature. They remain unaffected once disposed of and thus not compatible with the environment. Because of their durability and visibility in litter, plastics (polymers) have attracted more public and media attention than any other component of the solid waste stream. The hazard of discarding waste plastic, so-called ‘‘white pollution’’, is becoming more and more severe (Shah, 2007). Plastic waste is released during all stages of production and post-consumption; and disposed of through landfilling, incineration and recycling. Both the quantity and quality of plastic waste cause environmental problems. Thousands of tons of waste polyurethane products are disposed of in landfills and incinerators every year throughout the world, which causes significant environmental and resource depletion problems (Zheng *et al*., 2005).

The persistence in landfills is adding to the growing water and surface waste litter problems, which has raised concerns about non-degradable products and promoted increased interest in the development of new alternatives for the reduction of waste (Kawai, 1995). Improperly disposed plastic materials are a significant source of environmental pollution, as plastics (e.g. PUR) play important role for many ‘‘short live’’ applications such as packaging and these represent the major part of plastic waste, potentially harming life. The plastic sheets or bags do not allow water and air to go into earth which causes infertility of soil, preventing degradation of other normal substances, depletion of underground water source and danger to animal life (Muller *et al*., 2001; Shah, 2007). Municipal waste cannot be incinerated because of the presence of plastic in it and open burning releases pollutants into the air that could cause various health problems e.g. the burning of polyurethanes produce toxic irritant products and lead to immune and enzyme disorders, thus are classified as possible human carcinogens (Shah, 2007).

2.4.1 Health hazards

In many countries, plastics are disposed of through open, uncontrolled burning and landfilling. Open burning releases pollutants into the air that could cause various

health problems, in addition, the burning of polyvinylchloride (PVC) plastics produces persistent organic pollutants known as furans and dioxins and that of PUR produces toxic irritants products. These pollutants circulate globally and have been associated with a number of adverse effects in humans, including immune and enzyme disorders and chloracne, and they are classified as possible human carcinogens (Shah, 2007).

Health may be affected by the polymer itself, by chemicals added to the plastic to make it more flexible, stable or flame retardant, or by colouring agents. These substances may also be released to the air when the plastics are heated. When plastics are heated to form final products, monomers, additives and degradation products can be released; small amounts of these may also be present in the resins before heating. They can affect the health of the workers who use, clean or maintain the processing equipment (Jayasekara *et al*., 2005).

Despite the fact that plastics have been tested and found safe for use in a wide variety of products but the unhygienic use and disposal of plastics and its effects on human health has become a matter of concern. In most industrialized countries, coloured plastics have been legally banned because, these coloured plastics are harmful as their pigments contains heavy metals such as copper, lead, chromium and cadmium which are highly toxic (Shah, 2007).

2.4.2 Environmental hazards

Discarded, non-degradable polymers such as PUR show several undesirable environmental problems. These polymers create a threat to diverse animal populations. They have a direct impact on marine ecosystems and are believed to be responsible for the death of a very large number of birds by ingestion and strangulation (Scott, 1990). Polymers found in the ocean have a considerable effect on marine life, and if ingested cause intestinal blockages in small fish or suffocation of other marine animals (dolphins and turtles). The amount of litter at sea seems to be increasing despite control measures. It is estimated that one million tonnes of plastics are dumped in the sea annually. Litter is also a danger to terrestrial wildlife by tangling or by blocking digestion pathways (Whitney *et al*., 1993). Non-degradable polymers also have the

capacity to act as disease foci because they persist in the environment for a very long period of time enabling organisms to accumulate (Jayasekara *et al*., 2005).

2.5 Degradation of Polyurethane

Any physical or chemical change in polymer as a result of environmental factors, such as light, heat, moisture, chemical conditions or biological activity is termed degradation. The overview of this degradation pathway is shown in Figure 2.2. After years of production of PURs, manufacturers found them to be susceptible to degradation (Howard, 2002). Variations in the degradation patterns of different samples of PURs were attributed to the many properties of PURs such as topology and chemical composition (Pathirana and Seal, 1983). The regularity in synthetic polymers allows the polymer chain to pack easily, resulting in the formation of crystalline regions. This limits accessibility of the polymer chains to degradative agents.

Photodegradation means the degradation of polymer by light. The photodegradation is first initiated by the absorption of light energy by the appropriate group present in the polymer molecule. The initial act of light absorption results in the scission of the polymer material at an appropriate position of the chain leaching to smaller fragments (Shah, 2007). These fragments eventually mix with dust or the smaller volatile fragments escape to atmosphere; so photodegradable polymers require an in-built photo responsive group in the chain or an additive.

Thermal degradation means degradation of polymer by heat energy. It generally receives support from oxygen of the atmosphere, and is therefore, known as thermo-oxidative degradation. The primary act in this process is the rupture of bonds of the macromolecules resulting in radical sites. These radical sites react with oxygen present in the air to form peroxy radicals. Thus, again the long chain polymer molecules are converted into smaller fragment and volatiles.

Biodegradation follows a different path. Since most of the synthetic polymers are resistant to microbial attack, biodegradation of polymers may be achieved by two major paths viz. design of a polymer from monomers which are vulnerable to

microorganisms and incorporation of biodegradable additives or groups in the polymer (http:// www.envis-icpe.com). According to Martens and Domsch (1981), the microbial degradation of synthetic polymers has been studied in the past from two points of view; the first concerns the destruction of plastic materials by microbial degradation which could affect the intended use of the polymers and the second concerns the important necessity of incorporating waste polymers into the biocycle by the action of microorganisms. So far, information from these extensive studies indicates that majority of plastics produced are largely resistant to microbial attack and hence cannot be recycled into the environment while some can be attacked by microorganisms but the degradation is slow and superficial (Martens and Domsch, 1981).

Biodegradation or biological degradation consists of those processes resulting from the attack of a determined compound by a live organism, for example, bacteria, fungi, insects and rodents. However, this term is usually used only for degradation caused by microorganisms (Cangemi *et al.*, 2008). Plastics biodegradation can be profoundly affected by the conditions of the environment, the presence of additives in its composition and the kind of polymer. The action of microorganisms in polymeric materials can occur in three different forms:

- A biophysical effect, in which cellular growth can be caused by the loss of mechanical resistance in polymers;
- A biochemical effect, in which substances produced by microorganisms can act on the polymers; and
- A direct enzyme action, in which microbial enzymes attack the components of plastic products, promoting oxidative breaks in the polymeric chain (Cangemi *et al.*, 2008).

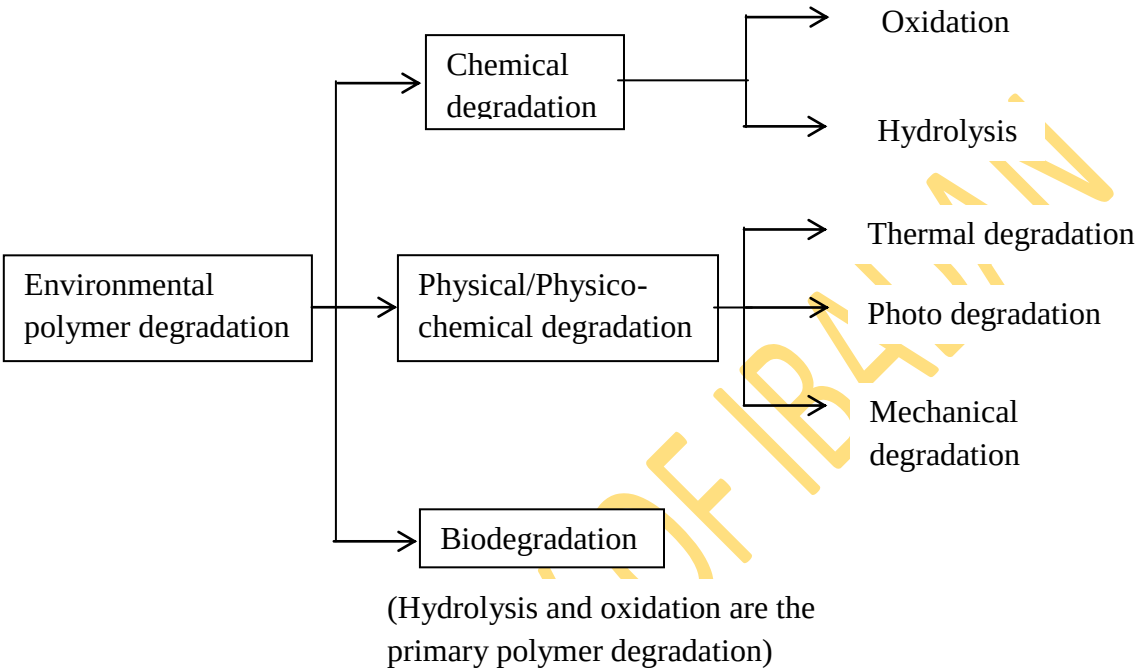


Figure 2.2: Overview of degradation of polymers
Source: Arutchelvi *et al*., 2008

2.6 Biodegradation of Polyurethane

Microbial degradation of PUR is dependent on the many properties of the polymer, such as molecular orientation, crystallinity, cross-linking and chemical groups present in the molecular chains which determine accessibility to degrading enzyme systems (Howard, 2002). In fact, biological degradation of polymers is generally influenced by a number of factors. Besides its nature as polymeric substance, the kind of organisms involved in biodegradation and the environmental conditions such as nutrient supply, temperature and pH are known to drastically influence their degradation rate. Darby and Kaplan (1968) first reported the biodegradation of PUR and found out that the polyester-type PUR were more degradable than the polyether-type PUR. Since then, a number of fungi have been isolated and characterized in terms of their ability to degrade polyester PURs but only a few reports on bacteria that degrade this material have been published (Nakajima-Kambe *et al*., 1995). In all these cases, however, the fungi could degrade polyester-type PUR only if provided with additional carbon sources. Both PUR-degrading fungi (Barrat *et al*., 2003; Sabev *et al*., 2006) and bacteria (Kay *et al*., 1991; Howard *et al*., 1999) have been isolated from soil buried PUR, indicating that there are potential reservoirs of PUR-degrading organisms widespread in the environment.

Biodegradation according to Wiley *et al.* (2008) has at least three outcomes which include a minor change in an organic molecule leaving the main structure still intact; fragmentation of a complex organic molecule in such a way that the fragments could be reassembled to yield the original structure and complete mineralisation, which is the transformation of organic molecules to mineral forms. The biodegradation of polymeric materials includes several steps (Figure 2.3) and the process can stop at each stage. Nathalie *et al.*, (2008) reported the several steps to involve the following; the combined action of microbial communities, other decomposer organisms or/and abiotic factors which fragment the biodegradable materials into tiny fractions. This step is called biodeterioration (Eggins and Oxley, 2001; Walsh, 2001). Then the microorganisms secrete catalytic agents such as enzymes and free radicals which are able to cleave the polymeric molecules reducing progressively their molecular weight.

The process generates oligomers, dimers and monomers and the step is called depolymerisation. These molecules which are depolymerized are then being recognised by receptors of microbial cells and can go across the plasma membrane. The other molecules stay in the extracellular surroundings and can be the object of different modifications.

In the cytoplasm, the transported molecules integrate with the microbial metabolism to produce energy, new biomass, storage vesicles and numerous primary and secondary metabolites and this step is called assimilation. Concomitantly, some simple and complex metabolites may be excreted and reach the extracellular surroundings (e.g. organic acids, aldehydes, antibiotics). Simple molecules as CO_2, N_2, CH_4, H_2O and different salts from intracellular metabolites that are completely oxidised are released into the environment. This is the mineralisation stage.

Two processes have been found to increase the activity of microorganisms during bioremediation/biodegradation which are biostimulation and bioaugmentation. Biostimulation involves the addition of nutrients and/or a terminal electron acceptor to increase the scant activities of indigenous microbial populations while bioaugmentation involves the addition of external microbial strains (indigenous or exogenous) which have the ability to degrade target toxic molecules (Odokuma and Dickson, 2003; Li *et al.*, 2009). The term 'biodegradation' indicates the predominance of biological activity. 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.
The general mechanism of plastic biodegradation under aerobic conditions is as shown in Figure 2.4.

Factors affecting biodegradability

Arutchelvi *et al*. (2008) reported that biodegradability of polymer is essentially determined by the following important physical and chemical characteristics such as:

- Availability of functional groups that increases hydrophobicity

- Size, molecular weight and density of the polymer
- Amount of crystalline and amorphous regions
- Structural complexity such as linearity or presence of branching in the polymer
- Presence of easily breakable bonds such as ester or amide bonds as against carbon-carbon bonds
- Molecular composition (blend) and
- Nature and physical form of the polymer such as whether it is in the form of films, pellets, powder or fibres

2.6.1 Abiotic methods in degradation of polymers

Polymeric materials that are exposed to outdoor conditions such as weather, ageing and burying can undergo mechanical, light, thermal, and chemical transformations. These exposure changes the ability of the polymeric materials to be biodegraded. In most cases, 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 *et al*., 2006). It is necessary to study the involvement of these abiotic conditions in order to have better understanding of the durability and degradation of the polymeric materials.

2.6.1.1 Mechanical

Mechanical degradation can take place due to compression, tension and/or shear forces. The causes of these forces are numerous. It includes a range of constraints during material installation, ageing due to load, air and water turbulences and bird damages. Frequently, at the macroscopic level, damages are not visible immediately but at the molecular level degradation could have started. Mechanical factors are not predominant during biodegradation process, but mechanical damages can activate it or accelerate it (Briassoulis, 2005). In field conditions, mechanical stresses act in synergy with the other abiotic parameters including temperature, solar radiations and chemicals.

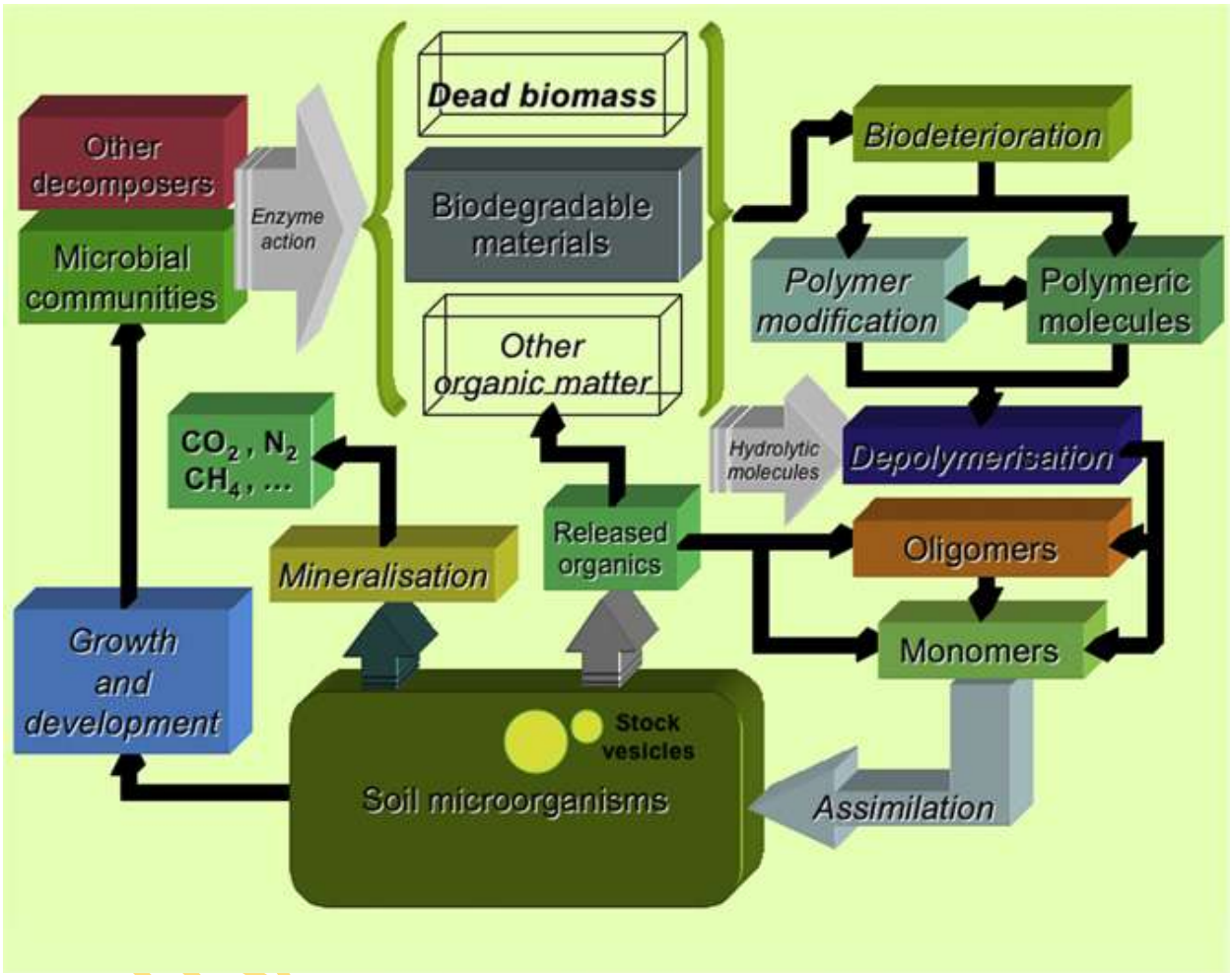


Figure 2.3: Polymer biodegradation scheme.
Source: Nathalie *et al.*, 2008.

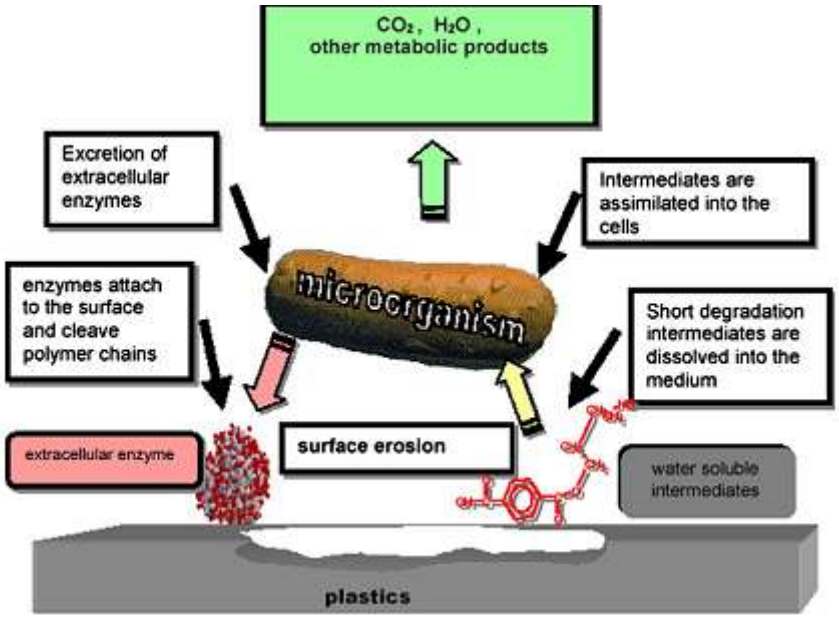


Figure 2.4: General mechanism of plastic biodegradation under aerobic conditions
Source: Muller, 2003.

2.6.1.2 Light

Several materials are photosensitive. The energy carried by photons can create unstable states in various molecules. Energy transfer can be accomplished by photoionization, luminescence, fluorescence and thermal radiation. Sometimes, involuntarily, the resistance of the material can be affected by impurities that are present in manufactured products. In other cases, photosensitive molecular structures are added intentionally such as by simple addition or copolymerisation into the polymer framework to induce a macromolecular degradation by light.

For example, prooxidants agents that can be activated depending on the light intensity and time of exposure can be added into a polymer (Kounty *et al*., 2006; Wiles and Scott, 2006). This strategy is used by polyolefin manufacturers to enhance degradability of plastic bags, packaging and agricultural films (Weiland *et al*., 1995; Schyichuk *et al*., 2001). The action of light radiation is one of the most important parameters in abiotic degradation.

2.6.1.3 Thermal

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 exhibit melting temperatures near to environmental conditions, this is the case for the thermophile stage of composting. Otherwise, temperature may influence the organisation of the macromolecular framework. Biodegradable polymers possess amorphous and crystalline regions (Wyart, 2007). Structural changes take place at their glass transition temperature (Tg), and the mobility and volume of the polymeric chains are modified. Above Tg (rubbery state), the disorganisation of chains facilitate the accessibility to chemical and bio-logical degradations (Iovino *et al*., 2008).

2.6.1.4 Chemical

Chemical transformation is the other most important parameter to light in the abiotic degradation. Atmospheric pollutants and agrochemicals may interact with polymers

changing the macromolecule properties (Briassoulis, 2005). Among the chemicals provoking the degradation of materials, oxygen is the most powerful. 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 such as unsaturated links and branched chains. These oxidations can be concomitant or synergic to light degradation to produce free radicals such as peroxyl radicals resulting from the oxidative degradation leading to crosslinking reactions and/or chain scissions (Nathalie *et al*., 2008).

Hydrolysis is another way by which polymers can undergo chemical degradation (Muller *et al*., 1998; Tsuji and Ikada, 2000; Yi *et al*., 2004). To be split by water, the polymer must contain hydrolysable covalent bonds such as in groups' like ester, ether, anhydride, amide, carbamide (urea) and ester amide (urethane). Hydrolysis is dependent on parameters such as water activity, temperature, pH and time. Oxidative and hydrolytic degradations on a given material are more easily performed within disorganized molecular regions (amorphous domains) whereas organised molecular frameworks (crystalline domains) prevent the diffusion of oxygen and water, limiting in this way the chemical degradation.

2.6.2 Biological degradation and biodeterioration in polymers

Deterioration is a superficial degradation that modifies mechanical, physical and chemical properties of a given material. The biodeterioration process is mainly the result of the activity of microorganisms growing on the surface or/and inside a given material (Hueck, 2001; Walsh, 2001). Microbial development depends on the constitution and the properties of polymer materials; the specific environmental conditions such as humidity, weather and atmospheric pollutants are also important parameters (Lugauskas *et al*., 2003).

The biodeterioration of thermoplastic polymers could proceed by two different mechanisms, i.e., bulk and surface erosion (von Burkersroda *et al*., 2002; Pepic *et al*., 2008). In the case of bulk erosion, fragments are lost from the entire polymer mass and

the molecular weight changes due to bond cleavage. This lysis is provoked by chemicals e.g. acids, bases, transition metals and radicals or by radiations but not by enzymes. While in surface erosion, matter is lost but there is no change in the molecular weight of polymers of the matrix. If the diffusion of chemicals throughout the material is faster than the cleavage of polymer bonds, the polymer undergoes bulk erosion but if the cleavage of bonds is faster than the diffusion of chemicals, then it is surface erosion as the process occurs mainly at the surface of the matrix (von Burkersroda *et al*., 2002; Pepic *et al*., 2008).

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 that works in synergy provokes serious damages on different materials (Flemming, 1998; Gu, 2007). 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 have shown 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 the urban air on several polymer materials. These adsorbed pollutants may also favour the material colonisation by other microbial species (Tharanathan, 2003; Faÿ *et al*., 2007). Microorganisms act by physical/mechanical, chemical and/or enzymatic means (Gu, 2003).

2.6.2.1 Physical means

Microbial species can adhere to material surfaces due to the secretion of a kind of glue which is a complex matrix made of polymers (e.g. polysaccharides and proteins) (Capitelli *et al*., 2006). This slime matter infiltrates porous structures and alters the size and the distribution of pores and changes moisture degrees and thermal transfers. The function of the slime matrix is to protect microorganisms against unfavourable conditions (e.g. desiccation and UV radiations). Bonhomme *et al*. (2003) reported that

filamentous microorganisms develop their mycelia framework within the materials, causing penetration; increase in the pore sizes and provoke cracks, thus, weakening the resistance and durability of the material.

2.6.2.2 Chemical means

The extracellular polymers produced by microorganisms can act as surfactants that facilitate the exchanges between hydrophilic and hydrophobic phases. These interactions favour the penetration rate of microbial species. Moreover, according to Warscheid and Braams (2000) and Zanardini *et al*. (2000) the presence of this slime on the polymer increases the accumulation of atmospheric pollutants and this accumulation favours the development of microorganisms and accelerates the biodeterioration.

Chemolithotrophic bacteria use inorganic compounds such as ammonia, nitrites, hydrogen sulphide, thiosulphates and elementary sulphur as energy and electron sources (Regnault, 1990). They can release active chemicals as nitrous acid e.g. *Nitrosomonas* spp., nitric acid e.g. *Nitrobacter* spp. or sulphuric acid e.g. *Thiobacillus* spp. (Warscheid and Braams, 2000; Roberts *et al*., 2002; Crispim and Gaylarde, 2005; Rubio *et al*., 2006).

Chemoorganotrophic microorganisms use organic substrates as carbon, energy and electron sources (Alcamo, 1998). They release organic acids as oxalic, citric, gluconic, glutaric, glyoxalic, oxaloacetic and fumaric acids (Jenings and Lysek, 1996). Succinic acid, adipic acid, lactic acid and others, as well as, butanediol are released by abiotic and/or biotic hydrolysis of several polymers (Göpferich, 1996; Lindström *et al*., 2004; Trinh Tan *et al*., 2008). Water enters in the polymer matrix, which might be accompanied by swelling. The intrusion of water initiates the hydrolysis of the polymer, leading to the creation of oligomers and monomers. Progressive degradation changes the microstructure of the matrix due to the formation of pores, then oligomers and monomers are released. Concomitantly the pH inside the pores is modified by the degradation products, which normally have some acid–base characteristics (Göpferich, 1996). These acids have various ways of action; some can react with components of

the material and increase the erosion of the surface (Lugauskas *et al*., 2003). Organic acids released by some microorganisms are considered as one of the main causes of biodeterioration (Warscheid and Braams, 2000). Also, some microorganisms as filamentous bacteria and fungi are able to use these organic acids as carbon sources to extend their mycelia framework (Hakkarainen *et al*., 2000).

2.6.2.3 Enzymatic means

Some materials considered as recalcitrant polymers (e.g. polyurethane, polyvinylchloride and polyamide) are nevertheless subject to microbial biodeterioration (Shimao, 2001; Howard, 2002; Szostak-Kotowa, 2004; Shah *et al*., 2008). The microbial vulnerability of these polymers is attributed to the biosynthesis of lipases, esterases, ureases and proteases (Flemming, 1998; Lugauskas *et al*., 2003). Enzymes involved in biodeterioration require the presence of cofactors (i.e. cations present into the material matrix and coenzymes synthesised by microorganisms) for the breakdown of specific bonds.

2.6.3 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. The energy to accomplish scissions of the polymer bonds may be of different origins namely thermal, light, mechanical, chemical and/or biological. Microorganisms use different ways to cleave polymers such as secretion of specific enzymes or generation of free radicals. Enzymes are catalytic proteins that decrease the level of activation energy of molecules favouring chemical reactions. These proteins have a wide diversity and a remarkable specificity, but they are easily denatured by heat, radiations, and surfactants (Nathalie *et al*., 2008).

Endopeptidase, endoesterases accomplish their catalytic action along the polymer chain whereas exoenzymes catalyse reactions principally at the edges. Biofragmentation can occur through enzymatic hydrolysis, enzymatic oxidation and radicalar oxidation.

2.6.4. Assimilation

The 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) for the formation of the cell structure. Assimilation allows microorganisms to grow and to reproduce while consuming nutrient substrate such as polymeric materials from the environment (Nathalie *et al*., 2008). Naturally, assimilated molecules may be the result of previous (bio) deterioration and/or (bio) fragmentation.

Monomers surrounding the microbial cells must go through the cellular membranes to be assimilated. Some monomers are easily brought inside the cell through specific membrane carriers while 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 the cells, transported molecules are oxidised through catabolic pathways conducing to the production of adenosine triphosphate (ATP) and constitutive elements of cells structure. Frequently, these molecules can be used as carbon sources by other organisms, since they still have a reduction power and generally, mineral molecules released by microorganisms do not represent ecotoxicity risk as they follow the biogeochemical cycles.

2.7 Ways of decreasing Polyurethane degradation

Kanavel *et al*. (1996) initiated a research to elucidate whether additives to the chemical structure of PURs could decrease biodegradation and observed that sulphur-cured polyester and polyether PURs had some fungal inertness. However, they noted that even with fungicides added to the sulphur- and peroxide-cured PURs, fungal growth still occurred on the polyester PURs and most fungicides had adverse effects on the formulations. They also recognized the need for physical testing of the PURs after extended exposure to the activity of fungi. Santerre *et al*. (1994) varied the amount of degradation products released by varying the physical makeup of the polyester PURs, as coatings on glass tubes or as films. This implied that while urethane and urea groups are susceptible to hydrolysis, they are not always accessible to the enzyme and

degradation may never proceed past the polymer surface. Although the polyether PURs showed no significant degradation, they consistently showed higher radiolabel products release from soft-segment-labelled, enzyme-incubated samples than controls. The authors attributed these results to the shielding of ester sites by secondary structures and hydrogen bonding within the hard segment.

Santerre and Labrow (1997) tested the effect of hard segment size on the stability of PURs against cleavage. Analysis was performed with polyether PURs and their susceptibility to cholesterol esterase and the investigators concluded that an increase in hard segment size does lead to restrictions in polymer chain mobility. In the medical field, PURs show resistance to macromolecular oxidation, hydrolysis and calcification (Marchant, 1992). PUR elastomers are being used in place of other elastomers due to higher elasticity and toughness, and resistance to tear, oxidation and humidity (Dombrow, 1957; Saunders and Frisch, 1964; Ulrich, 1983). In addition, polyether derivatives are inexpensive to produce as prepolymers, which can lower the overall cost of polymer production (Howard, 2012). Huang and Roby (1986) tested the biodegradability of polyamide-urethanes for medical purposes. They synthesized PURs with long repeating units and alternating amide and urethane groups from 2-aminoethanol. The resulting partial crystalline fibres were observed to undergo hydrolysis less readily than polyamide esters with degradation proceeding in a selective manner. The amorphous regions on the PUR were being degraded prior to the crystalline regions. These fibres showed promise as absorbable sutures and implants where in vivo degradation is needed. The investigators also noted that PURs with long repeating units and hydrophilic groups would less likely pack into high crystalline regions as normal PURs, and these polymers were more accessible to biodegradation.

Tang *et al*. (1997) added surface-modifying macromolecules (SMM) containing fluorinated end groups to the base PUR to reduce the material's susceptibility to hydrolysis by lysosomal enzymes. Synthesized polyester urea-urethanes were radiolabeled with [14C] and coated onto small hollow tubes and biodegradation experiments were carried out. Results indicated that degradation was inhibited by the SMM surface. Different SMM formulations provided varying degrees of enzyme

resistance. It was noted that some SMM formulations were incompatible with the PUR and led to increased biodeterioration. In an attempt to increase biocompatibility and reduce bacterial adhesion on PURs surfaces, Baumgartner *et al*. (1997) synthesized phosphonated PURs. They used glycerophosphorylcholine (GPC) as the chain extender, which incorporated phosphorylcholine head groups into the PUR backbone. This gave the PUR surface some characteristics of a red blood cell surface. Physical tests on the PUR showed a small decrease in tensile strength and transition temperature with increasing GPC concentration. Water absorption by the PUR was increased with increased GPC content. To test bacterial adhesion to the PU, Baumgartner *et al*. (1997) used a radial flow chamber. They passed a culture of *Staphylococcus aureus* across phosphonated and unphosphonated PUR at a rate of 8 ml min−1. The phosphonated PUR showed a decrease in bacterial adhesion with increased GPC content.

2.8 Ways of increasing polyurethane degradation

Lack of degradability and increasing depletion of landfill sites as well as growing water and land problems have led to concern about plastics (Kawai, 1995). As more and more raw materials (e.g. crude oil) become in short supply for the synthesis of plastics, recycling of waste plastics has thus become important (Schnabel, 1981). Degradability problems promoted researchers to investigate modification or productions that led to either chemically degradable or biodegradable PURs.

Huang *et al*. (1981) derived polyester PURs from polycaprolactonediols in an effort to produce biodegradable PURs for use in the medical field. Several different PURs were made containing polyester subunits of various lengths. The polymers were subjected to degradation by an enzyme and two species of fungi. The enzyme and fungi degraded each PUR. In addition, it was also noted that there was an increase in the biodegradability of the polyester PURs with increase in the chain length of the polyesters.

Labrow *et al*. (1996) treated polyester PUR and polyether PUR with human neutrophil elastase and porcine pancreatic elastase. The polyester PUR was readily degraded by porcine pancreatic elastase at a rate 10 times higher than by human neutrophil elastase.

The rate of polyester PUR degradation by porcine pancreatic elastase was also 10 times higher than its activity against the polyether PUR but human neutrophil elastase had no significant activity against the polyether PUR.

2.9 Fungal biodegradation of PUR

Studies by Darby and Kaplan (1968) and Kaplan *et al*. (1968) reported the susceptibility of polyester PURs to fungal attack. Boubendir (1993) isolated enzymes with esterase and urethane hydrolase activities from the fungi *Chaetomium globosum* and *Aspergillus terreus*. These organisms did not grow solely on PUR and the enzymes had to be induced and the induction of the enzymes was accomplished by addition of liquid polyester PUR to the growth media. Crabbe *et al*. (1994) in their study, isolated four species of fungi, *Curvularia senegalensis*, *Fusarium solani*, *Aureobasidium pullulans* and *Cladosporium* sp. based on their ability to utilize a colloidal polyester PUR (Impranil DLN) as the sole carbon and energy source. They found that *Curvularia senegalensis* had a higher PUR-degrading activity. Russel *et al.* (2011) in their study, isolated endophytic fungi from plant stems collected in the Ecuadorian rainforest and screened for their ability to degrade polyurethane. They reported two isolates of *Pestalotiopsis microspora* with the ability to efficiently degrade and utilize PUR as the sole carbon source when grown anaerobically- a unique observation among reported PUR biodegradation activities.

In their study, Cosgrove *et al.* (2007) reported the isolation of soil fungal communities with the ability to degrade PUR under different environmental conditions. They however, observed more percentage of cultivable fungi from the acidic and neutral soil environment as putative PUR degraders than in the laboratory soil microcosm. Cosgrove *et al.* (2010) reported the use of putative PUR degrading fungi namely; *Penicillium ochrochloron*, *Geomyces pannorum*, *Penicillium viridicatum*, *Penicillium inflatum* and *Nectria hematococca* isolated by Cosgrove *et al.* (2007) for bioaugmentation on degradation of PUR buried in soil.

2.10 Bacterial biodegradation of PUR

In a large-scale test of bacterial activity against PURs, Kay *et al*. (1991) investigated the ability of 16 bacterial isolates to degrade polyester-PUR. Seven of the isolates tested degraded PUR when the media was supplemented with yeast extract while two isolates *Corynebacterium* sp. and *Pseudomonas aeruginosa* could degrade PUR in the presence of basal media. However, none of the isolates grew on PUR alone. Physical tests of the degraded polyester PUR revealed different but significant decreases in tensile strength and elongation for each isolate. In a further study, Kay *et al*. (1993) tested the chemical and physical changes in degraded polyester PUR. PURs taken from *Corynebacterium* sp. cultures had significant reductions in both tensile strength and elongation after three days of incubation. Infra-red spectrophotometer analysis revealed the ester segment of the polymer to be the main site of attack.

Halim El-Sayed *et al*. (1996) tested the growth of several species of bacteria on PUR military aircraft paint. The investigators isolated *Acinetobacter calcoaceticus*, two *Pseudomonas* sp., *Pseudomonas cepacia*, and *Arthrobacter globiformis*. In addition, the U.S. Navy supplied two strains of *A. calcoaceticus*, *Pseudomonas aeruginosa* and *Pseudomonas putida*. All species were capable of utilizing the polyurethane paint as a sole carbon and energy source with the exception of *P. cepacia*. In an additional study, Nakajima-Kambe *et al*. (1995) isolated *Comamonas acidovorans* strain TB-35 from soil samples by its ability to degrade polyester PUR. Solid cubes of polyester PUR synthesized with various polyester segments were completely degraded after seven (7) days incubation when they were supplied as the sole carbon source and degraded 48% when they were the sole carbon and nitrogen source. Analysis of the breakdown products of the PUR revealed that the main metabolites were from the polyester segment of the polymer. Further analysis of strain TB-35 revealed that the degradation products from the polyester PUR were produced by an esterase activity (Nakajima-Kambe *et al*., 1997).

Most reports in the literature on the degradation of PUR have focused on fungal attack with few studies addressing bacterial degradation of these polymers. Blake and Howard (1998) reported bacterial degradation of a polyester PUR (Impranil DLN) by a

species of *Bacillus*. The pattern of degradation involved the binding of cells to the polymer with subsequent floc formation, and the degradation of substrate. Several members of the genus *Pseudomonas* have been isolated for their ability to utilize polyester PUR as the sole carbon and energy source (Howard, 2002). Nakajima-Kambe *et al*. (1995; 1997) reported a strain of *Comamonas acidovorans* that could utilize solid polyester PUR as the sole carbon and nitrogen source. These authors indicated the role of an extracellular membrane bound esterase activity in PUR degradation.

The biodegradation is a natural complex phenomenon. Nature-like experiments are difficult to realise in laboratory due to the great number of parameters occurring during the biogeochemical recycling. Actually, all these parameters cannot be entirely reproduced and controlled in vitro. Particularly, the diversity and efficiency of microbial communities (e.g. the complex structure of microbial biofilm) and catalytic abilities to use and to transform a variety of nutrients cannot be anticipated. Nevertheless, biodegradability tests are necessary to estimate the environmental impact of industrial materials and to find solutions to avoid the disturbing accumulation of polymers. The augmentation of derived biodegradability tests, developed by different research groups (Pagga *et al*., 2001; Rizzarelli *et al*., 2004; Wang *et al*., 2004; Kim *et al*., 2006), has conduced to confused interpretations about biodegradation mechanisms. To compensate for this problem, it is necessary to explain the different phenomena involved in biodegradation (i.e. biodeterioration, biofragmentation and assimilation). In addition, each biodegradation stage must be associated with the adapted estimation technique.

For instance, abiotic degradation and biodeterioration are mainly associated to physical tests (e.g. thermal transitions and tensile changes). Biofragmentation is revealed by the identification of fragments of lower molecular weight (i.e. using chromatographic methods). Assimilation is estimated by the production of metabolites or the development of microbial biomass (e.g. macroscopic and microscopic observations). The unique proof that a polymer is consumed by microorganisms is the release of carbon dioxide. Naturally, this method is suitable if the polymer is the sole carbon

source into the media. However, in soil, in compost or any other complex matrix, this test is unsuitable because the released carbon dioxide may come either from the polymer, or from the matrix, or from both (Nathalie *et al*., 2008).

CHAPTER THREE
MATERIALS AND METHODS

3.1. Sample collection and handling

Polyurethane (PUR) samples "Take away packs" were randomly collected from open air domestic refuse sites of Students' canteens and Staff homes in the University of Ibadan, Ibadan, Nigeria as well as two Fast foods outlets wastebins (Tantalizers, Mr. Biggs') located in Bojida area of Ibadan. These packs were transported in plastic containers to the Environmental and Biotechnology Laboratory of the Department of Microbiology, University of Ibadan, Nigeria, for microbiological analysis.

3.2 Sample preparation and burial

The packs were washed to remove the oils and spread out to air dry. After drying, they were divided into two sets- one set for the laboratory and pilot field study and the other set were taken to the field- fallow land with no farming activity for burial within the Department of Microbiology, University of Ibadan, Nigeria. Different burial points of different depths were made in the soil using digger and shovel and meter rule to take measurements. The area used for this study was 5 m x 5 m and five depths used were 15, 30, 45, 60 and 70 cm respectively. The surface area of each depth was 45 cm x 45 cm and the packs were placed at the bottom of each depth and closed back with soil to level and allowed to stay for two years. The burial points were marked and pegs placed for identification. The packs were exhumed at different time intervals of three, five, seven, twelve and twenty-four months for microbial isolation.

The set for the laboratory and pilot field study was also divided into two parts; one part was cut into small cubes using scissors and the second part was blended using electric home blender (Germatic YT-1831) and these packs were prepared in these ways so as to

increase the surface areas for enhancement of microbial adhesion to the PUR packs.

3.3. Sterilization of apparatus and media

All the glassware used for the experiments were thoroughly washed with detergent and rinsed with tap water. They were allowed to air dry before sterilizing in the oven at 160°C for 3 hrs. All the media employed (Appendix 1) were prepared and sterilized by autoclaving at 121°C and 1.05 g/cm^{2}Hg for 15 minutes.

3.4. Isolation of microorganisms

The buried PUR samples were exhumed at different time intervals as listed (Section **3.2)** for microbial isolation. At each time interval, the samples were exhumed, cut into small cubes and placed in 500 ml flasks and washed in three changes of sterile water (400 ml) with vigorous shaking to remove soil particles. After this, the samples were divided into two parts; one part was plated out directly while the second part was surface sterilized with 70% ethanol and finally rinsed with sterile water before plating out. After these sample preparations, a Mineral Salts Medium (MSM) as described by Urgun-Demirtas *et al.* (2007) containing K_2HPO_4, KH_2PO_4, NH_4NO_3, Glucose, $MgSO_4 \cdot 7H_2O$; $FeSO_4.7H_2O$; $ZnSO_4.7H_2O$; $CuSO_4.7H_2O$; $MnSO_4.7H_2O$, and Agar (Appendix 1) was used for both bacteria and fungi isolation employing the pour plate technique.

The plates were incubated at a temperature of approximately 28°C ± 2°C and monitored daily for microbial growth.

3.5. Screening for bacteria isolates utilising Polyurethane

The MSM by Urgun-Dermitas *et al*., (2007) was used for the PUR-utilising microbial screening. Microbial isolates obtained from above (Section **3.4**) were subjected to screening to select organisms that will utilize PUR as a sole carbon and/or nitrogen sources.

To screen for isolates that will utilise PUR as sole carbon source, the glucose component of the MSM (Appendix 1) was removed and replaced with PUR and the isolates were streaked on the media. For isolates that will utilise PUR as sole nitrogen source, the ammonium nitrate (NH_4NO_3) component of the MSM was removed and replaced with PUR and the isolates were streaked on the media. For isolates that will utilise PUR as sole carbon and nitrogen sources, both the glucose and NH_4NO_3 components of the MSM were removed and the quantities replaced with PUR and the isolates were streaked on the media. They plates were incubated at a temperature of approximately 28^{o}C ± 2^{o}C and monitored daily for growth rate.

Isolates that grew on the modified MSM using PUR as both carbon and nitrogen sources were selected for further studies and used for the degradation studies.

3.6. Culture preservation

Selected isolates were picked and streaked onto already prepared Nutrient agar (LabM®, IDG diagnostics, UK) plates to monitor their growth pattern and morphological characteristics. The pure cultures of the bacterial isolates were preserved in nutrient agar slants and put in the refrigerator until they are ready for use. The stock culture was prepared in duplicates, one serving as working culture while the other was the master stock.

3.7. Characterization of bacterial isolates

Pure cultures of the selected bacteria isolates were characterized and identified using various morphological and biochemical tests according to Probabilistic Identification of Bacteria Windows (PIBWin) software (Bryant, 2004) and Advanced Bacterial Identification Software (ABIS, 2012).

3.7.1 Morphological and biochemical characterisation tests

The selected bacteria were subjected to different morphological and biochemical tests to determine their probable identities.

3.7.1.1 Gram's staining reaction

Isolates reaction to Gram's dyes were determined as described by Skerma, (1967). Cells from 18 hour old pure culture of isolates were emulsified in normal saline on clean grease free glass slides with an inoculating loop. It was heat fixed by passing it through the Bunsen burner/ spirit lamp several times. It was stained with Crystal violet dye for 60 seconds after which it was rinsed with sterile distilled water. Gram's iodine was added for 60 seconds and was rinsed with distilled water. Then 95% alcohol was used to decolourise the stain and was rinsed with sterile water. Finally, it was counter stained with Safranin dye for 60 seconds. Then the slides were rinsed with distilled water and blot dry with filter paper (Whatmann No.42). The stained slides were examined under oil immersion objective lens (x100) in a compound microscope for the cell morphology and cell's reaction to Gram's staining.

3.7.1.2 Catalase test

This test was carried out as described by Olutiola *et al*. (2000) to test ability of the isolate to decompose hydrogen peroxide (H_2O_2) by the production of catalase enzyme. Using an inoculating loop, 18 hour old bacterial cells from a pure culture was placed on clean grease free glass slide and mixed in a 3% hydrogen peroxide solution with a sterile glass rod. The observation of effervescence due to the release of oxygen was looked out for.

3.7.1.3 Motility test

This was carried out as described by Olutiola *et al*. (2000). Semi solid media of Nutrient agar (half strength) was prepared and dispensed into tubes. These were sterilised and allowed to set on slope. Each of the tubes was inoculated using a sterile inoculating needle to make a simple stab down the center of the tubes to about half the depth of the medium. The tubes were incubated for 48 hours and examined for diffuse growth from the line of stab.

3.7.1.4 Starch hydrolysis

Ability to breakdown starch was carried out as described by Olutiola *et al*. (2000). One gram of white soluble starch was added to 100 ml Nutrient Agar, the mixture was homogenized and sterilised. It was allowed to cool and dispensed into sterile petri dishes. Each isolate was streaked across the starch agar plate and incubated for 48 hours. Un-inoculated starch agar plate served as control. After the incubation period, the plates were flooded with Gram's iodine and observed for presence of clear zones.

3.7.1.5 Spore staining

Screening for endospore forming bacterial cells was determined using the Schaeffer-Fulton staining technique. A thin smear of bacterial cells from 18 hour old culture was made on a clean grease free glass slide. It was heat fixed by passing it through a Bunsen burner flame. Malachite green was used to flood the glass slide. It was kept over a beaker of boiling water equipped with a staining loop. The water was heated until the stain steams for 5 minutes. The stain was continuously added so that the slide did not dry up. Later, it was rinsed with water and finally counter stained with safranin for 30 seconds and rinsed with water. The slide was air dried and observed under the oil immersion objective of a compound microscope. The presence, position and shape of the bacterial spores were observed.

3.7.1.6 Oxidase test

The ability of the isolates to produce cytochrome oxidase was determined as described by Steel (1961). The oxidase reagent tetramethyl-p-phenylene diamine hydrochloride was used to soak filter paper (Whatman No.1). Inoculating loop was used to pick a colony of 24 hour old bacterial culture. It was smeared on the soaked filter paper and observed within 10 seconds for a positive reaction. A deep purple colouration shows positive for oxidase while a delayed colour change or no colour change was taken as negative.

3.7.1.7 Methyl-Red test

Isolates grown in glucose-phosphate broth consisting of 0.5g peptone; 0.5g glucose and 0.5g di-potassium hydrogen phosphate (K_2HPO_4) dissolved in 100 ml of distilled water for 48 hours were used for the test as described by Cheesebrough (1984). After 48 hours, 5 drops of methyl red solution were added to the broth and observed for colour change.

