# ISOLATION OF PLASTIC-DEGRADING FUNGI FROM LANDFILL AND DETERMINING THE SELECTED PLASTICS BIODEGRADATION CAPABILITY

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**FACULTY OF SCIENCE**
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# ISOLATION OF PLASTIC-DEGRADING FUNGI FROM LANDFILL AND DETERMINING THE SELECTED PLASTICS BIODEGRADATION CAPABILITY

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**ISOLATION OF PLASTIC-DEGRADING FUNGI FROM LANDFILL AND DETERMINING THE SELECTED PLASTICS BIODEGRADATION CAPABILITY**


## ABSTRACT

Polyethylene Terephthalate (PET), Low Density Polyethylene (LDPE) and High Density Polyethylene (HDPE) are the most important polymers used predominantly in present world. Since synthetic polymers hardly undergo degradation and remain in the environment for a very long time, up to 1000 years, these drawbacks increase the percentage of solid wastes in landfill and threaten the environment. To date, Municipal Solid Waste (MSW) landfill is the most favored disposal method especially among the developing countries. The aim of present study is to evaluate the potential of fungus isolated from landfilled plastics to degrade PET, LDPE and HDPE. Besides, effect of different concentrations of the selected inoculum was carried out to investigate the biodegradation of PET, LDPE and HDPE polymers. A total of eight fungi strains were isolated from untreated dumped plastics. In this study, two liquid media (BOD and Bushnell Haas) were used to determine the most desirable broth media by the fungus to perform their degradation activity. The biodegradation of the plastics were confirmed by average radial diameter of fungi colonies and weight loss of the incubated polymers after 30 days of incubation period at $28^0$C. Based on the results obtained, the maximum degradation of mixed plastics (PET, LDPE and HDPE) was attributed to *Aspergillus fumigatus* (FI 5) strain in both BOD and Bushnell Haas media with an average weight loss of 1.76% and 2.03% respectively. Considering the cost and biodegradability potential of the isolates in the broth media, BOD media was selected over Bushnell Haas media to evaluate the suitability of the media for the fungus to perform the biodegradation activities. On treatment with different concentrations of *A. fumigatus,* 1% (w/v) inoculum gave highest weight loss for PET plastics (1.5%), while 5% (w/v)

inoculum showed highest weight loss for LDPE (21.9%) and HDPE (1.31%) films after 30 days of incubation period. The concentrations of inoculum strongly suggest that the factor has a great effect on biodegradation by fungi. Hence, further FTIR analysis was conducted to study the structural changes of the plastic films with maximum reduction in weight loss treated with *A. fumigatus*. The results showed the potentiality of *A. fumigatus* to consume these polymers (PET, LDPE and HDPE) as carbon and energy source and the effectiveness of the strains to perform their biodegradation activity can be clearly seen during the optimal condition.

**Keywords**: Fungus, Polyethylene Terephthalate (PET), Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE), degradation.

**PENGASINGAN KULAT DEGRADASI PLASTIK DARI TAPAK PELUPUSAN SAMPAH DAN MENENTUKAN KEUPAYAAN BIODEGRADASI PLASTIK TERPILIH**


**ABSTRAK**

*Polyethylene Terephthalate* (PET), *Low Density Polyethylene* (LDPE) dan *High Density Polyethylene* (HDPE) adalah polimer terpenting yang digunakan terutamanya dalam dunia masa kini. Kerana polimer sintetik sukar menjalani biodegradasi dan kekal dalam alam sekitar untuk masa yang sangat lama, sehingga ke 1000 tahun, kelemahan ini meningkatkan peratusan sisa pepejal di tapak pembuangan sampah dan mengancam alam sekitar. Setakat ini, tapak pelupusan sampah sisa pepejal perbandaran (SPP) adalah kaedah pelupusan paling disukai terutamanya di kalangan negara-negara membangun. Oleh itu, membolehkan penurunan polimer sintetik mengunakan kulat membantu mengurangkan sisa-sisa lengai dalam tapak pelupusan sampah tersebut. Semasa kajian bertujuan untuk menilai potensi kulat yang diasingkan daripada sisa untuk merendahkan berat plastik PET, LDPE dan HDPE. Selain itu, kesan kepekatan berbeza inokulum yang terpilih telah dijalankan untuk menyiasat biodegradasi daripada polimer PET, LDPE dan HDPE. Lapan jenis kulat yang diasingkan daripada plastik terbuang digunakan sebagai sumber karbon semata-mata. Dalam kajian ini, dua media cecair (BOD dan Bushnell Haas) telah digunakan untuk menentukan media yang paling sesuai dengan kulat untuk lakukan aktiviti biodegradasi mereka. Biodegradasi plastik yang telah disahkan oleh purata diameter jejarian koloni kulat dan penurunan berat polimer tersebut selepas 30 hari tempoh pengeraman pada 28$^0$C. Berdasarkan keputusan yang diperolehi, penurunan maksimum campuran plastik (PET, LDPE dan HDPE) dicapai oleh *Aspergillus fumigatus* (FI 5) dalam BOD dan Bushnell Haas media dengan penurunan berat sebanyak 1.76% dan 2.03% masing-masing. Memandangkan kos dan potensi biodegradasi isolat dalam media, BOD media telah dipilih untuk menilai

kesesuaian media untuk kulat untuk melaksanakan aktiviti-aktiviti biodegradasi mereka. Rawatan dengan kepekatan berbeza *A. fumigatus*, 1% (w/v) inokulum menunjukkan penurunan maksima berat plastik PET (1.5%), manakala 5% (w/v) inokulum menunjukkan penurunan berat tertinggi bagi LDPE (21.9%) dan HDPE (1.31%) polimer selepas 30 hari tempoh pengeraman. Kepekatan inokulum mencadangkan bahawa faktor ini mempunyai kesan yang besar terhadap biodegradasi oleh kulat. Oleh itu, analisis FTIR lanjut telah dijalankan untuk mengkaji perubahan struktur plastik yang menunjukkan pengurangan berat badan maksima yang dirawat dengan *A. fumigatus*. Hasil kajian menunjukkan kemampuan daripada ketegangan yang terpencil untuk mengguna polimer ini (PET, LDPE dan HDPE) sebagai sumber karbon dan tenaga dan keberkesanan kulat untuk melakukan aktiviti biodegradasi mereka boleh jelas dilihat dalam keadaan optimum.

**Kata kunci**: Kulat, *Polyethylene Terephthalate* (PET), *Low Density Polyethylene* (LDPE), *High Density Polyethylene* (HDPE), biodegradasi.

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## LIST OF SYMBOLS AND ABBREVIATIONS

| | | |
|---|---|---|
| µ | : | Micro |
| % | : | Percentage |
| < | : | Less than |
| Ag | : | Silver |
| BOD | : | Biochemical Oxygen Demand |
| BPA | : | Bisphenol A |
| $CH_4$ | : | Methane |
| CFU | : | Colony Forming Units |
| cm | : | Centimeter |
| $CO_2$ | : | Carbon dioxide |
| DEHP | : | Diethylhexyl phthalate |
| DCW | : | Dry Cell Weight |
| DNA | : | Deoxyribonucleic Acid |
| DNS | : | Dinitrosalicylic acid |
| EPS | : | Extracellular Polymeric Substances |
| FTIR | : | Fourier-transform infrared spectroscopy |
| g | : | gram |
| h | : | hour |
| HDPE | : | High Density Polyethylene |
| $H_2O$ | : | Hydrogen dioxide |
| IEEP | : | Institute for European Environmental Policy |
| Kg | : | Kilogram |
| L | : | Liter |
| LDPE | : | Low Density Polyethylene |

| | | |
|---|---|---|
| LLDPE | : | Linear Low Density Polyethylene |
| m | : | Meter |
| $m^3$ | : | Cubic meter |
| ml | : | Milliliter |
| mm | : | Millimeter |
| MSW | : | Municipal Solid Waste |
| NaoH | : | Sodium Hydroxide |
| NCBI | : | National Center for Biotechnology Information |
| ng | : | Nanogram |
| $N_2$ | : | Nitrogen |
| OH | : | Hydroxide |
| $O_2$ | : | Oxygen |
| PBDEs | : | Polybrominated diphenyl ethers |
| PCBs | : | Polychlorinated biphenyls |
| PCDD/FS | : | Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans |
| PCR | : | Polymerase Chain Reaction |
| PDA | : | Potato Dextrose Agar |
| PDB | : | Potato Dextrose Broth |
| PE | : | Polyethylene |
| PET | : | Poly Ethylene Terephthalate |
| PFOA | : | Perfluorooctanoic acid |
| PHB | : | Poly (hydroxy butyrate) |
| PLA | : | Polylactic acid |
| POP | : | Persistent Organic Pollutants |
| PP | : | Poly-Propylene |
| PS | : | Polystyrene |

| | | |
|---|---|---|
| PUR | : | Polyurethane |
| PVC | : | Polyvinyl chloride |
| rDNA | : | Ribosomal Deoxyribonucleic Acid |
| rpm | : | Rotation per minute |
| SwCorp | : | Solid Waste and Public Cleansing Management Corporation |
| USEPA | : | United States Environmental Protection Agency |
| UV | : | Ultra-violet |
| VOCs | : | Volatile Organic Compounds |
| w/v | : | Weight per volume |
| w/w | : | Weight per weight |

# CHAPTER 1: INTRODUCTION

## 1.1 General introduction

Tremendous increase in the production and utilization of plastics over the last decade has driven to a voracious appetite and over-consumption of plastic products. Plastic is a man-made synthetic polymer with undeniable behavioral propensity such as versatile, durable, light weight, moisture resistant, improved barrier resistance and high aesthetic appeal (Asmita *et al*., 2015). The low production cost and simple process have inflated global plastic demands in many sectors (Ojha *et al*., 2017; Russell *et al*., 2011). In 1950s, the global plastics production was only 1.5 million metric tonnes annually, but in 2017 the figure has escalated to 348 million metric tonnes per year (Statista, 2019).

