Chapter -III EXPERIMENTAL PLAN,EXPERIMENTAL METHODS AND ANALYTICAL TECHNIQUES

3.1. PLANNING OF EXPERIMENTS:

◊Various modes of polymer degradation were studied and the most suitable method was selected.

◊The effects of various plasticizers were studied and the most suitable with respect to availability, ease of processing and cost were selected.

◊Most conventional polymers used for the manufacture of carry bags were selected.

◊Microorganisms exhibiting the highest susceptibility towards the biodegradation were selected.

◊The selected polymers were processed by the conventional intermittent extrusion process.

◊The mechanical strength was compared with those of conventional materials.

◊Various standard test methods for the assessment of biodegradation were listed.

◊The loss in viscosity, mechanical strength was measured to assess the biodegradability.

◊The films manufactured were subjected to the microorganisms exhibiting the highest susceptibility towards the biodegradation.

◊ The microorganisms from the specimens showing highest degradability were isolated and the grown separately to study the structure of the microorganism.

◊The comparison of the structure of microorganism grown due to the biodegradable polymer with that of the original microorganism was carried out.

◊The polymer exhibiting good degradability and ease of processing was identified.

◊Films were manufactured on large scale on a conventional screw extruder to assess the ease of processing.

◊The effects of plasticizers on the processing parameters were recorded and the cost analysis of the film is carried out.

◊The films were subjected to the plant toxicity test to assess the effect of biodegradable polymer on the production of crops.

◊The soil analysis was also carried out to assess the fertility of the soil after land fill.

3.1.1 SELECTION OF POLYMER DEGRADATION METHOD:

VARIOUS MODES OF POLYMER DEGRADATION:

◊Thermal degradation

◊ Mechanical degradation

◊Ultrasonic wave degradation

◊High energy radiation degradation

◊Photo degradation

◊Chemical degradation

◊Bio degradation

Out of various modes of polymer degradation, BIODEGRADATION mode was selected. The other factors were left uncontrolled and unmeasured.

3.1.2 SELECTION OF FACTORS AFFECTING BIODEGRADABILITY:

FACTORS AFFECTING BIODEGRADABILITY:

Synthetic polymers are inherently resistant to biological attack. But susceptibility to biodegradation varies and is affected by:

◊Additives

◊Plasticizers

◊The type of chemical bond

◊Water uptake

◊Crystallinity and molecular weight

◊pH

◊Copolymer composition

◊Enzymatic degradation

Out of various factors affecting polymer degradation, the technique of BLENDING OF POLYMER WITH PLASTICIZER was selected. The other factors were left uncontrolled and unmeasured.

3.1.3 SELECTION OF POLYMER:

Thousands of polymers are available for various applications in various fields. Out these polymers the most conventional polymers used to manufacture microfilms for packaging were selected. The following polymers were selected for the experimental work:

◊LDPE ---- Low density polyethylene

◊HDPE ---- High density polyethylene

◊PS ---- Polystyrene

◊PP ---- Polypropylene

3.1.4 SELECTION OF PLASTICIZER:

Amongst the most susceptible plasticizers, following plasticizers were selected on the basis of availability and cost effectiveness:

◊Groundnut oil and

◊Soybean oil

3.1.5 SELECTION OF MICROORGANISMS:

The microorganisms associated with the study of biodegradation of polymers belong to different groups of fungi and bacteria. Six known, defined strains of microorganisms were selected on the basis of availability and susceptibility towards the biodegradability, as per the literature (Leonard I. Nass 1976, R. Gatcher et al 1985). They are:

◊PSEUDOMONAS(BACTERIA)

◊STAPHYLOCOCCUS(BACTERIA)

◊E-COLI(BACTERIA)

◊ASPERGILLUS NIGER(FUNGI)

◊RHIZOPUS(FUNGI)

◊CONSORTIUM – mixed culture

3.1.6 TEST METHODS FOR ASSESSMENT OF BIODEGRADATION:

Out of various test methods suggested in Ch.II 2.2 following most conventional methods were employed to measure the extent of biodegradability.

◊Screening of biodegradation of specimen in open air to assess the property loss.

◊Screening of biodegradation of specimens in open air

◊Growth ratings ASTM G-21

◊Plant toxicity test

The following test results were recorded:

◊Initial tensile impact strength, tensile strength, percent elongation and viscosity of all specimens were measured.

◊Loss in weight was carried out at the interval of 6, 8, 10 and 12 weeks.