3.7.1.8 Voges-Proskauer test

This test was carried out as described by Barrit (1936). Isolates were cultured on glucose phosphate broth for 48 hours. One millilitre of the culture broth was dispensed into clear test tubes. To the broth culture, 0.5 ml of a 6% α-naphthol solution and 0.5 ml of potassium hydroxide (KOH) were added. The tubes were shaken and observed usually within 5 minutes for production of acetyl methyl-carbinol from the fermentation of glucose phosphate broth.

3.7.1.9 Indole test

This was carried out as described by Olutiola *et al*. (2000). Tryptone broth was prepared and introduced into screw capped tubes. The tubes were inoculated with a loopful of the test organism and incubated at 37^{o}C for 5 days. After 5 days, 3 ml of KOVAC's reagent was added to 6 ml of culture fluid and observed for about 10 minutes for colour change.

3.7.1.10 Citrate utilisation

This was carried out as described by Olutiola *et al*. (2000) using Simmon's citrate medium (consisting of NaCl- 5g, $MgSO_4.7H_2O$- 0.2g, $NH_4H_2PO_4$- 1g, K_2HPO_4- 1g, Citric acid- 2g, Distilled water-1000 ml, bromothymol blue-0.008% (i.e. 20 ml of 0.4% aqueous solution per litre) and Agar-2%). Each test tube of the sterilized medium was inoculated with a loopful of the bacterial isolates. The tubes were then incubated for 48 hours and afterwards observed for colour change. An un-inoculated citrate medium served as control.

3.7.1.11 Oxidation and Fermentation (O – F) test

This test was used to differentiate bacterial group that oxidise carbohydrate (aerobic utilisation) from others that ferment carbohydrate (anaerobic utilisation) and tested as described by Hughes and Leifson (1953). The medium's composition per 100 ml of distilled water: Peptone- 0.2g; NaCl- 0.5g; K_2HPO_4- 30mg; bromothymol blue- 0.1 ml; Agar- 1.5g. The carbohydrate component (sucrose) was prepared separately as 10% solution and sterilized for 10 minutes. The basal medium was dispensed into test tubes and sterilized for 15 minutes. The sterile carbohydrate solution was then added aseptically to the sterile basal medium at final concentration of 1% prepared in duplicates.

After inoculation, the surface of the medium in one tube was covered with sterile paraffin. The tubes were incubated and examined daily for up to 7 days. Fermentative organisms will produce acid in both tubes while oxidative organisms will produce acid only in tubes without paraffin.

3.7.1.12 Urease test

Ability of the isolates to secrete urease enzyme was determined. The medium (consisting of the following: Peptone- 0.1g, NaCl- 0.5g, KH_2PO_4- 0.2g, D(+) Glucose- 0.1g, Phenol red (0.2% in 50% ethanol)- 0.6 ml , Agar- 2.0g, Distilled water- 100 ml) was distributed into bottles and sterilized. The urea incorporated into the medium was filter sterilized and added when the temperature of the medium was approximately 45^{o}C to give a final concentration of 2% urea. The bottles were slanted and allowed to set in position. A 24 hour old culture was stabbed and also streaked over the surface of the slant and incubated. Colour change was observed to indicate the breakdown of urea to ammonia by the organisms. It was carried out as described by Olutiola *et al*. (2000).

3.7.1.13 Nitrate (NO_3) reduction

Many microorganisms are capable of reducing nitrate to nitrite or even further to hydroxylamine, ammonia or nitrogen. These end products are used by the organism for amino acid synthesis. Thus, an intermediary in the reaction is nitrite and the first test

applied is for its presence. If this proves to be negative, the medium is tested for residual nitrate by the addition of zinc dust. If the addition of zinc dust does not result in the development of colour, no nitrate remains and this will only mean that the nitrate has been reduced by the organism beyond the nitrite stage. The presence of gas in the Durham tube indicates the formation of gaseous nitrogen and therefore complete reduction of the nitrate. This was carried out as described by Barrow and Feltham, (2003).

The nitrate medium consisting of – 0.1g KNO_3, 1.5g Peptone and 100 mls Distilled water was distributed into test tubes, each with an inverted Durham tube and sterilize. A loopful of the 24 hour old bacteria culture was inoculated into the nitrate medium and incubated for 5 days at room temperature. At the end of the incubation period, the Durham tubes were examined for the presence of nitrogen gas and presence of nitrite was tested using Griess Ilosvay's reagent (8g sulphanilic acid in 11 5N acetic acid and 5g naphthylamine in 11 5N acetic acid) for colour change and addition of zinc dust for confirmation.

3.7.1.14 Production of hydrogen sulphide (H_2S)

Ability of isolates to reduce inorganic sulphur compounds to produce hydrogen sulphide was tested as described by Olutiola *et al*. (2000). Thiosulphate broth was prepared by adding 0.01% sodium thiosulphate into peptone water. Strips of filter paper soaked in saturated solution of lead acetate served as indicator paper for the presence of hydrogen sulphide gas. The strips were dried and sterilized. A loopful of a 24 hour old bacteria culture was used to inoculate the thiosulphate broth. The plug from the tubes was removed and the indicator paper strips were placed at the mouth of each tube. It was placed such that the strip was above the broth and tightly screwed to the plug. The un-inoculated control also contains the indicator paper. The tubes were incubated at 37^{o}C for 3-5 days examining the indicator paper for blackening.

3.7.1.15 Casein hydrolysis

Utilisation of casein as a source of nitrogen due to the presence of protease enzyme was determined according to Wiley *et al.* (2008). Ten grammes of skimmed milk were added

to 100 ml of Nutrient agar. It was homogenised in a hot water bath, sterilized and poured into sterile petri dishes. Twenty four hour old cultures of the isolates were streaked across each petri dish and incubated at 37^{o}C for 7 days. A test reagent, Frazier's reagent (containing 15% Mercuric chloride in 1N HCl) was used to flood the growing bacterial cells and observed for presence of clear zone around the isolates.

3.7.1.16 Gelatin hydrolysis

Gelatin agar (consisting of Gelatin- 4g; Nutrient agar- 28g; Distilled water- 1000 ml) was prepared, poured into plates and allowed to solidify. A 24 hour culture of the test isolate was inoculated on the gelatin agar plate and incubated for 3 days. After the incubation period, the plate was flooded with 5-10 ml acid mercuric chloride solution (Frazier's reagent) and observed for clear zone around the isolates. This was carried out as described by Barrow and Feltham (2003).

3.7.1.17 Arginine Dehydrolase

Arginine agar (consisting of peptone- 1.0g; NaCl- 5.0g; K_2HPO_4- 0.3g; 1.0% aq. Soln. Phenol red- 1.0 ml; L (+) arginine hydrochloride- 10.0g ; Agar- 3.0g; Distilled water 1000 ml) was prepared. Five ml of the solution was distributed into tubes and sterilised. A 24 hour old culture of the test isolate was stab-inoculated into arginine agar and 2 ml of sterile paraffin oil was pipetted onto the surface layer of the inoculated tube and incubated at 30°C for 5 days with daily observation for colour change. This was carried out as described by Barrow and Feltham, (2003).

3.7.1.18 Tween 80 hydrolysis

This was carried out as described by Barrow and Feltham, (2003). Tween 80 medium (consisting peptone-10 g; NaCl-5 g; $CaCl.2H_2O$-0.1 g; agar-20 g; distilled water-1000 ml) was prepared. Tween 80 was sterilized separately and added aseptically to the sterile medium to give a final concentration of 1% and then distributed into plates. A 24 hour old culture of the test isolate was streak-inoculated on the surface of Tween 80 medium and

incubated at 30^{o}C for 7 days. The plate was examined daily for the formation of an opaque halo precipitate around the isolate growth line.

3.7.1.19 Dnase activity

This was carried out as described by Barrow and Feltham, (2003). DNase agar (containing Tryptose- 20g, Deoxyribonucleic acid- 2g, NaCl- 5g, Toluidine blue- 0.1g, Agar- 20g, Distilled water- 1000 ml) was prepared and poured into petri dishes. Using a 24 hour old culture, spot-inoculation was made on the DNase plate and incubated for 2-3 days at 30^{0}C. After incubation, the plate was flooded with 1N HCl and observed for appearance of clearing around the colonies within 5 minutes.

3.7.1.20 Sugar Fermentation test

Isolates ability to metabolize a large variety of sugars as carbon source was determined. The sugar fermentation test was carried out as described by Olutiola *et al*. (2000). A culture broth containing 1% peptone, 0.1% NaCl and 1% fermentable sugar with phenol red indicator was used. 10 ml of the solution was dispensed into test tube. A Durham tube was inverted and inserted into each test tube and sterilized for 10 minutes. Each of the test tubes was inoculated with the isolate and incubated at 37^{o}C for up to 7 days observing daily for colour change and gas production in the Durham tube by presence of air bubble.
The sugars tested include monosaccharides- glucose, fructose, galactose; disaccharides- maltose, sucrose, lactose; ribose- xylose, arabinose, sorbose; sugar alcohol- mannitol, inositol, sorbitol and polysaccharides- raffinose.

3.8 Physiological studies for selected bacteria isolates

3.8.1 Determination of optimal growth temperature of the bacteria isolates

Test tubes containing 10 ml of MSM each were inoculated with loopful of 24 hour old culture of the bacteria isolates and incubated separately at 25°C, 30°C, 37°C, 42°C and 50°C, respectively and monitored for five days. The bacterial growth was determined by measuring the optical density at wavelength of 600 nm using UV –Visible Spectrophotometer (Jenway- Model: 6405, UK).

3.8.2 Determination of optimal growth pH of the bacteria isolates

Test tube containing 10 ml each of MSM prepared in phosphate buffer of pH 6.0, 6.5, 7.0, 7.55, 8.0 and 8.5 were inoculated separately with loopful of 24 hour old cultures of the bacteria isolates and incubated at 30^{o}C and monitored for five days. The bacterial growth was determined by measuring the optical density at wavelength of 600 nm using UV – Visible Spectrophotometer (Jenway- Model: 6405, UK).

3.9 Biodegradation studies

3.9.1 Natural flora biodegradation study in field condition

PUR samples buried in the field (fallow land of the Department of Microbiology, University of Ibadan, Nigeria) at five different depths (Section **3.2**) were exhumed and observed for natural biodegradation after one and two year's intervals respectively. The exhumed samples were washed severally with sterile distilled water to remove the loosely attached soil materials and dried. They were analysed for degradation using FTIR (Fourier Transform Infrared Spectroscopy) and observed for structural changes in the polymer.

3.9.2 Laboratory study in broth

Selected PUR-utilising bacteria isolated in this study (Section **3.5**) were used in this laboratory set up. The isolates were used singly and in combination of twos. The cut and pulverised PUR packs (Section **3.2**) were sterilised by immersion in 70% (vol/vol) ethanol and rinsed with several changes of sterile distilled water. The PUR-utilising bacteria isolates were cultured in 250 ml conical flasks containing 75 ml MSM with the sterilised PUR packs added as sources of carbon and nitrogen in duplicates (one set containing the cut PUR pieces while the other set contains the pulverised PUR). They were left at room temperature for one (1) month and three (3) months intervals.

Effect of agitation was monitored by incubating a set of the conical flasks above in G24 Environmental incubator shaker (New Brunswick Scientific Co.Inc., Edison USA) at 180 rpm for one month. After these incubation periods, the PUR samples were removed, washed in several changes of distilled water, dried and analysed to detect degradation. The

analysis carried out includes weight loss using a digital analytical balance (A&D Model GR 200; capacity 210/0.0001g) before and after incubation as well as the structural changes using FTIR.

3.9.3 Bioaugmentation and biostimulation studies of PUR in soil samples under laboratory and field conditions

3.9.3.1 Bacterial isolates used for bioaugmentation and biostimulation and inoculum preparation

Six selected PUR-utilising bacteria from the laboratory experiment (Section **3.9.2**) above were used in combination of twos and a consortium of the six for bioaugmentation studies based on the ability of the bacteria isolates to utilise PUR in the broth. All these bacteria isolates were confirmed to be able to utilise solid PUR during growth as monocultures on the surface of PUR modified MSM. Three combinations of twos and a consortium of six were used. The combinations include;

Combination A= *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B= *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= Combination A + Combination B + Combination C.

The bacteria isolates were cultivated in the modified MSM (PUR as both C & N sources) of 150 ml Erlenmeyer flasks to produce large quantities of inoculum. In the combinations, 50 mls volume of inoculum was used, i.e. 25 mls from each culture while in the consortium 60 mls volume of inoculum was used, and i.e. 10 mls from each culture for the set up.

3.9.3.2 Substrates used for biostimulation and preparation

The substrates used for biostimulation in these studies were agro waste products; cassava peels and potato peels. Fresh peels of cassava and potato were collected in plastic containers from local processing mill in Ajibode area of Ibadan and transported to the Environmental Microbiology and Biotechnology laboratory, Department of Microbiology,

University of Ibadan, Nigeria. The peels were sundried prior to use in the experimental set up with each set up containing 3g of either cassava or potato peel.

3.9.3.3 Soil samples used for biostimulation and bioaugmentation studies

To test the effect of bioaugmentation and biostimulation on the biodegradation of PUR; the selected bacteria isolates, agro waste peels and PUR packs were used in both the field pilot study and laboratory. The garden soil behind the nursery house of Department of Botany, University of Ibadan, Nigeria was used for the field pilot study and also excavated for the laboratory study as well. The soil samples were collected with plastic containers and taken to the laboratory where they were used for the biodegradation study.

3.9.3.4 The Laboratory set-up (*ex situ*) (Laboratory controlled soil experiment)

The soil samples (Section **3.9.3.3**) were weighed (200g) into laboratory pots and sterilised by autoclaving. The PUR samples (prepared in Section **3.2**) were sterilised by immersion in 70% (V/V) ethanol and rinsed with several changes of sterile distilled water prior to burial in laboratory pot soil. To each sterile pot of soil, 50 ml of inoculum (Section **3.9.3.1**) of each combination, a set of the PUR samples (0.4g) and 3g of agro waste peels were mixed thoroughly and incubated at a temperature of $28^{o}C \pm 2^{o}C$ for 1 month (30 days).

This was done for the two biostimulation substrates (cassava and potato peels) and in duplicates (one set with cut PUR and the other with pulverised PUR). For the laboratory pots without biostimulation, the PUR samples were buried without the cassava or potato peels but with the different combinations. Laboratory pots with the different substrates, PUR samples and MSM but without bacterial isolates served as controls for the different set-ups. After the incubation period, the experiment was terminated and PUR samples recovered from the laboratory pots, washed, dried and the extent of degradation was determined using weight loss, visual observation and structural changes in the functional groups present in the polymer using FTIR spectrum.

3.9.3.5 Field Pilot Study (*in situ*)

The field pilot study was carried out in the garden soil behind the nursery house of Department of Botany, University of Ibadan, Nigeria using a completely randomized block design. An area of 5 m x 5 m was used and eight (8) agricultural beds/ridges of 300 cm x 60 cm each were made and the combined materials buried in different points on each bed to have sixty-four (64) burial points marked with pegs for identification (Appendix A1). The bottom of each burial point was lined and the materials added before finally covering with top soil and left for 1 month (30 days). After 30 days, the PUR samples were exhumed, washed, dried and extent of degradation was determined using weight loss, visual observation and structural changes in the functional groups present in the polymer using FTIR spectrum. The field pilot study was compared with the laboratory controlled soil study.

3.9.3.6 Determination of soil pH

Ten grammes of the garden soil sample were mixed with 40 ml of sterile distilled water in conical flask and the pH was determined with a portable laboratory pH meter 'Mettler Toledo AG- Switzerland'. The pH meter was calibrated with buffer 4.0, 7.0 and 9.0.

3.10 Assessment of biodegradation potential of the bacterial isolates

3.10.1 Determination of Weight Loss

The recovered PUR materials from the field and laboratory set ups were thoroughly washed with distilled water and dried. They were reweighed using digital analytical balance (A & D Model GR 200; with capacity to weigh 210 g / 0.0001 g) and the weight loss determined by subtracting the final values from initial values before biodegradation study.

The percentage weight loss of the film samples was calculated using the following equation:

$$\text{Weight loss (\%)} = \frac{W_i - W_f}{W_i} \times 100$$

where W_i = initial weight of the sample before biodegradation study (g)

W_f = final weight of the sample after biodegradation study (g).

3.10.2 Macroscopic Examination

Macroscopic/visual examination of both experimentally biodegraded and control PUR samples for presence of cracks was done using the method of Ibrahim *et al*. (2009). These materials were observed to see if there were presence of cracks/pits on the experimentally biodegraded samples.

3.10.3 Fourier Transform Infrared (FTIR) Spectroscopic studies for structural changes in experimentally biodegraded PUR samples

FTIR spectra allowed for qualitative and quantitative determination of functional groups. Fourier transform infrared (FTIR) spectrophotometer was used to assess the changes in the structure of PUR samples that can be attributed to the effect of the bacteria isolates at the termination of the experiment. This test was used to examine the differences in the structural bonds of experimentally biodegraded PUR compared to un-degraded PUR control samples.

The pulverised PUR samples recovered from the laboratory and field studies after termination of the experiments were thoroughly washed with four changes of sterile water to remove all materials attached to the samples. The samples were dried in the oven at 50^{o}C overnight until a constant weight was got, packaged into sterile packs and transported to Chemistry Laboratory of Redeemer's University, Ogun State, Nigeria (RUN) for FTIR analysis. Since PUR is not soluble in standard organic solvents used in FTIR analysis, they were incorporated with potassium bromide (KBr) and then made into KBr-discs and analysed as was described by Pathirana and Seal (1985a). Spectroscopic grade KBr (Sigma –Aldrich) was used in the pellet preparation and was dried in the oven at 105^{o}C for 4 hours and cooled in the desiccator before using it for the preparation due to its high hygroscopic nature. The discs were prepared by weighing 2 mg of the pulverised PUR sample and grinding it with 100 mg of the dried KBr and pressed into a disc. The KBr discs were fixed to the FTIR machine sample plates holder and the infrared spectra recorded on the IR in 400- 4000 cm^{-1} region with a resolution of 4 cm^{-1} using Spectrum 400 IR system (8400S, Shimadzu, Japan). These KBr discs were used in obtaining spectra

of the experimentally biodegraded and un-degraded control PUR samples. The changes in the functional groups of the experimentally biodegraded and control PUR samples were compared in the spectra obtained.

3.11 Statistical analysis

The changes in the characteristic functional groups of the PUR degraded samples from all the treatments were compared with the control sample and statistically analyzed using one-way Anova (Duncan) from SPSS version 16.

3.12 Quality control and assurance measures

The following quality control measures were taken to minimize random and systematic errors in the analysis carried out in the work.

- The weighing balance used in weighing the salts was calibrated with standard weight.
- The pH meter was calibrated using buffer 4.0, 7.0 and 9.0 from Mettler Toledo AG.
- Salts, media and reagents used in this work were of analytical grade with low levels of impurities.
- In the FTIR, the potassium bromide (KBr) was of spectroscopic grade; dried in the oven at 105^{o}C for 4 hours and cooled in the desiccator before use.
- The Fourier Transform Infra-Red (FTIR) machine was calibrated using potassium bromide (KBr).

Table 3.1: Some wavenumbers and their corresponding functional groups/bonds

Wavenumber (cm^{-1})	Corresponding functional groups/bonds
466.79	C-H out of plane bending vibration of substituted amide group
619.17	C-H bend of alkyne group
621.1	C-H bend of alkyne group
669.32	O-H out of plane bend
696.33	O-H out of plane bend
796	skeletal C-C vibrations
842.92	skeletal C-C vibrations
904.64	skeletal C-C vibrations
966.37	hydrogen bonded O-H out of plane bending
1028	primary amine (CN) stretch
1155.4	alkyl substituted ether (C-O) stretch
1068.6	alkyl substituted ether group
1180.47	alkyl substituted ether C-O stretch
1269.2, 1290.42	O-H in plane bend
1313	O-H in-plane bend
1329	C-H bending vibrations of methylene alkyl group
1338.64	bending vibration of a carboxylate group of carbonyl
1371	symmetric methyl ($-CH_3$) bend
1541.18	secondary amide (NH) bend
1583.61	secondary amine (NH) bend
1647.26	urethane (NH) group
1662.69	urethane (NH) group
1683.91	amide group of carbonyl
1747.57	carbonyl group
1869.08, 1872	anhydride of carbonyl of the amide
2196.99	cyanates (-OCN) asymmetric stretching vibration

Table 3.1 continued

Wavenumber (cm^{-1})	Corresponding functional groups/bonds
2258.72	cyanates (-OCN) asymmetric stretching vibration
2274.15	isocyanate (-N=C=O) asymmetric stretch
2704.29, 2787.23	C-H stretching vibrations of a methyl group
2850.88	methoxy methyl ether
2924.18	asymmetric C-H stretch of methylene group
3230.87, 3302.24, 3358.25, 3387.11	polymeric O-H stretch
3242.45	hydrogen bonded O-H stretch
3394.83	primary amine N-H stretch
3414.12	polymeric O-H stretch
3443.05	hydrogen bonded O-H stretch
3452.7, 3387.11	dimeric O-H stretch
3454.62	dimeric O-H stretch
3566.8	internally bonded O-H stretch
3616 – 3620.6	tertiary alcohol O-H stretch
3649.44, 3726.24, 3865.48	O-H stretching vibration of intramolecular hydrogen bond
3676.45, 3751.67	O-H stretching vibrations of intramolecular hydrogen bond

CHAPTER FOUR
RESULTS

4.1 Bacteria and Fungi isolates obtained from the exhumed polyurethane samples

A total of one hundred and six (106) bacteria isolate and seven (7) fungi isolates were obtained from PUR samples buried and exhumed at the five different periods of 3, 5, 7, 12 and 24 months and depths of 15, 30, 45, 60 and 70 cm. There were more bacteria isolate from PUR samples exhumed at 70 cm depth (26 %) compared to the other four depths and also at 5 months burial period (33 %) than other periods sampled and this is shown in Table 4.1 which showed the frequency of bacteria isolates during the different sampling periods and depths used for the experiment.

4.2 Screening for PUR-utilising isolates

The obtained isolates (Section **4.1**) were screened for their ability to utilize PUR as carbon, nitrogen and both carbon/nitrogen sources. The Fungi isolates could not utilise PUR as sole carbon source, however, ninety-four (94 %) out of the 106 bacteria isolates obtained were able to utilise PUR as either carbon or nitrogen source while only 35 (33 %) of the isolates utilised PUR as both nitrogen and carbon sources (Table 4.2).

4.2.1 Selection of best PUR-utilising isolates based on growth rate

The thirty-five (35) bacteria isolates able to utilise PUR as both carbon and nitrogen sources (Section **4.2**) were further screened based on their growth rate on modified MSM medium containing PUR as both carbon and nitrogen sources. Fifteen (42.8 %) out of the 35 bacteria isolates, with profuse growth on modified PUR medium (Table 4.3) were selected, characterised and used for the experimental biodegradation set ups.

Table 4.1: Frequency of bacteria isolates obtained from the five different depths of burial within the five different sampled periods.

Burial depths ↓ (cm)	Burial periods (months)					Total number of bacteria isolates obtained
	A	B	C	D	E	
15	4	10	2	4	2	22
30	4	5	2	4	2	17
45	3	8	3	4	2	20
60	4	7	2	4	2	19
70	9	5	3	7	4	28
Total	24	35	12	23	12	**106**

Key:

A= 3 months- September, 2010

B= 5 months- November, 2010

C= 7 months- January, 2011

D= 12 months- June, 2011

E= 24 months- June, 2012

Table 4.2: Number of PUR-utilizing bacteria obtained at different depths by substituting PUR as carbon (C), nitrogen (N) or both C/N sources in the Mineral Salts Medium (MSM) during first year of burial

Periods (Months) ↓	PUR substituted as C source in MSM						PUR substituted as N source in MSM						PUR substituted as both C &N sources in MSM					
	A	B	C	D	E	**Total**	A	B	C	D	E	**Total**	A	B	C	D	E	**Total**
	Depths (cm)						Depths (cm)						Depths (cm)					
3	2	2	1	2	3	**10**	3	2	1	3	3	**12**	1	1	-	2	2	**6**
5	3	2	4	6	5	**20**	6	4	4	6	5	**25**	-	1	2	5	2	**10**
7	1	1	1	2	3	**8**	2	1	2	2	3	**10**	-	1	1	-	3	**5**
12	3	4	4	4	5	**20**	4	4	4	4	7	**23**	2	1	2	3	6	**14**
Total																		**35**

Key: Alphabets represent the different depths at which isolates were recovered

A= 15 cm

B= 30 cm

C= 45 cm

D= 60 cm

E= 70 cm

- = none

Table 4.3: Qualitative screening based on growth rates of the bacteria isolates obtained from Table 4.2 within 24-48hrs of incubation on modified MSM (PUR as both C/N sources)

Isolates codes	Growth grade
$A_1$1	+
$A_4$1	++
$A_4$3	+
$B_1$3	+
$B_2$2	+++
$B_3$1	+++
$B_4$1	++
$C_2$1	++
$C_2$4	+++
$C_3$2	+++
$C_4$1	++
$C_4$2	++
$D_1$1	+
$D_1$2	+++
$D_2$1	+++
$D_2$2	++
$D_2$3	+++
$D_2$5	+++
$D_2$6	++
$D_4$1	++
$D_4$2	++
$D_4$4	++
$E_1$2	+++
$E_1$4	+++
$E_2$1	+++
$E_2$5	++
$E_3$2	+++
$E_3$2A	+++
$E_3$2B	+++
$E_4$1	+++
$E_4$2	++
$E_4$3	++
$E_4$4	+
$E_4$7	+
$E_4$8	++

key:
+++= Profuse growth; ++= Scanty growth; += Thin growth; Alphabets A-E represent soil burial depths where the isolates are coming from: A-15 cm, B- 30 cm, C- 45 cm, D- 60 cm and E- 70 cm depth. Subscripts represent sampling periods1st$_{=1,}$2nd$_{=2,}$ 3rd$_{=3,}$ 4th$_{=4}$.

4.3 Biochemical characterisation and identification of the selected bacteria isolates

The results of the biochemical characterisation and identification of the selected bacteria isolates used in this study are shown in Tables 4.4 and 4.5.

From the 30 cm depth, 2 bacteria isolates were amongst the selected with codes $B_2$2 and $B_3$1 and tentatively identified as *Corynebacterium* sp. and *Pseudomonas pseudoalcaligenes* respectively. *Corynebacterium* sp. $B_2$2 was obtained from the second sampling period (5 months) and is a Gram positive fermentative rod, which was positive for catalase, urease, citrate, Voges Proskauer and nitrate reduction but negative for oxidase, indole, Dnase, methyl red, hydrolysis of starch, casein, gelatin, arginine and Tween 80. It fermented all the sugars tested which were glucose, fructose, galactose, maltose, sucrose, lactose, xylose, arabinose, sorbose, mannitol, inositol, sorbitol and raffinose with gas and acid productions. $B_3$1 showed 92 % similarity with *Pseudomonas pseudoalcaligenes* and was obtained from the third sampling period (7 months) and is a Gram negative oxidative rod, which was positive for catalase, urease, hydrogen sulphide production, nitrate reduction and arginine hydrolysis but was negative for citrate, indole, MRVP, Dnase and hydrolysis of starch, gelatin, casein, Tween 80 and did not ferment any of the sugars tested. Also from 45 cm depth, 2 bacteria isolates were amongst the selected with codes $C_2$4 and $C_3$2 and were tentatively identified as *Bacillus thuringiensis* and *Vibrio* sp. with percentage similarities of 89 and 83 % respectively. *Bacillus thuringiensis* $C_2$4 was obtained from the second sampling period and is a Gram positive fermentative motile rod with sub terminal spores; positive for catalase, oxidase, methyl red, nitrate reduction, Dnase, hydrolysis of starch, casein, gelatin, arginine and Tween 80 but negative for urease, indole, VP, hydrogen sulphide production and did not ferment any of the sugars tested except glucose. *Vibrio* sp. $C_3$2 was obtained from the third sampling period and is a Gram negative fermentative rod which was positive for catalase, oxidase, urease, indole, hydrogen sulphide production, nitrate reduction, hydrolysis of starch, casein, gelatin and Tween 80 but negative for MRVP, Dnase and arginine hydrolysis. It fermented glucose, fructose, maltose, lactose and raffinose with acid production.

Four bacteria isolates were from 65 cm depth; had codes $D_1$2, $D_2$1, $D_2$3 and $D_2$5 and were tentatively identified as *Enterobacter amnigenus*, *Providencia pseudomallei*, *Comamonas acidovorans* and *Providencia pseudomallei* with percentage similarities of 90, 73, 87 and 73 % respectively. *Enterobacter amnigenus* $D_1$2 was obtained from the first sampling period (3 months) and is a Gram negative fermentative rod; positive for catalase, citrate, VP, nitrate reduction, hydrolysis of Tween 80 and arginine but negative for oxidase, urease, indole, MR, Dnase, hydrolysis of starch, casein and gelatin. It fermented all the sugars tested with gas and acid productions except lactose which had only acid production and inositol and sorbitol which were not fermented. *Providencia pseudomallei* $D_2$1 was obtained from second sampling period and is a Gram negative fermentative rod; positive for catalase, oxidase, citrate, nitrate reduction, hydrolysis of Tween 80 and arginine. It did not ferment any of the sugars tested except raffinose. *Comamonas acidovorans* $D_2$3 was obtained at second sampling period of November, 2011 and is a Gram negative rod positive for catalase, oxidase, nitrogen reduction and hydrolysis of Tween 80while. It did not ferment any of the sugars tested. *Providencia pseudomallei* $D_2$5 was obtained from second sampling period, it is a Gram negative fermentative rod positive for catalase, oxidase, urease, citrate, nitrogen reduction and hydrlolysed arginine but did not ferment any of the sugars tested except raffinose.

The remaining seven bacteria isolates amongst the selected were obtained from 70 cm depth and tentatively identified as *Micrococcus* sp. $E_1$2, *Pseudomonas alcaligenes* $E_1$4, *Comamonas testosteroni* $E_2$1, *Pseudomonas aeruginosa* $E_3$2, *Arthrobacter* sp. $E_3$2^A, *Enterobacter cloacae* $E_3$2^B and *Pseudomonas fluorescens* $E_4$1. *Micrococcus* sp. $E_1$2 and *Pseudomonas alcaligenes* $E_1$4 were obtained from first sampling period; *Comamonas testosteroni* $E_2$1 from second sampling period, *Pseudomonas aeruginosa* $E_3$2, *Arthrobacter* sp. $E_3$2^A and *Enterobacter cloacae* $E_3$2^B were from third sampling period while *Pseudomonas fluorescens* $E_4$1 was from the fourth sampling period (12 months). $E_1$2 showed 57 % similarity with *Micrococcus* sp. and is a Gram positive coccus which was positive for catalase, oxidase, Dnase and hydrolysed casein and gelatin but negative for urease, citrate, indole, MRVP, nitrate reduction, hydrogen sulphide production and

hydrolysis of starch, Tween 80 and arginine. It did not ferment any of the sugars tested except xylose. $E_1$4 showed 98 % similarity with *Pseudomonas alcaligenes* and is a Gram negative rod positive for oxidase, citrate, hydrogen sulphide production, nitrate reduction, and hydrolysed Tween 80 and arginine but negative for urease, indole, MRVP, Dnase and hydrolysis of starch, casein and gelatin. It did not ferment any of the sugars tested. $E_2$1 showed 92 % similarity with *Comamonas testosteroni* is a Gram negative rod positive for catalase, oxidase, nitrate reduction and hydrolysed Tween 80 and did not ferment any of the tested sugars. $E_3$2 showed 75 % similarity with *Pseudomonas aeruginosa* and is a Gram negative rod positive for catalase, oxidase, urease, nitrate reduction, hydrolysis of Tween 80 and arginine and fermented galactose, xylose, arabinose and raffinose with acid production.

Arthrobacter sp. $E_3 2^A$ is a Gram positive rod; positive for catalase, oxidase and urease and did not ferment any of the tested sugars. *Enterobacter cloacae* $E_3 2^B$ is a Gram negative fermentative rod positive for catalase, oxidase, urease, citrate and hydrolysed Tween 80 and arginine. It fermented all the sugars tested with acid and gas productions except sorbose. $E_4$1 showed 76 % similarity with *Pseudomonas fluorescens* and is a Gram negative rod positive for catalase, oxidase, urease, citrate and hydrolysed Tween 80 and arginine and was obtained from the fourth sampling period (12 months). It fermented fructose, maltose, sucrose and raffinose with acid and gas productions while glucose, galactose, xylose and arabinose with acid production only. In all, 3 of the isolates were Gram positive rods; eleven (11) isolates were Gram negative rods and the remaining one (1) was Gram negative cocci; thus Gram negative rods bacteria dominated in the selected isolates.

Nine (9) of the isolates belong to the Gamma (γ) Proteobacteria group which constitute the largest subgroup of proteobacteria with extraordinary variety of physiological ways and are of the genera *Pseudomonas* with 4 strains, *Providencia* with 2 strains, *Enterobacter* with 2 strains and a strain of *Vibrio*. The *Pseudomonas* is the most important genus in the order *Pseudomonadales*. *Pseudomonas* is a straight or slightly curved Gram negative rod

that is an exceptionally heterogenous taxon currently composed of about 60 species. They have great practical impact in several ways, including degrading an exceptionally wide variety of organic molecules and thus are very important in the mineralisation process (microbial breakdown of organic materials to inorganic substances) in nature. Two (2) of the isolates belong to Beta (β) Proteobacteria group and are of the genus *Comamonas*. Also, three (3) of the isolates belong to the Actinobacteria group and are of the genera *Corynebacteria, Arthrobacter* and *Micrococcus* with a strain each while one (1) of the isolates belongs to the Firmicutes group and is of the genus *Bacillus*. The genus *Bacillus*, of the family- *Bacillaceae*, is the largest in the order *Bacillales. Bacillus* is a gram positive, endospore forming, chemoheterotrophic rod that is usually motile. Many species of *Bacillus* are of considerable importance medically and industrially. Table 4.6 shows the percentage similarity of the bacteria isolates to the organism in database and their phylogenetic group in the classification of bacteria.

From the 15 selected bacteria isolates based on growth rates on modified MSM, six (6) (**3.9.3.1**) showed good degrading abilities in the laboratory broth experiment and were further studied. The optimal growth temperature and growth pH was studied and they were used for the biodegradation studies following.

Table 4.4: Biochemical tests of the bacteria isolates and tentative identities

Isolates Code	Shape	Gram's Reaction	Catalase	Oxidase	Urease	Citrate	Indole	Methyl Red	Voges- Proskauer	Starch hydrolysis	Motility	H_2S	NO_3	O-F test	Dnase test	Casein hydrolysis	Gelatine hydrolysis	Tween 80 hydrolysis	Arginine hydrolysis	Spore stain	Tentative identity of isolates
1. $B_2$2	R	+	+	-	+	+	-	-	+	-	-	+w	+	F	-	-	-	-	-	-	*Corynebacterium* sp.
2. $B_3$1	R	-	+	+	+w	-	-	-	-	-	-	+	+	O	-	-	-	-	+	nd	*Pseudomonas pseudoalcaligenes*
3. $C_2$4	R	+	+	+	-	+w	-	+	-	+	+	-	+	F	+	+	+	+	+	ST	*Bacillus thuringiensis*
4. $C_3$2	R	-	+	+	+	+w	+	-	-	+	-	+	+	F	-	+	+	+	-	nd	*Vibrio* sp.
5. $D_1$2	R	-	+	-	-	+	-	-	+	-	-	+w	+	F	-	-	-	+	+	nd	*Enterobacter amnigenus*
6. $D_2$1	R	-	+	+	-	+	-	-	-	-	-	-	+	F	-	-	-	+	+	nd	*Providencia pseudomallei*
7. $D_2$3	R	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	nd	*Comamonas acidovorans*
8. $D_2$5	R	-	+	+	+	+	-	-	-	-	-	-	+	F	-	-	-	-	+	nd	*Providencia pseudomallei*
9. $E_1$2	C	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	nd	*Micrococcus* sp.
10. $E_1$4	R	-	-	+	-	+	-	-	-	-	-	+	+	-	-	-	-	+	+	nd	*Pseudomonas alcaligenes*
11. $E_2$1	R	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	nd	*Comamonas testosteroni*
12. $E_3$2	R	-	+	+	+	-	-	-	-	-	-	+w	+	O	+	+	+	+	+	nd	*Pseudomonas aeruginosa*
13. $E_3$2^A	R	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*Arthrobacter* sp.
14. $E_3$2^B	R	-	+	+	+	+	-	-	-	-	-	-	-	F	-	-	-	+	+	nd	*Enterobacter cloacae*
15. $E_4$1	R	-	+	+	+	+	-	-	-	-	-	-	-	O	-	-	-	+	+	nd	*Pseudomonas fluorescens*

Key:
R = rod; + = positive; nd= not determined, ST- Sub terminal position
C = cocci; - = negative; +g = positive with gas production, +w = weakly positive

Table 4.5: Sugar Fermentation tests of the bacteria isolates obtained

	Bacteria Isolates	Shape	Glucose	Fructose	Galactose	Maltose	Sucrose	Lactose	Xylose	Arabinose	Sorbose	Mannitol	Inositol	Sorbitol	Raffinose	Identity of Isolates
1.	$B_2$2	R	+g	+g	+g	+g	+g	+g	+g	+g	+g	+g	+g	+g	+g	*Corynebacterium* sp.
2.	$B_3$1	R	-	-	-	-	-	-	-	-	-	-	-	-	-	*Pseudomonas pseudoalcaligenes*
3.	$C_2$4	R	+	-	-	+w	-	-	-	-	-	-	-	-	-	*Bacillus thuringiensis*
4.	$C_3$2	R	+	+	-	+	-	+	-	-	-	-	-	-	+g	*Vibrio* sp.
5.	$D_1$2	R	+g	+g	+g	+g	+g	+	+g	+g	+g	+g	-	-	+g	*Enterobacter amnigenus*
6.	$D_2$1	R	-	-	-	-	-	+w	-	-	-	-	-	-	+g	*Providencia pseudomallei*
7.	$D_2$3	R	-	-	-	-	-	-	-	-	-	+w	-	-	-	*Comamonas acidovorans*
8.	$D_2$5	R	-	-	-	-	-	-	-	-	-	-	-	-	+	*Providencia pseudomallei*
9.	$E_1$2	C	-	-	-	-	-	-	+	-	-	-	-	-	-	*Micrococcus* sp.
10.	$E_1$4	R	-	-	-	-	-	-	-	-	-	-	-	-	-	*Pseudomonas alcaligenes*
11.	$E_2$1	R	-	-	-	-	-	-	-	-	-	-	-	-	-	*Comamonas testosteroni*
12.	$E_3$2	R	-	-	+	-	-	-	+	+	-	-	-	-	+	*Pseudomonas aeruginosa*
13.	$E_3$2^A	R	-	-	-	-	-	-	-	-	-	-	-	-	-	*Arthrobacter* sp.
14.	$E_3$2^B	R	+g	+g	+g	+g	+g	+g	+g	+g	-	+g	+	+g	+g	*Enterobacter cloacae*
15.	$E_4$1	R	+	+g	+	+g	+g	-	+	+	-	-	-	-	+g	*Pseudomonas fluorescens*

Key:
+ = positive for acid production
- = negative (no acid production)
+g = positive for acid and gas production
+w = weakly positive for gas production

Table 4.6: Biochemical tests match (Highest similarity) using ABIS and PIBWIN databases.

Isolates Code	% similarity	Name of the organism	Phylogenetic group
$B_3$1	92	*Pseudomonas pseudoalcaligenes*	γ-Proteobacteria
$E_1$4	98	*Pseudomonas alcaligenes*	,,
$E_3$2	75	*Pseudomonas aeruginosa*	,,
$E_4$1	76	*Pseudomonas fluorescens*	,,
$D_2$1	73	*Providencia pseudomallei*	,,
$D_2$5	73	*Providencia pseudomallei*	,,
$D_1$2	90	*Enterobacter amnigenus*	,,
$E_3$2B	84	*Enterobacter cloacae*	,,
$C_3$2	83	*Vibrio* sp	,,
$C_2$4	89	*Bacillus thuringiensis*	Firmicutes
$D_2$3	87	*Comamonas acidovorans*	β–Proteobacteria
$E_2$1	92	*Comamonas testosteroni*	,,
$B_2$2	-	*Corynebacterium* sp	Actinobacteria
$E_1$2	57	*Micrococcus* sp	,,
$E_3$2A	-	*Arthrobacter* sp	,,

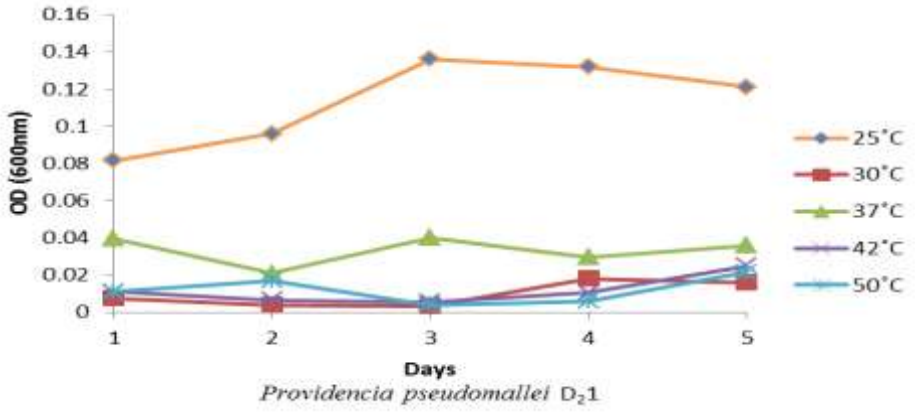
4.4 Optimal growth temperature for the bacteria isolates in MSM

The results of the different growth temperatures for which the six (6) bacteria isolates used for the biodegradation study in soil (*in situ* and *ex situ*) were grown are shown in Figure 4.1 (a-f).

From the figures, *Providencia pseudomallei* $D_2$1 (Fig. 4.1a) showed optimum growth temperature at 25^{o}C with highest OD reading on day 3 but showed decrease in OD reading on the other temperatures (30, 37, 42 and 50) tested. *Providencia pseudomallei* $D_2$5 (Fig. 4.1b) also showed optimum growth temperature at 25^{o}C with highest OD reading on day 3 and this was followed by 50^{o}C where it had continous increase in OD readings from day 1 to day 5 of the experiment. *Pseudomonas alcaligenes* $E_1$4 (Fig. 4.1c) showed optimum growth temperature with 30^{o}C with highest OD reading on the third day and followed by 37^{o}C with continous increase in OD readings from day 1 to day 5. It equally showed good OD readings at 42^{o}C and 50^{o}C respectively. *Pseudomonas aeruginosa* $E_3$2 (Fig. 4.1d) showed optimum growth temperature at 25^{o}C with highest OD reading on day 1 and this was followed by 42^{o}C whose OD reading had a drop on day 2 and peaked until the fifth day. *Enterobacter amnigenus* $D_1$2 (Fig. 4.1e) showed optimum growth temperature at 37^{o}C with highest OD reading on the third day while *Vibrio* sp. $C_3$2 (Fig. 4.1f) showed optimum growth temperature at 42^{o}C with continuous increase in OD readings and had the highest on the fifth day.

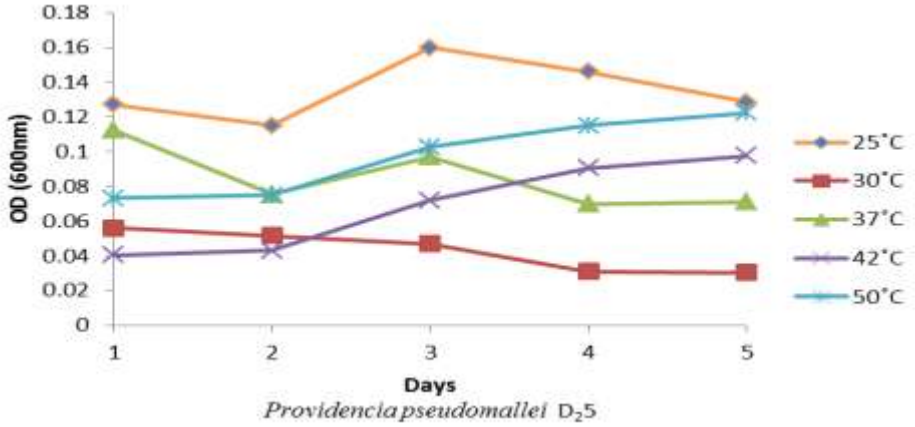
From above, 3 of the isolates had their optimum growth temperature at 25^{o}C while one each had their optimum growth temperature at 30^{o}C, 37^{o}C and 42^{o}C. Also, 4 of the isolates showed highest OD readings on third day of the experiment (day 3) while one each showed it on the first day (day 1) and last day (day 5) of the experiment.

(a)



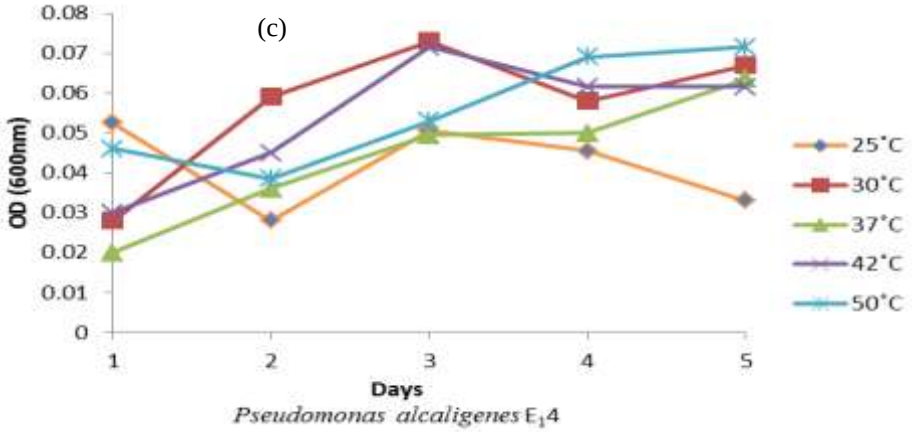
Providencia pseudomallei $D_2$1

(b)



Providencia pseudomallei $D_2$5

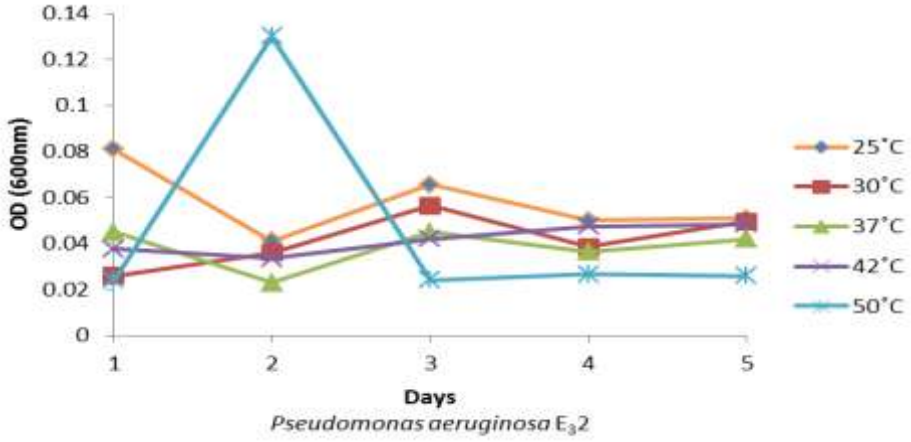
(c)



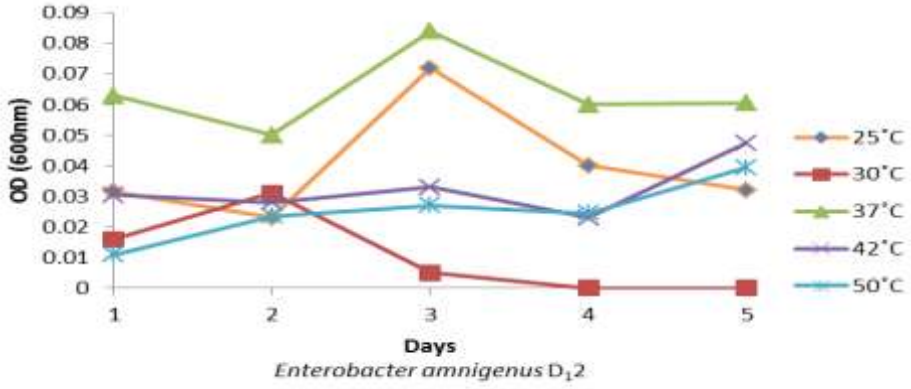
Pseudomonas alcaligenes $E_1$4

Figure 4.1 (a-c): Growth pattern of the selected bacteria isolates in MSM at different temperatures over a period of 5 days.

