Biodegradation of Low Density-Polyethylene (LDPE) by biosurfactant producing bacteria isolated from plastic polluted dumpsites

THESIS

SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW

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ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy

IN

ENVIRONMENTAL SCIENCE

Submitted by

Shailja Singh

ENROLLMENT NO. 155/13

Under the Supervision of

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DEPARTMENT OF ENVIRONMENTAL SCIENCE
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2021

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UNDER THE SUPERVISION OF
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(A CENTRAL UNIVERSITY), LUCKNOW-226025, INDIA
2021


Affectionately
Dedicated to
My
Teachers
And
Family

DECLARATION

I **Shailja Singh**, declare that the thesis entitled "**Biodegradation of Low Density-Polyethylene (LDPE) by biosurfactant producing bacteria isolated from plastic polluted dumpsites**" which is being submitted to department of Environmental Science, School of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow in fulfilment of the degree of Doctor of Philosophy in Environmental Science has previously not formed the basis for award of any such degree by any university. This is my original research work carried out during 2015-2021 and also declared that the thesis is essentially free from all kinds of plagiarism.

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Place: Lucknow

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CERTIFICATE

This is to certify that the thesis entitled "**Biodegradation of Low Density-Polyethylene (LDPE) by biosurfactant producing bacteria isolated from plastic polluted dumpsites"** submitted by **Ms. Shailja Singh** is an original research work and has not been previously submitted in part or full, for the award of any other degree or this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow, satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) Regulation-1999 as amended in 2008/2010/2015 and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the university.

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Preface

Plastic pollution has become one of the major issues of global concern. Among all the synthetic plastic used, Low density polyethylene (LDPE) is used frequently in number of applications due to their bio-inertness, excellent mechanical properties and low cost. The plastic debris accumulating in the biosphere, cause its harmful effect on all major types of biomes. All kinds of terrestrial ecosystems are being affected by the pollution of polyethylene such as deserts, forest, grassland and polar regions. Polyethylene wastes have shown deleterious effect on aquatic environment also as evident by a reduction in marine fauna population.

Several strategies are now being adopted to get rid of the LDPE-based waste. In this regard, burning, recycling, and bioremediation have been frequently practiced to manage LDPE waste. Burning of plastics is harmful due to a direct impact on the environment and human health. Recycling of polymer appears to be a little challenging as proper approaches need to be adopted to screen the waste products. Thus, the most efficient and eco-friendly approach for the reduction of plastic burden seems to be bioremediation.

In bioremediation, the polymers are broken down aerobically or anaerobically to much simpler monomers or oligomers by certain enzymes released by microorganisms. The end products of the aerobic breakdown of the contaminants are carbon dioxide and water while methane, carbon dioxide, and water are generated from anaerobic degradation. The necessity of environment friendly disposal policies designed for the biodegradation of synthetic plastics is tremendously crucial and need attention in the present scenario, considering the hazardous impact of widely used packaging material such as Low Density Polyethylene (LDPE) on the environment. Hence, it is recommended to degrade the recalcitrant LDPE by microorganisms as the microbial degradation of LDPE appears to be environment-friendly and degraded products are non-toxic.

In the aforesaid context, the aim of the research work entitled "**Biodegradation of Low Density-Polyethylene (LDPE) by biosurfactant producing bacteria isolated from plastic polluted dumpsites**" was to determine the LDPE degradation potential of the bacterial strains isolated from plastic polluted site in the presence and absence of the biosurfactant extracted by them.

The First objective was done to isolate and screen the bacteria having biosurfactant production capability. The isolated bacteria were then identified using 16s rRNA molecular analysis. The biochemical characterization was also performed for the

bacteria.

The Second objective was aimed at optimizing the biosurfactant production parameters using Response Surface Methodology. Confirmation experiment was performed for optimized parameters. The biosurfactant produced by the bacteria were then characterized using total protein content, total lipid content, FTIR, TGA and DSC analysis.

The Third objective was aimed at examining the LDPE degrading capability of the isolated bacteria long with their biosurfactant produced. The LDPE samples were treated with the bacteria and their biosurfactants inn different combination. The LDPE samples were then recovered after the treatment period and analyzed for the degradation using FTIR, SEM-EDX, TGA, XRD and DSC analysis.

The Fourth objective was aimed at examining the effect of different concentration of compost on the LDPE degradation. The LDPE sample were buried under the compost in pots and then recovered after specific period of time for assessing the degradation of LDPE using FTIR, SEM-EDX, TGA, XRD and DSC analysis.

The Fifth objective was aimed at determining the effect of three chemical surfactants *viz,* CTAB, Tween 80 and SDS on LDPE degradation. The LDPE sample were treated with the chemical surfactants in different concentrations for specific period of time. The LDPE samples were then recovered and examined for the degradation using FTIR, EDX and XRD analysis.

The Sixth objective was aimed at determining the effect of Solar radiation on LDPE degradation. The LDPE samples were kept under Sunlight for specific period of time after which samples were analyzed for degradation pattern.

So, the overall aim of the study was to investigate the different methods of LDPE degradation having cost effective and eco-friendly approach.

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Place: Lucknow **Shailja Singh**
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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATION

ACMS	Advanced center for material science
ANOVA	Analysis of variance
BLAST	Basic Local Alignment Search Tool
BS	Biosurfactant
BSA	Bovine Serum Albumin
CBI	Carbonyl bond index
CCRD	Central composite rotational design
CEC	Cation exchange capacity
CI	Carbonyl index
CLPE	Cross Linked Polyethylene
CTAB	Cationic (cetyltrimethylammonium bromide
Df	Degree of freedom
DSC	Differential scanning calorimetry
EDX	Energy dispersive X-ray
EI	Emulsification index
Exo-SAP	Exonuclease I -Shrimp Alkaline Phosphatase
FTIR	Fourier transform infra-red spectroscopy
g/l	Gram per litre
GPC	Gel permeation chromatographic
HDPE	High density polyethylene
IDBI	Internal double bond index
KCBI	Keto-carbonyl bond index
LDPE	Low density polyethylene
LLDPE	Linear low density polyethylene
MDPE	Medium density polyethylene
mg/l	Milligram per liter
Mt	Metric tones
NA	Nutrient agar
NCBI	National Centre for Biotechnology Information
PBA	Poly (butylene succinate/adipate)
PBA/T	Poly (butylene adipate- co -terephthalate)

PBDE	Polybrominated diphenyl ethers
PBS	Poly (butylene succinate)
PBT	Polybutylene Terephthalate
PCL	Poly (e-capro- lactone)
PEM	Polymethyl methacrylate
PET	Poly Ethylene Terephthalate
PHA	Poly-β-hydroxyalkanoates
PNA	Polynuclear aromatics
POP	Persistent organic pollutants
PP	Poly-Propylene
PS	Polystyrene
PU	Polyurethane
PUR	Polyurethane
PVC	Polyvinyl Chloride
rDNA	Ribosomal deoxy ribonucleic acid
rpm	Rotation per minute
rRNA	Ribosomal Ribo nucleic acid
RSM	Response surface methodology
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscopy
TGA	Thermal gravimetric analysis
UMTS	Universal mechanical testing system
USIC	University Scientific Instrument Centre
UV	Ultra violet
VBI	Vinyl bond index
XRD	X-ray diffraction

Background

Plastics are the most versatile synthetic 'man made' substance created from fossil fuel resources, which has enabled several industrial and technological revolutions **(Tribedi *et al.*, 2012)**. In the past 25 years, plastic materials have been used widely in number of applications, such as food, clothing, transportation, construction, medical, shelter and leisure industries. Plastics are made up of petroleum-based materials called resins, like polythene and polypropylene materials, which are resistant to biodegradation. Due to this resistance, plastics, which are disposed in the landfills, remain in their original form in environment **(Wang & Yang, 2010)**. Plastics offer many advantages over alternative materials being lightweight, extremely durable, low cost and relatively unbreakable **(Shimao, 2001)**. Production of plastics has increased significantly in the last 30 years with an annual growth rate of 10%. However, use of plastic materials has numerous disadvantages, among which the most important is that they do not break down easily in the environment. Commonly used plastics are polyethylene (LDPE, MDPE, HDPE and LLDPE), Poly Ethylene Terephthalate (PET), Polybutylene Terephthalate (PBT), nylons, Poly-Propylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC), and Polyurethane (PUR). Due to their buoyancy and long-term persistence in the marine environment, plastic waste poses number of hazards to the marine life **(Yamada *et al.*, 2001)**. Among the types of plastics mentioned above polyethylene is used extensively. Polyethylene polymer is of many types among which low-density polyethylene is commonly used for various purposes. In the recent years, there has been growing public concern over environmental deterioration associated with the disposal of conventional plastics. Discarded plastics like polyethylene, besides being highly visible are a rapidly increasing percentage of solid waste in landfills, resistant to biodegradation leading to pollution besides being

harmful to the natural environment. Polymers can be degraded using chemical, thermal, and photo or biological degradation **(Sivan *et al.,* 2006)**. When discarded in the environment, any physical (like weight loss of sample, tensile strength) or chemical change (like carbon dioxide production) in the plastic material suggests physico-chemical and biological degradation by microorganisms **(Nikolopoulou *et al.,* 2013a)**. However, the biological degradation of polymers in the environment takes thousands of years to disintegrate **(Webb *et al.,* 2012)**. The degradation of a polymer is affected by many factors like temperature, moisture, oxygen, sunlight, stress, living organisms and contaminants **(Xu *et al.,* 2018)**. Microorganisms are not able to use polyethylene due to its high hydrophobic nature and high molecular weight. It has been reported that if the pre-oxidation step is followed it may increase the hydrophilic nature of polyethylene by the introduction of polar groups like carbonyl groups which may cause the biodegradation process. Photo-oxidation of polyethylene could be achieved in the presence of sunlight or using U.V. light at temperature above 50 □C **(Russel *et al.,* 2011)**. But, pre or photo-oxidation through U.V. light and chemicals cannot be considered cost effective one. So, there is a need to find other economic way to increase the bioavailability of polyethylene to the microbes **(Garlotta, 2002)**. Oxidation of polyethylene using bio-surfactant can cause similar change in the chemical structure of polyethylene as it is reported in case of abiotically oxidised polyethylene. Surfactants are the amphiphilic molecule **(Nikolopoulou *et al.,* 2013b)**. The hydrophobic part of the bio-surfactant is attached with the hydrophobic surface of the polyethylene while the hydrophilic part remains projected towards the aqueous solution. This method increases the availability of polyethylene to dissolve oxygen, which further leads to oxidation of polyethylene.

In the light of above context, the explicit aim of this study entitled "**Biodegradation of Low Density-Polyethylene (LDPE) by biosurfactant producing bacteria isolated from plastic polluted dumpsites**" was to determine the LDPE degradation potential of the bacterial strains isolated from plastic polluted site in the presence and absence of the biosurfactant extracted by them. Apart from microbial degradation a comparison with other methods of LDPE degradation such as photo-oxidative degradation, composting and effect of chemical surfactant on the polymer have also been studied. The production of biosurfactant was also optimized using response surface methodology. So, the overall aim of the study was to investigate the different methods of LDPE degradation having cost effective and eco-friendly approach.

- Isolation, screening and characterization of biosurfactant producing bacteria isolated from plastic polluted dumpsite.
- Optimization, characterization and production of biosurfactant.
- Evaluation of LDPE degradation by biosurfactant and its producing bacteria.
- Evaluation of LDPE degradation under controlled composting.
- Analysis of the chemical alterations induced in the LDPE exposed to chemical surfactants.
- Analysis of photo-oxidation of LDPE.

2.1 Plastics and their classification

In the year 1830, the discovery of several chemical process utilized for developing synthetic polymers from crude oil was a breakthrough, in field of Chemistry and Material Science **(Lugauskas *et al.,* 2003; Arutchelvi *et al.,* 2008)**. It paved the way for the production of the most versatile group of materials that was ever produced. These new materials having combined features of exhibiting strength, light-weight, flexibility and low-cost production, were extremely durable and considered as the most non-biodegradable synthetic material. Such traits facilitated the usage of plastics to any agricultural, industrial or domestic market **(Caruso, 2015)**. For example, soil mulching using polethylene (PE) in agriculture is a commonly applied practice.

The most commonly used polymer products include light-weight and tough beverage bottles which are made up of polyethylene terephthalate (PET), flexible garden hoses composed of polyvinyl chloride (PVC), insulating food containers that is made up of foamed polystyrene, and shatterproof windows manufactured using polymethyl methacrylate (PEM) **(Chun *et al.,* 2013; Mani *et al.,* 2016)**.

Plastic debris hance contains several different polymers. Their unique chemical ingredients might make some types of polymer more hazardous as compared to others when their chemical products are bioavailable to organisms. For instance, polyvinyl chloride (PVC), polyurethane, polycarbonate, and polystyrene (PS) are made up of hazardous monomers like vinyl chloride, bisphenol-A, styrene and contains hazardous additives such as Polybrominated diphenyl ethers (PBDEs), phthalates and lead **(Garrett *et al.,* 2008)**.

Plastics are composed of Carbon, Oxygen, Hydrogen, Silicon, Chloride and Nitrogen. Oil, coal and natural gas are utilized for extraction of the basic raw materials of

polymers **(Dey *et al.*, 2012)**. The polymers of various forms like polycarbonate, polyethylene, nylon, polyethylene terepthalate, poly tetra flouroethylene, polyurethane (PU), polypropylene (PP), polystyrene, polyvinyl chloride are continuously used in our daily life **(Gerard *et al.*, 2014)**.

Polymers are classified into two major classes: thermoplastics and thermosets. Thermoplastics like polyethylene and polystyrene can be molded and remolded again and again, while thermosets can never be reprocessed upon reheating. During the initial process, thermosetting resins undergo a chemical reaction, which results in an infusible and insoluble network. Annual worldwide production of thermoplastics has been increased from two million tonnes in 1950 to 245 million tonnes in the last decade **(Ishi *et al.*, 2007)**.

Major types of plastics, which are commonly used, are polyethylene (PE), poly-ethylene terephthalate (PET) and polypropylene (PP). These polymers have contributed to the development of contemporary human society. They are typical oil-based non-biodegradable plastics whereas most of the oil-based plastics are considered as non-biodegradable. Poly (e-capro-lactone) (PCL), poly (butylene adipate-co-terephthalate) poly (butylene succinate (PBS) and poly (butylene adipate) (PBA), are biodegradable in nature **(Kathiresan, 2003)**.

Among the various types of plastic polymers, the most popular and convenient polymers include polyethylene. There are several different grades in polyethylene among which the most important grades used are High-density polyethylene (density greater or equal to 0.941 g/cm^{23}), Linear low-density polyethylene (density range of 0.915–0.925 g/cm^{23}) and LDPE density is 0.910–0.94 g/ cm^{23}. Polythene is made

from the cheap petrochemical stocks extracted from oil or gas through efficient catalytic polymerization of ethylene monomers **(Leja & Lewandowicz, 2010)**.

LDPE is a polymer, which is highly branched having weak intermolecular forces and tensile strength but have higher resistance. Polythene has been very useful in our daily lives, to meet our required needs. It is utilized for wrapping of the goods, food material, cosmetics, medicines and various scientific instruments. Due to its excellent feature usage of polyethylene is increasing every day. Among the synthetic polymer based waste produced, polythene contributes about 64%. The high rate of production of polyethylene based plastic items is due to its low cost, good mechanical properties and easy processability **(Priyanka & Archana, 2011)**. LDPE is prepared using gaseous ethylene under very high pressure (about 350 megapascals) and high temperature (about 350 °C) in the presence of oxide initiators. These processes produce a polymer structure having both long and short branches. LDPE is known to be very flexible material because the branches prevent the polyethylene molecules from packing closely together in hard and crystalline arrangements. Its melting point is approximately 110 °C (230 °F) **(Porebski *et al.*, 1997).**

During the last few decades, the global demand for these polymers has increased sharply. Accumulations of polyethylene products in the environment adversely affect the ecosystem. Polyethylene is not biodegradable in nature. Accumulation of polyethylene in the environment has been 25 million tons per year. In 2015, it has been estimated that approximately 60 to 99 million metric tonnes (Mt) of plastic waste were produced globally **(Premraj & Doble, 2005)**.

The increased levels of LDPE waste; decreased capacity of landfill and very slow rate of LDPE degradation in natural environment has caused many problems in processing

the amount of waste. Thus, the rapid degradation of plastic has been an area of interest in management of waste **(Panjiar *et al.*, 2015)**.

2.2 Impacts of low density polyethylene on environment

Plastic once discarded get into the form of litter and enters the running water in different ways according to the nature and ultimately pollutes the aquatic environment. The rate of proliferation for plastic materials is very fast, and mostly such wastes throughout the globe affect the aquatic bodies **(Santo *et al.*, 2013)**. Plastic waste causes eight complicated problems in the aquatic environment: (1) plastic trash pollutes, (2) plastic entangles the aquatic life, (3) ingestion of plastic material by aquatic animals, (4) biodegradation of plastic polymers is time-consuming, (5) Microplastics and its pellets disturb the food web, (6) interference with sediment inhabitants, (7) destroys the primary habitat of new emerging organism and (8) marine plastic litter cause major damage to vessels **(Obi *et al.*, 2016)**.

Plastic can release harmful additives and oxidants in soil, which affects groundwater ecosystems. Sunlight and seawater embrittle plastic, and the eventual break- down of larger objects makes it available to zooplankton and other small marine animals **(Tribedi & Sil, 2013)**.

Polyethylene, is believed to be carcinogenic. Phthalates and other products, associated with them cause hormonal disturbances, cancer, developmental issues, decreased sperm count, weakened immunity and infertility. The chemicals present in them are also responsible for birth and genetic conditions (Ali *et al.,* 2014). After incineration of the polyethylene waste harmful gases such as dioxins and furans are released which cause bronchitis, deafness, skin disease, vision problems, digestion and liver-related problems. Other hazardous chemicals released during the polymer's life cycle, such as

heavy metals and additives may pose irreversible life-long health threats **(Tribedi & Sil, 2014)**.

2.3 Degradation of Plastics

Most common method for disposal of municipal solid waste is land filling. Increasing amount of polymer waste produced has resulted in an increase in interest for polymer biodegradation. Natural factors such as heat, light, moisture, chemical conditions, and biological activity result in polymer's bond scission. During polymer degradation the formation of structural homogeneities and new functional groups occurs **(Albertsson, 1978).**

Based upon the nature of causing agents, polymer degradation has been categorized as (1) Photo-oxidative degradation, (2) Thermal degradation, (3) Ozone-induced degradation, (4) Mechano-chemical degradation, (4) Catalytic degradation, (5) Biodegradation and composting. However, the main degradation has been considered either to be photo-degradation, biological degradation or thermal degradation **(Okoh & Atuanya, 2014)**.

2.3.1 Photodegradation

Polyolefins are consistently used for outdoor purposes. Due to the effect of weather conditions, the material change its properties as it undergoes embrittlement, color changes, cracking of the surface etc. Weathering of polymers may be caused by various factors as for example mechanical stress, oxidation, heat or biodegradation **(Araujo *et al.*, 2008)**. One of the most important factors contributing to degradation is ultraviolet radiation.

The degradation, which is carried out in presence of light, is called as

photo-degradation. It is first initiated by the absorption of light energy by the correct functional group present in the polymer backbone. The absorption of light causes the scission of the polymer molecule at a proper position of the chain, which leads to the conversion of polymer molecule into smaller fragments **(Orhan & Buyukgungor, 2000)**. A degradable polymer in which the degradation occurs from the action of solar radiation is called a photodegradable plastic. Many synthetic polymers are prone to degradation initiated by UV radiation and visible light. Generally, the near-UV radiations (290-400 nm) in the solar radiation determine the lifecycle of polymeric materials in outdoor uses. UV radiations have sufficient energy to cleave carbon-carbon (C-C) bond. Photodegradable polymers generally require an in-built photosensitive group in the chain or an additive **(Restrepo-Florez *et al.*, 2014)**. Photodegradation of polymer involves UV degradation and oxidation. In UV degradation, the UV light degrades the end product and during the oxidation process, heat disrupts the plastic. The photo-induced degradation process initiates either by the absorption of photon by the polymer chain or by the additive present in the product. The degraded sites then act as stress concentrators and crack will appear when the polymer is subjected to stress **(Zhang *et al.*, 2009)**. Thus, they reduce the tensile strength and cause the mechanical destruction of the material. The above effects cause change in tensile properties. In photoactive-pigments like TiO_2, CdS or ZnO, formation of electron-hole pair over the pigment surface in presence of oxygen and water produces reactive species, which can ultimately cause oxidation of polymer **(Shah *et al.*, 2008)**. The direct effect of radiation on plastic is usually limited to the surface region, due to light absorption by the pigment or the degraded material itself. Photo-oxidation causes alterations in the physical and optical properties of the polymer. The most severe effects are the visual effect like yellowing of polymer, loss

in mechanical properties of the polymers, alterations in molecular weight and its distribution. Polyethylene films when exposed to solar UV radiation lose their mechanical strength along with an overall decrease in average molecular weight **(Weiland *et al.*, 1995)**.

2.3.1.1 Photodegradability of polyethylene

Pure polyethylene is generally considered a relatively stable polymer material under the ultraviolet radiation in the absence of oxygen. The polyethylene material after long exposure to UV radiation of short wavelength (254 nm) in vacuum of a nitrogen atmosphere, undergo chain scission and hydrogen abstraction, crosslinking and evolution of hydrogen **(Nayak & Tiwari, 2011)**.

Crystallinity influences the degradation of polyethylene to a greater extent. Branched polyethylene gets oxidized faster than linear polyethylene, and it has been observed that its oxidation rate is generally proportional to the amount of amorphous portion present in the polymer. This indicates that the oxidation of semi-crystalline polyethylene is limited to its amorphous region. It was subsequently stated that the crystalline region absorbs basically no gas, which indicates that oxygen is mainly not available in the crystalline portion. Based upon such facts, polyethylene having low crystallinity has higher rate of carbonyl formation **(Hwang *et al.*, 2007)**.

During extensive studies of photo-degradation and photo-oxidation of polyolefins in the past decade, the initiation mechanism has been discussed in connection with various chromophoric species such as carbonyl groups, hydroperoxides, metallic impurities, polynuclear aromatics (PNA), oxygen-polymer charge transfer complexes, and so on. The order for importance of chromophoric impurities as given by Scott for the photo-oxidation of low density polyethylene is given below **(Sen & Raut, 2015)**:

-OOH > >C=O > [>C=C<.....O_2] complex

The Carbonyl species have been considered as the main chromophores for the photo-oxidation of polyolefins **(Suzuki *et al.*, 1973).**

2.3.1.2 Mechanism of photodegradation

In photo-oxidative degradation, firstly short-lived singlet state is changed to long-lived triplet state. Excited triplet states then break the polymer chains due to which radical pairs (Norrish Type I reaction) are formed or pairs of saturated and unsaturated chain end are formed by hydrogen transfer (Norrish Type II reaction) **(Nicolas *et al.*, 2018)**. The polymer radicals formed incorporate molecular oxygen in triplet ground state to peroxy radicals. These peroxy radicals are known to abstract hydrogen and then hydroperoxide groups are formed, which can absorb UV light and become excited by energy transfer. The weak O-O bonds then breaks up and pairs of alkoxy as well as hydroxyl radicals are formed which can react in various ways by chain scission, rearrangement, hydrogen abstraction, etc. and increase the photo-degradation rate **(Tokiwa *et al.*, 2009)**.

In photo-oxidative degradation of polymer, the mechanism involves an auto-oxidation cycle which comprise of various steps as shown below (Figure 2.1) **(Muthukumar & Veerappapillai, 2015)**:

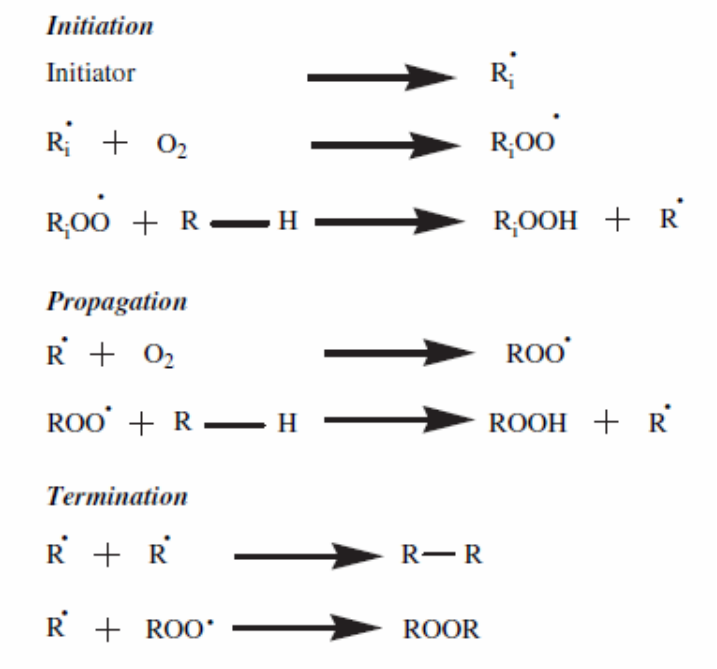


Figure 2.1: Mechanism of Photo-oxidative degradation

(a) Initiation

Different initiation steps under different conditions have been observed in different polymers **(Nie *et al.*, 2010)**.

(i) Photosensitized cleavage: Photosensitizers are considered to be highly photosensitive, and they readily get excited on exposure to radiation and are generally used to bring about effective homolysis of the polymeric structure, which otherwise can not undergo sufficient photo-excitation at the frequency of light that is available to the system **(Tribedi & Dey, 2017)**.

(ii) Catalyst residues as a source of radical generation: Some of the metal salts and their oxidation products when added to the plastics act as a catalyst to produce free radicals. Polymerization catalysts like transition metals (Ti) may remain in polyolefins at a concentration of 2-100 ppm, depending on catalyst efficiency. These residues have been involved in both photo as well as thermal stability problems. For

example, TiO_2 is a celebrated photosensitizer used for polyamide and polyolefin degradation and absorbs at 480 nm **(Nakajima *et al.*, 1999)**. Photosensitization generally involves the formation of highly reactive species which includes atomic oxygen, hydroperoxyl radical, hydroxyl radical and superoxide anion. The primary process includes the promotion of the Ti electron to the conduction band of the semiconductor in order to form an electron-positive hole pair. The presence of water determines the relative proportions of the reactive species. TiO_2 sensitization will not be observed unless both the oxygen and water are present **(Sudhakar *et al.*, 2008)**.

(iii) Introduction of carbonyl groups: Carbonyl groups that are formed by mild oxidation of polymer during the synthesis, act as chromophores and they become source of the initiation radicals. Carbonyl chromophore can absorb near-UV radiation. They form radicals following Norrish Type I, Norrish Type II and H-atom abstraction processes **(Whang *et al.*, 2008)**.

(iv) Incorporation of peroxides and site of unsaturation: In this step the peroxides or C=C sites become source for formation of initiation radicals. The near UV component of sunlight in the range of 280-390 nm is energetic enough to break C-C bond and C-heteroatom bonds. In unsaturated polymers, light generated singlet oxygen $^1O_2$ reacts with an unsaturated site by the method of an ‘‘ene’’ reaction and starts chain oxidation **(Mukherjee *et al.*, 2016)**.

(b) Propagation reaction

The propagating reactions under the auto-oxidation cycle are common for all carbon polymers. These reactions produce hydroperoxide species. They do not directly cleave the backbone structure but are the key intermediates of further reactions. The Hydroperoxide species formed in propagating step cause backbone degradation via

cleavage of hydroperoxide O-O bond followed by β- scission. The scission process then forms two chain ends, which are free to restructure, and lead to increase in crystallinity as the oxidative degradation proceeds **(Thomas & White, 1989)**.

(c) Termination reactions

This reaction occurs naturally by the combination of free radicals or assisted by the use of stabilizers in the plastic. Peroxide radicals ultimately terminate by reaction with other radicals to produce dialkyl peroxides, alcohols or carbonyl species **(Montazer *et al.*, 2019)**.

2.3.1.3 Methods for photo-degradation

(a) Natural weathering

Outdoor exposure is basically performed on samples by mounting it on testing racks, under standard conditions in order to expose the polymer to the full radiation spectrum, besides the humidity and temperature of that location **(Cornell *et al.*, 1984)**. For observing the aging of the polymer, it is characterized on the basis of mechanical properties (elongation at break, impact strength or tensile properties) and visible characteristics, like crack formation, changes in color and chalking. The alterations induced in the polymeric materials after exposure to solar radiation can be characterized by FTIR spectroscopy or ultra violet/visible (UV/vis) spectroscopy **(Horn *et al.*, 2012)**.

(b) Artificial weathering/laboratory test

Artificial weathering or pure laboratory testing basically involves using environmental chambers along with the artificial light sources to almost replicate the outdoor conditions but with a less test time under highly and carefully controlled

conditions. Laboratory testing could quickly assess the relative stability of polymers but also has the major demerit that the quicker the test is done the lower is the correlation as compared to the real behavior in the field **(Gajanand *et al.*, 2014)**.

2.3.2 Thermal degradation

Thermo degradation refers to the degradation of plastic material or polymer by heat energy. This process generally receives support from oxygen present in the atmosphere and is also known as thermo-oxidative degradation. The initial stage of thermal degradation is the rupture of the bonds of macromolecules that result in the formation of radical sites. Thermal degradation usually involves alterations in the molecular weight of polymer **(Skariyachan *et al.*, 2016)**.

Under normal conditions, both the photochemical and thermal degradations are similar and are classified as oxidative degradation. The main difference between the two method is determined by the sequence of initiation steps that leads to auto-oxidation cycle. Another important difference is that thermal reactions can occur throughout the bulk of the polymeric material, whereas, in photochemical reactions the process generally occurs only on the surface. Thermal degradation in polymers occurs via the random chain degradation, which is initiated by thermal radiation and the UV light **(Huang, 2012)**. Many addition polymers depolymerize at high temperatures for example PE decomposes into long olefinic structures actually producing little monomer. Thermal degradation above 200°C can cause chain scission and it largely depends on factors such as unsaturation sites or head-to- head units. Polyolefins are sensitive to thermal oxidation, because of the impurities produced during their manufacture at higher temperatures **(Tajvidi & Takemura, 2010)**.

2.3.3 Ozone-induced degradation

Both ozone and sunlight quickly attack the polymers, which can notably reduce the service life of a polymer. Mainly polymers having high unsaturation will suffer from ozone degradation, due to the double bonds present in unsaturated polymers that readily react with ozone **(Bhatnagar & Kumari 2013)**. Though, ozone is also known to reacts with saturated polymers but comparatively at a slower rate. The reaction of ozone with the double bonds basically causes chain scission. Oxidation and chain scission cause decrease in molecular weight and its distribution (thermoplast), and a change in its composition (oxidation). This results in decline in the mechanical properties of the polymer.

The aging is greatly speeded by stress. Surface cracks can usually be observed in the direction perpendicular to the strain applied when the critical stress value is exceeded. At low stress values and just above the critical value, long and deep cracks can be observed, whereas, at a very high stress values, the ozone cracks increase in number and are finer in size. In case of rubber goods, the microscopic disintegration causes dulling and a bluish sheen of the surface **(Shah, 2008)**. This phenomenon is commonly known as "frosting" because it resembles actual frost. It is significantly accelerated by heat and humidity. Frosting can be reduced or avoided by some types of high melting point waxes or by anti-ozonants like para-phenylenediamines and derivatives of dialkyl para-phenylenediamines **(Kumar *et al.*, 2007)**. The resistance to ozone cracking mainly depends on the chemical composition of the polymer. Elastomers are mainly susceptible to ozone attack, particularly those having electron donating side groups like methyl groups in isoprene, whereas polymers with electron-withdrawing side groups such as chlorine in neoprene are noticeably less susceptible to the attack of ozone because of the deactivating effect of halogen on the double bond **(Sivan, 2011)**.

The extent of ozone degradation depends upon the composition of the atmosphere and the temperature. Typically, the ozone concentration is rather low. Nevertheless, even low values of ozone can cause significant degradation over the period of time **(Singh *et al.*, 2012)**.

2.3.4 Mechano-chemical degradation

In Mechano-chemical degradation the degradation of polymer occurs under mechanical stress and by strong ultrasonic irradiations. The mechano-degradation of polymers occurs by means of free radical processes. Generated radicals are formed from the cleavage of the main backbone section of the polymer chains present in the stressed amorphous regions that connects crystallites **(Bhardwaj *et al.*, 2012a)**. The gel permeation chromatographic (GPC) studies of the degradation of polymer such as LDPE under excessive shear conditions have shown that major changes in molecular weight distribution and the long-chain branching have occurred from thermo-oxidative or thermal degradation. The orientation of solid polymers under extreme shear conditions (high draw rates and comparatively low temperatures) has produced the oxidation products due to shear process. Ultrasound is mainly responsible for the cleavage of macromolecular C-C bonds whereas termination reactions of the mechano-radicals occur as disproportionation or combination reactions. These reactions get suppressed in the presence of radical scavengers **(Siqueira *et al.*, 2010)**.

2.3.5 Catalytic degradation

The catalytic transformation of polymer waste into hydrocarbons having higher commercial value is of great interest. Polyolefins gets catalytically degraded into oils and gases. For catalytic degradation of polyolefins thermal gravimetric analysis (TGA) can be used as a potential method for screening the catalysts. It has also been

found that the presence of catalyst can cause decrease in the apparent activation energy. For plastic degradation, several types of catalysts have been reported which include Pt-Co and Pt-Mo supported over SiO_2, transition metal catalysts (Cr, Ni, Mo, Co, Fe) supported on Al_2O_3, SiO_2 zeolite catalysts and non-zeolite catalysts, zeolite and zirconium hydride **(Bhardwaj *et al.,* 2012b)**.

2.3.6 Biodegradation of polymers

Polymers degradation is a process of altering the strength and color of polymeric substance under controlled conditions. Disruption in the chain length starts the primary breakdown (aging), and numerous external factors like temperature and chemicals enhance the rate of degradation of polymer **(Albertsson *et al.,* 1998)**. The term "aging" is generally used for the change in properties. Currently, the recycling process of plastic material is increasing but the rate of recycling is very low for many polymers because of the usage of higher number of additives during their manufacturing **(Balasubramanaian *et al.,* 2010)**. The recycling rate of thermosets is extremely low but thermoplastics can easily be recycled. Plastic waste comprises 70–80% of litter. Its persistence and discharge into the surroundings impose harmful effects on wildlife, agriculture and forestland. Persistent organic pollutants (POPs) such as furans and dioxins are formed by the burning of polymers such as polyethylene and polyvinylchloride (PVC).

Research throughout the world has been focusing on the biodegradation of plastic. Biodegradation (microbial mineralization) is more eco-friendly as compared to other waste management methods. Bioremediation could serve as a best way to handle waste material without imposing any deleterious effect on nature. Pollution load is increasing constantly because of inappropriate waste management strategies. Such kind of waste comes from industrial part and community activities. Both prokaryotic

(bacteria) and eukaryotic (fungi, algae, plant), are engaged in the bioremedition process. Some of the prominent microbial agents used in bioremediation process include *Pseudomonas*, *Bacillus*, *Streptomyces*, *Corynebacterium*, *Lysinibacullus*, *Arthrobacter*, *Micrococcus* and *Rhodococcus* **(Antoniou *et al.*, 2015)**.

2.3.6.1 Mechanism of polymer biodegradation

Microorganisms are able to break down the compounds into simpler compounds via biochemical transformation (Figure 2.2). Bio-degradation of polymer can be described as any change in the polymer properties such as reduction in molecular weight, loss of mechanical and surface properties. It can also be described as the breakdown of material into smaller fragments through microbial digestion. Degraded particles are probably non-toxic to the environment. Microorganisms can form catalytic enzymes for the biodegradation process **(Kostka *et al.*, 2011)**. This approach is excellent for environmental waste management, and the microorganisms engaged in this process can serve as a perceptible alternative mode in order to maintain the healthy environment. Microorganisms via different enzymatic activities and bond cleavage accomplish the degradation process **(Kumar *et al.*, 2007)**. This degradation is known to occur in four sequential steps:

(1) Bio-deterioration, which involves an alteration in the physico-chemical as well as physical properties of the polymer.

(2) Bio-fragmentation, which include breakdown of polymer in to simpler form via enzymatic cleavage.

(3) Assimilation generally involves uptake of molecules by the microorganisms.

(4) Mineralization involves production of oxidized metabolites such as CO_2, CH_4, H_2O after degradation.

Mineralization of polymers can undergo in both aerobic and anaerobic conditions. Under aerobic conditions, carbon dioxide and water are formed, while in anaerobic conditions, methane, carbon dioxide and water are. Many microbial communities are able to use polyester and polyurethane at a very slower rate. Starch or cellulose based polymers are biodegradable **(Barnes *et al.*, 2002)**. They can be degraded easily through composting; this can reduce the burden on landfill sites and resolve the waste management problem. Biodegradation with the assistance of microorganisms is an amicable way to reduce such plastic waste **(Huang *et al.*, 2005)**. Microorganisms are able to exploit synthetic polymers, but still the composition of the polymeric material and its manufacturing method needs to be defined for the microbial activity on the polymers. Biodegradability of these synthetic polymers having chemical groups that are prone to microbial attack can be carried out with the polymers like polycaprolactone, poly-β-hydroxyalkanoates (PHA), oil-based polymers. Enzymes produced by microorganism are used to control plastic pollution and contribute in developing an eco-friendly environment. Distinct forms of microflora are known to use them through the mineralization process **(Heydari & Pessarakli, 2010)**.

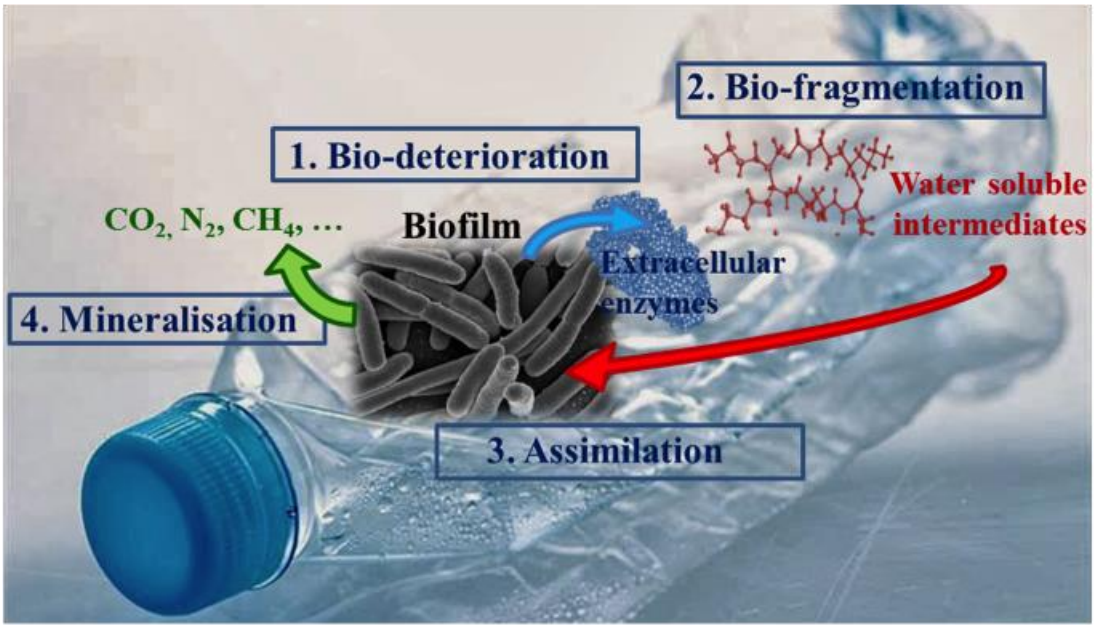


Figure 2.2: Mechanism of Biodegradation via four stages

2.3.6.2 Methods for biodegradation

(a) Pure culture method

In pure culture method, the pre-weighed plastic films are disinfected and then aseptically transferred to sterilized culture medium. Films in culture medium are incubated for 24 h under shake flask condition. The presence of microorganism can be confirmed by using a microscope **(Chahal *et al.*, 1992)**.

(b) Soil burial method

Soil burial method is among the frequently used methods for determining the biodegradability of plastics. This method involves the test of biodegradation under natural or laboratory conditions. Samples with specific weight and dimension are buried under the soil up to a specific depth for different interval of time **(Zahra *et al.*, 2010)**.

(c) Compost method

Composting can be described as a waste management process in which organic wastes are transformed into somewhat stable organic end products along with the production of carbon dioxide (CO_2). The physical, chemical, and biological conditions of the composting process are considerably different from those encountered in aquatic and soil environments **(Chakraborty *et al.*, 2019)**. These differences are significant because compost can affect the fate of degradable polymeric material in ways that cannot be anticipated from test results achieved in aquatic and soil media. Thus, the final composting products represent the biodegradability of polymer material better than the other methods. Even though biodegradation test method is used widely, more research is necessary for understanding the possible influence after the biodegradation

process and during the degradation process **(Carrier *et al.*, 2012)**. It is important to develop and validate the methods for a proper detection. Not only the conformation and configuration of the polymer such as molecular weight, chemical structure, crystallinity and orientation but also their surface area and morphology of the surface should affect their biodegradation behavior **(Bellia *et al.*, 1999)**.

2.4 Microorganisms involved in polyethylene degradation

Biodegradation of polyethylene is a complex process. There are two approaches for the mechanism of degradation. In first approach, degradation studies of polymers have been performed utilizing pure strains that are able to degrade it. The benefit of this approach is that it uses pure strain and it could be the suitable way to examine the metabolic pathway and to estimate the effect of different environmental conditions upon the degradation of polyethylene **(Berlemont & Martiny, 2013)**. A demerit of this method is that it overlooks the possibility that biodegradation of polyethylene could be due to the cooperative process among the different species. This can be avoided by another strategy, in which the use of complex environments alongwith the microbial communities are applied. Marine water, soil or compost can be the examples of the environments where polyethylene biodegradation has been examined under the second approach **(Calabia *et al.*, 2004)**.

The type of polymer used as the substrate can also influence the structure of a microbial community isolated on a polyethylene surface during biodegradation experiments **(Dey & Tribedi, 2018)**. In numerous studies it has been confirmed that the physicochemical nature of the surface decides the capability of microorganisms to form the biofilm structures. The common types of polyethylene are: Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE), Linear Low Density

Polyethylene (LLDPE) and Cross Linked Polyethylene (CLPE). They differ from each other in their density, availability of functional groups on the surface and degree of branching. It is important to mention that polyethylene is mixed with additives like pro-oxidants and starch. Both of them are used for improving the biodegradability of the polymer. Such additives can affect the kinds of microorganisms, that colonize the surfaces of the polymer **(Moriyama *et al.*, 1993)**.

A number of microbial strains have been discovered for their ability to interact with the polymer causing deterioration. So far, the richness of microorganisms to degrade the polyethylene is limited to 17 genera of bacteria, 9 genera of fungi. The numbers of microorganism can be increased by sensitive isolation and characterization techniques, which is based on sequencing of rDNA. This technology allows for a broader approach for assessing the composition of a community, including the non-culturable microorganisms that are not visible by using traditional microbiological methods **(Brune *et al.*, 2000)**.

2.5 Effect of microbial activity on polyethylene

Microorganisms are known to colonize the surfaces of polyethylene. This has diverse effects on its properties. Seven different characteristics are generally observed for change in order to determine the degree of biodegradation of the polymer. These characteristic properties are: different functional groups present on the surface, crystallinity, hydrophobicity/hydrophilicity, mechanical properties, surface topography, mass balance and molecular weight distribution. Modifications of the surface chemistry are the evidence of interactions by microorganisms with surface; though, more convincing proof of polymer degradation can be achieved when polymer consumption is determined by the course of experiments **(Cai *et al.*, 2015)**.

(a) Functional groups on the surface

The occurrence and type of functional groups on the surface of polyethylene can usually be studied by FTIR spectroscopy. For the analysis of the polymer's spectral information after the degradation, emphasis is given specifically on the presence and absence of following functional groups: carbonyls (1715 cm^{-1}), vinyls (1650 cm^{-1}), esters (1740 cm^{-1}), and double bonds (908 cm^{-1}). Changes in these groups are generally occur when any biological activity happens on the polymer's surface. The degradation activity in the presence of microorganisms led to the decrease in concentrations of these surface functional groups. The decrease in the functional groups can be confirmed by calculating carbonyl indices. The other evidence for the biodegradation of polymer is that there should be decrease in the number of double bonds during the microbial activity **(Chrissafis *et al.*, 2005)**.

Though, FTIR findings may seem to be contradictory at first glance but they show the biodegradation of polyethylene to be a complicated process that could differ for different microorganisms. The fact that is certainly true regarding functional groups is that incubation with microorganisms alters the functional groups present at polymer's surface because of their consumption or production. In complex microbial community, abiotic factors are also responsible for affecting the chemistry of the polymer, so, the effect observed (increase or decrease of functional groups) depends on the balance of rates of oxidation and the degradation, which in turn depends on the nature of the microorganism present **(Gu, 2003)**.

The analysis of the chemical change on the polymer surface is important; because oxidized groups are easily attacked by microorganisms and the oxidized groups regulate the microbial attachment by enhancing the hydrophilicity of the surface. This

implies that the polyethylene degradation can be enhanced if more oxidized surface is used as substrate **(Andrady *et al.*, 1993)**.

(b) Hydrophobicity and Hydrophilicity

The hydrophobicity and hydrophilicity of a surface depends on the nature, exposition and concentration of the functional groups present in the polymeric material. In polyethylene degradation two phenomena has been observed which depends on the relation of oxidation and reduction of oxidized groups by microorganisms. If the extent of oxidation process due to effect of abiotic factors like UV light or enzymes activity is greater than the extent of utilizing functional groups, then an increase in the hydrophilicity is observed. Conversely, if the rate of utilization of functional groups is greater than the rate of oxidation then an increase in the hydrophobicity is observed. Hydrophobicity is a significant property of the polymeric surface in biodegradation studies, because the relation between the surface and the microorganism's hydrophobicity determines the level of colonization over the polymer substrate. It has been stated that more the hydrophilic surface of the polymer, easy is the colonization by the microorganisms **(Jailawi *et al.*, 2015)**.

Hydrophobicity is mostly determined by the contact angle of the surface making use of a probe liquid like water. More hydrophilic surface implies the smaller contact angle with water. Another approach to determine the hydrophilicity of the surface is the YoungeDupré equation by which estimation of energy of adhesion to the solid as well as its acid can be determined **(Kaseem *et al.*, 2012)**.

(c) Crystallinity

Polyethylene is basically a semi-crystalline polymer, which consists of crystalline microstructures that are surrounded by amorphous regions. It has been evident

through the experiments, that amorphous regions are consumed first by the microorganism because they are more accessible to microorganisms. Experimentally it has been observed that an initial increase in percentage crystallinity results due to the consumption of amorphous portions. Yet there is no sufficient research to state definitively what exactly happens after the consumption of amorphous regions. However, it has been anticipated that once the amorphous portion have been consumed, microorganisms will then consume the smaller crystals present in the polymer, which result in an increase in the fraction of larger crystals **(Guo *et al.*, 2013)**.

(d) Molecular weight distribution

High molecular weight of polyethylene is one of its main limitations toward the biodegradation. One of the common effects that is observed after microbial attack, is an increase in the molecular weight which results due to consumption of the low molecular weight chains. However, this result is not universal, as some of the authors have observed only a slight change in the molecular weight distribution. Some other authors have concluded that the main factor that affects the molecular weight is the effect of abiotic factors like UV irradiation rather than direct attack by the microorganisms **(Cawoy *et al.*, 2015)**.

Two different strategies have been used to determine the molecular weight distribution. The most common method is the using size exclusion chromatography techniques at high temperature.

The other strategy is using rheological measurements, which correlate indirectly with the distribution of molecular weight **(Mathur *et al.*, 2011)**.

(e) **Surface topography**

Colonization of the polyethylene surfaces by the various microorganisms usually causes changes in the surface topography. This has been proven significantly in different research papers. Development of micro-colonies of different microorganisms on the surface of the polymer as well as penetration of hyphae structures has been reported as common features after microbial attack **(Saleem *et al.,* 2017)**. The changed surface in topography of the polymer after biodegradation requires more study to be conducted but there is enough evidence which can proves that some superficial damage can be observed after polyethylene surfaces have been exposed to biodegradation **(Charoenpanich *et al.,* 2006)**.

(f) Mechanical properties

Thin films of polyethylene have been focused much for studying the polyethylene biodegradation because the results show that in the form of film as substrate, deterioration in the mechanical properties like distortion is common. Oxidation-induced changes in crystallinity and in the average molecular weight cause the brittleness in polyethylene films. The modification in the mechanical properties is caused due to oxidation effects which induces change in crystallinity and in average molecular weight **(Gupta *et al.,* 2016)**. Rheological analysis be conducted to estimate the storage and loss modulus of the polyethylene, but for studying the biodegradation many researchers have opted for the use of a Universal Mechanical Testing System (UMTS) for evaluating the mechanical properties of the polymer specimen.

The effects of microbial activity on polyethylene have been studied mostly in thin films. The change in the mechanical properties caused due to microbial activity is still an active area for research **(Korenblum *et al.,* 2012)**.

(g) Consumption of the polymer

Consumption of the polymer by microorganisms provides the indication of its assimilation, but the slowness of the process could make it difficult to detect it. Some studies have reported the reduction in the weight of polymer samples, which was determined by gravimetric measurements. Among various techniques available, commonly used technique to determine the polyethylene consumption by microorganisms is CO_2 evolution **(Hanson *et al.,* 1993)**. In this technique, it is being assumed that polymer used by microorganism as a sole source of carbon will be finally converted to CO_2 during respiration process and therefore, it can be used as an indirect measurement for determining the amount of polyethylene that has been utilized by microorganisms. CO_2 evolution monitored continuously not only allows the determination of the total consumption of the polyethylene but also the rate of biodegradation. Some of the researchers have used this technique successfully for verifying the ability of some of the microbial strains to degrade the polyethylene of very low molecular weight **(Karlsson *et al.,* 1988)**.

2.6 Challenges in polyethylene degradation

There are many evidential proofs that the current methods of polymer degradation are not very effective, so, scientists are finding some other alternative ways where microbes can be utilized to degrade these long chain synthetic polymers into their respective monomers. This process of converting polymer into its monomer can also be termed as reverse flow to generate simple hydrocarbons because these polymers are commonly produced from different petrochemical products. Thus, we can be able to get those simple monomers, which can be the alternative source of energy and may act as the next generation fuel **(Kale *et al.,* 2015)**. This could decrease the

dependency of humankind on the limited petroleum reserves, as we could be able to recycle these monomers to again produce the polymer. Hence, there is a huge demand for exploring such microbes which are able to grow in different conditions and, under specific stress condition, and able to use carbon polymers as their source of energy, thus degrading these synthetic polymers. As our earth is a great natural source of varied types of microorganisms, scientists are trying to explore and exploit them for such activities. However, the extracellular substance such as biosurfactant produced by the bacteria could also be used to enhance the degradation process as they have tendency to reduce the surface tension of the aqueous medium. Moreover, use of biosurfactant is eco-friendly method of oxidation of polyethylene as compared to the chemical surfactants and other chemicals used in pre-oxidation step **(Hankermeyer *et al.*, 1999)**.

This chapter encompasses the general materials and methods followed throughout the course of the present study. However, materials and methods specific to a given study has been described in relevant chapters

3.1 Weight loss measurement

The dry weight of residual LDPE obtained after the treatment process was calculated. The LDPE surface was washed off using 2% (v/v) of aqueous sodium dodecyl sulphate (SDS) solution. After washing with SDS, the films were washed further with distilled water. The washed LDPE films were collected on the filter paper and dried overnight at room temperature. After drying recovered LDPE films, they were weighed and their weight loss or weight reduction was calculated by using the formula: WL (%) = $[(m_i - m_r)/m_i] \times 100$, where, WL stands for weight loss of samples (%), m_i is the initial weight of sample (g), and m_r is the retention weight of LDPE samples after testing (g) **(Jailawi *et al.*, 2016)**.

3.2 Fourier transform-infrared spectroscopic analysis (FTIR)

The formation or disappearance of the functional groups during the process of degradation can be observed by using FTIR spectroscopic analysis. Spectra in the frequency range of 400–4000 cm^{-1} were used at a resolution of 2 cm^{-1}. The relative absorbance intensities of keto carbonyl bond (1715 cm^{-1}) and internal double bond (908cm^{-1}) to that of the methylene bond at 1465 cm^{-1} were evaluated using the following formulae: Keto- carbonyl bond index (KCBI) = I_{1715}/I_{1465}; and Internal double bond index (IDBI) = I_{908}/I_{1465} (Albertsson et al., 1987). The crystallinity (%) of the LDPE films was measured and calculated using the following formula **(Ambika *et al.*, 2015)**:

Crystallinity % = 100 − [{1 − (I_a/1.233I_b)/1 + (I_a/ I_b)} × 100], where Ia and Ib stands for absorbance values from the groups at 1474 and 1464 cm^{-1} or at 730 and 720 cm^{-1} individually.