◊Loss in tensile strength was measured at the interval of 6, 8, 10 and 12weeks.

◊Loss in percent elongation was measured at the interval of 6, 8,10and 12weeks.

◊Loss in viscosity was carried out at the interval of 6, 8, 10 and 12weeks.

◊Weight loss of specimen with individual microorganism was measured after 16 weeks.

◊Percent growth of microorganism on the specimen was observed.

◊Structure of microorganism in the adopted culture was observed.

◊Soil analysis before and after the growth of crop was carried out.

3.2. EXPERIMENTAL WORK:

The experiments were carried out by preparing plasticized specimen, from various conventional polymers used to manufacture carry bags viz. LDPE, HDPE, PS and PP. The specimens were assessed for

strength and viscosity. The specimens were then subjected to microorganisms as per the ASTM standards, to assess the biodegradability of the specimen. The results were recorded as the loss in weight, loss in strength, loss in viscosity with time.

The microorganisms from the specimens showing highest degradability were isolated. There specie and the structure were identified.

The specimen showing the highest degradation, ease of processing, good surface quality and better strength (in comparison to the carry bags made from conventional polymer) were selected for the commercial production. The processing parameters, the energy consumption and the cost of the film were estimated. The estimation was compared with that of the conventional carry bags.

The film prepared on the commercial production basis was assessed for the plant toxicity and the soil analysis was carried out to assess the effect of land fill on the fertility of the soil. Thus the soil burial analysis of the film was carried out to assess the biodegradation of the film.

3.2.1 SPECIMEN PREPARATION:

◊ An intermittent extruder with slit die was fabricated for the manufacture of cast film from LDPE, HDPE, PS and PP.

◊ Cast films from LDPE, HDPE, PS and PP without addition of plasticizer was prepared.

◊ 100 g of LDPE granules were taken. 5 percent by weight of groundnut oil was added.

◊ The mixture was fed to the barrel of intermittent extruder and allowed to melt at 155 °C temperature.

◊ The molten LDPE and groundnut oil mixture was then extruded at constant pressure from the slit die and wound at 12 rpm.

◊ The process steps 3-5 were repeated for preparing five different specimens using varying amount of groundnut oil viz., is 5%, 10%, 15%, 20% and 25% (w/w).

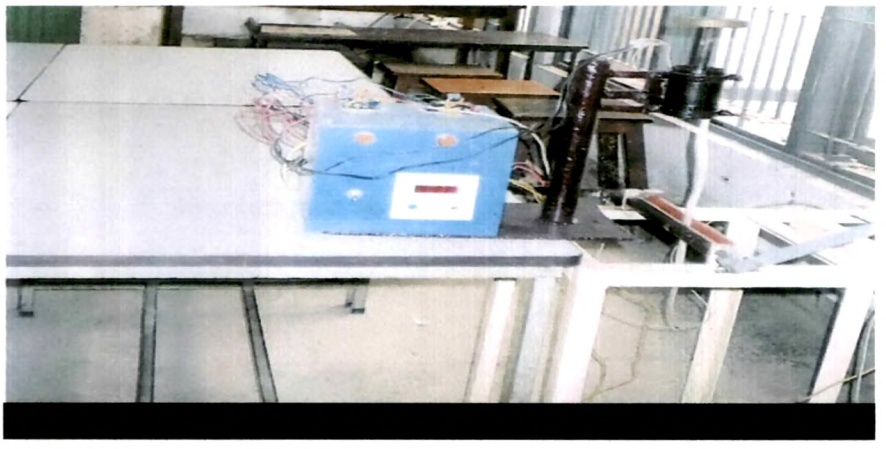
◊Process steps 3-6 were repeated for preparing five different specimen using soybean oil as plasticizer.

