Development of physico-chemical pretreatments to enhance the biodegradability of synthetic low-density polyethylene film

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by

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Abstract

Plastics discarded as litter are both a nuisance to man and a danger to wildlife. Low density polyethylene (LDPE) is one of the most widely used plastics but its environmental degradation is very slow. This may be in part due to its hydrophobicity making microbial colonisation difficult. Treatments were carried out to modify the surface of LDPE film in order to reduce its hydrophobicity with the aim of increasing microbial attachment, colonisation and subsequent microbial degradation.

Surfactant and vegetable oils were applied, chemical oxidation, ultra-violet light exposure and corona discharge pre-treatments were used to enhance microbial colonisation. Changes to tensile strength, extension, free surface energy and surface chemistry (by FTIR) were measured in addition to studies on microbial colonisation and degradation. Corona discharge treatment (CDT) was found to be the most successful treatment. It reduced hydrophobicity and enhanced colonisation and degradation. Results indicated that modification by CDT was only to the very surface of the LDPE film leaving its usage unaffected. Practical problems associated with most of the other treatments meant CDT was the treatment with the most potential for commercial exploitation.

After CDT there was visible biodegradation when LDPE film was exposed to aerobic compost for 100 days, but only when backing paper was attached to the film. Untreated film and film without backing paper were not visibly affected. LDPE-degrading microorganisms were isolated from the degraded LDPE film by repeated incubation in media with LDPE as the sole carbon source. Two fungi (tentatively identified as Aspergillus fumigatus and Acremonium charticola) and a Gram negative coccus were isolated. Significant biodegradation was not observed when each isolate was separately re-inoculated on to fresh CDT polyethylene film. However CDT and untreated LDPE film were degraded by the consortium of organisms in the culture using LDPE as a carbon source. The CDT-treated film showed significantly greater degradation.

It was concluded that it is possible to make LDPE film more suitable for microbial attachment and colonisation by surface modification and to thus enhance the rate of biodegradation of the film.
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2000年5月 松永昌之
Abbreviations

ATR       attenuated total reflectance
BSA       bovine serum albumin
CDT       corona discharge treatment (or treated)
CDT-LDPE  corona discharge treated LDPE
CDT-LDPE/P corona discharge treated LDPE with backing paper
FTIR      Fourier-transform infrared spectroscopy
LDPE      low-density polyethylene
MEA       malt extract agar
NA        Nutrient agar
PE        polyethylene
R.O. water reverse osmosis water
SEM       scanning electron microscopy
UV        ultra violet
Discarded plastic films are miserable in natural environment... (Ch.1, 12)

Environmental degradation is occurring to plastic film, but the speed is very slow... (Ch.1)

Rapid biodegradation was observed for surface modified LDPE film (Ch.9)

Methods of surface modification are proposed (Ch.2-8)

"Plastic degrading microorganisms" are purified and isolated (Ch.10-11)

Dr Whitney and the author with beautiful (?) Mt. Fuji (Mar 2001).
Chapter 1

General introduction

Plastic is a representative material of the 20th century as a key industrial material, compared to steel and coal which predominated the previous century. The unique characteristics of plastic such as lightness, toughness, forming-flexibility and chemical and biological stability have satisfied human demands and conveniences. However, people had to realise a new type of environmental problem that plastic waste has become a nuisance due to its biological stability. The majority of organic materials take part of the natural carbon cycle whereas most plastic wastes are only slowly involved in the carbon cycle, which results in unpleasant accumulation in the environment. Research is therefore taking place to seek microbes with an affinity for synthetic plastics. Mechanisms to enhance the biodegradability of plastic and production of new biodegradable materials are of scientific importance.

1.1. Author’s view

The 20th century was the era where the ‘mass production and mass consumption system’ developed and supported human life as well as the global economy systems. People's lifestyle has clearly been changed and become more convenient, however, we have to be aware of the serious waste problems caused by the global human consumption.
Before the synthetic technology developed, wastes only consisted of natural polymers (e.g. foods, paper, fibre etc.) and a small amount of metals and inorganics. The consistency and quantity of wastes made it possible for living organisms (macro- and micro-organisms) to degrade them. But nowadays, the quantity of waste has become enormous, and the components have become more complicated, containing synthetic or toxic compounds as well as natural or biodegradable components. The selection of waste treatment has also become difficult and complex. A number of attempts have ever been made to simplify and reduce the total amount of waste. Simplification and weight reduction of packaging materials, and the development of recycling technology can be categorised as direct methods, which contribute to saving resources. On the other hand, development of technology to classify waste types, development of environmental consciousness among consumers, and replacement of synthetic materials with biodegradable or recyclable ones can be categorised as indirect methods.

What is the best way to maximise recycling? The answer may be that classification of the wastes should be made at the very first stage of waste production, therefore consumers and industries possess the responsibility for domestic and industrial wastes, respectively. To date, recyclability has been increased significantly for paper, steel and glass materials, and high quality and high purity recycled materials can be obtained.

On the other hand, there are the other types of wastes which are not suitable for recycling. Hygiene and toxic wastes are obvious examples, but food packaging polymer film may also be defined as non-recyclable material. Shopping bags are suitable for ‘re-using’ but are difficult to recycle. Many plastics contain additives depending on their use in packaging, which will cause impurity when recycled. Plastic materials can be made from a single type of molecule and possess some level of
thickness or hardness which are only suitable for recycling (usually marked 'recyclable' e.g. drinks bottles). Plastic film can be shredded and recycled if clean and pure. Plastic discarded as litter is likely to be of mixed types, dirty and too widely spread to make collection economically feasible. The increase in composting of municipal waste has also increased the interest in the biodegradation of plastics. Therefore, methods to treat (thin) waste plastic film, as non-recyclable material, were investigated in this thesis. Biological waste treatment, to increase the biological sensitivity on this material, has mainly been considered.

1.2. Development of the plastic industry

The use of plastics in packaging has increased markedly over the last few decades, particularly for foods and drinks. The reasons for this are (Paine and Paine, 1983):

(a) Lower costs than other materials
(b) Lower energy content
(c) Wide range of properties
(d) More scope in forming and shape
(e) Light-weight coupled with strength
(f) Easier disposal after use

More than 30 different plastics are used in packaging but the most common ones are polyolefins, polyvinyls and polyesters. They may be divided into thermosetting and thermoplastic resins. The oldest of the purely synthetic plastics are the phenol-formaldehyde resins, of which Baekeland's Bakelite was the first commercial product (Baekeland, 1909). The first commercialised use of styrene was in synthetic rubbers made by copolymerisation with dienes in the early 1900's. Polystyrene was produced commercially in Germany in about 1930 and successfully in
the United States in 1937. Large-scale production of vinyl chloride-acetate resins began in the early 1920's. Table 1.1 shows the approximate dates of introduction of some of the synthetic plastics of greatest commercial interest (Billmeyer, 1984).

**Table 1.1. Some commercially important polymers and their dates of introduction.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Polymer</th>
<th>Date</th>
<th>Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1930</td>
<td>Styrene-butadiene rubber</td>
<td>1943</td>
<td>Silicones</td>
</tr>
<tr>
<td>1936</td>
<td>Poly (vinyl chloride)</td>
<td>1944</td>
<td>Poly (ethylene terephthalate)</td>
</tr>
<tr>
<td>1936</td>
<td>Polychloroprene (neoprene)</td>
<td>1947</td>
<td>Epoxies</td>
</tr>
<tr>
<td>1936</td>
<td>Poly (methyl methacrylate)</td>
<td>1948</td>
<td>ABS resins</td>
</tr>
<tr>
<td>1936</td>
<td>Poly (vinyl acetate)</td>
<td>1955</td>
<td>Polyethylene, linear</td>
</tr>
<tr>
<td>1937</td>
<td>Polystyrene</td>
<td>1956</td>
<td>Polyoxymethylene</td>
</tr>
<tr>
<td>1939</td>
<td>6,6-Nylon</td>
<td>1957</td>
<td>Polypropylene</td>
</tr>
<tr>
<td>1941</td>
<td>Polytetrafluoroethylene</td>
<td>1957</td>
<td>Polycarbonate</td>
</tr>
<tr>
<td>1942</td>
<td>Unsaturated Polymesters</td>
<td>1964</td>
<td>Ionomer resins</td>
</tr>
<tr>
<td>1943</td>
<td>Polyethylene, branched</td>
<td>1965</td>
<td>Polyimides</td>
</tr>
<tr>
<td>1943</td>
<td>Butyl rubber</td>
<td>1970</td>
<td>Thermoplastic elastomers</td>
</tr>
<tr>
<td>1943</td>
<td>6-Nylon</td>
<td>1974</td>
<td>Aromatic polyamides</td>
</tr>
</tbody>
</table>


LDPE accounts for the biggest proportion of the plastics used in packaging. One of the reasons for its widespread use is its versatility. It can be extruded into film, blown into bottles, injection moulded into closures and dispensers of all sorts, extruded as a coating on paper, aluminium foil or cellulose film, and made into large tanks and other containers by rotational casting. LDPE is relatively inert chemically and almost insoluble in all solvents at room temperature. Some softening and swelling can occur with hydrocarbons and chlorinated hydrocarbons. Permeability is low for water vapour but many organic vapours and essential oils pass rapidly through LDPE. Its permeability to oxygen is fairly high so where oxidation is likely to be a problem, LDPE is not suitable.
1.3. Food packaging plastic films and functionalised plastics

Before synthetic plastic technology was developed, natural polymers such as paper and wood were used for food packaging. Introduction of plastic materials in the food packaging industries have greatly influenced and made possible many changes in a diverse variety of both consumer and industrial packaging applications (Childs, 1974) and long distance distribution and longer preservation of foods became easier. Generally, food can easily deteriorate under biological, chemical and physical stress, which results in reducing the quality, but this deterioration can be minimised if appropriate packaging material is utilised. Food packaging grade LDPE, which has been a subject of this project, has been the most rapidly growing segment in all the packaging industry. It is expected that this growth will continue in the next century.

Many varieties of polymer were discovered and synthesised during the development of the polymer industries, not only using the pure plastic alone but also in plastic composites and with other materials. For example, combination of more than two types of synthetic plastics, combination of organic polymers and non-organic monomers, composites of natural and synthetic polymers (such as paper and plastics to increase wet strength for milk cartons), synthetic polymer having some other additives (such as carbon black or titanium dioxide colouring pigments). These polymers may be called “functionalised polymers” and were used for particular purposes to maximise the benefits of packaging or to make the product more attractive to consumers.

As an example, ‘Anti-fogging’ polymers have widely been used in fresh food packaging industries (Hasegawa, 2000; Kubotsuka, 1998; 1999; Nakamura, 1998). Fogginess on fresh food packaging develops when small water droplets attach to plastic film due to sudden changes in
temperature or humidity. It may reduce visibility of the contents (foods), and dripping of attached water droplet will cause deterioration or discolouration of the contents. In greenhouses, the fogginess of the constructive (outer) film reduces light-transparency and may cause inferior growth of the crops in the greenhouse. The fogginess happens because the surface of plastic films is generally hydrophobic (Kubotsuka, 1998; Nakamura, 1998) hence vapour adhered to the film tends to form a droplet. Shifting the surface condition to be hydrophilic can therefore minimise the formation of such water droplets, and a number of surface treatments have been attempted. Surface modification using surfactant is a major method to obtain a hydrophilic surface instantly. Applying non-ionic surfactants is common because many of them, such as the glyceric ester of fatty acid (Figure 1.1), are safe for human consumption and thus can be used for food packaging purposes (see Section 4.4. for details). Some of the methods of the ‘anti-fogging’ treatments gave ideas for this project because the enhancement of the surface hydrophilicity was considered to be important as well. Decreasing hydrophobicity may facilitate colonisation by microorganisms and enhance biodegradation.

\[
RCO-O-CH_2CH(OH)CH_2OH
\]

*Figure 1.1. Chemical structure of glycerine ester of fatty acid.*
1.4. Disposal of synthetic plastics

Modern polymer technology has mainly aimed to produce stable materials giving protection against changes in the surrounding environment. Durability against high temperature, strong pressure and other mechanical stresses against oxidation or water absorption, chemical stability, and macro- and micro-biological attack were required. Therefore, polymers having such stabilities have been recognised as high quality’ materials. However, people had to realise that the ‘quality’ becomes a nuisance when the polymers are discarded into the natural environment. Global environmental problems due to the stability (or non-degradability) of waste plastic have been indicated. Plastic wastes contribute a major part of domestic waste (Table 1.2).

<table>
<thead>
<tr>
<th>Composition</th>
<th>UK</th>
<th>USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper</td>
<td>35-60</td>
<td>32.0</td>
</tr>
<tr>
<td>Garden waste</td>
<td>2-35</td>
<td>14.8</td>
</tr>
<tr>
<td>Food waste</td>
<td>2-8</td>
<td>8.5</td>
</tr>
<tr>
<td>Metals</td>
<td>6-9</td>
<td>6.3</td>
</tr>
<tr>
<td>Glass</td>
<td>5-13</td>
<td>6.4</td>
</tr>
<tr>
<td>Plastics</td>
<td>1-2</td>
<td>11.8</td>
</tr>
<tr>
<td>Textiles/Woods</td>
<td>1-3</td>
<td></td>
</tr>
<tr>
<td>Rubber/Leather</td>
<td>1-3</td>
<td>19.3</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

(Scragg, 1999)

Plastics constitute an increasing problem for disposal, and the MARPOL (marine pollution) agreement in 1992 banned the disposal of plastic at sea (McCall, 1996). The report indicated that unsuitably discarded plastic materials sometimes caused serious damage to wildlife (Guillet, 1995). Suffocation, entanglement or obstruction in the guts of animals due to eating such discarded plastic materials and this happens both in aquatic...
and terrestrial environments. Also plastic materials become water reservoirs where mosquito and other disease vectors breed, which is recognised as an indirect problem that plastic wastes cause. The latter indirect problem is more serious especially in tropical islands such as Fiji, thus waste control is strictly managed. No international agreement has been made yet for the treatments of plastic wastes, and the waste methods vary depending on the political and geographical situation in each country.

Wastes can be classified into three types in terms of the restriction or allowance of the treatment (Figure 1.2). Plastic waste forms part of 'solid domestic, industrial and agricultural waste', and it has no restriction on environmental disposal because the material itself does not normally produce either toxic or harmful compounds in landfill or aerobic digestion. Landfill and incineration are the most common treatment for plastic waste, but recycling is becoming more popular. Also, composting treatment, which utilises biological activity to degrade waste (which includes plastics) under aerobic condition, is considered as a new type of waste treatment. Features of these common waste treatments are described as follows.

![Diagram of waste treatment methods](image)

**Figure 1.2.** Methods for the treatment and disposal of solid domestic waste (including plastics), excess sewage sludge and toxic liquid waste (Scagg, 1999).
1.4.1. Landfill

Perhaps the oldest method of disposal is burial. This treatment has been applied in countries or areas where enough suitable landfill sites can be found. Because biodegradation of plastics is so slow or non-existent under anaerobic conditions, the burial treatment of plastic or other wastes made from petroleum resources can be considered to return the resources into land as fixed carbon. Which means that no carbon dioxide is released into the atmosphere with this treatment. Because the production of packaging material is increasing faster than the processes of recycling and reuse, the amount of packaging waste is rapidly increasing. Most of this is at present going to landfill sites. Suitable sites are therefore either filled already or rapidly becoming filled. Initially most landfill sites were uncovered so that any leachate derived from the degradation of the wide variety of contents could be dispersed in the surrounding soil, and then contaminate the groundwater. Nowadays there are stricter regulations to avoid contamination of the groundwater and the sites have to be lined with an impermeable barrier or membrane. Materials such as clay and plastics such as polyethylene, polyvinylchloride and rubber have been used as barriers, based on compacted soil (Scragg, 1999). The waste is compacted by purpose-built vehicles and each day's waste must be covered with soil or ash. The compaction controls the air and water transfer, reduces the volume and reduces the possibility of spontaneous combustion by reduction of oxygen. Also little waste degradation by microorganisms can be expected because the covered wastes tend to become anaerobic very rapidly, and no photo-oxidation and photo-degradation is expected since sunlight cannot reach deeply into the landfill. Figure 1.3 shows the changes in gases in a landfill site over a number of years (Scragg, 1999). Waste matter becomes anaerobic soon after being capsulated and produces a steady level of methane typically for two years.
A landfill site may be used for construction or agricultural purpose after the accumulated waste reaches a certain level and the site is closed. However, a certain period of time is needed before the area can be used for another purpose. Landfill sites under construction will be active for up to 100 years before the site is suitable for alternative use and is issued with a Certificate of Completion in the United Kingdom. To obtain this certificate the biodegradable content of the landfill must have reached a steady state (stabilised) and metals and toxic contaminants flushed out. Thus landfill sites need careful management and landfills should be regarded as anaerobic reactors rather than just sealed disposal systems. There are some ethical arguments with the landfill treatment of domestic
wastes. Landfill sites are usually built up in suburbs or places where normally people do not live or visit. Therefore, tonnes of wastes produced in cities are only moved to such hidden places away from human attention. These wastes surely remain on the very top of the strata of the earth for a long time, and our descendants may suffer from these wastes. There is a proverb in Japanese, “lid on something smelly (indirectly meaning ‘hush up the scandal’)”, and the author believes that this proverb can be applied to this landfill treatment. It is often difficult to stabilise a land fill site. For example, there was a tragedy in the Philippines where loaded domestic wastes in a city collapsed and a number of people died (Asahi shinbun, 2000). The landfill treatment may no longer be practical under recent economic systems supported by the ‘mass production and mass consumption’.

1.4.2. Incineration

Incineration can be used for industrial, domestic and toxic wastes, since incineration is known as a safe waste treatment because even toxic compounds can be degraded under the high temperatures of combustion. Plastics have higher calorific value than coal, and it may be logical to use plastic wastes instead of paper or other lower calorific wastes. However, public opinion is turning away from burning waste because of the perceived dangers of toxic emissions. Incineration is not seen by the public to be a safe process and this conception may be difficult to reverse. On the other hand, incineration has been done traditionally in Japan. Plastics are burnt with other garbage and the collected heat is effectively used as electric energy. For example, heat energy produced from incineration plants is used for heating water and air in swimming pools and is also used as the local electric energy supply.

The critical factors for effective combustion are temperature, the maintenance of high temperature, and the mixing system of the waste to supply enough air for complete combustion. There are a number of
combustion systems which will operate at 1500–3000 °C, the
temperature required to break down organic wastes; these include rotary
kilns, liquid injection, fluidised beds and multiple hearth designs. The
advantages of incineration are the reduction in volume, the ability to treat
toxic materials and the fact that incineration can be constructed in areas
where landfill disposal is not available. The disadvantages of incineration
are that the process can be expensive to operate and to construct, it is not
a complete disposal solution as the resulting ash will need to be disposed
of in a landfill site and this ash may contain high levels of metals. Also
incineration produces particulates and flue gases. The flue gases from
incineration can contain hydrogen chloride, sulphur oxides, nitrogen
oxides which can be removed by wet scrubbing, carbon monoxide which
can be formed by incomplete combustion, and carbon dioxide which
increases the level of greenhouse gases. The incineration of chlorinated
compounds at low temperatures (300–500 °C), which can happen with
excess air, will allow the production of both toxic and non-toxic dioxins
(Figure 1.4) some of which are extremely toxic and/or carcinogenic. The
formation of particulates is also to be avoided and electrostatic
precipitation will need to be incorporated in incinerator systems (Scragg,
1999; Takeuchi et al., 1996).

Figure 1.4. Chemical structure of dioxins (Takeuchi et al., 1996).
1.4.3. Recycling

Recycling is another option in the hierarchy of waste reduction and can involve the recycling of material produced either during the manufacturing process or from the product itself after manufacture (Figure 1.5). Recycling makes considerable sense. Recycling metals and glass can save 95% of the energy that would be needed to mine new metals and make new glass from sand. Most recovery and recycling systems concentrate on the reuse of metals, glass and paper. For these materials, a number of recycling systems have been developed, including the use of two or three bins to segregate the waste at collection. However, systems to recycle plastic material have not been developed so much. Plastic is potentially recyclable but not suitable when contaminated with other materials. To overcome this problem, there is an interesting example of a recycling system which is successfully running in Taiwan and Korea (Asahi Shinbun, 2000): a deposit system has been introduced for all types of plastic bottles. Consumers bring used plastic bottles (which must be washed to remove any contamination) to retailers or recycling stations to have the deposit back. Also the same system is applied between retailer and manufacturers.

1.4.4. Composting

Composting can be defined as ‘the biological stabilisation of wastes of biological origin under controlled aerobic conditions’. In composting organic compounds contained in waste can be decomposed by microorganisms under aerobic conditions. Temperature and moisture are also controllable and the speed of the microbial degradation is much faster than that in landfills. The composting system may be uncovered to allow enough oxygen to maintain aerobic condition, and ventilation or mixing system may be added if necessary. Alternatively, it may be covered in one of various forms of bioreactor.
Conventional design

Design for recycle and reuse

Figure 1.5. Industrial designs for conventional processes and those incorporating recycling during manufacture and after product use (Redrawn from Hill, 1997).
The open system is cheaper but the closed system eliminates potential problems with vermin and gives closer control over the composting conditions, which usually results in more rapid composting of the waste. Due to rapid metabolic activities of microorganisms under aerobic conditions, the temperature in the system sometimes exceeds 50 °C. This high temperature inactivates many pathogenic organisms (Plat et al., 1994), and is important in the chemical oxidation of many of the components of composted waste. Depending on the waste that has been composted, the process can produce a material suitable for application to agricultural land as a fertiliser or a microbiologically safe material of reduced volume to go to landfill.

1.5. Trend of biodegradable materials

Since people recognised that human industrial activities have affected the global ecosystems. Environmental consciousness and regulations have become stricter especially for such materials that have a high impact on the environment. Plastic is a material that has supported modern industries due to its high durability against environmental deterioration. However, its durability has a down side. Being “non-biodegradability” or of “low-biodegradability” has negative environmental impact. Introduction of “biodegradable materials” boomed in the 1990s not only for plastic materials but also for many industrial materials, and they were commercialised as “environmentally friendly” materials.

There is a trend to introduce such “environmentally friendly biodegradable materials”, however, it may be required to establish certain definition of “biodegradable materials”. Establishing definitions is also required when estimating biodegradability of a plastic material scientifically.

According to the latest version of the British Standard (also European
Standard), "ultimate biodegradability" is defined as: "breakdown of an organic chemical compound by micro-organisms in the presence of oxygen to carbon dioxide, water and mineral salts of any other elements present (mineralisation) and new biomass or in the absence of oxygen to carbon dioxide, methane, mineral salts and new biomass" (BS EN 13432, 2000). In another words, this definition means that biodegradation has not been completed if a tiny amount of solid (= polymer) remains.

However, the author and Whitney et al. (1993) suggest that most of the environmentally negative impacts can be removed if the plastic is embrittled (or collapsed), because most of the problems are caused by the physical characters and not the chemical nature of the plastic. Thus, such materials do not need to be totally mineralised (considered to be the ultimate biodegradation). Also, the release of carbon dioxide into air, as a result of the mineralisation of polymer, will contribute to the global warming of the earth, which results in another type of environmental problem. Therefore, the author appreciates the other type of definition for the biodegradation, that is, "molecules of a material are reduced to environmentally benign subunits under normal environmental forces (including biological decomposition, photodegradation and hydrolysis)" (Whitney et al., 1993). However, here are alternative, more practical and realistic definitions to help deal with the disposal of plastic wastes.

When plastics or other fossil-originated materials are considered, what gives the negative environmental impacts is not the single molecular structure but the macro formula after casting to make products such as bottles or bags. It is therefore possibly that the negative environmental impacts can be significantly reduced if the macro structural formula is collapsed in to small enough subunits to no longer impact on the environment. As this research followed this fundamental concept, the main purpose of this project was established to find methods and mechanisms for plastic materials to be embrittled to environmentally
benign subunits rather than to be decomposed totally and mineralised. Further mineralisation could happen by microbial metabolism and natural carbon cycles.

As a solution to enhance the embrittlement of plastic materials, methods of blending natural polymers (e.g. starch) into synthetic polymers have been performed (Whitney and Williams, 1976; Griffin, 1985) and widely accepted in the recent industrial market. This material can be embrittled easily in a bioactive environment because the blended natural polymers are basically biodegradable and can be attacked by microorganisms. The molecules of the natural polymers are scattered into that of the synthetic ones to form a matrix, so that the overall mechanical and physical properties will be significantly changed even if only the natural polymer phases are consumed by microorganisms. As a result of fragmentation of the overall structure, the synthetic polymers form small enough subunits to be environmentally benign.

Apart from the natural-synthetic blended polymers, other biodegradable polymers have been produced. These include biopolymers (producing by living organisms), chemically modified synthetic polymers and modify natural polymers (such as chitosan or cellulose) to produce plastic-like materials. However, it is difficult to produce and commercialise such “man-made biodegradable” plastics in terms of cost. Usually the prices of producing the biodegradable polymers are much more than that of producing the current range of synthetic polymers. Therefore, the biodegradable materials tend to be used for special purposes like medical uses, educative or enlightening purposes for environmental campaigns, or business strategic purposes. Recently, Sony Co., Ltd. reported that all plastic-cases for music tapes, video tapes and CD will be replaced with biodegradable materials in early 2001 in Japan. The author hopes that every company using plastics (regardless of the quantity) should make such efforts even though it may cost more.
Using natural polymers instead of synthetic ones sounds more positive in the environmental aspect, however, it is not practical to expect that all synthetic plastics are replaced by the natural polymers. If all food-packaging plastic films in the world were replaced with paper materials, enormous areas of forest would be needed to provide the amount of wood required (Scott and Gilead, 1995). Secondarily here is a factor regarding the cost and energy effectiveness of plastics in packaging, which was initially less apparent but which is equally important in the context of the environment. Polyethylene can protect goods more effectively than twice the weight of paper. Mosthaf (1990) estimated that the weight and energy consumption of polyethylene and paper, for example: 1.8 tonnes of paper is needed to produce 50,000 sheets of shopping bags consuming 69.0 GJ of electric energy, whilst 0.9 tonnes of polyethylene is needed consuming 38.0 GJ of the energy. Therefore, it has to be remembered that producing a clean environment may involve considerable energy utilisation.

1.6. Biodegradability of synthetic plastics

Researchers have principally focused on producing new types of biodegradable materials to overcome the existing environmental problems that synthetic polymer produce. What about the biodegradability or environmental affinity of existing synthetic plastics? Are they totally inert to any biotic reactions? Synthetic plastic has been recognised as a biologically inert material but recent scientific articles have reported the biodegradability of several synthetic plastics.

In the 1960's plastic was believed to be chemically and biologically inert, but a few researchers started to doubt if the theory was perfectly true. At that time, polyurethane was widely used for fibre, synthetic rubber and foamed plastics etc. (Arai et al., 1993). It was expected that 40 million kg of rigid urethane foams would be produced in 1968 (Dombrow, 1965).
Kaplan et al. (1968) investigated moulded shelter construction and fabric fuel storage tanks coated by polyurethanes. A survey of the microbial aspects of polyurethanes was presented and concluded that at least polyester polyurethanes were fairly resistant to fungal attack but were biodeteriorated in an outdoor condition after a long period of exposure (Darby and Kaplan, 1968). The susceptibility of the polyurethanes was related to the number of adjacent methylene groups in the polymer chain. The same research group eventually concluded that high molecular weight synthetic polymers such as polyethylene were resistant to biodegradation (Kaplan, 1975).

In the 1970's research was more developed and a number of reports suggesting the biodegradability of synthetic plastics were published. Wallhauser (1972) noted that polyethylene film retrieved from his garbage dump excavation had deteriorated physically with some indication of bacterial presence. Many scientists might have been encouraged with this report since polyethylene was known as one of the most environmentally stable materials.

Besides, Potts et al. (1972) reported that the biodegradability can only be applied in lower molecular regions of the molecule and no biodegradability exists in higher molecular regions. A molecular weight of around 500 can be the border to decide whether or not the polymer is biodegradable. They added that high molecular weight polyethylene can be broken down due to photo-degradation if exposed to sunlight.

Hosoya et al. (1978) reported that synthetic polymers, especially water-insoluble ones, are difficult to be taken up and metabolised by microorganisms. It can be anticipated, however, that the oligomers of the water-insoluble polymers become soluble and assimilable by microorganisms, and that the oligomers can induce polymer degrading enzymes. Similarly, Cornell et al. (1984) investigated the biodegradability
of general polyolefin like polyethylene, polypropylene and concluded that only the oligomer fractions derived from high molecule polyolefin by UV irradiation supported microbial growth, but the high polymers gave minimal or no growth.

Gradually, biodegradability of synthetic polymers which have carbonyl or carboxyl parts in the molecular such as polyurethanes (Parahirana and Seal, 1983, 1984, 1985), polyester (Kay et al., 1993), poly (vinyl acetate) (Nieder et al., 1990; Trejo, 1988) has been proved and their correspondent degrading microorganisms were also identified.

Polyethylene is one of the most abundantly used materials among all types of synthetic polymers. If the biodegradability of this material is confirmed, a new type of biological waste treatment will be practically introduced e.g. sanitary composting systems (see Chapter 11). It is now widely known that many microorganisms can utilise hydrocarbon (paraffin) as a carbon source. Reports for hydrocarbon degradation by marine fungi and bacteria have been presented for the requirements to investigate the bioremediation of crude oils spilled from tankers (Bentham et al., 1987; Coony et al., 1995; Kirk et al., 1991; Kirk & Gordon, 1988; Pierce et al., 1975; Scott, 1993).

Biodegradability of oxidised polyethylene (at least in powder condition) was confirmed by an identified stain of Penicillium simplicissimum, YK (Tani et al., 2000), which exists in many types of environment all over the world.

Albertsson's group are pioneers for their studies on biodegradability of LDPE films. They investigated its biodegradability in laboratory and field models with a long span of experiments. A unique experimental method was applied by Albertsson (1980) that $^{14}$C labelled polyethylene was used for a biodegradation study, and evidence of the mineralisation due to the microbial catabolism was detected with measuring $^{14}$CO$_2$ levels. With this
experiment, only film originated carbon can be detected in the system, whereas other carbon sources exist such as respiration by the microorganisms. The same group also examined the biodegradation of high-density (linear) polyethylene (HDPE) film (MW 93,000) for two years and found that the short-chain oligomeric fraction contained in HDPE film was the main degraded component (Albertsson & Banhidi, 1980).

In another approach to investigate the biodegradability of polyethylene films, lignin-degradable fungi were applied since both lignin and polyethylene have rigid chemical structures and are normally unaffected by microbial attack. A significant reduction in tensile strain (elongation) of LDPE film was observed on an *IZU-157* strain, which is an unidentified strain isolated from rotted wood in Japan and is known as a lignin-degrading fungus (liyoshi et al., 1998). It was suggested that ligninolytic activity of lignin-degrading fungi appeared as a secondary metabolic event, and nutritional nitrogen or/and carbon limitation allowed extensive degradation of lignin, therefore, the ligninolytic activity was related to polyethylene degradation (Gold and Alic, 1993; Keyser et al., 1978; Kirk et al., 1978). The degradative enzyme was purified from the strain and was identified as a type of manganese peroxidase. Rapid enzymatic degradation was also again confirmed (Ehara et al., 2000-a; liyoshi et al., 1998).
Ohtake's group examined the effect of microbes on several polymer samples buried under bio-active soil for over 32 years (Ohtake et al., 1996-a, 1996-b, 1998-a, 1998-b). They found that LDPE films degraded considerably under biotic condition, while no evidence was obtained for the degradation of urea formaldehyde resin, polystyrene, and poly (vinyl chloride). The part of LDPE which was directly in contact with bioactive soil was severely degraded and characterised by whitening as a result of the detection of carboxylic acids (−COOH) and ketones (including >C=O) by FTIR analyses (see Section 2.3.2. and Section 3.3. for details of FTIR). On the other hand, the part which was not in contact with soil was still transparent. By comparison of the results of the biodegradation of LDPE under well controlled environments, they concluded that the degradation of the clear parts proceeds by the normal oxidative process, while degradation of the whitened parts was explained by the complex mechanism of oxidative degradation and biodegradation. Their results show that even the high molecular weight LDPE is biodegradable (to form low molecular weight compounds by a biotic oxidation process) if they were concealed under bioactive soil for several tens of years. It was also indicated that production of bioactive aerobic sites such as compost might be essential for the acceleration of the biodegradation of LDPE. There is little evidence of biodegradation of LDPE (and most other plastics) under anaerobic conditions.

The history of the research on the investigation of synthetic plastic materials now confirms the biodegradability of low-density polyethylene. However the degradation period is still slow if compared with the human life cycle or the speed of the production of virgin materials.
Chapter 2

Research outlook and materials

2.1. Research outlook

As explained in Section 1.5, Ohtake's group reported that more than 30 years of biotic exposure was required for significant LDPE biodegradation in a bioactive and aerobic soil environment. But how should this "30 years" be appreciated? Some may think that it is very short for the degradation period because all synthetic polymers had historically been recognised as biologically inert. Moreover, it falsifies the existing theory that synthetic polymer wastes would last semi-permanently without being degraded in any environment and our descendants would suffer from the cumulated plastic wastes produced in the last few decades. Others may think that the 30 years for incomplete degradation is still too long. It is reasonable to compare the production rate of virgin LDPE films and degradation rate of the waste ones. If a biodegradable material is produced, the degradation speed should be related to the rate of production. Thus the biodegradation of LDPE should be considered to be very slow. Furthermore, not all discarded plastic materials can be exposed to suitable environments where biotic, aerobic or rich in sunlight exposure (for inducing initial photo-degradation) conditions are ensured. Materials discarded into unsuitable environments will keep their formula without being degraded, or be degraded even more slowly and may therefore be considered semi-permanent.
Treatments that enhance the rate of degradation after use but do not affect the physical properties of films whilst in use are clearly important, especially for those films most resistant to (bio-) degradation. Progress of microbial colonisation on plastic films was considered as a minimum requirement to initiate any degradation. The degradation proceeds subsequently and the process mechanisms can be divided into several individual ‘steps’. Attempts were made to establish adequate pretreatment at each ‘step’ in order to accelerate the total biodegradation process.