3.3 Scanning electron microscopy and Energy dispersive X-ray (SEM-EDX)

The LDPE films were recovered after the treatment process to observe the surface erosion over the LDPE films. The samples were washed using 0.01 M phosphate buffer (pH 7.2). After first step of washing, for analyzing the surface erosion, the LDPE samples were again washed using 2% SDS followed by distilled water to completely remove the particlesthat were adhered to surface. The LDPE samples were fixed using 2% glutaraldehyde, washed twice for 30 min each in 50% ethanol and then incubated overnight in 70% ethanol. LDPE samples were finally washed again three times using 100% ethanol. After the fixation process, the samples were dried and coated with platinum and scanned under the instrument foe SEM-EDX **(Mendez *et al.*, 2007)**.

3.4 X-Ray diffraction (XRD)

The X-ray diffraction patterns of the LDPE films were measured using a X-ray diffractometer kept at ACMS, IIT, Kanpur. It was operated using Cu Kα radiation (λ = 1.5418 A°). The radiations scattered was registered at the angular interval (2⊖) from 5° to 80°. All the diffraction patterns were examined at the room temperature under constant operating conditions **(Jakubowicz, 2003)**.

3.5 Thermal gravimetric analysis (TGA)

TGA was performed using Shimadzu's TGA- 50 series thermo gravimetric analyzers Dynamic measurements kept Central Instrumentation Facility Centre, Jiwaji

University, Gwalior. LDPE samples were executed from 25 to 800°C at a heating rate of 10°C/min under an argon atmosphere (flow rate was 200 ml/min) **(Huang, 2012)**.

3.6 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was performed using Shimadzu Modal no. DSC-60 Plus kept at Central Instrumentation Facility, Jiwaji University, Gwalior. This technique was used to determine the melting temperature of the sample in the temperature range of 30°C to 300°C at heating rate of 10°C/min. The melting temperature was determined from endothermic peaks **(Mathur *et al.*, 2011)**.

4.0 Introduction

Plastics form an important part of our life for many decades. All the wastes that are generated from human activities finally go into the aquatic and terrestrial ecosystem. Plastic is known for its non-biodegradable nature; it can stay in condition for a long time and arranging plastic waste at a landfill sites are risky because dangerous chemicals drain out of it and contaminate the underground water bodies. Generally, polyethylene, which is a commonly used polymer are resistant to natural degradation processes and consequently, it does not break down readily in the environment. For degrading the polyethylene microbial degradation of plastic can be a promising and eco-friendly strategy, which provides a great chance to manage plastic materials waste with minimum adversarial impacts **(Mendez *et al.,* 2007)**. When the polyethylene waste is discarded it persist for the longer period of time in the environment before the microorganism naturally affects its properties **(Kannahi & Sudha, 2013)**. The soil adhered to such discarded plastic waste for a long time develops certain kind of microorganisms, which can have the potential to degrade the polyethylene. Due to certain biological conditions, the microorganisms developed in that condition are more suitably acclimated to the hostile conditions and possess complex characteristic features of adaptation **(Maric & Vranes, 2007)**. Furthermore, the microorganisms are the powerful tools to reduce the polyethylene waste by biodegradation process. Thus, the bacteria isolated from the plastic polluted sites are supposed to be better utilized for the production of biosurfactant producing microorganism in the process of bioremediation of low-density polyethylene (LDPE) **(Kameshwar & Qin, 2016)**. The isolation and screening of the microorganism is continuous process which lead to the identification of best microbial strain effective in polyethylene degradation **(Montazer *et al.,* 2020)**. The present study was done with an aim to screen, identify

and characterize the bacterial strains, capable of degrading low-density polyethylene in the laboratory conditions.

4.1 Materials and methods

4.1.1 Sampling

For sampling partially degraded polyethylene samples along with adhered soil were collected in sterile bags from the various plastic waste dumped sites of Lucknow city, Uttar Pradesh, India. Each sample collected was labeled and all the information, including date of sampling, location and sites were maintained for record and it was brought to the laboratory under the sterile conditions. The collected samples were then processed immediately for isolation of biosurfactant producing bacteria that could be employed for degrading LDPE.

4.1.2 Isolation of bacteria

From the collected waste samples 1 gm of polyethylene along with the adhered soil was weighed and inoculated to the sterile 100 ml synthetic media. The composition of synthetic media included: NH_4NO_3(1.0 g/l), $MgSO_4.7.H_2O$(0.2 g/l), K_2HPO_4 (1.0 g/l), $CaCl_2.2H_2O$(0.1 g/l), KCl(0.15 g/l). The pre-weighed partially degraded polyethylene samples were then added into the medium. The samples were then incubated at 30°C for one week under shake flask condition. After completion of the incubation period, the bacteria were isolated and purified using spread plate technique and subsequent purified cultures were then maintained in Nutrient Agar (NA) slants **(Akbari *et al.*, 2018)**.

4.1.3 Screening for Biosurfactant (BS) production

4.1.3.1 Oil Spreading Technique

For oil spreading technique, 50 ml of distilled water was poured into a large Petri dish of 15 cm diameter, followed by an addition of 20 µl of mustard oil over the surface of water, and finally 10 µl supernatant of culture broth was poured over the mustard oil droplet in the distilled water filled petri dish **(Akbari *et al.,* 2018)**.

4.1.3.2 Emulsification Index (EI_{24})

Emulsification index was estimated by adding 2 ml of mustard oil and 2 ml of cell free supernatant in test tube. The tubes were then vortexed for at least 5 minutes. They were then kept for 24 h at room temperature. EI after the time period of 24 h was calculated by using the following formula **(Cameotra & Makkar, 2010)**:

Emulsification index= 100(height of the emulsion layer/the total height)

4.1.3.3 Phenol-Sulphuric Acid Method

1 ml of 5% (v/v) phenol was added to 1 ml of cell free supernatant. 2.5 ml of concentrated sulphuric acid was then added drop by drop to this mixture, until the characteristic color was developed. Development of orange colour indicated the presence of biosurfactant glycolipids **(Chaprão *et al.,* 2018)**.

4.1.4 Molecular identification of the positively screened bacterial isolates using 16s rRNA Technique

The chromosomal DNA was extracted by using spin column kit. Bacterial 16S rRNA gene (1500 bp) was amplified using polymerase chain reaction in a thermal cycler and was purified using Exonuclease I -Shrimp Alkaline Phosphatase (Exo-SAP). Purified

amplicons were sequenced by Sanger method in ABI 3500xL genetic analyzer (Life Technologies, USA). Basic Local Alignment Search Tool (BLAST) analyzed sequences obtained with closest culture sequence retrieved from the National Centre for Biotechnology Information (NCBI) database that finds regions of local similarity between sequences. The sequences of the identified bacteria were then submitted to NCBI and the accession number was obtained **(Gautam *et al.*, 2007)**.

4.1.5 Morphological and biochemical characterization of bacterial isolates

Gram staining was done to identify the gram stain nature of the isolated bacteria. The biochemical parameters like protease, amylase, catalase, glucose oxidase and laccase were done using HiMedia biochemical test kit **(Hanson *et al.*, 1993)**.

4.2 Result and discussion

4.2.1 Isolation of bacteria

Total fifteen bacteria were isolated from the polyethylene samples collected from plastic polluted dumpsites (Plate 4.1, 4.2). They were labeled as ENV1, ENV2, ENV3, ENV4, ENV5, ENV6, ENV7, ENV8, ENV9, ENV10, ENV11, ENV12, ENV13, ENV14, and ENV15.

4.2.2 Screening of isolated bacteria

4.2.2.1 Oil Spreading Technique

According to oil spreading technique only two bacteria ENV1 and ENV4 was found to be positive for the presence of biosurfactant (Plate 4.3). Other isolates showed negative result for the production of biosurfactant. In oil spreading test, a clear zone was observed at the oil water interface, which confirmed the presence of biosurfactant

by both the bacteria. The zone of displacement for ENV1 was approximately 2 cm whereas that of ENV4 was approximately 2.5 cm **(Muthezhilan *et al.*, 2014; Diaz *et al.*, 2016)**.

4.2.2.2 Emulsification Index (EI_{24})

In emulsification index, the heights of each layer were taken and changes in heights were noted after the settling period. The emulsification activity was observed using mustard oil. The emulsification index for ENV1 was 51 % and ENV4 was 59%, which was highest among all the bacterial isolates and showed low percent values of emulsification index **(Cameotra & Makkar, 2010; Sachdev & Cameotra, 2013)**.

4.2.2.3 Phenol-Sulphuric Acid Method

The phenol-sulfuric acid test was done to identify the type of biosurfactant produced by the bacteria. Only two bacteria were ENV1 and ENV4 was found to be positive for the production of biosurfactant as they developed orang color upon adding the phenol and Sulphuric acid. Both the strains of bacteria produced orange color in the presence of crude extract (Plate 4.4). The phenol-sulfuric acid test confirms the presence of rhamnolipid biosurfactant produced by both the strains of bacteria. The other bacterial isolates were considered negative for this test because of absence of biosurfactant **(Ibrahim *et al.*, 2013; Joshi *et al.*, 2014)**.

4.2.3 Identification analysis of the positively screened bacterial isolates

Sequence alignment revealed that ENV1 was highly related (99% identity) to *Bacillus* species, such as *Bacillus paramycoids* (NR_157734) and *Bacillus anthracis* (CP007666). Therefore, bacterium ENV1 was identified as one of the novel strain of *Bacillus* species and named as *Bacillus* sp strain 530F_seq260_Env 1. The nucleotide

sequences were submitted to NCBI and the accession number is MN559801-MN559803. In the neighbor-joining tree, *Bacillus* sp strain 530F_seq260_Env1 was clustered with most known strains of *Bacillus* species (Figure 4.1). The other bacterial isolate ENV 4 showed closest homology (99% identity) to *Lysinibacillus macroids* (KY643638) and *Lysinibacillus macroids* NR_114920. Therefore, the bacterium ENV 4 was identified as one of the novel strain of *Lysinibacillus* species and named as *Lysinibacillus* sp strain DESBBAU2. The nucleotide sequences were submitted to NCBI. The accession number is MN715876-MN715869. In the neighbor-joining tree, *Lysinibacillus* sp strain DESBBAU2 was clustered with the strains of *Lysinibacillus* species (Figure 4.2) **(Mukherjee *et al.*, 2011)**.

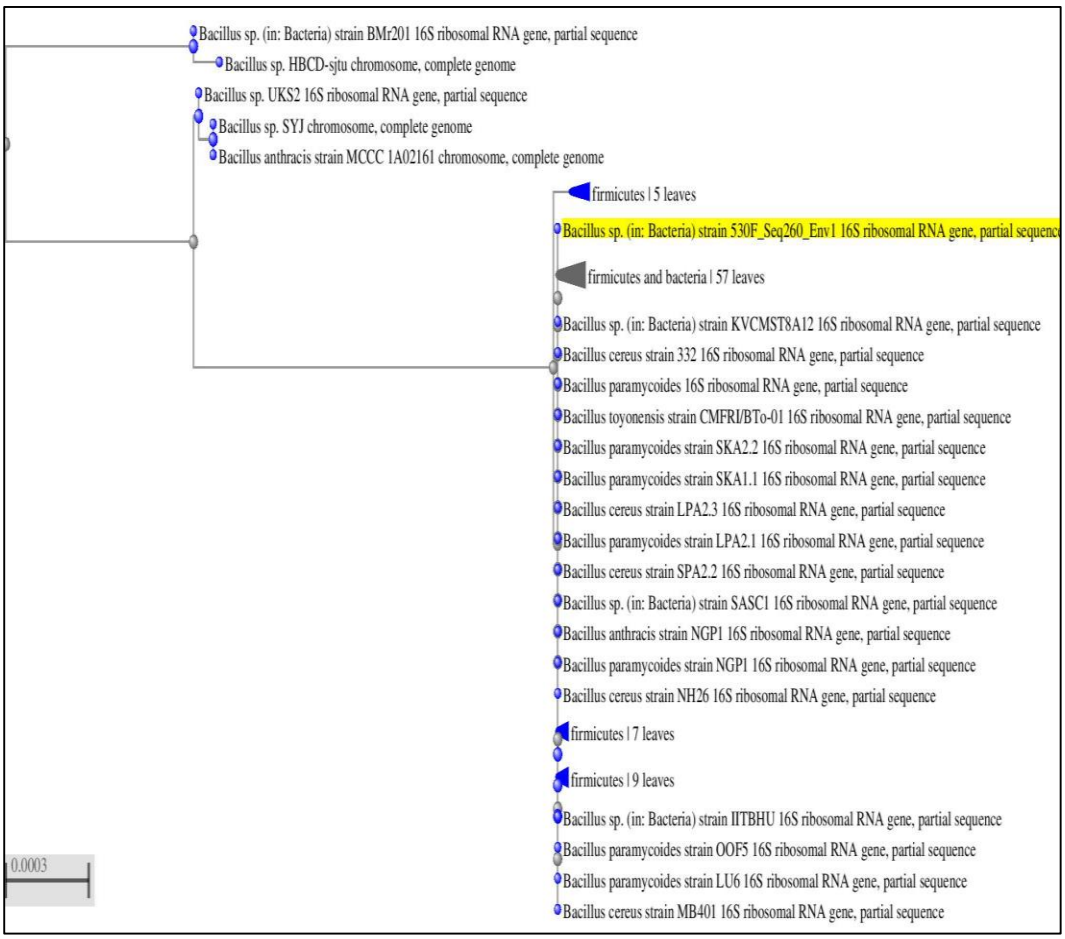


Figure 4.1: Phylogenetic tree of *Bacillus* sp strain 530F_seq260_Env1

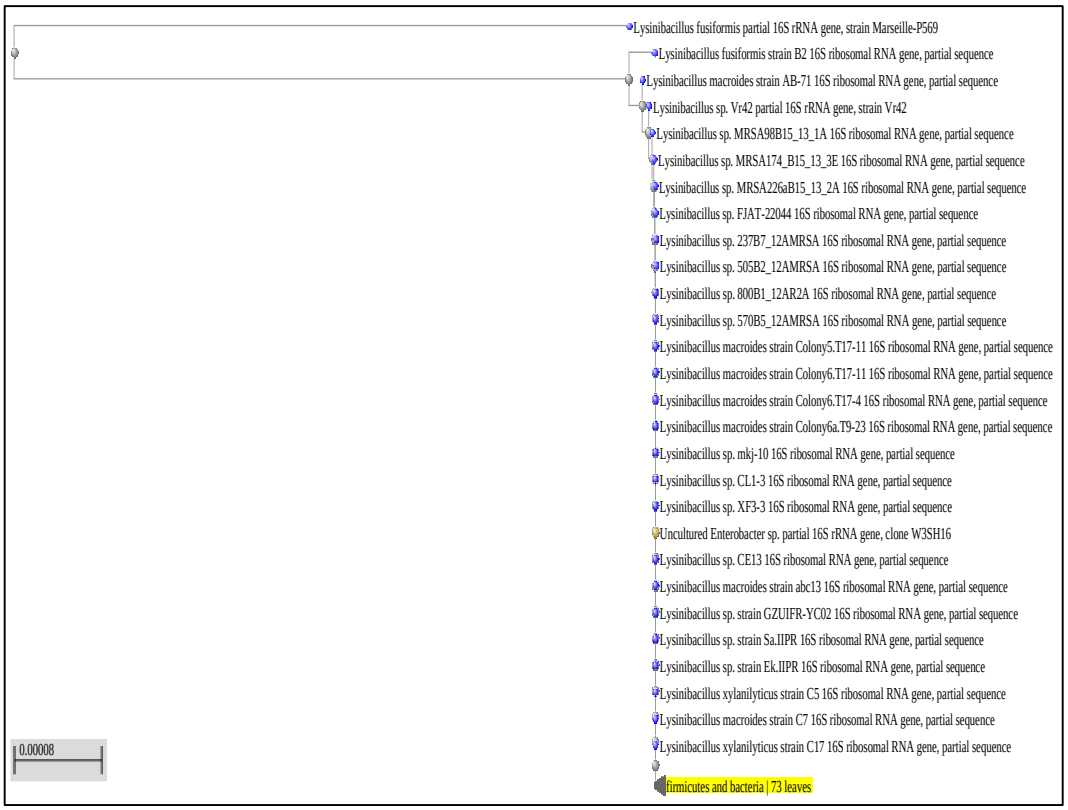


Figure 4.2: Phylogenetic tree of *Lysinibacillus* sp strain DESBBAU2

4.2.4 Morphological and biochemical characterization of bacterial isolates

Morphological characterization was done on the basis of gram staining method, which revealed that *Bacillus* sp strain 530F_seq260_Env1 and *Lysinibacillus* sp strain DESBBAU2 both are gram positive in nature (Plate 4.5).

The biochemical characterization test for both the bacteria revealed that *Bacillus* sp strain 530F_seq260_Env1 gave positive results for protease, amylase, catalase and glucose whereas *Lysinibacillus* sp strain DESBBAU2 was found to be positive for oxidase and laccase (Table 4.1) **(Mukherjee *et al.*, 2015)**.

Table 4.1: Morphological and biochemical characterization of isolated bacteria

Characteristics	***Bacillus* sp. strain 30F_Seq260_Env1**	***Lysinibacillu* sp. strain DESBBAU2**
Gram stain	Positive	Positive
Cell Shape	Rod	Rod
Colony Shape	Rough	Smooth
Colony margin	Filamentous	Circular
Colony elevation	Flat	Elevated
Protease	Positive	Negative
Amylase	Positive	Negative
Catalase	Positive	Negative
Indole production	Negative	Negative
Glucose	Positive	Negative
Oxidase	Negative	Positive
Deamination of phenylalanine	Negative	Negative
Laccase	Negative	Positive

4.2.5 Conclusion

For isolating the biosurfactant producing bacteria waste polyethylene samples were collected from plastic polluted dumpsites. Total fifteen bacteria were isolated which were then screened for the production of biosurfactant on the basis of oil spreading test, emulsification index test and phenol-sulphuric acid test. The biosurfactant produced are basically rhamnolipid in nature as indicated by phenol-sulphuric acid test. Among all the isolated bacteria only two bacteria ENV1 and ENV4 were screened positively for the production of biosurfactant. These two bacteria were further identified as the novel bacteria strains of *Bacillus* sp strain 530F_seq260_Env1 and *Lysinibacillus* sp strain DESBBAU2. These two isolated strains were further used for LDPE degradation.

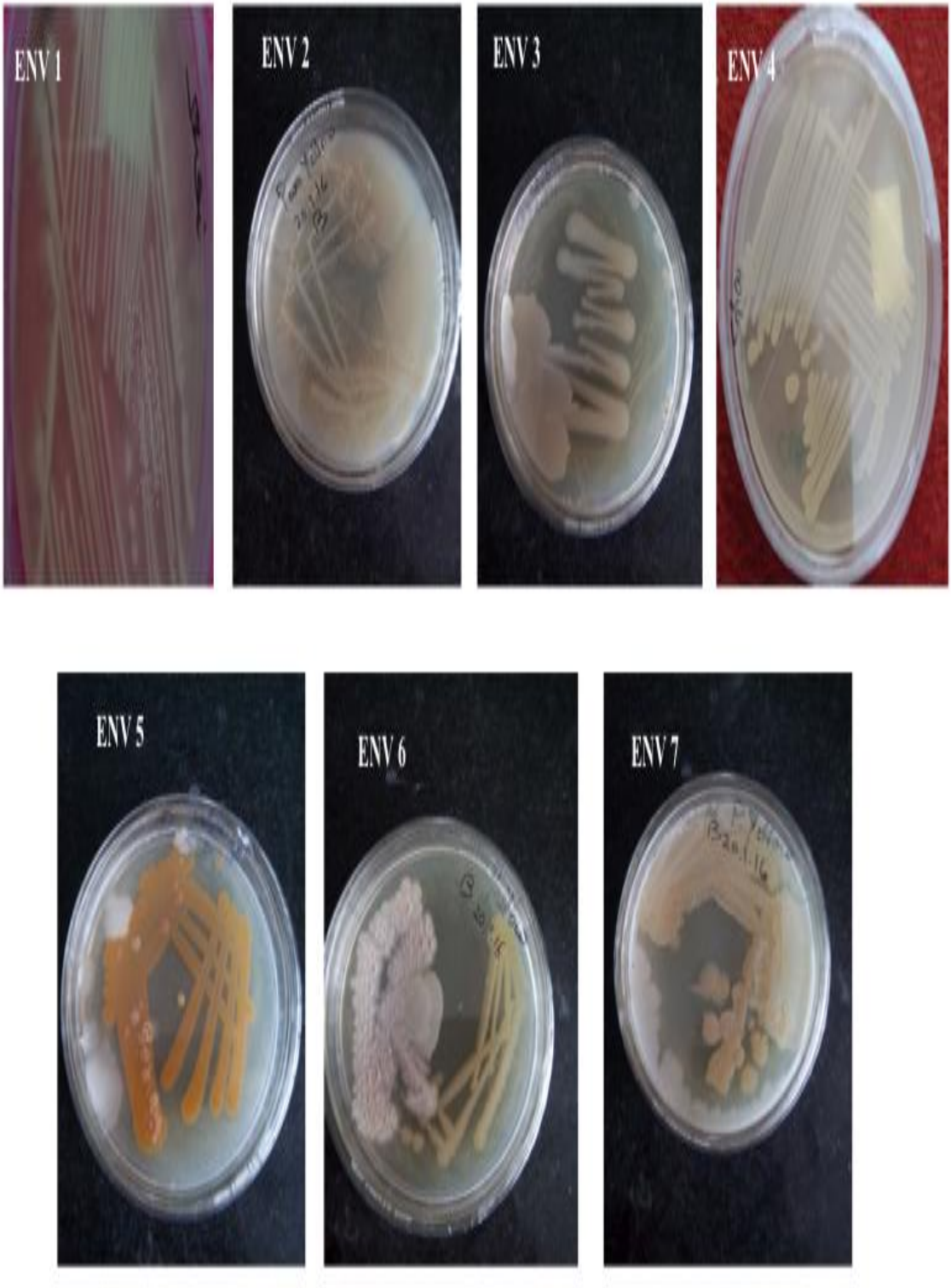


Plate 4.1: Bacteria isolated from plastic polluted dumpsites (labeled from ENV1 to ENV7)

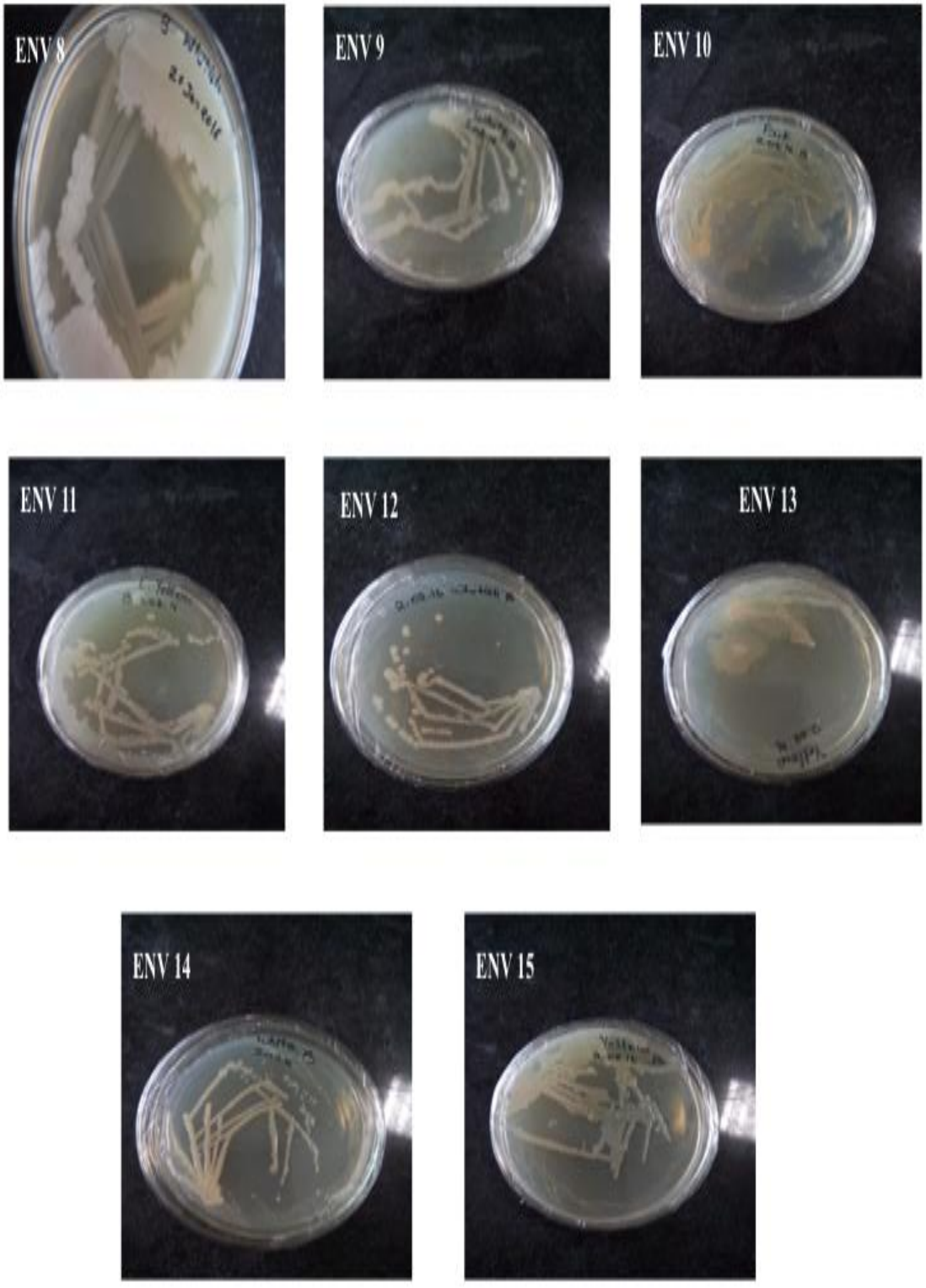


Plate 4.2: Bacteria isolated from plastic polluted dumpsites (labeled from ENV8 to ENV15)

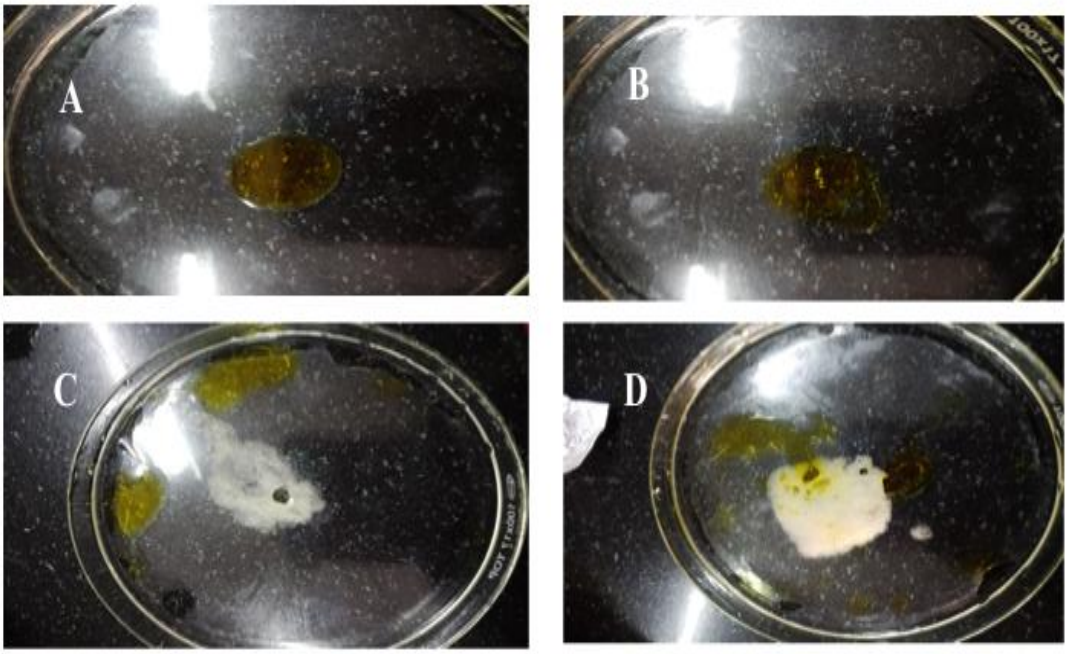


Plate 4.3: Oil spreading test (A) Oil drop over the water (B) Negative oil drop test for water (C) Oil spread by *Bacillus* sp (D) Oil spread by *Lysinibacillus* sp

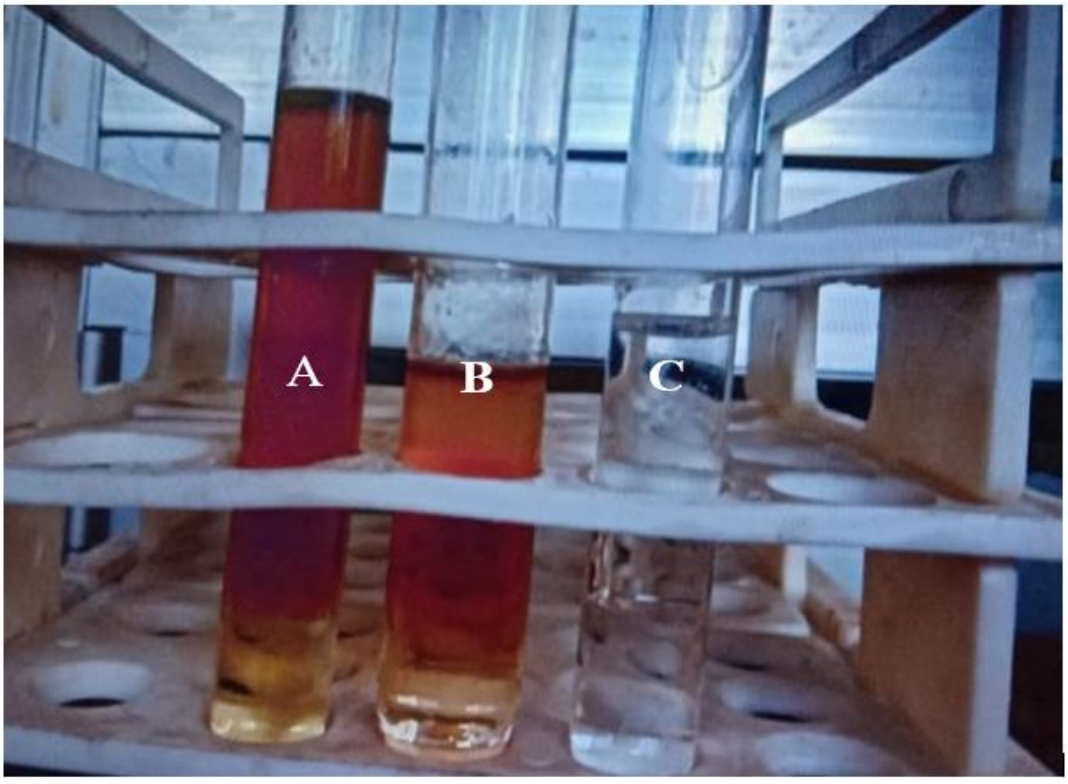


Plate 4.4: Phenol sulfuric acid test of both the biosurfactant (A: ENV1 and B: ENV4, C: Control) extracted from bacteria

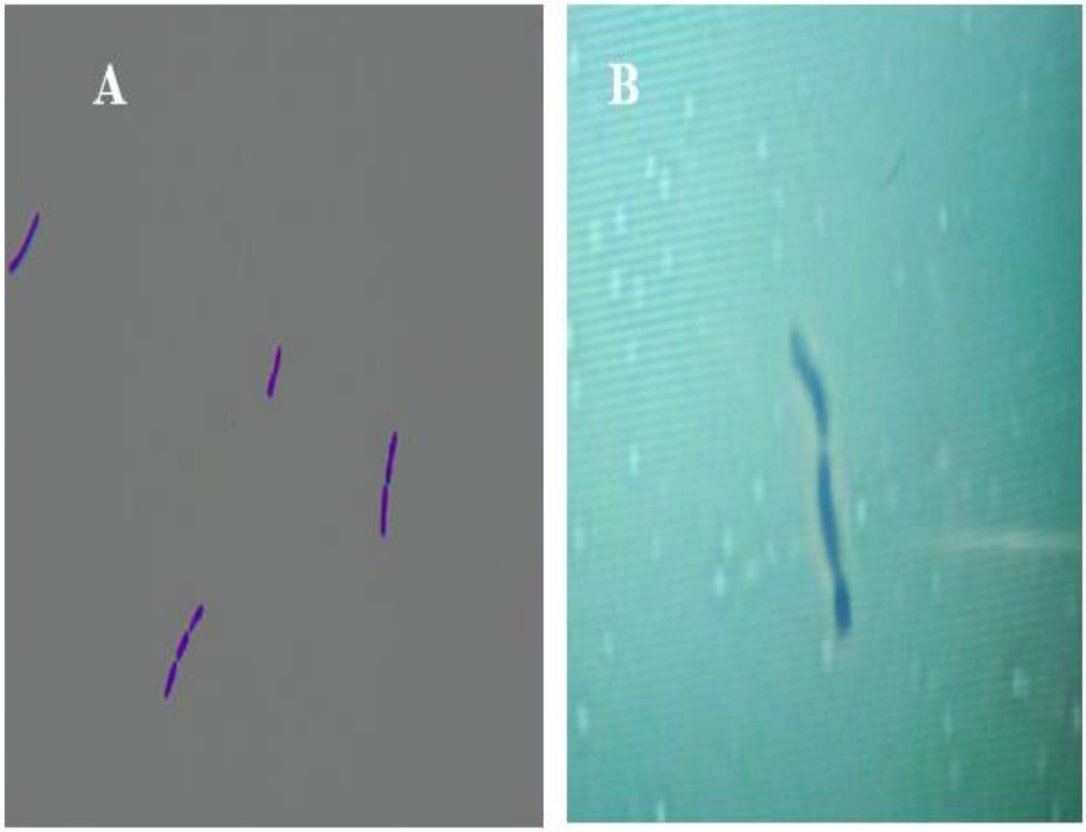


Plate 4.5: Gram staining images (A) *Bacillus* sp strain 530F_seq260_Env1 (B) *Lysinibacillus* sp strain DESBBAU2

5.0 Introduction

Surface-active agents that are produced by different groups of microorganisms are commonly known as biosurfactant **(Akbari *et al.,* 2018)**. Biosurfactants are known to reduce the surface tension in both aqueous and hydrocarbon mixture. Biosurfactant have capability to get aggregated at the interface between fluids of different polarities, like water and oil, leading to the decrease of interfacial tension. Biosurfactants possess hydrophobic and hydrophilic moieties **(Chaprao *et al.,* 2018)**. The hydrophilic moiety of a biosurfactant is made of carbohydrate, an amino acid or a peptide, and the hydrophobic moiety is made of saturated or unsaturated fatty acid **(Das *et al.,* 2014)**. According to this biosurfactants are classified as glycolipids, lipopeptides, lipoproteins, particulate, and polymeric biosurfactants **(Diaz *et al.,* 2015)**. Due to the adverse environmental impacts of chemical surfactants, researchers are focusing upon the replacement of these chemicals by biosurfactants. Biosurfactants have several benefits over chemical surfactants, like better biodegradability, low toxicity, environmental compatibility, and highly specific activity under extreme temperature, pH and **salinity (Meena & Kanwar, 2015; Sharma *et al.,* 2015)**. Despite such advantages, biosurfactants are not very competitive with their synthetic counterparts because of the high production costs. Therefore, many surfactants that are commercially available are made from the petrochemical industry, which presently accounts for 70–75% of all surfactants that are used in industrialized nations **(Santos *et al.,* 2016)**. Though, with the increased awareness for the environmentally friendly compounds, industries are now seeking to replace the chemical based surfactants with sustainable biosurfactants **(Ferradji *et al.,* 2014)**. As a result, the global market for the biosurfactants has been increased in recent years. In order to reduce the costs and increase the competitiveness for the

biosurfactant production there is a need to develop the economical process for producing the biosurfactant **(Jemil *et al.*, 2016)**. Response surface methodology (RSM) has been efficiently employed to decrease the production cost of biosurfactants **(Costa *et al.*, 2010)**. A Central Composite Rotational Design (CCRD) is generally used with the RSM to assess the relationship between one or more response variables and the set of quantitative experimental factors **(Akbari *et al.*, 2018)**. As the correlation between the response and independent variables is unknown at the beginning of a process, the first step in RSM needs to approximate the function *i.e.*, response by analyzing the factors *i.e.*, independent variables **(De Rienzo *et al.*, 2016)**. So, the aim of the present study was optimization, characterization and production of biosurfactant by the two positively screened bacteria *Bacillus* sp Strain 530F_seq260_Env1 and *Lysinibacillus* sp. strain DESBBAU2 under batch experimentation using the combination of CCRD and RSM.

5.1 Materials and methods

5.1.1 Optimization of biosurfactant production

The traditional method of optimization does not ensure the optimal values of selected variables in biosurfactant production. Use of an optimal design eliminates the classic designs from consideration; hence, a statistical optimization strategy, which is based on RSM, was applied **(Duddu *et al.*, 2015)**. This is a statistical and mathematical method used for analyzing the effects of independent variables on the response of a system. Components of the culture medium and environmental conditions greatly influence the amount of biosurfactant produced **(Hassan, 2014)**. Therefore, the optimization can be performed using mathematical and statistical tools to increase the production yield and lower the production cost. For optimization of the growth culture

and determination of the optimal conditions for maximum production of biosurfactant, a central composite rotational design (CCRD) was used. Four factors in five levels were considered **(Ibrahim *et al.*, 2013)**. Four factors taken for the study were temperature, pH, peptone concentration and beef extract concentration. The effect of the selected variables on biosurfactant yield and their interactions were evaluated using Design-Expert software version 10. A total of 30 experiments were done using a CCRD with 8 factorial points, 6 axial points and 4 central points **(Mukherjee *et al.*, 2009)**.

5.1.2 Characterization of biosurfactant

5.1.2.1 Estimation of protein

Estimation was done using Bradford protein assay **(Lowry *et al.*, 1951)**.

Reagents

- 2% Na2CO3 in 0.1 NaOH
- 0.5% CuSO4.&H2O in 1% Sodium Potassium Trtarate
- 1 Part of reagent A + 1 Part of reagent B
- 1 Part of Folin reagent + 2 Part of Distilled water

Stock Bovine Serum Albumin (BSA) Solution (10ppm)

1o mg of BSA was dissolved in 10 ml of 0.1 N NaOH

Method

About 0.5 mg of the sample was homogenized in 10 ml of 80% acetone and centrifuged at 5000 rpm for 15 min. The supernatant was discarded and pellet was dissolved in 10 ml of 0.1 N NaOH by heating for 10 min and was centrifuged at

5000 rpm for 15 min. 0.5 of supernatant was taken in clean dry test tube and 5 ml of reagent C was added and left for 10 min. After 10 min 0.5 ml of reagent D was added rapidly, the solution turns lue in color. The OD was taken at 700 nm by spectrophotometer.

5.1.2.2 Estimation of lipids

Lipid estimation was done according to the gravimetric method, which involved following steps atmosphere **(Nikolopoulou & Kalogerakis, 2008)**:

- For the estimation of lipid in the sample, 100 mg of the sample was homogenized with 10 ml of chloroform: methanol.
- Then the extract was mixed with 1ml of distilled water and was allowed to separate in two distinct phases.
- The upper layer was removed by micropipette and the lower was collected in a dish and the weight was measured.
- The content was then dried and the final weight was taken. The final weight subtracted from the initial weight was taken as the total lipid content of the sample.

5.1.2.3 FTIR of biosurfactant

FTIR spectroscopy was used for the determination of chemical structures of the components present in the crude biosurfactant sample. One milligram of lyophilized biosurfactant was grounded with 100 mg of KBr to get translucent pellets. The KBr pellet was used for the background reference. The IR spectra were then recorded on a Perkin Elmer FTIR instrument **(Mani *et al.*, 2016)**.

5.1.2.4 Thermal gravimetric analysis

Thermal gravimetric analysis (TGA) is known as a method used to characterize materials. This technique generally measures the amount and change in rate in the mass of the analyzed sample as a function of time or an increasing temperature (at a constant heating rate) and determines the thermal stability as well as compositional characteristics of the material. About 10 mg of the biosurfactant sample was placed onto a platinum pan and its weight loss was recorded at a heating rate of 10° C min^{-1} from 30–800° C. The test was performed under nitrogen atmosphere **(Nikolopoulou & Kalogerakis, 2008)**.

5.1.2.5 Differential scanning calorimetry

Differential Scanning Calorimetry (DSC) was performed using Shimadzu Modal no. DSC-60 Plus kept at central instrumentation facility, Jiwaji University, Gwalior. This technique was used to determine the melting temperature of the sample in the temperature range of 30°C to 300°C at heating rate of 10°C/min. Approximately 15 mg of biosurfactant were taken in sample holder and inserted inside the instrument for the analysis and The melting temperature was determined from endothermic peaks **(Pornsunthorntawe *et al.*, 2008)**.

5.1.3 Extraction and production of biosurfactant

The bacterial broth (10 ml) was inoculated into the medium nutrient broth along with the addition of mustard oil (1% v/v) and the pH value was adjusted to 7.0 (Plate 5.1). Incubation was carried out at 30°C and 150 rpm, for 72 h. The extraction technique was the combination of acid precipitation and the solvent extraction process (Plate 5.2). The broth culture sample was then centrifuged (at 4°C using 8,000 rpm for 15

min). The obtained supernatant was then treated by acidification to pH 2.0 by using 6 M HCl. The acidified supernatant was kept overnight at 4°C for complete precipitation of the biosurfactants. Then methanol and chloroform was added in 2:1 ratio in the supernatant mixture and stirred well for 1 h on magnetic stirrer. The mixture was kept for 24 h to get the aqueous and the organic layer separated from each other. After 24 h the aqueous layer was removed and organic layer was retained for the solvent extraction process (Plate 5.3). It was then evaporated to dryness using rotary evaporator. The obtained residue was then dried and weighed **(Patowary *et al.*, 2017)**.

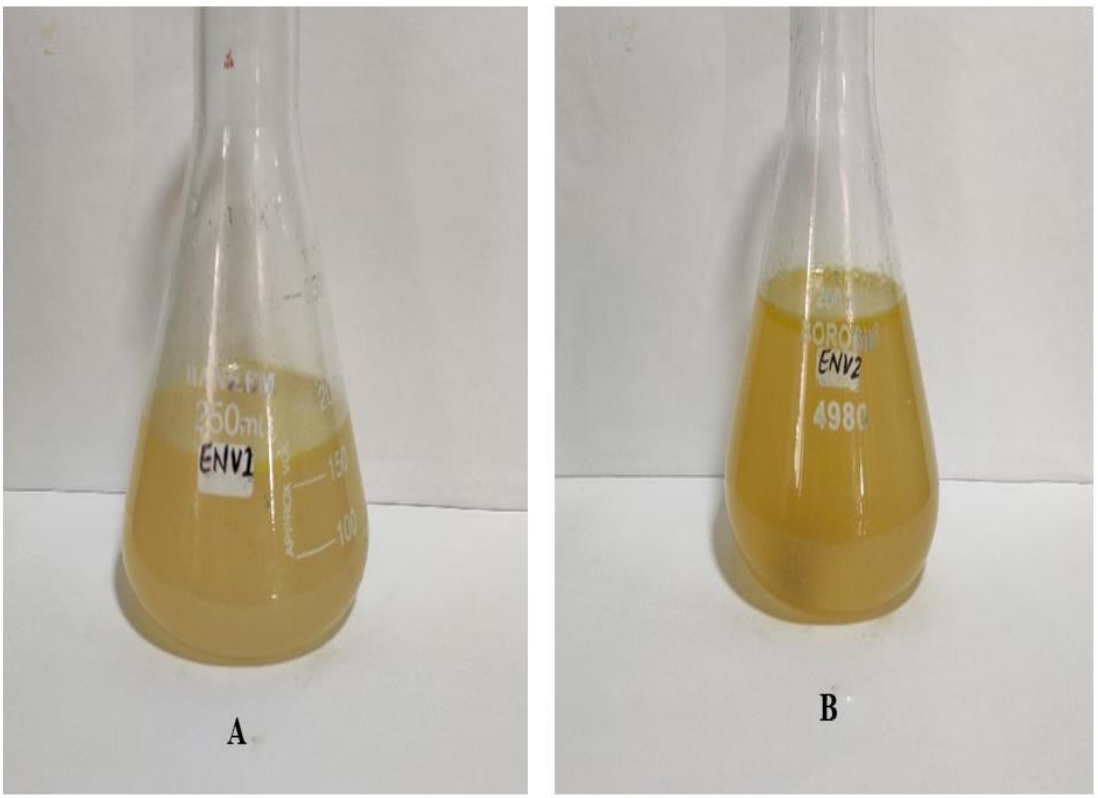
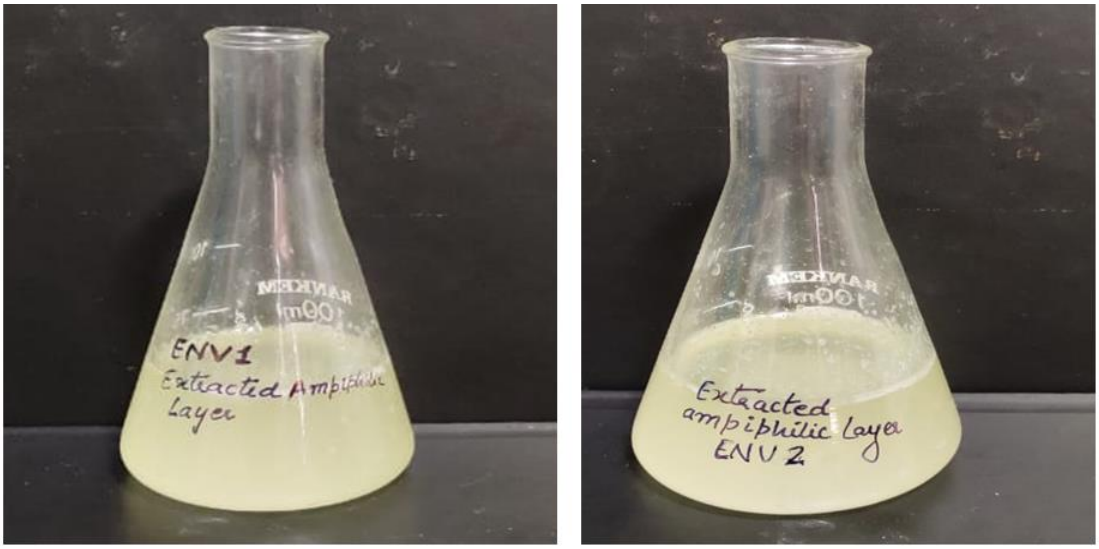
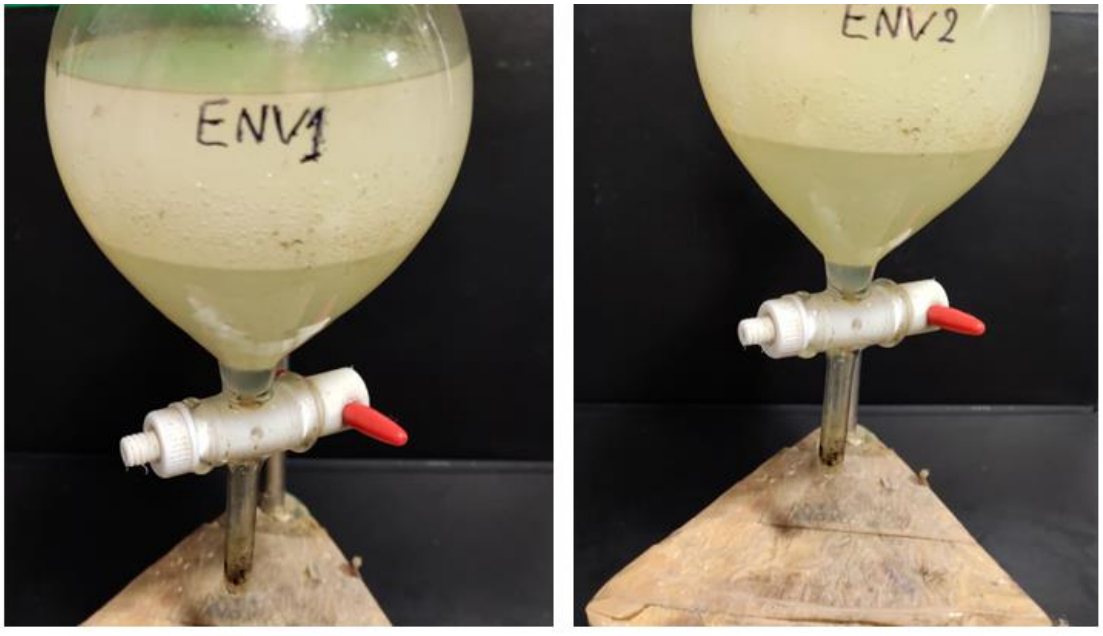


Plate 5.1: Media of Nutrient Broth inoculated with (A) *Bacillus* sp (ENV1) (B) *Lysinibacillus* sp (ENV2)



A **B**

Plate 5.2: Extracts obtained after solvent extraction and acid precipitation (A) *Bacillus* sp (ENV1) (B) *Lysinibacillus* sp (ENV2)



A **B**

Plate 5.3: Solvent extraction of the Biosurfactant (A) *Bacillus* sp (ENV1) (B) *Lysinibacillus* sp (ENV2)

5.2 Results and Discussion

5.2.1 Process optimization for *Bacillus* sp Strain 530F_seq260_Env1

5.2.1.1 Statistical modeling

Table 5.1 lists the experimental design and its results. The experimental results obtained were fitted to a modified quadratic polynomial model. The regression coefficients were calculated and fitted to the following quadratic model **(Randhawa & Rahman, 2014)**:

Emulsification index (%)=

32.34-2.03*A+0.2683*B+6.00*C+2.13*D+4.82*AB+6.33*AC+0.1525*AD

+1.28*BC-10.80*BD+4.70*CD

A, B, C and D denotes the actual pH, temperature, concentration of beef and concentration of peptone respectively. Analysis of variance (ANOVA) at a p-value < 0.05 was executed to assess the effect of all the process variables on the emulsification index of the biosurfactant as the response of the system.

Table 5.1: Experimental deign matrix for optimization of biosurfactant optimization produced by *Bacillus* sp according to CCRD.

Std	Run	Factor 1 A:pH	Factor 2 B:Temperature	Factor 3 C:Beef mg/l	Factor 4 D:Peptone mg/l	Response 1: E_{24}
7	1	6	50	10	5	32
8	2	8	50	10	5	52
24	3	7	40	7.5	12.5	40
29	4	7	40	7.5	7.5	52.34
3	5	6	50	5	5	48
26	6	7	40	7.5	7.5	51
16	7	8	50	10	10	51.2
27	8	7	40	7.5	7.5	32
25	9	7	40	7.5	7.5	20
2	10	8	30	5	5	11
21	11	7	40	2.5	7.5	18
20	12	7	60	7.5	7.5	21
13	13	6	30	10	10	57.36
4	14	8	50	5	5	29
1	15	6	30	5	5	23
30	16	7	40	7.5	7.5	17
23	17	7	40	7.5	2.5	42
11	18	6	50	5	10	17
5	19	6	30	10	5	15
12	20	8	50	5	10	13
10	21	8	30	5	10	22
6	22	8	30	10	5	13.4
19	23	7	20	7.5	7.5	30
15	24	6	50	10	10	21
28	25	7	40	7.5	7.5	35
14	26	8	30	10	10	48
18	27	9	40	7.5	7.5	23
17	28	5	40	7.5	7.5	36
22	29	7	40	12.5	7.5	51
9	30	6	30	5	10	49

5.2.1.2 Statistical analysis

The Table 5.2 shows the results of ANOVA for the effect of process variables on the emulsification index of biosurfactant, which in turn signifies the yield of biosurfactant. The p- value (<0.0034), lack of fit (0.9768) and high R^2 value (0.9899) indicates that the modified quadratic model correlated well with the experimental values **(Roy, 2017)**. Figure 5.1 shows the plots of the results predicted by the model versus the actual results of emulsification index. Most of the points occurred around the line, which means that there was good conformity between the experimental data and values predicted by the model. Variables with p-values of ≤0.05 were considered significant. Factor C, beef concentration, had the greatest effect on response **(Costa *et al.*, 2010; Marchant & Banat, 2012).**

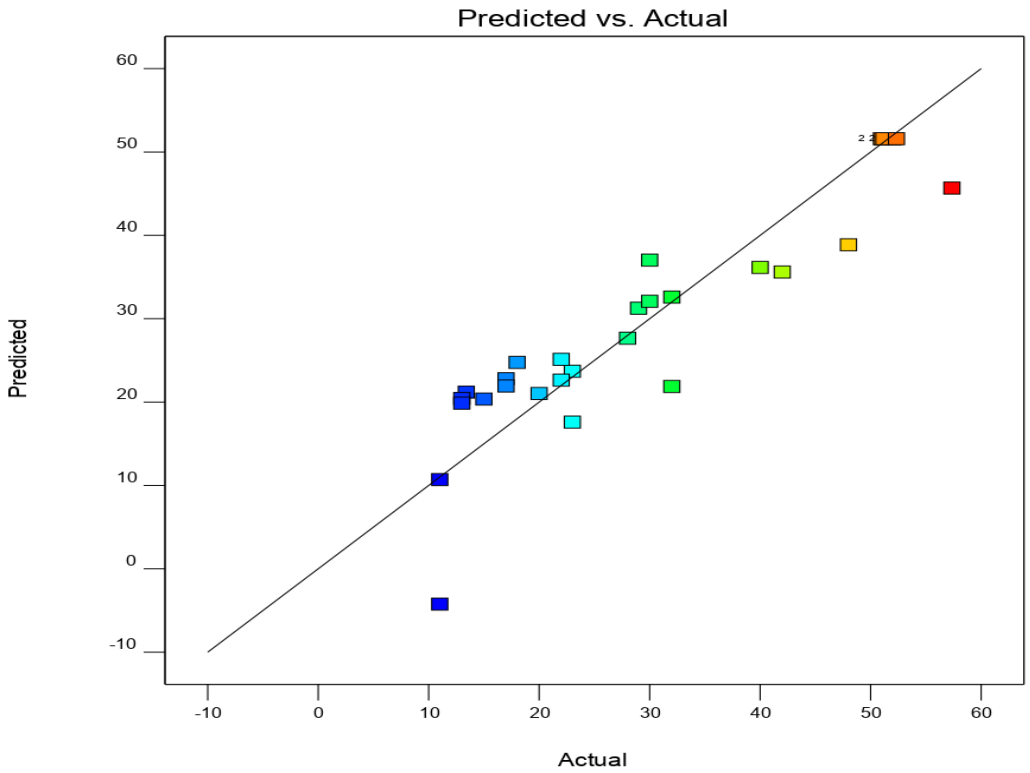


Figure 5.1: Actual vs predicted emulsification index of biosurfactant

Table 5.2: Analysis of variance for response surface quadratic model regarding surface tension achieved with biosurfactant produced by *Bacillus* sp

Sources	Sum of squares	df	Mean square	F-value	p-value
Model	4330.99	10	433.10	4.23	0.0034
A-pH	99.08	1	99.06	0.9668	0.3378
B-Temperature	1.73	1	1.73	0.0169	0.8980
C-Beef extract	863.52	1	863.52	8.43	0.0091
D-Peptone	109.06	1	109.06	1.06	0.3152
AB	372.10	1	372.10	3.63	0.0719
AC	640.60	1	640.60	6.25	0.0217
AD	0.3721	1	0.3721	0.0036	0.9526
BC	26.11	1	26.11	0.2548	0.6195
BD	1865.38	1	1865.38	18.20	0.0004
CD	353.06	1	353.06	3.45	0.0790
Residual	1946.92	19	102.47		
Lack of Fit	833.42	14	59.53	0.2673	0.9768
Pure Error	1113.50	5	222.70		
Cor Total	6277.91	29			

5.2.1.3 Effective parameters interaction

Figure 5A-F shows the 3D plots for maximum emulsification index (i.e., maximum biosurfactant production) and reveals the interactions of the independent variables, two by two.

