A STUDY OF DISPOSAL OF PLASTICS BY BIOLOGICAL METHOD

AKE-ANONG JANGBUA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
(POLYMER SCIENCE)

IN

FACULTY OF GRADUATE STUDIES

MAHIDOL UNIVERSITY

1996

Thesis
entitled

A STUDY OF DISPOSAL OF PLASTICS BY BIOLOGICAL METHOD

Ake-anong Jangbua
Candidate

Krisda Suchiva, Ph.D.
Major Advisor

Apinya Assavanig, Ph.D.
Co-advisor

Sauvarop Bualek, Dr.rer.nat
Co-advisor

Monthree Chulasamaya
M.D., Ph.D.
Dean
Faculty of Graduate Studies

Pranee Phinyocheep
Doctorat de l'Universite' du Maine
Chairman
Master of Science Program
in Polymer Science
Faculty of Science

Thesis
entitled

A STUDY OF DISPOSAL OF PLASTICS BY BIOLOGICAL METHOD

was submitted to the Faculty of Graduate Studies,
Mahidol University for the degree of
Master of Science (Polymer Science)
on
January 12, 1996

Ake-anong Jangbua
Candidate

Krisda Suchiva, Ph.D.
Chairman

Apinya Assavanig, Ph.D.
Member

Taweechai Amornsakchai, Ph.D.
Member

Monthree Chulasamaya
M.D., Ph.D.
Dean
Faculty of Graduate Studies

Pornchai Matangkasombut
M.D., Ph.D.
Dean
Faculty of Science

BIOGRAPHY

NAME	Ake-anong Jangbua
DATE OF BIRTH	January 29, 1971
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	Mahidol University, 1989 Bachelor of Science (Chemistry)
	Mahidol University, 1992 Master of Science (Polymer Science)

ACKNOWLEDGEMENTS

The author would like to express her deepest sincerely gratitude to her supervisor, Dr. Krisda Suchiva, for his kind supervision, valuable guidance and encouragement throughout this work. His kindness will always be remembered. Her appreciation goes to Dr. Apinya Assavanig and Dr. Taweechai Amornsakchai for their valuable comments and suggestions. This thesis cannot be accomplished without their help.

For the cooperation, she would like to thank Dr. Nuth Keerathibuth, Department of Entomology, Faculty of Science, Kasetsart University, who assists in termites field study, Mrs. Supasara Chansri, Department of Biotechnology, Faculty of Science, Mahidol University, who assists in microbiological techniques.

The author also wishes to thank her colleagues who are not mentioned here for their help in various ways.

Finally, she takes this opportunity to express her gratitude to her family for their kindness and encouragement.

Ake-anong Jangbua

ชื่อวิทยานิพนธ์ การศึกษาการกำจัดพลาสติกโดยวิธีการทางชีวภาพ

ผู้วิจัย เอกอนงค์ จางบัว

ปริญญา วิทยาศาสตรมหาบัณฑิต (วิทยาศาสตร์พอลิเมอร์)

คณะกรรมการควบคุมวิทยานิพนธ์

กฤษฎา สุชีวะ, Ph.D.

อภิญญา อัศวนิก, Ph.D.

ทวีชัย อมรศักดิ์ชัย, Ph.D.

วันที่สำเร็จการศึกษา 12 มกราคม พ.ศ. 2539

บทคัดย่อ

งานวิจัยนี้เป็นเป็นการศึกษาการจำกัดพลาสติกโดยกระบวนการทางชีวภาพ โดยศึกษาความสามารถในการย่อยสลายของพลาสติกซึ่งเตรียมจากการผสมแป้งข้าวโพดเข้ากับโพลีเอททิลีน และถุงพลาสติกที่สามารถย่อยสลายได้ซึ่งใช้ในเชิงการค้า โดยนำพลาสติกทั้งสองชนิดนี้มาทดสอบกับเชื้อจุลินทรีย์ ได้แก่ *Bacillus subtilis*(Bs) and *Pseudomonas aeroginosa*(Pa) สำหรับพลาสติกซึ่งเตรียมจากการผสมแป้งข้าวโพดเข้ากับโพลีเอททิลีนนั้น ยังได้นำไปศึกษาความเป็นไปได้ในการกำจัดพลาสติกโดยอาศัยปลวก นอกจากนี้ยังได้ศึกษาการเปลี่ยนแปลงโครงสร้างที่ผิวโดยอาศัยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดและคุณสมบัติในการรับแรงดึงของพลาสติกหลังจากผ่านการทดสอบแล้ว

ผลการทดลองพบว่า *Pseudomonas aeroginosa* เท่านั้นที่สามารถเจริญเติบโตในสภาวะแวดล้อมที่ใช้ทดสอบ กล่าวคือ มีเพียงพลาสติกเป็นแหล่งอาหารเท่านั้น และพลาสติกซึ่งผ่านการทดสอบกับ *Pseudomonas aeroginosa* มีการเปลี่ยนแปลงโครงสร้างที่ผิวของพลาสติก อย่างไรก็ตามได้มีการรายงานว่า *Pseudomonas aeroginosa* ไม่สามารถย่อยสลายคาร์โบไฮเดรทโมเลกุลใหญ่ เช่นแป้ง ได้ ดังนั้นจึงไม่สามารถสรุปได้ว่า แป้งซึ่งเป็นส่วนผสมในพลาสติกย่อยสลายได้ ได้รับการย่อยสลายโดย Pa ส่วนคุณสมบัติในการรับแรงดึงของพลาสติกหลังจากผ่านการทดสอบแล้วไม่เปลี่ยนแปลงมากนัก

สำหรับผลการทดลองภาคสนามของการศึกษาการกำจัดพลาสติกโดยอาศัยปลวกพบว่า พลาสติกบางส่วนสูญหายไปกล่าวคือ พลาสติกซึ่งมีแป้งเป็นส่วนประกอบ 25%โดยน้ำหนัก พบว่า พลาสติกถูกกัดกินไป 11.7%โดยน้ำหนัก หลังจากผ่านการทดสอบภาคสนามเป็นเวลา 69 วัน, พลาสติกซึ่งมีแป้งเป็นส่วนประกอบ 50%โดยน้ำหนัก พบว่าพลาสติกถูกกัดกินไป 21.5%โดยน้ำหนัก หลังจากผ่านการทดสอบภาคสนามเป็นเวลา 224 วัน และ พลาสติกซึ่งมีแป้งเป็นส่วนประกอบ 75%โดยน้ำหนัก พบว่าพลาสติกถูกกัดกินไป 13%โดยน้ำหนัก หลังจากผ่านการทดสอบภาคสนามเป็นเวลา 175 วัน

จากผลการทดลอง ชี้ให้เห็นว่ามีความเป็นไปได้ในการกำจัดพลาสติกโดยอาศัยปลวก ทั้งนี้ควรทำการศึกษาเพิ่มเติมเพื่อให้ได้ข้อมูลมากขึ้น

Thesis Title A Study of Disposal of Plastics by Biological Method

Name Ake-anong Jangbua

Degree Master of Science (Polymer Science)

Thesis Supervisory Committee

Krisada Suchiva, Ph.D.

Apinya Assvanig, Ph.D.

Taweechai Amornsakchai, Ph.D.

Date of Graduation 12 January B.E.2539(1996)

ABSTRACT

The present work involved study of biodegradation of starch-plastics blends and commercially-established type of biodegradable plastics, by the action of microorganisms, namely *Bacillus subtilis*(Bs) and *Pseudomonas aeroginosa*(Pa), and studies of degradation of starch-plastics blends by the action of termites. Surface morphology and tensile properties changes of the material subjected to the test were also studied. Evidences of surface morphology changes were obtained from scanning electron microscopic study of the specimens. It was also found that Pa could grow in the liquid medium which contained starch-plastics blends as nutrient source, whereas Bs could not. However, Pa has been reported to be incapable of hydrolysing of starch. Therefore, it was not

clear whether Pa could actually cause biodegradation of starch-plastics blends.

For the studies of action of termite on starch-plastics blends, starch-plastics blends having different starch contents (25%, 50%, and 75% by weight) were prepared and subjected to field test. Significant losses of plastics mass were observed. For 25% starch content, the weight loss was 11.7% after 69 days; for 50% starch content, the weight loss was 21.5% after 224 days and for 75% starch content, the weight loss was 13% after 175 days. The results obtained indicated that disposal of plastic waste by the action of termites might be feasible and further study might be worth undertaken.

TABLE OF CONTENTS

	PAGE
Biography	I
Acknowledgments	II
Abstract in Thai	III
Abstract in English	V
Table of contents	VII
List of Tables	X
List of Figures	XI
CHAPTER I INTRODUCTION	
1.1 Introduction	1
1.2 Method of plastics waste treatment	5
1.2.1 Landfilling	5
1.2.2 Incineration	5
1.2.3 Pyrolysis	6
1.2.4 Chemical conversion	8
1.2.5 Recycling	8
1.2.6 Degradable plastics	9
1.3 Biodegradation of plastics	13
1.3.1 Defining biodegradation	13
1.3.2 Inherently and partially biodegradable polymer	15
1.3.3 Type of biodegradable materials	15
1.3.4 Polyethylene-starch plastics	17

1.3.5 Studies of biodegradation 21
1.3.5.1 Factors influencing the biodegradability of synthetic polymers 23
1.3.5.2 Test methods 26
1.3.6 Mechanism of biodegradation of polymer-starch system 29
1.4 Macrobiological degradation 34
1.4.1 Factors influencing the damages of materials by termites 35
1.4.1.1 Physical factors 35
1.4.1.2 Chemical factors 36
1.4.1.3 Biological factors 36
1.5 Scope of the thesis 39
CHAPTER II EXPERIMENTAL
2.1 Chemicals and materials 40
2.2 Bacteria 41
2.3 Preparation of PE-starch films 41
2.4 Preparation of media for bacterial cells 43
2.4.1 Preparation of nutrient agar slant and nutrient broth 43
2.4.2 Preparation of nutrient-salt media 43
2.5 Preparation of bacterial cell suspension 45
2.6 Test procedures 45
2.6.1 Biological studies 45
2.6.2 Physical studies 48

2.7 Studies of biodeterioration of starch-filled polyethylene sheet by termites 48

2.8 Characterisation of commercial degradable plastics packaging films using Fourier Transform Infrared(FTIR) and Thermogravimetric Analysis(TGA) Techniques 50

CHAPTER III RESULTS AND DISCUSSION

3.1 Preparation of PE-starch blends 51

3.2 Studies of microbial degradation of PE-starch blends 54

3.3 Studies of biodegradation commercial degradable plastics 59

3.4 Studies of disposal of PE-starch blends by termites 65

CHAPTER IV CONCLUSION 76

Bibliography 78

LIST OF TABLES

TABLES		PAGE
1.1	1984 Plastics production and projected waste generation (in the United States)	2
1.2	1984 Plastics waste generation classified by application (in the United States)	2
1.3	Composition of plastics in municipal waste (wt%)	4
1.4	Energy equivalent value and combustion ethalpy of some common plastics	6
1.5	Principle products of pyrolysis of domestic plastics waste (wt%)	7
3.1	Control experiment for Procedures A, B and C	55
3.2	Results of biodegradation test experiments after 55 days of incubation	55

LIST OF FIGURES

FIGURES		PAGE
1.1	Plastics in packaging (by type)	3
1.2	Plastics in industrial, institutional and consumer packaging (by application)	3
1.3	Method for producing plastics containning granular starch	19
1.4	Method for producing plastics composites containing gelatinised starch	20
1.5	Postulated mechanism of degradation by microorganism	31
1.6	Diffusion path for starch-plastics films immersed in water	32
1.7	Diffusion path for starch-plastic films immersed in water	32
1.8	Influence of film morphology on diffusion paths in starch-plastics film	33
2.1	Tensile specimen	47
2.2	Test specimen placed in used-beverage can subjected to macroorganism exposure	50
3.1	SEM micrographs of PE-starch blend (50% starch) (magnification x150)	53

3.2 SEM micrograph of control specimen containing 50% starch incubated at 37°C for 60 days without microorganism in nutrient-salt liquid media (magnification x150) 57

3.3 SEM micrograph of specimen containing 50% starch incubated at 37°C for 60 days without microorganism in nutrient-salt liquid media (magnification x150) 58

3.4 FTIR spectra of reference bag and commercial UV&bio-degradable bag 59

3.5 SEM micrograph of UV&bio-degradable specimens before subjected to the test (magnification x100) 62

3.6 SEM micrograph of the control specimen incubated at 37 °C for 60 days without microorganism in nutrient-salt liquid media (magnification x100) 62

3.7 SEM micrograph of the UV&biodegradable specimen incubated at 37 °C for 60days with Pa in nutrient-salt liquid media (magnification x100) 64

3.8 Modulus at 300% eloongation of control specimens and specimens exposed to Pa 66

3.9 Tensile strength of control specimens and specimens exposed to Pa 67

3.10 Percentage elongation at break of control specimens and specimens exposed to Pa 67

3.11 Specimens recovered from termites field studies 70

3.12 Specimens placed in the beverage-can after recovered from the field study shows formation of fungi and colouration on the sample surface 72

3.13 Specimens placed in the beverage-can after recovered from the field study shows the routes which termites made to their food source. 73

3.14 Specimen recovered from the field study without cleaning 74

Chapter I
Introduction

1.1 Introduction

Today plastics are common materials in everyday life and commerce. They have become ubiquity in use because of their low bulk density, light weight, wide-ranging, performance and favourable cost. While the growth in the use of plastics material is accelerated due to their advantageous properties and values, the plastics materials are threatened by their visible impact on the environment. Thus, their widespread uses regularly receive caution from a number of public domain.

