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Biodegradation of polyurethane by bacterial consortium

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ABSTRACT

The present study deals with the isolation of polyurethane (PU) degrading microorganisms from the soil, analysis of biodegradation and optimization of various factors (temperature, pH and by using different co-metabolites). Soil sample was collected from dumping area, Sector H/8, Islamabad, Pakistan and was used as a source for isolation of polyurethane degrading bacteria. Sterilized polyurethane films were buried in soil for three months. The PU pieces were taken from the soil, washed with sterilized distilled water and enriched in liquid MSM for a period of one month. Six different bacterial strains were isolated through enrichment technique, which were identified on the basis of standard morphological and biochemical tests. The study yields that the bacteria with the ability to degrade polyurethane were isolated from soil. It is therefore concluded that soil contains the potential candidates for bioremediation of plastic wastes.

Introduction

Polyurethane is a synthetic polymer that is formed by condensation polymerization reaction between polyisocyanate and polyol having intramolecular urethane bonds. Urethanes are derivatives of carbamic acids which exist only in the form of their esters.

Including polyol and polyisocyanate other raw materials used in the synthesis of polyurethane are catalysts and auxiliary materials. Auxilliary materials include chain extension agents (e.g short-chain diols), crosslinking agents (e.g short chain polyol with three or more hydroxyl groups) that react with isocynate groups, addition agents for the PU manufacture process and improvement agents (e.g silicone compounds used as antifoams and aromatic esters as flexibilizers).

Polyol, can either be of ester or ether type. The PU polymer that contains an ester type polyol is known as polyester PU, whereas the PU polymer that contains an ether type polyol is known as polyether PU. Similarly, polyisocyanate can also be either aliphatic or aromatic type (Gautam et al., 2007). Among these, tolylene diisocyanate and diphenylmethane diisocynate are the most commonly used. In all types of PU polymers, the polymer backbone mainly contains urethane bonds.

Applications of Polyurethane

PU is used in a variety of industrial applications, including insulating foams, fibers, and synthetic leather and rubber goods. Auto manufacturers are replacing latex rubber in car seats and interior padding with PU foam because of lower density and greater flexibility (Ulrich, 1983). Polymer is widely used in liquid coating and paints, adhesives, sealants, flexible and rigid foams, and elastomers (Center for polyurethanes industry 2007). Polyurethane can be used in furniture, constructional materials, and synthetic skins (Howard, 2002). PU has increased tensile strength and melting points making them more durable (Bayer, 1947). Rigid PU foam is used as water heaters, insulation for buildings, commercial and residential refrigeration, for floatation and for energy management. Flexible PU foam is irreplaceable in the industry of furniture, bedding, carpet, packaging and machinery. Thermoplastic PU is highly elastic, flexible and resistant to many environmental factors, that is why it has found its applications in footwear production, wire and cable coatings, architectural glass lamination, auto-body side molding, medical tubing and biomedical apparatus etc. (Szycher, 1999).

Hazards of Polyurethane

Health effects of isocyanate exposure include irritation of skin and mucous membranes, chest tightness, and difficult breathing. Isocyanates include compounds classified as potential human carcinogens and known to cause cancer in animals. The main effects of hazardous exposures are occupational asthma and other lung problems, as well as irritation of the eyes, nose, throat, and skin.

Objectives

The objectives of the study are:

1. To isolate and identify polyurethane degrading bacteria from soil.
2. To check the activity of enzymes (esterase's and lipases) involved in degradation of polyurethane.
3. To study the effect of pH, temperature and presence of co-metabolites on the biodegradation of PU by bacterial consortium.

