CHANGES IN THE PROPERTIES OF LOW DENSITY POLYETHYLENE (LDPE) DURING BIODEGRADATION IN SOIL

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF BOTANY, UNIVERSITY OF DHAKA, BANGLADESH FOR THE AWARD OF THE DEGREE OF MASTER OF PHILOSOPHY

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CERTIFICATE

This is to certify that the thesis entitled "Changes in the Properties of Low Density Polyethylene (LDPE) During Biodegradation in Soil" has been prepared and submitted to the Department of Botany, University of Dhaka by Tabassum Mumtaz under my supervision.

It is further to certify that the research work embodied in the thesis, is original and suitable for awarding an M. Phil. degree in Botany.

02/05/05

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Dedicated to
those devoted workers in the field of Microbiology,
whose endless perseverance has contributed to the
understanding of the nature and power of 'microbes'.

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This rather ambitious project was undertaken with a scientific zeal but there were so many limitations. We had to move from lab to lab, from person to person and from library to library. We received help, support and assistance from so many and in so many ways that it is really difficult to acknowledge and thank in a single paragraph. We decided to record our gratitude and debt in a few separate statements.

The present work was carried out in the Laboratory of Microbiology, Department of Botany, University of Dhaka with the financial support from BCSIR, Dhaka and the author is grateful to the authorities.

The changes in the material properties of the PE films before and after treatment were analyzed with FTIR simultaneously at BCSIR Laboratories, Dhaka (where Mr. Moajjem Hossain, P.S.O. Analytical Division, did the work) and at a Private Firm. The author expresses her gratitude to the authorities and personnels who helped directly and indirectly.

At a stage, the total organic carbon (TOC) was estimated with a modern Shimadzu 5000A analyzer and the kind cooperation of the respective personnels are highly appreciated.

Mechanical and Dielectric properties of the control and treated samples were tested at the Polymer Physics Laboratory, Department of Physics, Dhaka University. Professor K. M. Mannan, the founder and in-charge of the laboratory, not only operated the sophisticated equipments, but he also worked throughout the summer vacation (2003) and interpreted the data. The author is deeply indebted and grateful to Professor Mannan for hosting her in his laboratory and for his help and all out cooperation.

The possible changes in the crystalline and amorphous structure of the selected PE samples before and after treatment were examined by JEOL Diffractometer Model JDX 8P which is in use at the Bangladesh University of Engineering and Technology (BUET), Dhaka. Professor Haseeb and Mr. Yosouf Khan helped in the operation. Their kind assistance and help is gratefully acknowledged.

In view of the fact that, not a single Electron Microscope is in working condition in Bangladesh, we tried desperately with the several laboratories in India. Finally, Dr. Radhaballav Bhar of the Department of Instrumentation Science, Jadavpur University agreed to help us. The author expresses her heartfelt gratitude to Dr. Radhaballav Bhar, Mr. Pallab Dasgupta and all the staff members of the Department of Instrumentation Science, Jadavpur University who helped us so much.

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The author

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List of abbreviations

ASTM	American Society for Testing and Materials
AT	Advanced technology
ATCC	American Type Culture Collection
ATR	Attenuated Total reflection
BF	Bright field
CAB	Commonwealth Agricultural Bureau
CPU	Central processing unit
d	day
DAP	Data Analysis Package
DEF	Direct Epifluorescence
DF	Dark field
DSC	Differential scanning calorimetry
e.g.	Exempli gratia (for example)
Ed	Editor
ed	Edition
et al.	et alibi (and others)
etc.	Etcetra
Fig.	Figure
FT-IR	Fourier transform infrared
g	Gram
HDPE	High-density polyethylene
i.e.	that is
IBM	International business machine
ISO	International Organization for Standardization
kHz	Kilo Hertz
kV	Kilovolt
LCD	Liquid crystal display
LCR	Inductance-Capacity-Resistance
LDPE	Low-density polyethylene
LED	Light emitting diode
LLDPE	Linear low density polyethylene
mg	Milligram
mm	millimeter
NA	Nutrient Agar
No.	Number
°C	degree celsius

OCST	Octanoated starch
pp.	pages
PBS	Poly butylene succinate
PC	Personal computer
PDA	Potato Dextrose Agar
PE	Polyethylene
PES	Poly (ethylene succinate)
PET	Poly (ethylene terephthalate)
pF	pico farraday
pH	Negative logarithm of hydrogen ion concentration
PHB	Poly hydroxybutyrate
PHBV	Poly (3-hydroxybutyrate-co-3-valerate)
PID	Personal information device
PLA	Poly (lactic acid)
PLLA	Poly (L-lactic acid)
PP	Polypropylene
ppm	Parts per million
PTFE	Poly (tetrafluoro ethylene)
PTMS-H	Poly (tetramethyl succinate) - high melting point
SEM	Scanning Electron Microscope
sp.	species (Singular)
spp.	Species (plural)
TEM	Transmission Electron Microscope
TOC	Total Organic Carbon
UV	Ultra violet
U LDPE	Ultra low density polyethylene
viz.	Videly (namely)
VLDPE	Very low density polyethylene
XRD	X-ray diffraction
XT	Extended technology
%	percentage
&	and
Ω	ohm
µm	micron
α	Alpha
β	beta
δ	delta
ε	epsilon
γ	gamma

ABSTRACT

In an effort to examine the fate of polyethylene films under natural soil burial conditions, an outdoor soil burial test was performed during December 2001 to October 2003 in an experimental plot of the Dhaka University Botanical Garden. Four commercial polyethylene samples (Black PE film-PE_B; white PE film PE_W; thick transparent-$PE_{Tr\text{-}HW}$ and thin, transparent film- $PE_{Tr\text{-}LW}$) differing in thickness and colour were buried vertically under well made soil. Thus the polyethylene samples were exposed to natural weathering processes including activity of indigenous soil microflora. Prior to experiment, the pH, texture and moisture holding capacity of experimental soil were determined. No extra nutrient was added for soil enrichment. Samples periodically removed from the plot were maintained for weight loss study. Changes in optical characters and fragmentation were assessed by visual estimation and more thoroughly by using bright-field, dark-field, phase-contrast, fluorescent and scanning electron microscopes. The photographs were arranged sequentially and the gradual degradation was found to be evident. The experiment continued for 22 months and the resulting degradation was assessed by FT-IR spectra, tensile properties, dielectric properties and X-ray diffraction patterns.

Out of the four tested samples, the thick transparent one ($PE_{Tr\text{-}HW}$) was found to degrade and disintegrate almost totally within the experimental period. The sample became completely opaque after 11 months and started fragmenting within 17 months. 10% reduction of the gross weight of the sample was noticed after 01 year of exposure. On the other hand, the black polyethylene sample was found to be more resistant than the others. Irrespective of thickness and colour, load-extension curves of all samples reflect complete and partial destruction of plastic and elastic region after 15-17 months. IR spectra after 15 months revealed the absorption of the region from 1400-1800 cm^{-1}, characteristic of carbonyl peak. Samples exhibit remarkable rise in power factor and loss curve shows fairly intense transition peaks after degradation. XRD graphs were inconclusive in terms of the crystallinity measurement but somewhat reflected that the amorphous region is affected. The overall picture reveals degradation of LDPE films during soil burial under the prevailing conditions including the effect of soil microflora.

Promising results were obtained in laboratory experiments with the isolated microorganisms. Enhancement of growth in presence of 0.1% wt/vol. of suspended polyethylene in carbon free medium and more than 50% surface growth of fungal isolate in plate test indicated possible utilization of polyethylene by these isolates.

Chapter - 1

INTRODUCTION

Introduction

"Everything will be destroyed except Allah"
- Al Quran (Para: 20, Sura: Kasas', Ayat: 88)

The greatest philosophers of all ages thought and talked about the beginning and ending of this universe but so far, very little is understood. Here, on this planet, every organism including ourselves and every material come and exist for a certain period of time. On the basis of our liking and necessities, sometimes we long to extend this period of stay as the Pharao's wanted to live through their second life but at other times we appeal to make it short (as in the cases of our own corpses and waste materials).

Macromolecules already exist in nature e.g. starch, cellulose, lignin, chitin, rubber etc. form the very foundation of life and intelligence and provide much of the food on which man exists. On the other hand, man-made macromolecules i.e. synthetic polymers, being essential to clothing, shelter, transportation and communication as well as to the conveniences of modern living, become truely indispensable to mankind (Billmeyer 1984).

Polyethylene, with all its inherent qualities came as a blessing to the mankind. However, everything has its merits and demerits. Durability is one of its characters which makes polyethylene adorable but at the same time it becomes a real problem if disposed off improperly or not recycled in a safe, eco-friendly manner.

This very realization prompted us to design and execute experiments which could possibly shed some light on the understanding of the existing problem with polyethylene. Before that, a discussion on the history of polyethylene may be considered as relevant.

History of polyethylene

It was not until 1930's that the science of high polymers began to emerge (Swallow 1957). Between 1931 and 1935 the study of the effects of very high pressures on chemical reactions was being actively pursued, and from this work the discovery of polyethylene was made. The history of polyethylene thus provides an unusually clear-cut instance of the unexpected results that may come from research, and of the importance of the role of chance in such work. In the years 1935 to 1939, a manufacturing process was devised and

particular uses for which the product was uniquely suitable were developed. Between 1939 and 1945 attention was principally given to the production of the material in the UK and USA specifically for the high frequency applications in radar cables and ancillary equipment for wartime uses (Swallow 1957).

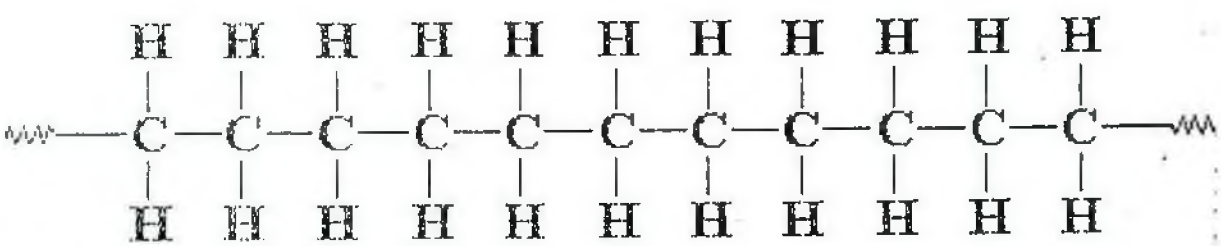
Until the mid-1950's all commercial polyethylene was produced by high pressure processes. In 1954, two other routes were developed, one using metal oxide catalysts (e.g. the Phillips process) and the other aluminium alkyl or similar materials (the Ziegler process). By these processes polymers could be prepared at lower temperature and pressures. These materials are known as high-density polyethylenes (HDPE), while the earlier materials are known as low-density polyethylenes (LDPE). At the end of the 1970's considerable interest developed in what became known as linear low density polyethylene (LLDPE). The LLDPE materials were rapidly accepted by industry particularly in the manufacture of film.

By the mid-1990's capacity for polyethylene production was about 50000000 tonnes per annum (Brydson 1999), much greater than for any other type of plastic material. No other modern plastic material has developed in such spectacular manner. By type, this market is shared between LDPE, HDPE and LLDPE approximately in the ratio 40:36:24. In the early 1990's it was estimated that the geographical breakdown for production capacity was Western Europe 26%, North America 33%, Japan 8%, Eastern Europe 8% and rest of the World 25% (Brydson 1999).

Polyethylene – Structure and classification:

Polyethylene is the simplest hydrocarbon polymer. A molecule of polyethylene is nothing more than a long chain of carbon atoms, with two hydrogen atoms attached to each carbon atom (**Fig. 1.1**).

However, it may be easier to understand the structure of polyethylene with only the chain of many thousands of carbon atoms:



There are many varieties of polyethylene:

LDPE- LDPE stands for low density polyethylene. LDPE has a random long branching structure, with branches on branches. The short branches are not uniform in length but are mainly four/two carbon atoms long. The ethyl branches probably occur in pairs and there may be some clustering of other branches (Whiteley 1992). LDPE is produced by the high pressure (approximately 1500 atmospheres) polymerization of ethylene, using oxygen as initiator (Billmeyer, 1984).

LDPE melts at 110-125^{0}C and is only 40% crystalline. The density is around 0.91-0.92g/cc (Billmeyer 1984). The molecular mass distribution (MMD) is moderately broad. This set of branched polymers is less crystalline than HDPE. Members are flexible and clear, with moderate toughness.

LDPE films are mainly used for packing and wrapping frozen food, textile products, and so on. LDPE's inertness to chemicals and resistance to breakage is made use of in 'squeeze bottles' and in many attractive containers. Pipes made of LDPE are used for both agricultural and domestic water line connections. The non-polar nature of the polymer makes it ideal for providing insulations to electric cables (Billmeyer 1984).

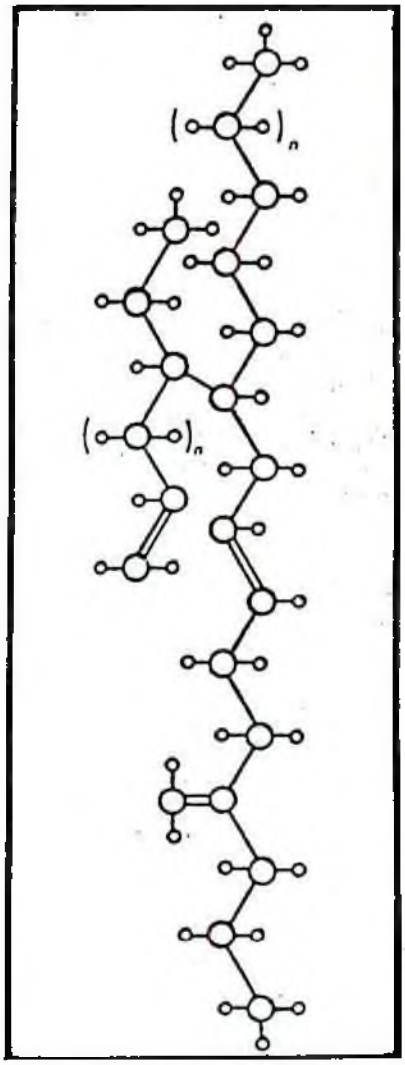


Fig 1.1 Polyethylene molecule showing some characteristic structures. Large circles represent carbon atoms; small circles, hydrogen atoms; double lines represent double bond (after Kräuter 1957).

HDPE- HDPE stands for high density polyethylene. HDPE is essentially free of both long and short branching, although very small amounts may be deliberately incorporated to achieve specific product targets. The MMD depends on the catalyst type but is typically of medium width (Whiteley 1992).

High density polyethylene is 90% crystalline and possesses densities as high as 0.965 g/cc. It has a melting point in the range of 144-150^{0}C. It is much stiffer than LDPE and has a higher tensile strength and hardness. Chemically, it is more resistant than the low density variety and has a significantly lower gas permeability (Billmeyer 1984).

This set of highly crystalline polymers is used in moulding and film applications, with milk jugs being a prime example. These polymers are less flexible than other polyethylenes.

LLDPE- These are copolymers of ethylene with modest amounts of butane, hexane or octane linear α- olefins. It has branching of uniform length which is randomly distributed along a given chain, but there is a spread of average concentrations of between chains, the highest concentrations of branches being generally in the shorter chains (Whiteley 1992). Grades are available for films and for moulding. Films typically show good toughness. Some grades are suitable for fibres.

VLDPE/ULDPE- This refers to very low density polyethylene or ultra low density polyethylene. These are also copolymers of ethylene and linear α-olefins ; however, they have densities less than 0.915 g/cm^3. They have improved toughness and sealability compared to LLDPE (DeLassus and Whiteman 1999).

Polyethylene - A unique packaging material

The present position of polyethylene as a general purpose thermoplastic material is due in no small measure to the low cost and easy processability of the polymer. Other characteristics of polyethylene, which leads to its widespread use are as follows (Brydson, 1999):

- Excellent electrical insulation properties
- Excellent chemical resistance
- Toughness and flexibility even at low temperatures
- Reasonable clarity of thin films
- Freedom from odour and toxicity
- A sufficiently low water vapour permeability for many packaging, building and agricultural applications.

The limitations of the polymer are:

- The low softening point
- The susceptibility of low molecular weight grades to environmental stress cracking
- The susceptibility to oxidation (however, polyethylene is better in this respect than many other polymers)
- The opacity of the material in bulk

- The wax-like appearance
- The poor scratch resistance
- The lack of rigidity (a limitation in some applications but a virtue in others)
- The low tensile strength
- The high gas permeability.

LDPE retains its position as a preferred packaging material because of its limp feel, transparency, toughness and the ability to rapidly take up the shape of the contents of the bag. Most LDPE film is produced by the film blowing process, but flat film extruded onto chilled rolls is also made, particularly in USA (Whiteley 1992).

Originally, bags were made directly from tubular film by welding one end, but the tendency now is to make wide film on large machines and fabricate the bags by welding and cutting. Apart from packaging film and heavy duty sacks, increasing quantities of polyethylene are used for impermeable or stabilizing membranes in civil engineering construction. LDPE is also used extensively for coating cardboard, paper and aluminium for milk cartons etc (Whiteley 1992).

Polyethylene in disposable packaging - consequences

The greatest challenge to polyethylene is not from other materials but arises as a consequence of its own success. There are many products, which have a relatively short use-life (weeks, months) following which the articles are no longer needed and discarded. At this point, the durability and persistence of man made, polyethylenes become disadvantageous (Billingham *et al.* 2002). The vast quantities of polyethylene used each year for packaging have resulted in an undesirable level of litter. However, the use of disposable packaging is being increasingly called into question. In the 1970s and 1980s, the concern was with the unsightness of a material, which was littering the environment and did not degrade (Whiteley 1992). As a result of public resistance and, in some countries, legislation, growth in the use of polyethylene for packaging have been constrained.

Whiteley (1992) summarized some of the options available to deal with the problem of disposable packaging. However, the relative merits of the different methods of managing plastic waste materials are eventually related to cost and legislation (Amass *et al.* 1998).

Table 1.1 Polyethylene packaging disposal strategy.

Disposal Method	Advantages	Disadvantages
Incineration	Avoids landfill	Generates CO_2 without benefits
Incineration with energy recovery	Displaces existing fossil fuel use	Loss of fabrication costs
Biodegradation	Removes unsightly refuse	Doubts about completeness of degradation: generates CO_2without benefits
Promoted degradation	Removes unsightly refuse; lower labour costs for agricultural uses	Degradation slow away from sunlight; generates CO_2 without benefits
Recycling	Makes use of scarce resource	Some deterioration in quality
Reuse	Saves on fabrication costs	PE may not be the best material

The need for a biodegradable plastic, particularly in packaging industry, has initiated a new surge of investigation. As the existing plastics seem to be either non-biodegradable or degraded very slowly, indeed, two alternatives remain: new biodegradable or self destructing plastics must be developed, or organisms must be found which can attack existing polymers (Kavelman and Kendrick 1978).

Polyethylene in Bangladesh

The use of polyethylene does not have a long history in Bangladesh. Mass use of polythene bags started in 1982 (Star Weekened Magazine 23/06/2000). Before that, people used to shop with jute bags in our country. These bags were used again and again for shopping. No one took them as something primitive. Sadly, this scenario has changed dramatically. Now, polythene bags are certainly the most popular carrying container in our country. The reasons are quite simple. They are cheap, easy to handle, easier to collect and convenient. In a poor country like Bangladesh, it is only natural that low price of polythene bag is more than enough reason for its being the favoured one. Every time one goes to the market, comes back with a number of polybags. These bags are so cheap that it is possible to get one bag for each of the items we buy in the market.

Manufacturing of polythene bags is highly profitable because of low production cost. According to the environment ministry, there are about 800 polythene factories all over the country. The preliminary investment in a polythene bag factory is only about Tk 2 lakh. The production cost of each polybag is about Tk 0.05 to Tk 0.07 while manufacturers sale these to the whole sellers for Tk 0.30 to Tk 0.35(The Daily Star, 24/12/2001).

Inspite of all its utility, it cannot be denied that polythene has caused serious problem in our environment. We are accustomed to throwing everything anywhere and everywhere as soon as their need is over and polythene is no exception. Dhaka is the worst victim of the polythene hazard in Bangladesh. As the number of people in the city grow, so do their need for polybags and the habit of indiscriminate disposal (Star Weekened Magazine 10/03/2000).

There is no proper method of disposing polythene in Dhaka and other parts of the country. Everyday about one crore polythene bags are used and 90 lakh of them are dumped haphazardly in the capital alone. About 20% of the bags are recycled while the rest 80% are being dumped into rivers, canals, drains, sewarage system, water bodies and in open spaces. Moreover, recycling of polythene is not taken in the technical sense. The waste pickers mainly 'Tokais' collect waste polybags and sell it to the Recycle Industries at Islambagh in old Dhaka. Such recycling process emits poisonous smoke and causes serious public health hazards(The Daily Star 24/12/2001). There, it is burnt and new bags are produced from the raw materials made from them. Usually these bags are washed with water and sold to the shops. In many instances, it is cheaper to make plastic bags from raw materials than from used bags since the latter consumes more energy.

Problems faced by the inhabitants

The rampant use of polythene bag has led to the clogging of the Buriganga river, lakes in the Dhaka city, and other water bodies. The dredging of Buriganga has been seriously hampered due to polythene bags accumulated at the riverbed. In addition, these also block urban drainage systems. This was vividly illustrated after the flood of 1998, when polythene bags, in particular, were widely blamed for making the drainage system of Dhaka City inoperational. When the flood water receded after 80 days, the inhabitants were left with millions of stinking polybags.

Considering the negative impact of polyethylene on our environment, on December 23, 2001, the parliament cabinet of the Government of People's Republic of Bangladesh approved the decision to ban the use and marketing of polythene bags in Dhaka city from January 01, 2002. Government also planned to phase out the use, marketing and production of polythene bags (20 micron thick) across the country. The ban has been implemented under the Environment Preservation Law, 1995 (The Daily Star,

24/12/2001). On the other hand, PE films for packaging and wrapping frozen foods and varieties of materials are still going on. While developed countries are engaged in developing biodegradable plastics, we are far behind from the proper disposal system. Moreover, conventional polyethylene bags used for the past 20 years have to be treated in a scientific manner.

Polyethylene disposal problem could be resolved in one of the two ways:- one approach is to **develop biodegradable plastics**, another one is to **develop systems to enhance biodegradation** (Cameron *et al.* 1988).

A number of standard authorities have sought to produce definitions for **'biodegradable plastics'** (Calmon-Decriaud *et al.* 1998); a few of which are presented below:

ISO 472-1988 : A plastic designed to undergo a significant change in its chemical structure under specific environmental conditions resulting in a loss of some properties that may vary as measured by standard test methods appropriate to the plastic and the application in a period of time that determines its classification. The change in the chemical structure results from the action of naturally occurring microorganisms.

ASTM sub-committee D20-96: A degradable plastic in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi and algae.

DIN 103.2-1993 German working group: A plastic material is called biodegradable if all its organic compounds undergo a complete biodegradation process. Environmental conditions and rates of biodegradation are to be determined by standardization test methods.

E.C.N (May 1993): A degradable material in which the degradation results from the action of microorganisms and ultimately the material is converted to water, carbon dioxide and/or methane and a new cell biomass.

Japanese Biodegradable Plastic Society(1994): Polymeric materials which are changed into lower molecular weight compounds where at least one step in the degradation process is through metabolism in the presence of naturally occurring organisms.

Approaches to degradable plastics:

Recent attempts to develop new biodegradable polymeric materials have centred into three areas (Calmon-Decriaud *et al* 1998):

- **Modified Natural polymers-** several new polymeric materials have been prepared by biological and/or chemical modification, such as cellulose acetate, lignocellulose esters, polylactic acid polyalkanoates, of which the most promising is the copolymer polyhydroxybutyrate/valerate (PHB/HV).
- **Biodegradable synthetic polymers-** although common low-cost thermoplastics such as polyethylene, polypropylene, polystyrene and PVC resist biodegradation, aliphatic polyesters, such as polycaprolactone, polyethylene adipate, and polyglycolic acid are readily attacked. Polyurethanes, block copolymers and graft copolymers containing aliphatic ester groups are similarly susceptible to biodegradation.
- **Composite materials-** which combine biodegradable particles (e.g., starch, regenerated cellulose or natural gums) and a synthetic biodegradable polymer (e.g.,starch –vinylalcohol-copolymer)

In an attempt to increase biodegradability, biodegradable fillers have been incorporated to polyethylene. One such filler is corn starch, a natural polymer composed of glucose monomers. To incorporate starch into the polyethylene in the manufacturing process, corn starch granules were chemically treated to render surfaces hydrophobic, dried to < 1% water content and added to molten polyethylene at a processing temperature <230°C. The average diameter of corn starch granules is 15 μm whereas the thickness of the starch-incorporated plastic films tested ranged from 28 μm to 99 μm. Therefore, some of the incorporated starch was embedded in the film surface and exposed directly to an external environment even though the starch was other-wise evenly distributed throughout the matrix, while the remaining starch was essentially encapsulated in the polyethylene matrix (Maddever and Chapman 1987). Other fillers include straight chain alkenes such as dotriacontane, $C_{32}H_{66}$, (Albertsson and Ranby 1979).

Researches on degradable PE- Regarding degradation of degradable PE/Plastics, some research articles have already been published. Spore and Bethea (1972) studied the

thermooxidative degradation of a linear polyethylene over the range of 75-200^{0}C. Andrady (1990) studied weathering of LDPE and enhanced photo-degradable PE in marine environment. Byngtae *et al.* (1991) demonstrated the ability of lignocellulose degrading *Streptomyces* spp. to attack polyethylene in heat treated degradable plastics in pure shake flask culture studies. Lee *et al.* (1991) demonstrated biodegradation of degradable starch-PE by lignin degrading microbes *viz. Streptomyces sp* and *Phanerochaete* sp. Goheen and Wool (1991) focused on the aerobic microbial degradation of corn starch and LDPE binary blends in soil. Barak *et al.* (1991) examined biodegradation of PHBV & Starch-PE in soils. David *et al.* (1992) studied photodegradation of polyethylene (LDPE and LLDPE). Main chain scission was found to be the dominant process in natural weathering conditions. Pometto *et al.* (1992) reported production of extracellular polyethylene degrading enzyme by *Streptomyces* sp. Johnson *et al.* (1993) confirmed degradation of degradable starch-plastic bags in a compost environment. Weiland *et al.* (1995) have investigated the biodegradability of thermally oxidized PE using a wide range of biotic conditions. There was some evidence of oxygen uptake in the respirometric method using liquid suspensions of compost microorganisms and very small concentration of substrate. Foust *et al.* (1997) developed a cellulose – PE blend polymer film for degradative susceptibility to brown-rot or cellulosic fungi. Naturally occuring fungi were harvested from decaying wood and cultured on nutrient agar. In their test, *Trichoderma viridae* showed 100% surface area growth or LDPE blended with 8% (v/v) cellulose in 90 days. El-Shafei *et al.* (1998) demonstrated biodegradation of disposable Starch-PE bags by fungi and *Streptomyces* sp. Bikiaris *et al.* (1998) reported biodegradation of octanoated starch (OCST) and its blend with LDPE.

Limitations of degradable plastics

Increased cost-The main constraint on the use of biodegradable plastics/polymers is the difference in the price of these polymers compared with that of bulk-produced, oil-based plastics. The cost of BIOPOL is approximately 8000 pound per tonne, compared with the UK price of commodity polymers of between 500 pound per tonne for PVC and PP and 600 pound per tonne for HDPE and high-impact PS (Amass *et al.* 1998).

Moreover, Taylor 1979 pointed out a number of obstacles to the commercial use of environmentally degradable plastics:

- **No positive contribution to the solid-waste disposal-** In the case of landfills, degradability can cause complications-settling, generation of gases, and possible contamination of ground water. In case of incinerator performance substitution of degradable for nondegradable plastics would probably have no significant impact- either positive or negative - in most cases.

- **Increased risk of contamination, damage or spoilage of packaged goods-** Existing packaging materials are useful precisely because of their strength, toughness, low permeability, and chemical inertness. Sacrificing any of these characteristics in the name of "enhanced degradability" will tend to reduce the probability that the packaged item will reach the ultimate consumer in the desired form.

- **Possible release to the environment of additives or degradation products having unknown environmental impact-** Degradation processes can permit the release of degradation-promoting additives, stabilizing additives, plasticizers, etc., which would be 'protected' from such release under normal circumstances. Although many of these degradation products will be harmless, one cannot be sure that all of them are environmentally acceptable. Surely it would be foolish to pollute water, air, or land in an attempt to reduce litter.

- **Degradability may complicate recycling-** In comparison to scrap from manufacture of nondegradable plastics, scrap generated during the manufacture of degradable products will tend to be less suitable for re-use, either by direct reprocessing or by conversion into secondary products. Furthermore, the greater difficulty of retrieving and separating partially degraded materials will hinder attempts to recover useful materials from mixed plastic wastes. Introduction of degradable plastics would probably have no significant effect - either positive or negative—on energy recovery via incineration or pyrolysis of mixed refuse.

- **Encouragement of littering-** A consumer may be more likely to discard items carelessly if he has been told they are degradable.

However, **conventional polyolefins** are still much the best solution for many applications requiring tough films, because polyethylene (PE) and polypropylene (PP) are cheap, easy

to process and both mechanically tough and bio-inert. A particularly good application is as agricultural films, which are widely used in the form of greenhouses, tunnels, mulch and silage films, and bale wrap, to improve crop cultivation and protect agricultural products after harvesting. Around 2.8 million tonnes of agricultural plastics are consumed annually world-wide and cover more than 5 million hectres of land. The majority of this surface is covered by mulch film, accounting for 4.5 million hectares (Billingham *et al* 2002).

Although both PE and PP will degrade naturally, the timescale is too long for them to be considered environmentally 'friendly' and the increasing demand for such materials requires ways of converting them into water-wettable, mechanically weak material in short periods. The solution lies in accelerating the natural oxidative degradation of the polymers (Billingham *et al.* 2002).

Chemical structure of the substrate is critically important for any enzymatic attack and the creation of a new kind of enzyme such as, for example, one which will degrade polyethylene, is probably not possible by the induction of biosynthesis. Enzymatic induction is just a triggering of an existing gene to produce a number of molecules of the corresponding enzyme. Thus a purely enzymatic degradation of a long straight olefin chain dependent on the enzymatic cleavage of the C-C bond should not be expected, since such an endoenzyme does not occur in nature. Oxidation is instead the critical initial step in the degradation of many rather inert organic molecules (Albertsson and Karlsson 1994).

The biological susceptibilities of major types of synthetic polymers like PCL, PHB, PHBV, PHAs, PLA etc. have been established by many scientists (Fields *et al* 1974, Margaert *et al.* 1993, Jendrossek *et al.* 1993, Mac Gillivray and Shiaris 1993, Mas-Castella *et al.* 1995, Pranamuda *et al.* 1997).

Degradation as defined by ASTM is 'a deleterious change in the chemical structure, physical properties, or appearance of a plastic.

Hatada *et al.* 1996 (IUPAC Recommendations 1996) termed degradation as 'chemical changes in a polymeric material that result in undesirable changes in the values of in-use properties of the material'.

Biodegradation of organic polymers was defined by Potts(1984) as 'the degradation and assimilation of organic polymers by the action of living organisms, primarily fungi and bacteria'. Biodegradation involves a biological agent utilizing the organic polymer as a substrate for growth and energy, so that the end product of complete biodegradation will be microbial biomass and, in an aerobic environment, CO_2 and water. Some substrates may not serve directly as either C or energy sources, but instead may be cometabolized in the presence of another C source to form alternate compounds; these products of cometabolism may be either assimilable, and therefore decomposed to CO_2 and water, or unassimilable (Alexander 1961), in which case no reduction in the amount of plastic in the waste stream occurs.

Biodegradation is an event which takes place through the action of enzymes and /or chemical decomposition associated with living organisms (bacteria, fungi, etc.) or their secretion products (Albertsson and Karlsson 1994). Microbiological deterioration can be achieved by exo- or endo-enzymes or by products secreted biochemically or chemically from these. Macroorganisms can also eat and, sometimes digest polymers and give a mechanical, chemical or enzymatic ageing. There are many different degradation modes that in nature combine synergistically to degrade polymers.

Biodegradation might be better used as a term only when it is essential to distinguish clearly between the action of living organisms and other degradation modes (e.g., **oxidation, photolysis** or **hydrolysis**).

- **Oxidation:** The initial step in the oxidation of polymer films may occur without any biodegradative attack, although both microbes and fungi may accelerate the process. The rate of initial oxidation of polymers is likely to decrease when air is limited, e.g., in the body of landfill sites (1-2 m below the surface). However, certain microorganisms can utilize oxygen in chemically bound form (e.g., nitrate, sulphate, carbonate) to biodegrade the polymer in anaerobic conditions.

- **Photodegradation:** Polymers are susceptible to electromagnetic radiation, with both γ-irradiation and light having an adverse effect on polymer properties; e.g. poly(α-ester)s show reduced molecular weight. Photo-oxidation has a profound effect on the biodegradability of certain polymers. Polyethylene without photo-oxidation has been shown to degrade by only 0.2% over a 10 year period, but with

42 days prior oxidation, a 2% weight loss was obtained after soil incubation for a similar time period (Albertsson and Karlsson 1994).

- **Hydrolysis:** Chemical and microbial hydrolysis mechanisms are the most important biodegradation reactions involving polyesters and water-soluble polyethers (Amass *et al* 1998). However, the degradation of polyethylene can not be carried out by hydrolysis.

The necessary enzymes for anabolism (biosynthesis) and catabolism (biodegradation, mineralization) have probably already been provided by nature to ensure the recycling of the basic elements of life (C,H,O,N,S,P, etc.) and only rather small modifications are considered by some scientists to be feasible through gene mutations, if one believes in the chances for spontaneous further development of the biosphere in contrast to human inventions.

Albertsson *et al* (1987) worked out the mechanism of biodegradation of LDPE in both abiotic and biotic environment. They found synergistic effect between environmental parameters and biodegradation.

Biodegradation test methods-

Standard tests for degradation of polymers under various conditions have been the object of many organizations such as ASTM (American Society for Testing and Materials), OECD (Organization for Economic Cooperation and Development), ECN (the European Committee of Normalization), the Japan Biodegradable Plastics Society, etc. (Calmon-Decriaud *et al* 1998).

Available biodegradation tests may largely be classified as liquid culture, solid culture, soil burial and weathering tests (Pankte 1996). Many test methods with fungi and bacteria have been developed as most damage are caused by these organisms. While pure cultures are mainly used for liquid culture and solid medium tests, the soil burial tests and weathering tests are performed using a natural microbial flora. Liquid culture and solid medium tests are conducted for a few days up to several weeks. Soil burial tests are usually carried on for several weeks or months. Weathering tests under field conditions require one or even several years.

Some test methods used for testing plastics against microbial attack are listed below:

1. Liquid culture tests
   - Tests of the microbial utilization of liquid components
   - Tests of the biocidal effectiveness of liquid components
   - Determination of mycelial dry weight
   - Determination of colony counts
   - Determination of turbidity
   - Determination of oxygen consumption
2. Solid medium tests
   - Tests of the microbial utilization
   - Tests of biocidal effectiveness
   - Determination of colony diameter
   - Determination of oxygen consumption
3. Soil burial tests
4. Weathering tests
   - Field tests
   - Tests in a tropical chamber
   - Laboratory tests, moist chamber.

Soil burial test - the perspective:

Polyethylene is resistant to physical, chemical and weathering agents but the possibility of microbial degradation still remained to be tested. For testing the unimaginable potentiality, initially, one has to study under natural conditions and in their own habitats. While the habitat was being considered, a choice had to be made.

Void is definitely not a suitable place for an organism to live and flourish. Keeping it aside, there are only three major components of this lonely planet and these are **air, water** and **soil.**

Again, **Air** cannot offer the basic needs like shelter and food at least not in any considerable amount. **Water,** we have plenty but mostly in the ocean (ca. 97%). Polar ice caps have ca. 2.1%, ground water ca. 0.3-0.8%, lakes 0.009% and rivers 0.00009% (Lynch and Pool 1979). Most of these are too cold, to deep, too dark or unstable and thus not very favourable for microbial growth and activity. We are left with only **soil**, the solid upper crust of the earth, which shows great variation in structure and composition.

Rocky and sandy soil may not offer favourable conditions but in Bangladesh, the mature alluvial soil is very rich and offers an ideal habitat for microbial growth and activity. The soil mostly contains a vast population of bacteria, fungi, alga, protozoa etc. It is one of the most dynamic sites of biological interactions in nature and it is the region in which occur many of the biochemical reactions concerned in the destruction of organic matter, weathering of rocks and in the nutrition of crops (Alexander 1961). While organisms in this region synthesizes carbon and nitrogen compounds, the degraders are too busy to break down whatever they are offered with. Man made recalcitrant molecules like polyethylene may be new to our environment but it is quite possible that the indigenous microorganisms have their ability or may become equipped with the enzymes and mechanisms to break down the notorious chemicals. Most of the workers selected soil for not only its very rich population but also for the most ideal natural condition where physical, chemical and biological agencies all interplay. It is quite logical to select and carry out the soil burial test for its promise and possibility.

Objectives

Humid tropical country like Bangladesh poses greater possibility of nurturing potent microbes responsible for degradation of xenobiotic compounds including Polyethylene. The groundwork for studying the biodegradation of polyethylene has been carried out in the Laboratory of Microbiology, Department of Botany, Dhaka University. The findings have been submitted in the form of a thesis for the partial fulfilment of M. Sc. degree. Previous work has shown the association of spore former bacilli with sample strips (Khan *et al.* 2000). Buried strips in natural soil showed extensive filamentous growth. Through the analysis of the mechanical behaviour (*viz.* tensile strength, elongation at break values) of polyethylene film as well as it's morphological changes, it was possible to assess biodegradation under controlled laboratory conditions. Other parameters like acid release,

dye release, change in dissolved oxygen, and optical density values also suggested the resulting degradation (Tabassum Mumtaz *et al.* 2001). Moreover, *Basil* saplings were found to exhibit good growth in presence of polyethylene pieces in pot soil (Khan and Tabassum 2003).

The enormous potentiality of the work demands a continued effort and the proposed investigation was expected to address the following:

- To assess the combined effects of natural agencies including physical, chemical and microbiological on polyethylene films buried under soil.
- Periodic examination of samples after retrieval from soil by visual and microscopic observation.
- Record changes in surface morphology as revealed under bright field, dark field, phase contrast and fluorescent microscope,.
- To observe selected samples under electron microscope.
- To measure the loss of average weight of each sample before and after soil exposure.
- To examine changes in mechanical properties during soil burial.
- To monitor changes in chemical structure of samples by FT-IR analyses.
- To examine changes in dielectric properties during soil burial.
- To observe changes in crystallinity by X-ray diffraction analysis.

Chapter - 2

SOIL BURIAL EXPERIMENT

Soil burial experiment

2.1 Introduction

To monitor degradation of polyethylene films in natural conditions, soil burial test was carried out in the Botanical Garden of Curzon Hall, Dhaka University Campus. Outdoor burial provides a realistic environment with seasonal changes, less control of soil wetness, temperature and influence of microbial activity. The study was performed during December 2001 to June 2003.

2.2 Materials and Methods

2.2.1 Experimental plot

A rectangular plot (76 cm wide and 354 cm long) was selected and weeded out for the purpose. The soil bed was conditioned for a few weeks prior to use and no extra nutrients were added to the soil. The texture, pH and moisture holding capacity of experimental soil were determined in the Department of Soil, Water and Environment, Dhaka University. The plot was divided into four sectors and within each sector; two columns were made for burying twenty strips of each type of PE samples. Soil was occasionally wetted to maintain optimum moisture content in field capacity. To avoid external interference, the plot was covered with dry, thorny twigs of rose plants.

2.2.2 Sample preparation

All the tested samples were purchased from Dhaka New Market and were in the form of film* used as carrier bags and banned from 01 January 2002. Four different types of polyethylene samples (**Fig. 2.1a**) used for the present study were coded as to the polymer name with additional letters in subscripts referring to their appearance.

Thus white polyethylene film was coded as PE_W which was opaque. PE_B referred to black polyethylene film. $PE_{Tr\text{-}HW}$ stands for transparent thick heavy duty film while $PE_{Tr\text{-}LW}$ was the code for transparent, light weight polyethylene film.

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*Film has been arbitrarily defined by ASTM as sheeting having nominal thickness not greater than 0.25 mm (0.010 in).

Table 2.1. Characteristics of four types of polyethylene samples used for the study.

Sample code	Colour	Thickness (mm)	Melting point (^{0}C)	Optical characters
PE_W	White	0.019	124	Opaque
PE_B	Black	0.010	126	Opaque
$PE_{Tr\text{-}HW}$	Colourless	0.050	118	Transparent
$PE_{Tr\text{-}LW}$	Colourless	0.015	120	Transparent

Samples were manually cut into 20 cm x 5 cm strips, weighed and then buried vertically downward below 7.5 cm from the top (**Fig. 2.1b**). The weight of each specimen was tagged on the top of it with adhesive tape. The soil bed containing samples were maintained for a period of two years.

2.2.3 Determination of soil properties

2.2.3.1 Soil pH

For the determination of soil pH (Jackson 1973), 10g of soil sample was placed in a 50 ml glass beaker and 25 ml of distilled water was added. The suspension was stirred for 30 minutes. pH was measured with an electric pH meter (Model: Jenway 3310). The soil suspension was stirred well just before the electrode was immersed.

2.2.3.2 Percentage Moisture content

Moisture content of experimental soil was measured by gravimetric method (Chowdhury *et al.* 1969). In this method, aluminium cup with moist and oven dried (at 110^{0}C for 8 hours) soil was weighed and the moisture content was calculated from the equation below:

b-c/b-a x 100 , where

a = wt. of Aluminium cup;

b = wt. of Aluminium cup + moist soil

c = wt. of Aluminium cup + dry soil (after heating at 110^{0}C)

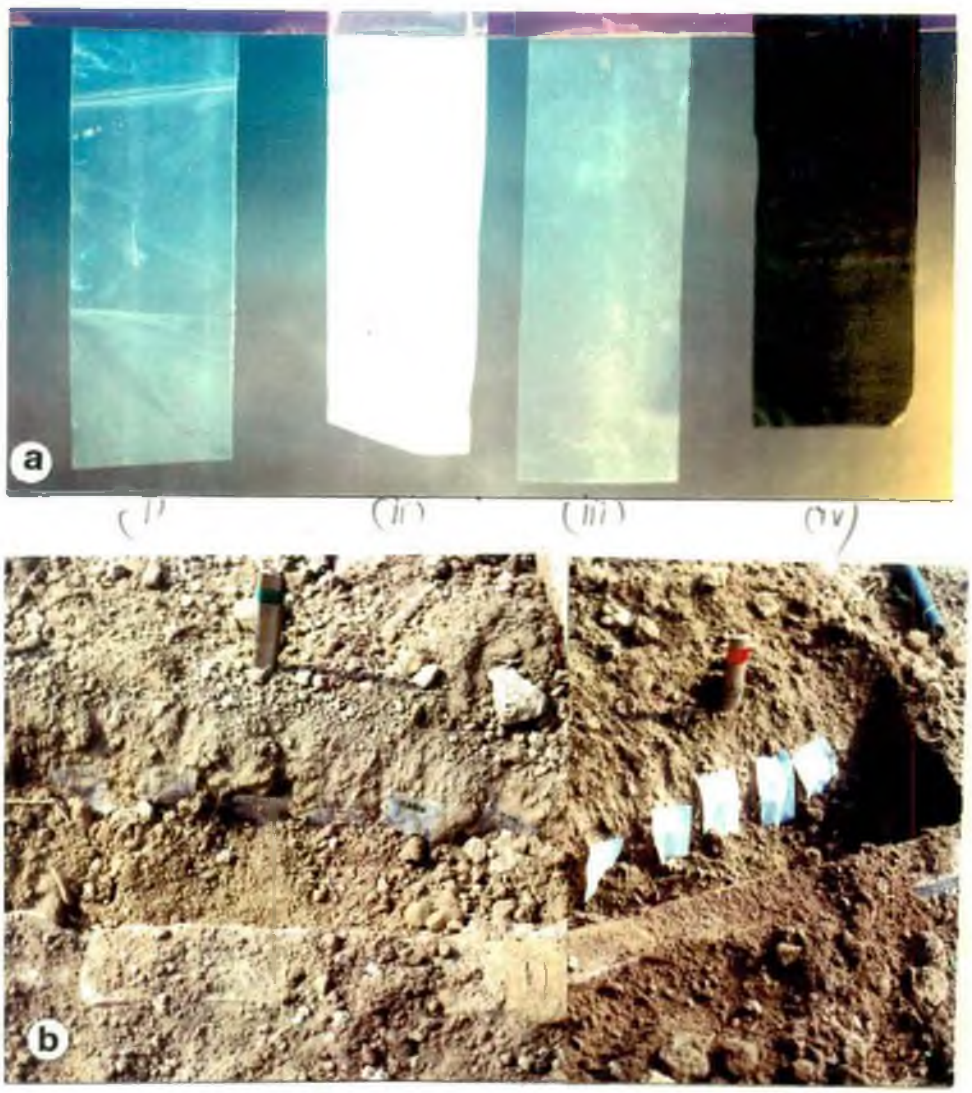


Fig. 2.1 (a,b) Photographs showing the pre cut polyethylene samples as used in the soil burial expt. (a) From left to right (i) thin transparent ($PE_{Tr\text{-}LW}$) (ii) white-opaque (PE_W) (iii) thick transparent ($PE_{Tr\text{-}HW}$) and (iv) black thin (PE_B) polyethylene films (b) PE samples arranged and about to be buried vertically under garden soil.

2.2.3.3 Field Capacity

To determine the field capacity of the soil sample, a plastic cup was perforated at the bottom and a filter paper was used to cover the holes (Chowdhury *et al.* 1969). Two-third of the cup was filled with soil. Water was added to the surface of the soil in such quantity that the first few centimeters of the surface soil is wet and there must be dry soil beneath the wetting front. The pot was then covered with a piece of a paper to prevent loss by evaporation and was allowed to drain for 48 hours. After that, approximately 10g of surface soil was taken and moisture content was calculated in the way mentioned previously.

2.2.3.4 Soil texture

Mechanical analysis of soil was determined by hydrometer method (Chowdhury *et al.* 1969). In this method, 50g oven dry soil sample was placed in a 600 ml beaker and 50-100 ml of 6% H_2O_2 solution was added to it. The beaker was covered with a clock-glass. After a while, the beaker was placed on a boiling water bath. When the organic matter was decomposed completely, the beaker was allowed to cool. About 1000 ml of 5% Calgon (Na-hexametaphosphate) solution was added and the beaker was kept covered overnight.

The whole content was transferred to a measuring cylinder and was made up to 400 ml with distilled water. The cylinder was placed in settling position and the hydrometer was inserted to start the test. Records were taken just after 4 minutes and 2 hours of settlings. Hydrometer reading was then corrected by running a blank test in parallel. The calculation was done by the following way:

2.2.3.4.1 Calculation

Corrected Hydrometer readings = (t-19.4) x 0.3

where, t = temperature of the suspension in ^{0}C during experiment; 19.4 = room temperature

Percent sand = 100 – (corrected hydrometer at 4 minutes x 2)

Percent clay = Corrected hydrometer reading at 2 hours x 2

And Percent silt = 100 – (percent sand + percent clay)

The textural class of the experimental soil was then determined from its mechanical composition by plotting the results of mechanical analysis in the "Textural triangular diagram" (**Fig. 2.2**).

2.2.4 Film harvest

Samples were removed after exposure periods of 01,03,05,07,09,11,13,15,17,19 and 22 months. After removal, samples were put in a tray and washed in slow running tap water to remove as much dirt and soil particles from the film as possible. After thorough washing, samples were dried in an oven at 45^0C overnight. Test pieces were then equilibrated at room temperature in a desiccator. Average weight loss of the polyethylene samples during soil burial was measured with an analytical balance until constant value was reached.

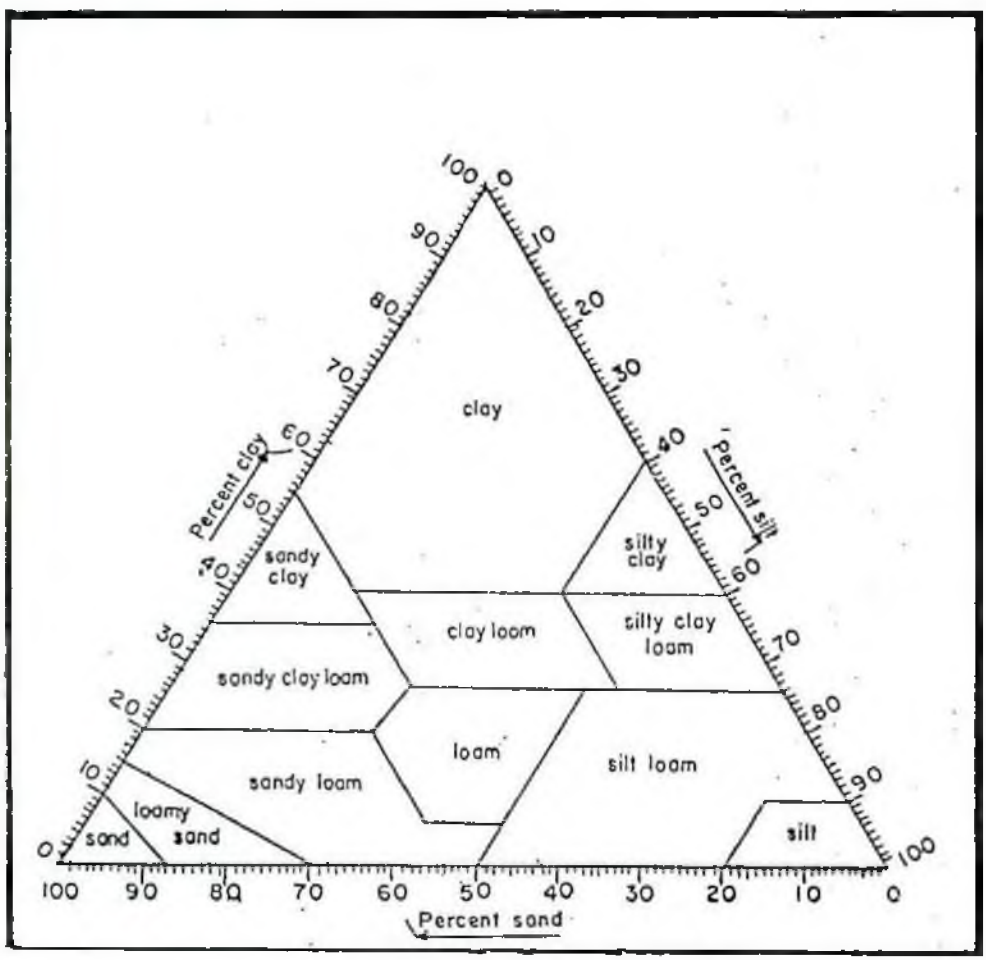


Fig. 2.2 The textural triangular diagram (after Chowdhury *et al.* 1969).

2.3 Results

2.3.1 Soil properties

The soil pH was found to be 6.83 (measured in water, 1:2.5). Moisture content of the soil in field capacity was 12.46%. Experimental soil was silty loam having 19.92% sand, 22.58% silt and 57.5% clay.

2.3.2 Average weight loss

During the two years study, polyethylene samples buried under natural soil were disinterred and weighed after thorough washing. Gross weight of each sample was measured for several times and the mean value was taken for weight loss measurements.

The changes in the average weight of the four polyethylene samples before and after soil burial for nearly two years are shown in Table 2.2. A slight gain of the weight was noticed for all samples at the beginning of the expt. Weight gain was found in PE_W sample up to 07 months of soil exposure. A negligible amount of weight was reduced afterwards and the value became significant after one year. However, weight loss didn't progressed after 15 months. This may be due to the fact that a single sample was not used and the weathering effect may not be identical for all samples. After one and half year, samples could not be retrieved with label so that initial weight could not be discerned. Taking a mean initial weight (0.1607 for PE_W) or the lowest observed value (0.1367) into concern, the weight reached to approximately 0.7279% or 0.2925% after 17 months. Similarly, it can be assumed that 0.8195% or 0.369% loss occurred after 19 months of soil burial.

In case of black sample PE_B, weight gain was noticed up to 15 months. However, no comments could be made whether sample lost their initial weight during the study or not.

Thin transparent sample behaved in the same way as sample no. 01. A slight loss of wt. was observed after 13 months which gradually progressed to 0.15% after 15 months. Weight loss after that can only be calculated by considering that the initial weight was in between the tested samples i.e. the mean of the samples taken out. Now if the sample weight was 0.2168 g or 0.2022 g, then the weight loss accounted for 0.4661% or 0.1395% respectively. The calculation becomes more complicated as the weight after 19 months of burial is very close to the previous value recorded after 17 months.

Table 2.2. Average weight loss of four types of polyethylene samples during soil burial test.

Sample Code / Exposure Period (in months)	PE_W		PE_B		$PE_{Tr\text{-}HW}$		$PE_{Tr\text{-}LW}$	
	Before burial	After burial	Before burial	After burial	Before burial	After burial	Before burial	After burial
01	0.1811	0.1813	0.0787	0.0781	0.5364	0.5364	0.2285	0.2293
02	0.1556	0.1550	0.0741	0.0750	0.5139	0.5126	0.2023	0.2023
03	0.1772	0.1786	0.0711	0.0737	0.6202	0.6190	0.2076	0.2075
04	0.1724	0.1737	0.0756	0.0796	0.6545	0.6550	0.2155	0.2171
05	0.1444	0.1454	0.0616	0.0636	0.5961	0.5953	0.2174	0.2185
06	0.1749	0.1758	0.0856	0.0880	0.5599	0.5594	0.2148	0.2176
07	0.1631	0.1638	0.0581	0.0602	0.6447	0.6424	0.2279	0.2269
09	0.1692	0.1680	0.0817	0.0865	0.5579	0.5497	0.2125	0.2131
11	0.1540	0.1522	*	*	0.6638	0.6036	0.2286	0.2312
13	0.1397	0.1045	0.0613	0.0671	0.6427	0.5410	0.2022	0.1993
15	0.1367	0.1339	0.0737	0.0813	0.6622	0.5145	0.2277	0.2211
17	*	0.1153	*	0.0609	*	0.3188	*	0.1953
19	*	0.1097	*	0.0702	*	0.0804	*	0.1992

*Could not be determined due to the loss of identifying marks.

Sample no. 03 showed gradual weight loss starting after 05 months of soil burial. The percentage weight loss of the sample plotted against exposure period is shown in **Fig 2.3**. Weight loss was about 0.5% after 09 months and reached to ca. 4% of the initial weight after 11 months of soil exposure. About 10% of the original weight was lost during 15 months of exposure.

After 17 months, the sample started fragmenting. The whole strip disintegrated into three larger and four smaller pieces (**Fig. 2.4a**). Larger pieces weighed 0.3190g, which is roughly half of the original weight. On the other hand, weight of smaller, individual disintegrated pieces ranged from 0.0036-0.0071g. After 19 months, only 0.0804g of sample was recovered (**Fig. 2.4b**).

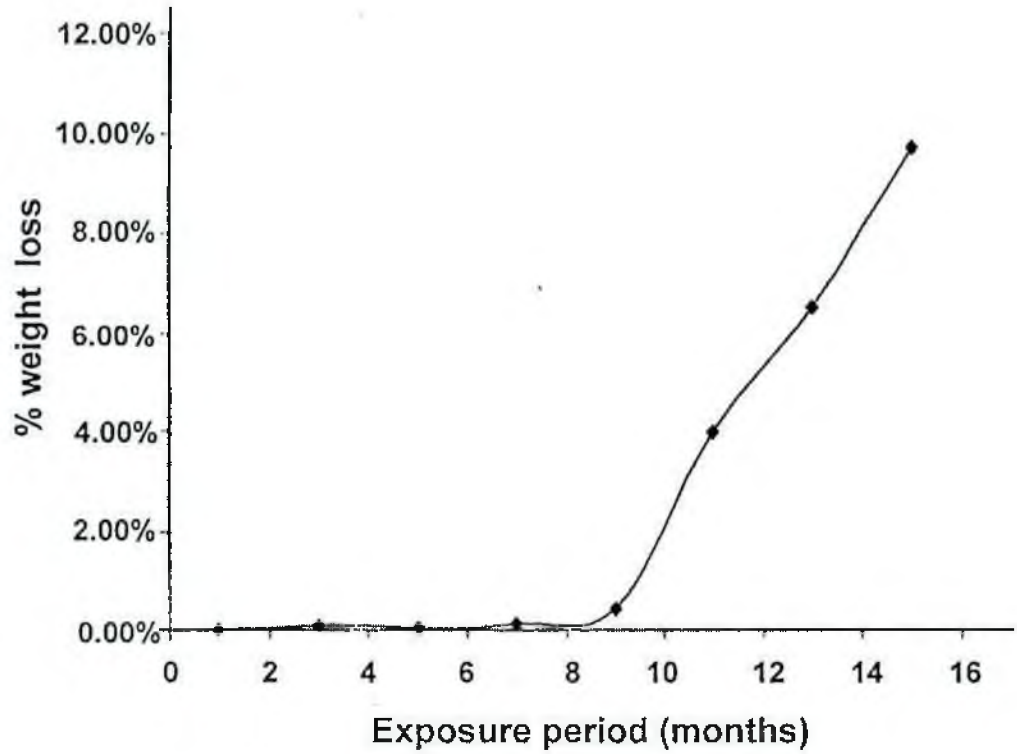


Fig. 2.3. Average weight loss of sample no. 03 ($PE_{Tr\text{-}HW}$) after soil burial.

At the end of the experiment when the whole plot was excavated, disintegrated pieces were found attached with soil particles (**Fig. 2.4c**). The entire soil from the bed was sieved through 2-mm pore under slow running tap water. Gravels, twigs were separated manually before primary sieving. The soil particles were further screened with less than 1mm pore sieve. The sieve was then oven dried at 45^0C overnight and very minute fragments were collected from the sieve (**Fig. 2.4d**).

2.3.3 Changes in Optical Characters

Optical changes in course of time were monitored visually. Effect of soil burial on the optical properties of polyethylene samples are shown in **Figs. 2.5-2.8d**.

Sample no. 01, PE_W

Sample no. 01 was white, opaque film with smooth, glossy surface. Upon exposure for 07 months, the sample showed no change in its optical character. After 09 months the soil particles adhered on the surface and the sample became slightly coloured which could be seen when placed against a light source (**Fig. 2.5a**). The glossiness of the film retained

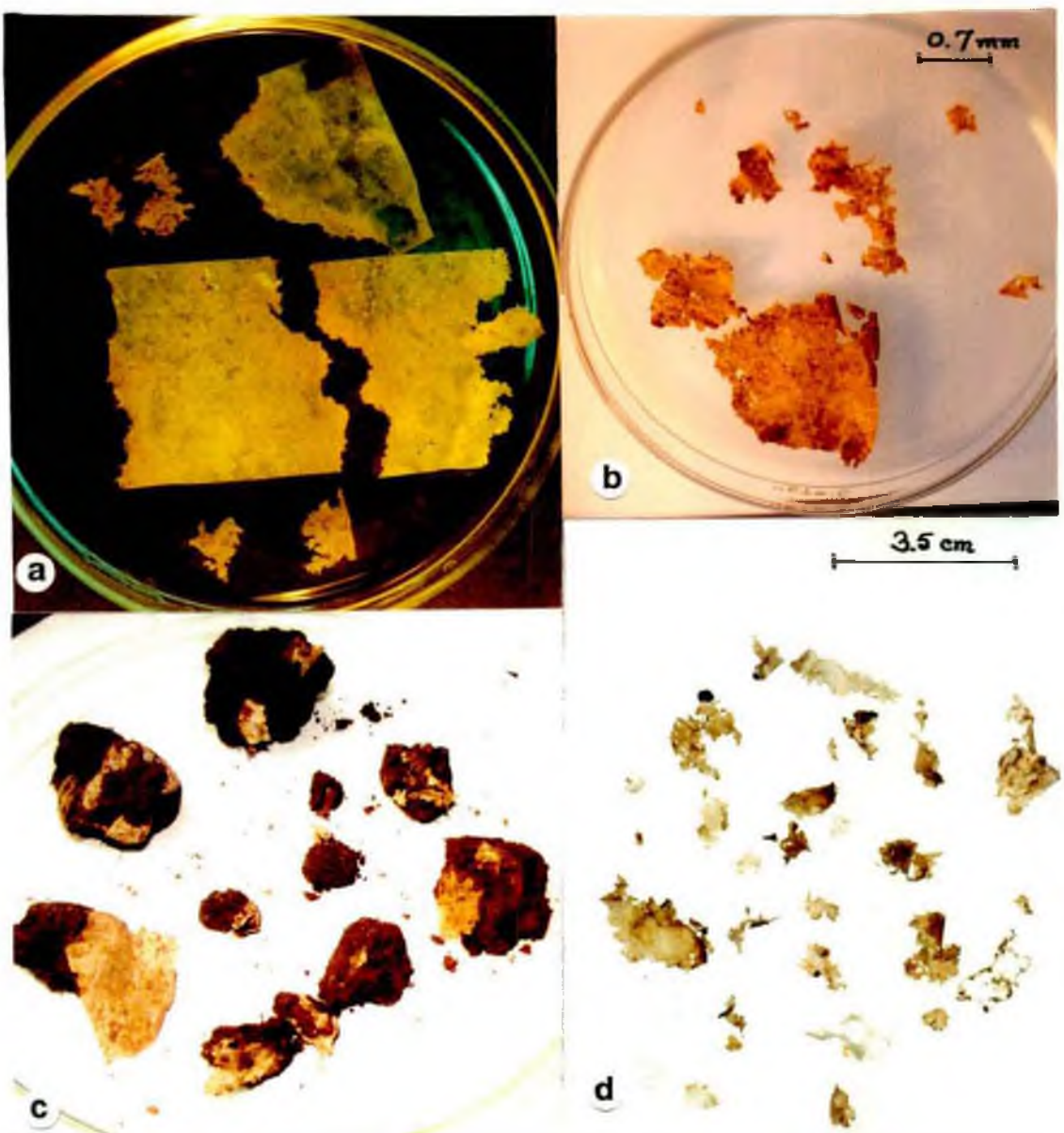


Fig. 2.4 (a,b) Photographs showing fragmentation and disintegration of thick, transparent sample ($PE_{Tr\text{-}Hw}$) after 17 and 19 months, respectively. (c,d) Photographs showing PE samples after 22 months (c) fragments of PE adhered and embedded in soil aggregates and (d) very tiny pieces of PE recovered after screening through a sieve of 1 mm pore size.