Figure 2.1 illustrates the key scheme of this research showing a table of ‘biodegradative conditions’ of LDPE films and the changes in molecular weight at different degradative stages.

When a high polymer material is exposed to the soil surface environment, a number of natural processes affect the material. For example, ultraviolet from sunlight, lead to photo-oxidation or photo-degradation, and heat from sunlight lead thermal-oxidation or thermal-degradation as a physical process. In composting, most of the material will be protected from ultraviolet light (none will be exposed to UV in a closed compost system) but substantial microbial heating is likely to occur. All these factors are expected to play a role to embrittle the polymer material and to alter the material conditions (especially for the surface) to be more susceptible for microorganisms to attach and colonise. After microbial colonisation, further biotic degradation proceeds due to hydrolysis or other biochemical reactions.

After the polymer becomes degraded to form smaller molecular compounds, microorganisms then start to take up the compound to metabolise as their nutrient (mainly carbon) sources. Eventually the polymer is mineralised and the smallest compounds (e.g. carbon dioxide and water for polyethylene) are released.
Film's condition

Production ↓
  In use ↓
  Wasted/discarded ↓
  Microbial colonisation ↓
  Hydrolysis ↓
  Metabolised by microorganisms ↓
  Formation of visible holes etc. ↓
  Mineralisation

High Polymer

Embrittlement

Low molecule

Mineralisation

MW

Discarded, waste disposal or incinerated

Exposed to abiotic (or anaerobic) environment

1

Slow aerobic degradation

2

Exposed to abiotic (or anaerobic) environment

Incinerated (instant release of CO₂)

Exposed to abiotic (or anaerobic) environment

3

Accelerated aerobic degradation (with adequate pretreatment, and exposed to suitable compost)

4

In use

Exposure time

Figure 2.1. Key scheme of this research.
However, the degradation rate varies due to the conditions where the polymer is exposed. On the right hand side of Figure 2.1, degradation progresses are illustrated with respect to exposure time.

**Curve One.** If a polymer is exposed to non-biotic and/or anaerobic environment, even the initial microbial colonisation can hardly be progressed, which results in “zero-degradation” in the environment. Polymers discarded to such environments last indefinitely.

**Curve Two.** If a polymer is exposed to a suitable environment for biodegradation such as biotic and aerobic conditions, biodegradation processes can progress although the degradation speed is still very slow (Ohtake *et al.*, 1996-a).

**Curve Three.** ‘Degradation’ can proceed most quickly if incinerated. However, incineration is not feasible for plastic discarded at low density such as litter (see Section 1.4. for details of incineration).

**Curve Four.** *Accelerated biotic degradation* (which is being proposed).

Degradation following curve one (anaerobic) will not take place in the litter or compost environments. Curve two illustrates a rate that has considerable environmental impact (as previously discussed), and is too slow for composting. Whilst curve three (incineration) though desirable in some situations is not without its problems and is not always possible. Thus, curve four was drawn as the most desirable option. This curve means that initial embrittlement can be completed rapidly to form smaller fragments of the polymer. The small fragment is still an intermediate composite if the total degradation is considered, but they no longer have a significant impact on the environment. Small enough fragments of plastic material in the environment are just like sand grains...
in soil, and are not an environmental nuisance. Many disposal problems of plastic materials are caused by their bulk rather than their actual volume or chemical formula. This shortens the capability of a landfill as well as creating problems when it is inappropriately discarded. Therefore, many environmental problems can be avoided if the plastics are fractured into small enough fragments. Such rapid embrittlement does not usually occur in natural environments but appropriate and adequate pretreatments may accelerate this process.

Formulations already exist which cause the plastic to become brittle at predetermined times after manufacture. However the desired working life of the plastic film is usually indeterminate. (It is often stored for long periods before use and the applications are likely to call for different periods of use.) Therefore treatments to accelerate degradation must not only leave the physical and chemical properties unaffected but must not accelerate degradation until after the useful life of the plastic i.e. when it is discarded. After forming small fragments of plastics, however, a rapid degradation should no longer be needed, in fact low mineralisation means a slower rate of carbon dioxide release. Degradation mechanism of some plant materials may be adapted to this degradation curve (curve four). For example, wood can quickly deteriorate by attack from living organisms or other physical processes (e.g. rainfall, sunshine exposure and temperature changes), but not completely decomposed for a long time. The non-degraded compounds also work to improve soil structure.
2.2. Experimental environment

The general concept of this research was to observe microbial phenomena on food packaging grade plastic films after being exposed to a biotic environment. Food packaging grade low-density polyethylene (LDPE) film was used in a series of experiments. The reasons for the selection of low-density polyethylene film were that:

1) Polyethylene used to be believed as one of the most inert materials for any biological reactions of all synthetic polymers. On the other hand, a number of researchers have reported the biodegradability of LDPE film (albeit very slow), hence studying the biodegradability of LDPE film from a different approach of investigations plays great scientific importance.

2) Polyethylene has many desirable properties including: strength, flexibility, waterproofness, ease of fabrication and forming, and low price (Table 2.1). For these reasons, polyethylene is widely used for food packaging as well as other packaging purposes and discarded wastes into the environment demonstrate more negative roles than other plastic materials.

Table 2.1. Plastic packaging materials used in the UK

<table>
<thead>
<tr>
<th></th>
<th>Films</th>
<th></th>
<th>Rigids</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Qty (kt) %</td>
<td>Qty (kt) %</td>
<td>Qty (kt)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyethylene</td>
<td>753  50.8</td>
<td>179  12.1</td>
<td>932</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypropylene</td>
<td>61   4.1</td>
<td>116  7.8</td>
<td>177</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polystyrene</td>
<td></td>
<td>124  8.4</td>
<td>124</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVC</td>
<td>15   1.0</td>
<td>120  8.1</td>
<td>135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyester</td>
<td>3    0.2</td>
<td>87   5.9</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>8    0.5</td>
<td>17   1.1</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>840  56.6</td>
<td>643  43.4</td>
<td>1483</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Packaging and Industrial Films Association (1995)
3) Polyolefin is now widely recognised as a relatively ‘environmentally friendly material’ whereas using chlorinated material such as poly(vinyl chloride) is warned against after the dioxin problem was recognised. The chlorinated plastics have therefore tended to be replaced by polyolefins including polyethylene for many purposes.

![Figure 2.2. Molecular arrangement of HDPE, LDPE and LLDPE.](image)

4) Low-density polyethylene (LDPE) is more widely used than high-density (HDPE) or linear-low-density polyethylene (LLDPE). Figure 2.2 shows the molecular arrangement of HDPE, LDPE and LLDPE. LDPE is rather more readily environmentally degraded than HDPE (Figure 2.3).

![Figure 2.3. Relative changes of tensile strength of plastic films (polypropylene and polyethylene) exposed to bioactive fertiliser up to 12 months (Source: Ohtake et al., 1999).](image)
Since the suggested pretreatments were proposed to modify the surface of polyethylene film, it was expected that most of the chemical compositions would not change significantly, thus there will be no significant reduction in recyclability. On the other hand, synthetic polymer mixed with natural polymers such as starch (mentioned in Chapter 1.5) is not suitable for recycling although such materials are more easily biodegraded. This is because the natural polymer contaminates purity of recycled materials. As recycling is recognised as the most economical and environmental-friendly way of treatment, it is necessary that any materials retain their recyclability, even if they have biodegradability. In the same way, chemically modified synthetic plastics work to reduce the quality and purity of the recycled material when they are involved with other abundant synthetic plastics.

Some of the suggested pretreatment in this study such as the exposure treatments retain the bulk chemistry (but only targeted to the surface) so that it is expected that the pretreated materials are also suitable for recycling. However, some of the treatments used in this thesis have a major effect on the bulk chemistry of the plastics, so much so that they may no longer be considered by recyclers as a known material.

### 2.3. Backgrounds to the estimation techniques used on surface modified films

To accelerate the degradation speed, attention was mainly paid to the surface condition of LDPE film because it was considered that low surface energy of the substrate (hydrophobicity) results in poor microbial colonisation. Methods of surface modification were investigated which did not damage the mechanical properties, such as tensile or water-repellent properties. A number of pretreatments and their subsequent microbial colonisation were investigated. These included pretreatment
with, surfactant, oils (Griffin, 1985), ultra-violet light and corona discharge treatment. This latter pretreatment seemed to be the most promising and was therefore most fully investigated. All the details (background, method, results and discussions) are individually considered in Chapters 4–8.

After these pretreatments, the conditions of the films were estimated with investigating the following categories. These are described briefly in the following text (the correspondent experimental methods are mentioned in Chapter 3)

- **Surface energy** (by means of water contact angle measurement)
- **Chemical changes** on the surfaces (by means of FTIR measurement)
- **Mechanical strength** (by means of tensiometry measurement)
- **Surface roughness** (by means of SEM analysis)
- **Microbial colonisation** (by means of visible observation and protein assay measurement)
2.3.1. Surface energy

Changes of surface energy of the substrate (LDPE film) due to applied pretreatments were investigated. It is considered that there is a correlation between microbial attachment and the surface free energy of the substrates. The higher the surface energy becomes, the more the microbial attachment usually develops. Normally the surface energy of LDPE film is extremely low hence the susceptibility by colonisation to microbes is low (Table 2.2). It is therefore expected that the microbial susceptibility can be increased if certain surface modifications are applied to increase the surface energy. Free surface energy of a substance affects the water contact angle with it. Low free surface energy produces a high contact angle (Table 2.2)

<table>
<thead>
<tr>
<th>Surface</th>
<th>Water contact angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylene (low density)</td>
<td>94</td>
</tr>
<tr>
<td>Poly (vinyl chloride)</td>
<td>87</td>
</tr>
<tr>
<td>Poly (vinylidene chloride)</td>
<td>80</td>
</tr>
<tr>
<td>Poly (vinyl fluoride)</td>
<td>80</td>
</tr>
<tr>
<td>Poly (vinylidene fluoride)</td>
<td>82</td>
</tr>
<tr>
<td>Poly (tetrafluoroethylene)</td>
<td>108</td>
</tr>
<tr>
<td>Poly (ethylene terephthalate)</td>
<td>81</td>
</tr>
<tr>
<td>Poly (methyl methacrylate)</td>
<td>80</td>
</tr>
<tr>
<td>Nylon 6-6</td>
<td>70</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>91</td>
</tr>
<tr>
<td>n-Hexatriacontane</td>
<td>111</td>
</tr>
<tr>
<td>Paraffin</td>
<td>108</td>
</tr>
</tbody>
</table>

Source: Arai et al., in Polymer Chemistry, 1993.

Surface energy of the substrate in terms of the ability of microbial attachment is discussed and in some scientific reports (Fletcher and Pringle, 1985; Skvarla, 1993; Dexter et al., 1975). They basically
discussed microbial attachments from the view of electrostatics for both substrate and the microbes. There may be another indirect effect to determine surface condition in terms of the wettable area where microorganisms can survive and attach the plastic, which is considered in this thesis. Water is one of the essential elements necessary for any living organism to grow, thus richer microbial growth could be expected on a substrate where water could easily spread. Therefore, water contact angle measurement has importance in order to estimate surface condition for microbial growth as well as observing surface energy.

When the correlation between water contact angle and “wetting area” was examined with a constant small volume of water, applied as a droplet on different types of surfaces, a hypothesis can be confirmed. When 6 µl of water droplet is formed, a trend shown in Figure 2.4 was observed. The wetting surface area became about double if contact angle is reduced from 90° (non-treated LDPE film) to 40°, and became four times wider if reduced to 20°. Thus increasing the proportion of the surface available for microbial attack (this is clearly illustrated by the results shown in Figure 2.4). Increasing the wettability could therefore be recognised as an

![Figure 2.4. Changes of wetting surface area of surfaces having different water contact angle (data obtained from preliminary experiment).](image)
essential factor to obtain susceptible surface conditions for microbial colonisation and degradation. There have been many scientific studies investigating the relationship between surface energy and microbial attachment and growth. Most of these studies were intended to *minimise* microbial attachment by *reducing* the surface energy of the substrate because microbial attachment to substrate is usually considered to be negative factor for industrial materials (so called 'biodeterioration'). In the process of biodeterioration, a biofilm is usually formed on the surface of the substrate and then adhered microorganisms attack the substrate to deteriorate. The term 'biofilm' is usually applied to the development of a microbial population, or more typically a community on a surface which is submerged in an aqueous environment (Chamberlain, 1997). The formation of biofilms or microbial adhesion on the surface of industrial materials leads to serious problems. Microbial adhesion inside pipe networks (fresh water, sewage, oil pipes, etc.) reduces the smooth flow of liquid. For mineral water bottling, the formation of biofilm results in reducing the quality of the contents and reducing the expiry period of the product. In low nutrient environments, such as in mineral water, the growth of microbial cells may be enhanced at the solid:liquid interface in response to the presence of adsorbed organic nutrients, resulting in biofilm formation (Kefford *et al*., 1982; Zobell and Anderson, 1936). Investigation of the surface physico-chemical properties of mineral water bottles was important for the distribution manufacturer to select materials possessing low surface energy (Jones *et al*., 1999).

Surface energy also plays an important role in defining the possibilities of supra- and subgingival plaque formation. Most bacteria in oral cavities can only survive and form plaque if they adhere to the hard surfaces, such as teeth, filling materials, dental implants, or prostheses. A reduction in surface free energy of the substratum results in a decrease in plaque growth rate (Quirynen, 1994; Quirynen and Bollen, 1995).
practice, fluoridation is widely applied as it works to reduce the surface energy at the surface of teeth.

These examples could guide this project to find methods to enhance microbial attachment. Several methods to increase the surface energy were therefore considered.

When actual environmental exposure of the pretreated LDPE films is considered, the enhanced surface energy of the film needs to last for a certain period of time until microbial colonisation has initiated on the surface. Surface aging tests were therefore carried out.

An estimation of surface energy on solid surface is usually made by estimating contact angles that from when a small droplet (called Sessile droplet) of liquid (normally water) lands on a surface (Zisman, 1964), and this method was applied in this research. For flat, smooth and water-proof substrates, surface energy can be defined only by the measurement of contact angles. Surface roughness does not have an influence on contact angle if the distance between bumps is > 0.1 μm (Busscher et al., 1984).

Contact angles are measured on microscopically smooth, planar surfaces by placing a droplet of liquid or solution on the surface and determining the contact angle by any of a number of techniques (Adamson, 1976). Nearly 200 years ago Thomas Young (1805) proposed treating the contact angle of a liquid as the result of the mechanical equilibrium of a drop resting on a plane solid surface under the action of three surface tensions (Figure 2.5). $\gamma_{LV}$ at the interface of the liquid and vapour phases, $\gamma_{SL}$ at the interface of the solid and the liquid, and $\gamma_{SV}$ at the interface of the solid and vapour. Hence, an equilibrium of

$$\gamma_{SV} - \gamma_{SL} = \gamma_{LV} \cos \theta$$

............... (1)
can be obtained.

Figure 2.5. Contact angle of a sessile droplet.

The concept of the contact angle and its equilibrium was valuable because it gave a definition to the notion of wettability and indicated the surface parameters needing measurement. Today when we say that a liquid is non-spreading, we simply mean that $\theta \neq 0^\circ$; and when we say that a liquid wets the solid completely and spreads freely over the surface at a rate depending on the liquid viscosity and solid surface roughness, we say that $\theta = 0^\circ$. A host of early experiments revealed that every liquid wets every solid to some extent, that is, $\theta \neq 180^\circ$. Another way to express this point is that there is always some adhesion of any liquid to any solid. On a homogeneous solid surface, angle $\theta$ is independent of the volume of the liquid drop. Obviously, since the tendency for the liquid to spread increases as $\theta$ decreases, the contact angle is a useful inverse measure of spreadability or wettability. Young's equation is deceptively simple; but, there are conceptual and experimental difficulties; and Equation (1) has been the source of many arguments. In the definition of $\gamma_{SL}$ and $\gamma_{SV}$, neither of which we can conveniently and reliably measure, there is the difficulty that any tensile stresses existing in the surface of a solid would rarely be in a system in equilibrium. Solids are rare whose surfaces are
free of stresses which have arisen from below the surface layer. Lester (1961) has given a sophisticated treatment of Young's equation and has shown that it is correct so long as the drop of liquid rests on a solid which is not too deformable (Zisman, 1964). For these reasons, contact angles should be measured immediately after a drop of the test liquid has been placed on the surface.

The contact angle can be measured directly by use of a microscope fitted with a goniometer eyepiece or by photographing the droplet. Indirect measurement of the contact angle can be done by measuring the height, \( h \), and the diameter, \( d \), of the droplet and, assuming a spherical shape, by using the relation

\[
\tan \frac{\theta}{2} = \frac{h}{2d}
\]

(Bartell and Zuidema, 1936). However, obtaining a valid, reproducible contact angle is more complicated and difficult than it appears, for a number of reasons:

1. Contamination of the droplet by adsorption of impurities from the gas phase tends to reduce \( \theta \) if \( \gamma_{LV} \) and/or \( \gamma_{SL} \) is reduced and \( \gamma_{SV} \) remains more or less constant.
2. A solid surface, even when apparently smooth, may have impurities and defects that vary from place to place on the surface and from sample to sample. Roughness reduces \( \theta \) when the value on a smooth surface is <90°, and increases it when the value there is >90°.
3. The contact angle may show hysteresis. In this case the advancing contact angle will always be greater than the reducing contact angle, sometimes differing by as much as 60°. Contact angle hysteresis is always present when the surface is not clean or when it contains considerable amounts of impurities. However, even when the surface is clean and the substrate pure, it may still show hysteresis. For
example, stearic acid becomes more wettable (shows a smaller contact angle) after being in contact with water. The explanation has been advanced that there is a change in orientation of the surface molecules in the presence of water, with more of the molecules becoming oriented with their carboxylic acid group facing the water, thus decreasing the interfacial free energy. Other reasons for low receding angles are penetration of the wetting liquid into the substrate, removal of an adsorbed surface film from the substrate by the wetting liquid, and microscopic surface roughness (can be ignored if < 0.1 μm). Therefore, contact angle measurement should be done immediately after the water droplet is formed.

Experimental methods of water contact angle are described in Section 3.2.

2.3.2. Surface oxidation and other chemical changes

It is expected that the applied pretreatments may cause a degree of chemical changes on LDPE film especially on the surface. It is assumed that there is a strong correlation between chemical condition of the substrate and microbial attachment and growth. Oxidation may be the most important chemical reaction when microbial colonisation and subsequent degradation are considered (Albertsson et al., 1987; Karlsson and Albertsson, 1995). Surface oxidation is also effective in increasing surface energy, and could lead to oxidative-degradation that plays a role as a trigger to initiate and assist biodegradation. Estimations of the chemical changes were made both on the interfacial and internal structures according to the level and type of treatment.
2.3.2.1. FTIR analysis

Infrared (IR) analysis is one of the absorption spectrometric methods which has developed relatively recently. The absorption spectrometry is the determination of the degree to which electromagnetic radiation (light and light-energy) is absorbed by a substance over a range of wavelength. The record of energy absorption versus wavelength is the absorption spectrum. The usual wavelength range for IR spectrometry is from 2 microns to 16 microns (5000 cm\(^{-1}\) to 60 cm\(^{-1}\) in wavenumber or frequency).

In all forms of absorption spectrometry, an interpretation of a spectrum is a description of the ways by which light energy is absorbed by the molecules of the sample. In the infrared range, energy absorption is explained by an increase in the amplitude of various molecular vibrations. The frequency of the light absorbed, equals the frequency of the molecular vibration, and thus the higher-frequency vibrations will absorb the larger quanta, or the shorter-wavelength light (Ault, 1998). The simplest use of infrared spectra is to determine whether two samples are identical or not. If the samples are the same, their IR spectra, obtained under the same conditions, must be the same. If the samples are different, their spectra will be different. Also infrared spectra are used to detect functional groups of the sample. Certain structural features can be established fairly easily. For example, if a substance contains only C, H, and O, the oxygen can be present only as C=O, O–H, or C–O–C (or a combination of the these, such as the ester or carboxylic acid group). The presence or absence of absorption in the carbonyl region (∼5.8–6.0 microns; ∼1730–1670 cm\(^{-1}\)) or O–H region (∼2.7–3.0 microns; ∼3700–3300 cm\(^{-1}\)) can serve to eliminate or establish some of these possibilities. The nature of a functional group involving nitrogen can be inferred in a compound containing only C, H, and N in a similar way. Typical IR absorption and structural correlations are shown in Table 2.3.

Polyethylene film has (CH\(_2\))\(_n\) structure, and oxidation is expected due to applied pretreatments. Therefore, the presence of oxygen atoms forming C=O, C–O, and O–H were carefully observed.
Analysis of FTIR (Fourier-transform infrared) spectra is effective for the observation of new functional groups of substrates. Attenuated total reflection (ATR) FTIR analysis, which allows the direct recording of the IR spectrum of interfacial conditions of samples, was applied to observe chemical components of the surface.

2.3.2.2. Index presentation

The FTIR method was applied in order to compare the spectra between non-treated and pretreated LDPE films. However, the difference between the two spectra is usually not very significant and it is difficult to detect the characterising differentiation. The reason is because LDPE (non-treated) already has a unique and steep curve, thus the detective peak cannot be observed obviously. Therefore, an index presentation method, which allows comparing the two curves relatively with zeroing the non-treated spectrum, was applied. The FTIR system is connected to a computer to display the obtained chart. Scanned data at the detector is converted to a numeric (digital) system to give each integral wave number (usually 4000 cm\(^{-1}\) to 500 cm\(^{-1}\)). Therefore every single wavenumber has absolute value of the spectrum. A calculation of:

\[
\pi = \frac{T_0 - T}{T_0}
\]

was carried out at every wavenumber, where \(\pi\) is the peak index, \(T_0\) is the absolute data for non-treated sample, and \(T\) is the absolute data for pretreated sample.

Experimental methods of FTIR are described in Section 3.3.
Table 2.3. Infrared absorption – structure correlations

<table>
<thead>
<tr>
<th>Structure</th>
<th>Range (cm⁻¹)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C–H stretching vibrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkane</td>
<td>2962–2853</td>
<td>m–s</td>
</tr>
<tr>
<td>Alkene</td>
<td>3095–3010</td>
<td>m</td>
</tr>
<tr>
<td>Alkyne</td>
<td>3300</td>
<td>s</td>
</tr>
<tr>
<td>Aromatic</td>
<td>3030</td>
<td>v</td>
</tr>
<tr>
<td>Aldehyde</td>
<td>2900–2820</td>
<td>w</td>
</tr>
<tr>
<td></td>
<td>2775–2700</td>
<td>v</td>
</tr>
<tr>
<td>C–H bending vibrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkane</td>
<td>1485–1365</td>
<td>v</td>
</tr>
<tr>
<td>Alkene of which monosubstituted (vinyl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disubstituted, cis</td>
<td>1420–1410</td>
<td>s</td>
</tr>
<tr>
<td>disubstituted, trans</td>
<td>1300–1290</td>
<td>w–s</td>
</tr>
<tr>
<td>disubstituted, gem</td>
<td>995–985</td>
<td>s</td>
</tr>
<tr>
<td>disubstituted, gem</td>
<td>915–905</td>
<td>s</td>
</tr>
<tr>
<td>trisubstituted</td>
<td>690</td>
<td>s</td>
</tr>
<tr>
<td>Aromatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 adjacent H atoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 adjacent H atoms</td>
<td>750</td>
<td>v, s</td>
</tr>
<tr>
<td>3 adjacent H atoms</td>
<td>700</td>
<td>v, s</td>
</tr>
<tr>
<td>2 adjacent H atoms</td>
<td>750</td>
<td>v, s</td>
</tr>
<tr>
<td>1 isolated H atoms</td>
<td>780</td>
<td>v, m</td>
</tr>
<tr>
<td></td>
<td>830</td>
<td>v, m</td>
</tr>
<tr>
<td></td>
<td>880</td>
<td>v, w</td>
</tr>
<tr>
<td>N–H stretching vibrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amine, not hydrogen bonded</td>
<td>3500–3300</td>
<td>m</td>
</tr>
<tr>
<td>Amide</td>
<td>3500–3140</td>
<td>m</td>
</tr>
<tr>
<td>O–H stretching vibrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohols and phenols</td>
<td></td>
<td></td>
</tr>
<tr>
<td>not hydrogen bonded</td>
<td>3650–3590</td>
<td>v, sh</td>
</tr>
<tr>
<td>hydrogen bonded</td>
<td>3750–3200</td>
<td>v, b</td>
</tr>
<tr>
<td>Carboxylic acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hydrogen bonded</td>
<td>2700–2500</td>
<td>w</td>
</tr>
<tr>
<td>C–O stretching vibrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>formates</td>
<td>1200–1180</td>
<td>s</td>
</tr>
<tr>
<td>acetates</td>
<td>1250–1230</td>
<td>s</td>
</tr>
<tr>
<td>propinates, etc.</td>
<td>1200–1150</td>
<td>s</td>
</tr>
<tr>
<td>benzoates; phthalates</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1310–1250</td>
<td>s</td>
</tr>
<tr>
<td></td>
<td>1150–1100</td>
<td>s</td>
</tr>
</tbody>
</table>
**Table 2.3. continued**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Range (cm⁻¹)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C=C stretching vibrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated alkene</td>
<td>1669–1645</td>
<td>v</td>
</tr>
<tr>
<td>Conjugated alkene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C=C conjugated</td>
<td>1600</td>
<td>m–s</td>
</tr>
<tr>
<td>C=O conjugated</td>
<td>1647–1621</td>
<td>m–s</td>
</tr>
<tr>
<td>phenyl conjugated</td>
<td>1625</td>
<td>m–s</td>
</tr>
<tr>
<td>Aromatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>1580</td>
<td>m</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>m</td>
</tr>
<tr>
<td>C=O stretching vibrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldehydes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>saturated aliphatic</td>
<td>1740–1720</td>
<td>s</td>
</tr>
<tr>
<td>α,β-unsaturated aliphatic</td>
<td>1705–1680</td>
<td>s</td>
</tr>
<tr>
<td>Ketones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>saturated acyclic</td>
<td>1725–1705</td>
<td>s</td>
</tr>
<tr>
<td>saturated 6-ring and larger</td>
<td>1725–1705</td>
<td>s</td>
</tr>
<tr>
<td>saturated 5-membered ring</td>
<td>1750–1740</td>
<td>s</td>
</tr>
<tr>
<td>α,β-unsaturated acyclic</td>
<td>1685–1665</td>
<td>s</td>
</tr>
<tr>
<td>aryl alkyl</td>
<td>1700–1680</td>
<td>s</td>
</tr>
<tr>
<td>diaryl</td>
<td>1670–1660</td>
<td>s</td>
</tr>
<tr>
<td>Corboxylic acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>saturated aliphatic</td>
<td>725–1700</td>
<td>s</td>
</tr>
<tr>
<td>aromatic</td>
<td>1700–1680</td>
<td>s</td>
</tr>
<tr>
<td>Corboxylic acid anhydrides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>saturated acyclic</td>
<td>1850–1800</td>
<td>s</td>
</tr>
<tr>
<td></td>
<td>1790–1740</td>
<td>s</td>
</tr>
<tr>
<td>Acyl halides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chlorides</td>
<td>1795</td>
<td>s</td>
</tr>
<tr>
<td>bromides</td>
<td>1810</td>
<td>s</td>
</tr>
<tr>
<td>Esters and lactones (cyclic esters)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>saturated acyclic</td>
<td>1750–1735</td>
<td>s</td>
</tr>
<tr>
<td>saturated 6-ring and larger</td>
<td>1750–1735</td>
<td>s</td>
</tr>
<tr>
<td>α,β-unsaturated and aryl</td>
<td>1730–1717</td>
<td>s</td>
</tr>
<tr>
<td>vinyl esters</td>
<td>1800–1770</td>
<td>s</td>
</tr>
<tr>
<td>Amides and lactams (cyclic amides)</td>
<td>1700–1630</td>
<td>s</td>
</tr>
<tr>
<td><strong>Triple-bond stretching vibrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C=N</td>
<td>2260–2215</td>
<td>m</td>
</tr>
<tr>
<td>C=C</td>
<td>2260–2100</td>
<td>v, m</td>
</tr>
</tbody>
</table>

Abbreviations: w = weak absorption; m = medium absorption; s = strong absorption; v = variable intensity of absorption; sh = sharp absorption; b = broad absorption. (Bellamy, 1975)
Table 2.4. Useful sources of test methods

1. International (ISO) Standards
   Usually re-issued as National Standards

2. National Standards
   (a) British Standards (BS)
   (b) American Society for Testing and Material Standards (ASTM)
   (c) Japan Industrial Standard (JIS)

3. Trade associations’ publications
   (a) Technical Association for the Pulp and Paper Industry Standard (TAPPI), where TAPPI Standards have been developed by ASTM, the latter reference has been used.
   (b) Federation Européenne des Fabricants de Carton Ondulé Test Methods (FEFCO)
   (c) Technical Section of the British Paper and Board Industries Federation Test Methods (BPBIF)
   (d) Plastic Film Manufactures’ Association (PFMS)

2.3.3. Mechanical strength

All industrial materials are required to have a certain level of mechanical strength, which depends on the purpose of use. Several standard test methods have been established nationally and internationally (Table 2.4) and usually all products must pass such standard tests before being sold commercially. The choice of test method depends on (1) the objectives (is the test required for comparison or checking of materials); (2) the relevance to performance (test properties should be correlated to actual performance in use); (3) the precision required (Paine & Paine, 1992). The more precise and statistically valid the results required, the more sophisticated the test equipment and its calibration; the better qualified the test staff and supervisory staff must be; the greater the number of replicate tests; the stricter the sampling procedure; and hence the more expensive the tests. It therefore follows that, whether we are comparing material or checking materials against a specification, we must decide on
the order of difference that will matter. On this depends the precision with which the test must be done (Table 2.5) and hence the cost of the test. Where the order of difference is large, the ‘test’ may be no more than a simple visual inspection. To find a small difference, repeated tests using accurate equipment and statistical techniques may be required. The results could be official information that customers and users know as the ‘reliability’ of the product.

There are a number of methods applicable for flexible packaging materials such as thin plastic films. Since commercialised food packaging plastic films are actually used in the project, the product should have agreed with some type of standard method (no information obtained from supplier, though). Even after pretreatments on the film were performed, no significant deterioration in categories shown in Table 2.5 should appear. For food packaging or shopping bag utilisation, reduction in tensile strength could be the most critical damage. For example, tearing the package (mainly happens in transportation) could lead to subsequent bacterial or chemical contamination and result in destroying contained food, and eventually losing the reliability of the products and the supplying company.

Measurement of tensile stress–strain properties is the most common mechanical measurement on most polymer materials. The principle is to stretch a test specimen until it breaks and measure the force and elongation at various stages. Even when the application of the material is in shear or compression, tensile properties are commonly measured as a general guide to quality, which probably owes much to the relative convenience and simplicity of the geometry (Brown, 1999). Because the results are at least to some degree dependent on test piece geometry, the tensile properties measured are generally considered arbitrary rather than absolute.
Table 2.5. Properties of flexible packaging materials

<table>
<thead>
<tr>
<th>Protective properties</th>
<th>Production properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Water vapour permeability</td>
<td>• Strength properties</td>
</tr>
<tr>
<td>• Gas permeability</td>
<td>• Resistance to blocking</td>
</tr>
<tr>
<td>• Thermal conductivity</td>
<td>• Freedom from static electricity</td>
</tr>
<tr>
<td>• Compatibility with product</td>
<td>• Forming or creasing properties</td>
</tr>
<tr>
<td>• Freedom from taint, odour, toxicity</td>
<td>• Flammability</td>
</tr>
<tr>
<td>• <strong>Strength properties</strong></td>
<td>• Thickness variation</td>
</tr>
<tr>
<td>• Puncture strength</td>
<td>• Heat shrinkability</td>
</tr>
<tr>
<td>• Tear strength</td>
<td>• Dimensional stability</td>
</tr>
<tr>
<td>• Stiffness</td>
<td>• Air permeability</td>
</tr>
<tr>
<td>• <strong>Tensile strength and stretch</strong></td>
<td>• Sealing temperature, pressure and dwell time</td>
</tr>
<tr>
<td>• Impact Strength</td>
<td>• Adhesion properties</td>
</tr>
</tbody>
</table>

**Appearance properties**

| • Printability                                              | • Attraction of dust by static chances                    |
| • Haze and gloss                                            |                                                           |
| • Resistance to abrasion                                    |                                                           |
| • Resistance to aging                                       |                                                           |
| • Resistance to fading                                      |                                                           |

Arai et al., Polymer chemistry (1994)
Tensile testing of plastic materials is covered by the various standard methods such as ISO 527 (Part 1, 2 and 3), BS 2782, JIS K 7127 and JIS Z 1702 (ISO 527-1,2,3 is relevant to BS 2782-321,322,326E). Different categories of the standard refer to different forms of plastic, such as general moulding and extrusion compounds, films, general purpose composites, high-performance composites, etc. Thin plastic films methods are linked by ISO 527- Part 1 (also BS 2782-3-321) which sets out the general principles to be applied, whatever the specific form of material to be tested. Test pieces are most often in the form of flat dumbbell shapes. Dumbbells have the basic attribute of being a way of concentrating the stress so that failure takes place in the narrow portion and not preferentially where the test piece is gripped, although this is not always successful in practice. For textiles a dumbbell would mean that some threads at the ends were not supported, and no advantage over a flat strip has been found for many plastics films. The detailed shape of dumbbell varies between standards (see Figure 3.4 and Table 3.3 in Section 3.4), and there is also a range of sizes. The shapes are to a degree arbitrary, but in some cases they result from theoretical predictions or practical experiments with the aim of optimising stress distribution. The different sizes largely result from the need to cater for circumstances where only small amounts of materials are available. Because results are likely to depend on the geometry and the size, comparisons should strictly only be made where the same type of test piece has been used (Brown, 1999).