In Malaysia, with increase in population and advance technology, plastics are broadly use in wide applications to meet consumer requests (UNEP, 2009). A statistics analysis conducted by Malaysian Plastics Manufacturers Association (MPMA) in 2015 reported, over the past eight years (2005 to 2013), the global resin consumption grew by 45 million metric tonnes to 202 million metric tonnes, approximately 3.2% of annual consumption of plastics. In 2015, Malaysia's per plastic consumption averaged 71 kg, compared to around 68 kg/person in 2011. The demand for plastic rises in many sectors and becomes the mother industry in the production of automotive components, electrical and electronics parts, components for the telecommunication industry, construction materials, housewares products, packaging materials and toys.

## 1.2 Polymer classification

Plastic are materials that are composed of any synthetic or semi-synthetic organic polymer that can be divided into two major categories which are thermosets and thermoplastics. Roughly, 80% of the global plastic production is contributed by

thermoplastics particularly in packaging application and non-plastics sectors including textile fibers and coating (Dewil *et al.*, 2006). Based on the polymer market, thermoplastics are further segmented into six groups including polyethylene (PE), Poly Ethylene Terephthalate (PET), nylons, Poly-Propylene (PP), Polystyrene (PS), Polyvinyl Chloride (PVC) and Polyurethane (PUR) (Alshehrei, 2017). Among other commodity of plastics, PET and PE plastics are predominantly used in packaging application. As stated by Euromonitor, 480 billion PET bottles have been produced globally and it is projected to reach 583.3 billion by 2021 (Laville and Taylor, 2017). In 2010, 690,000 tonnes of high density polyethylene (HDPE) plastic bags were produced in United States (United States Environmental Protection Agency, 2011). Approximately, € 72 to 108 billion of material value is lost in plastic packaging sector per annum although single uses of the products have low perceived value (World Economic Forum, 2016).

### 1.3 Plastic wastes management

Over consuming of plastics, have resulted to discarding, littering and accumulation of high amount of waste plastics which create big challenges for environmental and solid waste disposal (Jecu *et al*., 2012). The global cumulative waste generation of plastics between 1950 and 2015 was 6300 metric tonnes (Geyer *et al*., 2017). Only, about 800 metric tonnes and 600 metric tonnes of plastic wastes have been incinerated and recycled, respectively (Sunday Times of Malta, 2017). The remaining plastic wastes were discarded in landfill (Geyer *et al*., 2017). Solid Waste and Public Cleansing Management Corporation (SWCorp) (2017) stated that the generation of wastes in Malaysia was 6,935,000 tonnes/year in 2005 which further increased to 13,537,000 tonnes/year in 2016. In 2010, Malaysia generated one million tonnes of mishandle

plastic wastes, of which 0.14 to 0.37 million tons may have ended up in the seas (Abdullah, 2018).

High volume of plastic wastes are dominated by single-use plastic products such as plastic drink bottles, food wrappers, plastic bottle caps, straws and stirrers, plastic bags and plastic lids (Institute for European Environmental Policy (IEEP), 2016). In 2011, PET plastics were the most abundant wastes collected worldwide, reaching as high as approximately 7.5 million tonnes (Academy of Science Malaysia, 2015). Inefficient separation of wastes have resulted in more than 30% potentially recyclable materials such as paper, plastic, aluminum and glass, loss of in landfills (SWCorp, 2016). Synthetic plastics becomes a bigger nuisance because it resists to break down in natural environment due to the complexity of their structure, molecular size, surface topography and xenobiotic nature (Schlemmer *et al*., 2009).

According to The HINDU (2014), 19 million metric tonnes of PET have been manufactured and only one in six of the total number of PET were recycled or reused globally while the remaining 80% were sent to landfill (The HINDU, 2014). To date, Municipal Solid Waste (MSW) landfill is the most favored disposal method especially among developing countries. In Malaysia, approximately 95% of the wastes is directly disposed in landfills (Agamuthu *et al*., 2011). Landfilling of mixed wastes has become a growing public concern as biological, chemical and physical degradation poses environmental risk such as the release of harmful gas emission and leachate (Norkhadijah *et al*., 2013; Manaf *et al*., 2009) and pollute surface and groundwater (Suthar & Singh, 2015).

## 1.4 Biodegradation of plastic wastes

Biodegradation using microbes is an alternative option for efficient disposal of plastic wastes (Webb *et al*., 2013). Currently, more enzymatic degradation studies for plastic wastes treatment has been carried out. Attempts to raise the susceptibility of plastic, biodegradation approach using microorganism can be a more effective method than recycling, land filling and incineration (Kumar *et al*., 2011). Microbial degradation by bacteria and fungi play key role in biodeterioration of plastics (Gu, 2003).

Polymer degradation by potential fungal strains were focused as they are robust organisms and able to grow and deteriorate a variety of compounds, organic contaminants and polymeric materials (Chiellini *et al*., 2003). The extra- cellular enzymes secreted by microorganisms causes depolymerization of polymers into monomers by forming biofilm (Gu, 2003; Jumaah, 2017). The monomers will be subsequently broken down into smaller fragments that are easily absorbed and metabolized by intracellular enzymes (Gu *et al*., 2000b).

Several factors need to be accounted during biodegradation process such as properties of polymer, types of fungi, and nature of pretreatment. The characteristics of polymer such as mobility, crystallinity, molecular weight, functional groups, chain flexibility, and cross-linking structure contribute to enzymatic degradation of plastics (Artham and Doble, 2008). Varieties of physical and biological forces are required to breakdown large structure of polymers into smaller fragments (Swift, 1997). Introducing physical forces such as heating, cooling, freezing, thawing, wetting or drying, can cause mechanical damages by forming cracks on the polymers (Kamal and Huang, 1992).

Biodegradation mechanism is initiated by the excretion of extracellular enzymes by microorganisms to depolymerize polymers into smaller fragments prior to be absorbed and biodegraded within the fungi cells. The small size of monomers able to pass through semi-permeable outer membranes. The monomers will then be used as carbon and energy sources by fungi (Gu, 2003). A study stated that mycelium of fungus can cause small-scale swelling and bursting, as the fungi get penetrated in the polymer solids (Griffin, 1980) followed by breaking down of the chemical bonds during biodegradation process. Mineralization is the last step in biodegradation when the end products such as carbon dioxide, water, or methane are produced (Frazer, 1994; Hamilton *et al.,* 1995).

## 1.5 Problem Statement

The prevalence of and dependence on plastics in our modern life caused ubiquitous presence as litter in the environment. Pile-up of plastics in the environment primarily dependent on either inadvertently during use or upon disposal as wastes. Resilience to degrade naturally has caused plastics to remain in the environment for a very long time, up to 1000 years (Pramila & Ramesh, 2011). Thus, it is crucial to have a systematic waste management to minimize the loss of plastic to the environment.

Landfill, incineration and recycling methods are the most common techniques employed to manage the solid wastes in both developed and developing societies. However, poorly managed landfills have been reported to cause adverse impacts on the soil and surrounding environment. Chemical leaching from plastics into soil and water becomes a serious concern as the release of toxic chemicals such as, bisphenol A, phthalates and brominated flame retardants may threat the life of living organism (Environmental Health News, 2017). It also prevents break down of other normal wastes and affects soil fertility (Rinku Verma *et al.*, 2015).

In addition, plastic wastes dumped in poorly managed landfill along with littering activity have high potential to be carried away to sea by flood water and wind thus resulting to existence of 165 million tonnes of plastic debris floating in the oceans. Numerous documents have been reported on the impacts of plastic debris in marine environment notably on the entanglement and ingestion by marine species (Kuhn *et al*., 2015).

Poor maintenance of incineration of plastics waste releases hazardous substances (dioxins, furans polychlorinated-biphenyls) into the atmosphere causing dire impacts to the environment and human health. Although, recycling method can be advantageous over landfill and incineration, however it is difficult to collect and sort the plastic wastes. Reuse and recover of plastics become more promising in many developing countries to prevent reckless littering of plastic wastes into rivers and waterbodies. The existence of plastics debris in the environment was first reported by Carpenter and Smith in 1970's (Carpenter *et al*., 1972; Carpenter and Smith, 1972). Since then, many reports have been documented on the accumulation of plastic pellets in river, marine water and terrestrial area (Colton *et al.*, 1974; Eriksen *et al*., 2014). Therefore, considering the huge amount of plastics in the environment, biodegradation by microorganisms can be a new approach to manage plastic wastes in the environmental (Witt *et al*., 1997).

Biodegradation is an alluring option in contrast to current practices for waste disposal, as it is commonly a less expensive conceivably substantially more effective procedure and does not create secondary pollutants (Pieper and Reineke, 2000). Plastic degradation by potential fungal strains should be investigated as fungi are robust organisms and able to grow and deteriorate a variety of compounds, organic contaminants and polymeric materials (Chielline *et al*, 2003). Thus, a search for, and

isolation of fungus capable of degrading plastics could lead to solutions to the disturbing accumulation of plastics in the landfills.