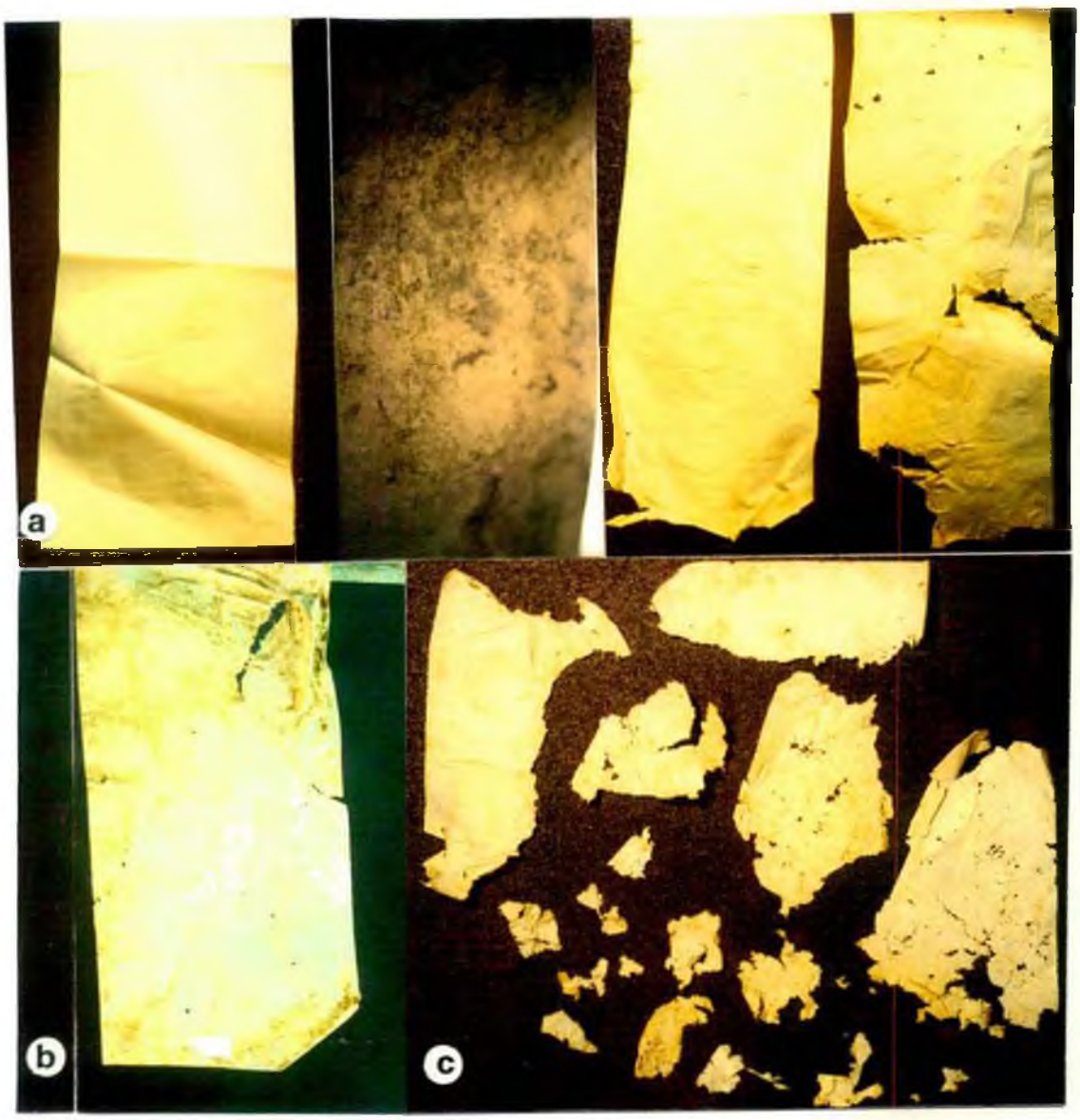


Fig. 2.5 (a-c) Photographs showing changes in white opaque sample (PE_W) during soil burial. (a) Gradual changes in visible characters after 0, 09, 13 and 15 months (from left to right) (b) surface soiled and matted after 13 months and (c) disintegration of sample after 22 months.

even after 13 months although the lower portion was torn off. After 15 months, sample had more nicks on the surface (**Fig. 2.5a**). The surface gradually became rough and had a paper like feeling after 19 months of soil burial (**Fig. 2.5b**). The sample had perforations mostly on the edge of the film and could not be retrieved intact. Disintegration of the sample was evident at the end of the study within 22 months (**Fig. 2.5c**).

Sample no. 02, PE_B

PE_B sample remain unchanged from the beginning up to 11 months. After 13 months, macroscopic holes were common over the film indicating grass-root penetration. Accumulation of soil particles over the surface was not prominent until 19 months. The otherwise resistant sample was found to fragment after 22 months (**Fig. 2.6**).

Sample no. 04, $PE_{Tr\text{-}LW}$

In case of transparent samples, loss of transparency was noticed with the exposure period. Sample no. 04 retained considerable glossiness and clarity even after 09 months. However, white spots were evident in the lower portion of the film. With the progress of time, the white spots developed sparsely near the edge, spread throughout the film. The lower half of the sample was invaded with whitened area after 15 months. Sample exposed for 19 months was found to have colouration and disintegration occurred after 22 months (**Fig. 2.7**).

Sample no. 03, $PE_{Tr\text{-}HW}$

The decrease in transparency hence transmittance was most pronounced in case of sample $PE_{Tr\text{-}HW}$ which showed considerable weight loss too. After 07 months, the originally transparent, thick film was invaded by white spots. The spots became more prominent in the lower deeper portion in the soil after 09 months. The whole sample became completely opaque within 11 months of exposure (**Fig. 2.8a**). The loss of transparency was not uniform throughout the film. Differences in the thickness of the film caused by grooves and pits on the surface were also noticed (**Fig. 2.8b**). After 13 and 15 months, the opaque sample was found to be deformed and wrinkled in appearance. The samples also developed cracks, fissures and irregularly broken edges (**Fig. 2.8c**). The sample could not be recovered intact afterwards and disintegrated pieces were retrieved after 17 months (**Fig. 2.4a**). The degree of disintegration progressed and only a small fraction of the whole sample could be retrieved after 19 months (**Fig. 2.4b**).

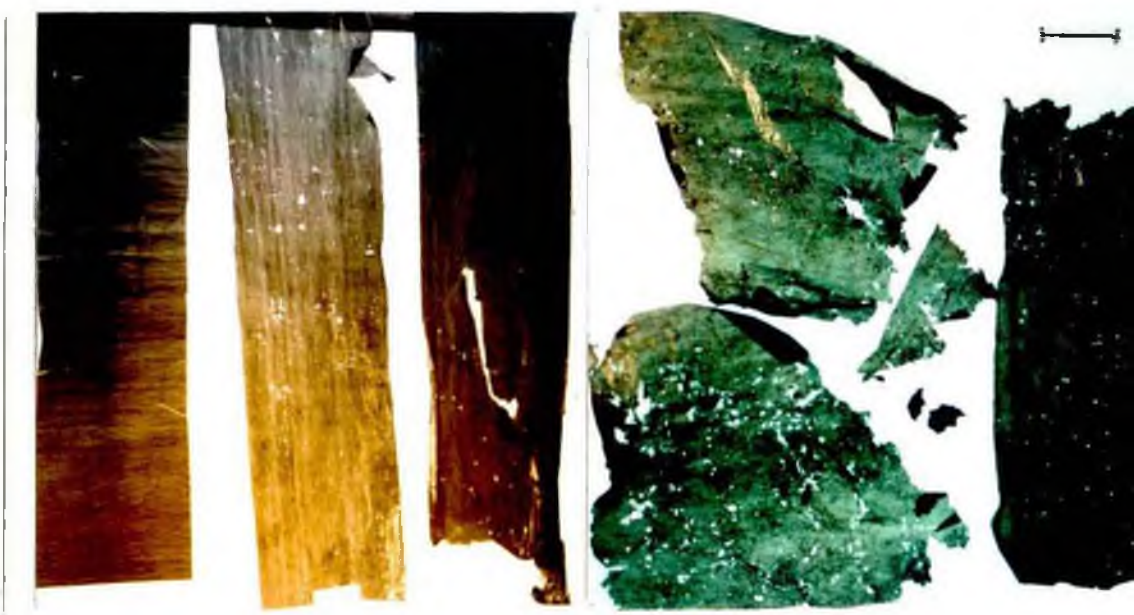


Fig. 2.6: Photographs showing how the black, thin polyethylene sample (PE_B) appeared after 0, 13, (from left to right).

Fig. 2.7: Sequentially arranged photographs showing the fate of thin transparent sample ($PE_{Tr\text{-}LW}$) after months (from left to right) of soil burial.

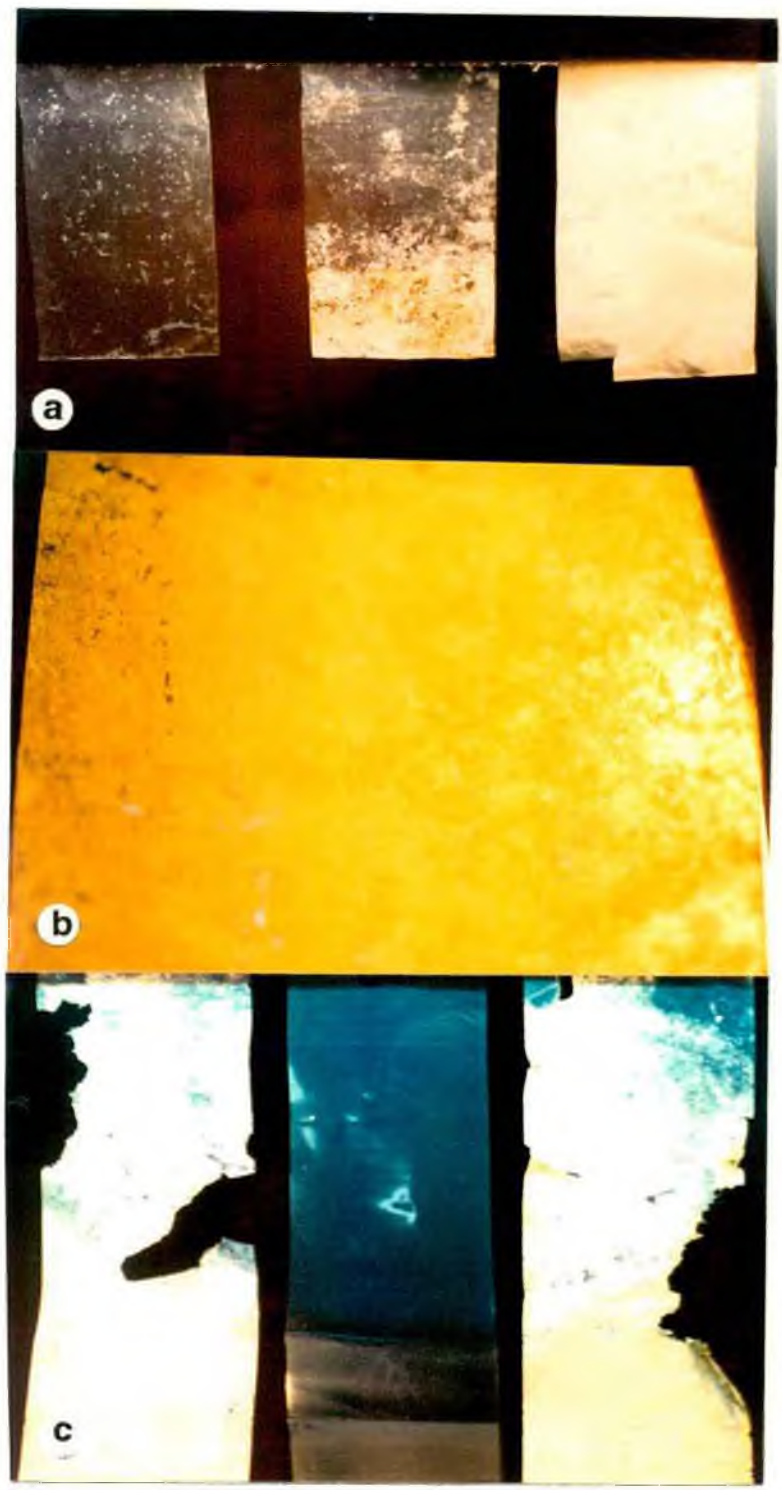


Fig. 2.8 (a-c) Photograph showing changes in the optical and physical characters of thick transparent sample ($PE_{Tc\text{-}HW}$) during soil burial. (a) films after 07, 09 and 11 months (from left to right). White spots on film gradually appeared, increased and covered the whole film within 11 months (b) a close-up view of the sample after 11 months showing colonization and mutiliation. (c) Comparing the opacity and integrity of samples after 13 months (left) and that after 15 months (right) with control sample (centre).

Soil may be considered as the final destination of all our materialistic integrity. Anything left on the surface may be obtrusive and eyesore but once buried under, it goes not only out of our sight but at the same time it is subjected to the influence of all conceivable agents of weathering and mineralization. Above all the omnipotent microbes with all their armours attack whatever comes in their vicinity and they can catch hold of. Thus it was thought to be logical to study the course of possible biodegradation of polyethylene using soil burial experiment.

Chapter - 3

OPTICAL AND ELECTRON MICROSCOPIC STUDIES

Optical and electron microscopic studies

3.1 Introduction

Microscopic studies are quite helpful in estimating some of the changes which take place in the structure and composition of materials exposed to various natural processes including physical, chemical and biological degradation. The rate of such destruction or degradation is dependent upon a number of factors including the type of organism and their enzymes, the nature and composition of the polymer concerned etc. Degradation processes are slow and their results may not be visible at the beginning but in the long run, the result will be evident. Initially there may not be any visible change but microscopic studies can be used to follow the course of changes including the initial site of attack, pattern and nature of the degradative activity of microbes.

To study the surface structure and erosion characteristics during biodegradation a range of different microscopy-based techniques have been used by several workers (Benedict *et al.* 1983, Janik 1998, Pearce *et al.* 1994).

3.1.1 Types of microscopic techniques

The following microscopic techniques were used during the present study:

- Optical Microscopy
  - Bright field/Transmission light microscopy (BF)
  - Dark field Microscopy (DF)
  - Phase contrast Microscopy (Ph)
  - Oblique light Microscopy (OLM)
- Fluorescent Microscopy
  - Direct Epifluorescence (DEF)
- Electron Microscopy
  - Scanning Electron Microscopy (SEM)

3.2 Optical Microscopy

When the rate of change is really slow, it may not be possible to estimate visually and at times may be virtually impossible in short time. In those difficult cases, examination through an advanced microscope proved to be useful to study and follow the possible course of degradation which the sample had undergone in soil.

During the present study, Bright-field, Dark-field and phase contrast microscopy were simultaneously used to reveal the exact nature of changes--- starting from the initial attack through the colonization and gradual processes of degradation resulting from microbial growth and activity.

3.2.1 Preparation of Slides

For microscopic analysis, a thin piece from the sample was cut with a scalpel and was mounted with distilled water or Acridine orange solution on a microscope slide. Cover slip was placed and sealed by nail polish to keep the test piece wet for longer period.

The test pieces were examined under 4x, 10x, 40x and 100x oil immersion objective lenses. Different light adjustments were employed to get the detailed view of the film, surface characteristics, textural changes, edge, degrees of microbial growth etc. The observation made throughout the study were arranged serially to follow the course of degradation.

3.2.2 Results

Microscopic views of the samples are shown in **Figs. 3.1-3.4**.

Sample no. 01, PE_W

Fig. 3.1 shows the changes in the surface characteristics of sample no. 01, PE_w. Under the optical microscope, the control sample presented a uniform homogenous appearance (**Fig. 3.1a**). After three months, no appreciable change was observed (**Fig. 3.1b**). Considerable colonization after 09 months was discernible under fluorescent microscope using blue filter (**Fig. 3.1c**). Colonization process progressed with the increase of fluoresced body in greater number covering more surface area.

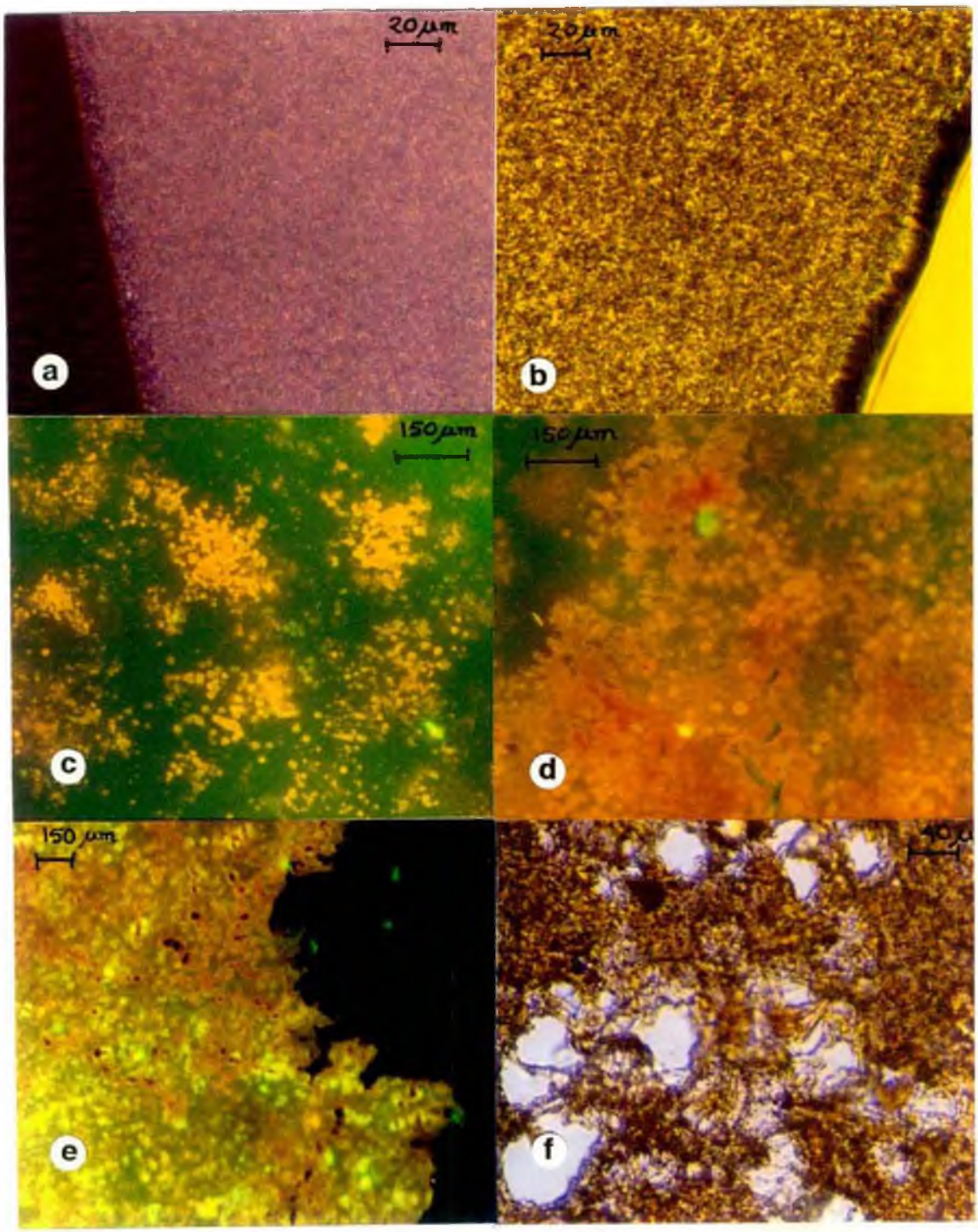


Fig. 3.1(a-f): Photomicrographs showing changes in the structure and appearance of sample no. 01, PE_W. a) control sample presenting homogenous granular appearance (b) no change after 03 months (c) Fluorescence microscopy revealed microbial colonization after 09 months (d) increased number of colonies developed after 11 months and (e, f) an irregular edge with its disrupted area magnified after 13 months.

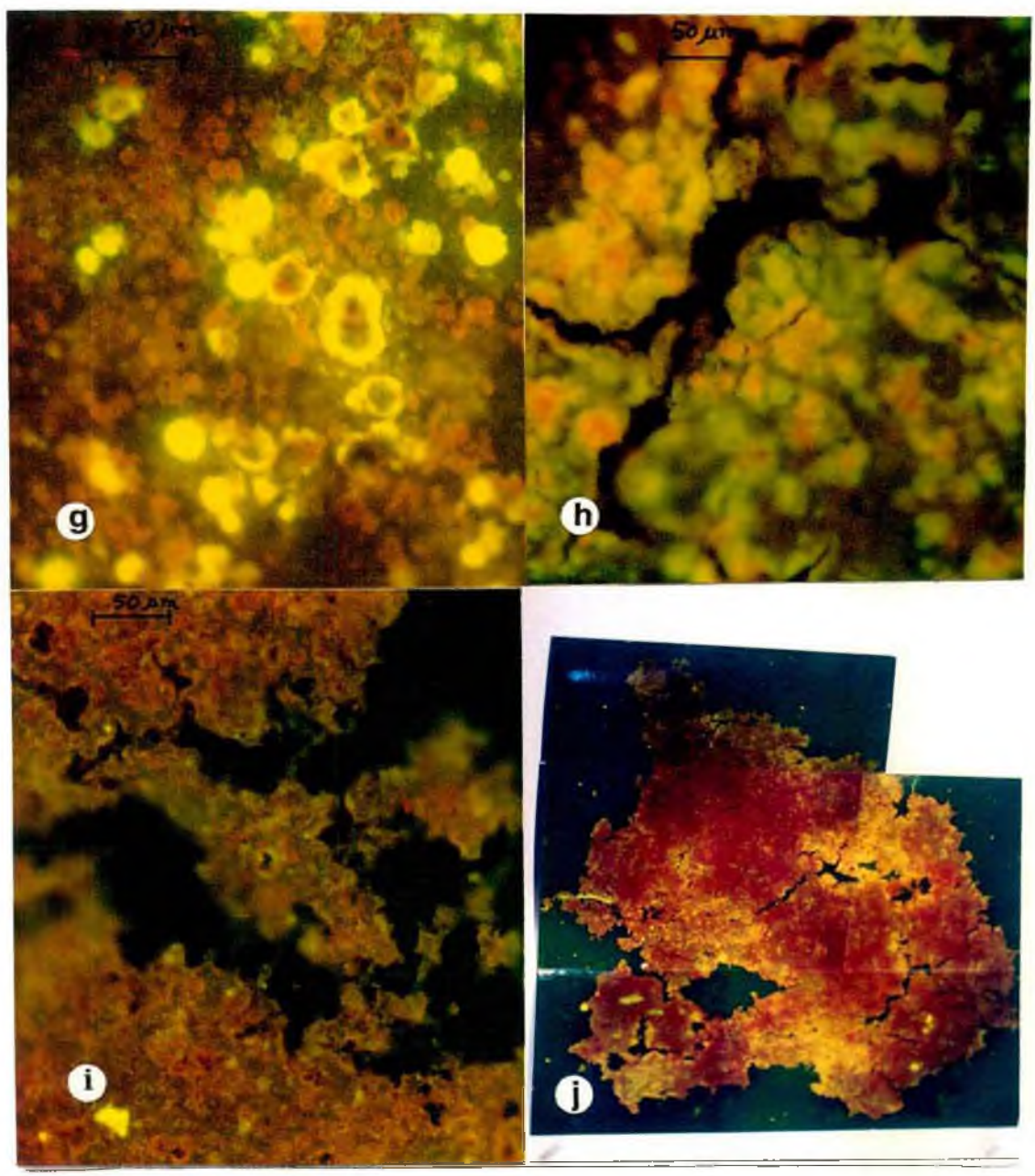


Fig. 3.1 (g-j) : Series continued to show the gradual development and fate of white PE film over 2 years time: (g-h) showing numerous cracks and holes as developed within 15 months (i,j) the sample as it appeared after 22 months.

After 13 months of soil exposure, the surface become heavily colonized; no smooth areas were left (**Fig. 3.1e**). Upon higher magnification, loosening of the PE network was evident (**Fig. 3.1f**). After 15 months, small pits and cracks on the surface were noticed (**Fig. 3.1g**). Cracks developed at the centre of microbial biomass initiates fragmentation of the film (**Fig. 3.1h**). The integrity of the film deteriorated thereafter and breaking apart of irregularly sized pieces were visible after 22 months (**Fig. 3.1i**). **Fig. 3.1j** is the photomicrograph of a fragmented piece under fluorescent microscope using blue filter showing its own internal injuries.

Sample no 02 PE_B

The black polyethylene film was found to be more resistant to degradation process which can be evident in **Figs. 3.2a**-f. The control film appeared smooth, uniform under light microscope (**Fig. 3.2a**). Hardly any irregularity in the surface morphology was noticed up to 09 months. After 11 months, some sort of microbial growth was visible (**Fig. 3.2c**). While the extent of microbial growth on film was relatively less, penetration of grass-root caused macroscopic holes on the film (**Fig. 3.2d**). The surrounded area of the macrohole was more populated than the inner smooth surface. After 15 months, colonization progressed with textural changes and surface erosion was evident (**Fig. 3.2e**). When the experiment was terminated, the sample showed dense colonization along with loosening surface texture (**Fig. 3.2f**).

Sample no. 03, $PE_{Tr\text{-}HW}$

Figs. 3.3 (a-h) presents the different views of the experimental transparent, thick polyethylene film. In **figs. 3.3a&b** both the control film and the film after 03 months are observed as a continuous sheet. Microbial colonies on the film appeared after 07 months. After 09 months, these colonies further developed and were found to adhere forming clusters (**Fig. 3.3c**). With the progress of time, number of colonies increased and covered almost the whole surface of the film (**Fig. 3.3d**). After 13 months, microbial invasion proceeded with the disruption of surface texture (**Fig. 3.3e**). **Fig 3.3f** shows the magnified views of these growing colonies under phase contrast microscope. After 15 months, the edges of the films are seen as heavily eroded and the film became friable (**Fig. 3.3g**). **Fig. 3.3h** represents a rare view of separation of smaller fragments evident after 17 months.

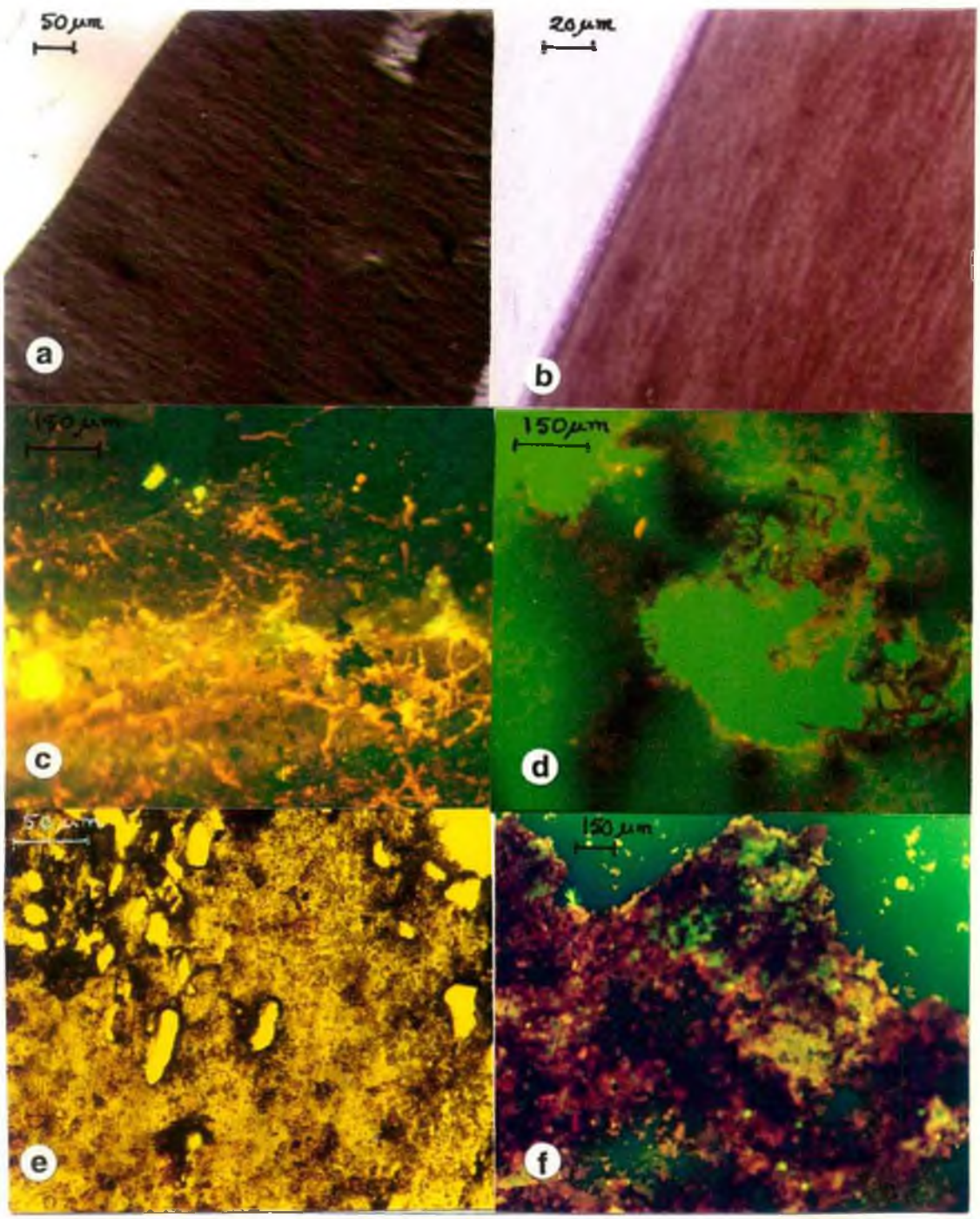


Fig. 3.2 (a-f): Photomicrographs showing changes in the structure and appearance in sample no. 02 (a) control film (b) film after 03 months (c) sparse colonization after 11 months (d) formation of macroholes by grass-root penetration after 13 months (e) interrupted surface with performations resulting after 15 months. (f) heavy growth with discontinuity of integrity at the end of the study.

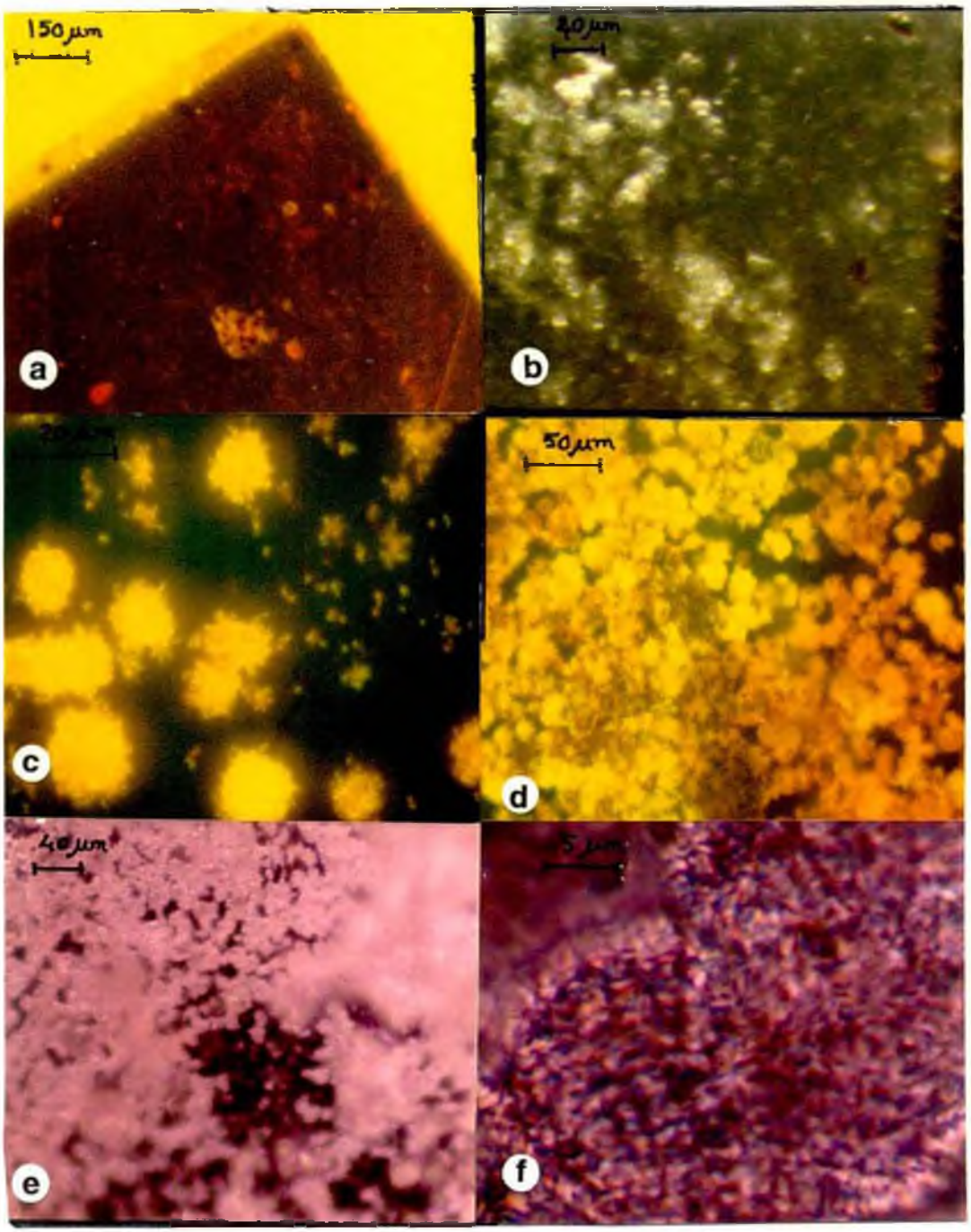


Fig. 3.3 (a-h): Photomicrographs showing surface changes in sample no. 03. (a) control film (b) film after 03 months showing no appreciable change (c) microbial colonies revealed under fluorescent microscope after 9 months (d) almost covered surface with microbial growth after 11 months (e&f) deeply eroded film after 13 months; magnified view of the whiter portion revealed clusters of bacterial cells.

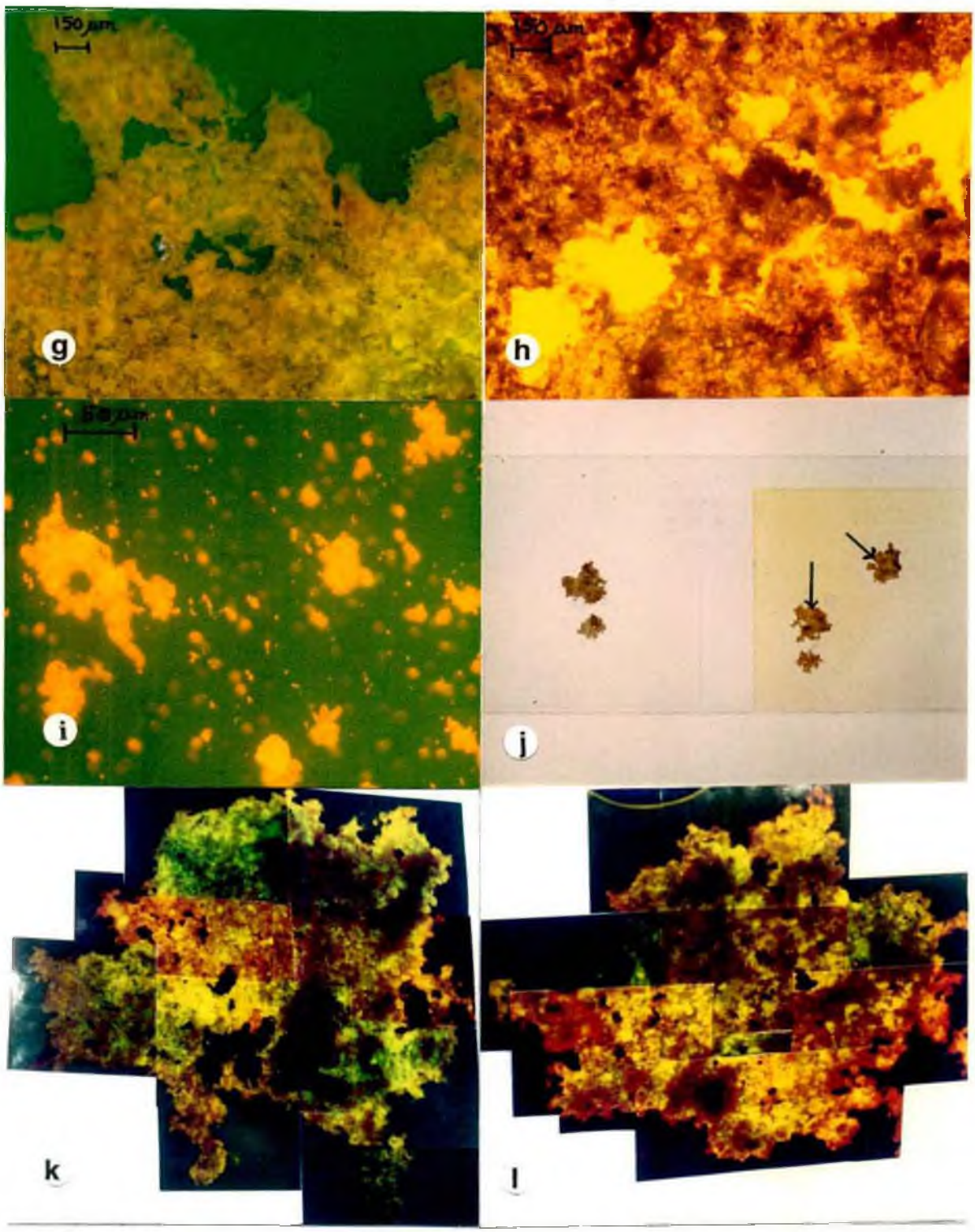


Fig. 3.3 (g-l) Series continued to show further deterioration of the sample (g) Initiation of fragmentation after 15 months (h) sample pieces tend to breaking apart after 17 months ((i) very minute pieces found floating on the wet mount slide after 19 months and (j-l) sample after 22 months of soil burial (j) Pieces taken on slide for microscopic observation (k,l) Photomicrographs joined together to present view of the entire small pieces (arrow).

Sample no. 04, $PE_{Tr\text{-}LW}$

Thin, transparent LDPE sample behaved in the similar way as no change was visible from the beginning up to 7 months. Bacterial cells were observed on the film after 07 months (**Fig. 3.4c**) and traces of colonization was evident after 09 months (**Fig. 3.4d**). After 11 months, colonies were consieerable in number (**Fig. 3.4e**) and under high power objective, these colonies were viewed as an elongated, dense clumps (**Fig. 3.4f**). The inside of these structures were filled. After 15 months, no. of colonies increased, intermingled and cracks were seen to separate the structure (**Fig. 3.4g**). The development of cracks were prominent under higher resolution (**Fig. 3.4h**). Degradation of the film further proceeded with the disintegration of the film and the formation of irregular pits (**Fig. 3.4 i,j**).

Through microscopic analysis, one can observe exactly how the biodegradation of polyethylene takes place in natural soil conditions. The entire process took about 22 months under the prevailing environmental conditions.

3.3 Scanning Electron Microscopy

After the initial studies carried out with the Optical and Fluorescent microscopes available in the lab., attempt was made to extend our vision through Electron Microscopy.

The surface characters of any material is rather clearly visible under Scanning electron microscopc and several workers employed Scanning Electron Microscopy to study the degradative changes of polymeric materials during different kinds of treatments (Imam *et al.* 1995; Nishida and Tokiwa 1993, Tansengco and Tokiwa 1998, Pranamuda *et al.* 1995, 1997, Amass *et al.* 1998). In the present study, SEM analysis was performed in the Department of Instrumentation Services, Jadhavpur University, Kolkata, India. The surfaces of the control and 17- 22 months treated samples were examined under a JEOL Scanning Electron Microscope (model JSM 5200) shown in **Fig. 3.5a,b**.

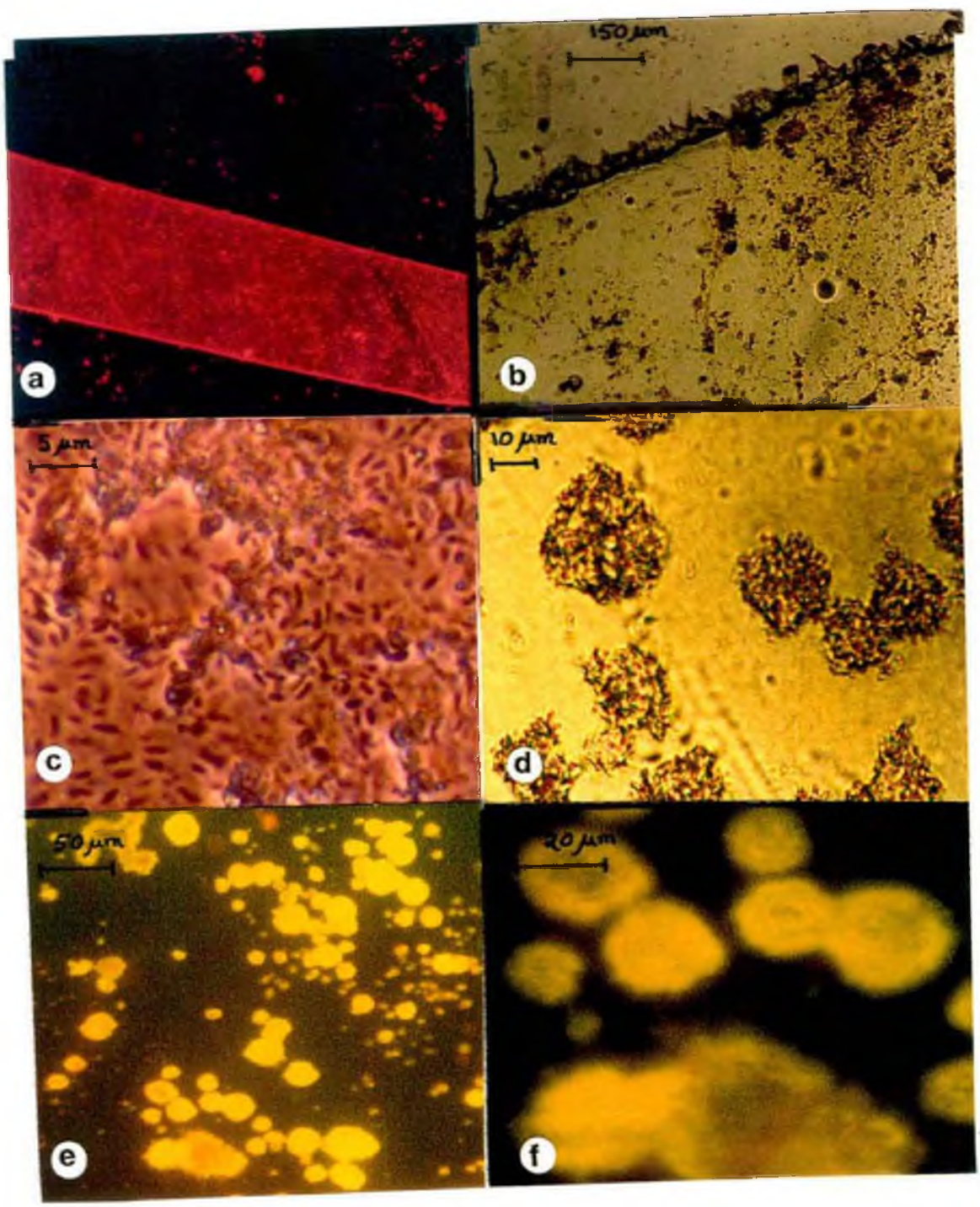


Fig. 3.4 (a-f): Photomicrographs showing changes in sample no. 04 as observed during soil burial. (a) film before soil burial (b) film after 03 months (c) bacterial cells and spores over the film after 07 months ((d) clusters formed as colonization progressed after 09 months. ((e&f) well-developed colonies intermingled indicating the initiation of microbial invasion after 11 months.

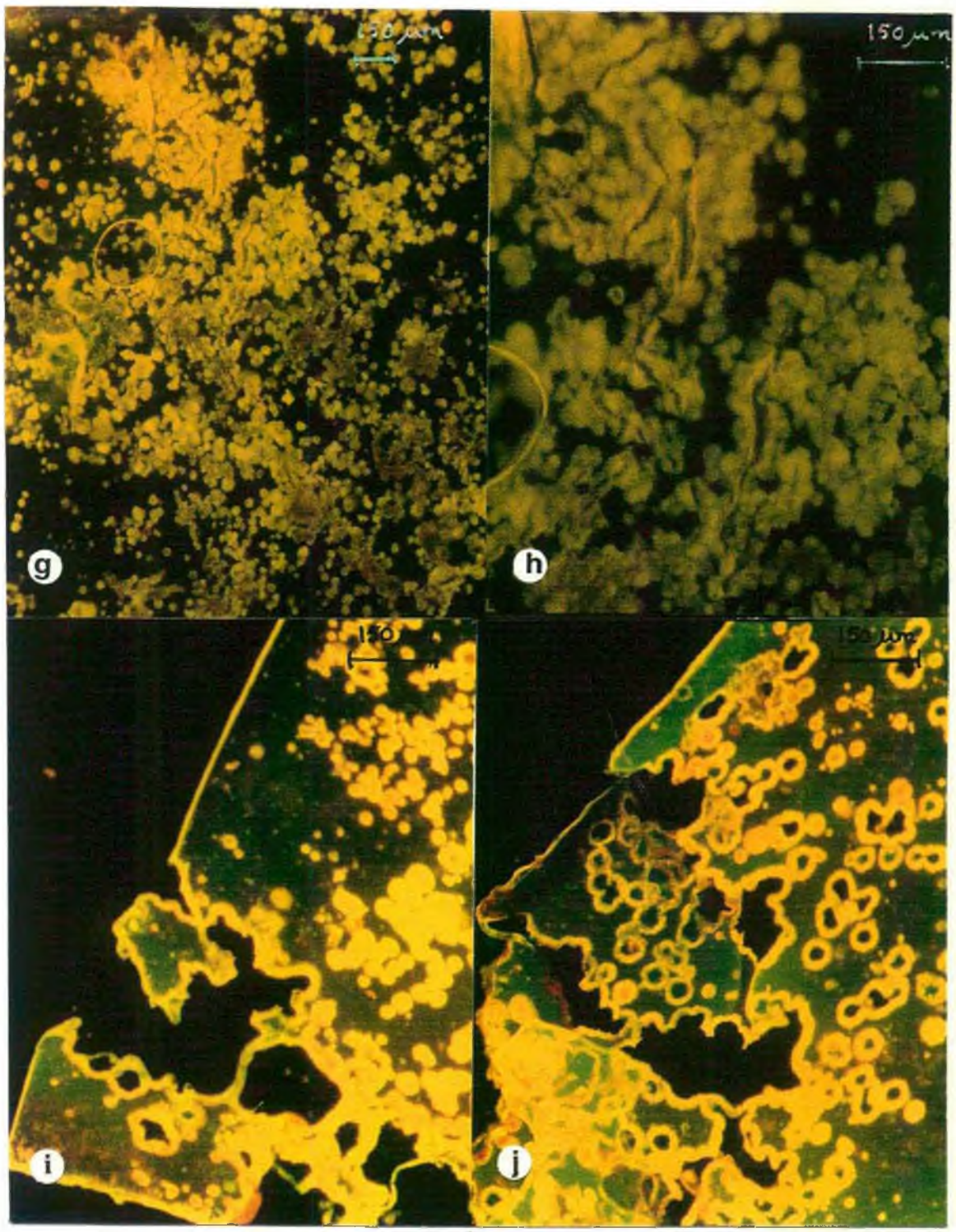


Fig. 3.4 (g-j): Series continued to show further damage on sample no. 04. (g&h) film integrity weakened after 15 months as cracks developed (i&j) film undergoing fragmentation after 19 months.

Fig. 3.5 (a-c): Photographs showing the SEM and shadow-casting equipments at the department of Instrumentation Science, Jadavpur University, India. (a) the Scanning Electron Microscope, (b) same showing the monitor screen, (c) the shadow casting equipment.

3.3.1 Preparation of specimens

The polyethylene samples, both untreated and treated were cut into 10 mm x 10mm pieces as per instruction. These pieces were then fixed on the top of the specimen holder and were coated with gold-palladium (**Fig. 3.5c**). After coating the films, the specimen holders were placed in position in the main body tube of the microscope for viewing. Through trials it was found that for our experimental materials, 20 kV and a magnification of 2000x most suitable to reveal the structural details.

At first, the control films were scanned and photographed. Then the test samples were examined. For the treated materials, multiple exposures were taken from different areas. Nova 125 ISO, NP22 black and white films were used.

3.3.2 Results

Scanning electron microscopy of the coated samples revealed crispy clear views and it was quite easy to follow the destructive effect of degradation during soil burial experiment.

Sample no. 01, PE_W

In case of sample no. 01, the control sample was found to be finely granular and homogenous sheet in appearance (**Fig. 3.6a**) while samples in **Figs. 3.6b&c** showed exfoliation, peeling and holes in the film structure. In case of **Fig. 3.6c** *i.e.* sample treated for 22 months, the peeling and loosening of the film were visible.

Sample no. 02, PE_B

The sample no. 02 showed a slightly different look. But still, there was uniformity in the film appearance. It was slightly wavy but a continuous, uninterrupted sheet (**Fig. 3.7a**). After 17 months, fractures on the surface are seen; eventhough a good portion of the film was found to be uninterrupted. At the end of the study fissures become more conspicuous which leads to its further deterioration into disintegration (**Fig. 3.7c**).

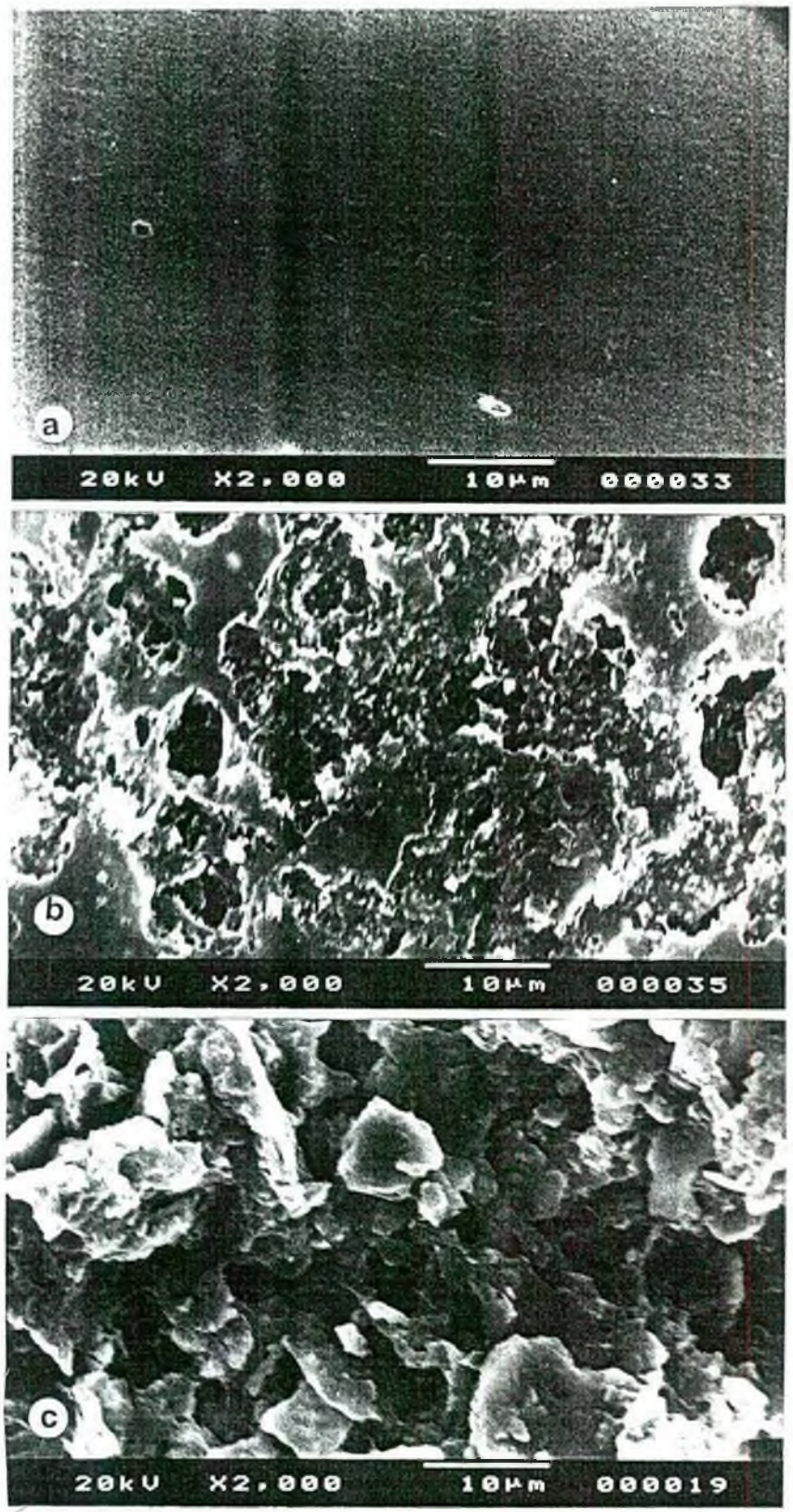


Fig. 3.6 (a-c): Scanning electron micrographs showing the structure and appearance of the sample no. 01 (PE_W) as revealed at 20 kV and x 2,000 (a) the control sample showing uniform and homogenous structure (b) numerous deeper holes formed after 17 months and (c) sample showing complete destruction of film integrity after 22 months. Loosening of portions is clearly visible.

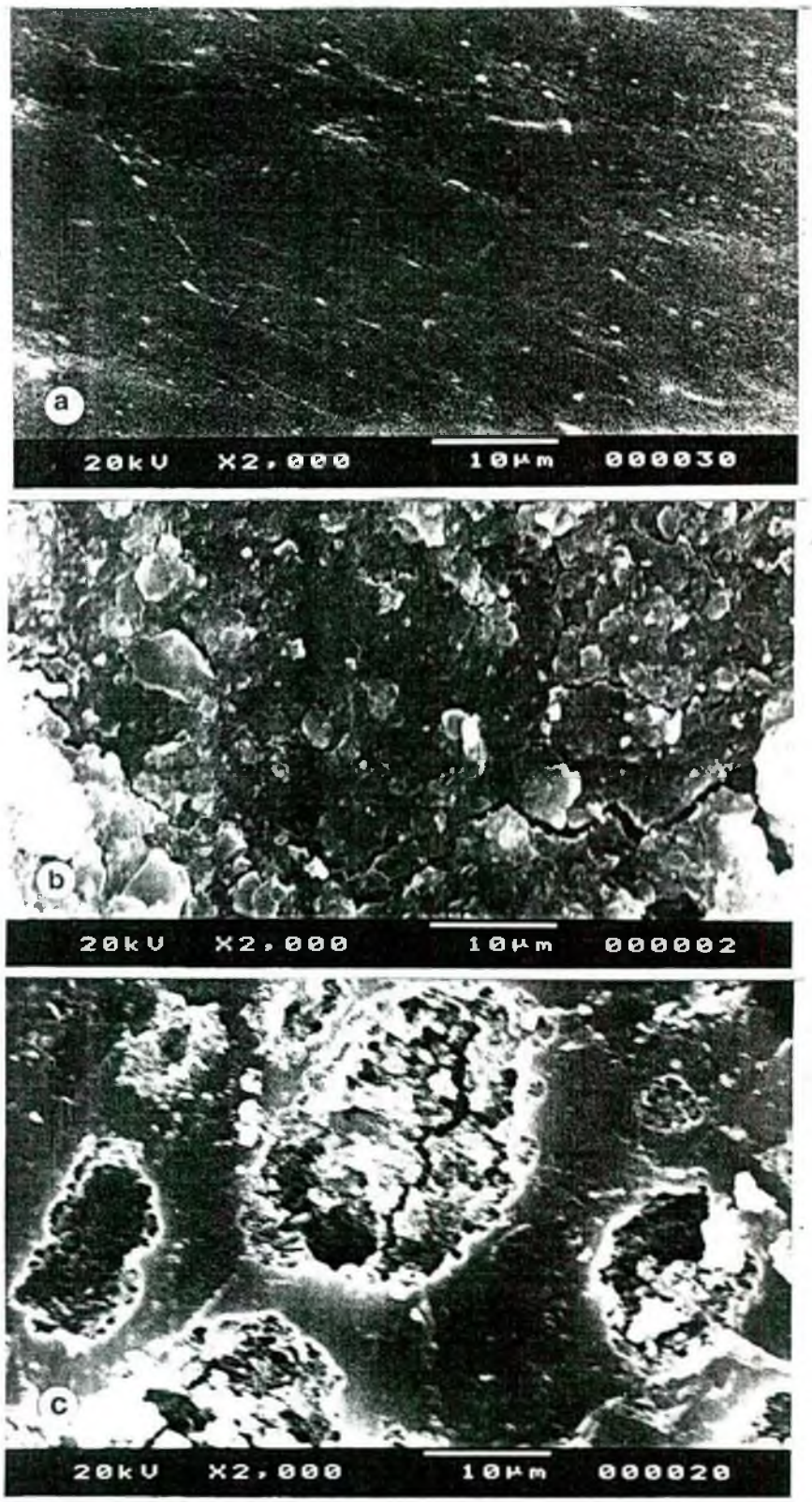


Fig. 3.7 (a-c): SEM images showing changes in Sample no. 02 (PE_B) during soil burial. (a) control film-showing uniform appearance (b) considerable fractures appeared on film after 17 months (c) cracks developed at the centre of globular patches resulted from microbial colonization.

Sample no. 03, $PE_{Tr\text{-}HW}$

In sample no. 03, the destructive process of degradation was very prominent. **Fig 3.8a** shows very finely granular and smooth appearance of the untreated sample. After 17 months, the original film surface become very rough with no planar areas left. **Figs. 3.8b** show gross upheaval of the surface structure. **Figs. 3.8c** presents much more devastation resulting from degradation which continued for 22 months in soil.

Sample no. 04, $PE_{Tr\text{-}LW}$

Sample no. 04 also shows various stages of degradation. **Fig. 3.9a** presents the general appearance of the untreated film, similar to other samples. **Fig. 3.9b** shows perforations which ultimately progressed with time. After 22 months, fractures resulted into fragments of different types (**Fig. 3.9c**).

Thus during soil burial experiment, the experimental polyethylene films degraded which was so vividly observed under Scanning Electron Microscope.

3.3.3 Discussion

According to Nishida and Tokiwa (1993), one of the mechanism of microbial degradation of PHB is the non-preferential spherical degradation of the PHB surface by bacteria. Colonization of hydrolyzing bacteria caused deep spherical holes on the surface.

Tansengco and Tokiwa (1998) established thermophilic microbial degradation of PES (Polyethylene succinate). They found that, microbial degradation of PES film surfaces proceeded uniformly with the formation of cracks.

Pranamuda *et al* (1995) evaluated the biodegradability of PTMS in soil environments. Microbial degradation were observed with the formation of cracks and hemispherical holes with a scanning electron microscope at various stages of cultivation.