Literature Review

Biodegradation of polyurethane

Different samples of polyurethane have different degradation patterns due to many properties of polyurethane such as molecular orientation, crystallinity, cross-linking, and chemical groups presented in the molecular chains which determine the accessibility to degrading enzyme systems (Pathirana and Seal, 1983). The microbial degradation process of PU can roughly be divided into the degradation of urethane bonds and into the degradation of polyol segments, which are the special constituents of PU, and degradability is mainly influenced by the chemical structure of the polyol segment (polyester type or polyether type) (Nakajima-Kambe *et al.*, 1999). PU with long repeating units and hydrolytic groups would be less likely to pack into high crystalline regions as normal polyurethanes, and these polymers were more accessible

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to biodegradation. Attack on PU could be through enzymatic action of hydrolases such as ureases, proteases, and esterases (Evans and Levisohn, 1968; Hole, 1972; Fillip, 1978; Griffin, 1980).

The urethane bond in PU has been reported to be susceptible to microbial attack. The hydrolysis of ester bonds in PU is postulated to be the mechanism of PU biodegradation. The breakdown products of the biodegradation are derived from polyester segment in PU when ester bonds are hydrolyzed and cleaved (Nakajima-Kambe *et al.*, 1999). Three types of PU degradations have been identified in literature: fungal biodegradation, bacterial biodegradation and degradation by polyurethanase enzymes (Howard, 2002). Darby and Kaplan (1968) were the first to report degradation of PU by fungi and their results indicated that polyester-type PU were more degradable than polyether-type PU.

Polyester PU are more susceptible to fungal attack than other forms, whereas polyether type is too highly resistant to microbial degradation (Darby and Kaplan, 1968; Kaplan et al.,1968; Ossefort and Testroet, 1966). Pathirana et al., (1984a, b, 1985a, b) isolated certain polyester degrading fungi (Gliocladium roseum, Chaetomium globosum, Penicilium citrinum, Aspergillus fumigatus) (Pathirana and Seal 1984a, b, 1985a, b). Four species of fungi, Curvularia senegalensis, Fusarium solani, Aureobasidium pullulans and Cladosporium sp. were isolated on their ability to degrade colloidal polyester PU (impranil DLNTM) as the sole carbon and energy source (Crabbe et al., 1994).

Although there are few reports on PU degrading bacteria, both gram positive and gram negative bacteria have the ability to degrade PU (Nakajima-Kambe et al., 1999). The bacterial isolates that can degrade PU includes Comamonas acidovorans, Pseudomonas flourescens, P. chlororaphis, and Bacillus subtilis (Nakajima-Kambe et al., 1995, 1997; Howard and Blake, 1998; Ruiz et al., 1999; Howard et al.,1999, 2001b; Rowe and Howard, 2002). Bacillus pumilus strain NMSN-Id isolated from polyurethane contaminated water can grow in high salt concentration (NaCl 10%, w/v) and degrade Impranil-DLN, water-dispersible polyurethane. Only Comamonas acidovorans has the ability to utilize solid PU as a carbon source (Nakajima-Kambe et al., 1995).

Cervantes et al. (2007) isolated two bacterial strains from decomposed soft foam. These strains were grown in a minimal medium supplemented with commercial surface-coating polyurethane as the carbon source. Both strains were identified by comparative 16S rRNA gene sequence analysis as Alicycliphilus sp. Nuclear magnetic resonance, fourier transform infrared (FTIR) spectroscopy, and gas chromatography-mass spectrometry analysis of PU showed that it was a polyester type which also contained N-methylpyrrolidone (NMP) as an additive. Alicycliphilus sp. utilizes NMP during the first stage of growth used it as the sole carbon and nitrogen source. Esterase, protease and urease activities related to PU degradation were tested by differential media and activity assays. Induction of esterase activity in the inoculated media, but not that of protease or urease activities, was observed at 12 h of culture. The ability of Alicycliphilus sp. to degrade PU was confirmed by changes in the PU IR spectrum and by the many holes produced in solid PU observed by scanning electron microscopy after bacterial culture. Changes in PU IR spectra show that in PU degradation esterase activity is involved.