## 1.6 Research Objectives

The overall objectives of the research is to study the biodegradation of three different types of plastics (PET, LDPE and HDPE) using fungus isolated from Jeram Sanitary landfill. The aims of this study are:

a) To isolate and identify potential plastic-degrading fungus from plastic samples in Jeram Sanitary Landfill.
b) To study the films biodegradation potential of cultured fungus.
c) To optimize the biodegradation of plastic films by the selected fungus.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Introduction to plastics

In present generation, one can hardly visualize daily life in the absence of plastics. The demand for plastics increases along with the global population. The history of plastics began with the introduction of the first man-made plastics by Alexander Parkes in 1862 (Pathak *et al*., 2014) which the material could be molded into different shapes like rubber, but at cheaper price. To further advance the technology of plastic, new inventions were made by several influential chemists and inventors (Pathak *et al*., 2014). In 1909, first synthetic plastic, Bakelite was introduced by a Belgian chemist, Leo Baekeland (Pathak *et al*., 2014). The development of brittle plastics made from formaldehyde and phenol marked the beginning of the Polymer Age. However, plastics began to be used globally after the World War II (Geyer *et al.*, 2017). One factor of plastics that draw the world's attention was its cost. The cost-effectiveness of plastics allow the manufacturers to produce in larger scale with different characteristics and functions. The word "plastic" originated from Latin word "plasticus" and Greek word "plastikos" both meaning "capable of shaping or molding". Plastics are made up of repeated monomers to form large complex molecules. The easy molding of plastics into desired color and shape allow its widespread usage in packaging, clothing, electric and electronic appliances, automotive parts, agriculture, industrial applications, biomedical and aerospace. Plastics can be categorised into two groups: natural and synthetic polymer.

### 2.1.1 Natural plastics

Natural polymer can be extracted from renewable sources and are made up of components from nature such as plants, animals and algae. All living organisms consist of polymer chain (Susan Freinkel, 2011). They are grouped into three different types of polymer such as polynucleotides, polyamides and polysaccharides. For example, plant cell walls are made up cellulose (polysaccharides) which is a polymer. So do, animals and humans muscle, skin and genetic molecule (DNA) are made up by proteins (polyamides) which is also a polymer. Other examples of natural polymer include enzymes, starch and rubber. Polylactic acid (PLA) and poly (hydroxy butyrate) (PHB) are examples of synthetic natural polymers made up of natural products such as proteins and polysaccharides with the help of various chemical polymerization techniques (Dai & Fan, 2014). Natural plastics are well known for their biodegradable and eco-friendly properties.

### 2.1.2 Synthetic plastics

Repeating units of monomers make up synthetic polymers which are joined together through polymerization process. The lesser dense synthetic polymers are manufactured using petrochemical feedstock (Hopewell *et al.,* 2009). They are composed of carbon, hydrogen, silicon, oxygen, chloride, nitrogen and other additives including lubricant, filler, plasticizer, stabilizer, catalysts, and coloring material to stabilize and improve their mechanical strength (Alshehrei, 2017). Synthetic plastics can be further divided into two broad categories namely thermoplastics and thermosetting polymers.

### *2.1.2.1 Thermoplastics*

Thermoplastics are plastics that can be repeatedly molded or shaped into new products with the application of heat without changes in their chemical properties. Most common thermoplastics that are used in many production sectors are polyethylene (HDPE and LDPE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and poly vinyl chloride (PVC). Table 2.1 shows the thermoplastic types and their properties.

**Table 2.1**: Types of thermoplastics and their properties

| Resin Code | General Properties |
|---|---|
|  | 1) Clear smooth surfaces for oriented films and bottles<br>2) High impact capability and shatter resistance |
|  | 1) Excellent resistance to most solvents<br>2) Higher tensile strength compared to other forms of polyethylene |
|  | 1) High impact strength, brilliant clarity, excellent processing performance<br>2) Resistance to grease, oil and chemicals |
|  | 1) Excellent resistance to acids, bases and vegetable oils<br>2) Toughness, flexibility and relative transparency |
|  | 1) Excellent optical clarity in biaxial oriented films and stretch blow molded containers<br>2) Low moisture vapor transmission |
|  | 1) Excellent moisture barrier for short shelf life products<br>2) Low thermal conductivity and excellent insulation properties in foamed form |

**Polyethylene terephthalate (PET)** belongs to polyester family and the molecules are loosely arranged making it semi-crystalline. PET plastics are manufactured through polycondensation of PTA with ethylene glycol. The semi-crystalline property of PET enable them to be more flexible, strong, chemically and thermally stable, causing them to be easily manufactured (Awaja & Pavel, 2005). It has a durable property which makes it resistant to weather, chemical, moisture and high energy radiation. PET is widely used to produce water bottles, automotive parts, houseware products, lighting products, photographic applications, X-ray sheets and textiles. Figure 2.1 shows the molecular arrangement of PET plastic.

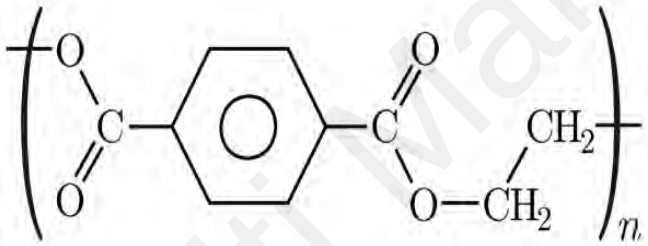


**Figure 2.1** : Molecular structure of PET

**High density polyethylene (HDPE)** belongs to polyethylene (PE) family and is made up of ethylene using catalytic process. Less branching properties as display in Figure 2.2 allow this polymer to closely pack to each other making them denser and harder (Carraher, 2003). Aaron *et al*. (2010) reported that HDPE polymers have molecular weight ranging from 5,000 to 250,000 Da and higher crystallinity which enable them to withstand high temperature (120° C) for short period of time. HDPE is characterized by resistance to impact, water, chemical corrosion and have hydrophobic property in nature. Generally, HDPE is commonly used to pack juices, soft drinks bottles, bags and industrial wrappings, detergents and cosmetics containers and other household products.

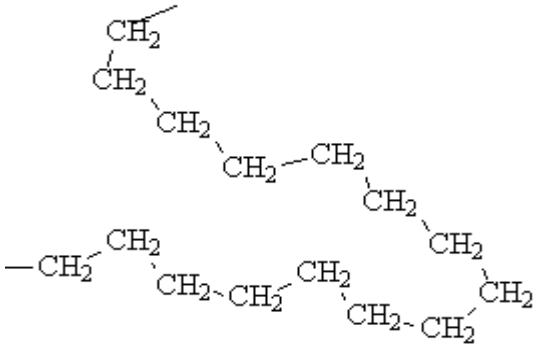


**Figure 2.2** : Molecular structure of HDPE

**Poly vinyl chloride (PVC)** is a synthetic resin made from polymerization of ethylene and vinyl chloride. High contents of chloride make it resistance to fires, oils and chemicals (Naqwi, 2006). The amorphous structure of PVC with polar chlorine atoms is shown in Figure 2.3. PVC has strong intermolecular interaction allowing them to be rigid at room temperature (Rahmah *et al.,* 2017). However, PVC polymer is unable to resist high temperature and decompose at 140°C. It is highly resistant to oxidative reactions due to durability properties. PVC is used in making hundreds of products including plumbing pipes and fittings, roof sheeting, cosmetic containers, raincoat, window and door frames. PVC is widely used in these applications due to its inexpensive and desirable physical and chemical properties.

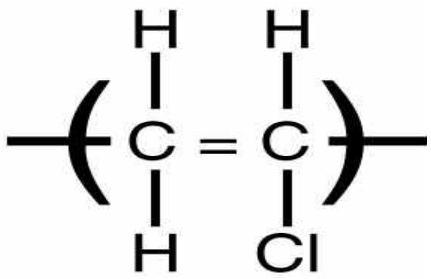


**Figure 2.3** : Molecular structure of PVC

**Low density polyethylene (LDPE)** is also another member of PE family. Highly branching property makes this polymer molecular arrangement less pack closely causes reduction in crystallinity (Peacock, 2000). LDPE has milky white texture when thick and becomes transparent when it is thin. LDPE are known for their low water absorption rate, low tensile strength, chemically inert and able to undergo combustion at high temperature (Yin *et al.,* 2015). The soft and flexible characteristics allow them to be used in manufacturing of carrier bags, packaging material, food boxes, flexible piping and hosepipes and agricultural plastic (mulching films). Figure 2.4 demonstrates the chemical structure of LDPE polymer.

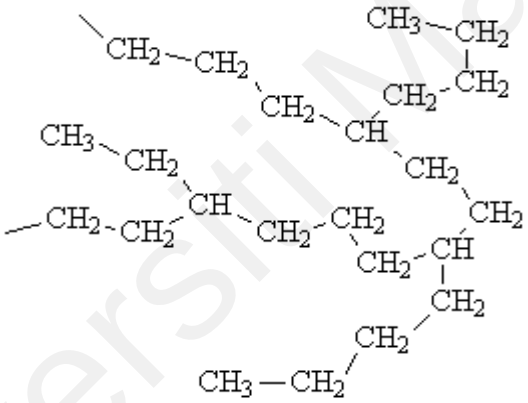


**Figure 2.4** : Molecular structure of LDPE

**Polypropylene (PP)** is the lowest density of plastics made from polymerization of propylene in presence of titanium chloride catalyst. The presences of methyl groups in the PP chain allow it to have crystalline structure (Van Schooten *et al*., 1961). The structural molecular is represented in Figure 2.5. PP is resistance to corrosion, moisture, chemicals and temperature up to 200°C. These characteristics allow it to be manufactured through many processes including injection molding and extrusion. However, PP is not suitable to be used at temperature lower than 0°C. Lightweight of the polymer makes it suitable for the production of trays, microwave meal trays, kettles,

garden furniture, lunch boxes, prescription bottles, funnels, pails, bottles, carboys and instrument jars.