(d)



(e)



(f)

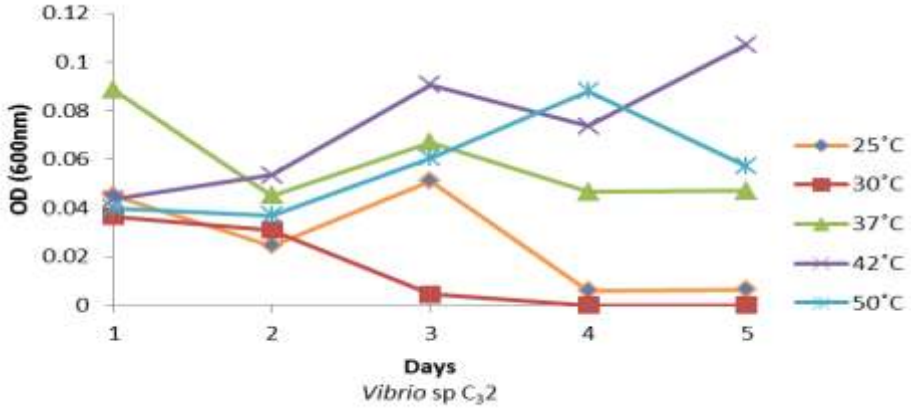


Figure 4.1 (d-f): Growth pattern of the selected bacteria isolates in MSM at different temperatures over a period 5 days.

4.5 Optimum pH for the growth of bacteria isolates in MSM

The results of the different growth pH for which the six (6) bacteria isolates used for the biodegradation study in soil (*in situ* and *ex situ*) were grown are shown in Figure 4.2 (a-f).

From the figures, *Providencia pseudomallei* $D_2$1 (Fig. 4.2a) showed optimum growth at pH 7.0 with highest OD reading on the second day of the experiment and this was followed by day 3 of pH 6.5 and 8.5. *Providencia pseudomallei* $D_2$5 (Fig. 4.2b) showed optimum growth at pH 6.0 with highest OD reading on the second day and this was followed also by day 2 of pH 7.0 and 7.55. *Pseudomonas alcaligenes* $E_1$4 (Fig. 4.2c) showed optimum growth at pH 7.55 with highest OD reading on the third day and this was followed also by day 3 of pH 7.0. *Pseudomonas aeruginosa* $E_3$2 (Fig. 4.2d) showed optimum growth at pH 6.0 with highest OD reading on the fourth day. *Enterobacter amnigenus* $D_1$2 (Fig. 4.2e) showed optimum growth at pH 8.5 with highest OD reading at day 3. *Vibrio* sp. $C_3$2 (Fig. 4.2f) showed optimum growth at pH 7.0 on the third day and this was followed also by third day of pH 6.5.

From the above, 3 of the isolates showed highest OD reading on the third day of their optimum growth pH; 2 of the isolates had their highest OD reading on the second day while one isolate had the highest OD reading on the fourth day of the experiment. Also, 2 of the isolates each showed optimum growth at pH 6.0 and 7.0 while one each showed optimum growth at pH 7.55 and 8.5.

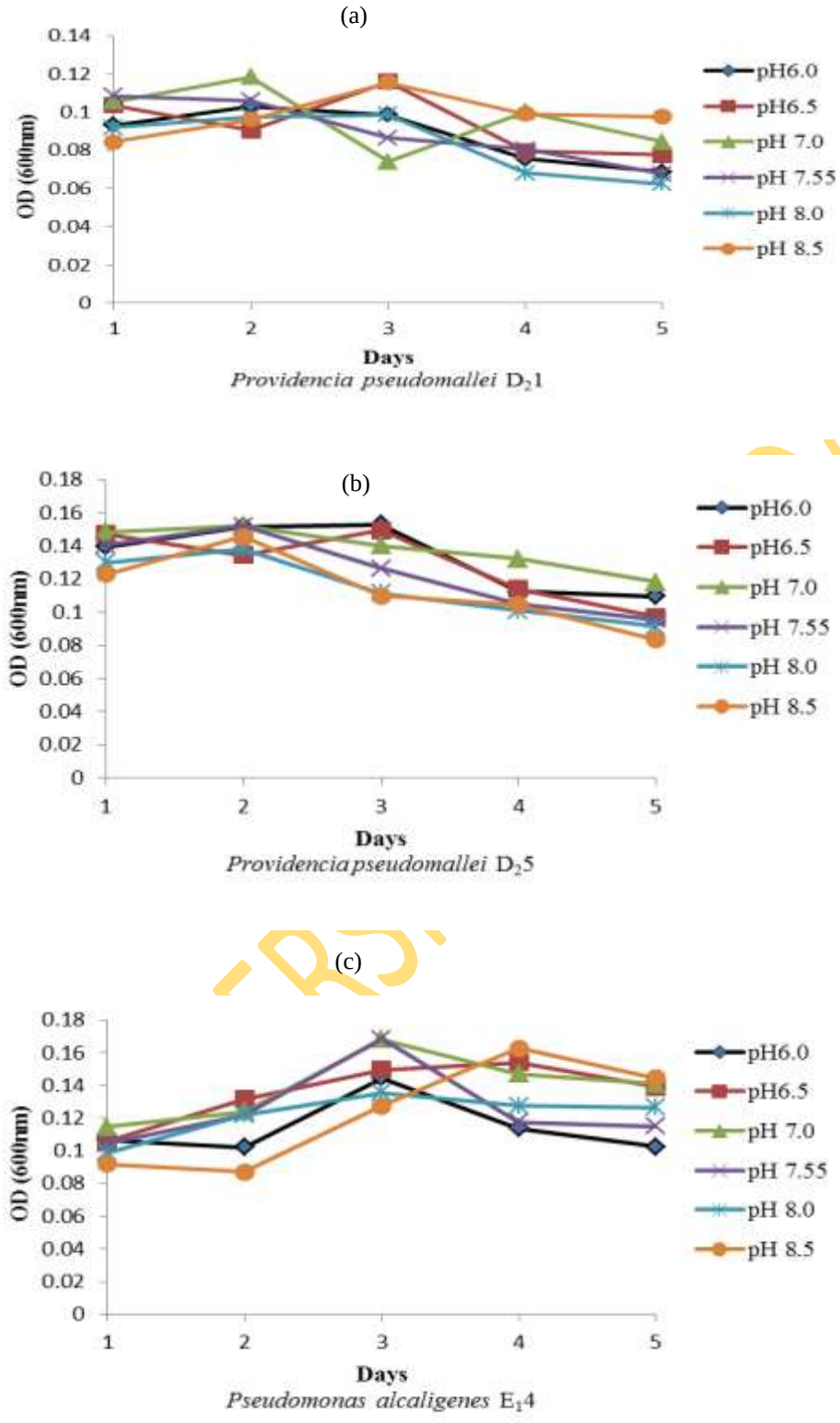
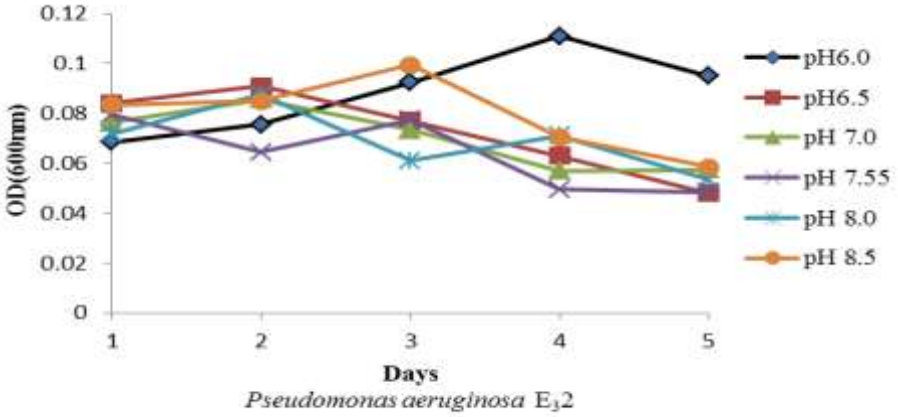


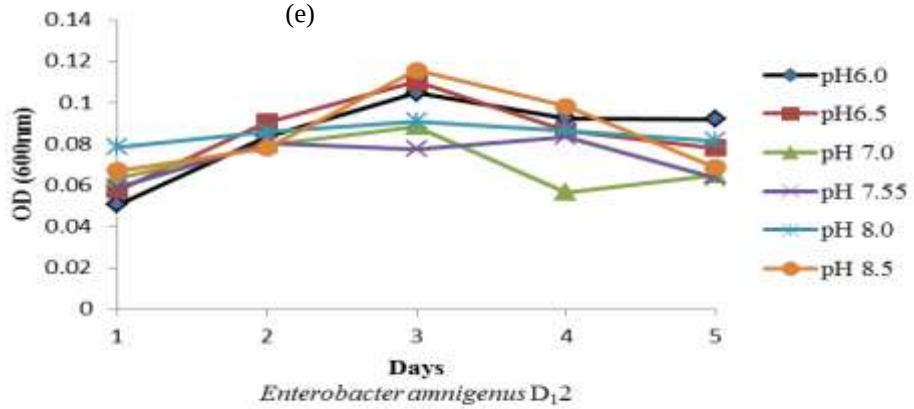
Figure 4.2 (a-c): Growth pattern of the selected bacteria isolates in MSM at different pH over a period of 5 days.

(d)



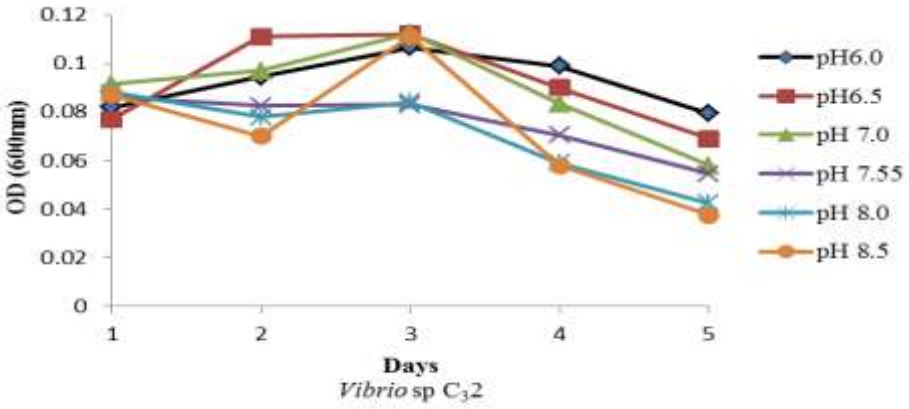
Pseudomonas aeruginosa E_32

(e)



Enterobacter amnigenus D_12

(f)



Vibrio sp C_32

Figure 4.2 (d-f): Growth pattern of the selected bacteria isolates in MSM at different pH over a period of 5 days.

4.6 Fourier Transform Infrared Spectroscopy (FTIR) analysis of PUR samples

The FTIR analysis carried out on the experimentally biodegraded and control PUR samples showed that some of the isolates singly and/or in combined form were able to show some changes in some wavenumbers which were signs of degradation/breakdown of the polymer in the IR spectrum. The FTIR spectra analysis used to monitor the functional groups changes of the PUR material during the biodegradation studies are shown below. From the FTIR analysis of the experimentally biodegraded samples, it was observed that the isolates were able to cause some functional groups changes in the PUR material as there were disappearances of some old peaks; formation of some new peaks and also shifts either to the right or left of the spectrum of existing peaks in relative to the control sample. The transmittance (%) in the experimentally biodegraded samples were drastically reduced as compared to the control sample. Some peak areas were reduced as a result of degradation while some increased and are attributed to accumulation of degradation/breakdown products.

4.6.1 Comparison of 1 month and 3 months FTIR analysis of experimentally biodegraded PUR samples in broth under static condition

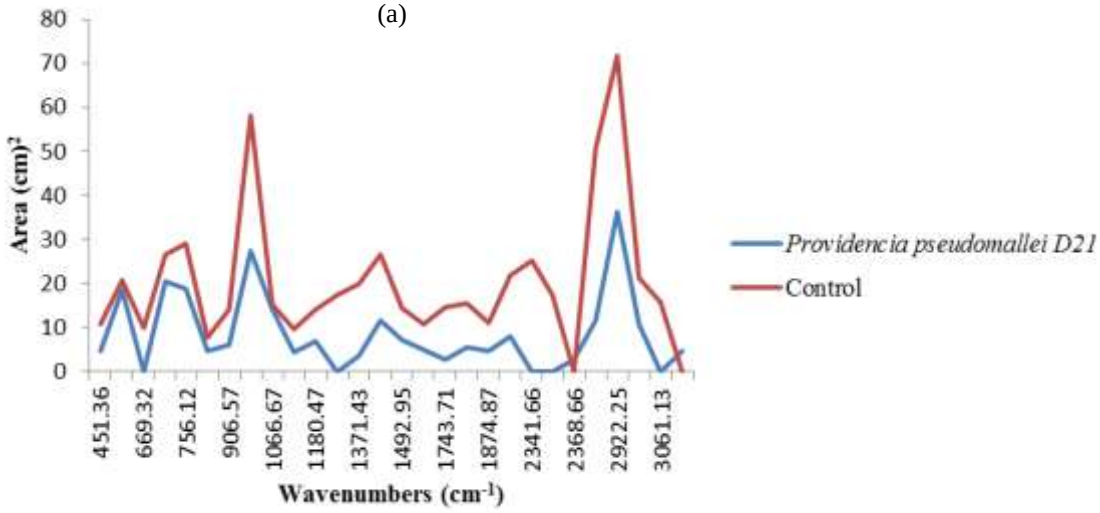
The comparison of the 1 month and 3 months FTIR spectral analysis of the experimentally biodegraded PUR samples incubated in MSM broth are shown in Figures 4.3a-p.

Figure 4.3a-b showed the comparison between 1 month and 3 months of experimentally biodegraded PUR samples using *Providencia pseudomallei* $D_2$1. In the first month FTIR spectral analysis of PUR samples biodegraded with *Providencia pseudomallei* $D_2$1 (Fig.4.3a), it showed removal of peaks at wavenumbers (cm^{-1}) 1329 corresponding to C-H bending vibrations of methylene alkyl group; 3676.45 and 3751.67 corresponding to O-H stretching vibrations of intramolecular hydrogen bond; 1647.26 corresponding to urethane (NH) group, 669.32 corresponding to O-H out of plane bend, 2345.52 and 3061.13 when compared to the control. It also showed formation of a new peak within the range of wavenumbers 2359- 2368.66. In the shifts to the right of the spectra, *Providencia pseudomallei* $D_2$1 caused shifts in peaks at wavenumbers 1155.4 corresponding to alkyl

substituted ether (C-O) stretch to 1153.47, 842.92 and 3443.05 corresponding to skeletal C-C vibrations and hydrogen bonded O-H stretch respectively to 840.99 and 3439.19, 466.79 corresponding to C-H out of plane bending vibration of substituted amide group and 1747.57 corresponding to carbonyl group to a range of 451-464.9 and 1743.71 respectively. In the shifts to the left of the spectra of existing peaks, it shifted peaks at wavenumbers 904.64 corresponding to skeletal C-C vibrations to 906.57 and 1869.08 corresponding to anhydride of carbonyl of the amide to 1871.01 when compared to control while in the third month FTIR spectral analysis of PUR samples biodegraded with *Providencia pseudomallei* $D_2$1 (Fig.4.3b), it showed removal of peaks at wavenumbers (cm^{-1}) 451.36 corresponding to C-H out of plane bending vibration of substituted amide group; 621.1 corresponding to C-H bend of alkyne group; 2196.99 and 2258.72 corresponding to cyanates (-OCN) asymmetric stretching vibration; 3566.5 corresponding to internally bonded O-H stretch; 3649.44, 3726.24 and 3865.48 corresponding to O-H stretching vibration of intramolecular hydrogen bond; 3103.57, 3163.36 and 3082.35 when compared to the control.

Figure 4.3c-d showed the comparison between 1 month and 3 months of experimentally biodegraded PUR samples using *Providencia pseudomallei* $D_2$5. In the first month FTIR spectral analysis of PUR samples biodegraded with *Providencia pseudomallei* $D_2$5 (Fig.4.3c), it showed removal of peaks at wavenumbers (cm^{-1}) 1329 corresponding to C-H bending vibrations of methylene alkyl group; 3676.45 and 3751.67 corresponding to O-H stretching vibrations of intramolecular hydrogen bond; 1647.26 corresponding to urethane (NH) group; 1541.18 corresponding to secondary amide (NH) bend; 1068.6 corresponding to alkyl substituted ether group; 842.92 corresponding to skeletal C-C vibrations and 2345.52 when compared to the control. It also showed formation of a new peak within the range of wavenumbers 2359-2368.66. In the shifts to the right of existing peaks in the control, it caused shifts in peaks at wavenumbers 466.79 corresponding to C-H out of plane bending vibration of substituted amide group and 1747.57 corresponding to carbonyl group to a range of 451-464.9 and 1743.71 respectively. Also peaks at wavenumbers 1155.4 corresponding to alkyl substituted ether (C-O) stretch was shifted to

(a)



(b)

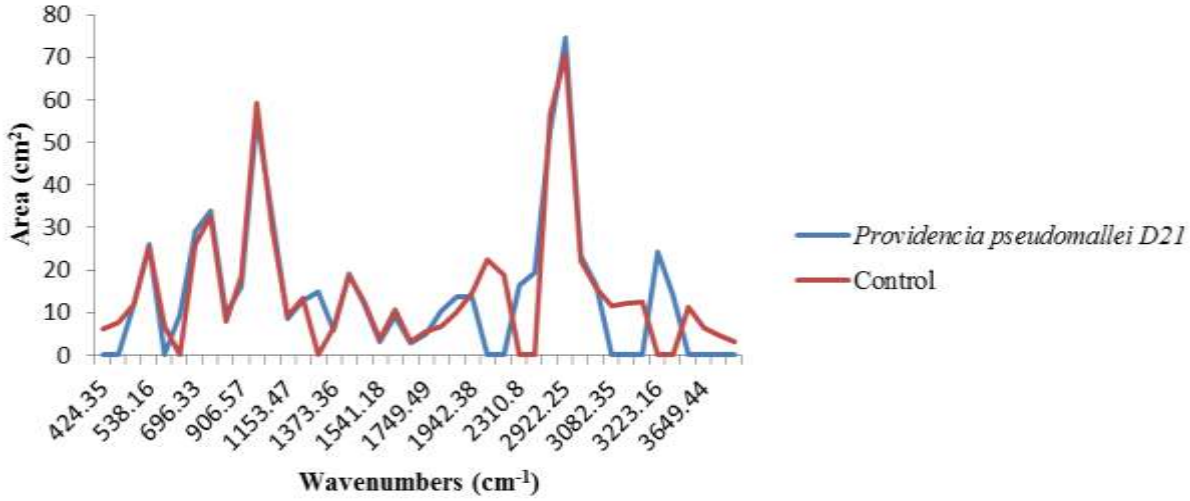
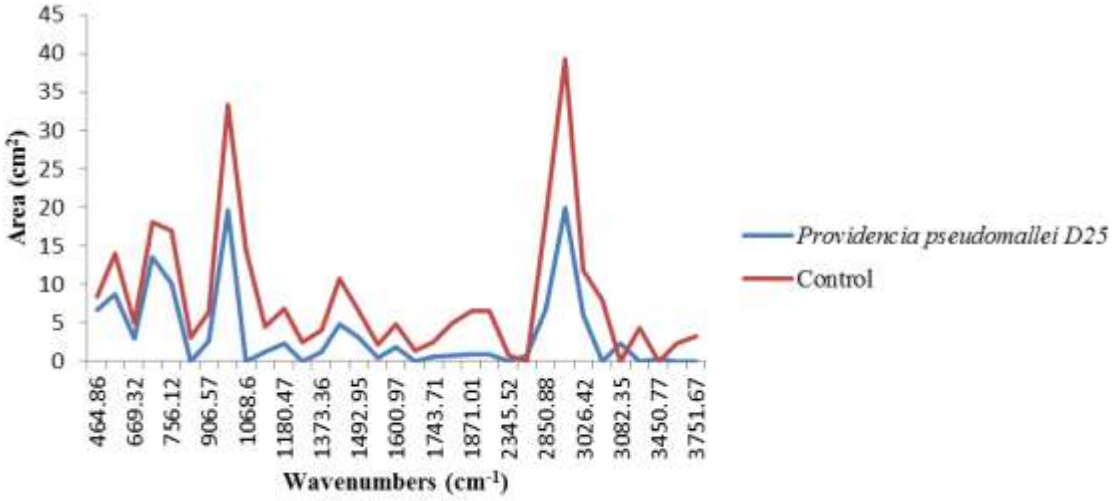


Figure 4.3 (a-b): Comparison of FTIR spectra analysis between 1 month (a) and 3 months (b) of experimentally biodegraded PUR samples using *Providencia pseudomallei* $D_2$1.

1153.47 and 1375.29 corresponding to symmetric C-H bend was shifted to 1373.36. In the shifts to the left of the spectra, it caused shifts in peaks at wavenumbers 3061.13 to 3082.35; 3443.05 corresponding to hydrogen bonded O-H stretch to a range of 3446 - 3456.6 when compared to the control while in the third month FTIR spectral analysis of PUR samples biodegraded with *Providencia pseudomallei* D_25 (Fig.4.3d), it showed removal of peaks at wavenumbers (cm^{-1}) 2189.28 corresponding to cyanates (-OCN) asymmetric stretching vibration, 2515.26, 2723.58 and 3448.84 corresponding to hydrogen bonded O-H stretch; reduced peaks at wavenumbers 842.92 to 840.99 and 464.86 to 451.36 corresponding respectively to skeletal C-C vibrations and C-H out of plane bending vibration of substituted amide group. It also caused a shift to the left of spectra of existing peak at wavenumber 1653.05 corresponding to urethane (NH) group to 1647.26 and equally to the right of spectra of peak at wavenumber 1541.18 corresponding to secondary amide (NH) bend to 1543.1 when compared to the control.

Figure 4.3e-f showed the comparison between 1 month and 3 months of experimentally biodegraded PUR samples using *Pseudomonas alcaligenes* E_14. In the first month FTIR spectral analysis of PUR samples biodegraded with *Pseudomonas alcaligenes* E_14 (Fig.4.3e), it showed removal of peaks at wavenumbers (cm^{-1}) 1329 corresponding to C-H bending vibrations of methylene alkyl group; 3676.45 and 3751.67 corresponding to O-H stretching vibrations of intramolecular hydrogen bond; 1541.18 corresponding to secondary amide (NH) bend; 3061.13 and 2345.52 when compared to the control. It caused a shift to the right of the spectra of peaks at wavenumbers 466.79 corresponding to C-H out of plane bending vibration of substituted amide group and 1747.57 corresponding to carbonyl group to a range of 451-464.9 and 1743.71 respectively. Also, peaks at wavenumbers 1155.4 corresponding to alkyl substituted ether (C-O) stretch to 1153.47 and 1375.29 corresponding to symmetric C-H bend to 1373.36 when compared to control. In the shifts to the left of the spectra, it caused shifts in peaks at wavenumbers 904.64 corresponding to skeletal C-C vibrations to 906.57; 3443.05 corresponding to hydrogen bonded O-H stretch to a range of 3446 -3456.6; 1647.26 corresponding to urethane (NH) group to a range of 1653 -1664.7 and 1869.08 corresponding to anhydride of carbonyl of

(c)



(d)

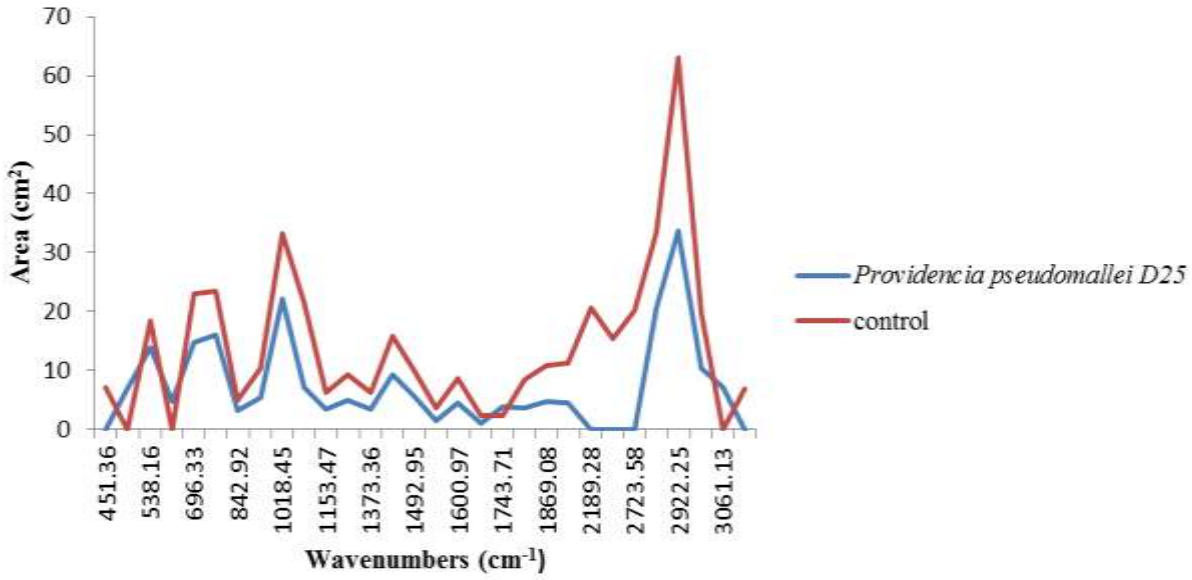
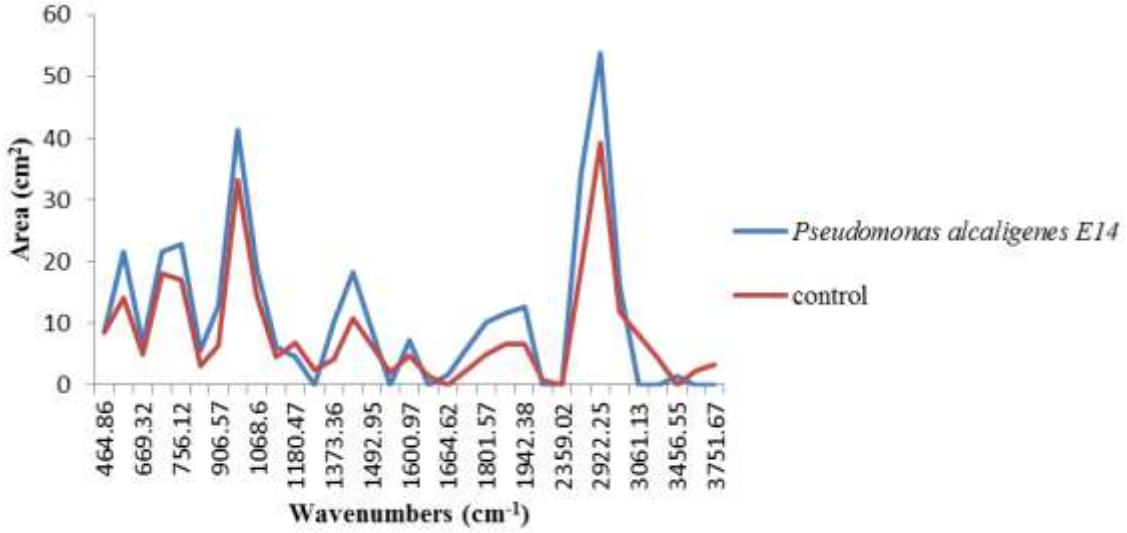


Figure 4.3 (c-d): Comparison of FTIR spectra analysis between 1 month (c) and 3 months (d) of experimentally biodegraded PUR samples using *Providencia pseudomallei* D_25.

the amide to 1871.01 when compared to the control while in the third month FTIR spectral analysis of biodegraded PUR samples with *Pseudomonas alcaligenes* $E_1$4 (Fig.4.3f), it showed removal of peaks at wavenumbers (cm^{-1}) 1541.18 corresponding to secondary amide (NH) bend; 2189.28 corresponding to cyanates (-OCN) asymmetric stretching vibration, 2515.26, 2723.58 and 3448.84 corresponding to hydrogen bonded O-H stretch. It also caused shifts in peaks at wavenumbers 842.92 to 840.99 and 464.86 to 451.36 corresponding respectively to skeletal C-C vibrations and C-H out of plane bending vibration of substituted amide group

Figure 4.3g-h showed the comparison between 1 month and 3 months of experimentally biodegraded PUR samples using *Pseudomonas aeruginosa* $E_3$2. In the first month FTIR spectral analysis of PUR samples biodegraded with *Pseudomonas aeruginosa* $E_3$2 (Fig.4.3g), it showed removal of peaks at wavenumbers (cm^{-1}) 1329 corresponding to C-H bending vibrations of methylene alkyl group, 3676.45 and 3751.67 corresponding to O-H stretching vibrations of intramolecular hydrogen bond and 3061.13. It caused a shift to the right of the spectra of peaks at wavenumbers 466.79 corresponding to C-H out of plane bending vibration of substituted amide group and 1747.57 corresponding to carbonyl group to a range of 451-464.9 and 1743.71 respectively when compared to control. In the shifts to the left of spectra of existing peaks, it caused shifts in peaks at wavenumbers 904.64 corresponding to skeletal C-C vibrations to 906.57; 3443.05 corresponding to hydrogen bonded O-H stretch to a range of 3446 -3456.6 and 1647.26 corresponding to urethane (NH) group to a range of 1653 -1664.7 when compared to the control while in the third month FTIR spectral analysis of biodegraded PUR samples using *Pseudomonas aeruginosa* $E_3$2 (Fig.4.3h), it showed removal of peaks at wavenumbers (cm^{-1}) 451.36 and 464.86 corresponding to C-H out of plane bending vibration of substituted amide group; 621.1 corresponding to C-H bend of alkyne group; 1749.49 corresponding to carbonyl group; 2196.99 and 2258.72 corresponding to cyanates (-OCN) asymmetric stretching vibration; 3082.35, 3061.13,3103.57 and 3163.36 were removed when compared to the control.

(e)



(f)

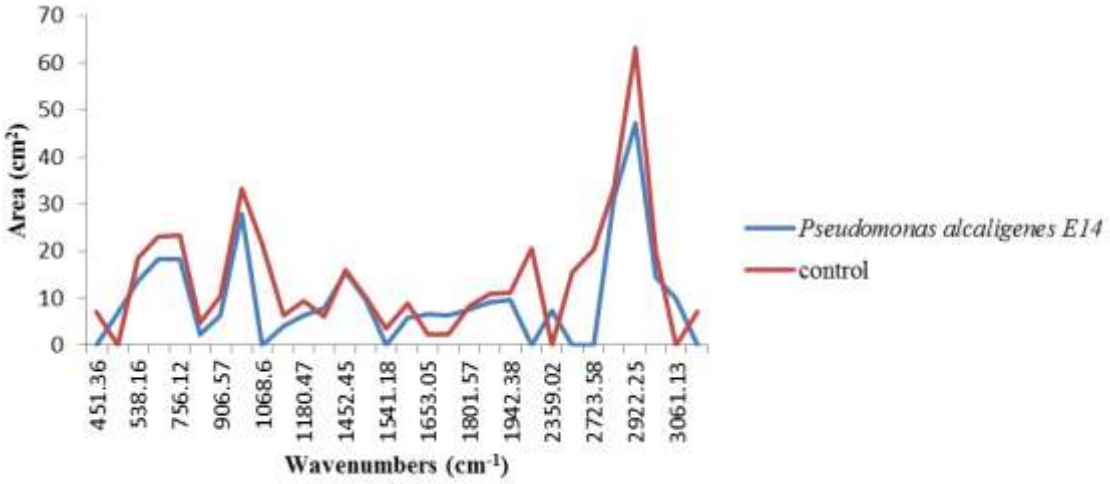
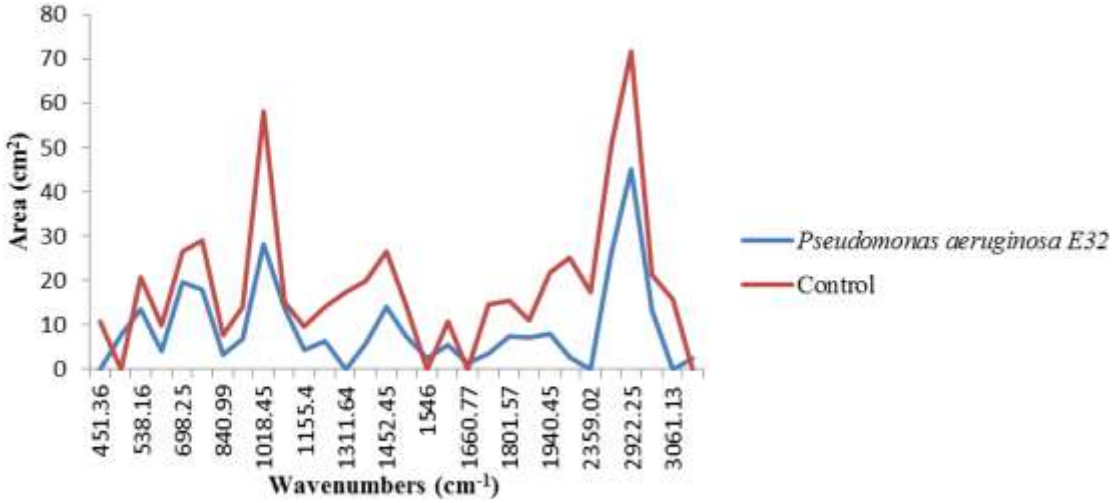


Figure 4.3 (e-f): Comparison of FTIR spectra analysis between 1 month (e) and 3 months (f) of experimentally biodegraded PUR samples using *Pseudomonas alcaligenes* $E_1$4.

(g)



(h)

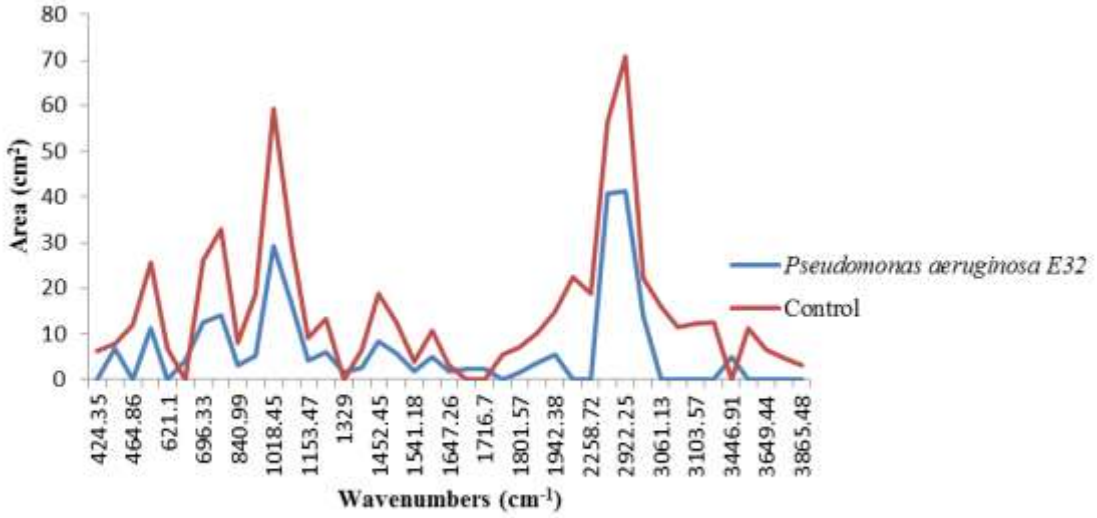
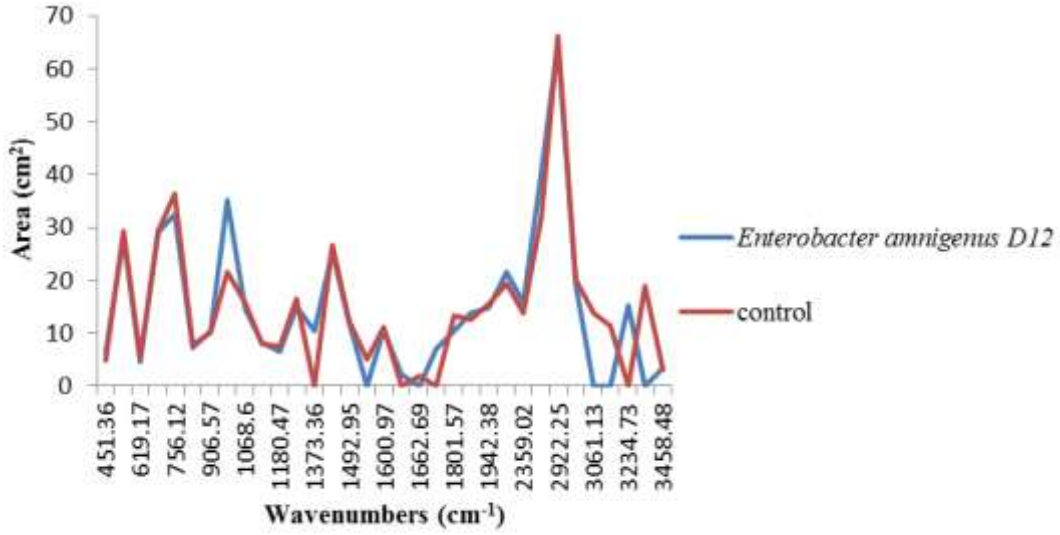


Figure 4.3 (g-h): Comparison of FTIR spectra analysis between 1 month (g) and 3 months (h) of experimentally biodegraded PUR samples using *Pseudomonas aeruginosa* E_32.

Figure 4.3i-j showed the comparison between 1 month and 3 months of experimentally biodegraded PUR samples using *Enterobacter amnigenus* $D_1$2. In the first month FTIR spectra analysis of PUR samples biodegraded with *Enterobacter amnigenus* $D_1$2 (Fig. 4.3i), it showed removal of peaks at wavenumbers (cm^{-1}) 1541.18 corresponding to secondary amide (NH) bend; 3061.13, 3103.57 and 3275.24 when compared to control. A new peak at wavenumber 3234.73 was observed. In the shifts to the right of spectra of existing peaks, it caused a shift in peak at wavenumber 1662.69 corresponding to urethane (NH) group to 1654.98 and also caused a shift to the left of the spectra of peak at wavenumber 3443.05 corresponding to hydrogen bonded O-H stretch to a range of 3446-3462.34 when compared to control while in the FTIR spectral analysis of biodegraded PUR samples after 3 months using *Enterobacter amnigenus* $D_1$2 (Fig. 4.3j), it showed removal of peaks at wavenumbers 451.36 corresponding to C-H out of plane bending vibration of substituted amide group; 3448.91 corresponding to hydrogen bonded O-H stretch; 3736.24 corresponding to O-H stretching vibrations of intramolecular hydrogen bond; 2274.15 corresponding to isocyanate (-N=C=O) asymmetric stretch and 3234.73. It also showed formation of a new peak at wavenumber 3398.69 corresponding to polymeric O-H stretch in comparison to the control. In the shifts to the right of the IR spectrum of some existing peaks, it caused a shift in peak at wavenumber 2343.59 to a range of 2332-2341.66 and in the shifts to the left of the spectrum, it caused shifts in peaks at wavenumbers 2359.02 to 2360.95; 1743.71 corresponding to carbonyl group to a range of 1747-1749.5 when compared to the control.

Figure 4.3k-l showed the comparison between 1 month and 3 months of experimentally biodegraded PUR samples using *Vibrio* sp. $C_3$2. In the first month FTIR spectral analysis of PUR samples biodegraded with *Vibrio* sp. $C_3$2 (Fig. 4.3k), it showed removal of peaks at wavenumbers (cm^{-1}) 3061.13, 3103.57 and 3275.24; with formation of a new peak observed at wavenumber 420.5 when compared to the control. In the shifts to the right of spectra of existing peaks, it caused a shift in peak at wavenumber 1662.69 corresponding to urethane (NH) group to 1654.98 and also caused shifts to the left of peaks at wavenumbers 3443.05 corresponding to hydrogen bonded O-H stretch to a range of 3446-

(i)



(j)

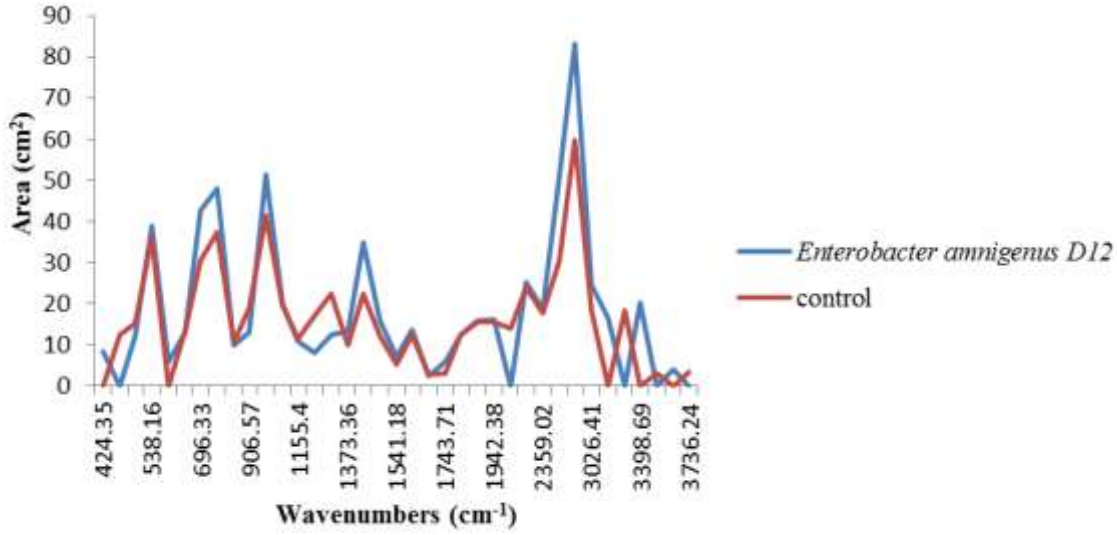
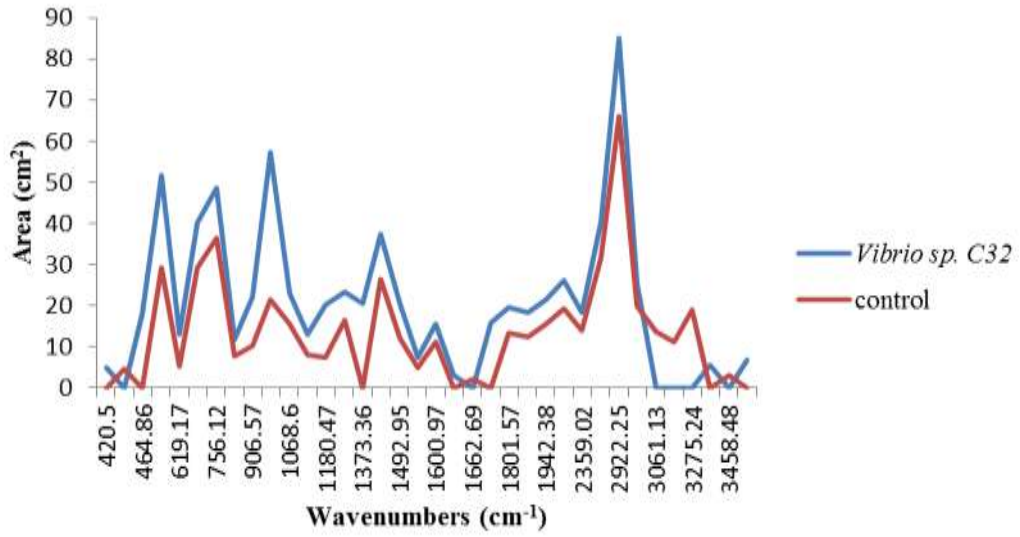


Figure 4.3 (i-j): Comparison of FTIR spectra analysis between 1 month (i) and 3 months (j) of experimentally biodegraded PUR samples using *Enterobacter amnigenus* D_12

3462.34; 451.36 corresponding to C-H out of plane bending vibration of substituted amide to a range of 464-466.8; 1541.18 corresponding to secondary amide (NH) bend to 1543.1; 2341.66 and 2359.02 to 2343.59 and 2360.95 respectively when compared to the control while in the FTIR spectral analysis of biodegraded PUR samples after 3 months using *Vibrio* sp. $C_3$2 (Fig. 4.3l), it showed removal of peaks at wavenumbers 451.36 corresponding to C-H out of plane bending vibration of substituted amide group; 669.32 corresponding to O-H out of plane bend; 1313.57 corresponding to O-H in-plane bend; 2274.15 corresponding to isocyanate (-N=C=O) asymmetric stretch and 3234.73. It also showed the formation of a new peak at wavenumber 1340.57. In the shifts to the right of the IR spectrum of some existing peaks, it caused shifts in peaks at wavenumbers 2343.59 to 2332.06; 1155.4 and 1654.98 corresponding to alkyl substituted ether (C-O) stretch and urethane (NH) group respectively to 1153.47 and 1653.06 and in the shifts to the left of the spectrum, it caused shifts in peaks at wavenumbers 2359.02 to 2360.95; 464.86 corresponding to C-H out of plane bending vibration of substituted amide group to 466.79 and 3448.91 corresponding to hydrogen bonded O-H stretch to 3460.41 when compared to the control.