For the tensiometry tests, using commercialised machines is essential and common. The basic elements are grips to hold the test piece, a means of applying a stress, a force-measuring element, an extensometer (ISO 5893). The machine is connected to a chart recorder (electrical transducer for modern machines) to record.
Figure 2.6 shows typical stress-strain curves for tensile strength measurement. The strain (elongation or extension) is shown as $\varepsilon$, which is defined as "increase in length per unit original length of the gauge" and is expressed as a dimensionless ratio, or as a percentage ($\%$). Tensile stress $\sigma$ is the tensile force per unit area of the original cross-section within the gauge length, carried by the test specimen at any given moment (gauge length: initial distance between the gauge marks on the central part of the specimen, usually shown as $L_0$). The following definitions were applied in ISO 527-1, and the same definitions were used in this report (see Figure 2.6).
> Tensile stress at break ($\sigma_B$) is the tensile stress at which the specimen ruptures.

> Tensile stress at yield (or "yield stress", $\sigma_y$) is the first stress at which an increase in strain (extension) occurs without an increase in stress (tensile load).

> Tensile strain (extension) at break ($\varepsilon_B$) is the tensile strain at the tensile stress at break.

> Tensile strain (extension) at yield ($\varepsilon_y$) is tensile strain at the yield stress.

<table>
<thead>
<tr>
<th>Code</th>
<th>Statement</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Low elongation without yield point</td>
<td>Brittle material</td>
</tr>
<tr>
<td>b</td>
<td>with yield point ($\sigma_B &gt; \sigma_y$)</td>
<td>Tough yielding material</td>
</tr>
<tr>
<td>c</td>
<td>with yield point ($\sigma_B &gt; \sigma_y$)</td>
<td>Fairly tough yielding material</td>
</tr>
<tr>
<td>d</td>
<td>without yield point but has enough elongation</td>
<td>Weak yielding material</td>
</tr>
</tbody>
</table>

There are four possible stress–strain curves (on Figure 2.6) and characteristics of the tested materials can roughly be defined. Table 2.6 links the type of curves and the characteristics of the material. This could guide to judge whether or not initial embrittlement has occurred. Using the code (a, b, c and d) was applied in the result sections of this thesis to explain the phenomena of test samples.

Tensiometry measurement can also be used to assess the biodegradability of thin films. Tensile strength is a sensitive mechanical
property, thus is widely used in the evaluation of degradative effects of polyolefins (Kay et al., 1993). Whitney and Williams (1976) reported that the loss of tensile strength of starch-filled polyethylene film was roughly exponential (Figure 2.7), whatever the rate of degradation before mineralisation takes place. A straight line was given with percentage change in tensile strength plotted against the logarithmic exposure time. From this line, the tensile-half-life could be predicted by extrapolation (Figure 2.8), and the biodegradability of a particular object in a specified environment could be quantified (Whitney et al., 1993). In practice, this method was applied to measure the biodegradability of biologically exposed LDPE films (in Chapter 9 and Chapter 10).

Experimental methods of tensiometry are described in Section 3.4.

2.3.4. Surface roughness

It is expected that a certain level of surface roughening develops due to the applied pretreatments on the exposure treatments (UV exposure and corona discharge treatment). Roughness of the surface is considered to play a key role in determining the potential for microbial colonisation. It was reported that surface roughness, in particular the scale of surface topographical features, is the most important physicochemical surface characteristic determining the distribution of the autochthonous microflora in mineral water bottles (Jones, 1999). Very few studies on surface roughness, however, have been reported while many chemical studies have already reported on the functional groups determined by X-ray photoelectron spectroscopy (XPS) noticing only the contributions of chemical changes of the surface (Andreopoulos et al., 1993; Chaoting et al., 1993; Gao and Zeng, 1993; Tissington et al., 1991). Surface roughness can be defined in various ways; one of the definitions is to evaluate the ratio of roughness curve ($R_j$):
Figure 2.7. Degradation of prototype starch-filled polyethylene in sandy loam, measured by loss of tensile strength (Whitney and William, 1976).

Figure 2.8. Prediction of tensile half-life of prototype starch filled polyethylene in sandy loam. Linear relationship could be found between changes of tensile strength and the logarithm of biotic exposure time (Whitney and William, 1976).
where $l_0$ is the adopted linear distance in roughness curve, and $l$ the real length along with roughness curve, shown in Figure 2.9 (Ogawa et al., 1999). These surface analyses can be made using XPS, atomic force microscopy (AFM) and scanning electron microscopy (SEM). Using AFM and SEM were considered best suited for the morphological estimation of LDPE surfaces (Vancso et al., 1996). SEM was selected in the experiments.

![Figure 2.9](image)

Figure 2.9. One of the definition of surface roughness.

SEM is widely used to obtain very detailed information of specimen, especially to obtain three-dimensional images (Goldstein et al., 1981). The mechanism is that the specimen is coated with a thin film of a heavy metal such as gold and then an electron beam from the SEM is then directed down on the specimen and scans back and forth across it (In the absence of a coating layer, non-conductive specimens examined at optimal instrumental parameters invariably exhibit charging phenomena which result in image distortion and thermal and radiation damage which can lead to a significant loss of material from the specimen). Electrons scattered by the metal are collected, and they activate a viewing
screen to produce an image. In the SEM, even fairly large specimens can be observed, and the depth of field is extremely good. A wide range of magnification can be obtained with the SEM, from as low as 15x up to about 100,000x, but only the surface of an object can be visualised. All electron microscopes are fitted with cameras to allow a photograph, called an electron micrograph, to be taken (Madigan et al., 1997).

The experimental methods of surface roughness are described in Section 3.5.

2.3.5. Microbial colonisation

Microbial adhesion starts with the transport of microorganisms toward a conditioned surface, which can be mediated by gravity (sedimentation), diffusion or convection, on active swimming or growth. Colonisation can then begin. The initial microbial adhesion was explained by Busscher's group (1990); once a microorganism is within the range of the interaction forces, the actual adhesion process commences. The first forces to become operative are Lifshitz-van der Waals forces, generally attractive and long range in character (Figure 2.10). As they approach closer, a microorganism will experience repulsive electrostatic interactions. Although most known microbial strains carry a net negative charge, yielding repulsive electrostatic interactions, localised positively charged domains on the cell surface may also yield attractive electrostatic interactions (Busscher et al., 1990). Contact angles reflect the overall hydrophobic character of a microorganism (van Loosdrecht et al., 1989) and are likely to be influenced greatly by the presence of a localised hydrophobic group. Therefore, these parameters are expected to be useful mainly to describe the first two steps in the adhesion process (Figure 2.10). Electrostatic interaction between a microorganism and the substrata before being attached to each other. However, after these processes are completed, biological affinity becomes more important. Ideally, the microorganism can utilise the substrate as a nutrient source.
Long range Lifshitz-van der Waals interactions (>50nm)

- Repulsive electrostatic interactions (10–20 nm)
- Repulsive and locally attractive electrostatic interactions (2–10 nm)
- Interfacial water poses a barrier for specific local interactions and is removed by hydrophobic groups (0.5–2 nm)
- Specific interactions (<0.5 nm)

Figure 2.10. Various stages in the process of adhesion of a microorganism to a solid substratum. The distances indicated are approximate. "H" denote localised hydrophobic groups on the cell surface, acting as brooms to remove interfacial water (Busscher et al., 1990).

2.4. Aims of project

The hypothesis of this project was that low-density polyethylene is fundamentally biodegradable (but very slowly) and thus methods to enhance the biodegradability have been considered. As already mentioned in Section 2.3.1. ("Surface energy") the characteristic of the poor microbial adhesion is considered the main cause of poor biodegradability. Therefore, improvement of the surface characteristics was aimed as a key issue in order to increase the microbial affinity and the biodegradability.
In this section, methods of observational and quantitative analyses of microorganisms colonised on pretreated LDPE films are shown. A protein assay was applied to quantify the amount of microorganisms.

The experimental methods of the estimation of the **microbial colonisation** (visible and quantitative) are described in Section 3.6.
In the following chapters, five different types of surface modifications (surfactant, vegetable oils, UV light exposure, corona discharge treatment, and chemical treatment) to LDPE film are reported. In this chapter, commonly used estimation techniques for the investigation of the effects of the pretreatments are explained as well as mentioning the properties of LDPE film used in this study.

3.1. Information on LDPE film

3.1.1. General information of plastic film used in this project

Supplied food packaging grade LDPE film, manufactured for Booker Belmont Wholesale Ltd., which passed BS 5750, was used in the series of experiments. Properties of the film are described in Table 3.1 (unfortunately, no additional information was available regarding the chemical nature of the material).
3.1.2. FTIR scan on the LDPE film as a preliminary study (the methodology)

As a preliminary experiment, an FTIR experiment was carried out for the LDPE test sample, comparing pure and oxidised polyethylene for diagnostic purposes. For the pure polyethylene LDPE powder (polyethylene, powder, spectrophotometric grade, ALDRICH) was used, and for the oxidised LDPE oxidised polyethylene pellet (ACROS) was used. To obtain comparative conditions for the FTIR analysis, thin transparent films were prepared using a casting method. A mass of 0.5 g of each sample was placed on a metal plate and heated at 145 °C for 1 h (melting point of polyethylene is 137–141 °C). Then the melted sample was force (quickly) cooled with water (about 20 °C) to obtain transparent film being highly non-crystalline. If cooled spontaneously (gently) at room temperature, the degree of crystallinity becomes high, therefore, non-transparent (whitened) film that is not suitable for transparency FTIR analysis is obtained. (see Section 3.3 for detailed methods of FTIR measurement.)

### Table 3.1. General information of the film sample.

<table>
<thead>
<tr>
<th>Category</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product name</td>
<td>HAPPY SHOPPER FOOD BAG 30</td>
</tr>
<tr>
<td>Produced for</td>
<td>Booker Belmont Wholesale Ltd.</td>
</tr>
<tr>
<td>Material</td>
<td>low-density polyethylene (LDPE)</td>
</tr>
<tr>
<td>Density</td>
<td>0.950 g/cm³ (by author)</td>
</tr>
<tr>
<td>Thickness</td>
<td>18 µm (by author)</td>
</tr>
<tr>
<td>Colour</td>
<td>Transparent</td>
</tr>
<tr>
<td>Initial tensile strength</td>
<td>2.29 ± 0.22 MPa (by author) *</td>
</tr>
<tr>
<td>Initial contact angle</td>
<td>92 ± 2 ° (by author) **</td>
</tr>
</tbody>
</table>

* see details of tensile strength in Section 3.4.
** see details of contact angle in Section 3.2.
3.1.3. FTIR estimation of the LDPE film

The FTIR charts shown in Figure 3.1 analysed from the three types of samples had identical peaks around 1720, 1560 and 1450 cm\(^{-1}\). A peak around 1720 cm\(^{-1}\) indicates oxidation at alkane chain (>C=O) and the formation of this type of oxidation is recognised as an important stage in polymer degradation studies. Almost the same level of oxidation in a polyethylene chain was obtained for LDPE test film and diagnostic oxidised LDPE powder. A peak around 1450 cm\(^{-1}\) indicates alkane bending (C–H), and the result shows that a higher number of C–H bonds were contained in LDPE test film. The reason was considered to be that the LDPE test sample had a lower degree of branching than diagnostic LDPE powders. (The more the branch develops in LDPE, the less hydrogen atoms are needed, relatively.) A peak around 1560 cm\(^{-1}\) was uniquely observed for LDPE test film, thus a possibility of some additive in the manufacturing process was considered possible. This peak could show carboxylic acid according to Table 2.3. However, this peak level was not considered since the relative amount of the unknown compound was very low.
3.2. Water contact angle

Water contact angle measurement was applied to evaluate the surface energy and wettability of the LDPE film before and after the pretreatment; as the theoretical background to this was discussed in Section 2.3.1.

3.2.1. Preparation of test samples

Pretreated LDPE film samples were cut into 50 x 15 mm sections immediately after the treatment was finished (normally within 20 min. after finishing pretreatments except for aging measurement) and then adhered to a flat and smooth surface (e.g. a glass slide) using double-sided sticky tape. Care was taken not to trap air bubbles between the film sample and tape, and tape and glass slide. Films having any scratches, holes, other irregularities or dust were rejected because these factors may influence significantly on readings of contact angles. Also, the measuring surface was kept untouched.

Figure 3.2. Contact angle measurement apparatus (left) and water droplet forming stage (right). (Black LDPE film is placed instead of transparent one for photographing.)
3.2.2. Method of measuring surface energy

The contact angle instrument (Research Instruments Ltd.) comprised a cylinder with a needle, a stage (to place test samples) and a goniometer placed on a flat stage (Figure 3.2). All measurements were carried out at ambient temperature (20 ± 4 °C). Air flow was avoided as much as possible because it may affect the reading of the contact angle of the water droplet.

The cylinder (as water reservoir) was filled up with R.O. (non-sterilised) water. Care was taken not to produce air traps in the cylinder and the needle, because it will result in forming air bubbles when developing a water droplet from the cylinder, causing inaccurate volumes of liquid to be produced. A test sample was then placed on the stage, and the distance between the testing surface and the point of the needle adjusted to about 3 mm so that both phases are able to be observed from the scope of the goniometer. The stage was lit using an external light source in order to observe the phase of water droplet clearly (using a fibrescope light as shown in Figure 3.2 was effective to obtain a clear view). Just before starting to develop a water droplet, any traces of dust on the test sample were removed using a jet of compressed air from a disposable cylinder designed to clear dust from optical equipment.

A water droplet of volume 6 μl was then ejected from the point of the needle, and gently placed on the test sample by moving the needle (about 1 mm towards the test surface). Normally, the water droplet can be removed from the needle immediately after attaching to the sample. If not, the needle was moved gently to be separated from the droplet. The tangent to the point of contact at the solid/liquid/vapour interface (see Figure 2.5) was then measured using the goniometer to give a (advancing) contact angle (θ). Only advancing contact angles were evaluated. At least five replicates were measured at different points for each test sample. Observed replicate contact angles usually agreed within ± 1°.
3.2.3. Evaluation of aging of the surface modified LDPE film

Pretreated LDPE films were placed in different environmental conditions for various period of time to investigate aging effects. No aging tests were, however, carried out for chemically treated LDPE films because this treatment was only used to compare the levels of oxidation with the corona treated film.

3.2.3.1. Aging test for surfactant and vegetable oil treatments (chemical applications)

_The durability of applied chemicals against water rinsing was tested._

Pretreated film (dried) was submerged into a large volume (> 2 L) of R.O. water (non-sterile) and gently stirred for 10, 20, 30, 40 and 50 min. The film was air-dried for approximately 30 min. under dust free conditions. After being dried, the film was adhered to a flat-smooth stage using double-sided sticky tape, and the water contact angle measured (see Section 3.2.1. for sample preparation and Section 3.2.2. for the method of contact angle measurement). At least five replicates for each test condition were made.

3.2.3.2. Aging test for UV and corona discharge treatment (exposure tests)

_The durability of applied enhanced energetic conditions against air-exposure treatments was tested._

Pretreated LDPE film was adhered to a flat-smooth stage (e.g. glass stage) using double-sided sticky tape as described in Section 3.2.1, and then exposed to four different types of air-exposure environments (see Table 3.2) for up to 44 days. After the exposure treatment, the water contact
angle of the test sample was measured. (see Section 3.2.1 for the sample preparation and Section 3.2.2. for the method of contact angle measurement). At least five replicates for each test condition were made.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Existing aging element(s)</th>
<th>Exposing place</th>
</tr>
</thead>
<tbody>
<tr>
<td>Room air</td>
<td>Oxygen, Moisture, Air dust,</td>
<td>Laboratory without covering</td>
</tr>
<tr>
<td>Dust free</td>
<td>Oxygen, Moisture,</td>
<td>Laboratory with covering</td>
</tr>
<tr>
<td>Moisture free</td>
<td>Oxygen,</td>
<td>Desiccator</td>
</tr>
<tr>
<td>Oxygen free</td>
<td>Moisture</td>
<td>Anaerobic chamber</td>
</tr>
</tbody>
</table>

### 3.3. FTIR analysis

The phenomenon of oxidation and other chemical changes due to applied pretreatments was estimated by FTIR (Fourier-transform infrared) analysis. The theoretical background was discussed in Section 2.3.2.

#### 3.3.1. Preparation of test sample

Film samples to be tested were kept dry in a desiccator at room temperature for at least 24 h before analysis. This was because moisture attached to the sample may give significant influences on obtained IR spectra especially for –OH readings. As this FTIR analysis was aimed at detecting minor chemical changes (especially at the surface), any considerable outer factors such as moisture and dusts, which may give critical errors in readings, were avoided where possible. Also care was taken to select samples without any visual scratches, holes and other physical damages.

For the measurement of transparency FTIR, film sample was cut into approximately 20 x 20 mm sections and fastened with a folder designed for the FTIR apparatus. For the measurement of reflection FTIR, film
sample was cut to approximately 10 x 10 mm sections and set into KRS-5 reflection element, which allows the reflected IR spectra to pass onto the detector of the FTIR apparatus.

### 3.3.2. FTIR analysis

A Perkin Elmer System 2000 FT-IR Instrument was used. Samples were prepared accord to the methods described in Section 3.3.1. After setting up the sample, the IR beam, from 4000 cm\(^{-1}\) to 500 cm\(^{-1}\), was passed through (or reflected at the surface of) the sample for the transparent analysis (or reflection analysis). Sixteen scans were made for each sample, and the average was displayed on a computer monitor.

### 3.3.3. Editing the IR chart

Obtained analogue FTIR spectra were digitised at the connected computer and saved as Microsoft Excel\(^\text{®}\) (*xls.*) files so that every single wavenumber had a transparency value, which facilitated the presentation of the data as an index, shown in Sub-Section 2.3.2.2. See the method of the calculation in the Section.
3.4. Tensiometry (for mechanical strength)

Tensiometry tests (tensile strength and elongation at break) were measured to estimate mechanical strength of the pretreated LDPE films. The theoretical background was mentioned in Section 2.3.3.

3.4.1. Preparation of test specimen

Dumbbell-shape test specimens (Type A in Figure 3.3) were prepared for the tensiometry tests. (There is another type of standard specimen, shown as Type B in Figure 3.3, but not applied in this experiment.) Table 3.3 shows the size of specimen compared to some standard methods. In order to obtain exactly the same size of specimens at every time of sample preparation, a cutter having two blades (Figure 3.4) was prepared and used. Test specimens can be obtained when the cutter is pressed down on a film sheet. Care was taken to press the cutter with strong enough pressure. The edges of the specimen were masked by using tape so that all tensile stress can be applied to the specimen without slipping of the specimen from the grip of the tensiometer.

Figure 3.3. Type of specimen for tensile measurement. Marks are relevant to the codes in Table 3.3.
### Table 3.3. Comparison of the size of specimen with standard methods

<table>
<thead>
<tr>
<th>Code</th>
<th>Statement</th>
<th>Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Experiment</td>
</tr>
<tr>
<td>A</td>
<td>Overall length</td>
<td>75</td>
</tr>
<tr>
<td>B</td>
<td>Width at ends</td>
<td>25</td>
</tr>
<tr>
<td>C</td>
<td>Length of narrow parallel sided portion</td>
<td>28</td>
</tr>
<tr>
<td>D</td>
<td>Width of narrow parallel-sided portion</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>Initial distance between grips</td>
<td>52</td>
</tr>
<tr>
<td>G</td>
<td>Gauge length</td>
<td>10</td>
</tr>
<tr>
<td>r</td>
<td>Small radius</td>
<td>10</td>
</tr>
<tr>
<td>R</td>
<td>Large radius</td>
<td>-</td>
</tr>
<tr>
<td>t</td>
<td>Thickness</td>
<td>0.018</td>
</tr>
</tbody>
</table>

**Figure 3.4.** Illustration of prepared cutter for the tensiometry studies. Aluminium was used for the grip. The blades are renewable using the loosening screws. The lengths are shown in [mm] unit.

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Material, methods and techniques

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3.4.2. Method of measurement

An Instron Tensiometer (Figure 3.5) was used for the tensiometry tests. The grips at the ends of the test specimen were operated by compressed air. The initial distance between the grips was 30 mm, and the specimen was pulled at a constant extension speed at 10 mm per minute. Data of tensile measurement tended to vary so more than ten replicates were carried out for each type of sample tested. Dumbbell specimens that break at inappropriate places such as the shoulders were not recorded.

3.4.3. Calculation of the reading data of tensile strength

Reading data of the tensile strength were given in [g] or [kg] units. These figures were converted to an SI unit (Système International d'Unités) of pressure, [Pa (pascal)]. As “pressure” is defined as “force per unit area”, and “force” (using [N (newton)] in SI unit) is defined as “the product of mass [kg] and acceleration [ms⁻²], the following equation can be obtained:

\[ 1Pa = 1Nm^{-2} = 1\left(\frac{kg}{ms^{-2}}\right)m^{-2} \]

however, “acceleration” can be ignored if the specimen was extended at a constant speed, thus a more simple equation can be obtained:

\[ 1Pa = 1kgm^{-2} \] (when acceleration is zero)

The test specimen had a width of 10 mm in the narrow sided portion and a thickness of 0.018 mm, the area at a section of the specimen will be 0.18 mm², which is \(1.8 \times 10^{-7} \) m². Therefore, pressure using [Pa] will be given by the calculation of [data obtained / area]. Usually, the calculated figure is given as MPa (mega pascal).
Figure 3.5. Intron tensiometry apparatus
3.5. Scanning electron microscopy

The surface of corona discharge treated LDPE film was observed using scanning electron microscopy (SEM) technique. The theoretical background to roughness was discussed in Section 2.3.4.

3.5.1. Preparation of test stubs

All test samples were air dried and then left at ambient temperature in a desiccator for at least 24 h before the coating treatment. The film was cut into approximately 10 x 10 mm sections leaving the required part for the scan untouched and the piece of film was mounted on a stage (called "stub") using double-sided sticky tape. The stubs were fixed on a suitable holder and treated with an Edwards-Sputter Coater S150B to develop a thin gold ion layer over the specimen. Treatment was under inert-gas-filled condition (argon) for about 5 min. at 30 keV (kilo electron volt). The electric discharge was applied between the electrodes one of which is made of gold to allow the gold ions to deposit (sputter) onto the sample.

3.5.2. SEM analysis

The sputter-coated sample was then placed into a low vacuum atmosphere and monitored by a HITACHI SCANNING ELECTRON MICROSCOPE S–3200N. Required SEM images were obtained by altering the tilt of the stage, magnification and resolution, automatically or manually. Obtained images were saved digitally Tagged Image File Format (TIFF). (Each image had approximately 700 KB.)

SEM analysis was also effective for estimating biodegraded LDPE films after the compost exposure (mentioned in Chapter 9).
3.6. Microbial colonisation

The phenomena of microbial colonisation on pretreated LDPE films was determined by visible and quantitative methods in order to evaluate the microbiological affinity of the pretreated LDPE films. The theoretical background was discussed in Section 2.3.5.

3.6.1. Diagnostic fungal selections and the maintenance

Fourteen fungal isolates, listed in Table 3.4, were used as diagnostic microorganisms for the microbial colonisation and degradation studies. The fourteen isolates were classified into two groups (primary and secondary) depending on the purpose of the experiments. All of these fungi were isolated from a soil environment and can widely be found in a large area in the world. They are therefore considered to be suitable as diagnostic fungi to study the phenomena of microbial colonisation and degradation. The growth of these fungi was maintained by subculturing on fresh malt extract agar (MEA) medium every month. Slant cultures (using MEA) were also prepared and kept at 4 °C.

Table 3.4. List of diagnostic fungi utilised in this project and their source

<table>
<thead>
<tr>
<th>Class in this project</th>
<th>Name of fungi</th>
<th>Culture collection code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary (five types of fungi*)</td>
<td>Aspergillus niger</td>
<td>DSM 1957</td>
</tr>
<tr>
<td></td>
<td>Chaetomium globosum</td>
<td>DSM 1962</td>
</tr>
<tr>
<td></td>
<td>Paecilomyces variotii</td>
<td>DSM 1960</td>
</tr>
<tr>
<td></td>
<td>Penicillium funiculosum</td>
<td>CBS 62866</td>
</tr>
<tr>
<td></td>
<td>Trichoderma longibrachiatum</td>
<td>DSM 768</td>
</tr>
<tr>
<td>Secondary (nine types of fungi**)</td>
<td>Chaetomium sp.</td>
<td>001</td>
</tr>
<tr>
<td></td>
<td>Curvularia sp.</td>
<td>002</td>
</tr>
<tr>
<td></td>
<td>Trichoderma sp.</td>
<td>003</td>
</tr>
<tr>
<td></td>
<td>Fusarium sp.</td>
<td>004</td>
</tr>
<tr>
<td></td>
<td>Fusarium sp.</td>
<td>005</td>
</tr>
<tr>
<td></td>
<td>Chaetomium sp.</td>
<td>006</td>
</tr>
<tr>
<td></td>
<td>Fusarium sp.</td>
<td>007</td>
</tr>
<tr>
<td></td>
<td>Corynascus sepedonium</td>
<td>008</td>
</tr>
<tr>
<td></td>
<td>Stachybotrys sp.</td>
<td>009</td>
</tr>
</tbody>
</table>

* Supplied from: culture collection of AKZO Chemical GmbH, Germany
** Supplied from: culture collection of Biological Resource Unit, University of Surrey, Surrey, UK
3.6.2. Observation of fungal growth (visible analysis)

Tensiometry test specimens were prepared from sheets of pretreated LDPE films (see Section 3.4.1. for how to prepare the tensiometry test specimen). As Figure 3.6 describes, the test specimen was placed at on one side on a 1/10 strength MEA (containing 1/10 malt extract and solidified with 1.5 % agar) plate and a non-treated film specimen was placed on the other side (as a control). 1/10 MEA was considered satisfactory to supply a minimum level of nutrient for fungi to grow and reach the film sample as well as supplying adequate humidity. A small block of fungal inoculum was placed at the middle of the medium and incubated for 2 or 4 weeks at 25 °C. Observation of colonised fungi was then demonstrated and the level of growth coded by the development of the fungal colonisation as Table 3.5 shows. At least 10 replicates were made for each sample.

![Figure 3.6. Illustration of test method to observe fungal growth after surface of LDPE films are treated.](image)

<table>
<thead>
<tr>
<th>Code</th>
<th>Growth from the edge</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>No growth on film</td>
</tr>
<tr>
<td>+</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>++</td>
<td>5 - 10</td>
</tr>
<tr>
<td>+++</td>
<td>11 - 15</td>
</tr>
<tr>
<td>++++</td>
<td>16 - 20</td>
</tr>
<tr>
<td>+++++</td>
<td>21 - 25</td>
</tr>
<tr>
<td>(++++)</td>
<td>(&gt; 25)</td>
</tr>
</tbody>
</table>

Material, methods and techniques 69
3.6.3. Quantitative analysis of microbial colonisation

After the visible estimation, quantitative analysis was also made on the film. The original inoculum block placed at the centre of the film was carefully removed, and the test film submerged into 10 ml of 0.5 M NaOH in a Universal bottle. The bottle was shaken for 24 h (at 100 rpm) in order to solubilise all protein from the microorganisms adhering to the film. The extract was centrifuged at 10,000 rpm for 10 min at 4 °C, and the supernatant tested by the Coomassie Brilliant Blue G-250 Dye binding method, described in the following. A volume of 0.1 ml of the supernatant was mixed with 1:5 diluted dying reagent (Biorad®). Then the absorbance at 595 nm was measured using a spectrophotometer. The obtained spectroscopic absorbance data was referred to two standard lines from known concentrations of bovine serum albumin (BSA) and casein solution. BSA and casein are known to give high and low readings with the protein assay, respectively. Therefore, a standard for the mixture of microbial proteins was drawn as the mid-point between these two protein solution standards (Figure 3.7). A linear correlation was obtained for the mid-line up to 200 µg/l which concentration is considered to be high enough for the microbial solutions. Consequently, the amount of protein (derived from microorganisms) was calculated from the standard curve.
Chapter 4

Surfactant

4.1. Introduction

Surfactants are among the most versatile of products from the chemical industry, appearing in such diverse items as motor oils, the detergents used in cleaning our laundry, our homes and place of work, the drilling muds used in prospecting for petroleum, and the flotation agents used in the beneficiation of ores, and pharmaceuticals we take when we are ill. The last decade has seen the extension of surfactant applications to such high-technology areas as electronic printing, magnetic recording, biotechnology, microelectronics, and viral research.

A surfactant (a contraction of the term *surface-active agent*) is a substance that, when present at low concentration in a system, has the property of adsorbing onto the surface reducing interfacial free energies of those surfaces (or interfaces). The term *interface* indicates a boundary between any two immiscible phases; the term surface denotes an interface where one phase is a gas (usually air) (Rosen, 1989).

The molecules at a surface have higher potential energies than those in the interior. This is because they interact more strongly with the molecules in the interior of the substrates than they do with the widely spaced gas molecules above it. Energy is therefore required to bring a molecule from the interior to the surface.
Surface-active agents have a characteristic molecular structure consisting of a structural group that has very little attraction for the solvent, known as a hydrophobic group if the solvent is water, together with a group that has strong attraction for the solvent (water), called the hydrophilic group. This is known as an amphiphilic structure. The terminology can also be used the other way round with a water loving hydrophilic group being referred to as a lipophobic group, fat hating and a hydrophobic group being called lipophilic, fat loving. As water is the most common solvent the former terminology is usually used. When a surface-active agent is dissolved in a solvent, the presence of the lyophobic group in the interior of the solvent may cause distortion of the solvent liquid structure, increasing the free energy of the system. In an aqueous solution of a surfactant this distortion of the water by the hydrophobic group of the surfactant, and the resulting increase in the free energy of the system when it is dissolved, means that less energy is needed to bring a surfactant molecule to the surface. The surfactant therefore concentrates at the surface. Since less work is now needed to bring molecules to the surface, the presence of the surfactant decreases the work needed to create a unit area of surface (the surface free energy per unit area, or surface tension). On the other hand, the presence of the hydrophilic group prevents the surfactant from being expelled completely from the solvent as a separate phase, since that would require dehydration of the hydrophilic group. The amphiphilic structure of the surfactant therefore causes not only concentration of the surfactant at the surface and reduction of the surface tension of the water, but also orientation of the molecule at the surface with its hydrophilic group in the aqueous phase and its hydrophobic group oriented away from it (Rosen, 1989).
Surface condition of LDPE film is extremely hydrophobic as its molecular structure is constructed from a simple lipophilic unit of CH₂. This hydrophobicity may cause the inhibition of microbial growth on the surfaces, and the surface modification was attempted using surfactant. It was hypothesised that the surfactant could create hydrophilic and 'biophilic' surface conditions because of the amphiphilicity.

4.2. Methods of treatment

An anionic surfactant, NIAPROOF (27% sodium 7-ethyl, 2-methyl, 4-undecyl, sulfate; from SIGMA) was selected due to its strong detergency. It was diluted to 5%, 10%, 20%, 30%, 40% and 50% with R.O. water to give a final concentration of 1.35%, 2.7%, 5.4%, 8.1%, 10.9% and 13.5% of surfactant. Ahead of the surfactant treatment, LDPE film (50 x 50 mm) was briefly washed with acetone and then R.O. water to remove attached dust or oil, which may affect surface analysis. (FTIR analysis showed this cleaning treatment to have had no detectable chemical effect on the surface of the film.) The film was then submerged in 50 ml of the surfactant solutions for 10 min. with gentle stirring and dried in a HEPA (high efficiency particulate arrester) clean air-cabinet at room temperature. Surface contact angles were measured at various times up to 60 min. and different concentrations of surfactant after 10 minute.

To observe aging effects of surfactant in aqueous environments, the surfactant treated LDPE films were rinsed by suspension in 2 L of sterile R.O. water for 10 min. with gentle agitation, and again dried in a dust-free condition. The rinsing treatments were repeated up to 5 times (up to 50 min.), and water contact angle measurement was performed at each stage of the rinsing treatment.
4.3. Methods of estimation

Water contact angle measurements, tensile strength analysis and microbial colonisation observation on treated film were carried out to estimate the effect of the surfactant treatment (all theoretical and technical details of these estimations are described in Section 3.2).