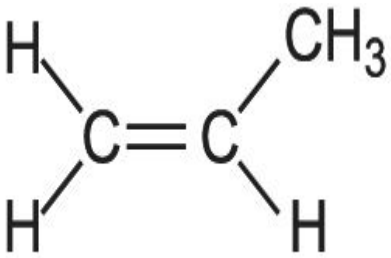


**Figure 2.5** : Molecular structure of PP

**Polystyrene (PS)** is a glassy polymer with presence of aromatic polymer of the monomer styrene shown in Figure 2.6. PS has great tensile strength and transparency (Benning, 1983; Kader *et al.,* 1989; Abdel-Bary, 2003). Mostly, PS is produced for a single use purpose as it has poor weathering properties and are not recommended for outdoors as it does not last long (Yousif & Haddad, 2013). Thus, PS can only be used to produce outdoor products with adequate coating on the surface of the polymer. PS can be utilized as electrical insulator due to its property being resistance to water absorption. Generally, PS is used in production of low-cost application such as plastic models, disposable cutlery, packaging foam, desk trays CD and DVD cases.

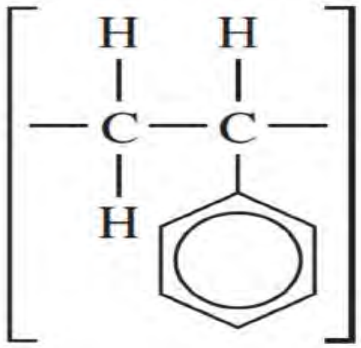


**Figure 2.6** : Molecular structure of PS

### *2.1.2.2 Thermosetting polymer*

Thermosets are plastics that cannot be reshaped after they have been solidified. Although they can be molded into various shapes but they cannot be softened again with repeated heat treatment. Examples of thermosets polymer include phenolic formaldehyde, urea formaldehyde, melamine formaldehyde, silicon and multilayer plastics (Plastics Europe, 2016).

## 2.2 Worldwide production of plastics

Global success story of plastics in many industries have contributed to rapid consumption of plastics globally over the past 50 years ranging from 1.5 million tonnes in 1950 to 322 million tonnes in 2015 (Plastics Europe, 2016). Figure 2.7 demonstrates the world production of plastics from 1950 to 2015.

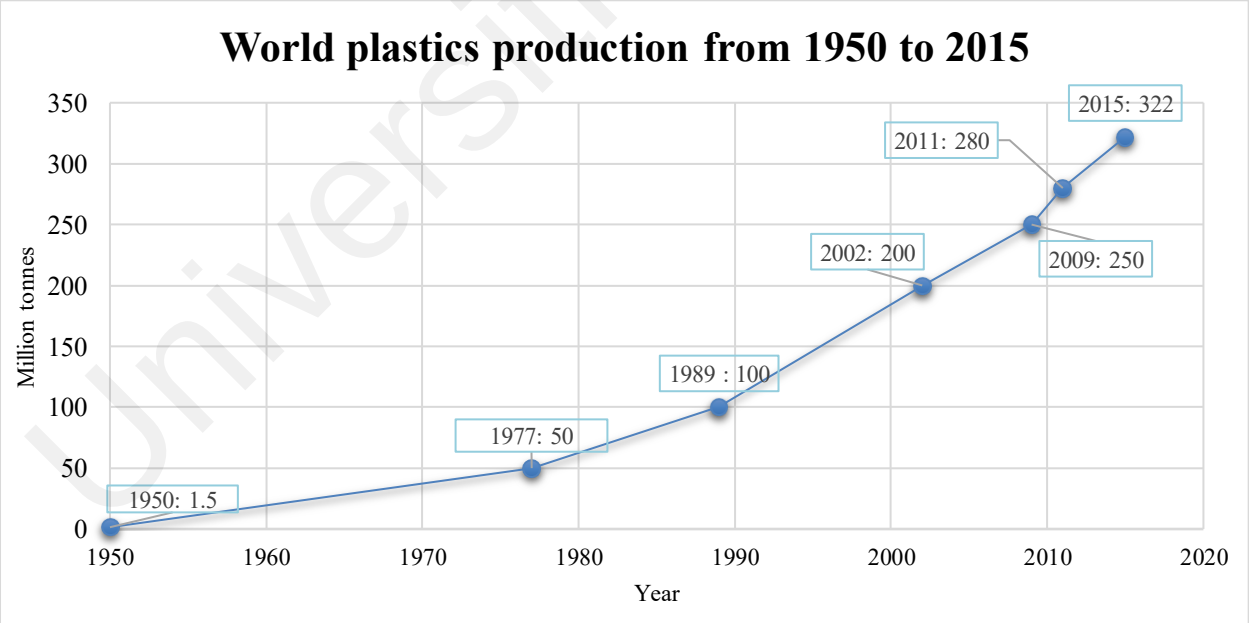



**Figure 2.7**: World production of plastics from 1950 to 2015. (Source: Plastics Europe, 2016)

The cumulative annual growth rate of plastics production from 1950-2015 was reported to be 8.4 % (Geyer *et al.,* 2017). According to World and Turkish Plastics Industry report (2016), 25% of the total production of plastics in 2015 was achieved by China followed by Europe (21%), NAFTA (20%), Asian Countries except China (16%), Middle East and African countries (8%) and Latin America (7%). The total plastic production by main countries in 2015 is presented in Figure 2.8.

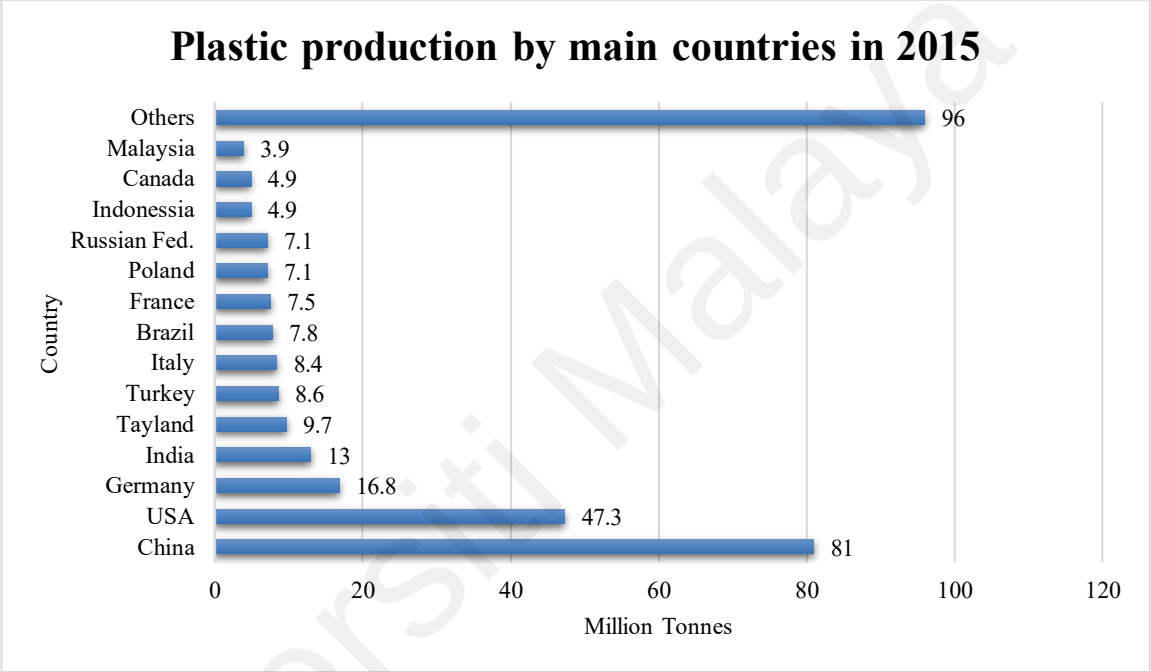



**Figure 2.8**: Total plastic production by main countries in 2015 (Source: World and Turkish Plastics Industry Report (2016))

In 2006, Europe was the main producer of plastics which accounted for 25% of the total worldwide 245 million tonnes of plastics production (Plastics Europe, 2008). However, in 2013, plastic production was led by Asia Pacific region constituting more than 40% by weight of the total market volume in which China surpassed Europe in plastic production continuing the shift from the West to Asia (Plastics Today, 2013). In 2015, China alone has become the dominant player in global plastic production reaching around 81 million tonnes (World and Turkish Plastics Industry Report, 2016). The rise in per-capita income of the middle class population resulted in increase of domestic

consumption for plastics within the country and makes China plays prominent role in plastic global market. Thus, Chinese government introduced "Plan to New Materials" in September 2012 to meet the plastic demand by producing domestically (Plastics Today, 2013). Europe represents second highest global plastic producer with 49 million tonnes (Plastics Europe, 2016). Germany is the third highest country producing 16.8 million tonnes of plastics representing European production followed by Italy (8.4 million tonnes), France (7.5 million tonnes) and Poland (7.1 million tonnes) (Plastics Europe, 2016). Slight decline in plastic production made Europe the second ranked producer (Plastics Europe, 2010). North America produced 19.4% of global plastics representing large part by the United States whereas Central and South America only accounted smallest part of the global plastic production with 4.8% in 2015 (Plastics Europe, 2014). Availability of low cost natural gas becomes an advantage for North America to continuously perform as one of the top producer of plastics worldwide. Besides China, three main countries that contributed in global plastic production in Asia are India, Indonesia and Malaysia with production of 13 million tonnes, 4.9 million tonnes and 3.9 million tonnes, respectively.