Lauzier *et al* (1992) have examined the structure of PHB granules isolated from bacterial cells using SEM and identified the outer lamellar crystalline coating and non-crystalline core. Miyata and Masuko (1997) reported the use of TEM to examine the morphology and structural features of PLLA grown from acetonitrile solution by an isothermal crystallization technique.

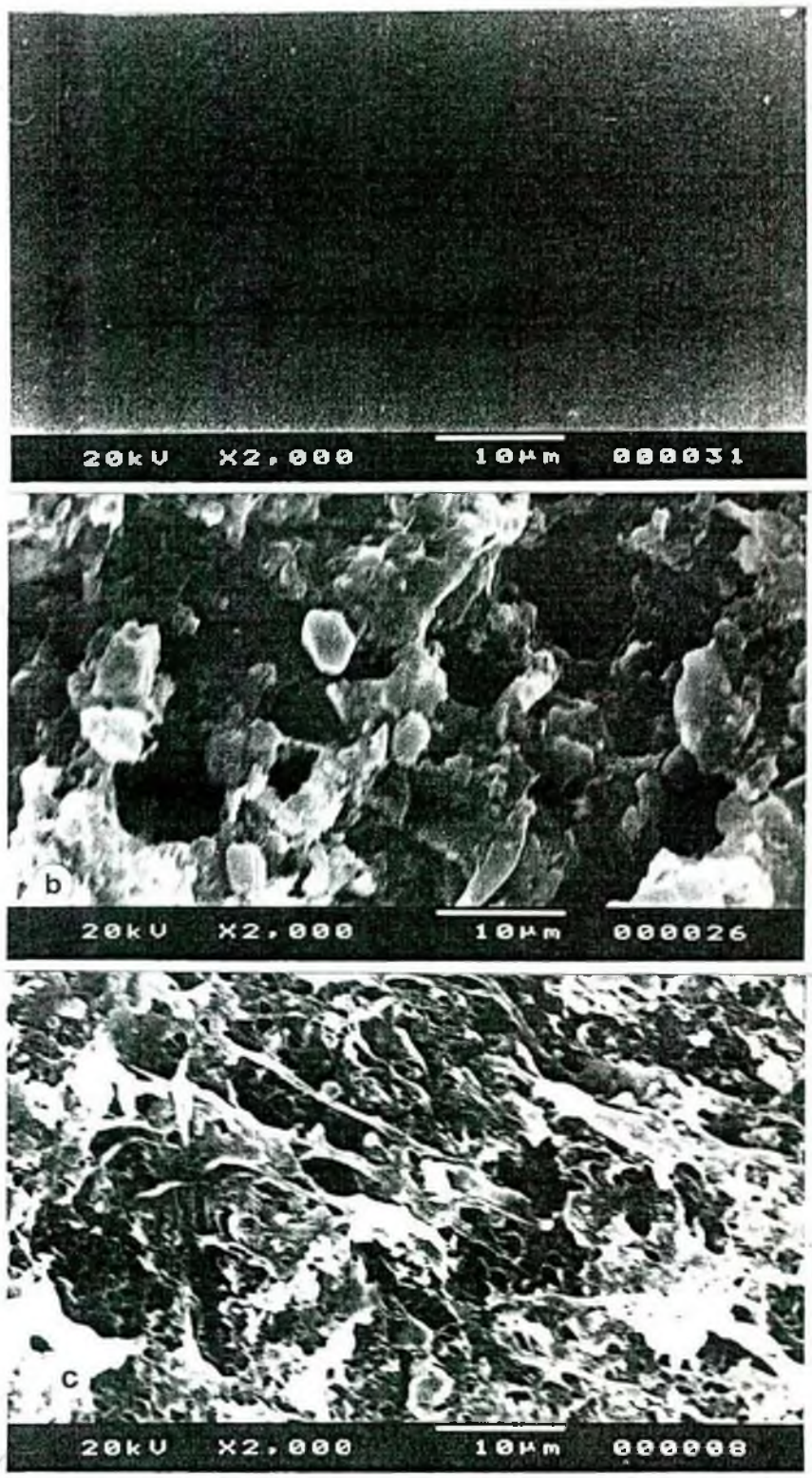
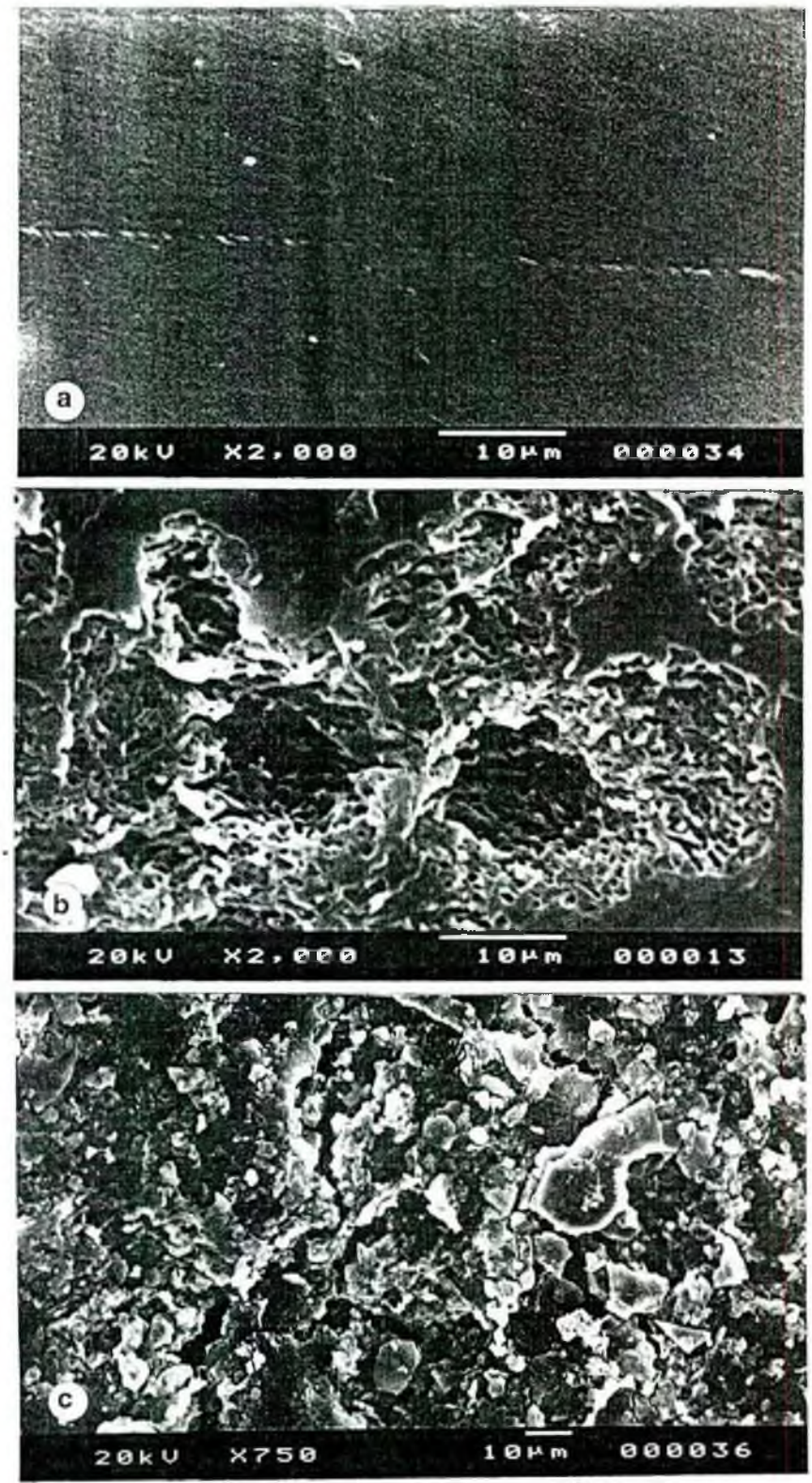


Fig. 3.8 (a-c): Electron micrographs showing changes in sample no. 03 ($PE_{Tr\text{-}HW}$) (a) untreated film having uniform structure, (b) breaking of smaller fragments (exfoliation) after 17 months and (c) fringe like appearance after 22 months.



of sample no. 04 (PE_{Th-LW})

Fig. 3.9 (a-c): Scanning electron micrographs showing changes in surface morphology after prolonged soil exposure; (a) the control sample appeared as continuous sheet, (b) sample showing irregular cavities on film after 17 months and (c) fissures progressed with fragmentation after 22 months.

During the present study, exposed LDPE samples exhibit progressive changes towards degradation. It took about seven to nine months for initial/primary colonization after which the process speeded up following microbial invasions, formation of cracks of film leading to disintegration. SEM images reveal distinct pattern of exfoliation as expected in the latter stages of degradation.

3.4 Conclusion

The results obtained throughout the experiments were very interesting and clearly convincing. The microscopic techniques used during the experiment were excellent and revealing. The results presented here explain beyond doubt that at least some polyethylene materials undergo degradation when remain buried under soil for a considerable period.

Chapter - 4

MECHANICAL PROPERTIES

Mechanical properties

4.1 Introduction

The study of mechanical properties of materials becomes an important consideration to correlate the response of different materials under a range of conditions in order to predict the performance of these polymers in practical applications. The properties of materials under the action of stresses and strains for various environments are important. The macroscopic properties of materials when subjected to these stresses and strains are called mechanical properties (Sultana 2000).

4.2 Nominal Stress-Strain Properties in Simple Tension

A specimen is subjected to an axial tensile force, P, by means of testing machine (**Fig 4.1**). At various increments of force, the change in length, l, of the specimen for an initial gauge length L_O are measured by a strain gauge. Then the initial force per unit of the original specimen cross-sectional area, is given by,

$$S = P/ A_O \qquad \text{---------- 4.1}$$

The stress is usually measured in Newton per square meter.

The tensile force produces stretching or elongation of the specimen. For the intermediate gauge length L of the specimen, the stress will be assumed to be uniformly distributed for all points of each cross-sections and for all cross-sections. This uniform stress will be assumed to produce a uniform elongation l. For the uniform elongation assumed, the deformation may be expressed by the elongation per unit length, or strain, ad can be expressed as,

$$\varepsilon = l/ L_O \qquad \text{---------- 4.2}$$

With the values of the stress, S and strain, ε known for various tensile load values, a diagram showing the relation between the stress and strain, called the stress-strain diagram. As the specimen stresses, the cross-sectional area/are reduces and the gauge length increases in value. The stress and strain values, as defined by equation 4.1 and 4.2, do not include these changes in area and gauge length but are based on the original values of area A_O and gauge length L_O. For this reason the stress and strain are called the nominal stress and strains.

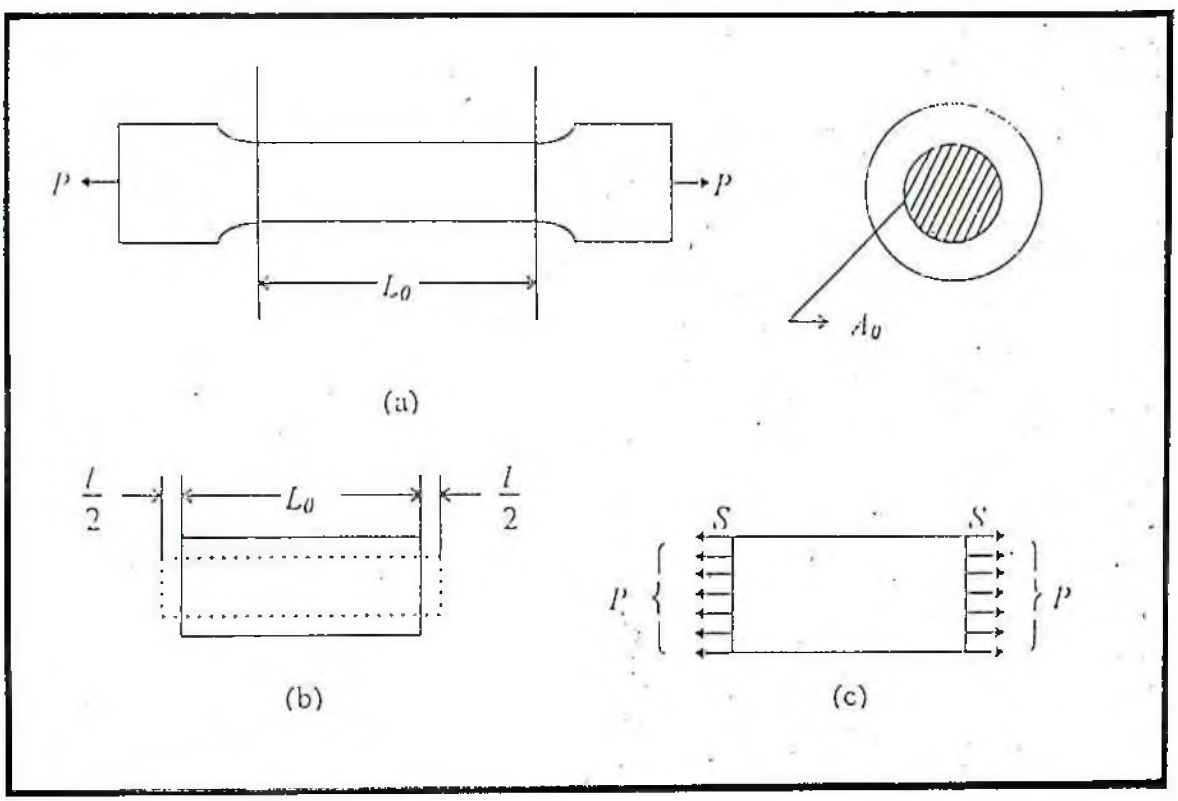


Fig. 4.1. Tension specimen.

There are two general ranges included in the stress-strain diagrams- an initial elastic range for which Hook's law generally applies, and a subsequent plastic range where the strain becomes large and Hook's law no longer applies. In the **figure 4.2**, the region OA is the elastic range and the region BE is the plastic range.

4.3 Young's Modulus

The ratio of tensile stress to strain is called the Young's Modulus, denoted by E and defined by

$$E = \text{STRESS/STRAIN}$$

E is measured in Newton per square meter.

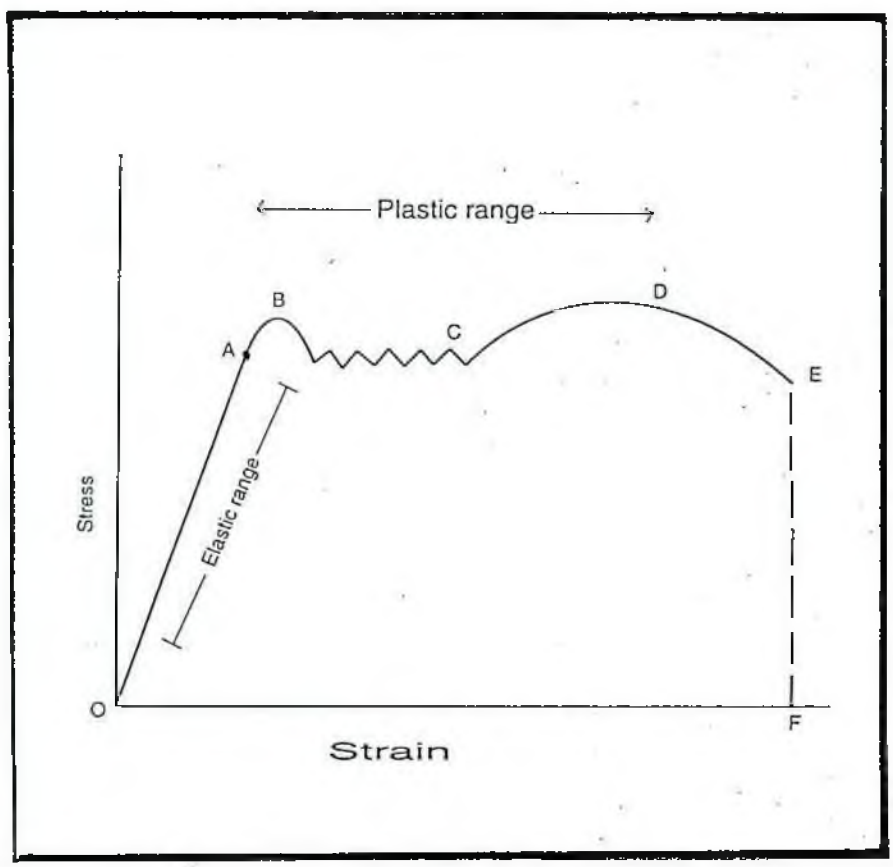


Fig. 4.2. Schematic diagram of a load-extension curve.

4.4 Mechanical Properties for the Elastic Range

The tensile mechanical properties of materials for the elastic range are those which represent the ability to resist loads and deformations and capacity to absorb energy in the elastic range.

4.4.1 Elastic Strength

A material has a high elastic strength if it resists loads without yielding and without being permanently deformed. Elastic strength is measured by the stress which represents the tension form the elastic range (where the strains are small and recoverable) to the plastic range (where the strains becomes large and are partly non-recoverable).

4.4.2 Elastic Limit

The elastic limit is defined as the maximum stress that can be applied to material without producing a permanent plastic deformation when the load is removed. In the **Fig. 4.2** the point B in the load –extension diagram denotes the elastic limit.

4.4.3 Hook's Law

"In an elastic limit the strain is directly proportional to the stress." It is applicable only for small strain. When the strain is large, this law is no longer applicable.

4.4.4 Work done within Elastic Limit or resilience

Work has to be done in stressing the material to 'destruction' under an increasing stress or tension. Up to the elastic limit, it remains stored up in the form of potential energy. Beyond the elastic limit, work done is converted into heat energy. The capacity of a body to resist a mechanical shock is known as resilience of the body. It is measured by the amount of work done in straining the body up to the elastic limit. The total work done is represented by the area (the area OABCDE in **fig. 4.2**) under the load-extension diagram. Resilience is measured in Joule.

4.5 Mechanical Properties for the Plastic Range

The tensile plastic properties of a material are the properties that define the ability to resist loads and deformations and the capacity to absorb energy in the plastic range. These mechanical properties are called plastic strength, durability and toughness and correspond respectively to elastic strength, stiffness and resilience in the elastic range.

4.5.1 Plastic Strength

A material has a high plastic strength if it resists loads without fracture. The plastic strength may be designated by either the ultimate strength corresponding to the ultimate stress or the rupture, breaking or fracture load divided by the original cross-sectional area of the specimen.

4.6 Experimental Method

The tensile behaviour of LDPE films buried under soil for more than 1 year was studied in details. The test was carried out at a test speed of 40 min/m.

4.6.1 Preparation of Specimens

The test specimens (LDPE Films) consisted of strips of uniform width and thickness. Strips were prepared by cutting the films into 50 mm long and 5mm wide pieces. Utmost care was taken to avoid nicks and tears in each specimen to minimize premature failure. On an average, 3-5 specimens were tested from each sample.

4.6.2 Test Procedure

Tensile strength of each specimen was tested by using a "LLOYD INSTRUMENTS LRX materials testing machine" (**Fig. 4.3**). The tests were made according to ASTM 882-91 specification. During each test, the test speed was kept constant. The specimen was placed in the grips of the machine taking care to align the long axis of the specimen with an imaginary line joining the points of attachment of the grips of the machine. The grips were tightened evenly and firmly to minimize the slipping of the specimen during the test. The cross-head was moved according to the set-up and the load and extension values were displayed on the LCD during the test.

4.6.2.1 LRX Materials Testing Machine

LLOYD INSTRUMENTS LRX materials testing machine (**Fig. 4.4**) is one of the versatile and comprehensive materials testing machines. It can be used as a stand-alone machine or it can be linked up to a remote computer where one can run data analysis software. All machines include an R-232 computer interface as standard. LRX machine is a very flexible and sophisticated instrument. It has been designed to meet the needs of both the research engineer and the quality assurance department. The power of the

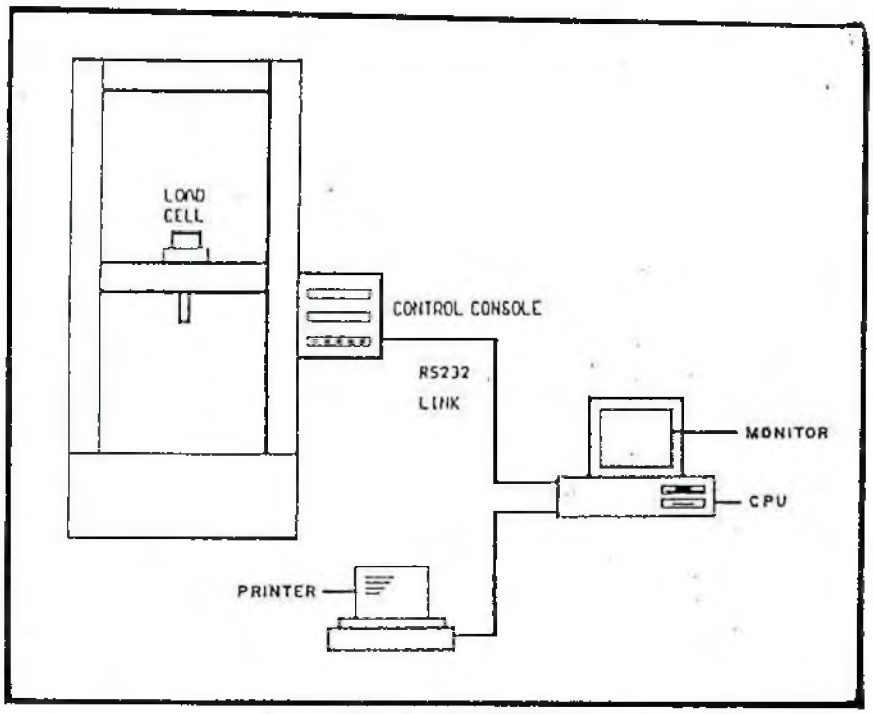


Fig. 4.3. Block diagram of test arrangement

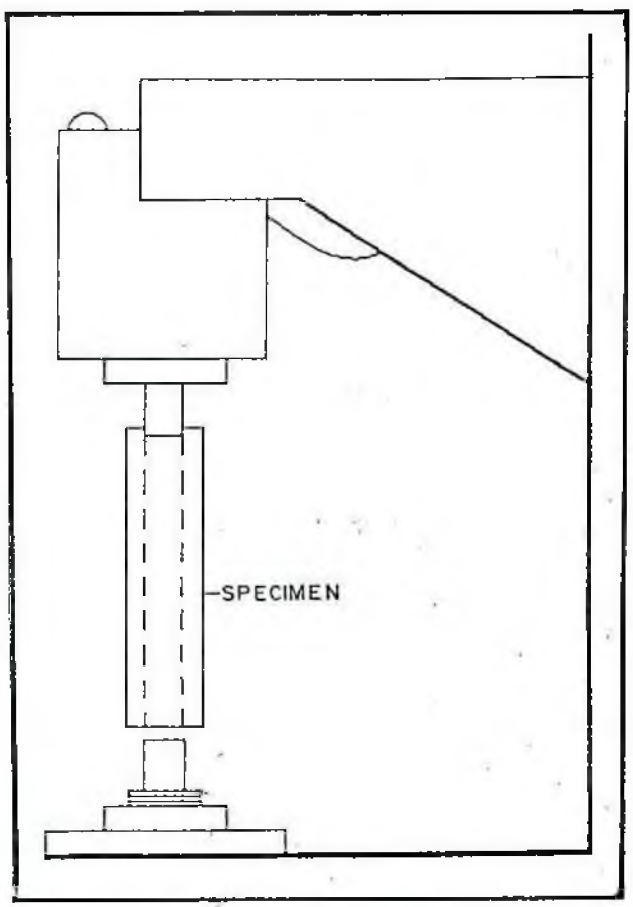


Fig. 4.4. LLOYD Instrument

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POLYETHYLENE AND PLANT GROWTH – A CASE STUDY

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Key words : Basil, Plant growth, Polyethylene

Abstract

Basil plants (*Ocimum basilicum* L. cv. Thai) were grown in eight plastic pots filled with garden soil to study the effects of polyethylene on their growth. In one set of pots, transparent polyethylene in the form of flakes were thoroughly mixed with soil; another set served as control. Two seedlings were grown in each pot and the growth rate was measured in terms of plant height, fresh- and dry weight. Plant height was recorded after every week and the experiment was continued for six weeks. Growth was not affected in the presence of polyethylene. In contrary to the general assumption that polyethylene pieces in soil affect plant growth adversely, present results showed little or no effect on the growth of basil plants due to the presence of polyethylene flakes in the rhizosphere soil.

Introduction

Since its discovery in 1933 and the initial boom in Industries, polyethylene found its use in radar, insulation cables, corrosion-proof constructions, packaging, laminating, house ware items and many other different fields. It was hailed for its unique properties like high dielectric strength, toughness, durability, flexibility, chemical inertness, moisture resistance etc.(1) But lately, it has earned a very bad reputation for being notoriously resistant to the natural processes of degradation including chemical, physical, photo- and biological degradation. When the use of polyethylene increased manifolds, the inevitable result was in the accumulation of waste. Tremendous amount of plastic wastes now account for about 10% of solid municipal waste.(2) The figure is increasing day by day. However, new technologies have been developed for getting rid of this environmental hazard. Besides proper disposal scheme, several alternatives have been proposed to make a better use of waste-polyethylene.

Industrial Consultancy and Marketing Services of India(3) has developed technology to use waste-plastic in construction. Brown *et al.*(4) recycled waste-polyethylene into food protein by chemical oxidation followed by microbial fermentation. Japan incinerates 70% and Sweden and Switzerland over 50% of

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their municipal waste to generate steam, hot water or electricity.[2] Karthigesan and Brown[5] used pyrolysis at 525 - 625°C for waste-polyethylene. Pyrolised polyethylene yielded 0.47g dry weights of cells of *Candida tropicalis* per gram pyrolysate utilized in batch culture and 0.339g per gram pyrolysate in continuous culture.

Waste polyethylene in soil obstructs sunlight reaching the soil, makes obstacle to the porosity and permeability thus decreasing the compactness and strength of soil. This reduces fertility of the soil and also renders it poorer for massive construction. These consequences can be visualized for sheets of polyethylene but the role of polyethylene chips or flakes should be examined carefully before any generalization. If shredded and mixed with the soil, then, probably the same polyethylene will increase the productivity of the soil providing plenty of air space within the soil particles and thus making it easy for root penetration and the uptake of nutrients. Ford[6] hypothesized the very fact and mentioned that the introduction of another xenobiotic constituents *viz.* polyethylene in natural spoil like earth will matter in the least.

Nowadays, millions of polybags are used for nursery propagation and transportation in Bangladesh as well as in India. Joshi of Pune, India has revolutionized Rose-growing and Joshi's 'Polybag method' has already become a common practice.[7] Introduction of UV-stabilized polybags in India for propagation has replaced the clay pots entirely.[8]

The effects of chemicals, growth regulators etc. on the growth of various plant species has been the major focus of study of horticulturists, plant physiologists and agronomists.[9-11] However, in Bangladesh, information on the plant-polyethylene interactions is virtually lacking. This preliminary study is an effort to support the view of Ford[6] and to examine the effects of polyethylene on the growth and morphology of Thai variety of basil plant.

Materials and Methods

Basil (*Ocimum basilicum* L., Bangla: Babui tulsi, English: common basil) is a member of family Lamiaceae and has a chromosome number 2n = 48.[12] Some cultivars of basil are highly prized and used for culinary purposes. Their medicinal uses are also well established.[13]

The experiment was conducted during December 2001 to January 2002 in the Botanical Garden of the Department of Botany (Curzon Hall Campus), Dhaka University. The experimental soil was a silty loam with pH - 6.83. Seeds of basil were brought from San Francisco, USA (Lilly Miller, 2002 Lot E-1) in September

2001. The seeds were sown at first in seedbed. When the seedlings were approximately 5 - 7 cm tall, 16 of them were carefully selected, uprooted and transplanted two plants per pot. Plastic pots were 16 cm high and 14 cm wide at the mouth.

In each of the four pots (pot nos. 1, 2, 3, 4), the soil was mixed with 5 grams of polyethylene flakes, purchased from a local industry at Urdu Road, Dhaka (Fig. 1). Another four pots served as control where only soil was used. Plastic pots were designated as P1, P2, P3 and so on. Plants were encoded as P1/1, P1/2, P2/1 etc. where the first No. was for the pot and the last one was for plants in each pot. Initial plant height was measured from soil surface to the apical tip just after plantation.

All the pots were exposed to natural condition and care was taken to keep the plants free from weed or insect infestation. The potted plants were watered regularly. Observations were made on the phenological developments of plants. Plant height was recorded at seven days interval. After six weeks, plants were taken out of soil carefully with root system, washed in tap water and the surface moisture was removed by pressing gently between tissue papers. Fresh weight of each plant (root + stem + petiole + leaves) was measured. Plants were dried in an electric oven at 80°C constant temperature for 24 hours. After drying, samples were weighed in a top loading electric balance (Sartorious 2355). Plant dry weight was measured on three consecutive days and an average was considered as the final dry weight in each case.

Results and Discussion

Figs. 2a and 2b show the growth of basil plants after six weeks. Records of plant height were plotted against the number of observations (in days). The bar graph in Fig. 3 shows a linear correlation except for P2/1 and P3/2. Plant height was highest (31.5 cm) in P1/1 and P7/1 followed by P3/1, P4/2, P7/2 and P8/1. Plant height was lowest in plant no. P2/1 (23.8 cm). Highest plant height was recorded in the pot one with polyethylene *i.e.*, P1/1 and another without polyethylene (in P7/1). Similarly, second highest values were obtained from both control and polyethylene treated plants.

Table 1 shows the data of fresh weight as well as dry weight of basil plants after six weeks. Fresh weight was 36.34 g in plant no. P4/2 which was the highest and had polyethylene flakes in the soil. Fresh weight ranged from 11.44 - 36.34 g while dry weight was in between 1.472 and 5.118 g. The ratio between fresh and dry

weight was in the range of 6.07 - 7.841. Fresh weight was six - eight times greater than the corresponding dry weight. Our results indicated that polyethylene flakes applied in the study did neither retard plant growth nor posed any deleterious effect on its phenological characters.

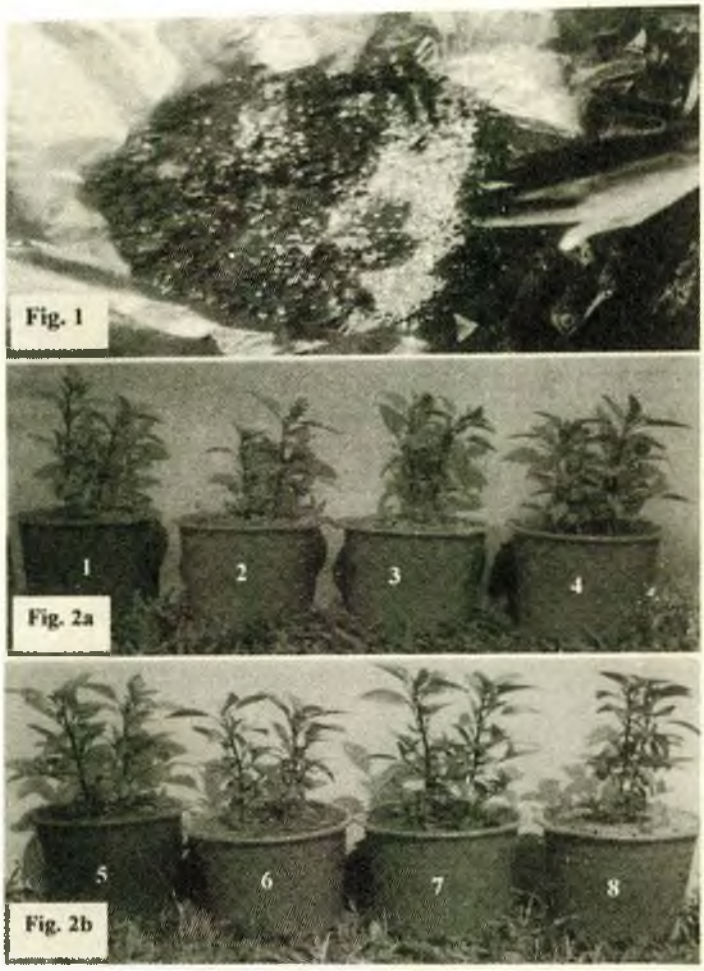

Fig. 1. Polyethylene flakes are being mixed with soil. Fig. 2 Basil plants grown for six weeks in pot culture. (a) Pot 1 - 4 had polyethylene flakes in soil. (b) Pot 5 - 8 had only garden soil.

In the last two decades, the use of polyethylene mulch in vegetable crop production has increased dramatically throughout the United States. Orzolek *et al.*[14] of Horticulture Department at Pennsylvania State University USA, investigated the effect of coloured polyethylene mulch on the yield of squash, tomato and cauliflower. They found that for all vegetables, coloured polyethylene mulch treatments produced significantly more yield than the bare ground treatment.

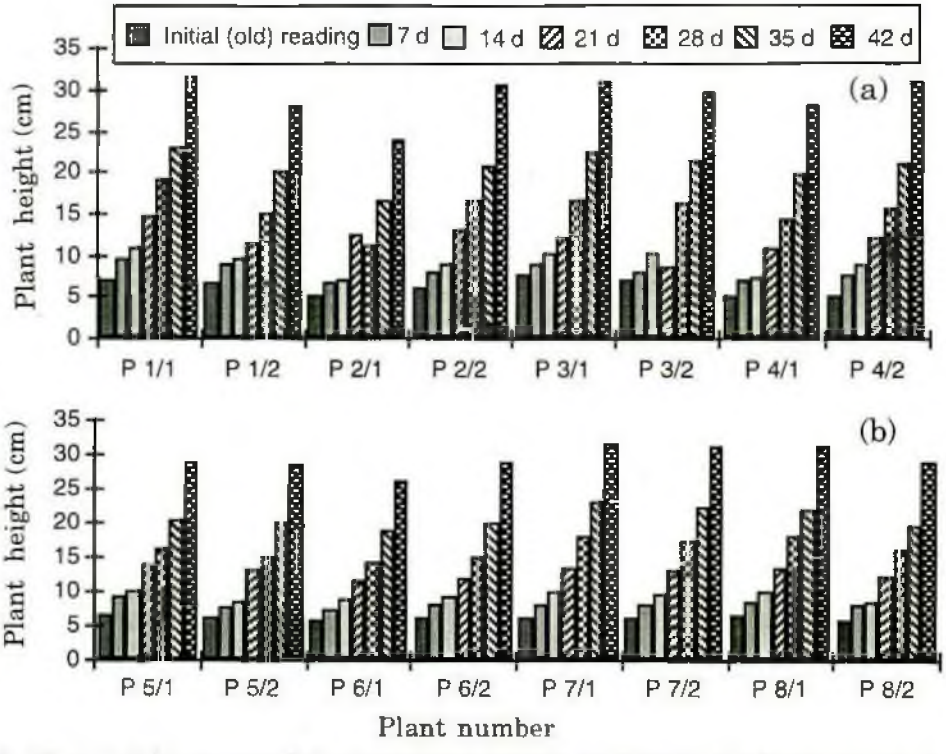

Fig. 3. Plant height as recorded at weekly intervals. (a) Height of plants growth in soil with polyethylene. (b) Height of plants growth in soil with no polyethylene.

Table 1. Fresh weight, dry weight and the ratio between these two of the experimental basil plants after six weeks.

Pot No.	Plant code	Fresh weight (g)	Dry weight (g)	Ratio between fresh/dry weight
1	P 1/1	27.32	4.5	6.07
	P 1/2	26.99	3.54	7.6
2	P 2/1	11.44	1.47	7.77
	P 2/2	27.08	3.86	7
3	P 3/1	22.59	3.06	7.37
	P 3/2	25.97	3.31	7.84
4	P 4/1	25.04	3.38	7.4
	P 4/2	36.34	5.11	7.1
5	P 5/1	30.63	4.44	6.89
	P 5/2	33	4.39	7.5
6	P 6/1	26.49	3.53	7.5
	P 6/2	27.47	3.94	6.97
7	P 7/1	32.2	4.59	7.01
	P 7/2	31.21	4.74	6.58
8	P 8/1	25.63	3.93	6.51
	P 8/2	21.13	3.05	6.91

At the end of six-week-experiment, it was interesting to observe that the polyethylene flakes added to the soil had no adverse effects on the grwoth of basil plants in terms of appearance, height, fresh- and dry weight. Moreover, growth was even better in some pots where no polyethylene was added to the rhizosphere soil.

Acknowledgements

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BIODEGRADATION OF POLYTHENE UNDER NATURAL CONDITIONS AND THE POSSIBLE ROLE OF ASSOCIATED BACTERIA

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Key words: Bacteria, Biodegradation, polythene

Abstract

Polythene samples randomly collected from garden refuses, open spaces and garbage dumps were inoculated in nutrient media (pH 7.0) for the enhancement of the growth of microorganisms which could have possibly started a process of colonization and adaptation. On prolonged incubation, one of the samples showed perforations and distintegration of polythene strips. Spore former bacilli were found to be dominating. The perforation and disintegration of the polythene strips in the presence of bacteria indicated the biodegradation of polythene.

Introduction

Inspite of innumerable uses in our modern civilized life, polythene has become an environmental problem of global magnitude. It poses particular threat to a third world country like Bangladesh where appropriate disposal system does not exist. Polythene is one of those non-biodegradable polymers which has already made its way in every nooks and corners of our home, society and environment. Nowadays, it is offered at any shop, be it a posh departmental store or a road side vendor. Once rejected, it clogs drains, occupies open spaces and becomes a part of the landfills posing all sorts of problems. In this country, raw materials for polypropylene (PP), polythene (PE) and polyvinylchloride (PVC) are imported in the form of granules. Small industries use these granules to produce toys, house hold items, packing materials, shopping bags, etc. Most of the shopping bags are rejected immediately after a single use. Garbage collectors collect these and sell for recycling while a significant amount goes to the refuse dumps, landfills and drains.

Lately workers are reporting biodegradation of compounds like polycyclic aromatic hydrocarbons, polyester, poly-β-hydroxybutyrate-co-valerate (PHBV), etc. (Pometto *et al.* 1992, Imam *et al.* 1995, El-Shafei *et al.* 1998, Johnson *et al.* 1993, Premanuda et al. 1997). But so far no specific report has been published on the biodegradation of unamended polythene. Polythene has been studied using a ^{14}C technique to determine its rate of breakdown in the soil (Allsopp and Seal 1968). Johnson *et al.* (1993) conducted several degradation experiments on degradable starch-polythene plastics in compost environment. El-Shafei *et al.* (1998) demonstrated biodegradation of disposable starch (6%) polythene by fungi and *Streptomyces* sp. In the present study we emphasized on the

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microflora growing on and around polythene under natural environmental conditions and their possible biodegradation activities on polythene over a period of time.

Materials and Methods

Polythene materials were collected from different areas of Dhaka metropolitan City specially from garden refuses, garbage dumps and open spaces. The collected samples were maintained at room temperature in the laboratory. The samples were cut into thin strips and were inoculated in Nutrient agar (pH 7.0) and Sloppy agar (Agar 0.5%) with added Tetrazolium chloride (0.001%). A set of culture flasks (250 ml) containing Nutrient broth inoculated with polythene strips were incubated at 37°C. The flasks were shaken regularly by using a Gyratory shaker (MRK Recipro Box Shaker). The strips were examined regularly and diferent types of microscopy including bright field, dark field, phase contrast and direct epifluorescence (Nikon Optiphot and Nikon Microphot) were used.

Results and Discussion

Polythene strips directly placed on Nutrient agar medium yielded growth within 24 hours while after 48 hours red visible bacterial growth appeared on Sloppy agar (Fig. 1A). Organisms adjacent to the test strips were isolated on respective media and were preserved for detail study. Initially, the polythene strips had sharp edges (Fig. 1B) but after 40 days strips from one of the culture flasks showed a wavy undulated margin with adhered bacterial growth (Fig. 1C), which could be considered as a sort of colonization. On the surface of the test strips, masses of bacterial cells were also noticed as globular patches of different sizes. Some of the observed globular patches had small perforations at their centres (Fig. 1D). In some cases the perforations were quite large which could be considered as an evidence of degradation. The globular patches when examined closely under phase contrast microscope, revealed the presence of bacterial spores (Fig. 1E). Finally, after 70 days of incubation, small disintegrated pieces of polythene were observed in the sediment which were made visible with direct epifluorescence using acridine orange and a blue filter (Fig. 1F)

Scientists all over the world (Mas-Castella *et al.* 1995, Mergaert *et al.* 1993, Byrom 1993, Ohtaki *et al.* 1998) are reporting about biodegradation of different tough materials and the volume of information is growing everyday. But so far no specific report has come up on the biodegradation of polythene. Shirahama *et al.* (1996) observed small cavities of 200 nm diameter to appear in the polyester poly [(R)-MOHEL-ron-CL] after 14 days of treatment with aclimated sludge. Imam *et al.* (1995) demonstrated significant disrupption of surface layers and spherical holes in Poly β–hydroxybutyrate-co-valerate (PHBV) with no starch blend after 0, 14 and 35 days of exposure to activated sludge.

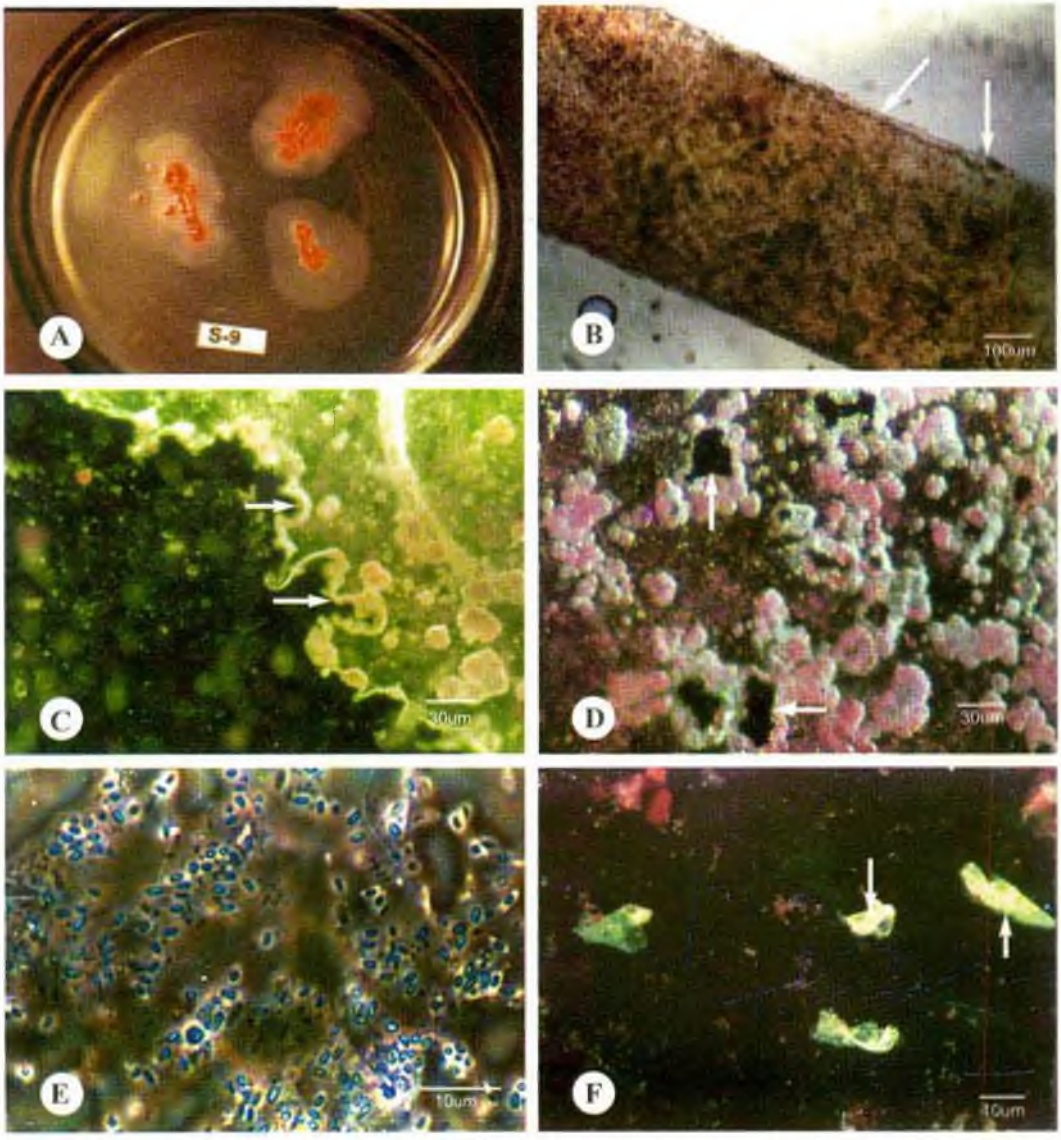

Fig. 1A-F. A. Bacterial growth (red) around polythene strips, B. Poythene strip with sharp edge (arrow) before culturing, C. Test strip with wavy margin (arrow) and bacterial cells, D. Bacterial colonies with perforations (arrow) on polythene strip, E. Bacterial spores under phase contrast microscope, F. Fragments of polythene film observed under direct epifluorescence (acridine orange and blue filter)

Premanuda *et al.* (1997) reported hemispherical holes on the surface of poly lactic acid (PLA) films in the inoculated culture of *Amycolatopsis* sp. which is quite in line with

our present work on polythene. The three major findings were, (i) presence of spore-former bacilli with polythene, (ii) perforations of the inoculated polythene strips and (iii) disintegration of the inoculated strips indicate the degradation of polythene in the presence of bacteria. Isolated bacteria are to be studied in detail. Their identity and possible role in the biodegradation of polythene will be published in near future.

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BIODEGRADATION OF POLYETHYLENE UNDER LABORATORY CONDITIONS

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Key words: Biodegradation, Microorganisms, Polyethylene

Abstract

After the initial observation of polyethylene degradation under natural conditions, several experiments were carried out and the results are reported in the present paper. The experiments included observations on preliminary adherence, dye release, acid release, oxygen consumption, loss of transparency, extensive mycelial growth and disintegration of polyethylene films. The results were indicative of biodegradation.

Introduction

Polyethylene has been identified as a recalcitrant macromolecule and ordinarily non-biodegradable in nature. Once it is rejected, it starts being accumulated in the surounding. It not only spoils the aesthetics of our landscapes, but also occupies space making it unusable for construction work and cultivation. In the drain and sewage systems, it may cause blockage resulting in the breakdown of the system. Like any other material of common human use, polyethylene should also have some means and ways for removal, decomposition and recycling. But, it is not affected by common environmental factors for degradation. Hence, it has already been termed as non biodegradable.

In Bangladesh locally made polyethylene shopping bags were first produced in 1982 (Star magazine, 10 March 2000) and since then it is being imported in the raw forms of low density polyethylene (LDPE) and high density polyethylene (HDPE). These raw materials are sold in the wholesale market. From there go to the retailers and then to the thermoplastic industries of the country.

Amongst numerous uses of polyethylene, the use of shopping bags is possibly the most common and extensive. Moreover, in most of the cases, these polybags are thrown away after a single use causing one of the greatest environmental problem of our time. Concerted efforts of scientists and engineers are needed for the safe and effective disposal of waste polyethylene and if possible, to find out its better and cost effective uses. Some sort of degradation of polyethylene was observed with associated microorganisms under natural conditions (Khan *et al.* 2000). The present study was under taken to examine the process of biodegradation in further detail under laboratory conditions.

Materials and Methods

Commercially available polyethylene pellets and granules were purchased from Chawk Bazar, the wholesale market of Dhaka, while the polybags were bought from Dhaka New market. The materials were sterilized with ether and dried before charging with the selected microorganisms. Naturally occurring microbial consortia were also employed for the degradation studies.

Changes in pH, optical density (O.D.) and dissolved oxygen (D.O.): For observing the changes in pH, O.D. and D.O. values, six conical flasks (1 litre) containing 540 ml of medium (diluted Nutrient broth 1:10, plus soil extract and phosphate buffer solution) and 1g of polyethylene in the form of thin films were inoculated with 60ml each of the selected bacterial inocula (7/1^{II} and 7/2^{II})

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and incubated at 30° C in a water bath. For aeration, air was passed through a column of glass wool and purged through the culture. The flask without polyethylene film served as film control while the flask without bacterial cells served as cell control. After every two weeks, 10 ml aliquots of culture broth were withdrawn from each of the flasks with sterile syringes and were analyzed for the changes in pH, O.D. and D.O. values. Optical density was measured at 550 nm wavelength. The used instruments were Jenway pH meter 3310, Lutron D.O.-5510 oxygen meter and Shimadzu UV 120-02 Spectrophotometer.

Dye release: Two sets of conical flasks (250ml) containing 90 ml modified glucose asparagine broth (supplemented with beef extract, pH 7.0) was inoculated with 10ml of selected bacterial inocula and incubated at room temperature on a shaker (MRK Recipro Box Shaker). Blue and orange coloured pellets were used as test samples.

Flask culture experiment: In this experiment conical flasks (250 ml) containing 100ml nutrient broth (pH 7.2) were inoculated with mixed bacterial cultures obtained from sample no. 7 and incubated at room temperature. To enhance microbial growth and activity, the flasks were shaken for an hour everyday. The polyethylene strips were examined periodically under the microscope for any change in appearance, texture or optical characters.

Petri dish culture experiments I and II: In experiment I, polyethylene discs were floated in petri dishes containing motility broth (nutrient broth having 0.001% TZC) were inoculated with strains 6/1and 6/2. The inoculated dishes were incubated at room temperature.

In experiment II transparent polyethylene granules were added to motility broth, inoculated with isolate no. 7/1^{II} and incubated at a room temperature.

Soil burial experiment: Six plastic pots were filled with garden soil and 12^{II} x 2^{II} polyethylene films were buried. Three of the pots were covered with liner bags while three were kept open. No extra nutrient was added. The pots were kept at room temperature. In accordance with the work of Yabbanavar and Bartha (1994), a six months period was allowed for incubation after which the polyethylene films were retrieved and tested microscopically for any change in their surface characters (transparency, biofilm, texture, etc.) and the mechanical properties *viz.* tensile strength and percentage elongation at break values were tested with computerized INTRON (Model 1011, England) machine at AERE, Savar, Dhaka.

Experiment with microbial consortia: Thin strips of polyethylene films were placed in conical flasks and pond water as such was added just to submerge the films. The flasks were kept at room temperature and the films were observed periodically using different types of microscopy.

Results and Discussion

During the course of incubation, both D.O. and pH values showed a declining tendency after initial rise (Table 1). The initial rise of dissolved oxygen could be due to the aeration. The air was gradually consumed by growing organisms. The increase of pH value at the onset could be due to the metabolites produced by the organisms but subsequently decreased due to acids released from the substrate. In case of O.D., which is considered to be a measurement of growth (biomass), the values first rose reflecting the utilization of culture media. With the gradual consumption of nutrients, the value dropped. As organisms started utilizing the substrate (polyethylene), it showed an upward trend.

Colourless polyethylene granules showed adherence of bacterial cells, which appeared red due to the reduction of tetrazolium chloride (Fig. 1a). Blue and orange coloured pellets, when acted upon by selected bacterial strains, showed a remarkable change in colour intensity. The pellets became lighter and dull in appearance which was clearly an indication of dye release (Fig. 1b). Polyethylene strips and discs inoculated with bacteria showed considerable changes in the surface

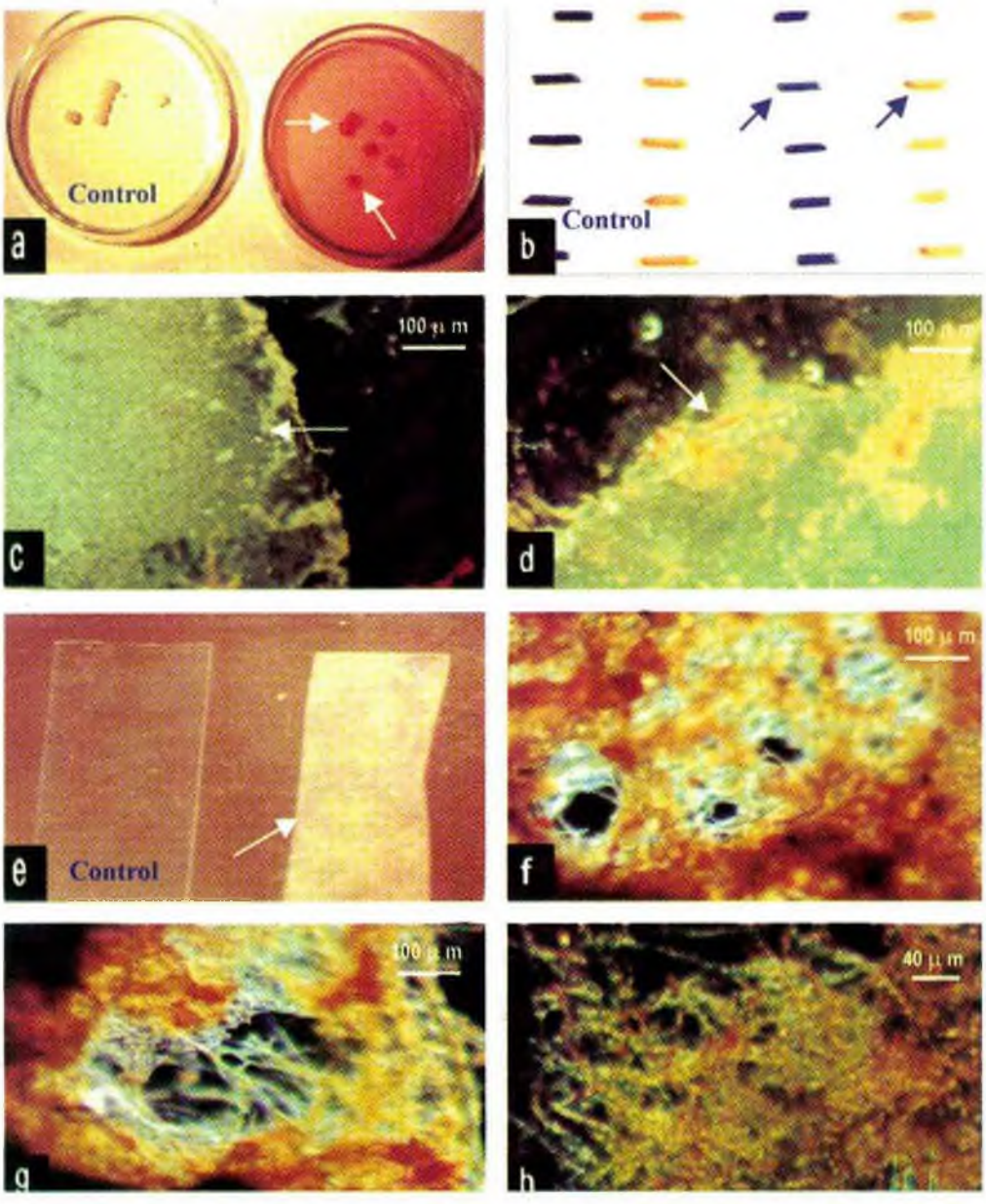


Fig. 1. Photographs showing process of degradation: a) Microbial growth adhered to polyethylene granules-red colour due to the reduction of tetrazolium chloride. b) Loss of colour of the blue and orange polyethylene pellets c) & d) Polyethylene discs showing dentations and associations of microorganisms. e) Loss of transparency of the polyethylene film after six months of soil burial. f-h) Photomicrographs showing extensive mycelial growth and stages in the degradation of polyethylene film.

texture. The polyethylene discs floated on nutrient broth showed clear dentations along the margin which also had microbial growth (Figs. 1c, d)

Table 1. Changes in dissolved oxygen, pH and optical density.

Parameters	Readings	7/1**		7/2**		Control	
		+ PE	– PE	+ PE	– PE	+ PE	– PE
Dissolved oxygen	Initial	7.9	8.0	7.9	8.0	7.5	7.5
	First	8.1	9.0	9.1	9.1	8.2	8.3
	Second	7.4	8.1	6.5	8.4	8.6	8.3
pH	Initial	7.27	7.25	7.25	7.23	7.24	7.24
	First	7.47	7.55	7.54	7.54	7.32	7.26
	Second	7.54	7.6	7.58	7.55	7.38	7.11
	Third	7.19	7.39	7.31	7.11	7.00	6.92
Optical density	Initial	0.1313	0.1549	0.1524	0.1555	0.0310	0.0310
	First	0.5361	0.1765	0.2660	0.4659	0.1979	0.0154
	Second	0.1118	0.1266	0.1079	0.2839	0.0846	0.0259
	Third	0.3893	0.4400	1.113	1.3665	0.4023	0.1938

*Third reading could not be recorded due to the malfunctioning of D.O. meter. Note: + PE = with polyethylene, –PE= without polyethylene.

Test strips retrieved after 6 months of incubation showed reduction in both tensile strength and elongation at break values. Buried strips lost transparency (Fig. 1e) and there was also an extensive mycelial growth on the film.

The polyethylene films treated with pond water developed visible microbial growth (Fig. 1f-h) during two years time. Polyethylene strips become so soft and weak that it was difficult to pick those intact. Under microscope, disintegrated pieces appeared nothing but a continuous mat of mycelial growth. On slightest disturbance, pieces came apart proving loss of integrity due to effective biodegradation.

Biodegradation of a recalcitrant polymer like polyethylene is indeed a very slow and lengthy process and also extremely difficult to observe the process in action. World's leading authorities like The American Society for Testing and Material (ASTM), Organization for Economic Co-operation and Development(OECD) and European committee of Normalization (OECD) have proposed some specific experiments to study the process of biodegradation of polymer materials.

Standard test methods recommended the use of plastic samples in the shape of films, fragments, pieces or formed articles for degradation experiments (Calmon-Decriaud *et al.* 1998). In the present study, polyethylene films in the form of discs, strips, pellets and granules were used for experimental purposes. Incubation at room temperature served two purposes-- good reproducibility and simulation of environmental condition.

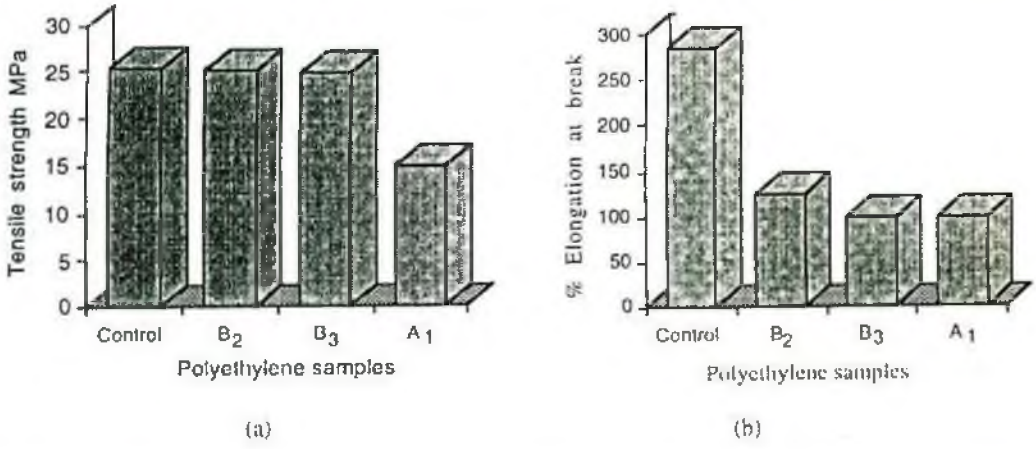
Shirahama *et al.* (1996) found that biodegradation under aerobic condition is preferable than anaerobic condition for polyester poly-[(R)-MOHEL-*ran*-CL]. Most of our experiments were designed to assess aerobic biodegradability of polyethylene in liquid culture conditions.

In some experiments, biodegradation was monitored by microscopic analysis. All available types of microscopy were employed to examine the surface texture as well as the margin of polyethylene materials. Janik (1998) also recommended the application of microscopy in estimating biodegradation. He pointed out that microscopic techniques allow to follow the changes on the surface of the materials as well as along the margin. Optical transmission microscopy having facilities like bright-field, dark-field, phase-contrast , as well as UV microscopy using fluorescent dye, *e.g.;* acridine orange were frequently used during the course of the present study. Mas-Castella *et al.* (1995) used SEM for the analysis of physical properties of the poly-β-

hydroxyalkanoates (PHA) film. ISO, ASTM also recommended microscopy for the visual assessment in the Petri dish screen test. Now-a-days, a range of different microscopy based techniques have been used to study the surfaces structure and erosion characteristics during biodegradation (Amass *et al.* 1998).

Tetrazolium chloride was used to visualize and monitor bacterial growth in association with polyethylene materials. Polyethylene discs in nutrient broth with added tetrazolium chloride showed marked dentations of the edge. On the other hand, after a period, transparent polyethylene granules found coated with bacterial film a sign of colonization. Flemming (1998) explained five mechanisms through which the structure and function of synthetic polymeric materials can be damaged by biofilms.