(a) The Figure 5.2A shows the effect of pH and temperature on biosurfactant production at fixed beef extract and peptone concentration *i.e.*, 7.5 mg/l and 7.5 mg/l respectively. It can be observed that by increasing the temperature above 40 °C the biosurfactant production got decreased whereas in case of pH, an alkaline pH discouraged the production of biosurfactant .

(b) The Figure 5.2B shows the effect of pH and concentration of beef extract at fixed temperature and peptone concentration of 40 °C and 7.5 mg/l respectively. By increasing the concentration of beef extract from 5 mg/l to 10 mg/l the emulsification index got increased which signifies that the higher amount of carbon source in the medium gives good production of biosurfactant.

(c) The Figure 5.2C shows the effect of peptone concentration and temperature on production of biosurfactant at fixed pH and beef extract concentration of 7 and 7.5 mg/l respectively. By increasing the peptone concentration, the emulsification index got increased.

(d) The Figure 5.2D shows the effect of peptone concentration and beef concentration at fixed pH and temperature of 7 and 40°C respectively. It can be presumed that higher peptone and beef concentration can enhance the biosurfactant production.

(e) The Figure 5.2F shows the effect of beef extract concentration and

temperature on production of biosurfactant at fixed pH and peptone concentration of 7 and 7.5 mg/l respectively. The graph shows that by increasing the beef concentration and by decreasing the temperature up to 30 °C, the biosurfactant production got increased.

(f) The Figure 5.2E shows the effect of peptone concentration and pH on production of biosurfactant at fixed temperature and beef concentration of 40°C and 7.5 mg/l respectively. It can be deduced that the combination of higher peptone concentration (10 mg/l) and higher pH (7.5) may enhance the biosurfactant production.

5.2.1.4 Conformation experiment

The model predicted the maximum emulsification index of 57.36% at optimum beef concentration (10 mg/l), peptone concentration (10 mg/l), pH 6 and temperature (30 °C). Conformation testing was accomplished in triplicate to validate the model at the predicted optimal points. The actual value for emulsification index was 59.71%, about 3% deviation as compared to the predicted emulsification index. The minimum and maximum values at the 95% confidence interval were 28.48 and 36.21, respectively **(Hu *et al.*, 2015)**.

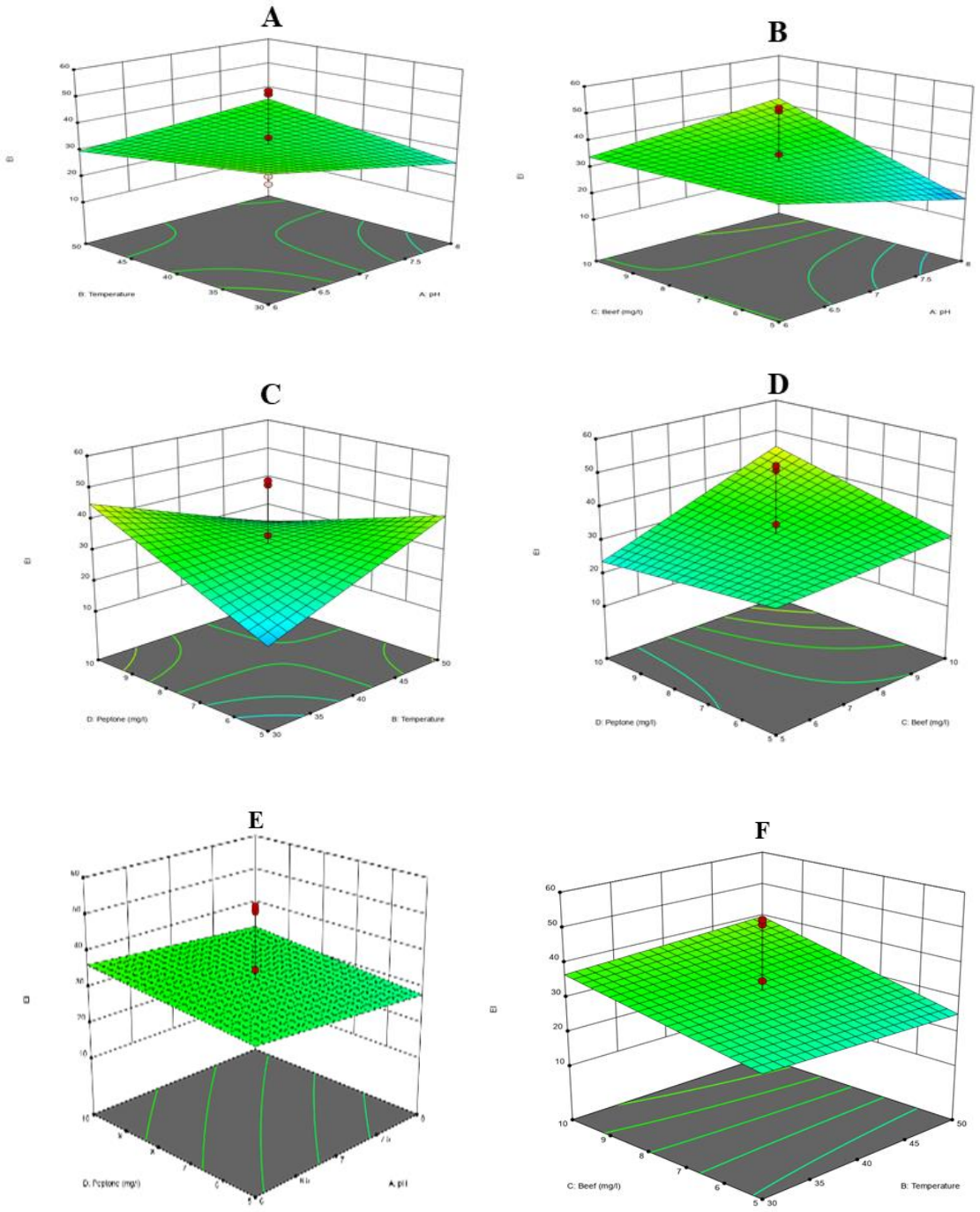


Figure 5.2: Response surface plots of biosurfactant production from *Bacillus* sp (A) Interactive effect of pH and temperature(B) Interactive effect of Beef extract and pH(C) Interactive effect of Peptone concentration and temperature (D) Interactive effect of Peptone concentration and Beef extract (E) Interactive effect of Peptone concentration and pH(F) Interactive effect of Beef extract and temperature

5.2.2 Process optimization for *Lysinibacillus* sp strain DESBBAU2

5.2.2.1 Statistical modeling

Table 5.3 given below shows the experimental design and its result. The experimental data were then fitted by stepwise regression using the design expert software. A multivariate quadratic regression model was achieved:

Emulsification index= +46.50+3.42*A-12.75*B-1.08*C+0.8333*D-1.75*AB+0.7500*AC+1.50*AD+1.25*BC+1.00*BD+0.00*CD-4.77*A^2-0.7708*B^2 -0.2708*C^2+0.1042*D^2

A, B, C and D denotes the actual pH, temperature, concentration of beef and concentration of peptone respectively. The model F-value of 6.72 implies the model is significant. There is only a 0.04% chance that an F-value this large could occur due to noise **(Ismail *et al.*, 2013)**.

5.2.2.2 Statistical analysis

For a good statistical model, there should be R^2 = 0–1.0. A R^2 value that is closer to 0 is an indicative of a better model and the actual value which is closer to the predicted value predicts a better model. The regression equation that was obtained by ANOVA showed that the established model had R^2 = 0.8432. Additionally, the model predicted showed the high statistical significance. The correction coefficient was: R^2adj = 0.7341; that is, the model could explain 17.40 % of variation in the response value. The prediction coefficient was: R^2pre = 0.3234; the lack of fit items was not significant (F = 0.2187, p = 0.0004 > 0.05), indicating that the model fully reflects the actual situation (Table 5.4). The above data demonstrated good fit of the model.

Hence, the proposed model can be used for analyzing and predicting the effects of pH, temperature, beef extract concentration, peptone concentration on the biosurfactant production from *Lysinibacillus* sp strain DESBBAU2. Figure 5.3 shows the plots of the results predicted by the model versus the actual results. Most of the points occurred around the line, which means that there was good conformity between the experimental data and values predicted by the model **(Das *et al.*, 2014; Joshi & Shekhawat, 2014)**.

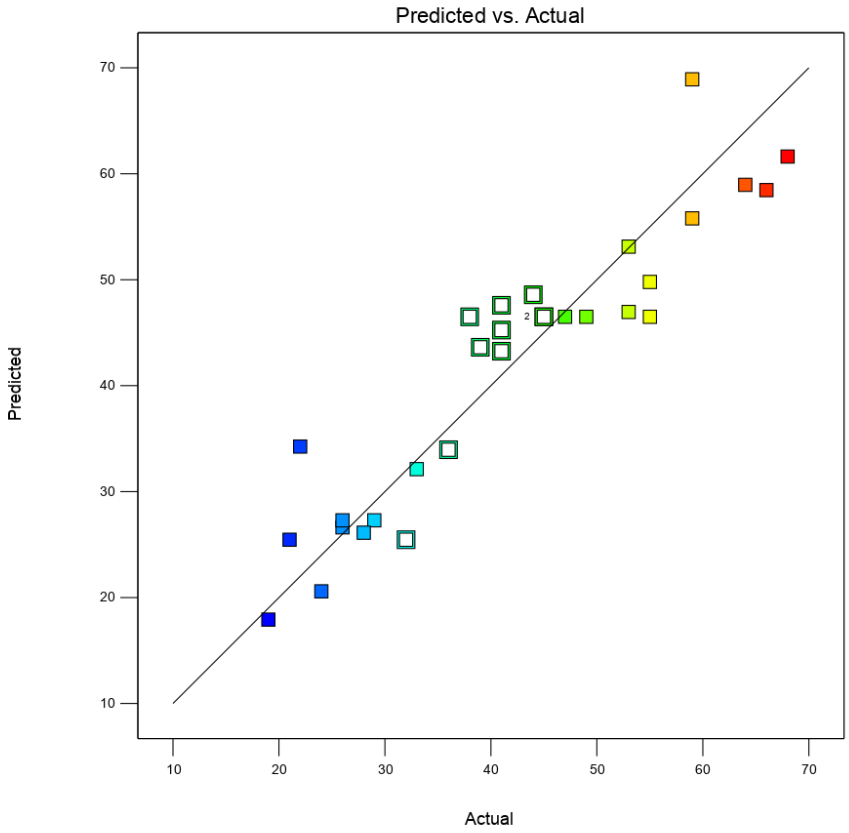


Figure 5.3: Actual vs predicted emulsification index of biosurfactant

Table 5.3: Experimental deign matrix for optimization of biosurfactant optimization produced by *Lysinibacillus* sp according to CCRD.

Std	Run	Factor 1 A:pH	Factor 2 B:Temperature	Factor 3 C:Beef mg/l	Factor 4 D:Peptone mg/l	Response 1: E_{24}
2	1	8	30	5	5	64.53
1	2	6	30	5	5	53.24
24	3	7	40	7.5	12.5	44.83
21	4	7	40	2.5	7.5	41.69
22	5	7	40	12.5	7.5	41.32
27	6	7	40	7.5	7.5	38.67
11	7	6	50	5	10	26.37
6	8	8	30	10	5	59.78
17	9	5	40	7.5	7.5	24.81
26	10	7	40	7.5	7.5	55.21
4	11	8	50	5	5	32.18
5	12	6	30	10	5	53.68
20	13	7	60	7.5	7.5	19.62
7	14	8	50	10	5	21.54
10	15	6	30	5	10	68.11
14	16	7	30	10	10	62.78
19	17	6	20	7.5	7.5	59.04
29	18	8	40	7.5	7.5	47.39
25	19	8	40	7.5	7.5	45.02
13	20	7	30	10	10	39.78
28	21	7	40	7.5	7.5	45.68
9	22	7	30	5	10	55.29
23	23	6	40	7.5	2.5	41.13
16	24	7	50	10	10	36.14
15	25	6	50	10	10	28.88
12	26	8	50	5	10	33.45
18	27	9	40	7.5	7.5	22.53
3	28	6	50	5	5	26.85
8	29	8	50	10	5	29.62
30	30	7	40	7.5	7.5	49.37

Table 5.4: Analysis of variance for response surface quadratic model regarding surface tension achieved with biosurfactant produced by *Lysinibacillus sp*

Sources	**Sum of squares**	**df**	**Mean square**	**F-value**	**p-value**
Model	5007.28	14	357.66	6.72	0.0004
A-pH	280.17	1	280.17	5.26	0.0366
B-Temperaure	3901.50	1	3901.50	73.26	<0.0001
C-Beef extract	26.17	1	28.17	0.5291	0.4782
D-Peptone	16.67	1	16.67	0.3131	0.5841
AB	49.00	1	49.00	0.9204	0.3526
AC	9.00	1	9.00	0.1690	0.6868
AD	36.00	1	36	0.6762	0.4238
BC	25	1	25	0.4696	0.5036
BD	16	1	16	0.3005	0.5916
CD	9.095	1	9.095	1.708	1.0000
A^2	624.30	1	624.30	11.73	0.0038
B^2	16.30	1	16.30	0.3061	0.5882
C^2	2.01	1	2.01	0.0378	0.8485
D^2	0.2976	1	0.2976	0.0056	0.9414
Residual	1946.92	19	53.24		
Lack of Fit	833.42	14	64.31	2.07	0.2187
Pure Error	1113.50	5	31.10		
Cor Total	6277.91	29			

5.2.2.3 Effective parameters interaction

(a) The Figure 5.4A shows the effect of pH and temperature on biosurfactant production at fixed beef extract and peptone concentration of 7.5 mg/l and 7.5 mg/l respectively. It can be presumed that an increase in temperature has decreased the biosurfactant production whereas the higher pH has encouraged the biosurfactant production.

(b) The Figure 5.4B shows the effect of pH and beef extract concentration on biosurfactant production at fixed temperature and peptone concentration of 40 °C and 7.5 mg/l respectively. It can be presumed that an increase in beef extract concentration from minimum to mid level has increased the biosurfactant production and then decreased from mid level to maximum.

(c) The Figure 5.4C shows the effect of pH and peptone concentration on biosurfactant production at fixed temperature and beef extract concentration of 40°C and 7.5 mg/l respectively. Figure revealed that the combination of minimum peptone (5 mg/l) concentration and maximum ph (7) led to the higher biosurfactant production.

(d) The Figure 5.4D shows the effect of temperature and beef extract concentration on biosurfactant production at fixed pH and peptone concentration of 7 and 7.5 mg/l respectively. The graph shows that the higher biosurfactant production could be reached when beef extract concentration was at maximum level (10 mg/l) and temperature (30°C) was at minimum level.

(e) The Figure 5.4E shows the effect of temperature and peptone concentration on biosurfactant production at fixed pH and beef extract concentration of 7 and 7.5 mg/l respectively. It can be predicted that the higher biosurfactant

production can be achieved with the combination of maximum peptone concentration of 10 mg/l and minimum temperature of 30°C.

(f) The Figure 5.4F shows the effect of beef extract concentration and peptone concentration on biosurfactant production at fixed pH and temperature of 7 and 40°C respectively. The graph shows that the equal concentration of beef extract and peptone may lead to higher biosurfactant production.

5.2.2.4 Conformation experiment

The model predicted the maximum emulsification index of 64.53% at optimum beef concentration (5 mg/l), peptone concentration (5 mg/l), pH 8 and temperature (30 °C). Conformation testing was accomplished in triplicate to validate the model at the predicted optimal points. The actual value for emulsification index was 62.81%, about 2% deviation as compared to the predicted emulsification index. The minimum and maximum values at the 95% confidence interval were 40.15 and 52.85, respectively **(Marchant & Banat, 2012; DeRienzo \ 2016)**.

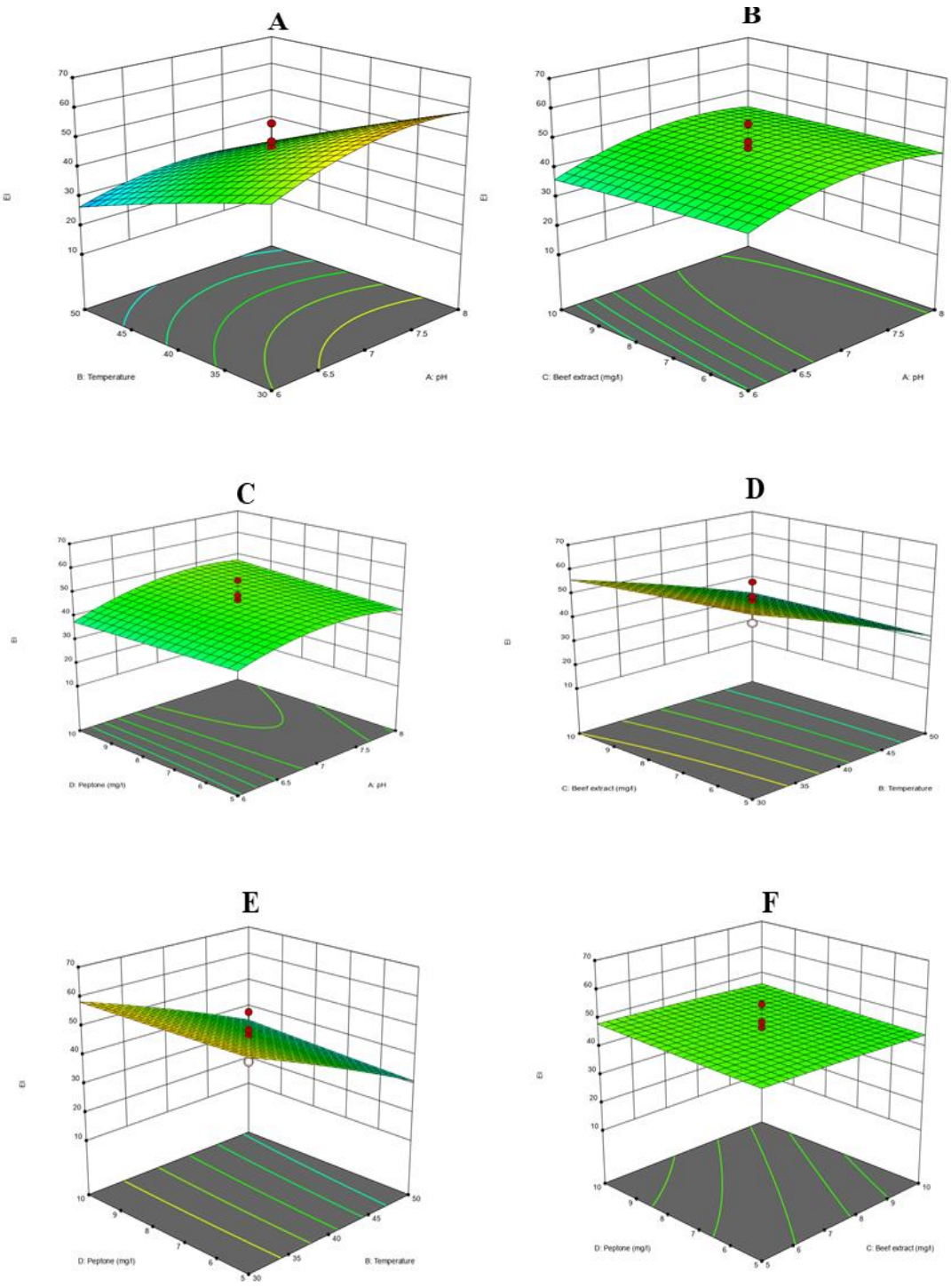


Figure 5.4: Response surface plots of biosurfactant production from *Lysinibacillus* sp (A) Interactive effect of pH and temperature (B) Interactive effect of Beef extract and pH(C) Interactive effect of Peptone concentration and pH (D) Interactive effect of Temperature and Beef extract (E) Interactive effect of Peptone concentration and temperature (F) Interactive effect of Beef extract and Peptone concentration

5.2.3 Characterization of biosurfactant

5.2.3.1 Total protein content

The protein content estimation of the biosurfactants produced by bacteria was done by bradford protein assay. The protein content of biosurfactant obtained by *Bacillus* sp strain 530F_seq260_Env1 was 5.981 µg/ml and by the biosurfactant obtained by *Lysinibacillus* sp strain DESBBAU2 was 6.940 µg/ml **(Muthezhilan *et al.*, 2014)**.

5.2.3.2 Total lipid content

The lipid content estimation of the biosurfactant produced by both the bacteria was done by gravimetric method. The results of the gravimetric method revealed the total lipid content of 53 mg (71.62%) by the biosurfactant produced by the *Bacillus* sp strain 530F_seq260_Env1 and 59 mg (75.64%) by the biosurfactant produced from *Lysinibacillus* sp strain DESBBAU2 (Figure 5.5) **(Rani *et al.*, 2020)**.

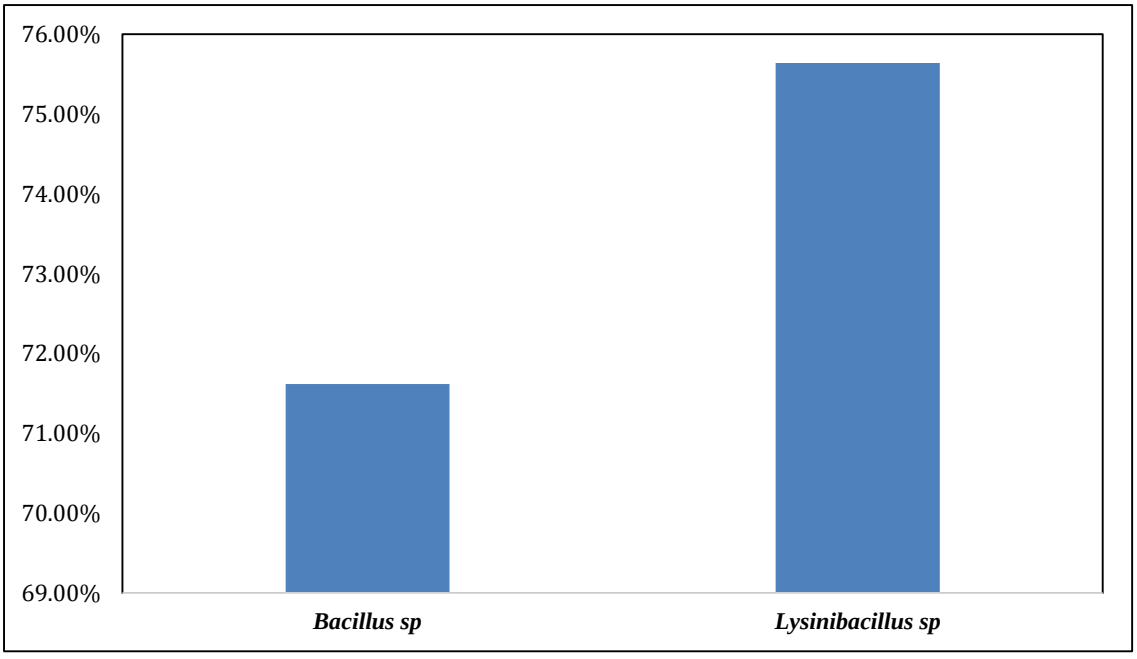


Figure 5.5: Total lipid content of both the biosurfactant

5.2.3.3 FTIR-characterization of the produced biosurfactant

The use of FTIR spectroscopy was done to elucidate the chemical structures of the chemical components present in the unknown biosurfactant by finding the types of chemical bonds or functional groups that are present in their chemical structures. Figure 5.6 represents the FTIR spectra of the biosurfactant produced by both the bacteria. A strong and broad band was observed at 3417.6 cm^{-1} that corresponded to the O-H stretching due to hydrogen bonding **(Diaz *et al.*, 2015)**.

The peaks at 2926.3 cm^{-1} and 2854.8 cm^{-1} represent the stretching of the aliphatic bonds CH_3 and CH_2 respectively. The peak at approximately 1648.5 cm^{-1} corresponds to the stretching of unsaturated C=C bonds **(Pornsunthorntawe *et al.*, 2008)**. The stretching due to carbonyl (-C=O) group was observed at 1743 cm^{-1} having strong intensity peak, while another small intensity peak due to the C=O absorption observed at approximately 1242–1243 cm^{-1} corresponds to the ester functional group **(Patowary *et al.*, 2017)**. Another small intensity peak at approximately 1150 cm^{-1}, might be due to the C-O-C stretching **(Nikolopoulou & Kalogerakis, 2008)**. Moreover, other medium intensity peaks found at 1475–1380 cm^{-1} were recognized as the bending of the hydroxyl group (OH), which confirmed the existence of carboxylic acid functional groups (-CO_2H) **(Ismail *et al.*, 2013)**. Based on the FTIR spectroscopy results of the crude biosurfactant produced by the newly isolated strains, *Bacillus* sp. Strain 530F_seq260_Env1 and *Lysinibacillus* sp strain DESBBAU2 it could be predicted that the biosurfactant produced belongs to the glycolipid type.

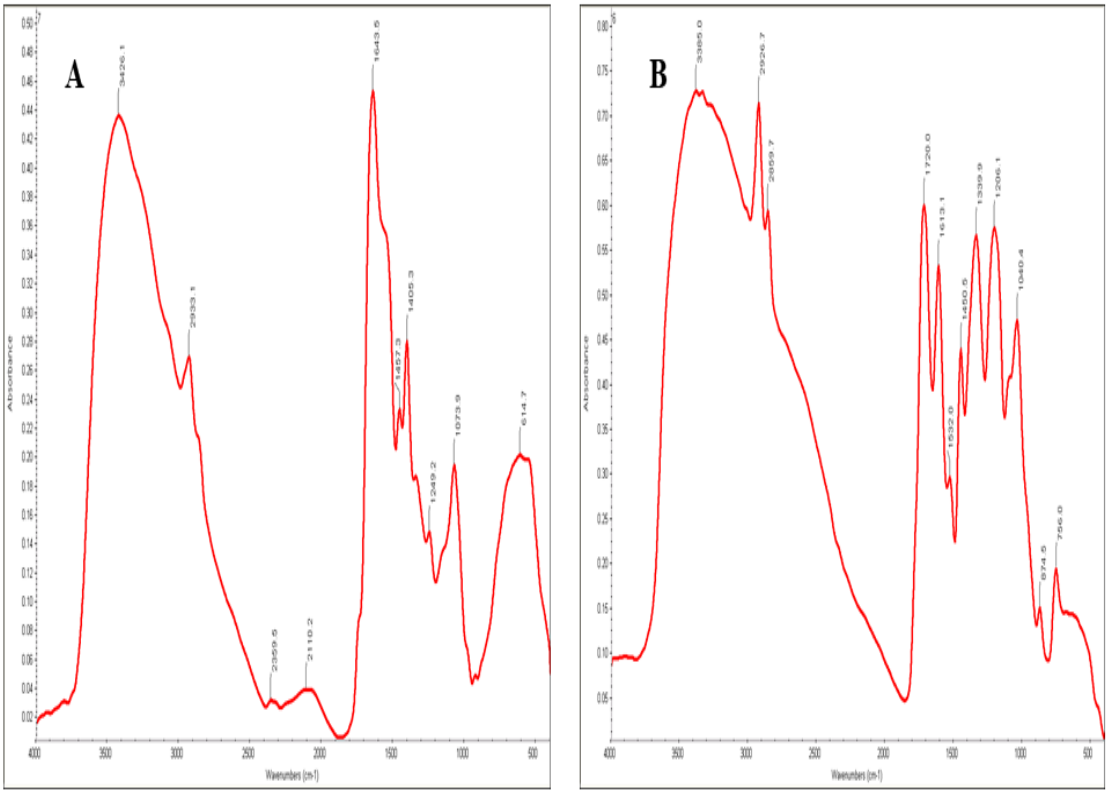


Figure 5.6: FTIR spectra of biosurfactants (A) *Bacillus* sp biosurfactant and (B) *Lysinibacillus* sp biosurfactant

5.2.3.4 Energy dispersive X-Ray of biosurfactant

Energy dispersive X-ray was done to determine the weight percent of different elements in the biosurfactant produced. From the XRD spectra given in Figure 5.8 various elements were detected. The result revealed that both biosurfactant produced are mainly composed of Carbon, Oxygen, Chlorine, and Sodium. Figure 5.7 shows the weight percent of the element in both biosurfactant **(De_Zeil *et al.*, 2000; Hu *et al.*, 2015).**

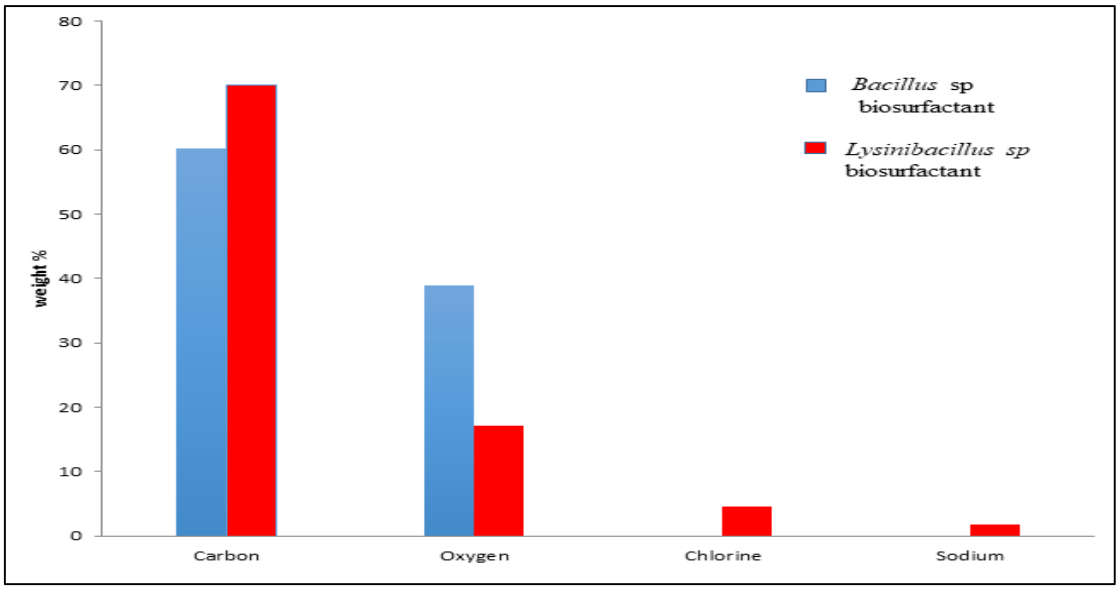


Figure 5.7: Weight percent of different elements in both the biosurfactant

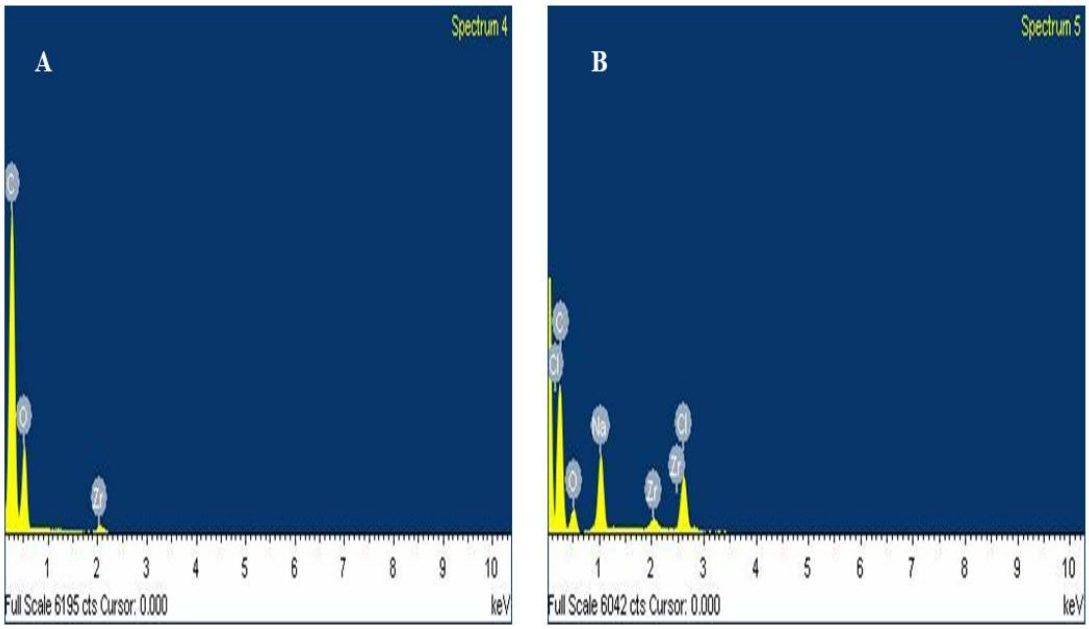


Figure 5.8: EDX spectra of biosurfactants (A) *Bacillus* sp and (B) *Lysinibacillus* sp

5.2.3.5 Thermal gravimetric analysis (TGA) of biosurfactant

The commercial application of any BS depends on its thermal stability at extreme temperatures. As determined from the TGA graph shown in Figure 5.9 the degradation temperature of biosurfactant extracted from *Bacillus* sp was found to be 215°C while complete weight loss was observed after 350°C. For biosurfactant that was extracted from *Lysinibacillus* sp., the degradation temperature was 255°C and final decomposition temperature was 450°C. As it can be seen in the Figure 5.9, a constant slope to 200°C denotes the absence of trapped moisture molecules in the structure of the biosurfactants and indicates that the biosurfactants was truly anhydrous **(Nikolopoulou & Kalogerakis, 2013a; Deepa, 2019)**. Both of the biosurfactant was found to be thermally stable beyond 250°C and the biosurfactant produced by *Lysinibacillus* sp was more thermally stable than *Bacillus* sp (Figure 5.10).

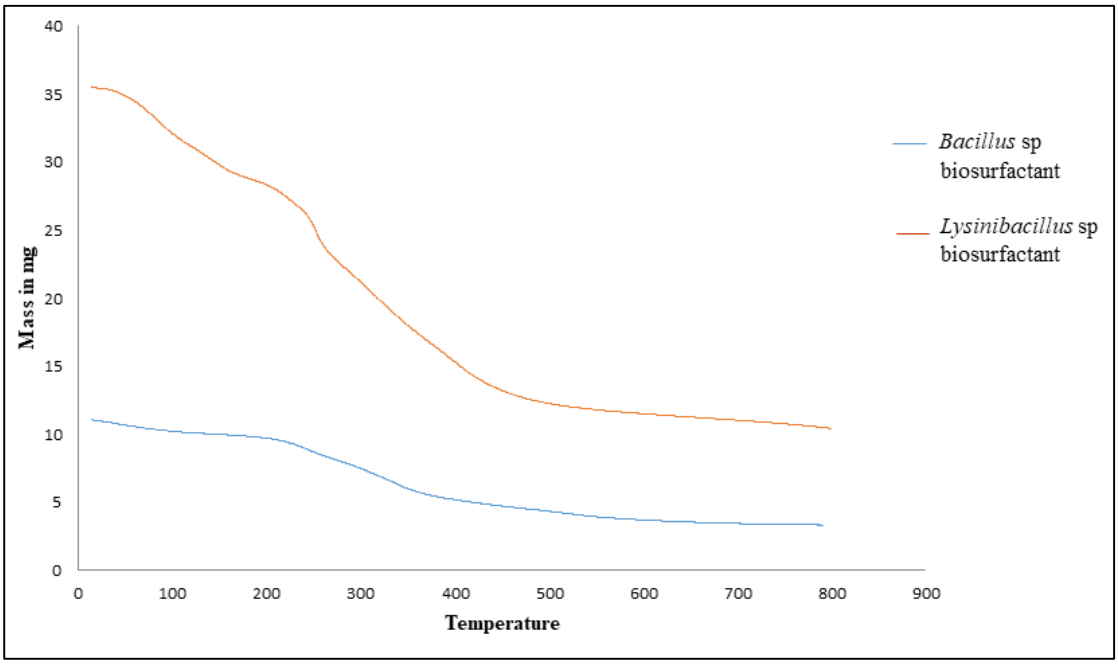


Figure 5.9: Thermal gravimetric curve of biosurfactant

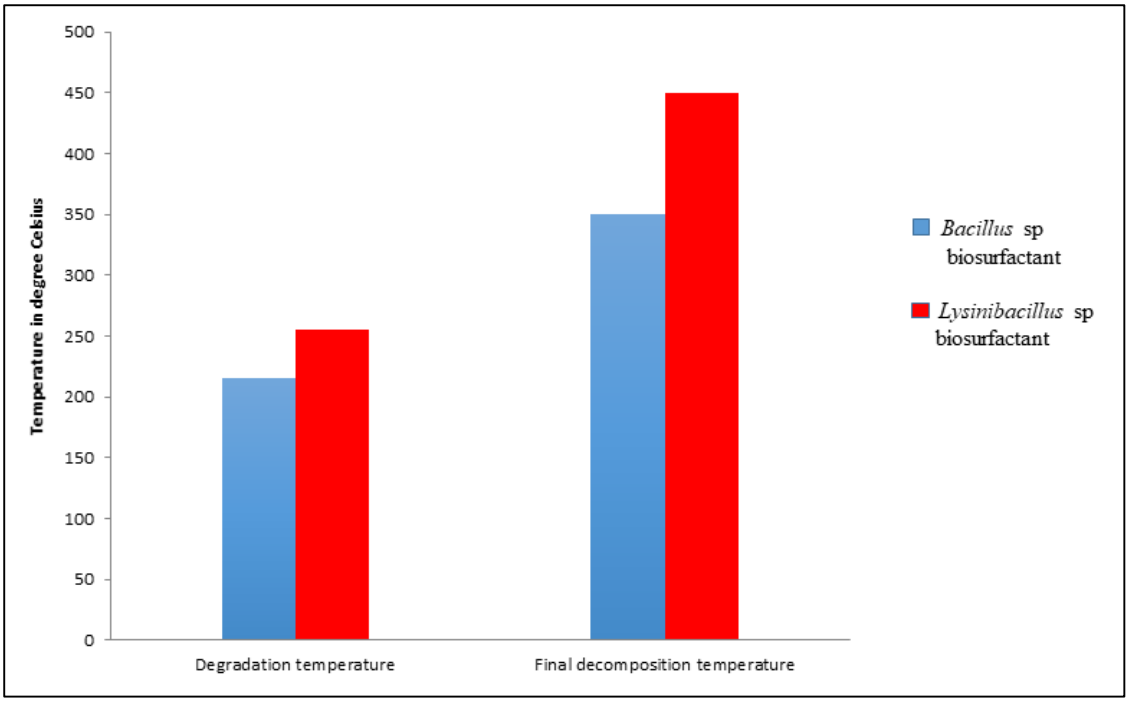


Figure 5.10: Thermal Gravimetric analysis parameters of biosurfactants

5.2.3.6 Differential scanning calorimetry (DSC)

DSC curve was obtained for both the surfactants isolated from *Lysinibacillus* sp and *Bacillus* sp (Figure 5.11). DSC thermogram of both the biosurfactants showed the difference in the melting transition temperature. The biosurfactant extracted from *Lysinibacillus* sp. bacteria showed the melting transition temperature 107°C, 171°C and 204°C whereas the biosurfactant extracted from *Bacillus* sp showed the melting transition temperature at 47°C and at 111°C. The melting temperature of *Lysinibacillus* sp extracted biosurfactant wass greater than the *Bacillus* sp. extracted biosurfactant. The DSC data reveals that the biosurfactant of *Lysinibacillus* sp is more thermostable which is important for a biosurfactant to be used in commercial application **(Akbari *et al.*, 2018; Deng *et al.*, 2020)**.

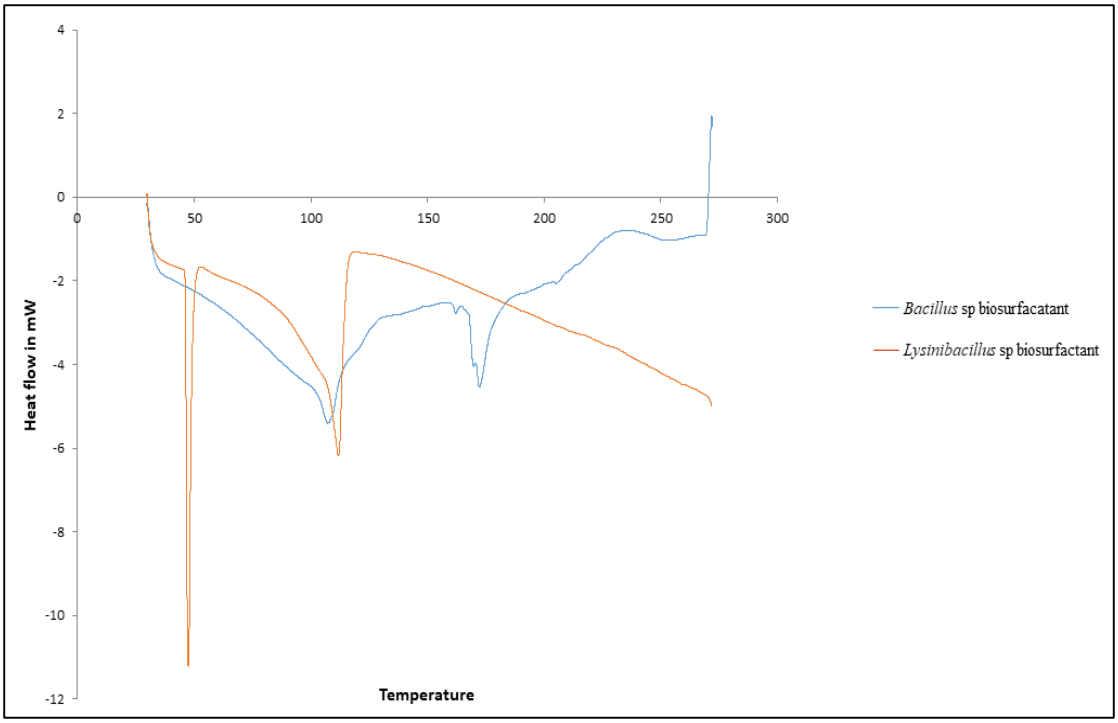


Figure 5.11: Differential Scanning Calorimetry analysis curve of biosurfactants

5.2.4 Extraction and production of biosurfactant

The dried form extracted biosurfactant by both the bacterial strains were weighed in order to determine the yield (Plate 5.4). Results for dry weight of biosurfactants revealed that the highest biosurfactant producer was *Lysinibacillus* sp strain DESBBAU2 that produced 1.48 g/l of the crude biosurfactant, followed by *Bacillus* sp strain 530F_seq260_Env1 that produced 1.36 g/l **(Duddu et al., 2015; Jemil *et al.*, 2016)**.

Plate 5.4: Dried form of biosurfactants obtained from isolated bacteria

5.3 Conclusion

The produced biosurfactant from both the bacteria was found to be of glycolipid type after the structure analysis by FTIR, EDX, DSC and TGA analysis. Both the biosurfactant showed significant stability under extreme conditions of temperature, which is very important for the commercial application of biosurfactant. The CCD conformed the optimal growth conditions for both the bacteria to maximize biosurfactant yield. The model generated for both the bacteria for biosurfactant production was found to be suitable for defining the response of the system as the experimental data was in good conformity with the values that were predicted by the model. The R^2 and p- value (<0.0001) of the model were significant. The model was also validated by the conformation test at the predicted optimum points. The value predicted by the model was compared with the experimental value and showed about 3% and 2% deviation for *Bacillus* sp 530F_seq260_Env1 and *Lysinibacillus* sp strain DESBBAU2.

6.0 Introduction

Polyethylene is an essential commodity polymer, categorized as high- density and low density polyethylenes (HDPE and LDPE), the latter of which is characterized by the toughness, flexibility, resistance to chemicals and clarity. LDPE are made up of thousands of repeating $–CH_2–$ units, which render them hydrophobic in nature and also too large to pass via microbial cell membranes. These factors have led to the prevalence and accumulation of polyethylene in the environment, which accounts nearly 21 million tonnes annually **(Ammala *et al.*, 2011)**.

The plastic pollution has become one of the important concerns for researchers and several studies have been performed to explore a way for polyethylene degradation. Many research has revealed that biotic, i.e., microbial degradation of polyethylene can be one of the eco-friendly methods **(Hu *et al.*, 2007)**. Microbial degradation is primarily achieved by the formation of a bio-film over the polyethylene surface. Many microbes such as *Aspergillus niger*, *Rhodococcus ruber*, *Penicillium pinophilum*, have been reported for the microbial degradation of polyethylene. To improve the microbial attachment to the polymer surface, the hydrophobic properties of the polyethylene must be changed into hydrophilic ones through the abiotic oxidation **(Blouzard *et al.*, 2007)**.

Another approach to increase the degradation rate is by increasing the contact surface between polymer and water by the addition of surfactant in the biodegradation system. Surfactants are the wide varieties of surface-active amphiphilic molecules **(Bezza & Chirwa, 2015)**. Surfactants possess ability to decrease the surface tension and interfacial tension of the solution. The bioavailability of non-soluble and hydrophobic substance is improved by the reduction of surface tension. Surfactants also possess the ability to improve the solubility of petroleum hydrocarbons. Surface tension reduction

property of surfactants can be utilized to increase the biodegradation of polymer. Some of the polyethylene biodegradation studies have made use of chemical surfactant in the system **(Hoang *et al.*, 2007)**. Better biodegradation of pre-oxidized LDPE has been reported in the soil after the addition of a surfactant in the system. In another study, adding Tween 80 into a polyethylene biodegradation system containing *Pseudomonas aeruginosa* has seen enhanced bio-film formation. Another type of surfactant commonly known as bio-surfactant is produced by different microbes **(Alajlani *et al.*, 2016)**. The main benefits of bio-surfactants over the use of chemical surfactants are their eco-friendly nature and their bio-degradability **(Akola & Jones, 2003)**. One of the bacterium known to be effective producer of biosurfactant is *Pseudomonas aeruginosa* and its bio-surfactant is called rhamnolipid. This specific bacterium has been utilized to study the biodegradation of other polymer such as polypropylene. The other bio-surfactant producing bacteria are *Bacillus pumilus*, *Bacillus halodenitricans*, and *Bacillus cereus* **(Hong *et al.*, 2014)**. All have been reported for their ability to biodegrade pre-oxidised polyethylene **(Carvalheiro *et al.*, 2008)**. In this study, two newly isolated strains of Bacillus and *Lysinibacillus* named as *Bacillus* sp Strain 530F_seq260_Env1 and *Lysinibacillus* sp strain DESBBAU2 respectively, have been examined for their ability to degrade low density polyethylene in the presence and absence of their extracted biosurfactant. The two novel strains have been tested as the effective producer of biosurfactant and they have not been reported elsewhere for their LDPE degradation potential.

6.1 Materials and method

6.1.1 Test material

For the test material 0.01 mm thick, transparent, white, LDPE films were used. The LDPE film was washed thoroughly with distilled water and surface sterilized before the treatment process.

6.1.2 Microbial culture

Microbial culture of *Bacillus* sp strain 530F_seq260_Env 1 and *Lysinibacillu* sp strain DESBBAU2 were preserved on separate nutrient agar plates. For the treatment process fresh culture broth of both the bacterial strains were made. For the preparation of culture broth following steps was performed **(Kim *et al.*, 2017)**:

1) Nutrient broth media was weighed (0.8 gm per 100 ml).
2) The media (100 ml) in two different erlenmeyer flask was autoclaved for 15 min at 121 psi.
3) For inoculation, when the media was cooled at room temperature a loop full of bacteria was taken from nutrient agar plate and transferred to the flask. The step was done aseptically under laminar flow.
4) The culture was then incubated at for 24 h for at 30 °C.

6.1.3 Bio-treatment process

Bio-treatment process was carried out in nutrient broth medium. Four setups were maintained for each bacterium for 45 days for observing the degradation pattern of LDPE. The LDPE samples were incubated in different sets of combinations for the two positively screened bacteria **(Mukherjee *et al.*, 2011)**.

First set contained LDPE sample, bacterium and its biosurfactant in nutrient broth

medium *i.e.,* LDPE+Bacteria+Biosurfactant. Second set contained LDPE sample and Bacterium only in broth medium *i.e.,* LDPE+Bacteria. Third set contained LDPE sample and biosurfactant only in broth medium *i.e.,* LDPE+Biosurfactant. Fourth set was maintained as negative control having LDPE only in nutrient broth media. Fifth set was maintained as positive control having bacteria only in broth medium. Separate flasks were maintained for the two positively screened bacteria identified as strains of *Bacillus* and *Lysinibacillus* species.

6.1.4 Characterization of degraded LDPE

6.1.4.1 Weight loss measurement

The dry weight of residual LDPE was obtained after the bio-treatment process to determine the degradation of LDPE by various combination of bacteria and biosurfactant and it was calculated according to method given in section **3.1** of Materials and Methods in **Chapter 3**.

6.1.4.2 Fourier Transform-Infrared Spectroscopic analysis (FTIR)

The formation or disappearance of the functional groups during the process of degradation can be observed by using FTIR spectroscopic analysis. The FTIR was done according to method given in in section **3.2** of Materials and Methods in **Chapter 3**.

6.1.4.3 Scanning Electron Microscopy and Energy Dispersive X-ray (SEM-EDX)

The LDPE films were recovered from the culture medium after the treatment process to observe the bacterial colonization and surface erosion over the LDPE films. SEM-EDX was done according to method given in in section **3.3** of Materials and Methods in **Chapter 3**.

6.1.4.4 X-Ray diffraction (XRD)

The X-ray diffraction patterns of the LDPE films were measured using a X-ray diffractometer which was operated using Cu Kα radiation (λ = 1.5418 A°). XRD was done according to method given in in section **3.4** of Materials and Methods in **Chapter 3**.

6.1.4.5 Thermal gravimetric analysis (TGA)

TGA was performed for determining the decomposition temperature of the LDPE samples. TGA was done according to method given in in section **3.5** of Materials and Methods in **Chapter 3**.

6.1.4.6 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was done for analyzing the melting temperature of the LDPE samples. DSC was done according to method given in in section **3.6** of Materials and Methods in **Chapter 3**.

6.2 Results and Discussion

6.2.1 Biological Degradation of polyethylene incubated with *Bacillus* sp Strain 530F_seq260_Env1

In order to determine the extent of degradation in LDPE samples various analysis such as FTIR, SEM, EDX, TGA and XRD were performed. Every analysis showed changes occurred in the LDPE samples. The analyses performed are described below.