## 2.3 Worldwide consumption of plastics

The consumption pattern by country varies according to their main market sectors. Considering European countries, about 39.9% of the total plastic productions find its ways into the packaging sector in 2015 (Plastics Europe, 2016). The demand for plastics is favored by packaging segment due to the fact that more than 50% of all products are packed in plastic (Plastics Europe, 2017). Central and South United States are fastest growing regions showing strong demand in construction (plastic products such as pipes, roofing and door frames) and beverage industry (containers, plastics bottles and film). Similarly, consumption patterns in Asia shows continues growth in automotive (reduce car-weight and fuel consumption) and packaging industry. For instants, in 2012,

Indonesia consumed 3.6 million tonnes of plastics and roughly 70% of the total plastics usage was accounted by food and beverage packaging sector (Elliott, 2015). In Indonesia, approximately 28% of the total plastic production was dominated by packaging application followed by electronic (15%), construction (14%) and automotive (8%). Likewise, highest demand for plastics was accounted by packaging sector in Vietnam and the remaining exigency was achieved by household appliances (29%), construction (18%) and technical products (15%) (Plastech Vietnam, 2018)

As for the demands for plastic commodity, PE, PP, PVC, PUR and PET are most widely used polymers worldwide. These polymers enjoy commanding positions in the world market sector due to their unique and valuable properties. The different types of global polymer consumption in 2015 are presented in Figure 2.9.

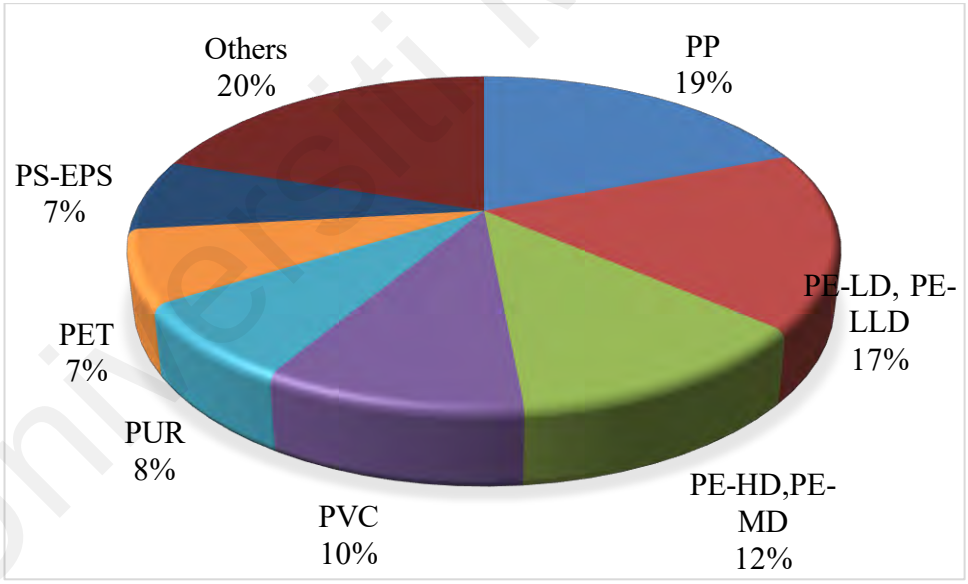



**Figure 2.9**: Global resin types consumption in 2015 (Source: Plastics Europe, 2015)

In 2015, PE (HDPE, LDPE and LLDPE) accounted for large share of about 29% of total plastic consumption followed by PP, PVC, PUR and PET with 19%, 10%, 8% and 7%, respectively (Nerland *et al*. 2014). More than 50% of the market share of PE plastic was dominated by Asia Pacific in 2016 with a total global consumption of 160 Million

tonnes. The rise in use of construction and container products is expected to promote the growth of HDPE polymer in 2018. The rising demand for PE has been highly impacted by use in recurrent markets of flexible packaging, construction products and rigid packaging. The demand for PP as a second highest polymer is anticipated to be the dynamic factor behind the growth markets of construction, packaging, automotive and agriculture. The construction activities involves the use of rigid films, pipes and fittings, tubes and cables have been the strong segment to advance in production of PVC polymer (Plastic world market, 2016).

Over the past 10 years, PET global consumption has increased more than 4% per year (IHS Markit, 2018). The greater demands for production of polyester fiber and packaging products become the major drivers for manufacturing of PET polymer especially in Asia with China representing 30.8% of the global total in 2017 (Plastics Insight, 2019). An analysis of plastic consumption on a per capita basis shows the consumption of plastics differs markedly from country to country reaching 136 kg/ person in Western Europe, NAFTA with 139 kg/person, Japan with 108 kg/person and Asia with 36 kg/person (Statista, 2018). China is one of the top consumers of plastic products and the per capita consumption of the products grew from 22 kg/person in 2005 to 46 kg/person in 2010 (Liao, 2011). Larger population growth countries unsurprisingly used more plastic products. Overall, the usage of plastic gets more attention predominantly in developing countries due to cheaper unit cost and improvement in quality of the plastics promote its substitution for other materials like glass and metal (Narayan, 2001).

## 2.4 Plastic consumption in Malaysia

Malaysia is a fast growing nation with plastics manufacturing industry becoming one of the most vibrant growth sectors contributing to its economic growth. Each year, plastics are manufactured with a volume of 2 million tonnes in Malaysia (MPMA, 2011). Increase in growth of consumer societies for better quality of plastic products have contributed to the rise in plastics production and consumption. Table 2.2 displays the plastics consumption and average per capita plastic consumption.

**Table 2.2**: Total and average per capita plastics consumption from 2011 -2015

| Year | 2011 | 2012 | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|
| Resin consumption (Million Metric Tonnes) | 1.98 | 2.04 | 2.10 | 2.15 | 2.22 |
| Per capita resin consumption (kg/ person) | 68 | 69 | 70 | 70 | 71 |

Plastic consumption had expanded from 1.98 million metric tonnes in 2011 to 2.22 million metric tonnes in 2015 (MPMA, 2016). One of the prime factors that contributed to the rise in plastic consumption is the demands towards plastic products. This scenario can also be explained with growth in per capita plastic consumption from 68 kg/person in 2011 to 71 kg/person in 2015 (MPMA, 2016). In conjunction with that, production of HDPE and LDPE polymers were dominated in Malaysia with proportion of 24% each and accompanied by PP and PET both with 13% (MPMA, 2016). In 2013, packaging accounted for the largest share of the Malaysia's market segment with 40% to make rigid and flexible packaging products (MPMA, 2016). The remaining fraction was distributed between electronic (26%), automotive (10%), construction (8%), household (7%) and others (9%) (MPMA, 2016). The second largest consumer is electronic industry which continues to grow due to the high demand on the production of

television and air-conditioner sets (MPMA, 2016). Surge in the usage of polymer in pharmaceutical sector also benefited the plastics market in the forecast period.

## 2.5 Generation of plastic wastes

The short lifespan of the products that are made from plastics could be a factor that contributes to surge in plastic wastes. According to Hopewell *et al*. (2009), roughly 50 % of plastics are manufactured for single-use application (packaging, disposable user items) and duration of these products usage could be shorter. Geyer *et al*. (2017) reported approximately 8.3 billion metric tonnes of Municipal Solid Waste (MSW) were generated globally in 2015 which 6.3 billion metric tonnes of the wastes were plastics waste. Only 9% was recycled and the remaining wastes were discarded in landfill (Geyer *et al*., 2017). Japan has the highest plastic recycling rate in the world accounting up to 77% of the total plastic wastes generated in the country (The Guardian, 2011).

Likewise, in Europe and United States, 8% of the total MSW generation comprised of plastic wastes (Malik & Roy, 2015). Ireland alone represents the highest producer of plastic wastes generating 61 kg/ person/ year (Jambeck *et al*., 2015). The second country from Europe continent is Luxembourg with 52 kg plastic wastes/person followed by Estonia (46 kg/person/year), Germany (37 kg/person/year) and Portugal (36 kg/person/year) (Malik & Roy, 2015). In Malaysia, more than 30 000 tonnes of MSW are discarded each day (Fauziah & Agamuthu, 2009) with 11% of the total MSW being plastic wastes and recycling was only 7 kg/person/year. China is one of the top producer of plastic wastes with an annual generation about 38,060,000 tonnes (Jambeck *et al*., 2015). In China, the production of plastic wastes by urban and rural domestic sector does not differ much with generation 20,000,000 tons and 18,060,000 tons, correspondingly (Yanga *et al.,* 2012). The imports of low-cost and secondary plastic material wastes from developed countries such as US (21%), Japan (18%), Germany

(12%) and United Kingdom (9%) to China makes it the largest generator of the polymer wastes in the world (Yanga *et al.,* 2012).

## 2.6 Impacts of plastic wastes in the environment

The accumulation of plastics in ecosystem becomes a global issue that threatens the environment and human health. The abundance of plastics can result from primary or secondary sources. Primary sources involve the disposal of plastics from anthropogenic activities while secondary sources encompass fragmentation of plastics into microplastics (<5mm) in size due to exposure to sunlight and physical abrasion (Arthur *et al.,* 2009). The profusion of these macroscopic plastics and microplastics causes impact to terrestrial and marine ecosystem. As stated by Jambeck *et al.* (2015), since only a small volume of plastic wastes are recycled worldwide, they will find the way to soils or marine environment.

### 2.6.1 Terrestrial effect

Existence of microplastic in the terrestrial environment can be strenuous to monitor and may have profound effect than macro-plastics. The secondary pollutants of plastics might be emanated from landfill and other human activities causing deposition of the microplastics on soil and land. Several factors that contributed to the input of smaller fragments of plastics on land are poor waste handling, accident loss of particles and production of polluted soils and aerosols (Bussi *et al.,* 2016). The plastic and microplastic contamination on the terrestrial system becomes promising as light density of these particles can be carried away easily from the point source to the environment. The abundance of microplastics in the shoreline can alter the physical properties of the sediment by lessening the heat conductivity and increased the grain size and water permeability (Wang *et al*., 2016). Few researches have reported that the pollution of microplastics can be four to 23 times more than marine microplastic pollution

(Anderson *et al*., 2017). Pollutants are made up of volatile compounds such as ethyl benzenes, trimethyl benzenes and BPA causing release of hazardous gases into the environment (Svenson *et al*., 2009). Besides, release of these chemicals in the landfill particularly BPA can contaminate the leachate and foster growth of sulphate-reducing bacteria in soil populations (Teuten *et al.,* 2009). Thus, increase in concentrations of hydrogen sulphide over the limit can potentially be life-threatening.