Changes in mechanical properties of polyethylene film during soil burial test are shown in Figs. 2a-b. Our results are in accordance with the findings of Yabbanavar and Bartha (1994) and Pometto *et al.* (1992). Bikiaris *et al.* (1998) studied octanoated starch blend LDPE during soil burial for 6 months and obtained a reduction in mol. wt. as well as in the mechanical properties such as tensile strength and elongation at break.



Figs. 2a-b. (a) Changes in the tensile strength and (b) percentage elongation at break values of polyethylene samples after six month of soil burial.

Our experimental results suggested that under the prevailing humid tropical conditions of Bangladesh polyethylene undergoes biodegradation by the activity of indigenous microflora. During past decades, workers have reported on the biodegradation of polymers and macromolecules like nylon, polyester, PHB, dyes, pesticides etc. but no report was available so far on the unammended polyethylene. In our experiments the observed results with acid release, dye release, loss of transparency, reduction in tensile-strength and elongation break values, dentation, surface erosion, extensive mycelial growth and irregular disintegration all are indicative of biodegradation of polyethylene. Biodegradation of polyethylene under natural conditions with the indigenous microflora was studied in detail with different types of microscopy including Bright-field, Dark-field, Phase-contrast and Fluorescent and our findings have already been reported (Khan *et al.* 2000). The work is still in progress and more information are expected to be published in due course.

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materials system is increased dramatically when the LRX machine is used with R control software which gives access to many more powerful features of LRX machine including cycling, constant load, creep testing, constant load rate and constant strain rate. The machine itself provides very comprehensive facilities for both tensile and comprehensive testing over very wide load and speed ranges. Data output in local mode is available via a highly legible LCD and to an optional XY plotter (LLOYD instrument PL 3/A 148). When used with R control software in remote mode, data output is both to a VDU (monochrome or color graphics) and also to a dot matrix printer (EPSON compatible). When using a remote computer, LLOYD instrument data analysis software package, DAPMAT, can be used to give plastics, metals, tear and peel, springs and other general testing standards.

4.6.2.2 Data Analysis Software

LLOYD instruments data analysis packages (DAP) software acquires, records, analyses, stores and prints test data instantaneously without operator skill or manual effort. Data analysis software simplifies the process of analyzing test results. It removes the guess work form graph interpretation and perform calculations such as psi, area under the graph, offset yields etc. In second, software provides a very powerful tool for analyzing the data produced by the LLOYD instruments materials testing machine. The LLOYD instruments data analysis packages run on most IBM, PC, XT or AT computers. DAP software are suitable for testing plastic, metals, tear and peel, stress relaxation and many more.

Available Options of the software Operation
Maximum load
Maximum stress
Extension/Deformation at maximum load
Strain at maximum load
Load at break/rupture
Stress at break/rupture
Extension/Deformation at break/rupture
Percentage Elongation after Fracture
Area under curve
Modulus of Elasticity
Maximum strength
Strength at break
Pass/fail limit
Sample Dimensions
Static loads
Static Extensions

Table 4.1 LRX Specification

LRX SPECIFICATION	
Maximum Force (Tension & Compression)	2.5 kN (550 lbf)
Overall force range using interchangeable load cells	0.5-2.5 kN
Force measuring system	Meets BS 1610:1992, BSEN10002-2: 1992 Grade 0.5, ASTM, DIM 51221
Force measuring accuracy	Better than 0.5%
Cross-head speed range	0.1-1000 mm/min (0.01-40 in/min)
Cross-head speed accuracy	+0.5% of set speed
Clearance between starting axis and column	125 mm (5 in)
Maximum cross head displacement	750 mm (30 in)
Cross head range setting	1-750 mm (0.05-30 in)
External extensometer connection	Optional
Analogue Load and Extension outputs for plotter/recorder	Standard
Internal extensometer accuracy	Better than 0.5% of range set
Frame stiffness without load cell	Greater than 2.5 kN/mm
Supply voltage	220V/240V ac 50 Hz (Fuse 5A anti-surge) or 110V/120V ac 60 Hz (Fuse 10A anti-surge)
Power consumption	500 watts maximum
Main Frame dimensions	Height 1233.0 mm (48.54 in) Depth 379.5 mm (14.94 in) Width 564.0 mm (22.20 in)
Console dimensions	Height 390.0 mm (15.4 in) Width 340.0 mm (13.4 in) Depth 80.0 mm (3.2 in)
Mass	44 Kg

4.7 Results

The results for Load-Extension curve, Maximum Stress and Strain at max. Load (%) were measured following ASTM specification D 882-91. The measured data for tensile properties of four tested Polyethylene samples are given in Table 4.2- 4.5.

Figure Nos. 4.5 - 4.8 are the load extension curves for polyethylene samples after 0, 01, 03, 15 and 17 months of soil burial. In these curves, AB≡ elastic region, BC≡plastic region and CE ≡ break. It is seen from the load-extension curves of four samples that,

Table 4.2. Experimental data for mechanical properties of **Sample no. 1** after soil burial expt.

Duration in Months	Max.Load (N)			Max.Stress (N/m. m^2)			Ext.@MaxLoad (mm)			Strain@Max Load (%)			Stress@Break (N/mm^2)		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
0	24.28	3.16	1.82	127.8	16.6	9.58	66.7	47.73	27.55	430.0	305.93	176.63	7.853	6.884	3.97
1	24.41	1.73	1.22	139,.5	9.9	7.0				415.7	31.2	22.06	99.98	49.32	34.87
3				134.73	78.87	39.44				344.95	281.65	140.82	42.05	49.0	24.5
15	12.0	0.29	0.21	72.09	1.73	1.22	1.218	0.042	0.029	7.611	0.263	0.185	0.00	0.00	0.00
17	13.12	1.74	0.87	71.33	41.72	20.86				8.601	5.021	2.51	0.00	0.00	0.00

Table 4.3. Experimental data for mechanical properties of **Sample no. 2** after soil burial expt.

Duration in Months	Max. Load (N)			Stress (N/m m^2) Max.			Ext.@MaxLoad (mm)			Strain@Max Load (%)			Stress@Break (N/mm^2)			S
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	M
0	25.09	1.54	0.889	250.9	15.4	8.891	57.93	9.48	5.47	371.4	60.8	35.10	18.99	4.7	2.71	4
1	23.80	0.86	0.49	238.0	8.6	4.96				73.53	12.37	7.14	16.28	0.00	0.00	1
3	17.50	1.00	0.50	218.7	12.5	6.25				165.7	49.9	24.95	24.16	19.20	9.6	1
15	14.85	0.29	0.20	148.5	2.9	2.05	5.804	0.35	0.25	36.27	2.17	1.534	0.00	0.00	0.00	0
17	13.35	2.81	1.26	133.5	28.1	12.56				26.60	20.04	8.962	15.46	18.29	8.18	5

Table 4.4. Experimental data for mechanical properties of **Sample no. 3** after soil burial expt.

Duration (Months)	Max. Load (N)			Stress (N/m.m^2) Max.			Ext.@MaxLoad (mm)			Strain@Max Load (%)			Stress@Break (N/mm^2)		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
0	36.49	3.41	1.97	192.0	18.0	10.39	251.6	38.2	22.05	1613	244.9	141.39	5.71	4.95	2.86
1	21.36	0.86	0.61	47.47	1.92	1.36				26.29	15.15	10.71	13.11	3.20	2.26
3	24.62	1.44	23.19	48.29	2.62	1.51				401.4	338.6	195.49	13.56	9.07	5.24
15	20.85	4.71	2.35	52.13	11.79	5.89	2.351	1.592	0.796	14.69	9.95	4.98	1.017	2.035	1.017
17	14.0	3.86	1.73	23.33	6.43	2.87				7.934	2.455	1.097	1.628	2.229	0.996

Table 4.5. Experimental data for mechanical properties of **Sample no. 4** after soil burial expt.

Duration (Months)	Max.Load (N)			Max.Stress (N/m.m^2)			Ext.@MaxLoad (mm)			Strain@Max Load (%)			Stress@Break (N/mm^2)		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
0	18.99	0.85	0.49	126.6	5.6	3.23	57.37	62.73	36.21	367.8	402.1	232.15	9.042	8.287	4.784
1	12.41	1.44	1.018	62.05	7.19	5.08				8587	5.21	3.68	13.22	7.19	5.081
3	18.17	2.38	1.06	82.61	10.83	4.42				87.13	56.72	23.16	10.48	11.62	4.74
15	14.85	2.59	1.83	49.51	8.63	6.1	6.632	0.265	0.187	41.45	1.66	1.17	2.713	3.836	2.712
17	17.63	0.23	0.132	64.12	0.85	0.49							9.864	12.32	7.113

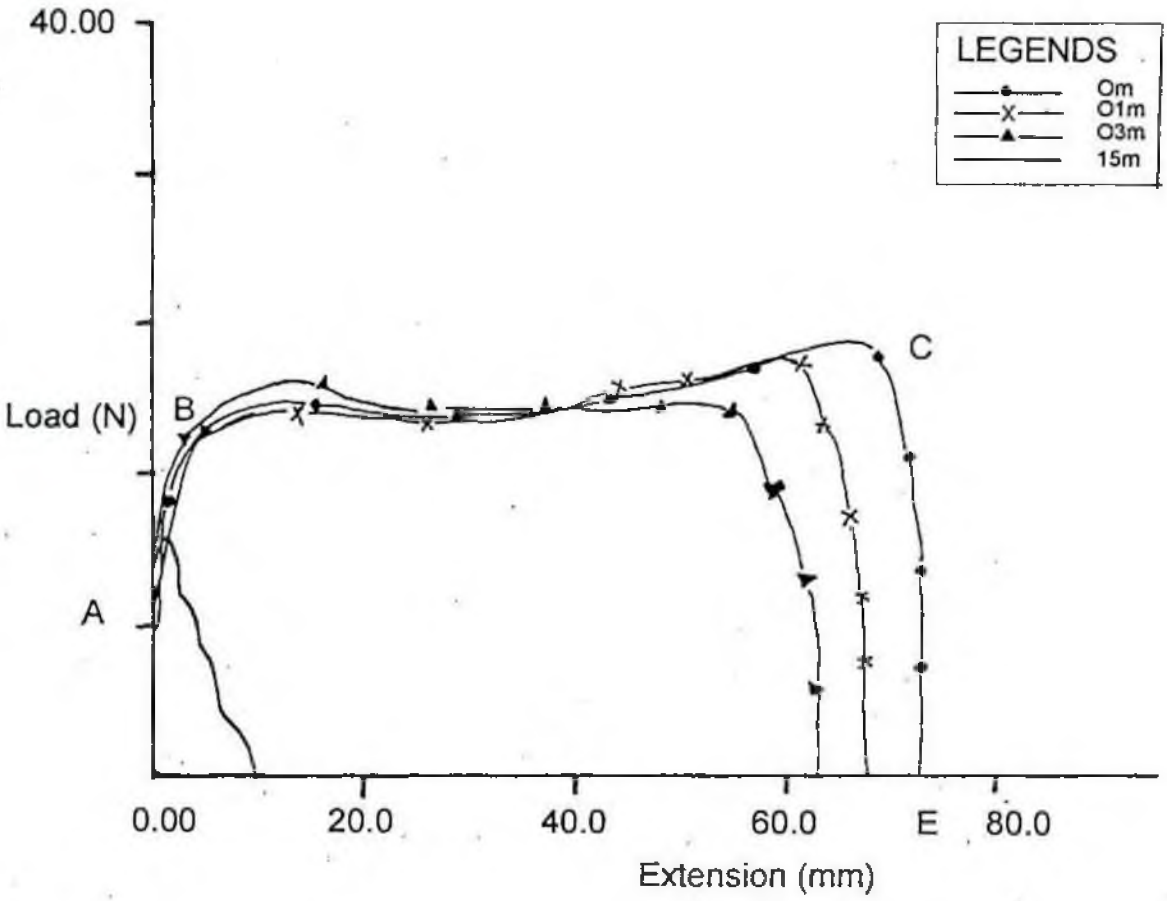


Fig. 4.5. Load-extension curve of LDPE sample No. 01 after O (—●—), 01 (—×—), 03 (—▲—) and 15 (—) months of soil burial.

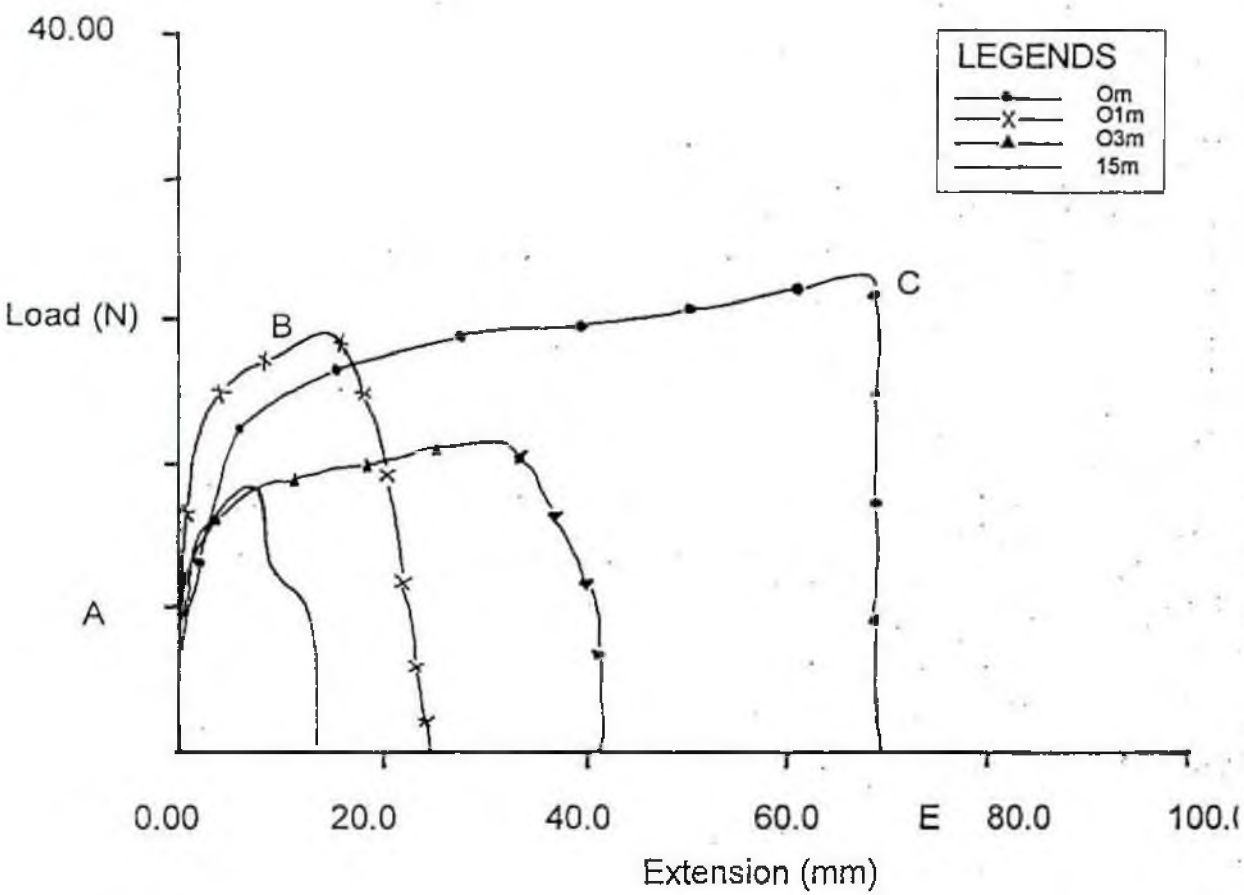


Fig. 4.6. Load-extension curve of LDPE sample No. 02 after O (—●—), 01 (—×—), 03 (—▲—) and 15 (—) months of soil burial.

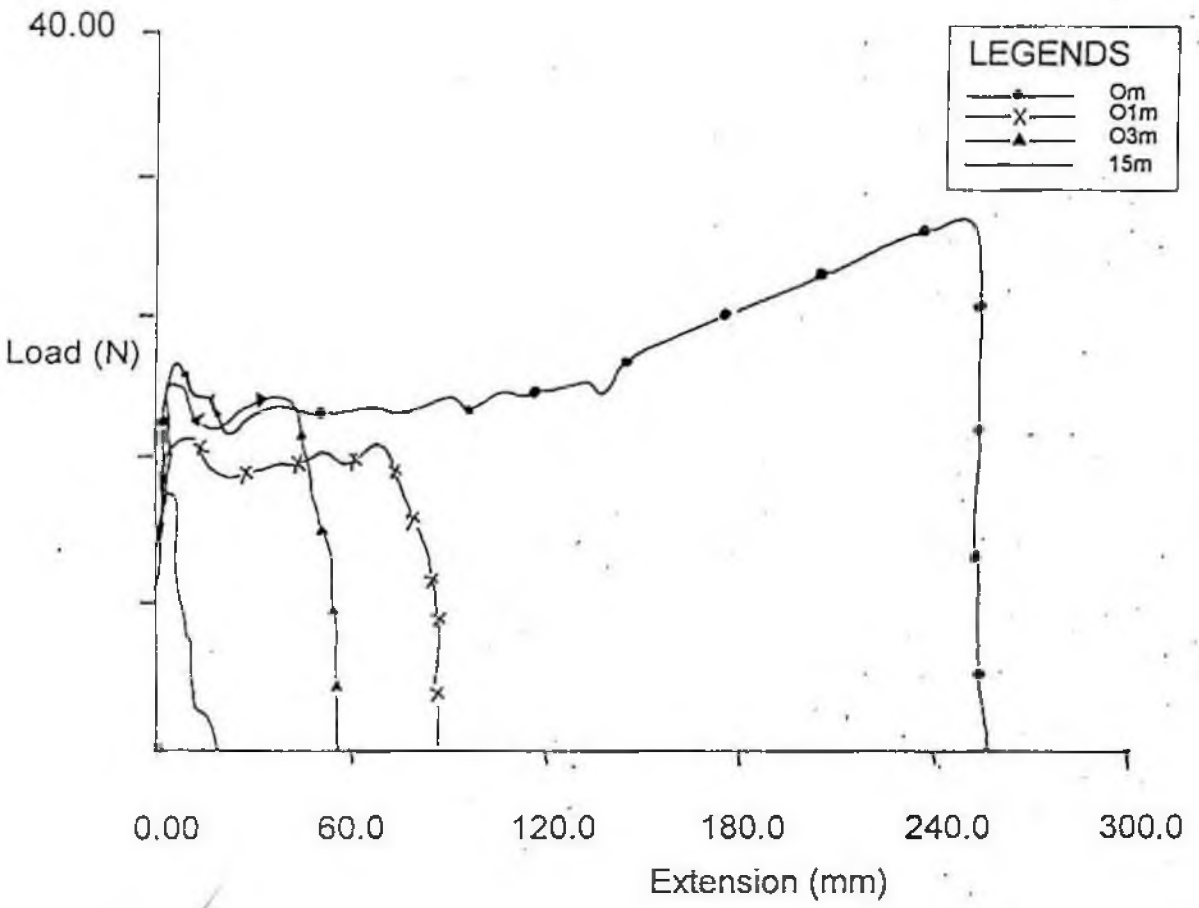


Fig. 4.7. Load-extension curve of LDPE sample No. 03 after O (—●—), 01 (—×—), 03 (—▲—) and 15 (—) months of soil burial.

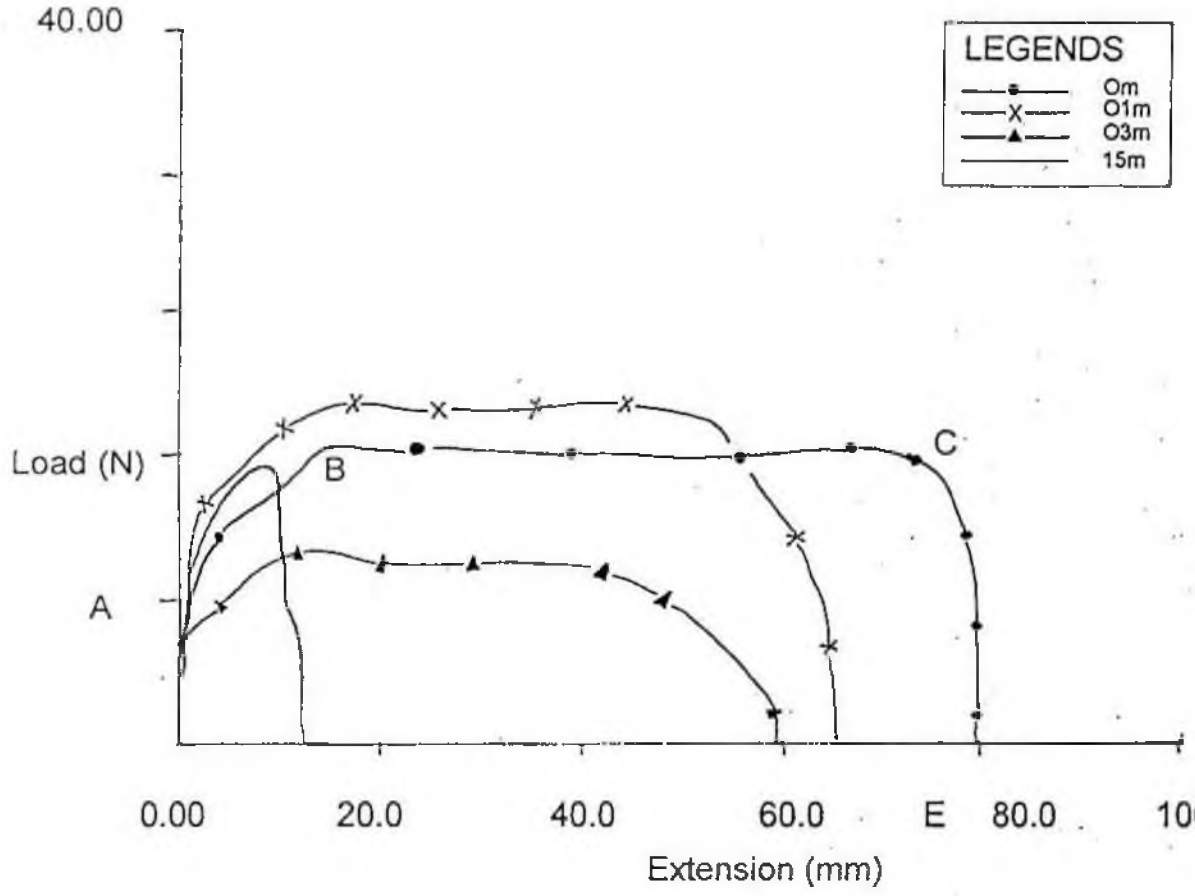


Fig. 4.8. Load-extension curve of LDPE sample No. 04 after O (—●—), 01 (—×—), 03 (—▲—) and 15 (—) months of soil burial.

break occurs earlier as soil burial time increases. In case of Sample no.01 and 03, complete destruction of elastic and plastic region is observed after 15 months. Samples gradually deteriorated by the reduction of elastic and plastic region with the progress of exposure period. For sample no.02 and 04, graphs after 15 months of soil burial showed almost a complete destruction of elastic and plastic region.

Percentage Strain at Maximum load and Maximum Stress (N/mm^2) against burial time are plotted in **Fig Nos. 4.9-4.12**. For Sample no. 01 Percentage Strain at Maximum load gained minimum values after 15 months of soil burial whereas max. stress value plateaud near about 13 months. The stress-strain curve of polyethylene sample no. 02 attained minimal values after 17 months. Sharp drop in the values of Percentage Strain at Maximum load and Maximum Stress (N/mm^2) for sample no. 03 occurs within 03 months. Strain tends to zero by 15 months of soil burial. Sample no. 04 behaved in the same way as sample no. 01 as minimum values are observed for both specimens after 15 months of soil burial.

4.8 Conclusion

- The load-extension curve for each sample reveals progressive disappearance of plastic region with the exposure period.
- Complete disappearance of plastic portion results after 17 months of soil burial and the specimens become highly brittle.
- After 17 months of soil burial, all specimens had a tensile elongation of 5% or less; therefore, these are degraded to the brittle point according to ASTM D3826-91.

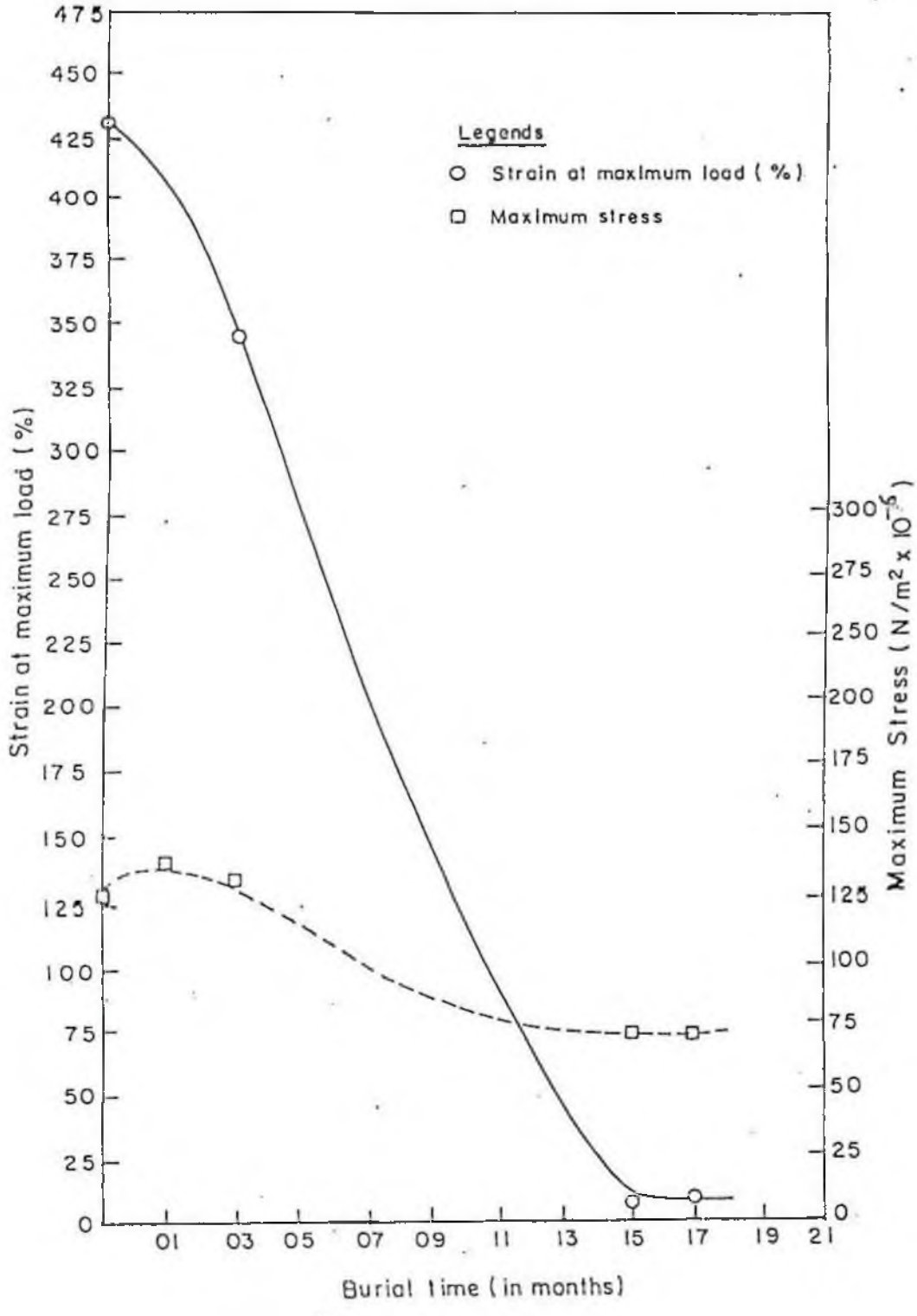


Fig. 4.9. Graph showing maximum stress versus burial time in months (□) and % strain at maximum load vs. burial time in months (O) for sample no. 01 (LDPE).

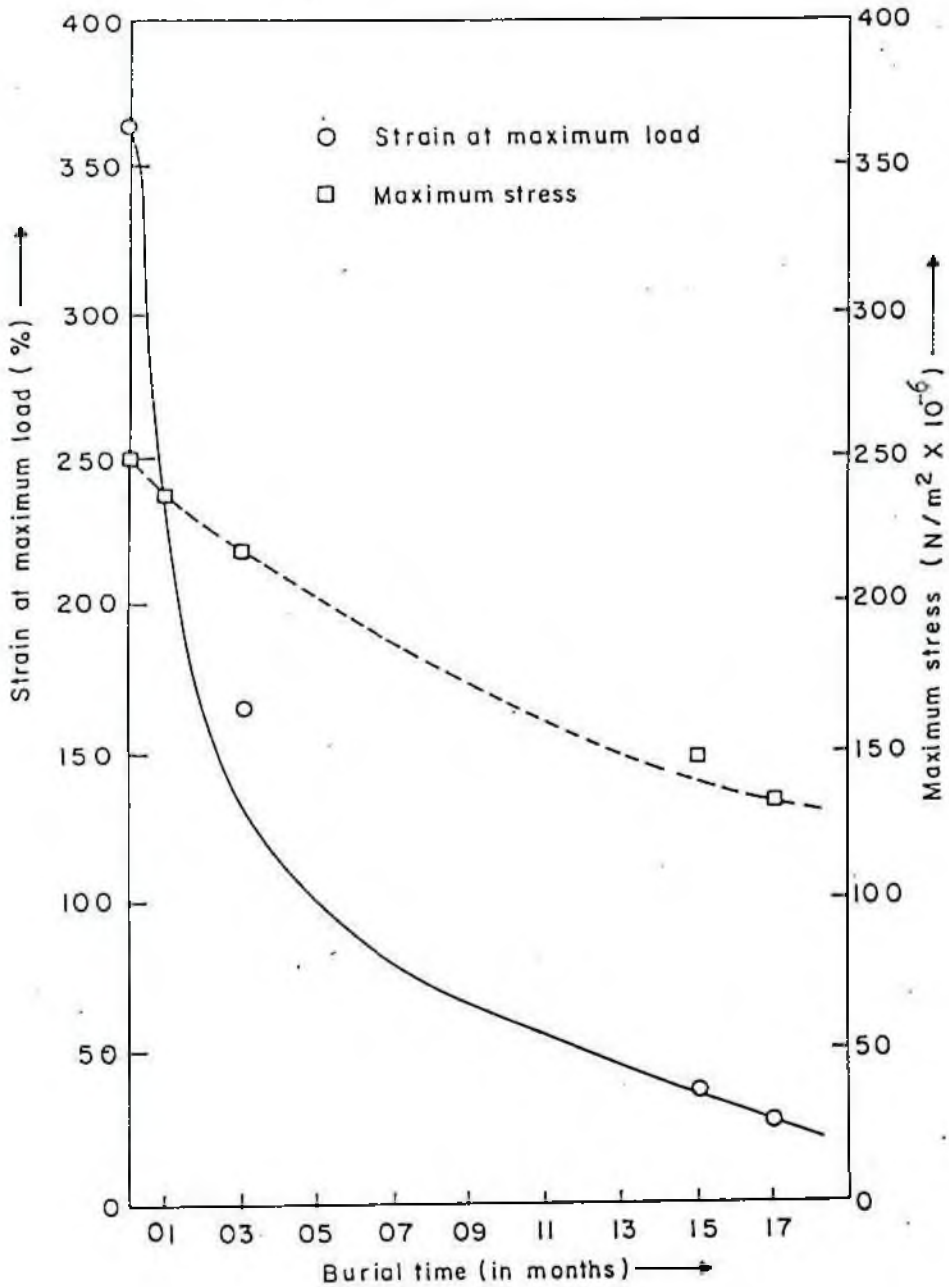


Fig. 4.10. Graph showing maximum stress versus burial time in months (□) and % strain at maximum load vs. burial time in months (O) for sample no. 02 (LDPE).

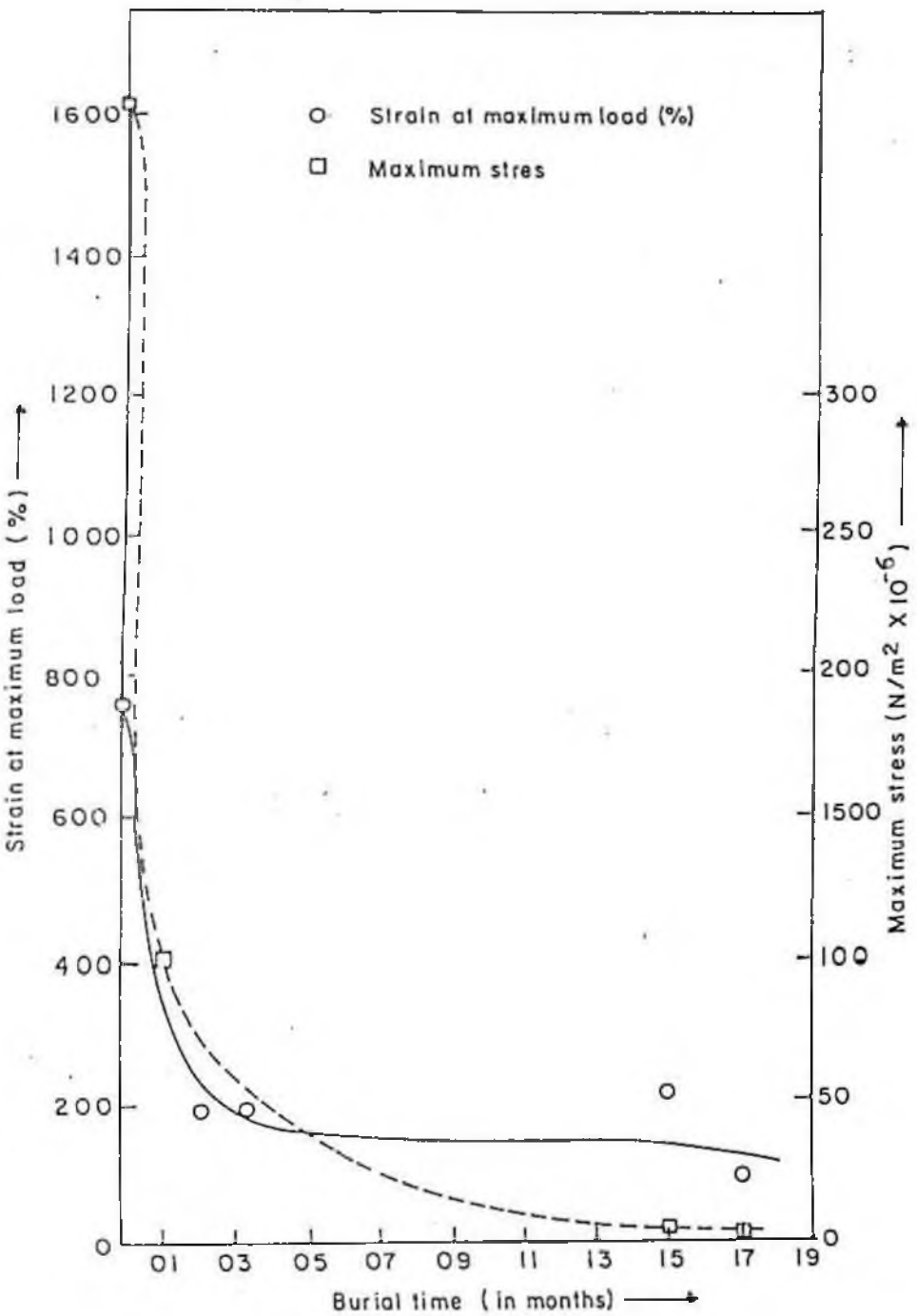


Fig. 4.11. Graph showing maximum stress versus burial time in months (□) and % strain at maximum load vs. burial time in months (O) for sample no. 03 (LDPE).

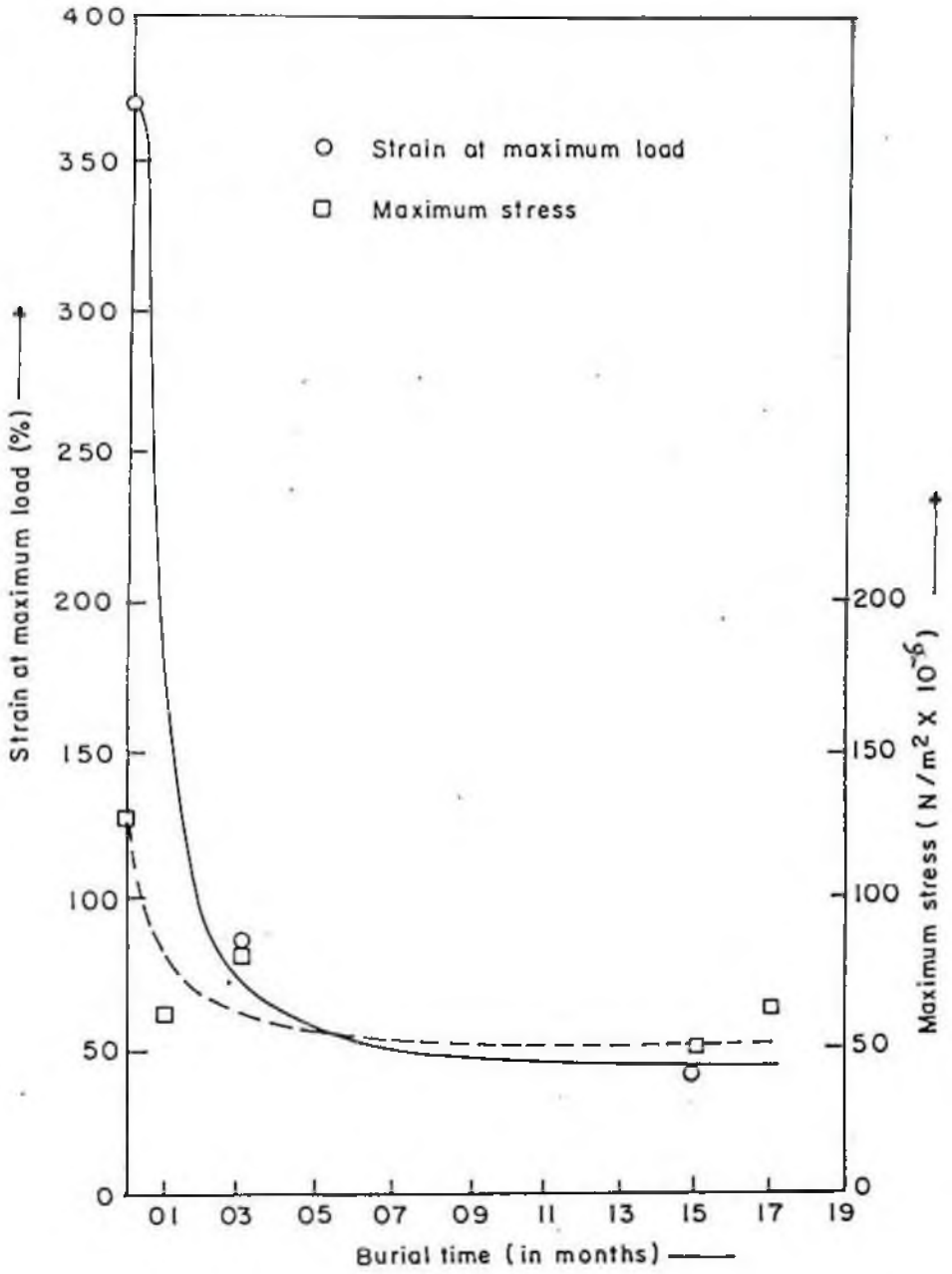


Fig. 4.12. Graph showing maximum stress versus burial time in months (□) and % strain at maximum load vs. burial time in months (O) for sample no. 04 (LDPE).

Chapter - 5

DIELECTRIC PROPERTIES OF POLYETHYLENE

Dielectric properties of polyethylene

5.1 Introduction

Most Plastic materials may be considered as electrical insulators, i.e., they are able to withstand a potential difference between different points of a given piece of material with the passage of only a small electric current and a low dissipation energy (Brydson 1999). Insulating properties of polyethylene compare favourably with those of any other known dielectric material. Polyethylene is a well-behaved dielectric and is probably better understood than any other commercial dielectric material (McCall 1957).

5.1.1 What is dielectric?

An atom consists essentially of a positively charged nucleus surrounded by a cloud of light negatively charged electrons which are in motion around the nucleus. In the absence of an electric field, the centres of both negative and positive charges are coincident and there is no external effect of theses two charges (**Fig 5.1a**). In a molecule, we have a number of positive nuclei surrounded by overlapping electron clouds. In a truly covalent molecule the centres of negative and positive charges again coincide and there is no external effect (Brydson 1999).

If an atom or covalent molecule is placed in an electric field, there will be a displacement of the light electron cloud in one direction and a considerably smaller displacement of the nucleus in the other direction (**Fig 5.1b**). The effect of the electron cloud displacement is known as **electron polarization**. In these circumstances the centres of negative and positive charge are no longer coincident.

One can consider a condenser system in which a massive quantity of a species of atom or molecule is placed between the two plates, i.e., forming the dielectric. The condenser is a device for storing charge. If two parallel plates are separated by a vacuum and one of the plates is brought to a given potential, it will become charged (a conduction charge). This conduction charge will induce an equal but opposite charge on the second plate. For a condenser, the relationship between the charge Q and the potential difference V between the plates is given by:

$$Q = CV \quad \text{----------(5.1)}$$

Where the constant of proportionality C is known as the **capacitance** and is a characteristic of a given condenser (**Fig. 5.1c**).

If a slab of dielectric composed of a mass of atoms or molecules is inserted between the plates, each of the atoms or molecules will be subject to electron polarization. In the centres of the dielectric, there will be no apparent effect but the edges of the slab adjacent to the metal plates will have a resultant charge, known as a **polarization charge.** Since the charge near each metal plate is of opposite sign to the conduction charge, it tends to offset the conduction charge in electrostatic effects. (**Fig. 5.1d**). This includes the potential difference V between the plates which is reduced when polarization charges are present. In any use of a condenser, it is the conduction charge Q that is relevant and this is unaltered by polarization . From Eqn (**5.1**), it can be seen that since the insertion of a dielectric reduces the potential difference but maintains a constant conduction charge the capacitance of the system is increased (**Fig. 5.2**)

The influence of a particular dielectric on the capacitance of a condenser is conveniently assessed by the **dielectric constant**, also known as the **relative permittivity** or rarely **specific inductive capacity.** The dielectric behaviour of plastics is characterized by the complex Dielectric constant (permitivity) $\varepsilon^* = \varepsilon'(\omega) - \mathbf{i}\,\varepsilon''(\omega)$, where ω is angular frequency, $\varepsilon'(\omega)$ the dielectric constant (permitivity), and $\varepsilon''(\omega)$ the dielectric loss factor and $\mathbf{i} = \sqrt{-1}$. The ratio $\varepsilon''/\varepsilon'$ is equal to the tangent of the phase angle between the voltage and the current : $\tan\delta = \varepsilon''/\varepsilon'$ (Carlowitz *et al.* 1992)

Measures of the fraction of energy absorbed per cycle by the dielectric from the field are the power factor and dissipation factor. These terms arise by considering the delay between the changes in the field and the change in polarization which in turn leads to a current in a condenser leading the voltage across it when a dielectric is present. The angle of lead is known as the phase angle and given by the symbol θ. The value $90-\theta$ is known as the loss angle and is given the symbol δ . The power factor is defined as **Cos**θ (**or Sin** δ) and the dissipation factor as **tan**δ (**Cot** θ). When δ is small the two are equivalent. Loss factor is numerically the product of the dissipation factor and the dielectric constant.

5.2 Electrical properties of polyethylene

As polyethylene is a non-polar material, properties such as power factor and dielectric constant are almost independent of temperature and frequency. Dielectric constant is linearly dependent on density and a reduction of density on heating leads to a small reduction in dielectric constant.

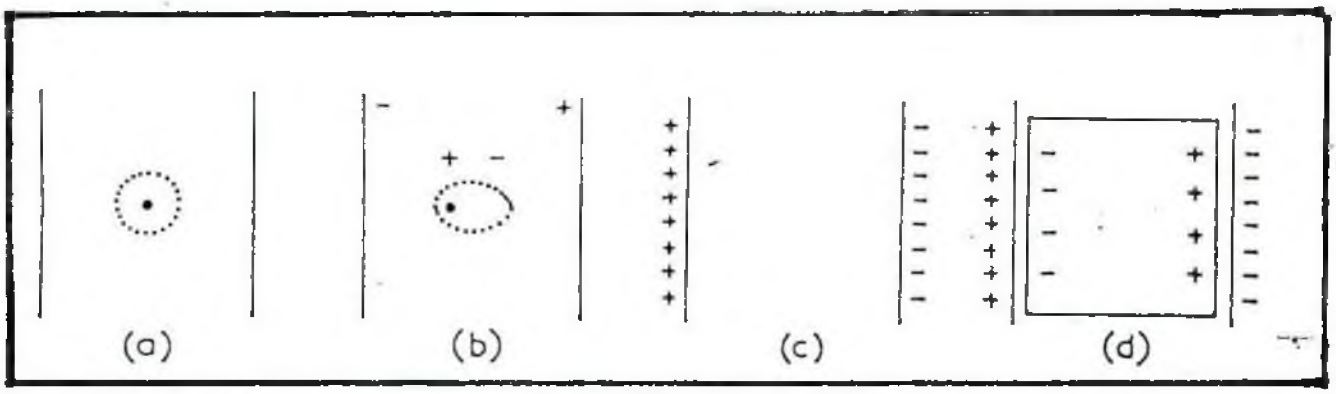


Fig. 5.1. (a) Atom not subjected to external field. Centre of electron cloud and nucleus coincident, (b) Electron cloud displacement through application of external field, (c) Charged condenser plates separated by vacuum, (d) Condenser plates separated by dielectric (after Brydson 1999).

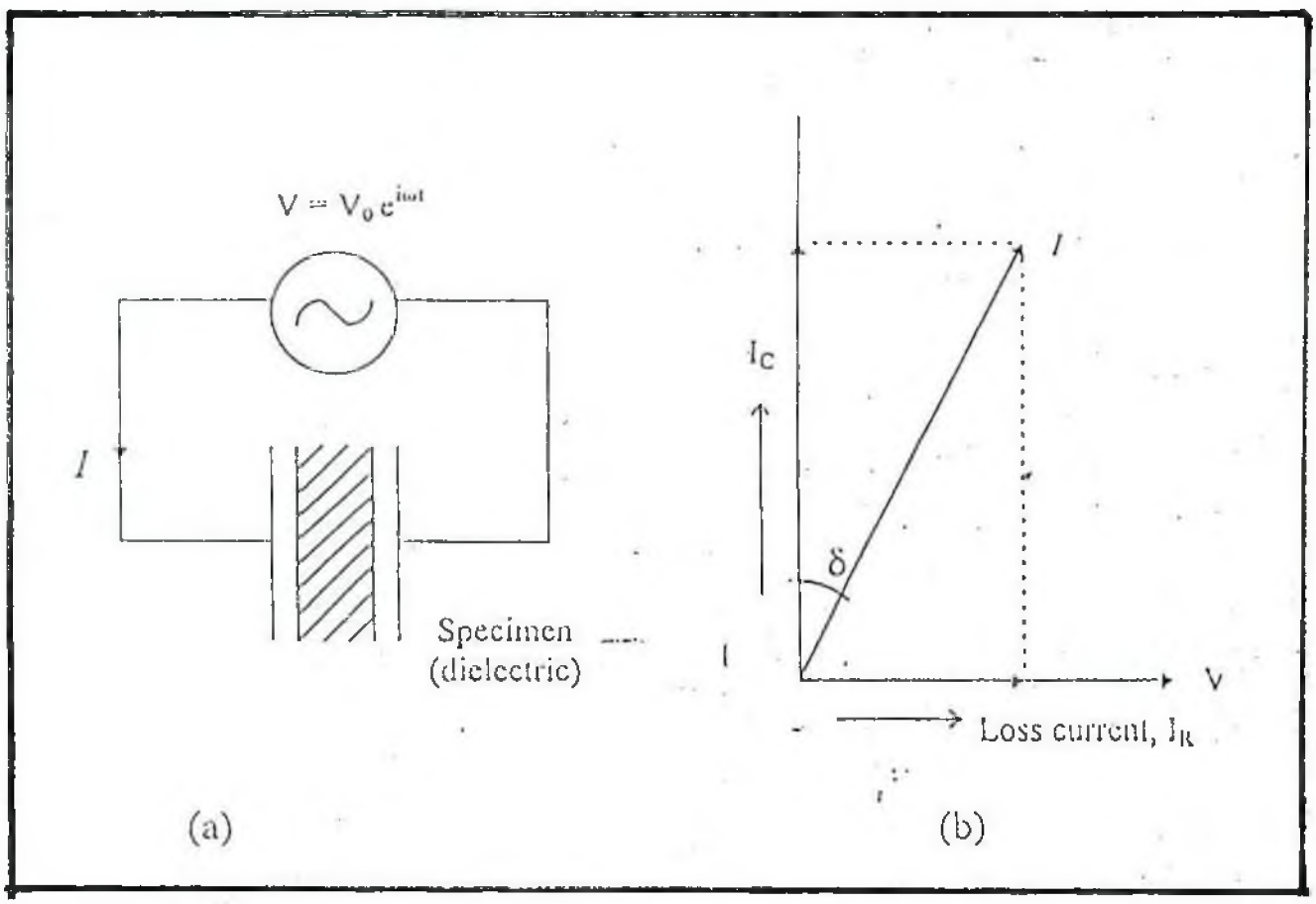


Fig. 5.2. AC losses in a dielectric (a) circuit diagram, (b) Argand diagram of complex current-voltage relationship.

Typical values of some electrical properties of polyethylene are given below (Brydson 1999):

Volume resistivity		$>10^{12}$ Ωm
Dielectric strength		700 kV/mm
Dielectric constant		
	Density=0.92g/cm^3	2.28
	Density=0.96g/ cm^3	2.35
Power factor		~1-2 x 10^{-4}

5.3 Principles of Dielectric Spectroscopy

Dielectric spectroscopy is based on the interaction of electromagnetic radiation with the electric dipole moments of the material under test. The frequency range of the radiation is usually between 10^{-6} Hz and about 10^{10} Hz. Dielectric spectroscopy, introduced by Debye in 1931 has been used since then to determine molecular dipole moments and to study liquid and solid structures (Hedvig 1977).

Dipole moments originate from the symmetry of the positive and negative charge densities of the system. Positive charges arise from nuclei: they are localized. Changes in the positive charge density result from structural transformations of the molecule (isomeric transitions, rotation of groups or that of the solid. e.g. recrystallization, local motions, rotation of groups, vibration etc). Negative charge densities arise from the electronic system, which is delocalized.

The total dipole moment of a molecule is

$$\mu = \int \mathbf{r} \, [\, Q_e(\mathbf{r}) + Q_n(\mathbf{r}) \,] d\mathbf{r} \quad \text{----------(5.2)}$$

where, $Q_e(r)$ and $Q_n(r)$ are the electronic and nuclear charge densities, respectively (Hedvig 1977).

The response of a material to an external electric field is essentially a statistical effect. It is not possible to observe the orientation of the individual moments; only the bulk polarization of the assembly can be measured. This means that dielectric spectroscopy is based on statistical thermodynamical considerations.

5.4 Dielectric Relaxation in Solid Polymers

Several distinct dielectric relaxation processes are usually present in a solid polymeric material (**Fig. 5.3**). As the temperature is raised, molecular mobilities of various types become successively energized and available for dipolar orientation. By convention the dielectric relaxation processes are labeled as α, β,...and so on, beginning at the high-temperature end. The same relaxation processes are generally responsible for dispersions in mechanical properties too (Blythe 1979).

In case of wholly amorphous polymers, there is always a high-temperature α-relaxation associated with the micro-Brownian motion of the whole chains and, in addition, at least one low-temperature (β, γ etc.) subsidiary relaxation. The high frequency β, γ..... subsidiary peak in amorphous polymers are characteristically very broad. Graphs of isothermal scans of dielectric constant and loss as a function of frequency 'f' are sometimes called dielectric spectra.

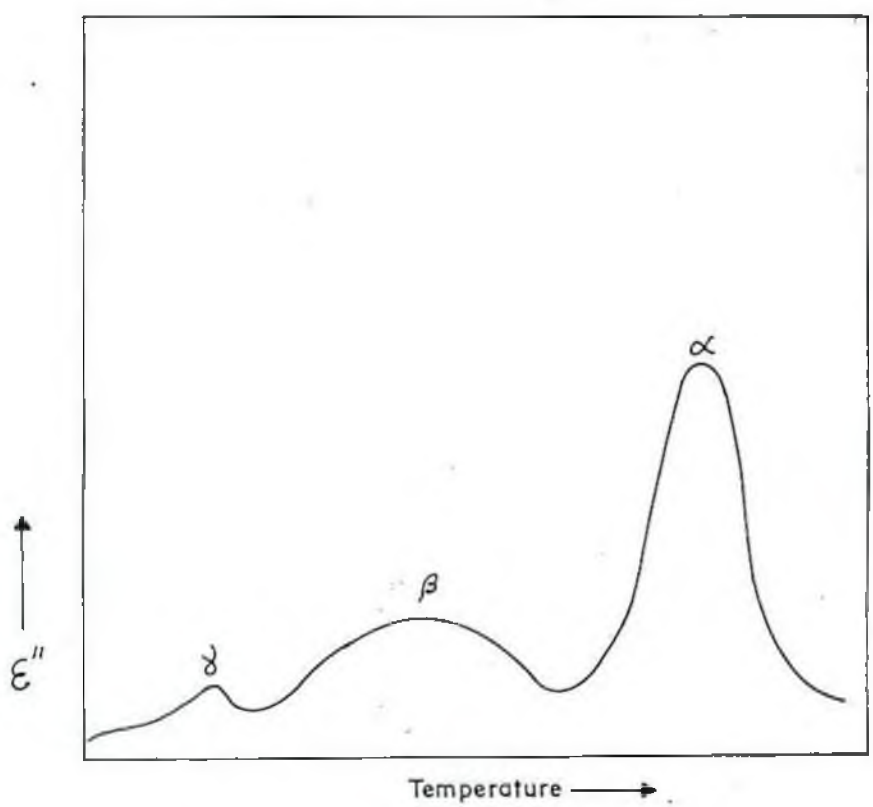


Fig. 5.3. A schematic dielectric loss curve (after Blythe 1979).

The relative strength of the α- and β-dielectric relaxation depend on how much orientation of the dipolar groups can occur through the limited mobility allowed by the β-process before the more extensive mobility of the α-process comes into play. The mechanism of a β-type process may be one of several different types depending on the nature of the dipole group concerned and its position on the polymer chain. The most important mechanisms include:.

- *Rotation of a side group about a C-C bond.*
- *Conformational flip of a cyclic unit*
- *Local motion of a segment of the main chain* (Blythe 1979).

5.5. Mawxell- Wagner- Sillars (MWS) Effects (Blythe 1979)

A material is always likely to have regions of non-uniformity and impurities may be present as a second phase. A common form of discontinuity can be a missing lattice site, a dislocation or an interface of a crystallite.

In the presence of an external field, a dipole is formed at structural defects. This will be referred to as a defect dipole. A defect dipole formed by the polarization of a Wannier excitation (Wannier 1937) at a lattice point is schematically illustrated in **Fig. 5.4a**.

Any inhomogenity in the permittivity of materials should result in a charge and correspondingly in the formation o f dipoles in the presence of an external field. This phenomenon has long been known as Maxwell-Wagner-Silllars (MWS) effect, since Maxwell, Wagner and Sillars were the first to consider them theoretically.

Maxwell(1892) examined the effect of a field applied across a specimen consisting of layers of two different materials with different dielectric constants and conductivities. His results showed that charges will accumulate in time at the interfaces between the layers to build up interfacial dipole polarization (**Fig. 5.4b**). Wagner (1914) gave an approximate treatment of the important practical case where a very highly insulating dielectric suffers from inclusions of conductive impurities.

Sillars (1937) demonstrated the importance of the shape of conductive inclusions. The loss peak is enlarged and shifted to lower frequencies by any elongation in the direction of the applied field.

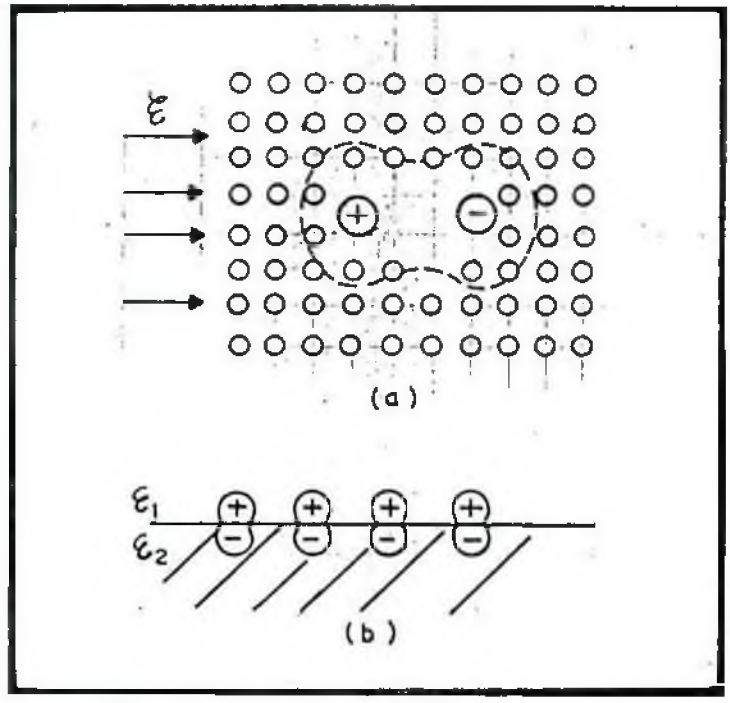
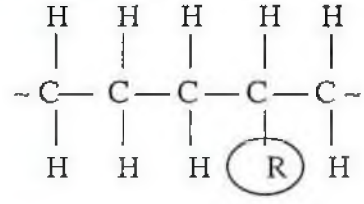


Fig. 5.4. Defect dipole moment in the solid (a) at missing lattice (b) at the interface of the two media (after Hedvig 1977).

5.6 Dielectric spectrum of polyethylene

Hedvig (1977) categorized polyethylene in three basic forms. These are LDPE, HDPE and LPE.

LDPE- Low density polyethylene (LDPE) is polymerized by a free radical mechanism with peroxide initiators under high pressure. Its chemical formula is:



The side branches R are mainly methyl or ethyl groups; they are statistically distributed along the main chain. In LDPE, the average number of methyl groups per 1000 carbon atoms is 21.5; that of ethyl groups is 14.4; its density is typically 0.91-0.92 g/cm^3, and its crystallinity is about 60%. The crystalline melting temperature of LDPE is 105-120 ^{O}C.

HDPE- Polyethylene produced at low pressure by Ziegler-Natta catalysts. The corresponding polymer has high density (0.93-0.94 g/cm^3) and high crystallinity (87%); it is referred to as low-pressure or high density polyethylene (HDPE). The number of

methyl groups per 100 carbon atoms in HDPE is 3, that of ethyl groups is 1. The crystalline melting temperature of HDPE is between 120 and 140 ^{O}C.

LPE- The third typical polyethylene is prepared by a specific catalyst (Phillips) which produces a linear polyethylene (LPE) of high density (0.96 g/cm^3) and high crystallinity (93%) exhibiting few side branches < 1 per 1000 carbon atoms. The crystalline melting temperature of LPE is 140 ^{O}C.

Fig. 5.5 shows the dielectric spectra of all types of polyethylene at a frequency of 100 kHz. The highest temperature α_c peak is found in all three types: it is thought to be due to motion at the crystalline surfaces. The position and intensity of the α_c peak is dependent on the crystallinity and crystallite size, and so this peak is sensitive to heat treatments. The lower temperature α_a-peak which is often referred to as the β-peak, is found in LDPE, is less intense in HDPE and is absent in LPE. The α_a-peak has been attributed to the motion of the side branches in the amorphous phase.

The same relaxation processes are generally responsible for dispersions in mechanical properties too, although a particular molecular rearrangement process may produce a stronger dielectric than mechanical effect or vice versa. **Fig 5.6** compares the dielectric depolarization spectrum of slightly oxidized low-density polyethylene with the corresponding high-frequency (10 kHz) dielectric and low frequency (10Hz) mechanical spectra. The depolarization spectrum has been measured after polarization of the sample at 100^{0}C for 30 minutes. The rates of heating and cooling were 2^{0}C/min. It was seen that in the depolarization spectrum the α_a and α'_a peaks were resolved much better than in the mechanical and in the dielectric spectra (**Fig. 5.6**) (Hedvig 1977).