Plastics disposal is regarded to be one of the problems in waste treatment. The main feature is that plastics are not degradable in nature or if they really are, the time lag is usually long, typically 15-20 years or more.

The available data on plastics consumption given in Tables 1.1, 1.2, and 1.3, although not updated, provide perspective to the plastics waste problem.

Table 1.1 1984 Plastics production and projected waste generation (in the United States) [1].

Type of plastics	Production ($\times 10^6$tonnes)	Discarded (wt%/year)	Plastics Waste ($\times 10^6$tonnes)	Total (%)
Polyethylene	6.00	65.6	3.94	65.3
Polystyrene[a]	2.69	38.1	1.03	17.1
Polypropylene	1.86	27.5	0.51	8.5
Poly(vinylchloride)	3.05	17.9	0.55	9.1

[a] Includes PS, ABS, and other styrene copolymers

Table 1.2. 1984 Plastic waste generation classified by application (in the United States) [1].

Use	Approximate Life (in years)	Discarded (%/year)	1984 Use ($\times 10^6$tonnes)	Estimated Waste ($\times 10^6$tonnes)
Packaging	1	100	5.62	5.62
Transportation	5	20	0.96	0.19
Furniture and housewares	10	10	0.96	0.10
Electrical and electronic	10	10	1.25	0.13
Building and construction	50	2	4.39	0.09
Total				6.13

In 1986 in the United States, 11.5 billion pounds used in packaging consisted of the following plastics, as shown in Figure 1.1 [2].

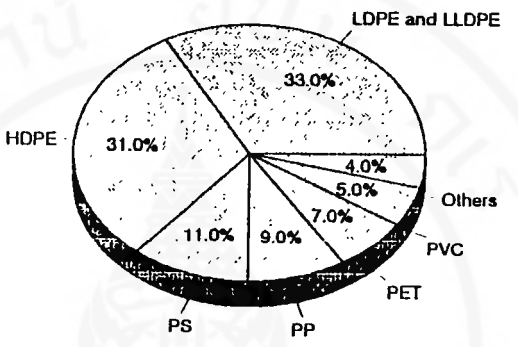


Figure 1.1 Plastics in packaging (by type)

These plastics were used in the following industrial, institutional, and consumer packaging application, as shown in Figure 1.2 [2].

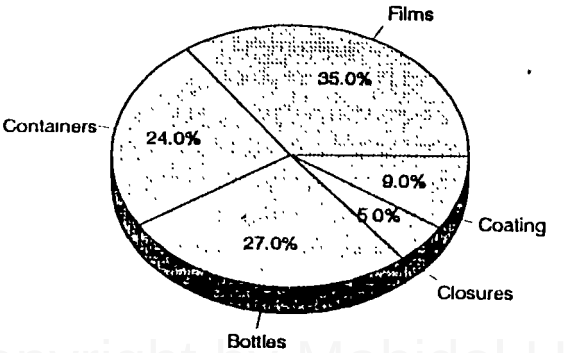


Figure 1.2 Plastics in industrial, institutional and consumer packaging (by application)

The composition of plastics in municipal wastes, as reported for several countries , is given in Table 1.3 [1].

Table 1.3 Composition of plastics[a] in municipal waste (wt%)

Country	Year	PO[a]	PS[a]	PVC[a]
Great Britain	1970	66.2	19.8	8.2
Germany	1983	70.2	15.3	11.7
Japan	1985	57.3	25,9	13.8
United States	1970	55	20	11
United States	1973	74	19	5

[a] PO = polyolefins, PS = polystyrene, PVC = poly (vinylchloride)

Note:These data are likely to have changed since the dates of the report because of shifts in packaging technology and consumer habits.

In Thailand, the problem of plastics waste is also likely to increase. At present, the disposal technology is growing very slowly compared to other countries. Landfilling and recycling appear to be the two major methods use presently. In Great Britain, landfilling has accounted for more than 85% of the total waste generated, whilst the remaining waste is incinerated. In most other European countries incineration accounts for between 25% to 40% of waste disposal. In the United States incineration has started to play a more significant role in waste management practice, particularly in some Eastern Seaboard States, where landfill capacity is no longer

available. The situation in Japan, where land is at a premium, has seen a marked increase in the use of incineration [3].

1.2 Methods of plastics waste treatment

1.2.1 Landfilling

While the volume to weight ratio of plastics provides a distinct advantage for their use in packaging , it also proves to be a disadvantage for disposal via landfilling because the number of landfill sites are diminishing [4]. However, landfill will still continue to be the major disposal option since other methods of plastics waste treatment are less efficient with respect to the volume of plastics waste they are capable of disposing.

1.2.2 Incineration

Incineration is the controlled combustion of waste. It is being developed as a mean of utilizing the energy content of plastics as a fuel. Energy can be generated by using waste plastics as a source of fuel or chemicals, or conserved by substituting plastics for materials which are more energy intensive in their construction or use [1,3].

The energy equivalent values of common plastics and their combustion enthalpies are given in Table 1.4 [1].

Table 1.4. Energy equivalent value and combustion enthalpy of some common plastics.

Plastics	Energy equivalent (MJ/kg)[a]	Combustion enthalpy (MJ/kg)[a]
Polyethylene	69-72	43
Polypropylene	73	44
Polystyrene	80	40
Poly(acrylonitrile-co-styrene) (SAN)	84	87
Poly(vinylchloride)	53	18
Nylon(6) or (6,6)	154-156	29
poly(ethylene terephthalate)	84	31
Polycarbonate	107	29

[a] To convert MJ/kg to Btu/lb. , multiply by 431.2

1.2.3 Pyrolysis

The basic process of pyrolysis involves heating either mixed or single plastics materials in a controlled atmosphere (i.e. in an oxygen free environment) and thereby recovered by distillation, oil, gas and various chemicals

by-products [3,4,5]. Pyrolysis of PE between 660 and 810 °C produces H_2, CH_4, C-2 and C-3 hydrocarbons, and cyclic and aromatics hydrocarbons. Pyrolysed waste plastics from a sorting plant, containing ca 68 wt% PE, less than 3 wt% other plastics, and the remainder paper, metal and dirt, give the product mixture shown in Table 1.5 [1].

Table 1.5. Principal products of pyrolysis of domestic plastics waste (wt%)

Product	Pyrolysis temperature	
	630 °C	790 °C
Methane	13.57	16.50
Ethane	5.37	3.42
Ethylene	11.15	11.37
Propylene	4.49	1.80
Carbon dioxide	2.03	2.93
Carbon monoxide	3.22	3.92
Hydrogen	0.59	0.63
Benzene	9.83	12.37
Toluene	2.46	3.76
Naphthalene	1.07	2.39
Water	5.62	4.59
Tars	12.96	13.92
Other solid	16.90	11.49
Other products	10.74	10.91

1.2.4 Chemical conversion

The process of chemical conversion involves the use of methanolysis, glycolysis and hydrolysis by condensation reactions that depolymerised polymers into their respective monomers [3]. For example, recovery of monomers from PET waste can be achieved by high pressure methanolysis giving pure dimethyl terepthalate and ethylene glycol. Glycolysis and hydrolysis of PET waste are also available but the purification of monomers are difficult. Another example is polyurethane foam used in automobile seats, it can be recovered by hydrolysis in an extruder. The products recovered depend on the polyester or polyether used to make the foam [1].

1.2.5 Recycling

Recycling of plastics materials from consumer solid wastes is often impeded by the collection of plastics and the need to separate individual plastics. In recycling technology, the methods used for reprocessing plastics are well known and generally involve melt extrusion technology and have been used for a number of years [3,6].

1.2.6 Degradable plastics

Many plastics products are not suitable for recovery and recycling, such as short-life packaging films, containers, and agricultural plastics waste. These products have caused the litter problems in the environment. Another alternative to ease such problems is to promote plastics with accelerated environmental degradation.

Degradable plastics are plastics which undergo significant change in its chemical structure under specific environmental condition resulting in losses of some properties [7].

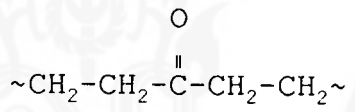
There are many types of degradable plastics; those which are broken down by exposure to sunlight (photodegradable) or by the action of microorganism (biodegradation) or by combination of both systems [8]. Biodegradation can lead to complete removal of polymers from environment, whereas photodegradation are fragmentation processes leading to reduced molecular weight, and small particles that either are biodegraded or remain in the environment [9].

(i) Photodegradable plastics

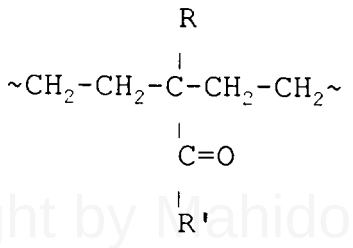
Photodegradable plastics undergo photooxidative degradation upon exposure to sunlight. Catalysts are normally used to accelerate the breaking of the polymer

chain by a series of UV-initiated free radical reactions [9].

The technology of photodegradation can be divided into two basic categories [10,11,12]. The first category is named copolymer technology, i.e. polymerizing carbon monoxide or ketone functional groups with ethylene, styrene or propylene monomers. Several companies in the USA - DuPont, Union Carbide and Dow - are producing specialty copolymers of polyethylene with carbon monoxide. Its structure is as follow:



The polymer is often known as E-CO polymer. Another commercial product employing copolymer technology is sold as Ecolyte, marketed by Eco Plastics Ltd. In this case, the polymer is modified by incorporating ketone groups into polymer chain. With polyethylene, the structure would be:



where R and R' are alkyl or aryl substituents which can be modified to control the rate of degradation. The

copolymers, on absorption of UV-light, undergo chain cleavage adjacent to the >C=O group, leading to a reduction in molecular weight, a loss in mechanical properties, and eventual disintegration. The degradation process is by Norrish type I and II reaction [10,13,14].

The other category is to add a small level of various photodegradable additives to conventional thermoplastics. The additives act as catalysts, sensitise the polymer to UV degradation. The most commonly used additives is a dual system of organometallic compounds with transition metals, for example Cu, Mn, Co, Fe, Cr or Ni. The use of organometallic complexes like ferric dibutyl dithiocarbamate has been patented by Scott [12,14]. And in conjunction with Gilead, Scott has developed a two-component system of certain metal salts to give more effective control of photodegradation process known as antioxidant-photoactivators under the tradename of Plastigone, which involves a minimum of two metal complex additive. At high concentrations, these salts stabilise polyolefins by decomposing peroxides, but at lower concentrations they catalyse photodegradation. The antioxidant-photoactivator in Plastigone is an iron complex, used together with at least one nickel or cobalt complex to act as a metal ion deactivating compound and also a photostabiliser. Relative concentrations are controlled such that after a predetermined induction period, the complexes break up, providing metal ions which

catalyse decomposition by Norrish types I and II reactions [10,15]. The fundamentals of Scott-Gilead system were fully described in the published patents [16].

The advantages and disadvantages of these photodegrable technologies depending on the particular application can be noted [11]. For the copolymer technology, degradation end point will be more programmable because its oxidation potential can be engineered into the polymer through the concentration of monomer in the synthetic plastics. However, its reaction virtually stops as the UV radiation is removed. On the contrary, the use of metal salt compounds which works on utilizing the oxidative degradation mechanism, degradation continues even if the UV radiation source is removed.

(ii) Biodegradable plastics

The primary degradation mechanism of biodegradable plastics is through the action of microorganisms such as bacteria, moulds, alga, yeasts [12]. It is a biochemical transformation of compounds by microorganism. Ultimate biodegradation is a complete loss of its structure as an effect of rapid decrease in molecular weight, resulting in mineralisation or incorporation into microbial biomass [17,18].

To function in the environment as an effective solution for the disposal crisis, both photodegradation and biodegradation must work synergistically towards

degradation or deterioration of polymeric materials upon exposure. That is as photodegradation triggers at the polymer chains, their structures are weakened, resulting in bonds breaking and increasing chain ends. Assimilation of carbon source from polymer chains by microorganism can then occur. Meanwhile, biodegradable part in the materials are biodegraded by living organism present in the environment. The embrittlement of the polymers allows susceptibility of environmental degradation, thus enhance degradation rate of the materials.

1.3 Biodegradation of plastics

1.3.1 Defining biodegradation

Several attempts have been made to define the terms "degradation" and "biodegradation" within the context of environmental application [18,23,24,25,26].

The term "degradation" is used in a broad sense in polymer chemistry to mean a change in properties of a polymer due to changes in the chemical structure which brought about by environmental factors such as light, heat, water, or oxidative reactions. Degradation involves chain scission or extensive crosslinking, resulting in loss of mechanical integrity of the polymer ensures in its disintegration, fragmentation or embrittlement.

The term "biodegradation" indicates a degradation process brought about by living organism during environmental exposure. It generally involves a biochemical transformation of compound by microorganism, which causes chain scission of polymer, a change in molecular weight distribution, and a change in degree of polymerisation. Biodegradation of a material results in mineralisation or incorporation into microbial biomass. Mineralisation of organic compounds will yield carbon dioxide and water under aerobic conditions and methane and carbon dioxide under anaerobic condition. Chemical hydrolysis, photooxidation or physical disintegration can enhance subsequent biodegradation of polymer by increasing surface area for microbial colonisation or by reducing molecular weight.

There are usually misconception between biodegradation and biodeterioration. The distinction between the two terms is important. Whereas biodegradation results in both chemical and physical changes in polymer as mentioned above, biodeterioration is the decay of materials caused by living organisms (such as fungi, bacteria, insects), and environmental effects, (such as water, radiation, or mechanical forces). Biodeterioration results in only physical changes of the polymer. Such physical changes lead to a loss of mechanical integrity of a material, result in a fragmentation of polymer material. Its process does not involve biochemical transformation of

compound by microorganism into biomass as the process of biodegradation does.