Usage of microplastic beads in personal care products, clothing and other household applications resulted in increase in volume of the fragmented particles in sewage (Mason *et al.,* 2016). Approximately 80% to 90% of the microplastics found in sewage are channeled into sludge (Talvitie & Julie, 2017). Utilization of sewage sludge as agricultural fertilizer implied accumulation of microplastics in the soils (Nizzetto *et* al., 2016). Apart from reducing soil fertility, microplastics caused huge impacts to terrestrial species. However, up to now, not many examinations have been reported on the uptake of microplastics by terrestrial organisms. Another researcher, Ugolini *et al.* (2013) conducted an experiment on sand hopper *Talitrus saltator* to demonstrate the consumption of microplastics. The organism was fed with dry fish food together with 10% (w/w) of PE microspheres size ranging from 10–45 μm (Ugolini *et al*., 2013). Within 24 hours, most of the microspheres were defecated and others were removed in less than one week (Ugolini *et al*., 2013). Similar research was conducted by Zhu *et al.,* (2018) on springtails in which their biological activity, particularly, the action of microbiomes on their guts was affected by changes in biophysical environment. Microplastics were also observed in the freshwater birds whereby their ingestion may be result from food chain or through accidental consumption (Zhao *et al.,* 2016, Gil-Delgado *et al.,* 2017; Holland *et al.,* 2016). Microplastics were found to be (0.9 particles/g) in soil, (14 particles/g) in earthworm casts and (129 particles/g) in chicken feces as a consequences of trophic transfer (Huerta Lwanga *et al.,* 2017).

Several studies on the effects of microplastic on the marine biota had been reported, however the studies related to accumulation of microplastics in the soil surface and their effects remain to a great extent unexplored (Nizzetto *et al.,* 2016).

### 2.6.2 Marine effect

Similar occurrence was observed in marine ecosystem whereby the marine biota are at great risk due to the abundance of plastics. Therefore, the ubiquitous plastics debris in the ecosystem have caught public attention on the impacts of the microplastic on living organisms. Plastics also act as source of habitat to many microorganisms and invertebrates. Reisser *et al*. (2014), reported that barnacles, dinoflagellate, isopods, marine worms, marine insect eggs, bacteria, cyanobacteria and fungi were found on plastics surface. Plastic fragments give new homes to the biota that have benefits over the plenitude of microplastics in the marine water. Moore *et al*. (2001) gathered PE plastic fragments from North Atlantic and noticed colonized pathogens on the outside of the plastics. As per Majer *et al*. (2012) and Goldstein *et al.* (2012), plastic pellets are utilized as eggs laying spots by *Halobates micans* with normal 5 to 48 eggs for each pellet. The transportation of this invasive organism by drifting plastics can threaten to other marine species (Barnes, 2002). Eventually, the plastics debris could alter the existing environmental conditions that may cause impacts to the balance of other living marine organisms (Chisholm *et al.,* 2011).

Utilization of microplastics by a marine organism are conceivable by filter feeding, direct engulf, ingestion of suspension materials, water intake and ingestion of lower trophic species which have consumed microplastics (Baulch & Perry, 2014; Depledge *et al*., 2013). Marine organisms are often exposed to microplastics through filter feeders which include expansive range of marine species ranging from lower trophic level species to high trophic level species (Andrady, 2011). Marine organisms often mistook

plastics as their prey and consumed them. As a result, the organisms suffer from reduction intake of food intake and blockage in their digestive tracts (Laist, 2006). Since, plastics cannot be digested by the organisms, they remain in the body. In addition, a study conducted in North Pacific Ocean revealed that marine plankton often mistook white and lightly-colored small sized plastics as food particles (Shaw & Day, 1994; Wang *et al*., 2016). Since, enzymatic pathway is available to digest the microplastics, the ingested plastic remains bio-inert in the organism system. Accumulation of microplastics in the species system makes them vulnerable due to the release of toxic chemicals from a) chemicals leaching from the microplastics and b) adsorption of external pollutants to microplastics (Cole *et al.,* 2011). Innumerable studies have been conducted to demonstrate the uptake of microplastics by the marine biota. Table 2.3 shows the list of several studies conducted to demonstrate the ingestion of microplastics by marine species.

**Table 2.3**: Microplastics ingestion by marine organisms

| Microorganisms | Type of microplastics | Sampling location | References |
|---|---|---|---|
| Ciliate *CycZidium sp*. | Microspheres (0.6 pm diameter) | Kaneohe Bay, Oahu, Hawaii | Pace and Bailiff, 1998 |
| Planktivorous mesopelagic fish | Fragments (94%), film (3%), fishing line (2%) and Styrofoam (< 1%) | North Pacific central Gyre | Boerger *et al*., 2010 |
| *Nephrops norvegicus* | 5 mm PP filaments | Clyde Sea | Murray and Cowie, 2011 |
| Blue mussels (*Mytilus edulis*) | Polystyrene (PS) beads (100 nm) | Dutch Wadden Sea | Wegner *et al*., 2012 |
| *Balaenoptera physalus* | Microplatics (9.67 pieces/m3) | Coasts of Italy | Fossi *et al.*, 2012 |
| Shore crab (*Carcinus maenas*) | 0.5 µm PS | River Exe estuary, Devon (UK) | Farrell and Nelson, 2013 |

**Table 2.3,** continued.

| Microorganisms | Type of microplastics | Sampling location | References |
|---|---|---|---|
| Zooplankton | *Centropages typicus* and *Temora longicornis* able to consume 7.3, 20.6, and 30.6 µm polystyrene bead. | Coastal site located in the western English Channel 12 km south of Plymouth, UK | Matthew *et al.*, 2013 |
| Centropages typicus | Ingested Polyamide-6 Nylon powder (µ = 30 µm), Polyethylene microbeads (µ = 20 µm) and artificial rope fibres (µ = 14.76 µm) | North Atlantic and Mediterranean coastal waters | Craig, 2014 |
| Mussels (Halifax Wild ) | Polypropylene lines | McCormack's Beach and Rainbow Haven Beach back lagoon | Mathalon and Hill, 2014 |
| *Dicentrarchus labrax* | Egestion of PE microbeads from 10 to 45 µm | Marine farm Aquastream (Ploemeur, France) | Mazurais *et al.,* 2015 |
| *Galeus melastomus* | Cellophane (33.33%) and Polyethylene Terephthalate PET) (27.27%) | Balearic Islands | Carme and Salud, 2017 |

The ingested microplastics can be consumed by the marine organisms and transported to the digestive tracts through translocation. Browne *et al.* (2008) carried out an experiment to exhibit the translocation of microplastic in marine biota. A model, *Mytilus edulis* was presented with fluorescent microspheres and the creature could ingest 2µm and 4µm of microplastics, following 3 days, 3 µm and 9.6 µm of microplastics was seen in the circulatory liquid of *M. edulis* (Browne *et al.,* 2008). Although, harmful effects weren't seen earlier in ingested microplastics by the mussels, on the other hand nodular irritation was seen in the stomach related organs (Kohler, 2010). Kiyama *et al.* (2012) performed an experiment to reveal the adsorption of microplastics by terrestrial organism, *nematode Caenorhabditis* was able to engulf polystyrene beads with high potential introduction of these microplastics into terrestrial food webs. At the same time, the effects of ingestion of non-polluted microplastics are yet to be distinguished (Zarfl *et al.,* 2011).

Engulfment of small plastics can result in physical, chemical and biological changes in the organism. The ingested microplastics can clump and cause blockage to maxilliped by preventing the passage of food into the intestinal tracts (Murray & Cowie, 2011). The physical damage eventually decreased the ability of the organism to uptake foods (Tourinho *et al*., 2010). Hamer *et al*. (2014) reported the intake of PE microplastics by *Idotea emarginata* over seven weeks of monitoring period caused reduction in food consumptions. The introduction of microplastics in the marine organism caused the availability of microplastics in the food chain of the marine systems. Eriksson and Burton (2003) revealed the uptake of 2mm to 5mm microplastics by fur seals from Macquarie Island which were assumed to be secondary plastics consumed by prey, *Electrona subaspera*. The exposure of nano-PS to algae has resulted in reduction of cellular chlorophyll-a content and growth likely to be caused by hindrance of $CO_2$ and nutrient directional flow by the adsorbed PS in the algae (Bhattacharya *et al.,* 2010;

Besseling *et al.,* 2014b). Long term exposure of microplastics may cause growth and reproduction effects in an organism.

Although, ingestion of microplastics poses harm to marine species, however some organisms have the ability to excrete the plastic fragments from the body without posing any threat. *Eurytemora affinis* copepods exposed to microplastics for a 12 hours, later excreted more than half of the ingested microplastics from the system (Setala *et al.,* 2014). Similarly, Thompson *et al*. (2004), recorded *polychaete* worms was able to expel consumed microplastics via their fecal casts.