Figure 4.3m-n showed the comparison between 1 month and 3 months of experimentally biodegraded PUR samples using combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5). In the first month FTIR spectral analysis of PUR samples biodegraded with combination A (Fig. 4.3m), it showed removal of peaks at wavenumbers (cm^{-1}) 619.17 corresponding to alkyne bend; 1541.18 corresponding to secondary amide (NH) bend; 1662.69 corresponding to urethane (NH) group; 2341.66, 2345.52, 3103.57 and 3275.24 when compared to the control. It caused shifts to the left of spectra of existing peaks at wavenumbers 1869.08 corresponding to anhydride of carbonyl of the amide to 1871.01; 3443.05 corresponding to hydrogen bonded O-H stretch to a range of 3446-3462.34; 451.36 corresponding to C-H out of plane bending vibration of substituted amide to a range of 464-466.8 and 2359.02 to 2360.95 when compared to the control while in the FTIR spectral analysis of PUR samples biodegraded with combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5) after 3 months (Fig.4.3n)

(k)



(l)

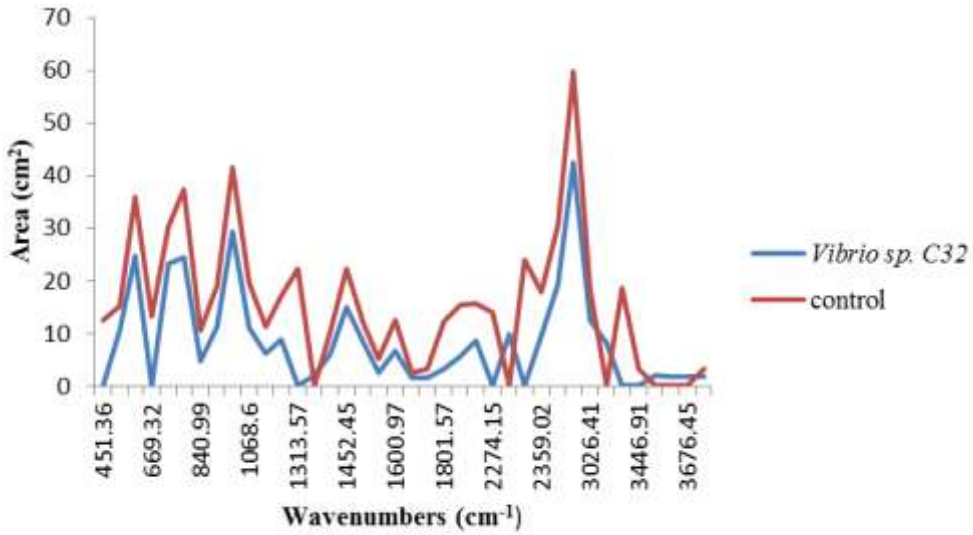
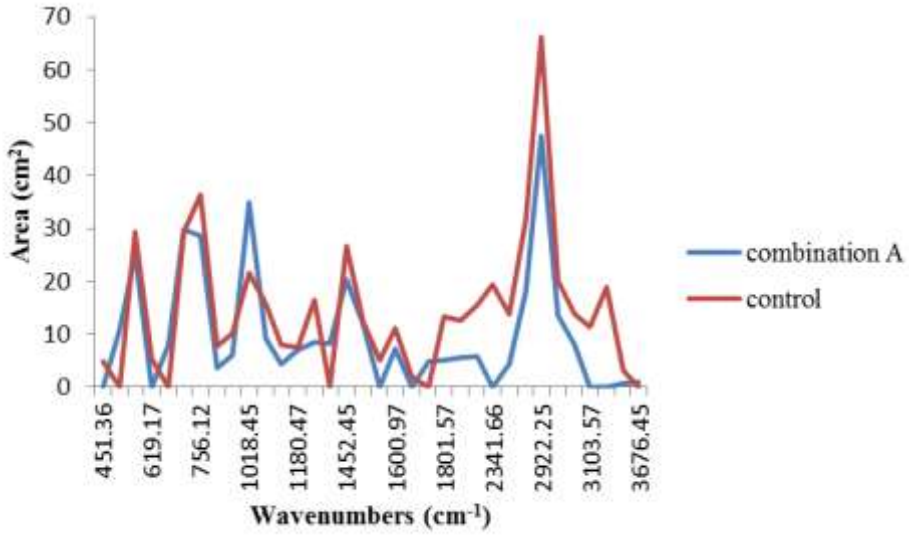
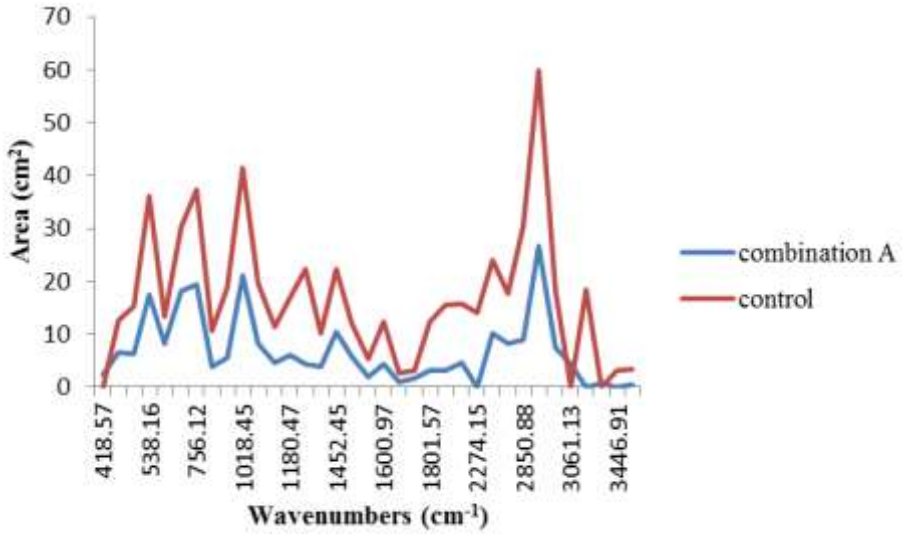


Figure 4.3 (k-l): Comparison of FTIR spectra analysis between 1 month (k) and 3 months (l) of experimentally biodegraded PUR samples using *Vibrio* sp. $C_3$2.

(m)



(n)



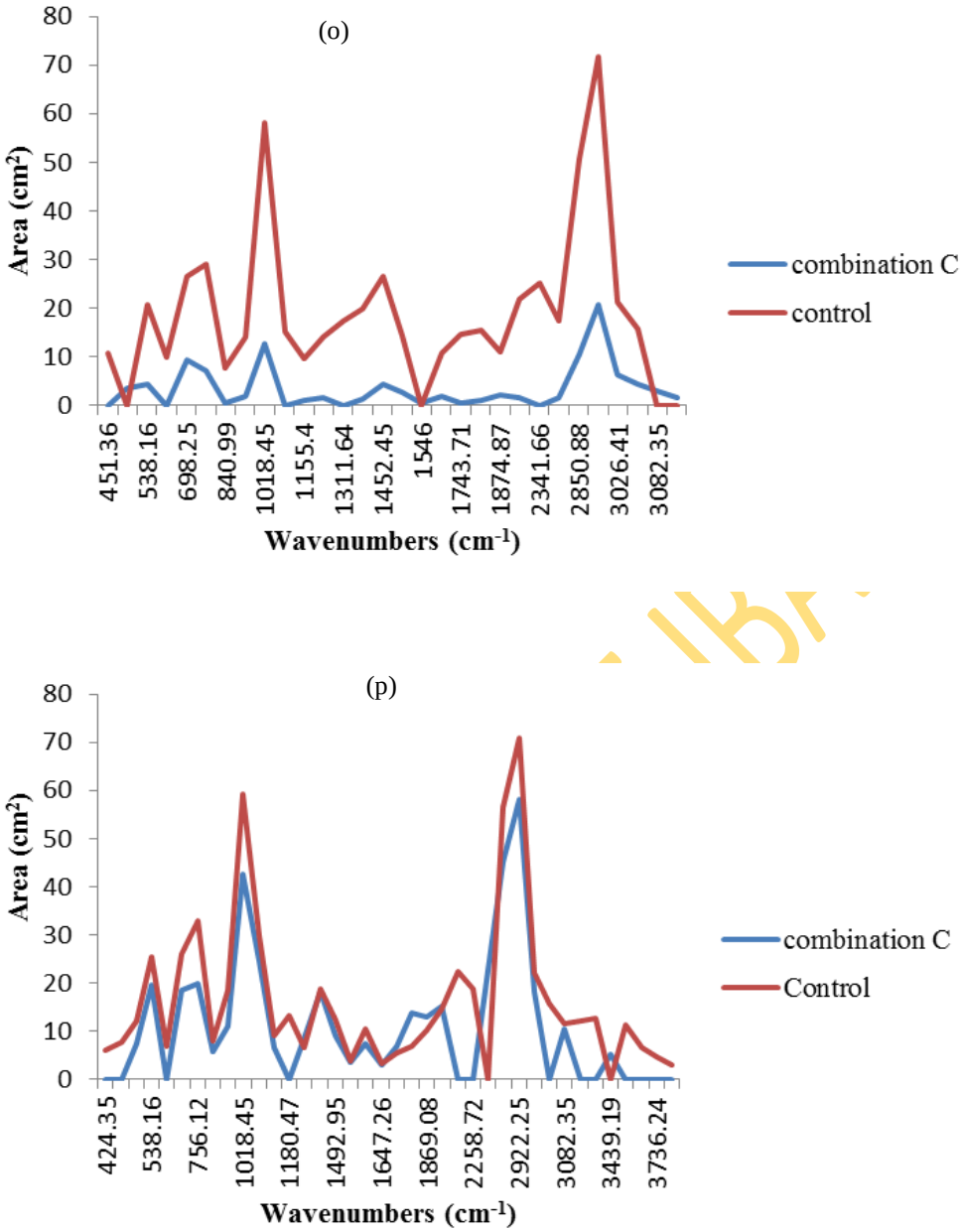
Key:

Combination A= *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Figure 4.3(m-n): Comparison of FTIR spectra analysis between 1 month (m) and 3 months (n) of experimentally biodegraded PUR samples using combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5).

it showed removal of peaks at wavenumbers 2274.15 corresponding to isocyanate (-N=C=O) asymmetric stretch; 3448.91 corresponding to hydrogen bonded O-H stretch and 3234.73. It equally showed formation of a new peak at wavenumber 3414.12 corresponding to polymeric O-H stretch. In the shifts to the right of the spectrum of existing peaks, it caused a shift in peak at wavenumber 2343.59 to 2341.66 and in the shifts to the left of the spectra, it caused shifts in peaks at wavenumbers 2359.02 to 2360.95; 1743.71 corresponding to carbonyl group to 1749.49; 464.86 corresponding to C-H out of plane bending vibration of substituted amide group to 466.79 and 1541.18 corresponding to secondary amide (NH) bend to 1545.03 when compared to the control.

Figure 4.3o-p showed the comparison between 1 month and 3 months of experimentally biodegraded PUR samples using combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1). In the first month FTIR spectral analysis of PUR samples biodegraded with combination C (Fig. 4.3o), it showed removal of peaks at wavenumbers (cm^{-1}) 669.32 corresponding to O-H out of plane bend; 1068.6 corresponding to alkyl substituted ether group; 1329 corresponding to C-H bending vibrations of methylene alkyl group; 1647.26 corresponding to urethane (NH) group; 3676.45 and 3751.67 corresponding to O-H stretching vibrations of intramolecular hydrogen bond and 2345.52. It equally showed formation of new peak within the range of wavenumbers 2359-2368.66 when compared to the control. It caused shifts to the right of the spectra of peaks at wavenumbers 1155.4 corresponding to alkyl substituted ether (C-O) stretch to 1153.47; 466.79 corresponding to C-H out of plane bending vibration of substituted amide group and 1747.57 corresponding to carbonyl group to a range of 451-464.9 and 1743.71 respectively. In the shifts to the left of existing peaks, combination C caused shifts in peaks at wavenumbers 3061.13 to 3082.35; 904.64 corresponding to skeletal C-C vibrations to 906.57 and 3443.05 corresponding to hydrogen bonded O-H stretch to a range of 3446 -3456.6 when compared to control while in the FTIR spectral analysis of PUR samples biodegraded with combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1) after 3 months (Fig. 4.3p), it showed removal of peaks at wavenumbers (cm^{-1}) 424.35; 451.36 corresponding to C-H out of plane bending vibration



Key:

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Figure 4.3 (o-p): Comparison of FTIR spectra analysis between 1 month (o) and 3 months (p) of experimentally biodegraded PUR samples using combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1).

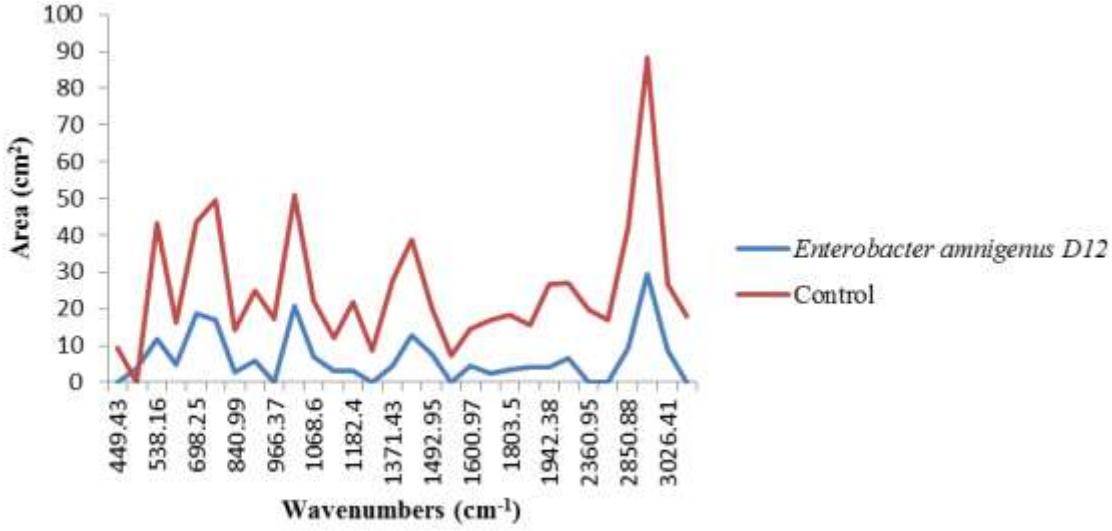
of substituted amide group; 621.1 corresponding to C-H bend of alkyne group; 1180.47 corresponding to alkyl substituted ether C-O stretch; 2196.99 corresponding to cyanates (-OCN) asymmetric stretching vibration; 3566.5 corresponding to internally bonded O-H stretch; 3649.44, 3726.24 and 3865.48 corresponding to O-H stretching vibration of intramolecular hydrogen bond; 3103.57 and 3163.36.

4.6.2 FTIR spectra analysis of degraded PUR samples after 1 month in broth under agitation

In the FTIR spectra analysis of PUR samples experimentally degraded under agitation in the laboratory broth, it showed that the PUR samples degraded with *Enterobacter amnigenus* $D_1$2 (Fig. 4.4a) had disappearances and alterations of some peaks when compared to the control. It showed removal of peaks at wavenumbers 966.37 corresponding to hydrogen bonded O-H out of plane bending; 1329 corresponding to C-H bending vibrations of methylene alkyl group; 1546.96 corresponding to secondary amide (NH) bend; 2787.23 corresponding to C-H stretching vibrations of a methyl group and also 2360.95 and 3061.13. It caused shifts to the right of IR spectra of some existing peaks such as peaks at wavenumbers 449.43 corresponding to C-H out of plane bending vibration of substituted amide to a range of 464-466.79; 1371 corresponding to a symmetric methyl (-CH_3) bend to a range of 1373-1375.3 and 538.16 to 540.09. In the shifts to the left of the spectra, it caused shifts in peaks at wavenumbers 698.25 corresponding to O-H out of plane bend to 696.33; 2924.18 corresponding to asymmetric C-H stretch of methylene group to 2922.25; 1872.94 corresponding to anhydride of carbonyl of the amide group to 1869.08 and 1803.5 to 1801.57 when compared to the control.

In the FTIR spectra analysis of agitated degradation experiment using *Vibrio* sp. $C_3$2 (Fig. 4.4b), it showed removal of peaks at wavenumbers 966.37 corresponding to hydrogen bonded O-H out of plane bending; 2787.23 corresponding to C-H stretching vibrations of a methyl group and 2341.66. It caused shifts to the right of peaks at wavenumbers 698.25 corresponding to O-H out of plane bend and 2924.18 corresponding to asymmetric C-H

(a)



(b)

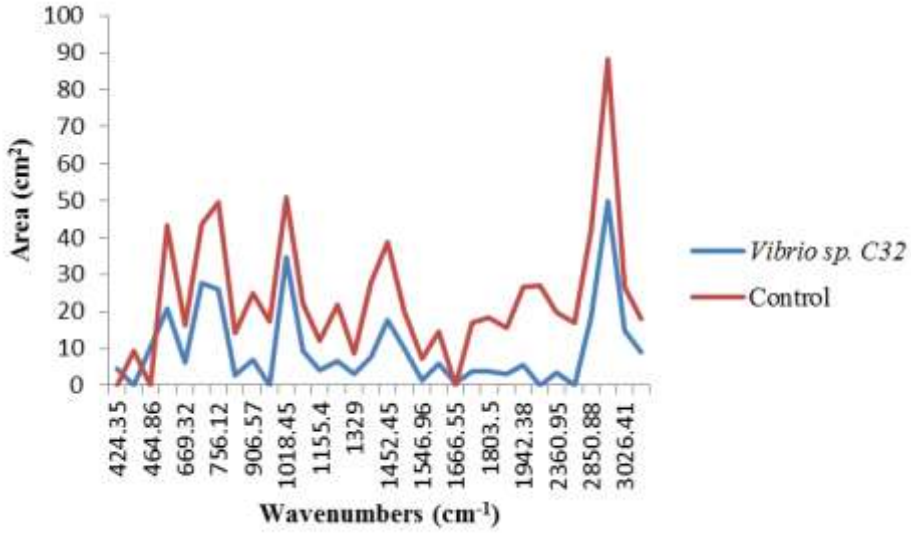


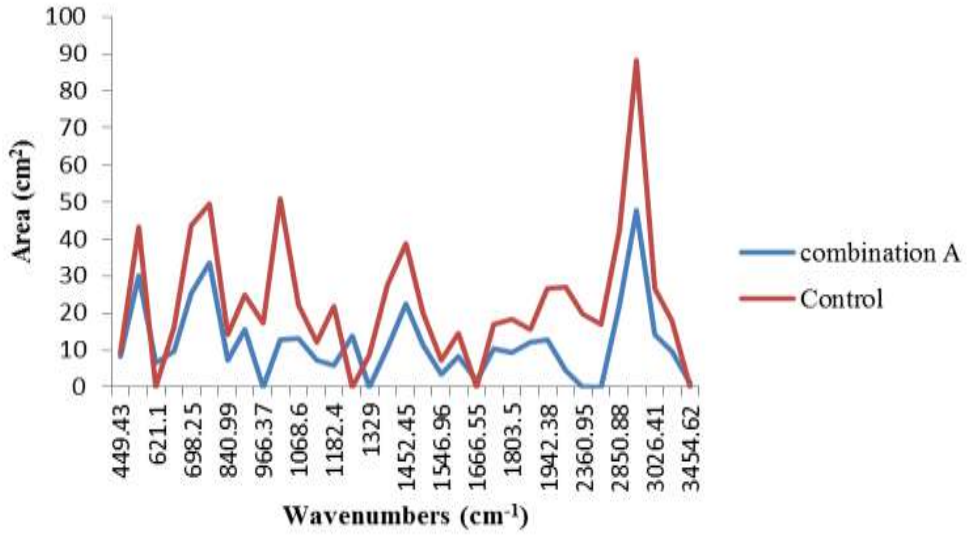
Figure 4.4 (a-b): FTIR spectral analysis of PUR samples degraded with *Enterobacter amnigenus* $D_1$2 (a) and *Vibrio* sp. $C_3$2 (b) after 1 month under agitation in comparison to control.

stretch of methylene group to 696.33 and 2922.25 respectively and shift to the left of peak at wavenumber 449.43 corresponding to C-H out of plane bending vibration of substituted amide to a range of 464-466.79. The spectra equally showed formation of new peak within the range of wavenumbers 418-424.35 and also at 1666.55 corresponding to urethane (NH) group when compared to the control.

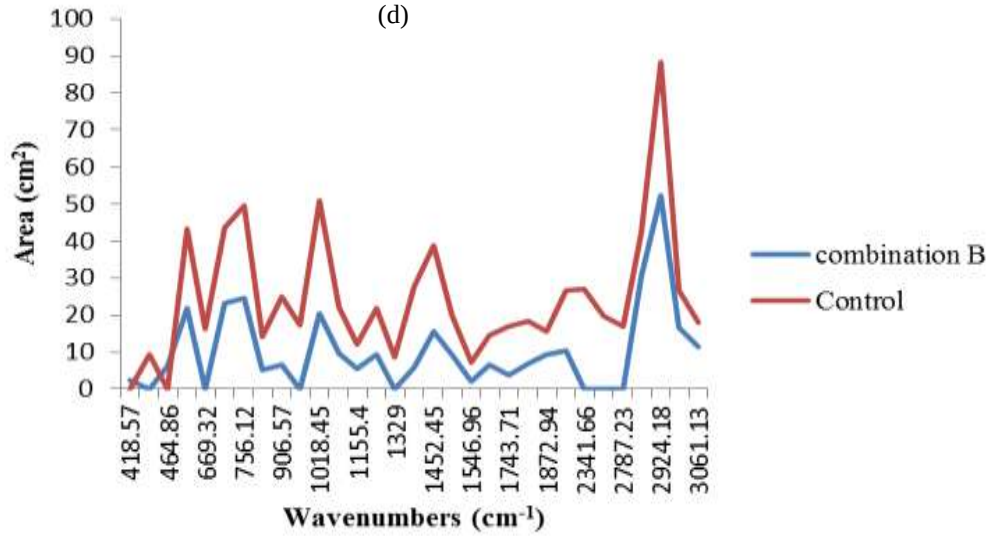
In the FTIR spectra analysis of agitated degradation experiment using Combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5) (Fig. 4.4c) it showed removal of peaks at wavenumbers 966.37 corresponding to hydrogen bonded O-H out of plane bending; 1329 corresponding to C-H bending vibrations of methylene alkyl group; 2787.23 corresponding to C-H stretching vibrations of a methyl group and 2360.95. It also showed the formation of new peaks at wavenumbers 621.1 corresponding to C-H bend of alkyne group; 1311 corresponding to O-H in-plane bend; 1666.55 corresponding to urethane (NH) group and 3454.62 corresponding to dimeric O-H stretch. In the shifts to the right of the spectra, Combination A caused shifts in peaks at wavenumbers 698.25 corresponding to O-H out of plane bend to 696.33; 2924.18 corresponding to asymmetric C-H stretch of methylene group to 2922.25; 1182.4 corresponding to alkyl substituted ether (C-O) stretch to 1180.47 and 2341.66 to 2335.87 when compared to the control. It caused a shift to the left of peak at wavenumber 449.43 corresponding to C-H out of plane bending vibration of substituted amide to a range of 464-466.79.

In the FTIR spectra analysis of agitated degradation experiment using Combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) (Fig. 4.4d) it showed removal of peaks at wavenumbers 669.32 corresponding to O-H out of plane bend; 966.37 corresponding to hydrogen bonded O-H out of plane bending; 1329 corresponding to C-H bending vibrations of methylene alkyl group; 2787.23 corresponding to C-H stretching vibrations of a methyl group; 2341.66 and 2360.95. It equally showed formation of new peak within the range of wavenumbers 418-424.35. Combination B caused shifts to the right of the spectra of peaks at wavenumbers 698.25 corresponding to O-H out of plane bend to 696.33; 1546.96 corresponding to secondary amide (NH) bend to 1541.18; 1872.94

(c)



(d)



Key:

Combination A= *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B= *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

Figures 4.4 (c-d): FTIR spectral analysis of PUR degraded with combination A (c) and combination B (d) under Agitation after 1 month in comparison to control

corresponding to anhydride of carbonyl of the amide group to 1869.08; 2924.18 corresponding to asymmetric C-H stretch of methylene group to 2922.25 and 1803.5 to 1801.57. It also caused shifts to the left of peaks at wavenumbers 449.43 corresponding to C-H out of plane bending vibration of substituted amide to a range of 464-466.79; 1371 corresponding to a symmetric methyl (-CH_3) bend to a range of 1373-1375.3 and 1743.71 corresponding to carbonyl group to 1749.49 when compared to the control.

4.7 Field biodegradation studies using bioaugmentation and biostimulation

4.7.1 FTIR spectra analysis of experimentally degraded PUR samples using the isolates combinations biostimulated with cassava peels in the field after 30 days of soil burial

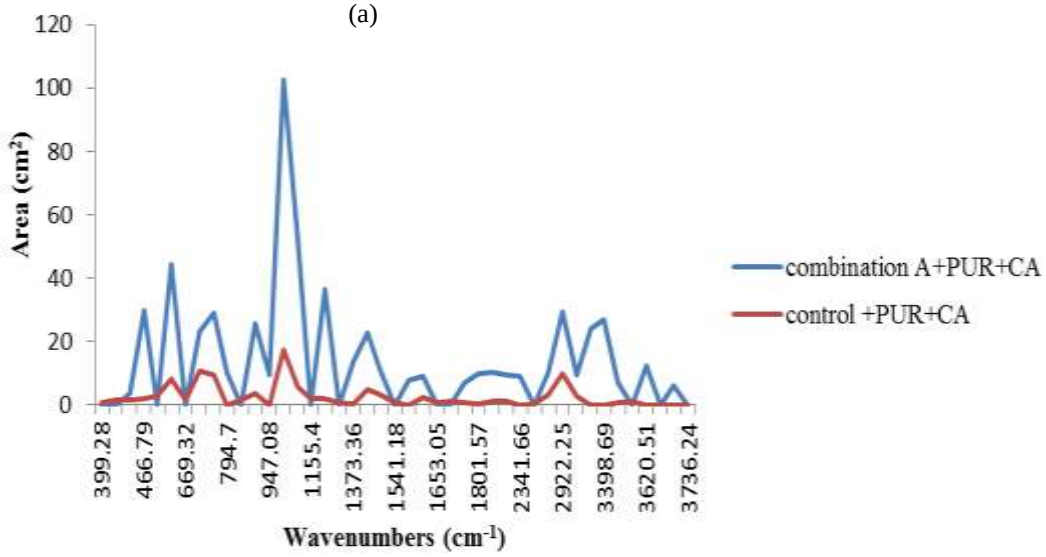
The FTIR spectra analysis of PUR samples experimentally degraded with combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5) biostimulated with cassava peels after 30 days in the field (Fig. 4.5a) showed removal of peaks at wavenumbers 399.28, 418.57 and 520.8; wavenumbers 669.32 corresponding to O-H out of plane bend; 839.06 corresponding to skeletal C-C vibrations; 1153.47 corresponding to alkyl substituted ether (C-O) stretch; 1338.64 corresponding to bending vibration of a carboxylate group of carbonyl; 1541.18 corresponding to secondary amide (NH) bend; 1653.05 corresponding to urethane (NH) group; 1683.91 corresponding to amide group of carbonyl; 3458.48 corresponding to dimeric O-H stretch; 3657.16 and 3736.24 corresponding to O-H stretching vibration of intramolecular hydrogen bond when compared to the control. It equally showed the formation of new peaks at wavenumbers 794.7, 1583.61, 3398.69, 3433.41, 3620.51 and 3697.66 corresponding to skeletal C-C vibrations, secondary amine (NH) bend, primary amine N-H stretch, polymeric O-H stretch, tertiary alcohol O-H stretch and O-H stretching vibrations of intramolecular hydrogen bond respectively. Also new peaks were formed at wavenumbers 947.08 and 3271.38. It caused shifts in the right of some existing peaks at wavenumbers 443.64 to 432.07; 538.16 to 536.23; 1180.47 corresponding to alkyl substituted ether (C-O) stretch to 1178.55; 1369.5 corresponding to a symmetric methyl (-CH_3) bend to 1365.65 and 2357.09 to 2341.66.

The spectra equally showed shifts to the left of the spectra of some existing peaks at wavenumbers 908.5 corresponding to skeletal C-C vibrations to 910.43; 1028.09 corresponding to primary amine (CN) stretch to 1030.02; 1068.6 corresponding to alkyl substituted ether group to 1078.24; 1734.06 corresponding to carbonyl group to 1741.76; 1869.08 corresponding to anhydride of carbonyl of the amide to 1872.94 and 2920.32 corresponding to asymmetric C-H stretch of methylene group to 2922.25 when compared to control.

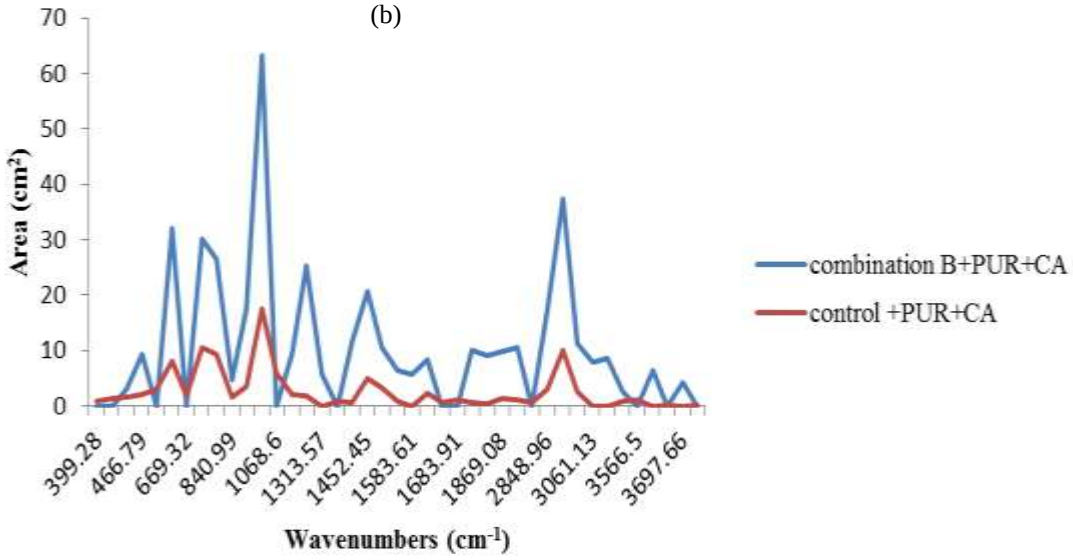
The FTIR spectra analysis of PUR samples experimentally degraded with combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) biostimulated with cassava peels after 30 days in the field (Fig. 4.5b) showed the removal of peaks at wavenumbers 399.28, 418.57, 520.8, 2357.09; 669.32 corresponding to O-H out of plane bend; 1068.6 corresponding to alkyl substituted ether group; 1338.64 corresponding to bending vibration of a carboxylate group of carbonyl; 1653.05 corresponding to urethane (NH) group; 1683.91 corresponding to amide group of carbonyl; 3458.48 corresponding to dimeric O-H stretch; 3566.8 corresponding to internally bonded O-H stretch; 3657.16 and 3736.24 corresponding to O-H stretching vibration of intramolecular hydrogen bond when compared to the control. The spectra also showed the formation of new peaks at wavenumbers 1311.64, 1583.61, 3433.41, 3616.58 and 3699.59 corresponding to O-H in plane bend, secondary amine (NH) bend, polymeric O-H stretch, tertiary alcohol O-H stretch and O-H stretching vibrations of intramolecular hydrogen bond respectively and also at 947.08 and 3271.38 in comparison to the control.

The analysis equally showed that there were shifts to the left of the spectra of some existing peaks at wavenumbers 462.93, 839.06, 1369.5, 1734.06 and 2920.32 corresponding to C-H out of plane bending vibration of substituted amide; skeletal C-C vibrations; symmetric methyl (-CH_3) bend; carbonyl group and asymmetric C-H stretch of methylene group to 470.65, 840.99, 1371.43, 1743.71 and 2922.25 respectively. It also caused shifts to the right of the spectra of peaks at wavenumbers 443.64 to 430.14; 538.16 to 536.23 and 1541.18 corresponding to secondary amide (NH) bend to 1539.25.

(a)



(b)



Key:

Combination A: *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B: *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

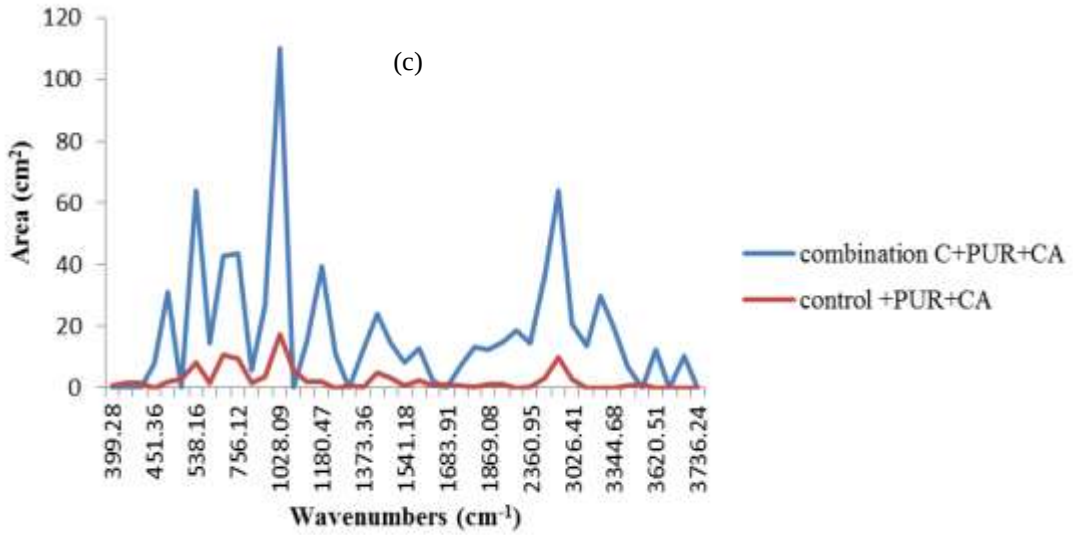
PUR= Polyurethane sample

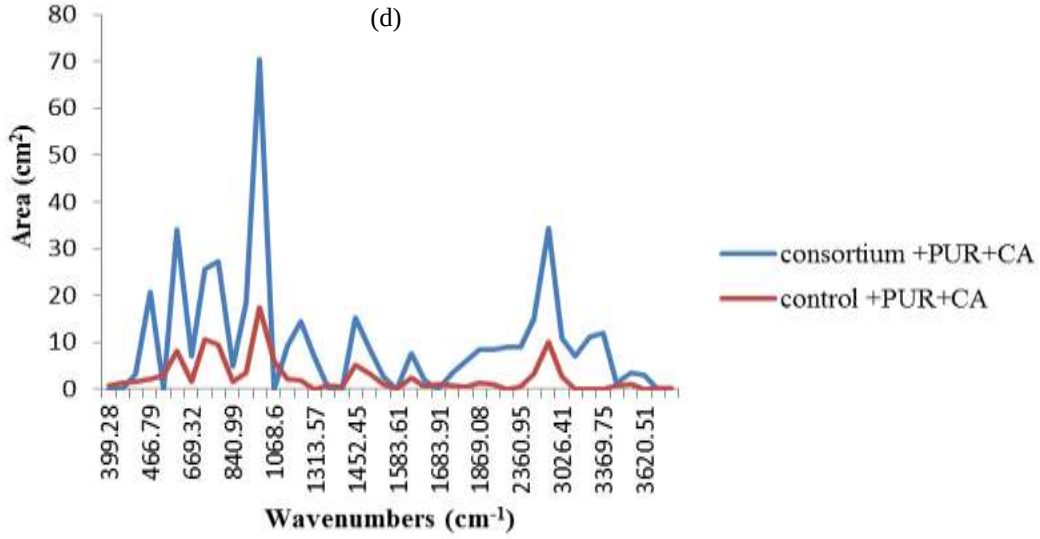
CA= Cassava peels

Figure 4.5 (a-b): FTIR spectra analysis of PUR samples degraded with combination A (a) and combination B (b) stimulated with cassava peels after 1 month in the field in comparison with the control.

The FTIR spectra analysis of PUR samples experimentally degraded with combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1) biostimulated with cassava peels after 30 days in the field (Fig. 4.5c) showed the removal of peaks at wavenumbers 399.28, 418.57, 520.8; 1068.6 corresponding to alkyl substituted ether group; 1338.64 corresponding to bending vibration of a carboxylate group of carbonyl; 1683.91 corresponding to amide group of carbonyl; 3657.16 and 3736.24 corresponding to O-H stretching vibration of intramolecular hydrogen bond when compared to the control. The spectra also showed formation of new peaks at wavenumbers 1313.64 corresponding to O-H in-plane bend; 2341.66; 3061.13; 3265.59; 3344.68 corresponding to polymeric O-H stretch; 3620.51 corresponding to tertiary alcohol O-H stretch and 3697.66 corresponding O-H stretching vibrations of intramolecular hydrogen bond. It caused shifts to the left of the spectra of peaks at wavenumbers 443 to 451; 2357.09 to 2360.95; 462.93, 839.06, 1369.5, 1541.18, 1734.06 and 2920.32 corresponding to C-H out of plane bending vibration of substituted amide; skeletal C-C vibrations; symmetric methyl (-CH_3) bend; secondary amide (NH) bend; carbonyl group and asymmetric C-H stretch of methylene group were shifted to 466.79, 840.99, 1373.96, 1543.1, 1743.71 and 2922.25 respectively. It equally caused shifts to the right of spectra of peaks at wavenumbers 756.12 to 754.19 and 3458.48 corresponding to dimeric O-H stretch to 3446.91 in comparison to the control.

The FTIR spectra analysis of PUR samples experimentally degraded with consortium (combination A + combination B + combination C) biostimulated with cassava peels after 30 days in the field (Fig. 4.5d) showed the removal of peaks at wavenumbers 399.28, 418.57, 520.8; 1068.6 corresponding to alkyl substituted ether group; 1338.64 corresponding to bending vibration of a carboxylate group of carbonyl; 1683.91 corresponding to amide group of carbonyl; 3657.16 and 3736.24 corresponding to O-H stretching vibration of intramolecular hydrogen bond when compared to the control. There were formations of new peaks at wavenumbers 1313.57 corresponding to O-H in-plane bend; 2332.02; 3061.13, 3244.38 corresponding to hydrogen bonded O-H stretch; 3369.75 corresponding to polymeric O-H stretch and 3620.51 corresponding to tertiary alcohol O-





Key:

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= combination A + combination B + combination C

PUR= Polyurethane sample

CA= Cassava peels

Figure 4.5 (c-d): FTIR spectra analysis of PUR samples degraded with combination C (c) and consortium (d) stimulated with cassava peels after 1 month in the field in comparison with the control.

H stretch. The spectra showed that there were shifts to the right of the spectra of some existing peaks at wavenumbers 443. 64 to 432.07; 1653.06 corresponding to urethane (NH) group to 1647.26; 2850.88 corresponding to methoxy methyl ether group to 2848.96 and 3458.48 corresponding to dimeric O-H stretch to 3446.91. The analysis also showed that the consortium caused shifts to the left of the spectra of peaks at wavenumbers 3566.5 corresponding to internally bonded O-H stretch to 3568.43; 2920.32 corresponding to asymmetric C-H stretch of methylene group to 2922.25; 2357.09 to 2359.02; 1734.06 corresponding to carbonyl group to 1749.49; 1541.18 corresponding to secondary amide (NH) bend to 1543.1; 1369.5 corresponding to a symmetric methyl (-CH_3) bend to 1373.38; 1028.09 corresponding to primary amine (CN) stretch to 1030.02; 839.06 and 462.93 to 840.99 and 468.72 respectively when compared to the control. The FTIR spectra of the different combinations and consortium (Fig. 4.5a-d) above showed increase in the peak areas of the experimentally degraded PUR samples compared to the control.

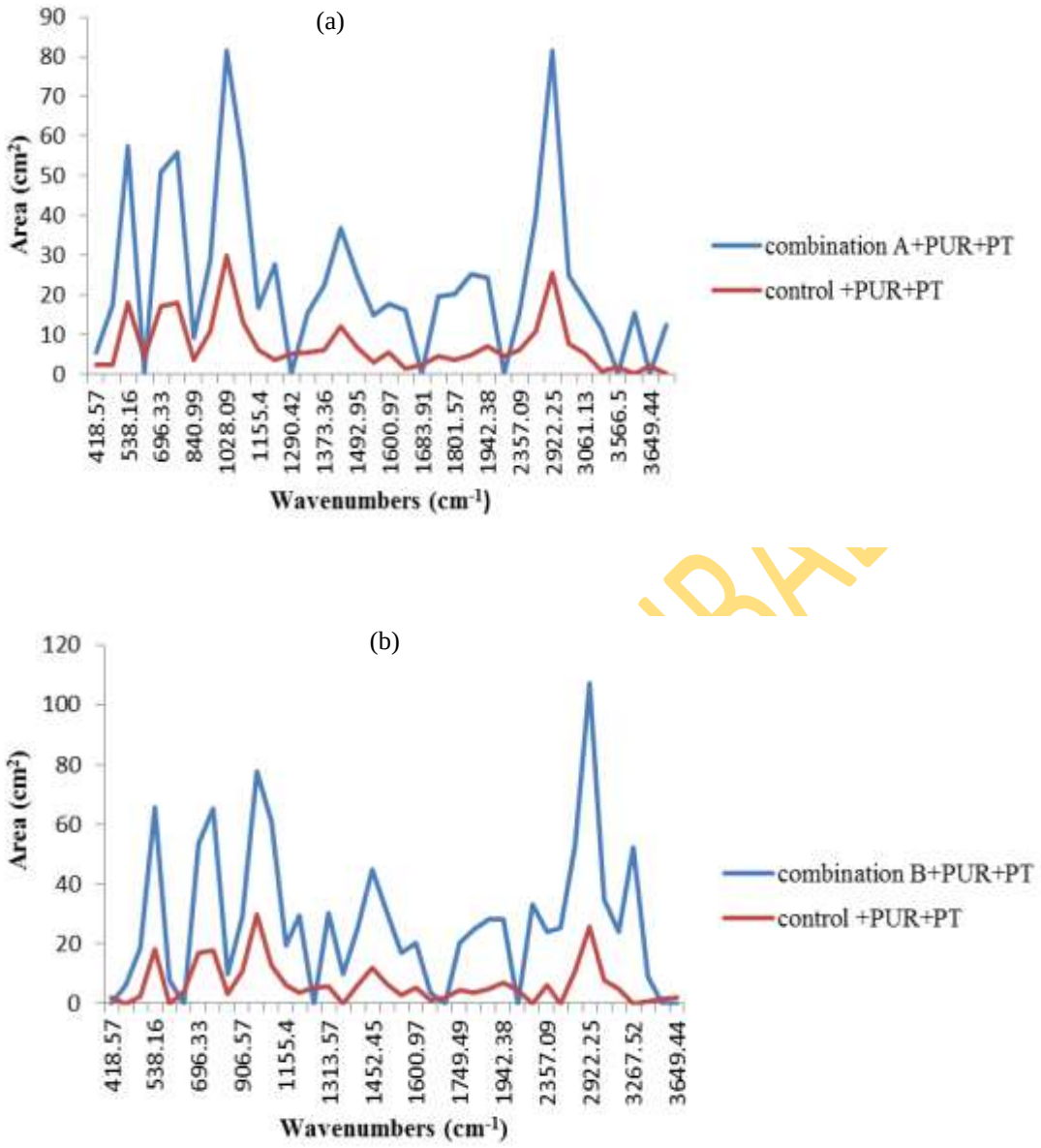
4.7.2 FTIR spectra analysis of experimentally degraded PUR samples using the isolates combinations biostimulated with Potato peels in the field after 30 days of soil burial

The FTIR spectra analysis of PUR samples experimentally degraded with combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5) biostimulated with potato peels after 30 days in the field (Fig. 4.6a) showed removal of peaks at wavenumbers 669.33 corresponding to O-H out of plane bend; 1290.42 corresponding to O-H in plane bend; 1683.91 corresponding to amide group of carbonyl; 3566.5 corresponding to internally bonded O-H stretch; 3649.44 corresponding to O-H stretching vibration of intramolecular hydrogen bond and 2314.66 when compared to the control. The spectra also showed formation of new peaks at wavenumbers 3620.51 corresponding to tertiary alcohol O-H stretch and 3699.59 corresponding to O-H stretching vibration of intramolecular hydrogen bond. In addition, it caused shifts to the left of the spectra of some existing peaks at wavenumbers 1653.05 corresponding to urethane (NH) group to 1660.77; 1869.08 corresponding to anhydride of carbonyl of the amide to 1871.01;

3446.91 corresponding to hydrogen bonded O-H stretch to 3452.7 and 2357.09 to 2359.02. In the shifts to the right of spectra, it showed that the peaks at the following wavenumbers were shifted to the right and they include; 422.42 to 418.57; 470.65 corresponding to C-H out of plane bending vibration of substituted amide group to 466.79; 758.05 to 756.12; 842.92 corresponding to skeletal C-C vibrations to 840.99; 1313.57 corresponding to O-H in-plane bend to 1311.64; 1373.36 corresponding to a symmetric methyl (-CH_3) bend to 1371.43 and 1749.49 corresponding to carbonyl group to 1745.64 in comparison to the control.

The FTIR spectra analysis of PUR samples experimentally degraded with combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) biostimulated with potato peels after 30 days in the field (Fig. 4.6b) showed removal of peaks at wavenumbers 669.33, 1290.42, 1683.91, 3566.5, and 3649.44 corresponding to O-H out of plane bend; O-H in plane bend; amide group of carbonyl; internally bonded O-H stretch and O-H stretching vibration of intramolecular hydrogen bond respectively; 422.42 and 2314.66 when compared to the control. It equally showed formation of new peaks at wavenumbers 451.36; 619.17 corresponding to C-H bend of alkyne group; 1329 corresponding to C-H bending vibrations of methylene alkyl group; 2341.66; 2507.54 and 3267.52. The spectra also showed that combination B caused shifts to the right of the spectra of some existing peaks at wavenumbers 470.65 corresponding to C-H out of plane bending vibration of substituted amide to 466.79; 758.05 to 756.12; 842.92 corresponding to skeletal C-C vibrations to 840.99; 1373.36 corresponding to a symmetric methyl (-CH_3) bend to 1371.43; 1749.49 corresponding to carbonyl group to 1743.71; 2850.88 corresponding to methoxy methyl ether to 2848.96 and 3446.91 corresponding to hydrogen bonded O-H stretch to 3441.12 in comparison to the control. In the shifts to the left, it caused shifts in peaks at wavenumbers 536.23 to 538.16; 696.33 corresponding to O-H out of plane bend to 698.25; 1653.05 corresponding to urethane (NH) group to 1654.98 and 2357.09 to 2359.02.