1.3.2 Inherently and partially biodegradable polymers

A polymer is often considered to be biodegradable if the loss of its properties is due, at least in part, to living organism. Actually, a polymer should be labeled as "biodegradable" only if it is completely assimilated by microorganisms [26]. Thus, the polymers which are said to be inherently biodegradable are polymers from natural sources, such as cellulose and starch; or polymers synthesised by living organism such as bacterial polyester such as poly(hydroxybutyrate) (PHB). Polymers which are prepared on the principle of polymer composites (e.g. polyolefins blends with starch); introducing weak links into inert polymer to make them easier to hydrolyse; and introducing pro-oxidants in order to enhance degradation; should be considered as partially biodegradable plastics [18,27].

1.3.3 Type of biodegradable materials

At present there are three main classes of biodegradable materials corresponding to the three stages of development.

(i) Blend of polymers with natural food source

The first class of biodegradable plastics is consisted of the blend of a polymer with natural food source such as starch which can be rapidly consumed by microorganism. The classic example is the use of starch blends with polyethylene for the manufacture of biodegradable bags [28]. These blends will disintegrate when the starch has been biodegraded away, leaving essentially undegraded, but fragmented polyethylene. There is some spectroscopic evidence that polyethylene itself can be oxidized in natural environment [29], but it has never been shown to biodegrade fully in high molecular weight form.

Two technologies exist for production of PE-starch blend. One is the incorporating of granular starch into plastics, and the other is the incorporation of gelatinized starch. The details of these two technologies are giving in Section 1.3.4

(ii) Introduction of weak links to the polymer chains

The second class concentrates on the insertion of functional groups such as ester linkage or amide groups on the polymeric backbone, such as polycaprolactone, polyamides and polyurethane. These synthetic polymers are vulnerable groups susceptible to hydrolysis, photolysis or

oxidation. The resultant polymer fragments are lower in molecular weight and may be easily metabolised [17].

(iii)Naturally synthesized polymer

The third class is the development of naturally occurring processible polymers, such as poly (hydroxybutyrate)(PHB) [17,18]. This material is synthesised by bacteria as a carbon storage chemical decomposite inside certain bacteria during fermentation in an environment deficient in an essential nutrient like nitrogen, phosphorus, sulfur or oxygen [30]. Since these polymers are naturally occurring, being attacked by a wide variety of bacteria, they are truly biodegradable. Possible application of these materials are in medical, packaging and agricultural fields.

1.3.4 Polyethylene-starch plastics [31,32]

Starch is an inherently biodegradable polymer because it can be readily metabolized by a wide array of organisms. Polyethylene, on the other hand, is not readily metabolized by living organisms, and so must be considered to be essentially non-biodegradable. Plastic formulations containing both starch and polyethylene should, therefore, be considered as partially biodegradable plastics. Microbial removal of starch from starch-plastics composites can cause severe reduction in the mechanical

strength of the remaining, non-biodegradable portion, so that the manufactured plastics product disintegrates readily into smaller pieces.

If appropriate chemical catalysts (e.g. metal ions) are present, the non-biodegradable polymers can be chemically oxidized to lower molecular weight compounds, some or all of which may be further metabolisable by living organisms.

There are two different technologies for the production of starch-plastics composites:

(i) Granular starch

Use of granular starch as a filler in plastics began with the work of Griffin in the 1970's [33,34,35]. He developed a process for incorporating granular starch particles into plastics films (Figure 1.3) [31]. Since whole starch granules are used in this technology, the level of starch addition is generally limited to about 10% or less, by weight.

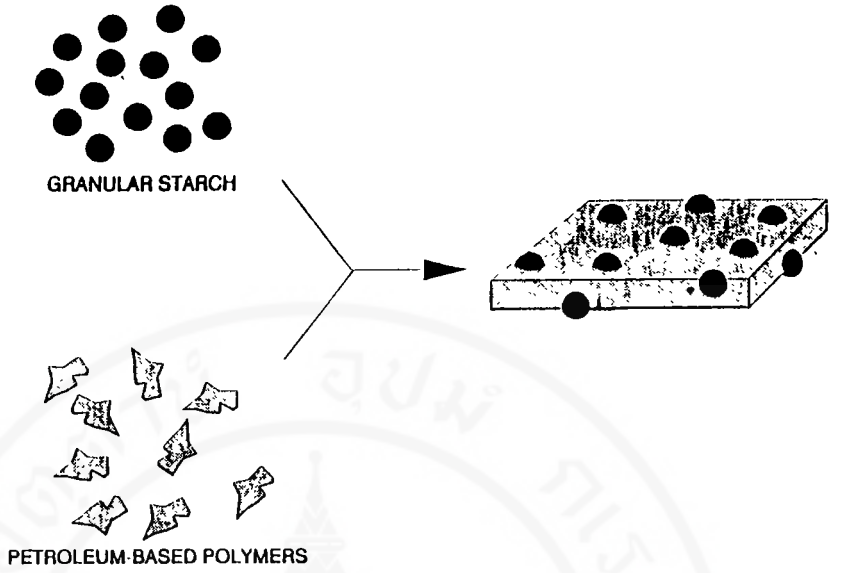


Figure 1.3 Method for producing plastics containing granular starch.

Starch granules have also been surface-treated (for example with silanes) to increase the compatibility of hydrophilic starch with hydrophobic plastics matrix. Pro-oxidant can also be added to promote degradation of the synthetic polymer.

(ii) Gelatinised starch

Gelatinisation is the disruption of molecular order within the granular structure leading to granule swelling, hydration and solubilisation of starch molecules. The processes are effected by heating granules that are slurred in water or other solvents, such as liquid ammonia [36,37].

Otey [31] developed a process for incorporating gelatinised starch into plastics films, yielding a more

uniform distribution of starch molecules throughout the starch-plastics matrix (Figure 1.4). Films produced with this technology typically contain 20-50% starch by weight.

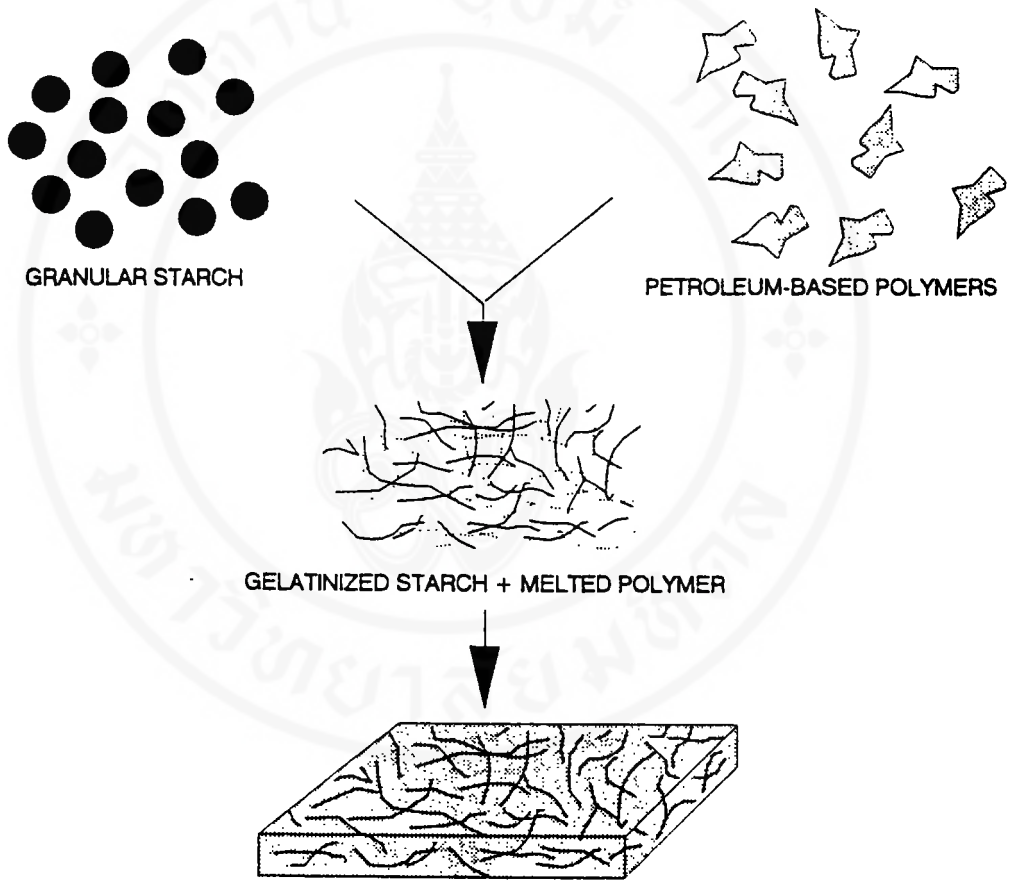


Figure 1.4 Method for producing plastics composites containing gelatinised starch

1.3.5 Studies of biodegradation

Degradation of polymer can be proceeded by direct and indirect biodegradation. Direct biodegradation involves a direct enzymatic scission of the polymer chains which may be followed by metabolisation of the cleavage products, or an enzymatic assimilation starting from the chain ends. Indirect biodegradation is oxidative cleavage of the macromolecule followed by metabolisation of the fragment [19].

To study biodegradation, there are actually two elements in biodegradability testing. The first is incubation in environment to allow living organism to attack polymer. The second is the measurement of the degree of degradation. Measurement of both chemical changes and performance properties are desirable [20].

The evidence for the breakdown of synthetic polymers in the natural environment under the influence of living organism can be studied in both field studies and laboratory testing.

(i) Field studies

Field studies involving soil burial are traditionally used because of the similarity to actual conditions of the use or disposal. At the end of exposure periods, changes in properties such as weight loss and mechanical strength are studied. Soil burial tests usually

require long time and have the problem of reproducibility of results due to the difficulty in controlling environmental condition and the microbial population.

(ii) Laboratory testing

Laboratory testing permits greater experimental control but at the expense of duplicating the actual exposure conditions. Biodegradation is studied by determining

- the production of CO_2,
- the consumption of O_2,
- the loss of weight,
- the increase in cell count or cell mass of the microorganism (if the polymer is the sole source of carbon),and
- chemical and physical examination of the sample for evidence of sample destruction.

The strategy of testing is to use the right organisms with the right conditions [21,22,23].

However, there are no universal test methods for determining biodegradability of polymers since there can be so many variables in the testing that the results cannot be used for comparative purposes. Degradation properties among different polymers can be determined only if they are studied by using the commonly available

methods such as physical, chemical and mechanical changes during and after exposure.

1.3.5.1 Factors influencing the biodegradability of synthetic polymers [40,41]

(i) Structural factors

Hydrophobicity is often regarded as a major obstacle to microbial attack on polymers. Thus, the following factors related to the nature of the polymers have important effects on biodegradability of the polymers.

(a) Steric hindrance

Since chemical structure influences the rate and extent of biodegradation, steric hindrance of polymer molecules, which is structural effects arise from spatial interactions between substituent groups, prevents the degradation of the polymer chains. Molecular weight is also another important factor. High molecular weight polyethylene are extremely durable, but low molecular weight samples (<500) are susceptible to degradation.

(b) Functional Groups

Functional groups like $-NH_2$, -COOH, -OH, and -NCO can improve the hydrophilicity of a polymer, thus making it more attractive habitat for microbes. The degree of susceptibility of some polymers to biodegradation

improves when the backbone contains both water-soluble and water-insoluble segments.

(c) Branching

Branching affects biodegradability adversely. Highly branched chains with an effective molecular weight of less than 400 have been found to be bioresistant.

(d) Crosslinking

Crosslinking restricts the mobility of polymer segments and consequently the accessibility of enzymes to susceptible point, thus leading to a decrease in biodegradability.

(ii) Physical factors

(a) Surface characteristics

It is oftenly found that rough surfaces are more susceptible to microbial attack than smoother surfaces. Perhaps the pits and crevices help retain moisture and thus promote microbial growth.

(b) Physical and morphological states

Physical and morphological states particularly whether it is crystalline or amorphous are important for biodegradability. In the case of partially crystalline polymers, amorphous regions are biodegraded preferably.

(iii) Environmental factors

(a) Temperature

Microbes need optimum temperatures for growth. At very high or very low temperatures, they are destroyed. Generally, fungi need temperature range of 20-28 °C and bacteria prefer 28-37 °C.

(b) pH

Each microorganism grows in specific pH ranges. Fungi can tolerate acidic pH but bacteria favour slightly basic pH.

(c) Water and humidity

Water is essential for the growth of microbes. Bacteria and fungi need humid habitat. For example, wood is attacked by fungi only when the moisture content is more than 20%.

(d) Oxygen

The availability of oxygen is an important factor for the growth of microbes. While fungi are generally aerobic, bacteria can be aerobic or anaerobic .

The following environmental factors are also relevant,

- the presence of other nutrients for growth of microorganism;
- microbial population, type and interactions of fungi and bacteria;

- surface-to-volume ratio, sample size and purity of the polymer sample;
- duration of the test;
- method of contact between the sample and the microorganism (whether in solution, or in gel or slurry, or in soil)

1.3.5.2 Test methods [25,42]

There are several test methods for assessment of biodegradability. These test methods can be divided into 4 main groups as follows.

(i) Monitoring biomass accumulation

The term "biomass" means growth and reproduction of microorganism. It involves the utilization of an organic substrate by microorganism. A significant part of the substrate, particularly carbon substrate, is used resulting in increase biomass. There are a number of practical techniques included, i.e.

- visual inspection;
- turbidity measurement;
- plate-count and direct counts;
- mineralisation of biomass;
- chemical techniques.