Shah et al. (2008) isolate bacteria from soil with the ability to degrade plastic polyurethane (PU). Bacterial strains attached on the polyurethane film, after soil burial for 6 months, were isolated and identified as Bacillus sp. AF8, Pseudomonas sp AF9, Micrococcus sp. 10, Arthrobacter sp. AF11, and Corynebacterium sp. AF12. In plate assay, zones of hydrolysis were visualized around the bacterial colonies on mineral salt agar plates containing polyurethane as a sole carbon source. The results of the Sturm test for degradability showed more CO2 production in the test than in control. The production of esterase was detected in the presence of polyurethane as a substrate. The Scanning Electron Microscopy and FTIR spectroscopy showed certain changes on the surface of PU film and formation of some new intermediate products after polymer breakdown.

Materials and Methods

Sample Collection

The present research was conducted to isolate the polyurethane degrading bacterial isolates from soil and production of degradative enzymes (lipases and esterases). For this purpose soil sample was taken from dumping area in Sector H/8, Islamabad. Soil sample was collected in sterilized polythene bags. The temperature and pH of the soil was observed. The large particles (pebbles, plastic pieces and stones) were removed. Soil samples were placed in a dark room in pots.

Material

Polyurethane, {Poly [4, 4'-methylenebis (phenyl isocyanate)-alt-1, 4-butanediol/poly (butylene adipate)]} was obtained from Aldrich Chemical Company, Inc. USA. Tetrahydrofuran was obtained from Panreac Quimica, SA.

Preparation of Polyurethane Film

One gram of polyurethane (pellets) was added to 100 ml tetrahydrofuran in 250 ml flask. The flask containing PU solution was sonicated for half an hour and then solution was poured into four Petri plates and allowed to dry for 48 hours in a desicator at room temperature. Dried PU films were carefully removed from Petri plates and stored at room temperature.

Isolation of Bacterial Strains

Two steps were followed for the isolation of PU degrading bacteria.

1: Soil burial

2: Enrichment technique

Soil Burial

Five polyurethane films of the same thickness were buried (6 inches deep) vertically in the pots containing soil and placed in dark at room temperature. At the time of burial 200 ml of mineral salt medium containing glucose was added to the pot, so as to meet the nutrient requirements of the microorganisms. One film was dug out after 3 months of burial, washed with sterilized distilled water and divided into three pieces.

Enrichment in Liquid Medium

Third piece was transferred to 250 ml flask containing nutrient broth placed in a shaker at 150 rpm and 37ºC for two days. Then PU film pieces were shifted to liquid MSM and incubated at 37oC at 150 rpm. Subculturing was done every week up to one month. In each transfer 2 ml of culture was added to the fresh medium. To get the pure colonies and count the total number of viable cells, colony forming units (CFU/ml) were calculated. Ten test tubes were taken for serial dilution. About 9 ml of distill water was sterilized in each of these ten test tubes. One ml of sample was serially diluted ten folds. 0.1 ml from selected dilutions was dispensed on nutrient agar plates. On fresh plates colonies and culture were observed separately.

Six types of bacteria were isolated, which were identified on the basis of standard morphological and biochemical tests on the basis of Bergey's Manual of Determinative Bacteriology.

Composition of Mineral Salt Medium:

Contents	Amount (g/l)
K_2HPO_4	0.5
KH_2PO_4	0.04
NaCl	0.1
$CaCl_2.2H_2O$	0.002
$(NH_4)_2SO_4$	0.2
$MgSO_4.7H_2O$	0.02
$FeSO_4$	0.001

The pH was adjusted at 7.0.

Biodegradation on Solid Medium

After 3 months of soil burial, formation of bio-film on the surface of PU piece was checked by observing growth of microorganisms. PU piece was washed with sterilized distilled water, placed on LB agar plate and incubated at 37ºC for 3 months.