The FTIR spectra analysis of PUR samples experimentally degraded with combination C



Key:

Combination A: *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B: *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

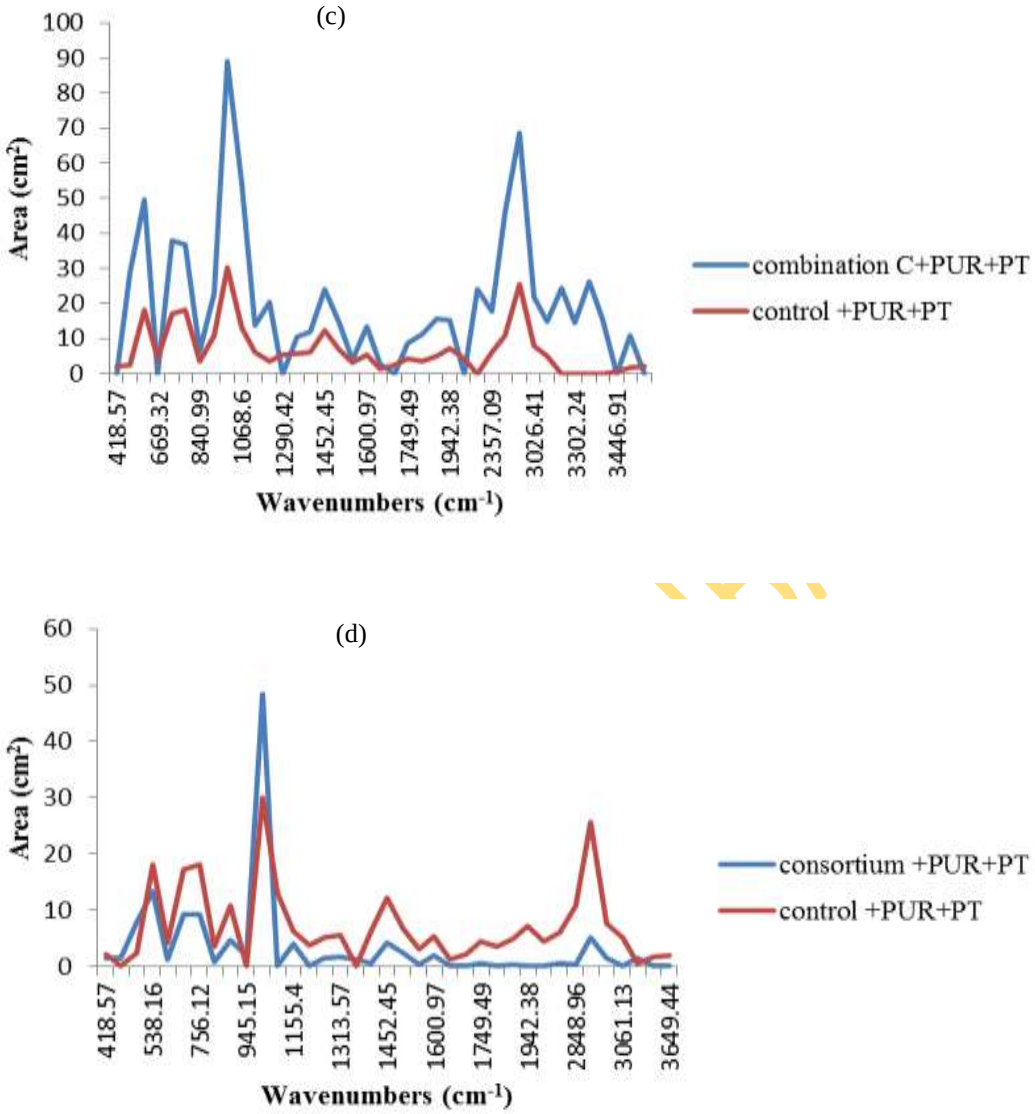
PUR= Polyurethane sample

PT= Potato peels

Figure 4.6 (a-b): FTIR spectra analysis of PUR samples degraded with combination A (a) and combination B (b) stimulated with potato peels after 1 month in the field in comparison with the control.

(*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1) biostimulated with potato peels after 30 days in the field (Fig. 4.6c) showed the removal of peaks at wavenumbers 669.33, 1290.42, 1683.91, 3446.91, and 3649.44 corresponding to O-H out of plane bend; O-H in plane bend; amide group of carbonyl; hydrogen bonded O-H stretch and O-H stretching vibration of intramolecular hydrogen bond respectively; 422.42 and 2314.66 when compared to the control. It also showed formation of new peaks at wavenumbers 3230.87, 3302.24, 3358.25 and 3387.11 corresponding to polymeric O-H stretch and 2341.66. In addition, the spectra showed that combination C caused shifts to the right of some existing peaks at wavenumbers 470.65 corresponding to C-H out of plane bending vibration of substituted amide to 466.79; 758.05 to 756.12; 842.92 corresponding to skeletal C-C vibrations to 840.99; 1653.05 corresponding to urethane (NH) group to 1647.26 and 1749.49 corresponding to carbonyl group to 1743.71. The spectra showed that combination C caused shifts to the left of spectra of peaks at wavenumbers 536.23 to 538.16; 1028.09 corresponding to primary amine (CN) stretch to 1030.02 and 2357.09 to 2360.95 when compared to the control.

The FTIR spectra analysis of PUR samples experimentally degraded with consortium (combination A + combination B + combination C) biostimulated with potato peels after 30 days in the field (Fig. 4.6d) showed the removal of peaks at wavenumbers 1068.6 corresponding to alkyl substituted ether group; 1180.47 corresponding to alkyl substituted ether (C-O) stretch; 1653.05 corresponding to urethane (NH) group; 1683.91 corresponding to amide group of carbonyl; 3566.5 corresponding to internally bonded O-H stretch; 3649.44 corresponding to O-H stretching vibration of intramolecular hydrogen bond; 2314.66 and 3061.13. It also showed the formation of new peaks at wavenumbers 445.57; 945.15 corresponding to hydrogen bonded O-H out of plane bending and 1338.7 corresponding to bending vibration of a carboxylate group of carbonyl when compared to the control. The spectra also showed that the peaks at wavenumbers following were shifted to the right of the spectra; 422.42 to 418.57; 470.65 corresponding to C-H out of plane bending vibration of substituted amide group to 468.72; 758.05 to 756.12; 842.92 corresponding to skeletal C-C vibrations to 840.99; 1290.42 corresponding to O-H in-



Key:

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= combination A + combination B + combination C

PUR=Polyurethane sample

PT= Potato peels

Figure 4.6 (c-d): FTIR spectra analysis of PUR samples degraded with combination C (c) and consortium (d) stimulated with potato peels after 1 month in the field in comparison with the control.

plane bend to 1280.78; 1313.57 corresponding to O-H in-plane bend to 1311.64; 1373.36 corresponding to a symmetric methyl (-CH_3) bend to 1361.79 and 1749.49 corresponding to carbonyl group to 1743.71 in comparison to the control. It caused shifts to the left of spectra of peaks at wavenumbers 906.57 corresponding to skeletal C-C vibrations, 1028.09 corresponding to primary amine (CN) stretch, 1543 corresponding to secondary amide (NH) bend and 2357.09 to 910.43, 1030.02, 1545.03 and 2359.02 respectively.

The FTIR spectra of the different combinations (Fig. 4.6a-c) showed increase in the peak areas of the experimentally degraded PUR samples compared to the control except consortium (Fig. 4.6d) which showed increase in area from 30.0 to 48.5 cm^{-2} at wavenumber 1030.

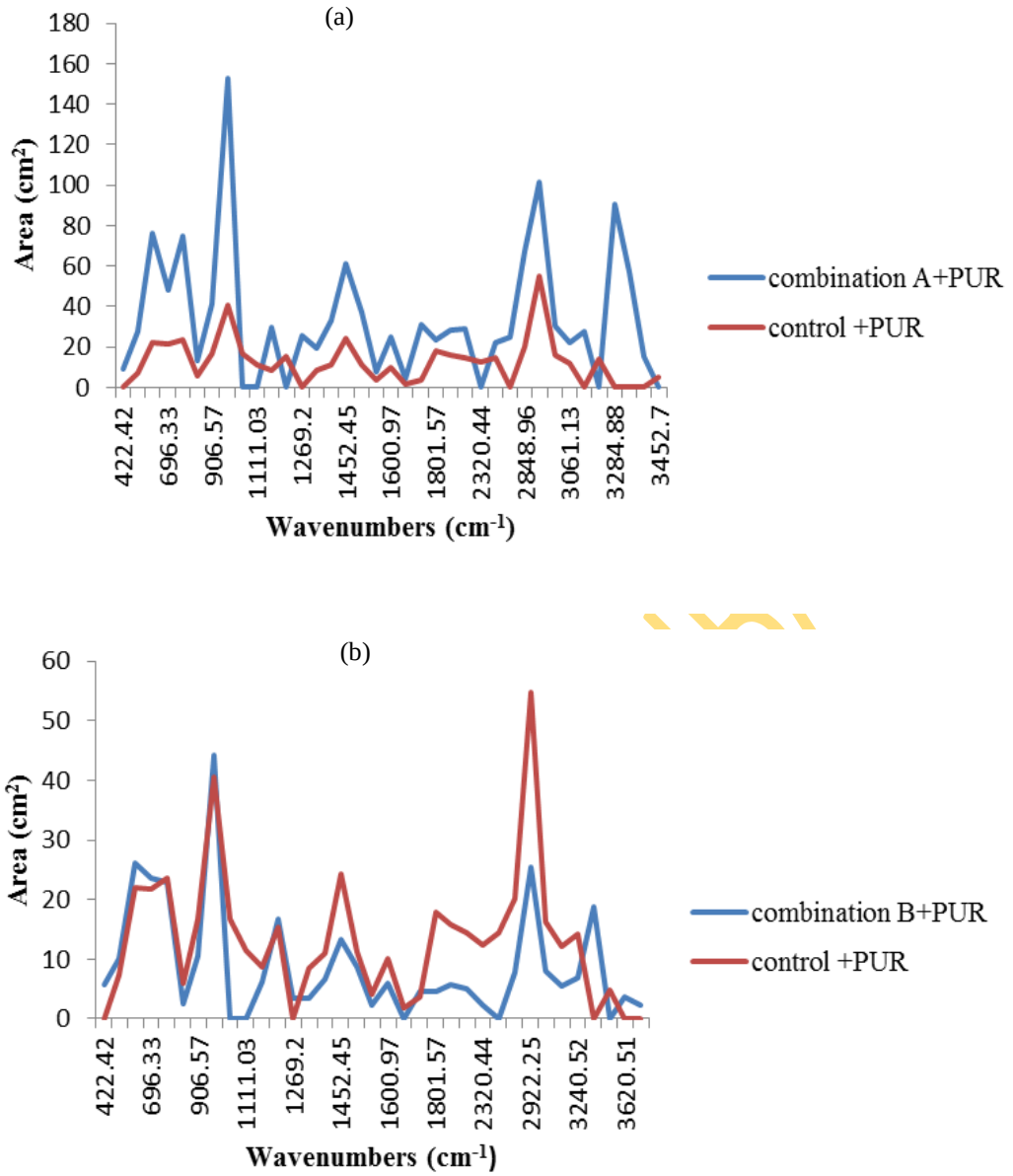
4.7.2 FTIR spectra analysis of experimentally degraded PUR samples using the isolates combinations without peels in the field after 30 days of soil burial

In this set up the bacterial isolates depended only on the nutrients in the soil microcosm within their reach; those in the growth medium used and also from the PUR samples for their activities. The FTIR spectra analysis of PUR samples degraded with combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5) without stimulation with any agro waste (Fig. 4.7a) showed removal of peaks at wavenumbers 1068.6, 1111.03 and 1180.47 corresponding to alkyl substituted ether (C-O) stretch; 2320.44; 3240.45 corresponding to hydrogen bonded O-H stretch and 3452.7 corresponding to dimeric O-H stretch; and formation of new peaks at 432.07; 1269.2 corresponding to O-H in-plane bend; 2704.29 corresponding to C-H stretching vibrations of a methyl group; 3284.88, 3346.61 and 3423.76 corresponding to polymeric O-H stretch and 3163.36 in comparison to control. The spectra equally showed that combination A caused shifts to the right of some existing peaks at wavenumbers 470.65 corresponding to C-H out of plane bending vibration of substituted amide, 1545.03 corresponding to secondary amide (NH) bend, 1660.77 corresponding to urethane (NH) group, 1743.71 corresponding to carbonyl group and 1801.57 to 466.79, 1539.25, 1651.12, 1741.78 and 1799.65 respectively. In the shifts to the left, it caused shifts in peaks at wavenumbers 754.19 corresponding to skeletal C-C

vibrations, 1028.09 corresponding to primary amine (CN) stretch, 1313.57 corresponding to O-H in-plane bend and 2357.09 to 756.12, 1030.02, 1329 and 2360.95 respectively when compared to control.

The FTIR spectra analysis of PUR samples degraded with combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) without stimulation with any agro waste (Fig. 4.7b) showed removal of peaks at wavenumbers 1068.6 and 1111.03 corresponding to alkyl substituted ether (C-O) stretch; 1660.77 corresponding to urethane (NH) group; 2357.09; and 3452.7 corresponding to dimeric O-H stretch. It also showed the formation of new peaks at wavenumbers 439.78; 1280.78 corresponding to O-H in-plane bend; 3402.54 corresponding to polymeric O-H stretch; 3620.51 corresponding to tertiary alcohol O-H stretch; and 3697.66 corresponding to O-H stretching vibration of intramolecular hydrogen bond when compared to the control. In addition, the spectra showed shifts to right of existing peaks at wavenumbers 470.65 corresponding to C-H out of plane bending vibration of substituted amide to 466.79; 1371.43 corresponding to a symmetric methyl (-CH_3) bend to 1369.5 and 2850.88 corresponding to methoxy methyl ether to 2848.96. In the shifts to the left, the spectra showed shifts in peaks at wavenumbers 754.19 corresponding to skeletal C-C vibrations to 756.12; 1313.57 corresponding to O-H in-plane bend to 1329; 1869.08 corresponding to anhydride of carbonyl of the amide to 1871.01; 2320.44 to 2339.73 and 3240.52 corresponding to hydrogen bonded O-H stretch to 3255.95 in comparison to the control.

The FTIR spectra analysis of PUR samples degraded with combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1) without stimulation with any agro waste (Fig. 4.7c) showed removal of peaks at wavenumbers 1111.03 corresponding to alkyl substituted ether (C-O) stretch and 3452.7 corresponding to dimeric O-H stretch. It showed formation of new peaks at wavenumbers 426.28; 669.32 corresponding to O-H out of plane bend; 2268.38 corresponding to isocyanate (-N=C=O) asymmetric stretch; 3103.57; 3358.19 and 3419.9 corresponding to the polymeric O-H stretch and 3697.66 corresponding to O-H stretching vibration of intramolecular hydrogen bond in comparison



Key:

Combination A: *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B: *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

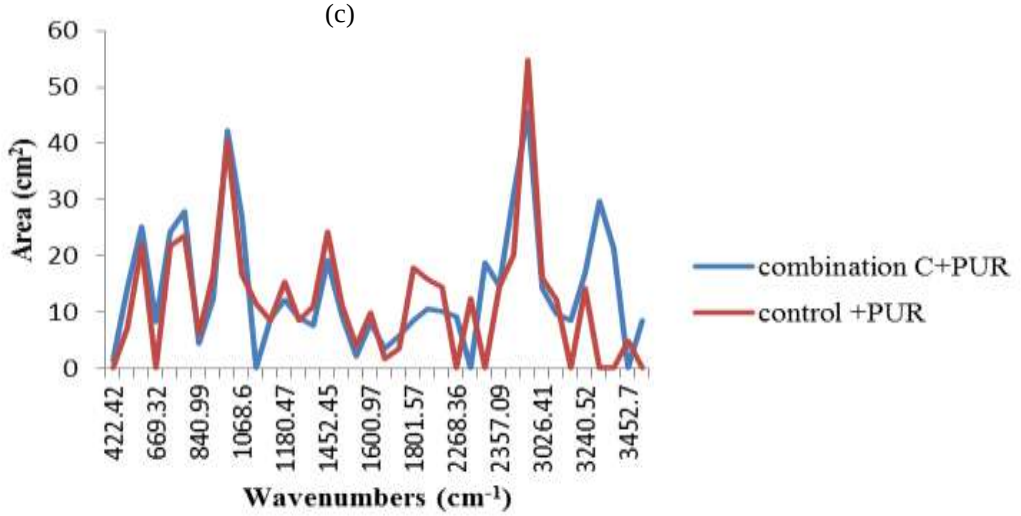
PUR= Polyurethane sample

Figure 4.7 (a-b): FTIR spectra analysis of PUR samples degraded with combination A (a) and combination B (c) without stimulation with any agro waste after 1 month in the field in comparison with the control.

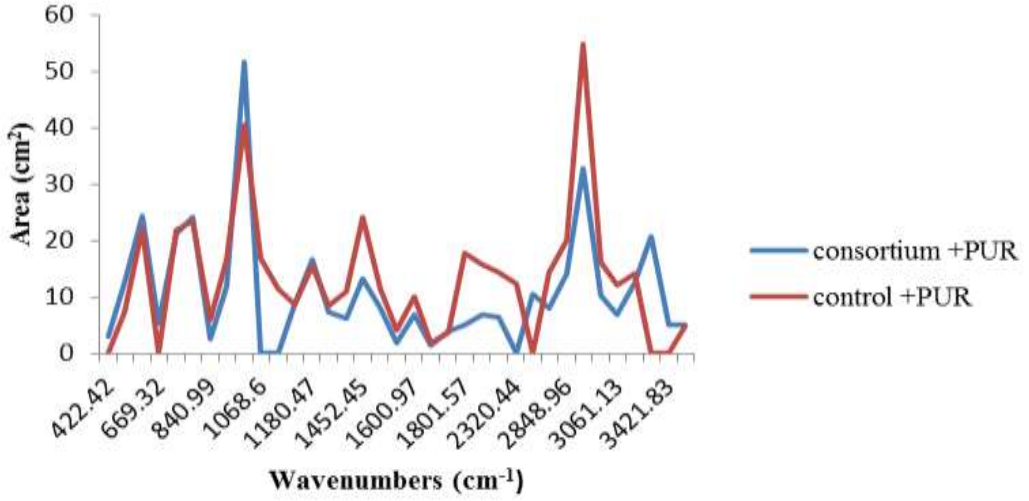
to the control. In the shifts to the left, it showed shifts in peaks at wavenumbers 754.19 corresponding to skeletal C-C vibrations to 756.12; 1371.43 corresponding to a symmetric methyl (-CH_3) bend to 1373.36; 2320.44 to 2341.66; 2357.09 to 2359.02 and 3240.52 corresponding to hydrogen bonded O-H stretch to 3255.95. The spectra equally showed shifts to the right of existing peaks at wavenumbers 470.65 corresponding to C-H out of plane bending vibration of substituted amide to 466.79; 1660.77 corresponding to urethane (NH) group to 1654.98 and 2850.88 corresponding to methoxy methyl ether to 2848.96 when compared to the control.

The FTIR spectra analysis of PUR samples degraded with the consortium (combination A + combination B + combination C) without stimulation with any agro waste (Fig. 4.7d) showed removal of peaks at wavenumbers 1068.6 and 1111.03 corresponding to alkyl substituted ether (C-O) stretch; and formation of new peaks at wavenumbers 418.57; 669.32 corresponding to O-H out of plane bend; 3365.9 and 3421.63 corresponding to polymeric O-H stretch when compared to the control. In the shifts to the left, it showed shifts in peaks at wavenumbers 754.19 corresponding to skeletal C-C vibrations to 756.12; 906.57 corresponding to skeletal C-C vibrations to 908.5; 1028.09 corresponding to primary amine (CN) stretch to 1030.02; 2320.44 to 2341.66; 2357.09 to 2359.02 and 3240.52 corresponding to hydrogen bonded O-H stretch to 3255.95. The spectra equally showed shifts to the right of existing peaks at wavenumbers 470.65 corresponding to C-H out of plane bending vibration of substituted amide to 468.72; 1545.03 corresponding to secondary amide (NH) bend to 1543.1; 1660.77 corresponding to urethane (NH) group to 1653.05; 2850.88 corresponding to methoxy methyl ether to 2848.96 and 3452.7 corresponding to hydrogen bonded O-H stretch to 3446.91 in comparison to the control.

(c)



(d)



Key:

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= combination A + combination B + combination C

PUR= Polyurethane sample

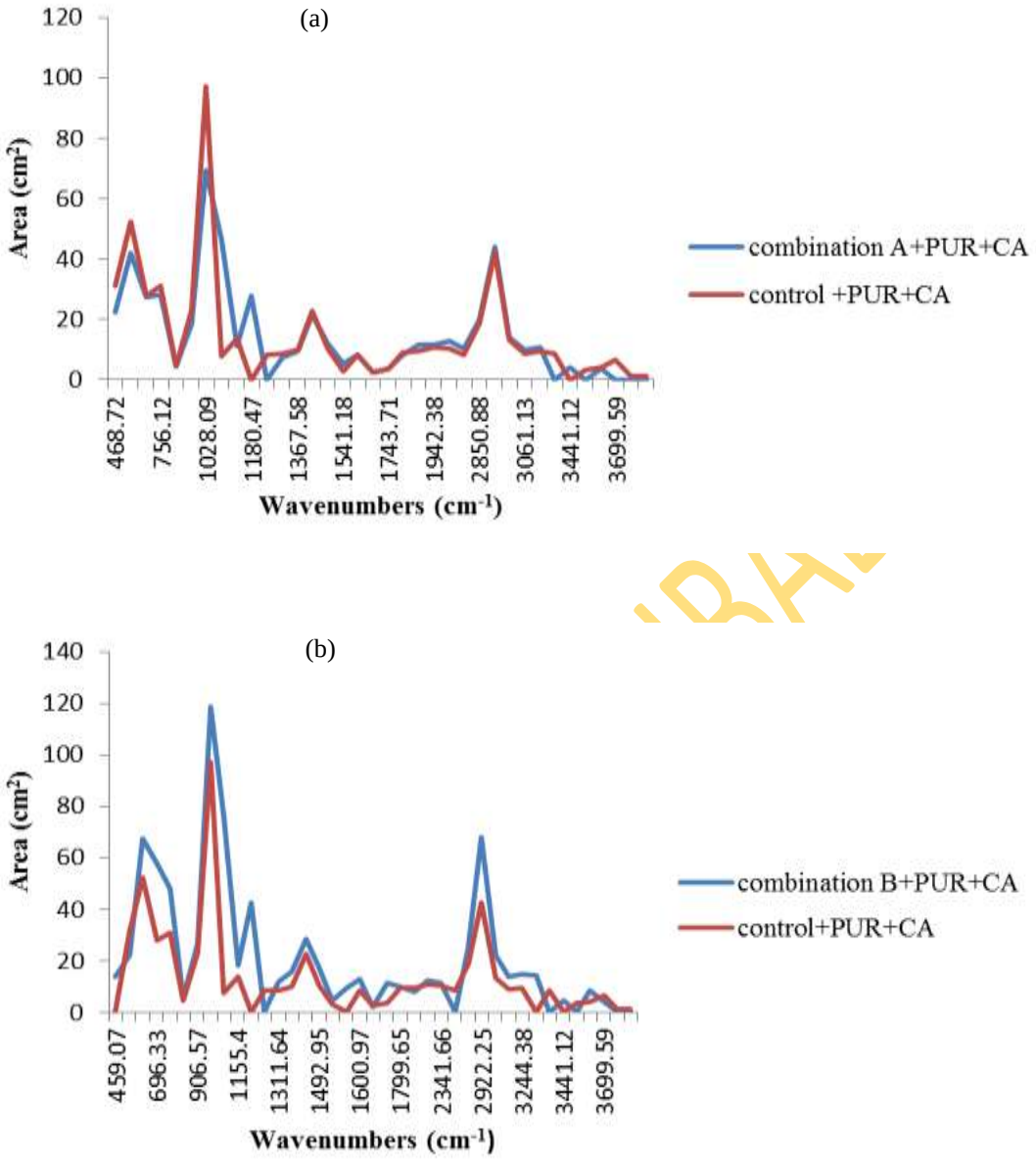
Figure 4.7 (c-d): FTIR spectra analysis of PUR samples degraded with combination C (c) and consortium (d) without stimulation with any agro waste after 1 month in the field in comparison with the control.

4.8 Laboratory biodegradation studies using bioaugmentation and biostimulation in sterilised soil

4.8.1 FTIR spectra analysis of experimentally degraded PUR samples using the isolates combinations biostimulated with cassava peels in sterilised soil after 1 month of burial in laboratory pots

The FTIR spectra analysis of PUR samples experimentally degraded with combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5) biostimulated with cassava peels after 30 days in laboratory sterilised soil (Fig. 4.8a) showed the removal of peaks at wavenumbers 1278.85 corresponding to O-H in plane bend; 3387.11 corresponding to dimeric O-H stretch; 3699.59, 3759.39 and 3863.55 corresponding to O-H stretching vibrations of intramolecular hydrogen bond in comparison to the control. The spectra also showed shifts to the right of peaks at wavenumbers 1070.53 corresponding to primary amine (CN) stretch and 3462.34 corresponding to hydrogen bonded O-H stretch to 1068.6 and 3446.91 respectively. It also caused shifts to the left of peaks at wavenumbers 1311.64 corresponding to O-H in-plane bend to 1313.57 and 1799.65 to 1801.57 when compared to the control.

The FTIR spectra analysis of PUR samples experimentally degraded with combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) biostimulated with cassava peels after 30 days in laboratory sterilised soil (Fig. 4.8b) showed formation of new peaks at wavenumbers 459.07 corresponding to C-H out of plane bending vibration of substituted amide; 1583.61 corresponding to secondary amine (NH) bend and 3323.46 corresponding to polymeric O-H stretch. It also showed the removal of peaks at wavenumbers 1278.85 corresponding to O-H in plane bend; 2360.95; 3387.11 corresponding to dimeric O-H stretch; 3759.39 and 3863.55 corresponding to O-H stretching vibrations of intramolecular hydrogen bond when compared to the control. It equally showed shifts to the right of peaks at wavenumbers 538.16 to 536.23; 1030.02 and 1070.53 corresponding to primary amine (CN) stretch to 1028.09 and 1068.6; 1373.36 corresponding to a symmetric methyl (-CH_3) bend to 1371.43; 1747.57 corresponding to carbonyl group to 1743.71; 1942.38 to 1940.45; 2341.66 to 2337.8; 3244.38 and 3462.34 corresponding to hydrogen bonded O-H



Key:

Combination A: *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B: *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

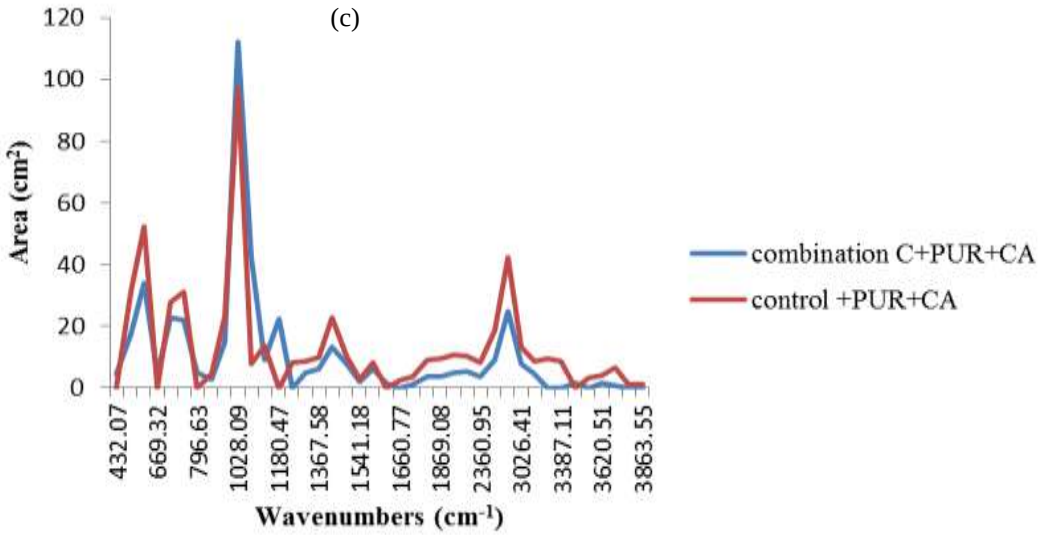
PUR=Polyurethane

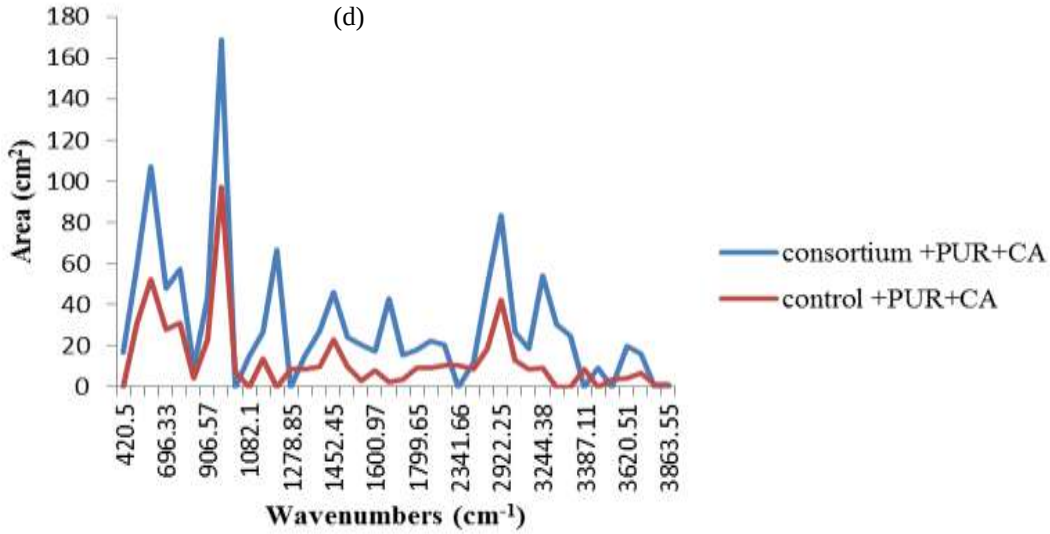
CA= Cassava peels

Figure 4.8 (a-b): FTIR spectra analysis of PUR samples degraded with combination A (a) and combination B (b) stimulated with cassava peels after 1 month in the laboratory sterilised soil in comparison with the control

stretch to 3242.45 and 3441.12 respectively. In the shifts to the left, it showed shifts in peaks at wavenumbers 464.86 corresponding to C-H out of plane bending vibration of substituted amide group to 468.72; 1541.18 corresponding to secondary amide (NH) bend to 1546.96; 1653.05 corresponding to urethane (NH) group to 1666.55; 1799.65 to 1801.57; 1869.08 corresponding to anhydride of carbonyl of the amide and 3620.51 corresponding to tertiary alcohol O-H stretch to 1872.94 and 3622.44 respectively in comparison to the control.

The FTIR spectra analysis of PUR samples experimentally degraded with combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1) biostimulated with cassava peels after 30 days in laboratory sterilised soil (Fig. 4.8c) showed removal of peaks at wavenumbers 1278.85 corresponding to O-H in plane bend; 3244.38 corresponding to hydrogen bonded O-H stretch; 3387.11 corresponding to dimeric O-H stretch; 3759.39 and 3863.55 corresponding to O-H stretching vibrations of intramolecular hydrogen bond and formation of new peaks at wavenumbers 432.07; 669.32 corresponding to O-H out of plane bend and 796.63 corresponding to skeletal C-C vibrations in comparison to control. The spectra also showed shifts to the left of peaks at wavenumbers 464.86 corresponding to C-H out of plane bending vibration of substituted amide group to 466.79; 840.99 corresponding to skeletal C-C vibrations to 842.92; 1541.18 corresponding to secondary amide (NH) bend to 1546.96; 1799.65 to 1801.57; 1869.08 corresponding to anhydride of carbonyl of the amide and 3620.51 corresponding to tertiary alcohol O-H stretch to 1872.94 and 3622.44 respectively. It (Fig. 4.8c) equally showed shifts to the right of peaks at wavenumbers 538.16 to 536.23; 1070.53 corresponding to primary amine (CN) stretch to 1066.67; 1373.36 corresponding to a symmetric methyl (-CH_3) bend to 1371.43; 1653.05 corresponding to urethane (NH) group to 1641.48; 1747.57 corresponding to carbonyl group to 1741.78; 1942.38 to 1940.45; 2850.88 corresponding to methoxy methyl ether to 2848.96 and 3462.34 corresponding to hydrogen bonded O-H stretch to 3441.12 in comparison to control.





Key:

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= combination A + combination B + combination C

PUR= Polyurethane sample

CA= Cassava peels

Figure 4.8 (c-d): FTIR spectra analysis of PUR samples degraded with combination C (c) and consortium (d) stimulated with cassava peels after 1 month in the laboratory sterilised soil in comparison with the control

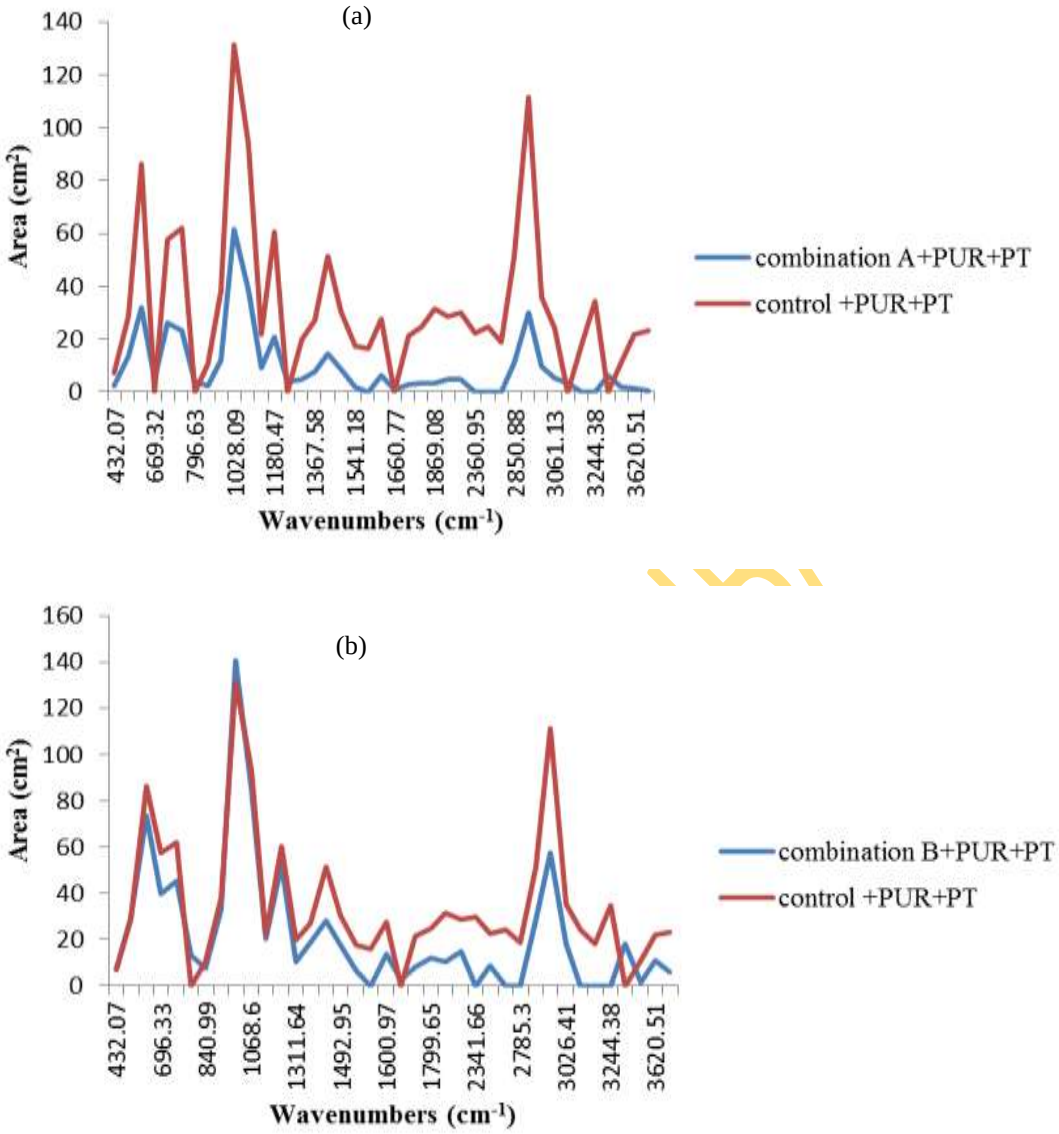
The FTIR spectra analysis of PUR samples experimentally degraded with consortium (combination A + combination B + combination C) biostimulated with cassava peels after 30 days in laboratory sterilised soil (Fig. 4.8d) showed removal of peaks at wavenumbers 1278.85 corresponding to O-H in plane bend; 2341.66; 3387.11 and 3462.34 corresponding to dimeric O-H stretch; 3759.39 and 3863.55 corresponding to O-H stretching vibrations of intramolecular hydrogen bond and formation of new peaks at wavenumbers 418.57; 3323.46, 3362.04, and 3423.76 corresponding to polymeric O-H stretch when compared to the control. The spectra also showed shifts to the right of peaks at wavenumbers 1373.36 corresponding to a symmetric methyl (-CH_3) bend to 1371.43; 1747.57 corresponding to carbonyl group to 1743.71; 2360.95 to 2359.02; 3244.38 corresponding to hydrogen bonded O-H stretch to 3242.45 and 3699.59 corresponding to O-H stretching vibrations of intramolecular hydrogen bond to 3697.66. In the shifts to the left of the spectra of existing peaks, the consortium caused shifts in peaks at wavenumbers 464.86 corresponding to C-H out of plane bending vibration of substituted amide group to 466.79; 1799.65 to 1801.57; 1070.53 alkyl substituted ether group and 2922.25 corresponding to asymmetric C-H stretch of methylene group to 1078.24 and 2924.18 respectively when compared to control.

4.8.2 FTIR spectra analysis of experimentally degraded PUR samples using the isolates combinations biostimulated with potato peels in sterilised soil after 30 days of burial in laboratory pots

The FTIR spectra analysis of PUR samples experimentally degraded with combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5) biostimulated with potato peels after 30 days in laboratory sterilised soil (Fig. 4.9a) showed removal of peaks at wavenumbers 1583.61 corresponding to secondary amine (NH) bend; 2360.95; 2515.26; 2785.3 corresponding to C-H stretching vibrations of a methyl group; 3103.57; 3242.45 corresponding to dimeric O-H stretch and formation of new peaks at wavenumbers 669.32 corresponding to O-H out of plane bend; 794.7 corresponding to skeletal C-C vibrations; 1280.78 corresponding to O-H in plane bend; 1666.55 corresponding to urethane (NH) group; 3082.3; and 3387.11 corresponding to polymeric

O-H stretch in comparison to the control. The spectra also showed shifts to the right of spectra of peaks at wavenumbers 432.07 to 430.14; 468.72 corresponding to C-H out of plane bending vibration of substituted amide group to 464.86; 1070.53 corresponding to alkyl substituted ether group to 1068.6; 1313.57 corresponding to O-H in-plane bend to 1311.64; 1942.38 to 1940.45; 2339.73 to 2337.8 and 2924.18 corresponding to asymmetric C-H stretch of methylene group to 2922.25. In the shifts to the left, combination A caused shifts in peaks at wavenumbers 536.23 to 538.16; 840.99 and 906.57 corresponding to skeletal C-C vibrations to 842.92 and 908.5; 1028.09 corresponding to primary amine (CN) stretch to 1030.02; 1541.18 corresponding to secondary amide (NH) bend to 1548.89; 1801.57 to 1803.5; 1869.08 corresponding to anhydride of carbonyl of the amide to 1872.94 and 3620.51 corresponding to tertiary alcohol O-H stretch to 3622.44 when compared to the control.

The FTIR spectra analysis of PUR samples experimentally degraded with combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) biostimulated with potato peels after 30 days in laboratory sterilised soil (Fig. 4.9b) showed removal of peaks at wavenumbers 1583.61 corresponding to secondary amine (NH) bend; 2339.73; 2515.26; 2785.3 corresponding to C-H stretching vibrations of a methyl group; 3061.13; 3103.57; 3242.45 corresponding to dimeric O-H stretch and formation of new peaks at wavenumbers 796.63 corresponding to skeletal C-C vibrations; 1651.12 corresponding to urethane (NH) group and 3340.82 corresponding to polymeric O-H stretch in comparison to the control. It also showed shifts to the right of spectra of peaks at wavenumbers 468.72 corresponding to C-H out of plane bending vibration of substituted amide group to 466.79; 1070.53 corresponding to alkyl substituted ether group to 1068.6; 1313.57 corresponding to O-H in-plane bend to 1311.64 and 3441.12 corresponding to hydrogen bonded O-H stretch to 3435.34. In the shifts to the left, it caused shifts in peaks at wavenumbers 432.07 to 435.95; 536.23 to 538.16; 696.33 corresponding to O-H out of plane bend to 698.25; 840.99 and 906.57 corresponding to skeletal C-C vibrations to 842.92 and 908.5; 1028.09 corresponding to primary amine (CN) stretch to 1030.02; and 3620.51 corresponding to tertiary alcohol O-H stretch to 3622.44 when compared to the control.



Key:

Combination A: *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B: *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

PUR= Polyurethane sample

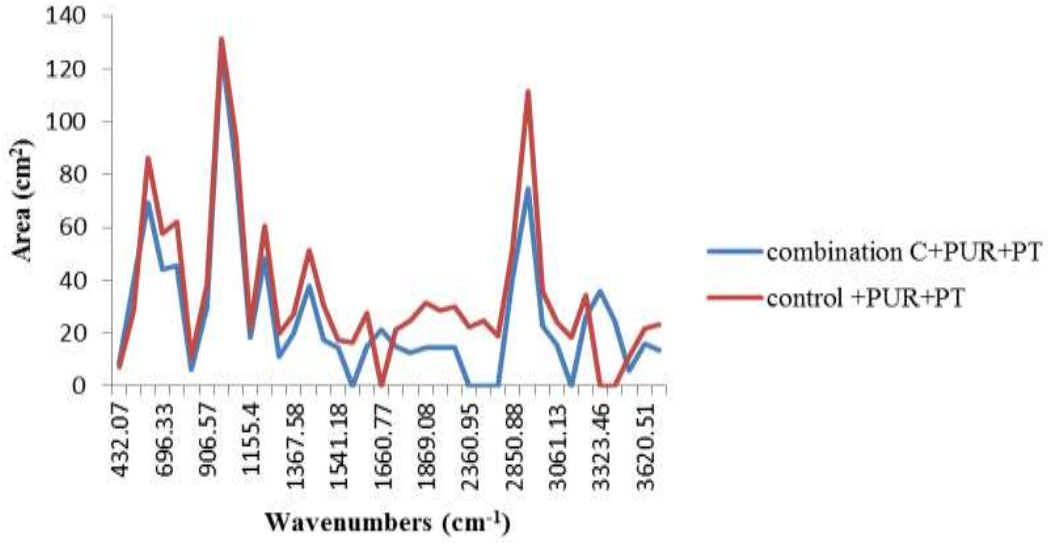
PT=Potato peels

Figure 4.9 (a-b): FTIR spectra analysis of PUR samples degraded with combination A (a) and combination B (b) stimulated with potato peels after 1 month in the laboratory sterilised soil in comparison with the control

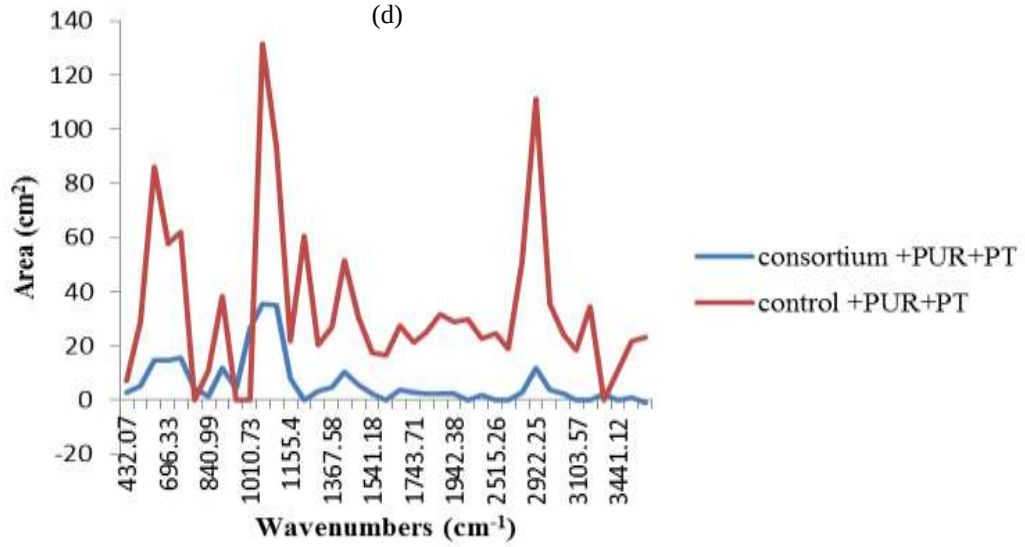
The FTIR spectra analysis of PUR samples experimentally degraded with combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1) biostimulated with potato peels after 30 days in laboratory sterilised soil (Fig. 4.9c) showed the formation of new peaks at wavenumbers 1653.05 corresponding to urethane (NH) group; 3333.1 and 3383.26 corresponding to polymeric O-H stretch and removal of peaks at wavenumbers 1583.61 corresponding to secondary amine (NH) bend; 2360.95; 2515.26; 2785.3 corresponding to C-H stretching vibrations of a methyl group and 3103.57 in comparison to the control. It also showed shifts to the right of spectra of peaks at wavenumbers 432.07 to 430.14; 468.72 corresponding to C-H out of plane bending vibration of substituted amide group to 464.79; 1070.53 corresponding to alkyl substituted ether group to 1068.6; 1313.57 corresponding to O-H in-plane bend to 1311.64; 2339.73 to 2337.8; 2924.18 corresponding to asymmetric C-H stretch of methylene group to 2922.25 and 3699.59 corresponding to O-H stretching vibration of intramolecular hydrogen bond to 3697.66. In addition, the spectra showed shifts to the left of spectra of peaks at wavenumbers 536.23 to 538.16; 696.33 corresponding to O-H out of plane bend to 698.25; 840.99 and 906.57 corresponding to skeletal C-C vibrations to 842.92 and 908.5; 1028.09 corresponding to primary amine (CN) stretch to 1030.02 and 3242.45 corresponding to hydrogen bonded O-H stretch to 3244.38 in comparison to the control.