### 2.6.3 Chemicals leaching from the plastics

Plastics are considered as plasticizer as plastic additives are added to alter the physical properties of plastics by increasing the shelf life of degradation, thermal resistance and resist to oxidative damage (Browne *et al*., 2007; Thompson *et al*., 2009b). Few studies reported approximately more than 50% of plastics are made of hazardous chemicals and additives (Lithner *et al*., 2011; Wang *et al*., 2016) to increase the extend of degradation causes leaching of these hazardous compounds to biota (Barnes *et al*., 2009; Lithner *et al.,* 2011; Talsness *et al.,* 2009). The common additives used in plastics are phthalates, polybrominated diphenyl ethers and constituent monomer bisphenol A. The use of these additives can results in hormone imbalance along with impacts on motility, reproductive abnormities and neurological development depending on the concentration of chemical engulfed by the organisms (Barnes *et al.,*2009; Lithner *et al*., 2009). Besides, proof of toxic contaminant adsorption by plastics has been reported. According to Bakir *et al*. (2014), microplastics can adsorb toxic contaminants from the aquatic environment thereby serving as scavengers and transporters of organic pollutants.

### 2.6.4 Adsorption of external pollutants

Persistent Organic Pollutants (POPs) are released into the marine water through wastewater from industrial chemicals and runoff of insecticides and pesticides (Wurl & Obbard, 2004). Small volume of POPs such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and perfluorooctanoic acid (PFOA) concentration ranging from 1 to 10,000 ng/g (Ogata *et al.,* 2009; Hirai *et al*., 2011) adhere to microplastics in marine water through partitioning process (Andrady, 2011). According to Ashton *et al.* (2010), adsorption of metal occurs when the cations of metals bind onto charged sites on the plastics surface. Greater Van der Waals force of attraction, larger surface area, and crystallinity properties promotes greater binding of organic contaminants and metals to the hydrophobic surface of small fragments compared to that in the marine water (Teuten *et al*., 2007; Ashton *et al*., 2010; Holmes *et al*., 2014). Besides, weathering of plastics and formation of biofilm increases the surface area and residence time which promotes the adhered of organic pollutants on microplastics (Mato *et al.,* 2001; Zettler *et al*., 2013). Tien and Chen (2013) reported concentration of metal sorption increased on the microplastics surface with aged biofilms. The biofilms alter the physical properties of microplastics surface making it more suitable or convenient for adsorption of metals (Artham *et al*., 2009).

Furthermore, the oxidation of UV on the surface of microplastics alter the physical and chemical properties of the plastics and resulted in noticeable fine cracks on the surface of the microplastics which allow accumulation of contaminants within the cracked areas (Severini *et al*., 1987; Satoto *et al*., 1997). Rochman *et al*. (2013) gave evidence that high concentration of PCBs and PBDEs adsorbed to the marine microplastics in the sampled fish compared to virgin plastics. Similarly, it was reported that the sorption of metals on the beached plastic pellet or contaminated pellet are

greater compared with pure plastic debris (Holmes *et al.,* 2012; Turner & Holmes, 2015) as the weathering of plastics increases the polarity of the plastics (Mato *et al*., 2001).

Although, the ingestion of the microplastics can lead to different problem, however the impacts of the dissolved POPs in microplastics increased environmental concerns (Bowmer & Kershaw, 2010). Ingestion of contaminated plastics by the marine biota facilitate the route of POPs into marine food web (Andrady, 2011) and can be transferred to higher trophic level (Anderson *et al*., 2014). Tanaka *et al*. (2013) conducted a study to demonstrate the transfer of organic contaminants from prey to predators. The study reported higher concentration of PBDEs found in the tissues of birds after ingestion of their prey, pelagic fish. The extent of POPs availability to the food web becomes promising as there is no enzymatic pathway to assimilate the microplastic in the marine species system (Wang *et al.,* 2016). Thus, the microplastic remains bio-inert in the marine biota and toxic substances are excreted out from POPs. However, the toxicity of the microplastics in the biota is dependent on the translocation and accumulation within tissue, ability to digest and excrete the pollutants and the potential to transfer to higher trophic level (Wright *et al*., 2013). A recent study by Avio *et al*. (2015) revealed that the uptake of Ag by zebrafish is at low concentration compared with the intake of Ag sorbed on the microplastics. Thus, the study shows microplastics have the potential to alter the bioavailability of absorbed pollutants.

### 2.6.5 Impacts on human health

The transfer of hazardous chemicals and microplastics into the food web poses threat to human health. Many studies on the ingestion of microplastics by wide range of marine organism have been reported which are generally consumed directly by humans (Cole *et al*., 2013). Few studies reported on the existence of microplastics in the food

consumed by human. De Witte *et al.* (2014) documented that mussels bought from Belgian stores contain microscopic fibers ranging from 200-1500 µm. Devriese *et al.* (2015) reported consumption of synthetic fibers by approximately 63% of brown shrimp caught in the Southern North Sea and Channel area. A study conducted in Belgium reported concentration of microplastics in *Mytilus edulis* and *Crassostrea gigas* are 0.36 ± 0.07 particles/g (wet weight) and 0.47 ± 0.16 particles/g (wet weight), respectively (Van Cauwenberghe and Janssen 2014). Liebezeit (2013) documented presence of colored plastic fragments (0 – 38/kg) and colored fibers (40/kg up to 660/kg) in 19 honey samples. The presence of microplastics in honey may result from the introduction of the particles by bees into the hive or accidental introduction during the manufacturing of honey. Another study documented the microplastic findings in five commercial sugars. The processed sugars contained plastic fragments with 32 ± 7/kg of sugar and plastic fibers ranged from 217 to 123/kg of sugar (Liebezeit and Liebezeit, 2013). Likewise, Yang *et al. (*2015) reported the presence of microplastics in rock salts (7 – 204 particles/kg), lake salts (43 – 364 particles/kg) and sea salts (550 – 681 particles/kg). Higher proportion of PET microplastics accounting more than 55% contaminated the sea salts followed by PE and cellophane (Yang *et al.,* 2015). Besides, Liebezeit (2014) revealed the appearance of microplastics in 24 German beer brands with volume of 2 to 79 fibers /L, 12 – 109 fragments/L and 2 – 66 granules/L.

The consumption of the infected food may be a route of transport of microplastics to humans (Van Cauwenberghe & Janssen, 2014). However, the risks of microplastics to human health dependent on the degree of uptake by enterocytes in the gut and the translocation in the infected organism tissue. Consumption of infected organism or food can cause direct toxicity from plastics that come from hazardous chemicals. Hussain *et al.* (2001) reported that consumption of PE particles can be transferred from gut to lymph and eventually to humans circulatory system. Wick *et al.* (2010) revealed a

finding from human *ex vivo* study that placenta can take up PS particle when the size was less than 240 nm and positively can led to cellular damage of human blood vessels. In an examination of 1455 adults, exposure of BPA in humans can result in cardio vascular disease and prevalent myocardial infarction (Lang *et al*., 2008). Toxicity chemical such as diethylhexyl phthalate (DEHP) and other toxins are possible to cause cancer, birth defects and immune system issues (Sutton *et al.,* 2016; Auta *et al*., 2017). Nevertheless, information on the consumption and biological impacts of microplastics resulting from ingestion of infected marine organism are yet available.

## 2.7 General plastic disposal methods

The changes in lifestyles especially among the urbanized citizen parallel with rapid economic growth have increased the quantum of plastics in the solid waste stream to a great extent. Collection and disposal of plastic wastes have emerged as an important environmental challenge to protect the public and environment from potential impacts of the waste. Thus, waste management becomes equally an important issue in both developed and developing countries. Two systems employed in waste management are formal and informal sectors. MSW waste collection by informal sectors (waste pickers) involves low and primitive methods such as collecting reusable or value added plastics waste such as PET bottles. However, development and employment of formal methods with advanced cleaner technologies are more crucial for positive environmental, resources and economic benefits (Cucchiella *et al*., 2016a; Cucchiella *et al*., 2016b). Thus, the wide range of waste-management prioritizations for the total MSW waste stream focused on landfill, incineration and recycling approach.

### 2.7.1 Landfill

The general approach employed in most of the nations in world is landfilling. Landfilling is a conventional method which allows the disposal of MSW. In 2008, 29.2 million tons of plastic wastes were disposed in landfills in the United States. In Malaysia, landfilling is the main technology for managing solid waste stream whereby, 80% to 90% of the total wastes generated ended up in the landfills (Ngoc & Schintze, 2009). In China, the low values of plastics resulted with their wastes are dumped in landfills rather than being recycled (Yang *et al.,* 2012). In the contrary, certain countries such as Belgium, Luxembourg, Netherlands, Germany, Denmark, Switzerland, Austria, Norway and Sweden forbid landfilling with only 10% of the plastic wastes are dumped in the landfill. Landfilling could threaten environment and humans when the landfills are poorly managed (Agamuthu *et al.,* 2004; Fauziah & Agamuthu, 2003). In certain countries, plastic waste can be carried away by flood and surface water into marine ecosystem. Plastic wastes hardly undergo degradation and could remain same in the soil for more than 100 years. Plastic wastes are made from toxic chemicals such as bisphenol A, phthalates, benzene, vinyl hydrochloride and other additives (Thompson *et al*., 2009). The leaching of phthalates chemical used as softener in plastic production is known to affect human fertility, disrupt endocrine glands, birth defects and other health problems (Verma *et al*., 2016). Inhalation of benzene and vinyl hydrochloride has been linked to cancer and potential to cause fatal (Falzone *et al*., 2016). Other drawbacks that have been reported are leakage of chemical constituent from plastic wastes, pollution of soils and underground water (Oehlmann *et al.,* 2009; Teuten *et al*., 2009).

### 2.7.2 Incineration

As an alternative method to overcome landfill limitations, incineration technology is routinely used. Incineration is able to recover energy from heat generated by generating electricity. However, due to poor maintenance, incineration of plastic releases harmful gases (dioxins (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans - PCDD/Fs), polycyclic aromatic hydrocarbons, polychlorinated biphenyls and various volatile organic compounds) into the environment (Blankenship *et al*., 1994). Gilpin *et al.* (2003) stated the presence of PVC and halogenated additives in the mixed waste leads to the release of toxic chemicals such as dioxins and polychlorinated-biphenyls into the environment. Further combustion of these wastes may lead to the release of toxic halogens into the atmospheres. Valavanidid *et al*. (2008) reported that the burning of PE can cause release of VOCs and semi VOCs including especially olefins, paraffin, aldehydes and light hydrocarbons. USEPA stated that plastics consist of plasticizers commonly of di (2-ethylhexyl) phthalate (DEHP)) which is known to be carcinogen to humans and have the potential to cause endocrine disruption and breathing difficulty (Verma *et al.,* 2016).