(ii) Monitoring depletion of reactants

The term "reactants" is defined to include not only the substrate but also oxygen associated in the assimilation of degradation products. Monitoring the loss of substrate during biodegradation by weight loss measurements is the simplest but the least accurate means. Specific analytical technique designed to quantitatively determine residual substrate level provides significant information. By measuring DOC (dissolved organic carbon) in the reaction medium and BOD (biochemical oxygen demand) caused by increase biomass production, indirect information on the end of the biodegradation process and total mineralisation of substrate are given.

(iii)Monitoring the reaction products

Ultimate or complete biodegradation of a molecule requires that the organic carbon trapped in its structure be mineralized into carbon dioxide, i.e.

$$\text{organic C,H,O} \dashrightarrow CO_2 + H_2O$$

Monitoring carbon dioxide thus provides direct reliable information on complete biodegradation.

(iv) Monitoring changes in properties of substrates

Biodegradation of a polymer substrate is usually associated with changes in its physical, chemical, and mechanical properties. These properties might be measured as a function of the duration of exposure to a biotic medium. Properties such as tensile strength, impact resistance, tear strength, and weight loss of the polymer are good indicators of property loss which is an indirect measurement of biodegradation. The extent of biodegradation claimed from these tests must be confirmed by direct quantitative methods, i.e. monitoring the reaction products. Choice of such properties should be relevant to the end-use of the polymer materials or provide fundamental information about the degradation process. For example, tensile properties has been emphasised for the use of biodegradable plastics in packaging applications. One of the most valuable measurement on the degrading polymer is perhaps the characterisation of its average molecular weight and molecular weight distribution.

1.3.6 Mechanism of biodegradation of polymer-starch system

There is no convincing evidence that unmodified, high molecular weight polyethylene can be utilised by microorganism, as a substrate and degraded at any measurable rate by living organism [41]. The blending of biodegradable polymer, such as starch, with inert polymers such as polyethylene, has received a considerable amount of attention for possible application in the waste disposal plastics.

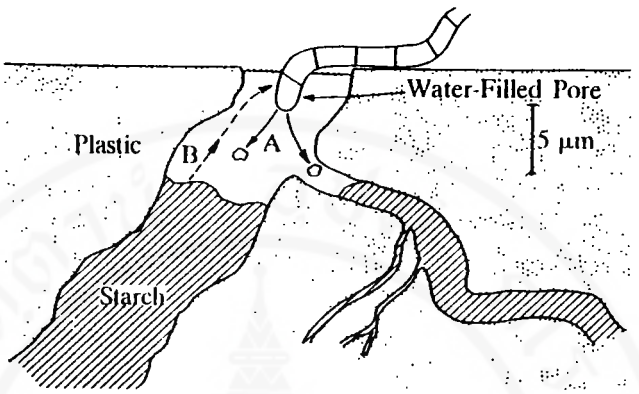
According to Griffin [43], corn starch together with a prooxidant formulation consisting of an unsaturated polymer, a transition metal salt, and a thermal stabilizer (all these additives in masterbatch) when mixed with Low Density Polyethylene (LDPE) gives materials with a greater susceptibility to degradation than pure LDPE.

Upon environmental exposure the following mechanism are intended to take place. The starch granules on the surface of the samples and those granules within the sample which are in direct contact with the surface granules are first biodegraded at the same time that the sample matrix is hollowed out, increasing the surface/volume ratio. Meanwhile sunlight, heat, oxygen, etc. trigger the chemically unstable prooxidant, generate free radical which can attack the molecule of LDPE. The

plastic or film should lose its integrity, disintegrate and disappear [44].

(i) Postulated mechanism of biodegradation of polymer-starch system [45].

The mechanism of starch degradation is possible in two ways. One could be diffusion of water-soluble substrate to the film surface, where degradation would occur. The other could be microbial production of enzymes in or near film surface, diffusion of the enzymes into the substrate and diffusion of soluble digestion products back to the surface where they are metabolised. The second mechanism would take place only when the pore diameter at film surface is too small to admit a microbial cell (i.e., at diameters <0.5 μm). Cole M.A. stated that none of the materials used to investigated starch degradation showed loss of starch even when soaked in water for extended periods with microbial inhibitor present. Therefore, he proposed that diffusion of enzymes to the substrate rather than diffusion of the substrate to the film surface is more likely to be the mechanism. Figure 1.5 shows postulated mechanism for degradation by microorganism of starch-plastics blends.



A: Diffusion of amylase to starch
B: Diffusion of digestion product to microorganism.

Figure 1.5 Postulated mechanism of degradation by microorganism

Under the conditions where films are buried in soil, lying on the soil surface, or lying on a solid culture medium (ASTM D1924-70), enzymes produced by organism on the film could only diffuse across the films and possibly into pores, as shown in Figure 1.6. By contrast, complete immersion of films (as would occur in water-saturated soil, flooded landfills, aquatic sediment, and in liquid microbial cultures) creates a situation in which enzymes produced by adherent organisms might diffuse away from the film, as shown in Figure 1.7. All produced enzymes have potential for contributing to film degradation.

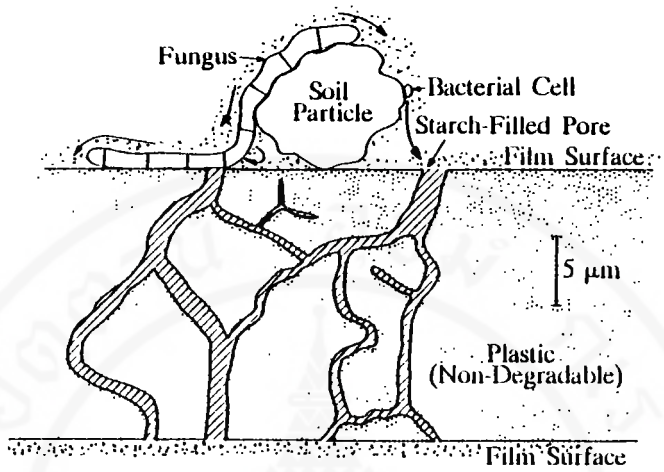


Figure 1.6 Diffusion path for starch-plastic film in a moist environment

Note: Lightly shaded area indicates thin water layer on surface , arrow show paths for enzymes produced by microbes on film surface.

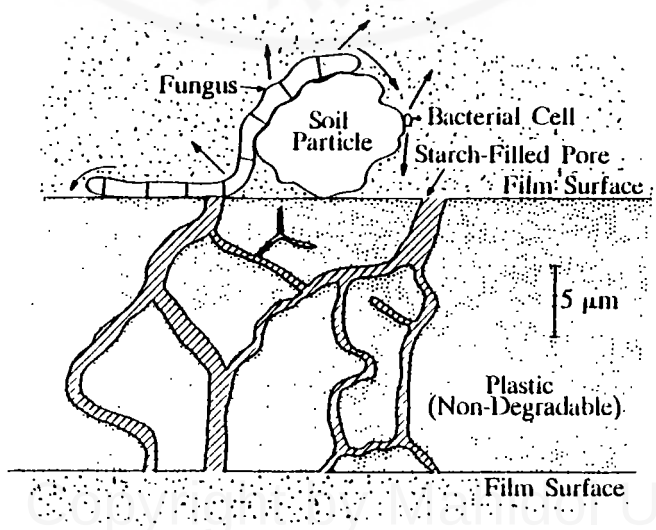


Figure 1.7 Diffusion path for starch-plastic films immersed in water

(ii) Critical film morphology that affects biodegradability

Degradation can be initiated only from the surface, thus the structure of the film should enable the starch to be suitably removed from the material. Figure 1.8 illustrates ideal film structure which influences biodegradability. Materials A and C are identical in composition but different in film morphology.

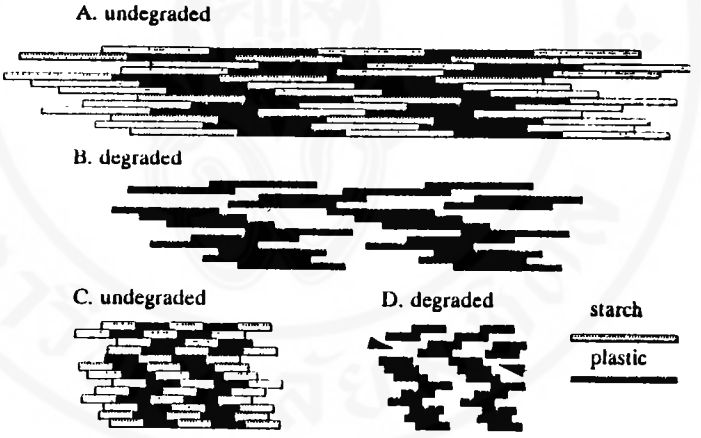


Figure 1.8 Influence of film morphology on diffusion paths in starch-plastic film.
A and C: intact film; B and D: after starch removal. Arrow indicate constructions that would control diffusion processes.

When starch is removed from the film surface, channels are created through which amylase would diffuse and digest the film inwards (Figure 1.8, B and D). In the degraded material D, there are a few site where

constructions occur (as indicated by arrows), and these sites would control enzymes digestion through the entire channel. By contrast, the degraded material B, does not have the severe construction in channels. Therefore, material A would likely to have greater biodegradability than material C.

Even though the surface porosity and diffusion path characteristics within the film seem to be major contributors to film biodegradability, the other factors mentioned in section 1.3.5 should also be considered.

1.4 Macrobiological degradation

Macrobiological degradation is caused by mechanical attack of plastics by living creatures larger than bacteria and fungi, such as rodents, insects, roaches. These macroorganism can masticate and digest the plastics [38,39]. The degradation probably followed by the action of microorganism accelerated by fragment of the material.

Termites are one of the main insects that destroy materials. Therefore, any degradation or deterioration of materials due to termites is of interest.

1.4.1 Factors influencing the damage of materials by termites [46]

1.4.1.1 Physical factors

Physical factors involved are environmental influences on the termites and properties of the materials.

(i) Environmental influences

Temperature : Most activities of insects depend on temperature. Many tropical species have their optimum feeding activity and development at around 30 °C. Temperature may also influence the properties of the material or the aging of preservatives.

Humidity : It is the other prerequisite for survival and activity of termites. In nature many termites need access to water supply or to wet wood. Under natural conditions, materials are more endangered by termites attack in the rainy season than during dry periods.

Weather condition and atmospherics : A correlation between food consumption and disturbances of magnetic field of the earth in a 27-day rhythm of the rotation of the sun is also significant. It is not proven that changes of the magnetic field determine termites activity, but the influences coming from sunspots are one parameter involved in termites reaction.

(ii) Material properties

Hardness : It is one of the important properties of material for an attack by termites. In the case of thermoplastics materials, the hardness depends on temperature. This may influence the results of tests which are carried out under different temperature.

Surface and internal structure : Termites cannot attack materials with a very smooth surface such as coating. They start to gnaw at rough spots and edges.

Thickness : The threshold of damage to materials by termites depends on the size of the termites species with dimension of the mandibles. However, termites can find a spot to start their attack on folded or deformed materials.

1.4.1.2 Chemical factors

Chemical factors involved are sensorial influence such as repellency, attraction and stimulation and toxic influences such as toxic efficiency, and aging of effective substances. These factors influence the termites' behaviour and thus results of bioassays in nature. Toxicity of the materials may also prevent attack.

1.4.1.3 Biological factors

Among biological factors influencing the results of laboratory and field tests are various behavioural patterns and differences in vitality of termite species

and races as well as the number of individuals and caste composition of groups. Biological and reaction of termites are also important for the performance of materials under service conditions.

(i) Behavioural influences

Gnawing activity : The continuous gnawing activity of termites also leads to damage of non-digestible material. Wood and other plant tissues constitute the normal food of termites. But they may attack rubber, synthetic textiles, plastic materials, paints, and may perforate even soft metals. The gnawing activity has to be induced and facilitated in laboratory testing procedure in order to be able to consider potential cases of damage in practice.

Influence of substrates : Matrix and surrounding conditions are important in a test. The composition of the matrix can fluence a laboratory test result remarkably. Also on a field tests consideration of overall conditions at the testing area is essentials. In order to attract termites and increase their number at test plot, feeder blocks or strips are employed. Another factor in testing is the size of testing specimens.

Choice or compulsory tests : These are two ways in which laboratory tests can be implemented. Compulsory conditions may lead to faster results, but the termites live under starvation if the material to be

tested has no nutritional value for them. Therefore the first days of the testing time with full activity of the insects constitute the most important period.

(ii) Various biological factors

Species and race of the termites : Species, even from the same genus, and geographical races of termites may react differently to materials. Geographical races can show variations in behaviour, vitality, activity and tolerance to toxic agents. Therefore, collections or cultures used for testing should be defined sufficiently in order to study variation in reaction and tolerance among species.

Number and composition of groups, vitality, and development stage of the colony : These are also significant and may influence the physiological reactions of the termites and thus the results.

However, various physical, chemical and biological factors influence the damage of materials under practice condition. The complexity of influences concerned together with the necessity to deal with sufficient numbers of different termite species require further research on the behaviour and reaction of various termites

1.5 Scope of the thesis

There have been in the past many studies concerning biodegradation of PE-starch plastics. However, the efficiency of degradation of starch-filled plastics has not clearly been reported. In Thailand, this type of study also has not been made. The present study was, therefore, undertaken in order to gain direct experience with the preparation and behaviours of PE-starch plastics.

The principal objective of the thesis was to study the possibility of disposing plastic waste by the action of insects, rodents or animals of this type. Termite was chosen for this study because of its well known capability of eating wood (a type of polymer).