5.7 Different methods for measuring dielectrics

The study of permittivity (ε′and ε″) and loss tangent (tanδ) dependence on frequency (ω) and temperature (T) can not be undertaken at all frequencies by the same methods. A wide varieties of techniques exist for measuring the electric properties and the nature of the materials in both a.c. and d.c. fields. The low frequency and steady state regime is covered by transient response methods, low and intermediate frequencies by bridge or resonant methods, and high and ultra-high frequencies by microwave techniques (Blythe 1979). The methods which are appropriate for the various frequency regimes are given in **Fig. 5.7**.

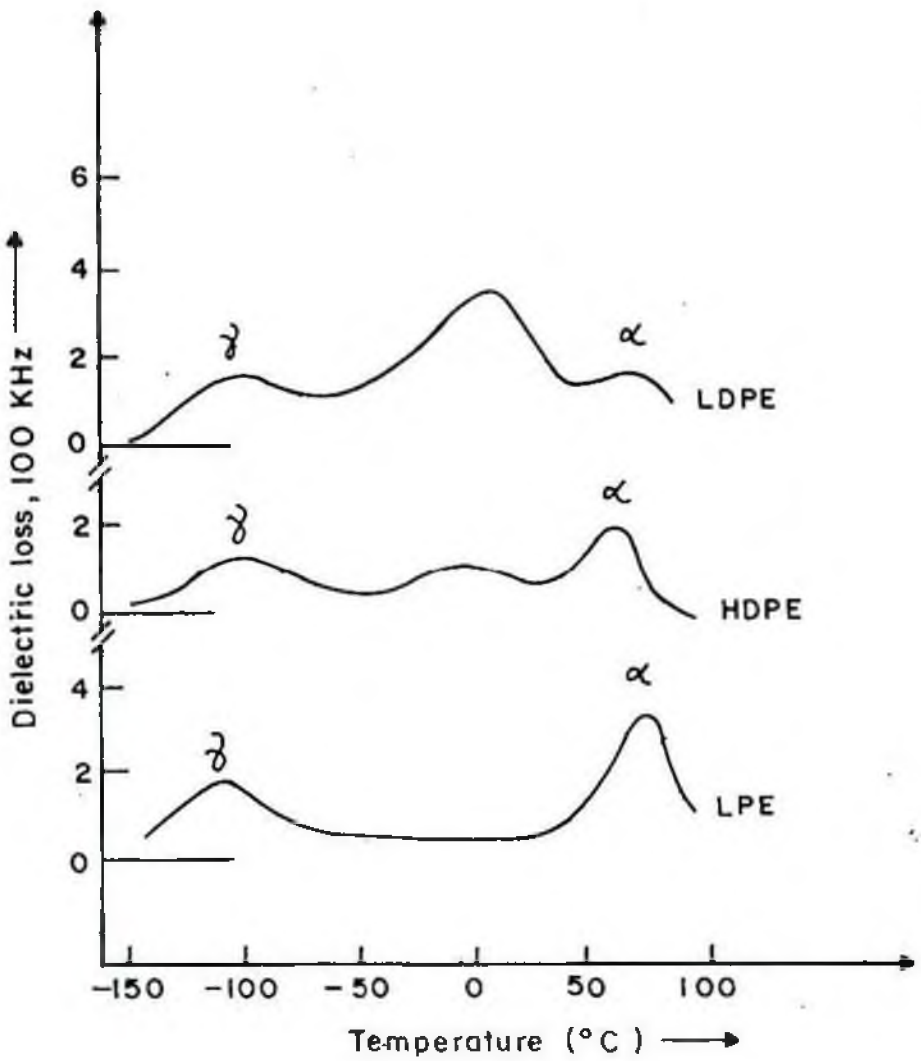


Fig. 5.5. Dielectric transitions in LDPE, HDPE and LPE (after Hedvig 1977).

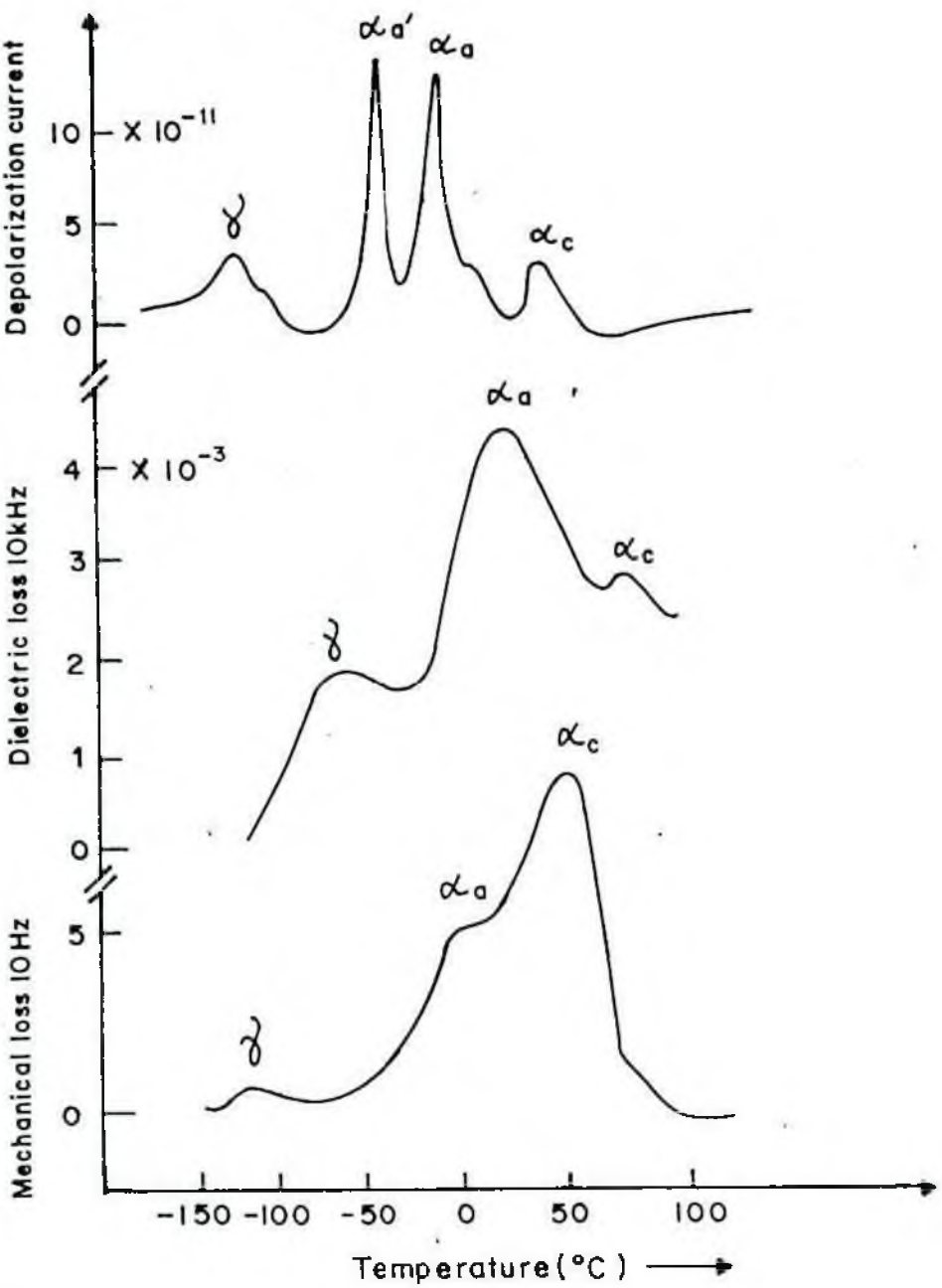


Fig. 5.6. Comparison of the dielectric depolarization, a.c. dielectric and mechanical relaxation spectra of LDPE (after Hedvig 1977).

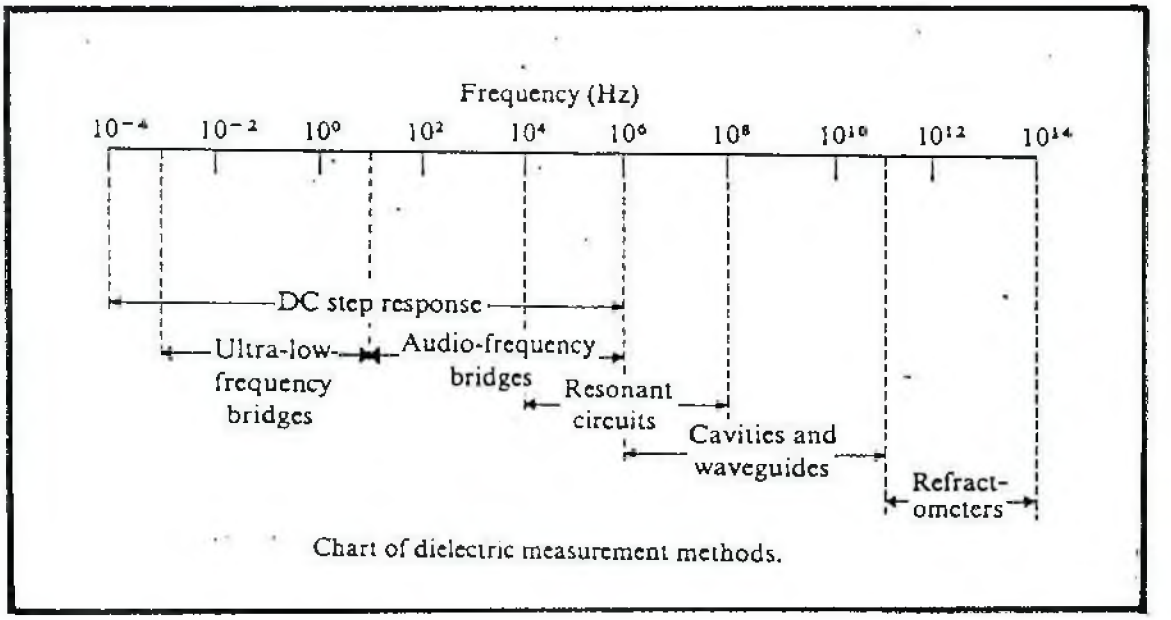


Fig. 5.7. Frequency ranges of dielectric measurement techniques (after Blythe 1979).

5.8 Materials and Methods

5.8.1 Operation of Type TO-19 Thermostatic Oven

Names and functions of the portions of temperature controller is given in the following:

1. **PID constant selection signal**

   Light flickering with about 1 sec. Period during automatic PID constant selection.

2. **Deviation value display**

   Displays deviation between adjusted set value and measured value within a range of ±10%. LED indication of 13-segment bar graph.

3. **Lower limit Alarm Lamp (LA)**

   Lights flickering with about 1 sec. Period when the measured value decreases below the lower limit alarm setting value.

4. **Higher Limit Alarm lamp (HA)**

   Lights flickering with about 1 sec. Period when the measured value increases above the higher limit alarm setting value.

5. **Data display**

Displays measured and set values with 4-digit LED digital indication.

6. **Display Selection Key (PV/SET)**

Depressed to switch over data display to PV (measured Value) or SET(various set modes).

7. **Set Mode Lamp**

Lights when display switch over key is set to SET mode.

8. **Auto/manual Key (AUTO/MAN)**

Depressed to switch over AUTO (automatic operation) or MAN (manual operation).

9. **Manual Lamp**

Lights when output is adjusted by manual operation.

10. **PID Selection Key (SELECT PID)**

Depressed to set PID constant to automatically stable condition. Automatic selection by selection key is classified into auto start with deviation value and manual start with entry key depressed.

11. **PID selection Lamp**

Lights PID constant is in automatically settable condition.

12. **Setting Digital switch**

A four-digit digital switch used to set various constants. At every depressing of push-button switch positioned to up or down each digit, number advances one by one facilitating to set the required value directly. Depressing of lower push-button switch facilitates advancing in 0→1→2… and so on, and that of upper switch in 0→9→9

13. **Entry Key**

Depressed to store various set values in memory and to start PID constant automatic manually.

14. Set mode selection switch

Selects eight set mode as follows:

SP→ Set point

Set point setting mode

LA→ Lower limit alarm/alarm release

Lower limit alarm setting mode which alarms lower limit deviation to set point. Alarm is released when setting [-] [0] [0] [0].

HA→ Higher limit alarm/alarm release

Higher limit alarm setting mode which alarm lower limit deviation to set point. Alarm is released when setting [-] [0] [0] [0].

P→ Proportion band/ sensor correction

This mode sets proportion constant manually, or confirms P value in automatic selection. By setting [-] [0] [0] [0] it turns to sensor correction setting mode which compensates for error by measurement sensor.

I→ Integrated time/output variation rate

This mode sets integrated tie manually, or confirms I value in automatic selection. By setting [-] [0] [0] [0] it turns to output variation rate setting mode which limits set output variation at each period under the set value.

D→ Differential time/remote input shift

This mode sets differential time in manual, or confirms D value in automatic selection . By setting [-] [0] [0] [0] when remote input specification (option) is added, it turns to remote input shift setting mode which shifts input value.

Out→ Output/lock switch

This mode displays set output value to set manual value for manual operation. By setting [-] [0] [0] [0] set data is locked to disable change of setting. By setting the same value again locking is released.

OL→ Output limiter (higher limit)/Output limiter (lower limit)

This mode limits higher limit of set output. By setting [-] [0] [0] [0] it turns to output limiter (lower limit) to limit lower value of set output (Fava 1980).

5.8.2 Operation of LCR Meter Model RC-535B

The LCR Meter Model RC-535B is designed to measure capacity C, inductance L, resistance R, dissipation factor D (in C measurement mode) or quality Q (in L measurement mode) and display, digitally the results of these measurements with high precision. Moreover, the LCR meter permits connection with external equipment through an optional I/O board.

5.8.2.1 Features

- Measure the values L, C, and R with precision as high as 0.3% (typical value)
- Measure frequencies of/at 100Hz, 120Hz and 1kHz .
- Range and mode selection can be accomplished automatically. A sample with a unknown value can be measured within the optimum range.
- Measured values of L, C, and R can be displayed.

5.8.2.2 Operations

♦ Operating switches	All operations are performed by use of self-illuminated LED touch keys.
♦ Guaranteed temperature range	23^0C $\pm5^0$C, with accuracy depending on this condition. At 5~4^0C, the critical value of an error will double. Operating temperature range 0- 50^0C.
♦ Power requirements	AC 100/115/215/230 ±10%, 50/60/400 Hz 25VA or less
♦ Dimensions and weight	Approx. 400 (width) x 100(height) x 310 (depth) mm, 6 Kg or less.
♦ Optional adapters	Usable with KC Series LCR meters - KCA-01 Socket Adapter - KCA-02 4-terminal lead Adapter - KCA-03 3-terminal lead adapter - KCA-04 Kelvin Clip Adapter

♦ Optional boards	KCB-11 BCD Board (BCD output, printer coupling, external control) KCG-12 GP-1B Board (Connection to GP-IB line)

5.8.2.3 Parts: Nomenclature and Functions

Front panel

Power Switch	This is a switch for applying power to the LCR meter.
Frequency switches	These are switches for setting the measuring frequency to 100 Hz, 120 Hz or 1kHz.
Circuit Mode switches	These are switches for setting the circuit mode to determine in which circuit mode, series (SER) or parallel (PRL) equivalent circuit, the specimen is to be tested. Depressing the AUTO switch will provide automatic setting and the optimum mode, SER or PRL, will internally be determined to perform measurement. Depressing the SER or PRL switch in the AUTO mode will provide manual setting automatically.
Range switches	These are range setting switches, With depression of the AUTO switch, the optimum range will automatically be selected. Depressing the MANU switch will provide manual setting. If the range is found unsuitable for the specimen, the LED lamp Range ADJ will flicker. UP and DOWN operations in the manual mode can be performed over the whole ranges to permit switching to any desired point while watching the decimal point and unit.
FUNCTION switches	These are L, C and R selector switches for selecting the desired measuring functions. From left, stands for inductance, C capacity and R resistance. Measuring terminals and short circuit metal: These are terminals for connecting the specimen. There are 4 terminals, i.e.; red terminals for H_{FORCE} on the signal impression HIGH side and H_{SENSE} on the signal detection HIGH side and black terminals for L_{SENSE} on the signal detection LOW side and L_{FORCE} on the signal

	impression LOW side. Addition of the GUARD terminal makes 5-terminal configuration. In the 2-or 3- terminal measurement mode, short-circuit metal is used to establish a short between respective terminals on the HIGH side and LOW side.
GUARD terminal	The GUARD terminal is a signal gland line of this LCR meter and it is connected to the enclosure gland to improve measuring accuracy, especially when testing the specimen of high impedance.
C ZERO for stray compensation	This is used to compensate the stray capacity in lead wire, jig, etc. across measuring terminals when the specimen is not connected in the capacity measurement mode. Turn the control with C in the minimum range and make adjustment so that the indicated value will read "00.0 pF".
Trigger lamp	The lamp will flicker continuously when repeated measurement is made.
LED for display of measured LCR values	Measured values will be displayed in decimal notation of $3^{1/2}$ digits with numerals 000 ~ 1999 and a decimal point. When the reading exceeds 1999, all numeral displays will go out.
LED for display of units	The LED lamps will illuminate to indicate respective unit symbols: "Ω" (ohm), "F" (farad), "H" (henry), "P" (pico), "n" (nano), "μ" (micron), "m" (mili), "k" (kilo), and "M" (mega).
LED for display of "BIAS"	When the switch "DC BIAS" found in the rear panel is set to the ON position, external bias will be applied and the LED lamp "BIAS" on the front panel will illuminate.
RANGE ADJ LED	This LED shows whether or not the specimen under test is being tested in an adequate range.
Measuring level display	When the "LEVEL (Cp)" switch found in the rear panel is changed over to 50mV, this lamp will illuminate. The lamp will go out in the modes other than parallel capacity (Cp) measurement.

LED for display of "Q" and "D"	"D" will be displayed when capacity "C" is being measured. When inductance "L" is being measured the LED lamp "D" will illuminate with the switch "L" found in the rear panel at "D"; the lamp "Q" will illuminate with the switch "L" at "Q". When a measurement is being made of resistance "R", both LED lamps will go out.
LED for display of "D" and "Q"	Dissipation factor "D" will be displayed when a measurement is being made of capacity "C". When inductance "L" is being measured, the dissipation factor will be displayed with the switch "L" found in the rear panel at "D"; the quality will be displayed with the switch "L" at "Q". All displays will go to when a measurement is made of resistance "R".
LED for display of GP-IB	When the optional "GP_IB" interface board is connected and the LCR meter is controlled with a GP-IB bus, this LED will show its status.

Rear panel

"EXT BIAS (Max 30V)" terminals	External DC bias voltage up to max. DC 30V can be applied from these terminals.Polarity ⊕ is for red terminal and ⊖ for black terminal.
DC BIAS switch	With the switch at "ON", bias voltage can be applied to the specimens. Normally, the switch is held at "OFF".
"TRIG/CONT" switch	When an optional board is not used, trigger signal for measurement will automatically be applied internally in the "LOCAL" mode, to repeat the measurement. In the "REMOTE" mode, trigger signal can be applied externally through the BCD board.
"LINE FREQ" switch	The object of this switching is to mitigate variation in the indication owing to external induction when the specimen under test has a high impedance. This phenomenon, however, develops only when the frequency is 1 kHz.

LEVEL (Cp) switch	The level of a measuring signal to be applied to the specimen can be switched over to "1V" to "50mV" by use of this switch only when the measuring mode is "PRL" during capacity measurement. Normally, the switch should be set to "1V". When measuring the capacity of a semiconductor, however, it is advisable to set the switch to "50mV".
"L" switch	When this switch is set to "Q" during measurement of inductance "L", the D,Q display on the front, right side will read quality "Q". When the switch is set to "D", dissipation factor "D" will be displayed.
"GND" switch	This terminal is connected to the enclosure of the LCR meter to make ground connection of the LCR meter.
POWER cord	This is a connected for plugging in the attached power cord. The pin found in the centre is connected to the enclosure of the LCR meter.
POWER fuse	The fuse can be taken out by turning the holder cap to the left. Use a glass-tube fuse with current capacity indicated in the rear panel.
Line voltage switch	Set the line voltage in the direction of the arrow.
Optional Board mounting hole	This is an optional GP-IB board or BCD board ounting hole. Normally, the hole is provided with a blank panel.
Measuring adapters and test leads	3-terminal measuring method: This method has an advantage of eliminating the effect of stray capacity produced across test lead wires or with nearby conductor, and is used to measure small capacity, high inductance and high resistance.
KCA –03	The KCA -03 is useful for high impedance measurement (small capacity, high inductance, high resistance) Measuring range 1kHz $R \geqq 20\Omega$; $C \leqq 20$ μF ; $L \geqq 2$ mH.

5.8.3 Operation of Type SE-70 Electrode

SE-70 is an electrode used to measure the dielectric constant and dielectric loss of solid specimens.

Specifications:

Operating frequency range	30 Hz to 3 MHz
Operating temperature	-70^0C to + 199^0C
Main electrode diameter of specimen	19 mmø to 53.7 mmø
Main electrode outside diameter	18 mmø
Guard electrode inside diameter	39, 55, 71 mmø
Thermostatic oven used	Type TO-19, TO-1 thermostatic oven
Dimensions and weight	Dimensions: Approx.140(H) x 129(W) x 120 (D) mm Weight: Approx. 2 kg

5.9 Preparation of specimens

Four types of thin LDPE films were cut into 18 mm discs with the help of a metal borer. Sample thickness was calculated by screw-gauge 3-5 times for each specimen.

The main electrode diameter is in the range from 19 to 53.7 mmø. This diameter was calculated by the following formula from the specimen thickness and estimated dielectric constant.

Main electrode diameter $\mathbf{D(cm) = \sqrt{14.39 C_x . t_x / \varepsilon}}$ ... (3)

where $\mathbf{t_x}$ = specimen thickness(cm)

ε = specimen dielectric constant

$\mathbf{C_x}$= electrostatic capacity to be obtained (pF)

5.10 Mounting of the specimen to the electrode

The main electrode was moved by turning the micrometer knob at the top. With the main electrode side upward, the specimen was placed at the centre of the counter electrode. Previously selected guard ring was placed on the surface. The main electrode was then lowered to come into contact with the specimen and the guard ring. For the purpose, the knob was turned counterclockwise until positive contact is obtained.

5.11 Measurement

To make measurements, the electrode body was plugged into the thermostatic oven and was connected to the dielectric loss measuring set. The dielectric constant (ε′), dielectric loss constant (ε″) and dielectric loss tangent (**tan**δ) can be calculated by the following formulas.

Dielectric constant $(\varepsilon') = 14.39/ D^2 C_x.t_x$ ...**(5.4)**

Dielectric loss constant $(\varepsilon'') = 14.39/ \omega D^2 G_x.t_x \times 10^{12}$ ...**(5.5)**

Dielectric loss tangent $(\tan\delta) = \varepsilon''/\varepsilon' = G_x/\omega C_x \times 10^{12}$ ...**(5.6)**

Angular frequency $\omega = 2\pi f$

where C_x = electrostatic capacity of the specimen, (pF)

G_x = conductance of the specimen, (S)

t_x = thickness of the specimen, (cm)

D = effective electrode diameter, (cm) = 1.8 cm

f = measuring frequency, (Hz)

5.12 Electrical connections

In the present study, the [HIGH, LOW] specimen connecting terminal of the oven is connected to the measuring terminal (6) of the front panel of the LCR meter using a 3-terminal KCA-03 Lead adapter, attached to the thermostatic oven. The specimen is mounted into the SE-70 type electrode (**Fig. 5.8**) and the electrode is mounted into the thermostatic oven . The electrical connections in this experiment is shown in **Fig 5.9**.

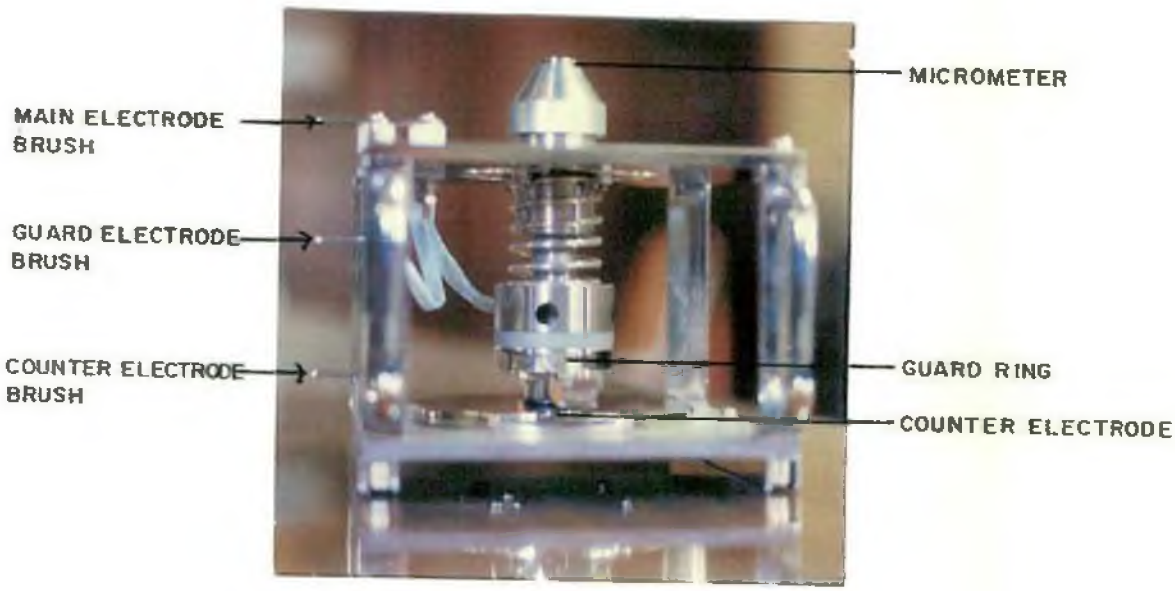


Fig. 5.8. Outside view of type SE-70 Electrode.

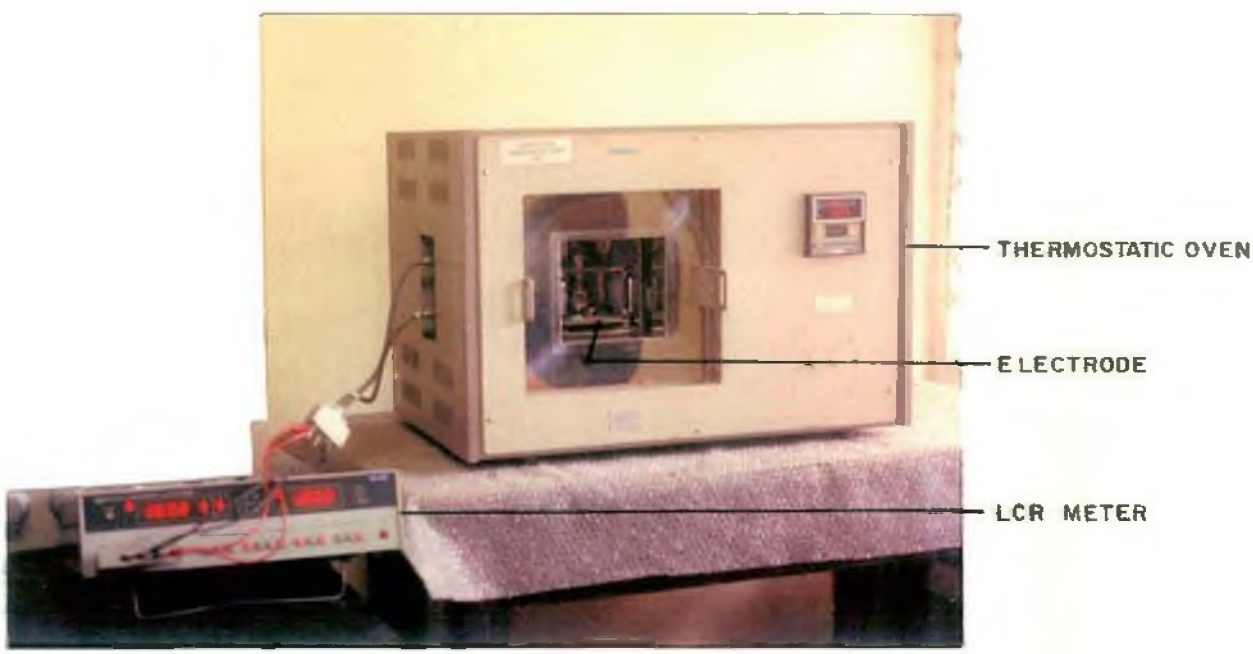


Fig. 5.9. Dielectric measurement unit.

5.13 Results

The dielectric measurements were made for constant frequency (1 kHz) at an approximate heating rate of 4^0C/min from 30^0C to 130^0C. The value of dielectric constant was calculated by using **Eqn. (5.4)**. Experimental data and the dielectric parameters of four low density polyethylene samples are given in **Table nos. 5.1-5.4**. The temperature dependence of the dielectric loss of four individual samples are shown in the form of graph (**Fig Nos. 5.10 to 5.13**).

In **Fig 5.10**, (a) corresponds to the thermal scan of the untreated, fresh Sample No. 1 and (b) to the same sample after 17 months of soil burial. It is seen that, the control **sample no. 1** already exhibits some polar groups during processing or manufacture for which α_c-peak appears between 60-90°C. Upon treatment for 17 months, the loss peak increases in intensity but is not shifted at. However, another weak loss peak at 35 °C appeared which is supposed to be α_a- or β- peak.

The peak in **Fig. 5.11 (a)** for control **sample no. 2** was found to be intensified after treatment. The α_a- transition peak was rather attenuated in **Fig. 5.11 (b)**,even though not shifted from its position.

Dielectric relaxation spectra of untreated, thick, transparent polyethylene **sample no. 3** is very weak as seen in **Fig. 5.12 (a)**. Upon soil burial for 15-17 months in Fig 5.12(b), the tanδ versus temperature spectra at 1 kHz reveals two well resolved peaks- one at a high temperature between 100-110 °C and another broad peak near 40-60°C. As the time progressed, the α_a- transition peak further intensified indicating gradual invasion in amorphous region of the sample.

Out of the four samples, the degree of degradation in sample no. 3 was greater in comparison. Unlike three other samples, relaxation spectra of this sample revealed α_c-transition peak after treatment. Appearance of the loss peak due to crystalline region suggests that the crystalline portion of the sample have also been affected by the soil treatment.

Sample no. 4 also showed weak dielectric relaxation spectra before treatment (**Fig. 5.13a**). However, sample recovered after 17 months of soil burial showed fairly intense α_a- transition peak below 40 °C (**Fig. 5.13b**).

Table 5.1 Experimental data for dielectric properties of Sample no. 01 measured at 1 kHz.

Temp. (°C)	Before burial			After 17 months of soil burial		
	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε'	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε'
30	153.5	0.007	1.3630	71.5	0.133	0.5569
34	155.3	0.008	1.3790	71.4	0.134	0.5549
36	155.2	0.008	1.3781	71.4	0.133	0.5549
38	155.1	0.008	1.3772	70.8	0.131	0.5502
40	155.0	0.008	1.3764	70.6	0.131	0.5487
42	155.0	0.008	13764	70.3	0.128	0.5463
44	154.8	0.008	1.3746	70.0	0.128	0.5440
46	154.6	0.009	1.3728	69.6	0.127	0.5409
48	154.6	0.008	1.3728	69.1	0.125	0.5370
50	154.4	0.008	1.3710	68.9	0.125	0.5354
52	154.2	0.009	1.3692	68.6	0.129	0.5331
54	154.1	0.008	1.3684	68.4	0.128	0.5316
56	153.9	0.008	1.3666	68.2	0.131	0.5300
58	153.8	0.009	1.3657	67.9	0.132	0.5277
60	153.6	0.009	1.3639	68.1	0.133	0.5292
62	153.5	0.009	1.3630	68.1	0.139	0.5292
64	153.3	0.009	1.3613	67.9	0.138	0.5277
66	153.3	0.010	1.3613	67.9	0.140	0.5277
68	153.2	0.009	1.3604	68	0.142	0.5284
70	153.1	0.010	1.3595	68	0.143	0.5284
72	153.0	0.011	1.3586	68	0.141	0.5284
74	153.0	0.010	1.3586	68.1	0.143	0.5292
76	153.0	0.010	1.3586	68	0.141	0.5284
78	152.9	0.010	1.3577	68	0.137	0.5284
80	152.9	0.010	1.3577	68	0.133	0.5284
82	153.0	0.009	1.3586	68.1	0.126	0.5292
84	153.0	0.008	1.3586	68.4	0.122	0.5316
86	153.0	0.008	1.3586	68.3	0.115	0.5308
88	153.0	0.007	1.3586	68.4	0.109	0.5316
90	153.1	0.006	1.3595	68.6	0.098	0.5331
92	153.1	0.005	1.3595	68.7	0.086	0.5339
94	153.2	0.004	1.3604	68.9	0.077	0.5354
96	153.4	0.005	1.3621	69.2	0.066	0.5378
98	153.4	0.004	1.3621	69.5	0.052	0.5401
100	153.5	0.004	1.3630	69.7	0.044	0.5417
102	153.6	0.004	1.3639	69.9	0.033	0.5432
104	153.7	0.004	1.3648	70.3	0.020	0.5463
106	153.9	0.004	1.3666	70.8	0.019	0.5502
108	154.0	0.004	1.3675	71.3	0.015	0.5541
110	154.1	0.003	1.3684	71.7	0.014	0.5572
112	154.3	0.004	1.3701	72.3	0.013	0.5619
114	154.4	0.004	1.3701	72.9	0.012	0.5665
116	154.5	0.004	1.3719	73.4	0.012	0.5704
118	154.7	0.003	1.3737	74.0	0.012	0.5751
120	154.8	0.003	1.3746	74.7	0.011	0.5805
122	155.0	0.004	1.3764	75.5	0.011	0.5867
124	155.1	0.004	1.3772	76.5	0.011	0.5945
126	155.3	0.004	1.3790	77.4	0.012	0.6015
128	155.7	0.004	1.3826	78.6	0.012	0.6108
130	156.0	0.003	1.3852	79.6	0.010	0.6186
132	156.2	0.004	1.3897	80.8	0.011	0.6279
134	156.5	0.004	1.3897	82.6	0.011	0.6419
136	156.8	0.004	1.3923	84.7	0.011	0.6582

Table 5.2 Experimental data for dielectric properties of Sample no. 02 measured at 1 kHz.

Temp. (°C)	Before burial			After 15 months of soil burial		
	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε′	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε′
30	150.5	0.017	0.8354	165.5	0.086	0.7349
32	150.4	0.017	0.8348	165.6	0.087	0.7354
34	150.4	0.017	0.8348	165.2	0.085	0.7336
36	150.3	0.016	0.8343	165.2	0.082	0.7336
38	150.2	0.015	0.8337	163.8	0.080	0.7274
40	150.1	0.014	0.8332	163.2	0.075	0.7247
42	149.9	0.012	0.8320	162.0	0.071	0.7194
44	149.8	0.011	0.8315	160.9	0.066	0.7145
46	149.6	0.010	0.8304	159.8	0.062	0.7096
48	149.4	0.009	0.8293	158.3	0.066	0.7030
50	149.2	0.007	0.8282	157.2	0.051	0.6981
52	149.0	0.006	0.8270	156.0	0.046	0.6927
54	148.9	0.006	0.8265	154.8	0.042	0.6874
56	148.6	0.005	0.8248	153.7	0.038	0.6825
58	148.4	0.005	0.8237	152.7	0.034	0.6781
60	148.2	0.004	0.8226	151.8	0.031	0.6741
62	148.1	0.004	0.8221	150.9	0.028	0.6701
64	147.9	0.004	0.8209	150.2	0.025	0.6670
66	147.8	0.004	0.8204	149.6	0.024	0.6643
68	147.7	0.004	0.8198	149.0	0.022	0.6617
70	147.6	0.003	0.8193	148.5	0.020	0.6594
72	147.6	0.004	0.8193	148.2	0.018	0.6581
74	147.6	0.004	0.8193	147.8	0.017	0.6563
76	147.7	0.003	0.8198	147.5	0.016	0.6550
78	147.7	0.003	0.8198	147.2	0.015	0.6537
80	147.8	0.003	0.8204	147.0	0.014	0.6528
82	147.9	0.003	0.8209	146.8	0.014	0.6519
84	148.0	0.004	0.8215	146.7	0.013	0.6514
86	148.2	0.004	0.8226	146.6	0.013	0.6510
88	148.3	0.003	0.8232	146.5	0.012	0.6506
90	148.5	0.003	0.8243	146.4	0.012	0.6501
92	148.8	0.003	0.8590	146.4	0.012	0.6501
94	149.0	0.003	0.8270	146.4	0.012	0.6501
96	149.2	0.003	0.8282	146.4	0.011	0.6501
98	149.5	0.003	0.8298	146.4	0.011	0.6501
100	149.8	0.003	0.8315	146.4	0.011	0.6501
104	150.4	0.003	0.8348	146.6	0.011	0.6510
108	151.1	0.003	0.8376	146.7	0.011	0.6514
112	152.0	0.003	0.8437	146.9	0.010	0.6523
116	155.8	0.003	0.8498	147.2	0.011	0.6537
120	154.4	0.003	0.8570	147.6	0.010	0.6554
124	155.8	0.004	0.8648	148.0	0.010	0.6572
128	157.6	0.003	0.8748	148.6	0.010	0.6599
130	158.7	0.003	0.8809	149.0	0.010	0.6617

Table 5.3 Experimental data for dielectric properties of Sample no. 03 measured at 1 kHz.

Temp. (^{0}C)	Before burial			After 15 months of soil burial			After 1
	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε'	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε'	Capacitance, C_x (pF)
30	80.5	0.029	1.9658	97.5	0.266	2.5935	69.0
32	80.5	0.030	1.9658	97.3	0.269	2.5881	69.1
34	80.4	0.028	1.9633	97.5	0.278	2.5935	69.3
36	80.3	0.029	1.9609	97.4	0.282	2.5908	69.3
38	80.3	0.030	1.9609	97.1	0.283	2.5828	69.4
40	80.2	0.028	1.9584	96.7	0.284	2.5722	69.5
42	80.1	0.028	1.9560	96.6	0.290	2.5695	69.5
44	80.1	0.027	1.9560	96.5	0.298	2.5669	69.5
46	80.0	0.028	1.9536	96.3	0.300	2.5615	69.5
48	80.0	0.029	1.9536	95.8	0.298	2.5482	69.5
50	80.0	0.029	1.9536	95.0	0.297	2.5270	69.5
52	79.9	0.028	1.9511	94.4	0.301	2.5110	69.4
54	79.7	0.027	1.9462	93.9	0.303	2.4977	69.3
56	79.7	0.028	1.9462	93.0	0.305	2.4738	69.2
58	79.6	0.029	1.9438	91.9	0.300	2.4445	69.1
60	79.6	0.029	1.9438	90.5	0.293	2.4073	68.9
62	79.4	0.027	1.9389	89.1	0.289	2.3700	68.7
64	79.4	0.029	1.9389	87.8	0.275	2.3354	68.5
66	79.3	0.028	1.9365	85.9	0.256	2.2849	68.2
68	79.1	0.023	1.9316	83.7	0.228	2.2264	68.0
70	79.1	0.028	1.9316	81.4	0.197	2.1652	67.6
72	79.0	0.028	1.9291	79.1	0.170	2.1040	67.4
74	78.9	0.026	1.9267	77.0	0.149	2.0482	67.0
76	78.7	0.023	1.9218	74.6	0.124	1.9843	66.7
78	78.6	0.024	1.9194	72.3	0.105	1.9231	66.3
80	78.6	0.025	1.9194	70.5	0.099	1.8753	66.0
82	78.5	0.024	1.9169	68.9	0.100	1.8327	65.7

Table 5.3 Contd.

Temp.	Before burial			After 15 months of soil burial			After
(^{0}C)	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε′	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε′	Capacitance, C_x (pF)
84	78.4	0.023	1.9145	67.5	0.089	1.7955	65.4
86	78.3	0.025	1.9120	66.1	0.081	1.7582	65.2
88	78.2	0.026	1.9096	65.1	0.073	1.7316	65.0
90	78.1	0.024	1.9072	64.8	0.080	1.7236	64.7
92	77.9	0.023	1.9023	64.4	0.079	1.7130	64.5
94	77.8	0.022	1.8998	63.8	0.067	1.6970	64.3
96	77.8	0.023	1.8998	63.4	0.061	1.6864	64.1
98	77.7	0.024	1.8974	63.2	0.066	1.6811	64.0
100	77.7	0.022	1.8974	63.2	0.067	1.6811	63.8
102	77.5	0.022	1.8925	62.8	0.060	1.6704	63.6
104	77.4	0.023	1.8901	62.5	0.055	1.6625	63.4
106	77.4	0.024	1.8901	62.7	0.065	1.6678	63.3
108	77.2	0.022	1.8852	62.7	0.069	1.6678	63.1
110	77.2	0.022	1.8852	62.3	0.054	1.6571	63.0
112	77.1	0.023	1.8827	62.2	0.052	1.6545	62.9
114	77.1	0.028	1.8827	62.4	0.061	1.6598	62.7
116	77.1	0.027	1.8827	62.4	0.062	1.6598	62.6
118	77.2	0.028	1.8852	62.3	0.056	1.6571	62.5
120	77.2	0.029	1.8852	62.2	0.049	1.6545	62.5
122	77.1	0.026	1.8827	62.7	0.060	1.6678	62.5
124	77.1	0.026	1.8827	62.7	0.054	1.6678	62.5
126	77.2	0.027	1.8852	62.9	0.053	1.6731	62.5
128	77.3	0.027	1.8876	63.4	0.055	1.6864	62.7
130	77.4	0.026	1.8901	64.3	0.062	1.7103	63.0
132	77.6	0.027	1.8949	65.1	0.053	1.7316	63.4
134	77.8	0.027	1.8998	66.6	0.046	1.7715	63.8
136	77.8	0.025	1.8998	68.5	0.057	1.8221	64.3
138	77.8	0.026	1.8998	71.1	0.053	1.8912	66.4
140	78.4	0.026	1.9145	74.3	0.046	1.9763	69.0

Table 5.4 Experimental data for dielectric properties of Sample no. 04 measured at 1 kHz.

Temp. (°C)	Before burial			After 17 months of soil burial		
	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε′	Capacitance, C_x (pF)	Loss tangent (tanδ)	Dielectric constant ε′
30	134.8	0.011	1.6459	191.8	0.622	2.3418
32	134.6	0.009	1.6434	192.1	0.624	2.3455
34	134.7	0.010	1.6446	191.4	0.623	2.3369
36	134.6	0.009	1.6434	189.0	0.616	2.3076
38	134.6	0.010	1.6434	183.5	0.600	2.2405
40	134.6	0.011	1.6434	178.5	0.577	2.1794
42	134.5	0.010	1.6422	171.5	0.545	2.0940
44	134.4	0.009	1.6410	163.0	0.500	1.9902
46	134.3	0.009	1.6398	153.3	0.440	1.8717
48	134.2	0.010	1.6385	145.0	0.364	1.7704
50	134.1	0.011	1.6373	136.5	0.302	1.6666
52	133.9	0.010	1.6349	130.0	0.225	1.5873
54	133.8	0.009	1.6336	124.6	0.168	1.5213
56	133.7	0.009	1.6324	120.6	0.118	1.4725
58	133.5	0.009	1.6300	117.6	0.078	1.4358
60	133.4	0.010	1.6288	115.7	0.056	1.4126
62	133.4	0.011	1.6288	114.3	0.040	1.3956
64	133.3	0.009	1.6275	113.1	0.030	1.3809
66	133.2	0.009	1.6263	112.2	0.025	1.3699
68	133.0	0.010	1.6239	111.5	0.021	1.3614
70	132.8	0.010	1.6214	111.2	0.019	1.3577
72	132.7	0.011	1.6202	111.0	0.028	1.3553
74	132.5	0.010	1.6178	110.0	0.025	1.3553
76	132.3	0.009	1.6153	110.5	0.024	1.3492
78	132.0	0.010	1.6117	110.4	0.025	1.3479
80	131.9	0.010	1.6104	110.4	0.025	1.3479
82	131.6	0.011	1.6068	110.3	0.025	1.3467
84	131.3	0.009	1.6031	110.3	0.023	1.3467
86	131.0	0.009	1.5995	110.4	0.024	1.3479
88	130.8	0.009	1.5970	110.4	0.023	1.3479
90	130.5	0.010	1.5934	110.4	0.022	1.3479
92	130.4	0.010	1.5921	110.4	0.020	1.3479
94	129.9	0.009	1.5860	110.4	0.021	1.3492
96	129.6	0.009	1.5824	110.5	0.021	1.3504
98	129.4	0.009	1.5799	110.6	0.020	1.3504
100	129.1	0.010	1.5763	110.6	0.018	1.3504
102	128.9	0.009	1.5738	110.6	0.019	1.3516
104	128.6	0.009	1.5702	110.7	0.020	1.3540
106	128.3	0.009	1.5665	110.9	0.019	1.3540
108	128.2	0.009	1.5653	110.9	0.017	1.3540
110	128.1	0.009	1.5641	111.0	0.017	1.3553
112	127.9	0.010	1.5616	111.1	0.018	1.3565
114	127.9	0.009	1.5616	111.1	0.018	1.3565
116	128.0	0.009	1.5628	111.2	0.015	1.3577
118	128.3	0.010	1.5665	111.4	0.017	1.3601
120	128.4	0.008	1.5677	111.5	0.017	1.3614
122	128.7	0.009	1.5714	111.6	0.007	1.3504
124	128.9	0.0	1.5738	111.7	0.006	1.3516
126	129.3	0.010	1.5787	112.0	0.007	1.3675
128	129.6	0.008	1.5824	112.3	0.007	1.3711
130	130.0	0.008	1.5873	112.6	0.006	1.3748
132	130.6	0.010	1.5946	113.0	0.006	1.3797
134	131.2	0.009	1.6019	113.8	0.007	1.3894
136	131.8	0.008	1.6092	115.2	0.007	1.4065
138	131.9	0.008	1.6104	116.5	0.006	1.4224
140	131.7	0.009	1.6080	119.2	0.006	1.4554

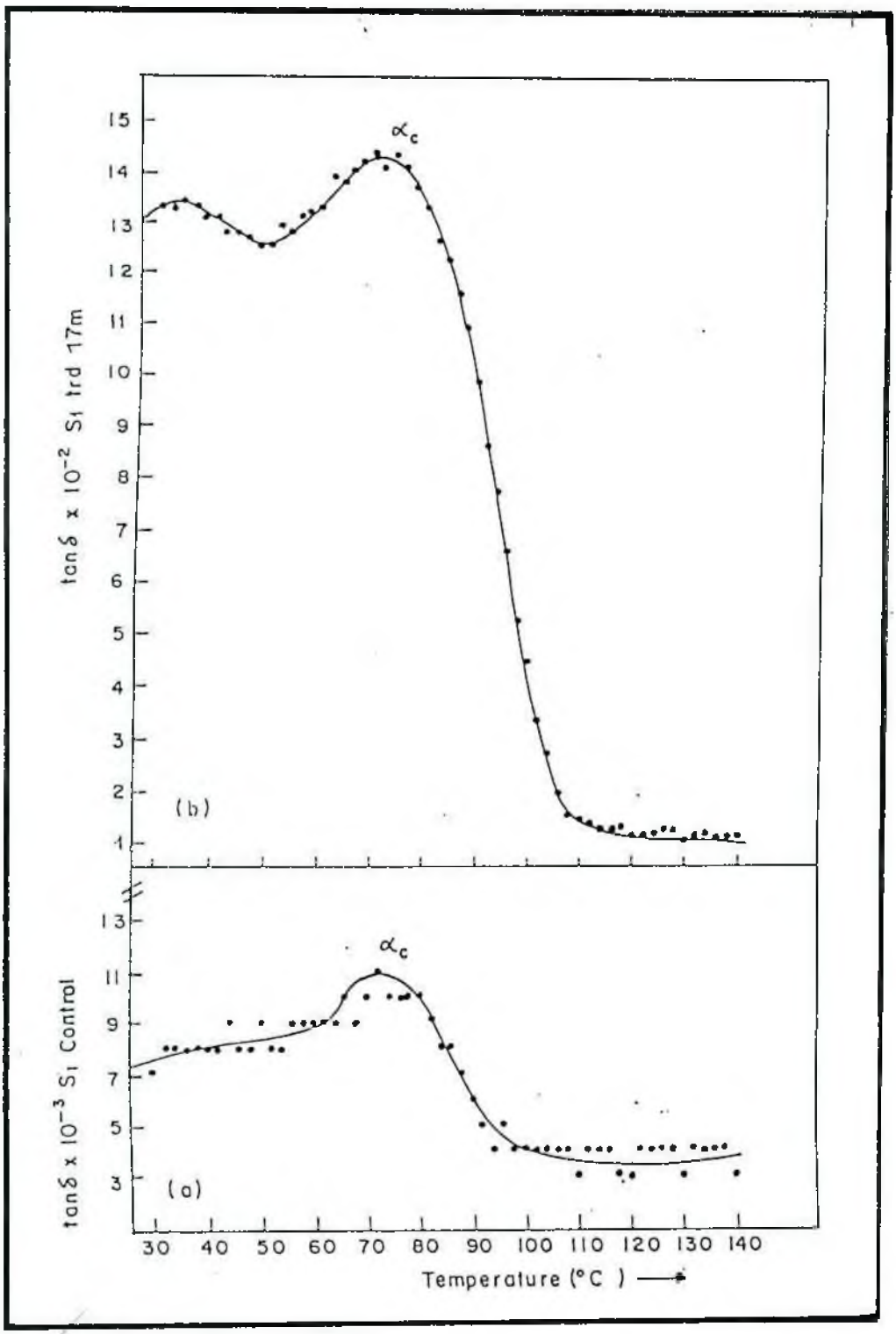


Fig. 5.10. Graph showing tanδ versus temperature at 1 kHz for LDPE sample No. 01 after 0 month (a) and 17 months (b) of soil burial.

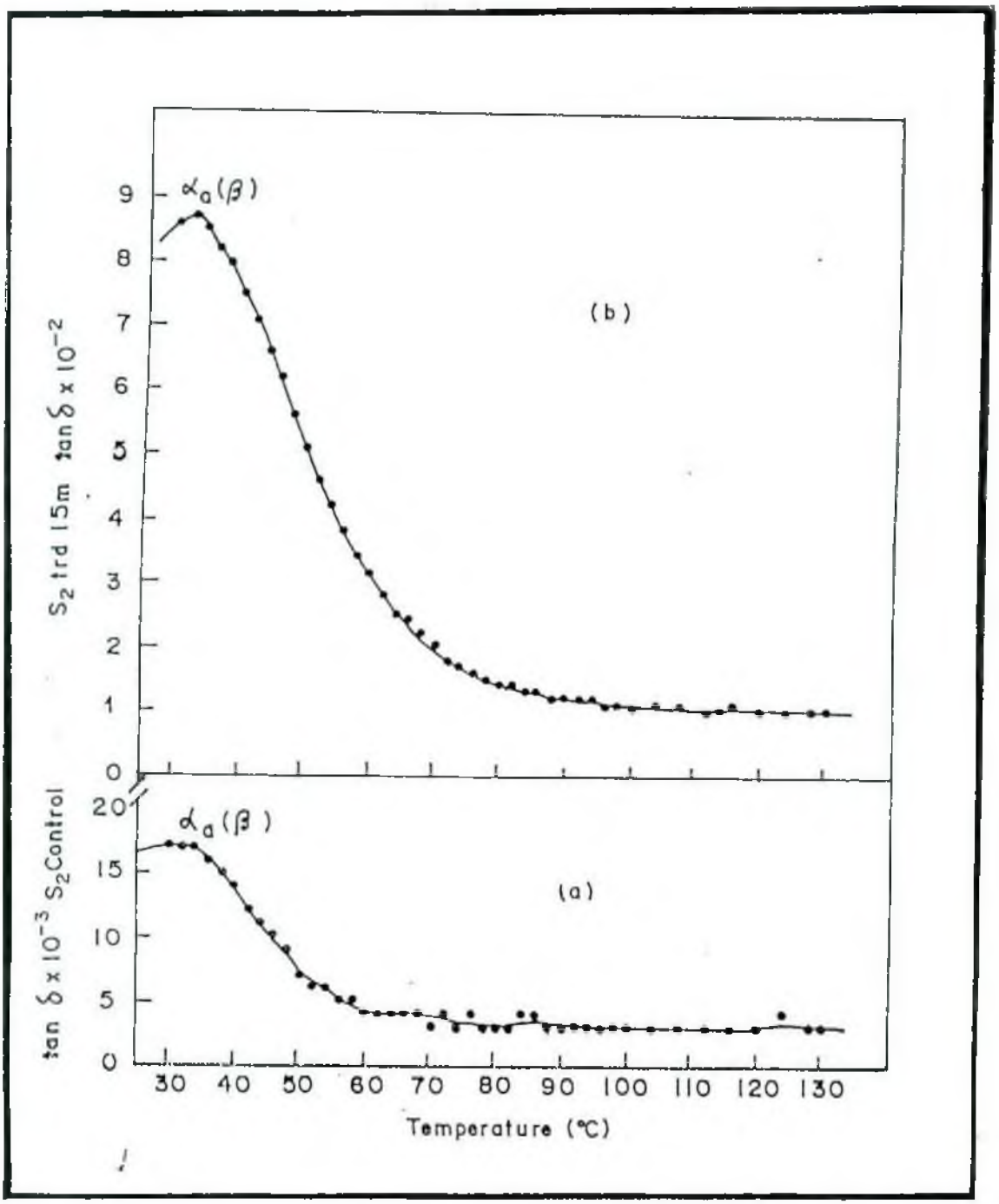


Fig. 5.11: Graph showing tanδ versus temperature at 1 kHz for LDPE sample No. 02 after 0 month (a) and 15 months (b) of soil burial.

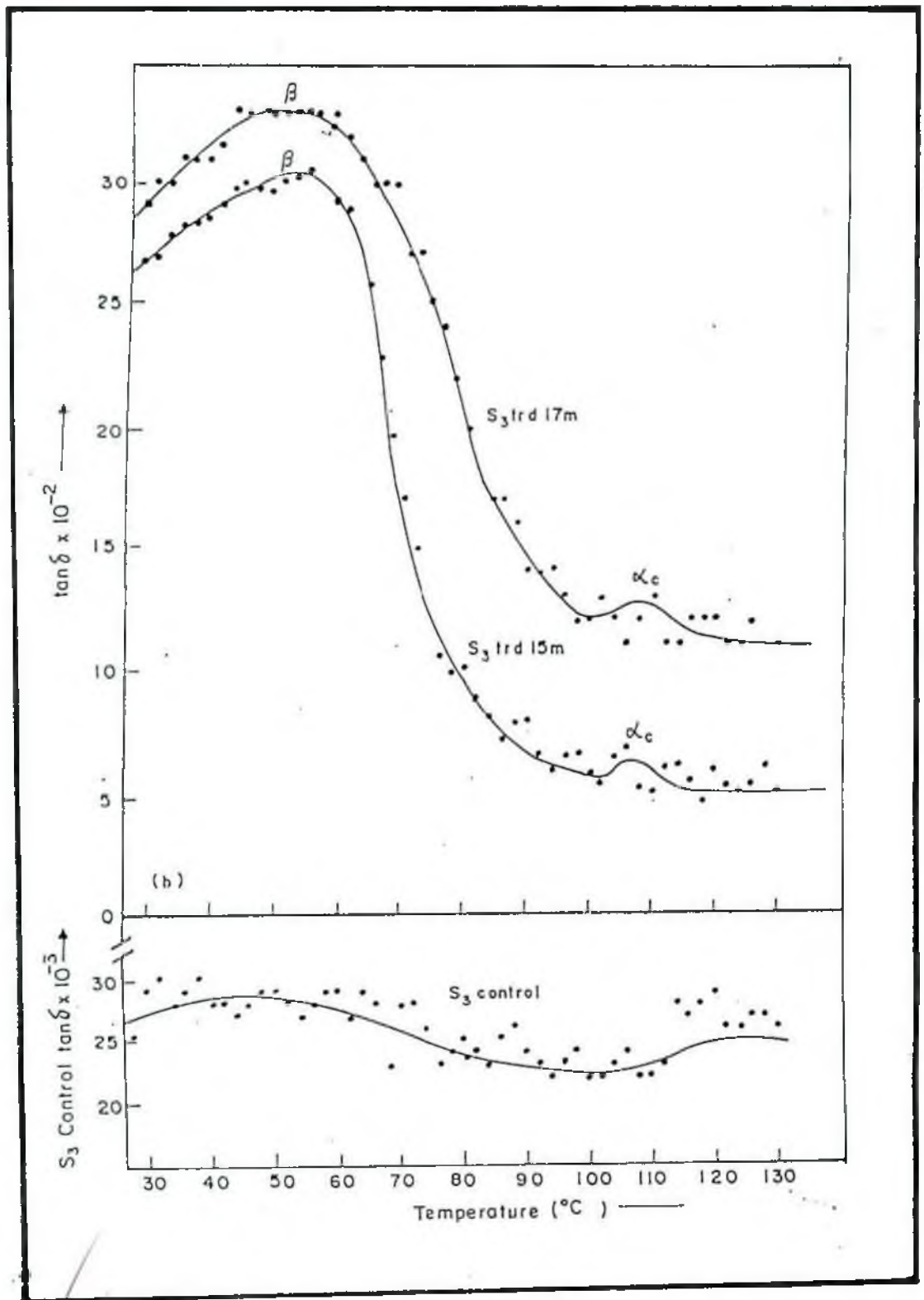


Fig. 5.12. Graph showing tanδ versus temperature at 1kHz for LDPE sample no. 03 after 0 month (a), 15 and 17 months (b) of soil burial.

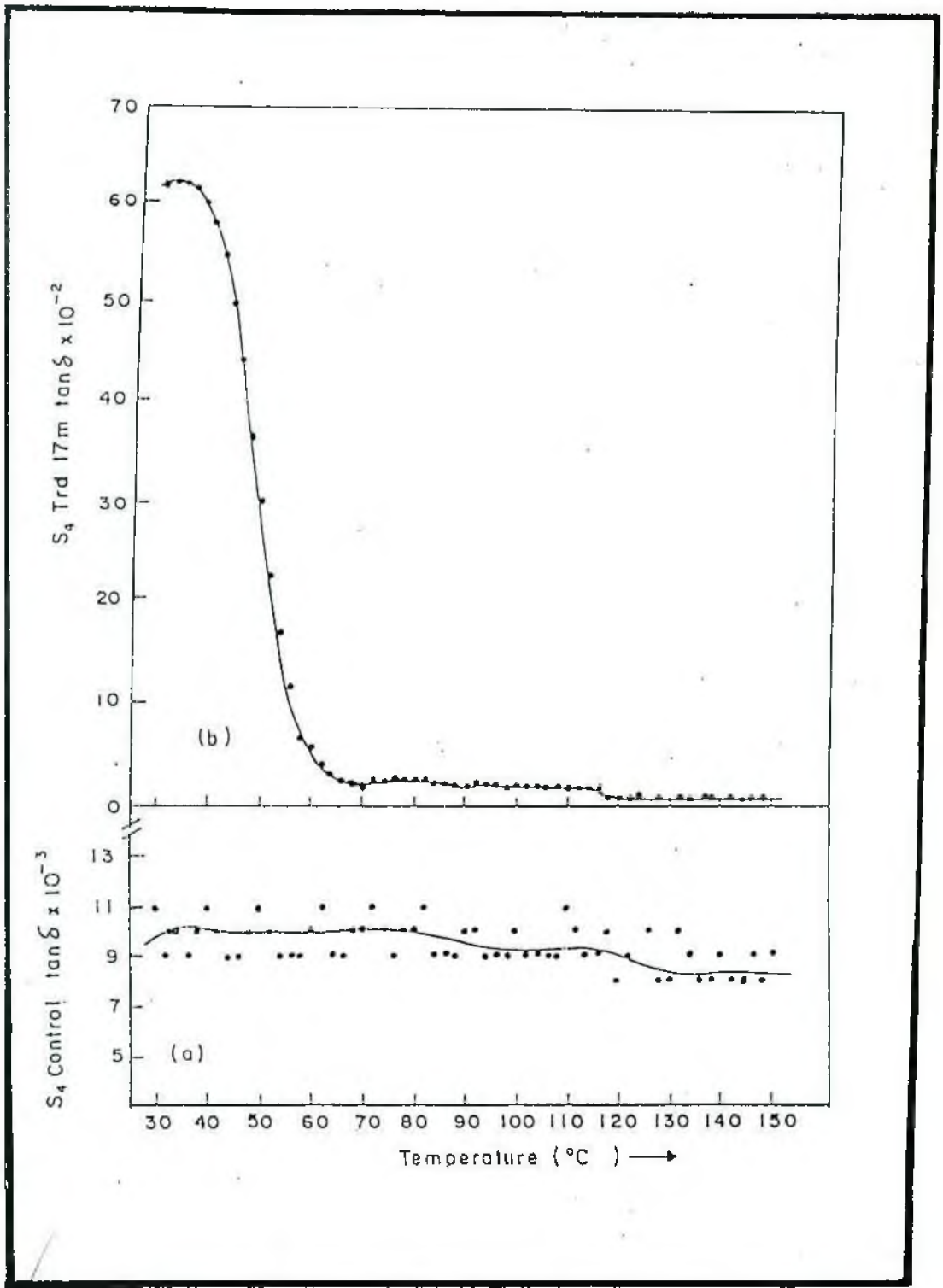


Fig. 5.13. Graph showing tanδ versus temperature at 1 kHz for LDPE sample No. 04 after 0 month (a) and 17 months (b) of soil burial.