6.2.1.1 FTIR analysis of biodegraded LDPE samples

FTIR spectra given in Figure 6.1 revealed significant changes in the LDPE samples treated with bacteria and its biosurfactant. Many new peaks appeared in the treated LDPE samples as compared to control LDPE sample. Maximum number of peaks emerged in the region 1400-2400 cm^{-1} in LDPE sample treated with bacteria and its biosurfactant only, followed by LDPE samples treated with bacteria and LDPE samples treated with biosurfactant separately. Peaks observed at 1740 cm^{-1} and 1650 cm^{-1} in the treated samples might be due to the formation of ketones, aldehydes and some of the unsaturated hydrocarbons. The transmittance of the peaks at 1460 cm^{-1}, 2919 cm^{-1}, 2853 cm^{-1} and 3435 cm^{-1} was observed to decrease in treated LDPE samples **(Artham & Doble, 2008; Raaman, 2012)**. The lowest transmittance at these peaks was recorded in the LDPE sample treated with bacteria and biosurfactant both followed by LDPE sample treated with bacteria only and LDPE treated with biosurfactant only. The Table 6.1 summarizes the number of peaks and their corresponding functional group in the LDPE samples **(Nanda & Sahu, 2010)**.

Table 6.1: Peaks and their corresponding functional group in Treated and Control LDPE samples

Origin	Peaks	Functional group	LDPE treated with bacteria and biosurfactant	LDPE treated with bacteria	LDPE treated with biosurfactant	Untreated control LDPE
CH_2	729-716	Methylene rocking	Present	Present	Present	Present
C-O-O-C	890-820	Peroxides, C-O-O stretch	Present	Present	Present	Absent
C-O-C	1150-1050	Alkyl-substituted ether, C-O stretch	Present	Present	Present	Absent
CH_3	1133	CH3 wagging, in-plane	Present	Present	Present	Present
C-O	1263	C-O stretch	Present	Present	Present	Present
C=O	1730-1725	C=O stretch, carbonyl	Present	Present	Present	Absent
CH_2	2848	Methylene symmetric stretch	Present	Present	Present	Present
CH_2	2915	Methylene asymmetric stretch	Present	Present	Present	Present

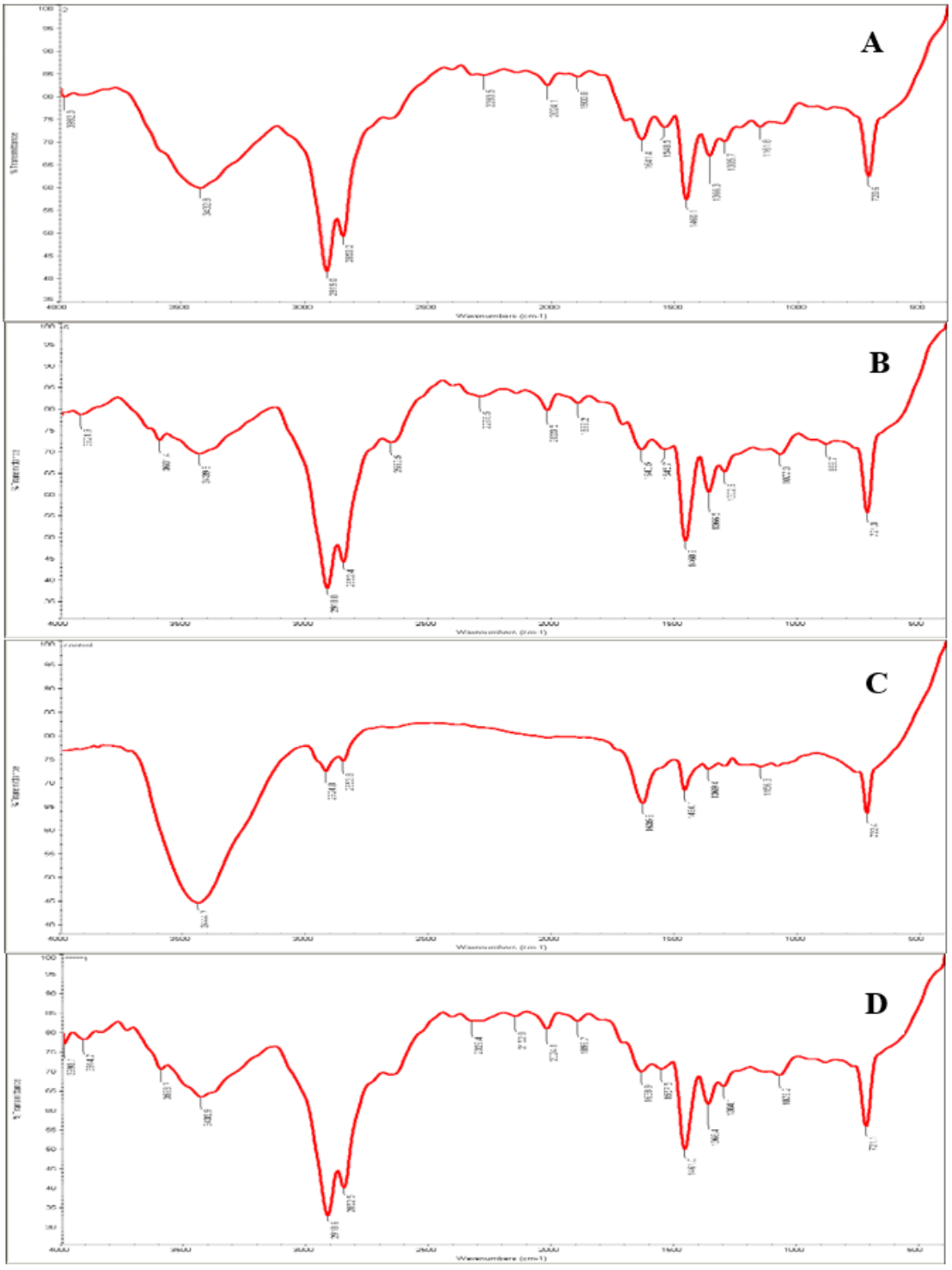


Figure 6.1: FTIR spectra (A) Control LDPE (B) LDPE treated with biosurfactant only (C) LDPE treated with bacteria and biosurfactant (D) LDPE treated with bacteria

6.2.1.1.1 Carbonyl Index, Double bond Index under FTIR Analysis

The LDPE biodegradation by bacteria and biosurfactant was further confirmed by an increase in the keto-carbonyl bond index and the internal double bond of FTIR spectra (Figure 6.2). The formation of terminal double bonds occurs due to exposure of polyethylene samples to biotic environment **(Salmah *et al.*, 2011)**. Carbonyl groups are considered as the major products formed in the presence of oxidoreductases. Maximum internal double bond index (DBI) and carbonyl bond index (CBI) was found in LDPE treated with bacteria and biosurfactant **(Cui *et al.*, 2008)**.

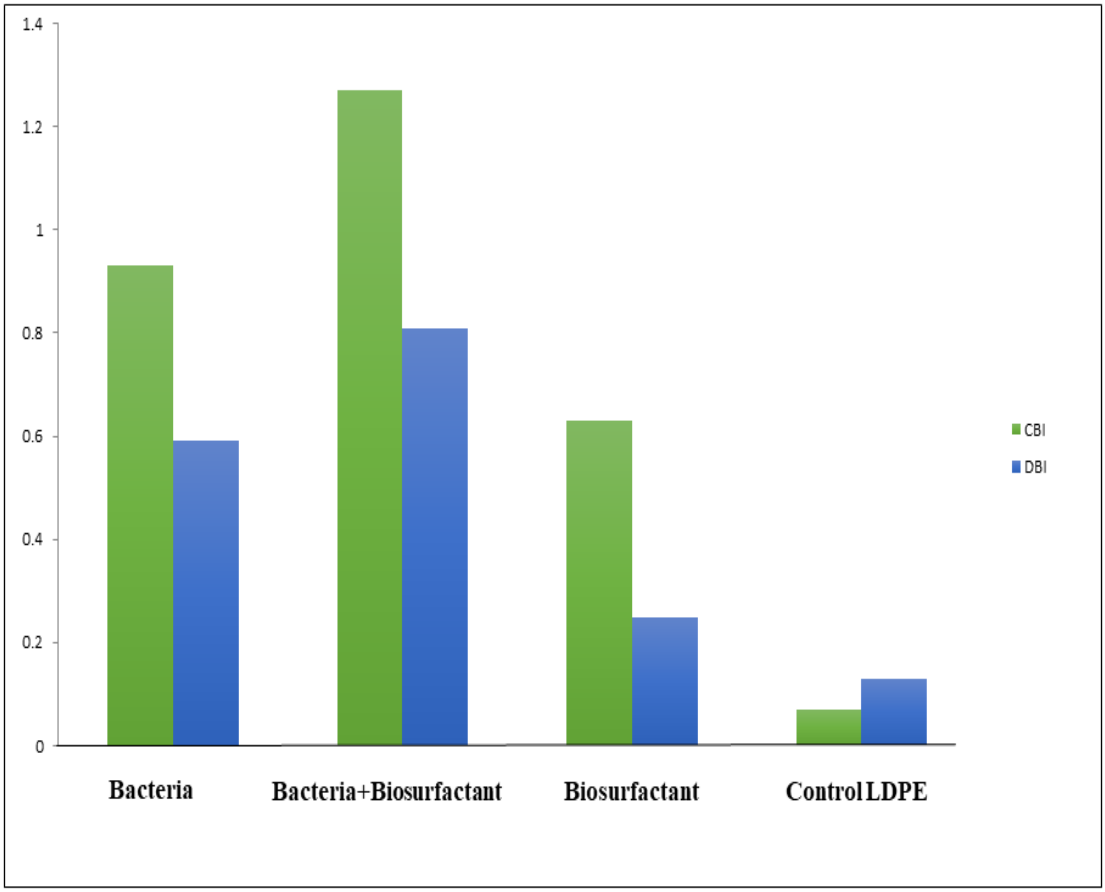


Figure 6.2: Carbonyl Index, Double Bond Index of Treated and Control LDPE samples

6.2.1.1.2 Crystallinity% under FTIR Analysis

The lowest crystallinity percentage was also reported in LDPE treated with bacteria and biosurfactant both (Figure 6.3). This might be due to the fact that addition of biosurfactant reduced the hydrophobic nature of the LDPE, which enhanced the better attachment of bacteria over the LDPE samples **(Albertsson, 1980)**.

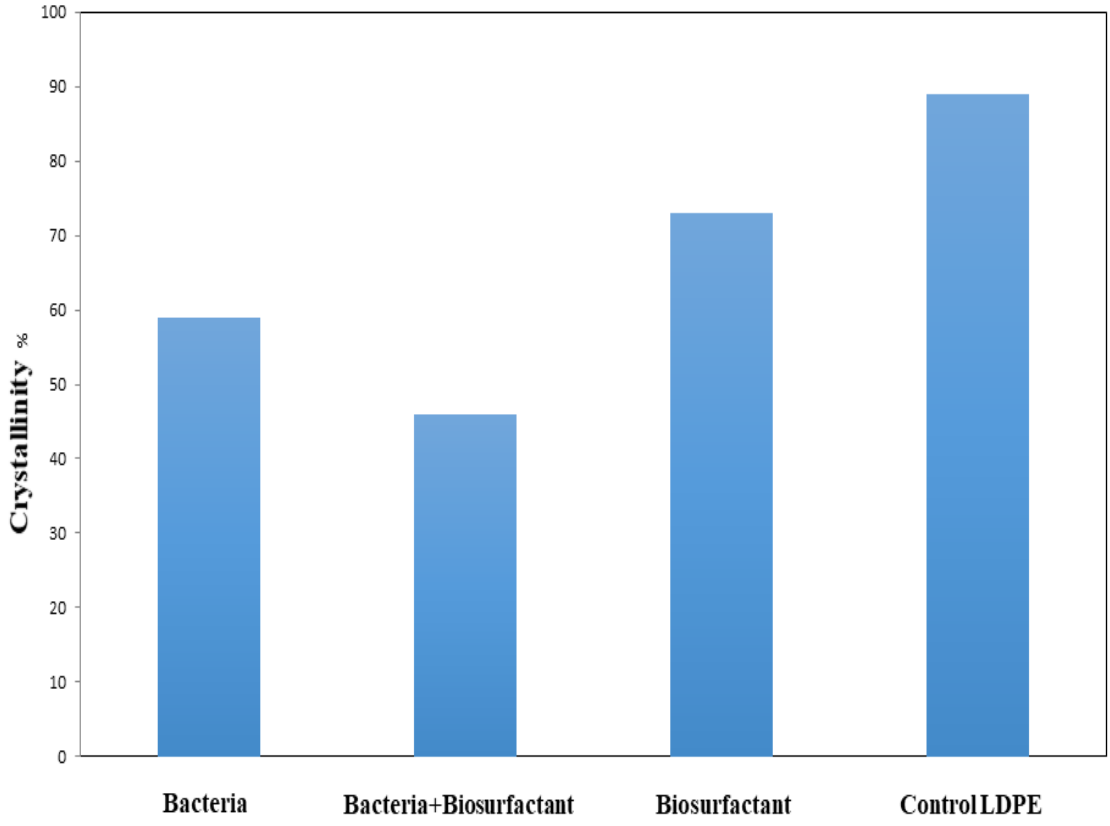


Figure 6.3: Crystallinity of Treated and Control LDPE samples

6.2.1.2 Scanning electron microscopy analysis

The SEM micrograph provides good evidence for analyzing biodegradation of test sample (Plate 6.1). The surfaces of the LDPE film that have not undergone any treatment showed smooth surface without any crack and it was free from any type of defect. Besides, the biodegradation of treated LDPE was clear through the cavity formation on the surface of polyethylene **(Bhatia *et al.*, 2014)**. All the treated samples of LDPE were compared on the basis of maximum disruption caused on the surface. Major damage to the surface can be observed in the LDPE sample treated with the bacteria and the biosurfactant both. The LDPE samples treated separately with bacteria only and biosurfactant only also showed damage over the surface but the extent of degradation was less than the LDPE treated with bacteria and biosurfactant both. The samples of polyethylene showed pitted and eroded surfaces. The surface of the polythene after the biological attack became physically weak and readily fragmented under mild pressure **(Babul *et al.*, 2013)**. These results suggest that treatment induces the oxidation and, hence, the polythene becomes brittle, which ultimately leads to cracks because of the action of bacteria and biosurfactant. Bacteria that colonize over the polymer surface can possibly adhere by means of extracellular polymeric substances, which are basically composed of polysaccharides. This forms a layer over the surface that is bonded to the polymer. This plays a significant role in transporting the enzymes to its surface. These changes can be possibly due to surface degradation **(Jirage *et al.*, 2011)**.

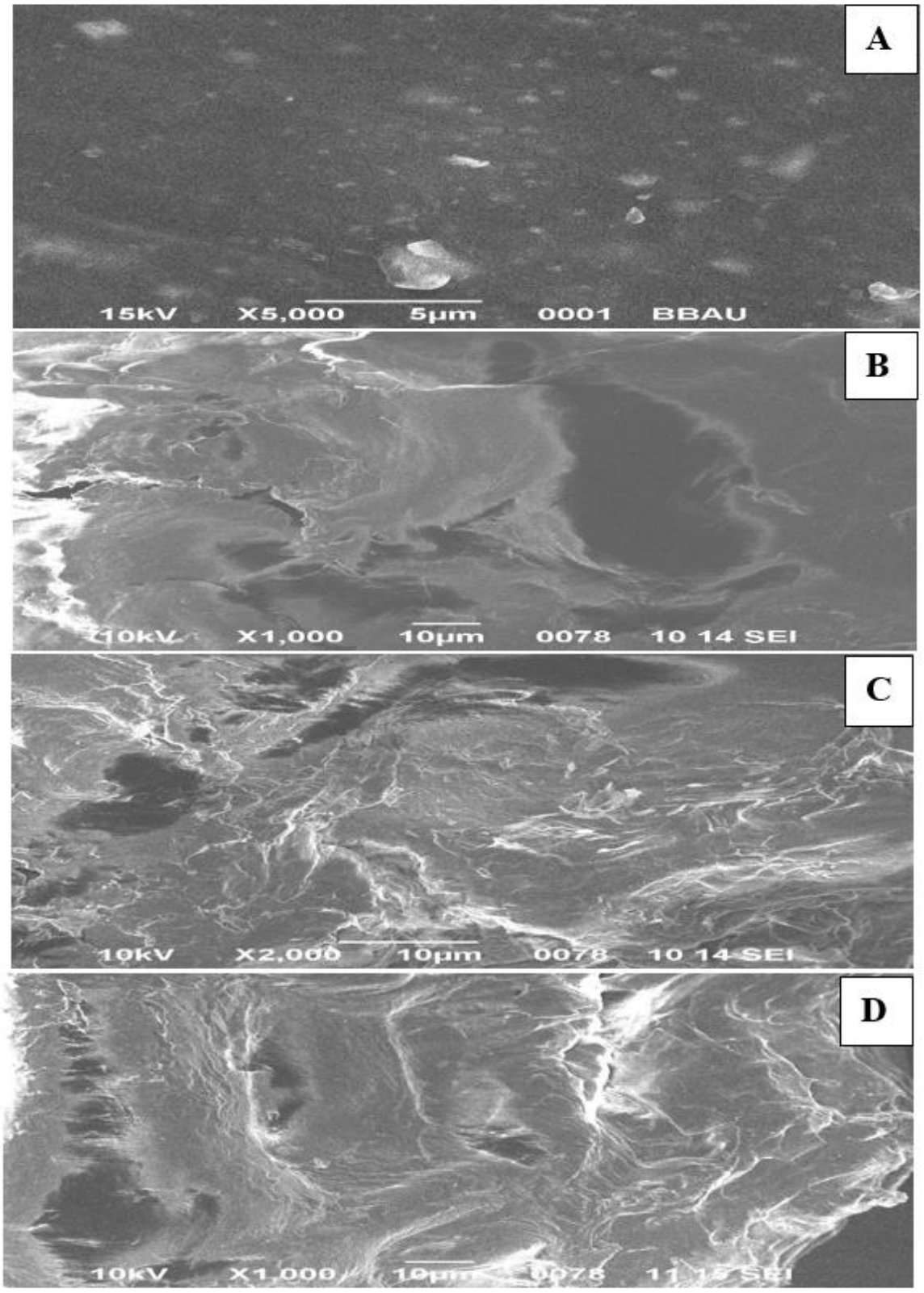


Plate 6.1: SEM micrographs of LDPE samples (A) Control LDPE (B) LDPE treated with biosurfactant only (C) LDPE treated with bacteria and biosurfactant both (D) LDPE treated with bacteria

6.2.1.3 Energy dispersive X-ray analysis (EDX)

EDX analysis was performed to determine the change in elemental composition of the LDPE film after the treatment. The Figure 6.4 shows the weight percent of carbon and oxygen in different LDPE samples undergone treatment with respect to untreated one. Maximum reduction of carbon content was seen in LDPE treated with bacteria and biosurfactant both whereas LDPE treated with bacteria and biosurfactant separately showed less reduction in carbon content with respect to untreated LDPE sample **(Bonhomme *et al.*, 2003)**. The decrease in carbon content can be due to the utilization of carbon by the Bacillus for their growth and development. The increase in oxygen content can be attributed due to the solubilization of the oxidation product into the liquid media **(Chen *et al.*, 2007)**.

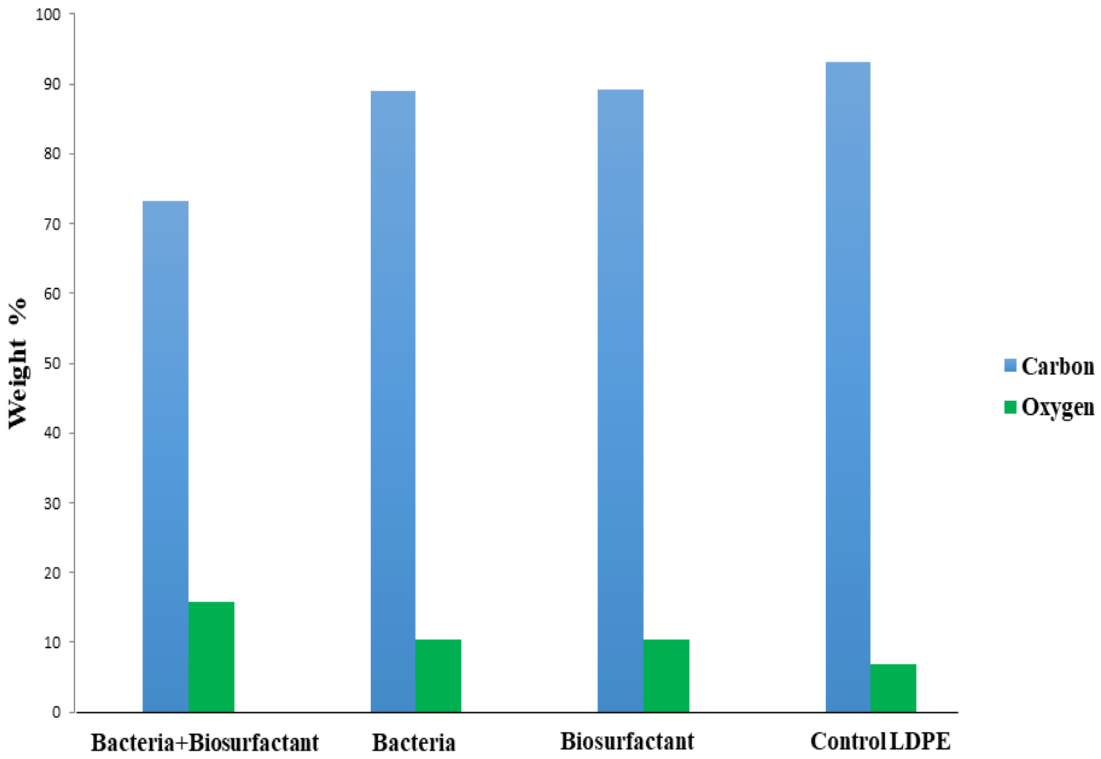


Figure 6.4: Carbon and Oxygen weight percent of Treated and Control LDPE samples

6.2.1.4 X-Ray diffraction analysis (XRD)

XRD analysis was done to determine the change in crystallinity pattern in the polymeric structure on the basis of appearance and disappearance of peaks at particular 2θ angle (Figure 6.5). In all the XRD spectra of LDPE samples the major peaks at 21° and 23.5° were found which are the characteristic peaks of the semi-crystalline polyethylene molecule **(Nanda *et al.,* 2010; Ambika *et al.,* 2015)**. Maximum number of peaks emerged in the LDPE samples incubated with biosurfactant and *Bacillus*. Lowest number of peaks among treated samples was observed in LDPE sample treated with biosurfactant only. The intensity of the peaks at 21° and 23.5° got decreased in all the treated LDPE samples as compared to the untreated control sample. Maximum reduction in the intensity was observed in LDPE sample treated with bacteria and biosurfactant both due to higher level of oxidation in LDPE as compared to other treated LDPE samples. This implies the major change in the crystallinity of polymer. XRD analysis provides the result in support of FTIR analysis, which implied the decrease in the crystallinity of LDPE samples **(Albertsson *et al.,* 1993)**.

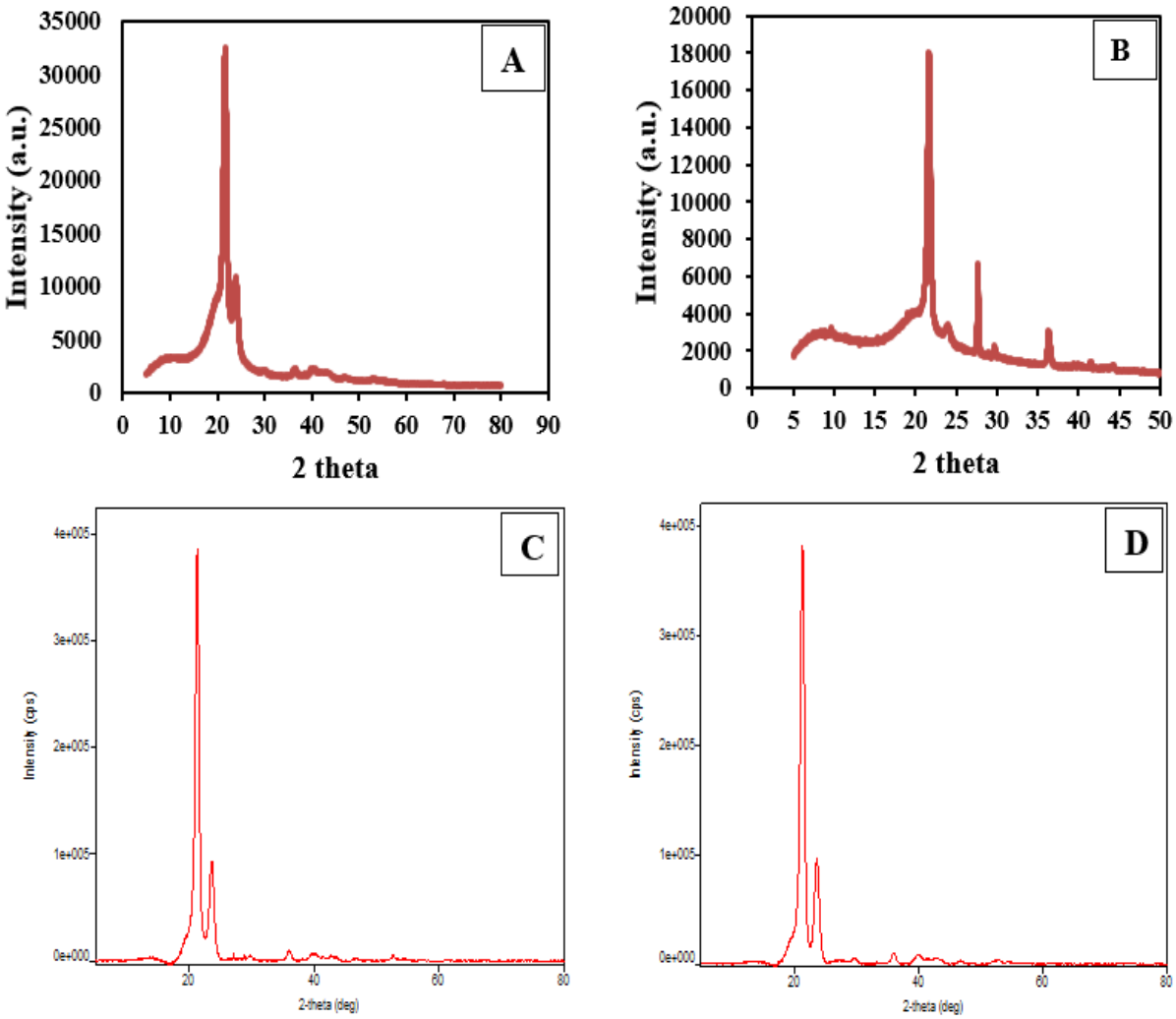


Figure 6.5: XRD spectra of LDPE samples (A) Control LDPE (B) LDPE treated with bacteria and biosurfactant (C) LDPE treated with bacteria only (D) LDPE treated with biosurfactant

6.2.1.5 Thermal gravimetric analysis (TGA)

The thermal stability of all the LDPE samples was studied using TGA. Figure 6.6 shows the behavior of mass-loss as a function of temperature for the treated and untreated control LDPE samples. All the polyethylene samples displayed similar thermograms with a single mass-loss zone which was centered on 460°C for LDPE samples. For all the treated LDPE samples no residue was left at decomposition temperature but in case of untreated LDPE some residue was left which is approximately 0.47 mg. The onset temperature (T_o) for treated LDPE samples got reduced as compared to untreated

control sample. The onset temperature was decreased in the treated LDPE samples due to the microbial erosion (Figure 6.7). Biotic induced oxidation produced low molecular weight products, which readily got degraded further in subsequent thermal degradation during TGA analysis **(Cheah & Cook, 2003)**. Because the oxidation is primarily confined to the amorphous portion of the polymer matrix, the remainder of the polymer gets more susceptible for the molecular reorganization, which may increase or decrease the crystallinity of the polymer containing pro-oxidant additive. Thus the thermal stability of biologically treated samples showed decrease in thermal stability when compared to control LDPE sample **(Basnett *et al.*, 2013)**.

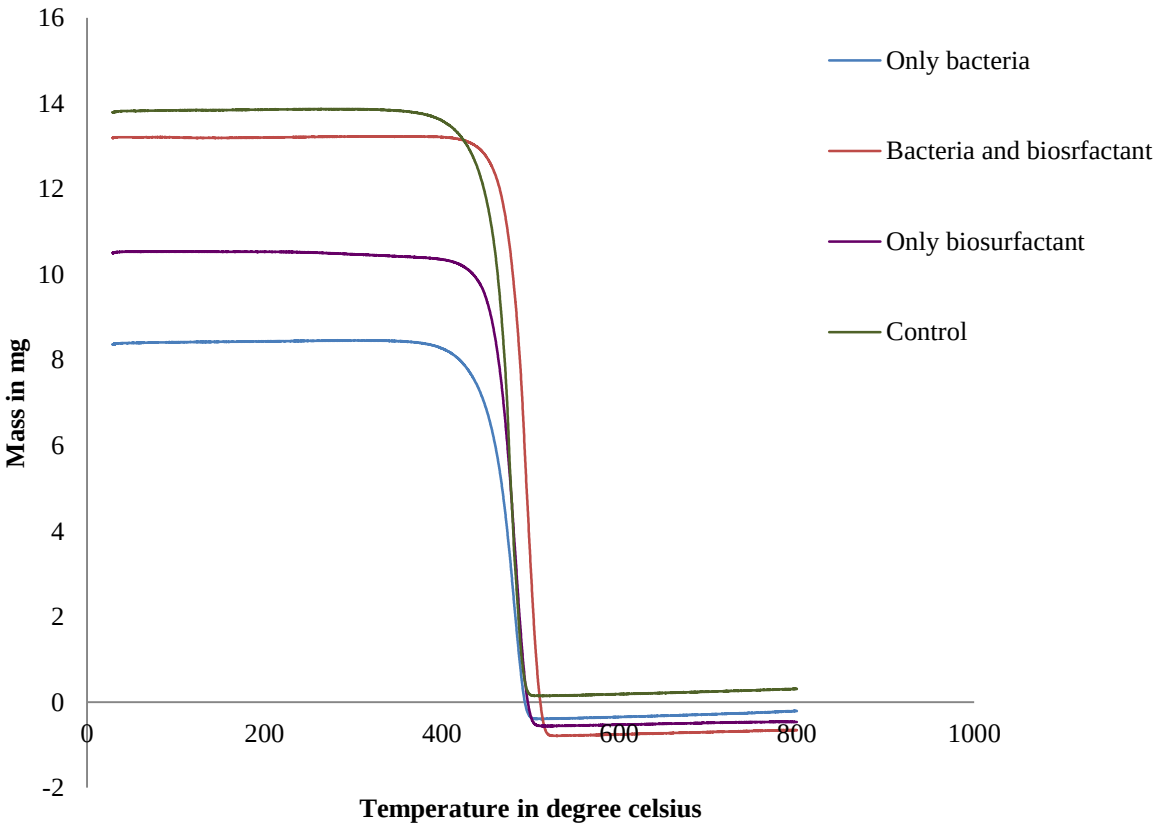


Figure 6.6: Thermal Gravimetric Analysis curve of Treated and control LDPE samples

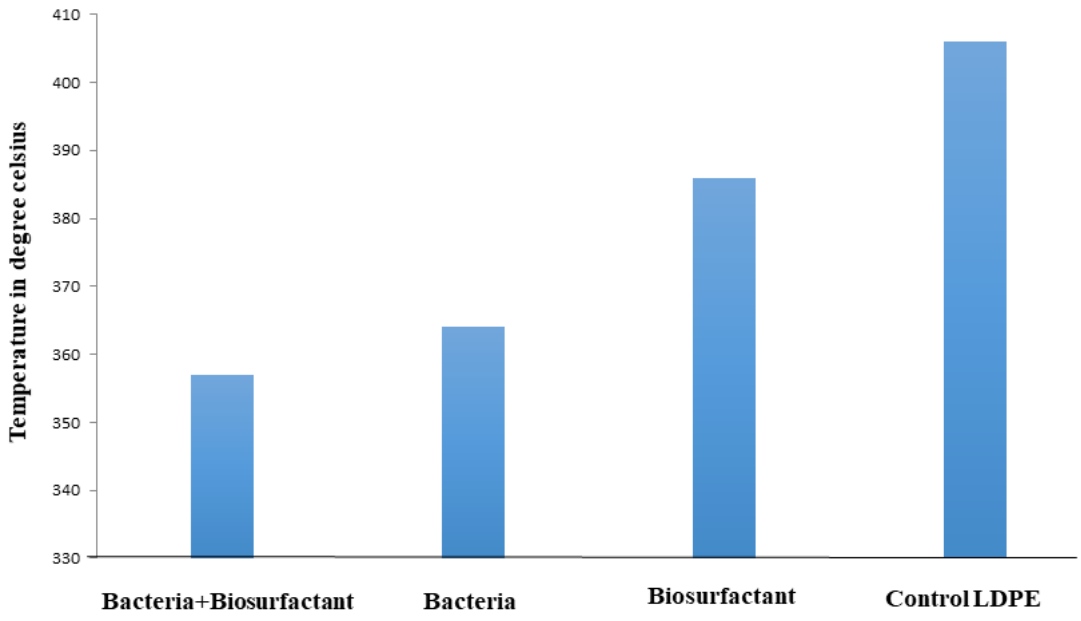


Figure 6.7: Onset Temperatures for Treated and Control LDPE samples

6.2.1.6 Differential scanning calorimetry (DSC)

DSC curve was obtained for all the treated and untreated LDPE samples. DSC thermogram of LDPE samples showed the difference in the melting transition temperature (Figure 6.8). The LDPE treated with biosurfactant, bacteria and biosurfactant+bacteria showed the melting transition temperature at 114 °C, 112 °C and 107 °C respectively. The melting temperature for untreated LDPE was obtained at 120 °C. So, the lowest melting temperature was observed in LDPE treated with biosurfactant+bacteria, which reveals the efficacy of biosurfactant and bacteria together to change the LDPE thermal properties, which could be the result of weakening of bonds in polymer chain in LDPE **(Hu *et al.*, 2007)**.

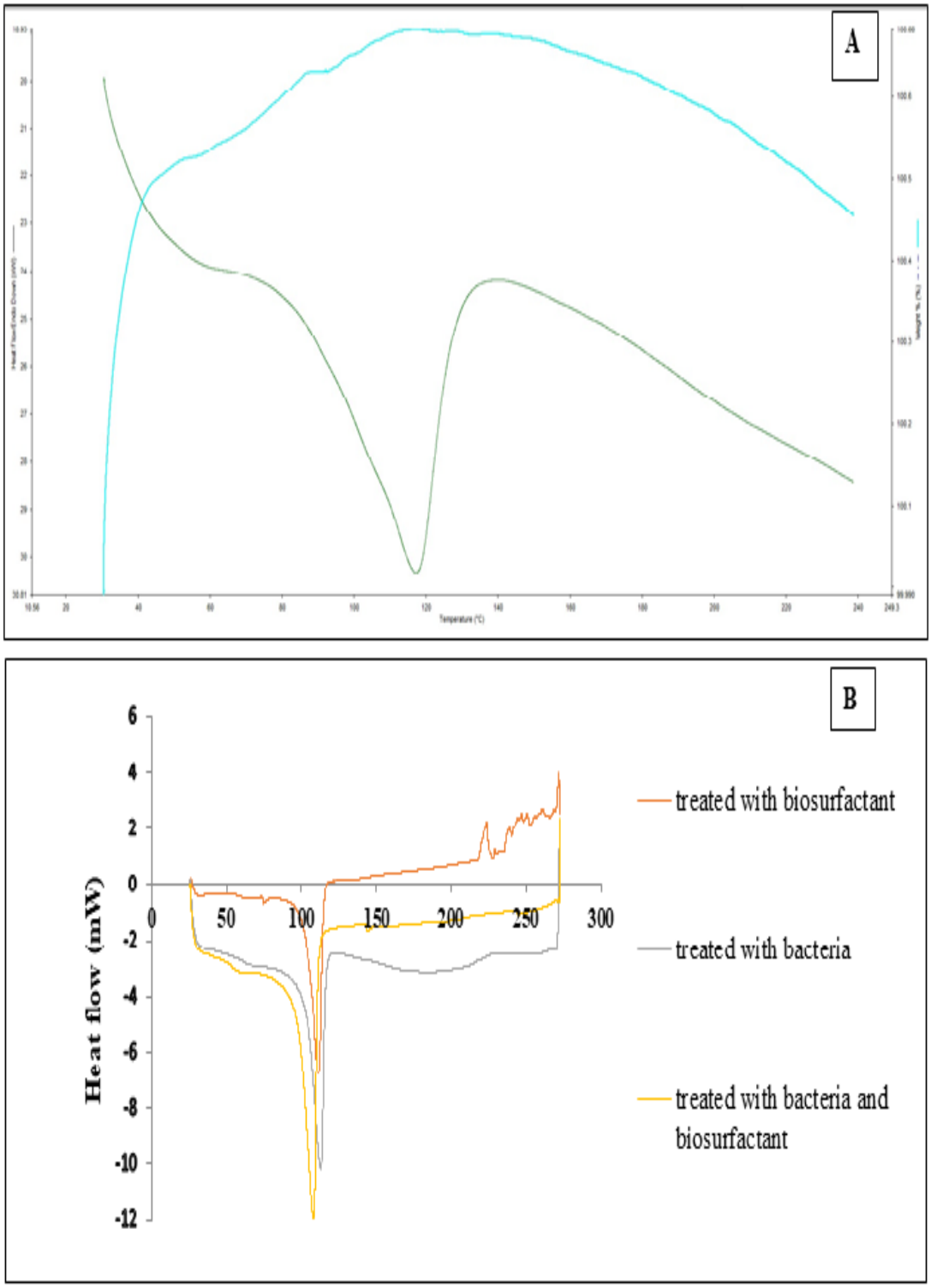


Figure 6.8: Differential Scanning Calorimetry curve of LDPE samples (A) Control LDPE sample (B)Treated LDPE samples

6.2.1.7 Weight reduction analysis

The weight loss analysis showed that the presence of *Bacillus* and its biosurfactant in the broth medium degraded the LDPE better than the all other combinations (Figure 6.9). Addition of biosurfactant in the culture medium enhanced the availability of LDPE molecules to the bacteria. Easier availability of carbon content in the molecules of polymeric chain supported the growth of bacteria in the medium while in other combination where the LDPE was treated only with bacteria the molecules of polymer were not readily available to the bacteria due to which growth was not proper and the degradation was slow **(Albertsson *et al.*, 1998)**.

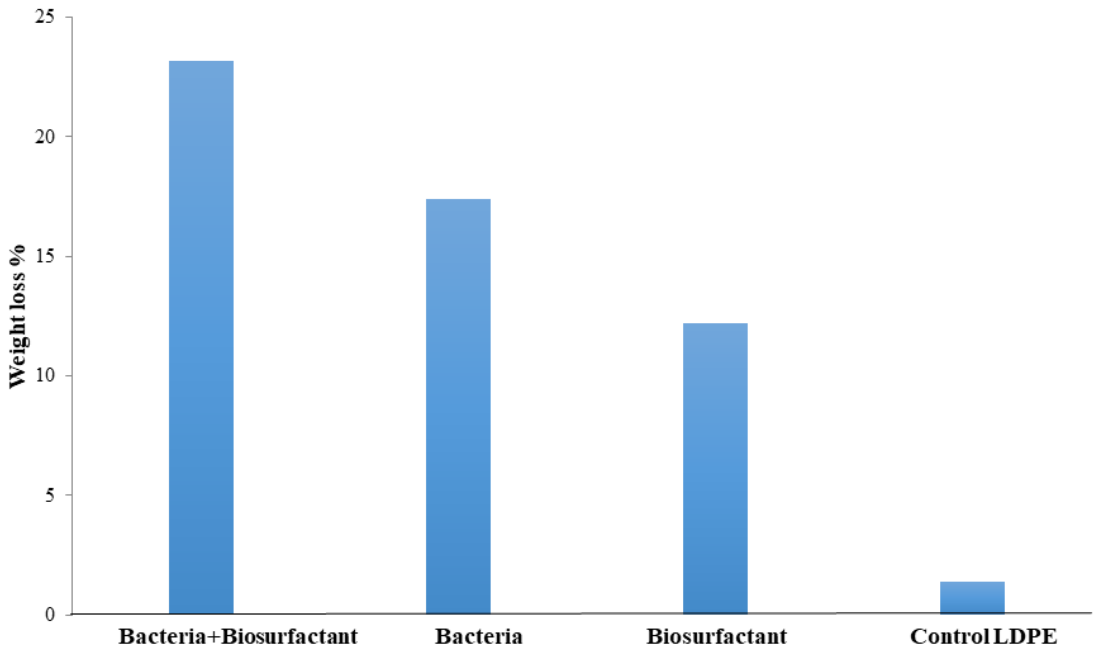


Figure 6.9: Weight percent reduction of Treated and Control LDPE samples

6.2.2 Biological degradation of polyethylene incubated with *Lysinibacillus* sp. Strain DESBBAU2

Lysinibacillus sp and its extracted biosurfactant was used in different combinations to determine the influence of bacteria and its biosurfactant on the degradation pattern of LDPE samples. The instrumental analysis such as FTIR, SEM, EDX, TGA, XRD and weight reduction analysis were performed to assess the extent of degradation in LDPE. Various analysis performed during the study are described below.

6.2.2.1 FTIR analysis

The structural analysis is an important parameter to know the structural changes appeared due to induced degradation responsible for weight loss. FTIR spectral analysis for the treated as well as untreated LPDE samples is given in Figure 6.10. FTIR spectra of the treated polyethylene samples were compared to that of untreated control samples. The emergence of new peaks in the 1800–1500 cm^{-1} region has been observed in all the types of treated polyethylene (Table 6.2). The emergence of new peaks specifies some structural changes in case of treated LDPE that indicated the breakdown of polymeric chain and presence of oxidation products of LDPE. Untreated control LDPE exhibited almost zero absorbance at those wave numbers. Absorbance at peaks 1710–1715 cm^{-1} (that corresponds to carbonyl compound), 1640 cm^{-1} (corresponding to —C=C—), decreased during the microbial treatment. Formation of bands at 1620–1640 cm^{-1} and 840–880 cm^{-1} are due to oxidation of polyethylene **(Ikada & Tsuji, 2000)**.

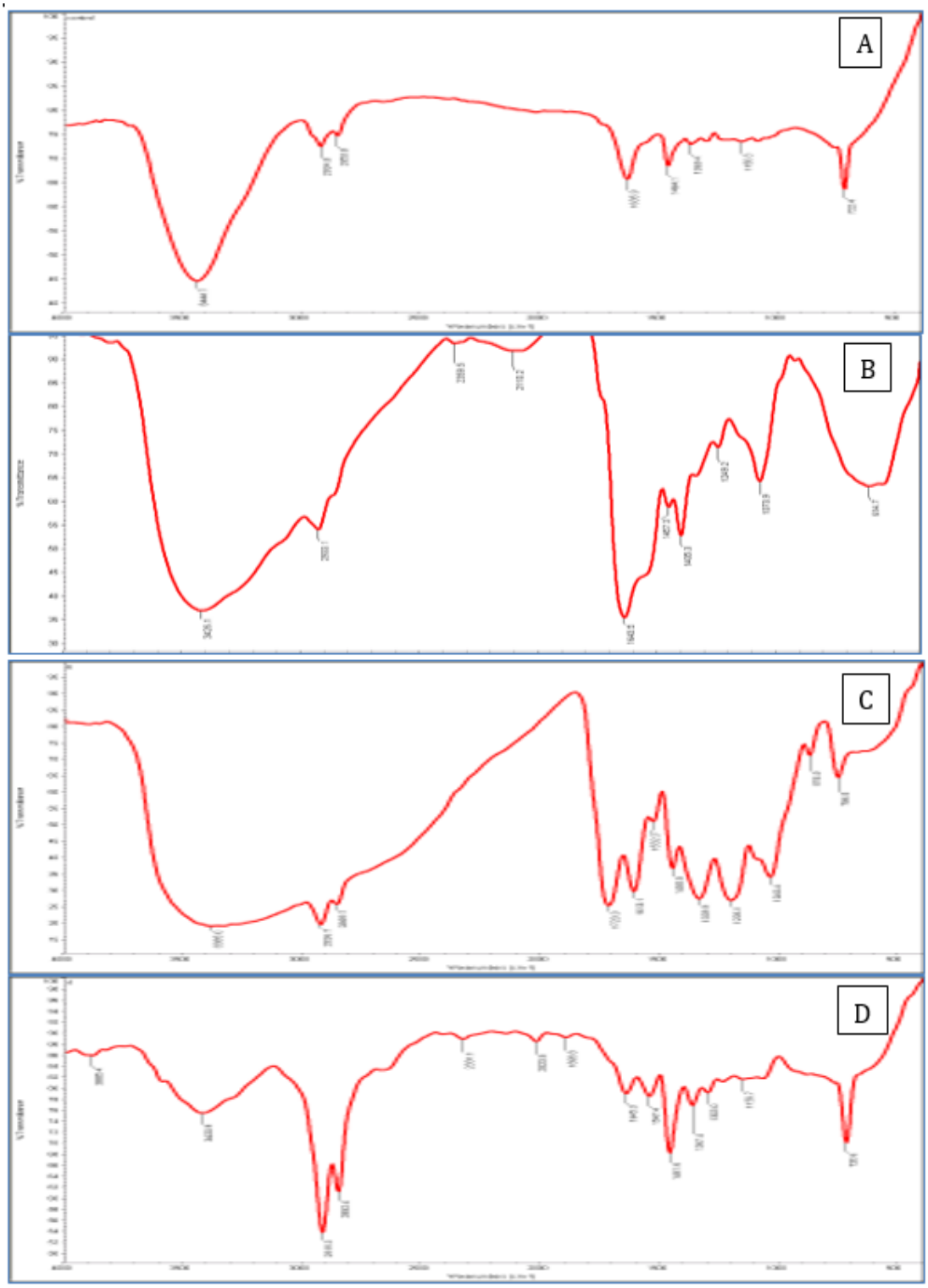


Figure 6.10: FTIR spectra (A) Control LDPE (B) LDPE treated with biosurfactant (C) LDPE treated with bacteria (D) LDPE treated with bacteria and biosurfactant

Table 6.2: Presence and absence of major functional groups observed in Treated and Control LDPE samples

Origin	Peak	Functional group	LDPE treated with bacteria and biosurfactant	LDPE treated with bacteria only	LDPE treated with biosurfactant only	Untreated control LDPE
N-H	3535.52 ,3410.15 cm^{-1}	stretching vibration of amine group.	Present	Present	Present	Present
=C-H	3142.04	stretch of alkyne group	Present	Present	Present	Present
(H-C-H)	2956.87 cm^{-1}	Asymmetric stretching of alkanes group	Present	Present	Present	Present
(H-C-H)	2922.16 cm^{-1}	symmetric stretching of alkanes group	Present	Present	Present	Present
N-H	1614.42 cm^{-1}	bend of amides	Present	Present	Present	Present
N=O	1462.04, 1379.10 cm^{-1}	N=O stretch of nitro group	Present	Present	Present	Present
C-O	1261.45 to 970.19 cm^{-1}	stretching vibrations of carbonyl group	Present	Present	Present	Absent

6.2.2.1.1 Carbonyl Index, Double bond Index under FTIR Analysis

The Carbonyl Index (CI) and Double Bond Index (DBI) of the treated LDPE were compared with that of untreated LDPE (Figure 6.11). Both the carbonyl and double bond index of the treated LDPE were observed to increase as compared to that of untreated control LDPE films. The formation of unsaturated hydrocarbons is considerably higher than that of the C=O bonds in all the treated control polyethylene, but the maximum value was obtained in case of LDPE treated with bacteria and biosurfactant both· Bio-surfactant is an amphiphilic molecule and it possess the ability to increase the solubilisation of hydrocarbons in the medium. The hydrophilic one projects towards the aqueous solution. This phenomenon improves the polyethylene's availability to dissolved oxygen, which results in the oxidation of LDPE **(Jakubowicz, 2003)**.

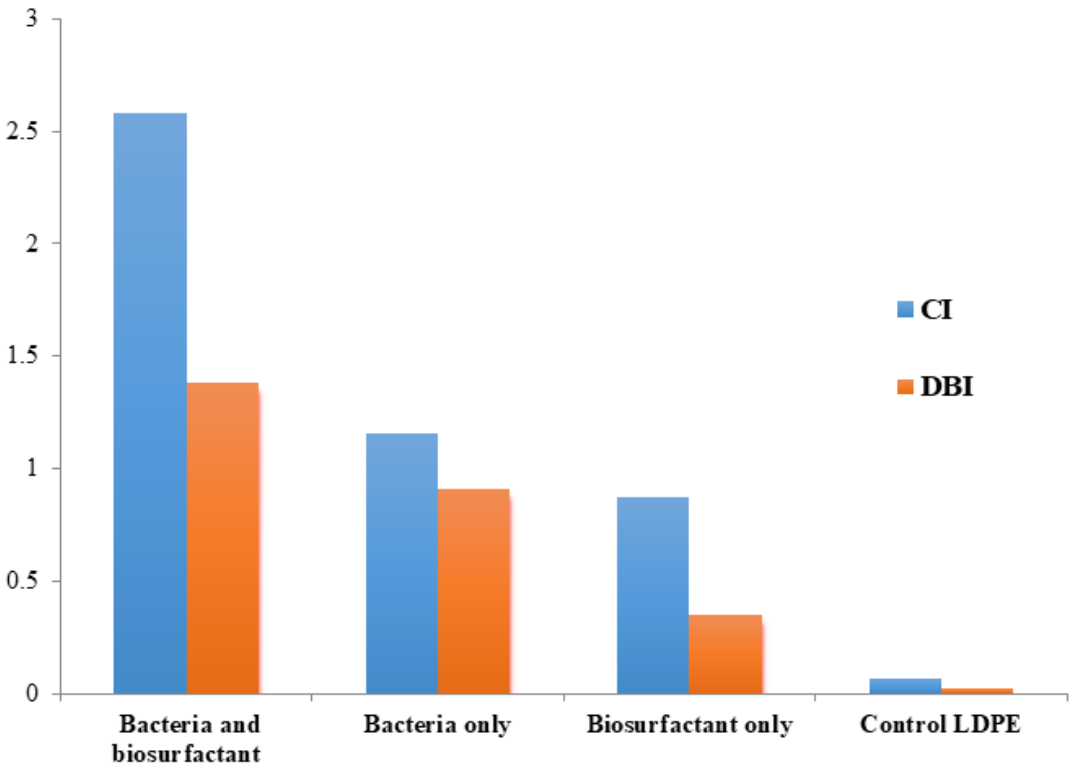


Figure 6.11: Carbonyl Index and Double Bond Index of Treated and Control LDPE samples

6.2.2.1.2 Crystallinity under FTIR Analysis

The crystallinity percent values defined the crystalline nature of the polymer. From the calculations of percent crystallinity, it has been found that the crystalline region of the LDPE got deteriorated in all the treated LDPE films as shown in figure 6.12. Lowest crystallinity percent of 31% was observed in the LDPE treated with bacteria and biosurfactant both. This could be due to the combined action of biosurfactant and bacteria as biosurfactant reduced the surface tension of the broth media and made the availability of carbon molecule present in the LDPE easier to the bacteria **(Koutny *et al.*, 2009)**.

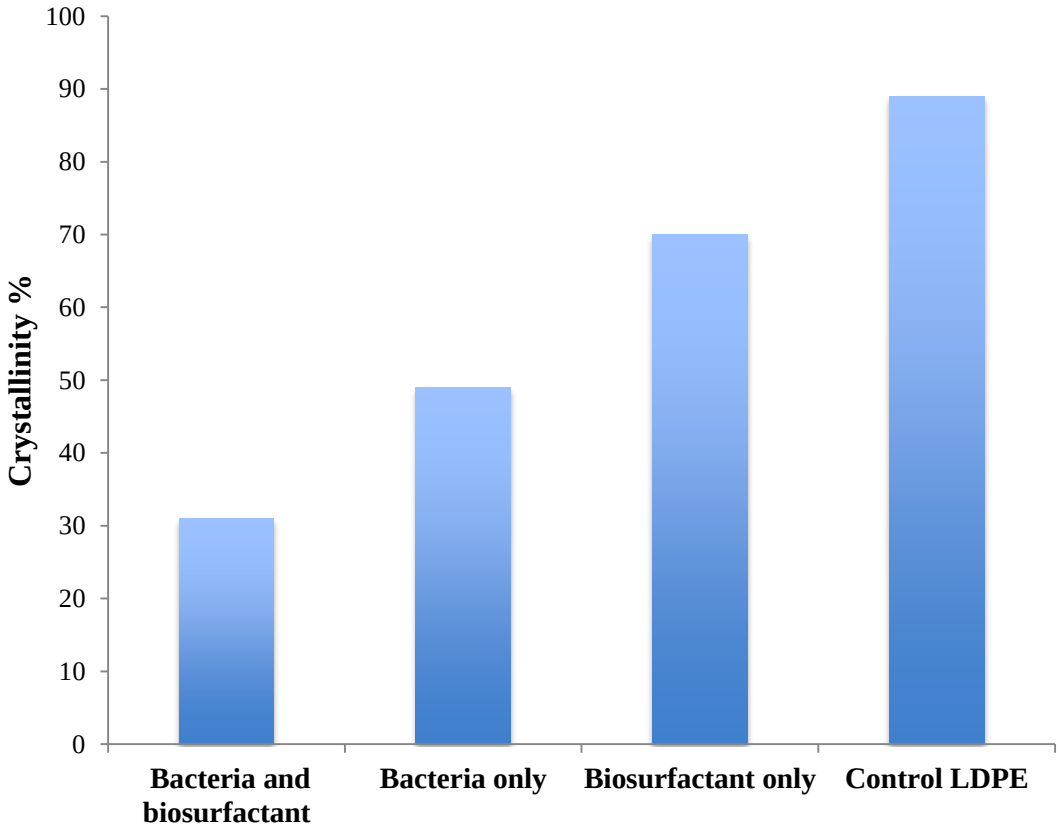


Figure 6.12: Crystallinity of Treated as well as Control LDPE sample.