4.3.1. Contact angle measurement (and aging evaluation)

Pretreated samples were attached to glass slides with double-sided sticky tape after the samples were totally dried. All other details of method are described in Section 3.2.

4.3.2. Influence of the surfactant on fungal growth

The applied pretreatments intended to encourage microbial colonisation of the plastic film surface, thus no applied chemicals should inhibit microbial growth. Influence of the surfactant on fungal growth was tested using known types of fungi. Two pieces of sterilised paper disks (\(\phi = 10\) mm) were dipped into sterilised 10 % surfactant (NIAPROOF) and then placed on a MEA plate as Figure 4.1 illustrates. Five types of soil fungi (listed in Table 4.1) were inoculated at the middle of the plate and then incubated at 25 °C. The fungal growth was observed for up to two weeks.

4.3.3. Influence on fungal growth on surfactant treated LDPE films

All methods were described in Section 3.6.
Figure 4.1. Illustration of test method to observe fungal growth on surfactant using paper disk technique (a), and on surfactant treated LDPE films (b).
4.4. Results and discussion

Enhancement of microbial colonisation of LDPE film was expected if the surface energy is increased by the surfactant treatment. The effect of surfactant treatment on surface energy was studied and this was related to microbial colonisation of LDPE film with and without surfactant treatment.

4.4.1. Surface energy

Figure 4.2 shows changes of contact angles when different surfactant treating-times were applied in order to establish the optimum treating time. 10 % (v/v) NIAPROOF diluted to give 2.7 % surfactant was used. Significant reduction of the contact angle was observed even for short duration of treatment, such as 5 min. This means that surfactant molecules tend to react with substrate (LDPE film) almost immediately. No further significant changes in contact angles were observed for longer treating times. It was therefore recognised that 10 min. treatment time was long enough to have sufficient detergency on the LDPE surface, thus this treating time was applied for all further experiments.

Secondly, correlation between concentration of the surfactant and changes of contact angles was examined and the result shown in Figure 4.3. The contact angle stabilised at around 35–40° when the concentration exceeds more than 10 %. Therefore 10 % of the concentration was applied for all further experiments as the standard.

The reason for the stabilisation of the concentration–contact angle over 10 % was that there was enough surfactant molecules to react with the substrate even in lower concentration of the solution, thus no further reaction was able to proceed even if the concentration increased. It is considered that 10 % of the surfactant concentration was enough to have steady chemical reaction with the substrate in the experiment.
Figure 4.2. Change of contact angles of LDPE film treated with surfactant with respect to the treating time. Bars show standard deviations.

Figure 4.3. Change of contact angle of surfactant treated LDPE films with respect to concentration of the surfactant. (Bars show standard deviation when not within symbol plotted.)
As the applied pretreatments were intended to enhance microbial colonisation and subsequent biodegradation, the effects of the pretreatments (such as having higher surface energy) should last a certain period of time until microbial attachment and colonisation develop. Observations of durability of applied pretreatment in an aqueous natural environment such as soil were therefore considered to be very important. This was modelled by rinsing the treated film with water.

Figure 4.4 shows the changes of contact angle when pretreated LDPE films were repeatedly rinsed with water to investigate the durability of the applied surfactant treatment. A significant recovery of the contact angle was observed only after rinsing for 10 min. ($\theta: 40.8^\circ \rightarrow 70.7^\circ$), and contact angle recovered slowly by the further rinsing treatment and eventually showed the final figure ($\theta=78.7^\circ$) as non-treated LDPE had ($\theta =92.0^\circ$) after 50 min. of the rinsing (total of 5 washes). It was concluded that the applied hydrophilicity was largely removed in an aqueous environment.
The mechanism for the initial development of surface energy (or decrease of contact angle) was that hydrophobic ends of surfactant molecules vertically bond to substrate, hence the hydrophilic ends shifted to an upright direction. Therefore, the surface condition shifted to be hydrophilic as if one extra hydrophilic layer was created (Figure 4.5). However, the chemical strength of the surfactant was not so strong that the surfactant layer was likely to be removed if other chemical forces such as rinsing with water are applied. The removal of the surfactant from the film occurred because the chemical force between the rinsing water and the surfactant was greater than that between the substrate and the surfactant. The aging plays a critical factor to reduce the effect of the pretreatment, thus further consideration is needed to overcome this problem.

Figure 4.5. Illustration of the probable mechanisms of attachment and removal (aging) of surfactant.
4.4.2. Microbial colonisation

As the second stage of the estimation of this pretreatment, the affinity between microorganisms and applied surfactant was evaluated (film is not involved). Table 4.1 shows the result of the observation of fungal growth on MEA medium on which wet paper disks treated with 10 % surfactant were placed. Apparent inhibition of fungal growth was observed for all types of fungi. As an example, a photograph is shown for Aspergillus niger in Figure 4.6. Development of fungal growth proceeds with avoiding the paper disks containing the surfactant. It also shows inhibition of spore formation. No inhibition was observed for the control where no surfactant (but water instead) was applied to the paper disks. It can be concluded that the surfactant inhibits fungal growth at high concentrations.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Surfactant</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>Strong inhibition</td>
<td>Vigorous growth</td>
</tr>
<tr>
<td>Penicillium funiculosum</td>
<td>Strong inhibition</td>
<td>Vigorous growth</td>
</tr>
<tr>
<td>Paecilomyces variotii</td>
<td>Strong inhibition</td>
<td>Vigorous growth</td>
</tr>
<tr>
<td>Trichoderma longibrachiatum</td>
<td>Weak inhibition</td>
<td>Vigorous growth</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>Weak inhibition</td>
<td>Vigorous growth</td>
</tr>
</tbody>
</table>

Figure 4.6. Growth of Aspergillus niger on MEA medium with surfactant (left) and without surfactant (right). Distance of the inhibition (edge of sporing fungus and the disk) was approximately 18 mm (as ➔ shows in the photograph).
Visible estimations of fungal growth on dried LDPE films after the surfactant treatments (10 %, 10 min) were made and the result of 14 days’ incubation is shown in Table 4.2. No fungal growths were observed of *Aspergillus niger* and *Chaetomium globosum*. Growths on surfactant treated LDPE films were observed for *Trichoderma longibrachiatum*, *Paecilomyces variotii* and *Penicillium funiculosum*, however richer growths were observed on non-treated LDPE films. For *T. longibrachiatum*, development of thin fungal hyphae was observed after one week of incubation and then colonisation on the surface of the film proceeded. In contrast, slower growth was observed on surfactant treated films (Figure 4.7). The same phenomena were observed for *Paecilomyces variotii* and *Penicillium funiculosum* (data not shown).

<table>
<thead>
<tr>
<th>Table 4.2. Fungal growth on surfactant treated LDPE film after 14 days of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of fungi</strong></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
</tr>
<tr>
<td><em>Chaetomium globosum</em></td>
</tr>
<tr>
<td><em>Paecilomyces variotii</em></td>
</tr>
<tr>
<td><em>Penicillium funiculosum</em></td>
</tr>
<tr>
<td><em>Trichoderma longibrachiatum</em></td>
</tr>
</tbody>
</table>

* NT: non-treated sample; ** SA: surfactant treated sample

A question has arisen as to how the surfactant inhibited fungal growth. A possible mechanism of the destruction of a cell membrane due to introduction of surfactant molecules was considered and a guide illustration is shown in Figure 4.8. Introduction of surfactant molecules between the phospho-lipid molecules of normal (or healthy) cell membrane causes weakening of the bonding strength with the neighbouring membrane molecules, and eventually the lipids part of one of the phospho-lipid molecules is covered by the surfactant molecules. This means that the phospho-lipid molecule is separated from the other member of the phospho-lipid bilayer and this will also lead to the overall destruction of the membrane and thus the death of the cell. It is to
be expected that the growth of fungal hyphae will be to avoid such destructive elements.

![Graph showing the growth of fungal hyphae for non-treated and surfactant-treated LDPE film.](image)

**Figure 4.7.** Colonisation of *Trichoderma longibachiatum* to surfactant treated LDPE film.

### 4.5. Chapter summary

It was concluded that surfactant does increase surface energy, however it was not suitable for enhancing microbial growth since it shows antimicrobial properties. It was discussed that the structure of the phospho-lipid bilayer that constructs the cell membrane of the microorganisms can be destroyed due to introduction of surfactant into the structure (Figure 4.8) and eventually inactivate the whole cell. The antimicrobial action and the transient effect of NIAPROOF make unsuitable as a treatment to enhance biodegradation. Although only one surfactant was tested the phenomena observed seems likely to occur with other ionic surfactants.
However, other types of surfactant such as nonionic ones may be useful. Surfactant application or adding the non-ionic surfactant to the polymer to increase the ‘anti-fogginess’ property was briefly described in Section 1.3. This treatment might also be interesting for a study to increase microbial affinity with films. For example, the utilisation of glycerine acid of ester or poly-glycerine acid of ester (Figure 4.9), which is approved to be safe as a food additive (Kubotsuka, 1999; Kubotsuka, 1998; Nakamura, 1998), applicable for food packaging films perhaps, might increase the microbial degradability of the film. The rapid leaching of surfactant from the surface of LDPE suggests it would need to be incorporated into the polymer. Utilisation of these safe non-ionic surfactants for the plastic film industry may therefore develop in the future.

![Phospho-lipid bilayer](image)

**Figure 4.8.** A possible mechanism for the destruction of a cell membrane due to introduction of surfactant molecules. (Left: structure of normal cell membrane. Right: partly damaged cell membrane with surfactant.)

\[
\text{RCO–O–CH}_2\text{CH(OH)CH}_2\text{OH}
\]

**Figure 4.9.** Chemical structure of glycerine ester of fatty acid
5.1. Introduction

Vegetable oils are sometimes used as die lubricants or additives in the production of plastic films. It has been suggested that they could be applied to starch-filled polyethylene to further enhance its biodegradability (Griffin, 1985). Vegetable oils are good nutrient sources for some microorganisms. In the metabolism of these oils peroxides and superoxides can be produced which could chemically attack the polyethylene film. It was therefore expected that total degradation speed of LDPE film might be enhanced if vegetable oils were coated over the surface. Microbial colonisation on LDPE films coated by vegetable oil is discussed in this chapter, and determination of suitable oils for enhancing microbial colonisation is reported.

5.2 Methods of treatment

Before treating with vegetable oils, 50 x 50 mm sections of the LDPE film sheets were washed with acetone and R.O. water to remove attached dust or sticky materials, which may affect subsequent surface analysis. Five types of food grade vegetable oils with different degrees of saturation (safflower-, corn-, soybean-, peanut- and coconut-oil) were purchased.
from SIGMA and used for this experiment. Approximately 50 ml of each type of oil was placed in 100 ml glass bottles and the LDPE films immersed in the oil at room temperature (approximately 20 °C) or the bottles were placed in a water bath at 40 °C or 70 °C for 24 hours. After the treatment, films were hung in an oven at 20, 40 or 70 °C, which respectively depending on the treating temperature, and the excess oils were drained off. This was to model their use as a die lubricant on hot polyethylene.

5.3. Methods of estimation

5.3.1. Observation of fungal growth on vegetable oils

A preliminary experiment was conducted to see if the test fungi (listed in Table 3.4) were able to utilise the vegetable oils as their nutrient sources. Carbon-free plates were prepared with White's Basal Salt Mixture (0.93 g/l) and 1.5 % (= 15 g per litre) agar (OXOID Bacteriological Agar). A volume of 100 μl of sterilised vegetable oil was overlaid using a sterile spreader and then diagnostic fungi (14 types; soil origin, listed in Table 3.4) were inoculated at the middle of the plate. The cultures were incubated at 25 °C for two weeks and the growth of fungi were observed.

5.3.2. Contact angle measurement

After the treatment, film samples were gently wiped using tissue to remove oil droplet, which may affect contact angle readings. All other methods used including “surface aging test” were described in Section 3.2.3.

5.3.3. Estimation of microbial colonisation

Both visible and quantitative analyses were performed for estimating the microbial colonisation. All the methods were described in Section 3.6.
5.4. Results and discussion

As a preliminary study, microbial growths were observed on media that vegetable oils were supplied as only carbon source. Positive growths were observed for all types of fungi although the data was not shown. Vegetable oils were then applied to the LDPE films and subsequent colonisation studies were carried out.

5.4.1. Surface energy

Table 5.1 shows the changes of contact angle at different treating temperatures with respect to the types of vegetable oils. For all types of oils, reductions of contact angle were observed with increasing the treating temperature, and the contact angles formed were approximately 50-60 ° for all types of vegetable oils at 70 °C. Exceptionally, peanut oil treated LDPE film showed a much lower contact angle (θ = 28.6 °).

Table 5.1. Changes of contact angle of LDPE films treated with vegetable oil at different temperatures.

<table>
<thead>
<tr>
<th>Type of oil</th>
<th>20 °C</th>
<th>40 °C</th>
<th>70 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>91.7</td>
<td>80.8</td>
<td>73.6</td>
</tr>
<tr>
<td>Corn oil</td>
<td>59.6</td>
<td>58.7</td>
<td>52.2</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>85.0</td>
<td>86.3</td>
<td>61.3</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>59.6</td>
<td>53.8</td>
<td>50.0</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>62.3</td>
<td>60.0</td>
<td>53.9</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>52.1</td>
<td>48.7</td>
<td>28.6</td>
</tr>
</tbody>
</table>

* Maximum standard error of mean was 2 °, therefore not given.
* Contact angle of non-treated LDPE film was 92.0 °.
Treating temperature at 70 °C seemed to be a suitable treating condition from the contact angle measurement, and also no visible damage due to the heat was observed. Therefore, the established treating temperature at 70 °C was applied for further experiments.

In order to see the durability of the vegetable oils remaining on LDPE films under aqueous environment, water-rinsing treatments were carried out up to 50 min. at room temperature. Figure 5.1 shows the result of changing the contact angle for LDPE films treated with the vegetable oil at 70 °C. For all types of vegetable oils, recovery of the contact angles was significant after the first 10 min. of rinsing and gradually increased for the rest of the rinsing treatments. The reason for the initial recovery of the contact angle was assumed to be that most of the treated vegetable oils were washed out by a single rinsing treatment hence the significant recovery in contact angle occurred. It should therefore be concluded that the vegetable oil treatment is not very durable in aquatic environments, thus not suitable for a long period of environmental exposure.

![Figure 5.1. Changes of contact angle after being washed with respect to the total washing times (vegetable oil treated at 70 °C).](image-url)
5.4.2. Microbial colonisation

Observations of the microbial colonisation were carried out by visible and quantitative methods. The result of the visible analysis is shown in Table 5.2. Richer growths were observed for all types of fungi compared to non-treated samples. Considering the five types of fungi, the growth of *Trichoderma longibrachiatum* with respect to the incubation time is shown in Figure 5.2. A significantly rapid growth was observed on safflower oil treated LDPE, and slower growths were observed for the other types of vegetable oils.

Quantitative analysis using the protein assay was also made for the fungi colonised films, shown in Table 5.3. The results were almost the same as that of visible analysis but some showed differences. Fungal growth on peanut oil treated film showed high level of microbial colonisation as high as that on safflower oil, which showed the highest in the visible growth.
Table 5.2. Microbial growth on water agar plates where vegetable oils were spread. *

<table>
<thead>
<tr>
<th>Type of fungus</th>
<th>Type of oil</th>
<th>Corn</th>
<th>Coconut</th>
<th>Soybean</th>
<th>Safflower</th>
<th>Peanut</th>
<th>Non-treat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Chaetomium sp. (1)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chaetomium sp. (2)</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
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</tr>
<tr>
<td>Corynascus sepedonium</td>
<td>++++</td>
<td>+++++</td>
<td>+++</td>
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<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Chaetomium sp. (2)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<td>-</td>
</tr>
<tr>
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<td>+++</td>
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<tr>
<td>Chaetomium sp. (2)</td>
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<td>+++</td>
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<td>++</td>
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<td>+++</td>
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<td>+</td>
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</tr>
<tr>
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<td>+++</td>
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<td>+</td>
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</tr>
<tr>
<td>Chaetomium sp. (2)</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chaetomium sp. (2)</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
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<td>+</td>
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<tr>
<td>Chaetomium sp. (2)</td>
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<td>++</td>
<td>+++</td>
<td>+++</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chaetomium sp. (2)</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Each code shows visible fungal growth from the incubated block in the centre; (–) for non-growth at all, (+) for 0–5 mm, (+++) for 6–10 mm, (++++) for 11–15 mm, (++++) for 16–20 mm, and (++++) for >21 mm. Calculated from the mean of 10 calorimetric estimations on each of five plastic samples (i.e. n=5)

Table 5.3. Amount of protein contained in fungi attached to LDPE films treated with vegetable oils at 70°C. (Unit: µg per TS specimen)

<table>
<thead>
<tr>
<th>Type of fungi</th>
<th>Type of oil</th>
<th>Corn</th>
<th>Coconut</th>
<th>Soybean</th>
<th>Safflower</th>
<th>Peanut</th>
<th>Non-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>2.75 ± 0.22</td>
<td>3.27 ± 0.45</td>
<td>4.93 ± 0.08</td>
<td>5.78 ± 0.72</td>
<td>5.08 ± 0.44</td>
<td>1.20 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>6.06 ± 1.33</td>
<td>14.86 ± 0.49</td>
<td>7.04 ± 0.07</td>
<td>12.87 ± 1.52</td>
<td>7.62 ± 1.29</td>
<td>0.96 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Paecilomyces variotii</td>
<td>5.02 ± 0.57</td>
<td>11.24 ± 2.44</td>
<td>3.03 ± 0.09</td>
<td>11.61 ± 1.42</td>
<td>3.18 ± 0.23</td>
<td>0.54 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Penicillium funiculosum</td>
<td>4.04 ± 0.49</td>
<td>1.19 ± 0.44</td>
<td>5.45 ± 0.20</td>
<td>7.56 ± 0.83</td>
<td>6.37 ± 0.32</td>
<td>1.06 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>

(± standard errors)
These results show that more dense or thicker fungal growth developed on peanut oil even though the growth of hyphae was not very rapid, while rapid but thinner fungal growth developed on safflower oil hence both quantitative data showed similar figures. Consequently, richer growths of microorganisms for all types of oils were observed and thus the efficiency of this vegetable oil treatment was confirmed.

5.5. Chapter summary

The results of the above experiments showed that the surfaces treated with oils remained sticky, even after the drying process. The stickiness temporarily disappeared after heating but reappeared when cooled. It gives an unpleasant feeling when handled and causes attachment of inappropriate materials such as air dust. It was considered that this problem might be solved if vegetable oil could be mixed with melting LDPE film before it is formed. This method has been applied industrially for food wrapping film products but not with the purpose of enhancing the microbial colonisation. For example, epoxidised vegetable oils have been mixed in PVC (poly vinyl chloride) as stabilising additives (Hitachi Fill-tech Ltd., personal information). Further details of technical information such as the ratio of the combination or temperature of the treatment were not supplied due to an industrial technical protection. Further investigation of vegetable oil treatment using this mixing technique might be meaningful for enhancing the microbial colonisation more effectively and practically.
Chapter 6

Ultra violet light exposure

6.1. Introduction

Two types of chemical application were discussed as surface modifications of LDPE film to increase sensitivity to microbial colonisation, however, there were several technical problems. One of the biggest problems was that applied surfactant inhibited microbial colonisation due to its toxicity. Although they increased microbial colonisation, the vegetable oils were also impractical as a pretreatment due to stickiness on the surface, which may be critical if an industrial application is considered. Another problem is that both of the applied materials were easily removed (aged) with gentle water rinsing treatments. These surface treatments should therefore be categorised as unsuitable methods as pretreatments applicable to packaging films. The problems may be solved if the methods of the applications were improved technically, e.g. the applied chemicals are blended into polyethylene mix. However, these treatments were not applied because this research is designed to find methods applicable to existing synthetic polymer formulations. What is needed as pretreatments are physical or physico-chemical methods which do not apply any chemicals to the LDPE films. In this chapter, UV light exposure to the LDPE film is discussed as a physical method using photolytic energy supply. Similar to the former chapters, general properties such as wettability, surface oxidation and
mechanical strength are discussed and finally microbial colonising phenomena are investigated.

Polymers are degradable by photolytic irradiation and the mechanism has been studied widely. Gijsman et al. (1999) reported that all polymers degrade in outdoor applications, and the greatest factor of the degradation was due to the energy from sunlight leading to photodegradation. Physical photo-degradation occurs after photo-oxidation has taken place (details are mentioned in Results and Discussion section) (Karlsson and Albertsson, 1995). It is known that degradation or oxidation occurs mainly when polymers absorb ultra violet light which is less than 290 nm of wavelength (Gijsman et al., 1999). The UV light is able to produce enough energy to cleave polymer chains and make polymers brittle.

UV light exposure to synthetic films has previously been studied to increase printability or wettability to adhesion with increasing surface energy (Brandrup and Immergut, 1989; Holmberg et al., 1993; Macmanus et al., 1999; Walton et al., 1997). No one has reported on any correlation between UV light exposed film and the phenomena of microbial colonisation, as far as the author knows. Microbial colonising phenomena on UV exposed LDPE film and the subsequent degradation were therefore discussed in this chapter after investigating basic physicochemical properties especially at the surface.

Surface changes, e.g. increasing surface energy, were expected to result from UV treatment, but it was also possible that exposure may reduce mechanical strength due to photo-degradation. General and physical properties were therefore investigated before testing microbial colonisation, and the best level of the UV exposing condition was established.
6.2. Methods of treatment

A lamp, "THORN 400 W MBF LAMP 50 Hz" purchased from Thorn Ltd., was used for the study of the ultra violet (UV) light exposure. This lamp was a mercury vapour lamp designed for lighting in warehouses, greenhouses etc., therefore, the range of the wavelength of this light was fairly similar to that of sunlight including significant amount of emission in the ultra violet part of the spectrum. A ‘dome shaped’ aluminium reflector was placed over flat surface, and the lamp was fixed at the top of the reflector 30 cm above the sample (Figure 6.1). The system was designed to allow effective air circulation with ventilation gaps at the top and the bottom of the reflector. An aluminium sheet was placed at the base in order to reflect the light efficiently. Test samples were placed on the aluminium sheet and exposed to the UV light up to 28 days continuously. Temperature was kept under 40 °C by allowing adequate airflow in order to minimise thermal influences on the films such as oxidation and thermal degradation.

![Figure 6.1. UV light exposure apparatus.](image-url)
6.3. Methods of estimation

6.3.1. Contact angle measurement

After the UV exposure treatment, the samples were gently washed with R.O. water and air-dried under dust free conditions. All other technical methods were described in Section 3.2. Changes during aging at room temperature after UV treatment were also studied.

6.3.2. FTIR analysis

FTIR analyses were carried out both on surface and inner-structural phases in order to observe the chemical changes due to the UV exposure. Methods and all other technical details were described in Section 3.3.

6.3.3. Estimation of microbial colonisation

Visible and quantitative analysis were performed to estimate the microbial colonisation. All the media methods were described in Section 3.6.

6.3.4. Tensiometry

The tensiometry tests were carried out in order to estimate the mechanical strength changes due to the UV exposure. Tensile strength and elongation at breaking point were measured. All technical methods were described in Chapter 3.4.
6.4. Results and discussion

6.4.1. Surface energy

Firstly, contact angle measurements were carried out on the UV treated LDPE samples with differing duration of exposure. Figure 6.2 shows the correlation between the total exposure time and the water contact angle. Before the exposure, the surface condition of the film showed highly hydrophobic properties ($\theta = 92.0^\circ$). The contact angles significantly decreased with increasing the duration of the exposure. The contact angle stabilised at $\theta = 70.0^\circ$, which indicates that the surface of the film became wettable and the surface energy increased. The contact angle, however, increased temporarily for the first 24 h of the exposure. A possible reason for this increase was considered that further polymerisation had occurred as an initial ‘impact’ due to the exposure (details will be discussed later).

Secondly, the durability of the modified surface energy was tested to estimate the aging properties on the UV treated LDPE films. The surface aging properties were tested for the UV exposed films for 14 ($\theta = 81.0^\circ$) and 28 days ($\theta = 69.4^\circ$). Figure 6.3 shows the result of the aging test performed under room conditions. No obvious surface energetic recoveries were observed for both samples, which indicates that the enhanced surface energy was reasonably stable. A little increase of the contact angle was, however, observed after 28 days of the aging test for 14-days UV treated LDPE film, but the change was still within the standard errors, hence insignificant. The aging tests were carried out under less strict conditions (dust free, moisture free, oxygen free as listed in Table 3.2), however, no greater aging effects were observed (data not shown). Thus the UV light exposure treatment should be categorised to be durable for aging and is potentially suitable for future industrial applications.
Figure 6.2. Change of contact angle of LDPE films exposed to UV light up to 28 days. Bars show standard deviations.

Figure 6.3. Change (recovery) of contact angles of UV treated LDPE films (aging test).
6.4.2. Surface oxidation and the other chemical changes

So what happened to the LDPE film by the UV exposure treatment? It was expected that certain chemical reactions (mainly oxidation) have been completed by the UV exposure, since the modified surface condition lasted a long time. If chemical reactions were not completed and temporarily free radicals were formed, the modified surface property might have recovered after the exposure and surface energy fallen close to the initial unexposed level.

To identify the chemical changes caused by the UV light exposure in air, FTIR analysis was carried out both for the inner-structure (by transmission FTIR) and for the surface (by reflectance FTIR). Figure 6.4 and Figure 6.5 show the peak index (i.e. relative IR peaks compared with non-treated LDPE) of the UV irradiated LDPE for the inner-structure and for the surface, respectively. The identical peaks are picked up in Table 6.1. Peaks at 3300, 1720, 1405 and 1160 cm\(^{-1}\) were significant for both charts, which attribute the increase of the compounds of hydroxyl (-OH), carbonyl (>C=O), alkane end (C-H) and ester (O-C=O), respectively (see Table 2.4 for infrared absorption). The reasons for the formation of each of the chemical compounds are considered as follows:

<table>
<thead>
<tr>
<th>Peak (cm(^{-1}))</th>
<th>Structure</th>
<th>(chem. bond)</th>
<th>Transmission</th>
<th>ATR (reflection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500-3000 (3200 peak)</td>
<td>Hydroxyl</td>
<td>(-OH)</td>
<td>Middle, Wide</td>
<td>Weak, Wide</td>
</tr>
<tr>
<td>1730-1700 (1720 peak)</td>
<td>Carbonyl</td>
<td>(&gt;C=O)</td>
<td>Strong, Sharp</td>
<td>Strong, Sharp</td>
</tr>
<tr>
<td>1420-1400 (1405 peak)</td>
<td>Alkane ends</td>
<td>(C-H)</td>
<td>Strong, Sharp</td>
<td>Weak, Sharp</td>
</tr>
<tr>
<td>1160</td>
<td>Ester</td>
<td>(O-C=O)</td>
<td>Middle</td>
<td>Strong, Sharp</td>
</tr>
</tbody>
</table>

Table 6.1. Peaks observed on UV exposure treated LDPE film and the affected chemical structures.

Ultra violet light exposure
Figure 6.4. Peak index of transmission FTIR charts of LDPE films exposed to UV light for 7, 14 and 28 days. Graph shows deviation from base line produced by non-treated sample. (The regular ripple in 7 and 14 day-treatments is thought to be an signal generation by the apparatus.)

Figure 6.5. Peak index of ATR (reflection) FTIR charts of LDPE films exposed to UV light for 7, 14 and 28 days.
C–H (alkane ends): smaller molecule compounds of polyethylene were created due to the result of degradation. Accordingly, the number of chain ends of polyethylene was increased. Thus, alkane ends (C–H) increased.

–OH (hydroxyl): existed as incomplete formula of the oxidation (reaction 6) and/or caused by water molecules around the substrate.

> C=O (carbonyl): a completed formula of the oxidation, which is considered to be an important intermediate on the total degradation process of polymers.

C–O (ester): result of ester-co-polymerisation, which is usually created from two activated oxidised polyethylene molecules (reaction 9).

A possible mechanism of the photolytic oxidising process of polyethylene (may also be adapted for other vinyl polymers) was proposed from the investigation of FTIR results and from other references (Karlsson and Albertsson, 1995; Lee, 1997).

\[
\text{O}_2 \text{ in air} \xrightarrow{\text{O}^*} \text{CH}_2\text{CH}^- \quad \text{(2)}
\]

\[
\text{Polyethylene} \rightarrow \text{Free radical appearance} \quad \text{(1)}
\]
A part of the molecular chain that absorbed photolytic energy ($h\nu$) is activated with forming a free radical (reaction 1). (* mark shows a free radical). This free radical plays a role in the activation of the substrate, and an oxygen molecule in air starts to react with the activated part (reaction 2). It then absorbs one hydrogen atom from the atmosphere (reaction 3), and eventually the part of molecular chain is oxidised with releasing one $H_2O$ molecule due to dehydration (reaction 4).

The oxidised formula of the polyethylene molecule represents a fairly active compound and could easily be involved in the next chemical reactions. When the oxidised compound absorbs more photolytic energy, it forms more unstable compounds (reaction 5).

This reaction is known as “Norrish type I reaction (or cleavage)”, which is recognised as an *initiator* of polymer degradation. Vink (1983) examined the absorption of oxygen with respect to the irradiation period under high concentrations of oxygen and a high level of UV light supply. Figure 6.6 shows the result (redrawn), which shows that the oxidation process develops linearly with the increase of the irradiation time.
On the other hand, polymerisation can also be produced from these unstable compounds. When two of the activated molecules react together, ester co-polymerisation occurs as shown in reaction 6. However, the level of the polymerisation is believed to be much lower than that of the degradation. Therefore, two contrasting reactions, 'degradation' and 'polymerisation' may occur from the same activated polyethylene molecules. It seems that the polymerisation level is stronger in the first 24 h of the UV exposure, hence the surface energy temporarily decreased as shown in Figure 6.2.

\[
\overset{\text{O}^*}{\text{CH}_2\text{CCH}_2} + \overset{\text{O}}{\text{CCH}_2} \rightarrow \overset{\text{O}}{\text{CH}_2\text{CCH}_2} \quad (6)
\]

The carbonyl formation as a result of oxidation of polyethylene film is considered as the most important chemical reaction for the subsequent biodegradation processes. It is possible for polyethylene films to be...
exposed to such strongly energetic condition, i.e. sunlight, for considerable period of time in the real environment which would enhance biodegradability. It is not considered that the application of UV is practical as a commercial pretreatment to enhance the biodegradability.

6.4.3. Microbial growth

It has been shown that the physico-chemical properties of the UV treated films were more suitable for obtaining microbial growth than untreated film. Biological tests were then carried out. Table 6.2 shows the result of the visible analysis of fungal growth using the test fungi grown on MEA (type I, listed in Table 3.4). No visible growth was observed for *Aspergillus niger* and *Chaetomium globosum*, while active growth was observed for *Paecilomyces variotii*, *Penicillium funiculosum* and *Trichoderma longibrachiatum*. These three strains were able to grow even on non-treated LDPE film (although the growth rates were very slow). It was therefore assumed that the UV treatment could only accelerate fungal growth when the fungi were originally able to colonise the substrate. Protein was assayed to give a quantitative measure of colonisation using the same samples, and the result was shown in Figure 6.7. A similar result to the visible analysis was, however, there was a measurable increase in the amount of protein on the surface (used as a measure of microbial colonisation) when inoculated with *Chaetomium globosum* even though there was no visible spread of the hyphae onto the film.

<table>
<thead>
<tr>
<th>Table 6.2. Visual analysis of microbial growth on UV light exposed LDPE film.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
</tr>
<tr>
<td><em>Chaetomium globosum</em></td>
</tr>
<tr>
<td><em>Paecilomyces variotii</em></td>
</tr>
<tr>
<td><em>Penicillium funiculosum</em></td>
</tr>
<tr>
<td><em>Trichoderma longibrachiatum</em></td>
</tr>
</tbody>
</table>

* Each code shows visible fungal growth from the incubated block in the central; (-) for non growth at all, (+) for 0–5 mm, (++) for 6–10 mm, and (+++) for 11–15 mm.
6.4.4. Mechanical properties

It has been confirmed that surface properties modified by the UV exposure were quite adequate both physically and chemically to enhance microbial colonisation, however, serious mechanical damage due to the exposure was a problem. The film became so brittle after 14 days of the exposure that the film could easily be torn by fingers and was obviously unsuitable for packaging.

The physical damage was confirmed by the tensiometry test before and after the UV exposure. Figure 6.8 shows the tensile strength, and Figure 6.9 shows the extension at breaking point. As suspected, the physical damage was so serious as to put into doubt if the UV treated film could be used for industrial purposes. Tensile strength became less than half and the elongation had nearly disappeared after 14 days of the UV exposure treatment. Clearly these figures do not satisfy the minimum requirement as packaging films.
Figure 6.8. Tensile strength of LDPE film treated with UV light. Bars at each point show standard errors. (n>10)

Figure 6.9. Elongation at breaking point of LDPE film treated with UV light. Bars at each point show standard errors (except where obscured by plot). (n>10)
A possible mechanism for the disintegration of the inner structure of LDPE film by the photolytic energy irradiation is illustrated in Figure 6.10. Photolytic energy supplied from a light source such as sunlight or artificial light is divided into two elements, "reflection" and "transmission", when the energy reaches the surface of a substrate. When the energy reflects at the surface, the surface molecules absorb part of the reflecting energy, and some work to labilise the surface structure which is called photo-degradation. On the other hand, when the energy passes through the film, the inside molecules absorb part of the energy, and some work to labilise the inner-structure of the substrate. Especially, ultra violet range of the light which contains a high energetic value hence causes the physico-chemical changes or disintegration of the structure. The LDPE film used for the experiment was transparent thus most of the energy passed through the film. Accordingly, the absorbed energy was more affected at the inner-structure rather than at the surface, which resulted in the inner-structural disintegration.