The FTIR spectra analysis of PUR samples experimentally degraded with consortium (combination A + combination B + combination C) biostimulated with potato peels after 30 days in laboratory sterilised soil (Fig. 4.9d) showed removal of peaks at wavenumbers 1180.47 corresponding to alkyl substituted ether (C-O) stretch; 1583.61 corresponding to secondary amine (NH) bend; 2339.73; 2515.26; 2785.3 corresponding to C-H stretching vibrations of a methyl group; 3103.57 and 3242.45 corresponding to dimeric O-H stretch. The spectra showed formation of new peaks at wavenumbers 796.9 corresponding to skeletal C-C vibrations; 945.15 corresponding to hydrogen bonded O-H out of plane bending and 1010.73 corresponding to primary amine (CN) stretch. It equally showed shifts to right of spectra in peaks at wavenumbers 756.12 corresponding to skeletal C-C vibrations, 1313.57 corresponding to O-H in-plane bend, 1371.43 corresponding to a

(c)



(d)



Key:

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1
Consortium= combination A + combination B + combination C
PUR= Polyurethane sample
PT= Potato peels

Figure 4.9 (c-d): FTIR spectra analysis of PUR samples degraded with combination C (c) and consortium (d) stimulated with potato peels after 1 month in the laboratory sterilised soil in comparison with the control.

symmetric methyl (-CH_3) bend and 1743.71 corresponding to carbonyl group, 3441.12 corresponding to hydrogen bonded O-H stretch and 3699.59 corresponding to O-H stretching vibration of intramolecular hydrogen bond to 754.19, 1311.64, 1369.5, 1741.78, 3435.34 and 3697.66 respectively. In shifts to the left, it showed shifts in peaks at wavenumbers 432.07 to 437.86; 468.72 corresponding to C-H out of plane bending vibration of substituted amide group to 470.65; 536.23 to 538.16; 906.57 corresponding to skeletal C-C vibrations to 910.43; 1028.09 corresponding to primary amine (CN) stretch to 1030.02 and 1070.53 corresponding to alkyl substituted ether group to 1078.24 when compared to the control.

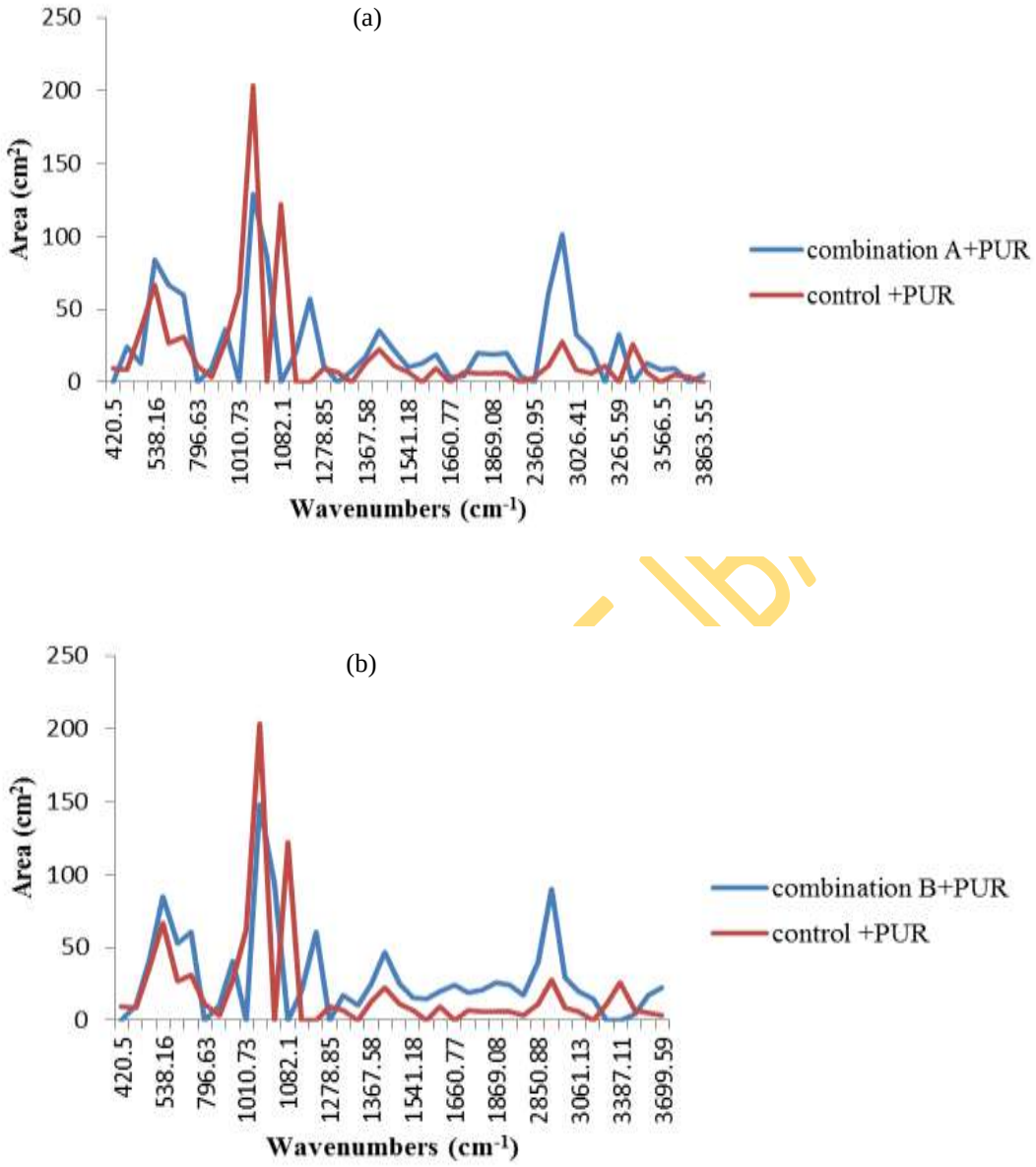
The FTIR spectra of the different combinations and consortium (Fig. 4.9a-d) showed decrease in the peak areas of the experimentally degraded PUR samples compared to the control.

4.8.3 FTIR spectra analysis of experimentally degraded PUR samples using the isolates combinations without stimulation with any agro waste in sterilised soil after 30 days of burial in laboratory pots

In this set up, the bacteria isolates depended only on the growth medium and PUR samples used for the degradation activity for their nutrient as the soil sample was sterilized. The FTIR spectra analysis of PUR samples degraded with combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5) without stimulation with any agro waste in laboratory sterilised soil (Fig. 4.10a) showed removal of peaks at wavenumbers 420.5; 796.63 corresponding to skeletal C-C vibrations; 1010.73 corresponding to primary amine (CN) stretch; 1309.71 corresponding to O-H in-plane bend; 3394.83 corresponding to primary amine (N-H) stretch and 3697.66 corresponding to O-H stretching vibration of intramolecular hydrogen bond and formation of new peaks at wavenumbers 1329 corresponding to C-H bending vibrations of methylene alkyl group; 1583.61 corresponding to secondary amine (NH) bend; 1653.05 corresponding to primary amine (N-H) bend; 3566.5 corresponding to internally bonded O-H stretch and 3853.9 corresponding to O-H stretching vibrations of intramolecular hydrogen bond in

comparison to the control. It also showed that there were shifts to the right of existing peaks in the spectra, and this includes peaks at wavenumbers 466.79 corresponding to C-H out of plane bending vibration of substituted amide group to 464.86; 756.12, 842.92 and 910.43 corresponding to skeletal C-C vibrations to 754.19, 840.99 and 908.5; 1030.02 corresponding to primary amine (CN) stretch to 1028.09; 1082.1 corresponding to alkyl substituted ether (C-O) stretch to 1066.57 and 2360.95 to 2339.73. In the shifts to the left of the spectra, it showed shifts in peaks at wavenumbers 435.93 to 439.78; 536.23 to 538.16; 1274.99 corresponding to O-H in plane bend to 1280.78; 1371.43 corresponding to a symmetric methyl ($-CH_3$) bend to 1373.36; 1799.65 to 1801.57; 2922.25 corresponding to asymmetric C-H stretch of methylene group to 2924.18; 3242.45 and 3441.12 corresponding to hydrogen bonded O-H stretch to 3265.59 and 3444.98 respectively when compared to the control.

The FTIR spectra analysis of PUR samples degraded with combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) without stimulation with any agro waste in laboratory sterilised soil (Fig. 4.10b) showed formation of new peaks at wavenumbers 1329 corresponding to C-H bending vibrations of methylene alkyl group; 1583.61 corresponding to secondary amine (NH) bend; 1658.84 corresponding to primary amine (N-H) bend and 3082.35 and removal of peaks at wavenumbers 420.5; 796.63 corresponding to skeletal C-C vibrations; 1010.73 corresponding to primary amine (CN) stretch; 1274.99 corresponding to O-H in-plane bend; 3242.45 corresponding to hydrogen bonded O-H stretch and 3394.83 corresponding to primary amine (N-H) stretch when compared to the control. The spectra also showed shifts to the right of some existing peaks at wavenumbers 435.93 to 430.14; 466.79 corresponding to C-H out of plane bending vibration of substituted amide group to 464.86; 910.43 corresponding to skeletal C-C vibrations to 908.5 and 1082.1 corresponding to alkyl substituted ether (C-O) stretch to 1068.6. It equally showed shifts to the left of peaks at wavenumbers 536.23 to 540.09; 1309.71 corresponding to O-H in plane bend to 1311.64; 1799.65 to 1801.57 and 2922.25 corresponding to asymmetric C-H stretch of methylene group to 2924.18 in comparison to the control.



Key:

Combination A: *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

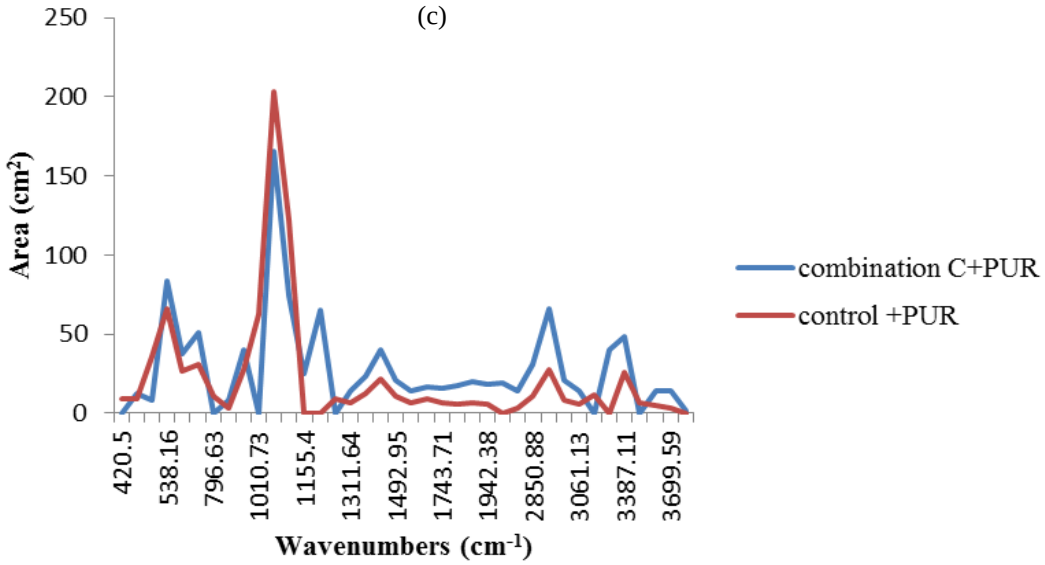
Combination B: *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

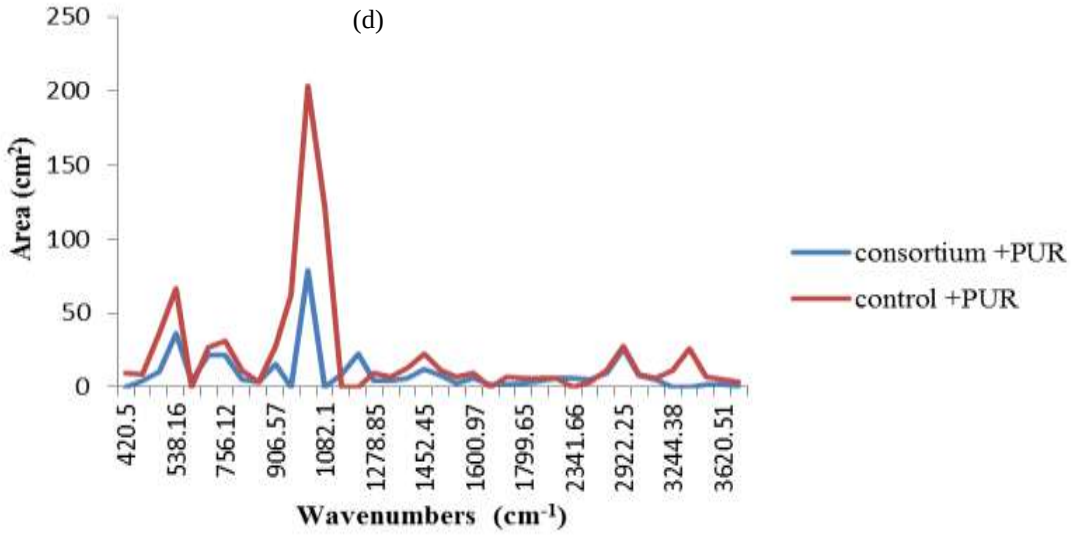
PUR=Polyurethane sample

Figure 4.10 (a-b): FTIR spectra analysis of PUR samples degraded with combination A (a) and combination B (b) without stimulation with any agro waste after 1 month in the laboratory sterilised soil in comparison with the control

The FTIR spectra analysis of PUR samples degraded with combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1) without stimulation with any agro waste in laboratory sterilised soil (Fig. 4.10c) showed removal of peaks at wavenumbers 420.5; 466.79 corresponding to C-H out of plane bending vibration of substituted amide group; 796.63 corresponding to skeletal C-C vibrations; 1010.73 corresponding to primary amine (CN) stretch and 3441.12 corresponding to hydrogen bonded O-H stretch and formation of new peaks at wavenumbers 3863.55 corresponding to O-H stretching vibrations of intramolecular hydrogen bond and 2341.66 in comparison to control. It also showed shifts to the right of some existing peaks at wavenumbers 435.93 to 434; 1541.18 corresponding to secondary amide (NH) bend and 3394.83 corresponding to primary amine (N-H) stretch to 1539.25 and 3387.11 respectively. In the shifts to the left, it showed that peaks at the following wavenumbers were shifted to the left and they include 536.23 to 538.16; 696.33 corresponding to O-H out of plane bend to 698.25; 1082.1 corresponding to alkyl substituted ether group to 1087.89; 1309.71 corresponding to O-H in-plane bend to 1311.64; 1799.65 to 1801.57 and 3242.45 corresponding to hydrogen bonded O-H stretch to 3265.59 when compared to control.

The FTIR spectra analysis of PUR samples degraded with consortium (combination A + combination B + combination C) without stimulation with any agro waste in laboratory sterilised soil (Fig. 4.10d) showed formation of new peaks at wavenumbers 669.32 corresponding to O-H out of plane bend; 1641.48 corresponding to primary amine N-H bend and 2337.8 and removal of peaks at wavenumbers 420.5; 1010.73 corresponding to primary amine (CN) stretch; 1082.1 corresponding to alkyl substituted ether group; 3242.45 corresponding to hydrogen bonded O-H stretch and 3394.83 corresponding to primary amine N-H stretch. The spectra also showed that there were shifts to the right of some peaks at wavenumbers 435.93 to 428.21; 466.79 corresponding to C-H out of plane bending vibration of substituted amide group to 462.93; 910.43 corresponding to skeletal C-C vibrations to 908.5; 1743.71 corresponding to carbonyl group to 1741.78; 1942.38 to 1940.45 and 2850.88 corresponding to methoxy methyl ether to 2848.96. The spectra equally showed shifts to the left of peaks at wavenumbers 536.23 to 538.16; 1274.99 and





Key:

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1
Consortium= combination A + combination B + combination C
PUR= Polyurethane sample

Figure 4.10 (c-d): FTIR spectra analysis of PUR samples degraded with combination C (c) and consortium (d) without stimulation with any agro waste after 1 month in the laboratory sterilised soil in comparison with the control

1309.71 corresponding to O-H in plane bend to 1280.78 and 1311.64; 1541.18 corresponding to secondary amide (NH) bend to 1548.89; 1799.65 to 1801.57; 1869.08 corresponding to anhydride of carbonyl of the amide to 1872.94; 3620.51 corresponding to tertiary alcohol O-H stretch and 3697.66 corresponding to O-H stretching vibrations of intramolecular hydrogen bond to 3622.44 and 3699.59 respectively in comparison to control.

4.9 Analysis of FTIR spectra of degraded PUR samples under natural biodegradation

4.9.1 FTIR analysis of PUR samples degraded under natural biodegradation after one and two year (s) of soil burial

The PUR samples were left to undergo biodegradation with the autochthonous/indigenous microorganisms in the soil microcosm where they were buried. After one (1) year of soil burial, the FTIR analysis (Fig. 4.11a) of exhumed PUR samples from the five different depths (15, 30, 45, 60 and 70 cm) showed the removal of peaks at wavenumbers 669.32 corresponding to O-H out of plane bend; 3676.45 and 3751.67 corresponding to O-H stretching vibrations of intramolecular hydrogen bond and formation of new peak within the range of wavenumbers 3620-3632.08 corresponding to tertiary alcohol O-H stretch when compared to control.

In addition, the FTIR spectra analysis (Fig. 4.11a) of PUR sample from 15 cm depth showed removal of peaks at wavenumbers 1541.18 corresponding to secondary amide (NH) bend and 3061.13. It showed shift to the right of peak at wavenumber 2345.52 to 2337.8 and shifts to the left of peaks at wavenumbers 1647.28 and 3444.98 corresponding to urethane (NH) group and hydrogen bonded O-H stretch to 1664.62 and 3450.77 respectively. The FTIR spectra of PUR sample from 30 cm depth showed removal of peak at wavenumber 3061.13; shifts to the left of peaks at wavenumbers 1180 corresponding to alkyl substituted ether C-O stretch to 1182.4 and 1647.28 corresponding to urethane (NH) group to 1670.41 and shifts to the right of peaks at wavenumbers 466.79 corresponding to C-H out of plane bending vibration of substituted amide group to 464.86; 698.25

corresponding to O-H out of plane bend to 696.33; 1871.01 corresponding to anhydride of carbonyl of the amide to 1869.08; 2345.52 to 2335.87 and 3444.98 corresponding to hydrogen bonded O-H stretch to 3439.19. The FTIR spectra of PUR sample from 45 cm depth showed formation of new peaks at wavenumbers 432.07; 1030.02 corresponding to primary amine (CN) stretch and 3871.26 corresponding to O-H stretching vibrations of intramolecular hydrogen bond; and removal of peak at wavenumber 1647.28 corresponding to urethane (NH) group. It also showed shifts to the left of peaks at wavenumbers 1180 corresponding to alkyl substituted ether C-O stretch to 1182.4 and 1743.71 corresponding to carbonyl group to 1747.57; and shifts to the right of peaks at wavenumbers 698.25 corresponding to O-H out of plane bend to 696.33; 1018.45 corresponding to primary amine (CN) stretch to 1010.73; 1871.01 corresponding to anhydride of carbonyl of the amide to 1869.08; 2345.52 to 2339.75; 2850.88 corresponding to methoxy methyl ether to 2848.96 and 3444.98 corresponding to hydrogen bonded O-H stretch to 3439.19 in comparison to control.

The FTIR spectra analysis (Fig. 4.11a) equally showed that PUR samples from 60 and 70 cm depths had removal of peaks at wavenumbers 1329 corresponding to C-H bending vibrations of methylene alkyl group; 1541.18 corresponding to secondary amide (NH) bend; 1647.28 corresponding to urethane (NH) group and 2345.52; shifts to the right of peaks at wavenumbers 466.79 corresponding to C-H out of plane bending vibration of substituted amide group to 464.86; 698.25 corresponding to O-H out of plane bend to 696.33 and shift to the left of peak at wavenumber 1801.57 to 1803.5. Also from 60 cm depth, it showed removal of peak at wavenumber 1068.6 corresponding to alkyl substituted ether C-O stretch; shift to the right of peak at wavenumber 1373.36 corresponding to symmetric methyl ($-CH_3$) bend to 1371.43 and shifts to the left of peaks at wavenumbers 1180 corresponding to alkyl substituted ether C-O stretch to 1182.4 and 3061.13 to 3082.36. From 70 cm depth, it showed removal of peaks at wavenumbers 1492.95 and 3061.13; shifts to the right of peak at wavenumber 2850.88 corresponding to methoxy methyl ether to 2848.96 and 3444.98 corresponding to hydrogen bonded O-H stretch to 3435.34 and shifts to the left of peaks at wavenumbers 1373.36 corresponding to

symmetric methyl (-CH_3) bend to 1384.94 and 1942.38 to 1944.31 when compared to the control.

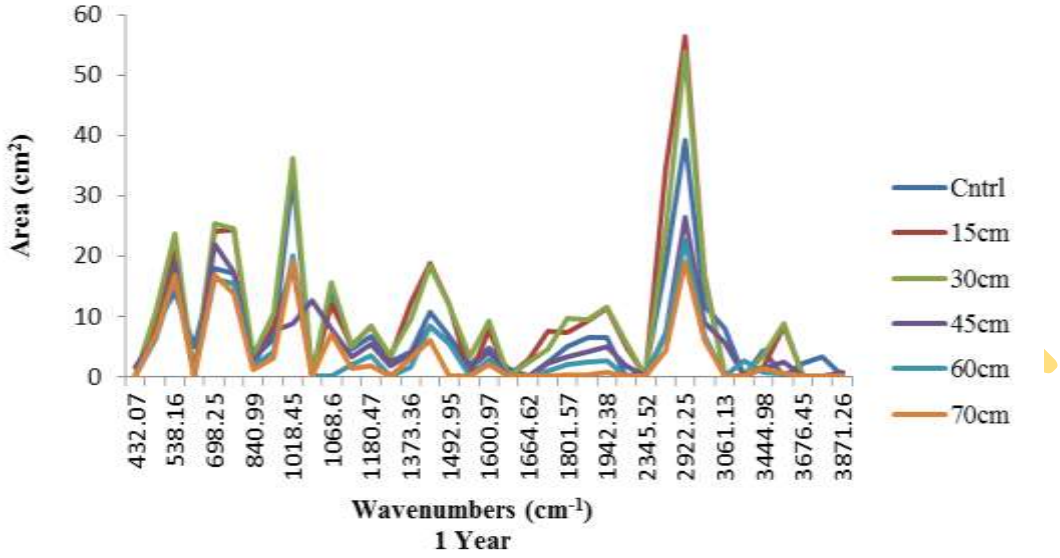
The FTIR analysis (Fig. 4.11b) of exhumed PUR samples from the five different depths (15, 30, 45, 60 and 70 cm) after 2 years of soil burial showed the removal of peaks at wavenumbers 3676.45 and 3751.67 corresponding to O-H stretching vibrations of intramolecular hydrogen bond; formation of new peak at wavenumber 2360.95 and shifts to the right of peaks at wavenumbers 698.25 corresponding to O-H out of plane bend to 696.33 and 1155.4 corresponding to alkyl substituted ether (C-O) stretch to 1153.47 in comparison to the control. In addition, the FTIR spectra analysis (Fig. 4.11b) of PUR sample from 15 cm depth showed formation of new peaks at wavenumbers 426.28; 3620.51 corresponding to tertiary alcohol O-H stretch and removal of peak at wavenumber 1647.28 corresponding to urethane (NH) group. It showed shifts to the left of peaks at wavenumbers 1018.45 corresponding to primary amine (CN) stretch to 1028.09 and 1541.18 corresponding to secondary amide (NH) bend to 1543.1; and shifts to the right of peaks at wavenumbers 1871.01 corresponding to anhydride of carbonyl of the amide to 1869.08; 2345.52 to 2341.66 and 3444.98 corresponding to hydrogen bonded O-H stretch to 3425.69. It equally showed that PUR sample from 30 cm depth had removal of peak at wavenumber 1647.28 corresponding to urethane (NH) group; formation of new peak at wavenumber 451.36 corresponding to C-H out of plane bending vibration of substituted amide group. It had shifts to the right of peaks at wavenumbers 1373.36 corresponding to a symmetric methyl (-CH_3) bend to 1371.43; 2345.52 to 2341.66 and 3444.98 corresponding to hydrogen bonded O-H stretch to 3443.05 and shifts to the left of peaks at wavenumbers 1018.45 corresponding to primary amine (CN) stretch to 1020.38; 1541.18 corresponding to secondary amide (NH) bend to 1543.1 and 1801.57 to 1803.5.

The spectra analysis (Fig. 4.11b) showed that PUR sample from 45 cm depth had formation of peak at wavenumber 3396.76 corresponding to primary amine N-H stretch and removal of peaks at wavenumbers 1329 corresponding to C-H bending vibrations of methylene alkyl group and 1541.18 corresponding to secondary amide (NH) bend. It

showed shifts to the right of peaks at wavenumbers 1068.6 corresponding to alkyl substituted ether C-O stretch to 1066.67; 1373.36 corresponding to a symmetric methyl (-CH_3) bend to 1371.43; 1647.28 corresponding to urethane (NH) group to 1639.55; 1743.71 corresponding to carbonyl group to 1741.78; 1942.38 to 1940.45 and 2345.52 to 2341.66; and shifts to the left of peaks at wavenumbers 840.99 corresponding to skeletal C-C vibrations to 842.92; 906.57 corresponding to skeletal C-C vibrations to 908.5; 1801.57 to 1803.5; 1871.01 corresponding to anhydride of carbonyl of the amide to 1872.94 and 3444.98 corresponding to hydrogen bonded O-H stretch to 3450.77 in comparison to control.

The spectra analysis (Fig. 4.11b) showed that PUR sample from 60 cm depth had removal of peak at wavenumber 2345.52; formation of a new peak at wavenumber 1583 corresponding to secondary amine (NH) bend; shifts to the right of peaks at wavenumbers 1871.01 corresponding to anhydride of carbonyl of the amide to 1869.08 and 3444.98 corresponding to hydrogen bonded O-H stretch to 3443.05 and shifts to the left of peaks at wavenumbers 906.57 corresponding to skeletal C-C vibrations to 908.5; 1018.45 corresponding to primary amine (CN) stretch to 1020.38 and 1743.71 corresponding to carbonyl group to 1745.64. It showed that PUR sample from 70 cm depth had removal of peaks at wavenumbers 1329 corresponding to C-H bending vibrations of methylene alkyl group; 1647.28 corresponding to urethane (NH) group; 3444.98 corresponding to hydrogen bonded O-H stretch and formation of new peaks at wavenumbers 626.89 corresponding to C-H bend of alkyne group and 1311.64 corresponding to O-H in-plane bend. It equally showed shifts to the left of spectra of peaks at wavenumbers 466.79 corresponding to C-H out of plane bending vibration of substituted amide group to 468.72; 1018.45 corresponding to primary amine (CN) stretch to 1026.16 and 1743.71 corresponding to carbonyl group to 1745.64; shifts to the right of spectra of peaks at wavenumbers 1541.18 corresponding to secondary amide (NH) bend to 1539.25; 1801.57 to 1799.65; 1871.01 corresponding to anhydride of carbonyl of the amide to 1867.16 1942.38 to 1940.45 and 2345.52 to 2341.66 in comparison to the control.

(a)



(b)

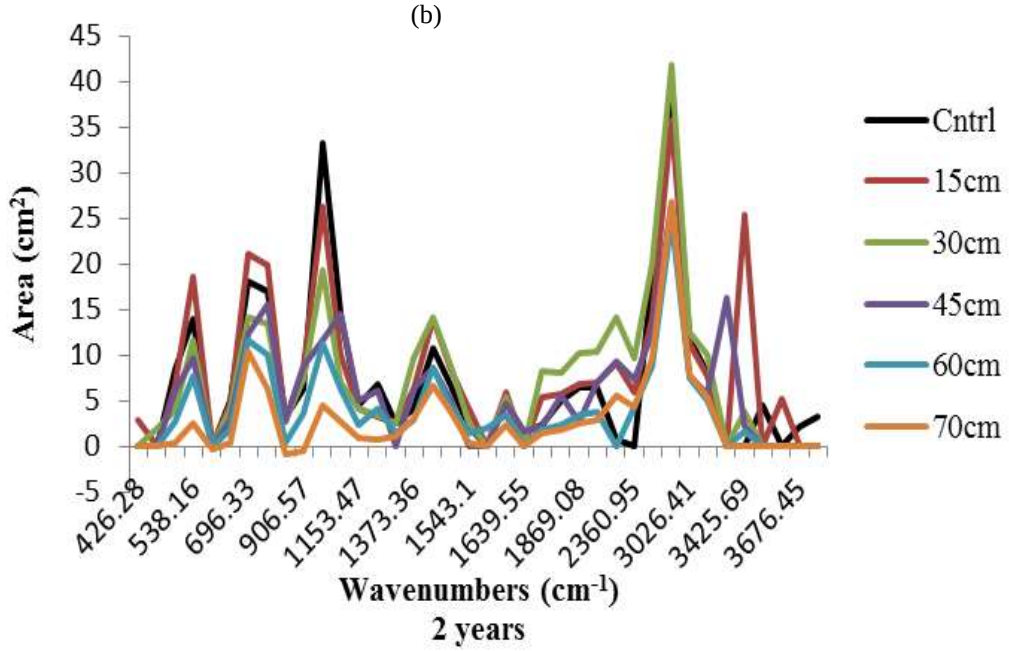


Figure 4.11 (a-b): Comparison of FTIR spectra analysis of PUR samples degraded under natural biodegradation in the field after 1 Year (a) and 2 Years (b) of soil burial in comparison to unburied control.

4.10 Changes in weights of degraded PUR samples

Changes in weights of degraded PUR samples after 1 month (30 days) and 3 months (90 days) intervals were calculated and the percentage change (loss) in weights of the samples equally determined (Tables 4.7 – 4.9). In the laboratory broth, *Enterobacter amnigenus* $D_1$2 had the highest percentage weight loss of 10.00 and 8.33 % under both static and agitated conditions. In the field with bioaugmentation and biostimulation, the consortium had the highest percentage weight loss of 22.5 % without peels while in the laboratory sterilised soil combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) and consortium had the highest value of 15.0 % when both were stimulated with cassava peels.

4.11 Percentage degradation of the PUR samples

After the FTIR analysis, the percentage degradation of the degraded PUR samples in the different experimental set ups were calculated (Table 4.10 – 4.12). From the FTIR spectra analysis, *Enterobacter amnigenus* $D_1$2 had the highest percentage degradation of 86.67 % under agitation while *Providencia Pseudomallei* $D_2$1 had the highest value of 72.18 % under static condition in the laboratory broth. In the field with bioaugmentation and biostimulation, combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) had the highest value of 76.27 % without peels while in the laboratory sterilised soil, the consortium had the highest value of 90.98 % stimulated with potato peels.

4.12 Macroscopic/Visual examination of exhumed PUR samples

The exhumed PUR samples showed presence of cracks and pits. The samples buried in the field under natural biodegradation had these cracks mostly on exhumed samples from 60 and 70 cm depths compared to other depths and more on 2 years of PUR burial than 1 year.

4.13 pH measurement of soil sample used in biodegradation study

The pH of the soil sample was found to be in the neutral range, as the soil sample had pH of 7.2.

Table 4.7: The percentage weight losses in degraded PUR samples in the laboratory broth

Isolates ↓	1 month (Static) %	3 months (Static) %	1 month (Agitated) %
Enterobacter amnigenus $D_1$2	10.00	3.33	8.33
Pseudomonas alcaligenes $E_1$4	nd	nd	6.67
Vibrio sp. $C_3$2	6.67	3.33	6.00
Combination A	6.67	3.33	6.67

Key:

Combination A= *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

nd= not determined

Table 4.8: The percentage weight losses of degraded PUR samples in field with bioaugmentation and biostimulation after 30 days

Isolates ↓	Biostimulationwith Cassava peels (%)	Biostimulation with Potato peels (%)	No Peels (%)
Combination A	7.5	5.0	5.0
Combination B	10.0	15.0	5.0
Combination C	2.5	10.0	17.5
Consortium	10.0	10.0	22.5

Key:

Combination A= *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B= *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= Combination A + Combination B + Combination C

Table 4.9: The percentage weight losses in degraded PUR samples in laboratory sterilised soil with bioaugmentation and biostimulation after 30 days

Isolates ↓	Biostimulationwith Cassava peels (%)	Biostimulation with Potato peels (%)	No Peels (%)
Combination A	2.5	5.0	2.5
Combination B	15.0	7.5	2.5
Combination C	0.0	5.0	5.0
Consortium	15.0	7.0	2.5

Key:

Combination A= *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B= *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= Combination A + Combination B + Combination C

Table 4.10: The percentage degradation in PUR samples degraded in laboratory broth after 1 month (Agitation) and after 1 and 3 month(s) (Static)

Periods ⟶ / Isolates ↓	1 month (Static) %	3 months (Static) %	1 month (Agitated) %
Enterobacter amnigenus $D_1$2	-14.07	-9.38	86.67
Pseudomonas alcaligenes $E_1$4	66.47	39.76	nd
Providencia pseudomallei $D_2$5	0.00	64.13	nd
Providencia pseudomallei $D_2$1	72.18	18.68	nd
Vibrio sp. $C_3$2	-47.47	60.63	80.75
Pseudomonas aeruginosa $E_3$2	64.86	54.95	nd
Combination A	64.68	72.29	60.39
Combination B	nd	nd	64.24
Combination C	80.32	28.74	nd
Consortium	39.04	60.14	23.11

Key:

nd = not determined

Combination A= *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B= *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= Combination A + Combination B + Combination C

Table 4.11: The percentage degradation in PUR samples degraded in the field after 1 month with bioaugmentation and biostimulation.

Isolates ↓	Biostimulationwith Cassava peels (%)	Biostimulation with Potato peels (%)	No Peels (%)
Combination A	0.00	0.00	0.00
Combination B	0.00	0.00	76.27
Combination C	0.00	0.00	34.36
Consortium	0.00	35.89	68.37

Key:

Combination A= *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B= *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= Combination A + Combination B + Combination C

Table 4.12: The percentage degradation in PUR samples degraded in laboratory sterilised soil after 1 month with bioaugmentation and biostimulation

Isolates	Biostimulationwith Cassava peels (%)	Biostimulation with Potato peels (%)	No Peels (%)
Combination A	-6.68	88.88	0.00
Combination B	-13.59	65.54	0.00
Combination C	33.01	57.44	0.00
Consortium	0.00	90.98	57.24

Key:

Combination A= *Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5

Combination B= *Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2

Combination C= *Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1

Consortium= Combination A + Combination B + Combination C

Table 4.13: Statistical Analysis of changes in areas of the four functional groups (C=O, NH, C-C, C-O) considered in polyurethane degradation in the FTIR analysis using one way Anova (Duncan) in the field degradation experiment after 30 days.

Treatments	Carbonyl (C=O)	Urethane (NH)	Alkyl (C-C)	Ether (C-O)
No Peels				
A+PUR	31.1410^{e}	15.5450^{c}	24.8600^{e}	0.0000^{a}
B+PUR	4.5310^{c}	18.7440^{d}	5.9120^{a}	0.0000^{a}
C+PUR	5.7430^{d}	21.3700^{e}	8.1000^{c}	26.9070^{c}
D+PUR	3.9480^{b}	5.1160^{b}	6.8190^{b}	0.0000^{a}
Control+PUR	3.5820^{a}	4.8540^{a}	10.0150^{d}	16.8100^{b}
Stimulation with Cassava Peels				
A+PUR+CA	7.0850^{c}	7.1680^{e}	9.2910^{d}	52.3370^{c}
B+PUR+CA	10.0310^{e}	2.4320^{c}	8.4380^{c}	0.0000^{a}
C+PUR+CA	7.2840^{d}	7.0410^{d}	12.9600^{e}	0.0000^{a}
D+PUR+CA	3.5370^{b}	1.3440^{b}	7.5720^{b}	0.0000^{a}
Control+PUR+CA	0.6780^{a}	0.8730^{a}	2.28630^{a}	5.8630^{b}
Stimulation with Potato Peels				
A+PUR+PT	19.4120^{d}	11.3420^{e}	17.7690^{d}	54.0700^{d}
B+PUR+PT	20.3000^{e}	9.1710^{d}	20.3680^{e}	60.8420^{e}
C+PUR+PT	8.5260^{c}	0.0000^{a}	13.2670^{c}	53.8830^{c}
D+PUR+PT	0.5390^{a}	1.4280^{c}	2.0310^{a}	0.0000^{a}
Control+PUR+PT	4.3680^{b}	0.6440^{b}	5.3740^{b}	13.0080^{b}

Key:

A= Combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5)

B= Combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2)

C= Combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1)

D= Consortium (Combination A + Combination B + Combination C)

PUR: Polyurethane samples

CA: Cassava peels

PT: Potato peels

Table 4.14: Statistical Analysis of changes in areas of the four functional groups (C=O, NH, C-C, C-O) considered in polyurethane degradation in the FTIR analysis using one way Anova (Duncan) in the laboratory sterile soil degradation experiment after 30 days.

Treatments	Carbonyl (C=O)	Urethane (NH)	Alkyl (C-C)	Ether (C-O)
No Peels				
A+PUR	4.2200^{b}	12.7720^{e}	18.8580^{d}	85.7830^{c}
B+PUR	19.0680^{e}	4.3550^{c}	20.1090^{e}	93.5900^{d}
C+PUR	15.5940^{d}	0.0000^{a}	16.4930^{c}	74.6120^{b}
D+PUR	1.9800^{a}	1.6400^{b}	6.1900^{a}	0.0000^{a}
Control+PUR	7.0500^{c}	6.9400^{d}	9.1200^{b}	121.91^{e}
Stimulation with Cassava Peels				
A+PUR+CA	3.7340^{c}	4.2760^{c}	8.4320^{c}	46.3410^{d}
B+PUR+CA	11.2050^{d}	4.6920^{d}	12.6910^{d}	76.0950^{e}
C+PUR+CA	1.2700^{a}	1.5600^{a}	6.1500^{a}	42.2400^{c}
D+PUR+CA	15.5200^{e}	9.2530^{e}	17.6270^{e}	15.0370^{b}
Control+PUR+CA	3.6170^{b}	3.3960^{b}	8.3060^{b}	7.6610^{a}
Stimulation with Potato Peels				
A+PUR+PT	2.7700^{b}	1.8400^{b}	6.2000^{b}	38.5500^{b}
B+PUR+PT	7.9970^{c}	1.4960^{a}	13.8780^{c}	85.4980^{d}
C+PUR+PT	15.1970^{d}	5.6590^{d}	15.0190^{d}	83.0710^{c}
D+PUR+PT	2.6560^{a}	2.1080^{c}	3.9240^{a}	34.8780^{a}
Control+PUR+PT	21.3700^{e}	10.9580^{e}	27.3860^{e}	93.8060^{e}

Key:

A= Combination A (*Pseudomonas alcaligenes* E_14 + *Providencia pseudomallei* D_25)

B= Combination B (*Enterobacter amnigenus* D_12 + *Vibrio* sp. C_32)

C= Combination C (*Pseudomonas aeruginosa* E_32 + *Providencia pseudomallei* D_21)

D= Consortium (Combination A + Combination B + Combination C)

PUR: Polyurethane samples
CA: Cassava peels
PT: Potato peels

CHAPTER FIVE

DISCUSSION

The ability of ninety four (94) isolated bacteria strains and seven (7) fungi isolates obtained within 3- 12 months of isolations in this study to utilise PUR as carbon, nitrogen, or carbon and nitrogen sources showed that the bacteria isolates utilised PUR. The fungi isolates could not utilise PUR as sole source of carbon and were not used in the biodegradation study. From the 94 bacteria isolates, 35 showed potential of utilising PUR as both carbon and nitrogen sources and this agreed with the study of Nakajima-Kambe *et al*. (1995) who isolated a bacterium, *Comamonas acidovoran* TB-35 with ability to utilise polyester PUR as sole source of carbon and nitrogen. Fifteen bacteria isolates selected from the 35 which were capable of utilising PUR as both carbon and nitrogen sources with profuse growth on the modified MSM were characterised and identified as members of the genera *Pseudomonas* (4), *Providencia* (2), *Enterobacter* (2), *Vibrio* (1), *Comamonas* (2), *Corynebacterium* (1), *Arthrobacter* (1), *Micrococcus* (1) and *Bacillus* (1). These bacteria strains have been reportedly isolated from PUR and other polymers in biodegradation studies by some researchers such as Kay *et al*. (1991) who reported the isolation of *Corynebacterium, Enterobacter, Pseudomonas, Serratia, Alcaligenes* and *Aeromonas* among 15 bacteria isolates from PUR samples buried in soil for 28 days while Cacciari *et al.* (1993) isolated *Vibrio* sp. in community soil sample and used it for the degradation of polypropylene samples. Nakajima-Kambe *et al*. (1995) reported the isolation of a strain of *Comamonas* from soil sample which has capability of utilising PUR. Blake and Howard (1998) and Rowe and Howard (2002) also reported the degradation of PUR by species of *Bacillus* isolated from soil while Shah *et al*. (2008) isolated species of *Bacillus, Pseudomonas, Micrococcus, Arthrobacter* and *Corynebacterium* capable of degrading PUR from polyurethane samples buried in soil for 6 months.

The seasonal variation in the bacteria isolated obtained showed that 87 % of the bacteria isolates were obtained in the rainy seasons of June, 2010 to June, 2012 with the remaining 13 % during the dry season. This may be attributed to higher interaction between the soil particles adherence to the PUR samples and physiological activities of the bacteria isolates as water activity was high and bacteria has higher water activity value and acted on the surface of the PUR samples for their growth and survival. Thirty-three per cent of the bacteria strains were obtained at the second sampling period of 5 months in November, 2010 compared to 23 % of 3 months, 11 % of 7 months, 22 % of 12 months and 11 % of 24 months. There were 26 % of bacteria isolates obtained from the 70 cm depth which is higher than 21, 16, 19, and 18 % obtained from 15, 30, 45 and 60 cm depths respectively. The PUR samples acted as substrate of support for the bacteria and provided source of energy and this explained why they could be isolated at such depth of 70 cm where nutrients would easily be washed off by rain percolating down the depth of the soil to underground water as against the top soil of 15 cm where the organisms use nutrient from leaf litter decay for sources of carbon and nitrogen required for their survival.

These bacteria isolates showed capability of degrading/utilising PUR as sole carbon and nitrogen sources without any addition of supplementary sources of either carbon or nitrogen source in the cause of this study and this is in contrast to reports of some previous researchers. For instance, Kay *et al.* (1991) in their investigation reported that the bacteria isolates *Corynebacterium* sp. and *Enterobacter agglomerans* in their study required organic nutrients like yeast extract in order to degrade polyester PUR and thus supplemented it in their medium to aid the degradation. Oceguera-Cervantes *et al*. (2007) used commercial surface-coating polyester PUR as carbon source and ammonium nitrate NH_4NO_3 as nitrogen source for 2 strains of *Alicycliphillus* sp. capable of degrading PUR while Shah *et al*. (2008) used ammonium sulphate $(NH_4)_2SO_4$ as nitrogen source and also glucose as a co-metabolite in their PUR degradation. The bacteria isolates obtained in this study therefore had better biodegradation ability by being able to grow on the PUR sample which was more recalcitrant than the polyester PUR studied by Kay *et al*. (1991). This study is the second report on ability of bacteria isolates to utilise PUR as sole carbon and nitrogen source, but first for polyether PUR as the first report on utilisation of PUR as sole

carbon and nitrogen source was in the study of Nakajima-Kambe *et al*. (1995) with *Comamonas acidovoran* TB-35 and polyester PUR as sole carbon and nitrogen source.

The bacteria isolates were able to degrade PUR samples in the laboratory experiments of both broth and soil and this showed that despite the fact that the isolates were from the natural environment, they showed degradation potential in the laboratory environment as well. Akutsu *et al*. (1998) reported that the degradation of PUR could be due to utilization of PUR as carbon and /or nitrogen source by microorganisms or co-metabolic biodegradation in the presence of other nutrients and substrates while Darby and Kaplan (1968) reported that PUR biodegradation in the laboratory under controlled conditions were mainly due to fungal attacks and this contrasts the findings of this study as only bacteria isolates were used for this study. Urgun-Demirtas *et al*. (2007) reported that PUR biodegradation mostly come from laboratory studies, in many cases, providing additional nutrients to microorganisms, and use of highly concentrated enzymes to promote biodegradation but in this study, both laboratory and pilot field studies were reported to advance knowledge in real life situation of biodegradation in the field. This also contrasts findings in biodegradation studies especially in oil biodegradation where organisms found to degrade oil in the laboratory were taken to the field but do not show the biodegradation potential.

In the assessment of potential of biostimulation and bioaugmentation as methods for accelerating the degradation of PUR waste in the environment in this study, the response of microbial communities in soil microcosms to (i) addition of nutrients-cassava peels or potato peels or (ii) influx of PUR-degrading bacteria (*Pseudomonas alcaligenes* $E_1$4, *Providencia pseudomallei* $D_2$5, *Enterobacter amnigenus* $D_1$2, *Vibrio* sp. $C_3$2, *Pseudomonas aeruginosa* $E_3$2, *Providencia pseudomallei* $D_2$1) showed that the biostimulation and bioaugmentation methods aided the degradation of PUR samples buried in the soil microcosms (Figs.4.5- 4.7) and caused degradation changes of 35.89 % with biostimulation with potato peels and 76.27 % with bioaugmentation with combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) (Table 4.11). This is in line with the work of Cosgrove *et al*. (2010) where PUR-degrading fungal isolates were used for bioaugmentation and impranil DLN (which is a base material for PUR) and/or yeast extract were used for biostimulation and reported that biostimulation with

yeast extract and impranil increased PUR degradation 62 % compared to control and bioaugmentation with *Nectria haematococca, Penicillium viridicatum, Penicillium ochrochloron and Mucormycotina* sp. increased PUR degradation further 70 %, hence suggesting that biostimulation and bioaugmentation were operating in concert to enhance PUR degradation. This study contrasts in materials used for biostimulation which in this study were cassava and potato peels and bioaugmentation with bacteria as against fungi. The isolated bacteria native to the site of PUR burial showed degradability in natural conditions and also exhibited biodegradation in laboratory conditions on synthetic media. This gave clues that these bacteria can be used in both natural and artificial conditions for the purpose of degradation of polymers. The biostimulation with potato peels gave a better degradation activity as was seen in both weight loss 15 % (Table 4.8) and reduced areas in the FTIR spectra of analysed experimentally degraded PUR samples (Fig. 4.6) compared to the cassava peels (Fig. 4.5) which may be attributed to potato peels having more soluble sugars than cassava. The consortium in most of the analysis had best degradation activity (Figs. 4.6d, 4.9d, 4.10d) and (Tables 4.8, 4.11, 4.12) and this may be attributed to co-metabolic activities of the bacteria isolates present in the consortium. This is similar to Kay *et al*. (1993) who reported that *Corynebacterium* sp. used in biodegradation of polyester polyurethane in medium supplemented with yeast extract utilised the test material as a result of co-metabolism.