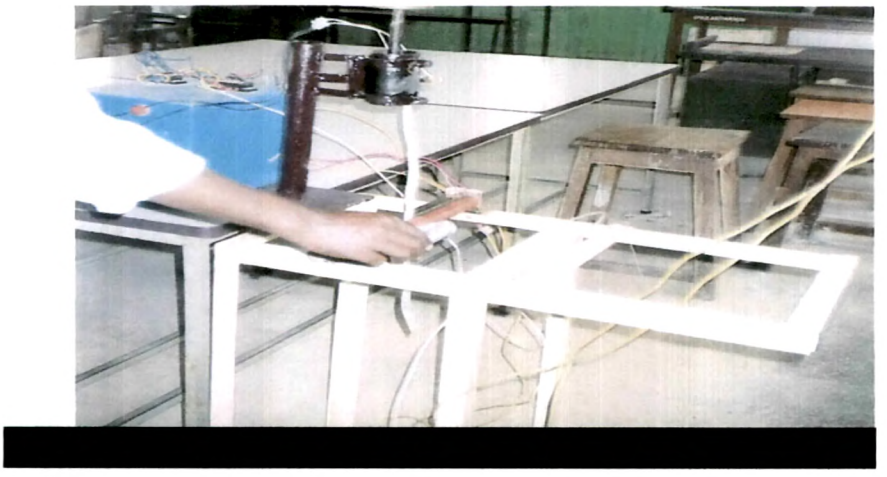
◊Thus one specimen without plasticizer, five specimens with varying amount of groundnut oil and five specimens with varying amount of soybean oil with LDPE as base polymer was prepared.

◊Process 2-8 were repeated to manufacture forty four different specimens using different plasticizer and various polymer viz., LDPE, HDPE, PS and PP. The specimens were given number as per the polymer, plasticizer and concentration of plasticizer.

Photograph no. 3.1

Experimental setup for producing film





3.2.2. SPECIMEN TESTING:

The specimens were tested for:

◊Tensile impact strength

◊Tensile strength

◊Tensile elongation

◊Viscosity

TENSILE STRENGTH, TENSILE IMPACT STRENGTH AND TENSILE ELONGATION:

The test results for the properties 1-3 were obtained from Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388120. Report was produced using NEXYGEN from Lloyd LR 30 K plus UTM.

VISCOSITY:

The solution viscosities of specimens were obtained using Redwood Viscometer. Xylene HPLC grade was used as the solvent.

PROCEDURE:

◊0.1 g of test specimen was dissolved in 10 ml. of solvent (Xylene HPLC grade) at 130°C temperature.

◊ The solution was poured in Redwood Viscometer.

◊ A constant temperature of 130°C was maintained in the viscometer using oil bath.

◊The solution was allowed to flow through the orifice.

◊The time for solution to pass through the orifice was measured using stopwatch.

◊Reduced viscosity was calculated using following equation:

(Vishu Shah 1998)

$$\eta = \frac{\text{Efflux time of solution in second}}{\text{Concentration of Solution in g/ml.}}$$

3.3. TESTING FOR BIODEGRADABILITY:

Forty four specimens prepared as per 3.2.1 were tested for biodegradation. The following test methods were employed.

3.3.1 SCREENING OF BIODEGRADATION OF SPECIMEN TO ASSESS THE PROPERTY LOSS:

PROCESS DESCRIPTION:

◊1 kg. Sewage soil was mixed with polymer powder and plasticizers and was allowed to stay for one week with regular watering so that the microorganism therein gets adopted with the nutrition made available from the polymer and plasticizer.

◊Soil with adopted microorganisms and pure sewage were mixed in equal amount and boiled for 15 minutes and allowed to cool.

◊Cooled mixture was decanted, filtered and mixed with tap water to prepare supernatant.

◊12 g NaCl+18g Peptone+6g sugar was added as nutrient to the supernatant prepared.

◊Long specimen approximately 6cm length was taken, weighed and the weight was recorded as initial weight.

◊Seven chains of 44 specimens each, was prepared by inserting a sample and a plastic bead alternatively in a plastic monofilament.

◊Consortium of five microorganisms: Pseudomonas (Bacteria), Staphylococcus (Bacteria), E- Coli (Bacteria), Aspergillus Niger (Fungi), Rhizopus (Fungi) was used as the medium for degradation.

◊Seven chains were placed in seven different bowls. The bowls were placed in open air under stagnant condition for 16 weeks.

◊During the stagnation period daily water was added in each sample to compensate the water deficiency due to the water consumption by microorganisms and evaporation.

◊After 6weeks, one chain from the first bowl was taken out. The specimens were weighed and the weight loss and percent weight loss was calculated.

◊The specimens were tested for tensile strength and percent elongation. The loss in tensile strength and percent elongation was calculated and recorded.

◊Visual observation for cracks, holes, and breakdown of specimen were carried out periodically.