![Figure 6.10. Illustration of diffusion of photolytic energy through a transparent film. Most of the energy tends to pass through the substrate hence the inner-structure absorbs a high level of the energy.](image-url)
This phenomenon was confirmed with comparing the carbonyl compounds of the inner-structural and surface molecules using the FTIR index analysis. As there was a strong correlation between polymer oxidation and degradation, it was hypothesised that the evaluation of the degradation rate could be made with estimating the rate of oxidation. Figure 6.11 compares the IR peak index of the inner-structural and the surface molecules at 1720 cm\(^{-1}\) where carbonyl formations were quantitatively detected. This can also compare the relative amount of oxidised molecules at inner-structural and interfacial phases. It was observed that oxidation significantly developed inside the LDPE structure rather than the surface. Oxidation does help the degradation process (and helps biodegradation), however, it also means that the inner structure itself was significantly damaged due to the photo-degradation.
A minor and temporary increase of the tensile strength was observed after a short period of UV exposure to the LDPE film (Figure 6.8). The reason was again considered to be that the ratio of the polymerisation might be superior to the degradation in the initial stage of the irradiation. The main process of the polymerisation may be the ester copolymerisation, which can make cross-linking networks. Similar initial increases were observed from contact angle measurements (Figure 6.2) for the same reason.

6.5. Chapter summary

It was considered that the UV exposure treatment was at least a more effective and practical method than previously described surfactant and vegetable oil treatments in term of the simplicity, the facility to handle, and no concern of chemical residue on the surface. However, the confirmed physical damage of the inner-structure is critical if industrial application was considered. Also the treating period seems to be too long for industrial applications. Therefore, improvement of this point, or finding alternative methods to enable to targeting on surface oxidation only may be required.
Chapter 7

Corona discharge treatment

7.1. Introduction

Corona discharge treatment was proposed in order to overcome some issues pointed out in the former chapters. In terms of the surface modification, the UV light exposure worked effectively, however, the critical deterioration of the inner-structure should be improved and the treatment time shortened for industrial application.

To overcome this issue, alternative exposure methods which only target to the surface molecules were desired. One of the required factors was that the energy to be exposed should be more concentrated (or compressed) so that the period of exposure would be much shorter than the UV exposure. Examples of such rapid treatment are plasma treatment (Comyn et al., 1996; Kazuya et al., 1998; Morra et al., 1992, 1993; Tan et al., 1993), ozone treatment and corona discharge treatment. The corona discharge treatment has widely been recognised in industrial fields, but no studies on the effect of this on subsequent microbial colonisation or biodegradation have previously been carried out.

7.1.1. What is corona discharge?

The term “corona” is used to describe the condition of a gas, usually air
between electrodes. Gases such as air are ordinarily good electrical insulators, but in a strong enough electric field its insulating properties break down, thus, its molecules become ionised and it conducts electricity. If there is a sudden electric discharge between two conductive electrodes, an arc usually develops (Zhang et al., 1998). However if the electrodes are insulators the charges arriving at the electrodes are not conducted away and they quench the potential difference between electrode and the ionised gas so that no further current flows in that localised area. By using an alternating polarity on the electrodes the charge moves back and forwards between the electrodes maintaining ionisation of the gas between the electrodes. This is called a corona discharge and the photons emitted make it clearly visible as a blue glow.

The dominant theory concerning corona discharge is that polar groups are introduced into the polymer surfaces, which leads to higher surface energy and better adhesion (one of the areas of commercial interest). In practice, if a solid film is placed inside the corona affecting area, free radicals are instantly formed on the surface and the surface energy is increased. At the same time, oxygen molecules in the air are also activated by the energy of the corona. Activated oxygen forms elemental oxygen (O), active oxygen molecule (O₂*) and/or ozone (O₃), which is vibrationally and electrically excited above its ground state energy (Bezigian, 1992; Hansen and Sharpe, 1965). After a very short period of time, the activated oxygen reacts with the activated surface to form a stable oxidised surface (Matsunaga and Whitney, 2000).

Polar chemical functional groups are created on the surface, which enhance the surface adhesion and wettability. It is known that corona discharge can also change the morphology of polymer films and affect their adhesion properties (Owens, 1975; Stradal and Goring, 1975). Corona discharge treatment in the presence of air entails substantial morphological and chemical modification on the surface region of a
polymer, such as alteration of external appearance and formation of functional groups (Blais et al., 1974; Mittal, 1982). Although the magnitude and the nature of these modifications seem to depend on the treatment techniques and polymers used, the overall aim of these processes is to enhance the adhesive properties of polymers (Kaelble, 1971). The treated surface is known to be changed both chemically and physically by the corona discharge treatment (Egitto and Matienzo, 1994; Mangipudi et al., 1995).

7.1.2. Chemical changes

Adhesion and wetting properties of substrates having low surface energy can be improved by the corona discharge treatment because of the introduction of polar groups on the surface. The main chemical reactions due to the corona discharge treatment is oxidation (Zhang, 1998). In addition, the crosslinking of the molecules at the surface has taken place with inhibiting mobility of the surface molecules which result in increasing the molecular weight. Various analytical techniques have been used to identify these chemical functional groups (Schrader and Loeb, 1992; Smith, 1991), such as electron spectroscopy for chemical analysis (XPS), second any ion mass spectroscopy (SIMS), infrared spectroscopy (IR), electron spin resonance (ESR). Of these, XPS and IR have been most widely used. In this project, FTIR techniques were used to detect the oxidation and other chemical changes at the surface or inner-structure of the films.

Apart from the oxidation effects, it has been noted that corona discharge treatment also leads to further increase in the molecular weight of polymeric materials (Farley and Meka, 1994; Briggs and Kendall, 1979; Schonhorn and Hansen, 1967). The introduced free radicals work to increase the molecular weight of the polymer surface enough to give strength, a higher melting point, and greater integrity.
7.1.3. Physical changes

It is known that corona discharge treatment can change the surface morphology of polyolefin films as well as introducing polar functional groups to the surface. The polar component of surface energy is the key to understanding the change in adhesive behaviour of the films during corona discharge treatment. However, changes in surface morphology caused by these processes can also affect the level of adhesion. The surface morphology is usually studied by means of scanning electron microscopy (SEM) (Schrader and Loeb, 1992; Smith, 1991).

7.1.4. Industrial applications

The main industrial utilisation of corona discharge treatment is to increase wettablility of inks or adhesives by modifying a substrate that has low surface energy (Brewis and Briggs, 1981; Kruse et al., 1995; Liston et al., 1993; Sun et al., 1998). Theories proposed for the increased adhesion of corona treated polymer surfaces include electret formation (Stradal and Goring, 1975), elimination of weak boundary layers (Schonhorn and Ryan, 1974), increased surface roughness due to pitting (Kim et al., 1971), and the introduction of polar groups due to oxidation and other chemical changes in the surface region (Briggs and Kendall, 1982; Gerenser, 1985; Owens, 1975). The common theory is that corona discharge treatment causes an increase in surface energy by the introduction of polar groups on the surface. This improves adhesion and wetting properties (Mangipudi et al., 1995; Podhajny, 1987).

7.1.5. What was the aim of these experiments?

No studies have been reported which correlate between the corona discharge treatment and the ability of microbial colonisation, as far as the author knows. For this reason, fundamental characteristics of the corona discharge treated LDPE films were primarily observed and the ability of microbial colonisation was investigated.
7.2. Methods of the treatment

7.2.1. Corona discharge apparatus

The corona discharge apparatus is made from three different parts: high voltage supply, the discharge-head where corona is produced, and a running system that sheet samples can be discharge treated equally. The latter part was built in the Workshop in the School of Biological Sciences, University of Surrey whilst the high voltage supply power is on loan from Ahlbrandt System (UK) Ltd. Figure 7.1 shows photographs of the corona discharge apparatus.

To elucidate operation of the system, an illustrated figure of the system is shown in Figure 7.2. The high frequency generator converts mains at 50 Hz (250 V) to 10–30 kHz (still at 250 V) and power is controlled by altering the frequency. The high frequency supply is fed to a high voltage transformer to give the required voltage (approximately 14 kV). Figure 7.3 and Figure 7.4 show waveforms for low and high frequency, respectively. Peak voltage and times at peak voltage were unaffected. The discharge head having two electrodes is connected to the transformer to produce corona when high voltage is supplied.

The samples were treated whilst pressed against a rotating earthed aluminium drum by two belts. Each belt moves round three rollers (one of them is the drive roller and one a tensioning roller) at a constant speed. The speed was fixed to move the belt at 1 m/sec, that is, the aluminium drum rotating at 154 rpm. The discharge head was placed at 1 mm from the earthed aluminium drum by the two transport belts that carry the samples through the gap. There are two electrodes each producing a corona about 5 mm wide (i.e. total corona width of 1 cm). At 1 m/sec treatment time, exposure at any point on the plastic film is $10^{-2}$ seconds.
(a) The apparatus with ventilation system (produced ozone and heat can be removed).

(b) The apparatus in a draft chamber (produced ozone can be removed).

Figure 7.1. The corona discharge apparatus. (Rotating drum system must be closed during the treatment for a safety reason.)
(c) The rotating system with a discharge head and a rotating drum. Whole system must be closed by aluminium cover when operated.

(d) Discharged "corona" from the discharge head. Left: high voltage (1.0 kW). Right: low voltage (100 W).

**Figure 7.1.** (cont’d) The corona discharge apparatus
Figure 7.2. Illustration of the corona discharge treatment apparatus.

Figure 7.3. Wave form at low frequency

Figure 7.4. Wave form at high frequency
Table 7.1 shows the correlation of the absolute total exposure time, number of passes, number of rotations of the drum and the machine operating time. Samples were attached to the drum using PVC tape in advance when a treatment exceeds 100 passes. To reduce heating and ozone accumulation, air was drawn between the two electrodes of the discharge head at approximately 1.25 m³/min.

The following procedure was used when operating the apparatus.

**When starting:** Start the ventilation system → Start the roller → Start to produce corona

**When stopping:** Stop producing corona → (10 s) → Stop the roller → (10 s) → Stop ventilation.

### Table 7.1. Correlation of the total corona discharge treating times and number of passes

<table>
<thead>
<tr>
<th>Time of exposure* (to corona) (seconds)</th>
<th>No. of passes</th>
<th>No. of drum rotation</th>
<th>Machine operating time** (min: sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>0.02</td>
<td>2</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>0.05</td>
<td>5</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>100</td>
<td>0:39</td>
</tr>
<tr>
<td>2.0</td>
<td>200</td>
<td>200</td>
<td>1:18</td>
</tr>
<tr>
<td>5.0</td>
<td>500</td>
<td>500</td>
<td>3:15</td>
</tr>
<tr>
<td>10.0</td>
<td>1000</td>
<td>1000</td>
<td>6:30</td>
</tr>
<tr>
<td>20.0</td>
<td>2000</td>
<td>2000</td>
<td>13:00</td>
</tr>
</tbody>
</table>

* For which corona discharge is purely applied to the sample (0.005 s per pass).

** Only applied for multiple (>100) treatment.
7.2.2. Preparation of test sample

To aid the transport of the film through the apparatus, the film was temporally attached to a sheet of printing paper when treated with the corona discharge treatment system. The film was cut into $150 \times 240$ mm sections, and the three sides of the film sheet taped using masking tape to an A4 size paper ($210 \times 297$ mm) as Figure 7.5 shows. One side of the film sheet was not taped in order to remove air between the film and the paper efficiently when passing through the running system.

![Diagram](image-url)

**Figure 7.5. Test specimen for corona discharge treatment.**
7.3. Methods of estimation

7.3.1. Contact angle measurement

In order to observe the changes of surface energy or hydrophilicity, contact angle measurements were conducted. Surface energy aging was also observed. All technical methods were described in Section 3.2.

7.3.2. FTIR analysis

Both reflective (ATR) and transparent FTIR analyses were carried out in order to observe interfacial and internal chemical changes due to the corona discharge treatment. Especially oxidation properties were observed by this analysis. All methods are described in Section 3.3.

7.3.3. Tensiometry

To investigate the mechanical properties, tensile strength and elongation at breaking point were measured for corona discharge treated LDPE films with respect to the level of the corona exposure. All technical methods were mentioned in Section 3.4.

7.3.4. Surface morphology

A certain level of surface roughening was expected due to the corona discharge treatment as an exposing treatment, thus electron microscopical analysis was carried out using SEM. Details of the SEM analysis were described in Section 3.5.

7.3.5. Microbial colonisation

In order to investigate the microbial colonisation on the corona discharge treated LDPE films, both visible and quantitative analyses were performed. All technical methods were described in Section 3.6.
7.4. Results and discussion

7.4.1. Establishment of the best treating condition

Before discussing general physico-chemical and biological properties of corona discharge treated LDPE films, a couple of preliminary investigations were made in order to establish the most effective treatment conditions for the treatment times and discharging voltages. Firstly, determination of the best treating condition (i.e. the highest voltage and the highest number of passes but no physical damage to the film) was attempted. As shown in Table 7.2, the level of visible damage was marked from A to D, and only the samples marked “A” were utilised for the further experiments (however, the samples marked “B” were sometimes used if required). For samples marked C and D, the treatment condition was too strong for thin LDPE films, and critical physical damage such as melting due to the heat generated were observed. The condition of the corona discharge treatment selected was up to 500W for 500 passes (i.e. 5.0 s of total exposure time). This was the maximum effective treatment condition that did not cause physical damage.

7.4.2. Surface energy

Estimation of water contact angles at different conditions of the corona discharge treatment was carried out. Table 7.3 shows the result with respect to the voltage and number of passes. The surface energy or hydrophilicity of the LDPE film, which normally shows high hydrophobicity ($\theta = 92^\circ$), dramatically increased by the corona discharge treatment even at short periods of exposure (e.g. $\theta = 66.6^\circ$ at a single pass at 500 W). According to the effect of the treating wattage at a particular number of treating passes, the contact angles linearly decreased up to 500 W but no further decreases were observed for 800 and 1000 W.
Table 7.2. Visible mechanical damage on LDPE film with respect to treating time and strength of the electric power supply.

<table>
<thead>
<tr>
<th>No of passes</th>
<th>0</th>
<th>100</th>
<th>200</th>
<th>300</th>
<th>400</th>
<th>500</th>
<th>800</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>3</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>4</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>10</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>50</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>100</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>200</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>c</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>500</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>c</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>1000</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>2000</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td>d</td>
</tr>
</tbody>
</table>

a: good condition (no visible damage)  
b: small pits near taping places, however the film is applicable for further tests.  
c: large holes over the film, and film is no longer applicable for further tests.  
d: film (and base paper) seriously damaged and crumpled.

Table 7.3. Contact angles of water droplets on LDPE film with respect to treating time and strength of the electric power supply.

<table>
<thead>
<tr>
<th>No of passes</th>
<th>0</th>
<th>100</th>
<th>200</th>
<th>300</th>
<th>400</th>
<th>500</th>
<th>800</th>
<th>1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>92.0</td>
<td>88.6</td>
<td>87.8</td>
<td>77.2</td>
<td>79.2</td>
<td>66.6</td>
<td>65.4</td>
<td>66.1</td>
</tr>
<tr>
<td>2</td>
<td>92.0</td>
<td>84.0</td>
<td>68.0</td>
<td>63.2</td>
<td>57.0</td>
<td>63.6</td>
<td>64.5</td>
<td>62.8</td>
</tr>
<tr>
<td>3</td>
<td>92.0</td>
<td>74.0</td>
<td>61.0</td>
<td>58.4</td>
<td>56.4</td>
<td>55.8</td>
<td>54.6</td>
<td>56.8</td>
</tr>
<tr>
<td>4</td>
<td>92.0</td>
<td>70.0</td>
<td>63.2</td>
<td>59.4</td>
<td>56.0</td>
<td>57.2</td>
<td>53.0</td>
<td>53.4</td>
</tr>
<tr>
<td>5</td>
<td>92.0</td>
<td>65.0</td>
<td>63.4</td>
<td>62.8</td>
<td>59.2</td>
<td>56.6</td>
<td>53.7</td>
<td>52.0</td>
</tr>
<tr>
<td>10</td>
<td>92.0</td>
<td>-</td>
<td>54.3</td>
<td>-</td>
<td>52.3</td>
<td>53.8</td>
<td>52.0</td>
<td>52.0</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>92.0</td>
<td>56.6</td>
<td>49.5</td>
<td>47.4</td>
<td>52.3</td>
<td>48.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>200</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>500</td>
<td>92.0</td>
<td>55.5</td>
<td>49.2</td>
<td>45.4</td>
<td>48.1</td>
<td>42.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1000</td>
<td>92.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40.8</td>
<td>-</td>
</tr>
<tr>
<td>2000</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All contact angle data agreed within 2° of the standard error. "-": no measurement performed.
Also, physical damage was significantly observed at the treatment at 800 and 1000 W for multiple treatments, therefore, it was recognised that treating frequency at 500 W was the most effective condition.

According to the effect of number of passes of the treatment at a fixed electric frequency at 500 W, contact angles steeply decreased after only a single pass, and linearly (but less steeply) decreased up to 10 passes of the treatment. For the multiple passes of the treatment ($\geq$ 100 passes), the contact angle gently decreased and finally stabilised at $\theta = 42.8^\circ$ when passed 500 times at 500 W. When the relationship of contact angle and of the exposure time was plotted on logarithmic scale for the discharge time, a roughly linear correlation was observed as Figure 7.6 shows.

Effect of aging of the surface treatment was then investigated. Figure 7.7 shows the changes of the contact angles when different discharge levels of corona treated LDPE samples (1, 10 and 500 passes at 500 W) were exposed to the room-air environment. Recovery of the hydrophobicity was significant for LDPE samples treated only for a single pass, and its contact angle was recovered to nearly the same level of virgin LDPE film ($92 \pm 2^\circ$) after 7 days of the aging exposure. Even for LDPE sample passing for 10 times, most of the induced hydrophilicity had gone after 28 days of exposure. On the other hand, enough hydrophilicity left on 500 passes on LDPE film after 28 days of exposure, though a small level of recovery of the contact angle was observed in the first 7 days.

It was assumed that the mechanism of the surface modification of LDPE film of the corona discharge treatment was the same as that for the UV exposure treatment because both treatments were based on photolytic energy irradiation. Therefore, the process of the chemical changes was considered as Figure 7.8 shows (redrawn from 'reaction 1-4' in Chapter 6 for convenience).
Figure 7.6. Change of water contact angle for corona discharge treated LDPE films at 500 W of the discharge wattage.

Figure 7.7. Recovery of contact angle during air exposure.
It was considered that the initial free radical formation with accepting one unit of photolytic energy \((hv)\) contributed the significant decrease to the contact angle (i.e. increase in the surface energy). However, the surface condition at this stage is unstable and tends to revert to the original stable compound (Figure 7.8, reaction 1'). This condition relates to shorter frequency of the corona treatment (1 and 10 passes on Figure 7.7, in which sustainable oxidation has not been completed.

Figure 7.8. Possible mechanism for the formation of carbonyl groups on polyethylene molecules by photochemical irradiation.

On the other hand, sustainable treating effects without showing critical aging was observed when sufficient corona discharge treatment was applied repeatedly to LDPE film, e.g. 500 passes as Figure 7.7 shows. This result indicates that oxidation was completed on the surface and thus a sustainable chemical condition was obtained.
Such surface aging may not be so important when the corona discharge treatment is utilised to increase printability, ink- or adhesion-wettability because these processes are usually applied soon after the corona discharge treatment is made. However, surface aging would be a very critical factor when microbial colonisation and the subsequent microbial degradation are considered. Therefore, obtaining 'sustainable and active surface condition' was considered to be a suitable method of the pretreatment in this research.

The recovery of the applied surface energy for the short corona treated samples was remarkable, thus another investigation to determine the element(s) of the aging was attempted at different exposure conditions (as shown in Table 3.2). The test was performed under three different aging environments (air dust free [covered], oxygen free and moisture free) and the results were shown in Figure 7.9.

![Figure 7.9. Recovery of contact angle at different exposure condition. The films were corona discharge treated for 10 passes (0.1 s total exposure).](image-url)
As the graph shows, recovery of the hydrophobicity was greater in the order of:

ambient air environment > dust free > oxygen free > moisture free

thus the affecting elements were:

(dust + oxygen + moisture) > (oxygen + moisture) > (moisture) > (oxygen)

This result means that the effect of both oxygen and moisture in air was high, and moisture gives more effect on aging rather than oxygen. Consequently, the effect of the corona discharge treatment may be sustainable if the moisture level can be depressed.

It was considered that physicochemical factors for the aging (for plasma-treated LDPE though) were that decomposed low molecular weight polar substances were dispersed into air, and the grafted polar groups at the film surface were reversed into inert regions of the film (Gerenser et al., 1985; Stobel et al., 1992; Taru et al., 1986, 1996).

7.4.3. Surface oxidation and other chemical changes

Results of the FTIR analyses, which were performed in order to observe surface and inner-structural chemical changes, are shown in Figure 7.10 and Figure 7.11, respectively. Comments on the significant peaks shown in these figures are rewritten in Table 7.4 with reference to Table 2.3. Overall, similar peaks were observed both in the transparency and reflection IR, but the levels of the peak strength were different. The peak levels on the reflection IR were basically stronger than the inner-structural, which indicated that influences of chemical reaction due to the corona discharge treatment were higher at the surface than in the inner-structure.
Figure 7.10. Peak index of transmission FTIR charts of corona discharge treated LDPE films at different number (passes) of treatments.

Figure 7.11. Peak index of ATR-FTIR (reflection) charts for corona discharge treated LDPE films at different number (passes) of treatment (500W fixed).
Table 7.4. Peaks observed on corona discharge treated LDPE film and the affected chemical structures.

<table>
<thead>
<tr>
<th>Peak (cm⁻¹)</th>
<th>Structure</th>
<th>(chem. bond)</th>
<th>Transparency</th>
<th>ATR (reflection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3500-3000 (3200 peak)</td>
<td>Hydroxyl</td>
<td>(–OH)</td>
<td>Strong, Wide</td>
<td>Strong, Wide</td>
</tr>
<tr>
<td>1730-1700 (1720 peak)</td>
<td>Carbonyl</td>
<td>(&gt;C=O)</td>
<td>Strong, Sharp</td>
<td>Strong, Sharp</td>
</tr>
<tr>
<td>1620</td>
<td>Conjugated C=O</td>
<td>(C=O)</td>
<td>–</td>
<td>Middle, Sharp</td>
</tr>
<tr>
<td>1420</td>
<td>Alkane ends</td>
<td>(C–H)</td>
<td>–</td>
<td>Strong, Sharp</td>
</tr>
<tr>
<td>1190</td>
<td>Ester</td>
<td>(C–O)</td>
<td>Middle</td>
<td>Strong</td>
</tr>
</tbody>
</table>

For making quantitative analysis of the carbonyl compounds (which was recognised as an important intermediate on polymer degradation processes; peak at 1720 cm⁻¹), relative comparison for the FTIR index for the transparent and the reflection was made. As can be seen from Figure 7.12, approximately 5 % of the IR absorbance was observed for the transparency chart for 500 passes, whilst approximately 8.5 % was observed for the reflection chart. Therefore the formation of the carbonyl compounds were more active at the surface molecules than the inner-structural ones. For the UV treatment as Figure 6.11 already showed, the oxidation level was much greater inside the structure, thus structural deterioration was significant because the subsequent photo-degradation has also developed after the oxidation. For these reasons, it was expected that much less inner-structural damage occurred by corona discharge treatment, thus required film condition may be obtained.
7.4.4. Surface morphology

Figure 7.13 shows an image of surface roughness of the corona discharge treated LDPE film by means of SEM analysis. The film was treated by the corona discharge operator for 500 passes at 500 W. Small protuberances (bumps) were observed on the surface of the film while no such surface roughness was observed on non-treated LDPE film (the surface was so smooth that white-and-black contrast can not be obtained on the SEM analysis, thus the image was not shown). The diameter of the bumps were approximately 2–5 μm, and it was assumed that these bumps influenced measurement of the contact angle as well as the surface chemical changes. As Busscher et al. (1984) reports, surface roughness influences the contact angle if the distance between the bumps is more than 0.1 μm. Whichever the main effect of the changes of contact angle was, it was assumed that surface condition of LDPE film has become more suitable to microbial colonisation. Also no critical physical damage such as tears or holes were observed.
Figure 7.13. Scanning electron microscopy image (tilt: 70 °) of the surface of corona discharge treated LDPE film at 500 W for 500 passes. Small protuberances (e.g. circled with a white dotted line) were observed and the size of each protuberance was 2–5 μm in diameter.

Figure 7.14. Scanning electron microscopy image (tilt: 0 °) of the surface of corona discharge treated LDPE film at 800 W for 500 passes. Splinters or protuberances as surface damage were observed.
On the contrary, physical damage on the corona discharge treated film was observed when too strong corona discharge energy was applied. Figure 7.14 shows the surface image of a corona discharge treated LDPE film treated for 500 passes at 800 W. (From the visible analysis, this condition was marked as “level c” as Table 7.2 shows.) A large hole observed at top-right of the image was supposed to be a melted part where a highly compressed discharging energy was applied. Also, it was considered that sharp splinters observed over the film surface were created due to the extra discharge energy after bumps (as shown in Figure 7.13) were created. These splinters are observable like visible small pits, and they correlated with decreasing mechanical strength.

7.4.5. Mechanical strength

The tensiometry analyses were made in order to estimate the mechanical properties after the corona discharge treatment. Figure 7.15 shows the result of the tensile strength, and Figure 7.16 shows that of elongation at breaking point. According to the visible evaluation after the treatment, up to 500 passes – 500 W of the treating condition was accepted, and the corresponding results were obtained also for the tensiometry analyses. Firstly, the tensile strength showed the highest figure at 100 times of the pass at 100 W, and then gradually decreased with increase in the number of passes. The tensile strength also showed the highest figure at 100 passes at 500 W, but it significantly decreased at 1000 passes. The reason for the initial increase of the tensile strength up to 100 passes was considered to be that cross-linking might have happened in the polyethylene molecules. Whitney (1993) noted a similar initial increase in tensile strength in starch-filled polyethylene exposed to a range of different natural environments. He also thought this could be due to cleavage of linear chains allowing increase cross linking to take place. The reason for the dramatic decrease in the tensile strength at 800 W – 500 passes was considered to be due to structural damage to the polymer and possibly surface erosion of the film, shown by the slight dimpling of
the surface just visible in Figure 7.14. For 1000 W treatments, which is practically unrealistic since physical damage was so obvious, significantly low readings in the tensile strength were observed up to 100 passes.

![Figure 7.15. Tensile strength of corona discharge treated LDPE at different discharge wattage and number of treatments. Data over 500 passes for 1000 W treatment were not obtained due to critical physical damage.](image)

![Figure 7.16. Elongation at breaking point of corona discharge treated LDPE at different discharge strength and frequency. Data over 500 passes for 1000 W treatment were not obtained due to critical physical damage.](image)
Again this reason was considered to be due to the formation of small pits that results in decreasing mechanical strength. Note that it was unable to measure tensile properties for samples more than 500 passes at this electric frequency due to the critical mechanical damage.

For the analysis of the elongation at breaking point shown in Figure 7.16, there was no notable difference from the result of the tensile strength. However, the initial increase with 100 W and 500 W was not observed. This result indicates that the reason for the initial increase in tensile strength was due to formation of rigid cross-links, therefore, the flexibility (i.e. reading of the elongation at breaking point) decreased at this stage, and therefore the elongation properties also decreased.

### 7.4.6. Microbial colonisation

Tests for the visible and quantitative microbial colonisation were carried out to assess the biotic effects of corona discharge treatment of LDPE films. Table 7.5 shows the recorded result of the visible analysis of the fungal colonisation on LDPE films. Obviously, the rate of growth of the fungi increased with increasing number of the corona discharge treatments for almost all types of diagnostic fungi. Also the quantitative analysis using the protein assay could confirm this tendency as shown in Figure 7.17. For almost all types of fungi, the amounts of protein (e.g. the amount of the fungal growth) on corona discharge treated LDPE film were higher than non-treated films, though for *Chaetomium globosum* and *Trichoderma longibrachiatum* this was not significant.

The reasons for obtaining favourable biotic conditions on the corona discharge treated LDPE films were considered due to the increase of the surface electrostatic energy and the increase of the roughness of the surface, which correlate to the ability for microbial colonisation.
Table 7.5. Visible analysis of known types of fungi on LDPE films with respect to the exposure time of corona discharge treatment *.

<table>
<thead>
<tr>
<th>Type of fungi</th>
<th>Non-treated</th>
<th>Corona discharge treatment (passes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Chaetomium sp. (1)</em></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><em>Chaetomium sp. (2)</em></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><em>Chaetomium globosum</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Corynascus sepedonium</em></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><em>Curvularia sp.</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Fusarium sp. (1)</em></td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td><em>Fusarium sp. (2)</em></td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td><em>Paecilomyces varioti</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Penicillium funiculosum</em></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>Stachybotrys sp.</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Trichoderma sp.</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Trichoderma longibrachiatum</em></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Each code shows visible fungal growth from the incubated block in the central; (−) for non growth at all, (+) for 0–5 mm, (++) for 6–10 mm, (+++) for 11–15 mm, and (++++) for 16–20 mm.

---

![Graph showing amount of protein originated from test fungi attached to corona discharge treated LDPE film.](image)

**Figure 7.17.** Amount of protein originated from test fungi attached to corona discharge treated LDPE film.
7.5. Chapter summary

The corona discharge treatment was a more effective pretreatment than the other treatments applied (surfactant, vegetable oils and UV light exposure) in encouraging microbial colonisation. Also the corona discharge treatment was the most satisfactory in terms of mechanical strength and durability for aging. The speed, the facility and the continuity of the treatment made it the most suitable for industrial application. Moreover, this treatment is still applicable for food packaging purposes since it does not require any additional chemicals.
8.1. Introduction

The effect of the surface modification by the UV and corona discharge treatments was compared with the oxidation made by the treatment with some strong chemical oxidants. The chemical oxidation test was only proposed as a comparison of the oxidation level and further biotic tests were not carried out because some of the applied chemicals are toxic and they are all corrosive, therefore, commercial treatment is unlikely to be economic for a low cost packaging material.

Examples of chemical treatment procedures that have been used are chromium trioxide in sulfuric acid (Rasmussen et al., 1977), chromyl trifluoroacetate (Suggs and Yturate, 1986), potassium chlorate in sulfuric acid (Baszkin et al., 1976), potassium permanganate (Eriksson et al., 1984), potassium dichromate in sulfuric acid, and nitric acid (Bag et al., 1997, 1999; Tani et al. 2000). Again, these methods have mainly been studied only academically because the use of such toxic reagents such as chromium compounds makes these treatments unacceptable in any sort of commercial process (Bag et al., 1997).

In this experiment, LDPE films were treated with different concentrations of chromic acid (potassium dichromate in sulphuric acid) and nitric acid. Their contact angles, IR spectra and tensile properties were estimated.
8.2. Methods of treatment

8.2.1. Preparation of the chemicals

Approximately 5 g of potassium dichromate (K₂Cr₂O₇ = 294.19) and approximately 5 ml of R.O. water were mixed, and then 20 ml of concentrated sulphuric acid (approximately 18.5 molar) was added to obtain saturated chromic acid as supernatant. The chemical reaction here is:

\[ K_2Cr_2O_7 + H_2SO_4 \rightarrow K_2SO_4 \downarrow + H_2O + 2(CrO_3) \]

70 % nitric acid was used (undiluted concentrated nitric acid approximately 16.0 molar).

8.2.2. Treatment on LDPE films

Approximately 50 ml of the acid used was placed in a beaker and then 50 x 50 mm sections of LDPE film sheet was submerged into the reagent. The submerging periods of 1, 5, 30 and 180 min. were applied. After the treatment, the sample was washed with plenty of water (approximately 5 L) to remove attached chemicals, and then air-dried in a dust-free cabinet.

8.3. Methods of estimation

8.3.1. Contact angle measurement

Contact angle measurement to estimate surface energy/wettability was carried out immediately after the treatment. All methods are described in Section 3.2.
8.3.2. Surface oxidation

Reflective and transmission FTIR analyses were carried out to observe chemical changes. All methods are described in Section 3.3.

8.3.3. Tensiometry

Tensile strength was measured to observe mechanical strength. All methods are described in Section 3.4.

8.4. Results and discussion

Understanding the level of the surface modification by the chemical treated LDPE films using strong oxidants were conducted to compare to previously discussed corona discharge treatment and other pretreatments.

8.4.1. Surface energy

Changes of contact angles for chemical treated LDPE films using different concentration of chromic acid and nitric acid with respect to the treatment time are shown in Figure 8.1. It was observed that the ability of the surface energy enhancement was higher in chromic acid when the same concentration (70 %) of the chromic acid and nitric acid was compared. About a 30° decrease in contact angle was observed for chromic acid whilst only about 5° decrease was obtained for nitric acid. No statistical difference was observed between saturated and 70 % chromic acid. The reason was considered to be that enough treating effect continued even if the chromic acid was diluted to 70 % under this experimental condition (i.e. volume of the oxidant was much higher than the volume of the film).
The same level of the hydrophilicity ($\theta = 60^\circ$) was obtained from 180 min. of the chromic acid treatment and from 3–10 passes (or 0.015–0.05 s of total exposure time) of the corona discharge treatment of 100 passes (1.0 second of exposure) at 500 W of the electric strength (which does not damage the film).