6.2.2.2 SEM analysis

SEM analysis of the recovered LDPE sample was performed to determine the changes

occurred on the surface. The SEM micrographs of all the LDPE samples are given in Plate 6.2. The SEM micrographs of the untreated control LDPE showed a smooth surface morphology whereas the micrographs of treated LDPE sample revealed the occurrence of many non-uniformly scattered whitened areas and the erosional zones which illustrate the surface erosion mechanism involved in degradation which could be due to the enzymatic action. The worn-out regions with randomly scattered cracks and fissures show the disruption of the surface texture of LDPE film. The surface of the polymer became physically weak and eroded. Maximum change induced was observed in LDPE treated with bacteria and biosurfactant both as the maximum number of cracks and grooves were observed on its surface. The treated polymer films became rough, whereas the untreated control film retained a smooth surface even after 45 days of incubation under the same condition. Bacterial adhesion over the LDPE surface during the biofilm formation led to the corrosion on the surface of LDPE films **(Konduri *et al.*, 2010)**.

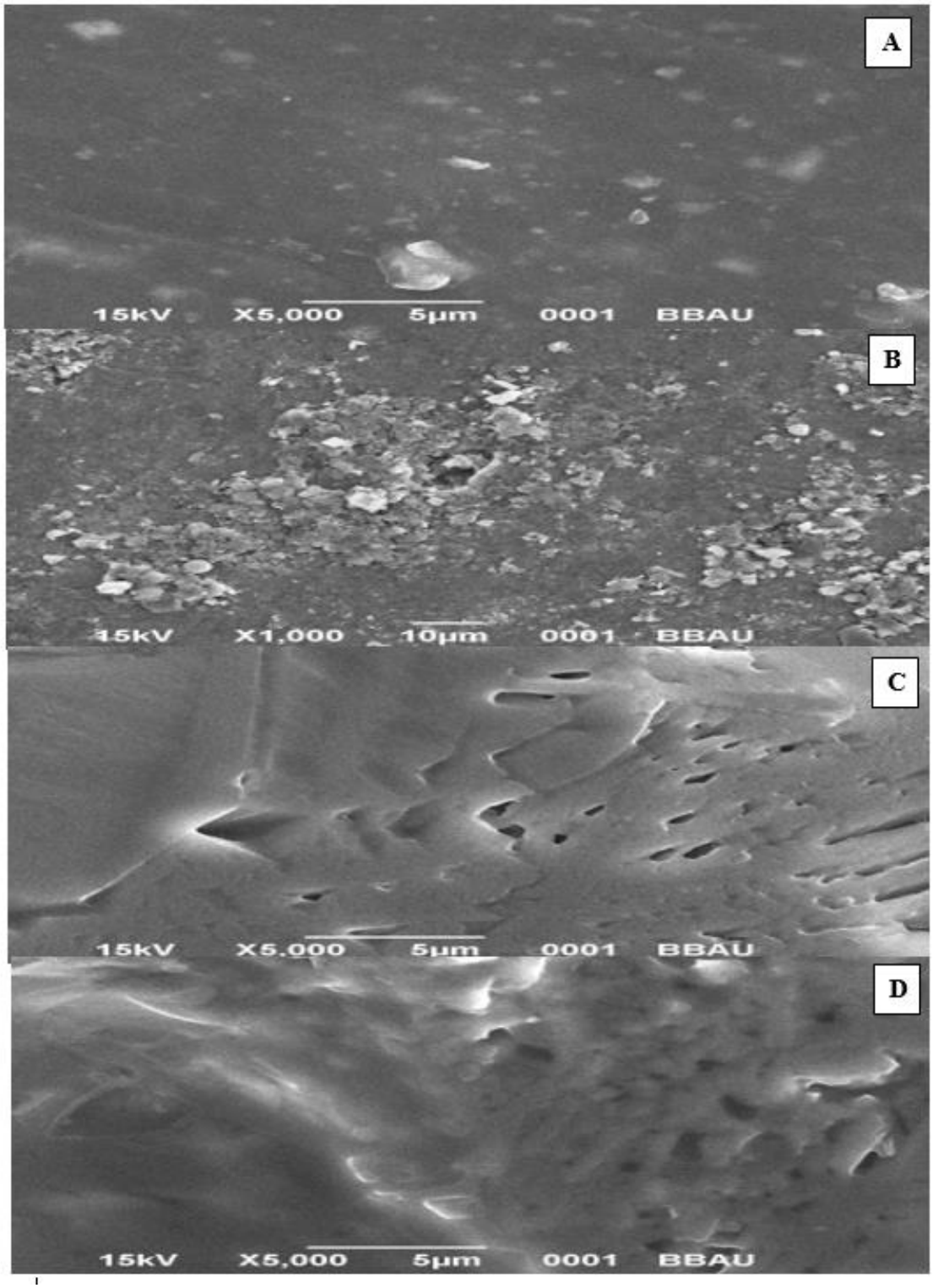


Plate 6.2: SEM micrographs of LDPE (A) Control LDPE (B) treated with bacteria and biosurfactant (C) treated with biosurfactant (D) treated with bacteria

6.2.2.3 EDX analysis

The EDX analysis revealed about the carbon and oxygen content in the polymer after degradation (Figure 6.13). Maximum reduction of carbon content was observed in LDPE treated with bacteria and biosurfactant both followed by LDPE treated with bacteria and with biosurfactant. The decrease in the carbon content might be due to the surface erosion, which is the primary cause of mass loss from the surface of the film **(Manzur *et al.*, 2004)**.

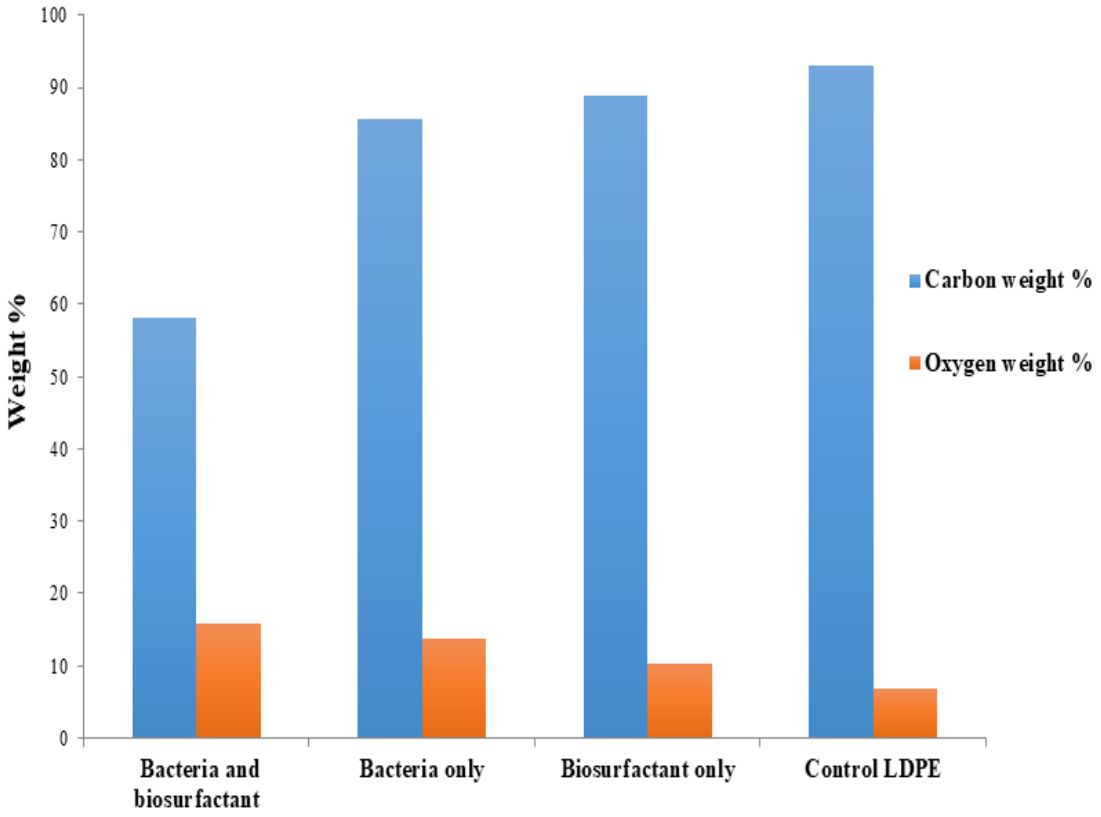


Figure 6.13: Carbon and Oxygen weight % of Treated and Control LDPE samples under EDX analysis

6.2.2.4 XRD Analysis

The XRD analysis was done to compare the level of crystallinity of treated and untreated LDPE samples. In case of biologically treated LDPE samples significant change in crystallinity were observed. Maximum number of peaks emerged in LDPE samples treated with bacteria and biosurfactant both followed by LDPE sample treated with bacteria and biosurfactant separately (Figure 6.14). The LDPE sample treated with bacteria and biosurfactant together showed 16 peaks at different 2 theta angles. The major peaks were observed at 21.371°, 23.718°, 29.81°, 36.035°, 54.73°, 57.19°. Synthetic polymers are generally known for their water insolubility which is due to their crystallinity **(Gautam *et al.,* 2007)**. When the polyethylene gets exposed to the biotic environment, the increase in uptake of water is accompanied by an increase in degree of crystallinity. In this case reduction in crystallinity was noticed as observed under FTIR analysis because experimentally there is an initial increase in percentage crystallinity owing to the consumption of amorphous portions but later, the depleted smaller crystals get consumed by microorganisms resulting in the decrease in crystallinity **(Kyaw *et al.,* 2012)**.

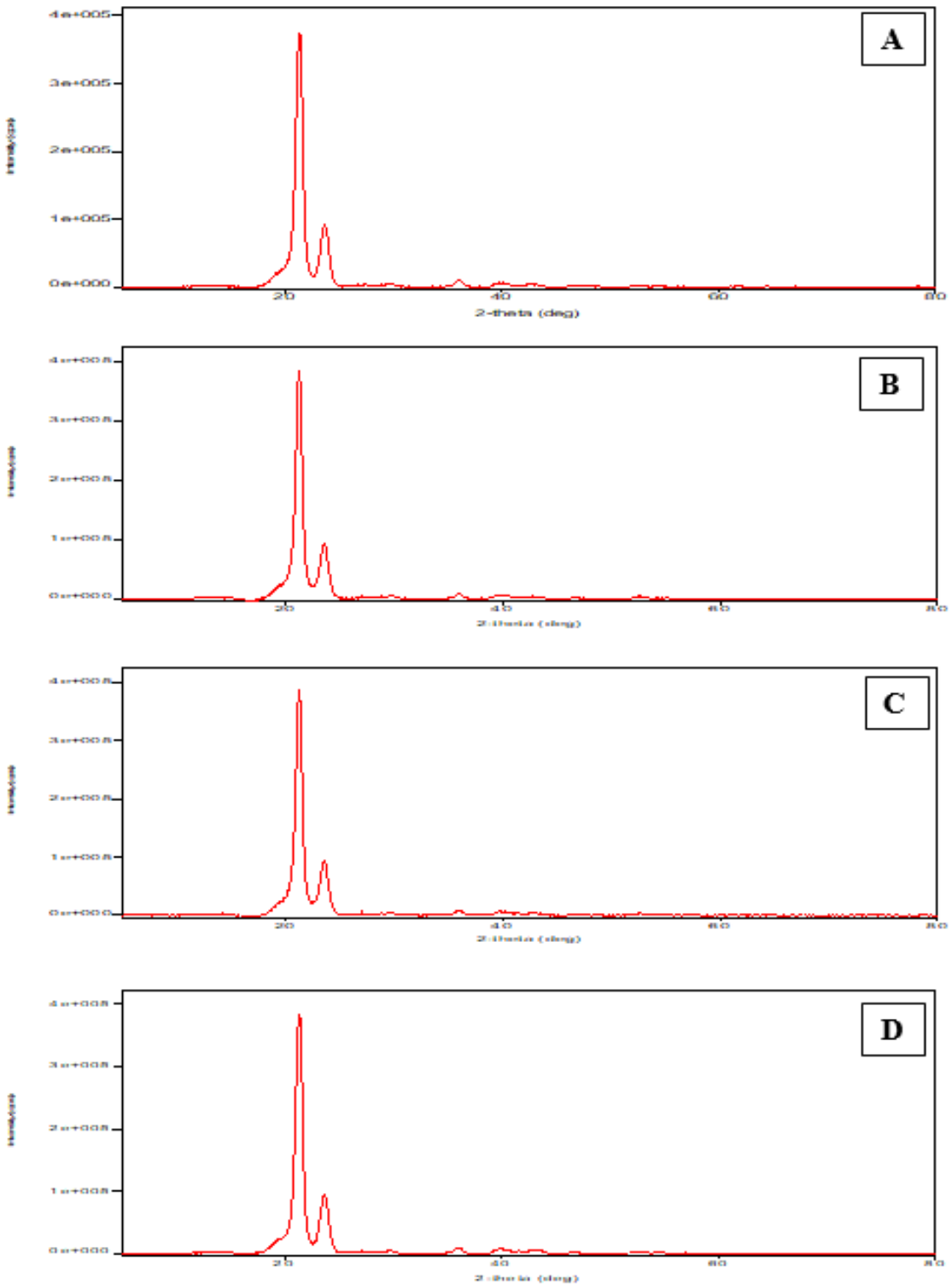


Figure 6.14: XRD spectra of (A) Control LDPE (B) LDPE Treated with bacteria only (C) LDPE with bacteria and biosurfactant (D) LDPE with biosurfactant

6.2.2.5 Thermal gravimetric analysis

From the TGA curves shown in the Figure 6.15, results of thermal analysis for treated and control LDPE samples, revealed the maximum decomposition temperature at 500 °C. The occurrence of endothermic effects in LDPE samples is the result of three processes namely: inter-molecular dehydrogenation, vaporization and the solid-state decomposition of some of the additives **(Mohammadi *et al.*, 2012)**. The total burning as well as the degradation of residual polymer backbone i.e., dehydrogenation of LDPE took place at a temperature interval of 200–500 °C. The onset temperature (T_o) for treated LDPE samples got reduced as compared to control sample. The T_O for the control was 406°C, for the LDPE sample treated with bacteria and biosurfactant T_o was 353°C, for LDPE samples treated with bacteria only T_o was 357 °C while the onset temperature for LDPE treated only with biosurfactant was 390 °C (Figure 6.16). No residue was left at final decomposition temperature for all the treated LDPE samples whereas for untreated control LDPE approximately 4.67 mg of residue was left at final decomposition temperature. The results of TGA analysis showed that decrease in the onset temperature for the LDPE sample treated with bacteria and biosurfactant can be due to the oxidation of polyethylene followed by its solubilisation into the aqueous media simultaneously by biosurfactant **(Hidayat & Tachibana, 2012)**. The second reason for the decrease in onset temperature could be the decrease of crystalline fraction of the biologically treated LDPE samples **(Lee *et al.*, 2018)**.

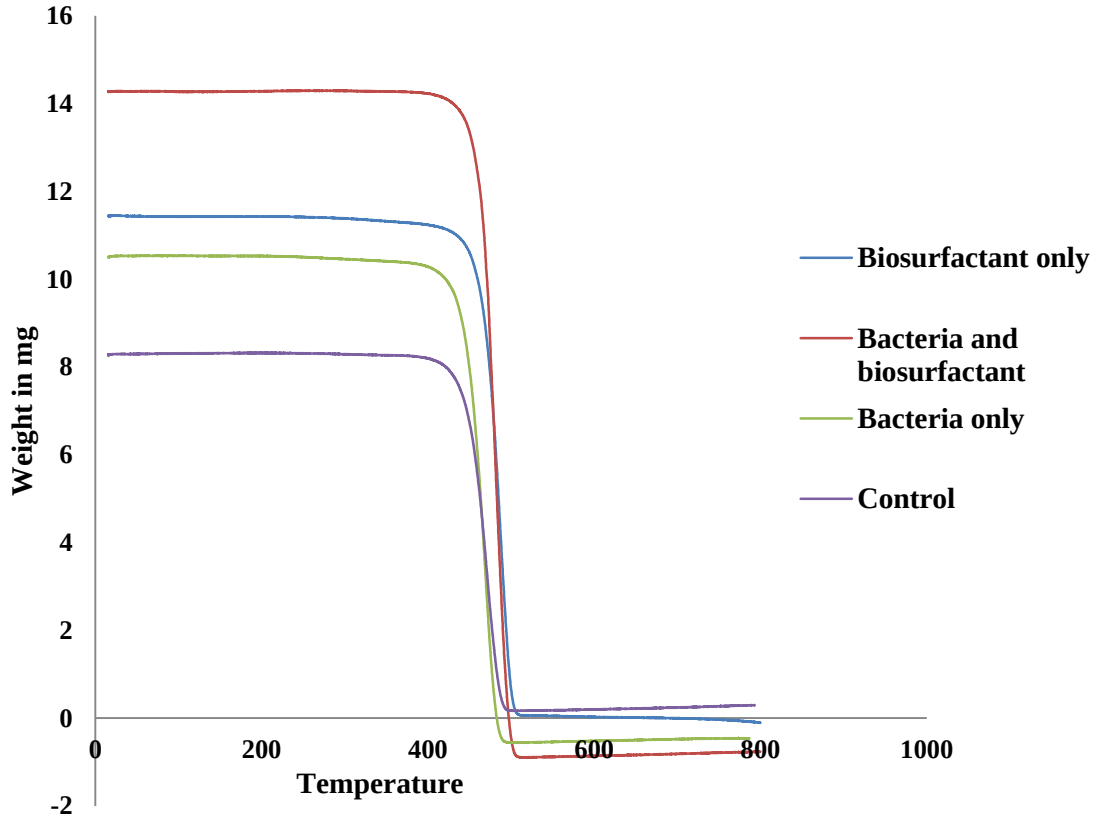


Figure 6.15: Thermal Gravimetric Analysis curve of Treated and Control LDPE samples

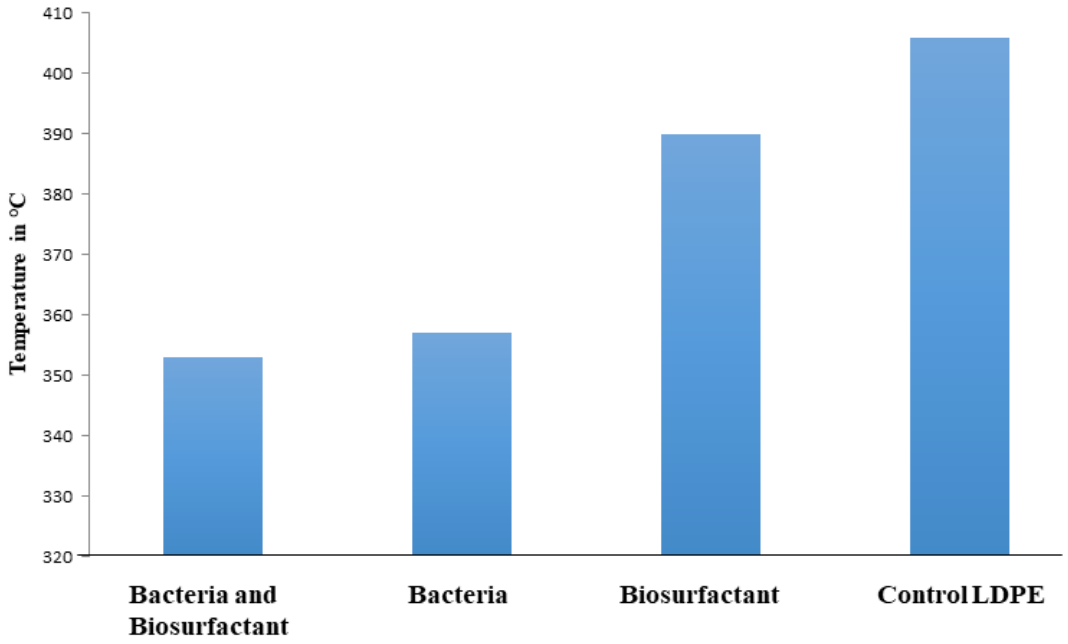


Figure 6.16: Decomposition temperature of Treated and Control LDPE samples

6.2.2.6 Differential scanning calorimetry

DSC curve was obtained for all the treated and untreated LDPE samples (Figure 6.17). DSC thermogram of LDPE samples showed the difference in the melting transition temperature. The LDPE treated with biosurfactant, bacteria and biosurfactant+bacteria showed the melting transition temperature at 124°C, 112°C and 105°C respectively. The melting temperature for untreated LDPE was obtained at 120°C. So, the lowest melting temperature was observed in LDPE treated with biosurfactant+bacteria. This clearly shows that bacteria along with the addition of biosurfactant deteriorate the polymer efficiently because biosurfactant makes the LDPE molecule easily available to the bacteria by reducing its hydrophobicity **(Lee *et al.*, 1991)**.

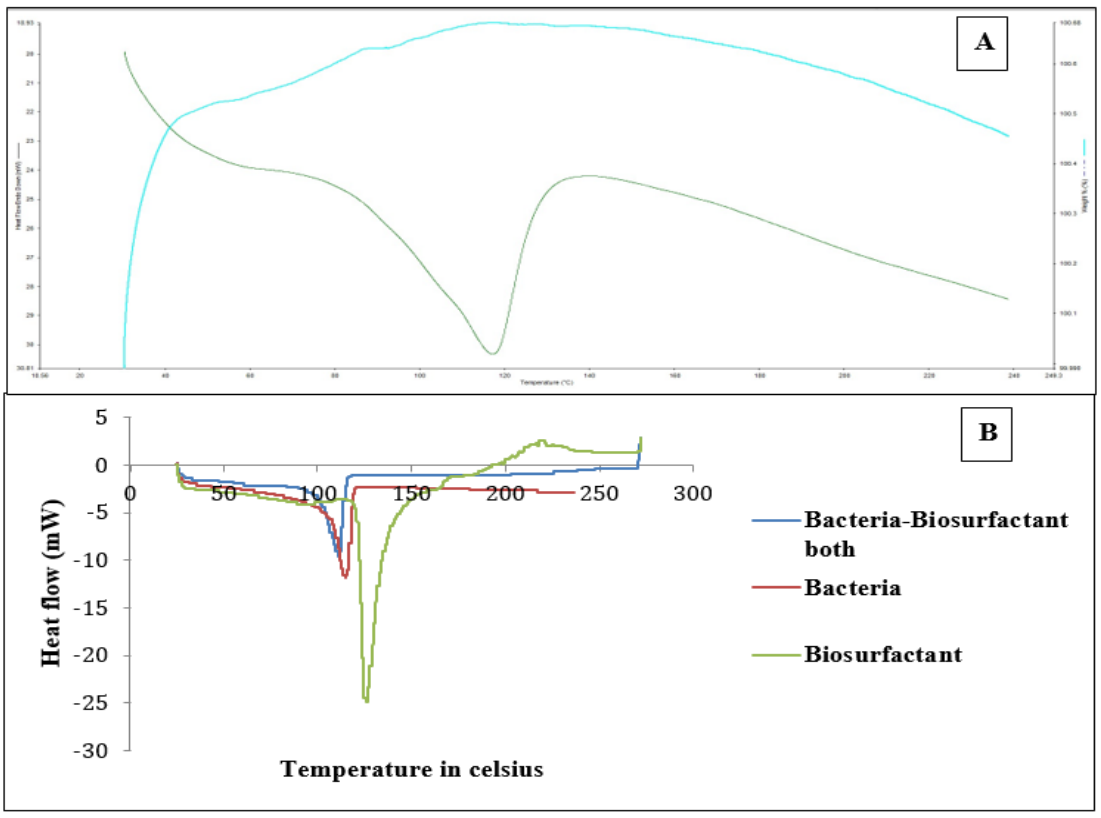


Figure 6.17: Differential Scanning Calorimetry curve of LDPE (A) Control LDPE (B)Treated LDPE

6.2.2.7 Weight reduction analysis of LDPE samples

After 45 days of incubation period, the percent weight reduction in case of LDPE treated with bacteria and biosurfactant together was approximately 29.32% whereas the LDPE treated separately with bacteria and biosurfactant, the weight reduction was 21.68% and 14% respectively (Figure 6.18) **(Nowak *et al.*, 2011)**. The graph for the weight reduction is shown in figure. Slight change in weight was observed in control LDPE sample. The weight reduction for the untreated control was 0.27%. The weight loss of the LDPE films can be attributed due to the breakdown of carbon backbone, which can be due to the enzymatic degradation of bacterium *Lysinibacillus* sp strain DESBBAU2. The reduction of weight in LDPE positively indicated the deterioration and breakage of polymer chains by the bacterium.

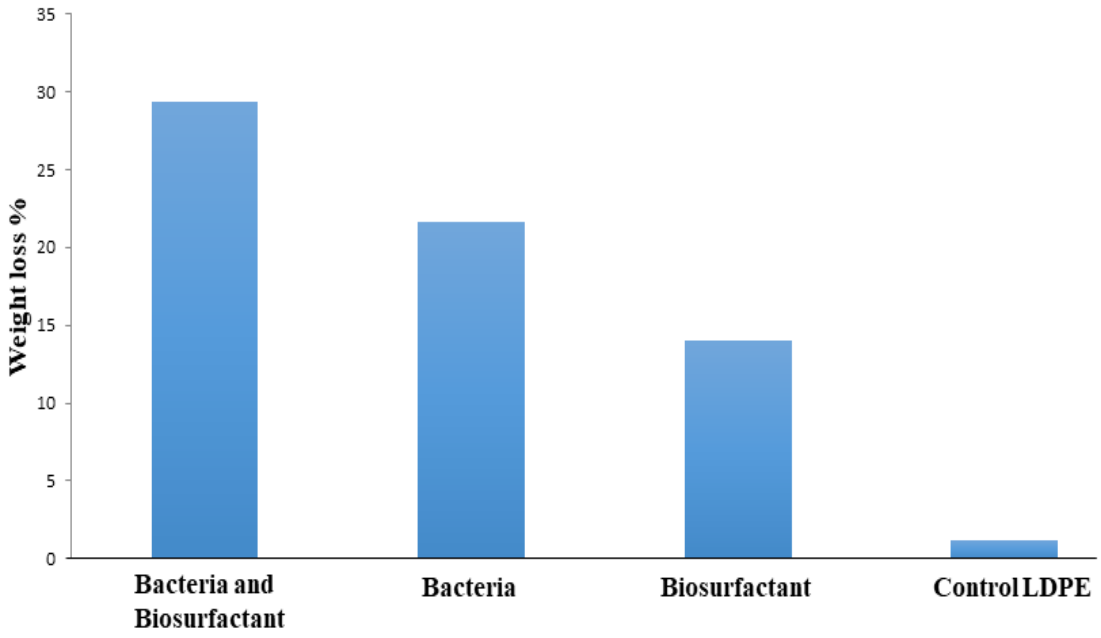


Figure 6.18: Weight loss percent of Treated and Control LDPE samples

6.3 Conclusion

The overall degradation results obtained for both the bacteria revealed that *Bacillus sp* and *Lysinibacillus* are effective in degrading the LDPE. However, *Lysinibacillus* sp has shown greater impact on the degradation of the polymer. Among all the combinations major form of bio-deterioration was observed in case of LDPE treated with *Lysinibacillus* sp in combination with its biosurfactant. The addition of biosurfactant in the culture medium enhanced the availability of the polymer molecules to the bacterial cells by reducing the surface tension. Polyethylene samples which were treated with *Lysinibacillus* along with bio-surfactant showed maximum weight loss of 29.32%. FTIR spectrum of LDPE film confirmed changes in the presence of chemical groups like amine, alkanes, phenols, and alcohol after degradation of LDPE films by *Bacillus* sp and *Lysinibacillus* sp The results affirmed that the isolates are capable of degrading LDPE. The two bacterial isolates are first time being reported for their LDPE degrading potential and also, they are the active producer of biosurfactant, which has several applications in bioremediation.

7.0 Introduction

Composting is basically a natural process by which organic material get decomposed into humus, which is a soil- like material. The major groups of microorganisms that are involved in composting are bacteria and fungi **(Hadad *et al.*, 2005)**. These microorganisms decompose the organic substances as their source of food. This process of degradation requires Carbon, Nitrogen, Oxygen, water and heat **(Kadouri *et al.*, 2002)**. Organisms decompose the organic substance to use carbon and nitrogen as the source of energy and for building their cell structure. Degradation of the polymers in compost environment occurs primarily through the mechanical, chemical, and thermal degradation. Of all the mechanisms of degradation, chemical degradation is considered as the most important for the polymers **(Das & Mukherjee, 2007)**. In compost method, the specific weight of the plastic is buried under the mixture of definite amount of mature compost and soil and then kept under the natural weathering conditions with maintained moisture content. Biodegradation of polymer is measured based on the amount of carbon present in the polymer converted to gaseous carbon dioxide **(Gulmine, 2003)**. Nature and type of compost affects the extent and degree of degradation of polymer. This is the natural way of decomposing the plastic in eco-friendly manner **(Das & Kumar, 2015)**. The increased accumulation of plastics in the environment have forced the researchers across the world to develop several degradation methods to deal with the plastic waste which do not create further stress on natural environment. The study presented here is one such attempt of dealing with plastic waste. In order to evaluate biodegradability under real disposal conditions, compost burial test of LDPE films was performed for a period of six months. In the experiment different concentrations of compost were used and kept in pots to degrade

the low-density polyethylene (LDPE) films. The investigations on the chemical and morphological changes in LDPE films revealed the extent of degradation.

7.1 Materials and methods

7.1.1 Experimental Setup

For composting method different concentration of compost 20%, 40%, 60%, 80% and 100% (w/w) was made. For this, approximately 1 kg of the compost and soil was maintained in different concentration in different pots. Experiment was done in triplicate. The LDPE films of area 5 cm^2 were buried under the compost in pots for the period of six months.

7.1.2 Characterization of degraded LDPE samples

After six months LDPE samples were taken out, washed and characterized using weight reduction, SEM, EDX, FTIR, TGA, XRD and DSC analysis.

7.1.2.1 Fourier transform Infra Red Spectroscopy (FTIR)

The detailed methodology for performing FTIR is given in Section **3.2** of Materials and Methods in **Chapter 3**.

7.1.2.2 X-Ray Diffraction Analysis (XRD)

The detailed methodology for performing XRD analysis is given in Section **3.4** of Materials and Methods in **Chapter 3**.

7.1.2.3 Thermal Gravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC)

The detailed methodology for performing TGA and DSC analysis is given in Section

3.5 and **3.6** of Materials and Methods in **Chapter 3**.

7.1.3 Physico-Chemical analysis of Compost

Major Physico-chemical parameters such as pH, electrical conductivity, cation exchange capacity, total organic carbon, total nitrogen and sulfur of the compost-soil mixture were also tested before six months and after six months.

7.1.3.1 pH analysis of Compost samples

1:5 soil:water suspension was prepared. 10 g air-dried compost was weighed into the beaker and 50 mL of deionized water was added. The solution was then mechanically shaken for 1 hour at 15 rpm. pH meter was calibrated using standard buffer. The electrode was washed thoroughly between measurements with deionized water. The electrode was then immersed into the soil suspension. The pH value was recorded for the samples **(Okoh & Atuanya, 2014)**.

7.1.3.2 Electrical conductivity (EC) of Compost samples

1:5 soil:water suspension was prepared by weighing 10 g of air-dried compost into the bottle. 50 mL of deionised water was added. Soil solution was mechanically shaken at 15 rpm for about 1 h to dissolve soluble salts. Conductivity meter was calibrated using the KCl reference solution to obtain the cell constant. The electrical conductivity of 0.01M KCl was measured at the same temperature as the soil suspensions. The conductivity cell was rinsed with double distilled water. Conductivity cell was then refilled without disturbing the settled soil. Value was recorded as indicated on the conductivity meter **(Pandey *et al.*, 2003)**.

7.1.3.3 Cation Exchange Capacity of Compost samples

Reagents used:

i. 1 M ammonium acetate (NH_4OAc) saturating solution: 57 ml of glacial acetic acid (99.5%) was diluted with 800 mL of distilled water in a 1 L volumetric flask. 68 mL of concentrated NH_4OH, was added in it and then mixed and cooled.

ii. 1 M KCl replacing solution: 74.5 g of KCl was dissolved completely in distilled water and diluted to a final volume of 1 L.

iii. Ethanol 95%.

Procedure

25 g of compost was added to a 500 mL of Erlenmeyer flask. Then added 125 mL of 1 M NH_4OAc, and shaked thoroughly and kept for overnight.

A Buchner funnel of 5.5 cm with retentive filter paper was fitted, and transferred the soil in it. Soil was washed gently four times with 25 mL of NH_4OAc allowing each addition to filter through it but not letting the soil to crack or dry. Washed the soil eight times with the additions of 95% ethanol to remove the excess saturating solution. Extracted the adsorbed NH_4 by leaching the soil with the addition of 25 ml of 1 M KCl. Discarded the soil and transferred the leachate to a 250 mL volumetric flask. Diluted the volume with the addition of KCl. Determined the concentration of NH_4-N in KCl extract by colorimetry **(Reddy, 2008)**.

Calculations:

Where NH_4-N is in mg/L:

CEC (cmolc/kg) = (NH_4-Nin extract - NH_4-Nin blank) / 14

Where NH_4-N is in mg NH_4/L:

CEC (cmolc/kg) = (NH_4-Nin extract - NH_4-Nin blank) / 18

7.1.3.4 Total Organic Carbon of compost samples

Total organic carbon was analyzed by well-known Walkley and Black method (1934).

Reagents

- $K_2Cr_2O_7$ (0.1 M)
- $FeSo_4.(NH_4)_2.H_2O$ (0.5 N)
- Concentrated H_2SO_4
- Concentrated H_3PO_4
- Diphenylamine indicator (DPA)

Method

For determining the total organic carbon, 1.0 g of air dried and sieved sample was taken in 250 ml conical flask and 10 ml of $K_2Cr_2O_7$ (0.1 M), 20 ml pf conc. H_2SO_4. 10 ml of H_3PO_4 was added and left for 30 min. After completion of reaction 200 ml of distilled water and 2 ml of DPA were added and titrate with 0.5 M ferrous ammonium sulphate solution till the color of the solution turn brown in colour. For the blank same procedure was followed.

Calculation: % Organic C in compost= (B-T) x N x 0.003 x 100/WS

Where, N = Normality of FAS, B= Blank burette reading (ml), T= Sample reading (ml), WS= Weight of Sample (g)

7.1.3.5 Available Nitrogen

Reagents

- $KMnO_4$ (0.32%)
- NaOH (2.5%)
- H_3BO_3
- H_2SO_4 (0.02 M)
- NaOH (0.1 M)

Method

The available N was analyzed by using the Kjeldahl flask (tube) and 20 ml of distilled water, 50 ml $KMnO_4$ (0.32%), 50 ml NaOH (2.5%) were added. Distillation was started by using automatic distillation unit for 9-12 min. The released ammonia was collected in 250 ml conical flask containing 20 ml boric acid (2%) at the distillation end. After completion of the reaction pinkish color of boric acid turns to light green color by the absorption of ammonia in the conical flask. The collected distillate was titrated with 0.02N H_2SO_4 till the color of solution turn pinkish. For the blank same procedure was followed without the sample.

Calculation: Available N (%) = (T-B) x 1.401 x N of HCl/WS

Where, T = Titrate value, B = Blank value, WS = Weight of sample

7.1.3.6 Available Sulphur

Available phosphorous content was measured by the method described by (**Tabatabi, 1982)**.

Reagents

- Acidified ammonium acetate (0.25 M)
- Acid seed solution (1 N HCl contain 20 ppm of sulphate)
- Crystals of $BaCl_2.2H_2O$

Stock Standard Sulphur Solution (100 ppm)

0.534 g of oven dried AR grade potassium sulphate (K2SO4) was dissolved in distilled water, and diluted the solution to 1 liter. From this solution 10 ml of 100 ppm standard S solution was transferred into 100 ml of volumetric flask and diluted with water up to mark and mixed. The standard solution contained 10 ppm of S and working standard was made from this solution.

Method

For determination of AS, 10 gm of air-dried sample was taken in 150 ml of conical flask and 25 ml of 0.25 M Acidified ammonium acetate was added. The mixture was put on to a horizontal shaker for 30 min. After the completion of shaking process, the suspension was passed through a Whatman filter paper No. 42. For the estimation of AS, 10 ml of aliquot was taken from the filtrate in a conical flask and 1 ml of seed solution and 0.5 g crystal of $BaCl_2$ were added. The solution was turn in turbid (white) color. The optical density (OD) was taken at 420 nm by spectrophotometer and the concentration was obtained with the help of calibration curve.

Calculation: Available S (mg/Kg) = S Concentration from curve x V/v x WS

Where WS = Weight of the sample taken, V = Volume of the extract, v = Volume of the aliquot taken for estimation.

7.2 Results and discussion

The LDPE films were subjected to the compost treatment of varying concentration from 20% to 100%. After the period of six months LDPE films were recovered from the compost and various analysis such as SEM, EDX, FTIR, TGA, DSC, XRD and weight reduction analysis was performed for determining the extent of degradability. The physico chemical parameter of compost sample was also estimated to observe the post treatment changes in the compost parameters. The results obtained for various analyses are detailed below.

7.2.1 Scanning Electron Microscopy

In SEM analysis (Plate 7.1) the untreated LDPE film appeared as a smooth, uniform and homogeneous sheet, whereas, other LDPE films that were kept under the treatment with different concentration of compost showed significant changes in the surface morphology. After six months of treatment, most prominent change was observed in LDPE film that was treated with 80% compost followed by 100%, 60%, 40% and 20% compost. In LDPE film treated with 100% compost the deterioration was observed over the surface but it was less prominent than 80% compost treated LDPE. In 60% and 40% compost treated LDPE some changes such as exfoliation of surface and peeling was observed. Least change was observed in 20% compost treated LDPE due to less amount of compost and more amount of dry soil sample. The LDPE film appeared as a continuous, uninterrupted sheet. SEM micrographs in this study revealed that upon exposure to compost, surface erosion took place and the matrix became perforated **(Eubeler *et al.*, 2009; Fontanella *et al.*, 2010)**. This ultimately resulted into a thinning if matrix with inconsistent properties which corresponds to the deterioration of the mechanical properties of the LDPE sheets. Exfoliation was clearly demonstrated

in the treated LDPE. This peeling of thin layer (exfoliation) can happen only if there is a major erosion of the more accessible amorphous regions in the polyethylene, leaving crystalline region that degrades very slowly. The SEM analysis clearly revealed the change in the surface structure of the LDPE and moreover the degradation was observed to be more prominent in 80% compost treated LDPE sample **(Iovino *et al.*, 2008; Gajendiran *et al.*, 2016)**.

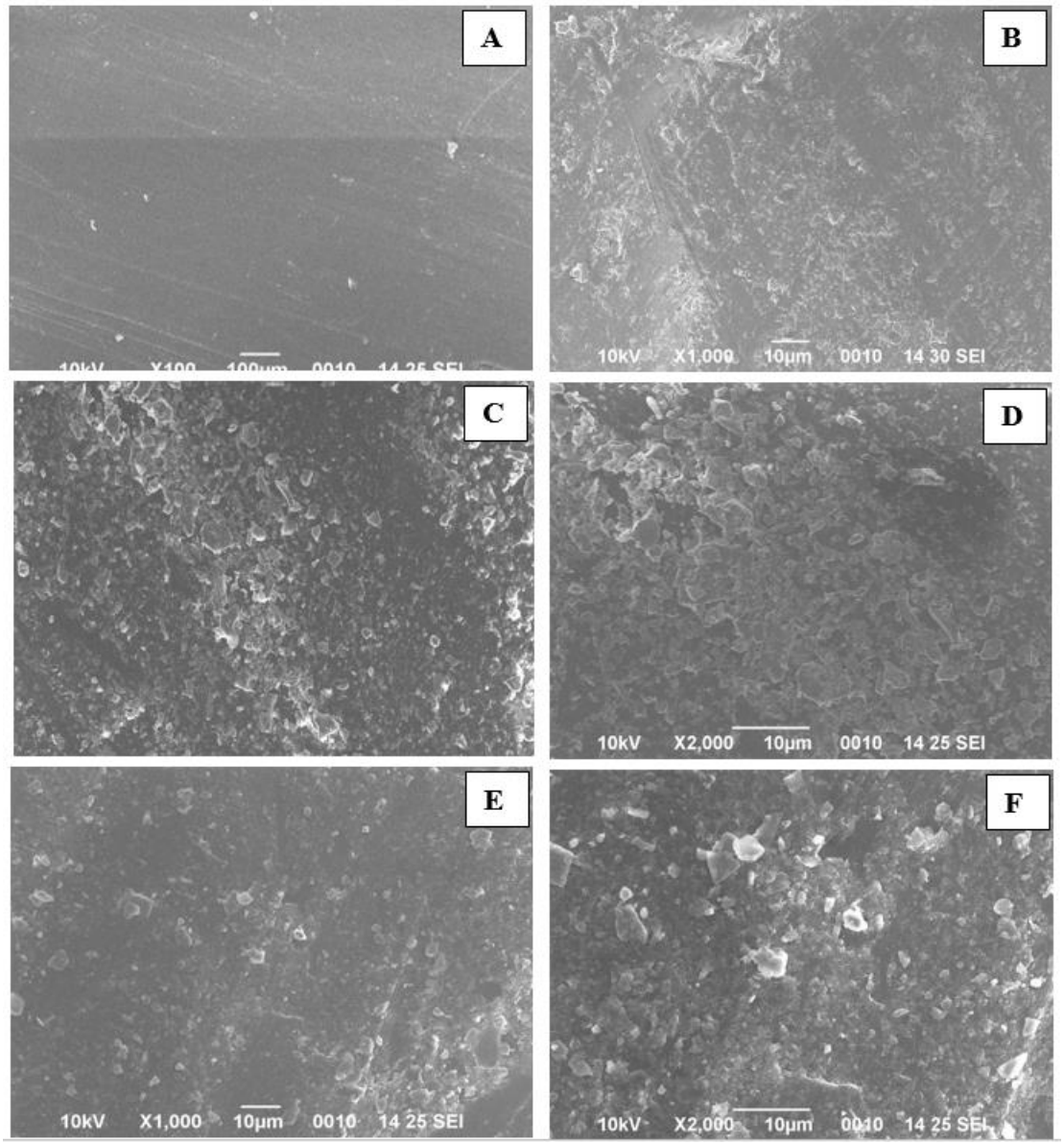


Plate 7.1: SEM micrographs of LDPE sample treated with different concentration of compost (A) Untreated LDPE (B) 20% (C) 40% (D) 60% (E) 80% (F) 100%

7.2.2 X-Ray Diffraction Analysis

The crystallinity of the polymer is directly in proportion to the diffraction peak intensity. The peaks at 21° and 23.5° are the characteristic peaks of the semi-crystalline polyethylene molecule. The intensities of the different compost treated samples were compared for the intensities at these two significant peaks. The change in crystallinity of treated and untreated samples was compared according to the change in X-Ray intensities at different 2 theta angles (Figure 7.1). The two obvious diffractive peaks of about 21° and 23.5° corresponds to typical crystalline plane (110), and (200) of orthorhombic phase, respectively. From the Table 7.1 it can clearly be seen that the lowest intensity at 21° and 23.5° was observed on 80% compost treated LDPE sample. Maximum intensity was found in untreated LDPE sample followed by 20%, 40%, 60% and 100% compost treated sample **(Reddy, 2008; Grover *et al.*, 2015)**.

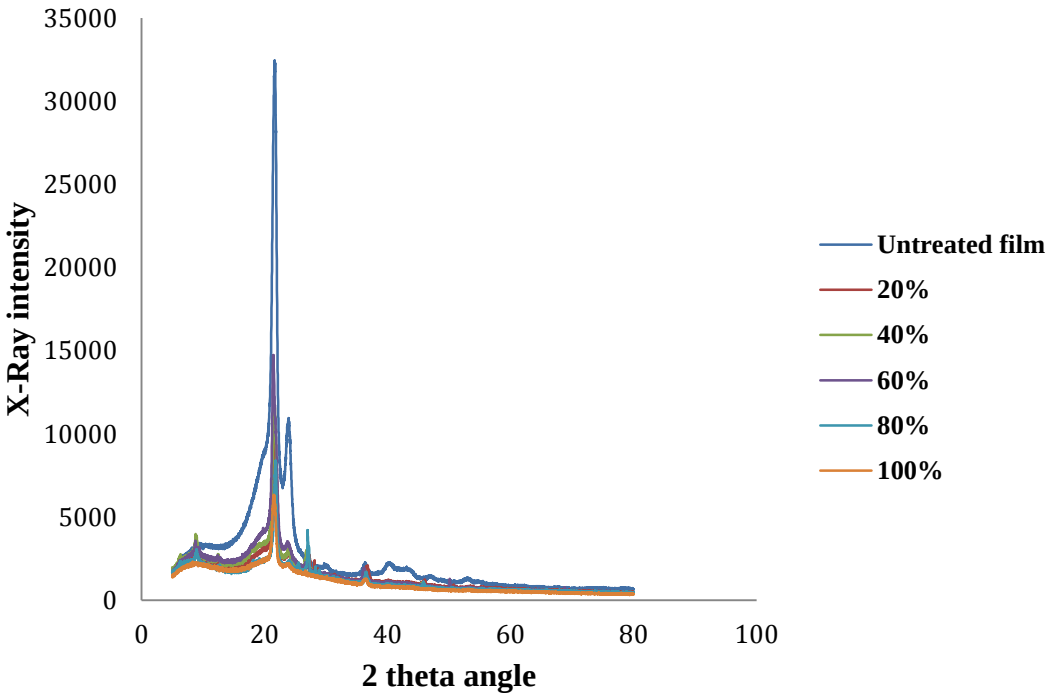


Figure 7.1: X-Ray Diffraction curve of LDPE treated with different concentrations of LDPE

Table 7.1: X-Ray intensities of LDPE samples treated with different concentration of compost

Samples	Intensity at 21°	Intensity at 23.5°
Untreated sample	20425	8831
20% compost	8445	3263
40% compost	7455	2748
60% compost	6225	2625
80% compost	3371	2100
100% compost	5740	2169

7.2.3 Energy Dispersive X-ray analysis

The weight percent of Carbon and Oxygen of LDPE treated with different concentration of compost is given in the Table 7.2. The EDX analysis revealed that lowest carbon content was observed in 80% compost treated sample and higher oxygen content was also found in the LDPE treated with 80% compost treated sample. This signifies that the microbial attack began at the surface of the polymer resulting in higher oxygen content on the surface. This step can be described as development of oxidation skin and as a result the polymers surface is damaged with fine cracks indicating morphological transformations after which degradation proceeds towards the inner part of the polymer depending on the diffusion rate **(Volke *et al.*, 2002; Okoh & Trejo-Hernandez, 2006)**.

Table 7.2: Weight percent of Carbon and Oxygen of LDPE treated with different concentration of compost

Sample	Carbon	Oxygen
Untreated LDPE	77.29%	22.71%
20% Compost treated	73.01%	26.42%
40% Compost treated	67.75%	32.25%
60% Compost treated	53.11%	44.20%
80% Compost treated	46.29%	50.26%
100% Compost treated	50.22%	46.32%

7.2.4 Thermal Gravimetric Analysis

After six months of incubation in compost, a significant drop in thermal stability of LDPE films were recorded with respect to untreated LDPE sample (Figure 7.2). Maximum reduction in thermal stability was observed in 80% compost treated LDPE. The onset temperature was found to be 403°C and final decomposition temperature was 486°C with no residue left (Table 7.3). Both the temperature for 80% compost treated LDPE obtained were found to be least among all the compost treated samples and untreated sample. TGA results showed the presence of endothermic effects in the compost treated sample. The endothermic effect was due to three processes, which are intermolecular dehydration, vaporization and solid-state decomposition. The total burning and degradation of the residual polymer backbone took place at temperature interval of 200°C to 500°C **(Islam *et al.*, 2011)**.

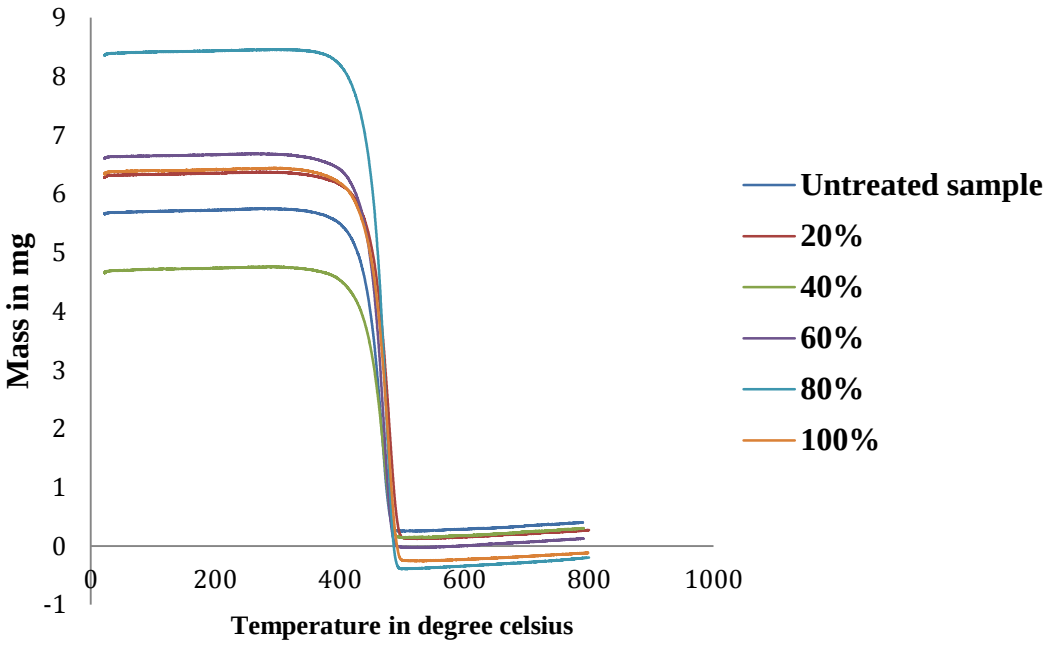


Figure 7.2: Thermal gravimetric analysis curve of LDPE samples treated with different concentration of compost

Table 7.3: Thermal gravimetric analysis parameters of LDPE samples treated with different concentration of compost

Samples	Onset temperature	Final temperature	Residue left
Untreated sample	426°	531°	0.26 mg
20% compost	425°	528°	0.148 mg
40% compost	421°	520°	0.129 mg
60% compost	410°	513°	0.02 mg
80% compost	403°	486°	No residue
100% compost	408°	500°	No residue

7.2.5 Differential Scanning Calorimetric Analysis

From the curve of DSC analysis (Figure 7.3) some of the parameters such as glass transition temperature (T_g), onset melting temperature (T_o), melting point temperature (T_m) and end melting point temperature (T_f) were recorded. The glass transition temperature, onset melting temperature, melting point temperature and end melting point temperature for 80% compost treated sample are 120°C, 395°C, 472°C and 507°C, respectively (Table 7.4). These values recorded for 80% compost treated sample are highest among all the compost treated and untreated samples. This signifies the increase in crystallinity of the sample **(Tribedi *et al.*, 2015)**. This might be due to the fact that biotic degradation by microbial communities on the LDPE sample attacked on the amorphous region, due to which crystalline region remained intact which increased the crystallinity of the polymer. DSC results clearly reveals the increased crystallinity, increased chain scissoring, oxidation and a minor decrease in tensile strength **(Reddy, 2008)**.