Preparation of Inoculums

Each of 6 isolated bacterial strains were inoculated (2 to 3 loops) in separate test tubes, each containing 5 ml of sterilized nutrient broth. These test tubes were placed in a shaker at 150 rpm at 37ºC. After 24 hours of incubation the 6 cultures were shift to a 100 ml flask and were mixed to form a bacterial consortium. This bacterial consortium was used as inoculums for degradation studies.

Optimization of Biodegradation of Polyurethane Film by Bacterial Consortium

Effect of pH, temperature, and co-metabolites on biodegradation of polyurethane by bacterial consortium was studied by inoculating the bacterial consortium in liquid MSM containing polyurethane film pieces as a sole carbon source. The samples were taken after every one week and were analyzed for the any physical or chemical change. Analysis was done by measuring CFU/ml, hydrolytic enzyme (lipases and esterase) analysis, surface topology by SEM and chemical change analysis by FTIR.

Effect of pH on Biodegradation of Polyurethane

Polyurethane film pieces were sterilized by exposing them to ultraviolet light for 2 minutes. Pieces (2-3) of PU were transferred to 5 flasks (250ml), each containing 150ml of MSM pH having 5, 6, 7, 8, and 9. The flasks were inoculated with 5% inoculum. All 5 flasks were incubated in a shaker at 150 rpm at 37ºC for a period of one month. From each flask 5 ml of sample was collected at zero time and after every one week for up to one month to check the bacterial growth (CFU/ml), and enzyme activity.

Effect of Temperature on Biodegradation of Polyurethane

Sterilized pieces of PU film were transferred to 3 flasks (250ml), each containing MSM (pH 7). These 3 flasks were inoculated (5% of inoculum) and were incubated at 30, 37, and 45ºC for one month at 150 rpm. About 5ml of each sample was taken at zero time and after every one week for up to one month to check the bacterial growth (CFU/ml), and enzyme activity.

Effect of Co-metabolites on Biodegradation of Polyurethane

Co-metabolites such as glucose, yeast extract, Tween 20 and Tween 80 were added to 250 ml flasks separately. Each flask contained 150 ml MSM (pH 7) and sterilized pieces of PU were added as substrate in each flask. All the flasks were inoculated with 5% inoculum and incubated at 37ºC at 150 rpm for a period of one month. About 5ml of each sample were taken at zero time and after every one week for up to one month to check the bacterial growth in the form of CFU/ml, and activity of enzymes (lipases and esterases).

Biodegradation Analysis

Analysis of biodegradation of polyurethane film by bacterial consortium was done by observing the increase in growth, enzyme assay.

Viable Cell Count (CFU/ml)

For bacterial growth determination (CFU/ml), viable cell count was performed. Ten test tubes were taken for serial dilution. About 9 ml of distill water was sterilized in each of these ten test tubes. One ml of sample was serially diluted ten folds. A total of 0.1 ml of each dilution was used to spread on nutrient agar plates. Glass spreader dipped in alcohol, flamed, and cooled, was used each time for spreading the sample on nutrient agar plates. These plates were incubated at 37ºC for 24 hours and then colonies were counted by using colony counter. Viable cells per ml were calculated by following formula:

$$\text{CFU/ml} = \frac{\text{number of colonies} \times \text{dilution factor}}{\text{Volume of inoculum}}$$

Hydrolytic Enzyme Assays

The samples were centrifuged and the cell free supernatant was used to detect the activity of the hydrolytic enzymes i.e. Lipases and Esterases.

Lipase assay

The method of Lesuisse *et al*., (1993) was used for the determination of lipase activity. Chromogenic substrate, *p*-nitrophenyl laurate was used as a substrate and the amount of *p*-nitrophenol produced as a product was used as lipase activity indication (U/ml).