8.4.2. Surface oxidation

ATR-FTIR (reflection) charts for LDPE films treated with 70 % chromic acid and 70 % nitric acid are shown in Figure 8.2 and Figure 8.3, respectively. These figures show the chemical changes of the surface molecules. Basically, similar charts were obtained for both oxidants, and their charts also resemble the peak of the corona discharge treated LDPE film. To compare the level of surface oxidation, Figure 8.4 shows the relative peak index for chromic acid and nitric acid at 1720 cm$^{-1}$. IR peak at 1720 cm$^{-1}$ indicates the level of carbonyl compound, thus refers to the level of oxidation.
Figure 8.2. Peak index of ATR-FTIR (reflection) charts for chromic acid (70%) treated LDPE films.

Figure 8.3. Peak index of ATR-FTIR (reflection) charts for nitric acid (70%) treated LDPE films.
No obvious evidence of the oxidation was observed for the nitric acid treated film, while there was about a 4 % decrease in the IR absorbance by chromic acid treated films. Therefore, chromic acid was more suitable to oxidise polyethylene. The level of the oxidation using 70% chromic acid for 180 min. on LDPE film was similar to that of corona discharged LDPE film treated for 500 passes (total of 5.0 seconds exposure) at 500 W. Since chromic acid is recognised as highly corrosive chemical, the use of corona discharge is a more practical treatment.

The transmission FTIR analyses were also carried out in order for both oxidants to observe the chemical changes at the molecules inside the film. However, no informative peaks were obtained (data not shown), which indicates that little chemical change has occurred within the film. It was therefore concluded that the oxidants only affected the surface molecules.

![Graph](image)

Figure 8.4. Relative peak index of ATR-FTIR of the peak at 1720 cm\(^{-1}\) for chromic acid and nitric acid treated LDPE films.
8.4.3. Tensile strength

Finally, the tensile properties of the chemical treated LDPE films were investigated. The tensile strength results were shown in Figure 8.5. No significant changes in tensile strength were observed for the nitric acid (70 \%) treated film. For the chromic acid treatment, an increase in the tensile strength was observed for 10 and 25 min. treatment times 100 \% chromic acid having a greater effect than 70 \% chromic acid. This is in agreement with the findings of Bag et al. (1997) who suggested this is due to the formation of polar groups resulting in hydrogen bonding on the surface of the treated film.

Figure 8.5. Changes of tensile strength of LDPE films treated with nitric acid (70 \%) and chromic acid (100 and 70 \%). Bars at plots show standard errors of the mean.
8.5. Chapter summary

Little effect of the surface modification of LDPE films were observed for nitric acid, whilst moderate surface-modifying effects were observed for chromic acid. However, the treating effect of the chromic acid was not as strong as the corona discharge treatment in terms of surface oxidation, although chromic acid (and also nitric acid) are generally recognised as strong oxidants. Also an agreement was made from the comparison of the chemical treatment and corona discharge treatment that the corona discharge treatment was more effective in terms of the treating speed. These chemical treatment investigations confirmed the efficiency of the corona discharge treatment for industrial applications. Also the corona discharge treatment does not need to use any chemicals hence be recognised as a safe treatment, whilst toxic chromic acid and corrosive nitric acid are unacceptable for most industrial applications.
9.1. Introduction

The most effective method, the corona discharge treatment, was selected for further study. The treatment worked most effectively in terms of the surface property without having critical inner-structural damages. This chapter evaluates the biodegradability of the corona discharge treated LDPE film in model environments.

Biotic soil and lake-water environments were selected as the places that surface modified LDPE samples were exposed, since plastic wastes tend to be discarded to these places in the real environment. Also, aerobic composts were prepared and the ability of the microbial colonisation and the biodegradability of the plastic films evaluated. As briefly mentioned in Chapter 1, composting treatment has been expected to emerge as a new clean technology for waste treatment (which may include synthetic plastics). Composting can be classified into two types; one is called “domestic compost” composting food and organic wastes. The other is called “agricultural compost” mainly comprising plant and animal wastes. What can be said for both types of compost is that plastic materials can easily be mixed with the other wastes, and they usually retard the total
degradation period. For example, food-packaging plastic materials are likely be contained in domestic composts with other food wastes, and packaging films of fertiliser can be contained in agricultural composts with other agricultural wastes. When the two types of compost are compared in terms of environmental impact, the agricultural wastes tend to be scattered widely whilst most domestic wastes are treated in a controlled manner. Thus it can be said that environmental impact of the agricultural compost is higher. The more careful attention was therefore paid to agricultural compost in this experiment rather than domestic compost. Model agricultural composts were prepared and the biodegradability of the LDPE film was evaluated in them.

In this biotic exposure treatment, influences of the backing paper, which was electro-statically adhered to the film by the corona discharge treatment, was also evaluated. These two materials (LDPE film and paper) were considered as a single product (PE/paper) and its biodegradability was evaluated.

After biotic exposure of treated film to the lake-water, the soil environment and the agricultural compost the following analyses were performed; 1) quantitative analysis of microbial colonisation, 2) tensile strength measurements and 3) visible and microscopical analysis of the films. The tensile strength and extension analysis are effective in determining the changes of polymer structure. Once physical damage such as producing holes or scratches are observed, visible and microscopical analyses were more effective to obtain information on the degradative processes.
9.2. Experiments

All external experiments were conducted in the summer (from June to September) because biotic activities are most dynamic in this season.

9.2.1. Selection and preparation of the model biological environments

9.2.1.1. Lake water
All the investigations of the lake water were made at the pond at Manor Farm, University of Surrey. Water samples were taken from the edge of the lake (where plastic wastes tend to accumulate). Approximately 100 ml of water was sampled from 10 cm depth in order to evaluate basic properties such as temperature, pH and amount of microorganisms.

9.2.1.2. Soil
A neutral sandy loam soil taken from Farncombe, Surrey was used. The soil was put in a tank (approximately 100 x 60 x 80 cm) and placed in the same greenhouse where the agricultural compost was located. Approximately 5-10 L of water was added to the soil twice a week (or more frequently if needed) in order to moisten the material. The surface of the soil was covered by dry newspaper to minimise evaporation. Soil samples were taken from approximately 25 cm depth from the surface and basic properties, such as temperature, pH, moisture content, amount of microorganisms and ratio of organic and inorganic compounds, were evaluated.
9.2.1.3. Compost
Approximately equal volumes (25 % each) of horse manure, wheat straw, fresh cut grass (green) and old cut grass (light brown) were well mixed, and enough water added to prepare a moist compost mixture. The mixture was then placed into a 1 m³ basket in the greenhouse (Figure 9.1). Extra water was supplied to re-moisten the mixture. The top of the compost mixture was covered by extra straw in order to avoid evaporation. Water was supplied twice a week (or more if needed). Samples of the compost mixture were taken from 25 cm depth from the surface and basic properties, such as temperature, pH, moisture contents, amount of visible organisms, calculation of the amount of microorganisms and oxygen contents, were measured.

Figure 9.1. Prepared agricultural composts. The size of the cages was approximately 1 m³. These composts were located in the greenhouse at the University of Surrey (Manor Farm).
9.2.2. Basic properties of the exposing environments

The following estimations were carried out for the three types of biotic environments.

- Lake water: temperature, pH, biotic activity,
- Soil (sandy loam): temperature, pH, bio-activity, composition (oxygen/moisture/organic/inorganic),
- Compost: temperature, pH, bio-activity, composition (oxygen/moisture/organic/inorganic),

9.2.2.1. Measurement of temperature

All the temperatures of the biological sites were measured at 6 pm. Temperature from 1.5 m height from the ground was measured to have air temperature in the greenhouse.

9.2.2.2. Measurement of pH

For the lake water, pH was measured directly from the sampled water. For soil and compost, on the other hand, suspension of 10 g (wet weight) of the solid matter was prepared in 100 ml R.O. water (pH = 7.1), and the pH was then measured.

9.2.2.3. Measurement of moisture content (for soil and compost)

Approximately 10 g of the soil and the compost matter was sampled and weighed to obtain the wet weight. Then, they were dried in an oven at 70 °C for 24 h and cooled in a desiccator for another 24 h to obtain dry weight. Moisture content (%) was obtained from the calculation of:

\[
\text{Moisture content (\%) = } \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100 (\%)
\]
9.2.2.4. Measurement of organic and inorganic compounds (for soil and compost)

Approximately 10 g of the soil and the compost matter was weighed to obtain the wet weight. Then, they were placed in a high temperature oven at 600 °C for 12 h to oxidise all organic compounds and cooled in a desiccator for 24 h to obtain the weight of inorganic matter. The ratio of the inorganic compound (%) was obtained from the calculation of:

\[
\text{Inorganic compound (\%) = } \frac{\text{Weight of inorganic matter}}{\text{Wet weight}} \times 100(\%)
\]

therefore, organic ratio (%) can be calculated from

\[
\text{Organic ratio (\%) = 100 - Inorganic ratio (\%)}
\]

9.2.2.5. Measurement of oxygen content

50 ml of R.O. water and 0.5 ml of mercuric chloride were placed in a 250-ml glass bottle. Mercuric chloride was added in order to kill any microorganisms growing in the liquid, which may affect the measurement of oxygen concentration. The top of the bottle was covered by filter paper so that only air could be transferred between inside and outside the bottle, and living organisms and the compost matter cannot contaminate the bottle contents. Two bottles were used, and one of the bottles was buried at the middle of the compost, and another one was buried at the edge of the compost (approximately 15 cm from an edge). Two of the bottles were placed at 25 cm depth from the surface. After five days of burial, the bottles were removed from the compost/soil and air-tight caps were immediately placed on top in order to terminate air exchange. The oxygen content was then measured using an Oxygen Meter (L.H. Engineering-500 Series III). When buried bottles were removed from the compost/soil, new bottles were placed at the same place where the old bottles were
buried, they were exposed for another five days. This cycle was repeated for five times, that is, the oxygen contents were measured up to 25 days (i.e. 5 days x 5 sets).

9.2.2.6. Measurement of colony forming unit (cfu) of microorganisms
To give a comparison of the biotic activity in these environments, cfu per gram or ml were estimated on a low nutrient medium as a simple model of their original environment. To measure the cfu of microorganisms living in the soil and compost, suspensions were prepared. 1 g (wet weight) of the soil or compost was suspended into 1 L of sterile R.O. water and settled for 2 h. A volume of 10 ml of the supernatant was then replaced into a sterile bottle which was considered to be the original suspension. Dilution series were then prepared up to $10^{-10}$. For the lake water, 10 ml of the water was directly sampled to a sterile Universal bottle, and then dilution series were prepared up to $10^{-10}$. A volume of 100 μl of each diluted liquid was placed on 1/10 MEA plate and spread. The plates were then incubated for 3 days at 25 °C. The number of colony forming unit (cfu) was counted to obtain the number of microorganisms in the soil and compost samples.

9.2.3. Methods and duration of exposure
LDPE films (untreated film, corona treated film, and corona treated film with backing paper) cut into 500 x 1000 mm sections were inserted into mesh bags (made from polypropylene; but the bag was regarded to be non-degradable), as shown in Figure 9.2 and they were exposed to the biotic environments. When measuring tensile strength, tensile dumbbell specimens (as explained in Section 3.4.1) were prepared and then inserted into these bags. The mesh bags with the test films were buried at 25 cm depth from the surface for the compost and soil treatment, and they were submerged at 10 cm depth from the surface of the lake water as the illustration shows in Figure 9.3. For the compost exposure, samples were exposed after the temperature settled at a stable level because the initial
increase in temperature was expected to cause some levels of physical (or thermal) damage. The samples were exposed to these environment for 50, 100, 150 and 200 days, and their biodegradation investigated.

Figure 9.2. A plastic mesh bag used for biological exposure.

Figure 9.3. Illustrated image of the environmental exposure of LDPE films into lake-water. Film samples contained in a mesh bag were placed at approximately 10 cm depth.
9.2.4. Methods of estimation

9.2.4.1. Microbial colonisation
In order to investigate the microbial colonisation on the films, the protein assay measurement was applied as a quantitative analysis. Samples cut into 50 x 50 mm sections were submerged into 10 ml of 0.5 M sodium hydroxide in a Universal bottle and then shaken for 24 h (at 100 rpm) in order to solubilise all protein adhered to the film originated with the microorganisms. The extract was centrifuged at 10,000 rpm for 10 min. at 4 °C. The supernatant was then tested by the Coomassie Brilliant Blue G-250 Dye binding method. 0.1 ml of the supernatant was mixed with 1:5 diluted dying reagent. Then absorbance at 595 nm was measured using a spectrophotometer. The obtained spectroscopic absorbance data was referred to two standard lines from known concentration of bovine serum albumin (BSA) and casein solution (details are shown in Section 3.6.4).

9.2.4.2. Visible and microscopic analysis
When visible degradation was observed for the films after biological exposure, surface and degraded edge were analysed using the SEM technique. Details of the SEM were described in Section 3.5. When observing the degraded edge of films, tilting methods were applied where appropriate in order to obtain “three-dimensional” images.

9.2.4.3. Tensile strength
Tensile strength and elongation at breaking point were measured for the biotic exposed LDPE samples (especially for those that had no visible damage after biological exposure). When backing paper was still attached to the films after the biotic exposure, it was carefully removed before measuring the tensile strength because the paper might affect the tensiometry measurement.
9.3. Results and discussion

9.3.1. Properties of the exposure environments

Evaluations of the exposure environments were made before LDPE film samples were exposed.

9.3.1.1. Temperature

Temperature shifts after preparing the environmental sites were measured for up to 50 days, and the results were shown in Figure 9.4. The dotted line in the graph shows room temperature in the greenhouse at 1.5 m above the ground level. Relatively stable temperatures were obtained for the soil (around 25 °C) and the lake-water (around 20 °C) whilst a significant initial increase of the temperature was observed for the compost soon after preparing the compost.

Figure 9.4. Changes of temperature at the bio-exposure environments (soil, lake water and compost). Measurements of the temperature were made at 6 pm.
The top temperature exceeded 60 °C soon after preparation of the compost, and the temperature gradually decreased to give a stable figure around 30 °C, eventually decreasing to around 25 °C. The initial increase of the temperature might have happened because thermophilic microorganisms were activated after enough oxygen, water and nutrient were supplied.

From the fact that high temperature condition (over 50 °C) remained for 3 days, it was expected that most pathogenic microorganisms living in the manure were killed (because most of them normally live at body temperature).

When an industrial or sanitary compost treatment is proposed as a method, which can treat wastes in a more environmentally-friendly manner (discussed in Chapter 12), the inside of the compost must be kept bio-active, however, any pathogenic organisms should be removed from the compost. This initial increase in compost temperature, which spontaneously occurs, might be effective to inactivate such organisms without affecting advantageous microorganisms too much for the plastic degradation. It was therefore recognised that the ‘preparation’ of the compost was completed after the initial heating is finished, i.e. four days after preparation.
9.3.1.2. pH

The shift of pH at each exposing site is shown in Figure 9.5. For the lake water, relatively stable pH was observed compared to the other biological sites because the lake was a more stable environment. On the other hand, changes in pH were significant for the soil and the compost environments since they were newly prepared for this experiment, hence the components were unstable. The soil showed low-alkali, but the pH gradually decreased and settled at around 7.0. The prepared compost showed strong-alkali but the pH gradually decreased. A possible reason for forming high pH was because urea contained in the horse manure was microbially converted to ammonia with increasing the pH. However, pH due to free ammonia and ammonium ions decreased when the components were exposed to aerobic conditions where nitrification could occur. The other possible reason was that some of the urea was leached out from the compost mixture when large amounts of water was supplied. In general, it is difficult to expect rich microbial growth in low-pH condition (Urushibara, 1994). In which aspect, the obtained condition (between pH = 6.5 to 8.5) may be suitable for microbial growth.

![Figure 9.5. Changes of pH of compost, soil and lake water where LDPE samples were exposed.](image-url)
9.3.1.3. Moisture and ratio of organic/inorganic materials

Percentage ratio of moisture/organic/inorganic compounds for soil and compost, which were obtained from moisture and ash contents, is shown in Figure 9.6. An obvious difference was observed between the soil and the compost: soil had a higher inorganic content whilst the organic fraction was richer in the compost mixture. According to the moisture content, the compost contained more nutrient rich water as it was prepared from nutrient rich materials such as manure and fresh cut grass. From these results, it could be said that the compost environment had a greater potential for microbial growth.

![Figure 9.6. Construction of moisture/organic/inorganic compounds in the soil and compost.](image)

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9.3.1.4. Oxygen concentration

It was considered that the oxygen concentration in the compost might greatly affect the degradative properties of LDPE films because chemical reactions differ greatly in aerobic and anaerobic conditions. Estimations as to whether the compost was aerobic or anaerobic was therefore important. Two bottles containing water and a tiny amount of mercuric chloride were buried in the compost, and gaseous exchange between the compost matter and the liquid inside the bottles was expected. After five days of burial, which was expected as enough duration to reach gaseous equilibrium between the two milieus, oxygen concentrations of the liquid inside the bottles were measured and the results are shown in Figure 9.7. The oxygen concentration was 76% at the middle of the compost for the first five days (date 1–5) of burial (78% at the edge). The oxygen level gradually increased and 83% was given for the last five days (date 21–25) of burial at the middle (85% at the edge). The oxygen concentration increased as the BOD declined with the microbial utilisation of the most easily metabolised components of the compost mixture.
These results showed that even the inside of the compost was highly aerobic. Thus, degradation may occur by the process suggested in this thesis. If the simplest degradation process of polyethylene is shown below, oxygen is needed when a alkane unit (repeat of CH₂) is degraded to produce water and carbon dioxide.

\[(CH₂)_n + \frac{3}{2} n O₂ \rightarrow n(H₂O) + n(CO₂)\]

It is therefore required that polyethylene film should be surrounded by plenty of oxygen molecules. In this concern, the prepared compost satisfied the requirement.

9.3.1.5. Amount of microorganisms

The amounts of microorganisms per unit volume of the biological matters (lake water, soil and compost) were measured in order to have information of biotic activities. For the lake water, dilution series were prepared directly from the original liquid. On the other hand, suspensions were prepared and then dilution series prepared for the soil and the compost. Therefore, comparison of lake water can not be made directly, however, these results were referred to in order to obtain a rough indication of the amount of microorganisms.

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**Figure 9.8.** Numbers of colony forming units in the lake water, soil and compost.
As a preliminary experiment, three days of incubation of these dilution series were carried out using un-diluted MEA plates, but too many colonies were formed. Therefore, a method using 1/10 diluted MEA plates were applied as explained in the experimental section. Figure 9.8 shows the result. While $10^6$ to $10^7$ colony forming units per gram and ml were observed for the lake-water and the soil, the compost showed about $10^9$ colony forming unit per gram, which is over 100 times more than in the other environments. The biological activity was the highest in the compost, but the activity in the lake and the soil was still high enough for them to be called *biologically active sites*.

**9.3.2. Microbial colonisation (from the quantitative analysis)**

Approximate amounts of microorganisms colonised on corona discharge treated LDPE films after 50 days of exposure to the environments were measured using the protein assay technique. Microbial colonising phenomena were also estimated for the corona discharge treated LDPE films with the backing paper (regarded as LDPE/paper composite). The results of the microbial colonisation for the lake water, the soil and the compost are shown in Figure 9.9, Figure 9.10 and Figure 9.11, respectively. The highest colonisation was observed on LDPE film exposed to the compost, while the lowest colonisation was observed on the one exposed to the lake water. It seemed that the absolute amount of microorganisms had a correlation between the soil and the compost. The reason for low microbial colonisation on the lake-exposed sample seemed to be that microbial adhesion was rejected and/or removed from the film due to the flow of the lake water. No microbial colonisation was detected by protein analysis for un-treated LDPE film exposed to the lake water environment as shown in Figure 9.9. The most considerable reason for this was due to the low surface energy, which prevents any microbial attachment to the film surface. Instead, microbial colonisation was observed for the corona discharge treated LDPE films although the colonisation level was not very high.
Figure 9.9. Amounts of protein originated from microorganisms colonised (attached) to corona discharge treated LDPE films (50 x 50 mm) with or without the backing paper in the lake water. Films were exposed for 50 days.

Figure 9.10. Amounts of protein originated from microorganisms colonised (attached) to corona discharge treated LDPE films (50 x 50 mm) with or without the backing paper in the soil. Films were exposed for 50 days.

Figure 9.11. Amounts of protein originated from microorganisms colonised (attached) to corona discharge treated LDPE films with or without the backing paper.
Especially for the corona treated film for only 10 passes, the colonisation level was significantly lower compared to the other LDPE films treated for 100 or 500 passes. It seemed that most of the modified surface properties had recovered on samples treated for short periods, removing free radicals early in the 50 days of environmental exposure. Higher levels of microbial colonisation were observed on the corona discharge treated LDPE films with backing paper. The reason was considered to be that microorganisms firstly attacked the backing paper since they could utilise cellulosic materials more easily rather than LDPE films, and secondly, these microorganisms adhered to the films because the density of microorganisms increased around the film (it seemed that microorganisms only adhered to films but not attacked).

Regarding the soil and the compost exposed LDPE films, the amount of colonising microorganisms increased with increasing number of the corona discharge treatments. The level of microbial colonisation of the LDPE film exposed to the compost was slightly higher than that exposed to the soil, however the differences were only just significant. A little colonisation on non-treated LDPE film was observed unlike the films exposed to the lake water. A possible reason for this microbial colonisation was that small soil grains pressed onto the film and physically damaged the surface and also became embedded into the plastic. The detected proteins might therefore be originated by the organisms attached to these grains (thus indirectly attacking the films). This surface damage and the attachment of small soil grains were observed.

For the films exposed to the compost on the other hand, the surface property was different from that of soil-exposed films. Sticky materials such as wet horse manure were attached to parts of the film, therefore organisms originating from the sticky matter were detected in the protein assay. Regarding the effect of the backing paper, the microbial
colonisation to the films with the backing paper was 28 to 90 % higher than the films without it. Degradation of the backing paper was clearly observed. Two things could consequently be concluded: the level of microbial colonisation (as measured by protein determination) increased with increasing the level of corona discharge treatment, and presence of the backing paper could enhance the level of microbial colonisation.

Effect of the backing paper was estimated by the SEM method, and Figure 9.12 shows a boundary part of the backing paper and the film phases. The film was corona discharge treated for 100 days at 500 W, and exposed to mature compost for 100 days with backing paper. Numbers of microorganisms were observed on the fibrous part, i.e. the backing paper. On the other hand, quite a few microbial attachments were observed on the smooth part, i.e. the film surface. As it is expected that the absolute amount of microorganisms existing on the film significantly correlates and relates to the biodegradability of the film, the backing played an important role in encouraging a range of microorganisms to get close to the film.
Figure 9.12. SEM image of corona discharge treated LDPE film with backing paper exposed to compost for 100 days. Richer microbial colonisation develops on cellulosic fibre (lower and left) rather than smooth LDPE film surface (upper right).
9.3.3. Visible and microscopic estimation

Environmental exposure of LDPE films was carried out at the bioactive sites up to 200 days. Varieties of the film conditions were obtained after the exposure depending on the exposing environment, the level of the corona discharge treatment, existence of the backing paper, and the number of days of exposure.

9.3.3.1. Lake water and soil

No degradation phenomena were observed on any types of film. The most likely reason is that this is due to the poor levels of microbial colonisation. However, several reports suggest that microorganisms (mainly fungi) living in aqueous environments (mainly marine) could degrade hydrocarbons and low-molecular synthetic polymers (Coony et al., 1995; Dexter et al., 1975; Kirk and Gordon, 1998; Kirk et al., 1991). It was also expected that some of the plastic materials discarded into ocean are photo-chemically and/or biologically degraded, because much more volume of plastic wastes have been discarded compared to the total volume of plastics found on seashores. A possible reason for this is that photo-irradiation that changed the surface condition occurred on the plastic wastes floating in the ocean, and physical deterioration of the long chain molecules eventually occurred. After such physical deterioration, the plastics were finally metabolised by particular microorganisms in the ocean. It is expected that the duration of the photo-irradiation in the practical environment is much longer than the experimental duration, which was 200 days at maximum. Therefore, the exposure levels both for the surface treatment (corona discharge treatment) and the environmental exposure were not strong/long enough to satisfy the microbial degradation.

No visible degradation was observed on soil-exposed LDPE films at any conditions probably for the same reason as for the lake water.
9.3.3.2. Compost
Sheets of polyethylene approximately 15 x 20 cm with and without backing paper were laid horizontally in compost approximately 20 cm below the surface of the compost. There was a significant degradation only on the corona discharge treated films with backing paper after 150 days of exposure. No obvious degradation was observed for 50 and 100 days of the compost exposure, and also no degradation was observed for the corona treated LDPE films without backing paper, or for untreated film with or without backing paper. Photographs and sketch (Figure 9.13) show that in places there is complete degradation of the backing paper (upper part of photograph) but the maximum degradation of the polyethylene (lower part of photograph) is where there is incomplete degradation of the backing paper. It would therefore appear that there is no correlation between the degradation of the LDPE film and that of the backing paper.

For the next estimation, the degraded parts of the films were observed using a light microscope, and the results are shown in Figure 9.14. Microbial colonisation near the degraded parts and attachment of the backing paper showed enhanced microbial colonisation especially on picture D. The degraded parts were relatively smooth, which indicates that the degraded parts/edges were created as a result of biological forces. However, it was still difficult to determine whether the degradation has been developed by micro- or macro-organisms, or other factors.

Finally, scanning electron microscopic analyses were made of the degraded parts and these results were shown from Figure 9.15: Figure 9.15 through to Figure 9.17 show the overlook of the degraded holes at lower magnification. Figure 9.15 shows that lots of microbial colonisation can be observed on un-degraded parts of the film, and Figure 9.16 (photographed obliquely) and Figure 9.17 (photographed from the top) show filamentous material crossing over the degraded holes. The filament was observed at
higher magnification as shown in Figure 9.18. The filament created a network and the joint part to the film indicates as if the filament grips the film tightly to absorb nutrient from the film. With these aspects, the filament was recognised as fungal hyphae.

Figure 9.19 shows the other part of the edge of the degraded LDPE film. A piece of the compost material (seemed to be a piece of straw) develops over the degraded edge, and richer microbial attachment can be observed on and around this material. It was expected that the organic compost matter assisted the microbial colonisation and subsequent degradation. Also, Figure 9.20 shows groups of microbial colonies surrounding the degraded edge of the LDPE film. Microbial degradation can be enhanced if there were richer microorganisms near the substrate.

The edge of the degraded LDPE film was observed using SEM at higher magnifications in order to determine how the degraded holes were created. After attempting a number of scans to a number of edges of the films, a picture was obtained which clearly shows the degraded edge as shown in Figure 9.21. Development of fungal networks were detected. It seems that the fungi and the film were unified to each other, which could mean that the hyphae adhered to the film in order to utilise it as nutrient source, which eventually leads to degradation of the film. It was observed from the other parts of the degraded edge of the film that these parts were very smooth which indicates that these parts were not created by macro-organisms (such as insects) thus, biting the film, but probably melted by the microbial activities.
Figure 9.13. Photographs of the degraded LDPE film (corona discharged for 500 passes and exposed to the compost for 150 days with backing paper) and the sketch. Degraded parts (A to D) are photographed using light microscopy shown in Figure 9.14.
Figure 9.14. Light microscopic photographs of the degraded parts of the LDPE film exposed to the compost for 150 days. Header (A) to (D) are relevant to the correspondent marks in the photograph in Figure 9.13. (Bars = 5 mm for all photos)
Figure 9.15. SEM of the degraded edge of LDPE film. Two holes seem to have developed due to microbial attack. This scan was made from a tilt 30°.
Figure 9.16. SEM of degraded edge of LDPE film. What appears to be bacterial cells and fungal filaments are visible on the surface of the film and filaments which could be a mixture of fungal hyphae and cellulosic fibres are bridging the hole that has been eroded in the film (tilt 70°).
Figure 9.17. SEM of degraded edge of LDPE film observed from the top (tilt 0°). This photograph is targeting the same degraded hole as shown in Fig 9.16. Fungal hyphae, possible cellulose fibres and bacteria are again visible.
Figure 9.18. SEM image of degraded edge of LDPE film observed from the top (tilt 0°). This photograph is targeting the same degraded hole as shown in Figure 9.17, and focusing the edge of the hole in higher magnification. The edge of the film is eroded rather than having a brittle fracture. The filaments may well be fungal hyphae growing on cellulose fibres remaining from the backing paper.
Figure 9.19. SEM image of the degraded hole of LDPE film. A piece of compost material (maybe a piece of cut straw which shows the sharp edges of a brittle fracture) crosses the degraded edge of the LDPE film and microbial colonisation can be observed on the compost matter. This scan was made from a tilt 70°. Bacteria which are very short rods are clearly visible on the left of the film.
Figure 9.20. SEM image of corona discharge treated LDPE film. Material adhering to the film is likely to be rich in microorganisms.
Figure 9.21. SEM image of an edge of the degraded LDPE film. Bacteria and fungal hyphae develop at the edge of the film. Erosion of the edges rather than sharp brittle fracture suggests it is utilised as nutrient sources by microorganisms. This scan was made from a tilt 70°.
9.4. Chapter summary

Since LDPE film is widely recognised as a very difficult material to biodegrade, the initial purpose of this research was aimed to enhance the microbial colonisation as a precursor to biodegradation. Therefore, observing such significant biodegradation within only 150 days was rather unexpected. It was considered that the degradation was due to a number of simultaneous factors such as the surface conditions of the film after the corona discharge treatment, role of attached backing paper and biological activity in the compost.

The other interesting thing was that the degradation did not occur all over the surface even though every part of the surface was touched by the compost material. This fact led to the conclusion that “degrading microorganisms” exist at particular places in the compost, and this fact should be considered on future applications of such compost treatments. In the following chapter, isolation and cultivation of the degradable microorganisms attached to the degraded film, and purification and identification of the organisms was conducted.
Chapter 10

Purification and identification of polyethylene degrading microorganisms

10.1. Introduction

Significant microbial degradation was observed on corona discharge treated LDPE film after being treated in the compost for 100 days. This result indicated that both surface condition of the film and microbiological environments that the film was exposed to were quite important to distinguish the speed of microbial degradation of synthetic plastic films. In this chapter, incubation, cultivation and identification of LDPE degrading microorganisms was attempted.

The basis of these experiments is related to Koch's postulates. Koch's postulates are designed to distinguish between causal organisms and coincidental or secondary organisms in a disease. They can also be applied to a situation such as biodegradation. To investigate the system using Koch's postulates it is necessary to (1) isolate organisms from degraded plastic or an environment in which degradation is likely, (2) grow the organism in isolated culture, (3) show the organism is capable of causing biodegradation of the plastic film and (4) ideally re-isolate the organism from this film and show it to be the same as that isolation in (2).
It was assumed that few types of microorganism could degrade LDPE film although many types of microorganisms attach to the LDPE film. Therefore the particular microorganisms that degrade LDPE have to be purified. The purification was made by repeated subculture and incubation in media in which polyethylene was supplied as the sole carbon source, so that only polyethylene degrading microorganisms were expected to multiply and not be diluted out. Powdered LDPE and oxidised LDPE powder were used as the carbon sources in these experiments. Oxidised polyethylene was used as this was considered to be more readily degraded by microorganisms. The method is based on that used by Ohtake's group which they call accumulative incubation method, which they used for purifying polyolefin-degrading microorganisms. They used photo-irradiated and pulverised LDPE as carbon source, however, because of difficulties in pulverising LDPE, even after freezing in liquid nitrogen, powdered and oxidised powdered LDPE was used. The other nutrients were provided using a minimal salt mixture. With regard to the ratio of such supplement, it is widely recognised that microbial growths especially for fungi are accelerated under low-nitrogen and high-carbon (LN-HC) condition (Ehara et al., 2000-b; Ehara et al., 1997; Katagiri et al., 1997). Ehara's group in Shizuoka University estimated that the best ratio was C:N = 30:1. However, the speed of the consumption of the LDPE powder was expected to be very slow compared to the other carbon source such as glucose, thus excess LDPE powder (C:N = 300:1 or more) was supplied in the incubation system. Low concentration of MEA or Nutrient agar was added to the media containing LDPE powder or oxidised LDPE powder when the initial culture was prepared from the degraded LDPE film. The reason for this was to accelerate the initial growth of microorganisms from the degraded polyethylene film into the culture medium.
Biodegraded LDPE films are cut into small sections. Degrading microorganisms still attach to the films.

Film sections are placed in a liquid medium (50 ml) containing LDPE powder and a small amount of Nutrient broth or MEA to support initial growth in the medium.

Purification of LDPE degrading microorganisms is repeated in liquid media containing LDPE (powder) as the sole carbon source.

Isolation and identification of single microorganisms,

As single isolates of degrading microorganisms

Re-inoculation to fresh LDPE (either non-treated or corona discharge treated) film

Figure 10.1. Illustrated diagram of methodology applied in this chapter. Re-inoculation tests are described in the following chapter.
It was expected that microorganisms merely growing on the surface as well as those attacking the polyethylene would also survive on the isolation plates, so a series of subcultures were carried out into media containing only polyethylene or oxidised polyethylene as the carbon source. Streak plate technique was used to isolate pure cultures of microorganism from the final incubations. Figure 10.1 shows the diagram for the process of this experiment. According to Koch's postulate, re-inoculation of the isolated microorganisms to fresh substrate is needed. This is discussed in the following chapter.