The methods currently used in testing microbiological degradation and deterioration of a wide range of PUR materials had been reviewed by Gu and Gu (2005) and Zheng *et al.* (2005). Among them, the most commonly practiced methods to assess the biodegradability of PUR in different environmental conditions are tensile strength, weight loss, change in FTIR signature and bacterial growth and these methods were used in this study to assess the activities of the bacteria isolates on the PUR samples except tensile strength which was not measured. The changes in FTIR signatures, weight losses, percentage degradation changes and also the bacteria growth on the materials were used to ascertain that the bacterial isolates can actually use the PUR materials as source of nutrients (carbon and nitrogen sources). The results of this study have shown that PUR materials of the polyether type which have been previously thought to be resistant to biodegradation by past researchers such as Darby and Kaplan

(1968); Martens and Domsch (1981) showed degradation by the isolated bacteria and thus these bacteria isolates can be used as potential PUR degraders.

The FTIR analysis of experimentally degraded PUR samples and un-degraded control in this study showed the presence of changes in chemical functional groups of the PUR such as disappearance of some old existing peaks, formation of some new peaks and shifts to both left and right of some peaks in the spectra in comparison to the control and this is in line with the reports of Sudhakar *et al.* (2007) who opined that these observations may be due to the process of hydrolysis and oxidation and Shah *et al.* (2008) who attributed it to hydrolysis of bonds. These changes observed in the FTIR contrast the findings of Urgun-Demirtas *et al*. (2007) who in their study noted that the PUR samples showed no change in their chemical signature and also no new peaks were formed in comparison to the control after 6 weeks of incubation.

The FTIR spectra analysis in this study showed removal of urethane group assigned to peak at wavenumbers 1647 and 1662 cm^{-1} in the laboratory broth (1 month) under static condition by isolates such as *Providencia pseudomallei* $D_2$1 and $D_2$5 (Figs. 4.3a &c); in the field bioaugmentation and biostimulation pilot study by combinations A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5) and B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) biostimulated with cassava peels (Fig. 4.5a-b), consortium (Combination A + Combination B + Combination C) biostimulated with potato peels (Fig. 4.6d) and combination B without agro waste stimulation (Fig. 4.7b) and in the field natural biodegradation at depths 45, 60 and 70 cm in the 12-month period and at depths 15, 30 and 70 cm in the 24-month period (Fig. 4.11a-b). This finding contrasts findings of some previous researchers such as Martens and Domsch (1981) who detected a limited amount of degradation products derived from the hydrolysis of urethane bonds after 3-month incubation of PUR foams in three different media: leachate from a refuse tip, composted municipal waste and soil. Santerre *et al*. (1994) reported that urethane bonds were not cleaved and remained in the degradation products in their biodegradation of PUR using hydrolytic and oxidative enzymes while Nakajima-Kambe *et al*. (1997) reported that low-molecular-weight urethane compounds remained after microbial degradation of PUR contrary to removal of urethane bonds in this study. Akutsu-Shigeno *et al*. (2006) opined that for complete

degradation of PUR, the cleavage of the urethane bond is necessary and this cleavage was observed in the FTIR spectra analysis of some of the experimentally degraded PUR samples in this study which showed shifts in the assigned urethane group peaks by isolates such as *Pseudomonas alcaligenes* $E_1$4 in 1 month laboratory broth (Fig. 4.3e); consortium biostimulated with cassava peels in the pilot field study (Fig.4.5d), and in the laboratory sterile soil by combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2) biostimulated with cassava peels (Fig.4.8b).

The FTIR spectra analysis in this study equally revealed shifts in peak at wavenumber 1747 cm^{-1} assigned to carbonyl group by all the bacteria isolates in both laboratory and pilot field studies and this observation is in line with the work of Pathirana and Seal (1985b) that attributed the change as indication of build-up of free carboxyl groups during degradation. The carbonyl peak area was significantly reduced by the isolates but of prominence is the 87.6 % reduction showed by the spectra of PUR sample degraded by the consortium (Combination A + Combination B + Combination C) biostimulated with potato peels in the laboratory sterile soil (Fig. 4.9d). The spectra of PUR samples experimentally degraded by *Pseudomonas aeruginosa* $E_3$2 in the laboratory broth after 3 months showed the removal of the carbonyl peak by this isolate (Fig.4.3h). Peaks at wavenumbers 2196 and 2258 cm^{-1} assigned to cyanates (-OCN) and wavenumber 2274 cm^{-1} assigned to isocyanates (-N=C=O) asymmetric stretching vibrations were removed by all the bacteria isolates in the PUR samples experimentally degraded in the laboratory broth for 3 months such as *Providencia pseudomallei* $D_2$1 (Fig. 4.3b), *Pseudomonas alcaligenes* $E_1$4 (Fig. 4.3f) and *Enterobacter amnigenus* $D_1$2 (Fig.4.3j). There was drastic decrease in the intensity of the spectra of experimentally degraded PUR samples when compared to the control sample and this reduction in intensity is similar to the findings of Filip (1978) who attributed the decrease in intensity of absorption bands to cleavage of N=C and C=O valency bonds of the isocyanate groups.

The cleavage of C-H bonds was shown by shifts in peak at wavenumber 2924 cm^{-1} assigned to asymmetric C-H stretch of methylene group in the FTIR spectra of PUR degraded with the bacteria isolates in the laboratory broth under agitation for 1 month (Fig.4.4a-d), in the pilot field study biostimulated with cassava peels (Fig.4.5a-d) and

in the laboratory sterile soil study with and without agro waste materials (Figs.4.8-4.10). This was in agreement with the report of Shah *et al*. (2008) and Pathirana and Seal (1985b) that attributed the shifts as indication of loss of $-CH_2-$ groups from the polyurethane. Pathirana and Seal (1985b) opined that these shifts in C-H stretch during degradation indicated a loss of diacid and dialcohol groups from the polymer, suggesting that hydrolysis was occurring. Also, there was formation of new peaks corresponding to polymeric O-H stretch assigned to peaks at wavenumbers such as 3230, 3367 and 3433 cm^{-1} by the isolates in both laboratory (Figs. 4.3n; 4.8b; 4.9c) and pilot field studies (Figs.4.5c; 4.6c; 4.7a) and this was attributed to be as a result of degradation and linked to diethyl glycol which is a degradation product in PUR degradation.

The FTIR spectra in this study also revealed a shift in peak at wavenumber 2850 cm^{-1} assigned to methoxy methyl ether to 2848 cm^{-1} by the isolate combinations in the laboratory sterile soil (Figs.4.8c; 4.10d), pilot field studies (Figs.4.5d;4.6b;4.7c) and field natural biodegradation experiment after 1 year in depths 45 and 70 cm (Fig.4.11a). Ether peak at wavenumbers 1068 and 1153 cm^{-1} assigned to alkyl substituted ether (C-O) stretch was removed by the isolates combinations in pilot field study but drastically by combinations B, C and consortium biostimulated with cassava in the field (Figs.4.5b-d). This contrast the works of Darby and Kaplan (1968); Martens and Domsch (1981) and Kawai *et al*. (1985) where the polyether polyurethane were relatively resistant to microbial degradation by both bacteria and fungi. Kawai *et al.* (1985) attributed this resistance to the degradation mechanism in polyether polyurethane which involves exo-type depolymerisation.

The highest weight loss of 22.5 % observed in this study was with the consortium (combination A+ combination B+ combination C) without agro waste stimulation in the field (Table 4.8) and may be attributed to complete dependence of the bacteria isolates on the PUR samples and nutrient within their reach in the soil microcosm while the consortium with either potato or cassava peels had 10.0 % weight loss as they equally obtained additional nutrients from the peels. This potential in the bacteria isolates suggested the production of extracelluar enzymes that possess specificity for PUR and or its components which acted on the fibres to make available to the

microorganisms the inherent nutrient requirement in the PUR fibres. The extracellular 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. The laboratory study on the other hand had its highest weight loss of 15.0 % each with combination B (*Enterobacter amnigenus* $D_1$2+ *Vibrio* sp. $C_3$2) and the consortium biostimulated with cassava peels. This was similar to Rotkowska *et al*. (2002) who reported that weight loss in PUR samples incubated in the laboratory medium decreased compared to PUR samples in natural environment. The lowest weight losses in the field and laboratory were 2.5 and 0.0 % respectively by combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1) biostimulated with cassava peels (Tables 4.8 and 4.9), thus showing that the cassava peels did not support combination C in its degradation activity as it showed higher weight losses in field and laboratory with potato peels and without stimulation with any agro waste. This may be attributed to potato peels having many soluble sugars while cassava has insoluble starch components such as cellulose which are not easily hydrolysed.

The percentage degradation changes showed that in the laboratory broth, *Enterobacter amnigenus* $D_1$2 under agitation for 1 month had 86.67 % degradation change and this was followed by *Vibrio* sp. $C_3$2 under agitation for 1 month, but in the static condition of 1 month and 3 months, it was observed that most of the bacteria isolates showed better percentage degradation under 1 month than 3 months (Table 4.10). Combination C with highest percentage degradation of 80.32 % in 1 month (static) had 28.74 % in 3 months set up and also *Providencia pseudomallei* $D_2$1 had 72.18 % in 1 month (static) and 18.68 % in 3 months set up, thus 1 month gave a better percentage degradation than 3 months and this may be attributed to accumulation of materials for a long time in the 3 months set up. In the biostimulation and bioaugmentation studies in both laboratory and field experiments (Tables 4.11 and 4.12), the highest percentage degradation was exhibited by the consortium biostimulated with potato peels in the field with 90.98 % and least by combination B biostimulated with cassava peels in the field with -13.59 %. The best percentage degradation change in the laboratory was exhibited by combination B without stimulation with any agro waste with 76.27 % and this was followed by consortium without stimulation with 68.37 %.

The growth of the six bacteria isolates in the temperature ranges of 25^{o}C- 50^{o}C showed their ability to thrive under different temperatures though with some fluctuations. From this study, three of the isolates had their optimum growth temperature at 25^{o}C while one each had their optimum growth temperature at 30^{o}C, 37^{o}C and 42^{o}C (Fig. 4.1a-f) after 3 day incubation period. This could be attributed to the isolates environmental sources as Kurian *et al*. (2003) reported a temperature range of 17^{o}C -39^{o}C in isolates from dumpsites while Ibrahim *et al*. (2009) reported a temperature range of 20^{o}C – 35^{o}C in soil isolate. Pathirana and Seal (1984) reported a temperature range of 20^{o}C – 45^{o}C for soil fungal isolates.

In optimum growth pH, the ability of the bacteria isolates to grow on the pH range of 6.0-8.5 showed their ability to thrive well in fluctuating pH. The optimum growth pH in this study was 6.0 and 7.0 though the isolates still showed growth at all the pH ranges tested and this was similar to the work of Ibrahim *et al.* (2009) who in their study had pH 7.0 as optimum and Pathirana and Seal (1984) had pH 7.0 and 6.0 as optimum in their soil fungal isolates. All the isolates showed varying degrees of growth throughout the pH and temperature ranges tested.

There were presence of pits and cracks on the exhumed PUR materials and these were more on the samples buried in 70cm depth of the soil. The presence of these pits and cracks show that the microorganisms are actually degrading the materials though it took about one year of soil burial for the PUR samples to show presence of this physical deformation. Equally, the PUR materials exhumed after two years of soil burial showed the same trend of cracks and pits in all the different depths but more effect was observed at 60 cm and 70 cm depths. This was in agreement with the report of Howard and Hilliard (1999) who noted that physical examination of PUR plugs for deterioration is considered as an important method for investigating biodeterioration of the PUR plugs because physical changes in the structure of PUR are more likely to occur before complete degradation of PUR takes place and Kay *et al*. (1991) who reported severe cracking of polyester PUR by *Corynebacterium* sp. in yeast extract supplemented media for 12 weeks.

Although there have been numerous investigations in degrading polyurethanes, the fate of these organic polymers in the environment and the time required for their complete mineralization to carbon dioxide needs to be fully understood, thus forming the basis for further work and also to find out if toxic substances are released into the environment when the polymer structure is degraded as there are strong objections to use of some monomers in plastic products.

CHAPTER SIX

CONCLUSIONS

These isolated bacteria from buried polyurethane samples showed that 33 % of the 106 bacteria isolates obtained from this study were able to utilize the PUR samples as both carbon and nitrogen sources. The isolates were able to degrade the PUR samples with removal of recalcitrant functional groups such as ether and carbonyl groups in laboratory and field studies. The isolates used in combinations- Combination A (*Pseudomonas alcaligenes* $E_1$4 + *Providencia pseudomallei* $D_2$5), Combination B (*Enterobacter amnigenus* $D_1$2 + *Vibrio* sp. $C_3$2), Combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1) and consortium (Combination A + Combination B + Combination C) showed effectiveness of these organisms synergistically in tackling environmental challenges posed by the PUR polymer.

Agro based wastes, cassava and potato peels were found to be effective for biostimulation in the degradation activities of the isolates and use of the bacteria isolates for bioaugmentation in both field and laboratory studies were also effective. The best isolate combination is the consortium (Combination A + Combination B + Combination C) biostimulated with cassava peels which resulted in weight losses of 22.5 % and 15 % in field and laboratory studies respectively.

Works done on this polymer were of the opinion that polyether type PUR are resistant to biodegradation but with the findings of this work it has been found that the polyether type of PUR can be susceptible to bacterial degradation both in the laboratory and in the field. The following conclusions can be drawn from above:

- The results of this study have shown that PUR materials of the polyether type which have been previously thought to be resistant to biodegradation by past researchers showed appreciable level of degradation by the isolated bacteria- *Pseudomonas alcaligenes* $E_1$4, *Providencia pseudomallei* $D_2$5, *Enterobacter amnigenus* $D_1$2, *Vibrio* sp. $C_3$2, *Pseudomonas aeruginosa* $E_3$2 and *Providencia pseudomallei* $D_2$1.

- The isolated bacteria were native to the site of PUR burial and might show some degradability in natural conditions, they also exhibited biodegradation in laboratory conditions on synthetic media. This gave prospects for the use of these bacteria in both natural and artificial conditions for the purpose of degradation of polymers.
- This study served as a baseline research into potential of the isolates for PUR degradation and management in our Nigeria environment where these polymers are used on daily basis and always discarded into the environment where they constitute environmental nuisance.
- Further works will be channelled towards elucidating the time required for their complete mineralization to carbon dioxide and also to find out if toxic substances are released into the environment when the polymer structure is degraded as there are strong objections to use of some monomers in plastic products.

RECOMMENDATIONS

Improperly disposed plastics are a significant source of environmental pollution, potentially harming life as the sheets do not allow water and air to go into the earth which causes infertility of soil, preventing degradation of other normal substances, depletion of underground water source and danger to animal life. With the observed widespread littering of the environment with polyurethanes and in view of the fact that these materials constitute environmental nuisance because of their persistence in our environments, deleterious effects on wildlife and on the aesthetic qualities of cities and forest, the following are therefore recommended:

- Policies should be formulated to enhance waste sorting which will aid in recycling of these plastic wastes and reduction of its unsightly presence and persistence in the environment.
- There should be incentives for plastic waste gathering as we now have for metal scraps as this will reduce its abundance in the environment.
- Policies should be made to ban use of PUR as packaging materials as majority of the wastes come from it and also use of polyether PUR should be banned due to its recalcitrance nature.
- Relevant regulatory agencies should be strengthened to enforce such policies.

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APPENDIX I

COMPONENTS OF MEDIA USED IN THIS STUDY

a. Nutrient Agar

Composition	**g/l**
Peptone	5.0
Beef Extract	3.0
Sodium Chloride	8.0
Agar	12.0
pH	7.2 ± 0.2

b. Mineral Salts Medium (MSM) for PUR (Urgun Demirtas *et al*., 2007)

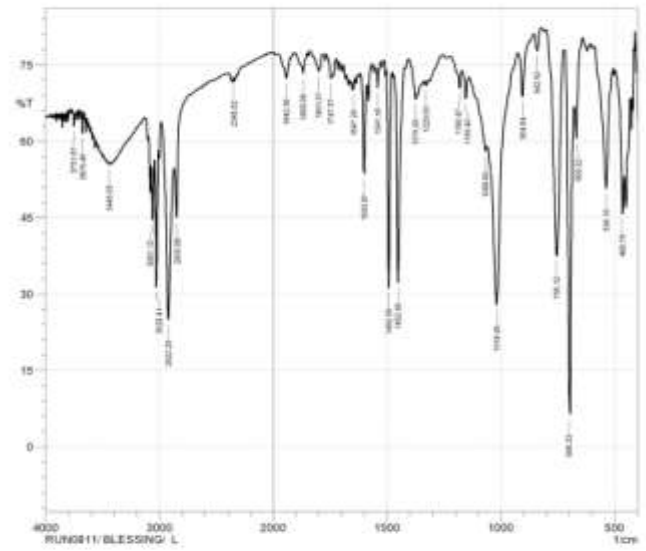
Component	**g/l**
KH_2PO_4	2.0000
K_2HPO_4	7.0000
NH_4NO_3	1.0000
Glucose	3.7500
$MgSO_4.7H_2O$	0.1000
$ZnSO_4.7H_2O$	0.0010
$CuSO_4.7H_2O$	0.0001
$FeSO_4.7H_2O$	0.0100
$MnSO_4.7H_2O$	0.0020
pH	7.2

(where glucose and/or ammonium nitrate is omitted when PUR is supplied as a sole carbon and/or nitrogen source)

APPENDIX II

FTIR SPECTRA OF CONTROL AND SOME DEGRADED PUR SAMPLES IN LABORATORY BROTH

SHIMADZU



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	466.79	45.73	11.372	499.58	469.07	6.444	0.225
2	538.16	60.748	24.42	590.24	513.08	14.035	4.823
3	669.32	60.588	9.201	677.04	642.32	4.982	0.288
4	696.33	6.496	64.955	719.47	678.97	18.037	12.203
5	756.12	37.449	42.25	800.49	721.4	17.018	9.295
6	842.92	77.777	3.812	896.42	823.63	3.151	0.273
7	904.64	68.825	9.449	925.86	873.78	5.324	0.843
8	1018.46	27.911	36.596	1058.96	925.86	33.295	10.886
9	1068.6	58.135	2.213	1139.97	1060.88	14.4	0.149
10	1155.4	68.479	4.766	1170.83	1139.97	4.464	0.311
11	1180.47	70.468	3.996	1222.91	1170.83	6.785	0.373
12	1329	70.867	1.003	1334.78	1317.43	2.531	0.046
13	1375.29	68.363	2.968	1392.65	1365.65	4.09	0.244
14	1462.45	32.277	41.161	1469.81	1423.51	10.771	4.62
15	1492.95	31.256	42.553	1504.53	1477.52	6.574	3.009
16	1541.16	70.548	3.738	1592.75	1537.32	2.093	0.111
17	1600.07	53.659	18.219	1614.47	1589.4	4.853	1.267
18	1647.26	70.203	1.378	1651.12	1641.48	1.417	0.023
19	1747.57	72.44	0.549	1764.93	1745.64	2.426	-0.011
20	1801.57	73.729	3.114	1822.79	1782.29	4.969	0.332
21	1869.08	73.333	3.977	1905.73	1851.72	5.53	0.5
22	1942.36	72.38	4.192	1977.1	1925.02	5.531	0.528
23	2346.52	72.059	0.2	2349.36	2343.58	0.816	0.003
24	2850.66	49.111	12.902	2872.1	2791.09	16.866	1.909
25	2922.25	25.004	33.58	2983.96	2874.03	39.271	13.988
26	3026.41	31.356	28.947	3043.77	3009.05	11.878	3.495
27	3061.13	44.581	11.013	3072.71	3048.7	8.067	1.14
28	3443.06	55.481	0.37	3462.7	3436.34	4.421	0.03
29	3676.46	61.894	3.309	3684.16	3672.59	2.246	0.092
30	3751.67	62.911	2.584	3757.46	3740.1	3.299	0.109

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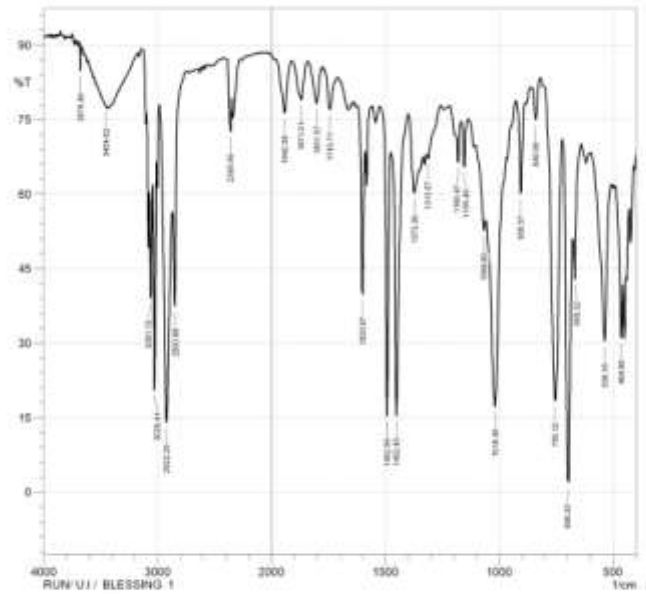

	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	464.86	31.088	14.265	489.94	469.07	10.778	1.538
2	538.16	30.42	33.362	598.02	511.15	25.123	9.13
3	669.32	42.852	12.28	677.04	642.32	7.939	0.844
4	696.33	2.032	98.741	723.33	678.97	29.842	19.918
5	756.12	18.431	56.601	819.77	725.26	28.499	17.694
6	840.99	74.945	7.3	859.42	819.77	3.648	0.567
7	906.57	60.213	16.397	927.79	885.36	6.01	1.575
8	1018.46	17.249	43.197	1058.96	970.23	34.957	15.786
9	1068.6	52.673	4.745	1105.32	1060.88	9.196	0.364
10	1155.4	65.504	9.269	1166.97	1136.11	4.457	0.573
11	1180.47	66.403	8.907	1219.06	1168.9	6.946	0.999
12	1315.57	67.089	1.758	1319.35	1257.83	5.576	0.142
13	1373.36	60.265	9.392	1398.44	1354.07	8.247	1.355
14	1452.45	55.461	55.334	1475.59	1400.37	20.279	9.672
15	1492.95	55.364	56.051	1525.82	1477.52	11.954	5.583
16	1600.07	39.846	31.707	1624.12	1589.4	7.319	2.564
17	1743.71	76.975	7.319	1766.85	1712.85	4.927	0.89
18	1801.57	78.232	7.616	1842.08	1780.36	5.068	1.041
19	1871.01	78.987	7.772	1911.52	1842.08	5.526	1.242
20	1942.36	76.298	10.618	1982.89	1911.52	5.867	1.548
21	2360.06	72.611	8.89	2391.81	2349.36	4.327	0.931
22	2850.66	37.529	23.018	2868.24	2752.51	18.089	3.272
23	2922.25	14.097	52.031	2985.91	2870.17	47.399	26.635
24	3026.41	20.563	43.263	3043.77	3009.05	13.509	6.76
25	3061.13	39.154	21.779	3072.71	3048.7	8.067	2.279
26	3454.62	77.721	0.048	3466.48	3452.7	0.633	0.001
27	3676.46	84.8	5.295	3689.95	3670.66	1.004	0.14

Comment:
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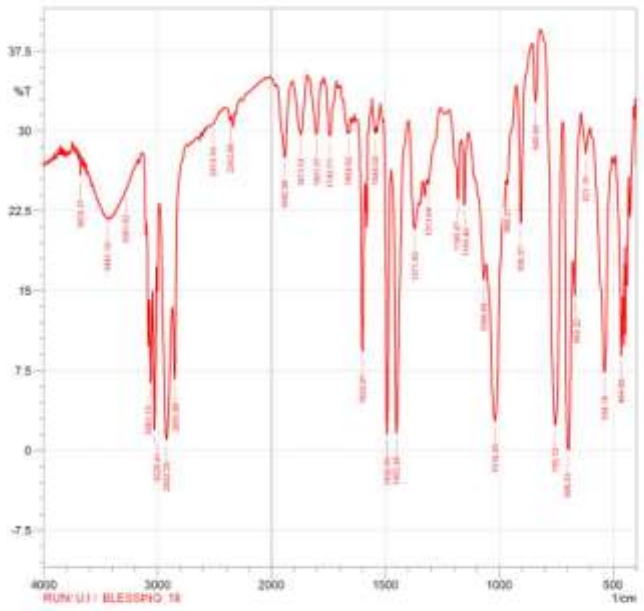
User: adebayot

SHIMADZU

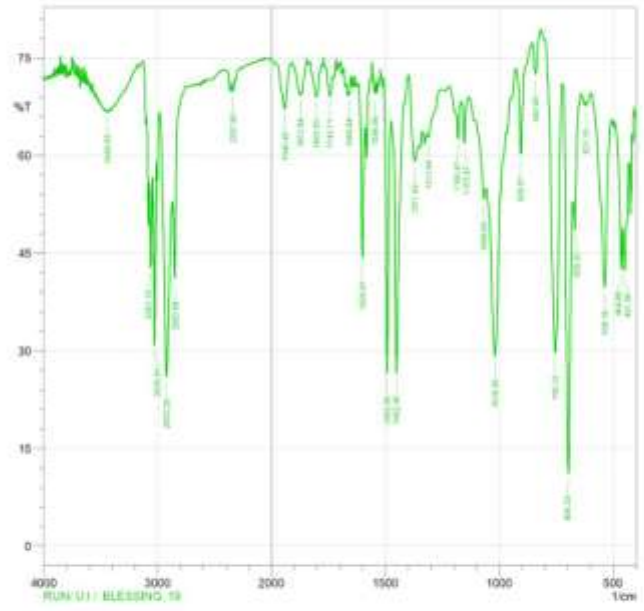


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	464.86	8.873	7.899	491.86	459.07	24.827	1.888
2	538.16	7.322	20.94	592.17	505.37	64.321	16.933
3	621.1	27.864	2.816	642.32	607.6	16.41	0.676
4	669.32	14.542	6.393	677.04	642.32	21.765	0.961
5	696.33	0.081	22.993	721.4	679.97	80.191	33.149
6	756.12	2.405	31.351	816.77	723.33	77.069	32.958
7	840.99	32.715	6.098	856.42	821.7	15.289	1.043
8	906.57	21.309	14.031	925.86	858.35	33.21	3.4
9	966.37	24.968	1.256	970.23	927.79	22.402	0.208
10	1018.46	2.773	18.379	1058.96	972.16	83.887	25.315
11	1068.6	16.001	2.715	1136.11	1060.88	47.881	0.579
12	1155.4	23.05	6.365	1168.9	1136.11	18.473	1.076
13	1180.47	23.637	6.123	1220.96	1168.9	29.771	1.892
14	1311.64	24.993	1.009	1317.43	1295.86	31.517	0.213
15	1371.43	20.784	5.507	1396.44	1354.07	27.742	2.293
16	1452.45	1.619	26.051	1473.66	1400.37	57.209	17.374
17	1492.96	1.539	27.295	1506.46	1475.59	28.436	10.537
18	1545.09	29.79	1.369	1558.54	1539.25	9.864	0.251
19	1600.97	9.278	17.883	1622.19	1589.4	22.887	4.636
20	1664.62	29.786	0.339	1670.41	1662.69	4.032	0.03
21	1743.71	29.522	5.339	1770.71	1716.7	26.505	1.784
22	1851.57	29.707	4.939	1830.51	1782.29	23.841	1.652
23	1871.01	29.668	5.357	1907.66	1844.01	31.309	2.274
24	1942.38	27.527	6.994	1979.03	1909.59	34.828	2.747
25	2362.88	30.967	0.682	2397.6	2349.38	24.106	0.129
26	2513.33	30.61	0.075	2515.26	2465.11	25.429	0.05
27	2850.88	6.668	8.924	2868.24	2742.87	85.944	5.657
28	2922.25	1.066	16.879	2983.98	2870.17	134.4	48.606
29	3026.41	1.949	14.013	3043.77	3009.05	39.998	12.339
30	3061.13	6.348	8.143	3072.71	3045.7	26.783	4.195
31	3281.02	24.304	0.186	3284.88	3209.66	44.65	0.146
32	3441.12	21.695	0.065	3448.84	3437.26	7.864	0.009
33	3678.37	25.78	2.027	3689.95	3670.66	10.938	0.227

Comment:
RUN/ U.I / BLESSING 19

Date/Time: 12/1/2011 6:44:04 AM
No. of Scans: 45
Resolution: 4 [1/cm]
Apodization: Happ-Genzel
User: adebayot

SHIMADZU



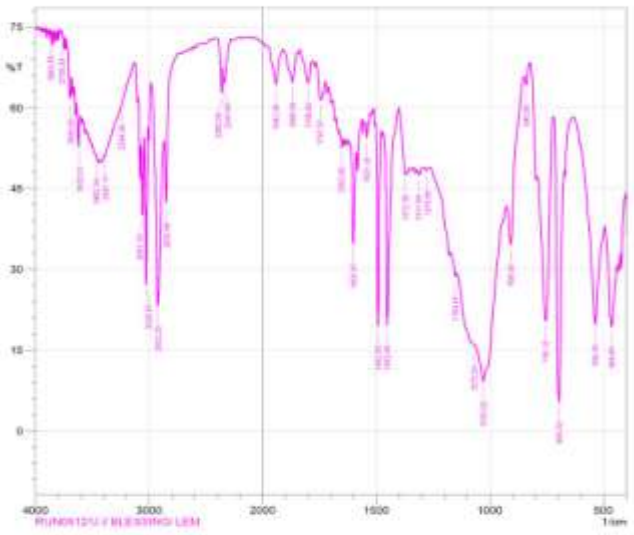
	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	451.36	42.397	9.524	459.07	434	7.882	1.166
2	464.86	42.663	7.489	493.76	461	8.612	0.509
3	538.16	39.767	27.109	594.1	507.3	21.771	6.882
4	621.1	67.619	0.834	624.96	599.88	4.127	0.074
5	669.32	48.647	7.955	677.04	642.32	7.569	0.428
6	696.33	11.281	48.626	723.33	679.97	20.73	11.192
7	756.12	29.793	44	802.41	729.26	22.277	12.333
8	840.99	72.902	5.634	856.42	817.85	4.577	0.485
9	906.57	60.257	14.1	925.86	858.35	9.93	1.587
10	1018.46	29.176	29.789	1058.96	970.23	29.625	9.475
11	1068.6	53.319	3.404	1105.25	1060.88	10.076	0.265
12	1153.47	61.979	6.823	1166.97	1136.11	5.496	0.454
13	1180.47	62.603	6.294	1219.05	1166.97	9.013	0.754
14	1311.64	62.791	1.129	1317.43	1295.7	10.841	0.091
15	1371.43	59.044	6.407	1396.44	1356	8.541	1.015
16	1452.45	26.685	40.693	1473.66	1400.37	18.641	6.968
17	1492.96	26.616	41.509	1506.46	1475.59	9.21	3.947
18	1546.96	69.58	3.043	1558.54	1541.18	2.586	0.216
19	1600.97	44.299	22.808	1616.4	1589.4	6.568	1.917
20	1658.84	69.296	1.382	1662.69	1653.05	1.467	0.046
21	1743.71	69.093	3.55	1763	1734.06	4.336	0.365
22	1803.5	69.026	5.127	1830.51	1780.36	7.314	0.805
23	1872.94	69.225	0.993	1909.59	1869.08	5.868	0.092
24	1940.46	67.172	6.886	1979.03	1917.31	9.218	1.172
25	2337.8	69.912	1.576	2349.38	2316.58	4.919	0.155
26	2850.88	41.175	15.137	2868.24	2795.3	19.212	2.276
27	2922.25	26.074	33.105	2983.98	2870.17	41.369	15.502
28	3026.41	30.948	28.841	3043.77	3009.05	12.246	3.993
29	3061.13	42.885	13.298	3072.71	3045.7	8.175	1.433
30	3443.05	66.769	0.102	3446.91	3441.12	1.013	0.003

Comment:
RUN/ U.I / BLESSING 19

Date/Time: 12/1/2011 6:51:13 AM
No. of Scans: 45
Resolution: 4 [1/cm]
Apodization: Happ-Genzel
User: adebayot

FTIR SPECTRA OF CONTROL AND SOME DEGRADED PUR SAMPLES IN LABORATORY STERILISED SOIL SUPPLEMENTED WITH CASSAVA/ POTATO PEELS

SHIMADZU

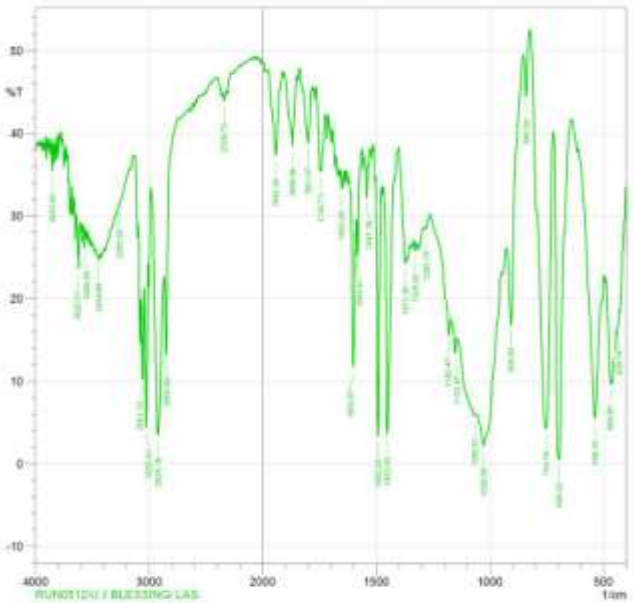


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	464.86	19.311	14.764	493.79	439.78	31.177	5.660
2	538.16	19.719	24.767	613.38	495.72	62.44	12.724
3	696.33	5.295	48.071	717.54	673.18	27.825	15.489
4	756.12	20.45	32.529	790.84	723.33	31.109	12.308
5	840.99	64.353	2.586	850.64	825.56	4.525	0.185
6	908.5	34.612	13.264	927.78	852.96	23.084	2.352
7	1030.02	8.177	13.596	1060.67	929.72	97.071	17.182
8	1070.53	15.847	0.131	1078.24	1058.6	7.661	0.014
9	1163.47	28.762	1.376	1174.89	1147.89	13.765	0.103
10	1278.85	48.362	0.615	1294.28	1267.27	8.439	0.073
11	1311.64	47.479	1.23	1321.28	1294.28	8.551	0.133
12	1373.36	47.458	3.198	1400.37	1365.88	9.896	0.415
13	1462.45	19.833	36.66	1467.88	1402.3	22.923	7.11
14	1492.95	16.537	36.624	1502.6	1477.52	10.217	3.913
15	1541.19	54.472	2.701	1546.96	1536.39	2.93	0.12
16	1600.97	34.788	17.515	1612.54	1589.4	8.306	1.78
17	1653.05	52.563	1.841	1656.84	1649.19	2.563	0.043
18	1747.57	61.502	0.713	1764.93	1745.64	3.617	-0.006
19	1799.65	64.884	4.602	1838.22	1784.21	9.237	0.744
20	1869.08	54.635	5.646	1905.73	1849.8	9.498	0.948
21	1942.38	64.35	6.753	1979.03	1913.45	10.639	1.142
22	2341.68	64.536	2.966	2347.45	2283.79	10.421	0.53
23	2360.95	62.743	5.856	2369.53	2348.38	8.406	0.691
24	2850.88	42.364	14.723	2888.24	2789.16	18.572	2.229
25	2922.25	25.228	35.592	2963.98	2870.17	42.433	16.305
26	3026.41	27.229	28.199	3043.77	3009.06	13.291	4.366
27	3061.13	40.118	13.458	3072.71	3045.7	8.799	1.511
28	3244.58	60.528	0.197	3246.51	3230.01	9.559	0.078
29	3367.11	61.191	0.188	3389.04	3358.18	8.726	0.039
30	3462.34	60.549	0.33	3470.06	3458.48	3.398	0.01
31	3620.81	52.731	3.183	3626.29	3610.86	4.039	0.183
32	3699.59	61.805	0.778	3716.95	3682.23	6.575	0.833
33	3759.39	72.865	0.71	3763.24	3755.53	1.042	0.014
34	3883.55	73.422	0.568	3887.4	3859.69	1.021	0.013

Comment:
RUN0512/U.I/ BLESSING/ LEM

Date/Time: 5/4/2012 2:29:15 AM
No. of Scans: 45
Resolution: 4 [1/cm]
Apodization: Happ-Genzel

SHIMADZU

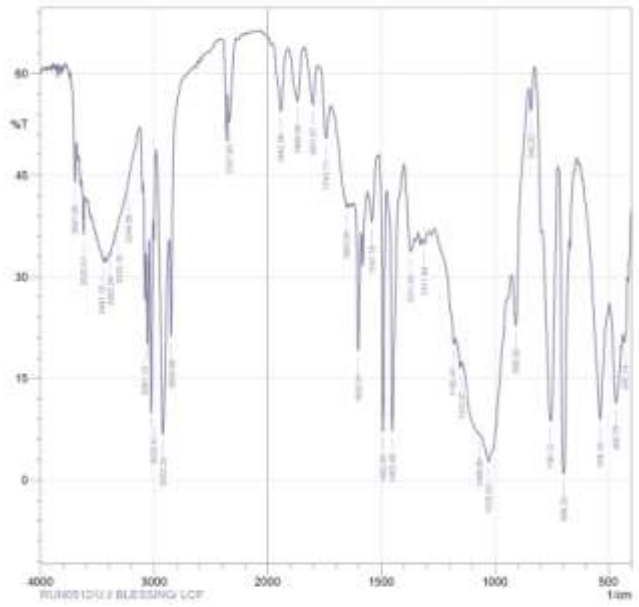


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	439.78	16.502	0.82	441.71	405.06	23.813	0.721
2	464.86	9.718	0.738	486.79	453.29	12.905	0.152
3	538.16	5.545	22.331	613.38	499.58	83.79	23.031
4	696.33	0.483	40.164	721.4	640.39	86.511	35.104
5	754.19	4.275	31.581	792.77	723.33	60.029	28.477
6	840.99	44.462	6.502	854.49	825.56	9.142	0.691
7	908.5	16.842	15.458	927.79	854.49	36.482	4.521
8	1028.09	2.234	8.799	1060.88	949.01	128.934	25.73
9	1066.67	5.662	0.969	1143.83	1062.81	85.783	2.956
10	1153.47	13.44	2.435	1170.83	1145.75	20.452	0.616
11	1180.47	15.583	2.976	1253.42	1172.76	56.969	0.672
12	1280.78	28.522	0.425	1284.63	1265.42	11.314	0.042
13	1329	25.774	1.156	1334.76	1321.28	7.631	0.132
14	1373.36	24.237	2.85	1400.37	1367.56	17.586	0.588
15	1462.45	3.556	30.147	1467.88	1421.58	35.507	13.794
16	1492.95	3.366	30.869	1504.53	1477.52	21.315	8.692
17	1541.19	32.207	5.448	1552.75	1529.6	10.42	0.801
18	1583.61	25.159	6.247	1589.4	1562.39	13.161	0.364
19	1600.97	11.754	19.649	1618.33	1589.4	18.859	4.392
20	1653.05	33.365	1.491	1656.84	1651.12	3.567	0.068
21	1743.71	35.462	0.498	1745.64	1735.99	4.22	0.016
22	1801.57	38.613	7.401	1838.22	1784.21	19.698	1.776
23	1889.08	38.57	8.404	1903.8	1851.72	19.198	2.127
24	1942.38	37.305	8.609	1979.03	1926.95	19.412	2.09
25	2339.73	43.961	0.284	2349.38	2337.8	4.063	0.014
26	2850.88	13.159	12.346	2868.24	2748.65	60.099	4.649
27	2924.18	3.508	24.304	2963.98	2870.17	101.024	37.2
28	3026.41	4.453	16.162	3043.77	3009.06	32.492	10.098
29	3061.13	10.262	10.189	3072.71	3045.7	22.109	3.576
30	3265.09	31.109	0.347	3269.45	3201.94	32.801	0.231
31	3444.98	24.66	0.735	3458.48	3437.26	12.772	0.158
32	3586.5	26.171	2.2	3572.29	3556.85	8.644	0.197
33	3620.51	23.668	3.034	3626.29	3610.86	9.114	0.317
34	3853.9	35.484	3.511	3859.69	3848.12	4.916	0.182

Comment:
RUN0512/U.I/ BLESSING/ LAS

Date/Time: 5/3/2012 12:52:26 AM
No. of Scans: 45
Resolution: 4 [1/cm]
Apodization: Happ-Genzel

SHIMADZU



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	430.14	20.335	1.775	432.07	418.57	8.756	0.62
2	466.79	11.369	10.858	497.65	447.5	39.118	6.761
3	538.18	8.881	21.604	613.38	505.37	89.423	17.04
4	698.25	0.901	39.969	723.33	673.18	44.074	24.302
5	756.12	8.665	32.914	790.84	725.26	46.322	20.041
6	842.92	54.543	4.471	852.56	827.49	6.064	0.405
7	908.5	22.741	13.984	925.86	852.96	30.074	3.105
8	1030.02	2.681	9.97	1064.74	949.01	127.932	27.193
9	1068.6	6.58	0.543	1147.68	1064.74	83.071	2.704
10	1153.47	16.482	1.736	1172.76	1147.68	18.423	0.308
11	1180.47	19.95	1.99	1266.35	1174.69	48.256	0.255
12	1311.64	34.827	1.173	1321.28	1296.21	11.269	0.177
13	1371.43	33.676	6.05	1400.37	1354.07	19.806	1.642
14	1462.45	7.219	36.66	1475.59	1402.3	37.676	11.959
15	1492.95	7.188	38.071	1508.38	1477.52	17.517	6.81
16	1541.19	38.001	6.234	1556.61	1518.03	14.589	0.914
17	1600.97	19.114	19.145	1618.33	1589.4	15.019	3.02
18	1653.05	40.118	1.395	1712.85	1649.19	21.109	0.27
19	1743.71	50.466	7.114	1780.36	1720.58	15.197	1.148
20	1801.57	55.4	6.771	1836.22	1782.29	12.654	1.261
21	1869.08	55.791	7.884	1911.52	1845.94	14.592	1.74
22	1942.38	54.371	9.648	1979.03	1913.45	14.593	1.904
23	2337.8	52.888	4.704	2347.45	2283.79	14.876	0.897
24	2850.88	21.112	17.785	2868.24	2744.8	40.723	4.052
25	2922.25	5.726	34.838	2983.88	2870.17	74.512	31.039
26	3026.41	9.911	27.002	3043.77	3009.06	22.931	7.927
27	3061.13	20.118	14.11	3072.71	3045.7	15.235	2.728
28	3244.38	42.56	0.229	3246.31	3169.15	26.128	0.027
29	3333.1	36.59	0.228	3335.03	3246.31	35.747	0.105
30	3383.26	33.377	0.588	3390.97	3336.96	24.728	0.18
31	3441.12	32.129	0.548	3446.91	3435.34	5.869	0.037
32	3620.51	36.275	6.614	3643.65	3603.15	15.83	1.004
33	3697.66	43.963	8.835	3728.53	3682.23	13.66	1.307

Comment;
RUN0512/U.I/ BLESSING/ LCP

Date/Time; 5/4/2012 3:29:32 AM
No. of Scans; 45
Resolution; 4 [1/cm]
Apodization; Happ-Genzel

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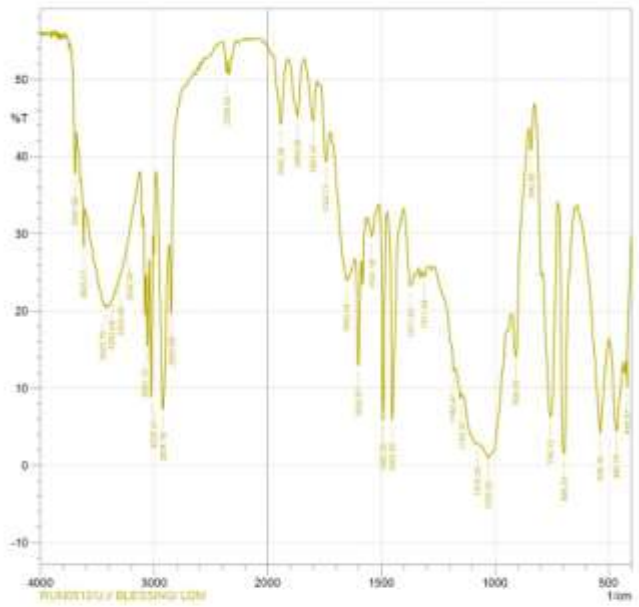