5.14 Discussion

The physical and chemical structure and the macroscopic properties of polymers change during processing, storage, and use. This change commonly referred to as aging, is evidently a very complex process involving chemical reactions, physical structural changes and changes in the mechanical, thermal and electric properties of the material. Even biological problems are involved in connection with the effect of some microorganisms on the polymeric materials. Dielectric spectroscopy has much to contribute to understanding chemical and physical aging processes (Hedvig 1977).

An important group of aging processes involves main chain scission. As a result of this the average molecular weight of the polymer decreases; the polymer degraded down to its monomer units.

The minimum zip-length during the thermal depolymerization for polyethylene is 0.01 and the percentage monomer yield is very negligible ca. 0.025. In case of polyethylene, the chain scission is practically a random process. In such polymers, the average molecular weight gradually decreases even in the first stage of degradation. The transition temperatures of randomly degrading polymers measured by dielectric or mechanical relaxation spectroscopy are expected to shift down to lower temperatures as degradation proceeds.

Oxidative degradation of polymers is, of course, a very complex process, which is not fully understood. Polymers in which degradation is not principally a chain reaction, the random chain scission are highly accelerated, if not induced, by the presence of oxygen.

Apolar polymers like polyethylene exhibits dielectric spectra as a result of polar groups formed by oxidation. The main transitions in polyethylene correspond to the crystalline and amorphous phases as follows: T_m-melting temperature, crystallite phase; α_c (α)-transition: crystalline phase, surface of crystallites; α_a(β)-transition: amorphous phase; γ-transition: crystallite defects and amorphous phase (Hedvig 1977).

Carlowitz (1992) illustrated the temperature curve of tanδ at 1 and 10 kHz for both HDPE and LDPE. He observed two maxima of the loss factor: the α absorption due to motion of the CH_2 dipoles and the γ-absorption due to relaxation in crystalline regions (**Fig. 5.14**). Branching in LDPE gives rise to a third absorption region, β absorption, which lies between the α and γ- absorptions in the tanδ curves.

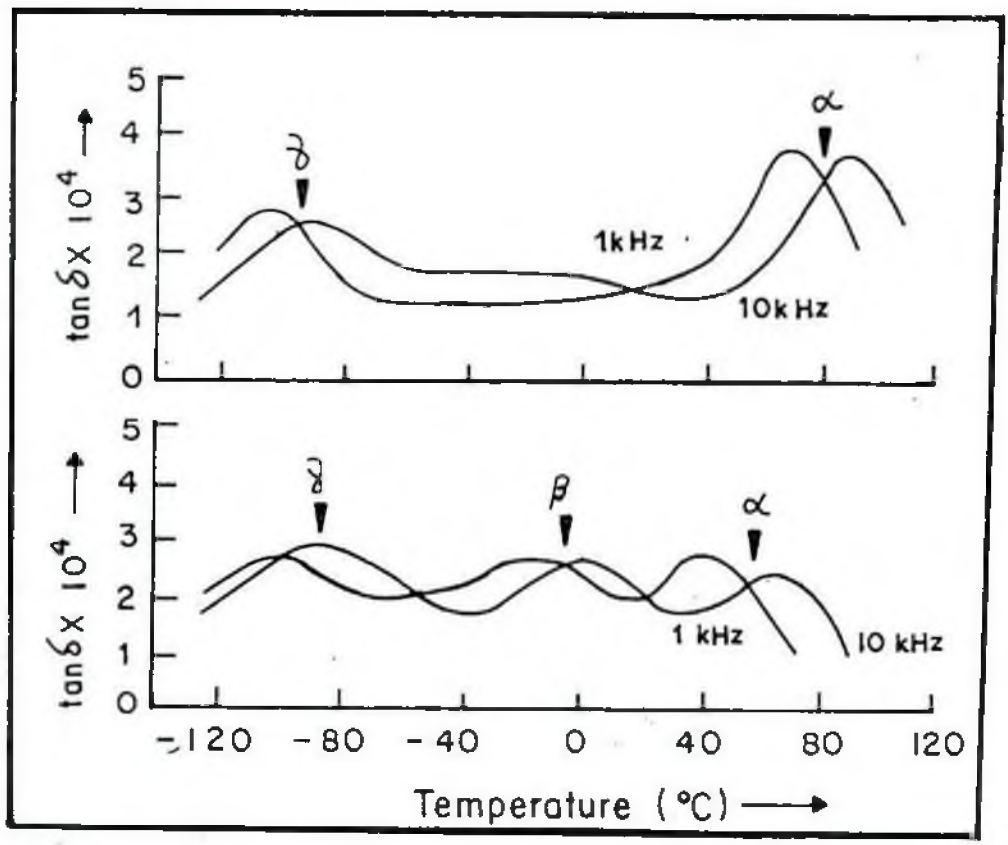


Fig. 5.14. Graph showing tanδ versus temperature at 1 and 10 kHz for HDPE (top) and LDPE (bottom) films (after Carlowitz 1992).

Oakes and Robinson (1954) established the fine structure of in the tanδ-temp curve for oxidized polyethylene. They identified three loss peaks and observed that the loss peaks are increased in intensity but are not shifted in frequency by oxidation.

According to Reddish and Berrie (1969) oxidation in LDPE is more likely to be higher in the amorphous phase than at crystallite surfaces. Correspondingly, most of the dielectric effect due to oxidation is expected in the α_a-region.

In the present study, the dielectric spectra of commercial formulations of fresh polyethylene samples were compared with those after soil burial. In all types of polyethylene samples, serious increase in the values of power factor was found which reflects the oxidation of films with the formation of carbonyl groups. It was also seen that with increasing exposure time, the oscillator strength of α_a- transition region increase at different rates indicating that the resulting degradation indeed proceeds preferably in the amorphous region.

Chapter - 6

INFRA-RED AND X-RAY DIFFRACTION ANALYSIS

Infra-red and X-ray diffraction analysis

6. 1 Infra-Red Spectral Analysis of Polyethylene

6.1.1 Introduction

Infrared radiation refers broadly to that part of the electromagnetic spectrum between the visible and the microwave regions. Of greatest practical use to the organic chemist is the limited portion between 4000cm^{-1} and 666cm^{-1}(2.5-15.0μ) (Silverstein and Bassler 1967).

Infrared radiation of frequencies less than about 100 cm^{-1} (wavelengths longer than 100μ) is absorbed and converted by an organic molecule into energy of molecular rotation. This absorption is quantized; thus a molecular rotation spectrum consists of discrete lines. Infrared radiation in the range from about 10,000- 100cm^{-1}(1-100μ) is absorbed and converted by an organic molecule into energy of molecular vibrational spectra appear as bands rather than lines because a single vibrational energy change is accompanied by a number of rotational energy changes.

The frequency or wavelength of absorption depends on the relative masses of the atoms, the force constants of the bonds, and the geometry of the atoms. Band positions in infrared spectra are presented either as wavelengths or frequencies. The common unit of wavelength in infrared spectrometry is the micron (μ), equal to the 10^3mm. Frequencies are usually expressed in terms of wavenumber (ν) whose unit is the reciprocal centimeter (cm^{-1}). In terms of this unit, the wavenumber is the reciprocal of the wavelength in centimeters. Or, when the wavelength is in microns, the wavenumber is $1/\mu \times 10^4$. The energy of radiation is directly proportional to the frequency.

Band intensities are expressed either as transmittance (T) or absorbance (A). Transmittance is the ratio of the radiant power transmitted by a sample to the radiant power incident on the sample. Absorbance is the logarithm to the base 10, of the reciprocal of the transmittance, $A = \log_{10}(1/T)$.

There are two types of molecular vibrations: stretching and bending. A stretching vibration is a rhythmical movement along the bond axis such that the inter-atomic distance is increasing or decreasing. A bending vibration may consist of a change in bond angles between bonds with a common atom, or the movement of a group of atoms with

respect to one another. For example, twisting, rocking and tortional vibrations involve a change in bond angles with reference to a set of coordinates arbitarily set up within the molecule.

Only those vibrations that result in a rhythmical change in the dipole moment of the molecule are observed in the infrared. The alternating electric field, produced by the changing charge distribution accompanying a vibration with the oscillating electric field of the electromagnetic radiation.

IR spectroscopy is used to determine the molecular composition of polymers by analyzing the characteristic vibrations of the functional groups (**Fig. 6.1**). The more recent applications of vibrational spectroscopy are the characterization of the microstructures and their relationships to the macroscopic properties of polymers. The principal advantage of spectroscopy is that it is a short range technique depending only on local chain conformation and packing. Therefore, orientation information obtained from spectroscopy is highly selective and can be used to characterize individual chain segmental orientation in both crystalline and amorphous regions (Booth and Price 1989).

6.1.2 IR Spectra of LDPE

Infra-red spectra of low density polyethylene (LDPE) show very strong bands which leave no doubt that the molecules consist for the most part of CH_2 chains (Bunn 1957). Absorption bands are observed corresponding to:

- C-H stretching bands of CH_2 groups (symmetric stretching) 2850 cm^{-1}
- C-H stretching bands of CH_2 groups (asymmetric stretching) 2926 cm^{-1}
- Symmetric deformation at 1464 and 1473 cm^{-1}
- Asymmetric deformation at 720 and 730 cm^{-1}

The infra-red absorption spectra of most specimens show also bands due to CH_3 groups, notably a symmetric stretching band at 2962 cm^{-1}, and a symmetric deformation band at 1378 cm^{-1}. For low density polyethylenes, the intensities of these bands are much greater showing that each molecule is branched. Absorption bands due to various types of unsaturated groups are also found. In low density polyethylenes, some four-fifths of the unsaturated groups are pendant methylene groups $R_1R_2C=CH_2$ (bands at 887 and 1640 cm^{-1}), the remainder being chain unsaturated groups $R_1CH=CHR_2$ (700 cm^{-1} cis, 964 and 1660 cm^{-1} trans) (Bunn 1957).

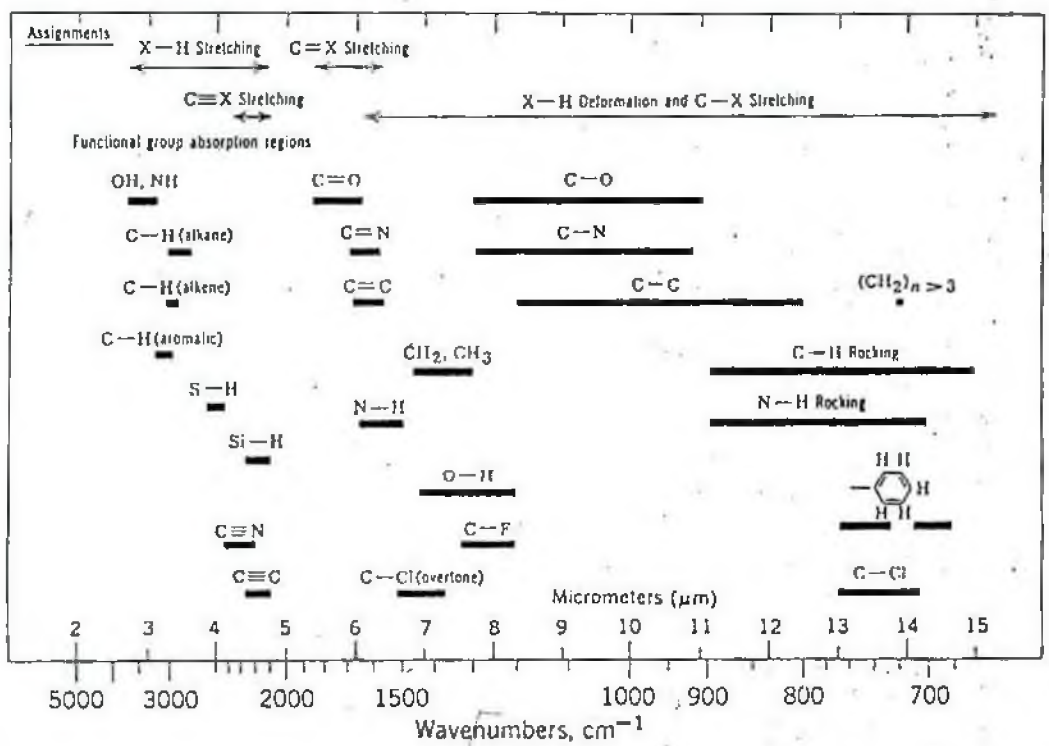


Fig. 6.1. Significant infra-red absorption bands in polymers (after Billmeyer 1984).

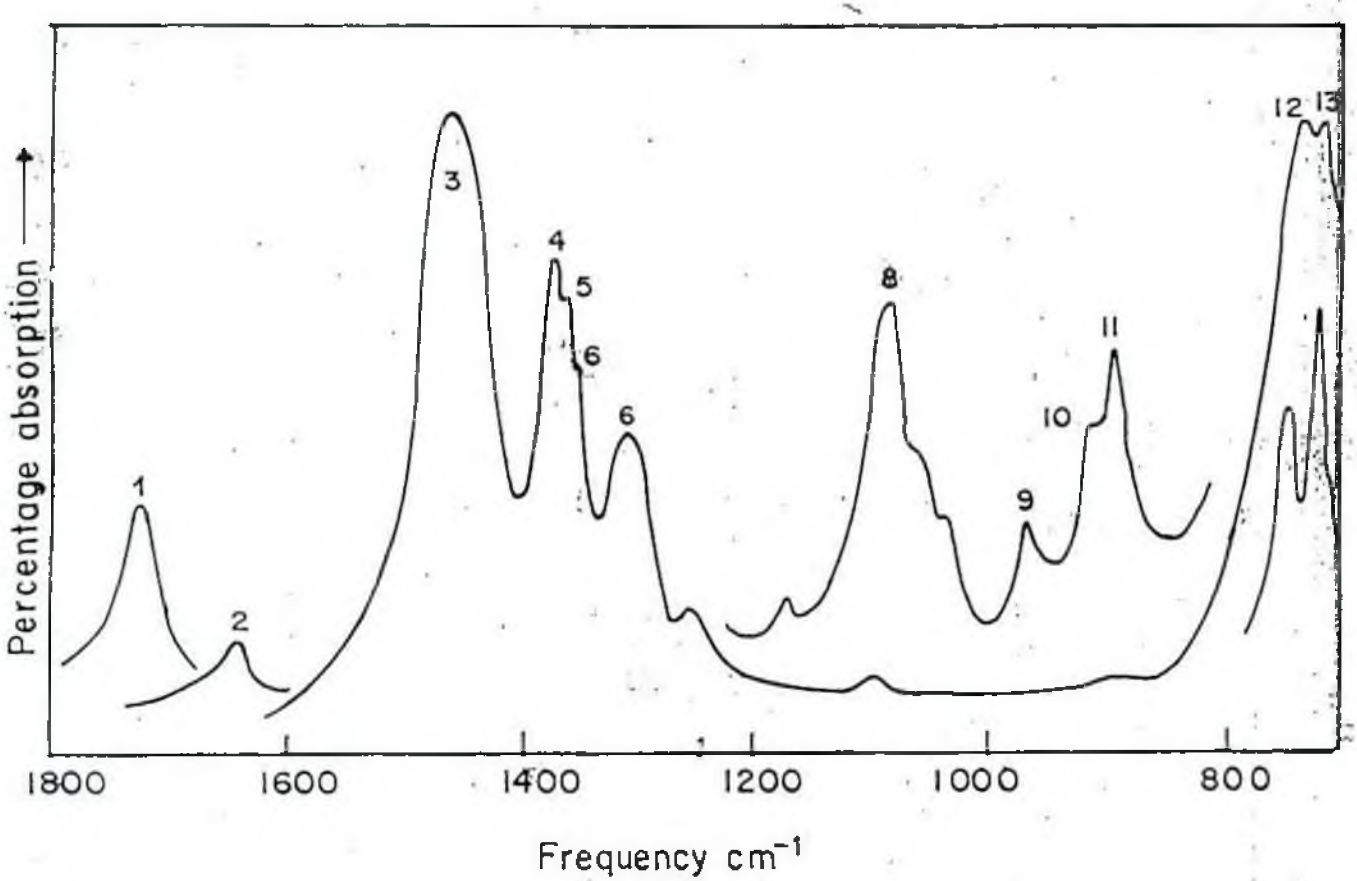


Fig. 6.2. Infra-red absorption spectrum of high pressure polythene (after Bunn 1957).

A weak band at 1720 cm^{-1} due to C=O stretching is sometimes found; the oxygen presumably comes from oxygen-containing catalysts used in the high-pressure process. A hydroxyl band at 3300 cm^{-1} is also sometimes found. **Figure Nos. 6.2-6.4** shows IR spectra of typical polyethylene (LDPE) taken as absorbance or transmittance units as cited by different authors(Goheen and Wool 1991, Prakash and Srivastava 1977 and Bunn 1957).

6.1.3 Materials and Methods

The Fourier transform infrared (FT-IR) spectrophotometer used was a Shimadzu instrument (Shimadzu, Japan) Model 8300 controlled by Hyper-IR software (**Fig. 6.5**). It is a high sensitivity FTIR with Dynamic Alignment Functions.

6.1.3.1 Operation of the FTIR 8300 spectrophotometer:

Features:

- Dynamic alignment functions ensure the most suitable interferometer alignment.
- The validation program assures the basic performance of the instrument.
- The complete file history from the measurement conditions to the data processing history is automatically recorded.
- The higher SN ratio is obtained by the adoption of a long- life bright ceramic light source and high sensitivity detector.
- Hyper-IR Software in Windows ™95 for complete Infrared Analysis.
- Provision of a Variety of Accessories from Macro to Micro.

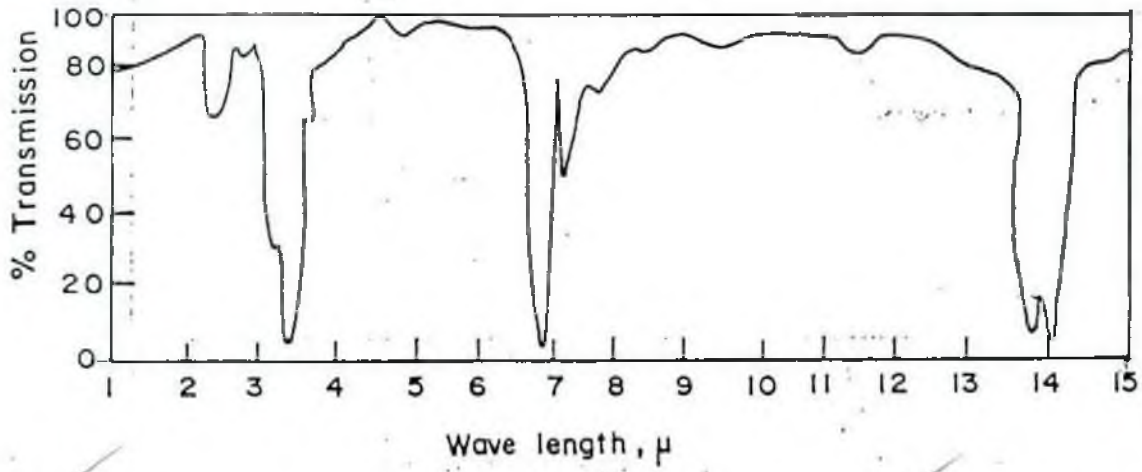


Fig. 6.3. Infrared spectra of polyethylene measured at 20°C (after Prakash and Srivastava 1977).

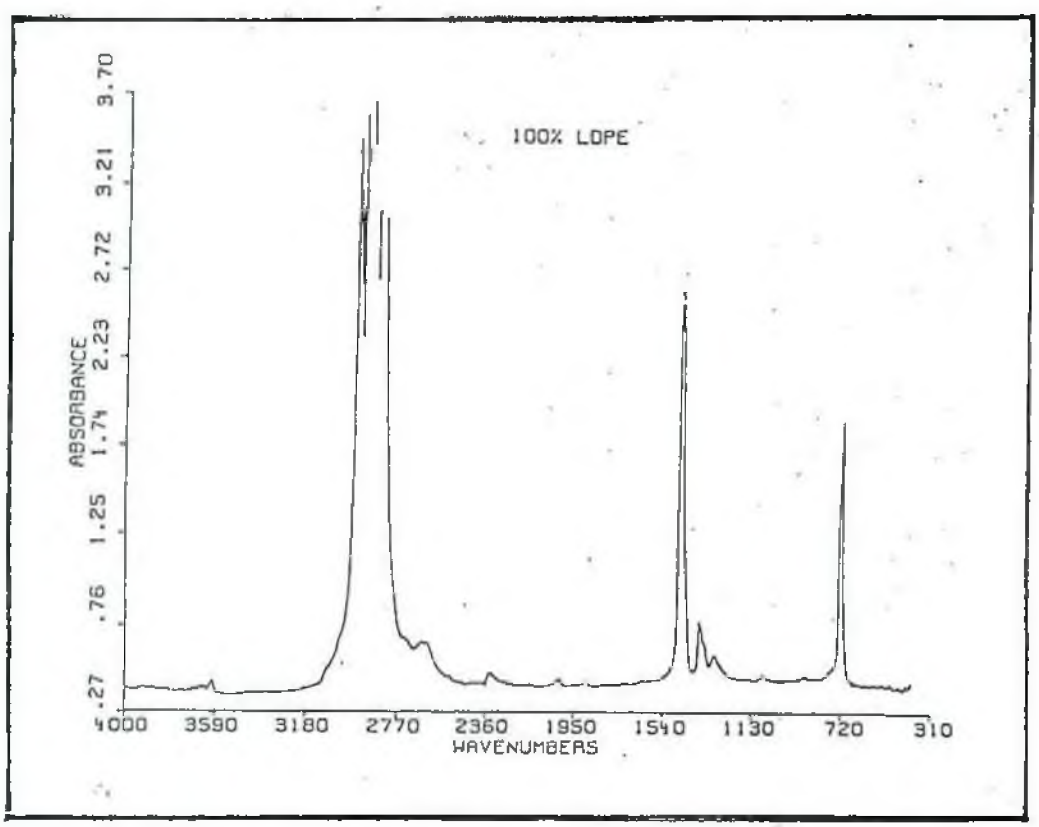


Fig. 6.4. Infra-red spectrum of 100% LDPE (Goheen and Wool 1991)

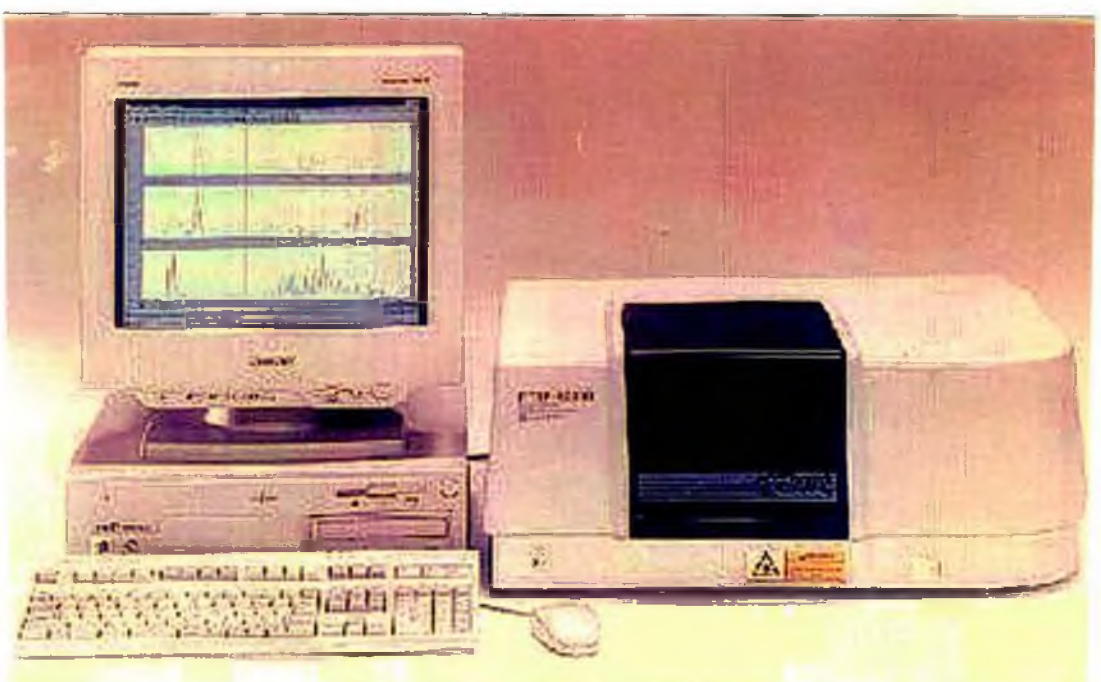


Fig. 6.5. Outside view of Shimadzu FTIR 8300 Spectrophotometer.

6.1.3.2 Specifications:

Interferometer	Michelson type with 30degree Incident Angle, Dynamic Alignment, Sealed and Dessicated.
Optical system	Single Beam Optics
Beam Splitter	Germanium coated KBr plate
Light Source	Ceramic
Detector	Temperature Controlled High Sensitivity Detector (DLATGS Detector)
S/N	Above 3200:1 (KBr plate, $4cm^{-1}$, for 4 seconds, around $2200m^{-1}$, P-P)
Wave Number Range	7800 cm^{-1} ~ 350 cm^{-1}
Resolution	1,2,4,8,16 cm^{-1}
Mirror Speed	3 Steps: 2.8 mm/sec, 5mm/sec, 9mm/sec
Data Sampling	By He-Ne laser
Sample Compartment	W200XD230XH170 mm
Data Processing Functions	Arithmatic calculation, peak pick, spectrum subtraction, smoothing, baseline correction, data cut , data correction, noramalize, Kubelka-Munk conversion, Kramers-Kroning analysis, ATR correction, Fourier transform, derivatives, transmittance-absorbance conversion, peak area integration, peak-ratio calculation, Emission correction, deconvolution, quantitation, multi-linear regression quantitation, spectrum search, JCAMP conversion, ASCII conversion, logarithmic conversion, wavelength wavenumber conversion, shift along X axis.
Optional Programmes	PLS quantitation, curve fitting, Mapping Programme, Macro program (BASIC)
Validation Program	Provided as standard. The polystryene auto-shutter for use in full automatic mode is provided as an option
Operating Conditions	Temperature 15^0C to 30^0C, Humidity: Below 70%
Required power source	AC 100V/120V/220V/230V/50/60Hz, 160VA

6.1.3.3 Preparation of the Specimens

Samples to be analyzed by FT-IR spectrometer were cut out from the exposed films into 15-20 mm x 35-40 mm strips with the help of an NT-cutter after thorough washing with water. The dimension of the standard plate for holding solid sample was typically of 10mm x 21 mm. The strips were then directly affixed to standard FT-IR plate with cellotape. At the beginning, the instrument was calibrated using Polystyrene as a standard (**Fig. 6.6**). The spectrum performed was from 500 to 4,000 wave numbers (cm^{-1}) for each sample.

6.1.4 Results

Samples retrieved after 17 months from soil burial were evaluated for changes by FT-IR spectroscopy. In case of Sample No. 03 changes after 11 months was also examined. The same FT-IR spectra were exhibited for all duplicate specimens. FT-IR spectra of individual samples before and after 17 months of soil exposure are shown in **Fig 6.7-6.16**.

Fig 6.7a,b shows IR spectra of white LDPE sample No. 01(25μm) before and after burial for 17 month, respectively. Besides the typical bands of polyethylene (C-H valence vibration at 2916 and 2850 cm^{-1}, C-H deformation vibration band at 1460 and 1471 cm^{-1} and $-CH_2-$ rocking vibration at 730 cm^{-1}), a few weak bands at 900 and 1000cm^{-1} and several medium strength bands between 1000-1200 cm^{-1} were observed in the control, untreated sample. These extra bands correlate with primary and secondary alcohols. However, after treatment, replaced band at 1076 cm^{-1} was found in the sample. Bands around 1100-1400 cm^{-1} flattened from the corresponding control film. The characteristic C-H stretching band, C-H deformation band and CH_2- rocking vibration are not affected after prolonged exposure in soil for 17 months. Transmittance is reduced considerably.

Upon soil burial, new bands at 1006-1031cm^{-1} appeared in black LDPE film (sample no. 02) whereas the corresponding control film demonstrated no change (**Fig. 6.8 a,b**). The typical bands in the sample remain unaltered although transparency decreased to certain extent. The carbonyl absorption bands remains same without any significant absorption at 1715$cm^{2)}$.

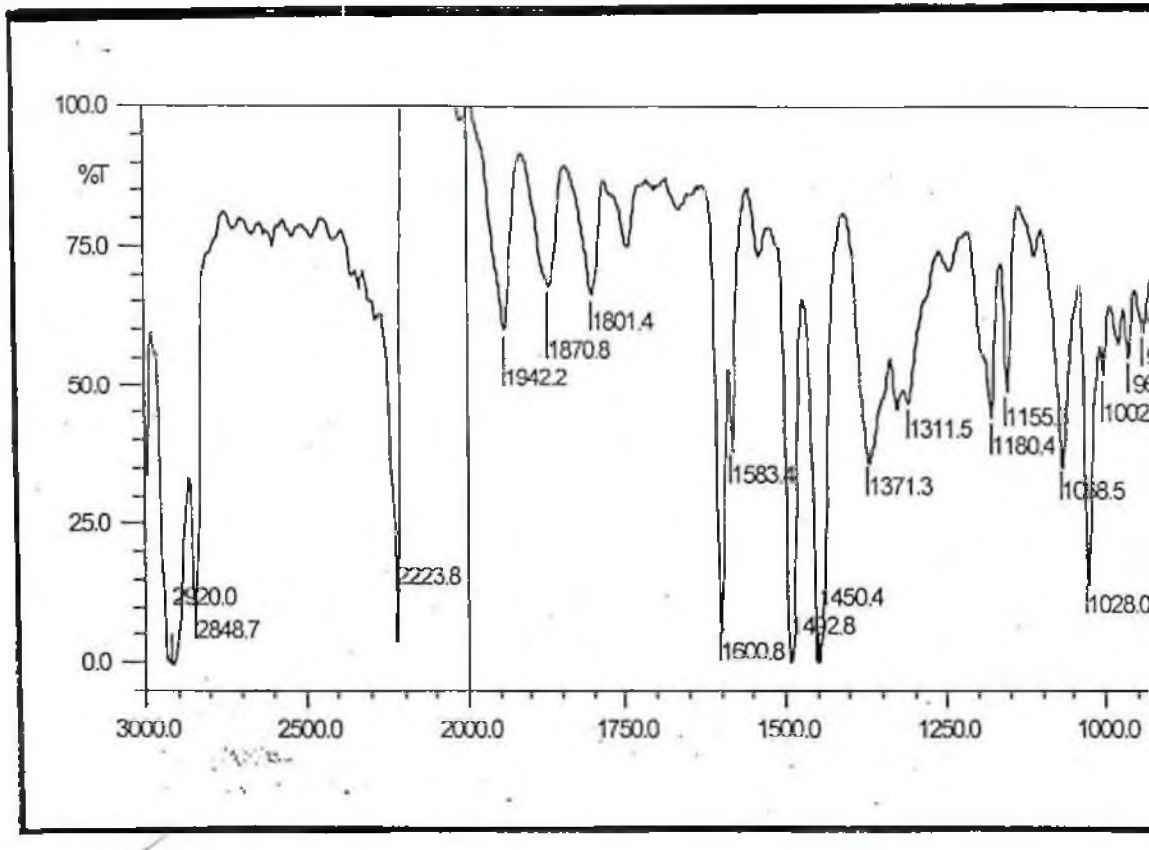


Fig. 6.6. Fourier-transform Infra-red (FTIR) spectrum of a standard polystyrene film (x
y-axis percentage of transmission).

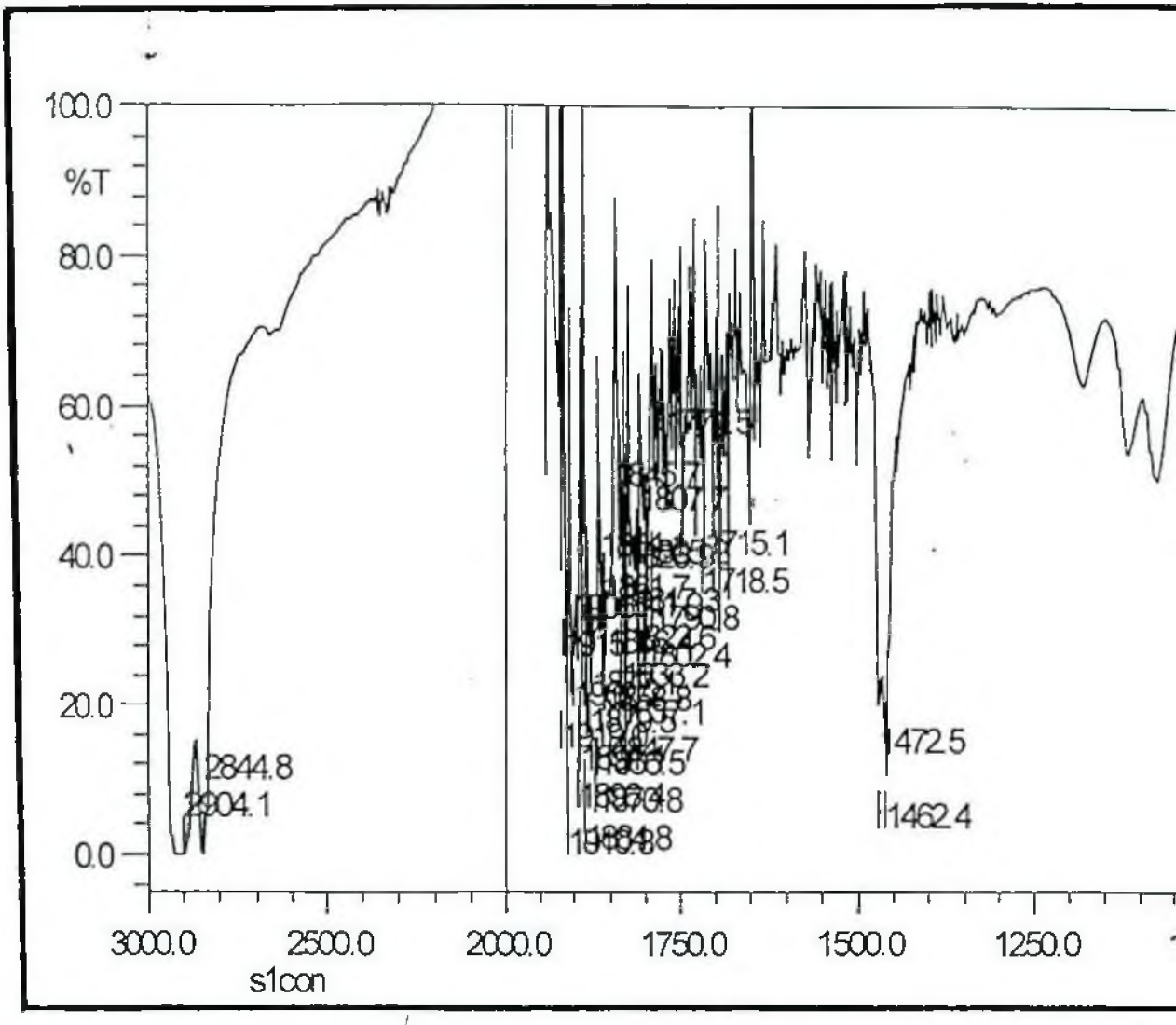


Fig. 6.7. FTIR spectra of LDPE sample no. 01 (a) before soil bur

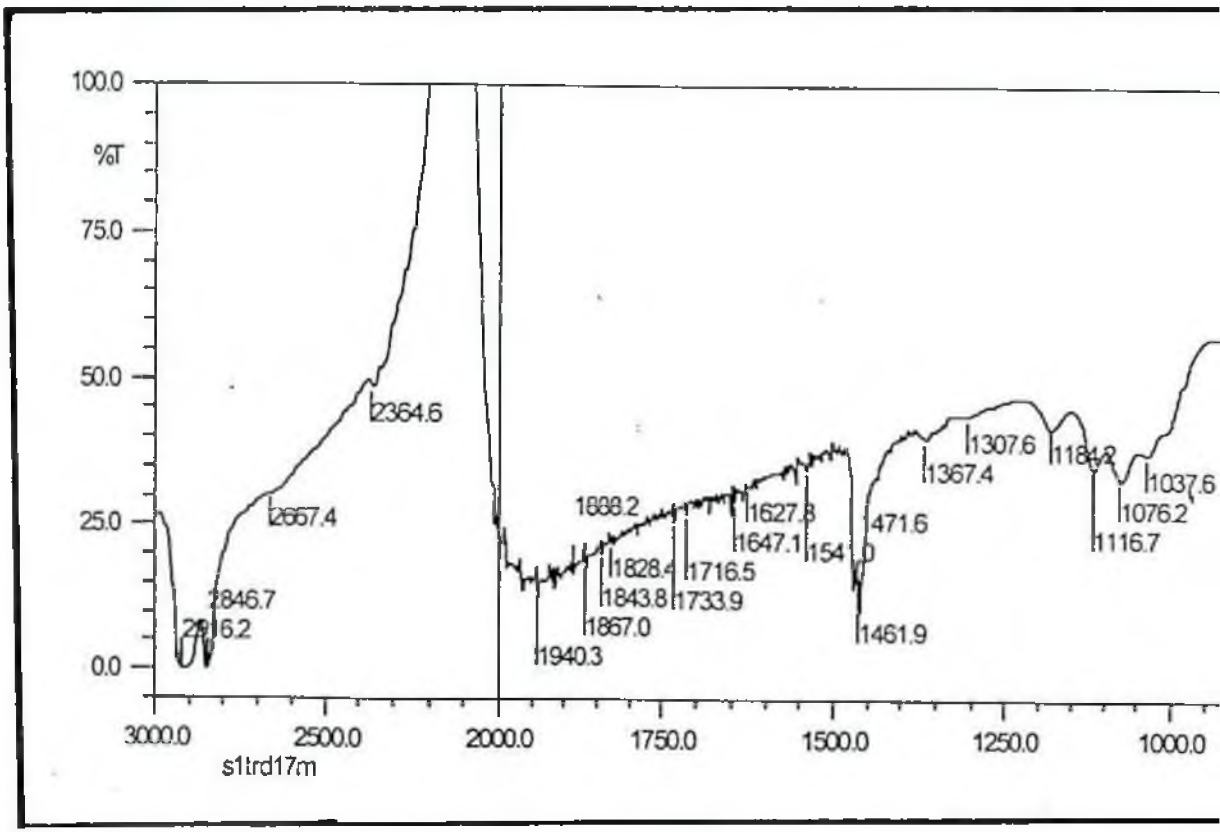


Fig. 6.7. FTIR spectra of LDPE sample no. 01 (b) after 17 months of expos

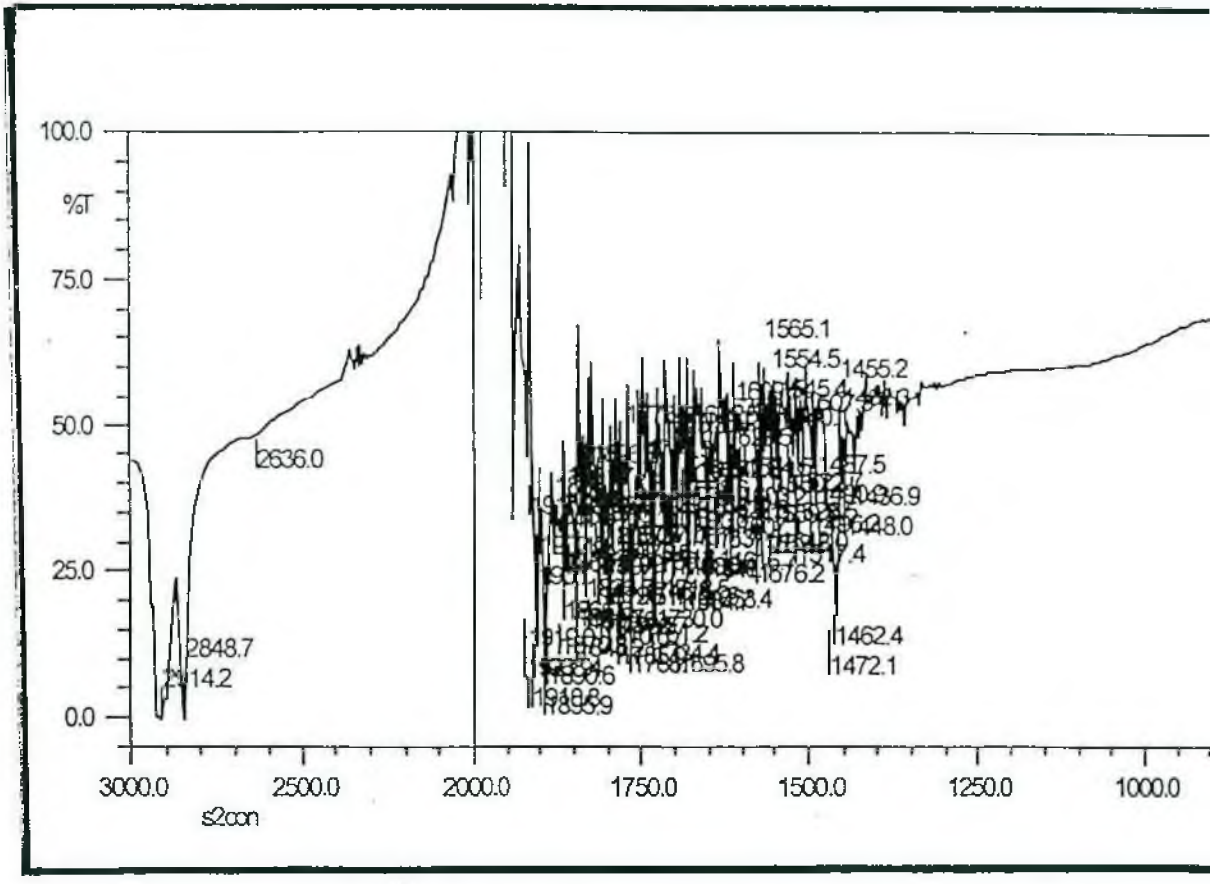


Fig. 6.8. FTIR spectra of LDPE sample no. 02 (a) before soil bu

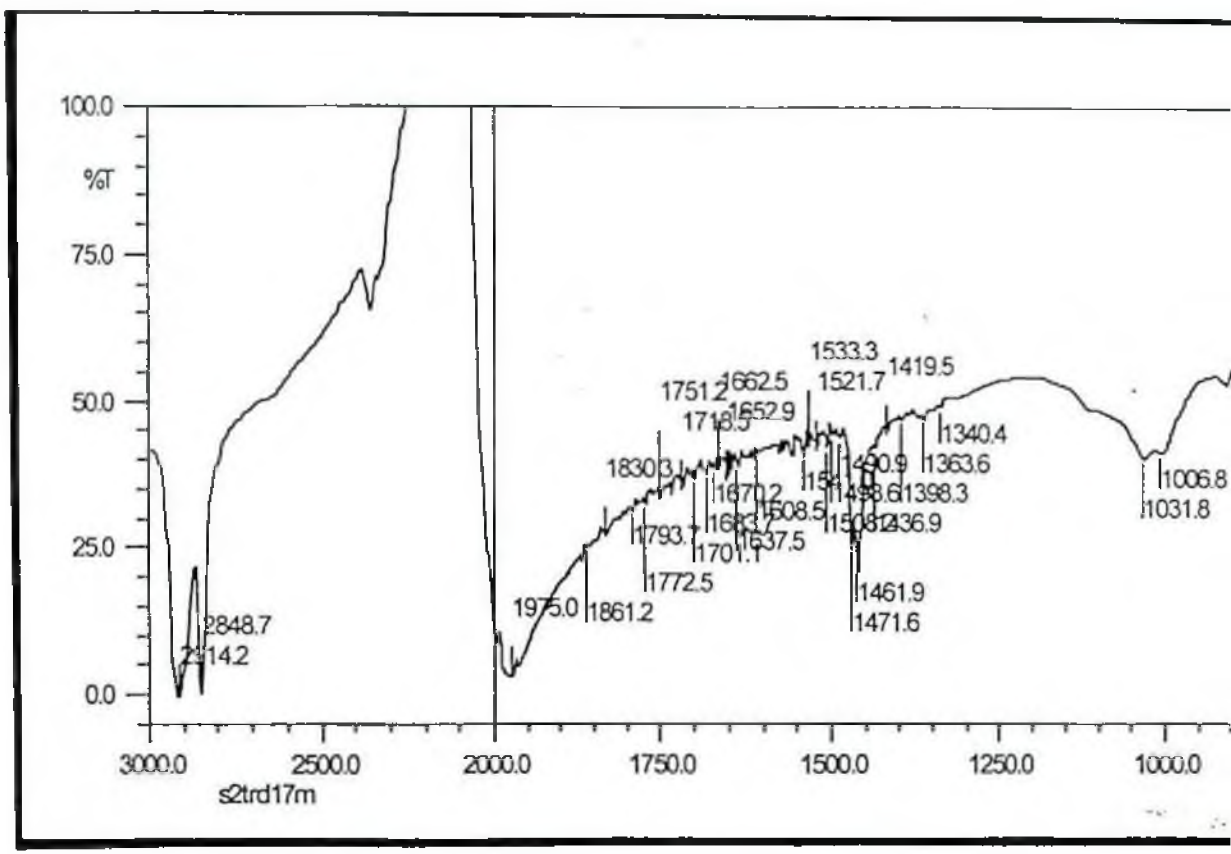


Fig. 6.8. FTIR spectra of LDPE sample no. 02 (b) after 17 months of expo

Fig. 6.9 is the IR spectra of control sample no.03 in the range of 400-2000 cm^{-1}. After 11 months of soil burial, the transmittance of the sample decreased to about 88.44 %. However, the absorption band at 1460 cm^{-1} due to CH_2 scisson and CH_2 rocking band at 720,730cm^{-1} were not changed (**Fig. 6.9 b**). **Fig. 6.10a,b** compares FT-IR spectra of sample no.03 before and After 17 months, the degradation of the sample proceeds with the complete disappearance of C-H deformation band, characteristic of polyethylene sample films. The absorption band due to CH_2 rocking vibration is greatly reduced. All bands corresponding to carbonyl absorption are almost suppressed.

Fig. 6.11a,b shows FT-IR spectra of thin, transparent polyethylene film (sample No. 04) before and after soil burial for 17 months. After prolonged exposure in soil, significant absorption at 1652 cm^{-1} in the control film was almost suppressed. The treated film illustrated an increase in the 1000-1100 cm^{-1} region. This broad complex absorption assigned to C-C-0 and C-O groups. The characteristic bands at 720, 739 cm^{-1}, 1460, 1471 cm^{-1} and 2850, 2920 cm^{-1} are not affected. However, a new medium to weak band near 2300 cm^{-1} was found in the treated sample.

6.1.5 Discussion

The FTIR spectroscopic technique does not directly measure the loss of materials as a result of biodegradation but, rather, semi quantitatively estimates the relative amounts of certain chemical species that remain in the test specimen after degradation.

FTIR techniques have been applied by many scientists to measure the overall changes in polymer film after degradation. Albertsson *et al.* (1987) studied the mechanism of biodegradation of polyethylene in details. LDPE films were exposed to abiotic and biotic environment. The films were UV irradiated for periods of 0,7,14,42 days before being mixed with water and soil. The degradation of the films was monitored with an IR instrument. During their study, PE films were examined after 10 years storage in a mixture of soil. They have calculated both carbonyl (the ratio of absorption peak at 1715 &1465) and the double bond index (ratio of absorption peak at 1640 & 1465) to follow oxidative degradation. The carbonyl index decreased with increasing biodegradation. They postulated that, carbonyl peak arised during both abiotic and biotic degradation but carbonyl index decreased with prolonged incubation time. A peak at 905-915 cm^{-1} was noted corresponding to double bond groups. This peak is missing in abiotic samples.

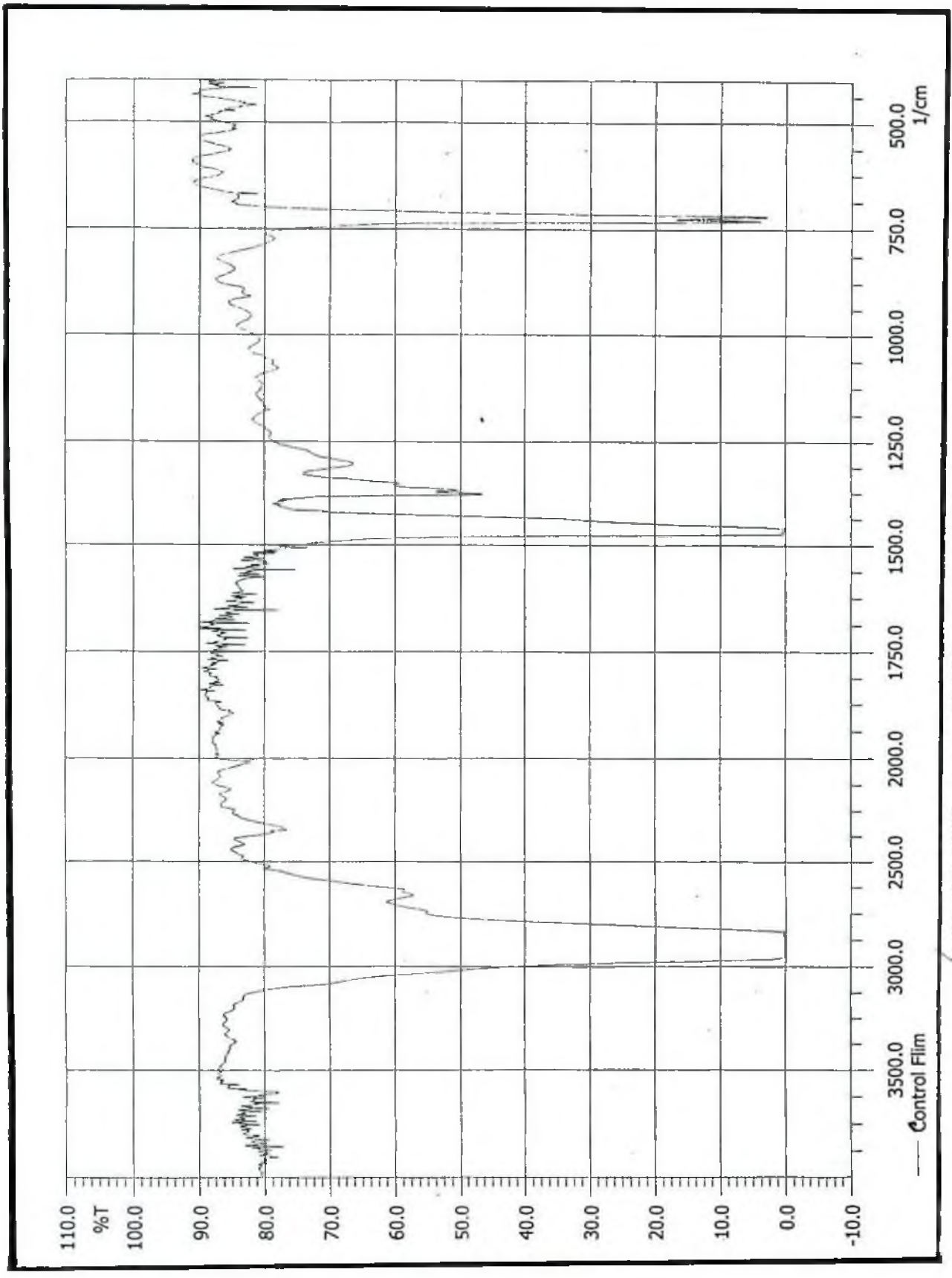


Fig. 6.9. FTIR spectra of LDPE sample no. 03 (a) before soil burial.

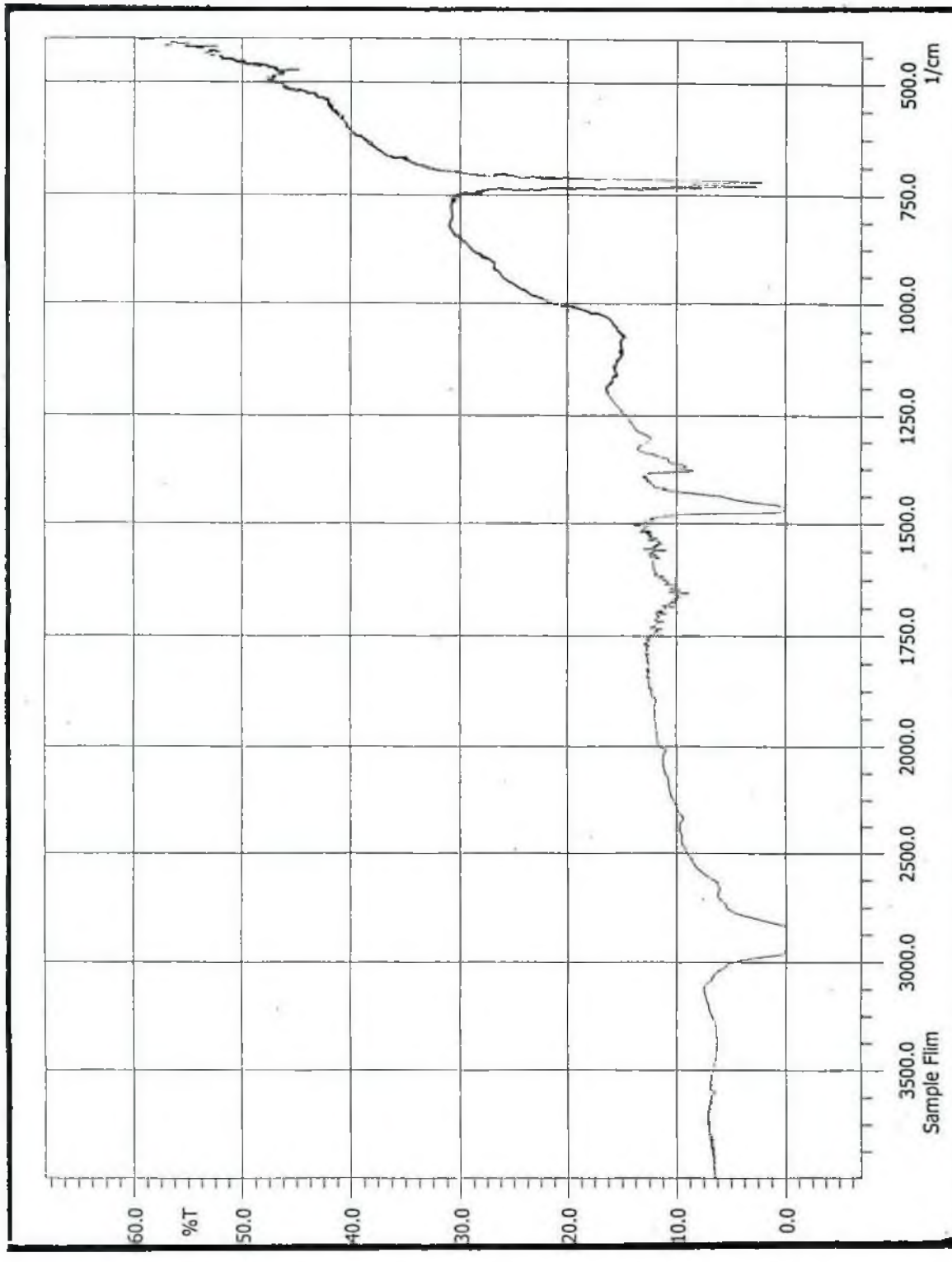


Fig. 6.9. FTIR spectra of LDPE sample no. 03 (b) after 11 months of exposure in soil.

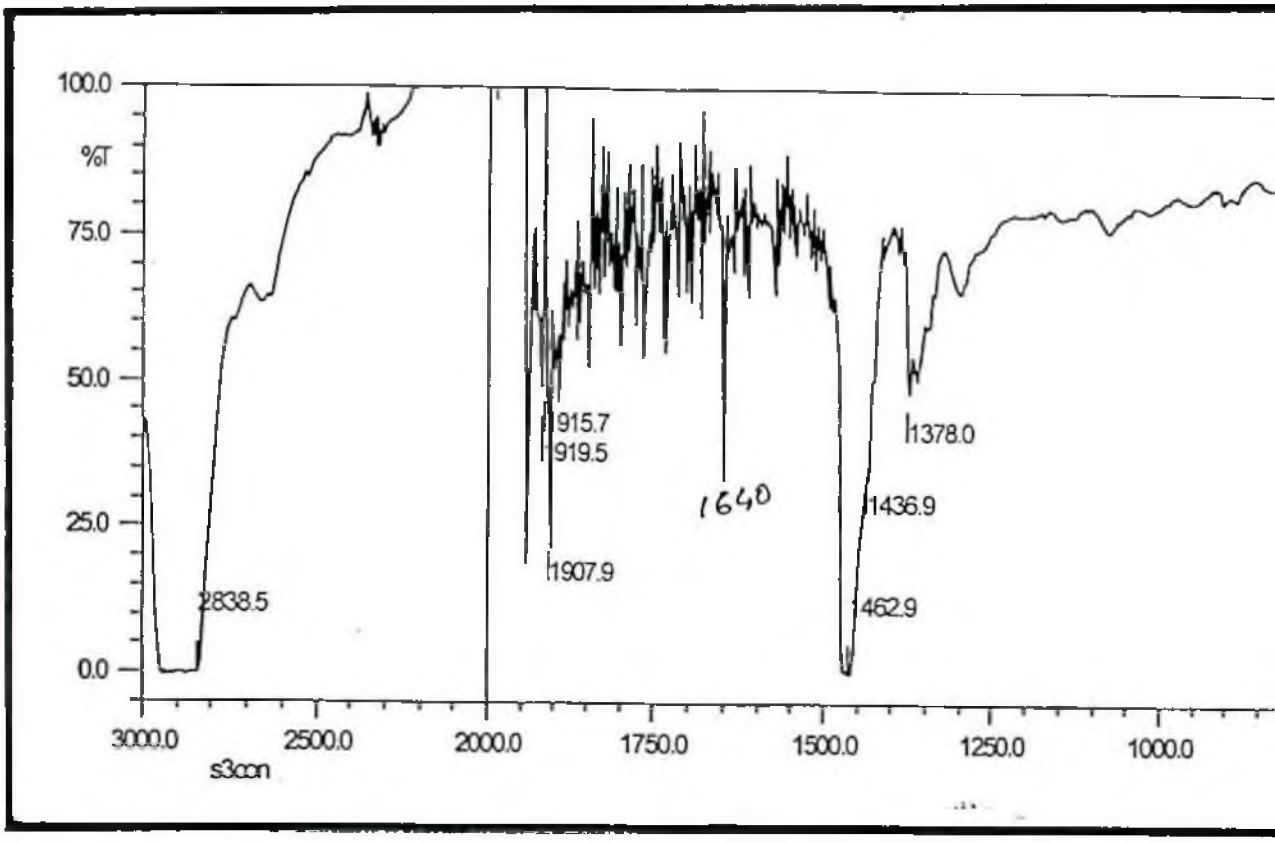


Fig. 6.10. FTIR spectra of LDPE sample no. 03 (a) before soil burial.