Since the idea of using termites to dispose of plastic waste was relatively new, it could not be certain that the study would be successful, it was decided that study of biodegradation of starch-plastics blends should also be made, in order to obtain direct experience with the commercially-established type of biodegradable plastics, the starch-blended plastics. Both self-prepared and commercial biodegradable starch-filled plastics were studied. Several test methods for study of degradation by microorganisms, including Bacillus subtilis and Pseudomonas aeroginosa, were studied. Surface morphology and tensile properties changes were also studied.

CHAPTER II
EXPERIMENTAL

2.1 Chemicals and materials

Chemicals and materials used in the present study are given below :

Chemicals and Materials	Manufacturers
Actidione	Fluka
Agar-agar	Merck
Ammonium nitrate	Merck
Calcium chloride	Merck
Manganese chloride	Merck
Sodium chloride	Merck
Cupric sulphate	Merck
Ferrous sulphate	Merck
Iron sulphate	Merck
Magnesium sulphate	Merck
Manganese sulphate	Merck
Zinc sulphate	Merck
Nutrient agar	Merck
Nutrient broth	Merck
Potassium dihydrogen orthophosphate	Merck
Potassium monohydrogen orthophosphate	Merck
Sodium monohydrogen orthophosphate	Merck
Mercuric chloride	Merck
D-glucose	Merck

Yeast extract	Difco
Malt extract	Difco
Corn starch	Maiza
Low density polyethylene (JJ 4324)	TPE (ThaiPolyethylene Co.,Ltd.)
UV&bio-degradable bags	Thai Rotary Plastic Co.,Ltd.

2.2 Bacteria

Bacteria used in this study were *Bacillus subtilis* (Bs) and *Pseudomonas aeroginosa* (Pa) which were kindly provided by the Department of Biotechnology, Faculty of Science Mahidol University. Bs and Pa were selected for study of biodegrdation of starch-plastic blend according to the work of Griffin [50], and [47].

2.3 Preparation of PE-starch films

The specimens were prepared by mixing LDPE and corn-starch in HAAKE Rheocord 90. Corn starch was dried prior to mixing in the oven at 60 °C for 24 hr and kept in dessicator if it was not used immediately after removal from the oven. The amount of corn starch in the specimens

was varied from 25% to 75% by weight of LDPE. The mixing condition is given as follow.

Mixing condition:	Rotor speed	60	rpm
	Set temperature	140	°C
	Mixing time	30	min
	Batch size	60	ml

After mixing, the specimens were granulated and pressed into thin sheets of approximate 1-2 mm in thickness by compression moulding. The moulding condition is given below.

Moulding condition:	Temperature	150	°C
	Pressure	150	psi
	Preheat	5	min
	Press	5	min
	Cool	5	min

The thin sheets were then cut into approximately 10 by 10 cm for exposure in the field study and in the form of small pieces of 2 by 2 cm for laboratory study.

2.4 Preparation of media for bacterial cells

2.4.1 Preparation of nutrient agar slant and nutrient broth

To prepare nutrient agar (NA) slant, the nutrient agar was suspended in distilled water (0.2 g/100 ml) and heated until dissolved. It was then tubed, plugged and autoclaved at 121 °C, 15 psi, for 15 min. The sterilized medium was allowed to cool and gel in a slanted position to afford an appropriate surface on which the bacteria may be cultured. In addition, the nutrient broth (NB) (0.8 g/100 ml) was also prepared in test tube (5 ml) and in 250 ml erlenmayer flask (50 ml), and autoclaved as previously described.

2.4.2 Preparation of nutrient-salt media

Nutrient-salt medium is a carbon difficient medium. Nutrient-salt agar was prepared by dissolving in 1 L of water the designated amounts of the following reagents:

Potassium dihydrogen orthophosphate (KH_2PO_4)	0.7 g
Potassium monohydrogen orthophosphate (K_2HPO_4)	0.7 g
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.7 g
Ammonium nitrate (NH_4NO_3)	1.0 g
Sodium chloride (NaCl)	0.005 g
Ferrous sulphate ($FeSO_4 \cdot 7H_2O$)	0.002 g

Zinc sulphate($ZnSO_4 \cdot 7H_2O$)	0.002 g
Manganese sulphate($MnSO_4 \cdot H_2O$)	0.001 g
Agar	15.0 g
Distilled water	1000.0 ml

Since nutrient-salt agar readily supports growth of fungi which may be present on the test specimens, fungal contamination was controlled by the addition of filter-sterilized 0.15% actidione (cycloheximide) to the test media after sterilized by autoclaving at 121 °C, 15 psi, for 15 min.

Nutrient-salt liquid medium was prepared by using all ingredients above except the agar and was sterilized under the same condition.

2.5 Preparation of bacterial cell suspension

The test organisms, Bs and Pa were cultured on nutrient agar(NA) slants and incubated at 37 °C for 24 hr, and then kept in refrigerator as stock cultures until used. Whenever the inoculum was needed at least two successive transfers of desired bacterial culture in nutrient broth(NB) were prepared as the following. One loopful of the bacterial culture on NA slant was transferred with aseptic technique to 5 ml NB tube and incubated at 37 °C for 18-20 hr. One ml of this broth culture was transferred to 50 ml NB flask, then it was placed in rotary shaker (160 rpm) and incubated as before.

The broth culture was centrifuged at 7000 rpm at 4 °C by using Kubota KR 20000T. The supernatant was decanted. The bacterial cells were washed twice with sterile normal saline solution(0.8% NaCl), and centrifuged at the same condition. The bacterial cells were resuspended in sterile saline solution, to obtain the concentration of approximately 10^8 cells/ml.

2.6 Test procedures

2.6.1 Biological studies

Preliminary studies to select test method and microorganism were tried with 3 procedures (namely Procedures A, B and C) and the 2 types of microorganisms, i.e. Bs and Pa

The test specimens used in Procedures A and B were prepared in laboratory (50% starch concentration, size 2 by 2 cm, thickness ~0.5 mm) as described in Section 2.3. For Procedure C, both self-prepared specimens (as used in Procedures A and B) and the specimen manufactured by Thai Rotary Plastic Co.,Ltd. as UV&bio-degradable bags were used. The latter specimens were cut into dumbbell shape (as shown in Figure 2.1) for which were measured tensile properties before and after subjected to the test.

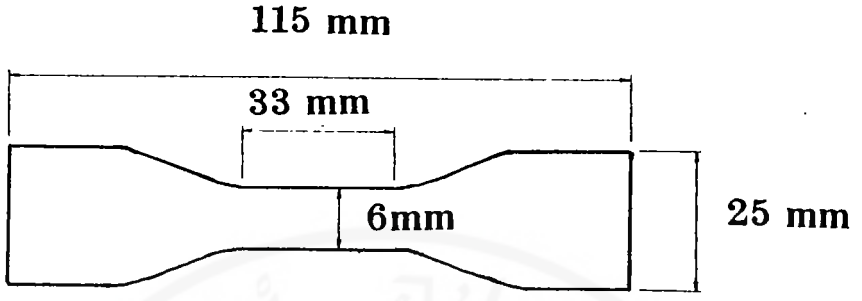


Figure 2.1 Tensile specimen

Procedure A

1. The specimens were disinfected prior to the test by soaking them in 0.3% hydrogen peroxide for about 15 min, and rinsing with sterile water 2-3 times. The experiment was performed with aseptic technique.

2. Inoculation: One ml of bacterial cell suspension, Bs or Pa was pipetted into 100 ml sterilized nutrient-salt agar, melted and cooled to about 45 °C. This inoculated agar was poured into sterile petri dishes to provide an agar layer about 1/4 inch in depth and allowed to solidified. Later, 4 pieces of the specimens were placed on the surface of the agar.

3. Uninoculated control specimens were prepared as described in the Procedure A-2, using uninoculated nutrient-salt agar.

4. Incubation: All dishes were covered and incubated at 37 °C for 60 days.

Procedure B

The experiment was performed as Procedure A, but after the specimens were placed on the surface of solidified agar, the inoculated agar (with Bs or Pa) was poured over the specimens and allowed to gel. Uninoculated control specimens were similarly prepared with uninoculated overlay agar. All dishes were incubated at 37 °C for 60 days.

Procedure C

1. One ml of bacterial cell suspension, Bs or Pa, was pipetted into 100 ml of sterilized nutrient-salt liquid medium. Ten pieces of the disinfected specimens were aseptically introduced to the medium.

2. Uninoculated control specimens were prepared as Procedure C-1 using uninoculated medium.

3. All flasks were incubated with shaking at 160 rpm at 37 °C for 60 days.

Viability of microorganisms during exposure time at every 15 days time interval was observed. One ml of each incubated flask was sampled each incubated flask, and serially diluted in sterile normal saline solution. A 0.1 ml of appropriate dilution was spreaded onto NA plate (in duplicate). Colony-forming unit (CFU) were counted after incubation at 37 °C for 18-20 hr.

2.6.2 Physical Studies

After each defined interval, the specimens were recovered from the incubated petri dishes and flasks, washed in an aqueous solution of 0.1% mercuric chloride for at least 5 min, rinsed in tap water, and air-dried overnight at room temperature. The specimens were stored in dessicator at room temperature prior to the study. The unexposured specimens were also ketp in the dessicator through out the test period.

The specimens were studied for surface morphology using Scanning Electron Microscope(SEM) and for tensile properties using Tensile Tester. Tensile strength, elongation at break and 300% modulus (stress at 300% elongation) were measured with 500 mm/min crosshead speed.

2.7 Studies of biodeterioration of starch-filled polyethylene sheet by termites

Field studies were performed to observe deterioration of specimen affected by macroorganism. In this experiment, the self-prepared specimens (0%, 25%, 50% and 75% starch concentration, size 10 by 10 cm and thickness ~0.5 mm) were exposed in the field study of termite at Industrial Entomology Research and Development Center, Kumpangsan Campus, Kasetsart University.

The specimens, three pieces of each starchconcentration, were used. Each peice was placed in open, used beverage can with a small hole at the bottom. The can was placed in inclining position (about 45°) in soil as shown in Figure 2.2. The specimens were removed from the field at required period. Visible and physical changes on specimens were examined.

Figure 2.2 Test specimen placed in used-beverage can subjected to macroorganism exposure

2.8 Characterization of commercial degradable plastics packaging films using Fourier Transform Infrared(FTIR) and Thermogravimatric Anylysis(TGA) techniques

Degradable plastics were examined by FTIR and TGA techniques. The instruments used were Perkin-Elmer System 200 NIR FT-Raman and Perkin-Elmer TGA7.

CHAPTER III
RESULTS AND DISCUSSION

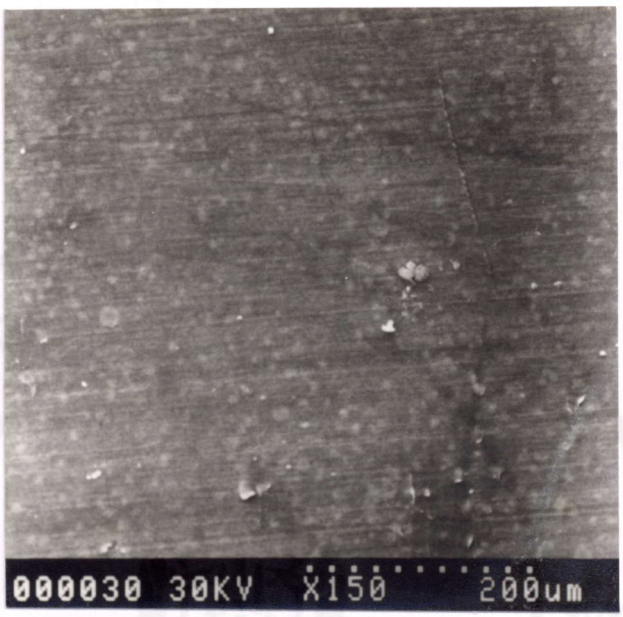
3.1 Preparation of PE-starch films

When polyethylene(PE)-starch samples were granulated and moulded into thin sheets approximately 1-2 mm in thickness by compression moulding, the sheet samples seemed to be homogeneous as observed by eyes but with scanning electron microscope(SEM) rough surface showing discrete starch granules was observed, as shown in Figure 3.1.

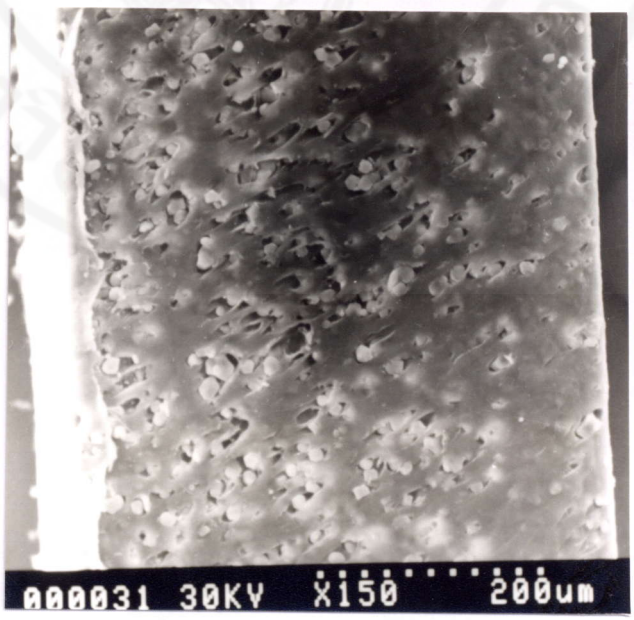
The PE and starch were immiscible showing PE matrix and starch granular filler. Cutting the film with blade to observe its cross-section could affect in fracture of PE as seen in Figure 3.1 (b). The immiscibility of the blend was the result of the influence of the hydrophobic and hydrophilic nature of the two components. To increase compatibility of hydrophilic starch with hydrophobic PE, surface-treated starch, such as process of starch gelatinisation [36,37], should come into consideration. Coupling agents such as silane would ease hydrophilic and hydrophobic influence and increase the compatibility of PE and starch.