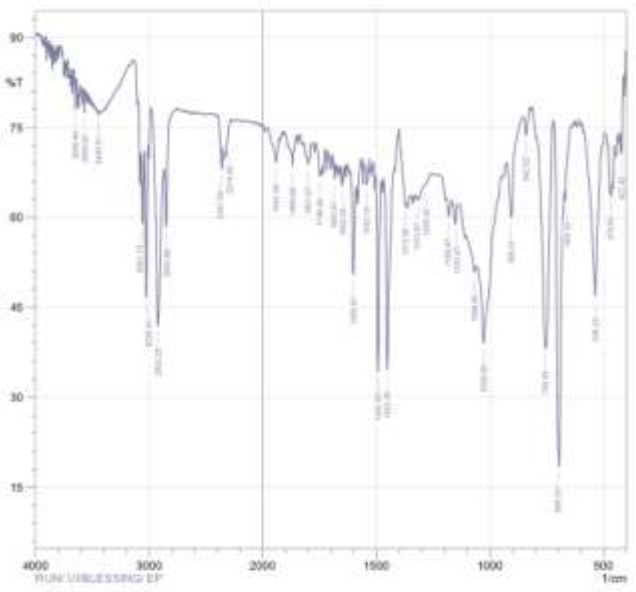
	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	418.57	10.154	7.405	424.35	401.21	16.809	0.745
2	466.79	4.378	9.878	485.72	439.78	59.039	11.521
3	538.18	4.173	16.971	636.53	501.51	107.328	22.376
4	696.33	1.501	27.841	721.4	673.18	47.895	21.988
5	756.12	6.286	23.349	790.84	723.33	57.164	21.147
6	840.99	40.832	4.273	852.56	827.49	9.127	0.48
7	908.5	13.988	11.786	927.79	852.96	43.399	4.111
8	1030.02	0.906	6.167	1064.74	949.01	168.596	34.295
9	1079.24	2.726	0.068	1084.03	1074.39	19.037	0.075
10	1153.47	8.711	1.275	1174.69	1147.68	28.669	0.465
11	1180.47	12.162	1.214	1266.2	1174.69	66.711	-3.706
12	1311.64	24.427	0.957	1321.28	1296.21	15.093	0.3
13	1371.43	23.268	4.657	1400.37	1354.07	27.063	1.822
14	1462.45	5.848	26.988	1473.66	1402.3	46.327	11.923
15	1492.95	5.998	27.483	1512.24	1475.59	24.342	6.868
16	1541.19	29.579	2.565	1554.68	1514.17	20.287	0.558
17	1600.97	12.931	13.613	1614.47	1589.4	17.827	3.189
18	1653.05	23.908	3.716	1724.42	1639.55	43.163	1.969
19	1743.71	39.334	6.319	1768.79	1726.35	15.52	1.252
20	1801.57	44.577	6.152	1840.15	1782.29	18.209	1.386
21	1869.08	45.162	7.53	1913.45	1840.15	22.462	2.064
22	1942.38	44.164	8.764	1980.96	1913.45	20.795	2.119
23	2359.02	50.751	2.165	2391.81	2349.38	11.848	0.341
24	2850.88	19.615	11.981	2868.24	2750.58	49.062	3.379
25	2924.18	7.215	25.803	2983.88	2870.17	83.213	28.186
26	3026.41	8.853	19.326	3043.77	3009.06	26.436	7.359
27	3061.13	15.463	10.011	3072.71	3045.7	18.605	2.617
28	3242.45	27.844	0.173	3244.38	3132.5	54.253	0.013
29	3323.46	23.504	0.102	3325.39	3275.24	30.532	0.13
30	3362.04	21.622	0.094	3363.97	3325.39	24.995	0.073
31	3423.76	20.401	0.099	3435.34	3421.83	9.253	0.007
32	3620.51	28.28	6.426	3645.58	3605.08	19.606	1.184
33	3697.66	37.771	8.918	3728.53	3682.23	16.209	1.519

Comment;
RUN0512/U.I/ BLESSING/ LDM

Date/Time; 5/4/2012 5:10:45 AM
No. of Scans; 45
Resolution; 4 [1/cm]
Apodization; Happ-Genzel

FTIR SPECTRA OF CONTROL AND SOME DEGRADED PUR SAMPLES IN FIELD SOIL SUPPLEMENTED WITH CASSAVA/POTATO PEELS

SHIMADZU

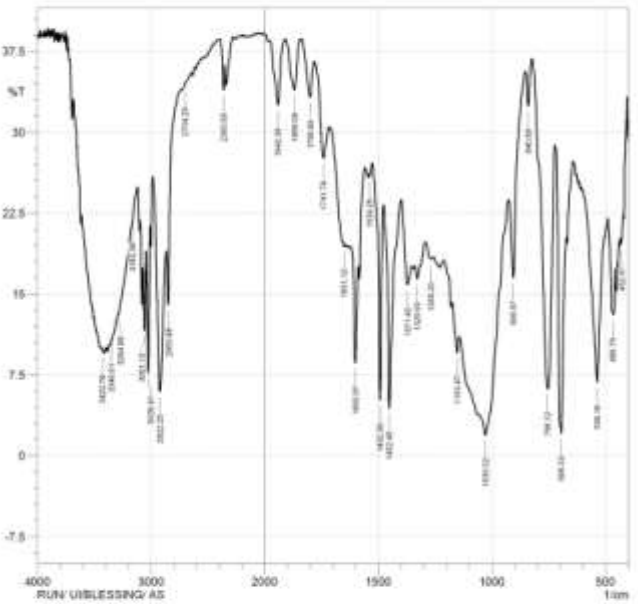


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	422.42	70.556	7.802	430.14	412.78	2.193	0.381
2	470.65	63.627	1.848	482.22	468.72	2.306	0.066
3	536.23	46.956	27.223	586.36	495.72	18.052	6.297
4	669.32	82.92	2.882	675.18	646.17	4.165	0.064
5	696.33	18.463	51.529	721.4	673.18	17.127	9.579
6	758.05	38.116	36.579	794.7	723.33	18.011	8.963
7	842.92	73.671	3.632	852.56	823.63	3.463	0.271
8	906.57	59.941	11.186	925.86	896.42	10.777	1.291
9	1028.09	39.067	16.882	1057.03	990.94	30.029	5.729
10	1068.6	50.868	2.077	1107.18	1058.96	13.008	0.227
11	1153.47	58.86	3.595	1166.97	1136.04	8.173	0.253
12	1180.47	60.209	2.155	1192.05	1174.69	3.647	0.111
13	1290.42	66.208	0.139	1292.35	1263.42	5.211	0.05
14	1313.57	62.81	1.129	1321.28	1292.35	5.801	0.074
15	1373.36	61.674	3.118	1400.37	1365.65	6.204	0.324
16	1452.45	34.673	32.395	1467.88	1421.58	12.182	4.246
17	1492.95	34.369	31.962	1502.6	1479.45	6.707	2.567
18	1543.1	65.411	2.996	1582.75	1535.39	2.999	0.143
19	1600.97	50.498	15.717	1612.54	1589.4	5.374	1.214
20	1653.05	65.188	2.318	1658.84	1651.12	1.347	0.046
21	1683.91	66.361	3.135	1691.63	1678.13	2.237	0.113
22	1748.48	66.792	3.407	1764.93	1737.92	4.368	0.237
23	1801.57	68.821	1.79	1806.43	1782.29	3.518	0.113
24	1869.08	68.695	3.909	1884.52	1851.72	4.818	0.281
25	1942.38	69.122	4.655	1977.1	1926.95	7.157	0.622
26	2314.66	72.293	0.255	2316.58	2281.87	4.407	0.012
27	2357.09	68.015	4.531	2391.81	2349.36	6.132	0.607
28	2850.88	56.405	11.961	2868.24	2798.8	10.807	1.433
29	2922.25	41.928	30.835	2983.98	2870.17	28.559	9.957
30	3026.41	46.671	24.902	3043.77	3009.05	7.733	2.698
31	3061.13	59.013	11.909	3072.71	3045.7	5.006	0.967
32	3446.91	77.152	0.313	3450.77	3444.98	0.844	0.008
33	3566.5	77.46	3.706	3574.21	3556.85	1.589	0.116
34	3648.44	78.001	5.793	3662.94	3639.8	1.971	0.209

Comment;
RUN/ UI/BLESSING/ EP

Date/Time; 4/18/2012 11:00:35 PM
No. of Scans; 45
Resolution; 4 [1/cm]
Apodization; Happ-Genzel

SHIMADZU

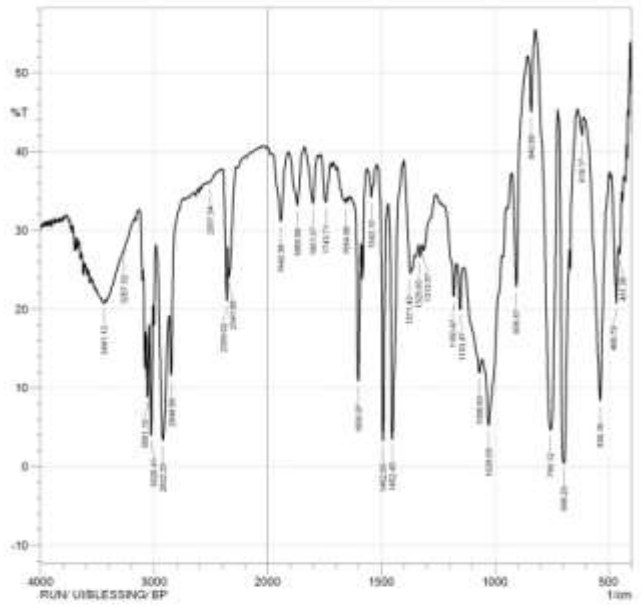


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	432.07	19.635	1.201	435.93	422.42	9.212	0.156
2	466.79	13.065	5.597	491.86	457.14	27.364	2.754
3	538.16	6.826	15.597	590.24	497.65	76.493	16.55
4	696.33	2.062	22.239	721.4	673.18	48.419	18.474
5	756.12	8.209	25.225	823.63	723.33	74.681	26.02
6	840.99	32.428	3.696	852.56	823.63	13.336	0.579
7	906.57	16.496	10.418	925.86	854.49	41.424	3.498
8	1030.02	1.906	6.789	1062.81	927.79	153.059	18.386
9	1153.47	9.567	2.437	1174.69	1143.83	29.389	1.41
10	1269.2	16.208	0.656	1292.35	1257.63	25.309	0.312
11	1329	16.389	1.224	1340.57	1315.5	19.239	0.326
12	1371.43	15.854	4.112	1398.44	1354.07	32.977	2.229
13	1462.45	4.406	16.898	1475.59	1400.37	60.67	13.467
14	1492.95	5.156	19.303	1527.67	1477.52	37.328	7.164
15	1539.25	25.944	0.302	1541.18	1527.67	1.798	0.03
16	1600.97	8.619	9.576	1618.33	1589.4	24.86	3.519
17	1651.12	19.442	0.262	1654.98	1649.19	4.102	0.026
18	1741.79	27.617	4.892	1762.29	1720.56	31.141	1.661
19	1799.65	33.243	3.883	1836.29	1784.21	23.381	1.188
20	1869.08	33.928	4.697	1909.59	1845.94	28.03	1.734
21	1942.38	32.526	6.046	1977.1	1911.52	29.149	2.014
22	2360.95	33.924	2.804	2399.53	2349.36	22.004	0.572
23	2704.29	34.443	0.048	2706.22	2652.21	24.677	0.022
24	2850.88	13.97	7.246	2868.24	2752.51	86.983	2.87
25	2922.25	5.949	16.098	2985.91	2870.17	101.682	25.728
26	3026.41	7.71	12.299	3043.77	3009.05	30.446	6.209
27	3061.13	11.623	6.445	3072.71	3045.7	22.309	2.339
28	3163.36	21.78	0.137	3166.29	3120.93	27.763	0.005
29	3284.88	13.199	0.188	3286.81	3169.15	90.591	0.208
30	3346.61	10.671	0.178	3350.46	3288.74	57.132	0.156
31	3423.76	9.647	0.093	3457.26	3421.83	15.545	0.051

Comment;
RUN/ UI/BLESSING/ AS

Date/Time; 4/19/2012 5:52:22 AM
No. of Scans; 45
Resolution; 4 [1/cm]
Apodization; Happ-Genzel

SHIMADZU

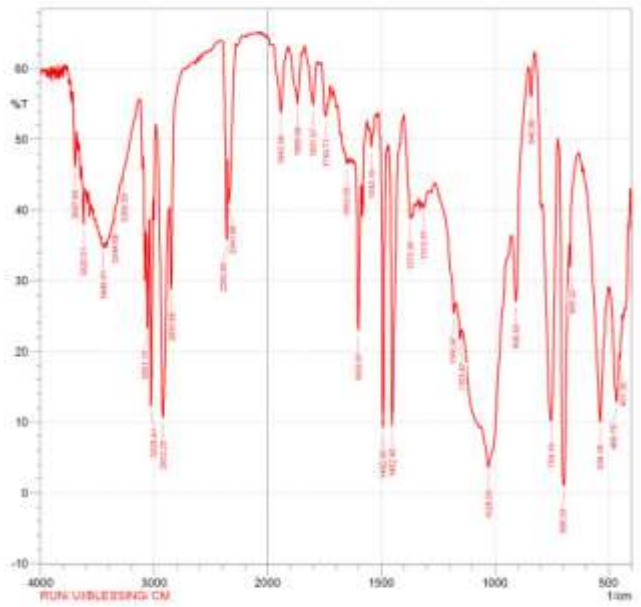


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	451.36	26.580	3.218	455.22	443.64	6.122	0.262
2	466.79	20.736	10.378	488.01	455.22	19.466	2.299
3	538.16	8.372	31.582	607.6	495.72	65.682	21.981
4	619.17	42.109	2.436	630.74	609.53	7.689	0.241
5	668.25	5.403	36.129	721.4	673.18	53.391	31.275
6	756.12	4.657	43.99	821.7	723.33	85.183	35.543
7	840.99	45.040	8.99	856.42	823.63	9.803	0.976
8	906.57	22.892	18.803	927.79	858.42	29.187	4.078
9	1028.09	5.2	12.804	1057.03	970.23	77.779	15.651
10	1068.8	11.875	3.129	1158.04	1058.96	60.642	2.825
11	1153.47	19.964	5.874	1166.9	1138.04	19.389	1.088
12	1180.47	21.834	5.475	1222.91	1170.83	29.435	1.544
13	1313.87	27.485	1.219	1319.35	1259.56	30.325	0.233
14	1529	26.602	1.532	1536.71	1519.35	9.746	0.162
15	1371.43	24.583	6.647	1400.37	1354.07	25.314	2.38
16	1452.45	3.427	31.344	1469.81	1402.3	44.95	15.078
17	1492.95	3.316	32.166	1518.03	1471.74	30.568	9.751
18	1543.1	34.235	3.774	1562.39	1523.82	16.914	0.707
19	1600.97	10.875	19.336	1620.26	1589.4	20.369	4.56
20	1654.98	33.436	0.609	1658.84	1651.12	3.639	0.029
21	1743.71	33.614	5.461	1766.85	1720.56	20.3	1.406
22	1801.57	33.379	6.484	1838.22	1780.36	24.69	1.843
23	1869.08	33.175	7.122	1905.73	1840.15	28.339	2.427
24	1942.38	31.115	8.682	1977.1	1913.45	28.337	2.862
25	2341.66	23.988	4.098	2347.45	2283.79	33.136	1.903
26	2389.02	21.104	9.004	2391.81	2349.38	23.863	3.027
27	2507.54	36.057	0.242	2515.26	2457.39	25.227	0.064
28	2846.66	11.618	10.639	2868.24	2785.3	52.54	4.712
29	2922.25	3.364	19.902	2963.98	2870.17	107.363	55.029
30	3026.41	3.953	19.16	3043.77	3009.05	34.777	9.847
31	3061.13	8.883	8.565	3072.71	3045.7	23.934	3.513
32	3267.52	27.286	0.243	3271.38	3173.01	52.157	0.038
33	3441.12	20.767	0.101	3443.05	3429.55	9.171	0.011

Comment;
RUN/ UI/BLESSING/ BP

Date/Time; 4/19/2012 3:54:47 AM
No. of Scans; 45
Resolution; 4 [1/cm]
Apodization; Happ-Genzel

SHIMADZU



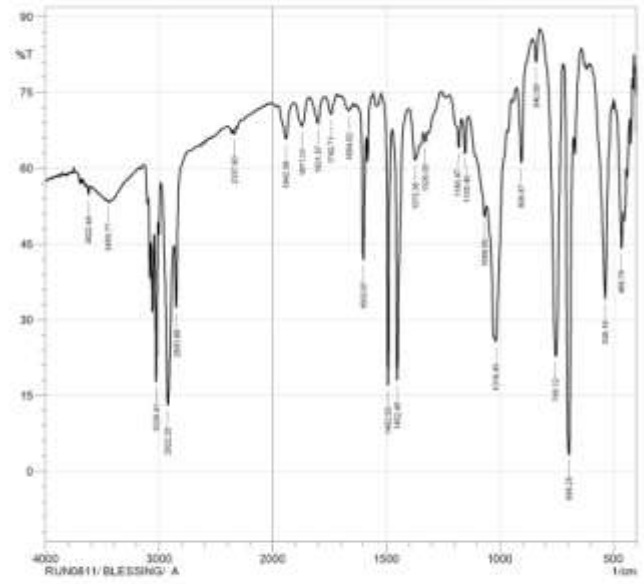
	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	451.36	18.941	1.638	453.29	441.71	7.801	0.249
2	466.79	12.85	9.759	495.72	453.29	30.937	4.36
3	538.16	10.019	23.866	613.38	505.37	63.927	16.169
4	669.32	32.021	4.408	673.18	632.67	14.612	0.15
5	696.33	1.03	41.126	721.4	673.18	42.661	24.462
6	754.19	10.223	35.826	792.77	723.33	43.524	19.731
7	840.99	56.02	5.189	852.56	827.49	5.813	0.48
8	908.5	27.043	15.652	927.79	854.49	26.963	3.247
9	1028.09	3.636	12.936	1062.81	949.01	110.217	24.882
10	1153.47	21.578	2.25	1172.76	1147.68	15.479	0.297
11	1180.47	25.436	2.592	1263.42	1174.69	39.396	0.272
12	1313.87	40.318	1.206	1321.28	1292.35	11.069	0.134
13	1373.36	38.76	3.634	1402.3	1365.65	12.679	0.496
14	1452.45	9.535	39.499	1467.88	1421.58	24.034	9.725
15	1492.95	9.282	40.372	1504.53	1477.52	14.475	6.218
16	1543.1	48.95	3.321	1552.75	1523.82	8.383	0.304
17	1600.97	23.042	21.83	1618.33	1589.4	12.96	2.978
18	1653.05	46.486	0.892	1656.84	1651.12	2.517	0.029
19	1743.71	53.301	3.268	1764.93	1735.99	7.264	0.416
20	1801.57	54.79	6.935	1840.15	1782.29	13.199	1.219
21	1869.08	54.937	7.575	1905.73	1851.72	12.433	1.444
22	1942.38	53.787	9.481	1979.03	1913.45	14.891	1.851
23	2341.66	41.116	6.557	2347.45	2283.79	18.645	1.824
24	2389.95	35.779	15.714	2393.74	2349.38	14.509	2.905
25	2850.88	28.733	14.465	2868.24	2746.73	36.694	2.953
26	2922.25	10.65	35.064	2963.98	2870.17	63.976	25.316
27	3026.41	12.314	27.882	3043.77	3009.05	20.91	7.169
28	3061.13	23.339	14.282	3072.71	3045.7	13.867	2.46
29	3265.59	45.496	0.152	3267.52	3171.06	29.679	0.047
30	3344.68	39.544	0.165	3346.61	3294.53	19.659	0.005
31	3448.91	34.69	0.139	3460.41	3444.98	7.041	0.021
32	3620.51	38.028	5.586	3643.65	3610.86	12.364	0.77
33	3697.66	46.048	7.513	3716.95	3682.23	10.536	1.188

Comment;
RUN/ UI/BLESSING/ CM

Date/Time; 4/19/2012 3:35:08 AM
No. of Scans; 45
Resolution; 4 [1/cm]
Apodization; Happ-Genzel

FTIR SPECTRA OF SOME DEGRADED PUR SAMPLES IN FIELD AFTER ONE YEAR OF SOIL BURIAL (NATURAL BIODEGRADATION)

SHIMADZU



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	466.79	44.179	13.041	489.94	457.14	8.162	1.021
2	538.16	34.192	40.854	601.81	507.3	20.685	9.557
3	698.25	3.305	69.76	721.4	677.04	24.174	18.037
4	756.12	22.753	60.083	825.56	723.33	24.301	16.525
5	840.99	91.547	5.632	856.42	827.49	2.184	0.377
6	906.57	61.141	17.668	927.79	896.42	8.416	1.74
7	1018.45	25.712	5.244	1024.24	970.23	18.183	0.518
8	1068.6	50.361	3.668	1108.11	1060.88	12.036	0.361
9	1155.4	82.96	7.005	1168.9	1138.04	5.241	0.46
10	1180.47	64.12	6.639	1222.91	1168.9	8.474	0.797
11	1329	66.203	1.942	1336.71	1319.35	3.113	0.111
12	1373.36	61.633	8.016	1404.22	1336.71	12.31	1.603
13	1452.45	18.093	50.761	1473.66	1404.22	18.811	7.928
14	1492.95	17.069	53.338	1521.89	1475.59	11.975	5.115
15	1600.97	41.897	26.14	1624.12	1589.4	7.649	2.09
16	1664.62	71.357	0.625	1670.41	1651.12	2.776	0.036
17	1743.71	70.663	3.79	1770.71	1716.7	7.438	0.522
18	1801.57	66.818	5.028	1830.51	1780.36	7.331	0.721
19	1871.01	66.325	5.212	1903.8	1842.08	9.201	0.948
20	1942.38	65.769	6.998	1977.1	1903.8	11.315	1.159
21	2337.8	96.748	1.117	2349.36	2320.44	4.961	0.1
22	2850.88	32.438	15.008	2870.17	2739.01	34.713	2.671
23	2922.25	13.074	37.104	2965.91	2872.1	56.37	22.534
24	3026.41	17.774	30.378	3043.77	3009.05	16.835	5.835
25	3450.77	53.465	0.078	3464.62	3448.84	1.57	0.002
26	3622.44	54.824	1.762	3639.8	3607.01	8.286	0.179

Comment:
RUN0811/ BLESSING/ A

Date/Time: 8/17/2011 1:47:21 PM
No. of Scans: 45
Resolution: 4 [1/cm]
Apodization: Happ-Genzel
User: adebayot

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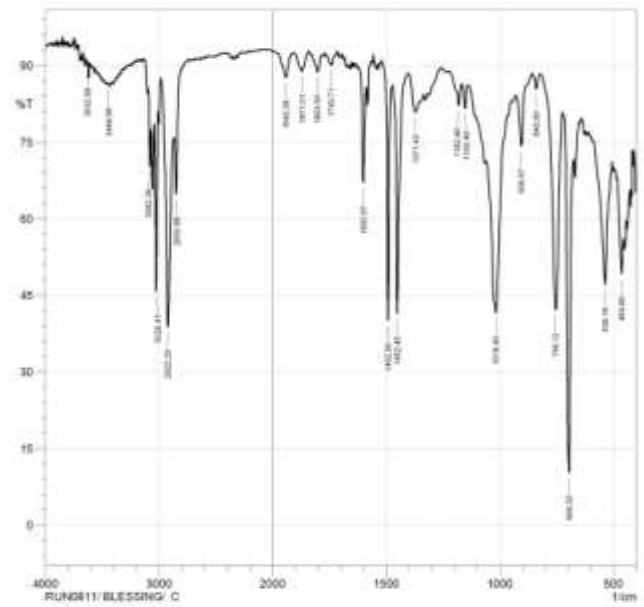
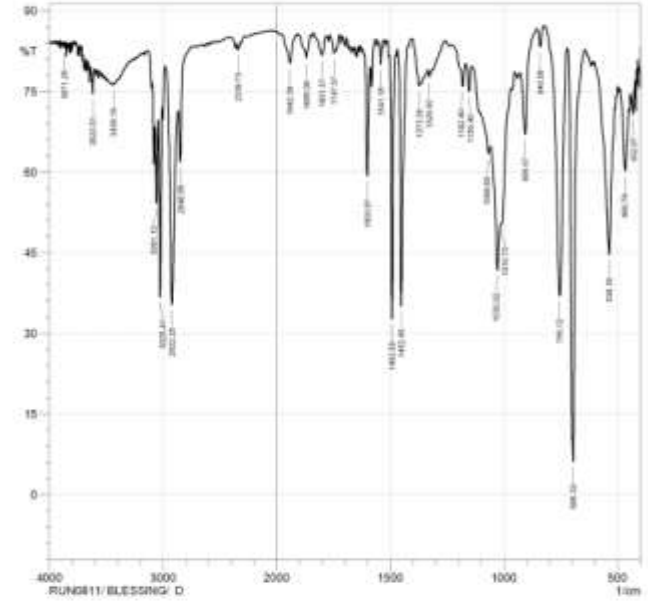


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	464.86	49.23	8.815	484.15	459.07	6.348	0.868
2	538.16	47.065	25.587	586.38	507.3	16.514	5.706
3	696.33	10.261	64.584	723.33	676.97	16.161	10.783
4	756.12	42.169	41.109	817.85	725.26	15.29	8.454
5	840.99	85.441	2.895	856.42	827.49	1.759	0.201
6	906.57	74.212	10.968	931.65	885.36	4.223	0.978
7	1018.45	41.528	33.866	1060.88	970.23	20.001	9.011
8	1155.4	81.618	4.635	1168.9	1141.9	1.973	0.238
9	1182.4	82.204	4.78	1222.91	1168.9	3.557	0.457
10	1371.43	80.941	0.234	1373.36	1354.07	1.677	0.013
11	1452.45	41.489	46.286	1471.74	1404.22	8.392	4.42
12	1492.95	40.195	47.873	1519.96	1473.66	5.405	2.913
13	1600.97	67.078	20.122	1616.4	1589.4	2.747	1.172
14	1743.71	90.125	0.785	1761.07	1737.92	0.974	0.051
15	1803.5	88.76	3.329	1826.58	1784.21	1.944	0.358
16	1871.01	88.848	3.469	1906.73	1847.87	2.471	0.467
17	1942.38	87.667	4.488	1962.89	1921.18	2.724	0.569
18	2850.88	64.665	14.57	2866.24	2800.73	6.808	1.545
19	2922.25	38.863	42.287	2965.91	2870.17	22.709	12.383
20	3026.41	45.778	34.132	3045.7	3009.05	6.672	3.301
21	3082.35	70.357	10.452	3095.85	3072.71	2.649	0.579
22	3444.99	86.262	0.073	3464.62	3443.06	0.733	0.002
23	3632.08	90.132	0.712	3639.8	3628.22	0.504	0.021

Comment:
RUN0811/ BLESSING/ C

Date/Time: 8/17/2011 1:19:10 PM
No. of Scans: 45
Resolution: 4 [1/cm]
Apodization: Happ-Genzel
User: adebayot

SHIMADZU



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	432.07	70.738	4.102	439.76	428.21	1.612	0.139
2	466.79	60.212	13.083	489.94	451.36	6.694	1.546
3	538.16	44.633	32.892	601.81	501.51	18.795	7.952
4	696.33	8.165	76.77	721.4	644.25	21.919	15.62
5	756.12	37.003	47.382	817.88	723.33	17.244	10.558
6	840.99	83.273	3.592	856.42	823.63	2.228	0.225
7	906.57	67.04	14	929.72	896.42	7.506	1.449
8	1010.73	50.301	2.267	1014.59	970.23	8.704	0.224
9	1030.02	41.757	13.608	1058.96	1014.59	12.609	1.723
10	1068.6	53.421	2.384	1107.18	1060.88	7.952	0.167
11	1155.4	75.048	4.813	1170.83	1139.97	3.268	0.26
12	1182.4	75.947	4.93	1228.7	1170.83	5.466	0.449
13	1329	77.746	1.264	1334.78	1317.43	1.638	0.089
14	1373.36	76.004	2.4	1402.3	1365.65	3.644	0.212
15	1462.45	35.102	47.733	1467.88	1423.51	8.281	4.689
16	1492.95	32.733	48.584	1504.53	1477.52	5.566	3.199
17	1541.18	80.046	3.423	1552.75	1537.32	1.274	0.086
18	1600.07	59.274	21.413	1620.26	1589.4	4.183	1.355
19	1747.57	82.106	1.791	1764.93	1737.92	2.128	0.115
20	1801.57	81.691	3.448	1826.65	1784.21	3.343	0.371
21	1869.08	81.234	4.314	1903.8	1849.8	4.193	0.536
22	1942.38	90.196	5.402	1977.1	1913.45	5.024	0.725
23	2339.73	82.785	1.115	2349.38	2326.23	1.845	0.061
24	2848.96	61.861	13.255	2866.24	2818.09	7.406	1.534
25	2922.25	35.341	39.822	2983.88	2870.17	26.42	12.426
26	3026.41	36.802	34.098	3043.77	3009.06	8.99	3.818
27	3061.13	54.23	14.711	3072.71	3045.7	5.568	1.228
28	3436.19	76.183	0.211	3444.98	3433.41	1.358	0.005
29	3620.51	74.564	2.894	3626.29	3606.98	2.427	0.134
30	3871.26	83.193	0.612	3877.05	3867.4	0.758	0.018

Comment:
RUN0811/ BLESSING/ D

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Apodization: Happ-Genzel
User: adebayot

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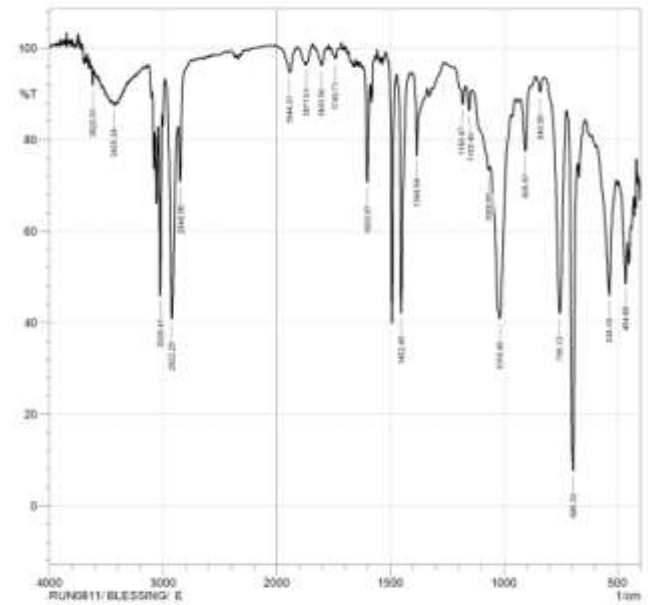

	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	464.86	48.518	12.051	484.15	457.14	6.808	1.243
2	538.16	46.006	27.88	592.17	507.3	16.787	6.063
3	696.33	7.684	71.933	723.33	677.04	16.909	12.466
4	756.12	42.055	46.58	821.7	725.26	13.702	9.198
5	840.99	90.388	3.277	862.21	827.49	1.199	0.218
6	906.57	77.565	12.721	929.72	885.36	3.061	1.077
7	1018.45	40.931	38.436	1060.88	970.23	19.265	10.306
8	1068.6	73.317	2.354	1141.9	1060.88	7.147	0.17
9	1155.4	86.474	4.716	1170.83	1141.9	1.364	0.209
10	1180.47	87.786	4.854	1222.91	1170.83	1.834	0.362
11	1384.94	76.537	16.02	1404.22	1352.14	3.062	1.109
12	1462.45	42.065	52.295	1471.74	1417.73	5.945	4.59
13	1600.07	70.701	22.906	1616.4	1589.4	1.967	1.242
14	1743.71	97.677	0.775	1749.49	1737.92	0.1	0.021
15	1803.5	96.193	3.515	1826.58	1780	0.362	0.329
16	1871.01	96.279	1.064	1907.66	1867.16	0.329	0.086
17	1944.31	94.7	5.037	1982.89	1921.18	0.643	0.58
18	2848.96	70.739	16.266	2866.24	2812.31	4.589	1.471
19	2922.25	40.825	46.719	2983.88	2870.17	18.904	12.442
20	3026.41	45.877	38.2	3043.77	3009.06	6.133	3.531
21	3435.34	87.461	0.299	3454.62	3431.48	1.286	0.009
22	3620.51	91.908	3.654	3626.29	3612.79	0.433	0.129

Comment:
RUN0811/ BLESSING/ E

Date/Time: 8/17/2011 12:57:11 PM
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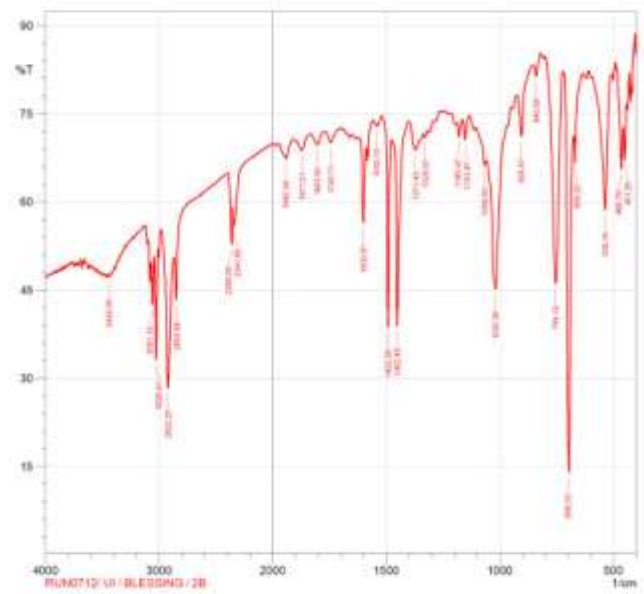
Resolution: 4 [1/cm]
Apodization: Happ-Genzel
User: adebayot

FTIR SPECTRA OF SOME DEGRADED PUR SAMPLES IN FIELD AFTER TWO YEARS OF SOIL BURIAL (NATURAL BIODEGRADATION)

SHIMADZU



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	451.36	67.244	7.122	457.14	443.64	1.969	0.273
2	466.79	65.82	10.462	486.08	457.14	3.918	0.756
3	538.16	58.627	22.929	594.1	509.22	11.611	4.073
4	669.32	66.738	6.237	675.11	640.39	4.044	-0.048
5	696.33	14.015	62.2	717.54	677.04	14.191	9.369
6	756.12	46.104	36.974	800.49	725.26	13.387	7.407
7	840.99	81.352	2.921	858.35	823.63	2.799	0.218
8	906.57	71.152	8.554	927.79	858.35	7.312	0.829
9	1020.38	46.175	24.414	1058.96	972.16	19.348	5.859
10	1066.6	66.166	1.844	1105.25	1060.88	0.998	0.064
11	1153.47	70.638	3.659	1165.04	1134.18	4.212	0.246
12	1180.47	71.064	2.586	1190.12	1165.04	3.468	0.142
13	1329	70.901	0.982	1336.71	1319.35	2.536	0.049
14	1371.43	68.857	3.904	1400.37	1336.71	9.617	0.788
15	1452.45	38.932	32.512	1473.66	1400.37	14.104	3.709
16	1492.95	38.668	33.46	1521.89	1475.59	8.687	2.251
17	1543.1	72.834	0.456	1558.54	1539.25	2.601	0.027
18	1600.97	56.449	13.397	1618.33	1589.4	5.409	0.93
19	1743.71	70.063	2.066	1772.64	1718.7	8.254	0.315
20	1803.6	69.693	2.053	1834.36	1780.36	8.133	0.339
21	1871.01	68.83	2.238	1909.59	1844.01	10.157	0.408
22	1942.38	67.35	2.956	1975.17	1911.52	10.28	0.585
23	2341.66	55.825	3.208	2347.45	2281.87	14.097	0.612
24	2360.95	52.846	7.815	2389.88	2349.38	9.58	1.128
25	2850.88	43.347	9.124	2868.24	2796.88	19.949	1.365
26	2922.25	28.372	24.336	2963.98	2870.17	41.755	10.205
27	3026.41	33.176	18.123	3043.77	3006.05	12.606	2.551
28	3061.13	42.59	7.554	3074.63	3043.77	10.095	0.828
29	3443.05	47.208	0.314	3446.91	3435.34	3.756	0.021

Comment;
RUN0712/ UI / BLESSING / 2B

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Apodization; Happ-Genzel

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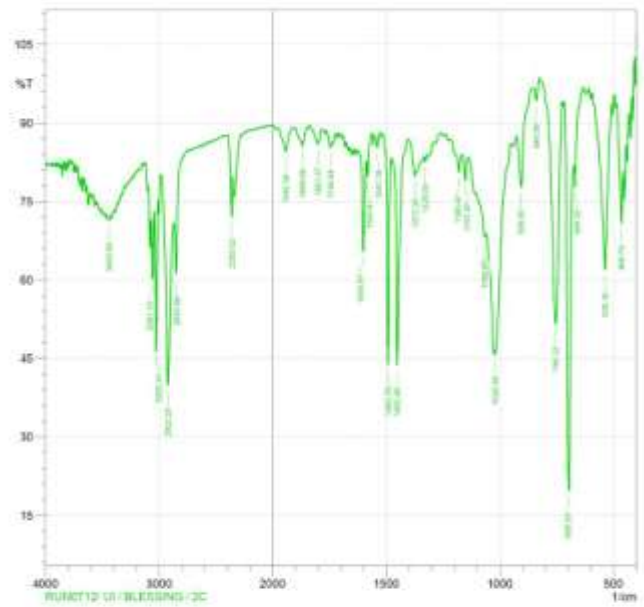

	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	466.79	70.856	13.355	486.01	459.07	2.624	0.876
2	538.16	61.929	31.736	588.31	507.3	7.699	5.404
3	669.32	77.807	6.172	675.11	634.6	1.822	-0.224
4	696.33	19.734	68.174	717.54	675.11	11.809	9.131
5	756.12	51.582	43.628	825.56	727.19	9.967	8.303
6	840.99	94.233	3.297	852.56	827.49	0.444	0.179
7	908.5	77.723	12.334	927.79	860.28	3.666	1.19
8	1020.38	46.024	1.385	1022.31	952.87	11.22	0.117
9	1066.6	68.164	1.652	1109.11	1062.81	6.335	-0.097
10	1153.47	79.131	4.563	1165.04	1138.04	2.332	0.239
11	1180.47	80.65	4.068	1220.98	1165.04	4.207	0.361
12	1329	82.639	1.089	1334.78	1317.43	1.377	0.044
13	1373.36	79.91	2.229	1402.3	1365.68	2.951	0.142
14	1452.45	43.797	39.786	1469.81	1406.15	8.802	3.967
15	1492.95	43.93	40.275	1504.53	1477.52	4.383	2.351
16	1541.18	85.413	2.432	1552.75	1529.6	1.432	0.129
17	1583.81	79.696	4.126	1589.4	1562.38	1.961	0.089
18	1600.97	65.454	17.939	1620.26	1589.4	5.487	1.09
19	1745.64	85.396	1.728	1764.93	1735.99	1.799	0.117
20	1801.67	85.886	2.859	1826.65	1786.14	2.395	0.294
21	1869.08	85.532	3.626	1906.73	1847.87	3.335	0.447
22	1942.38	84.554	4.508	1975.17	1913.45	3.706	0.599
23	2359.02	72.187	9.378	2391.81	2349.38	4.282	0.984
24	2850.88	61.189	13.638	2872.1	2808.45	8.656	1.47
25	2922.25	39.89	35.131	2963.98	2872.1	24.783	10.985
26	3026.41	46.402	27.72	3045.7	3009.05	7.584	2.805
27	3061.13	60.172	12.077	3074.63	3045.7	5.005	0.929
28	3443.05	71.491	0.365	3446.91	3435.34	1.671	0.017

Comment;
RUN0712/ UI / BLESSING / 2C

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Apodization; Happ-Genzel

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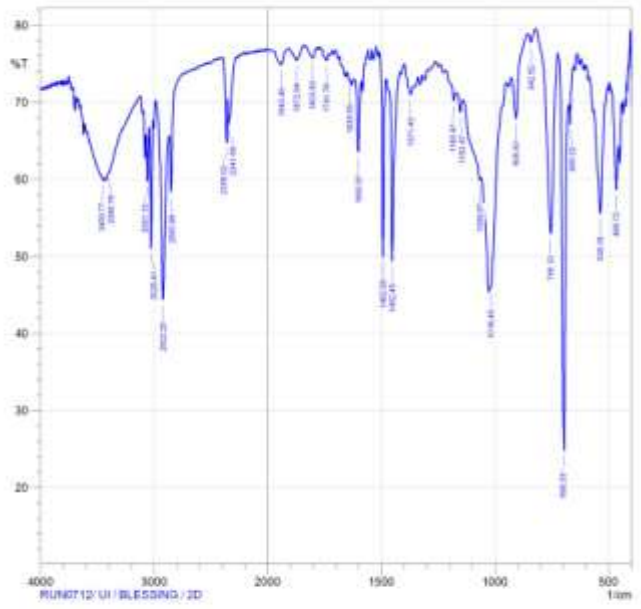

	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	468.72	58.65	8.293	480.94	457.14	6.137	0.532
2	538.16	58.57	13.793	585.18	516.87	9.578	2.212
3	669.32	67.141	4.021	675.11	655.82	2.851	0.111
4	696.33	24.823	47.559	721.4	677.04	12.277	6.106
5	756.12	52.969	24.492	817.85	723.33	15.594	5.336
6	842.92	77.77	1.285	852.56	825.56	2.841	0.097
7	908.5	67.875	6.909	927.79	860.28	6.812	0.775
8	1018.45	46.849	0.909	1020.38	970.23	11.583	0.059
9	1066.67	59.894	0.831	1138.04	1062.81	14.525	0.419
10	1153.47	68.726	1.957	1172.76	1139.97	5.04	0.117
11	1180.47	70.228	1.827	1215.19	1172.76	6.028	0.144
12	1371.43	70.958	2.114	1400.37	1356	6.225	0.292
13	1462.45	49.426	22.77	1466.95	1419.66	8.528	2.126
14	1492.95	49.932	24.705	1508.38	1479.45	5.1	1.44
15	1600.97	63.54	9.241	1616.33	1589.4	4.602	0.616
16	1639.55	72.687	0.419	1647.26	1635.69	1.584	0.02
17	1741.78	75.386	0.711	1751.42	1734.06	2.107	0.047
18	1803.5	75.719	1.475	1830.51	1782.29	5.637	0.219
19	1872.94	75.436	0.51	1890.3	1867.16	2.786	0.039
20	1940.45	74.839	1.967	1975.17	1917.31	6.962	0.324
21	2341.66	67.206	2.988	2347.45	2293.79	9.227	0.454
22	2359.02	64.764	6.64	2397.6	2349.38	7.365	0.79
23	2850.88	58.402	8.956	2868.24	2796.88	12.271	1.036
24	2922.25	44.383	23.343	2965.91	2870.17	26.694	7.221
25	3026.41	51.041	16.178	3043.77	3009.05	7.51	1.827
26	3061.13	59.774	6.438	3074.63	3045.7	5.998	0.523
27	3396.78	60.94	0.115	3398.69	3317.67	16.313	0.09
28	3460.77	59.957	0.162	3486.55	3446.91	2.138	0.007

Comment;
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Apodization; Happ-Genzel

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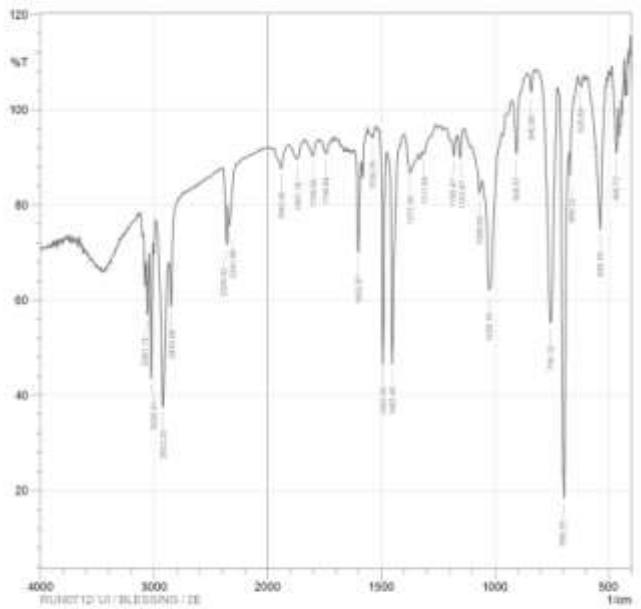

	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	468.72	90.786	11.536	480.29	459.07	0.333	0.561
2	538.16	74.805	23.223	557.48	515.01	2.44	2.143
3	626.89	105.189	0.561	636.53	624.96	-0.256	0.01
4	669.32	86.251	6.974	673.18	651.96	0.352	0.045
5	696.33	18.401	78.405	723.33	675.11	10.412	9.802
6	756.12	55.182	50.711	810.13	725.26	5.189	6.444
7	840.99	103.612	4.442	856.35	825.56	-0.865	0.215
8	906.57	90.768	12.878	927.79	858.35	-0.45	0.942
9	1026.16	82.066	2.93	1058.96	1022.31	4.53	-0.48
10	1068.8	82.456	4.015	1107.18	1058.96	2.707	0.168
11	1153.47	89.856	5.412	1165.04	1136.11	0.858	0.265
12	1180.47	90.312	3.705	1190.12	1165.04	0.807	0.149
13	1311.64	90.829	1.078	1319.35	1284.63	1.186	0.092
14	1373.36	86.563	6.426	1400.37	1336.71	3.03	0.948
15	1462.45	46.637	45.57	1473.66	1400.37	6.567	4.266
16	1492.95	48.567	47.068	1508.38	1473.66	5.661	2.663
17	1539.25	94.022	0.815	1541.18	1521.89	0.396	0.02
18	1600.97	70.044	20.017	1616.33	1589.4	2.401	1.12
19	1745.64	90.744	2.669	1763	1724.42	1.391	0.248
20	1799.65	90.26	3.275	1832.44	1782.29	1.825	0.379
21	1867.16	89.648	3.512	1911.52	1845.94	2.55	0.49
22	1940.45	87.732	4.694	1977.1	1911.52	2.899	0.616
23	2341.66	75.471	4.468	2347.45	2281.87	5.613	0.534
24	2359.02	71.676	9.89	2389.88	2349.38	4.281	1.064
25	2850.88	58.609	13.427	2872.1	2810.38	9.864	1.535
26	2922.25	37.486	34.255	2963.98	2874.03	26.839	11.203
27	3026.41	45.539	27.147	3043.77	3010.98	7.808	2.866
28	3061.13	57.001	11.722	3072.71	3045.7	6.319	0.86

Comment;
RUN0712/ UI / BLESSING / 2E

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Apodization; Happ-Genzel

APPENDIX TABLE A1

Appendix Table A1: The completely randomized block design used for the field (pilot) experimental set-up.

ROWS ▼	DIFFERENT COMBINATIONS OF BACTERIA ISOLATES, PUR SAMPLES AND CASSAVA/POTATO PEELS FOR STIMULATIONS							
1	AFc	BFs	CFp	EFs	DFc	CHp	Blank+F	AHp
2	BHp	DHc	EFp	CFs	AHc	EHs	DFp	CHs
3	DFs	CHc	AFp	EHc	BFc	Blank +H	BHs	EFc
4	AHs	DHp	BHc	AFs	DHs	EHp	CFc	BFp

Key:

A= Combination A (*Pseudomonas alcaligenes* E_4 + *Providencia pseudomallei* $D_2$5)

B= Combination B (*Enterobacter amnigenus* D_2 + *Vibrio* sp. $C_3$2)

C= Combination C (*Pseudomonas aeruginosa* $E_3$2 + *Providencia pseudomallei* $D_2$1)

D= Consortium (Combination A + Combination B + Combination C)

Blank= PUR samples alone

E= Control

F= Cut PUR samples

H= Pulverized PUR samples

$_P$= Stimulation with Potato peels

$_C$= Stimulation with Cassava peels

$_S$=PUR samples i.e. no stimulation