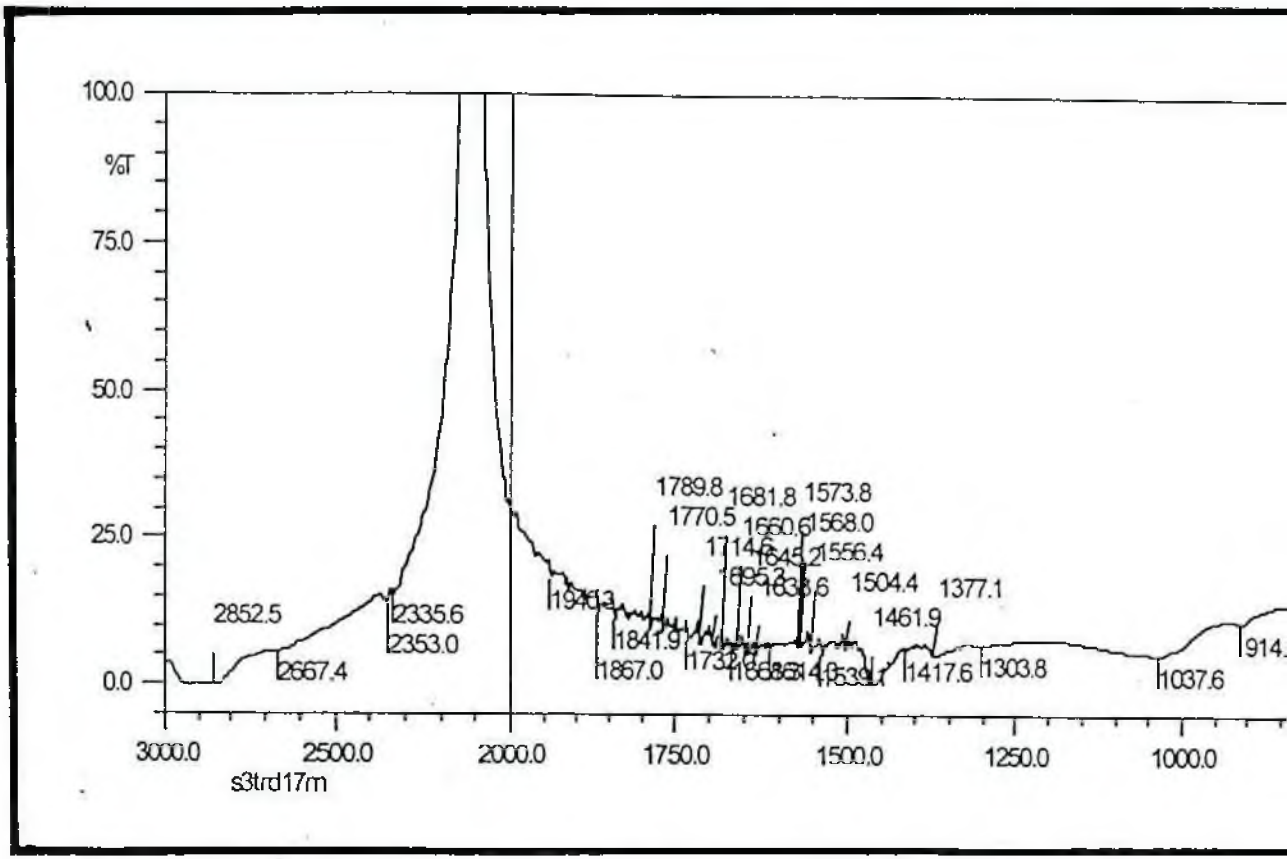


Fig. 6.10. FTIR spectra of LDPE sample no. 03 (b) after 17 months of exposure i

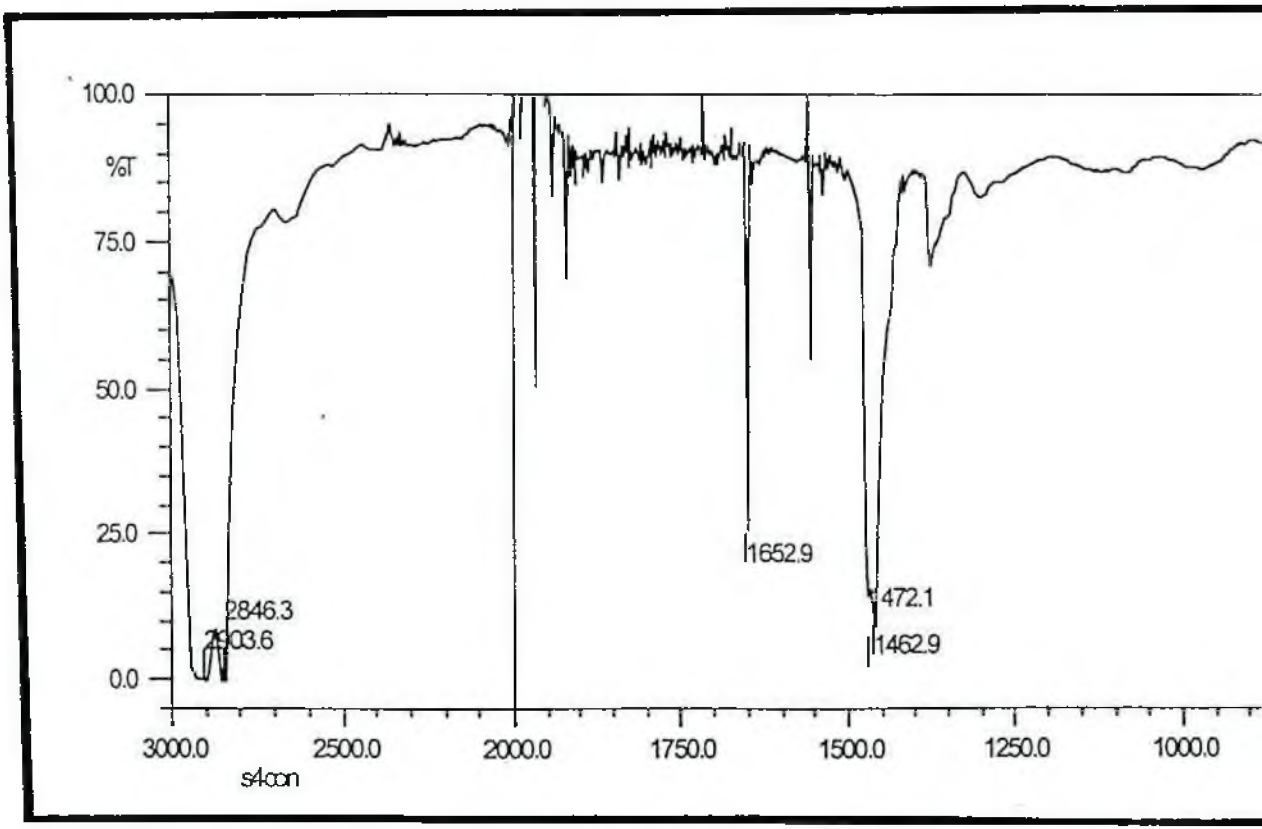


Fig. 6.11. FTIR spectra of LDPE sample no. 04 (a) before so

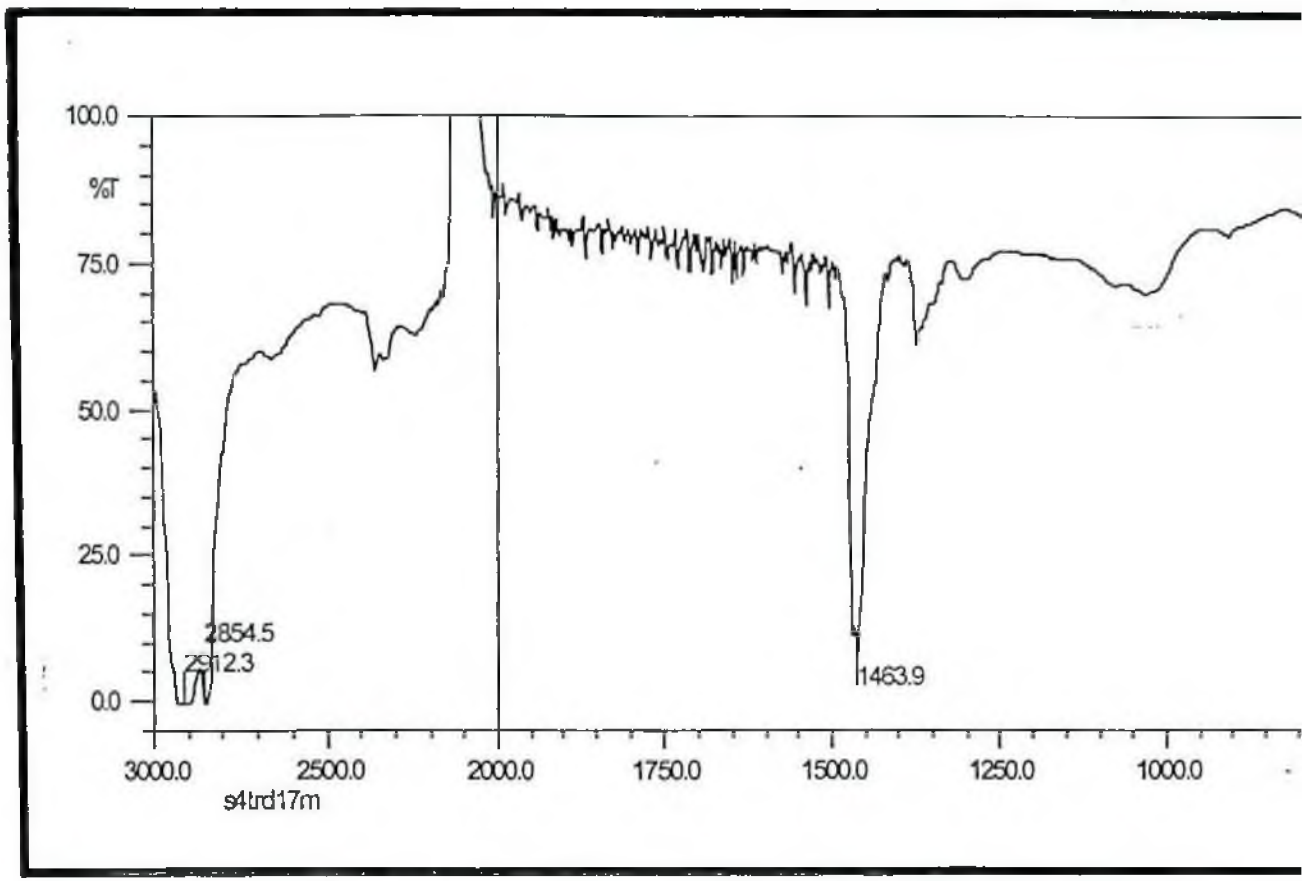


Fig. 6.11. FTIR spectra of LDPE sample no. 04 (b) after 17 months of exposu

Goheen and Wool (1991) followed Albertsson *et al.* (1987) to monitor soil effects on the degradation rates in polyethylene. Besides the two indices, bands at 1740 (the ester carbonyl-COO-), 905-915 (the double bond-CH_2=C-) and the doublet band at 720, 730 cm^{-1} were given importance. Pometto *et al.* (1992) demonstrated the activity of Polyethylene degrading enzyme by changes in the FT-IR spectra. David *et al.* (1992) studied photodegradation of polyethylene films in natural weathering conditions containing various commercial photoinitiator system. In their investigation, the absorbance of the carbonyl groups near 1715 cm^{-1} was used to monitor degradation. Imam *et al.* (1995) evaluated biodegradation of starch-PHBV formulations exposed to municipal activated sludge. They quantified changes in Starch/PHBV composition after biodegradation by FTIR spectroscopy.

In the present study, samples before treatment and those exposed in soil for 17 months were evaluated for changes in their chemical structure. As anticipated, all four samples exhibit a gross reduction in the complex overlapping bands between 1400-1900 cm^{-1} corresponding to carbonyl absorption. White opaque film (PE_W) demonstrated a peak at 1076 cm-1 after treatment. According to Bunn 1957, this is due to association of hydroperoxide group in the solid state of oxidized polyethylene. The degradation behaviour of thick, transparent polyethylene sample ($PE_{Tr\text{-}HW}$) was greater in comparison and FT-IR data reveals complete destruction of C-H deformation band as well as carbonyl and CH_2 rocking vibration bands after soil exposure. A peak at 914 cm^{-1} was found in the sample suggesting biotic changes during degradation (Albertsson *et al.* 1987). Indeed, during the biotic degradation of polyethylene, carbonyl peak decreases with the progress of degradation. Moreover, hydroperoxides formed are broken down into primary and secondary alcohols in the latter stage of degradation.

6.2 X-Ray Diffraction Analysis

6.2.1 Introduction

The X-ray diffraction method is a powerful tool for investigating orderly arrangements of atoms or molecules through the interaction of electromagnmetic radiation to give interference effects with structures comparable in size to the wavelength of the radiation (Billmeyer 1984). If the structures are arranged in an orderly array or lattice, the interferences are sharpened so that the radiation is scattered or diffracted only under specific experimental conditions. Knowledge of these conditions gives information regarding the geometry of the scattering structures. The wavelengths of X-rays are comparable to interatomic distances in crystals. Wide angle scattering is useful in obtaining informations about the spatial arrangements of the atoms. Low-angle scattering detects larger periodicities, which may arise from lamellar crystallites or from voids.

6.2.2 Principle of X-ray diffraction analysis

X-rays are usually produced by bombarding a metal target with a beam of high-voltage electrons. This is done inside a vacuum tube, the X-rays passing out through a beryllium or polyester film window in the tube in a well-defined beam. Choice of the target metal and the applied voltage determines the wavelength or wavelengths of x-rays produced. The diffracted x-rays may be detected by their action on photographic films or plates, or by means of a radiation counter and electronic equipment feeding data to a computer (Billmeyer, 1984).

6.2.3 XRD of polyethylene

The X-ray diffraction method rests on measurements of the intensities of diffuse scattering due to non-crystalline material and sharp scattering due to crystalline regions (Bunn 1957). The method allows calculation of the relative amounts of crystalline and amorphous material in a sample if it is possible to resolve the contributions of the two types of structure to the x-ray diffraction pattern. For example, in **Fig. 6.12** the scattering envelope of a polyethylene sample (outerline on the graph) has been resolved into contributions of strong crystal diffractions from 110 and 200 planes (4.1 and 3.7 Å) and a broad amorphous peak (the area under the main 4.5 Å peak). The amorphous halo may go up to ~15-25 Å Cukα, peak breadth FWHM ~6^0. Often only a few weak peaks viz. 110, 200 & 020 may be observed.

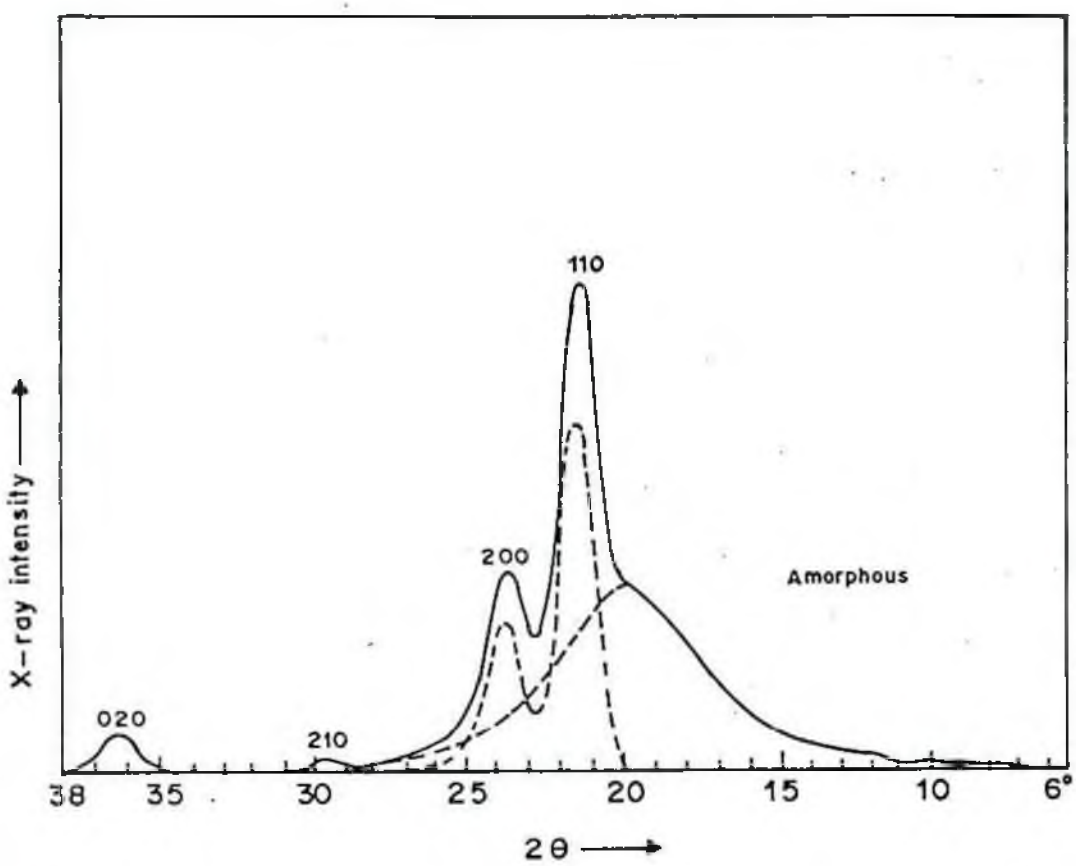


Fig. 6.12. The plot of X-ray intensity against scattering angle for polyethylene after subtraction of background intensity (after Bunn 1957).

Fig. 6.13 compares X-ray diffractrograms of high density and low density polyethylene with that of amorphous polystyrene (Hedvig 1977). In polymers, crystallinity is defined as the relative area of the sharp maxima with respect to the broad amorphous band. However, no information can be derived from the X-ray diffraction measurements about how the amorphous phase is distributed in a highly crystalline polymer.

6.2.4 Materials and Methods

X-ray equatorial diffraction profiles were collected by a JEOL diffractometer, Model JDX 8P, using CuKα radiation at the operating voltage and current of 30kV and 20mA, respectively, from 5^0 to 50^0 of the 2θ range at the scanning speed of 1^0/min. For x-ray diffraction studies, specimens were cut into 20 x 2 $6mm^2$ pieces.

6.2.5 Results

The results of the quantitative X-ray diffraction analysis of four Polyethylene samples before and after 17 months of soil burial are shown in **Figs. 6.14-6.17. Fig. 6.18** shows the diffraction pattern of glass slide under the same experimental condition.

Wide-angle diffraction pattern of all the control and treated specimens (shown in **Figs. 6.14 to 6.17**) are similar to the standard diffraction pattern as shown in **Fig. 6.12.**

So, upon treatment, there is no change in crystalline portion of the specimen. It may be inferred that initially the change takes place at the amonphous region. Confirmation cannot be made by XRD analysis. Percentage crystallinity dose not change much.

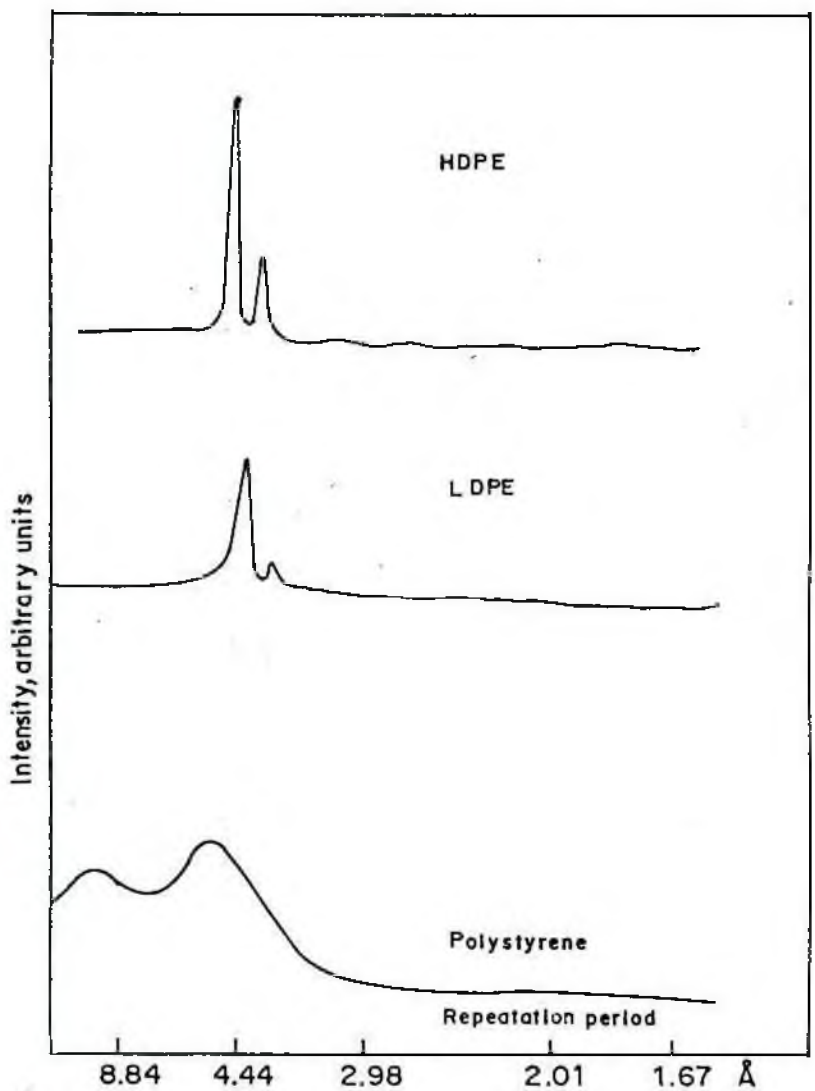


Fig. 6.13. X-ray diffraction pattern of high and low density polyethylene (HDPE and LDPE) and polystyrene (PS) (after Hedvig 1977).

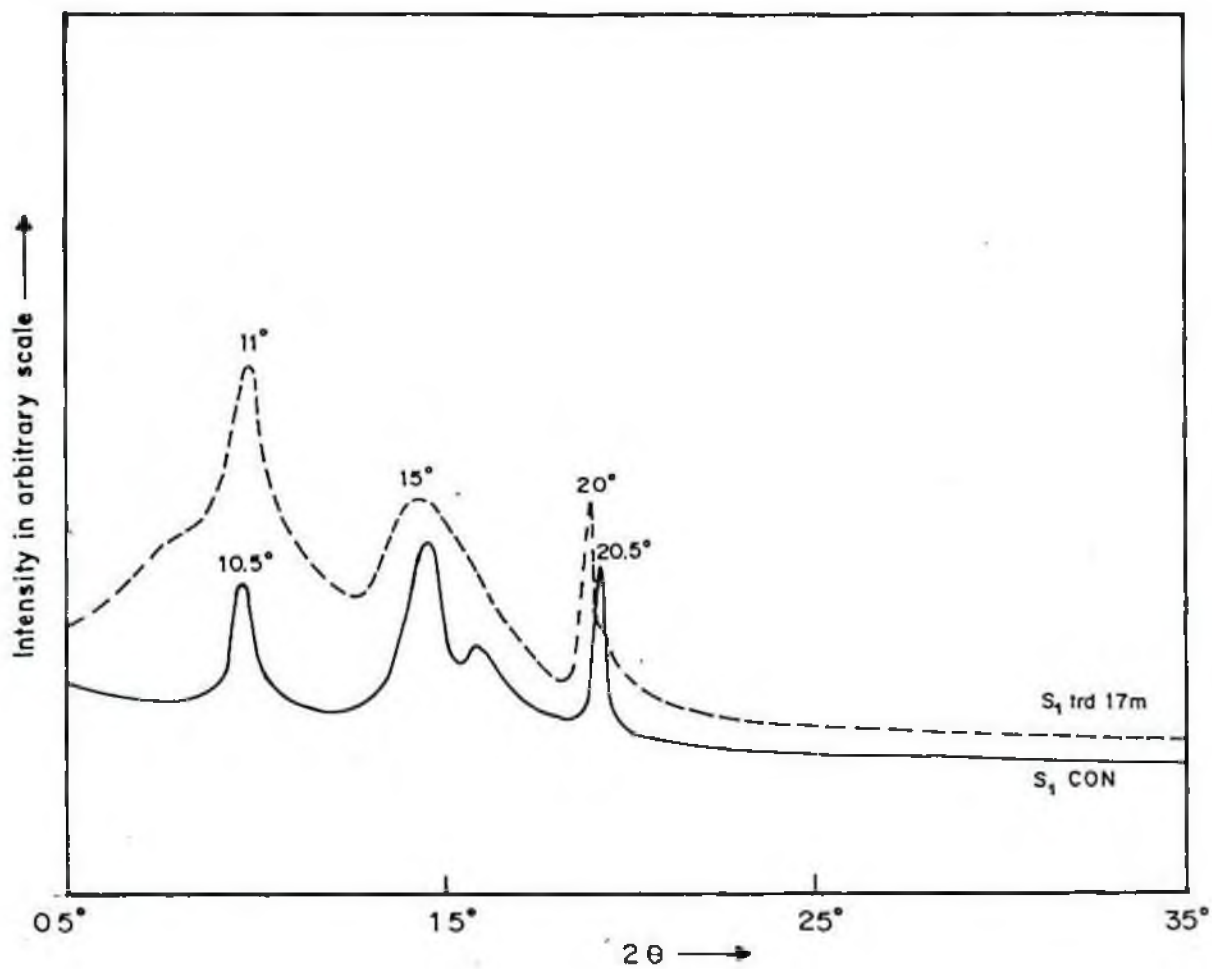


Fig. 6.14 Resolved equatorial X-ray diffraction pattern for LDPE sample no. 01 before (—) and after 17 months of exposure in Soil (----).

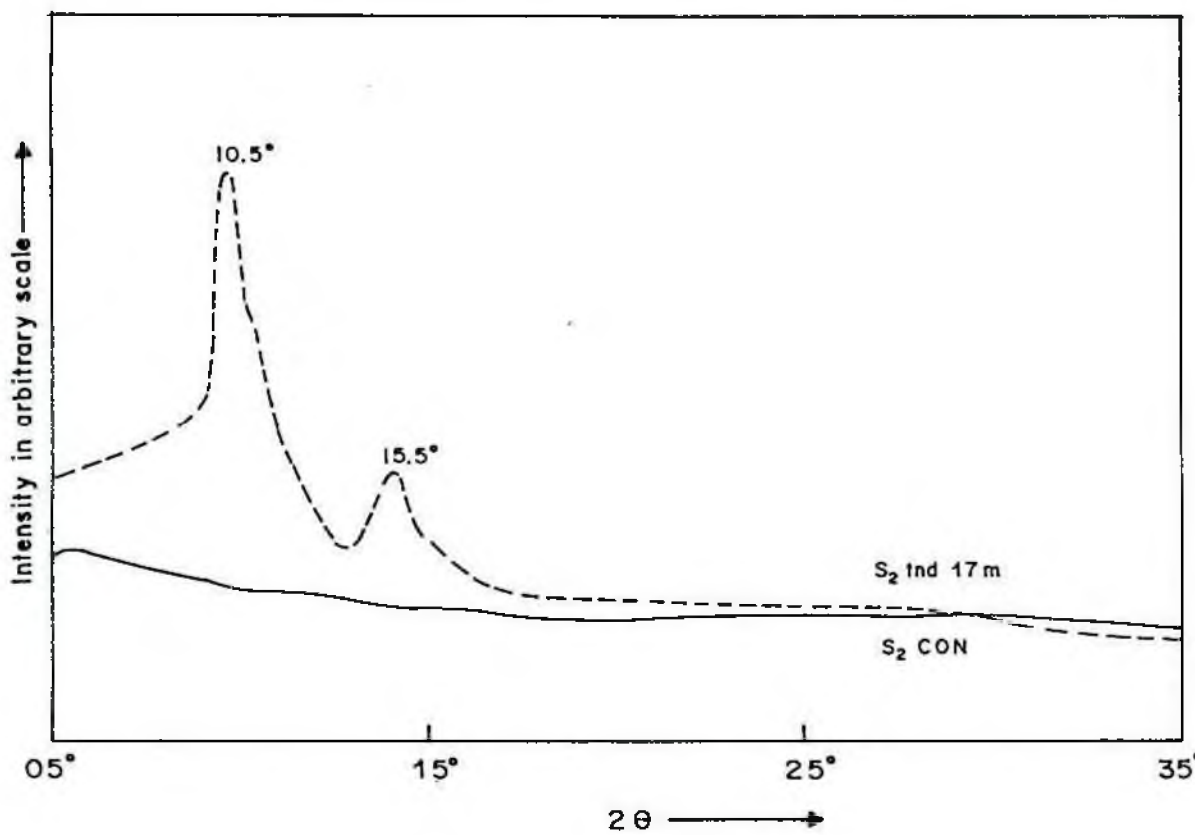


Fig. 6.15. Resolved equatorial X-ray diffraction pattern for LDPE sample no. 02 before (—) and after 17 months of exposure in Soil (----).

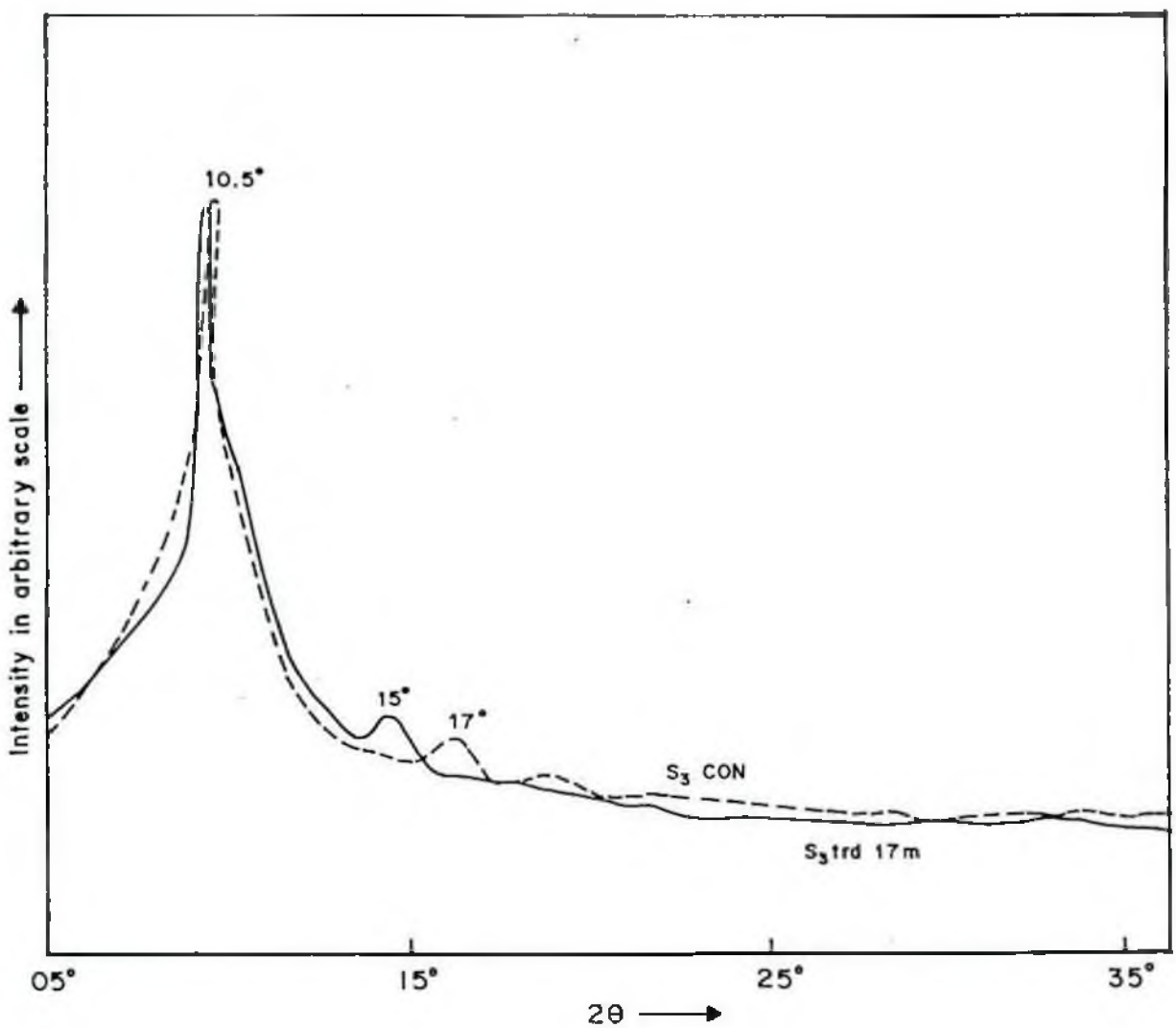


Fig. 6.16. Resolved equatorial X-ray diffraction pattern for LDPE sample no. 03 before (----) and after 17 months of exposure in Soil (—).

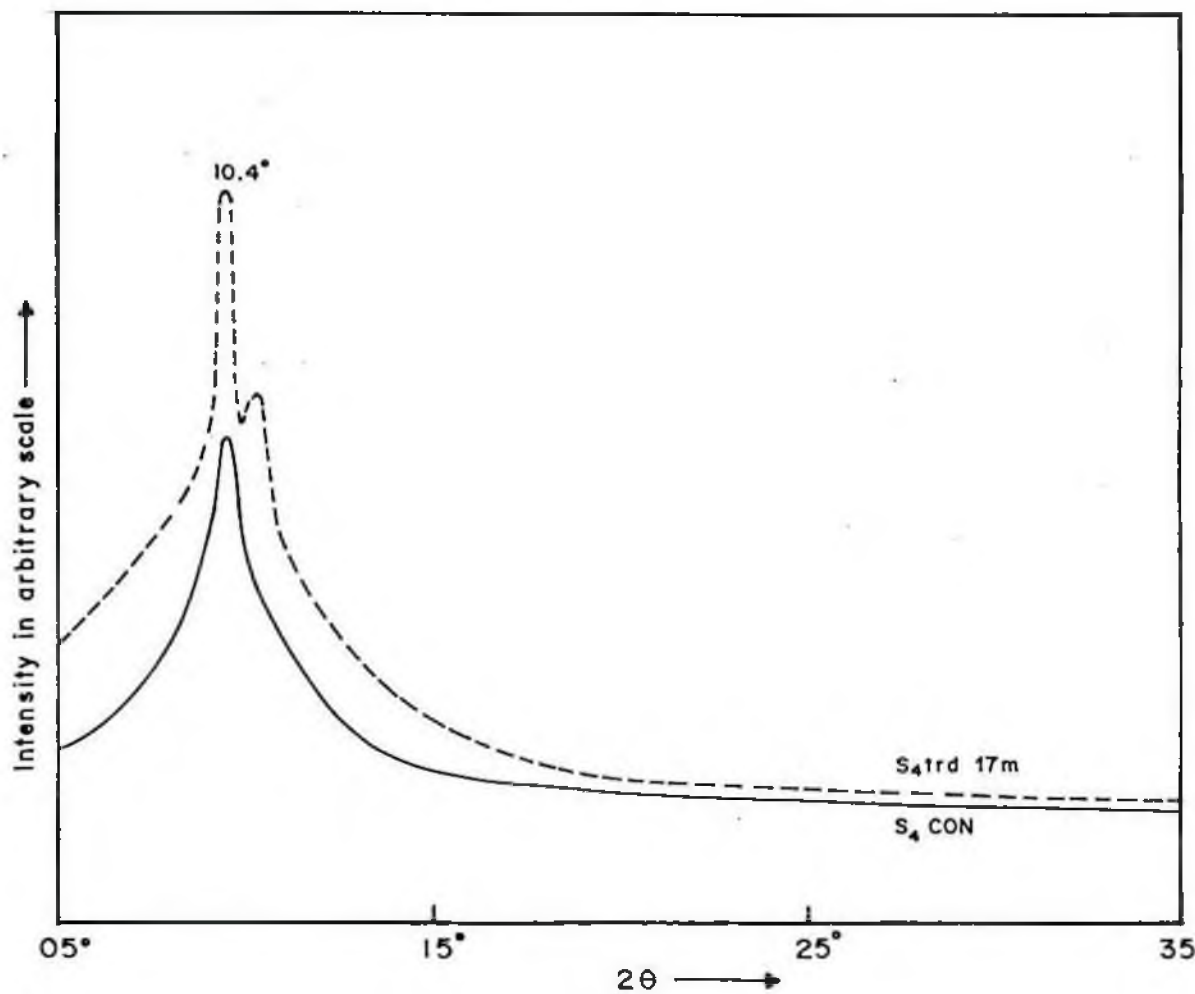


Fig. 6.17. Resolved equatorial X-ray diffraction pattern for LDPE sample no. 04 before (—) and after 17 months of exposure in soil (----).

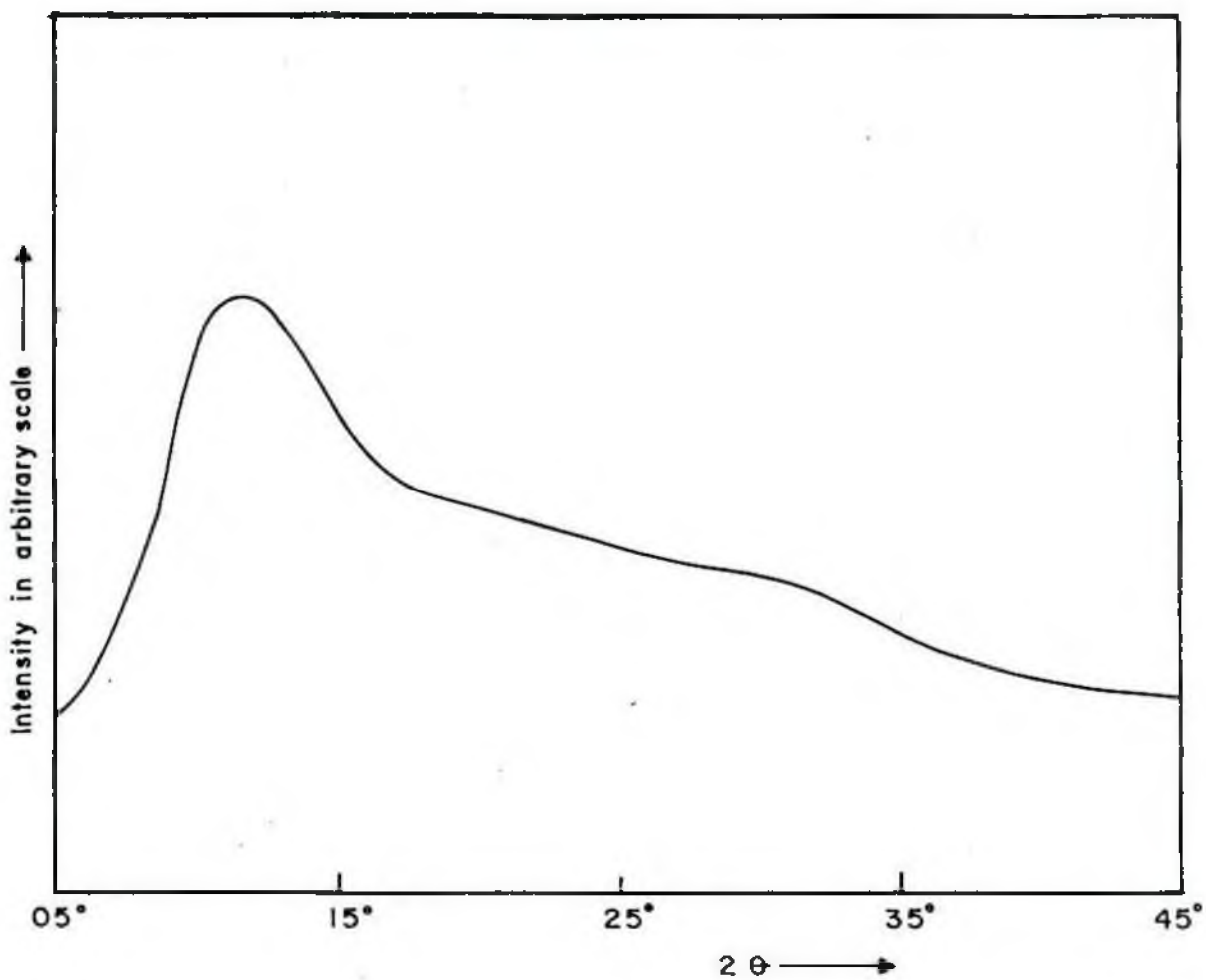


Fig. 6.18. X-ray diffraction pattern for Glass slide under identical experimental condition.

Chapter - 7

ADDITIONAL EXPERIMENTS SUPPORTING BIODEGRADATION OF POLYETHYLENE

Additional experiments supporting biodegradation of polyethylene

7.1. Introduction

In parallel to soil burial test a number of biological experiments were carried out during the present study. An experiment was set up to compare the fate of polyethylene films under submerged condition in pond water, but due to the reasons beyond our control the experiment could not be continued for long. The results obtained for up to 10 months discussed here. Other tests include **Liquid culture test with mixed population, Plate test with fungi** and **Clear zone assay with bacteria.**

7.2 Pond water experiment

A weathering experiment was set up to examine degradation of polyethylene under submerged condition in pond water.

7.2.1 Materials and Method

Three types of commercially available polyethylene films were used in this study. These were black, white opaque and thin transparent films. Samples were manually cut into 20 cm x 5 cm strips and were kept submerged in the pond of Shahidullah Hall (D.U.).

Each sample was enclosed in a nylon net jacket and was hanged with a nylon thread. A pebble was inserted in each nylon jacket to resist floating. Each thread with hanging sample was then stitched to a long thick nylon rope. Samples were tagged with rigid plastic pieces with corresponding numbers engraved on it. The two ends of the rope tightly bound with bamboo sticks were firmly dug in the mud of the pond.

Nylon net ensured minimal accumulation of biofilm and other floating debris on the sample while exposure in pond provided a biologically active liquid medium. The samples were withdrawn periodically and were subjected to critical observation under microscope.

7.2.2 Film harvest

After prolonged exposure in pond water, samples were taken out and brought in the laboratory. The outer nylon jackets was carefully removed and the samples were rinsed thoroughly with tap water. Attached biofilm and other debris were gently scrapped off the

film. A small portion of the film was taken on a microscope slide and was mounted with Acridine orange solution (see Appendix). The slide was then observed under fluorescent and advanced research microscope (Nikon Microphot and Nikon Optiphot).

7.2.3 Results

Black thin samples, PE_B

During the two months exposure black sample didn't undergo any significant change in appearance. No trace of colonization was noticed under microscope.

After 03 months, thick slimy biofilm developed over the nylon jackets. Inside the jacket, thin layer rich in green algae was observed on each sample. Sample became softer from the uninoculated control film. Microscopic observation revealed dominance of algal cells on the film (**Fig. 7.1**). After 5 months of exposure, extensive biomass growth is apparent throughout the film. Microbial mat consisted of algal filaments, green algal cells and bacteria.

White opaque sample, PE_W

After 06 months under pond water, the white sample was found to be colonized by microbial filaments. Colonization was denser at the edge though no physical change was observed under microscope. Resulting biofilm differed in fluorescent behaviour. Sample exposed in pond water for up to 10 months showed green algal film on the surface (**Fig. 7.2d-f**).

Thin transparent sample, $PE_{Tr\text{-}LW}$

After 03 months, thin transparent sample showed no change in its physical properties with the control despite microbial colonization. After 10 months samples had considerable growth of various organisms the identity of many of which could not be ascertained (**Fig. 7.2a-c**)

Samples used for this experiment were cut into different shapes to see whether it affects in its degradability or not. While some were perforated, others were cut in into comb like structure. Abrased sample showed greater surface growth than the other forms. Physical treatment like abrasion probably resulted into surface roughness which increased the chance of colonization.

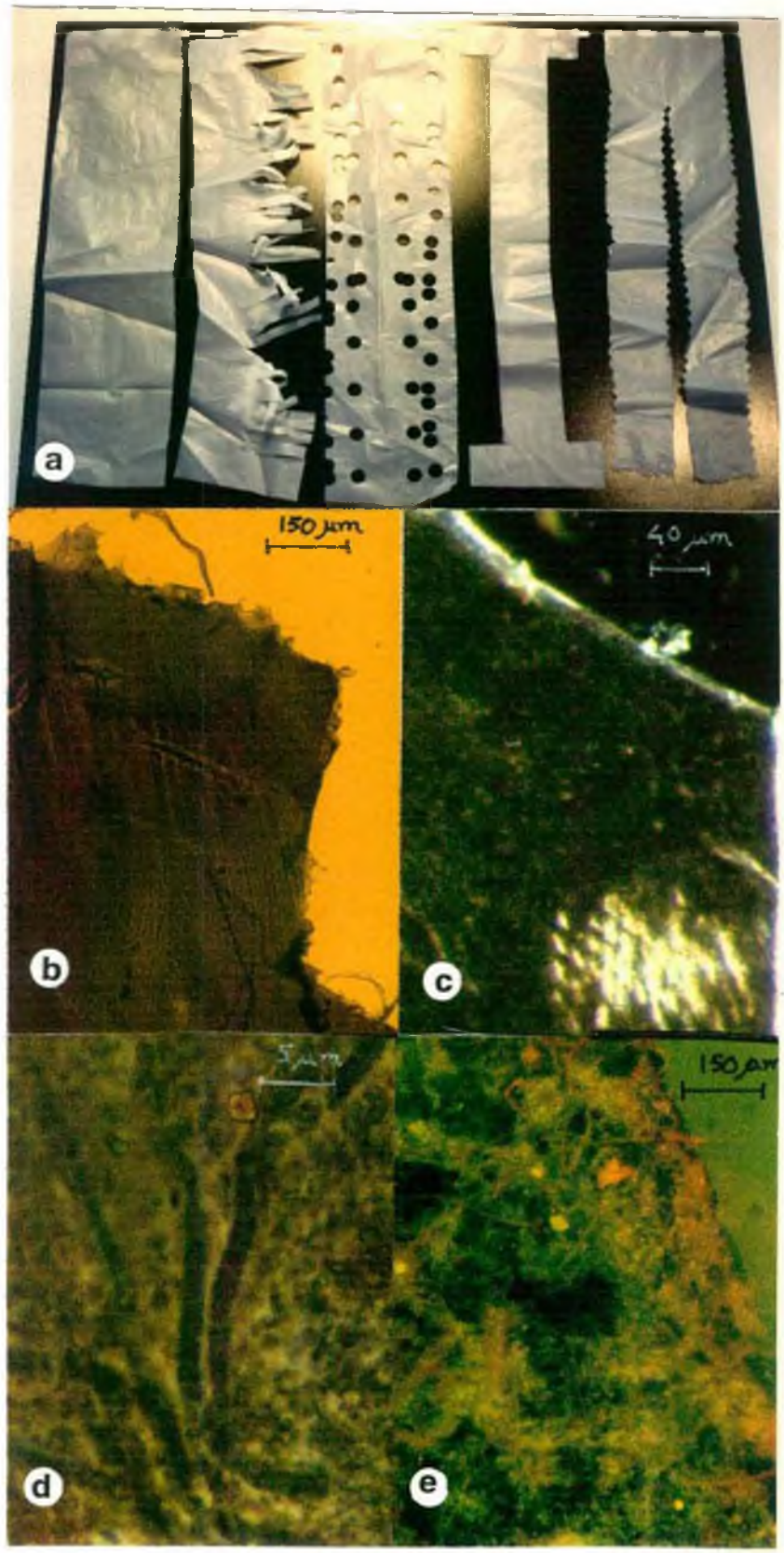


Fig. 7.1 a) Samples cut into different patterns for pond water experiment, b-e) Photomicrographs showing black thin sample submerged in pond water for 0, 02, 03 and 05 months, respectively.

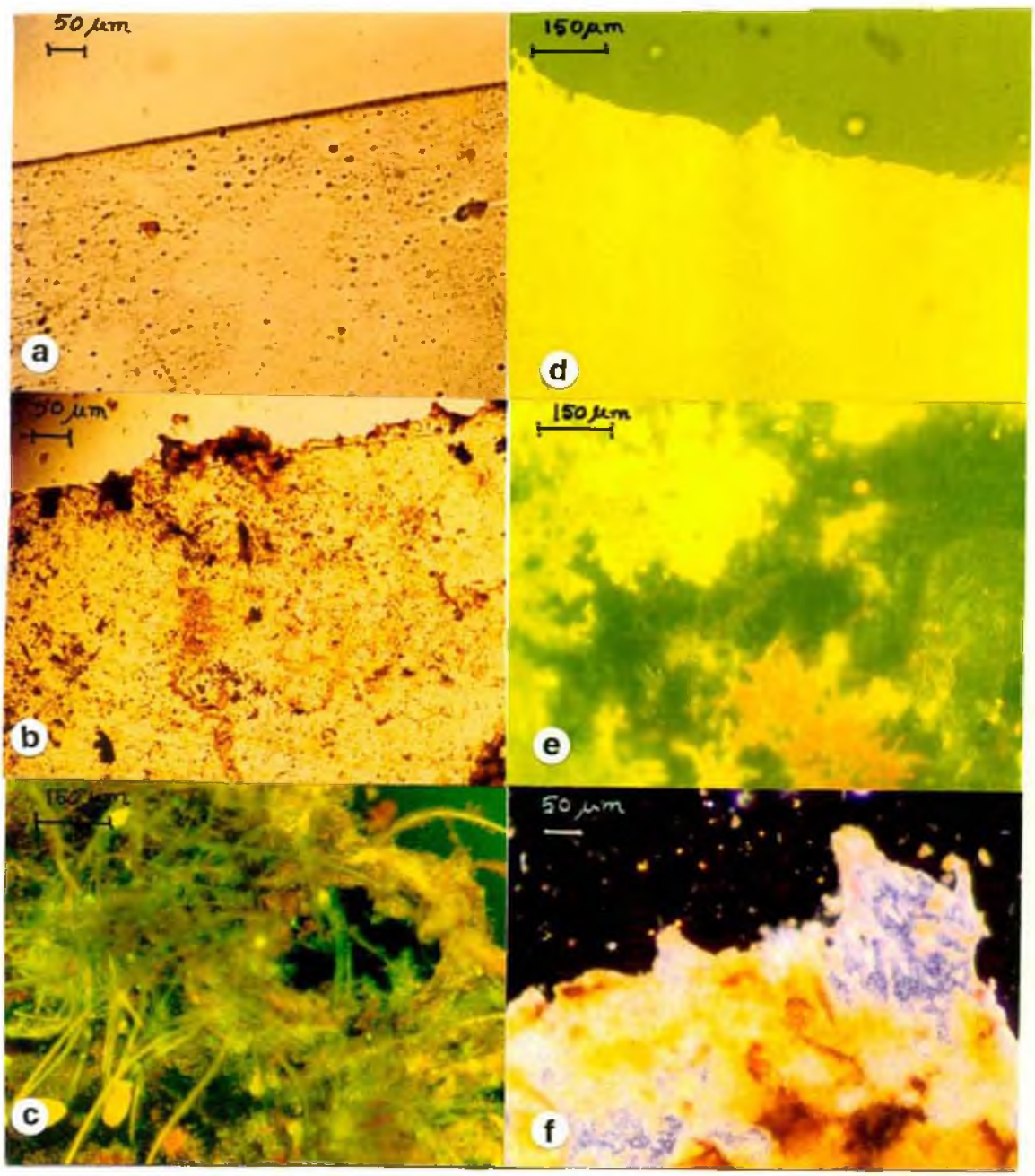


Fig. 7.2 (a-f) Microscopic view of polyethylene samples during pond water experiment. (a-c) Thin, transparent sample after 0, 03 and 10 months and (d-f) white, opaque sample sumerged in pond water for 0, 06 and 10 months.

Ten months is really not sufficient for the degradation of material like polyethylene, and no significant result was obtained during this short period. We could only observe the formation of biofilm rich in algal cells. Since, only physical properties were examined using fluorescent and optical microscopes, no comments could be made or other properties.

7.3 Examination of weathered polyethylene in laboratory conditions

Polyethylene films in conical flasks (2 L capacity) containing pond water had been kept at laboratory room temperature for more than 02 years. Biofilm developed in these flasks were examined under microscope for the microbial types as well as erosion characteristics of the film. Sample which showed considerable surface damage or deformities in surface morphology was selected for the subsequent cultivation and isolation of the dominant microorganisms.

7.3.1 Isolation of microorganisms from weathered sample

Sample from each flask was taken out and a small portion was cut from each strips for inoculation. For isolation two-types of media were used and were designated as SPYM and OASP media (see Appendix). pH was adjusted to 7.0 with 0.1 N NaOH solution. Freshly prepared agar plates were dried before inoculation. Sample pieces were placed on agar, incubated and checked for any growth. Growth appeared after 3-5 days were isolated and purified.

7.3.2 Results

Flask Nos. 3, 8, 14 & 15 were chosen for further study. Microbiofilm developed over polyethylene films in flasks consisted of blue green algal filaments, desmids, diatoms, some filamentous structure, bacterial cells and spores.

Chu's broth (Claus 1995) was chosen for the cultivation of BGA along with other microbial types. On the other hand, SPYM (ATCC 1992) and OASP plates revealed bacterial and fungal colonies. After purification, isolates were maintained on Nutrient Agar slants (for bacteria) and on Czapek-Dox agar (for fungi) (CAB 1968) having pH 7.2 and 6.5, respectively.

Three distinct types of fungal isolates and five bacterial isolates were cultured for further study. Table 7.1 and **Fig. 7.3** shows characteristics of fungal isolates studied during the present investigation. Colony characteristics, morphology of vegetative cells, spore and

sporangia of bacterial isolates are listed in Table 7.2. All bacterial isolates were found to be Gram positive rods of variable sizes (**Fig. 7.4**). Confirmation of endospores was performed by examination under Phase contrast microscope in wet mount slide. Out of five isolates, three were found to be spore former while the other two were non-spore former. Cultural and biochemical studies (in case of bacteria) were also performed to some extent (Table 7.3).

Table 7.1 Descriptions of fungal isolates.

Sl.No.	Isolate Nos.	Colony morphology	Vegetative and reproductive features
01	S_{14}/F_1	Colonies on potato dextrose agar restricted, attaining a diameter of 2-3 cm in 10 days at room temperature (27 ± 2°C), velvety with central area more or less raised. Reverse colourless or dull yellow. Colony azonate at first but gradually becoming definitely zonate with age, radiately furrowed. Thin growth of aerial hyphae at margin appearing somewhat floccose, mostly white through flesh to dull pink or lavender, becoming lighter with age. Odor lacking. Grow well on both PDA and Czapek Dox agar having pH of 4.5 to 6.5.	Long chains of conidia on short phiallides, phiallides 3-4 in number with reduced stalk.
02	S_{14}/F_2	Colony on PDA and Czapek Dox (pH 4.5 and 6.5) spreading, quick growing. Surface at first white then olive green to gray green. Reverse colourless. Sporulation halted on Nutrient agar medium (pH 7.0)	Mycelia speptate, hyaline erect. Conidiophores arise laterally, coloured, septot branched. Straight, conidia borne terminally, in chains pyriform.
03	S_3/F_3	Colonies on PDA (pH 4.5 to 6.5) restricted, slow growing. Growth at first cream coloured later dark green to black, tough basal felt like. Whitish superficial hyphae appears in old colonies. Reverse colourless.	Sporangiophores much branched, dark brown. All the branches terminated by sporangia. Sporangium globose, olivaceous translucent. Columella observed on old slides mounted with lactophenol and cotton blue. Growth enhanced at 37°C.

Table 7.2. Morphological studies of bacterial isolates.

Isolates Nos.	Growth on		Vegetative cells	Sporangium	Spore
	Agar colony	Agar slant			
S_3/B_1	Circular, entire, raised, glistening, cream coloured.	Filiform	Spindle shaped cells, non-chain former, end rounded.	Sporangial wall thin, sporangium not swollen	Cylindrical, subterminal
S_3/B_2	Irregular, opaque, erose, spreading type	Filiform	Medium, thick rod, chains of 4-5 cells common with rounded ends	Sprongium not swollen	Cylindrical, subterminal
S_8/B_1	Punctiform, entire, glistening, translucent, with bluish sheen	Effuse	Short, thin rods	-	-
S_{14}/B_1	Circular, erose, flat, opaque, dull, whitish	Filiform	Long rods, seldom in chains	Sporangium not swollen	Oval, subterminal
C_1	Punctiform, entire, opaque, slightly raised	Filiform	Medium, thin rods, do not form chains, bead-like structure found at distal ends of cells	-	-

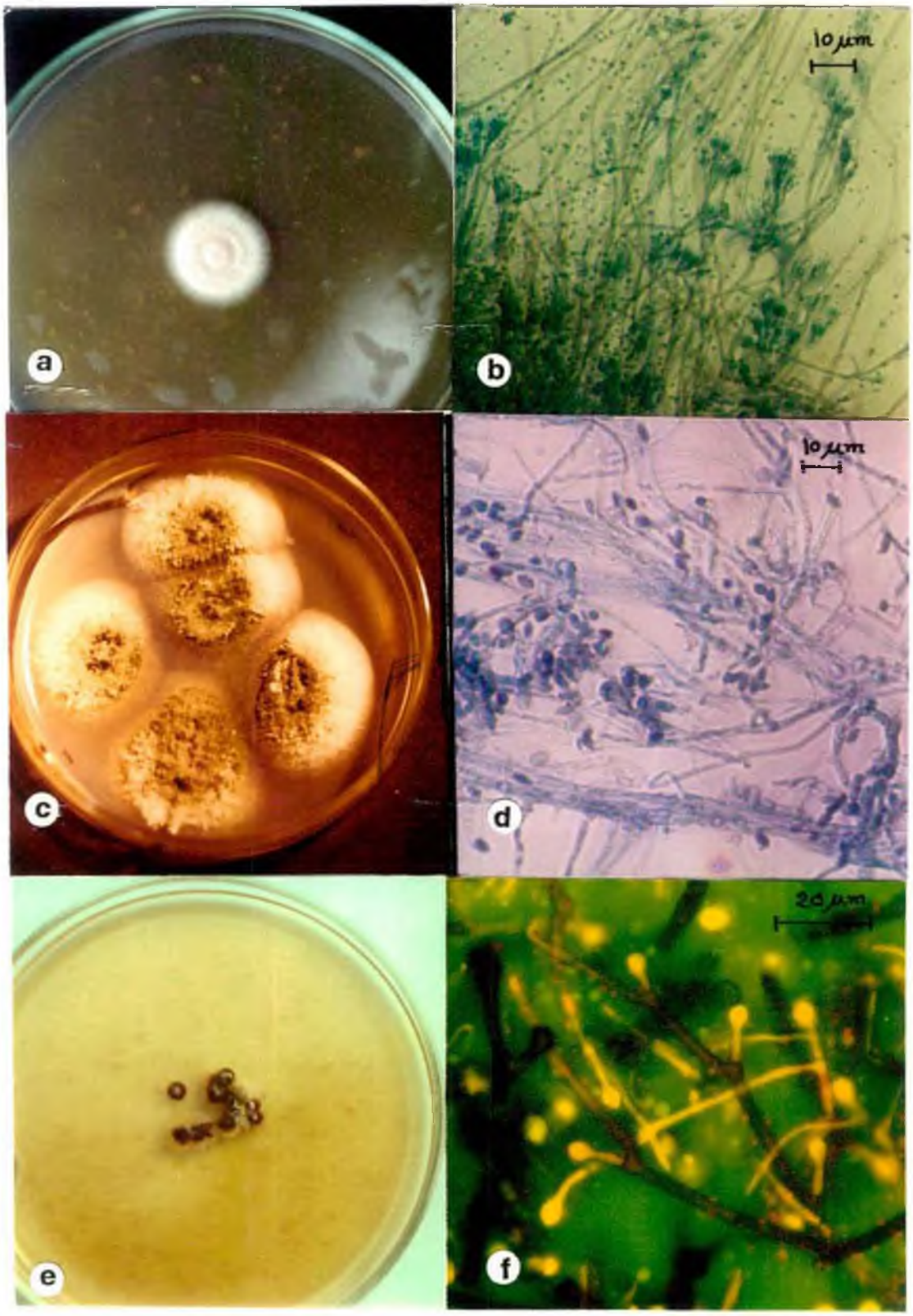


Fig. 7.3 (a-f) Colony morphology and microscopic view showing vegetative and reproductive structures of three fungal isolates. (a,b) S_1/F_1, (c,d) S_1/F_2 and (e,f) S_3/F_3.