Reagents:

- *p*-nitrophenyl laurate solution
  8.0 mM solubilized in isopropanol
- Reaction buffer
  0.1M potassium phosphate buffer pH 7.0
  0.1% gum Arabic
  0.2% sodium deoxycholate
- 3.0M HCl
- 2.0M NaOH

Procedure

About 880 µl reaction buffer was taken in a small tube, added 20µl crude enzyme extract and incubated for three minutes at 37°C in an incubator. The reaction was initiated by adding 100 µl of 8 mM substrate and stopped by adding 0.5 ml of 3 M HCl. The suspension formed was centrifuged for 10 minutes at 10,000 rpm and then 333 µl of the supernatant was taken in a separate tube; added 1 ml of 2 M NaOH and absorbance was noted at 420 nm. Standard curve was prepared with known concentration of *p*-nitrophenol.

Unit of Activity

The unit of activity is defined as the amount of enzyme that hydrolyzes 1 µmol substrate in 1 minute.

Esterase Assay:

The method of Lesuisse et al., (1993) was used for the determination of esterase activity. Chromogenic substrate, p-nitrophenyl acetate was used as a substrate and the amount of p-nitrophenol produced as a product is used as esterase activity indication (U/ml)

Reagents:

- p-nitrophenyl acetate solution
  8.0 mM solubilized in isopropanol
- Reaction buffer

0.1M potassium phosphate buffer pH 7.0
0.1% gum Arabic
0.2% sodium deoxycholate
_ 3.0M HCl
_ 2.0M NaOH

Procedure:

About 880 µl reaction buffer was taken in a small tube, added 20 µl crude enzyme extract and incubated for three minute at 37ºC. The reaction was initiated by adding 100 µl of 8 mM substrate and stopped by adding 0.5 ml of 3M HCl. The suspension formed was centrifuged for ten min at 10,000 rpm and then 333 µl supernatant was taken in a separate tube; added 1 ml of 2M NaOH and absorbance was noted at 420 nm wavelength. Standard curve was prepared with known concentrations of p-nitrophenol.

Unit of Activity

The unit of activity is defined as the amount of enzyme that hydrolyzes 1 µM substrate in 1 minute.

Results

Isolation and Identification of Polyurethane Degrading Bacterial Strains

Soil sample was collected from dumping area, Sector H/8, Islamabad, and was used as a source for isolation of polyurethane degrading bacteria.

Sterilized polyurethane films were buried in soil for three months. The PU pieces were taken from the soil, washed with sterilized distilled water and enriched in liquid MSM for a period of one month.

Six different bacterial strains were isolated through enrichment technique, which were identified on the basis of standard morphological and biochemical tests.

Due to shortage of time out of six only one microbe was identified, pseudomonas sp. For identification of other five further tests were required.

Optimization of Various Parameters for Biodegradation of PU Film

Effect of various culture conditions on the biodegradation of PU film by bacterial consortium in MSM was investigated. Viable cell count and production of hydrolytic enzyme (lipase and esterase) were determined.

Effect of pH

Effect of different pH (5, 6, 7, 8, and 9) on the biodegradation of PU pieces by bacterial consortium was investigated.

Viable Cell Count

Cell growth and viability was recorded in terms of CFU/ml after every seven day interval. It was evident from the results that maximum CFU/ml (93 × 107) was observed during the fourth week of incubation at pH 7. Maximum CFU/ml at pH 5 was 130 × 107 during 2nd week, at pH 6 was 150 × 107 in 2nd week, at pH 7 was 210 × 107 in 1st week, at pH 8 was 215 × 107 in 2nd week and at pH 9 it was 550 × 107 in third week.

Enzyme Activity

Optimum activities 3.61 U/ml and 12.51 U/ml, were observed in case of esterase and lipase, at pH 8 and 9, respectively.

At pH 5, after 8 days of incubation, lipase and esterase activities were 3.15 U/ml and 9.27 U/ml, respectively (Fig.8 and 9). Maximum lipase activity at pH 5, 6, 7, 8, and 9 was 3.15, 3.44, 3.32, 3.2 and 3.61 U/ml respectively. Maximum esterase activity at pH 5, 6, 7, 8, and 9 was 9.68, 8.62, 10.86, 12.51 and 10.97 U/ml respectively.