◊The solution viscosities of specimens were obtained using Redwood Viscometer. Xylene HPLC grade was used as the solvent to measure the solution viscosity using following procedure:

- 0.1 g of test specimen was dissolved in 10 ml. of solvent (Xylene HPLC grade) at 130°C temperature.
- The solution was poured in Redwood Viscometer.
- A constant temperature of 130°C was maintained in the viscometer using oil bath.
- The solution was allowed to flow through the orifice.
- The time for solution to pass through the orifice was measured using stopwatch.
- Reduced viscosity was calculated using following equation (Vishu Shah 1998)
- $\eta = \frac{\text{Efflux time of solution in second}}{\text{Concentration of Solution in g/ml.}}$

◊The loss in viscosity was calculated and recorded.

◊Steps 10-14 were repeated after 8weeks, 10weeks, and 12 weeks.

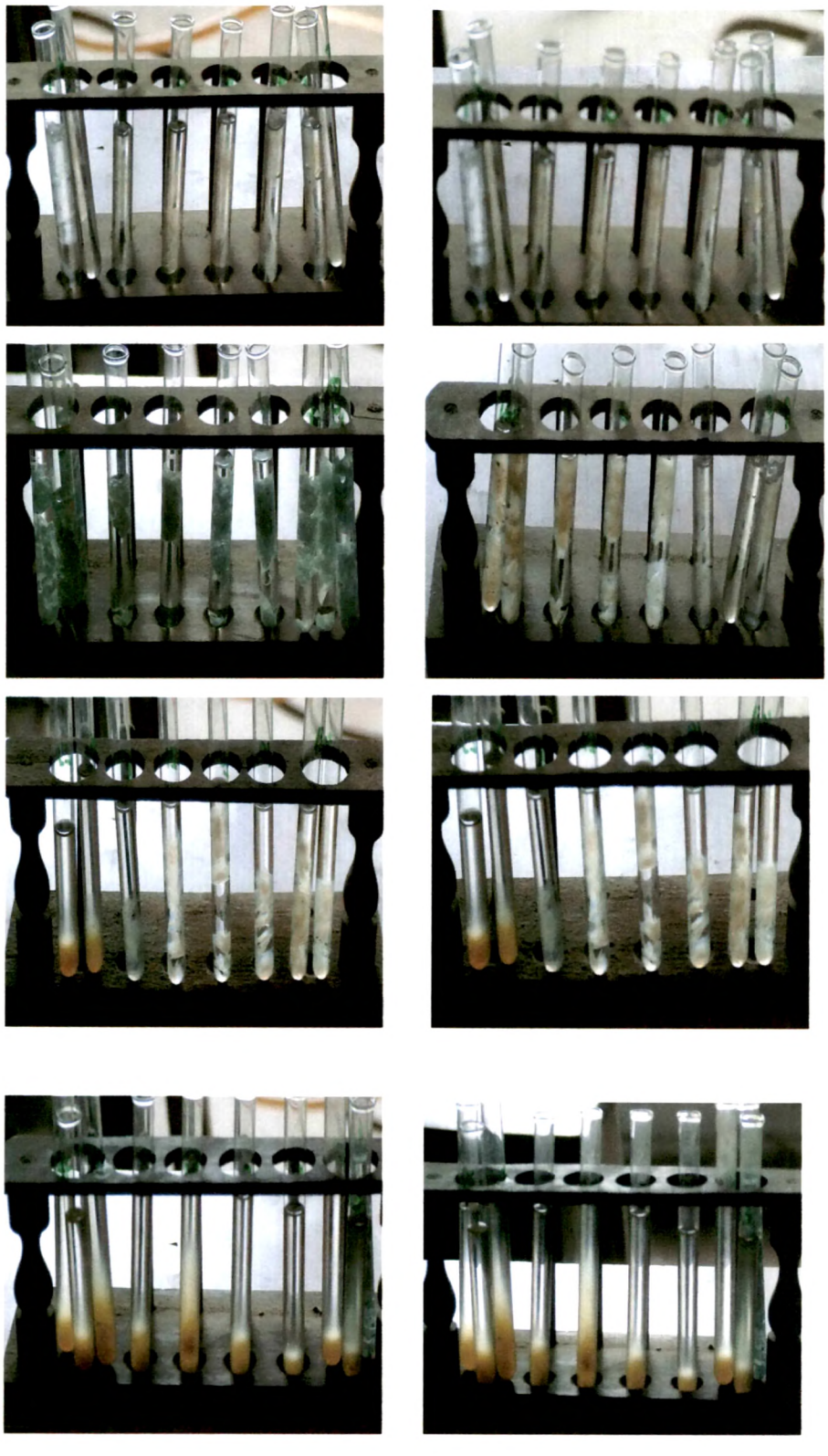
◊After 12 weeks the specimens were broken in to small fragments and since they were tied in a single chain all the specimens were mixed hence further results for property loss were not available.