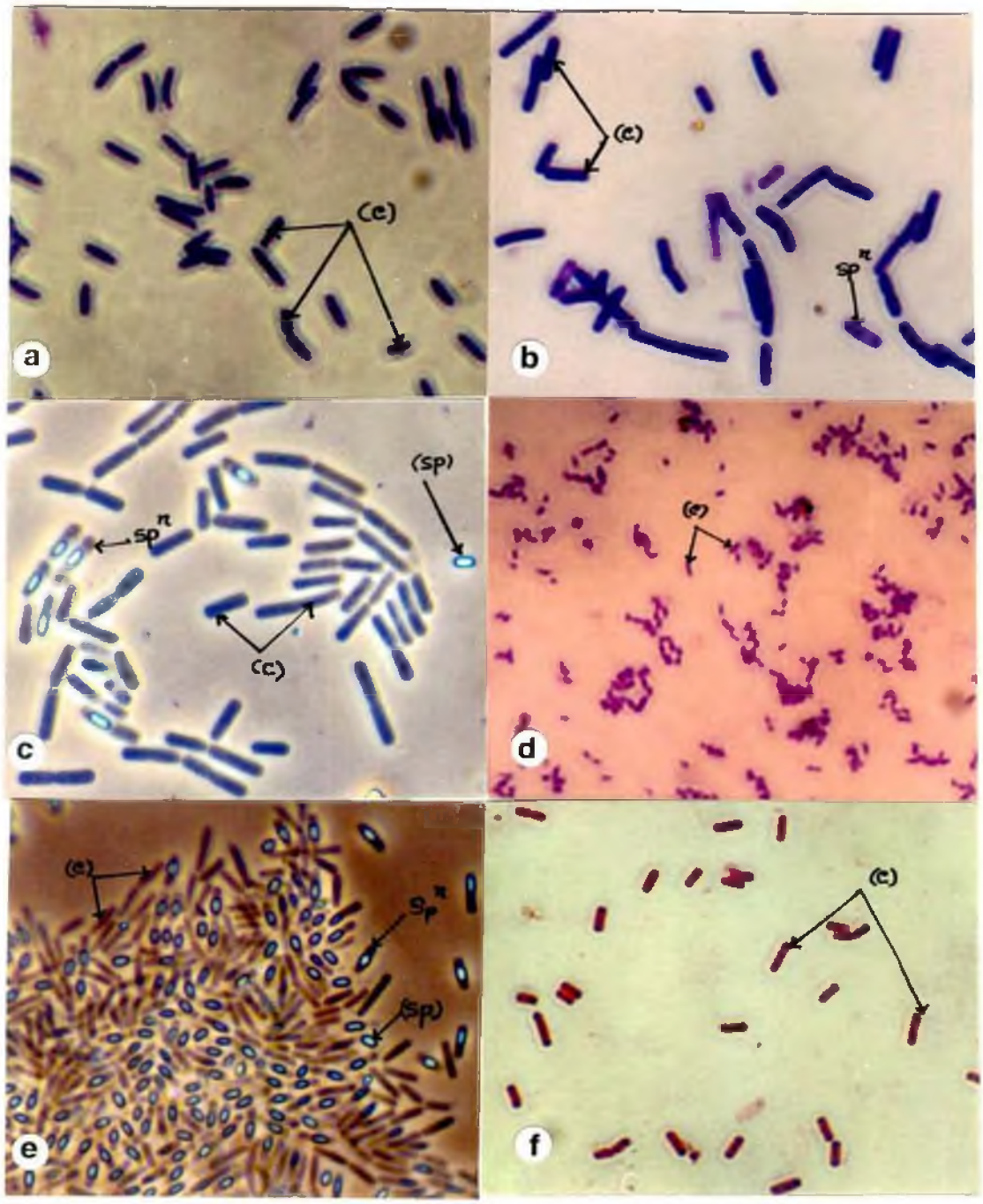


Fig. 7.4. Photomicrographs showing vegetative cells (c), sporangia (sp^n) and spores (sp) of bacterial isolates. Vegetative cells of a) S_3/B_1 and b) S_3/B_2, stained with crystal violet c) S_3/B_2 under phase contrast microscope d) S_9/B_1 stains with crystal violet e) S_{14}/B_1 under phase contrast microscope and f) vegetative cells of C_1 stained with basic fuchsin. (——— = 5 µm)

Table 7.3 Cultural and biochemical characteristics of bacterial isolates.

Sl. No.	Name of tests	Isolates No.				
		S_3/B_1	S_3/B_2	S_{14}/B_1	C_1	S_8/B_1
0-2	Surface growth in nutrient broth	Uniformly turbid without sediment	Thin, pellicle	Moderate pellicle, no sediment	Pellicle with no sediment	Heavy turbid, ring.
03	Starch Hydrolysis	Weak positive	+	+	+	-
04	Nitrate reduction (Result taken after 24 h)	+	+	+	+	+
05	Deep Glucose Agar (O_2 relationship)	Surface growth	Surface growth	Surface growth	Surface growth (pigment formed beneath the growth)	Surface growth
06	Motility	+	-	-	+	+
07	Methyl Red	-	+	+	-	-
08	Voges-Proskauer	-	-	+	+	-
09	Indole	-	-	-	-	-
10	Tyrosine degradation	-	+	-	-	+
11	Cellulase production	NG	+	+	+	-

NG = No growth.

7.3.3 Identification

Preliminary examination of bacterial isolates in accordance with Bergey's Manual of Systematic Bacteriology (Sneath *et al.* 1986), showed that all spore former strains belonged to the *Bacillus* group.

On the other hand, taxonomic identification of fungal isolates up to Genus level included examination with optical microscope at different magnifications and comparing illustrations with that of Gilman (1957). S_{14}/F_1 belonged to the genus *Penicillium* while S_{14}/F_2 was assigned to *Humicola*. Taxonomic position of S_3/F_3 could not be asertained.

7.4 Liquid Culture Test

To assess the biodegradability of materials, a liquid environment is currently used despite the fact that the fate of most films is soil, compost or landfill (Calmon-Decriaud *et al.* 1998). The general principle of these tests is mainly aerobic fermentation of polymers in an aquatic environment.

In the present study, biofilm developed on PE films during two years of incubation in pond water were charged with fresh PE flakes and degradation was quantified by the estimation of water soluble TOC and pH of the culture media.

To quantify resulting degradation in flask culture experiments with mixed population, water soluble total organic carbon (TOC) was measured at regular intervals of two weeks with TOC Analyzer (Shimadzu 5000A).

7.4.1 Material and Method

Chu's media (see Appendix) was used to cultivate algal cells in mixed culture. 0.01% yeast extract was added to the basal medium. The pH of the medium was adjusted to 7.2 with 0.1N NaOH before sterilization.

Microbial population consisted of BGA, bacteria and fungal cells adapted to grow in presence of polyethylene films submerged in pond water for nearly two years under laboratory conditions. Culture was grown in the previously mentioned medium for 48 hrs and was inoculated in the flasks (10% v/v).

Five hundred milliliter flasks plugged with cotton were labelled as M, M + C, M + PE, M + C + PE where, M stands for media, C for culture and PE for polyethylene. M + C served as cell control (no film) whereas M + PE served as film control (no inoculation). 0.1% (w/v) polyethylene flakes were used as substrate. Final volume in each flask was 200mL.

The experiment continued for six weeks and during each sampling, approximately 15 ml of culture broth was withdrawn from the flasks for the analysis of water soluble TOC and pH. Glasswares were chemically disinfected with 3M HNO_3 (see Appendix) solution prior to use. The reaction mixture containing TOC was filtered through membrane filter (pore size 0.45µm, later 0.2 µm were used) and was estimated with a TOC 5000 analyzer (Shimadzu Corp.).

7.4.2 Results

The value of measured TOC(in ppm) during the experiment are shown in the following chart:

Days of observation	TOC (in ppm)	
	M + C	M + C + PE
0d	289.6	284.8
14d	78.08	830.80
28d	453.3	218.0

Culture was carried out with 180 mL basal medium with 0.01% (w/v) yeast extract, 10% inocula and 200 mg of bleaching powder treated polyethylene flakes at room temperature for 28 days.

It was interesting to note that in the film control, (medium + cell) where the initial TOC value was 289.6 ppm, the microorganisms grew and utilized soluble carbon (yeast extract) of the medium. As a result, after 14 days the TOC value dropped down to 78.08 ppm. The subsequent rise of the TOC value up to 453.3 ppm after 28 days could be due to the result of autolysis and release of organic carbon in the medium. On the other hand, in the experimental flask with medium + cell + polyethylene, the initial TOC value (248.8 ppm) was close to the value of the control flask (289.6 ppm). After 14 days, the value rose upto 830.80 ppm. Possibly due to the release of carbon from polyethylene substrate due to microbial activity. After 28 days, the TOC value dropped down to 218.0 ppm which was even below the initial value. The only logical explanation was the microbial assimilation during this period. This also indicated the fitness for survival of these organisms in an environment where carbon was supplied from a recalcitrant polymer material like polyethylene.

7.4.3 Discussion

Tokiwa *et al* (1976) demonstrated degradation of polycaprolactone (PCL) by *Penicillium sp*. In their study, the time course of PCL degradation was recorded in parallel with the estimation of water-soluble total organic carbon (TOC) concentration in the culture broth. During the experiment, accumulation of TOC in the culture medium was noticed from 3-7 days while the pH dropped from 7.5 to 6.4.

Pranamuda *et al* (1995) evaluated the biodegradability of PTMS, an aliphatic polyester by a soil isolate. In a liquid culture test, rapid degradation of PTMS-L powder corresponded

to a remarkable rise in TOC within 4 days. After that, degradation became slower and the TOC decreased gradually. The pH dropped slightly during cultivation. Pranamuda *et al* (1997), in their later study confirmed the degradation of polylactide (PLA) or poly(lactic acid) by an *Amycolatopsis* sp. In a liquid culture test, in the absence of culture, TOC didn't increase significantly and pH drop was not observed. However, TOC value increased seven folds and pH value dropped from 7.0 to 6.0 after culturing with the bacteria. In flasks containing only culture, TOC decreased by the consumption of yeast extract present in the medium. pH remained unchanged. The time course of PLA degradation was also determined by measuring TOC and pH during liquid culture test.

Thus it appears that, polymer degradation by hydrolysis progresses with the increase of water soluble TOC in a liquid culture. This increase also reflects exponential phase of microbial growth. After that the value remains unaltered as the metabolic activity of microbial cells become slower. A drop of pH value was observed by the above workers after two weeks of incubation but no such change was noticed in the present experiment.

7.5 Biodegradation studies with pure culture

The key advantage of pure culture biodegradation assay is the ability to identify what portion of the degradation is due to chemical degradation and what can be attributed directly to biological degradation (El-Shafei *et al.* 1998). In the present study, fungal and bacterial isolates were subjected to grow on mineral agar medium with polyethylene as the only carbon source. The tests include plate test with fungi and clear zone assay with bacteria.

7.5.1 Plate test with fungi

ISO and ASTM authorities have sought few methods in solid media which includes Petri disk screen test and controlled composting test (Calmon-Decriaud *et al.* 1998). Petri disk screen test is a method for assessing the resistance of plastics to microbial degradation. Usually, the polymer is placed on the surface of a mineral salt agar in a Petri dish containing no additional carbon source. The test material and the agar media are sprayed with a standardized mixed inoculum of known fungi or bacteria. Petri dishes were incubated at a constant temperature for a few weeks. Degradation was monitored by visual assessment and weight loss of the material.

To test the ability of fungal strains to attack polyethylene films, three strains were cultured on Mineral Base Agar Medium as described by ASTM. The carbon free, synthetic medium was supplemented with 0.02% Yeast extract. The pH of the medium was adjusted to 6.5..

7.5.1.1 Materials

White, opaque polyethylene strips submerged in pond water for two years were used as test specimen (**Fig. 7.5a**). Strips were cut into 1 mm x 1 mm pieces and were sterilized with 5% aqueous solution of sodium hypochlorite (chlorox) for a few seconds in a pre-sterilized Petriplate (Pranamuda *et al.* 1997). Pieces weigh about 0.0007-0.0009 g. Each piece was then placed on dried, mineral base agar plates. Plates were inoculated with 3-7 days old fungal isolates. Fungal isolates were maintained on PDA (Potato dextrose agar) slants having pH of 5.5. Plate incubated at room temperature were sealed with adhesive tape to maintain internal moisture. These were observed at regular intervals and polyethylene pieces were transferred aseptically to fresh pre-dried agar plates after 28-35 days. Surface area covered by each fungus was measured visually using transparent grid (**Fig. 7.5b**). The experiment continued for 65 days.

7.5.1.2 Quantitative assessment

Degradative ability of three fungal isolates was examined by the quantitaive assessment of growth on polyethylene sample as illustrated in ASTM G21-90 specifications. ASTM has created a standard rating system for the evaluation of fungal growth on polymeric materials for biodegradation. The grades are 0 = no visible growth, 1 = less than 10% growth, 2 =10-30% growth, 3 = 30-60% growth, 4 = 60-100% growth covering the surface of the polymer film.

7.5.1.3 Results

Fungal plates were observed after 14 days. S_4/F_3 did not grow on mineral base agar (MBA) plates. *Penicillium spp.* (S_{14}/F_1) and *Humicola spp.* (S_{14}/F_2) showed extensive growth on culture media and over polyethylene pieces. **Fig. 7.5 (c-f)** shows growth of *Penicillium spp* (S_{14}/F_1) and *Humicola spp.* (S_{14}/F_2) on MBA plates.

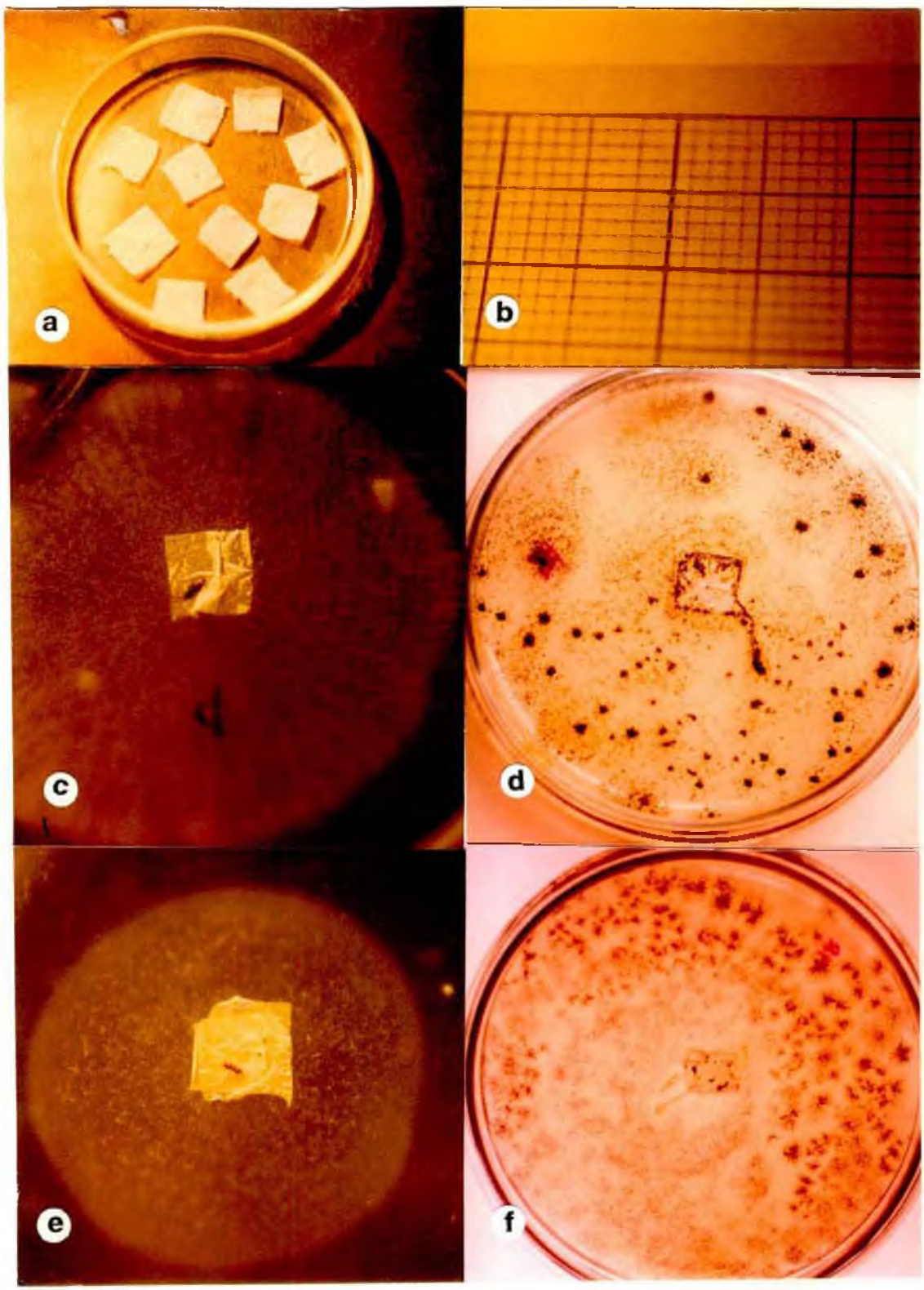


Fig. 7.5 (a-f): a) Polyethylene sample pieces as used in the plate test, b) Transparent grid for measuring the extent of growth and (c-f) observed surface growth of S_{14}/F_1 (c,d) and S_{14}/F_2 (e,f) after 2 weeks and 9 weeks of incubation at room temperature, respectively.

Growth of *Humicola* sp. on polyethylene sample varied form 44-81.25% with surface growth observed after 6-7 days of incubation . On the other hand, *Penicillium* sp. inoculated in triplicates showed 15-58.33% coverage only after 65 days. Whereas S_4/F_3 showed no growth on Polyethylene. The results were summarized in Table 7.4.

Plate tests are more likely to give an estimate of primary biodegradability and the resistance of plastic to microbiological degradation. This particular study indicated that the weathered polyethylene sample supported the growth of fungi and therefore might be damaged by these fungal isolates.

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Table 7.4: Growth rating of fungal isolates growing on polyethylene.

Name of Isolate	Plate no.	Incubation period (in Days)	Growth rating
Humicola sp.	04	38	4
		65	4
	05	38	3
		65	3
	07	38	3
		65	4
Penicillium sp	06	38	4
		65	3
	08	38	2
		65	3
	09	38	2
		65	1

7.5.2 Clear zone assay with bacteria

Clear zone assay is a common technique used for the isolation of microorganisms containing exo-depolymerases. In this technique, most insoluble polymers are sterilized, ground to fine powders and suspended in the molten agar. Alternatively, polymer could be blended homogenously with agar and allowed to cool, resulting in the desired turbid suspension. An increase in the biomass or formation of clear zones around the colonies on agar are endpoints for this assay (Mas-Castella *et al.*1995).

Nishida and Tokiwa (1993) pointed out that the clear zone technique is a powerful method for ecological investigation of plastic degradation. In addition, the procedure has the advantage that any inoculum from the soil suspension to a pure culture, can be tested for its ability to degrade any polymer that can be ground into a fine powder and incorporated into an agar matrix. This technique is qualitative and the size of the zone is regulated by (i) concentration of agar (ii) concentration of the polymer (iii) molecular weight of the enzymes involved (Mas-Castella *et al.*1995).

Five bacterial isolates were grown on polyethylene-emulsified agar plate. PE-emulsified agar was prepared as follows:

7.5.2.1 Materials and Methods

White polyethylene film was first cut into thin strips. 0.05g of strips were then dissolved in 10 mL Xylene at 110^0C. The solution thus formed became cloudy in appearance at room temperature. The suspension was then sterilized in an autoclave at 121^0C for 20 minutes. Mineral base agar prepared for the purpose was adjusted to pH 7.2. The media was sterilized separately in a flask. A magnetic bar was left in the flask and a few drops of Tween-80 was added to the flask prior to sterilization. After sterilization, polyethylene suspension was aseptically added to tempered agar media in flask and was stirred on a magnetic shaker for 15-20 minutes until polyethylene was homogenously dispersed into the agar media.

The resulting turbid media was then poured in sterilized Petriplates and was allowed to cool. Parallel sets of plate without polyethylene were also prepared. Well-dried plates were inoculated with bacterial isolates and was incubated at 37^0C for five days. To prevent

excessive desiccation, plates were sealed and later incubated at lower temperature i.e.; 32^0C. Plates were observed for growth at regular intervals.

7.5.2.2 Results

Among five bacterial isolates, S_{14}/B_1, S_3/B_1 and C_1 showed considerable growth on PE polyethylene emulsified agar plates (designated as M+PE) after only 4 days. For each isolate, growth appeared to be enhanced in presence of 0.1% polyethylene (**Fig. 7.6**). S_3/B_2 did not grow at all on M+PE plates. S_8/B_1 , on the other hand, showed faint growth after 04 days of incubation. But after 09 days, considerable growth occurred. The control plates were purple in colour due to the incorporation of Bromo-cresol purple a pH indicator in the media. M+PE plates turned to sea-green probably due to the slight increase in acidity due to xylene (**Fig. 7.6**).

Tansengco and Tokiwa 1998 confirmed degradation of polyethylene succinate (PES) by clear zone test. Clear zones were formed around the colonies of organisms on the turbid agar plates. Fields *et al.* 1974 stated that this happened when the polymer-degrading microorganisms excrete extra-cellular enzymes which diffuse through the agar and degrade the polymer to water soluble materials.

In our experiment, no such clear zones were observed, even though biomass increased in presence of polyethylene. Absence of clear zone may be due to a number of reasons (i) greater number of point inoculation per plate may cause complete consumption of polyethylene therefore no clear areas were left (ii) the concentration of the polymer in medium may be too low so that the media was not turbid enough to demonstrate clear areas around colonies and (iii) hydrolysis may not be the primary/principle route for the degradation of polyethylene.

The medium was carbon free. So, the growth of bacterial isolates on carbon free media with polyethylene as a sole source of carbon reflects the utilization of polyethylene by the isolates.

Recalcitrant polymers are unique in many ways and it is rather difficult to assess the initial degradation processes. But the experimental results obtained during the present study clearly demonstrated biodegradation of selected polyethylene materials under laboratory conditions.

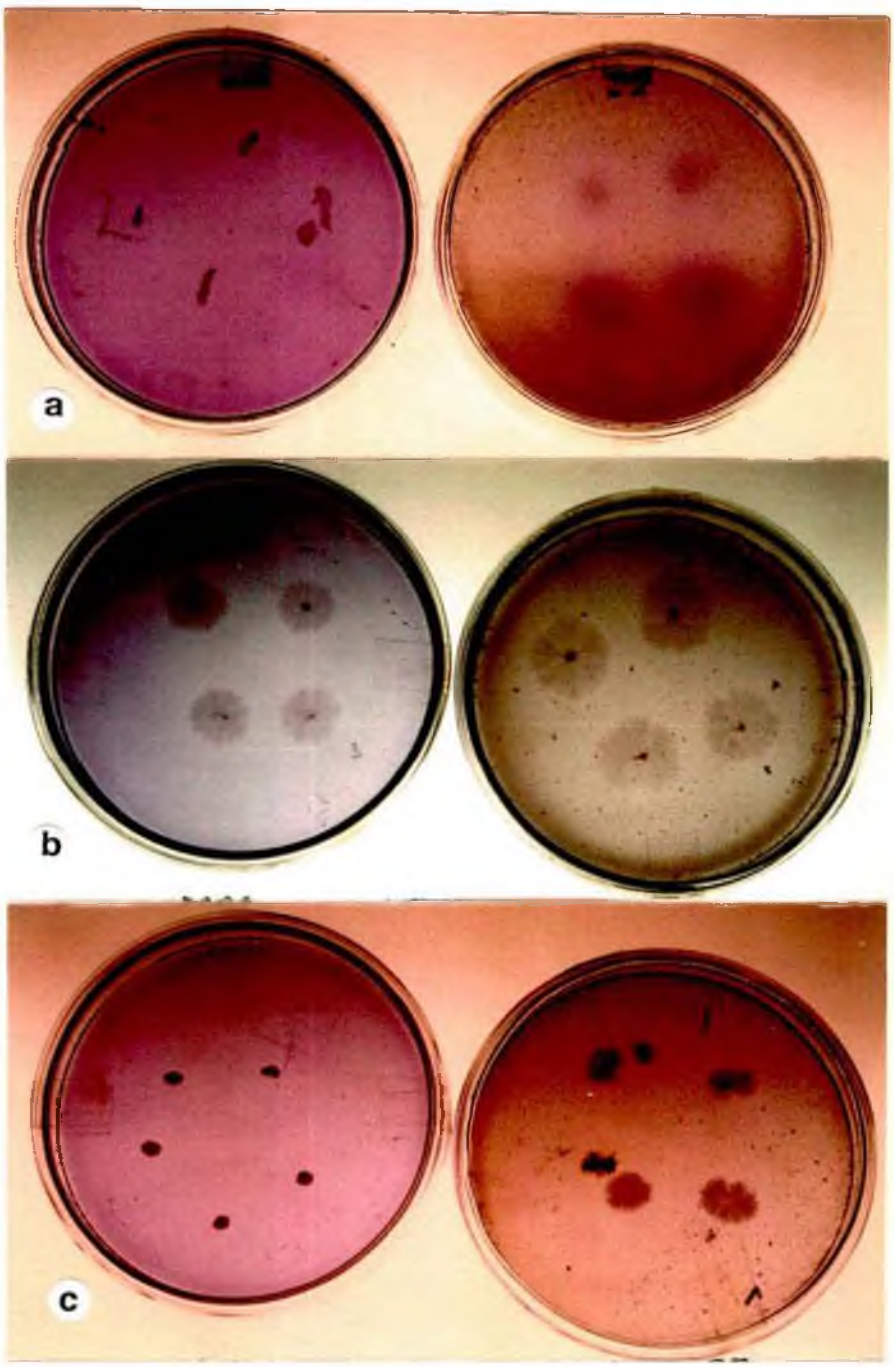

Fig. 7.6 (a-c) Enhanced bacterial growth observed in mineral base agar medium with emulsified polyethylene. Growth of a) S_3/B_1, b) S_{14}/B_1 and c) C_1 isolates in control (left) and media with polyethylene (right) plates (The observation was taken after 04 days).

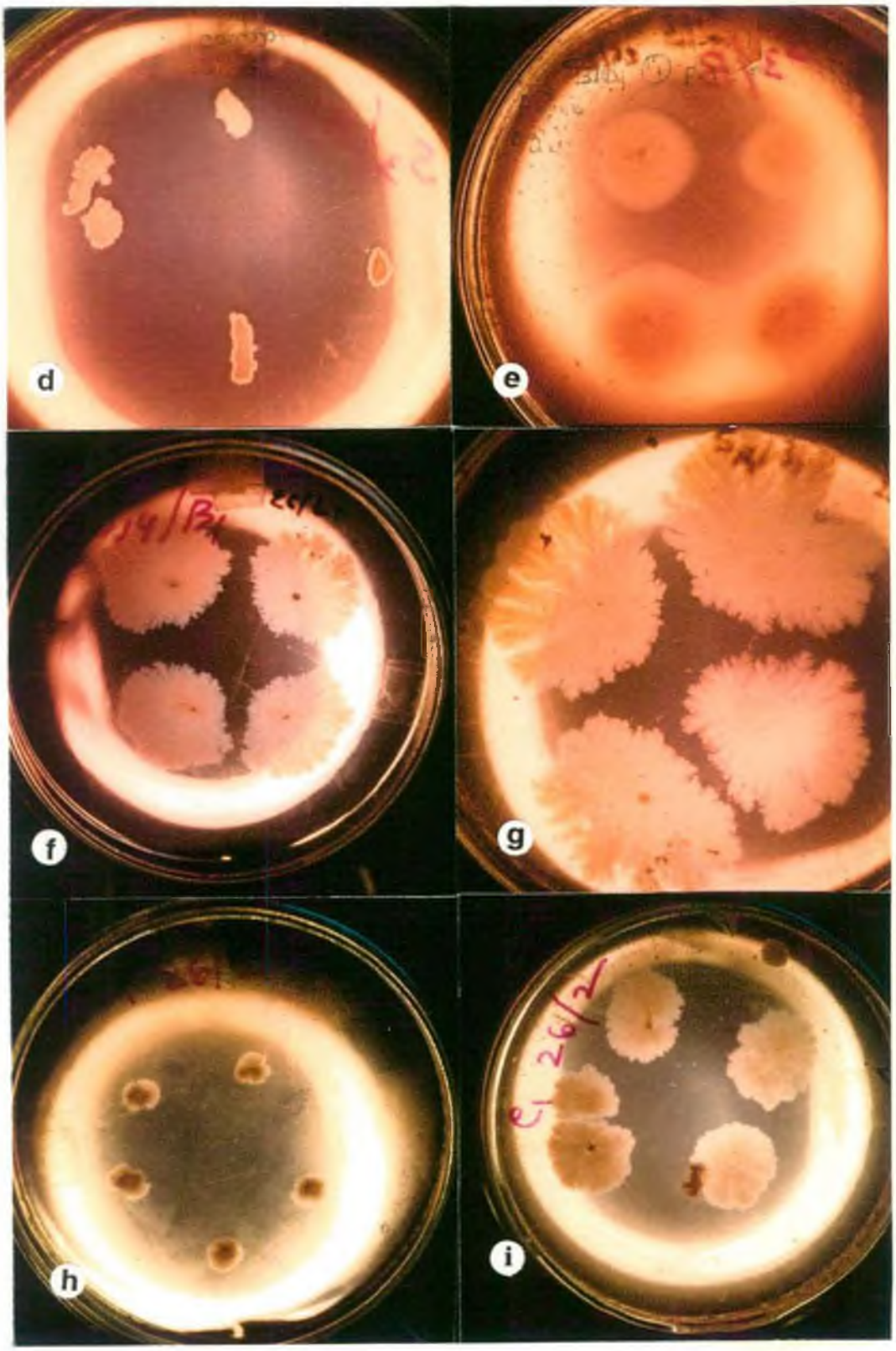

Fig. 7.6 (d-i) Bacterial isolates as observed after 09 days of incubation at 37°C. Growth of S_3/B_1 (d,e), S_{14}/B_1 (f,g) and C_1 (h,i) in control (left) and PE-emulsified media (right) plates.

Chapter - 8

DISCUSSION

Discussion

Natural polymers like cellulose, lignin, keratin and chitin are very stable substances which do not break down when exposed to environmental extremes of temperature, radiation and moisture. Man, seeking as usual to improve on nature; is producing synthetic polymers in great profusion. About forty families of these giant molecules are now in widespread use. Synthetic polymers are organic materials, in many cases similar to natural polymers. This means they could be susceptible to biodegradation (Kavelman and Kendrick 1978).

Stranger-Johanssen (1979) pointed out that plastic materials can be deteriorated by microbes, especially after longer ageing periods. The question is how long it will take under certain environmental conditions until the properties of plastic materials are altered to a net acceptable degree by microbes. However, it is believed that these substances are nonbiodegradable.

In biodegradable terms, plastic is forever. Neither wind, nor rain, nor water will wear it down. Scientists estimated that it will take about 200 years before polyethylene will start to decompose (Wilke 1988). In every survey of the biological degradation of synthetic polymers, it is fairly clearly stated that polyethylene is an inert polymer with good resistance to microbes.

However, Kaplan *et al.* (1968) stated that polyethylenes, nylons, Dacron and PP do not support fungal growth as polymers but various finishes or additives used for processing purposes may provide a substrate for microbial growth. Dolezel (1967) and Connolly (1972) have reported a change in tensile strength for polyethylene exposed to a biotic environment. Kestel'man *et al* (1972) demonstrated a higher water uptake in polyethylene overseeded with growing moulds. Potts and his collaborators (1972) found that polyethylene was not biodegradable, but the linear paraffin molecules below a molecular weight of about 500 were utilized by several microorganisms.

Scott (1975) concluded that attack by microorganisms is a secondary process. The step which determines the rate at which degradable polyethylene is returned to the biological

cycle appears to be the rate of the oxidation process which reduces the molecular weight of the molecule to the value required for biodegradation to occur.

Albertsson and Ranby (1975) estimated that polyethylene would degrade less than 0.5% over 100 year and 1% if exposed to sunlight for 2 years before biodegradation. Exposure of ultraviolet radiation that simulated 2 years outdoor exposure before incubation increased the amount of biodegradation to 0.5% during the course of 2 years. Addition of a chemical photosensitizer before irradiation increased weight loss as CO_2 to 1.7% during the same period. Extensive photodegradation had caused an additional 1.2% of the polyethylene to become susceptible to microbial degradation. Other studies (Stranger-Johanssen 1979; Cornell *et al*,1984) have shown that the biodegradable fraction is only a small portion of the low molecular mass fraction formed by photodegradation. In all cases, decomposition rates were highest in the first 100 to 200 days of incubation and decomposition rates were declining at the end of the study.

Griffin (1983) also questioned whether polyethylene is biodegradable. He emphasized the complex nature of the process proposed in view of the interacting synergistic autooxidation and biodegradation effects to which polyethylene is exposed in the great variety of natural environments available.

Karlsson *et al* 1988 tested biodegradation of LDPE and HDPE films that have been filled up to 20% by weight of starch. Removal of starch was evident from the different films by massive colonization of various organisms, and this is accompanied by a small but significant drop in the average molecular weight and decrement in mechanical strength of the films. Composting trial, on the other hand, produced small but detectable spectroscopic evidence of oxidation of the polyethylene matrix.

However, from a chemical point of view, it can be anticipated that microorganisms may be able to degrade PE since the chemical structure of polyethylene is similar to that of linear alkanes, which are known to be biodegradable (Albertsson *et al* 1987).

Many factors influence rates of biodegradation including: the environment (for example O_2, temperature, moisture, trace minerals and salts, nutrients and co-metabolites, pH, redox potential, pressure and light); the nature of the polymeric substrate (chemical bonds, branching, hydrophobicity, stereochemistry, molecular weight, crystallinity,

interactions with other components and surface area); and the organisms (appropriate enzymes and levels, extracellular or intracellular enzyme location, predators, the presence of inhibitors or inducers of enzymes) (Mayer and Kaplan 1994).

In the biomedical area, "biodegradation" refers to hydrolyses and oxidations that are the primary polymer degradation processes (Cha and Pitt 1990). On the other hand, for materials exposed to a natural environment, biodegradation means fragmentation, loss of mechanical properties or chemical modifications through the action of microorganisms. Thus many different degradation modes, either abiotic degradation (e.g., degradation due to agents such as oxygen, water and sunlight) or biotic degradation (e.g., degradation due to microorganisms), can synergistically combine in natural conditions to degrade polymers, leading to different degrees of degradability, (e.g., fragilisation, fragmentation, solubilisation) and each specific action is difficult to isolate (Calmon-Decriaud *et al.* 1998).

In a natural environment, microorganisms on a substrate tend to form a biofilm, consisting of bacteria and fungi in a highly hydrated (85-98% water) matrix of extracellular polymers. Both hydrolysis and oxidation of the substrate can be mediated by the biofilm, by release of extracellular enzymes or free radicals. The extracellular nature of these substances enable microorganisms to tolerate higher concentrations of toxic chemicals than would be possible if the compounds had to be brought into the cell before degradation. In addition, insoluble compounds that cannot cross a cell membrane are also susceptible to attack. Cell enzymes and particularly cytochrome P450 which is produced by many bacteria, continue peroxidation by reducing ground state oxygen to the free radical superoxide ($O_2^{\cdot-}$). When protonated, this species is converted to the much more reactive peroxyl radical and hydrogen peroxide, which can be reduced by transition metal ions in the polymer to give the highly reactive hydroxyl radical. $^{\cdot}OH$ initiates further peroxidation leading to continued biodegradation and ultimate bioassimilation to biomass and CO_2 so long as environmental oxygen and cell nutrients are available(Billingham *et al.* 2002).

Thus, macromolecules can be degraded using abiotic processes such that the molecular fragments become biodegradable. In the case of aliphatic polyesters, for example, the first stage in this two-stage process is hydrolysis. The second stage is bioassimilation of the

hydrolysis products. In the case of hydrocarbon polymers such as polyethylene, the first stage is oxidation initiated by heat, or UV light or mechanical stress. The oxidized fragments of the polymer chains are biodegraded in the second stage by the complex mixture of microorganisms found in soil, in composting or in landfill (Billingham *et al.* 2002).

Mechanism of biodegradation of polyethylene

In 1987, Albertsson and her collaborators presented the mechanism for the biodegradation of polyethylene involving initial abiotic oxidation.

There are several reasons why the initial degradation of polyethylene has to be abiotic. The biotic degradation of long chain alkanes by the β-oxidation mechanism cannot occur enzymatically beyond n-C_{44} because of steric hindrance. The initial step of C-C bond splitting cannot be carried out by any natural enzyme in either the side or main chain. With the increasing number of carbon atoms in the paraffin chain, both melting point and hydrophobic character are increased and make it more difficult for the substrate to fill the crevice of the active site in the large molecule of the enzyme, and thus establish contact with polar groups, a prerequisite for enzyme action. The combined effect of an abiotic oxidative step with consequent biotic action will eventually be slow but definite progressive mineralization.

The mechanism for the degradation of polyethylene (**Fig 8.1**) is similar to the typical β-oxidation of fatty acids and paraffins in man and animal. It is generally accepted that the key intermediates are hydroperoxides, which are always present because of oxidation during preparation or processing, and decompose under the influence of heat, light or transition metal catalysis to produce free radicals. Once radicals are produced they enter into a chain reaction with oxygen and C-H bonds in the polymer, to produce a range of oxidation products. Although the primary products are hydroperoxides, their decomposition yields alkoxy radicals which are responsible for many secondary products. Elimination of alkoxyl radicals competes with H abstraction, and leads to chain scission and formation of a variety of carbonyl products (Billingham *et al.* 2002). When carbonyl groups have been produced, these are attacked by microorganisms which degrade the shorter fragments of polyethylene chains to the end products, carbon dioxide and water (Albertsson *et al.* 1987). Thus, in the degradation of polyethylene, abiotic steps contribute to the total degradation.

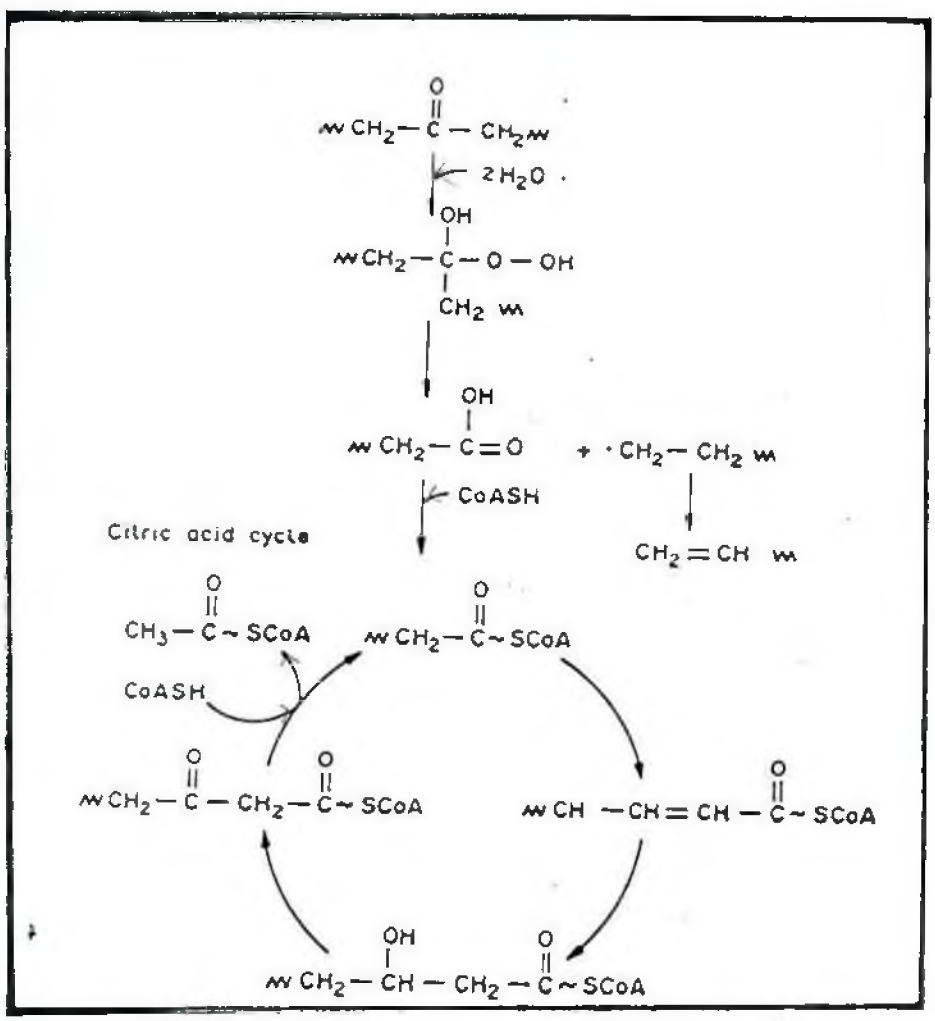

Fig. 8.1: The biodegradation mechanism of polyethylene.

Albertsson *et al.* 1987 have also shown that the biodegradation of polyethylene is affected by several factors *viz.* preliminary irradiation from a UV source, by the presence of photodegradative enhancers, by its morphology and surface area, by additives, antioxidants and by its molecular weight.

Hydrophobicity is often regarded as a major obstacle to microbial (biodegradation) attack on polymers. Addition of surfactants in degradation studies with polyethylene showed a considerable increase in the biodegradation rate compared with samples without surfactants (Karlsson *et al.* 1988). Ongoing studies incorporate surfactants in polyethylene showing promising results as observed by attenuated total reflection infrared spectroscopy, differential scanning calorimetry and scanning electron microscopy (Albertsson *et al.* 1993).

Husmark 1993 reported that *Bacillus cereus* (hay-bacterium) with a hydrophobic surface adheres most readily to hydrophobic surfaces such as silicone, polytetrafluoroethylene (PTFE), ethylene-propylene diene tercopolymers and low density polyethylene (LDPE).

Biodegradation test methods

Two criteria are required to demonstrate biodegradation: growth of microorganisms and polymer degradation (Mas-Castella *et al.* 1995). All organisms require a source of carbon, a source of nitrogen and certain inorganic ions, as well as water. The polymer typically serves as the only carbon source for anabolic reactions. Most common polymers lack nitrogen, which the organisms need for the synthesis of proteins and other nitrogenous compounds. Organisms are able to use inorganic nitrogen and /or organic nitrogen. Inorganic ions are also required as enzyme cofactors and as components of proteins, nucleic acids etc (Mas-Castella *et al.* 1995).

There are three basic approaches for the evaluation of biodegradability of synthetic polymers (Barak *et al.* 1991):

The first approach entails monitoring growth on C-deficient nutrient agar inoculated with either specific fungal or bacterial species or with a soil-derived microbial population, with the plastic as a sole C source. With such methods, the extent of microbial growth is either visually rated as in ASTM G21-70, ASTM G 22-76, and clear zone methods, or the number of microorganisms is counted, as in the plate count method.

In a second approach, weight loss of the polymer after incubation with either an organism or a consortium of organisms is measured; the weight loss of the polymer may be determined after incubation in either nutrient agar, as in the quantitative Petri dish method, or in whole soil, as in the soil burial and retrieval method.

A third approach is based upon measurement of CO_2-C evolved during the microbial activity on the polymer substrate in either nutrient medium or soil; such techniques may either measure the amount of CO_2-C evolved above the background respiration or CO_2-C measure CO_2-^{14}C evolved from radio-carbon-labelled plastics (Potts 1984; Ennis and Kramer, 1974).

To evaluate biodegradability of polymers in natural conditions, the habitat must first be considered. Amass *et al* 1998 pointed out that the degradation tests must incorporate realistic environment disposal conditions which are either terrestrial (landfill, soil, composting) or aqueous (groundwater, marine, waterways, sewage or sediment).

Discarded shopping bags and other materials made up of polyethylene eventually find their way to open dumping sites/spaces. They come in contact with soil and remain there for a couple of years. Our previous study on waste polyethylene has established association and role of bacteria with sample films. To get an in depth knowledge on the fate of polyethylene films under soil, an outdoor exposure or soil burial test has been performed for two years.

Soil burial test has been employed by many scientists working on biodegradation of synthetic polymers (Kavelman and Kendrick 1978, Barak *et al.* 1991, Goheen and Wool 1991, Ohtake *et al.* 1995, Mayer *et al.* 1996, Contat-Rodrigo and Ribes-Greus 1998). The obvious advantage is its simulation of the environmental conditions of interest, and the fact that the polymer can be prepared in a predetermined form.

In particular, soil burial tests with 50 mm wide strips samples are buried in cultivated well drained but moist garden soil with the top of the samples about 50 mm below the surface. The soil beds are normally conditioned prior to use and may be supplemented with organic fertilizer to encourage an active microbial flora. The soil beds containing the samples are incubated at a constant temperature between 28 days to 12 months. Samples are removed for the assessment of changes in their properties such as weight loss,

mechanical strength changes or a microscopic (light and scanning electron microscope) examination to assess surface damage and to look for the presence and nature of microbial growth. Physical factors such as fragmentation and embrittlement can also be assessed in these tests (Seal 1994).

There are few results in the literature related to very long periods of degradation of inert polymers like polyethylene. Long term soil burial tests and use of a broad spectrum of microorganisms have resulted in various interpretations. Connally (1971) found that after 8 yr soil burial, plastics had undergone some physical changes but he concluded that these changes could be caused by nonbiological as well as biological factors.

Colin *et al.* (1981) have also studied PE under soil burial conditions and interpreted that the small but significant embritterment of the PE could not be explained simply by thermal oxidation or microbial attack. Ohtake *et al* 1995 examined biotic degradation of LDPE, polystyrene (PS), poly (vinyl chloride) (PVC) and urea-formaldehyde resin (UF) buried in bioactive soil for over 32 years. In their study, only the LDPE showed any signs of degradation, as the surface in contact with the soil had whitened and was severely degraded. The inside of the LDPE was still transparent and differed in FT-IR spectrum from the surface. The oxidation products in the clear part were carboxylic acids and ketones, whereas the whitened surface showed some carbonyl absorbance but increased C=C (double bond) absorption. The usual oxidation mechanism was proposed for the clear regions and a more complex mechanism postulated for the whitened area. Under biotic conditions the alkoxyl radical scission may proceed through a γ-scission mechanism, form vinyl groups and volatile products, in addition to the β-scission mechanism.

In an attempts to accelarate the biodegradation of LDPE, UV radiation was used and several oxidizing agents were added to the LDPE and was left in bioactive soil for 12 months. It was found that UV radiation accelerated the biodegradation very effectively and the addition of vegetable oil, metal carboxylates and small amounts of dicumyl peroxide also promoted the degradation of LDPE (Ohtake *et al* 1996).

Albertsson *et al.* (1990) carried out extensive research on the mechanism of biodegradation of polyethylene. They also worked out the influence of abiotic and biotic environmental factors on polyethylene degradation. Using ^{14}-C labeling technique, they

were able to study microbial and oxidative effects in the degradation of polyethylene. The degradation curves obtained for polyethylene consisted of three stages. First there is a constant degradation rate, secondly a decline and thirdly an increase in the rate of degradation leading to a final destruction and mineralization of the material. They also pointed out that even after 10 years of inoculation in soil, it was possible to see only small indications of stage III and a coming mineralization point whereas for aliphatic polyesters, 2 years was sufficient to show all three stages.

In the present study, soil burial test was performed to determine the susceptibility of polyethylene materials to biodegradation. Samples were buried vertically downwards from 7.5 cm below the soil surface.

As water is a necessity for biodegradation and a prerequisite for microbial growth and activity, soil bed was wetted occasionally. For enrichment of the existing microflora of experimental soil, no extra nutrients were added. We decided to observe how polyethylene behaves in apparently less favourable conditions and relied on the capability of indigenous microflora present in the soil. Jones *et al.* (1974) also believed that natural soil must contain a variety of microorganisms capable of carrying out the biooxidation of polyethylene.

Polyethylene films, thus exposed to natural soil conditions were retrieved at regular intervals and examined for loss of average weight, changes in surface morphology by visual estimation as well as microscopic examination. Degradation was also assessed by FT-IR spectroscopy, tensile testing, dielectric loss measurements and x-ray diffraction analysis.

Despite of its multifarious application, polyethylene was blamed particularly for causing havoc in our country especially as shopping bags. On the contrary, Government imposed ban on polyethylene shopping bags having 20 μm thickness. Surely, polyethylene terephthalate (PET) bottles will be the next issue of environmental concern, but in the present context, polybag poses a major environmental problem. Taking this into account, commercial formulations of LDPE films used as shopping bags were considered as the material of choice for our project.

Polyethylene films used in soil burial test were not ground into powder or emulsified rather employed in their usual sheeting form. Calmond-Decriaud *et al.* (1998) mentioned that the best procedure is to measure the biodegradability on film strips of a standard size and thickness. Nevertheless, the shape of the sample influences the degradation rate. The same sample could be assessed as 'easily biodegradable', when in powder form and as 'inherently biodegradable' when in the form of a film.

The ribbon shaped test strips were of typically 5 cm wide and 20 cm long. Specimens were not cut in dumb-bell shape because, besides tensile determinations, a number of other parameters also had to be studied.

Weight loss

The most widely used test of polymer changes is weight loss. Generally, the polymer is prepared in suitable form, sterilized, weighed and exposed to the activity of microorganisms. These tests also provide information on the rate of biodegradation of the sample (Mas-Castella *et al.* 1995).

In our study, all four samples showed a weight gain during the 1st year of study (Table 2.2). The average weight of specimens increased up to 09 months in case of PE_w, 05 months for $PE_{Tr\text{-}HW}$ and 11 months for $PE_{Tr\text{-}LW}$. Black sample, on the other hand, did not exhibit any distinct pattern of weight loss since weight gain was noticed even after 15 months of soil exposure.

Weight gain was also noticed by El-Shafei *et al.* (1998) during pure shake flask culture studies with *Streptomyces* spp. on disposable starch-polyethylene bags. Out of the four tested samples, thick, transparent sample $PE_{Tr\text{-}HW}$ showed a progressive loss started form 05 months of exposure.

Gohcen and Wool (1991) conducted degradation experiment with PE-starch blends in soil for a period of over 08 months. Starch blend LDPE films lost 4% of the mass in 08 months while in the present study, similar results *i.e.*, reduction of average weight was obtained for sample $PE_{Tr\text{-}HW}$ after 11 months of exposure in soil. The induction period took about 04 months and then samples lost mass gradually and 10% of the original weight was lost during 15 months of exposure. Imam *et al.* (1995) stated that, weight loss can serve as a good indication of degradation.

Changes in visible character

Nykvist (1974) stated that packaging of polyethylene which have been in and on soil for five years show no visible change whereas in our experiment, changes in visual appearance of samples were noticed even after 1 year.

The thick, transparent sample lost its clarity completely even after 11 months of soil burial (**Fig 2.4a**). Fragmentation started after 17 months and after 19 months although the film could still be detected, it was heavily degraded and friable (**Fig 2.4b**). After 22 months, tiny pieces were retrieved a sieve of 1 mm mesh size.

While transparent samples turned opaque, white film became matt and perforations on the film was common in samples exposed for 22 months. The otherwise resistant black sample also underwent fragmentation at the end of the study.

Microscopic observation

To follow the possible course of degradation of polyethylene material under soil, samples were examined under microscope having facilities like Bright field, Dark field, Phase contrast and Fluorescence microscope. Surface morphology, erosion characteristics and the extent of microbial growth were monitored by microscopic examination.

Barak *et al.* (1991) observed no evidence of deformations, fissures, craters or perforations in starch incorporated LDPE samples during incubation in soil for 5 months. Similar results were obtained for LDPE samples when all of the samples did not undergo any significant change up to 07 months. After that, microbial colonization was evident by red fluorescent areas under blue filter which increased in number as time progressed. When the film was completely invaded by microbes, the difference in thickness was evident by closer eye estimation. Surface erosion was not uniform throughout the film nor it was confined at the edge. Cracks and fissures developed which resulted in the fragmentation of the samples.

SEM analysis

In majority of investigations on polymer degradation, workers frequently employed Scanning electron microscopy (SEM) to verify the extent of microbial degradation (Nishida and Tokiwa 1993, Pranamuda *et al.* 1995, Tansengco and Tokiwa 1998).

Unfortunately, Electron microscope is not available in our country. Even then it was proved beyond doubt that the techniques of optical microscopy was even better at least in some cases. Photographs taken during the study are informative enough to explain the pattern of changes took place during biodegradation.

It is mentioned earlier that SEM analysis of a few samples was performed at Jadhavpur University, Kolkata, India. After 17 months, one of the samples ($PE_{Tr\text{-}HW}$) started fragmenting. After 22 months, when the experiment was discontinued, the rest of the samples were retrieved as fragments of various sizes. To verify whether the observed change is due to microbial activity or not, samples exposed for 17 months and those buried under soil for 22 months were selected for SEM analysis.

SEM images revealed that, upon exposure in soil, surface erosion takes place and the matrix become heavily perforated. This ultimately results into a thinning of matrix with inconsistent properties which corresponds to the deterioration of the mechanical properties of the material. Exfoliation was clearly demonstrated. This peeling of thin layers (exfoliation) can happen only if there is a major erosion of the more accessible, amorphous regions in the polyethylene, leaving crystalline regions that degrade very slowly and can peel off.

All photos taken after 22 months showed that the polyethylene film integrity was completely destroyed after microbial invasion, probably by the oxidative etching away of the more accessible regions. There is the possibility that bacterial peroxidase enzymes generated free radicals that could catalyze the oxidative attack on the polyethylene film.

SEM evidence of rapid surface degradation during microbial degradation were also observed by Pranamuda *et al.* (1995) and Tansengco and Tokiwa (1998). Enzymatic degradation in PE/starch composite films with different fungal species on agar nutrients was investigated by Nakashima and Matsuo in 1996. Although the biodegradation of the starch and a decrease in the mechanical properties of the films were observed, no disruption of the polyethylene fibrous texture was discernible by SEM. The surface morphology as revealed under optical and electron microscope confirmed that microbial decomposition had taken place.

Changes in mechanical properties

Most of the workers monitored degradation by changes in tensile properties, particularly the tensile strength and percent elongation at break (Andrady 1990, Johnson *et al.* 1993, Imam *et al.* 1995 and El-Shafei *et al.* 1998). The value of each parameter was found to be lowered after degradation. Andrady (1990) observed marginal decline in tensile strength for LDPE homopolymers after 01 year of exposure in air, whereas, mean ultimate extension decreased to about 12-17%.

The load-extension data generated by Lloyd Instrument showed a common trend of the destruction of plastic region in progress of time. Around 95% of the elongation lost after 17 months of soil burial and the samples turned brittle. Billingham *et al.* (2002) postulated that, since linear polymers like LDPE derive their mechanical properties from chain entanglement, they can tolerate only limited scission before becoming brittle. It is well known that polyolefins that have undergone oxidative degradation, have hydrophilic surfaces and greatly reduced molar masses. Reduction of the molecular weight of polyethylene to around 40,000 combined with introduction of oxygen-containing functional groups leading to biodegradable products.

Strain at maximum load and maximum stress attained minimal values within 15 to 17 months. Thus it appears that, loss of mechanical strength of polyethylene materials occurs after less than one and half year of soil exposure.

Changes in dielectric properties

Measurement of power factor at high frequencies has been found useful to follow the rates of oxidation of polyethylene (Haywood 1957). This method is convenient, non-destructive and fairly rapid and this particular property is sensitive to small changes.

Serious oxidation of natural polyethylene takes place in a relatively short time at temperatures in excess of 120^0C. This results in a rapid rise in power factor, increase in melt viscosity and eventual extensive discolouration. However, at normal temperatures, the rate of oxidation is very slow.

Haywood (1957) showed that for 40 mm thick PE sample exposed in air at 75^0C, 100^0C and 150^0C, it took about 10 years for the power factor to rise five folds from 0.0002

(original value) to 0.001. Whereas, our experimental data showed that 10 times increase in the values of power factor occurred only after 15-17 months of exposure in soil. Compared to the unincubated control, buried samples exhibited good dielectric relaxation spectra reflecting oxidation of the samples. Smook *et al.* (1957) observed ten fold increase (0.00055-0.0055) in the power factor of polyethylene when the carbonyl oxygen was increase by 0.05%, or by one oxygen atom per 2,280 methylene groups.

Brydson (1999) stated that oxidation of polyethylene with the formation of carbonyl groups can lead to serious increase in power factor. Exposure of polyethylene to ultraviolet light causes eventual embrittlement of the polymer. Incorporation of carbon black into polyethylene can give substantial reinforcement to brittleness. Carbon black is cheap, inert and stable to light and heat. 2% carbon black incorporated into the polyethylene increases the power factor but the value remains steady even after 2,000 hours aging at 105^0C. That could be the reason for the resistance of black thin film (PE_B) to biodegradation which showed no change in dielectric spectra.

FT-IR analysis

Spectroscopic studies were employed to characterize the chemical composition of the sample before and after soil burial. Degradation rates could not be measured by carbonyl and double bond indices as suggested by Albertsson *et al.* (1990). The only significant trend that was observed was a rapid decrease in the carbonyl region 1400-1800 cm^{-1} over time. Additional peaks around 1076 cm^{-1} and at 905-915 cm^{-1} also appeared which corresponded to hydroperoxide group and double bond groups, respectively.

XRD analysis

It is known that LDPE is semi-crystalline and is only around 40% crystalline in nature (Gowariker *et al.* 1986). Our XRD data did not shed much light on the degradation. However, it was clear that the amorphous phase was affected rather than crystalline portion. Contat-Rodrigo and Ribes-Greus (1998) conducted an outdoor soil burial test on HDPE/PP blends with different biodegradable additives. They concluded that degradation seemed to start from the molecular chains of the amorphous phase or interfacial region rather than to affect the crystalline morphology.

Eventhough, it is frequently recognized that polyethylene films were disposed off under less favourable conditions, a study under adverse conditions was considered premature when even the inherent degradability of the materials was in doubt. This study was performed under generally favourable condition for biodegradation.

Two year is too short for material like polyethylene. Even within this short period, loss of integrity of the samples accompanied by the loss of mechanical properties was witnessed. Disintegrated pieces measured 1 mm resembling an early stage of mineralization. Microscopic observation revealed surface erosion while rapid increase in power factor reflected oxidation of the samples.

Biodegradation, in practice, can never be entirely separated from the purely physical and chemical – mainly autooxidative-progressive ageing. It is seldom due to a single cause, but a combined effect including heat, UV light, microorganisms, stress and water (Albertsson *et al.* 1987).

Domestic and industrial wastes have been considered as a problem for too long. With the microbe on our side, refuse could be seen as a valuable commodity – an untrapped source of solar energy, ready for use by man and effluent can be regarded as a culture medium. Microbes and the energy of life can help us overcome many of the problems which have no industrial answer. The fact that these most minute of living things can undertake tasks which the mightiest industrial complex fails to master shows how blind we are to its advantages and how biased we are against it (Dixon 1994).

'The simplest experiments are often the most accurate and revealing'

- Margaret Burnett

Chapter - 9

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APPENDIX

Acridine Orange Solution

Acridine Orange	120 mg
Distilled water	1000 ml

Chu's medium (Claus 1995)

$MgSO_4.7H_2O$	0.025 g
Na_2SiO_3	0.025 g
$NaCO_3$	0.020 g
K_2HPO_4	0.010 g
Ferric citrate	0.003 g
Citric acid	0.003 g
Distilled water	1000 ml
pH	7.2

Czapek (Dox) Agar (CAB 1968)

$NaNO_3$	2.0 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4.7H_2O$	0.01 g
*$ZnSO_4.7H_2O$	0.001 g
*$CuSO_4.7H_2O$	0.005 g
Sucrose	30.0 g
Agar	20.0 g
Distilled water	1000 ml
pH	6.5

*Applied only if glass-distilled water is used.

3M HNO_3 Solution

$$1M = \frac{\text{Mol. wt. x 100}}{\text{wt./ml x Declared \%}}$$

Mol. wt. of HNO_3 = 63
Declared % = 65; Wt/ ml. = 1.4 g/ml

Therefore, $$1\,M = \frac{63 \times 100}{1.4 \times 65} = 69.23 \text{ ml/1000}$$

3 M = (69.23 x 3) ml/1000
= 207.69 ml/1000
≈ 208 ml/1000

Thus, 3 M HNO_3 solution was prepared by adding 208 ml of supplied HNO_3 acid into 792 ml of distilled water.

Mineral Base Agar Medium (ASTM G21-90)

KH_2PO_4	0.7 g
K_2HPO_4	0.7 g
$MgSO_4.7H_2O$	0.7 g
NH_4NO_3	1.0 g
NaCl	0.005 g
$FeSO_4.7H_2O$	0.002 g
$ZnSO_4.7H_2O$	0.002 g
$MnSO_4.7H_2O$	0.001 g
Agar	15.0 g
Distilled Water	1000 ml

Nutrient Agar Medium (Bryan 1950)

Beef extract	3.0 g
Peptone	5.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	7.2

Oat-meal - Asparagine - Soil extract - Potato extract (OASP) Medium

*Oat meal	5.0 g
L-Asparagine	0.5 g
Soil extract	250 ml
Potato extract	250 ml
Distilled water	500 ml
Phosphate buffer	6.5
pH	7.0

*5 g of oat was heated in water for 10 minutes. The turbid suspension was filtered and made to 500 ml.

Potato Dextrose Agar (Claus 1995)

Potato	200 g
Dextrose	20.0 g
Agar	15.0 g
Distilled water	1000 ml
pH	4.5-6.5

Soil extract, Potato extract, Yeast extract, Malt extract (ATCC 1992)

*Malt extract	10.0 g
*Yeast extract	4.0 g
Potato extract	250 ml
Soil extract	250 ml
Distilled water	1000 ml
Phosphate buffer pH	6.5
Trace salt solution	1 ml
pH	7.0

Soil extract – 400 g of mixed with 1 litre of water. The suspension was bioled for 45 minutes and was filtered before use.

Potato extract – 400 g of soil was sliced potato was cooked for 15 min in 500 ml of water. Resulting extract was diluted to 1 litre and was filtered before use.

*For the present study, half strength of malt extract and yeast extract i.e. 5.0 g and 2.0 g were used, respectively.

Phosphate buffer pH 6.5 (Williams *et al.* 1971)

KH_2PO_4	2.72 g
K_2HPO_4	1.74 g
Na_2HPO_4	1.39 g
Deionized water	200 ml

Trace Salt Solution (Waksman 1967)

$FeSO_4.7H_2O$	0.1 g
$MnCl_2.4H_2O$	0.1 g
$ZnSO_4.7H_2O$	0.1 g
Distilled water	100 ml