It has been mentioned that the level of starch addition to the PE film is generally limited to about 10%

or less by weight in order to maintain film strength [32]. However, in this work, the concentration of starch in the films prepared was varied from 20-75% in order to see biodegradation by microorganisms clearly. Tensile testing of the films both before and after exposure to microbial degradation revealed that the strength of the film was so poor that tensile testing could not be carried out. As such, only surface morphology change of the films was studied.



(a) surface



(b) cross-section

Figure 3.1 SEM micrographs of PE-starch blend (50% starch) (magnification x150) (a) surface, (b) cross-section

3.2 Studies of microbial degradation

The results of the experiments of each Procedure are summerised in Tables 3.1 and 3.2.

The results shows that no growth of added microorganisms in all control experiments was observed and no changes or slightly surface erosion of the films of Procedures A, B, and C (Table 3.1). The similar results were also detected in the test experiments of Procedures A, B, and C

It was also found that the solid media used in Procedures A and B were dried and cracked after 30 days of incubation, which was before the end of the test period, whereas the liquid media used in Procedure C remained in the flask in sufficient amount through the end of experiment. In the test experiment of Procedure C, only Pa was present through out the experimental period, whereas Bs was absent after 15 days of incubation.

Table 3.1 Control experiment for Procedures A,B and C

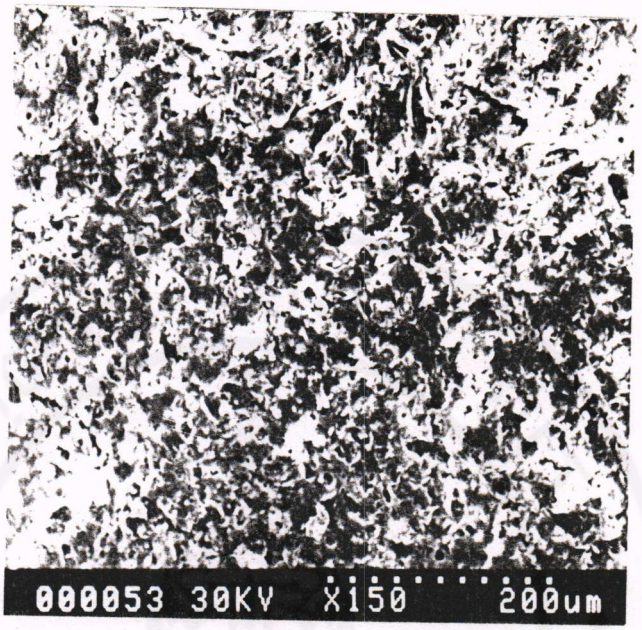
Procedure	Control Experiment	Results
A	Bs present only	No growth of Bs
	Pa present only	No growth of Pa
	Test specimen present only	No degradation or surface erosion observed on the specimen.
B	Bs present only	No growth of Bs
	Pa present only	No growth of Pa
	Test specimen present only	No degradation or surface erosion observed on the specimen.
C	Bs present only	No growth of Bs
	Pa present only	No growth of Pa
	Test specimen present only	No degradation but only surface erosion was observed.

Table 3.2 Results of biodegradation test experiments after 55 days of incubation.

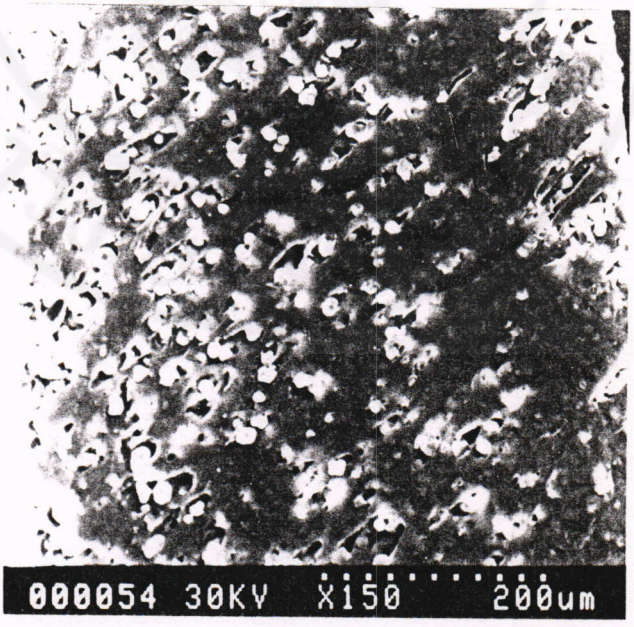
Procedure	Microorganism added	Results
A	Bs	No growth of Ba
	Pa	No growth of Pa
B	Bs	No growth of Ba
	Pa	No growth of Pa
C	Bs	No growth of Ba
	Pa	Growth of Pa

The results of physical changes observed from Procedure C were shown in Figures 3.2 and 3.3. Figure 3.2 shows SEM micrograph of the control specimen containing 50% starch incubated at 37 °C for 55 days without inoculating microorganism. It was observed that the morphology of the specimen changed (compared to Figure 3.1) even though microorganism was not introduced to the test medium. Holes as the result of extraction of starch granules from the PE-matrix were observed. This phenomena might be due mechanical force occurred by rotary shaking of liquid medium could loosen the water-soluble starch from PE matrix, leaving porous structure. Figure 3.3 shows SEM micrograph of the specimens containing 50% starch incubated with Pa at 37 °C for 55 days. Surface morphology of specimens changed in greater extent by comparing to the control specimen (Figure 3.1 and 3.2). Indicating the effect of presence microorganism.

Although many investigators reported several microorganisms, including *Bacillus subtilis* and *Pseudomonas aeroginosa* [47,48,49,50], that can degraded starch-plastic films, the evidence herein this study was not strongly support this statements. More experiments should be further carried out.



(a) surface

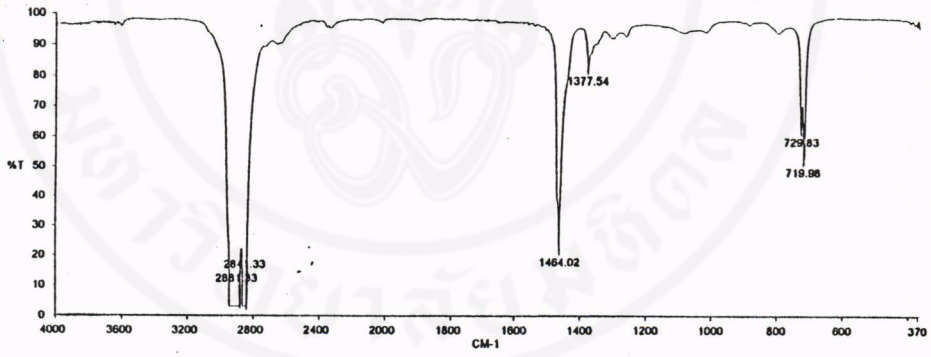


(b) cross-section

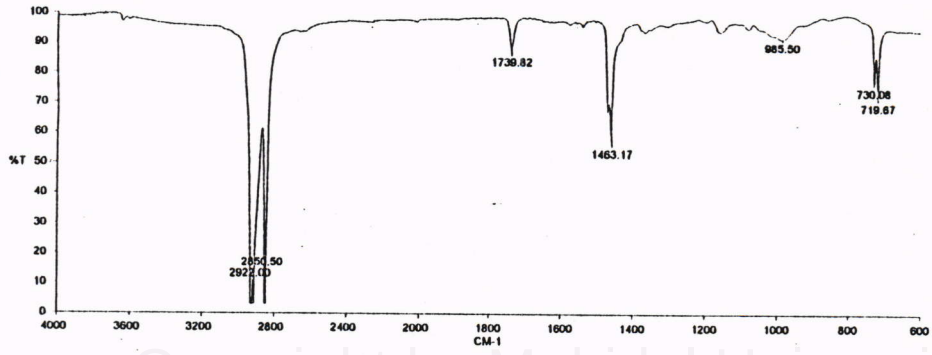
Figure 3.3 SEM micrographs of specimens containing 50% starch incubated at 37 °C for 60 days with Pa in nutrient-salt liquid media.
(magnification x150)
(a) surface and (b) cross-section

3.3 Study of commercial degradable plastic bags

The commercial plastics bag which was claimed to be UV&bio-degradable was examined by FTIR to prove the existence of biodegradable component, i.e. starch. Characteristic of hydroxyl group at wave number 3700 cm^{-1} to 3300 cm^{-1} indicated the presence of starch could not be observed. Conventional low-density polyethylene(LDPE) bag was used as reference. Figure 3.4 illustrates FTIR spectra of two types of the bags.



(a) reference bag



(b) commercial UV&bio-degradable bag

Figure 3.4 FTIR spectra of (a) reference bag (LDPE bag), and (b) commercial UV&bio-degradable bag

The ~3000-2800 cm^{-1} and ~1400 cm^{-1} absorbency characteristics of alkyl group (C-H stretching and C-H deformation) were observed in both spectra. The ~700 cm^{-1} absorbence presented in reference bag and UV&bio-degradable bag is believed to be characteristic of C-H deformation of aromatic compound [53]. The ~1740 cm^{-1} absorbence in the spectrum of UV&bio-degradable bag is believed to be characteristic of ester group [53]. The characteristics of hydrocarbon deformation and ester functional groups may results from additives present in the bag. However, absorbency characteristics of hydroxyl group were not found in the UV&bio-degradable bag as expected. Zobel H.F.[51] stated that modification of starch at hydroxyl groups to increase its compatibility in manufacture the bag by using silane as coupling agent, consequently reduces hydrophilic nature of starch. Therefore, hydroxyl group which is hydrophilic nature of starch could not be observed.

Since the self-prepared specimens (Section 3.2) could not be measured for tensile properties after recovered from the inoculum. Thus, UV&bio-degradable bags were used as test specimens and cut in dumbbell shape as described in Section 2.6.1. Procedure C was again carried out using this test specimens. The results showed that Pa survived through out the experimental period (90 days). Cell concentration of Pa remained in approximately the same amount as initial concentration until the end of

experimental period. Since *Pseudomonas aeroginosa*(Pa) could not hydrolse starch [54], therefore it would be possible that Pa might utilise any other ingredient in the test specimens.

Figure 3.5 shows SEM micrograph of UV&bio-degradable specimens before subjected to the test. Figure 3.6 shows SEM micrographs of the control specimen incubated at 37 °C for 60 days without added microorganism in nutrient-salt liquid media. Figure 3.7 shows surface morphology changes of the specimen incubated at 37 °C for 60 days with Pa in nutrient-salt liquid media. The results obtained in these UV&bio-degradable specimens were similar to those obtained in self-prepared specimens.

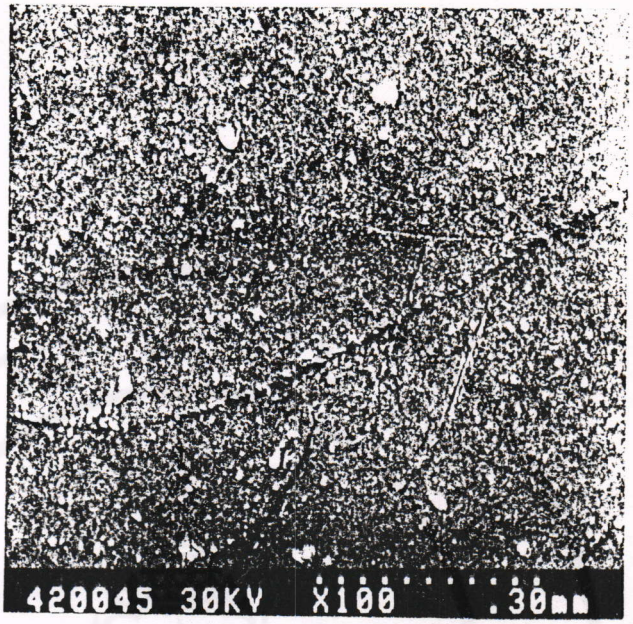


Figure 3.5 SEM micrograph of UV&bio-degradable specimens before subjected to the test (magnification x100)