10.2. Materials and methods

10.2.1. Obtaining suspension of LDPE degrading microorganisms

The biodegraded LDPE film (corona discharged and exposed to the compost with the backing paper) previously considered (Figure 10.2) was used in this study. Care was taken not to dry out the degraded film from which isolation was made, before starting this experiment because drying out of the film may kill the microorganisms degrading the film. The degraded parts of the film with paper backing were cut into pieces (not exceeding 30 x 30 mm). 50 ml of diluted White's basal salt mixture (Sigma, containing 46.5 µl in 50 ml, Table 10.1), 1 g of oxidised LDPE powder and either 0.01 % Nutrient broth or 0.01 % malt extract broth, were placed in a flask and sterilised at 100 °C for 30 minutes. Sterility was confirmed by plating uninoculated media onto MEA and Nutrient agar plates.

Figure 10.2. Degraded part of the corona discharge treated LDPE film exposed to the compost for 100 days with backing paper. It is believed that some of those microorganisms colonised to the film contributed to the degradation.
Table 10.1. Ingredient of White's salt mixture (mg/l)

<table>
<thead>
<tr>
<th>Major</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>KI</td>
</tr>
<tr>
<td>MgSO₄ + 7 H₂O</td>
<td>H₂BO₃</td>
</tr>
<tr>
<td>NaH₂PO₄ + H₂O</td>
<td>MnSO₄ + 4H₂O</td>
</tr>
<tr>
<td>Ca(NO₃)₂ + 4H₂O</td>
<td>ZnSO₄ + 7H₂O</td>
</tr>
<tr>
<td>KCl</td>
<td>CuSO₄ + 5H₂O</td>
</tr>
<tr>
<td></td>
<td>CoSO₄ + 6H₂O</td>
</tr>
<tr>
<td></td>
<td>Fe(SO₄)₃</td>
</tr>
</tbody>
</table>

*9.3 g of this mixture to be diluted with 10 L water. The pH usually showed 6.8 after being diluted.*

The pieces of the film were then placed in the liquid medium and incubated for 15 days at 25 °C with shaking the flask at 150 rpm. Low concentrations of Nutrient Broth or Malt Extract were added to these initial isolation medium to try to increase the total microbial population both polyethylene degraders and non-degraders (enrichment) before selection of LDPE degrading organisms on media with LDPE as the sole carbon source.

10.2.2. Purification of LDPE degrading microorganisms

The purification stage was carried out in liquid media having LDPE as sole carbon source. The medium was prepared with 50 ml of the diluted White's basal salt mixture (containing 0.93 g/l, Table 10.1) and 1 g of either oxidised or non-oxidised LDPE powder, and then sterilised at 100°C for 30 minutes. These liquid media were called either LDPE media or oxidised LDPE media. 1 ml of the initial isolation suspension was placed into each of the purification media (LDPE and oxidised LDPE media) and incubated for 15 days at 25 °C with shaking at 150 rpm. After the incubation, 1 ml of the incubated medium was placed into fresh LDPE medium or oxidised LDPE medium and incubated for a further 15 days at 25 °C. This was repeated for four times to purify LDPE degrading microorganisms.
10.2.3. Quantitative evaluation of microorganisms in purification stage

100μl of the incubated liquid was placed at each incubation stage, and spread onto either Nutrient agar plate or MEA plate with depending on the type of nutrient used at the initial incubation. These plates were incubated for 3 days at 25 °C, and then the colony forming units (cfu) were counted.

10.2.4. Isolation and identification of the LDPE degrading microorganisms

Drops of the final culture were spread on sterile Nutrient agar plate or MEA plate depending on the type of nutrient used in the first incubation) and streaked across the surface of the agar. Colonies of a single type of microorganism were obtained using this streak plate technique. Each colony was subcultured to a fresh medium to obtain isolated single culture. The isolates were then observed using optical microscopy with and without staining with lacto-phenol blue. Two fungi were isolated and identified, and photographed and one bacterium was isolated, Gram's stained and photographed.
10.3. Results and discussion

10.3.1. Experimental condition of the initial incubation and purification

There was a technical problem when the LDPE powder was autoclaved with the other ingredients to sterilise the medium at 120 °C under pressure. The LDPE formed a thin membrane on the surface of the medium. A new sterilising protocol was established for this study, and the condition was at 100 °C for 30 minutes. Under this condition the LDPE and oxidised LDPE remained as a powder. Sterilisation of the media was confirmed under this condition by plating uninoculated samples onto MEA and Nutrient agar and incubating for 7 days at 25 °C and no growth occurred.

10.3.2. Purification of LDPE degrading microorganisms

Figure 10.3 to Figure 10.6 shows the numbers of the colony forming units at every 15 days of incubation at different nutrient condition in the media. The triangle dots and the thicker line in Figure 10.3 show the change of cfu when LDPE powder was supplied as the sole carbon source. The dots in the graph show the calculated number of cfu if the initial cfu of microorganisms were diluted to the following incubation steps without growing and dying. The cfu observed in the media were more than this calculated figure, which means that some microorganisms could survive and grow in the media. After having 3 and 4 periods of incubation the numbers of cfu had stabilised or increased, which shows that the microorganisms utilised LDPE powder as carbon source. The square dots and the thinner line in the same figure show the change of cfu without supplying LDPE powder, which means that there was no carbon source except for the tiny volume of malt extract broth used in the initial incubation. As 0.005 g of malt extract in 50 ml liquid medium was diluted to 1:50 for four times, the remaining malt extract was $8 \times 10^{-10}$ g.
Figure 10.3. Changes of colony forming unit of PE-powder degrading microorganisms in LDPE media. 1% malt extract was added for the first 15 days as the initial incubation.

Figure 10.4. Changes of colony forming unit of LDPE powder degrading microorganisms in oxidised LDPE media. 1% malt extract was added for the first 15 days as the initial incubation.

For both figures, marks "×" in the graphs show expected number of microorganisms if the original number of microorganisms diluted at sub-culturing without any growth and death (1/50 diluted at sub-culturing).
Figure 10.5. Changes of colony forming unit of LDPE-powder degrading microorganisms in LDPE media. 1% Nutrient broth was added for the first 15 days as the initial incubation.

For both figures, marks “×” in the graphs show expected number of microorganisms if the original number of microorganisms diluted at sub-culturing without any growth and death (1/50 diluted at sub-culturing).
This result is puzzling because of the lack of carbon source. For Figure 10.4 (for oxidised LDPE powder selection medium and initially isolated with LDPE plus Malt Extract broth), Figure 10.5 (using LDPE powder selection medium and initially isolated with LDPE plus Nutrient Broth), and Figure 10.6 (for oxidised LDPE powder selection medium initially isolated with oxidised LDPE plus Nutrient Broth), similar results were obtained. The number of cfu was always higher than the calculated number, which strongly suggests that LDPE was used as nutrient source by certain types of microorganisms.

10.3.3. Isolation and identification of LDPE degrading microorganisms

Isolates were obtained as pure culture after using the streak-plate technique and re-inoculation on to sterile media. Two fungi and one bacterium were successfully isolated. Preliminary identification of the microorganisms was carried out by microscopic observations. One of the fungi having grey green spores and after microscopic observation was thought to be *Aspergillus fumigatus* (Figure 10.7). The other fungus produced colonies with black spores and after microscopic examination was more tentatively identified as *Acremonium charticola* (Figure 10.8). The isolated bacterium produced creamy-white coloured colonies. After Gram's staining the bacteria were pink coloured indicating they were Gram negative and they appeared to be cocci or very short rods (Figure 10.9 and Figure 9.19). No further identification has been completed yet.

These isolates are re-inoculated to fresh LDPE film as described in the following chapter.
Figure 10.7. Light microscopic images of the isolates as polyethylene-degrading fungi. The isolate was identified as *Aspergillus fumigatus*. 
Figure 10.8. Light microscopic images of the isolates as polyethylene-degrading fungi. The isolate was identified as Acremonium charticola.
Figure 10.9. Light microscopic images of the isolate bacterium from the biodegraded LDPE film. This bacterium seems to be Gram's negative coccus or very short rod.
10.4. Chapter summary

Purification, isolation and identification of LDPE degradable microorganisms were carried out with following the modified theory of Koch's postulate. To purify the LDPE degrading microorganisms, screening was carried out in limited nutrient condition, and only purposed microorganisms were obtained. Finally, two fungi and one bacterium were isolated, and their identification was progressed. *Aspergillus fumigatus* and *Acremonium charticola* were considered to be the purified fungi, however, identification of the bacterium was not completed.
Chapter 11.

Re-inoculation of microorganisms isolated from degraded LDPE film

11.1. Introduction

In the previous chapter isolation of LDPE degrading microorganisms was carried out using LDPE media or oxidised LDPE media (using only LDPE or oxidised LDPE as carbon source). It was recognised that the incubated liquid contained a mixture of LDPE degrading microorganisms. To test if these organisms were those that degraded the original LDPE samples (i.e. using Koch's postulates), the isolated single cultures were re-inoculated onto fresh LDPE films (corona discharge treated and non-treated), on an agar plate containing Whites mineral salt medium and also the group of LDPE degrading microorganisms onto LDPE films in Whites liquid medium. The physical changes of the films were measured by tensile strength and extension measurements. Any visible changes to the film were also noted. A laboratory modelled or miniature system of the compost environment was prepared using wheat straw, onto which the isolates and the group of LDPE degrading microorganisms were sprayed. Fresh LDPE films were placed in the compost and changes in tensile strength and extension at breaking point were estimated.
11.2. Materials and methods

11.2.1. Test LDPE films

For the corona discharge treated LDPE films the level of treatment was 500 W for 500 passes (5.0 s total exposure). Non-treated LDPE films were also tested. Films were cut into the tensile specimens (described in Section 3.4.1). Films were not sterilised, however care was taken that all preparatory work was conducted without attaching significant dust or grease (i.e. finger prints).

11.2.2. Re-inoculating LDPE film with isolated microorganisms

The tensile strength test specimen was placed on sterile agar plate made from diluted White's salts medium (0.93 g per litre). A block (about 5 mm³) of either fungal or bacterial culture was taken with agar from the plate and used to inoculate the middle of the specimen as illustrated in Figure 11.1. The Petri dish lid was then sealed using PVC tape in order to protect it from drying out during extended incubation. The plates were incubated at 25 °C for 15, 30 and 60 days. Tensile strength and extension at breaking point of the incubated LDPE specimens were measured at the end of these periods.

![Diagram](image)

**Figure 11.1.** Illustrated method to place microbial inoculum on a piece of LDPE specimen.
11.2.3. Re-inoculating the LDPE film with the mixture microorganisms from culture.

Ten pieces of tensile strength test specimen were placed into sterile liquid media prepared from dilute White's mineral salts mixture (0.93 g per L). The volume of the liquid was approximately 50 ml. A 1 ml aliquot of the final culture using LDPE or oxidised LDPE as a carbon source was re-inoculated into the sterile liquid media. Incubation was conducted at 25 °C with shaking at 150 rpm for 15, 30 and 60 days. Tensile strength and extension at breaking point of the incubated LDPE specimens was measured at the end of these periods.

11.2.4. LDPE film degradation in model compost environments inoculated with the mixture of microorganisms from culture

To construct model compost environments with rich LDPE degrading microorganisms, miniature compost environments were prepared. A dry mass of 10 g of wheat straw (cut into approximately 3 cm lengths) and 10 pieces of the tensile specimen of the LDPE film (either non-treated, or corona discharge treated with or without backing paper) were placed in a 500 ml flask. A volume of 10 ml of the final culture using LDPE as a carbon source was poured over the straw. No other liquid was added to the compost. The top of the flask was capped with a foam plug covered with aluminium foil. These model compost environments were then incubated at 25 °C at a constant humidity (at 60 %) for 15, 30, 60 and 90 days. After the incubation, tensile strength and extension at breaking point were measured in order to estimate any degradation of the films.
11.3. Results and discussion

11.3.1. Re-inoculation of a single isolate onto LDPE films

Three types of the LDPE degrading microorganisms were re-inoculated onto fresh LDPE films or CDT-LDPE films (with and without backing paper). There were no changes in tensile strength, extension or appearance for 15 and 30 days of the incubation (data not shown). Neither were there any significant changes to the tensile strength for all types of LDPE films caused by any of the microorganisms after incubation for 60 days. The results obtained for changes in tensile strength are shown in Figure 11.2. The results obtained for changes in the extension at breaking point is illustrated in Figure 11.3, which shows that average extension at breaking point was rather lower compared to the non-treated film. However, the error bars do overlap between the measurements before and after incubation, hence no statistical differences can be indicated. It was therefore considered that no significant degradation of the LDPE occurred on any of the re-inoculated plates.
**Figure 11.2.** Changes of tensile strength of LDPE films after being incubated with pure microbial isolates for 60 days.

**Figure 11.3.** Changes of elongation at break of LDPE films after being incubated with microbial isolates for 60 days.
11.3.2. Re-inoculation with the mixture of microorganisms from the LDPE culture.

LDPE films (both non-treated and corona discharge treated with and without backing paper) were placed in the same liquid media that the mixture of the LDPE degrading microorganisms existed. The result of the tensile strength measurements after incubation for up to 60 days is shown in Figure 11.4. Though no significant differences in the tensile strength were observed for non-treated LDPE film, statistically significant decreases in the tensile strength were observed for the corona discharge treated films (both with and without backing paper). The tensile strength decreased with increasing period of incubation. The levels in decreases were slightly more significant for corona discharge treated film with backing paper. Figure 11.5 shows the changes in extension at breaking pointing point for the corresponding experiments. There also had no significant change in extension for non-treated LDPE film, however the extension dramatically decreased soon after the incubation for corona discharge treated films.

What could be deduced from these results was that the mixture of the LDPE degrading microorganisms influenced only the corona discharge treated films, although no significant influences were observed from incubation with single fungal or bacterial inocula. This means that degrading reactions can occur only if the mixture of LDPE degrading microorganisms react as a consortium. Also, there was no doubt that surface modification of the LDPE film was very important to initiate the degradation process by increasing the ability of the microbial community to colonise the film. The backing paper enhanced the biodegradability (though the difference was not so significant), which indicates that the attached backing paper could enhance access of the degrading microorganisms to the film via backing paper. In general, it is believed that isolating particular LDPE degrading microorganisms is technically difficult.
Figure 11.4. Changes of tensile strength of LDPE films treated with the group of LDPE degrading microorganisms in liquid media.

Figure 11.5. Changes of extension at break of LDPE films treated with LDPE degrading microorganisms in liquid media.
11.3.3. LDPE degrading microorganisms in a model compost environment.

In order to observe the effect of the mixture of the LDPE degrading microorganisms in a model of a real application, the degrading phenomena of the LDPE films were tested in model (miniature) compost environments. It could be said that this compost was designed specially for LDPE degradation because the LDPE degrading microorganisms were inoculated at a high concentration onto the compost. Figure 11.6 shows the changes of tensile strength of the LDPE films after 90 days of compost exposure.

These results are similar to those in the previous section (liquid incubation), statistical changes of the decrease of the tensile strength were observed only on corona discharge treated LDPE films, and the decrease was more significant for the corona discharge treated film with backing paper than that without. Very similar statistical information was obtained from the results of the extension at breaking pointing point, which are shown in Figure 11.7. These results show that the degradation in the compost inoculated with organisms from the cultures using LDPE as a carbon source was a little inferior to the direct incubation in liquid media similarly inoculated, as shown in Figure 11.4 and Figure 11.5.

These results indicate that inoculation of compost with a suitable consortium of microorganisms can accelerate the rate of degradation of low density polyethylene by a significant amount.
Figure 11.6. Changes of tensile strength of LDPE films after being incubated in miniature compost containing mixture of the LDPE degrading microorganisms.

Figure 11.7. Changes of extension at break of LDPE films treated in miniature compost containing mixture of LDPE degrading microorganisms.
Chapter 12

General discussion and conclusion

12.1. Summary discussion

The ultimate target of this research was to enhance the speed of biodegradation of LDPE films. LDPE film was believed to be inert to microbial attack, however, recent studies have proved that it is biodegradable although the rate is very slow (Albertsson, 1989; Ohtake et al., 1996-a, 1998-b, 1999). The rate of biodegradation is slow when LDPE film is directly exposed to an environment, such as soil. However, it was considered that the rate of degradation may be enhanced if appropriate physico-chemical pretreatments were made to the film to enhance initial microbial attachments, or if the film was in a biologically more aggressive environment, such as compost.

12.1.1. The effect of surface modification of LDPE film

It takes more than 30 years until visible degradation of LDPE film can be observed when the film is exposed to an aerobic soil environment (Ohtake et al., 1998-b). It was suspected that it takes quite a long time for microorganisms to attach to the surface and to colonise the film. It was therefore hypothesised that improving microbial affinity to the surface could also improve the degradability of the films. The most probable reason for the slow microbial attachment to an LDPE surface is its low
surface energy. Thus surface treatments to increase the surface energy without changing other properties of LDPE film were considered, and some of these pretreatments were tested. Surfactant application was considered as one of the simplest methods to change surface properties of the film, however it was removed from the surface by washing and the surface returned to its original hydrophobic nature. Secondly, if left unwashed the surfactant on the film was toxic to microorganisms, which resulted in less microbial colonisation to the surfactant-treated LDPE film than expected. The surfactant application was questioned in terms of safety. As surface modified film could be used as food packaging purposes, applying such types of surfactant should be avoided It is possible that less toxic surfactants could be found but in view of the ease of reversal of the effect by washing this did not seem a useful pretreatment to pursue.

Previous workers (Griffin, 1985; Whitney, 1993) found application of vegetable oil could accelerate embrittlement of polythene in various environmental exposure experiments. Vegetable-oil application was therefore investigated. The oils were applied to the surface to provide nutrients with the aim of inducing microbial attachment and subsequent colonisation. This treatment worked effectively to increase microbial colonisation, however the stickiness of the surface was a problem. This problem could be solved if the vegetable oil is combined with polyethylene before the film is formed. Unfortunately this is likely to alter the characteristics of the film and it is not certain that it would then still enhance colonisation. This approach was also not pursued because the priority of these investigations was to find practical methods of pretreatment which can be applied in existing film production.

Methods of photo-energetic irradiation were investigated as well as chemical application methods. It was considered that irradiation was safer than chemical application because the chemicals required are
powerful oxidising agents which would cause problems in manufacturing film. Physical treatments leave no chemical residue remains on or inside the treated film.

Exposure to UV light was shown to increase the surface energy of LDPE film and also to accelerate microbial attachment and colonisation. However, UV light was not just a surface effect, it also effected the inner molecules of the film, reducing its mechanical strength. So although UV light exposure could enhance microbial colonisation after disposal it was not suitable as a pretreatment because the photo-degraded plastic was no longer strong enough for its designed purpose. Thus both vegetable oil application and UV light exposure may be suitable as treatments after the films are disposed of in a controlled manner, however, they are unsuitable as pretreatments prior to use to enhance microbial colonisation or degradation of discarded film.

Finally, corona discharge treatment was investigated. It was hoped that this treatment could solve the problems or disadvantages of the former three types of treatment. The corona discharge influences only the surface without damaging inner molecules of the film. Treatment levels can be controlled by altering the discharging levels or time of exposure. This determines whether the effect is temporary by inducing free radicals or if the effect is permanent by oxidising the surface molecules. The level of oxidation can be finely controlled using these pretreatment.

Corona discharge treatment was shown to both enhance colonisation and accelerate degradation of LDPE film under suitable aerobic conditions. As corona discharge treatment is already used to facilitate printing and the use of adhesives it seems an eminently practical way of making LDPE more environmentally friendly by accelerating its degradation after use.
12.1.2. Waste treatment in controlled manner

Plastics have gained a unique position in packaging technology for a number of different reasons. Initially they replaced paper and other cellulose-based products because of their better physical properties, notably strength and barrier properties against microbial attack which was the major reason for spoiling perishable commodities. This led to a revolution in the distribution of foodstuffs. However, this barrier property against microbial attack ironically became an environmental problem when waste plastic materials are discarded in the natural environment. Plastics can be recycled but there are a number of types of plastic products that are not designed to be recycled. Food-packaging thin plastic films are a typical example, which is difficult to be recycled especially when food wastes are attached to the films. Food packaging film is commonly discarded and often found in the natural environment.

Recent studies have proved that polyethylene is not totally non-biodegradable. This discovery may not be due only to the natural progress of research and development but may also be due to the natural adaptation of microorganisms. It has been reported that the degradation period was more than 30 years but this is very dependent on the environment. In anaerobic environments degradation of most organic material is very much slower than in aerobic environments. This will be particularly true for compounds like polyethylene. Thus the total degradation period must be considered to be very long if the huge quantity of virgin polyethylene being produced is considered. Therefore all methods of accelerating the degradation of discarded polyethylene are important. These include not only pretreatment of the film to accelerate its final degradation but the provision of an environment in which biodegradation will be more rapid.

Agricultural waste was composted in an attempt to create an active and controllable biotic environment. Microbial activity and oxygen content of
the compost were higher than in a normal soil environment. In order to maintain high oxygen levels inside the compost, a large proportion of straw was used, but there was another possible reason why oxygen levels were high in the compost. There were a large number of earthworms in the compost: more than ten earthworms were found in each 500 g of the mature compost. Their activities could have created air-tunnels.

The fact that corona discharge treated LDPE film had enough microbial degradation to the point of having holes in the film within 100 days of exposure to compost shows that compost has other hidden properties that have to be understood before industrial applications can be optimised.

12.2. Possibility of industrial applications

12.2.1. Corona discharge treatment

Corona discharge treatment has already been applied industrially, e.g. to increase printability (or ink attachment) to surfaces having low energy. However, correlation between the corona discharge treatment and any biotic phenomena including biodegradability has never been reported as far as the author understands. It is suggested that the use of corona discharge treated plastic film may be used for food packaging films, or other disposal purposes in order to increase the biodegradability.

Many people recognise the importance of environmental awareness and they are happy and proud to be using simple packaging or environmental-friendly materials. So corona discharge treated film could well be readily accepted by the market if the quality of the packaged material is guaranteed. Also, the use of low-glossy and low-transparency materials is popular in Japan especially for packaging or coating...
materials (Marui et al., 1995) so the combination of corona discharge treated polythene with other materials could be commercially feasible.

Composites are increasingly being used to get the best combination of characters by combining several different materials to get the benefits of each material. One example is the combination of thermoplastic films with paper to give the stiffness of paper or card combined with the waterproof character of thermoplastic. Corona discharge treatment could readily be combined with such a process. Extremely thin films of polyethylene are used for such composites. So thin that it is much more readily biodegradable. There is also some evidence from these studies that the degradation of polyethylene is accelerated by the presence of cellulosic materials such as paper. Corona discharge treated polyethylene/paper composites might therefore be biodegraded fairly rapidly in the litter (discarded waste) environment.

12.2.2. Compost

The management of solid waste is becoming a more serious issue in many countries. Municipal solid waste is a major component of the total solid waste generated by society, and the fraction of municipal solid waste made up of plastic materials continues to increase. The composting of plastic materials along with organic waste will help society to usefully recycle more of its solid waste and divert it from landfills and other less desirable waste-management options. Therefore, the use of composting has gained some attention, although the composting of municipal refuse is not yet widespread in countries where incineration is popular, such as treatment of municipal solid waste in Japan.

Not more than 30 composting plants are operating in Japan (Ohtaki et al., 1998-a, 1998-b). On the other hand, more than 60 full-scale aerobic composting plants were operating in 1983 with another 20 or so in the
design stage or under construction in the United States (Willson and Dalmat, 1983) and by now this number will be much higher because landfill disposal is a major priority in this country and the composting treatment was easily accepted as an alternative treating method. A possible reason that the Japanese do not accept such treatment may be that products from compost contain significant quantities of impurities, including undegraded plastic, which cannot be accepted by farmers as fertiliser. Separation processes of the materials unsuitable for the composting from municipal refuse are very costly, and even when an excellent separator is used, the compost products still suffer from some contamination by plastics. Accordingly, many kinds of biodegradable plastic materials have been developed (Doi and Fukada, 1994; Scott and Gilead, 1995), which are expected to effectively promote the composting of organic waste. Recently, studies have been conducted on the degradation of biodegradable plastic in composting processes. Choi and Park (1996) reported on the biodegradability of polycaprolactone/styrene-acrylonitrile copolymer blend in the composting process. By measuring the weight loss of the blend film, which contained more than 50% polycaprolactone by weight, they found that it became significantly degraded over the composting time. Gu et al., (1993) ascertained the rate of degradation of cellulose acetate films when exposed to composting materials in laboratory test vessels maintained at 53 °C by measuring their weight loss. Yue et al., (1996) measured the biodegradation of films composed of poly-hydroxybutyrate and a copolymer of hydroxyvalerate by observing weight loss and normalised thickness. They reported that the copolymer degraded significantly faster in a simulated municipal solid waste compost test at the constant temperature of 55 °C. However most of these highly biodegradable plastics lack the desirable properties of polyethylene, low cost, high strength, transparency and ease of moulding. Weiland et al., (1993) examined the degradability of thermally oxidised polyethylene film in the composting process by monitoring molecular weight and surface erosion and their results mostly support

General discussion and conclusion
the findings of this thesis. Bioassimilation of oxidised polyethylene films was obtained with observing increases in microbial colonisation and decrease in the molecular weight. In addition to these studies, other research has attempted to evaluate the degradability of biodegradable plastic during composting by measuring the weight loss of the film (Nakasaki, 1998; Ohtaki et al., 1998-a). Moreover, various test methods have been designed to determine the percentage of aerobic degradation of plastic materials when exposed to a controlled composting environment under laboratory conditions.

12.3. Future studies

This research investigated new methods to solve a serious environmental issue, that discarded synthetic plastic films damage the environment. The research investigated biological effects of physical and chemical treatments to the film that is already most widely used rather than studies on films designed to be biodegradable, which are used in small quantities. The author believes that these academic investigations could suggest directions to help solve such existing environmental issues, however there are usually difficulties in transferring information between the academic investigation and practical application. This study, to investigate methods to enhance biodegradability of LDPE film, is not the exception. As corona discharge treatment is already used to modify the surface properties of films in the manufacture of a number of products the transfer of this technology to reduce problems of disposal are likely to be less than for many academic findings. Despite this there are a number of issues to be considered before the surface modified synthetic film can be utilised as to make plastic films more biodegradable. The things to be considered are: 1) whether the equipment for corona discharge treatment can be readily added to existing machines 2) whether it is practical to give high enough levels of treatment to have a significant effect on subsequent
biodegradation. 3) whether corona discharge treated LDPE films are acceptable in the market especially in food packaging industries. If the film is going into a composting process for disposal this process needs to be optimised. Therefore, 1) further laboratory studies are needed to purify and identify the effective microorganisms degrading LDPE, 2) identify the combination in which they work most effectively, 3) the competitive and survival ability of the selected organisms, and 4) to investigate the condition in the compost such as, constituents of the compost, water content, aeration particle size (and its effect on aeration), temperature effect of inoculation with cultures of selected microorganisms or mature compost.

A collaborative project between the author, the University of Surrey and the Graduate School of Biological Sciences, Nara Institute of Science and Technology (Nara, Japan) has been initiated to continue the laboratory studies to purify and identify microorganisms suitable for the LDPE degradation is outlined in appendix 1.
Appendix 1

Collaborative studies

Degradation of corona discharge treated LDPE film by \textit{Penicillium simplicissimum} YK

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\textit{Nara Institute of Science and Technology, Nara, Japan}

and

Masashi Matsunaga, Philip J. Whitney

\textit{School of Biomedical and Life Sciences}
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(Project started in October 2000)
A1.1. Introduction

The author and Dr Whitney plan to carry a collaborative research with Professor Y. Tani and Dr K. Yamada-Onodera in Graduate School of Biological Sciences, Nara Institute of Science and Technology. They have investigated the degradation of LDPE using their isolated fungus, *Penicillium simplicissimum* YK (Yamada-Onodera et al., in press). They reported its ability to degrade polyethylene powder (Mp = 32,887) at Fifth International Symposium on Environmental Biotechnology (ISEB 2000) held in Kyoto in June 2000, but they have not yet investigated degradation of polyethylene film. The author suggested a collaborative project testing degradation of the film samples used in the research reported in this thesis using *Penicillium simplicissimum* YK. Corona discharge treated and untreated film samples were provided by the Dr. Whitney (University of Surrey) and laboratory experiments were carried out at NAIST. The degradation of the corona discharge treated and untreated LDPE film was monitored by high-temperature gel-permeation chromatography (HT-GPC) after different periods of incubation with the isolate. The average molecular weight, Mz+1, Mz, Mw (correspond directly to high-molecular weight polymers), Mp and Mn (correspond to number of molecules per sample), of the LDPE films were investigated.

A1.2. Materials and methods

A1.2.1. Film samples

LDPE film was corona discharge treated at 500 W for 500 passes (total 5.0 s). Corona discharge treated and untreated films were cut into pieces (each weight is approximately 400 micrograms) and then sterilised in 70 % ethanol before being placed in the liquid culture of *P. simplicissimum*. 
A1.2.2. Detection of polyethylene degradation in liquid culture

*P. simplicissimum* was cultured in 100 ml liquid mineral salts medium that had the LDPE film as the sole carbon source. Culture was in 500 ml flasks at 30 °C shaken at 150 rpm and inoculation was by spore suspension.

The inoculum for the tests was made by taking the spores from stock slant cultures (on 2 % agar containing 5 % malt extract, 0.3 % yeast extract and distilled water and the medium adjusted to pH 5.6) and inoculating them into 500-ml Erlenmeyer flasks containing 100 ml of 2 % agar medium containing 2 g of malt extract, 0.1 g of peptone, 2 g of glucose and distilled water. Incubation was at 30 °C without shaking. After 1 week of incubation 50 ml of 0.05 M sodium phosphate buffer (pH 7) containing 0.3 % Tween 80 was added. The mixture was stirred vigorously, and 10 ml of this spore suspension was used to inoculate 100 ml of medium D in a 500-ml Erlenmeyer flask, and was shaken for 1 week. Hyphae were harvested and homogenised by a mill (Labo Milser, Osaka Chemical Co., Osaka, Japan). 2.5 g wet cell weight of the hyphae was then used to inoculate 100 ml of medium containing 30 pieces of the corona discharge treated or non-treated LDPE film, 0.3 g of NH₄NO₃, 0.5 g of K₂HPO₄, 0.1 g of NaCl, 0.02 g of MgSO₄·7H₂O, 0.15 g of Triton X-100, and distilled water. The suspension of the hyphae autoclaved at 120 °C for 20 min. was used as a source of dead cells. The dead cells were subsequently checked for viability by plating on 2% agar medium D, and no growth was confirmed.

At intervals during the 8 weeks of cultivation, several pieces of the LDPE films were randomly picked from the culture. They were washed with sterile distilled water several times to remove cell constituents, and dried at 70 °C for 20 h. Each film was dissolved in an appropriate amount of o-dichlorobenzene. For the measurement of the distribution of molecular
weight of the LDPE, GPC analysis was performed with a HT-GPC apparatus (Model 150-CV, Waters, Tokyo, Japan) with two microStyrageLT linear columns (Waters) at an elution speed of 1 ml/min and with o-dichlorobenzene as the solvent. Temperatures were 135 °C for the injector, column and detector, and 50 °C for the pump. Polystyrene (Waters) was used as a standard for the calibration curve.

## A1.3. Results and discussion

HP-GPC was used to determine the changes of molecular weight distribution of the LDPE. Changes in Mw corresponded directly to high-molecular-weight polymers, whereas changes in Mn corresponded to the number of molecules per sample. Figure A1.1 shows the comparison of Mz+1, Mz, Mw, Mp and Mn of corona discharge treated and non-treated LDPE films during 8 weeks of the incubation with the isolate. As small pieces of LDPE films were used in the experiment, the molecular distribution of each piece might have a small difference. Both for corona discharge treated and non-treated LDPE films, decreases in Mz+1, Mz, Mw, Mp and Mn were observed, which leads that degradation of LDPE film occurred with the isolate. The level of the decrease in molecular weight was slightly higher for the corona discharge treated film than non-treated film after 8 weeks of the incubation.

Figure A1.2 shows the comparison of the molecular weight distribution between corona discharge treated and non-treated LDPE films after 8 weeks incubation. From the results of GPC analysis, both corona discharge treated and untreated film showed a decrease in the distribution of molecular weight but corona discharge treated film had decreased more than that of non-treated film. There was a 22 % decrease for corona discharge treated LDPE film and an 18 % decrease non-treated film. However this difference was not statistically significant.
This difference was not as great as that found with the isolated LDPE degrading microorganisms that were purified from the degraded corona discharge treated LDPE film after compost exposure. The NAIST biodegradability tests used GPC data, whereas the University of Surrey tests measured tensile strength and extension at breaking point. Also the conditions for biodegradation to occur were very different. So direct comparisons between these two sets of results are difficult.

The purpose of applying corona discharge treatment is to enhance microbial affinity with LDPE film in order to introduce rich microbial attachment and colonisation. Under such aqueous condition, however, microbial affinity becomes less important as the microbes are able to contact to the surface at anytime. It is proposed that tensile strength measurement will be carried out for LDPE samples treated with *P. simplicissimum* in liquid culture and molecular weight determinations carried out by GPC on LDPE samples incubated in inoculated straw compost.