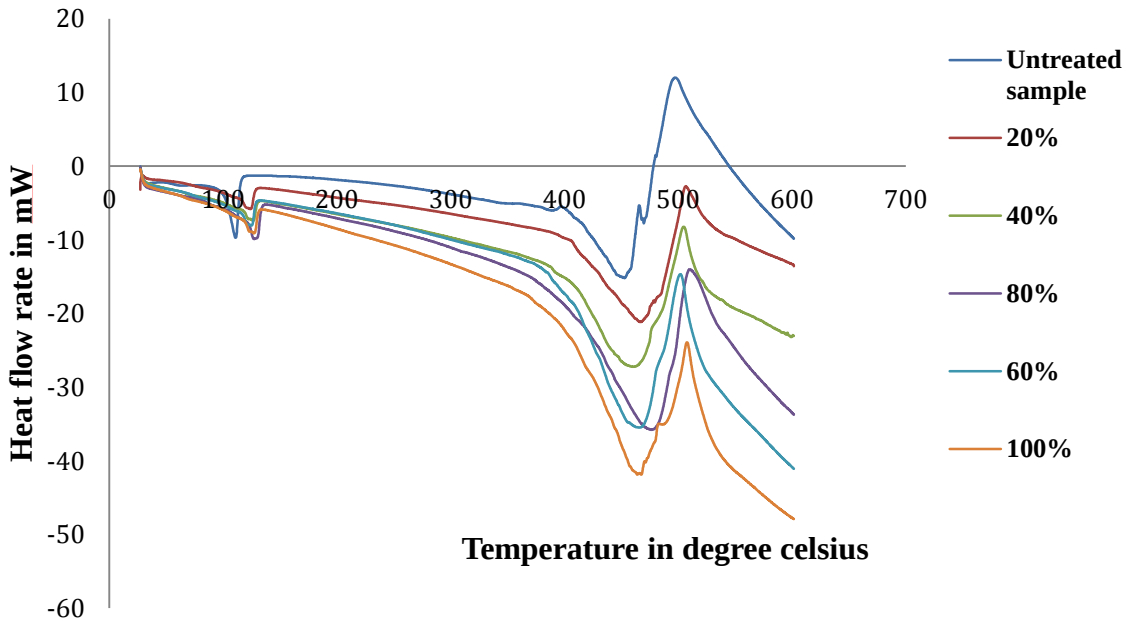


Figure 7.3: Differential calorimetry graph of different concentration of compost treated LDPE

Table 7.4: Differential scanning calorimetric (DSC) analysis parameters of different concentration of compost treated LDPE

Samples	T_o	T_m	T_f	T_g
Untreated sample	378°C	448°C	477°C	109°C
20% compost	389°C	453°C	489°C	113°C
40% compost	390°C	455°C	492°C	119°C
60% compost	390°C	459°C	497°C	125°C
80% compost	395°C	472°C	507°C	120°C
100% compost	392°C	462°C	506°C	121°C

7.2.6 Fourier Transform Infra Red Spectroscopic Analysis

FTIR study was conducted to determine the functional groups of the treated and untreated LDPE samples. The spectroscopic graphs are given in Figure 7.4. The overall trend obtained after aging LDPE by burying it under the different concentrations of compost is that there are higher transmittance values at certain wavenumbers like 2840 cm^{-1}, 2916 cm^{-1}, 1480 cm^{-1} and 719 cm^{-1}), representing an increase in C-H bond intensity, which can be explained by chain scissoring or increase of CH_3 endings (Table 7.5). All the compost treated samples composted formed new spectra at wavenumber of 1380 cm^{-1}, representing hydroxyl group. Furthermore, new transmittance peaks were also observed at wavenumbers 997 cm^{-1} and 933 cm^{-1}, indicating the formation of C=C bonds **(Yang *et al.*, 2004)**.

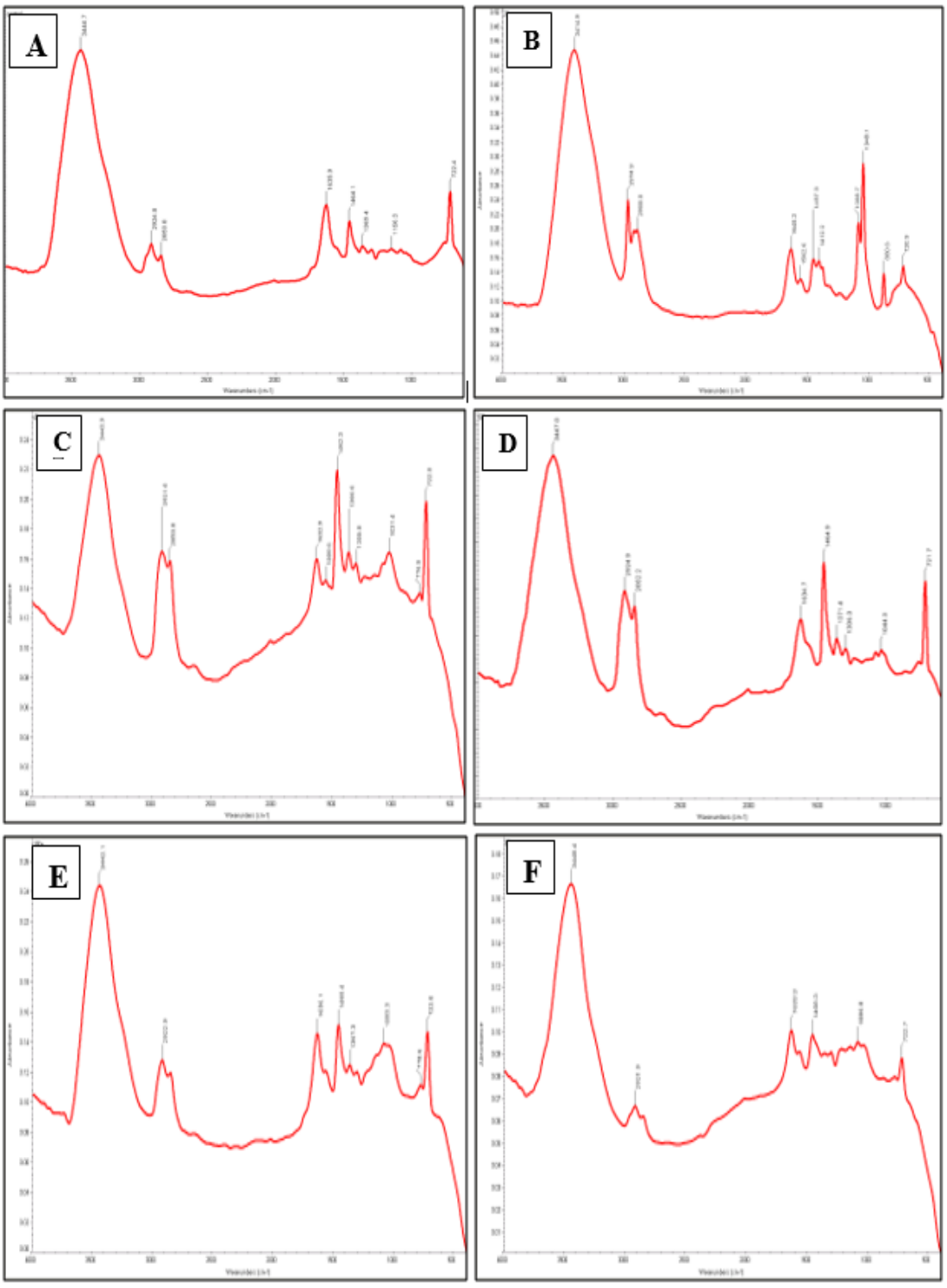


Figure 7.4: FTIR spectra of LDPE samples treated with different concentration of compost (A) Control LDPE (B) 20% (C) 40% (D) 60% (E) 80% (F) 100%

Table 7.5: Functional groups detected in LDPE samples treated with different concentration of compost

Origin	Peaks	Functional group	Detected sample
CH_2	2920 cm^{-1}	Alkyl asymmetric stretching	20%, 40%, 60%, 80%, 100% and control LDPE
CH_2	2850 cm-1	Alkyl symmetric stretching	20%, 40%, 60%, 80%, 100% and control LDPE
C=C	1473, 1462, 1437 cm^{-1}	Bending deformation of carbon double bond	60%, 80%, 100% LDPE
C-H	727 and 723 cm^{-1}	Alkane bend mono	20%, 40%, 60%, 80%, 100% and control LDPE
O-H	3200-3450 cm^{-1}	Hydroxyl stretching (intermolecular)	20%, 40%, 60%, 80%, 100% and control LDPE
O-H	1380 cm^{-1}	Hydroxyl bending	80% LDPE
C-O	1160 cm^{-1}	Carbonyl group	20%, 40%, 60%, 80%, 100% LDPE

7.2.6.1 Carbonyl Index and Internal Double Bond Index under FTIR analysis

The carbonyl bond index and internal double bond index was found to be maximum in the case of 80% compost treated LDPE. The formation of double bonds in the polymeric chain can be due to the Norrish type II reaction and increase in the concentration of the carbonyl group could be responsible for higher carbonyl bond index (Figure 7.5) **(Bode *et al.*, 2000)**.

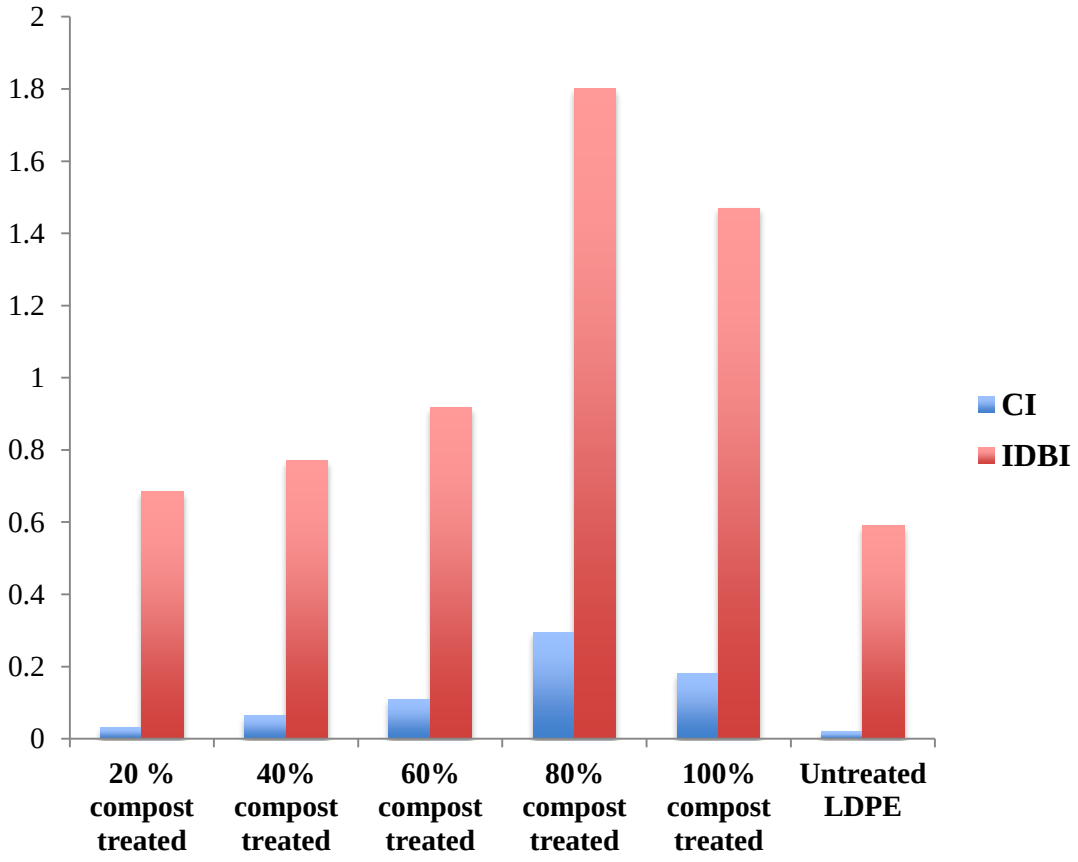


Figure 7.5: Carbonyl and Internal Double Bond Index of LDPE samples treated with different concentration of compost

7.2.6.2 Crystallinity % under FTIR analysis

Low density polyethylene is insoluble in nature due to their crystalline nature. In this study, there was an observed reduction in the crystallinity of the polyethylene after six months of incubation with the compost. The crystallinity percent for untreated LDPE and 20%, 40%, 60%, 80% and 100% compost treated samples were 86.32%, 81.67%, 79.51%, 74.91%, 65.48% and 71.13%, respectively (Figure 7.6). The lowest crystallinity value was observed in 80% compost treated LDPE. The decrease in crystallinity supports the conversion of crystalline structures of the LDPE films into an amorphous structure as a consequence of degradation **(Bode *et al.*, 2000)**.

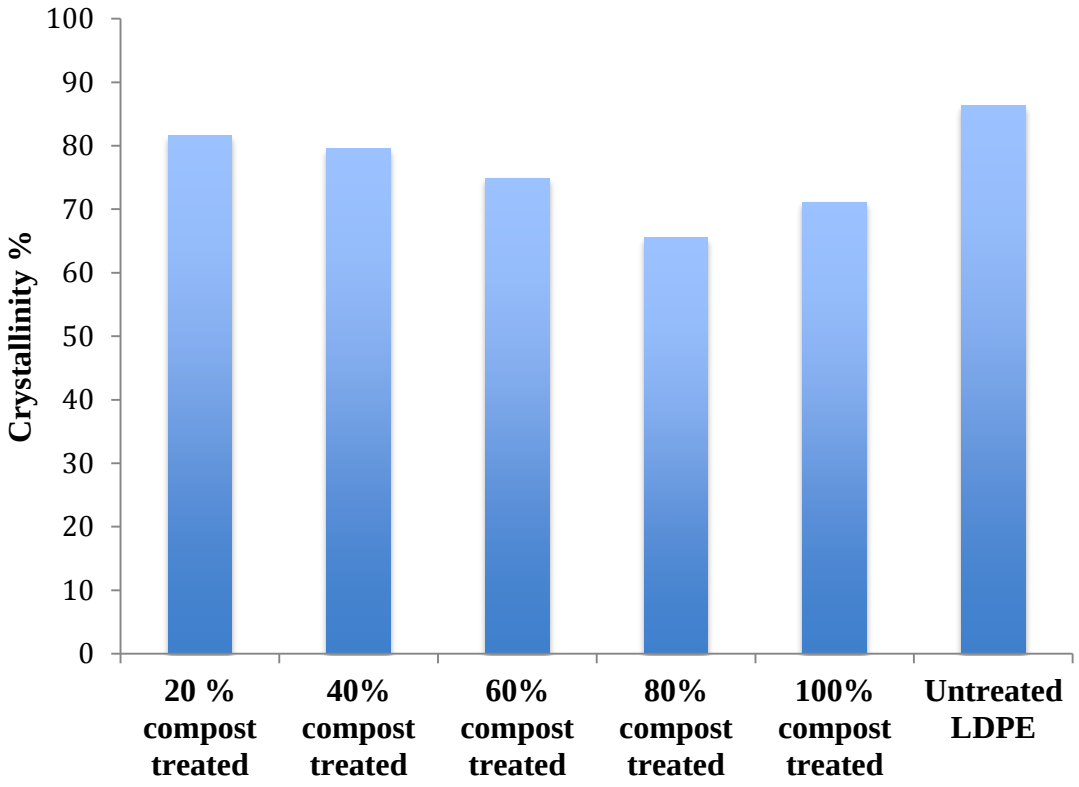


Figure 7.6: Crystallinity percent of LDPE samples treated with different concentration of compost

7.2.7 Weight reduction analysis

Polyethylene degradation was monitored by dry weight reduction of polyethylene films treated with different compost concentration. The present findings revealed that the compost treatment showed some deterioration effect on the LDPE samples. Among all the samples maximum weight reduction of approximately 17% was observed in 80% compost treated sample. For rest of the 20%, 40%, 60%, 100% compost treated samples, weight reduction obtained was 6.3%, 8.45%, 11.97% and 14.29% (Figure 7.7) **(Devi *et al.*, 2015)**.

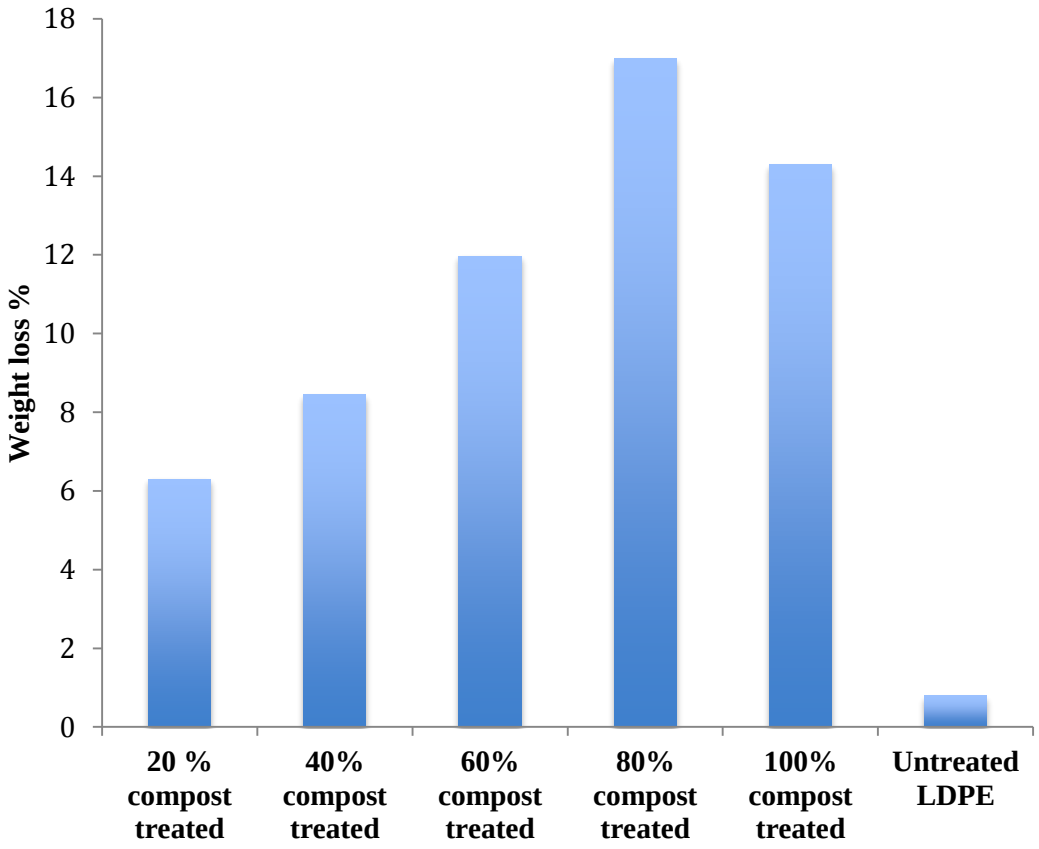


Figure 7.7: Weight loss percent of LDPE samples treated with different concentration of compost

7.2.8 Compost physical and chemical parameter

The parameters of different compost samples amended with the LDPE films showed significant changes in percentage of total organic carbon, nitrogen and sulfur before and after six months (Table 7.6). All these parameters were found to increase after six months. The addition of LDPE films resulted in an increase in the total organic carbon content due to mineralization of the LDPE films. The increased carbon and nitrogen content in the compost samples favored the growth of polyethylene degrading microorganism, which further provided the better conditions for mineralization of hydrocarbon **(Gulmine, 2003)**.

Table 7.6: Physico-Chemical Psarameters of Compost pre and post treatment period

Samples	Electrical conductivity		Organic carbon %		Available nitrogen(mg/kg)		Sulfur(mg/kg)		CEC		pH	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
20% compost	0.05	0.08	0.45	1.47	117.6	212.87	11.47	28.38	7	12	7.91	7.65
40% compost	0.08	0.14	0.6	3.9	120.6	242.87	19.66	93.23	8	12.2	7.74	7.92
60% compost	0.06	0.14	0.9	4.2	145.6	285.63	13.32	49.38	9.6	24.2	7.81	7.67
80% compost	0.11	0.16	1.53	8.25	158	298.09	49.41	54.01	13	34.2	7.75	7.51
100% compost	0.32	0.13	5.89	11.4	189.6	229.61	58.54	71.35	4.4	18.6	7.71	7.70

7.3 Conclusion

All the composted LDPE samples showed the changes induced in the polymer. Major changes were observed in LDPE samples treated with 80% compost. Maximum weight reduction of 17% was noted in 80% compost treated sample. Thermal gravimetric analysis revealed lowest onset and decomposition temperature with no residue left in LDPE sample treated with 80% compost treated sample. EDX analysis showed minimum carbon content of 46.29% and maximum oxygen content of 50.26% in 80% compost treated LDPE sample after six months of treatment. In FTIR analysis, maximum value of carbonyl and double bond index supported the greater extent of degradation in 80% composted LDPE.

8.0 Introduction

Surfactants are generally the organic compounds. They are amphiphilic, which contain hydrophobic as well as hydrophilic groups **(Abdel-Mawgoud *et al.*, 2010)**. Hence, a surfactant contains both the water-insoluble component and the fat-soluble component. Surfactants diffuse in water and adsorbs at the interface between oil and water, when water is mixed with oil or interface between air and water. The hydrophobic group, which is water-insoluble, might extend out of the bulk water phase, into the oil phase or into the air, while the water-soluble head group rests in the water phase **(Basnett *et al.*, 2012)**. There are basically 4 types of surfactants, which are classified, based upon the composition of polarity of the head group: anionic, cationic, nonionic and amphoteric. A non-ionic surfactant does not have charge groups on its head. Anionic surfactants comprise of anionic functional groups at its head, such as phosphate, carboxylates and sulfate. Cationic surfactant contains positively charged head **(Agrawal & Shahi, 2015)**. Zwitterionic or amphoteric surfactants have both cationic as well as anionic centers attached to the same molecule **(Kumar *et al.*, 2011)**. Chemical surfactants are synthesized chemically and are predictable to be much more effective than the bio-surfactant produced by microorganism for inducing oxidation of polyethylene. Some chemical surfactants are known to have very low critical micelle concentration, which means they have higher solubilization ability at very low concentration and they are also biodegradable **(Jeon & Baek, 2010)**. Such a chemical anionic surfactant like sodium dodecyl sulphate has been used for removal of crude oil from the soil at 50 $^{\circ}$C for 14 days **(Abbasian *et al.*, 2016)**. The chemical surfactants have been used greatly in oil removal application by increasing the solubilization of petroleum substance. Hydrocarbon solubilization capacity of biodegradable chemical surfactant has never been utilized for the

oxidation of polyethylene. This ability of chemical surfactant can be utilized during biodegradation of polyethylene to improve its oxidation level. Surfactants could be used at higher concentration than its CMC to achieve higher rate of hydrocarbon solubilization **(Chaisu *et al.*, 2015)**. Simultaneous oxidation as well as solubilization of oxidized polyethylene in the aqueous media during treatment process in the presence of chemical surfactant, might lead to greater amount of degradation at the surface of polyethylene. In the present study polyethylene samples were treated with all the three types of anionic (sodium dodecyl sulfate), Cationic (cetyltrimethylammonium bromide (CTAB)) and non-ionic (Tween 80) chemical surfactant separately at 60 °C for one month to study the effect of oxidizing ability and hydrocarbon solubilization ability of surfactant on polyethylene.

8.1 Materials and methods

8.1.1 Oxidation of polyethylene

Low density polyethylene (LDPE) samples were immersed in chemical surfactant solutions of different concentrations from 2-10% and were kept at 60 °C for 30 days. Chemical surfactants, Sodium dodecyl sulphate-SDS (anionic), cetyl trimethylammonium bromide-CTAB (cationic), and Tween 80 (non-ionic) were used in the experiment. All the treatment of LDPE samples was done in triplicates. After 30 days, polyethylene samples were recovered.

8..1.2 Characterization of LDPE samples

8.1.2.1 FTIR analysis

Fourier transform infrared spectroscopy was done to determine the change in the functional group of the LDPE samples before and after treatment. FTIR was done according to method given in Section **3.2** of Materials and Methods in **Chapter 3**.

8.1.2.2 XRD analysis

X-ray diffraction (XRD) analysis of the LDPE was done using X-ray diffractometer that is operated using Cu Kα radiation. XRD was done according to the method given in Section **3.4** of Materials and Methods in **Chapter 3**.

8.1.2.3 EDX analysis

EDX was done to determine the changes occurred in the elemental composition of the LDPE sample before and after the treatment with the chemical surfactants. EDX was done according to the method given in Section **3.3** of Materials and Methods in **Chapter 3**.

8.2 Results and Discussion

8.2.1 FTIR analysis

FTIR characterization of chemical surfactant treated polyethylene is shown in Figure 8.1. A broad transmittance peak at 1775-1725 cm^{-1} appeared in the FTIR spectra of LDPE treated with all the types of surfactant. This broad peak emerges due to overlapping of two or more than two peaks, which resulted due to the formation of various carbonyl groups, like 1733 cm^{-1} for aldehydes, 1740 cm^{-1} for ketones **(Chiellini *et al.*, 2003)**. The absorbance peak at 1715 cm^{-1} appeared in the FTIR spectra of LDPE treated with surfactant, due to the formation of acids or keto carbonyls. The absorbance intensity at this peak at 1715 cm^{-1} decreases with the LDPE treated with decreasing concentration of the surfactants. In case of LDPE treated with three different types of surfactants and oxidised thermally, the peak for carbonyl groups emerged at 1740 cm^{-1} and therefore only keto carbonyl bond index has been calculated **(Aboulkas *et al.*, 2010; Marchut-Mikolajczyk *et al.*, 2018)**.

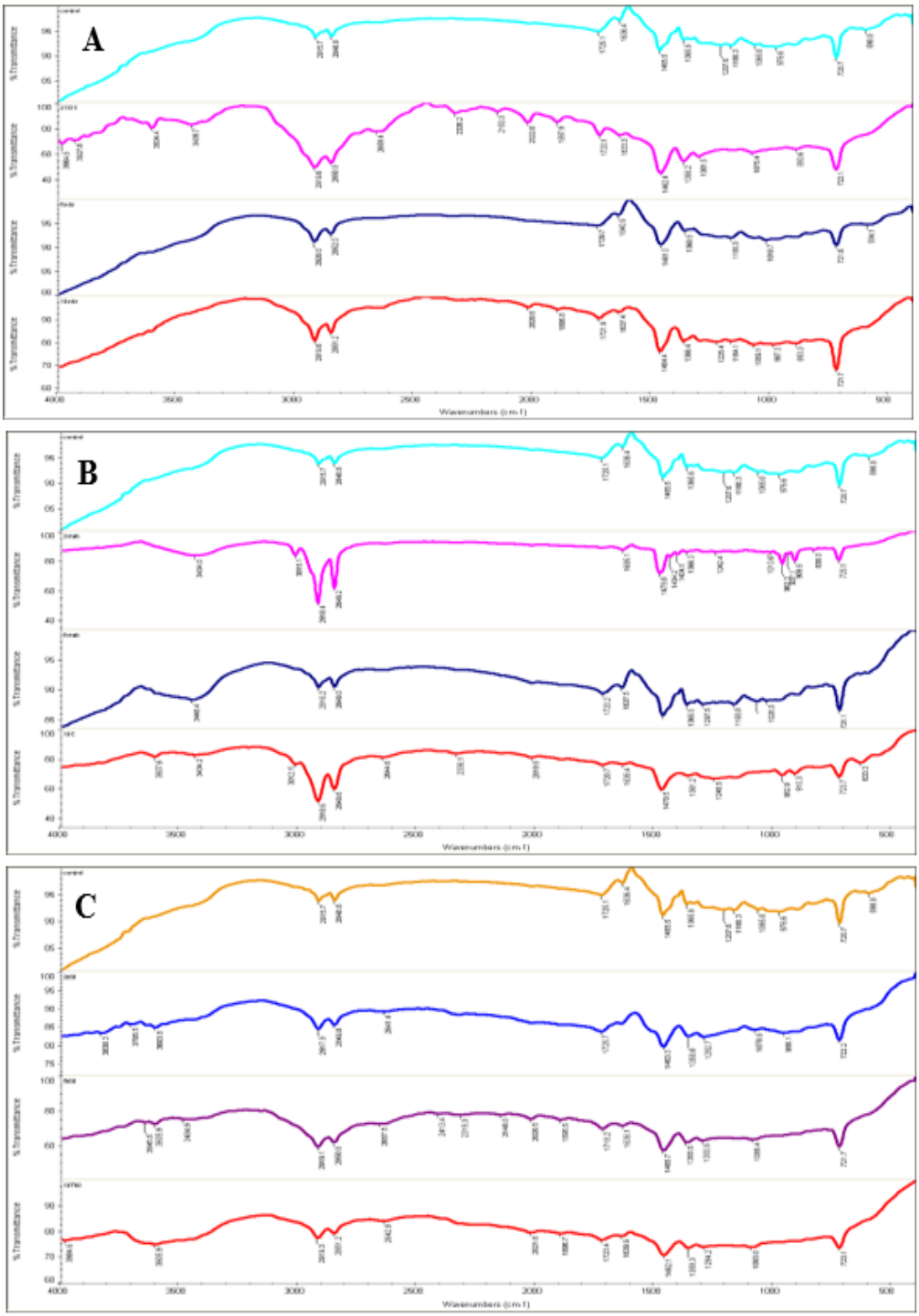


Figure 8.1: FTIR spectra of (A) CTAB treated LDPE (B) SDS treated LDPE (C) Tween 80 treated LDPE

8.2.1.1 Keto Carbonyl Index and Double Bond Index of LDPE under FTIR analysis

The Table 8.1 and 8.2 shows the keto carbonyl index and double bond index of LDPE samples treated with different surfactant. Results revealed that after chemical treatment of polyethylene in the presence of chemical surfactant i.e., anionic, cationic and non-ionic; keto carbonyl and double bond index of treated LDPE samples increased as compared to the unoxidized control polyethylene. However, carbonyl bond index and double bond index of LDPE treated with 10% SDS is greater than the LDPE treated with 2%, 6% and 10% of CTAB, and it has been reported that during the thermal oxidation of LDPE, chain scission occurs due to radical reaction which leads to the formation of unsaturated hydrocarbons. Commonly, this chain scission occurs at weak links due to existence of carbonyl groups and chain branching in the polymer, which are formed during the oxidation process. However, during the chemical treatment with the surfactant, LDPE is oxidized through autocatalytic mechanisms and it further proceeds via a free radical mechanism **(Meena & Kanwar, 2015; Okoh, & Trejo-Hernandez, 2006)**. In the presence of oxygen, chain scission is basically predominant. Further, it has been reported that primarily chain scission occurs in the chain branching and impurities which is integrated in the LDPE during the oxidation process. Because of this initial chain scission, free radicals are produced which are further used in random chain scissions or breaking **(Otake *et al.*, 1995)**. Amphiphilic anionic surfactant molecules in the thermal oxidation process of polymer get attached to the hydrophobic surface of the LDPE molecule having its hydrophilic end portion stretched into the aqueous solution **(Mukherjee *et al.*, 2015)**. In this way, the availability of the oxygen to the polyethylene molecule increases which results in enhancement of the chain scission, thus increasing the number of free radicals, which lead to increase in the polyethylene oxidation rate **(Pantazaki *et al.*, 2011)**. Therefore,

the amount of keto carbonyls, which are considered as the main product of oxidation process, increases as observed by the FTIR analysis of the treated LDPE sample. So, it is clear that 10% SDS can be said to be more effective for oxidation of LDPE polymer than the CTAB and Tween 80 **(Mukherjee *et al.*, 2009)**.

Table 8.1: Keto Carbonyl Index of different chemical surfactant treated LDPE samples

Sample	SDS	Tween 80	CTAB
2% Surfactant	0.25	0.19	0.13
6% Surfactant	0.32	0.41	0.29
10% Surfactant	0.48	0.35	0.37

Table 8.2: Double Bond Index of different chemical surfactant treated LDPE samples

Sample	SDS	Tween 80	CTAB
2% Surfactant	0.20	0	0.08
6% Surfactant	0.23	0.28	0.10
10% Surfactant	0.37	0.34	0.14

8.2.2 XRD analysis

XRD analysis (Figure 8.2) revealed that the crystallinity of the 10% SDS treated LDPE film was observed to be the highest among all the surfactant treated LDPE samples. Beside the peaks at 21° and 23°, which are the characteristic peak for defining the crystalline nature of the polymer, several other peak also emerged different 2 theta angle showing the changes induced in the 10% SDS treated LDPE sample **(Orr *et al.*, 2004)**. The number of peaks emerged in all the treated LDPE samples have been shown in Figure 8.3. Such increase in crystallinity of polyethylene treated with anionic surfactant SDS is due to secondary crystallization which resulted due to the annealing effect of the temperature, by chain scission and by the formation of intermolecular polar bonds between the carbonyl groups during the chemical treatment of polyethylene **(Raaijmakers *et al.*, 2010)**. Initially the deterioration of the amorphous region took place followed by the smaller regions of the crystalline portions after which the larger crystalline fragment were left behind and resulted in the increase in the value of percent crystallinity **(Aburas, 2016)**.

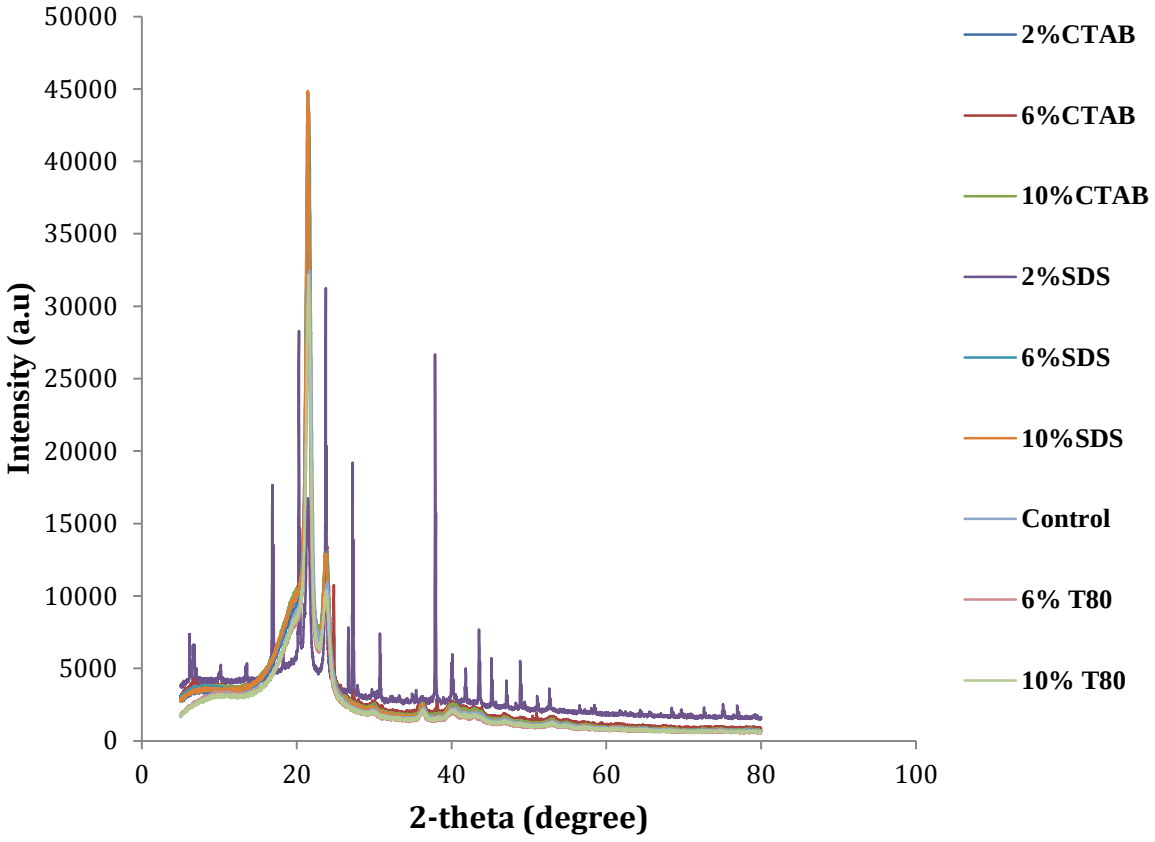


Figure 8.2: X-Ray Diffraction spectra of chemical surfactant treated LDPE

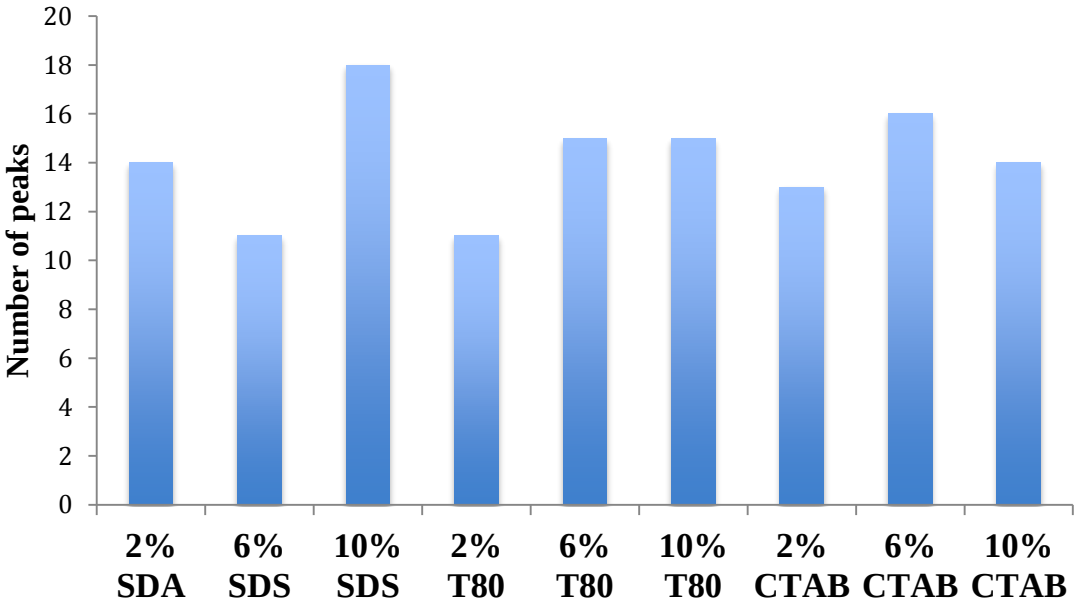


Figure 8.3 Number of peaks emerged in XRD spectra for chemical surfactant treated LDPE

8.2.3 EDX analysis

EDX analysis was done to observe the change in the composition of carbon and oxygen of oxidized as well as unoxidized LDPE samples. The table 8.3 shows the weight percent of carbon and oxygen of different LDPE samples. The lowest carbon and highest oxygen content was reported in 10% SDS treated LDPE sample. The carbon content was 70.47% and oxygen content was 25.02%. This can be attributed due to the fact that amphiphilic anionic surfactant (SDS) molecules present during the thermal oxidation of polyethylene get attached with the hydrophobic end stretched into the aqueous solution **(Phua *et al.*, 1987)**. In this way oxygen availability of the polyethylene molecule increased resulting to the enhancement of the random chain scission, thereby increasing the amount of free radicals, which lead to increment of the polyethylene oxidation rate **(Pandey *et al.*, 2003)**.

Table 8.3: Weight percent of carbon and oxygen of LDPE treated with chemical surfactant

Sample	Carbon weight %	Oxygen weight %
Untreated LDPE	100	0
2% SDS	88.08	11.92
6% SDS	85.92	12.88
10% SDS	70.47	25.02
2% CTAB	92.15	7.85
6% CTAB	89.01	10.99
10% CTAB	80.84	19.16
2%Tween 80	96.79	3.21
6%Tween 80	89.88	10.12
10%Tween 80	81.15	18.29

8.3 Conclusion

The oxidation level of polyethylene was found to be higher by anionic surfactant Sodium Dodecyl Sulfate at 60 °C for 30 days as compared to the cationic and non ionic surfactant CTAB and Tween 80 respectively. Carbonyl index and double bond index of polyethylene treated with SDS at 60 °C for 30 days was found to be higher than CTAB and Tween 80 oxidized polyethylene as observed in FTIR study. The maximum number of peaks in XRD analysis was observed for SDS treated polyethylene. This shows the effect of chemical surfactant on the crystallinity of the polymer. Such increase in crystallinity due to secondary crystallization resulted by annealing effect of the temperature, by chain scission and by formation of intermolecular polar bonds between carbonyl groups during thermal oxidation of polyethylene at 60 °C. Oxidation level of polyethylene treated by SDS was higher as the availability of soluble oxygen and chain scission increased due to the attachment of surfactant to the polyethylene surface. EDX analysis showed maximum decrease of carbon content (70.47 %) in 10% SDS treated polyethylene.

9.0 Introduction

Ageing of plastics has created a debate between many researcher teams in order to make a plastic material showing better performance and longer service lifetime **(Borah & Chaki 2011)**. But, on the other hand ecologists are concerned about the accumulation of plastic wastes that poses real threat to the balance of environment due to the fact that it takes somewhat long time for such type of material to degrade completely. Low-density polyethylene (LDPE) films are widely used owing to their number of characteristics, which make them useful in day-to-day life. Exposure of plastic materials to weathering conditions may cause deterioration of their physical, chemical and mechanical characteristics **(Sarkhel *et al.*, 2006; Roy *et al.*, 2008)**.

Exposure to solar or UV radiation have direct oxidation effect on plastic material that have a direct impact on the mechanical performance. The most evident chemical effect induced in the weathered LDPE is the development of vinyl group and carbonyl group. They are associated with clear loss in the mechanical properties. Both crosslinking and chains scission happen under natural weathering conditions which lead to molecular degradation **(Roy, 2009; Shamsuri & Daik, 2015)**.

During the photo-oxidation process, because of the effect of oxygen diffusion the resulting degradation is spatially heterogeneous (3-D) across the film thickness and over the film surface due to the attenuation of the ultra violet light that penetrate through the polymer **(Samyn & Schoukens, 2014)**. It has been assumed that ageing could probably affect the polymer directly exposed to solar radiation. Sunlight induced degradation of polymer has been an interesting area of research for number of the scientists **(Arnaud *et al.*, 2009)**. If the photo degraded polymer is further subjected to microbial degradation then it would be easier for the microorganisms to

attack on the hydrophobic carbon backbone, thus completely mineralizing the polymer into carbon dioxide and water. So, the present study was done with the aim to determine the changes induced in the LDPE films after they are exposed to the solar radiation.

9.1 Materials and method

9.1.1 Sunlight exposure

In order to examine the degradation of polyethylene due to solar radiation and the environmental factors, fresh untreated samples were obtained and exposed to natural conditions at Babasaheb Bhimrao Ambedkar University, Lucknow for a period of 90 days between May to July 2018. The LDPE samples were then clipped on rigid cardboards. The samples were elevated from the ground facing upward towards the sun that is, it was inclined at an angle of 0° with respect to the horizontal plane. The rigid cardboards were the cut to make a rectangular frame. The samples were clipped at the hollow center position of the frames in order to ensure that the large portion of the sample did not come in direct contact with the cardboard **(Petchwattana *et al.*, 2012)**.

9.1.2 FTIR analysis

9.1.3 Scanning Electron Microscopy (SEM) analysis

SEM analysis was done according to method given in Section **3.3** of Materials and Methods in **Chapter 3**.

9.1.4 Energy Dispersive X-ray (EDX)

EDX analysis was done according to method given in Section **3.3** of Materials and Methods in **Chapter 3**.

9.1.5 X-Ray Diffraction

XRD analysis was according to method given in Section **3.4** of Materials and Methods in **Chapter 3**.

9.1.6 Thermal Gravimetric analysis

TGA analysis was done to deer according to Section **3.5** Materials and Methods given in **Chapter 3**.

9.1.7 Differential Scanning Calorimetry

DSC analysis was done to deer according to Section **3.6** Materials and Methods given in **Chapter 3**.

9.1.8 Weight loss determination of polyethylene

Weight loss determination was done to deer according to Section **3.1** Materials and Methods given in **Chapter 3**.

9.2 Results and Discussion

9.2.1 FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) is a method to detect organic, polymeric as well as inorganic materials. The structural changes occurred due to photo-oxidation were explored by recording the FTIR spectra of the LDPE samples that were exposed to sunlight and comparing it with the standard FTIR spectra of unexposed LDPE film (Figure 9.1). Major changes were detected in sunlight exposed LDPE film. The most significant change in FTIR spectra was seen in carbonyl (1785-1700 cm^{-1}) peak **(Chrissafis *et al.*, 2006)**. After certain period of photo-oxidation slight increase in the intensity of absorption was observed at 1720 cm^{-1}, which represents the C=O stretch of saturated keto-carbonyl. Some studies have also reported the similar change, as they also showed an increase in the absorption intensity of carbonyl peak due to photo-oxidation of LDPE film **(Tamada & Lauger, 1993)**. FTIR analysis of LDPE film revealed number of peaks got disappeared in the exposed LDPE films and some new peaks got emerged due to addition or removal of specific functional group as well as chemical bonds in the LDPE samples. The peaks that were observed to be common in both the LDPE samples with and without exposure are: CH_2 asymmetric stretching (2919 cm^{-1}); CH_2 symmetric stretching (2851 cm^{-1}); CH_2 bending deformation at 1463 cm^{-1}; wagging deformation (1351 cm^{-1}) and rocking deformation (720 cm^{-1}) (Abrusci *et al.*, 2011). The spectral band that generated after the photo-oxidation of the LDPE samples were at 1738 cm^{-1} corresponding to carbonyl group (R-CO-OR'); at 1068 cm^{-1} (RCH_2-CHOH-CH_2R'); and CH_3 symmetric deformation at 1375 cm^{-1}. The band that was found absent in the

sunlight-exposed sample was at 1306 cm^{-1}, which corresponded to twisting deformation obtained after natural weathering **(Shamsuri *et al.*, 2014)**.

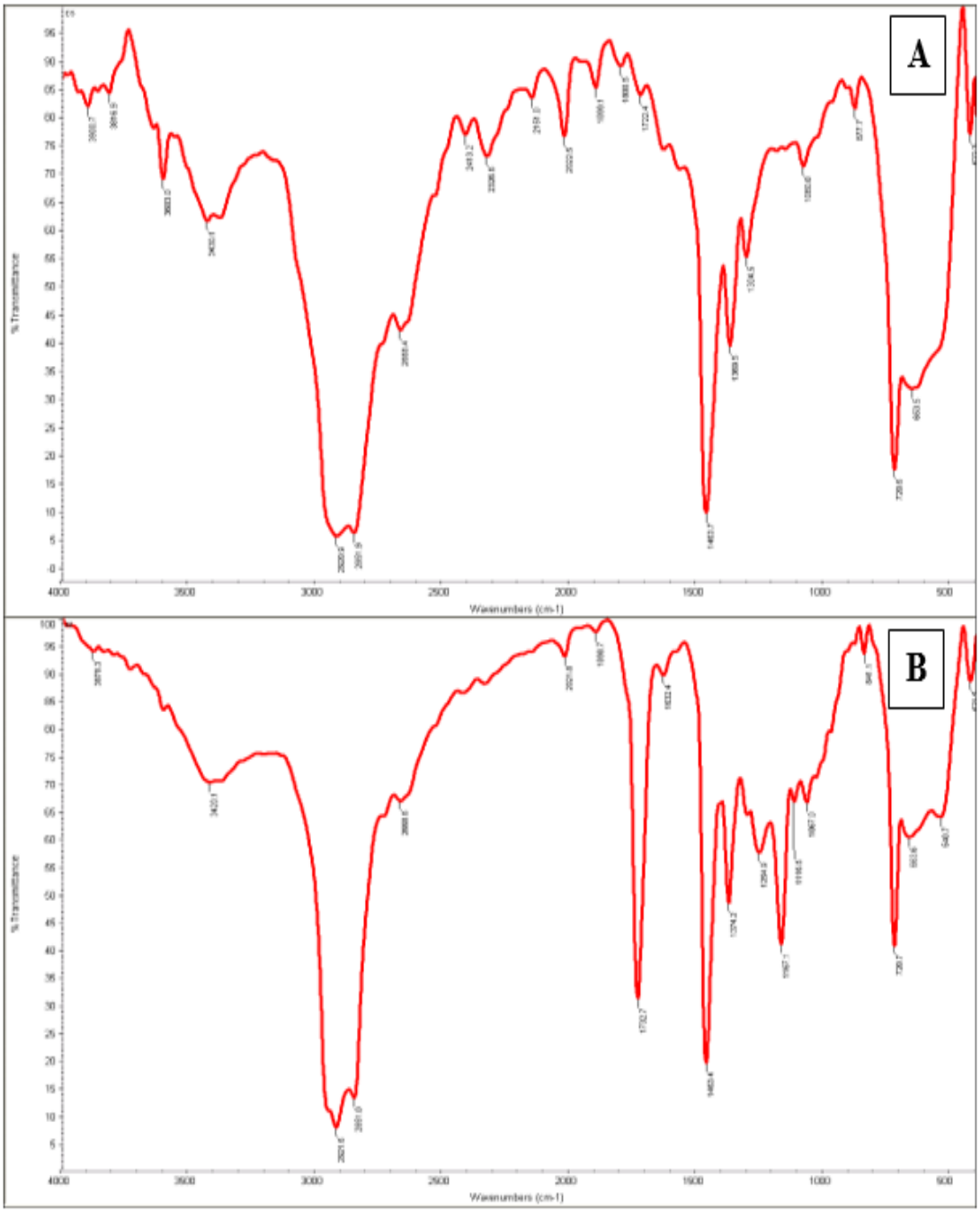


Figure 9.1: FTIR Spectra of LDPE samples (A) without exposure (B) with Exposure.

Number of parameters were also derived from the FTIR analysis. The LDPE films when exposed to solar radiation were rapidly oxidized, which is shown by the increase in carbonyl bond index and vinyl bond index (Table 9.1). The higher values of carbonyl bond index in the exposed LDPE indicated the photo-oxidation of solar radiation on polymer matrix. The carbonyl group belongs to ketone group because none of signature peaks of aldehydes and acid were noticed. In the weathered or exposed LDPE sample the KCBI, IDBI and VBI was observed to be increased (Figure 9.2). This verifies that some kind of degradation changes has been occurred on the structure of polymer owing to the weathering effect and solar radiation. The percentage crystallinity of the LDPE sample exposed to sunshine was observed to be more than that of unexposed LDPE films (Figure 9.3). The increase in the crystallinity of the LDPE can be explained due to introduction of the carbonyl group. This increase in the percentage of crystallinity made the plastic hard and brittle but lowered its impact resistance which means further it could be subjected to biodegradation for enhanced degradation process of polymers in nature.

Table 9.1: Various FTIR derived parameters of LDPE samples

Samples	**KCBI[a]**	**VBI[b]**	**IDBI[c]**	**Crystallinity(%)**
Without exposure	0	0	0.1	35
With exposure	0.6	0.093	0.04	37

KCBI[a]= Keto carbonyl bond index
VBI[b]= Vinyl bond index
IDBI[c]= Internal double bond index

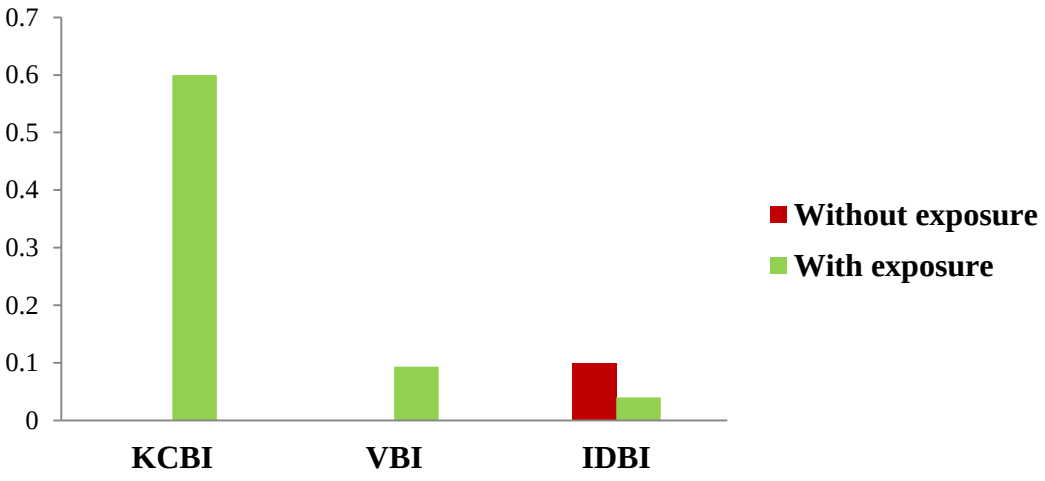


Figure 9.2: FTIR parameters of LDPE samples

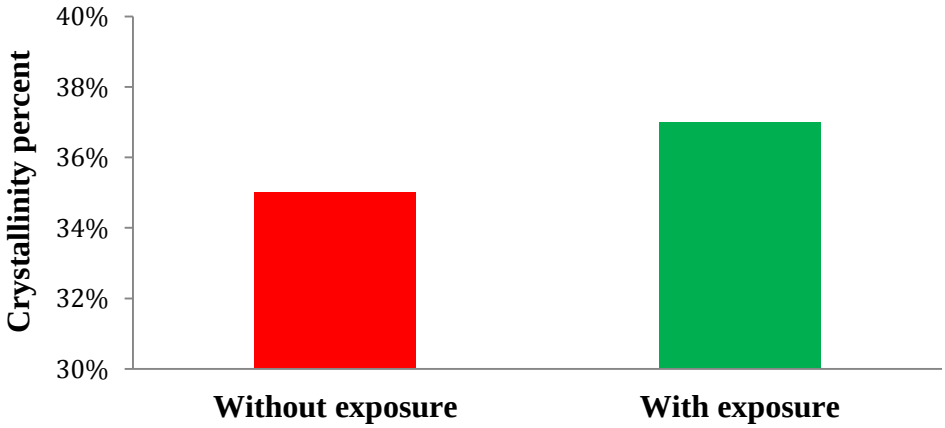


Figure 9.3: Crystallinity percent of LDPE samples

9.2.2 SEM analysis

LDPE samples that were subjected to solar radiation under natural weathering conditions were examined through Scanning Electron Microscopy (SEM). Major modifications occurred in the surface morphology of the LDPE samples exposed to solar radiation under natural weathering condition.

The SEM micrographs revealed that the surface of the unexposed LDPE sample was smooth and without any distortion (Plate 9.1). While, the LDPE sample exposed to

sunlight has shown some cracking and fissures on the surface. Some of the researchers have also stated the same findings such as grooves over the sunlight treated LDPE samples. The surface got eroded due to the effect of solar radiation and other weathering conditions. The increase in the roughness of the LDPE sample was also observed which clearly indicates that the photo oxidation process influences the degradation of the polymer that cause changes in the surface texture of the plastic, which makes it suitable for further degradation by microorganisms in nature **(Copinet *et al.*, 2004)**.