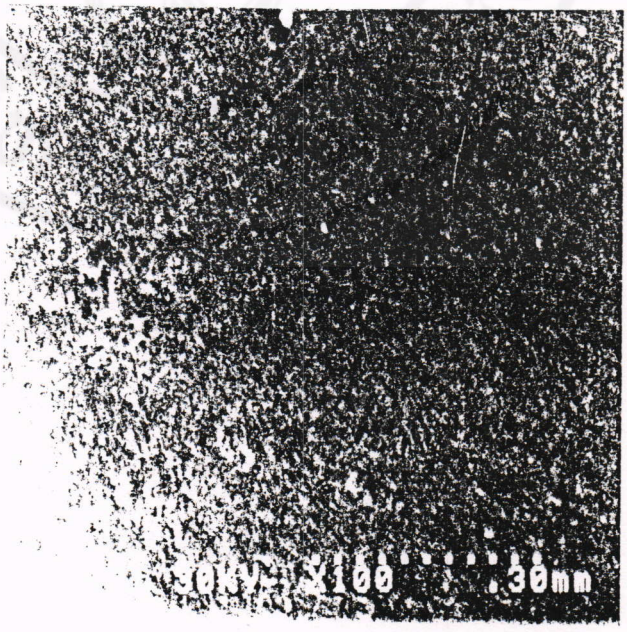
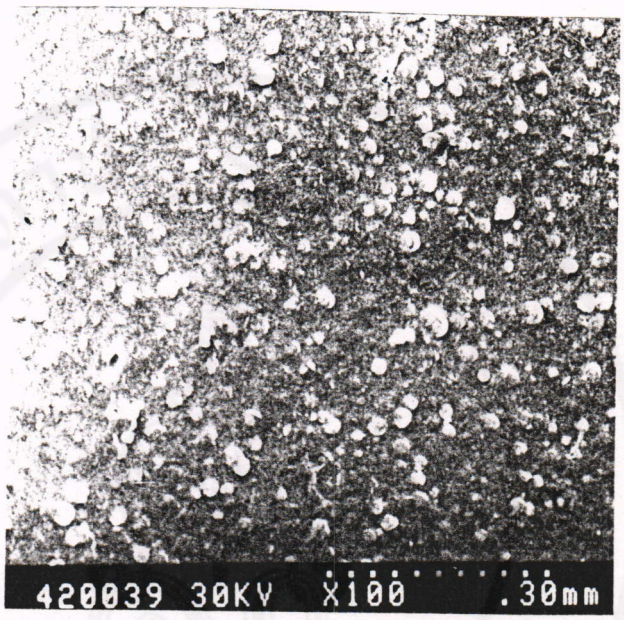
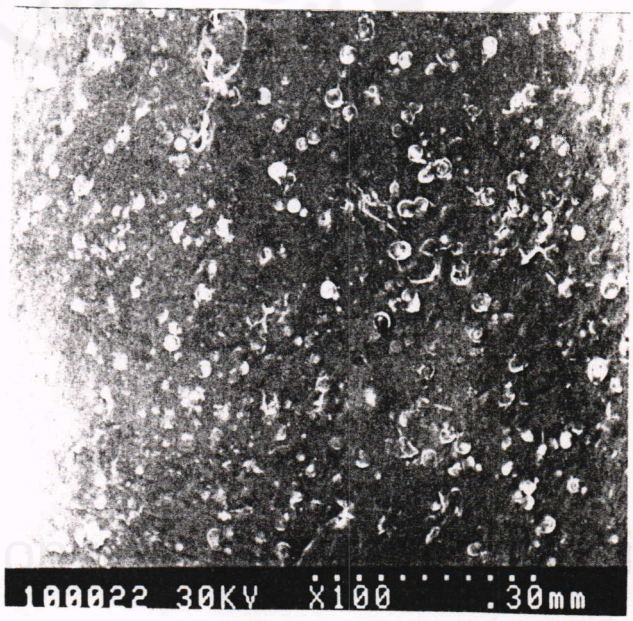


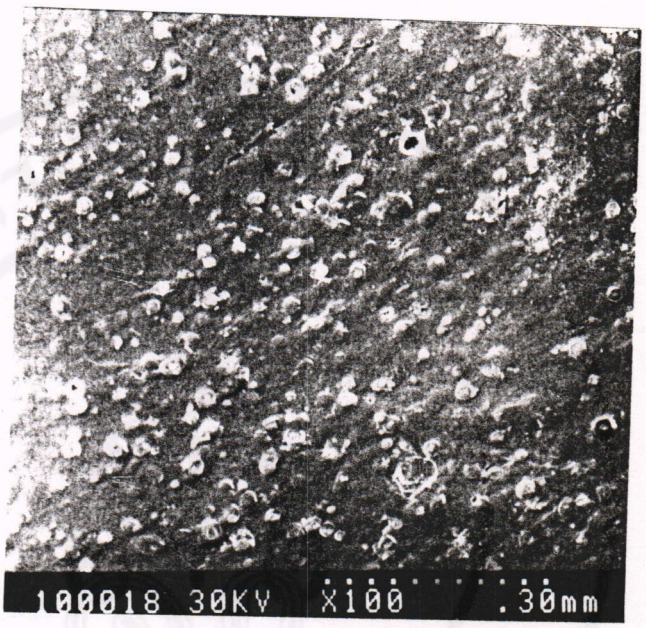
Figure 3.6 SEM micrographs of the control specimen incubated at 37 °C for 60 days without microorganism in nutrient-salt liquid media. (magnification x100)



(a) incubated for 30 days



(b) incubated for 60 days



(c) incubated for 90 days

Figure 3.7 SEM micrographs of UV&bio-degradable specimen incubated at 37 °C with Pa in nutrient-salt liquid media. (magnification x100) (a) incubated for 30 days, (b) incubated for 60 days, and (c) incubated for 90 days

3.4 Studies of Tensile properties of specimen after exposure period

Tensile properties of the UV&bio-degrdable specimens exposed to Pa were studied. The results obtained are illustrated in Figures 3.8, 3.9 and 3.10.

Figure 3.8 illustrates modulus at 300% elongation (M300%) of the control specimens (in which the sample was incubated without inoculation of microorganism),and the specimens exposed to Pa during exposure period. M300% of specimens exposed to Pa decreases during the first 15 days and remained rather steady until the end of the exposure period. M300% of the control sample decreased during the first 15 days of incubation. After day 15, its value could not be measured as the specimens broken before reaching 300% elongation (as shown in Figure 3.10)

Figure 3.9 illustrates tensile strengths(TS) of the control specimens, and specimens exposed to Pa. TS of both control specimens and specimens exposed to Pa decreased at the beginning of the period and remain rather steady until the end of the test period.

Figure 3.10 illustrates elongations at break of the control specimens, and specimens exposed to Pa. Percentage of elongation at break(%EB) of the specimen exposed to Pa tended to decrease as exposure time increased. For the control specimens, its %EB did not change during the first

15 days. After day 15, its %EB decreased rapidly from 450 %EB to ~50 %EB and increased to ~350 %EB after day 45.

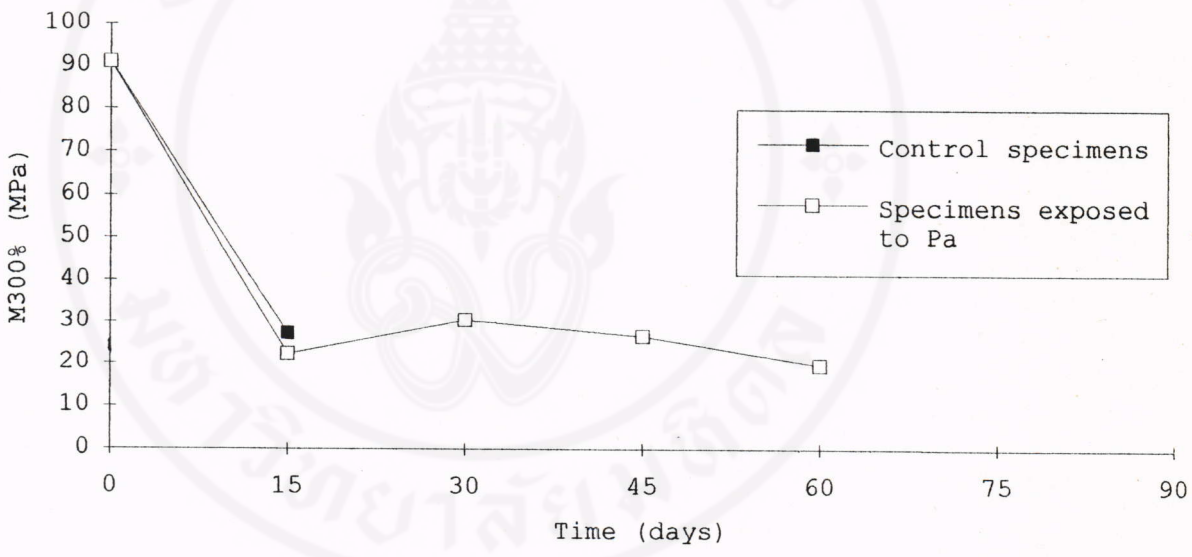


Figure 3.8 Modulus at 300% elongation of control specimen and specimens exposed to Pa

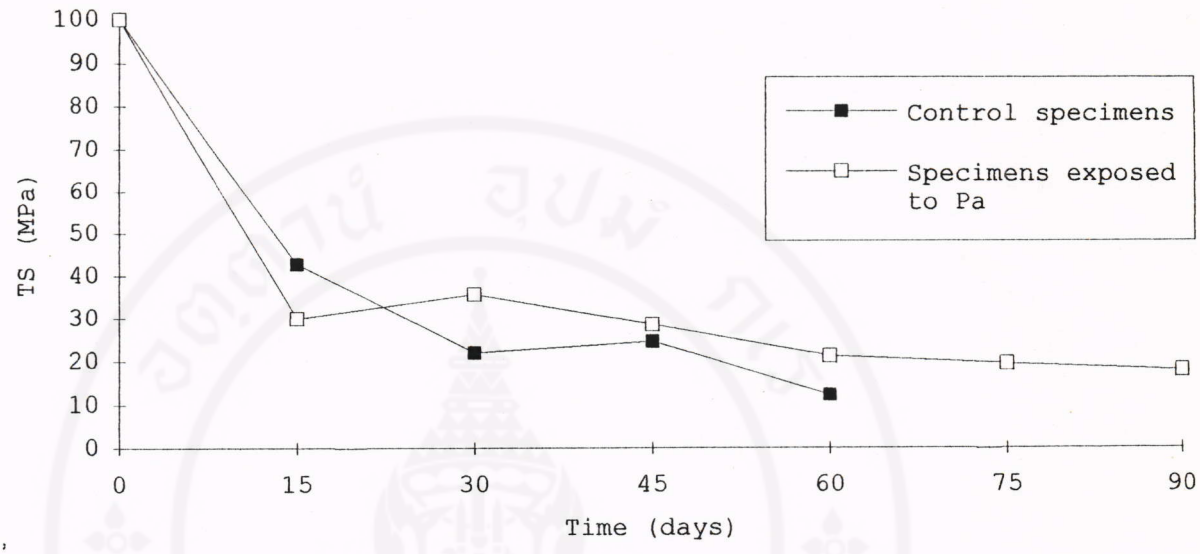


Figure 3.9 Tensile strength of control specimen, and specimens exposed to Pa

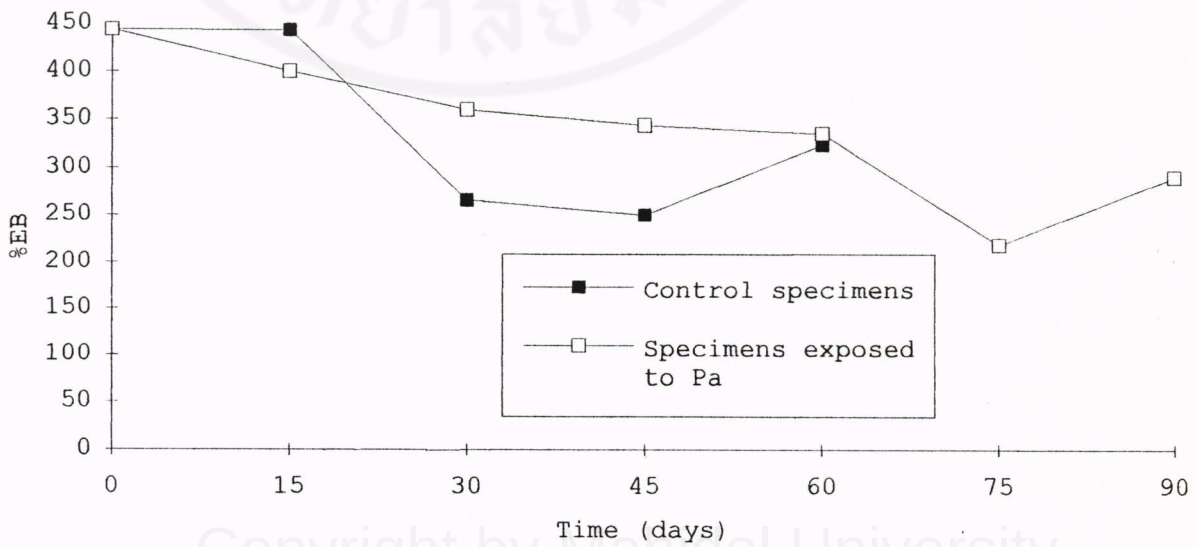


Figure 3.10 Percentage elongation at break of control specimens, and specimens exposed to Pa

The decrease in tensile properties of the specimens exposed to microorganism might resulted from migration of the ingredients in the matrix to the surface, enable the microbes to utilise such ingredient as the nutrient source. However, the experiment was performed by shaking the flasks to optimise the growth of microorganism in the test environment. Thus, mechanical force might also affect migration of the ingredients from the matrix, rendering poor physical properties of the films.

From the experiment, tensile properties of specimens exposed to microorganism seemed to be higher than those of the control specimen. It might be due to the influence of orientation of molecular segments in the samples, occurred during the blown-film process. Theoretically, tensile properties in the oriented direction are greater than those in perpendicular direction. As orientation increases, modulus and tensile strength will increase, and breaking extension decrease [52]. Directions of films were not controlled during cutting of the test specimens. Therefore, it might be possible that the control specimens were cut from unoriented or less oriented direction. Where as those from the specimens exposed to microorganism might be along the orientation direction.

From the results showed that, tensile properties changes of the specimen during the experimental period were not significant. However, there are no report on rate and extent of tensile properties changes of studies of

biodegradation of starch-filled plastics. But Griffin [13] reported that it took almost one year to observed significant changes of tensile properties of polyethylene which subjected to studies of biodegradation. Therefore, longer experimental period is required to obtain additional information of the tensile properties changes.

3.5 Studies of disposal of PE-starch plastics by termite

The specimens prepared in the laboratory containing different percentages of starch were exposed to termite in the field studies as describe in Section 2.7. The test duration was 6 months, started in July and ended in December 1994. The recovered specimens are shown in Figure 3.11.