Photograph no 3.2:

Experimental setup for screening of biodegradation of specimen to assess the property loss

Photograph no. 3.3:

Experimental setup for measuring viscosity of specimen



3.3.2 SCREENING OF BIODEGRADATION OF SPECIMEN IN OPEN AIR:

In the previous test the specimens were fragmented after 12 weeks. To identify the most susceptible microorganism and the corresponding biological system affecting the biodegradability, the specimens were subjected to concentrated cultures of specific microorganism. The following test procedure was employed.

◊Forty four specimens prepared as per 3.2.1 were tested for biodegradation.

◊ These forty four samples were tested for biodegradability with 6 different organisms: Pseudomonas (Bacteria), Staphylococcus (Bacteria), E- Coli (Bacteria), Aspergillus Niger (Fungi), Rhizopus (Fungi) and Consortium - Mixed Culture

◊1 kg. Sewage soil was mixed with polymer powder and plasticizers and was allowed to stay for one week with regular watering so that the microorganism therein gets adopted with the nutrition made available from the polymer and plasticizer.

◊Soil with adopted microorganisms and pure sewage were mixed in equal amount and boiled for 15 minutes and allowed to cool.

◊Cooled mixture was decanted, filtered and mixed with tap water to prepare supernatant.

◊12 g NaCl+18g Peptone+6g sugar was added as nutrient to the supernatant prepared.

◊Concentrated culture of microorganisms was prepared by taking colony of growth from individual microorganism slants.

◊Colony growth from individual microorganism slants was taken into six different containers.

◊Specimens were weighed and weight was recorded as initial weight of the specimen.

◊All 264 specimens were placed in separate container marked with sample number.

◊1/6th part of supernatant was mixed with the culture from 1st container and thoroughly mixed.

◊Approximately 10 ml of the mixed supernatant was poured in first 44 specimens.

◊Steps 9 and 10 were repeated for the remaining five concentrated cultures in the containers.

◊All 264 specimens were subjected to the microorganisms and placed in open air under stagnant condition for 16 weeks.

◊During the stagnation period daily water was added in each sample to compensate the water deficiency due to the water consumption by microorganisms and evaporation.

◊After 16 weeks the specimens were washed and allowed to dry for one day and weighed.

◊Weight loss and percent weight loss was calculated.

◊The specimens were visually observed for physical changes on the surface like holes, cracks, surface finish etc.

◊Medium showing typical color and growth were collected for further experimentation of growth rating and identification of microorganism.

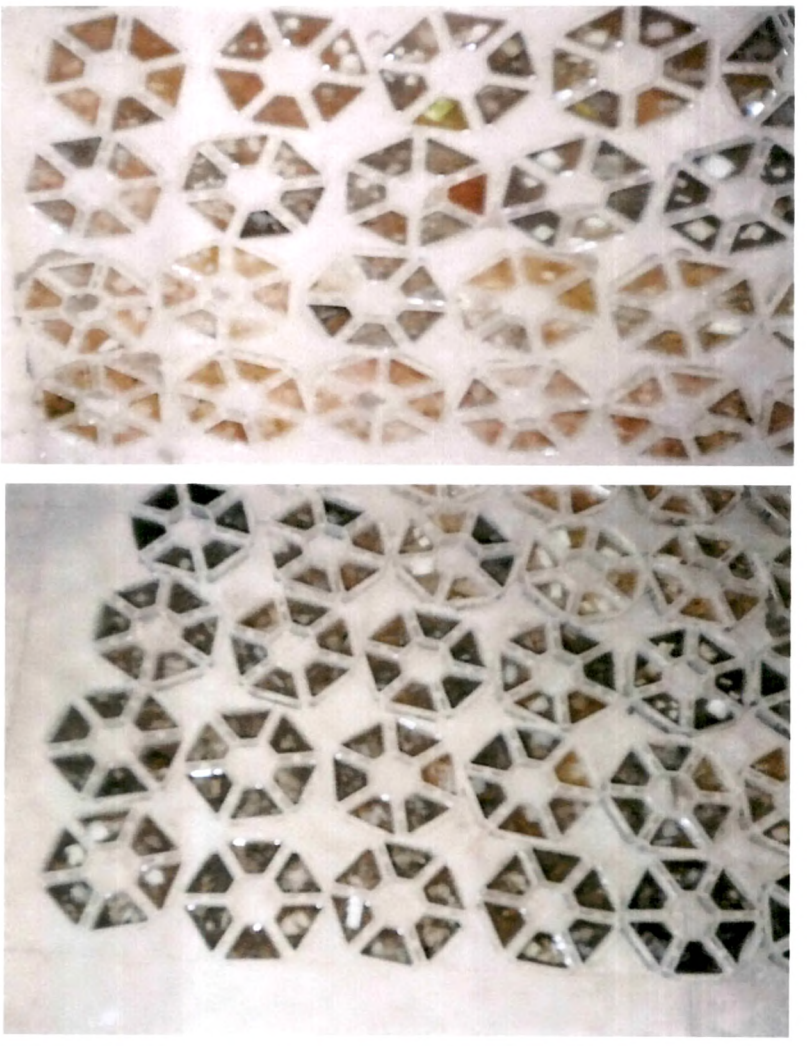
Photograph no. 3. 4:

Concentrated culture of microorganism

Photograph no. 3.5:

Experimental setup for screening of biodegradation of specimen in open air:

264 samples are placed in separate container marked with sample numbers.



3.3.3 GROWTH RATINGS ASTM G – 21

◊Medium from the plastic containers (of the preceding test 3.3.2) with specimen showing typical growth in the screening of biodegradation of specimen under stagnant condition in open air was collected and mixed in a container.

◊Four unmodified specimen and eight specimens with highest plasticizer concentration were selected as reference specimen i.e.

specimen LD00, LDG25, LDS25, HD00, HDG25, HDS25, PS00, PSG25, PSS25 PP00, PPG25 AND PPS25 were selected.

◊Sabouraud dextrose Himedia (M063) agar plates were prepared before night.

◊The selected specimens were dipped in the mixed medium and were placed in 12 different agar plates.

◊The growth of the microorganisms was observed and photographs were taken for reference.

◊After one week and four weeks the samples were observed for the percent growth of the microorganism on the specimens.

3.3.4 STUDY OF THE STRUCTURE OF VARIOUS MICROORGANISMS:

Individual microorganism slants were prepared from the growth in specimen No. 1- LD00, 2- LDG25, 5- HDG25, 9-PSS25, 10- PP00, 11-PPG25 and 12- PPS25 to develop colony of growth from individual microorganism. The structure of the microorganisms were studied and compared with that of the original specie with the help of photographs. The photographs were taken with 100x magnification and 450 x magnifications.

3.3.5 PLANT TOXICITY TEST:

◊From the results of preceding tests for biodegradability, quality of the film and ease of processing LDPE with 25% groundnut oil was considered for the mass production in conventional extruder.

◊The plasticized LDPE films were buried in soil (@ 30 cm. deep)

◊Sewage soil was dumped over the plasticized LDPE film.

◊Fenugreek was seeded in the sewage soil.

◊After 21 days the growth of fenugreek was seen and the photograph of degraded plasticized LDPE film was taken.

◊In the same soil palakh (a kind of leafy vegetable) was seeded over plasticized LDPE films.

◊After 3 months the growth of vegetable was seen and the photograph of degraded plasticized LDPE film was taken.

◊The original soil and the soil after degradation of the plasticized LDPE films was tested for the fertility i.e. soil value was measured.

3.4 FACTORS AFFECTING THE TEST RESULTS:

The ultimate aim of the experiment was to biodegrade the specimen hence following factors that improve the biodegradability were not controlled.

◊The specimens were prepared by intermittent extrusion process where the thickness control was not provided hence the surface to volume ration may vary with the specimen of same concentration.

◊Mixing of plasticizer in the polymer bulk was made manually. Non uniformity in the plasticizer concentration may occur in a specific area of the specimen.

◊Exudation and migration of plasticizer may be uneven especially with the crystalline polymer (HDPE, PS, and PP)

◊The specimens were immersed in supernatant containing the concentrated culture. Moisture absorption by the specimen may be revealed as increase in weight.

◊The specimens were placed in open air so that the environmental factors such as *uv* radiation, temperature variation, oxidation etc. help the biodegradation process.

◊Microorganisms from the environment may also be involved in the process of biodegradation because the experiments were not carried out in a contamination free environment.