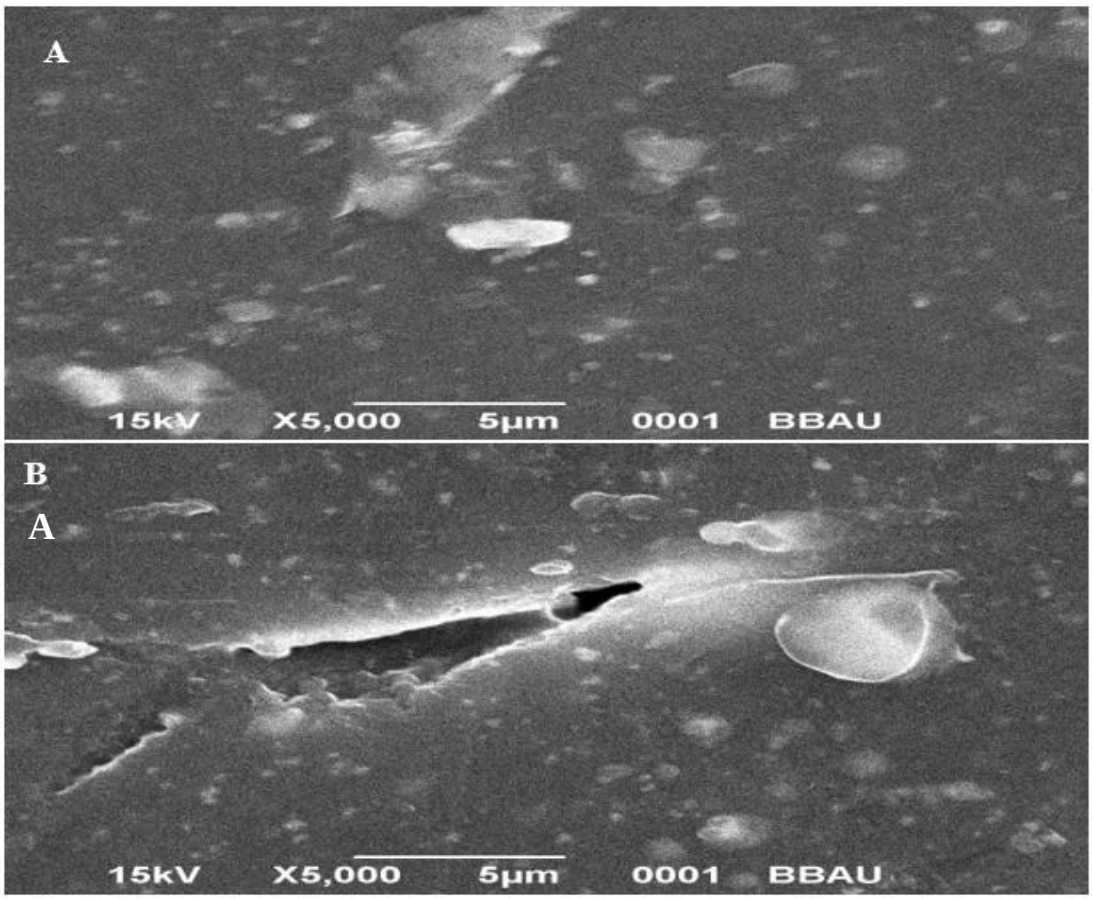


Plate 9.1: SEM micrographs of LDPE samples (A) without exposure (B) with exposure to sunlight.

9.2.3 Energy Dispersive X-Ray

The energy dispersive X-Ray analysis of LDPE samples was done to examine various elements in the polymer (Table 9.2). The results revealed that there was decrease in the carbon matter of the LDPE samples that was exposed to sunlight whereas no change was observed in carbon content of unexposed LDPE samples. On the other hand, oxygen content was also reduced in the exposed polymer (Figure 9.4). The reduction in the carbon content in the polymer was due to the main chain breakage and abstraction of side group during the photo-oxidation process. The reduction in the oxygen was due to the removal of certain oxygen containing compounds **(Lucas *et al.*, 2008)**.

Table 9.2 Elemental compositions of LDPE samples

Elements	**Without exposure (Weight %)**	**With exposure (Weight %)**
Carbon	29	21
Oxygen	52	44
Sodium	9.77	12
Phosphorus	6.84	16
Chlorine	0.24	5.92
Potassium	1.38	1.08
Titanium	0.27	0

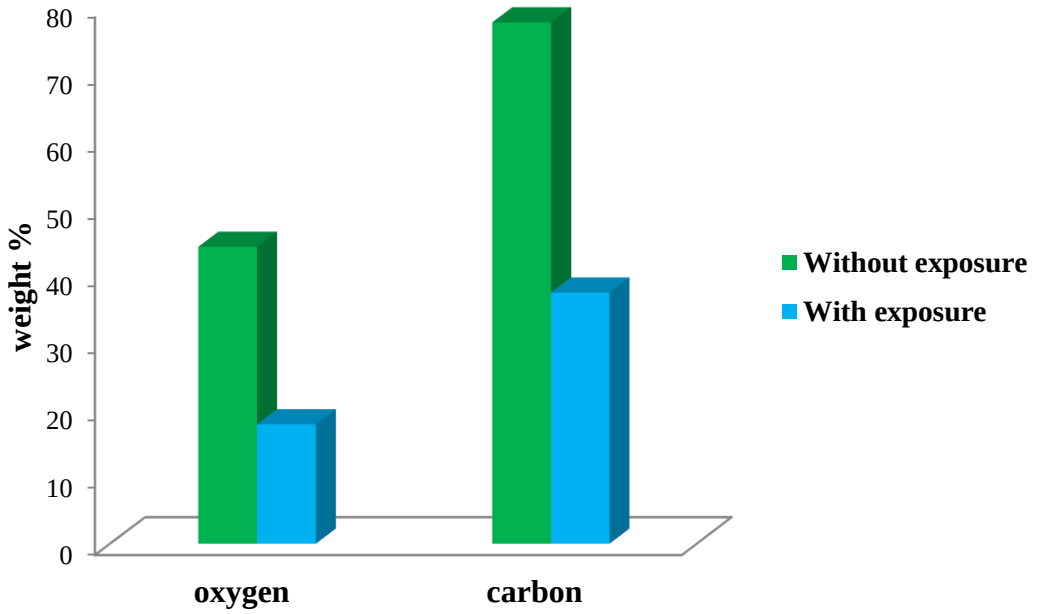


Figure 9.4: Carbon and Oxygen weight percent of LDPE samples

9.2.4 X- Ray diffraction analysis

XRD analysis was done to investigate about the modifications induced by the solar radiation such as Crystallite size and Crystallinity. The XRD pattern obtained clearly showed the presence of amorphous as well as crystalline region (Figure 9.5). The peak at 22° denotes the amorphous structure. Three crystalline peaks at 21.5°, 24.3° and 36.5° appeared during the experiment corresponds to the crystalline structure. These peaks were common in both the LDPE sample with and without exposure. But some of the new major peaks at 16° and 15° were found to appear in the LDPE sample exposed to sunlight. Other peaks centered at 26°, 28°, 30° and 35° have shown increase in the intensity as compared to the LDPE not exposed to sunlight. The increased and intense number of peak in the LDPE polymer with exposure marks the increase in the crystalline region of the polymer. This may be due to the fact that amorphous region is more prone to photo-oxidative degradation as compared to the

densely packed crystalline part. From FTIR it has been concluded that there has been an introduction of the carbonyl group in the exposed polymer, which indicates an increase in the Crystallinity of polymer. The orthorhombic phase of the peak located at 21.5°, 24.3°, 36.5° are 110, 200 and 210 respectively. These phases depict the orthorhombic crystal plane **(Kyrikou *et al.*, 2011)**.

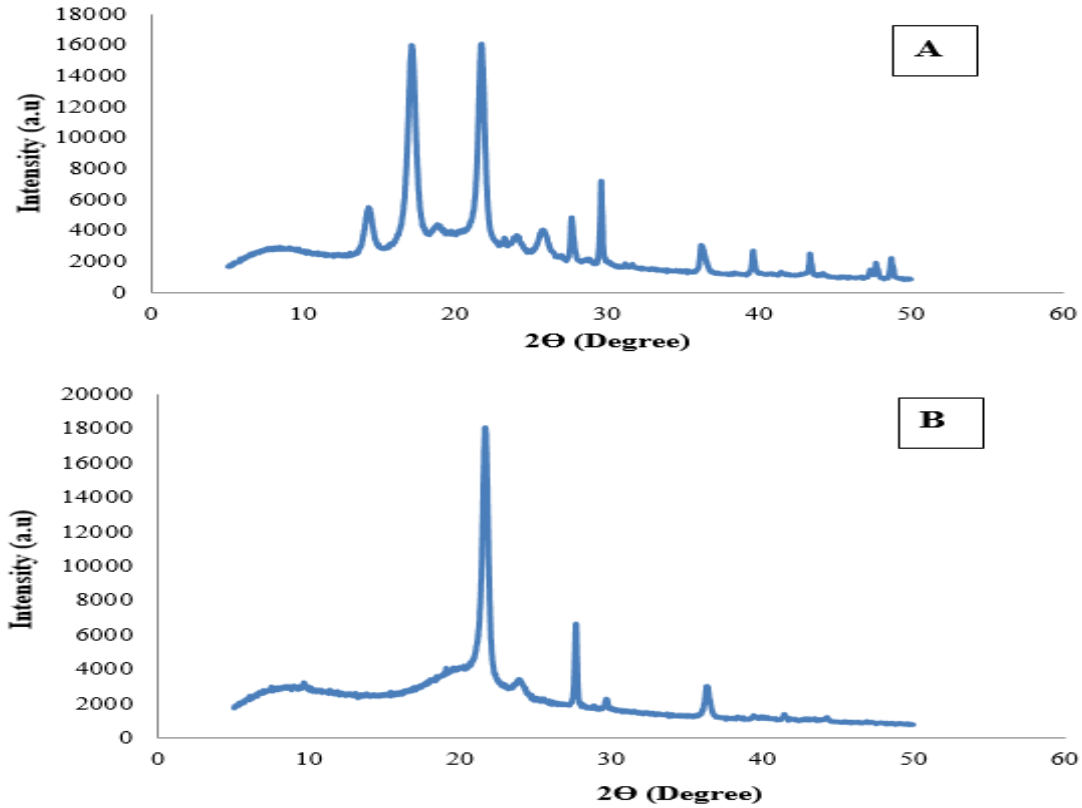


Figure 9.5: XRD spectra of LDPE samples (A) without exposure (B) with exposure.

9.2.5 Thermal Gravimetric Analysis (TGA)

The thermal profile of LDPE samples was tested for determining the effect of sunlight on exposed and unexposed LDPE sample. Thermal analysis for both the LDPE samples revealed the reduction in weight % of solar irradiated sample by 0.189% whereas increase in weight % by 0.459% was observed in LDPE sample that were not

kept under sunlight. In the exposed LDPE sample final mass was decreased whereas in the unexposed LDPE sample final mass was increased at the end temperature. The graph of weight percent vs temperature clearly shows the difference between weight percent and temperature (Figure 9.6). The final decomposition temperature (T_f) for solar-exposed LDPE (162°C) was obtained at higher temperature as compared to unexposed LDPE (138°C) **(Shibulal & Naskar, 2011)**. The drop in weight % of the exposed LDPE sample could be attributed due to the decrease in thermal stability of the polymeric sample and weakening of the bond between the polymeric chains because of the weathering effect. This clearly shows that the polymer becomes brittle in nature when subjected to solar radiation **(Howard, 2002)**.

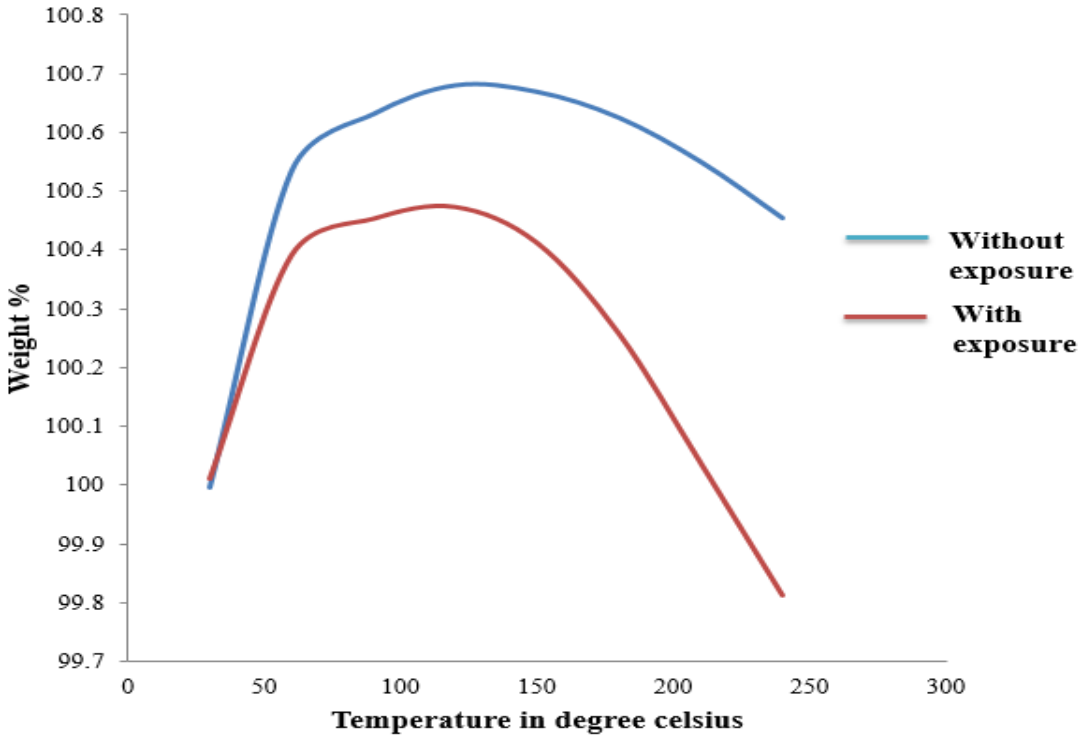


Figure 9.6: Thermal Gravimetric Analysis curve of LDPE samples.

9.2.6 Differential Scanning Calorimetry (DSC)

DSC thermogram of the LDPE samples revealed the various thermal parameters of melting and degradation process (Figure 9.7). In the calorimetric study some processes associated with melting as well as thermal decomposition were detected. For both the exposed and unexposed LDPE samples endothermic peaks were obtained.

Table 9.3 Melting and thermal decomposition process of LDPE samples.

Sample	**Melting**			**Decomposition**
	T_o(°C)	**T_p(°C)**	**Δh_f(J/g)**	**T_f(°C)**
Without Exposure	62	116	0.2	138
With Exposure	62	118	0.4	162

T_o- Onset temperature; T_p – Melting Peak temperature; Δh_f – Melting enthalpy
T_f – Final decomposition temperature

The onset temperature (T_o) was observed to be at 62°C for both the samples which signifies that the sample started decomposing at same temperature. However, no significant change in the melting peak temperature (T_p) was observed but an increase in the melting enthalpy and the final decomposition temperature (T_f) was noted in LDPE sample that was exposed to sunlight (Table 9.3). The area present under the melting curve was more in case of LDPE sample exposed to sunlight **(Sivasankari & Vinotha, 2014)**. The increase in the melting enthalpy of the exposed polyethylene may be due to the increase in the crystalline region of the polymer. The increase in Crystallinity can be stated due to the indirect result of the end chain scission of the polymer in the amorphous regions. The chain end scission allows the low molecular

weight region to crystallize or act as nucleating agents for enhancing the crystallization. The increase in crystallinity also contributes in the embrittlement of the polymer **(Giudicianni *et al.*, 2013)**.

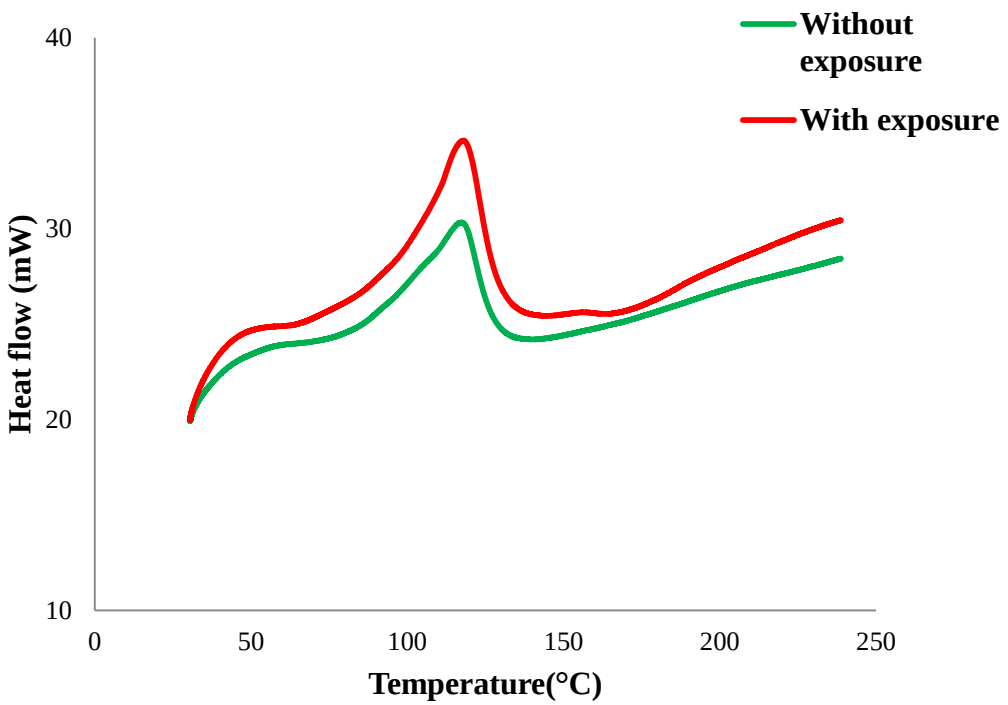


Figure 9.7: Differential Scanning Calorimetry thermogram of LDPE samples

9.2.7 Weight loss determination of polyethylene

Weight loss determination of LDPE samples was performed to study the photo-degradation effects on the LDPE samples. Figure 9.8 displays the weight loss of samples over a period of 90 days. The percent weight reduction increased with increase in exposure time, which is, 2.04%, 3.85%, 5.91%, 8.32%, 13.53%, 15.67% for 15, 30, 45, 60, 75 and 90 days respectively. The increase in weight reduction might be due to the breakage of polymeric chains **(Cornell *et al.*, 1984)**.

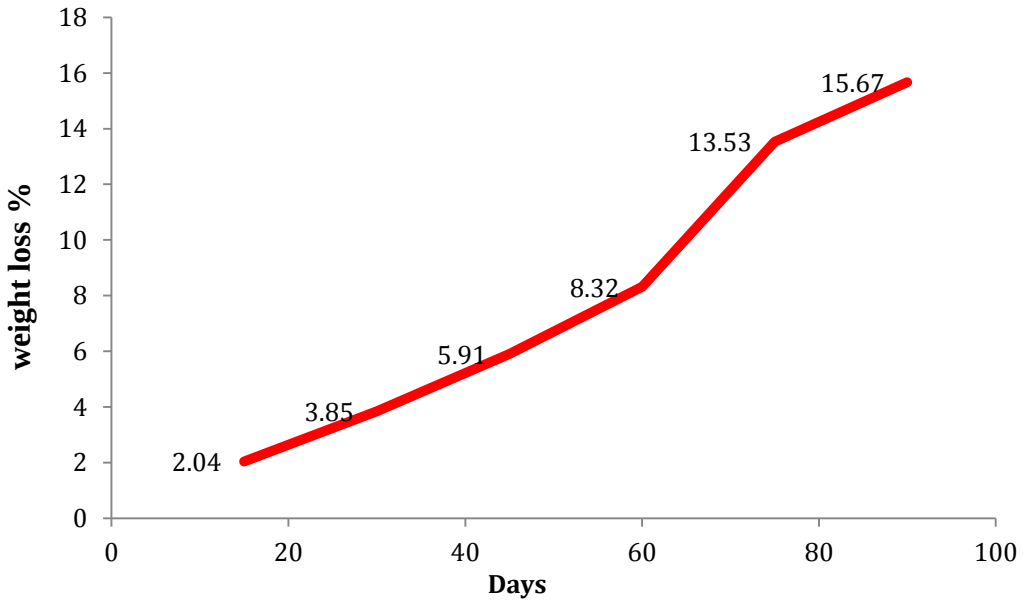


Figure 9.8: Weight loss percentage of LDPE over a period of 90 days

9.3 Conclusion

In order to manage the plastic waste, the study performed demonstrated that the conventional polyethylene gets affected when it is exposed to solar radiation. The degradation study was performed under the solar radiation in natural environment. SEM analysis signified that solar radiation altered the surface morphology of the polymer. Cracks and grooves were seen. The increased carbonyl index examined by FTIR analysis confirmed the photo oxidation of polymer upon exposure to sunlight. While changes in the elemental composition of the polymer confirmed the breakage in the polymer chain. Various analyses like XRD, TGA and DSC showed that once the polymer gets brittle their resistance power gets decreased and microbes can make use of the carbon in the polymer chains for their growth and development. The study performed will contribute in the management of the plastic waste in efficient and cost-effective manner.

10.0 Comparative analysis of all the degradation methods of LDPE

The present study aimed to isolate and identify a potent polyethylene degrading bacterial strains from the sample obtained from plastic polluted dumpsites and to study its degrading efficiency in a laboratory scale. The isolated strains were further screened for the production of biosurfactant. Two novel bacteria were isolated and identified as *Bacillus* sp strain 530F_seq260_Env1 and *Lysinibacillus* sp strain DESBBAU2. Both the strains showed capability of producing biosurfactant effectively. *Bacillus* sp showed 1.36 gm/l of biosurfactant production while *Lysinibacillus* sp showed 1.48gm/l of biosurfactant production. The optimization results revealed the maximum biosurfactant production for the *Bacillus* sp at pH 6, temperature 30 °C, peptone and beef extract concentration of 10 mg/l. However, for *Lysinibacillus* sp maximum biosurfactant production was obtained at pH 8, temperature 30 °C, peptone and beef extract concentration of 5 mg/l. Results for the degradation potential of LDPE revealed that *Lysinibacillus* sp is a more potent degrader of LDPE as compared to *Bacillus* sp. as defined by the characterization analysis of LDPE. The weight loss in LDPE treated by *Bacillus* sp and its biosurfactant was 23.18 % whereas LDPE treated with *Lysinibacillus* sp and its biosurfactant showed 29.32 % reduction in weight. Moreover, the crystallinity % for the LDPE subjected to *Bacillus* sp was 59% and that of *Lysinibacillus* sp treated LDPE was 31%. SEM micrographs showed morphological changes in the LDPE treated by both the bacterial strains. Similarly, EDX analysis showed higher change in weight percent of carbon and oxygen in bacterial treated LDPE. The carbon content for *Bacillus* sp treated LDPE was 83% whereas for *Lysinibacillus* sp treated LDPE carbon content was 73%.

Results for LDPE treated with different concentration of compost revealed that the major changes in LDPE were obtained when the samples were treated with 80% compost. LDPE subjected to 80% compost mixture, showed lowest crystallinity of 65% and weight reduction of 17%.

Results for LDPE treated with chemical surfactant showed maximum deterioration in LDPE treated with 10% SDS as the LDPE samples showed higher carbonyl index of 0.48 and double bond index of 0.37. XRD results showed maximum number of peaks in the spectra for 10% SDS treated LDPE. EDX analysis showed 70.47% of carbon content, which is lowest among all the LDPE samples, treated with the chemical surfactant.

Photo-oxidation results of LDPE revealed that the weight of the LDPE got decreased on exposure to sunlight. Weight reduction was 15.67%. The crystallinity of photo-oxidized sample was 37% higher than the unexposed sample whose value was 35%. The increased crystallinity may be due to the weathering influence on amorphous regions. The TGA and DSC results also revealed changes in the thermal character of the LDPE exposed to solar radiation.

11. General Discussion

Low density polyethylene films occupy a large portion of the waste in industrial and municipal waste because it is massively used in the packaging of items. Due to its exceptional thermal and mechanical property and low cost it is used frequently in number of applications. Once used, it is disposed of to the landfill sites, thus increasing the burden on the landfill sites. LDPE is resistant toward degradation in natural environment because it is highly inert in nature. So, littering of such type of plastic is a major environmental problem. The waste recycling of plastic is considered as one of the measures to reduce the waste but it has certain economic problem because recycling involves the huge investment of money. So, there is a need to find a better way to degrade the LDPE waste. There is a contradiction of views in terms of polymer degradation for polymer scientist and microbiologist. For polymer scientist, the degradation of polymer is mainly defined as loss in mechanical and physical properties, whereas for microbiologist the degradation means transformation of the polymer into biomass and carbon dioxide. Exploring the alternate ways for the LDPE degradation has been an interesting area of research. There are basically three ways of degrading the LDPE, which are: Biodegradation, chemical degradation and physical degradation. In the present study a comparison of three methods of degradation have been experimented to observe the degradation pattern of the LDPE.

For analyzing the biodegradation of the polymer two novel bacteria were isolated which were identified as *Bacillus* sp strain 530F_seq260_Env1 and *Lysinibacillus* sp strain DESBBAU2. These two novel isolates of bacteria were positively screened for the biosurfactant production. Not all the microbes present in the environment are active producer of biosurfactant. The biosurfactant producing bacteria generally thrive

upon hydrocarbon-contaminated sites. **Rani *et al.*, (2020)** have isolated bacteria from the sample collected obtained from oil batteries. The bacterium was identified as *Bacillus methylotrophicus* strain OB9 that is the active producer of biosurfactant. **Sohail & Jamil, (2020)** have isolated four bacterial strains among which only one bacterium was identified as prime biosurfactant producer and it was identified as *Pseudomonas aeruginosa* (MH142144). **Deng *et al.*, (2020)** isolated 10 strains of bacteria from oil-contaminated site and out of those ten bacteria only one found to produce biosurfactant. The bacterium was identified as *Achromobacter ruhlandii* strain MF988690.1. All the recent studies conducted shows that the possibility of isolating bacteria that is the active producer of biosurfactant is very less. In the present study total 15 bacteria were isolated, among which, two were successfully screened for biosurfactant production.

The production of biosurfactant can be improved by maintaining the optimal condition for growth and activity of bacteria. For the optimization Response surface methodology can be the best way for designing the optimization experiment**. Hu *et al.*, (2015)** has optimized the biosurfactant production for the bacterium *Vibrio* sp. 3B-2 by selecting the four variables, which were important for the nutrient medium and the optimal medium for producing the biosurfactant contained: 0.5% lactose, 0.1% disodium hydrogen phosphate 1.1% yeast extract, and 2% sodium chloride. In this study, four factors: temperature, pH, carbon source (beef extract) and nitrogen source (peptone) were chosen. For the *Bacillus* sp optimal conditions were- pH: 6, temperature: 30 °C, Peptone concentration: 10 mg/l, Beef extract concentration: 10 mg/l. For *Lysinibacillus* sp, optimal conditions were - pH: 8, temperature: 30 °C, Peptone concentration: 5 mg/l, Beef extract concentration: 5 mg/l.

The characterization of biosurfactant is the important step for its commercial application. **Patowary *et al.*, (2017)** has characterized the biosurfactant extracted from a potent bacterium *Pseudomonas aeruginosa* PG1. The FTIR spectrum of biosurfactant revealed some of the important bands at 2928, 3376, 1648, 1732, and 1038 cm^{-1}. The lipid content was 0.67 g/l. SEM-EDS analysis of biosurfactant revealed the existence of oxygen, carbon, sodium, chlorine, phosphorus, and potassium. The biosurfactant yield was 2.26 g/l by bacterium *Pseudomonas aeruginosa* PG1. According to the study presented here on the characterization of biosurfactant, FTIR analysis showed major functional groups such as O-H stretching, stretching of unsaturated C=C bonds, stretching due to carbonyl (C=O) groups, ester functional group, bending of the hydroxyl group (OH) and Sugar C-O stretch, which are the characteristic functional group depicting the biosurfactant. The EDX analysis showed the carbon content of 60.22% and 70.01% in *Bacillus* sp biosurfactant and *Lysinibacillus* sp biosurfactant respectively, whereas, the oxygen content was 38.9% and 17.1% for *Bacillus* sp biosurfactant and *Lysinibacillus* sp biosurfactant respectively. TGA results revealed that for biosurfactant that was extracted from *Lysinibacillus* sp, the degradation temperature was 255°C and final decomposition temperature was 450°C. The DSC analysis showed that the melting temperature of *Lysinibacillus* sp extracted biosurfactant is greater than the *Bacillus* sp extracted biosurfactant. The DSC data revealed that the biosurfactant of *Lysinibacillus* sp is more thermostable which is important for a biosurfactant to be used in commercial application. The yield of biosurfactant obtained from *Bacillus* sp and *Lysinibacillus* sp was 1.36 gm/l and 1.48 gm/l respectively.

Some of the research conducted on polymer degradation has shown that biological degradation, i.e., microbial degradation is the best method due to environmental

friendly method **(Kathiresan, 2003)**. Microbial degradation occurs due to formation of biofilm over the polyethylene. To enhance the attachment of microbes over the polyethylene film, the hydrophobic properties of polymer should be transformed into hydrophilic ones through oxidation. This can be done using biosurfactants as they are environment friendly. **Mukherjee *et al.*, (2015)** utilized two biosurfactant-producing bacteria *Bacillus licheniformis* and *Lysinibacillus fusiformis* for the degradation of polyethylene. They incubated the polyethylene with *B. licheniformis* for 2 months. The surfactant produced by bacteria oxidized both unoxidized as well as pre-oxidized polyethylene during the period of incubation. During the bio-degradation process of polyethylene, unsaturated hydrocarbons were formed **(Nanda *et al.*, 2010)**. The oxidation was confirmed through the reduction in crystalline property and erosioned polymer surface using SEM. The degradation also resulted in rapid loss of rapid loss of mechanical properties. Maximum deterioration was observed in case of polyethylene incubated with bio-surfactant for 2 months. In case of *Lysinibacillus fusiformis*, the polyethylene films that were treated with bacterium along with bio-surfactant showed highest weight loss. Maximum weight loss of 2.97 5 % was obtained in case of polyethylene that was treated with *Lysinibacillus* for 1 month and then treated with bio-surfactant extracted from it for 1 month, followed by treatment with the bacterium for another 1 month. Polyethylene got biodegraded through transformation of carbonyl groups into unsaturated hydrocarbon with the help of bio-surfactant and *Lysinibacillus* bacterium.

However, in the present study two novel strains of *Bacillus* and *Lysinibacillus* has been isolated which has never been reported for the ability to produce biosurfactant and polyethylene degradation. The degradation results obtained for both the bacteria revealed that *Bacillus* sp and *Lysinibacillus* sp are effective in degrading the LDPE.

However, *Lysinibacillus* sp has shown greater impact on the degradation of the polymer. Among all the combinations major degradation was observed in case of LDPE treated with *Lysinibacillus* sp in combination with its biosurfactant. The addition of biosurfactant in the culture medium had enhanced the availability of the polymer molecules to the bacterial cells by reducing the surface tension. Polyethylene samples which were treated with *Lysinibacillus* along with bio-surfactant showed maximum weight loss. FTIR spectrum of LDPE film confirmed changes in the presence of chemical groups like amine, alkanes, phenols, and alcohol after degradation of LDPE films by *Bacillus* sp and *Lysinibacillus* sp. The results affirmed that the isolates are capable of degrading LDPE. The two bacterial isolates have been first time being reported for their LDPE degrading potential and also, they are the active producer of biosurfactant, which has several applications in bioremediation **(Mohammadi *et al.*, 2012)**.

In composting method of degrading LDPE, definite weight of LDPE is subjected to mixture of certain amount of mature compost. Biodegradation of composted LDPE is measured on the basis of amount of material carbon transformed to s carbon dioxide **(Yang *et al.*, 2004)**. Nature of the compost decides the degree of LDPE degradation **(Bellia *et al.*, 1999)**. Beside this, the shape of the polymer sample and additives in it affects the plastic degradation in the compost **(Pandey *et al.*, 2003)**. **Okoh and Atuanya, (2014)** has studied the impacts of soil composting along with the poultry manure on biodegradation of polyethylene. According to their study, the percentage weight loss of polyethylene after the treatment in compost burial soil was 0.12-0.93%. In the present study of controlled composting treatment of LDPE, all the composted LDPE samples showed the changes induced in the polymer. Major changes were observed in LDPE samples treated with 80% compost. Maximum weight loss of 17%

was observed in 80% compost treated sample. Thermal gravimetric analysis revealed lowest onset and decomposition temperature with no residue left in LDPE sample treated with 80% compost mixture. EDX analysis showed minimum carbon content and maximum oxygen content in 80% compost treated LDPE sample after six months of treatment. In FTIR analysis, maximum value of carbonyl and double bond index supported the greater extent of degradation in 80% composted LDPE that denotes the change in the molecular structure of the polymer.

Chemical surfactants are synthesized chemically and have low critical micelle concentration (CMC), which means they have higher solubilization ability at a very low concentration. Chemical surfactants like sodium dodecyl sulphate have been utilized to remove crude oil from soil at 50°C. for 14 days **(Thomas & White, 1989; Urum *et al.*, 2004)**. Tween 80, which is a non-ionic surfactant, has been used during biodegradation of polyethylene. LDPE was treated with 0.5% of tween 80 and then subjected to biodegradation has enhanced the availability of the polyethylene to microbes **(Albertsson *et al.*, 1993)**. In the study presented here, three chemical surfactant were used, which are: SDS, CTAB and tween 80. The result showed, higher oxidation of polyethylene was induced by sodium dodecyl sulfate as compared to CTAB and tween 80. Carbonyl index and double bond index of polyethylene treated with SDS at 60 °C for 1 month was found to be higher than CTAB and Tween 80 oxidized polyethylene as observed in FTIR study. The maximum number of peaks in XRD analysis was observed for 10% SDS treated polyethylene. This shows the effect of chemical surfactant on the crystallinity of the polymer. Such increase in crystallinity due to the annealing effect of temperature, chain scission and formation of intermolecular polar bonds between carbonyl groups during thermal oxidation of polyethylene at 60°C. Oxidation level of polyethylene treated by SDS was higher as

the availability of soluble oxygen and chain scission increased due to the attachment of surfactant to the polyethylene surface. EDX analysis showed maximum decrease of carbon content (70.47 %) in case of 10% SDS treated polyethylene. This is the first study where all the three types of chemical surfactants i.e., anionic, cationic and non-ionic surfactants have been used and the study has shown the maximum oxidation of LDPE by 10% SDS which is an anionic surfactant.

Exposure of the polymer to solar radiation causes the significant changes in it. UV radiation in of the sunlight causes photo-oxidative degradation that results in breaking of polymer chains, generate free radical and decreases the molecular weight thus, causing deterioration in the mechanical properties of polymer. Weathering behavior **Andrady *et al.*, (1993)** studied the weathering and photooxidative effect on polyethylene using FTIR spectroscopy, gel-permeation chromatography to monitor structural changes accompanying photodegradation and change in tensile property. They revealed the changes in carbonyl index during degradation and change in the average molecular weight of the polyethylene. In the present study on photooxidation of LDPE, major changes were observed after the period of 90 days exposure to sunlight. SEM analysis signified that solar radiation altered the surface morphology of the polymer. Cracks and grooves were seen. The increased carbonyl index examined by FTIR analysis confirmed the photo oxidation of polymer upon exposure to sunlight. While changes in the elemental composition of the polymer confirms the breakage in the polymer chain. XRD analysis showed the emergence of new peaks in LDPE exposed to the sunlight. TGA and DSC have also shown the change in the thermal behavior of the LDPE after exposure to sunlight. that once the polymer gets brittle their resistance power gets decreased and microbes can make use of the carbon in the polymer chains for their growth and development. The study performed will

contribute in the management of the plastic waste in efficient and cost effective manner **(Urum *et al.,* 2004)**. The final melting temperature for the sunlight exposed LDPE was 162°C while that for unexposed one was 138°C. The increase in the temperature can be due to the crystalline region that was left behind after the disruption of amorphous region. The weight reduction obtained over the period of 90 days was 15.67%. So, the above analyses showed that upon exposure to sunlight major changes occur in the molecular structure of LDPE.

The overall study conducted compared the three types of degradation mainly biodegradation through microbes, biosurfactant and composting; chemical degradation by chemical surfactants; and physical degradation by solar radiation. All these three methods of degradation revealed significant changes but the highest changes were observed in biodegradation method using bacteria and biosurfactant. The two novel isolated strains of *Bacillus* and *Lysinibacillus* species proved to be the active producer of biosurfactant and effectively degraded the LDPE, elaborating futuristic scope of the isolates for better application performance.

12. General Conclusion

Disposal of LDPE waste has been the most important environmental issue. LDPE waste is strongly resistant toward degradation. Used LDPE is disposed of to the landfill sites and it is usually burned, that contaminates the air with toxic compounds, like dioxins, phthalates and polycyclic hydrocarbons. The recycling of LDPE is still an expensive way for reducing the plastic waste. So, there is a need to find the alternative ways for degrading the LDPE in cheapest and eco-friendly manner. With reference to aforesaid context the study was performed to investigate the degradation of LDPE by biological, physical and chemical method.

For isolation of biosurfactant-producing bacteria, waste polyethylene samples were collected from plastic polluted dumpsites. Total fifteen bacteria were isolated which were screened for the production of biosurfactant on the basis of oil spreading test, emulsification index test and phenol-sulphuric acid test. Only two bacteria were screened positively for the production of biosurfactant. These two bacteria were then subjected to molecular identification and they were identified as the novel bacteria strains. The bacteria ENV1 was identified as *Bacillus* sp strain 530F_seq260_Env1 with accession number MN559801-MN559803 and ENV4 was identified as *Lysinibacillus* sp strain DESBBAU2 having accession number MN715876-MN715869.

The biosurfactant production was then optimized using response surface methodology and central composite design for both the novel isolated bacteria. The CCD confirmed the optimal growth conditions for both the bacteria to maximize biosurfactant yield. The model generated for both the bacteria for biosurfactant production was found to be suitable for defining the response of the system as the experimental data was in

good conformity with the values that were predicted by the model. The R^2 and p-value (<0.0001) of the model were significant. The model was also validated by the conformation test at the predicted optimum points. The value predicted by the model was compared with the experimental value and showed about 3% and 2% deviation for *Bacillus* sp and *Lysinibacillus* sp. Biosurfactant obtained from both the bacteria was found to be of glycolipid type after the structure analysis by FTIR, EDX, DSC and TGA analysis. Both the biosurfactant showed significant stability under extreme conditions of temperature, which is very important for the commercial application of biosurfactant. Using the optimized parameter for both the bacteria biosurfactant was produced. The biosurfactant yield for *Bacillus* sp was 1.36 gm/l and for *Lysinibacillus* sp was 1.48 gm/l.

The degradation results obtained for both the bacteria revealed that *Bacillus* sp and *Lysinibacillus* sp are effective in degrading the LDPE. However, *Lysinibacillus* sp has shown greater impact on the degradation of the polymer. Among all the combinations major degradation was observed in case of LDPE treated with *Lysinibacillus* sp in combination with its biosurfactant. The addition of biosurfactant in the culture medium had enhanced the availability of the polymer molecules to the bacterial cells by reducing the surface tension. Polyethylene samples which were treated with *Lysinibacillus* along with bio-surfactant showed maximum weight loss. FTIR spectrum of LDPE film confirmed changes in the presence of chemical groups like amine, alkanes, phenols, and alcohol after degradation of LDPE films by *Bacillus* sp and *Lysinibacillus* sp. The results affirmed that the isolates are capable of degrading LDPE. The two bacterial isolates have been first time being reported for their LDPE degrading potential and also, they are the active producer of biosurfactant, which has several applications in bioremediation.

In case of controlled composting treatment of LDPE, all the composted LDPE samples showed the changes induced in the polymer. Major changes were observed in LDPE samples treated with 80% compost. Maximum weight loss of 17% was observed in 80% compost treated sample. Thermal gravimetric analysis revealed lowest onset and decomposition temperature with no residue left in LDPE sample treated with 80% compost mixture. EDX analysis showed minimum carbon content and maximum oxygen content in 80% compost treated LDPE sample after six months of treatment. In FTIR analysis, maximum value of carbonyl and double bond index supported the greater extent of degradation in 80% composted LDPE that denotes the change in the molecular structure of the polymer.

In case of chemical surfactant treatment of LDPE, higher oxidation of polyethylene was induced by sodium dodecyl sulfate as compared to CTAB and tween 80. Carbonyl index and double bond index of polyethylene treated with Sodium dodecyl sulphate (SDS) at 60 °C for 1 month was found to be higher than CTAB and Tween 80 oxidized polyethylene as observed in FTIR study. The maximum number of peaks in XRD analysis was observed for 10% SDS treated polyethylene. This shows the effect of chemical surfactant on the crystallinity of the polymer. Such increase in crystallinity due to the annealing effect of temperature, chain scission and formation of intermolecular polar bonds between carbonyl groups during thermal oxidation of polyethylene at 60°C. Oxidation level of polyethylene treated by SDS was higher as the availability of soluble oxygen and chain scission increased due to the attachment of surfactant to the polyethylene surface. EDX analysis showed maximum decrease of carbon content (70.47 %) in case of 10% SDS treated polyethylene.

The degradation study performed under the solar radiation in natural environment showed some positive results toward polymer degradation. SEM analysis signified

that solar radiation altered the surface morphology of the polymer. Cracks and grooves were observed over the film surface. The increased carbonyl index examined by FTIR analysis confirmed the photo oxidation of polymer upon exposure to sunlight. While changes in the elemental composition of the polymer confirms the breakage in the polymer chain. Various analyses like XRD, TGA and DSC shown that once the polymer gets brittle their resistance power get decreased and microbes can make use of the carbon in the polymer chains for their growth and development. The study performed will contribute in the management of the plastic waste in efficient and cost-effective manner.

All the degradation methods performed here showed significant changes in the polymeric structure. But the most promising method for LDPE degradation was through biodegradation using the two isolated bacteria and their biosurfactant. The amphiphilic nature of the biosurfactant enhanced the bioavailability of the polymer to the bacteria. Further, the application of biosurfactant in degrading the other types of polymer could also be an interesting area of research.

13. Summary

Petroleum based polymers are used widely in number of applications due to their excellent thermal and physico-chemical properties. Among all the polymers, low-density polyethylene (LDPE), which is a thermoplastic, is used commonly. Polyethylene is used in large quantity for the manufacture of carry bags, bottles, milk packets, garbage containers etc. Low cost of LDPE and its bio-inertness nature are among the prime reasons for its usage as a packaging material. However, the accumulation of LDPE waste in large amount is imposing a great threat to the terrestrial as well as aquatic organisms. LDPE also causes environmental pollution because it is resistant to biodegradation.

Several other chemicals are also used with the manufacturing of such plastic to provide it certain characteristic such as additives, phthalates and flame-retardants. They all have been identified as harmful chemicals, which affects the health of human, and animal by interrupting with their endocrine system. So, the problem of white pollution or plastic pollution has been a major concern for the mankind. Almost, all the parts of the world are suffering with negative consequences of the plastic pollution.

In the current era of globalization some efforts should be made to develop the alternative ways for dealing with such plastic waste, which have eco-friendly approach. So, in relation to the aforesaid context, for dealing with the degradation of LDPE, the objectives designed for the study entitled "**Biodegradation of Low Density-Polyethylene (LDPE) by biosurfactant producing bacteria isolated from plastic polluted dumpsites**" were:

1. Isolation and screening and characterization of biosurfactant producing bacteria isolated from plastic polluted dumpsite.
2. Optimization, characterization and productionof biosurfactant.
3. Evaluation of LDPE degradation by biosurfactant and its producing bacteria.
4. Evaluation of LDPE degradation under controlled composting.
5. Analysis of the chemical alterations induced in the LDPE exposed to chemical surfactants.
6. Analysis of the photo-oxidation of LDPE.

Initial objective was to isolate, screen and characterize the biosurfactant-producing bacteria. In this study plastic samples were collected from plastic polluted dumpsites. They were then used for isolating bacteria. In total, fifteen bacteria were isolated among which two bacteria were found to be the active producer of biosurfactant. These two bacteria named as ENV 1 and ENV 4 were then subjected to molecular identification. The analysis identified ENV1 as novel strain of *Bacillus* sp. and it was named as *Bacillus* sp strain 530F_seq260_Env1 whereas, ENV 4 was identified as the novel strain of *Lysinibacillus* sp and it was names as *Lysinibacillus* sp strain DESBBAU2. These two bacteria were found to be positive for the emulsification index test, oil spreading test and phenol-sulfuric acid test, which are the major test for detecting the production of biosurfactant. The morphological characteristic for both the bacteria showed that they were gram positive and rod shaped. For the biochemical test performed, *Bacillus* sp. was found to be positive for protease, amylase, catalase and glucose whereas *Lysinibacillus* sp was found to be positive for oxidase and laccase.

Second objective was to produce, optimize and characterize the biosurfactant. In optimization study, the optimized parameter for *Bacillus* sp were- pH: 6, temperature:

30 °C, Peptone concentration: 10 mg/l, Beef extract concentration: 10 mg/l. For *Lysinibacillus* sp, optimized parameter were- pH: 8, temperature: 30 °C, Peptone concentration: 5 mg/l, Beef extract concentration: 5 mg/l. The characterization of the both biosurfactant extracted from the bacteria showed the total lipid content of 53 mg (71.62%) in the biosurfactant produced by the *Bacillus* sp strain 530F_seq260_Env1 and 59 mg (75.64%) in the biosurfactant produced from *Lysinibacillus* sp strain DESBBAU2. The total protein content of biosurfactant obtained by *Bacillus* sp strain 530F_seq260_Env1 was 5.981 µg/ml and that by the *Lysinibacillus* sp strain DESBBAU2 was 6.94 µg/ml. The FTIR analysis showed major functional groups such as O-H stretching, stretching of unsaturated C=C bonds, stretching due to carbonyl (C=O) groups, ester functional group, bending of the hydroxyl group (OH) and Sugar C-O stretch, which are the characteristic functional group depicting the biosurfactant. The EDX analysis showed the carbon content of 60.22% and 70.01% in *Bacillus* sp. biosurfactant and *Lysinibacillus* sp biosurfactant respectively, whereas, the oxygen content was 38.9% and 17.1% for *Bacillus* sp biosurfactant and *Lysinibacillus* sp biosurfactant respectively. TGA results revealed that for biosurfactant that was extracted from *Lysinibacillus* sp, the degradation temperature was 255°C and final decomposition temperature was 450°C. The DSC analysis showed that the melting temperature of *Lysinibacillus* sp extracted biosurfactant is greater than the *Bacillus* sp extracted biosurfactant. The DSC data reveals that the biosurfactant of *Lysinibacillus* sp is more thermostable which is important for a biosurfactant to be used in commercial application.

For the production of biosurfactant, the yield of biosurfactant obtained from *Bacillus* sp and *Lysinibacillus* sp was 1.36 gm/l and 1.48 gm/l respectively.

Third objective was to evaluate the LDPE degradation by the biosurfactant and its producing bacteria. The two isolated bacteria alongwith their extracted biosurfactant were tested for their potential to degrade LDPE. Different types of analysis like weight reduction test, FTIR, XRD, SEM-EDX, TGA and DSC was performed on the LDPE samples after the treatment to identify the changes occurred in the morphological and chemical structure of the polymer. The different combinations of bacteria and biosurfactant along with the LDPE as a substrate were maintained in the separate conical flasks for both the bacteria. The different combinations were: (i) Bacteria and its extracted biosurfactant in dried form along with the LDPE in nutrient broth as a medium (ii) Bacteria and LDPE in nutrient broth as a medium (iii) extracted biosurfactant in dried form and LDPE in nutrient broth as a medium (iv) LDPE only in nutrient broth medium. In all the combinations of experiment performed separately for *Bacillus* sp and the *Lysinibacillus* sp the LDPE that was treated with bacteria alongwith its biosurfactant showed better degradation. Through EDX analysis carbon content was measured which revealed the major reduction of carbon after the degradation. *Bacillus* sp and its biosurfactant treated LDPE showed 73% of carbon and *Lysinibacillus* sp and its biosurfactant treated LDPE showed 58% of carbon content after the degradation, which is lesser than that of untreated LDPE for which the carbon content was 93%. The TGA and DSC analysis showed the lowest decomposition temperature and melting temperature for the LDPE treated with bacteria and biosurfactant together.

Fourth objective was to evaluate the LDPE degradation under controlled composting. In this experiment different concentration of compost with soil was made. The different concentrations of the compost made are: 20%, 40%, 60%, 80% and 100% (w/w). The total amount of compost and soil mixture was 1 kg for the different

concentrations of compost. The compost mixture was transferred to the different pots in which LDPE films were buried for the period of 90 days. After treatment period, LDPE films were recovered from the compost mixture and then subjected to various analysis. Significant change of degradation as shown by the analysis post treatment was observed in LDPE treated with 80% compost mixture. Maximum weight reduction of approximately 17% was observed in 80% compost treated LDPE sample followed by 100%, 60%, 40% and 20% compost mixture treated LDPE samples. FTIR analysis performed on the samples revealed the lower crystallinity percent (65%) and higher carbonyl (0.2) and double bond index (1.8) in case of LDPE subjected to 80% compost mixture. SEM analysis showed maximum distortion in case of 80% compost mixture treated LDPE sample. EDX analysis showed lowest carbon weight percent (46%) and highest oxygen weight percent (50%) in case 80% compost mixture treated LDPE. In XRD analysis the comparison of degradation for different LDPE samples was done by comparing the intensity of X-ray at peaks 21° and 23.5°. So, the lowest intensity at the two peaks i.e., 21° and 23.5° was recorded in LDPE subjected to 80% compost mixture. DSC analysis showed highest onset temperature (395°C) and melting temperature (472°C) for the 80% compost treated LDPE. TGA analysis revealed lowest onset (403°C) and final decomposition (486°C) temperature for the LDPE treated with 80% compost.

Fifth objective was to analyse the chemical alterations induced in the LDPE exposed to chemical surfactants. For this experiment, LDPE was subjected to three chemical surfactant which are: Sodium dodecyl sulphate-SDS (anionic), cetyl trimethylammonium bromide-CTAB (cationic), and Tween 80 (non-ionic). Solution of all the three surfactants in various concentrations ranging from 2 to 5% w/v was made separately. The LDPE samples were then transferred to these solutions in the conical flask and kept for chemical treatment at

a temperature of 60°C for the period of one month. After the treatment period of one month LDPE films were recovered and subjected to various analysis. From FTIR analysis highest keto carbonyl index (0.48) and double bond index (0.37) was observed in 10% SDS treated LDPE. In case of XRD analysis, maximum number of peaks emerged in LDPE treated with 10% SDS. EDX analysis revealed the carbon and oxygen weight percent of all the LDPE samples. The analysis showed the lowest carbon weight percent (70%) and highest oxygen weight percent (25%) in case of LDPE treated with 10% SDS. So, the maximum change post degradation was observed in LDPE that was treated with 10% SDS.

Sixth objective was to analyse the photo-oxidation of LDPE. The degradation study was done in the presence of sunlight in natural environment. The LDPE sheets cut into a specific dimension was kept over the terrace of a building so that it can be exposed to the maximum intensity of solar radiation for the period of 90 days. The LDPE films were recovered after the period of 90 days and various analyses was performed to check the degradation. SEM analysis signified that solar radiation altered the surface morphology of the polymer. Cracks and grooves were seen. The increased carbonyl index examined by FTIR analysis confirmed the photo oxidation of polymer upon exposure to sunlight. While changes in the elemental composition of the polymer confirms the breakage in the polymer chain. XRD analysis showed the emergence of new peaks in LDPE exposed to the sunlight. TGA and DSC have also shown the change in the thermal behavior of the LDPE after exposure to sunlight. that once the polymer gets brittle their resistance power gets decreased and microbes can make use of the carbon in the polymer chains for their growth and development. The study performed will contribute in the management of the plastic waste in efficient and cost-effective manner. The final melting temperature for the sunlight exposed

LDPE was 162°C while that for unexposed one was 138°C. The increase in the temperature can be due to the crystalline region that was left behind after the disruption of amorphous region. The weight reduction obtained over the period of 90 days was 15.67%. So, the above analyses showed that upon exposure to sunlight major changes occur in the molecular structure of LDPE.

In the study basically three forms of LDPE degradation *i.e.,* biodegradation, chemical degradation and physical degradation were compared. The data obtained showed that biodegradation of the LDPE is the better option for dealing with the LDPE waste in an eco-friendly manner. Under biodegradation two methods were used *viz,* composting and bacterial degradation using biosurfactant. Between these two methods, LDPE degradation using the two isolated novel strains of *Bacillus* sp strain 530F_seq260_Env1 and *Lysinibacillus* sp strain DESBBAU2 along with their biosurfactant proved better degradation of the LDPE samples compared to composting. Further the isolation of more such strains of bacteria capable of producing biosurfactant could be a promising way towards the easy and better degradation of LDPE.