Effect of co-metabolites

Effect of various co-metabolites on the biodegradation of PU pieces in liquid MSM by bacterial consortium was studied. Different co-metabolites included Tween 20, Tween 80, glucose and yeast extract.

Viable Cell Count

Maximum growth (CFU/ml) was observed after the second week of incubation in case of Tween 20 (610 ×107) whereas; in the case of Tween 80 it was highest during the third week of incubation (510 × 107). In case of yeast extract and glucose growth was highest during the second week (138 × 107 and 70 × 107 respectively).

Enzyme Activity

In case of esterase and lipase, maximum activities; 12.39 U/ml and 7.79 U/ml were observed in the presence of glucose. Maximum lipase activity was 4.38 U/ml in the 1st week, 4.20 U/ml after 1st week, and 4.49 U/ml in 3rd week in the presence of Tween 20, Tween 80 and yeast extract respectively. Maximum esterase activity was 13.40 U/ml after 2nd week of incubation, 10.52 U/ml after 4th week, and 10.61 U/ml after 4th week in the presence of Tween 20, Tween 80 and yeast extract respectively.

Effect of Temperature

Effect of different temperatures (30, 37 and 45oC) on the biodegradation of PU pieces by bacterial consortium in was studied.

Viable Cell Count

Maximum growth was observed after the third week of incubation (80×107) at 30oC. Where as maximum growth was observed after second week (80×107) and then decrease gradually after third and fourth week at 37oC. Maximum growth at 45oC was (10×107) after 2nd week.

Enzyme Activity

Maximum activities; 10.57 U/ml and 3.44 U/ml were observed in case of esterase and lipase at 30oC and 37oC respectively. Both esterase and lipase activity was low at 45oC (9.98 U/ml and 2.96 U/ml respectively).

Discussion

One of the serious problems, mankind is facing now, is the production and enormous use of the various types of man made polymers, which is a growing threat for the environment due to unabated dumping. Since the polymeric materials do not decomposed easily, that is why the environmental pollution and xenobiotic nature of these polymers is a worldwide problem (Pathirana and Seal, 1984a). Polyurethane is widely used in various fields, such as manufacture of plastic foam, cushions, rubber goods, synthetic leathers, adhesive, paints, fibers etc.

The present study deals with the isolation of polyurethane degrading microorganisms from the soil, analysis of biodegradation and optimization of various factors (temperature, pH and by using different co-metabolites). PU films were buried in soil for a period of 3 months and then used for the isolation of PU degrading microorganisms.

Polyurethanes are considered to be comparatively susceptible to microbial degradation (Morton and Surman, 1994). In our study, bacterial stains having the ability to utilize PU as a sole carbon source, were isolated after soil burial through enrichment in liquid medium. The adherence of Bacillus sp. on the polyurethane surface has also been reported by Robert et al. (1998). Kay et al. (1991) isolated 15 kinds of bacteria from polyester PU pieces following their burial in soil for 28 days. Shah et al (2008) also isolated 12 bacterial strains, 5 through

enrichment technique and 7 from buried PU films after six months. Soil organic carbon content and pH influence the structure of soil microorganisms (Garbeva et al., 2004). Attachment of microorganisms to buried PU is mediated by nonspecific hydrophobic interaction (Bos et al., 1999) and local environmental conditions also influence the surface hydrophobicity of fungi and bacteria (Smits et al., 2003). Therefore, the differences in the physiochemical properties of the soil may influence microorganisms which successfully colonize the surface of polyurethane.

In the present study, the biodegradation of PU was checked in liquid medium using different temperatures, pH and co-metabolites for a period of one month. The increase in CFU/ml indicated the increased activity of bacterial consortium against PU, i.e., its ability to utilize it as carbon and energy source. Biological degradation of polymers is generally influenced by a number of factors. Besides the nature of the polymer substances, the kind of organism involved in biodegradation and environmental condition (e.g. nutrient supply, temperature and pH) are known to drastically influence the degradation rate (Abou-Zeid, 2001).