It is believed that the weight losses of PE-starch blends were due to termite although the effect of other macroinvertibrates present in the field study such as ants and insects, could not be completely eliminated. Weight losses of specimen in Figure 3.11(b) is greater than those in Figure 3.11(c) even though the specimen in Figure 3.11 (c) contained greater value of starch and was exposed in greater period. This might due to population of termite which attacked the specimens were different. However, the specimens with greater exposure period shows colouration

of specimen. As observed on the specimen recovered was the formation of fungi on the surface and colouration of specimens as shown in Figure 3.12.

(a) 25% starch content, 69 days exposure, 11.7% weight loss

(b) 50% starch content,224 days exposure,21.5% weight loss

(c) 75% starch content,175 day exposure,13% weight loss

Figure 3.11 Specimens recovered from termite field studies

Figure 3.12 Specimen placed in the beverage can after recovered from the field study shows formation of fungi and colouration on the sample surface.

It could be reason that the colour was caused by hypha penetration of soil fungi into the film. Since life cycle of termite is closely associated with soil fungi in fungus garden. Therefore, when termites made their ways to the samples they also brought fungi with them. The routes which termite made to their food source were seen on the specimen recovered as shown in Figure 3.13. The soil fungi and bacteria at the place where termites are abundant can influence degradation of several materials including plastics. Fracture at the edges of the specimens, as shown in Figure 3.14, was believed to be biting characteristics of termite.

Figure 3.13 Specimen placed in the beverage can after recovered from the field study shows the routes which termite made to their food source.

Figure 3.14 Specimen recovered from the field study without cleaning. The specimen was in contact with soil and fracture at the edge of the specimen shows biting characteristics of termite.

Although the present studies could not be completely certain that only termite caused degradation of the plastics films, deterioration which is one of the crucial factor for complete degradation of the film, did occur. These results had important implications. Innovative method for disposing of plastics waste by microorganism was demonstrated. Studies such as these, couples with the results of studies of microbial degradation of plastics will provide the information needed to access the overall potential of PE-starch blends as biodegradation or naturally disposal plastics.

Chapter IV
Conclusion

From the studies made in the present thesis, the following conclusion could be made.

1. Preparation of starch-polyethylene blend using granular starch unaided with coupling agent resulted in plastics sheets or films having poor mechanical properties.

2. Commercial starch-polyethylene blends obtained from commercial UV&bio-degradable bags showed good mechanical properties. IR spectroscopic evidence showed that the starch used was modified.

3. *Pseudomonas aeroginosa*(Pa) could grow in liquid medium which contained starch-plastics blends or commercially-established type of biodegradable plastics, whereas *Bacillus subtilis*(Bs) could not. Since Pa has been reported to be incapable of hydrolysing starch, therefore it was not clear whether Pa could actually cause biodegradation of starch-plastics blends.

4. Prefer test method using nutrient-salt liquid media could be used for biodegradation study of starch-filled polyethylene.

5. Preliminary study of disposing plastics waste by the action of termites showed interesting promises. Significant losses of plastics mass were observed after disposing of the plastics samples to termites in field

studies. For 25% starch content sample, the weight loss was 11.7% after 69 days; for 50% starch content sample, the weight loss was 21.5% after 224 days and for 75% starch content sample, the weight loss was 13% after 175 days.

BIBLIOGRAPHY

1. Mark H.F., Bikales N.M., Overberger C.G., and Manges G., Encyclopedia of Polymer Science and Engineering, 2nd edition (1986), Vol.5, DISPOSAL AND REFUSE OF PLASTICS, p.103-129

2. Johnson R., AN SPI OVERVIEW OF DEGRADABLE PLASTICS, Proceedings of Symposium on Degradable Plastics, The Society of the Plastics Industry, Inc., Washington,D.C. (June 10,1987), p.6-12

3. Fox R.C., RECYCLING AND DISPOSAL OF PLASTICS PACKAGING, Plas/Tech Ltd. p.1-13

4. PLASTICS WASTE-DISPOSAL AND RECYCLING, PAST, PRESENT AND FUTURE IN JAPAN, Surveys & Public Relations Dept., Plastics Waste management Institute

5. Mark H.F., Othmer D.F., Overberger C.G., and Seaberg G.T., Encyclopedia of Chemical and Technology, 3rd edition (1982), Vol.19, RECYCLING OF PLASTICS WASTE, p.993-1002

6. Morrow D.R., Amini M.A., Adams J.C., and Merriam C.N., OVERVIEW OF PLASTICS RECYCLING, *Converting & Packaging* (Dec.1987), p.138-143

7. ASTM D 883-91a, Standard Terminology Relating to Plastics

8. TECHNICAL REPORT-BIODEGRADABLE PLASTICS, *Materials & Design* (1992), 13:5 ,p.299-304

9. Swift G.,BIODEGRADABLE POLYMERS IN THE ENVIRONMENT: A POLYMER WASTE-MANAGEMENT OPTION?, *Polymer News* (1994), 19, p.102-106

10. Hocking P.J., THE CLASSIFICATION, PREPARATION AND UTILITY OF DEGRADABLE PLASTICS, *J.M.S.-Rev.Macromol.Chem.Phys.*(1992), C32(1), p.35-54

11. Gage P., DEGRADABLE POLYETHYLENE FLIMS-THE FACT, *Tappi Journal* (Oct.1990), p.161-169

12. Robey M.J., Field G., and Styzinski M., DEGRADABLE PLASTICS, *Materials Forum* (1989), 13, p.1-10

13. Griffin G.J.L., Chemistry and Technology of Biodegradable Polymers 1st ed.(1994)

14. Gerald S., PHOTO-BIODEGRADABLE PLASTICS: THEIR ROLE IN PROTECTION OF THE ENVIRONMENT, *Polym. Degr. Stab.* (1990), 29, p.135-154

15. Gilead D., PHOTODEGRADABLE FILMS FOR AGRICULTURE, *Polym. Degr. Stab.*(1990), 29, p.65-71

16. Gilead D. and Gerald S., British Patent No.2099/78 ; US Patent No.4461853, 4519161

17. Huang J.C., Shetty A.S., and Wang M.S., BIODEGRADABLE PLASTICS: A REVIEW, *Advances in Polymer Technology* (1990), 10:1, p.23-30

18. Palmisano A.C. and Pettigrew C.A., BIODEGRADABILITY OF PLASTICS-CONSISTANT METHODS FOR TESTING CLAIMS OF BIODEGRADABILITY NEED TO BE DEVELOPED, *BioScience* (1992), 42:9, p.680-685

19. Griffin G.J.L., SYNTHETIC POLYMERS AND LIVING ENVIRONMENT, *Pure & Appl. Chem.*(1980), 52, p.399-407

20. Aminabhavi T.M. and Balundgi R.H., A REVIEW ON BIODEGRADABLE PLASTICS, *Polym.-Plast. Technol. Eng.*(1990), 29:3, p.235-262

21. Guillet J., ENVIRONMENTAL STUDIED ON PHOTODEGRADABLE PLASTIC PACKAGING , *Polym. Mat. Sci & Eng.* (1990), 63, p.946-949

22. Mayer J.M., Greenberger M., Kaplan D.L., Gross R., and McCarthy S., DEGRADTION KINETICS OF POLYMER FILMS IN MARINE AND SOIL SYSTEMS UNDER ACCELERATED CONDITION, *Polym.Mat.Sci&Eng.* (1990), 63, p.858-861

23. Kaplan D.l., Meyer J.M., Greenberger M., Gross R., and McCarthy S., DEGRADATION METHODS AND DEGRADATION KINETICS OF POLYMER FILMS, *Polym. Degr. Stab.*(1994), 45, p.165-172

24. Barenberg S.A., Brash J.L., Narayan r., and Redpath A.E., DEGRADBLE MATERIALS: PERSPECTIVES, ISSUES AND OPPERTUNITIES, CRC Press, Boca Raton, Florida, 1990

25. Coma V., Couturier Y., Pascat B., Bureau G., Guibert S., and Cuq J.L. in BIODEGRADABLE POLYMERS AND PLASTICS (Vert M., Feijen J., Albertsson A.C., Scott G., and Chellini E., Eds.), Royal Society of chemistry, Cambridge, England, 1992, p.242

26. Andrady A.L., ASSESSMENT OF ENVIRONMENTAL BIODEGRADATION OF SYNTHETICS POLYMERS, *J.M.S.-Rev. Macromol. Chem. Phys.* (1994), C34:1, p. 25-76

27. David C., De Kesel C., Lefebvre F., and Weiland M., THE BIODEGRDATION OF POLYMERS: RECENT RESULTS, *Die Angewandte Makromolekulare Chemie* (1994), 216, p.21-35

28. Griffin G.J.L., SYNTHETIC POLYMERS AND LIVING ENVIRONMENT, *Pure & Appl. Chem.*(1980), 52, p.399-407

29. ChielliniI E., Cioni F., Solaro R., Vallini G., Corti A., and Pera A., *J. Environ. Polym. Degrad.* (1993), 1, p.

30. HOLMES P.A., COLLINS S.H., and WRIGHT L.H., U.S. Pat. 4,477,654 (1984)

31. Gould J.M., Gorden S.H., Dexter L.B., and Swanson C.L. in BIODEGRADATION OF STARCH-CONTAINING PLASTICS, Agricultural and Synthetic Polymers: Biodegradability and Utilization (Glass J.E. and Swift G., Eds.), ACS Symposium Series 433, Washington, DC. 1990, p.65-75

32. Griffin G.J.L., BIODEGRADABLE FILLERS IN THERMOPLASTICS, *Adv. Chem. Ser.*(1974), 134, p.159-170

33. Griffin G.J.L., U.S. Patent No. 4,016,117 (1977)

34. Griffin G.J.L., U.S. Patent No. 4,021,388 (1977)

35. Griffin G.J.L., U.S. Patent No. 4,125,495 (1978)

36. Otey F.H., Westhoff R.P., Doane W.M., STARCH-BASED BLOWN FILMS, *Ind. Eng. Chem. Prod. Res. Dev.* (1980), 19, p.592-595

37. Otey F.H., Westhoff R.P., Doane W.M., STARCH-BASED BLOWN FILMS 2, *Ind. Eng. Chem. Res.*(1987), 26, p.1659-1663

38. Goheen S.M. and Wool R.P., DEGRADATION OF POLYETHYLENE-STARCH BLENDS IN SOIL, *J. Appl. Polym. Sci.*(1991), 42, p. 2691-2701

39. Peanasky J.S., Long J.M. and Wool R.P., PERCOLATION EFFECTS IN DEGRADABLE POLYETHYLENE-STARCH BLENDS, *J. Appl. Polym. Sci.*(1991), 29, p. 565-579

40. Satyanarayana D. and Chatterji P.R., BIODEGRADABLE POLYMERS: CHALLENGES AND STRATEGIES, *J.M.S.-Rev. Macromol. Chem. Phys.(1993)*, C33(3), p. 349-368

41. Lenz R.W., BIODEGRADABLE POLYMERS, *Adv. Polym. Sci.(1993)*, 107, p.1-40

42. Seal K.J. in TEST METHOS AND STANDARDS FOR BIODEGRDABLE PLASTICS, Chemistry and Technology of Biodegrdable Polymers, edited by Griffin G.J.L., 1st ed. 1994, p. 116-135

43. Griffin G.J.L., Internatioal Patent PCT/GB 88/00368(1988)

44. Albetrsson A.C., Barenstedt C., and Karlsson S., INCREADED BIODEGRADATION OF A LOW-DENSITY POLYETHYLENE (LDPE) MATRIX IN A STACH-FILLED LDPE MATERIALS, *J. Environ. Polym. Degr.* (1993), 1:4, p. 241-245

45. Cole M.A., in Constraints of decays of polysaccharide-Plastic blends, Agricultural and Synthetic Polymers: Biodegradability and Utilization (Glass J.E. and Swift G., Eds.), ACS Symposium Series 433, Washington, DC. 1990, p.77-95

46. Becker, Gunther in PHYSICAL ,CHEMICAL AND BIOLOGICAL FACTORS INFLUENCING THE DAMAGE OF WOOD AND OTHER MATERIAL BY TERMITES, Proceeding of the Third International Biodegradation Symposium (1976), edited by Sharply J.M. and Maplan A.M., Apllied Science Pulishers LTD.,London

47. ASTM G22-76, Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Bacteria

48. J. Guillet, ENVIRONMENTAL STUDIES ON PHOTODEGRADABLE PLASTICS PACKAGING, Polym. Mat. Sci.&Eng., 1990, V63,964-949

49. W. Sung, Z.l. Nikolov, Accelerated Degradation Studies of Starch-Filled PE Films, Ind. Eng. Chem. Res. 1992, 31, 2332-2339

50. G.J.L. Griffin, BIODEGRADABLE FILERS IN THERMOPLASTICS, Adv. Chem. Ser. 1974, 134

51. Zobel H.F., in STARCH CHEMISTRY AND TECHNOLOGY, Acadamic:Orlando Fl., 285-309 (1984)

52. Hearle J.W.S., in POLYMER AND THEIR PROPERTIES Vol.1: FUNDAMENTALS OF STRUCTURE AND MECHANICS, Ellis Horwood Limited, 220, 228, 349 (1982)

53. Viboonchan S., in SPECTROSCOPY DATA FOR ORGANICS SUBSTANCE ANYLYSIS (1989)

54. Bergey's Manual of systematic Bacteriology Volume 1, (Noel R. Krieg, Editor Volume 1, John G Holt, Editor in-Chief) Williams&Wilkins Baltimore/Condone