Abbasian, F., Lockington, R., Megharaj, M. & Naidu, R. (2016). A review on the genetics of aliphatic and aromatic hydrocarbon degradation. *Applied Biochemistry and Biotechnology*, 178, 224-250.

Abdel-Mawgoud, A.M., Lepine, F. & De_ziel, E. (2010). Rhamno lipids: diversity of structures, microbial origins and roles. *Applied Microbiology and Biotechnology*, 86, 1323-1336.

Aboulkas, A., Harfi, K.E. & Bouadili, A.E. (2010). Thermal degradation behaviors of polyethylene and polypropylene. Part I Pyrolysis kinetics and mechanisms. *Energy Conversion and Management*, 51, 1363-1369.

Abrusci, C., Pablos, J.L., Corrales, T., López-Marín, J., Marín, I. & Catalina, F. (2011). Biodegradation of photo-degraded mulching films based on polyethylenes and stearates of calcium and iron as pro-oxidant additives. *International Biodeterioration and Biodegradation,* 65, 451–459.

Aburas, M.M.A. (2016). Degradation of poly (3-hydroxybuthyrate) using *Aspergillus oryzae* obtained from uncultivated soil. *Life Science Journal*, 13, 51–56.

Agrawal, N. & Shahi, S.K. (2015). An environmental cleanup strategy-Microbial transformation of xenobiotic compounds. *International Journal of Current Microbiology and Applied Sciences*, 4, 429–46.

Akbari, S., Abdurahman, N. H., Yunus, R. M., Fayaz, F. & Alara, O. R. (2018). Biosurfactants a new frontier for social and environmental safety: a mini review. *Biotechnology Research and Innovation*, 2, 81–90.

Akola, J. & Jones, R., O. (2003). Branching reactions in polycarbonate: a density functional study. *Macromolecules,* 36, 1355–1360.

Alajlani, M., Shiekh, A., Hasnain, S. & Brantner, A. (2016). Purification of bioactive lipopeptides produced by *Bacillus subtilis* strain BIA. *Chromatographia,* 79, 1527–1532.

Albertsson, A.C. (1978). Biodegradation of synthetic polymers. II. A limited microbial conversion of 14C in polyethylene to 14CO2 by some soil fungi. *Journal of Applied Polymer Science,* 22, 3419–3433.

Albertsson, A.C. (1980). The shape of the biodegradation curve for low and high density polyethenes in prolonged series of experiments. *European Polymer Journal,* 16, 623–630.

Albertsson, A.C., Erlandsson., B., Hakkarainen, M. & Karlsson, S. (1998). Molecular weight changes and polymeric matrix changes correlated with the formation of degradation products in biodegraded polyethylene. *Journal of Environmental Polymer Degradation,* 6, 187-195.

Albertsson, A.C., Sares, C., Karlsson, S., 1993. Increased biodegradation of LDPE with nonionic surfactant. *Acta Polymer,* 44, 243-246.

Ali, M.I., Ahmed, S., Robson, G., Javed, I., Ali, N., Atiq, N. & Hameed, A. (2014). Isolation and molecular characterization of polyvinyl chloride (PVC) plastic degrading fungal isolates. *Journal of Basic Microbiology,* 54,18–27.

Ambika, D.K. & Lakshmi, B.K.M. & Hemalatha, K.P.J. (2015). Degradation of low density polythene by *Achromobacter denitrificans* strain s1, a novel marine isolate. *International Journal* of *Science* and *Research,* 6, 5454–5464.

Ammala, A., Bateman, S., Dean, K., Petinakis, E., Sangwan, P. & Wong, S. (2011). An overview of degradable and biodegradable polyolefin. *Progress in Polymer Science*, 36, 1015- 1049.

Andrady, A.L., Pegram, J.E. & Tropsha, Y. (1993). Changes in Carbonyl Index and Average Molecular Weight on Embrittlement of Enhanced-Photodegradable Polyethylenes. *Journal of environmental polymer degradation,* 1, 171-179.

Antoniou, E., Fodelianakis, S., Korkakaki, E. & Kalogerakis, N. (2015). Bio surfactant production from marine hydrocarbon-degrading consortia and pure bacterial strains using crude oil as carbon source. *Frontiers in microbiology*, 6. 13-25.

Araujo, J.R., Waldman, W.R. & De, P.M.A. (2008). Thermal properties of high density polyethylene composites with natural fibres coupling agent effect. *Polymer Degradation and Stability*, 93, 1770–1775.

Arnaud, R., Dabin, P., Lemaire, J., Al-Malaika, S., Chohan, S. & Coker, M. (2009). Photooxidation and biodegradation of commercial photodegradable polyethylenes. *Polymer Degradadation Stability*, 46, 211-224.

Artham, T. & Doble, M. (2008). Biodegradation of aliphatic and aromatic polycarbonates. *Macromolecular* Symposia, 115, 143-63.

Arutchelvi, J., Sudhakar, M., Arkatkar, A., Doble, M., Bhaduri, S. & Uppara, P.V. (2008). Biodegradation of polyethylene and polypropylene. *Indian Journal of Biotechnology*, 7, 9–22.

Babul, R.P.O., Connor, K. & Seeram, R. (2013). Current progress on bio-based polymers and their future trends. *Progress in Biomaterials*, 2, 1–16.

Balasubramanian, V., Natarajan, K., Hemambika, B., Ramesh, N., Sumathi, C.S., Kottaimuthu, R. & Rajesh, K.V. (2010). High-densitypolyethylene (HDPE)-degrading potential bacteria from marine ecosystem of Gulf of Mannar. *Indian Journal of Applied Microbiology*, 51, 205–211.

Barnes, D.K. (2002). Biodiversity: invasions by marine life on plastic debris. *Nature* 416, 808–809.

Basnett, P., Ching, K.Y., Stolz, M., Knowles, J.C., Boccaccini, A.R., Smith, C., Locke, I.C., Keshavarz, T. & Roy, I. (2013). Novel poly (3-hydroxyoctanoate)/poly (3-hydroxybutyrate) blends for medical applications. *Reactive and Functional Polymers*, 73, 1340–1348.

Basnett, P., Knowles, J.C., Pishbin., F., Smith, C., Keshavarz, T., Boccaccini, A.R. & Roy, I. (2012). Novel biodegradable and biocompatible poly (3-hydroxyoctanoate)/bacterial cellulose composites. *Advanced engineering materials*, 14, 330–343.

Bellia, G., Tosin, M., Floridi, G. & Innocenti, F.D. (1999) Activated vermiculite, a solid bed for testing biodegradability under composting conditions. *Polymer Degradation and Stability*, 66, 65-79.

Berlemont, R. & Martiny, A.C. (2013). Phylogenetic distribution of potential cellulases in bacteria. *Applied and environmental microbiology*, 79, 1545–1554.

Bezza, F.A. & Chirwa, E.M.N. (2015). Bio surfactant from Paeni bacillus dendritiformis and its application in assisting polycyclic aromatic hydrocarbon (PAH) and motor oil sludge removal from contaminated soil and sand media.

Process Safe. *Environmental protection*, 98, 354-364.

Bhardwaj, H., Gupta, R. & Tiwari, A. (2012a). Microbial population associated with plastic degradation. *Scientific Reports*, 1, 1–4.

Bhardwaj, H., Gupta, R. & Tiwari, A. (2012b). Communities of microbial enzymes associated with biodegradation of plastics. *Journal of Environmental Polymer*, 21, 575–579.

Bhatia, M., Girdhar, A., Tiwari, A. & Nayarisseri, A. (2014). Implications of a novel *Pseudomonas* species on low density polyethylene biodegradation: an in vitro to in silico approach. *Springer Plus*, 3, 1–10.

Bhatnagar, S. & Kumari, R. (2013). Bioremediation: a sustainable tool for environmental management a review. *Annual Research & Review in Biology,* 3, 974–993.

Blouzard, J.C., Bourgeois, C.D., Philip, P., Valette, O., Bélaïch, A., Tardif, C., Belaich, J.P. & Pagès, S. (2007). Enzyme diversity of the cellulolytic system produced by *Clostridium cellulolyticum* explored by two dimensional analysis identification of seven genes encoding new dockerin containing proteins. *Journal of Bacteriology*, 189, 2300–2309.

Bode, H., B., Zeeck, A., Plückhahn, K. & Jendrossek, D. (2000). Physiological and chemical investigations into microbial degradation of synthetic poly (cis-1,4-isoprene). *Applied and environmental microbiology*, 66, 3680–3685.

Bonhomme, S., Cuer, A., Delort, A.M., Lemaire, J., Sancelme, M. & Scott, G. (2003). Environmental biodegradation of polyethylene. *Polymer degradation and stability*, 81, 441-452.

Borah, J.S. & Chaki, T.K. (2011). Dynamic mechanical, thermal, physic-mechanical and morphological properties of LLDPE/EMA blends. *Journal of polymer research,* 18, 569–578.

Brune, A., Frenzel, P. & Cypionka, H. (2000). Life at the oxic anoxic interface: microbial activities and adaptations. *FEMS microbiology reviews,* 24, (5)691–710.

Cai, M., Nie, Y., Chi, C. Q., Tang, Y. Q., Li, Y. & Wang, X. B. (2015). Crude oil as a microbial seed bank with unexpected functional potentials. *Science Repor*,5, 16057.

Calabia, B.P. & Tokiwa, Y. (2004). Microbial degradation of poly (D-3-hydroxybutyrate) by a new thermophilic *Streptomyces* isolate. *Biotechnology Letters*, 26, 15–19.

Cameotra, S.S. & Makkar, R.S. (2010). Biosurfactant-enhanced bioremediation of hydrophobic pollutants. *Pure* and *Applied Chemistry*, 82, 97-116.

Carrier, M., Serani, A.L., Absalon, C., Aymonier, C. & Mench, M. (2012). Degradation pathways of holocellulose, lignin and a-cellulose from Pteris vittata fronds in sub- and super critical conditions. *Biomass and Bioenergy*, 43, 65–70.

Caruso, G. (2015). Plastic degrading microorganisms as a tool for bioremediation of plastic contamination in aquatic environments. *Journal of Pollution Effects & Control*, 3, 1–2.

Carvalheiro, F., Duarte, L.C. & Girio, F.M. (2008). Hemicellulose biorefineries a review on biomass pretreatments. *Journal of Scientific and Industrial Research*, 67, 849–864

Cawoy, H., Debois, D., Franzil, L., De Pauw, E., Thonart, P. & Ongena, M. (2015). Lipopeptides as main ingredients for inhibition of fungal phytopathogens by *Bacillus subtilis/amyloliquefaciens*. *Microbial. Biotechnology,* 8, 281–295.

Chahal, P.S., Chahal, D.S. & André, G. (1992). Cellulase production profile of *Trichoderma reesei* on different cellulosic substrates at various pH levels. *Journal of Fermentation and Bioengineering,* 74, 126–128.

Chaisu, K., Siripholvat, V. & Chiu, C.H. (2015). New method of rapid and simple colorimetric assay for detecting the enzymatic degradation of poly lactic acid plastic films. *International journal of life sciences biotechnology and pharma research,* 4, 57–61.

Chakraborty, P., Sarker, R.K., Roy, R., Ghosh, A., Maiti, D. & Tribedi, P. (2019). Bio augmentation of soil with *Enterobacter cloacae* AKS7 enhances soil nitrogen content and boosts soil microbial functional-diversity. 3 *Biotechnology,* 9, 253.

Chaprão, M. J., Rita De Cássia, F., Rufino, R. D., Luna, J. M., Santos, V. A. & Sarubbo, L.A. (2018). Production of a biosurfactant from *Bacillus methylotrophicus* UCP1616 for use in the bioremediation of oil-contaminated environments. *Ecotoxicology,* 27, 1310–1322.

Charoenpanich, J., Tani, A., Moriwaki, N., Kimbara, K. & Kawai, F. (2006). Dual regulation of a polyethylene glycol degradative operon by AraC-type and

GalR-type regulators in *Sphingopyxis macrogoltabida* strain 103. *Microbiology,*152, 3025–3034.

Cheah, K. & Cook, W.D. (2003). Structure-property relationships of blends of polycarbonate. *Polymer Engineering and Science*, 43, 1727–1739.

Chen, J., Zhang, Y., Du, G.C., Hua, Z.Z. & Zhu, Y. (2007). Biodegradation of polyvinyl alcohol by a mixed microbial culture. *Enzyme and Microbial Technology,* 40, 1686–1691.

Chiellini, E., Corti, A. & Swift, G. (2003). Biodegradation of thermally-oxidized, fragmented low-density polyethylenes. *Polymer Degradation and Stability*, 81, 341-351.

Chrissafis, K., Paraskevopoulos, K.M. & Bikiaris, D.N. (2005). Thermal degradation mechanism of poly (ethylene succinate) and poly (butylene succinate): comparative study. *Thermochemistry Act,* 43, 142–150.

Chrissafis, K., Paraskevopoulos, K.M. & Bikiaris, D.N. (2006). Effect of molecular weight on thermal degradation mechanism of the biodegradable polyester poly (ethylene succinate). Thermochemistry Acta, 440, 166–175.

Chun, K.S., Husseinsyah, S. & Osman, H. (2013). Modified cocoa pod husk-filled polypropylene composites by using methacrylic acid. *BioResources*, 8, 3260–3275.

Copinet, A., Bertrand, C., Govindin, S., Coma, V. & Couturier, Y. (2004). Effects of ultraviolet light (315 nm), temperature and relative humidity on the degradation of polylactic acid plastic films. *Chemosphere,* 55, 763–773.

Cornell, J.H., Kaplan, A.M. & Rogers, M.R. (1984). Biodegradability of photooxidized polyalkylenes. *Journal of Applied Polymer Science,* 29, 2581–2597.

Costa, S.G., Nitschke, M., Lepine, F., De_ziel, E. & Contiero, J. (2010). Structure, properties and applications of rhamnolipids produced by Pseudomonas aeruginosa L2-1 from cassava wastewater. *Process Biochemistry,* 45, 1511-1516.

Cui, Z., Lai, Q., Dong, C. & Shao, Z. (2008). Biodiversity of polycyclic aromatic hydrocarbon-degrading bacteria from deep sea sediments of the Middle Atlantic ridge. *Environmental microbiology,* 10, 2138-2149.

Das, K. & Mukherjee, A.K. (2007). Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum- oil contaminated soil from North-East India. *Bioresource Technology,* 98, 1339-1345.

Das, M.P. & Kumar, S. (2015). An approach to low-density polyethylene biodegradation by *Bacillus amyloliquefaciens*. 3 *Biotechnology,* 5, 81–86.

Das, P., Yang, X.-P. & Ma, L.Z. (2014). Analysis of biosurfactants from industrially viable Pseudomonas strain isolated from crude oil suggests how rhamnolipids con- geners affect emulsification property and antimicrobial activity. *Frontiers in Microbiology,* 5.

De Rienzo, M.D., Kamalanathan, I. & Martin, P. (2016). Comparative study of the production of rhamnolipid biosurfactants by B. thailandensis E264 and P. aeruginosa ATCC 9027 using foam fractionation. *Process Biochemistry,* 51,

820-827.

De_ziel, E., Le_pine, F., Milot, S. & Villemur, R. (2000). Mass spectrometry monitoring of rhamnolipids from a growing culture of Pseudomonas aeruginosa strain 57RP. *Biochemica Biophysica Acta*, 1485, 145-152.

Deepa, D. (2019). Biodegradation of Low Density Polyethylene by Selected *Bacillus* sp. *Gazi University Journal of Science***,** 32, 802–813.

Deng, Z., Jiang, Y., Chen, K., Li, J., Zheng, C., Gao, F. & Liu, X. (2020). One Biosurfactant-Producing Bacteria *Achromobacter* sp. A-8 and Its Potential Use in Microbial Enhanced Oil Recovery and Bioremediation. *Frontiers in microbiology*, 11, 247.

Devi, R.S., Kannan, V.R., Nivas, D., Kannan, K., Chandru, S. & Antony, A.R. (2015). Biodegradation of HDPE by *Aspergillus* spp. from marine ecosystem of Gulf of Mannar. *Marine Pollution Bulletin*, 96, 32–40.

Dey, S. & Tribedi, P. (2018). Microbial functional diversity plays an important role in the degradation of polyhydroxybutyrate (PHB) in soil. 3 *Biotechnology*, 8, 171.

Dey, U., Mondal, N.K., Das, K. & Dutta, S. (2012). An approach to polymer degradation through microbes. *Journal* of *Pharmaceutical* Sciences, 2, 385–388.

Díaz De Rienzo, M. A., Stevenson, P., Marchant, R. & Banat, I. M. (2015). Antibacterial properties of biosurfactants against selected Gram-positive and-negative bacteria. *FEMS Microbiology Letters*, 363, 224.

Duddu, M.K., Tripura, K.L., Guntuku, G. & Divya, D.S. (2015). Biodegradation of low-density polyethylene (LDPE) by a new biosurfactant-producing thermophilic *Streptomyces coelicoflavus* NBRC 15399T. *African Journal of Biotechnology*, 14, 327–340.

Eubeler, JP., Zok, S., Bernhard, M. & Knepper, T.P. (2009). Environmental biodegradation of synthetic polymers I. Test methodologies and procedures. *Trends in analytical chemistry,* 28, 1057–1072.

Fontanella, S., Bonhomme, S., Koutny, M., Husarova, L., Brusson, J.M. & Courdavault, J.P. (2010). Comparison of the biodegradability of various polyethylene films containing pro-oxidant additives. *Polymer Degradation and Stability*, 95, 1011-1021.

Gajanand, E., Soni, L.K. & Dixit, V.K. (2014). Biodegradable polymers: a smart strategy for today's crucial needs. *International Journal of Pharmaceutical Sciences,* 3, 1–70.

Gajendiran, A., Krishnamoorthy, S. & Abraham, J. (2016). Microbial degradation of low-density polyethylene (LDPE) by *Aspergillus clavatus* strain JASK1 isolated from landfill soil. 3 *Biotechnology*, 6, 1–6.

Garlotta, D. (2002) A literature review of poly (lactic acid). *Journal of environmental polymer*, 9, 63–84.

Garrett, T.R., Bhakoo, M. & Zhang, Z. (2008). Bacterial adhesion and bioflms on surfaces. *journal of Progress* in *Natural Science.*18, 1049–1056.

Gautam, R., Bassi, A.S., Yanful, E.K. & Cullen, E. (2007). Biodegradation of automotive waste polyester polyurethane foam using *Pseudomonas*

chlororaphis ATCC55729. *International Biodeterioration and Biodegradation*, 60, 245–249.

Gerard, T., Budtova, T., Podshivalov, A. & Bronnikov, S. (2014). Polylactide/poly (hydroxybutyrate-co-hydroxyvalerate) blends morphology and mechanical properties. *Express polymer letters*, 8, 609–617.

Giudicianni, P., Cardone, G. & Ragucci, R. (2013). Cellulose, hemicellulose and lignin slow steam pyrolysis: thermal decomposition of biomass components mixtures. *Journal of Analytical and Applied Pyrolysis*, 100, 213–222.

Grover, A., Gupta, A., Chandra, S., Kumari, A. & Khurana, S.P. (2015). Polythene and environment. *International Journal of Environmental Science*, 5, 1091–1105.

Gu, J.D. (2003). Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. *International Biodeterioration and Biodegradation*, 52, 69–91.

Gulmine, J.V. (2003). Degradation profile of polyehtylene after artificial accelerated weathering. *Polymer Degradation and Stability*, 79, 385-397.

Guo, W., Duan, J., Geng, W., Feng, J., Wang, S. & Song, C. (2013). Comparison of medium-chain-length polyhydroxyalkanoates synthases from *Pseudomonas mendocina* NK-01 with the same substrate specificity. *Microbiological research*,168, 231–237.

Gupta, P., Sarkar, S. & Das, B., Bhattacharjee, S. & Tribedi, P. (2016). Bioflm, pathogenesis and prevention-a journey to break the wall: a review. *Archives of Microbiology*, 198, 1–15.

Hadad, D., Geresh, S. Sivan, A. (2005). Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *Journal Applied Microbiology*, 98, 1093–1100.

Hankermeyer, C.R. & Tjeerdema, R.S. (1999). Polyhydroxy butyrate Plastic made and degraded by microorganism. *Reviews of Environmental Contamination* and *Toxicology*,159, 1-24.

Hanson, K., Desai, J.D. & Desai, A.J. (1993). A rapid and simple screening technique for potential crude oil degrading microorganisms. *Biotechnology techniques*,7, 745-748.

Heydari, A. & Pessarakli, M. (2010). A review on biological control of fungal plant pathogens using microbial antagonists. *Journal of Biological Science,* 10, 273–290.

Hidayat, A. & Tachibana, S. (2012). Characterization of polylactic acid (PLA)/kenaf composite degradation by immobilized mycelia of *Pleurotus ostreatus*. *International Biodeterioration and Biodegradation*, 71, 50–54.

Hoang, K.C., Tseng, M. & Shu, W.J. (2007). Degradation of polyethylene succinate (PES) by a new thermophilic Microbispora strain. *Biodegradation*, 18, 333–342.

Hong, S., Khim, J.S., Ryu, J., Kang, S.-G., Shim, W.J. & Yim, U.H. (2014). Environmental and ecological effects and recoveries after five years of the Hebei Spirit oil spill, Taean, Korea. *Ocean and Coastal Management*, 102, 522-532.

Horn, S.J., Kolstad, G.V., Westereng, B. & Eijsink, V.G. (2012). Novel enzymes for the degradation of cellulose. *Biotechnology and Biofuels,* 5, 1–12.

Howard, G.T. (2002). Biodegradation of polyurethane: a review. *International Biodeterioration and Biodegradation,* 49, 245–252.

Hu, X., Mamoto, R., Shimomura, Y., Kimbara, K. & Kawai, F. (2007). Cell surface structure enhancing uptake of polyvinyl alcohol (PVA) is induced by PVA in the PVA-utilizing *Sphingopyxis* sp. strain 113P3. *Archives of Microbiology*,188, 235–241.

Hu, X., Wang, C. & Wang, P. (2015). Optimization and characterization of biosurfactant production from marine Vibrio sp. strain 3B-2. *Frontiers in microbiology*, 6, 976.

Huang, F.Y. (2012). Thermal properties and thermal degradation of cellulose tri-stearate (CTs). *Polymers*, 4, 1012–1024.

Huang, Y.L., Li, Q.B., Deng, X., Lu, Y.H., Liao, X.K., Hong, M.Y. & Wang, Y. (2005). Aerobic and anaerobic biodegradation of polyethylene glycols using sludge microbes. *Process Biochemistry,* 40, 207–211.

Hwang, H.-M., Hu, X. & Zhao, X. (2007). Enhanced bioremediation of polycyclic aromatic hydrocarbons by environmentally friendly techniques. *Journal of Environmental Science and Health, Part C*, 25, 313-352.

Ibrahim, M., Ijah, U., Manga, S., Bilbis, L. & Umar, S. (2013). Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. *International Biodeterioration* & *Biodegradation*, 81, 28-34.

Ikada, Y. & Tsuji, H. (2000). Biodegradable polyesters for medical and ecological applications. *Macro molicule Rapid and Communication,* 21, 117–132.

Iovino, R., Zullo, R., Rao, M.A., Cassar, L. & Gianfreda, L. (2008). Biodegradation of poly (lactic acid)/starch/coir biocomposites under controlled composting conditions. *Polymer Degradation and Stability*, 93, (1)147–157.

Ishi, N., Inoue, Y., Shimada, K.I., Tezuka, Y., Mitomo, H. & Kasuya, K.I. (2007). Fungal degradation of poly (ethylene succinate). *Polymer Degradation and Stability*, 92, 44–52.

Islam, M.N. & Islam, M.S. (2011). Mechanical properties of chemically treated sawdust-reinforcedrecycled polyethylene composites. *Industrial & Engineering Chemistry Research,* 50, 11124–11129.

Ismail, W., Al-Rowaihi, I.S., Al-Humam, A.A., Hamza, R.Y., El Nayal, A.M. & Bououdina, M. (2013). Characterization of a lipopeptide biosurfactant produced by a crude-oil-emulsifying Bacillus sp. I-15. *International Biodeterioration & Biodegradation,* 84, 168-178.

Jailawi, M., Ameen, S.R. & Saraf A. Ali. (2015). Polyethylene degradation by *Pseudomonas putida* S3A. *International Journal* of *Advanced Research* in *Biological Sciences,* 2, 90-97.

Jakubowicz, I. (2003). Evaluation of degradation of biodegradable polyethylene (PE). *Polymer Degradation and Stability*, 80, 39-43.

Jemil, N., Ayed, H. B., Hmidet, N. & Nasri, M. (2016). Characterization and properties of biosurfactants produced by a newly isolated strain *Bacillus*

methylotrophicus DCS1 and their applications in enhancing solubility of hydrocarbon. *World Journal of Microbiology and Biotechnology,* 32:175.

Jeon, I.Y. & Baek, J.B. (2010). Nanocomposites derived from polymers and inorganic nanoparticles. *Materials,* 3, 3654–3674.

Jirage, A.S., Baravkar, V.S., Kate, V.K., Payghan, S.A. & Disouza, J.I. (2011). Poly-β-hydroxybutyrate: intriguing biopolymer in biomedical applications and pharma formulation trends. *International journal* of *pharmaceutical* and biological archive, 4, 1107–1118.

Joshi, P.A. & Shekhawat, D.B. (2014). Screening and isolation of biosurfactant producing bacteria from petroleum contaminated soil. *The European Journal of Experimental Biology*, 4, 164-169.

Kadouri, D., Burdman, S., Jurkevitch, E. & Okon, Y. (2002). Identification and isolation of genes involved in poly (β-hydroxybutyrate) biosynthesis in Azospirillum brasilense and characterization of a phbC mutant. Applied and environmental microbiology. 68, 2943–2949.

Kale, S.K., Deshmukh, A.G., Dudhare, M.S. & Patil, V.B. (2015). Microbial degradation of plastic: a review. *Journal of biochemical technology*, 6, 952–961.

Kameshwar, A.K.S. & Qin, W. (2016). Recent developments in using advanced sequencing technologies for the genomic studies of lignin and cellulose degrading microorganisms. *International Journal of Biological Sciences,* 12, 156–171.

Kannahi, M. & Sudha, P. (2013). Screening of polythene and plastic degrading microbes from Muthupet mangrove soil. *Journal of chemical and pharmaceutical research*, 5, 122–127.

Karlsson, S., Ljungquist, O. & Albertsson, A.C. (1988). Biodegradation of Polyethylene and the Influence of Surfactants. *Polymer Degradation and Stability*, 21, 237-250.

Kaseem, M., Hamad, K. & Deri, F. (2012). Thermoplastic starch blends: a review of recent works. *Polymer Science, Series B is a journal*, 54, 165–176.

Kathiresan, K. (2003). Polythene & plastics-degrading microbes from the mangrove soil. *Revista de Biología Tropical.* 51, 629-634.

Kim, M., Jung, J.-H., Ha, S.Y., An, J.G., Shim, W.J. & Yim, U.H. (2017). Long-term moni- toring of PAH contamination in sediment and recovery after the Hebei Spirit oil spill. *Archives of Environmental Contamination and Toxicology*,73, 93-102.

Konduri, M.K.R., Anupam, K.S., Vivek, J.S., Kumar, D.B.R. & Narasu, M.L. (2010). Synergistic effect of chemical and photo treatment on the rate of biodegradation of high density polyethilene by indigenous fungal isolates. *International Journal of Biotechnology and Biochemistry*, 6,157-174.

Korenblum, E., Souza, D. B., Penna, M. & Seldin, L. (2012). Molecular analysis of the bacterial communities in crude oil samples from two Brazilian offshore petroleum platforms. *International Journal of Microbiology*, 2012, 156537.

Kostka, J.E., Prakash, O., Overholt, W.A., Green, S.J., Freyer, G., Canion, A., Delgardio, J., Norton, N., Hazen, T.C. & Huettel, M. (2011). Hydrocarbon-

degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. *Applied* and *Environmental Microbiology*, 77, 7962-7974.

Koutny, M., Amato, P., Muchova, M., Ruzicka, J. & Delort, A.M. (2009). Soil bacterial strains able to grow on the surface of oxidized polyethylene film containing prooxidant additives. *International Biodeterioration & Biodegradation*, 63, 354-357.

Kumar, A.A., Karthik, K. & Arumugam, P.K. (2011). Properties of biodegradable polymers and degradation for sustainable development. *journal of Chemical engineering involves the application*, 2, 164-167.

Kumar, S., Hatha, K. & Christi, K.S. (2007). Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags. *International Journal of Tropical Biology*, 55, 777-786.

Kyaw, B.M., Champakalakshmi, R., Sakharkar, M.K, Lim, C.S. & Sakharkar, K.R. (2012). Biodegradation of low-density polythene (LDPE) by *Pseudomonas* species. *Indian Journal of Microbiology*, 52, 411–419.

Kyrikou, I., Briassoulis, D., Hiskakis, M. & Babou, E. (2011). Analysis of photo-chemical degradation behaviour of polyethylene mulching film with pro-oxidants. *Polymer Degradation and Stability*, 9, 2237-2252.

Lee, B., Pometto, A.L., Fratzke, A. & Bailey, T.B. (1991). Biodegradation of degradable plastic polyethylene by Phanerochaete and Streptomyces species. *Applied and environmental microbiology*, 57, 678–685.

Lee, D.W., Lee, H., Lee, A.H., Kwon, B.-O., Khim, J.S., Yim, U.H., Kim, B.S. &

Kim, J.-J. (2018). Microbial community composition and PAHs removal potential of indigenous bacteria in oil contaminated sediment of Taean coast, Korea. *Environmental Pollution*, 234, 503-512.

Leja, K. & Lewandowicz, G. (2010). Polymer biodegradation and biodegradable polymers a review. *Polish journal of environmental studies*, 19, 255–266.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265–275.

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F. & Navasaucedo, J.E. (2008). Polymer biodegradation: Mechanisms and estimation techniques. *Chemosphere*, 73, 429- 442.

Lugauskas, A., Levinskait, L. & Peciulyte, D. (2003). Micromycetes as deterioration agents of polymeric materials. *International biodeterioration and biodegradation*, 52, 233-242.

Mani, P., Dineshkumar, G., Jayaseelan, T., Deepalakshmi, K., Kumar, C. G. & Balan, S.S. (2016). Antimicrobial activities of a promising glycolipid biosurfactant from a novel marine *Staphylococcus saprophyticus* SBPS 15. *3 Biotech* 6, 163.

Manzur, A., Limo´n-Gonza´lez, M. & Favela-Torres, E. (2004). Biodegradation of physicochemically treated LDPE by a consortium of filamentous fungi. *Journal of Applied Polymer Science*, 92, 265–271

Marchant, R. & Banat, I.M. (2012). Biosurfactants: a sustainable replacement for chemical surfactants? *Biotechnology Letters*, 34, 1597–1605.

Marchut-Mikolajczyk, O., Drożdżyński, P., Pietrzyk, D. & Antczak, T. (2018). Biosurfactant production and hydrocarbon degradation activity of endophytic bacteria isolated from *Chelidonium majus* L. *Microbial Cell Factories,* 17, 171.

Maric, S. & Vranes, J. (2007). Characteristics and significance of microbial bioflm formation. *Periodicum Bilogorum,* 109, 115–121.

Mathur, G., Mathur, A. & Prasad, R. (2011). Colonization and degradation of thermally oxidized high-density polyethylene by (ITCC No.6052) isolated from plastic waste dumpsite. *Bioremediation Journal*, 15, 69–76.

Meena, K. R. & Kanwar, S. S. (2015). Lipopeptides as the antifungal and antibacterial agents: applications in food safety and therapeutics. *BioMed research international,* 2015, 473050.

Mendez, C.R., Vergaray, G., Vilma, R., Karina, B. & Cardenas, J. (2007). Isolation and characterization of polyethylene-biodegrading mycromycetes. *Peruvian Journal of Biology,* 13, 203–205.

Mohammadi, M., Yousefi, A.A. & Ehsani, M. (2012). Thermorheological analysis of blend of high and low density polyethylenes. *Journal of polymer research,* 19, 9798–9810.

Montazer, Z., Habibi, Najaf, M.B. & Levin, D.B. (2019). Microbial degradation of low-density polyethylene and synthesis of polyhydroxy alkanoate polymers. *The Canadian Journal of Microbiology,* 65, 224–234.

Montazer, Z., Habibi, Najaf, M.B. & Levin, D.B. (2020). Challenges with verifying microbial degradation of polyethylene. *Polymers*, 12, 123.

Moriyama, Y., Kimura, N., Inoue, R. & Kawaguchi, A. (1993). Examination of fungal deterioration on plasticized polyvinyl chloride by cryoscanning electron microscopy. *International Biodeterioration & Biodegradation,* 31, 231–239.

Mukherjee, K., Tribedi, P., Chowdhury, A., Ray, T., Joardar, A., Giri, S. & Sil, A.K. (2011). Isolation of a *Pseudomonas aeruginosa* strain from soil that can degrade polyurethane diol. *Biodegradation*, 22, (2)377–388.

Mukherjee, S., Chaudhuri, U.R. & Kundu, P.P. (2015). Biotic oxidation of polyethylene using a bio- surfactant produced by *B. licheniformis*: a novel technique. *RSC Advances*, 5,75089.

Mukherjee, S., Das, P. & Sen, R. (2009). Rapid quantification of a microbial surfactant by a simple turbidometric method. *The Journal of Microbiological Methods*, 76, 38-42.

Mukherjee, S., Roy, Chowdhuri, U. & Kundu, P.P. (2016). Bio-degradation of polyethylene waste by simultaneous use of two bacteria: Bacillus licheniformis for production of bio-surfactant and *Lysinibacillus fusiformis* for bio-degradation. *RSC Advances*, 6, 2982-2992.

Muthezhilan, R., Ragul, R., Pushpam, A. C. & Hussain, A. J. (2014). Production and purification of biosurfactant from marine yeast isolated from kelambakkam salterns. *Bioscience and Biotechnology Research,* 11, 59–67.

Muthukumar, A. & Veerappapillai, S. (2015). Biodegradation of plastics- A brief review. *International Journal of Pharmaceutical Sciences Review and Research*, 31, 204-209.

Nakajima, K.T., Shigeno, A.Y., Nomura, N., Onuma, F. & Nakahara (1999). Microbial degradation of polyurethane, polyester polyurethanes and polyether polyurethanes. *Applied microbiology and biotechnology*, 51, 134-140.

Nanda, S. & Sahu, S.S. (2010). Biodegradability of polyethylene by *Brevibacillus, Pseudomonas* and *Rhodococcus* spp. *The Annals of the New York Academy of Sciences is an academic journal,* 3, 95–98.

Nanda, S., Sahu, S.S. & Abraham, J. (2010). Studies on the biodegradation of natural and synthetic polyethylene by Pseudomonas spp. *Journal of applied science and environmental management,* 14, 57–60.

Nayak, P. & Tiwari, A. (2011). Biodegradation of polythene and plastic by the help of microbial tools: a recent approach. *International Journal of Biomedical and Advance Research,* 2, 344–355.

Nicolas, O., Charles, M. T., Jenni, S., Toussaint, V. & Beaulieu, C. (2018). Relationships between *Xanthomonas campestris* pv. *vitians* population sizes, stomatal density and lettuce resistance to bacterial leaf spot. *Canadian Journal* of *Plant Pathology*, 40, 399–407.

Nie, M., Yin, X., Ren, C., Wang, Y., Xu, F. & Shen, Q. (2010). Novel rhamnolipid bio- surfactants produced by a polycyclic aromatic hydrocarbon-degrading bacterium Pseudomonas aeruginosa strain NY3. *Biotechnology Advance,* 28, 635-643.

Nikolopoulou, M. & Kalogerakis, N. (2008). Enhanced bioremediation of crude oil utilizing lipophilic fertilizers combined with biosurfactants and molasses. *Marine Pollution Bulletin,* 56, 1855-1861.

Nikolopoulou, M., Pasadakis, N. & Kalogerakis, N. (2013a). Evaluation of autochthonous bioaugmentation and biostimulation during microcosm-simulated oil spills. *Marine Pollution Bulletin* ,72, 165-173.

Nikolopoulou, M., Pasadakis, N., Norf, H. Kalogerakis, N. (2013b). Enhanced ex situ bioremediation of crude oil contaminated beach sand by supplementation with nutrients and rhamnolipids. *Marine Pollution Bulletin,* 77, 37-44.

Nowak, B., Pajak, J., Drozd-Bratkowicz, M. & Rymarz, G. (2011). Microorganisms participating in the biodegradation of modified polyethylene films in different soils under laboratory conditions. *International Biodeterioration* & *Biodegradation,* 65, 757–767.

Obi, L. U., Atagana, H. I. and Adeleke, R. A. (2016). Isolation and characterisation of crude oil sludge degrading bacteria. *Springerplus,* 5, 1946.

Okoh, A. & Trejo-Hernandez, M. (2006). Remediation of petroleum hydrocarbon polluted systems: exploiting the bioremediation strategies. *The African Journal of Biotechnology*, 5.

Okoh, E.B. & Atuanya, E. (2014). Impacts of soil composting and poultry manure on biodegradation of polyethylene. *International journal of applied microbiology and biotechnology research*; 2, 18-29.

Orhan, Y. & Buyukgungor, H. (2000). Enhancement of biodegradability of disposable polyethylene in controlled biological soil. *International Biodeterioration & Biodegradation*, 45, 49–55.

Orr I.G., Hadar, Y. & Sivan, A. (2004). Colonization, bioflm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber*. The Applied Microbiology and Biotechnology, 65, 97–104.

Otake, Y., Kobayashi, T., Asabe, H., Murakami, N. & Ono, K. (1995). Biodegradation of low-density polyethylene, polystyrene, polyvinyl chloride, and urea formaldehyde resin buried under soil for over 32 years. *The Journal of Applied Polymer Science*, 56, 1789–1796.

Pandey, J.K., Kumar, A.P. & Singh, R.P. (2003) Biodegradation of packaging materials. Composting of polyolefins. *Macromolecular Symposia,*197, 411-420.

Panjiar, N., Sachan, S.G. & Sachan, A. (2015). Screening of bioemulsifier-producing micro-organisms isolated from oil-contaminated sites. *Annals of microbiology*, 65, 753-764.

Pantazaki, A.A., Papaneophytou, C.P. & Lambropoulou, D.A. (2011). Simultaneous pol- yhydroxyalkanoates an rhamnolipids production by *Thermus thermophilus* HB 8. AMB. Express 1, 17.

Parthipan, P., Preetham, E., Machuca, L. L., Rahman, P. K., Murugan, K. and Rajasekar, A. (2017). Biosurfactant and degradative enzymes mediated crude oil degradation by bacterium *Bacillus subtilis* A1. *Frontiers in Microbiology*, 8, 193.

Patowary, K., Patowary, R., Kalita, M.C. & Deka, S. (2017). Characterization of Biosurfactant Produced during Degradation of Hydrocarbons Using Crude Oil As Sole Source of Carbon. *Frontiers in microbiology*, 8, 279.

Petchwattana, N., Covavisaruch, S. & Chanakul, S. (2012). Mechanical properties, thermal degradation and natural weathering of high density polyethylene/rice hull composites compatibilized with maleic anhydride grafted polyethylene. *Journal of polymer research,* 19, 9921–9930.

Phua, S.K., Castillo, E., Anderson, J.M. & Hiltner, A. (1987). Biodegradation of polyurethane in vitro. *Journal of Biomedical Materials Research,* 21, 231-246.

Porebski, S., Bailey, L. G. and Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant molecular biology*, 15, 8–15.

Pornsunthorntawee, O., Wongpanit, P., Chavadej, S., Abe, M. & Rujiravanit, R. (2008). Structural and physicochemical characterization of crude biosurfactant pro- duced by Pseudomonas aeruginosa SP4 isolated from petroleum-contaminated soil. *Bioresource technology,* 99, 1589-1595.

Premraj, R. & Doble, M. (2005). Biodegradation of polymers. *Indian Journal of Biotechnology,* 4, 186–193.

Priyanka, N. & Archana, T. (2011). Biodegradation of polythene and plastic by the help of microorganisms: a way for brighter future. *Journal of Environmental & Analytical Toxicology,* 1, 111.

Raaijmakers, J.M., De Bruijn, I., Nybroe, O. & Ongena, M. (2010). Natural functions of lipopeptides from Bacillus and Pseudomonas: more than surfactants and anti- biotics. *FEMS microbiology reviews*, 34, 1037-1062.

Raaman, N. (2012). Biodegradation of plastic by Aspergillus spp. isolated from polythene polluted sites around Chennai. *Journal of academia and industrial research*, 1, 313–316.

Randhawa, S.K. & Rahman, P. (2014). Rhamnolipid biosurfactants-past, present, and future scenario of global market. *Frontiers in Microbiology*, 5, 454.

Rani, M., Weadge, J.T. & Jabaji, S. (2020). Isolation and Characterization of Biosurfactant Producing Bacteria From Oil Well Batteries With Antimicrobial Activities Against Food-Borne and Plant Pathogens. *Frontiers in microbiology*, 11, 64.

Reddy, R.M. (2008). Impact of soil composting using municipal solid waste on biodegradation of plastics. *Indian Journal Biotechnology,* 7, 235–239.

Restrepo-Florez, J.M., Bassi, A. & Thompson, M.R. (2014). Microbial degradation and deterioration of polyethylene a review. *International Biodeterioration & Biodegradation*, 80, 83–90.

Roy, A. (2017). Review on the biosurfactants: properties, types and its applications. Journal of *Fundamentals* of *Renewable Energy* and Applications, 8, 248.

Roy, P.K. (2009). Investigating the role of metal oxidation state on the degradation behaviour of LDPE. *Polymer Dagradation and Stability*, 94, 1033-1039.

Roy, P.K., Titus, S., Surekha, P., Tulsi, E., Deshmukh, C. & Rajagopal, C. (2008). Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. *Polymer Degradation and Stability*. 93, 1917-1922.

Russel, J.R., Huang, J., Anand, P., Kucera, K. & Sandoval, A., G. (2011). Biodegradation of polyester polyurethane by endophytic fungi. *Applied environmental and microbiology*, 77, 6076-6084.

Sachdev, D. P., and Cameotra, S. S. (2013). Biosurfactants in agriculture. *Applied Microbiology and Biotechnology*, 97, 1005–1016.

Saleem, M., Meckes, N., Pervaiz, Z. H. and Traw, M. B. (2017). Microbial interactions in the phyllosphere increase plant performance under herbivore biotic stress. *Frontiers in Microbiology*, 8, 41.

Salmah, H., Romisuhani, A. & Akmal, H. (2011). Properties of low-density polyethylene/palm kernel shell composites: effect of polyethylene co-acrylic acid. *Journal of thermoplastic composite materials*, 26, 3–15.

Samyn, P. & Schoukens, G. (2014). Morphological and thermal variability of patterned polymer films with poly (styrene-co-maleic anhydride). *Polymers*, 46, 820–845.

Santo M., Weitsman R., Sivan A. (2013) The role of the copperbinding enzyme-laccase-in the biodegradation of polyethylene by the actinomycete *Rhodococcus ruber International Biodeterioration* and *Biodegradation*, 84, 204–210.

Santos, D., Rufino, R., Luna, J., Santos, V. and Sarubbo, L. A. (2016). Biosurfactants: multifunctional biomolecules of the 21st century. *International Journal of Molecular Science*, 17:401.

Sarkhel, G., Banerjee, A. & Bhattacharya, P. (2006). Rheological and mechanical properties of LDPE/HDPE blends. *Polymer - Plastics Technology and Engineering*, 45, 713–718.

Sen, S.K. & Raut, S. (2015). Microbial degradation of low density polyethylene (LDPE) a review. *Journal of Environment and Chemical Engineering*, 3, 462–473.

Shah, A. (2008). Biological degradation of plastics a comprehensive review. *Biotechnology Advances*, 26, 246- 265.

Shah, A.A., Hasan, F., Hameed, A. & Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. *Biotechnology advances*, 26, 246-265

Shamsuri, A.A. & Daik R. (2015). Applications of ionic liquids and their mixtures for preparation of advanced polymer blends and composites: a short review. *Reviews on advanced materials science,* 40, 45–59.

Shamsuri, A.A., Azid, M.K.A. & Ariff, A.H.M. (2014). Influence of surface treatment on tensile properties of low-density polyethylene/cellulose woven biocomposites a preliminary study. *Polymers*, 6, 2345–2356.

Sharma, D., Ansari, M.J., Al-Ghamdi, A., Adgaba, N., Khan, K.A., Pruthi, V. & Al-Waili, N. (2015). Biosurfactant production by Pseudomonas aeruginosa DSVP20 isolated from petroleum hydrocarbon-contaminated soil and its physico- chemical characterization. *Environmental Science and Pollution Research*, 22, 17636-17643.

Shibulal, G.S. & Naskar, K. (2011). RFL coated aramid short fiber reinforced thermoplastic elastomer mechanical, rheological and morphological characteristics. *Journal of polymer research*, 18, 2295–2306.

Shimao, A. (2001). Biodegradation of plastics. *Current opinion in biotechnology*, 12, 242-247.

Singh, V., Dubey, M. & Bhadauria, S. (2012). Microbial degradation of polyethylene (low density) by *Aspergillius Fumigatus* and *Penicillium* sp. *Asian journal of experimental biological sciences*, 3, 498–501.

Siqueira, G., Bras, J. & Dufresne, A. (2010). Cellulosic binanocomposites a review of preparation properties and applications. *Polymers*, 2, 728–765.

Sivan, A. (2011). New perspective in plastic biodegradation. *Current opinion in biotechnology*, 22, 422-426.

Sivan, A., Szanto, M. & Pavlov, V. (2006). Biofilm development of the polyethylene-degrading bacterium *Rhodococcus ruber*. *Applied Microbiology and Biotechnology*, 72, 346–352.

Sivasankari, S. & Vinotha, T. (2014). In vitro degradation of plastics (plastic cup) using *Micrococcus luteus* and *Masoniella* spp. *Scholars Academic Journal of Biosciences*, 2, 85-89.

Skariyachan, S., Manjunatha, V., Sultana, S., Jois, C., Bai, V. & Vasist, K.S. (2016). Novel bacterial consortia isolated from plastic garbage processing areas demonstrated enhanced degradation for low density polyethylene. *Environmental Science and Pollution Research*, 23, 18307–18319.

Sudhakar, M., Doble, M., Murthy, P.S. & Venkatesan, R. (2008). Marinemicrobe-mediated biodegradation of low-and high-density polyethylenes. *International Biodeterioration and Biodegradation,* 61, 203–213.

Suzuki T., Ichichara, Y., Yamoda, M. & Tonomurak. (1973). Some characteristics of *Pseudomonas* 0-3 which utilizes polyvinyl alcohol. *Agricultural* and *Biological Chemistry*, 37, 747-756.

Tabatabai, M.A. (1982). Sulfur, In: Page, A.L., Miller, R.H., Keeney, D.R. (eds.), Methods of soil analysis, Part 2- Chemical and microbiological properties, 2nd. Edn., *Agronomy*. 9, 501-538

Tajvidi, M. & Takemura, A. (2010). Thermal degradation of natural fiber-reinforced polypropylene composites. *Journal of thermoplastic composite materials,* 23, 281–298.

Tamada, J. & Lauger, R. (1993). Erosion kinetics of hydrolytically degradable polymers. *Proceedings of the National Academy of Sciences*, 90, (2) 552-556.

Thomas, O.R. & White, G.F. (1989). Metabolic pathway for the biodegradation of sodium dodecyl sulfate by *Pseudomonas* sp. C12B. *Biotechnology and Applied Biochemistry*, 11, 318-327.

Tokiwa, Y., Calabia, P.B., Ugwu, U.C. & Aiba, S. (2009). Biodegradability of plastics. *International Journal of Molecular Sciences*,10, 3722 -3742.

Tribedi P. & Sil, A.K. (2013). Low-density polyethylene degradation by *Pseudomonas* sp. AKS2 biofilm. *Environmental Science* and *Pollution Research*, 20, 4146–4153.

Tribedi, P. & Dey, S. (2017). Pre-oxidation of low-density polyethylene (LDPE) by ultraviolet light (UV) promotes enhanced degradation of LDPE in soil. E*nvironmental Monitoring and Assessment,*189, 624.

Tribedi, P. & Sil, A.K. (2014). Cell surface hydrophobicity: a key component in the degradation of poly ethylene succinate by *Pseudomonas* sp. AKS2. *Journal of applied microbiology,*116, 295–303.

Tribedi, P., Gupta, A.D., Sil, A.K. (2015). Adaptation of Pseudomonas sp. AKS2 in bioflm on low-density polyethylene surface: an effective strategy for efficient survival and polymer degradation. *Bioresour Bioprocess,* 2, 14.

Tribedi, P., Sarkar, S., Mukherjee, K. & Sil, A.K. (2012). Isolation of a novel *Pseudomonas* sp. from soil that can efficiently degrade polyethylene succinate. *Environmental science and pollution research international,* 19, 2115–2124.

Volke, S.T., Saucedo, C.G., Gutierrez, R.M., Manzur, A. & Favela, T.E. (2002). Thermally treated low density polyethylene biodegradation by penicillium *Pinophilum* and *Aspergillus niger*. *Journal of Applied Polymer Science,* 83, 305–314.

Wang, Y. & Yang, J.F. (2010). Physical properties and biodegradation of acrylic acid grafted poly (caprolactone) /chitosan blends. *Journal of polymer research,* 17, 221–232.

Webb, K.H., Arnott, J., Gawford, J.R. & Ivanova, P.E. (2012). Plastic degradation and environmental implications with special reference to Poly (ethylene terephthalate). *Polymers,* 5, 1-18.

Weiland, M., Daro, A. & David, C. (1995). Biodegradation of thermally oxidized polyethylene. *Polymer Degradation and Stability*, 48, 275-289.

Whang, L.-M., Liu, P.-W.G., Ma, C. C. & Cheng, S. S. (2008). Application of bio-surfactants, rhamnolipid and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. *Journal of hazardous materials*, 151, 155-163.

Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q. and Jiang, P. (2018). Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. *Frontiers Microbiology,* 9, 2885.

Yamada, O.K., Mukumoto, H., Katsuyaya, Y., Saiganji, A. & Tani, Y. (2001). Degradation of polyethylene by a fungus, *Penicillium simplicissmum* YK. *Polymer Degradation and Stability*, 72, 323–327.

Yang, H.S., Yoon, J.S. & Kim, M.N. (2004). Effects of storage of a mature compost on its potential for biodegradation of plastics. *Polymer Degradation and Stability*, 84, 411-417.

Zahra, S., Abbas, S.S., Mahsa, M.T. & Mohsen, N. (2010). Biodegradation of low-density polyethylene (LDPE) by isolated fungi in solid waste medium. *Waste Management Research*, 30, 396–401.

Zhang, S., Li, F.X. & Yu, J. (2009). Preparation of cellulose/chitin blend bio-fibers via direct dissolution. *journal Cellulose Chemistry and Technology,* 43, 393–398.

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List of Publication

Research papers

- **Singh S.**, Shankar S., Shikha (2016). Biodegradation of plastic: A Review. International Journal of Scientific and Innovative research 4(1): 2347-4971.
- Shankar S., **Singh S.**, Ratnakar A. and Shikha (2018) Removal of synthetic dyes from textile wastewater using bioadsorbents: A Review. Indian journal of environmental protection. 48(2): 116-133.
- **Singh S**., Shankar S., Shikha (2020) Effects of photo-oxidative degradation on low-density polyethylene (LDPE). Asia pacific journal of multidisciplinary research. Vol. 8 (1), 56-62.
- **Singh S**, Chowdhary V.S., Singh D.P., Shankar S, Shikha, (2019) Evaluation of low-density polyethylene degradation by bacteria isolated from hydrocarbon-contaminated site. Indian journal of environment protection. Vol. 41 (1), 3-10.

- **Singh S**, Shankar S., Shikha (2020) Evaluation of LDPE degradation under controlled composting. Nature environment and pollution technology. Vol. 20 (3).

Book Chapters

- Shankar S., **Singh S.**, Rawat S., Ratnakar A. and Shikha (2017). Environmental contamination, toxicity profile and bioremediation approaches for detoxification of pulp and paper mill wastewater. (Bhargava R.N and Saxena G Eds) Springer Verlag. pp 181-206.

- **Singh S.** and Shikha (2019) Treatment and recycling of wastewater from oil refinery/petroleum industry. (Singh R.L., SinghR.P. (eds)), Springer nature, Singapore. pp. 303-332.

- Shankar S., **Singh S.**, Mishra A., Sharma M. and Shikha (2019) Microbial Degradation of Polyethylene: Recent Progress and Challenges. (Arora P. K. (ed.)), Springer nature, Singapore. pp. 245-262.

- Shankar S., **Singh S.**, Shikha, Mishra A. and Ram S. (2019) Strategic role of fungal laccases in biodegradation of lignin. (Narain R. (eds.)), Springer nature, Switzerland. pp. 119-135.

- Shikha, **Singh S**., Shankar S., (2020) Microbial metagenomics: Potential and challenges. Springer nature. pp. 109-120.

Conference and seminars attended

- **Shailja Singh** and **Shikha** (2015) National conference on climate change and sustainable development: Emerging issues and mitigation strategies (CCSD-2015). (Participation)

- **Shailja Singh** and **Shikha** (2017) Association of microbiologists of India (AMI-2017) International symposium on microbes for sustainable development: Scope and application (MSDSA-2017) (Poster presentation)

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