The optimum temperature, at which the bacterial consortium used the polyurethane as a source of carbon more efficiently, was found to be 30ºC. At 37oC esterase activity increased in the start of the month and then decrease in the last week whereas, lipase activity at this temperature increased in the last week. At 45oC both enzymes show low activity in the second and then in the last week. The best results are that of the temperature 30oC, at which the enzyme activity was maximum. Lipase activity was maximum during the second week of incubation at pH 9, whereas esterase activity was maximum on 8th day of incubation at pH 8. At pH 5 lipase activity increased in the start and then declined till end of the month. Both esterase and lipase activity at pH 6 and pH 7 decrease in the third week and then increased in the fourth week.

Although, Tween 80 (1%) has earlier been reported to increase the production of lipase from Bacillus sp. (Handelsmen and Shohan, 1999). Effect of co-metabolites (Tween 20, Tween 80, glucose and yeast extract) on the degradation of polyurethane was checked. Both esterase and lipase activity were maximum in the presence of glucose.

Glucose, fructose and arabinose supplementation has been reported to lower the extent of degradation while for the isolates it was almost not affected when lactose was supplemented in the medium (Manna and Paul, 2000). Lipase activity decline after 1st week till end in the presence of Tween 80 whereas esterase activity increased after 2nd week till end in the presence of Tween 80.

The loss of tensile strength, discoloration and cracking observed for soil buried in polyester polyurethane is a typical of the effects of degradation of polyester polyurethane as a result of soil burial (Dale and Squirrell, 1990). In our study, on the surface of the polyurethane film, there were discoloration, spots and cracking. Different patterns of degradation observed by fungi with different samples of polyurethane, were attributed to the many properties of polyurethanes such as topology and chemical composition (Pathirana and Seal, 1983). In addition, synthetic polymers generally have short repeating units. The regularity in synthetic polymers allows the polymer chains to pack easily, resulting in the formation of crystalline regions. This limits the accessibility of the polymer chains to enzymes. Huang and Roby (1986) observed polyurethane degradation proceeding in a selective manner, with the amorphous regions being degraded prior to the crystalline regions. It was also observed that polyurethanes with long repeating units and hydrophilic groups would be less likely to pack into high crystalline regions as normal polyurethanes and these polymers were more accessible to biodegradation.

Conclusions

The following results were concluded from this study.

1.Bacteria with the ability to degrade polyurethane were isolated from soil. It can be concluded that soil contains the potential candidates for bioremediation of plastic wastes.

2.Maximum activity of the enzymes (lipases and esterases) was observed at 37ºC, pH 9 and in the presence of 5% glucose as an additional carbon source.

3.Growth (CFU/ml) was maximum at pH 7, 30oC and in the presence of Tween 80.

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Table 1. Morphology of Bacterial Isolates

Morphology	SK1	SK 2	SK 3	SK 4	SK 5	SK 6
Size	Moderate	Small	Moderate	Small	Medium	Medium
Pigmentation	Off white	White	Orange	Off white	Greenish	Golden yellow
Margin	Undulate	Circular	Undulate	Entire	Undulate	Entire
Elevation	Raised	Convex	Convex	Umbonate	Convex	Umbonate
Motility	-	-	-	-	+	-

Table 2. Gram Staining of Bacterial Isolates

Staining	SK1	SK 2	SK 3	SK 4	SK 5	SK 6
Gram staining	-	-	+	-	-	-
Shape	Rod shaped	Cocci	Bacilli	Cocci	Rod shaped	Rod shaped

Table 3. Biochemical Tests for the Identification of Bacterial Isolates

Test Type	SK
Citrate utilization	+
Urease	-
Nitrate reduction	+
Indole	+
TSI	-
Catalase	+
Oxidase	+
Methyl red	-
Voges Proskauer	-
Identified strain	***Pseudomonas* sp.**