Also the use of Triton X-100 is questioned because it works as surfactant. It may have an effect on the ability of the media to wet the film and may well make it easier for fungus to colonise untreated film. This could reduce the difference between corona discharge treated and non-treated film. Incubation without containing Triton X will therefore be tested.

When these differences have been clarified or resolved it is hoped to publish the results as a joint publication in an appropriate journal.
Figure A1.1. The average molecular weight of corona discharge treated and non-treated polyethylene film after 8-week liquid immersion.
Figure A1.2. HT-GPC of corona discharge treated and non-treated LDPE films after 8 weeks of incubation in liquid media with *Penicillium simplicissimum* YK. (data provided from NAIST.)
Appendix 2

Published material (1)

Microbial degradation of corona discharge treated low-density polyethylene film

(Presentation number: P10-12)

Masashi Matsunaga and Philip J. Whitney

Published as a conference paper for the Fifth International Symposium on Environmental Biotechnology, (ISEB 2000)

At Kyoto International Conference Hall, Kyoto, Japan
9 – 13 July, 2000
Microbial Degradation of Corona Discharge Treated Low-Density Polyethylene Film

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LDPE film was corona discharge treated and its microbial affinity (colonisation and biodegradation) was tested in an aerobic agricultural compost. Significant biodegradation of the LDPE film was observed when backed by paper and buried in compost. Holes developed in the LDPE film after 100 days burial. The degraded edges were observed by SEM to identify whether the attack occurred by macro-organisms or by micro-organisms. The results indicated degradation was due to microbial attack. The oxidised surface condition of LDPE film due to the corona discharge treatment appears to be an important factor to help the acceleration of the biodegradability.

INTRODUCTION

Research to create biodegradable polymer using renewable polymer seems to be approaching its time of maturity, and some of the biodegradable polymers are actually commercialised. However, some problems have been indicated in terms of the costs of production and the utilisation of vast areas of agricultural land where raw materials of renewable polymer should be cultivated [1]. Production of synthetic plastic is still growing [2] despite its waste problem has been indicated and techniques to create biodegradable materials have been developed. Polyolefin films, which are likely to be used as disposable purposes, tend to be discarded into the environment. Also plastics could be nuisance even if collected suitably because wasted plastic materials remain in landfill when buried, produce carbon dioxide (or dioxin if containing chloride) when incinerated, and have difficulty to be recycled when contaminated with other biological or chemical materials. However, recent reports may give us positive prospects; biodegradabilities of synthetic polymer have been indicated. According to polyethylene film, studies of its biodegradation (including exposure to actual biological environment) have been carried out. For example Albertsson’s group has been studied biodegradability of polyethylene film for a long period of time, and eventually suggested the degradative mechanism with emphasising the importance of initial oxidation [3-4]. Lately Ohtake’s reports gave us more impact [5-6]. He and his colleagues investigated LDPE samples buried under aerobic and bioactive soil for more than 30 years, and found the evidence of biodegradation of LDPE with detecting lower molecular compounds. They also have attempted to isolate some degradative fungi.

With these backgrounds, we have focused on the biodegradability of LDPE films and investigations to establish methods of which biodegradability of LDPE films can be accelerated were discussed in terms of enrichment of the surface energy and oxidation of the surface. Some reports [7-10] say that less microbial corrosion or deterioration was found on most of substrates (such as plastics or steels) with which surface energy is low. That is, alteration of the surface condition of the substrate could be the method to obtain particular surface conditions that microorganisms grow either richly or poorly. Therefore, attempts were made to enhance
surface energy of the LDPE film with applying modification in order to obtain surface conditions that more microorganisms can grow. It has been confirmed that oxidation is required when any synthetic organic polymers are started to be degraded [4, 11]. However, rapid or stable oxidation can hardly be expected in the natural environment because most of oxidative factors are given by photo-oxidation (UV irradiation) from sunlight, which unstably supplied with depending on weather or the location such as latitude. Also less photo-oxidation is expected after polymers are buried under soil.

Corona discharged treatment is known as a new technique enables to modify surface condition of substrate with increasing their surface energy [12-15] and with oxidising the surface [16-18]. Although UV light irradiation has traditionally been applied as an artificial method of oxidation [19], corona discharge treatment is recognised as more practical and beneficial method because it can modify the surface much more quickly. Very few studies have been reported with regard to the correlation between the surface-modified synthetic plastic films and the ability of microbial colonisation or degradation as far as the authors comprehend. The corona treated LDPE films and those adhered with paper by the corona discharge treatment (LDPE/paper hybrid) were ultimately exposed to an agricultural compost and the biodegradabilities were examined.

**MATERIALS AND METHODS**

**Polyethylene film and paper**

Transparent food packaging grade virgin LDPE film was used in the series of study. Thickness and weight of the film were 19.7 μm and 18.5 g/m², respectively. Xerox “Premier” paper was used as a backing for LDPE film during corona discharge treatment. Thickness and weight of the paper were 104 μm and 80 g/m², respectively.

**Corona discharge and UV treatment**

Schematic illustration of the corona discharge apparatus constructed in the School of Biological Sciences workshop (University of Surrey) is shown on Figure 1. The apparatus comprising a ceramic faced corona discharge head connected to a high frequency, high voltage supply (maximum at 14kV at up to 12 kHz: Ahlbrandt Systems (UK) Ltd.) was placed 1mm from an earthed aluminium drum. Power could be adjusted by altering the period of the high frequency cycle that was at zero volts. Peak voltage and times at peak voltage were unaffected. In the series of experiments, the frequency of the supply was adjusted to supply 500W to the discharge head. Test samples are carried through the corona by transport belts at a known speed to give an even and reproducible treatment. The sample passed through two 5mm wide coronas at 1 m/sec giving each part of the sample 0.01 sec exposure at each pass. For longer exposures samples were attached to the drum and repeatedly passed through the coronas. (Total treatment ranged from 0.01 to 10 seconds). Air was drawn between the two halves of the discharge head at approximately 1.25 m³/minute in order to remove heat and ozone.

![Figure 1. Schematic illustration of the corona discharge apparatus.](image-url)
For the UV exposure treatment, a 300W lamp (ULTRA-VITRUX®, purchased from Amba Lamps) was used. The range of the wavelength of this light source is similar to that of sunlight. Test samples were exposed at 30 cm under the lamp, and the temperature above the films was kept under 40°C in order to avoid to progress thermal oxidation on the samples. This UV treatment was carried out up to 44 days.

Compost exposure

LDPE films pretreated by the corona and UV treatment were exposed to bioactive conditions in a compost (simulating an agricultural compost) consisting of approximately 25% horse manure, 25% fresh grass waste, 25% partially rotted plants material, and 25% straw (all by weight). Straw was added as poromeric (in order to keep aerobic condition inside the compost) and bulking purposes as well as supplying cellulosic source. All the components were well mixed with some added water. Corona treated and UV exposed LDPE films contained in mesh bags were placed in the middle of the composting mixture just after the peak heating period of the compost.

Estimation of microbial colonisation

After corona discharged and UV exposed LDPE films were buried in the agricultural compost, the samples were removed from the compost and microbial colonisation was estimated after 100 days of the exposure. The amount of colonised microorganisms was quantified by the protein assay method. A 50 x 50 mm of the test sample was placed into 10ml of 0.5M NaOH in a Universal bottle. The bottle was shaken for 24 hours to solubilise protein in the material attached to the film. The extract was centrifuged at 10,000 rpm for 10 minutes at 4°C, and its supernatant was tested by the Coomassie Brilliant Blue dye method. 0.1 ml of the test liquid was mixed with 1:5 diluted dying reagent, and then absorbance at 595nm was estimated. The obtained spectroscopic absorbance data was referred to two standard lines that were preliminarily prepared from known concentrations of bovine serum albumin (BSA) and casein solution. Linear correlation was obtained both from BSA and casein solution in a range up to 100 mg/l of the concentration. BSA and casein are known to give high and low readings with the Coomassie Brilliant Blue, respectively, and therefore a standard for the mixture of microbial proteins was taken as the mid-point between these two proteins.

Observation of composted LDPE films

Scanning electron microscopic (SEM) analysis was carried out in order to evaluate the images of biodegraded parts on corona discharged LDPE films using HITACHI SEM S-3200N. Test samples were previously coated by exposure to a gold ion beam sputter using Edwards Sputter Coater S150B.

RESULTS

Surface energy

Surface energy of corona discharged LDPE film was observed by estimating its water contact angle, and its result is shown in Figure 2. Non-treated LDPE film showed a strong hydrophobicity with a contact angle of 92° (±2°). After applying only a single corona discharge treatment for 10² sec, the film was significantly less hydrophobic and the contact

![Fig.2. Change of contact angle with respect to the duration of exposure to corona and UV light. The contact angle of non-treated LDPE film was 92.0° (±2.0°).](image-url)
angle was reduced to 67°. By the further exposure, the water contact angle was decreased until 100 passes of the treatment (i.e. 1 sec total exposure), and gave a water contact angle around 48°. Extra exposures caused little further decrease in contact angle. Although the corona discharge treatment showed a marked increase in surface energy (decrease in contact angle) with 0.01 sec of treatment, the effect of UV exposure was much slower. The water contact angle gradually decreased with increasing UV exposure, and finally the water droplet formed a 68° of the contact angle from an LDPE film exposed for 44 days (note on Figure 2 that time scale is logarithmic).

**Tensile Strength**

Neither the corona discharge treatment (even at 10 seconds exposure) nor UV exposure had a statistically significant effect on tensile strength or extension when measured with an Instron tensiometer.

**Surface oxidation**

ATR-FTIR spectra were analysed for detecting subsequent oxidation or other chemical changes due to short and long term exposure to the corona. Figure 3 shows peak index of the spectra for corona discharged LDPE films with respect to the number of exposure passes. The untreated LDPE film basal peak has been subtracted from all the peaks plotted. Significant peaks, as an evidence of oxidation of LDPE film surface, were observed; peaks at 3200 (wide), 1720, 1645, 1185 cm\(^{-1}\) are due to hydrogen bond of hydroxyl (O-H), carbonyl stretching (C=O), carbonyl un-stretching (C=O), and formation of ester (C-O), respectively. All the peaks increased with increasing the number of corona discharge treatments.

**Microbial colonisation**

Test samples were buried in agricultural compost for 100 days and the microbial colonisation on LDPE films was quantified using the protein assay method. The absorbance data was referred to the standard concentration of BSA and casein solutions, and amount of protein in attached microorganisms was quantified. It was observed that the amount of protein, which relates to the biomass of microorganisms attached on the films, was significantly higher in corona discharged and UV treated LDPE films (Figure 4). For UV treated film, the amount of protein was approximately double than that of non-treated LDPE film. For corona discharged films, the amount of protein increased with increasing exposure times with nearly thrice the amount of attached protein on the 5 sec total exposure film as compared to the untreated film.
Observation of biodegraded LDPE film

Figure 5 shows a picture of a 5 sec corona discharge treated LDPE film showing biodegradation after being exposed in the agricultural compost for 100 days. No macroscopically visible degradation was observed on LDPE films treated with corona discharge for short periods of time. The presence of backing paper (cellulose) seemed to further enhance the rate of degradation.

Figure 6(a) shows SEM image of biodegraded part of the LDPE. The filamentous growth inside the degraded hole seems to be fungal filaments that have been correlated to the degradation processes. Figure 6(b) is a higher magnified SEM image of the degraded part seen in Figure 6(a). The layer is the degraded LDPE film, and the degraded edge looks ‘dissolved’ or ‘melted’ rather than ‘bitten’ or ‘chewed’. This indicates that the degradation has occurred due to microbial attacks (not macro-organisms such as arthropods) possibly by enzymatic or hydrolytic reactions. SEMs show an apparent increase in thickness of the LDPE film. This does not appear to be due to the presence of surface mucilage as biofilm. Therefore swelling or hydration of the film as part of its biodegradation seems a real possibility.

Figure 6. SEM images of biodegraded corona discharge treated (5 sec) LDPE films after being exposed in the agricultural compost for 100 days. (a, left): Biodegraded hole with remains of fungal filament. (b, right): Higher magnification of picture (a) with focusing on the degraded edge. The smooth and ‘melted like’ degraded edge indicates more like being attacked by micro-organisms rather than macro-organisms.
DISCUSSION

It was observed that surface condition of LDPE film significantly changed on treated with corona discharge for short periods of exposure times. As far as the time scale is concerned, surface energy was observed as increase instantly even with a single treatment of 0.01 sec exposure time. Surface oxidation followed subsequently and the oxidation of the surface was progressed linearly with increasing corona exposure times. It has been reported that formation of microbial colonies on organic film has a strong relationship with its surface energy [7-10,20]. We have confirmed this phenomenon with observing richer microbial colonisation on surface activated LDPE films [20].

Oxidation of the surface was observed by FTIR analysis. The principle behind the oxidation due to corona discharge treatment is that free radicals are formed on the surface by the irradiation of high energy from the corona. Also oxygen molecules in the air closed to the discharger was activated to form unstable elemental oxygen (O), activated oxygen (O₂*) and/or ozone (O₃). The activated oxygen then reacts with the activated surface to complete surface oxidation. Formation of oxidative surface is believed to be an obligate intermediate for any organic polymer to initiate degradation (including biodegradation). Applying proper pretreatments to lead the oxidation of the surface without damaging original property of the substrate is therefore considered as a critical method so that the total period of degradation can be accelerated since long periods of time are required to complete oxidation in the natural environment [21]. Biodegradation of corona discharge treated LDPE film was observed after a remarkably short period of time especially in the presence of the backing paper. It is speculated that attached paper, having richer biodegradability, became a substrate for microorganism to colonise, and then some of the organisms attacked the surface activated LDPE film. LDPE/paper composite materials have already been used commercially for disposable uses such as milk cartons. The author suggests that the biodegradability of this hybrid deserves future studies.

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Appendix 3

Published material (2)

Surface changes brought about by corona discharge treatment of polyethylene film and the effect on subsequent microbial colonisation

Masashi Matsunaga and Philip J. Whitney

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Surface changes brought about by corona discharge treatment of polyethylene film and the effect on subsequent microbial colonisation

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Abstract

Microbial colonisation of synthetic plastic films is normally slow, which affects the total period of biodegradation. Correlation between the modified surface condition and the ability for microorganisms to colonise low-density polyethylene (LDPE) film was studied. Corona discharge treatment was applied to obtain enriched and activated surface condition of LDPE film. It was found from water contact angle and FTIR spectrum evaluations that surface energy was significantly increased due to production of free radicals. Stabilised oxidised LDPE surface was also obtained by further exposure to the corona which gave more suitable condition for subsequent colonisation. Results were compared with UV irradiated (photo-oxidised) LDPE films. Colonisation of corona discharged and UV treated LDPE films were tested in the laboratory environment using known fungal isolates and in a natural compost environment. More active microbial colonisation was observed in all cases for corona discharged and UV treated LDPE films. Far longer UV exposure was required to have the same physicochemical and biological effect as the corona discharge treatment. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Polyethylene film; Oxidation; Corona discharge treatment; Microorganism; Degradation

1. Introduction

Biodegradation of low-density polyethylene (LDPE) has been reported, but the rate is very slow. Albertsson found 0.2% weight loss for LDPE films buried in soil for 10 years [1]. Ohtake et al. tested LDPE bottles exposed in aerobic soil for over 30 years, and observed some evidences of biodegradation using SEM of the degraded parts. TOF-MS spectra also showed that a reduction of molecular weight had occurred [2,3]. When the total biodegradation process of any organic substrates is considered the formation of microbial colonies is critical to the initiation of biodegradation. Thus the duration of the microbial colonisation is an important factor that effects total degradation period [4]. In order to avoid microbial growth on the substrates, treatments to reduce their surface energy have commonly been applied [5,6]. As the surface energy of polyethylene film is very low formation of microbial colonies is likely to be poor. Methods of surface modification to enhance the microbial colonisation were therefore investigated. Corona discharge treatment is already widely used in industry such as adhesive bonding, printing, extrusion coating, composite and heat sealing [7]. Corona discharge treatment results in an increase in surface energy by introduction of polar groups on the surface, thus improving their adhesion and wetting properties [8-11]. However, little is known about the effects on subsequent microbial colonisation and degradation. In this series of studies, food packaging grade LDPE films were corona discharge treated and changes to the characteristics of the film were investigated. Surface energy, oxidation processes and mechanical strength were measured as well as the rate of microbial colonisation. All these characteristics were compared with those for UV exposed LDPE films.

2. Experimental

2.1. Materials and applied pretreatments

Transparent food packaging grade virgin LDPE film was used in these studies. Thickness and weight of the film were 19.7 μm and 18.5 g/m², respectively. Corona discharge treatment and ultra violet (UV) light exposure
treatment were performed as pretreatments in order to enhance the surface energy and oxidise the surface of LDPE films. The corona discharge apparatus was constructed in the School of Biological Sciences workshop (University of Surrey). The apparatus comprised a ceramic faced corona discharge head with two electrodes connected in parallel to a high frequency, high voltage supply [maximum at 14 kV at up to 12 kHz: Ahlbrandt Systems (UK) Ltd.]. The discharge head was placed 1 mm from an earthed aluminium drum driven by the transport belts that carried the samples through this gap. Power could be adjusted by altering the period of the high frequency cycle that was at zero volts. Peak voltage and times at peak voltage were unaffected. In the series of experiments, the frequency of the supply was adjusted to supply 500 W to the discharge head. The samples carried by the transport belts passed through two 5 mm wide coronas at 1 m/s giving each part of the sample 0.01 s exposure at each pass. For longer exposures, samples were attached to the rotating drum and repeatedly passed through the coronas at 1 m/s. Air was drawn between the two electrodes of the discharge head at approximately 1.25 m^3/min in order to remove heat and ozone.

For the UV exposure treatment, a 300 W lamp (Ultra Vitrux®, Amba Lamps) was used. The range of the wavelength of this light source is similar to that of sunlight. Test samples were exposed at 30 cm under the lamp, and the temperature above the films was kept under 40°C by ventilation in order to minimise thermal oxidation.

2.2. Estimation of the surface energy

Water contact angle measurements were carried out to measure the surface energy. Sessile water droplet method was applied using a Contact Angle Goniometer (Research Instruments Ltd.) containing SuperQ ion exchange purified water, and advancing angles were recorded. It has been reported that the hydrophobicity of corona discharged LDPE films recovers by aging [12]. Therefore, surface activated LDPE films were exposed to a room air condition (approximately 20°C), and contact angles were periodically measured up to 44 days after both corona and UV treatment being made in order to investigate the contact aging. Mean value of all contact angles data were obtained from more than five replicates.

2.3. Tensiometry

Tensile strength and elongation at fracture of thin film are sensitive mechanical properties for evaluating degradation of polymers [13–15]. A bench-top Instron Tensiometer adjusted at 100 mm per minute was used, and results were averaged from at least 10 replicates for each treatment.

2.4. Estimation of chemical changes

In order to evaluate the changes of chemical components on the surfaces (especially for detecting oxidation), attenuated total reflectance Fourier-transform infra-red spectroscopy (ATR-FTIR) measurements were performed using Perkin-Elmer System 2000 FT-IR.

2.5. Observation of fungus growth

Microbial colonisation is considered to be an obligate stage for initiating biodegradation. Observation of microbial colonising phenomenon was carried out with using a range of fungi commonly used for biodegradation studies (Table 2). Fifty-Millimetre square samples of LDPE, which had either been corona discharge treated for a total of 5 s (500 passes) at 500 W, UV treated for 3 weeks or untreated, were placed onto nutrient free agar (water agar) to maintain a humid environment. The samples were inoculated with 5 mm square blocks of Malt Extract Agar on which the test fungi had already grown. The inoculated plates were incubated at 30°C for 2 weeks and then visible observation of fungal colonisation on the films were made.

2.6. Preparation of agricultural compost

LDPE films pretreated by the corona and UV treatment were exposed to bioactive conditions in compost consisting of approximately 25% horse manure, 25% fresh grass waste, 25% partially rotted plants material, and 25% straw (all by weight). Straw was added as poromeric (in order to keep aerobic condition inside the compost) and bulking purposes as well as supplying a cellulosic source. All the components were well mixed with some added water. Corona treated and UV exposed LDPE films (50×50 mm) were placed in the composting mixture just after the peak heating period of the compost (in order to avoid heat induced oxidation or degradation taken place).

2.7. Quantification of colonised microorganisms

Corona discharged and UV exposed LDPE films were buried in the agricultural compost for 50 days after which microbial colonisation was estimated. The amount of colonised microorganisms was quantified by a protein assay method. A 50×50 mm of the test sample was placed into 10 ml of 0.5 M NaOH in a Universal bottle. The bottle was shaken for 24 h to solubilise protein from living material attached to the film. The extract was centrifuged at 10,000 rpm for 10 min at 4°C, and the supernatant tested by the Coomassie Brilliant Blue dyeing method. 0.1 ml of the supernatant was mixed with 1:5 diluted dyeing reagent. Then absorbance at 595 nm was estimated. The obtained spectroscopic absorbance
data was referred to two standard lines from known concentrations of bovine serum albumin (BSA) and casein solution. Linear correlation was obtained both from BSA and casein solution in a range up to 100 µg/l of the concentration. BSA and casein are known to give high and low readings with the protein assay respectively, and therefore a standard for the mixture of microbial proteins was drawn as the mid-point between these two protein solutions.

2.8. Microscopic analysis

Scanning electron microscopic (SEM) analysis was applied in order to observe microscopical images of the microbial growth or attachment on the surface of LDPE films and observed with a Hitachi S-3200N SEM. Test samples were coated by exposing to a gold ion beam sputter using Edwards Sputter Coater S150B.

3. Results and discussion

3.1. Estimation of surface energy and tensiometry of LDPE film

Table 1 shows changes of advancing contact angle with respect to the time of the treatments. Non-treated LDPE film showed a strong hydrophobicity with a contact angle of 92.0°. After applying only a single corona discharge treatment for 0.01 s, the film was significantly less hydrophobic and the contact angle was reduced to 66.6°. By further exposure, the water contact angle was decreased until 5.0 s of the exposure, and gave a water contact angle around 42.8°. Extra exposures caused little further decrease in contact angle. Although the corona discharge treatment showed a marked increase in surface energy (decrease in contact angle) with only 0.01 s of treatment the effect of UV exposure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total exposure time (s)</th>
<th>Contact angle (°)</th>
<th>Tensile strength (g/mm²)</th>
<th>Elongation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treated</td>
<td>0</td>
<td>92.0±0.55</td>
<td>206.5±0.65</td>
<td>488±32.1</td>
</tr>
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<td>Corona (0.1 s)</td>
<td>0.1</td>
<td>53.8±1.24</td>
<td>234.0±2.45</td>
<td>524±22.3</td>
</tr>
<tr>
<td>Corona (1.0 s)</td>
<td>1.0</td>
<td>48.4±0.40</td>
<td>217.5±1.30</td>
<td>509±28.6</td>
</tr>
<tr>
<td>Corona (5.0 s)</td>
<td>5.0</td>
<td>42.8±1.02</td>
<td>227.0±2.50</td>
<td>523±48.0</td>
</tr>
<tr>
<td>UV (7 days)</td>
<td>6.04×10⁵</td>
<td>87.6±0.81</td>
<td>262.5±2.20</td>
<td>480±25.3</td>
</tr>
<tr>
<td>UV (14 days)</td>
<td>1.21×10⁶</td>
<td>81.0±2.55</td>
<td>221.0±3.30</td>
<td>432±17.3</td>
</tr>
<tr>
<td>UV (21 days)</td>
<td>1.81×10⁶</td>
<td>72.2±0.49</td>
<td>95.0±4.45</td>
<td>185±12.1</td>
</tr>
<tr>
<td>UV (28 days)</td>
<td>2.42×10⁶</td>
<td>69.4±0.40</td>
<td>60.0±4.35</td>
<td>187±8.47</td>
</tr>
</tbody>
</table>

* Mean±standard errors.

Table 2

Visible analysis of known types of fungi on LDPE films with respect to the exposure time of corona and UV treatment*

<table>
<thead>
<tr>
<th>Name</th>
<th>Non-treated</th>
<th>Corona 0.1 s</th>
<th>Corona 1.0 s</th>
<th>Corona 5.0 s</th>
<th>UV 7 days</th>
<th>UV 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chaetomium sp.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chaetomium sp.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Corynascus sepedonius</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Curvularia sp.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fusarium sp. (1)</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fusarium sp. (2)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Paecilomyces varioti</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stachybotrys sp.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trichoderma longibrachiatum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Each code shows visible fungus growth from the inoculated block in the central; (−) for non growth at all, (+) for 0–10 mm, (+++) for 11–20 mm, (++++) for 21–30 mm and (++++++) for <31 mm in diameter.
was much slower. The water contact angles were gradually decreased with increasing the UV exposure, and finally the water droplet formed 69.4° of the contact angle from a film exposed for 28 days. Compared two treatments showed a massive difference in the rate of the reactions. The corona discharge has a significant effect on water contact angle a very short period of treatment but UV light did not have such a pronounced initial effect on the water contact angle. However, the effect increased steadily with increase exposure but only at about \(1 \times 10^{-8}\) of the rate produced by of the corona discharge treatment.

Table 1 also shows changes of tensile strength and elongation at fracture of treated and non-treated LDPE films. No significant differences were seen either for tensile strength or elongation at fracture before and after the corona discharge treatment of the LDPE films. It can be assumed therefore that the corona discharge was only effecting the surface molecules and the inner structure was not damaged. On the other hand, significant reductions of both tensile strength and elongation were observed for UV treated LDPE films. This reduction of the tensile strength means that the inner structure of the LDPE film had been influenced by the energy from the UV light.

3.2. FTIR analysis

ATR-FTIR spectra were analysed for detecting subseuent oxidation or other chemical changes on LDPE surfaces due to exposure to corona and UV light. Fig. 1 shows peak index of the spectra for corona discharged LDPE films with respect to the number of exposure passes. Significant peaks, as an evidence of oxidation of LDPE film surface, were observed. Peaks at 3200 (wide), 1720, 1645, 1185 cm\(^{-1}\) are due to hydrogen bond of alcohol (O–H), carbonyl stretching (C=O), carbonyl un-stretching (C=O), and formation of ester (C–O), respectively. All the peaks increased with increasing the number of corona discharge treatments, hence showing these peaks were originated from chemical reactions due to the corona discharge treatments. An ATR-FTIR spectrum of LDPE film corona discharged for at 500 W for 5 s was also compared with that of UV exposed for 21 days, and those peak index are shown in Fig. 2. It appears that corona discharge treatment leads almost the same chemical reactions as UV treatment. Forming carbonyl groups as a result of oxidation is known to be the type of oxidation that may help subsequent biodegradative processes [16].

3.2.1. Formation of carbonyl group

A possible mechanism of chemical reactions caused by corona discharge treatment on LDPE film is illustrated in Fig. 3 [17]. Applying energy by the corona discharge treatment of LDPE film, free radicals were instantly created and thus the surface energy was increased (as Table 1 shows). At the same time, oxygen molecules (in the air) are also activated by the energy from the corona, and elemental oxygen (O), activated oxygen molecule (O\(_2^+\)) and/or ozone (O\(_3\)) are created. After a very short period of time, the activated oxygen will react with the activated surface to form a stable oxidised surface. Considerably greater energy was needed to induce oxidation of the LDPE surface to produce stable carbonyl groups. The changes of surface energy and oxidation process with respect to the energy supplies are compared in Fig. 4 in order to evaluate the

![Fig. 1. Peak index of ATR-FTIR spectra for corona discharge treated LDPE films (at 500 W) with respect to the exposure time. Large and sharp peaks at 3000–2900 cm\(^{-1}\) were considered to be noises due to existence of CO\(_2\) in air, which could be ignored.](image-url)
Fig. 2. Peak index of ATR-FTIR spectra of corona discharged (at 500 W for 5 s) and UV exposed (for 28 days) LDPE films. Large and sharp peaks at 3000-2800 cm\(^{-1}\) are supposed to be noises due to existence of CO\(_2\) in air, which could be ignored.

Fig. 3. A considerable mechanism of free radical formation and subsequent oxidation on surface of polyethylene film.

Fig. 4. Changes of surface energy and oxidation process with respect to the number of corona discharge treatments. Surface energy increased immediately whereas oxidation process developed linearly by the number of treatments.
speed of the formation of free radicals and carbonyl groups. The surface energy increases rapidly with short periods of exposure whilst the carbonyl indexes (due to oxidation) increase gradually with increasing the corona exposure times.

Although the surface energy could be enriched by the corona discharge treatment in a very short time, the formed free radicals were still unstable intermediates. The stability of surface energy was examined by observing ‘aging’ of the surface energy. The enriched surface condition could not last permanently when the formation of free radicals was the sole factor to increase the surface energy because the free radicals can be absorbed by water or carbon particles in air [18]. Fig. 5 shows the contact angle aging of corona (0.1 and 5 s) and UV treated (3 weeks) samples when the samples were air-exposed for up to 28 days after the treatments. The Hydrophobicity had almost totally recovered by 28 days’ air-exposure for the shorter corona treated and UV exposed samples. It was considered that the enrichment of surface energy was only induced by formation of free radicals, which were unstable and removed by aging. On the other hand, less recovery was observed for the longer corona treated films. Enrichment of the surface energy due to the formation of oxidised compounds as well as that of free radicals on the surface appears to have taken place. Longer periods of corona discharge treatment lead to oxidation and the formation of stable carbonyl groups on the surface of LDPE. Oxidation is considered an essential chemical reaction to induce subsequent biodegradation [19]. An initial step in the degradation of polyethylene is known to involve photo-oxidation that increases the amount of low molecular weight material by breaking and increase the surface area through embrittlement. All these effects of oxidation promote the degradation of polymers [20]. Corona discharge treatment that oxidises surface of LDPE film instantly as well as enhancing the surface energy was likely to accelerate microbial colonisation, but does not cause embrittlement. The frequency of the discharge should be adequately enough to produce carbonyl groups, however too much treatment should be avoided due to causing mechanical damages of the films.

3.3. Observation of fungal growth

Visible growth of known fungi on surface-activated LDPE is described in Table 2. Very few of these fungi were able to significantly colonise non-treated LDPE films. Corona discharge treatment, on the other hand, resulted in greater colonisation of the film by all the fungi, and the longer the corona discharge treatment the more rapidly the fungi colonised the film. Longer and thicker growths were observed for the most types of fungi. Particularly, significant increases were observed for Fusarium sp., Paecilomyces variotii, and Trichoderma longibrachiatum. UV treatment resulted in similar though less dramatic increases in the rate of colonisation.

3.4. Quantification of microorganisms

After confirming positive fungal growths on corona treated LDPE film using modelled Petri dish cultures, test samples were buried in agricultural compost. After 50 days of exposure to the compost, the microbial colonisations on LDPE films were quantified using a
Fig. 6. Amounts of protein originated from microorganisms colonised on LDPE films after being exposed to the agricultural compost for 50 days. Bars show standard errors by five replicates of measurements.

protein assay method (Fig. 6). The amount of protein, which relates to the biomass of microorganisms attached on the films, was significantly higher in corona discharged and UV treated LDPE films. For UV treated film, the amount of protein was approximately double than that of non-treated LDPE film. For corona discharged films, the amount of protein increased with increasing exposure time. About three times the amount of protein attached to the film treated for 5 s was obtained with compared to the untreated film.

3.5. Microscopic analysis

In order to analyse morphology of microbial growth on corona discharged LDPE films, microscopic surface images were obtained using scanning electron microscopy. Corona discharged LDPE films exposed in the agricultural compost for 50 days were examined. Fig. 7(a) shows for non-treated LDPE surface, and Fig. 7(b) and (c) shows for corona treated (5 s at 500 W) LDPE film. There were significant differences between non-treated and corona treated LDPE films. Microbial colonies were scattered on the non-treated LDPE surface with long and thin filaments. For corona treated LDPE film, on the other hand, richer microbial colonisations were observed on the surfaces. Microorganisms created complex-networks on the film [Fig. 7(b)], and formation of biofilms was observed [Fig. 7(c)].

The formation of biofilm was considered to be induced as a result of moistness of the surface. Water could smoothly spread on the surface of corona discharged LDPE film because the surface condition has been modified to be hydrophilic. Therefore, microorganisms are also able to expand their colonies over the surface and form a biofilm of organisms and secreted mucilage. Settled and precipitated microorganisms attached to the surface and formed a biofilm after the water being dried out. The biofilm itself could then become an appropriate surface for the next wave of microorganisms to colonise, being a potential nutrient source for further microbial colonisation.

Fig. 7. SEM image of LDPE film after being exposed to the agricultural compost for 50 days: (a) non-treated; (b) and (c) corona treated at 500 W for 5 s.
4. Conclusion

The aim of this study was to investigate the effect of two physical treatments on the initial colonisation and possible subsequent biodegradation period on food packaging grade LDPE films. It was confirmed that the corona discharge treatment and the UV exposure did increase the surface energy and oxidise the surface of LDPE film. Enrichment of microbial colonisation was confirmed for both treatments. It seems that the corona discharge treatment was markedly more effective and more practical than UV exposure in terms of speed and quality of modification of the characteristics of LDPE films. We found that the corona discharge treatment of LDPE film has little effects both on tensile strength and elongation at fracture whilst the UV treatment caused serious physical deterioration of the film. Corona discharge treatment is thus a possible means of accelerating the microbial colonisation of plastic films likely to be discarded into the litter environment without affecting the recycling of the film should be practicable. Further studies are needed to determine if the accelerated colonisation has a significant effect in the overall rate of degradation.

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