### 2.7.3 Recycling of polymer wastes

In Europe, not greater than 30% of plastic wastes are recycled out of 25 million tonnes of plastic wastes generated each year (Plastics Europe, 2016). Recycling of plastic includes several steps including primary, secondary, tertiary and quaternary stage. The mechanical, chemical and thermal depolymerization techniques incorporate with the four different types of recycling of plastics (Hopewell *et al.,* 2009). Primary and secondary recycling involves mechanical recycling which involves the breaking down of the polymer wastes into smaller sizes using physical methods such as collection, sorting, washing and shredding. The plastic wastes are then proceeded with

tertiary recycling which involves depolymerization into their chemical constituents (Fisher, 2003). At this stage, chemical recycling is used to extract or recover the petrochemical components in the waste for reproduction of other synthetic plastics or chemicals. The recovery of energy takes place under quaternary recycling. Recycling of plastic has a handful of advantages such as reducing release of greenhouse gases, recovery of non-renewable petrochemicals, reduced energy utilization and many more (Al-Salem *et al.,* 2009).

However, recycling method seems not economical in many developing countries due to high cost for management and maintenance (Patel *et al.,* 2000). In fact, effectiveness of recycling techniques is highly dependent on public awareness and economic viability. Due to these factors, recycling rate for plastic is still low in many developing countries. Besides, release of toxic and other hazardous chemical compounds have been reported especially during the recycling process of converting the plastic wastes into new plastics (Yamashita *et al.,* 2009; He *et al.,* 2015). Thus, more attention to various methods of degradation of plastic has been taken place among the researches (Yang, 2004).

## 2.8 Degradation of plastics

Any changes in physical or chemical properties of plastics due to environmental factors (thermal, photo, chemical or biological activity) are called degradation process. The changes mainly involved bond scission, erosion, discoloration followed by chemical transformations of the plastic characteristics (Pospisil & Nespurek, 1997). Polymer degradation can either be initiated by biotic or abiotic route. The major abiotic degradation of plastic is generally induced by thermal degradation, photo-oxidative degradation and ozone-induced degradation.

### 2.8.1 Thermal degradation

Thermal degradation or also known as pyrolysis involves deterioration of plastic wastes at high temperature. The degradation process take place at temperature between 350°C and 900°C to break down the long chain of polymers which resulted in the reduction of molecular weight, ductility and other physical properties together with discoloration (Olayan *et al.,* 1996). Overheating the polymer leads to the recovery of hydrocarbon oil such as paraffins, isoparaffins, olefins, naphthenes and aromatics. However, the production of the hydrocarbon oil primarily depends on the nature of the polymer wastes and temperature used to process the wastes.

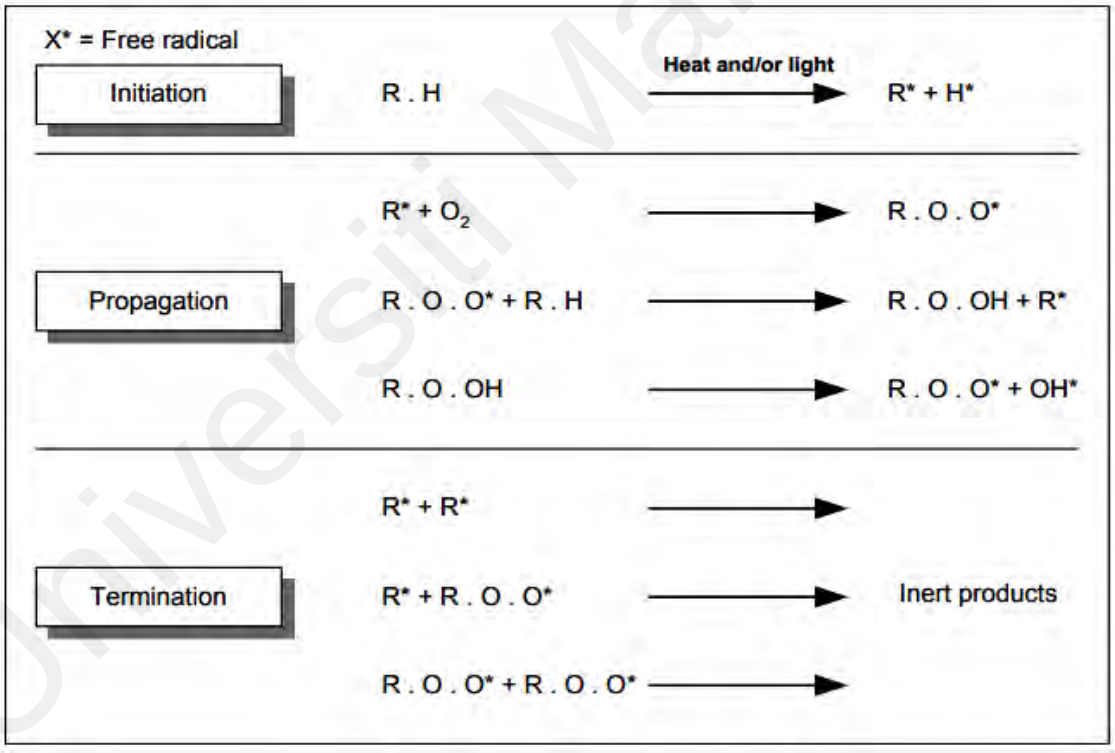



**Figure 2.10**: General mechanism for thermal degradation (Zeus Industrial Products, 2005)

### 2.8.2 Photo-oxidative degradation

Two methods adapted in photo-oxidative degradation are photodegradation (UV) and oxidation. The degradation of plastic in the presence of light is termed as

photodegradation. Generally, UV light is used to breakdown the polymer through absorption of light energy by certain molecule groups that exist in the polymer (Kyrikou & Briassoulis, 2007). The light energy thus causes depolymerization of the polymer into smaller fragments whereas for oxidation, heat is required to proceed with depolymerization. In PE, exposure to ultraviolet radiation causes autoxidation of polymer to form low molecular weight fragments (aliphatic carboxylic acids, alcohols, aldehydes and ketones) by the action of radical. Attachment of oxygen in this stage further promotes chain scission causing reduction in molecular weight making them more brittle (Rabek, 1995). Similarly, photo-oxidative initiate the degradation action in PET by cleaving ester bond formed between carboxylic acid group and vinyl group. The process is then continued by thermo-oxidative degradation.

### 2.8.3 Ozone-induced degradation

The availability of ozone in the atmosphere allows degradation of polymer under normal conditions. The polymer can still undergo rapid aging process with low concentration of ozone. The ozone degradation is initiated with the reaction between unsaturated double bond of the polymer with ozone element. This reaction results in formation of carbonyl products such as aliphatic esters, ketones and lactones. As the duration of subjection gradually increases, ether, hydroxyl and terminal vinyl groups are formed as a result of chain scission (Allen *et al*., 2003). These stress speed up the aging process causing cracks which are visible on the surface of the polymer. The formation of cracks evidenced reduction in density and other mechanical properties of the polymer.

## 2.9 Biodegradation of plastics

Biodegradation can be defined as mineralization of polymer by natural occurring biotic organism such as bacteria, fungi and algae. In short, microorganisms consume

nutrients from the polymer generating by products including gases ($CO_2$ and/or $CH_4$), water and biomass. The release of gases either $CO_2$ and/or $CH_4$ dependent on the availability of oxygen. Aerobic degradation takes place with presence of oxygen and releasing $CO_2$ as by product, whereas in the absence of oxygen, anaerobic degradation liberates $CH_4$. However, these conditions are exceptional under some circumstances.

### 2.9.1 Mechanism of biodegradation

Biodegradation is a process of mineralization of polymers into monomers by microorganism. Depolymerization is the first step in biodegradation process as the large size of plastics consists of hydrophobicity and larger fatty acid chain unable to enter the cell membrane of the microorganism (Swift, 1997). The breakdown of polymer into smaller units involves various mechanical forces and biological forces. The mechanical forces mainly involves heating, cooling, freezing, thawing, wetting, drying actions while biological process take place when microbes grow on the surface of the plastics which causes swelling and bursting (Kamal & Huang, 1992; Griffin, 1980). The actions of physical forces, photo-oxidation and abiotic hydrolysis are very crucial in primary biodegradation activity as they promote larger surface area for colonization of microbes on the surface of the polymers (Palmisano *et al*., 1992). The biodegradation process take place in sequential steps begins with bio-deterioration, bio-fragmentation, assimilation and mineralization.

Biodeterioration mainly involves action of enzyme-catalyzed chemical reactions on the surface of polymer (Lenz, 1993; Ranjith *et al.,* 2005) and can be caused by microbiological agents, macrobiological agents, and marine biological agents. Bio-fragmentation refers to catalytic action to break down the polymer into oligomers, dimers or monomers with the help of enzymes or free-radicals secreted by microorganism (Mohan & Srivastava, 2010). Assimilation involves the transport or

movement of the small polymer into the outer membrane of microorganism. Finally, mineralization completes degradation process releasing $CO_2$, $N_2$, $CH_4$, and $H_2O$ as an end products (Mohan & Srivastava, 2010).

In the initial stage of biodeterioration, biofilm forms around the polymer. Biofilms are layer of matrix which comprises polysaccharides, protein, EPS, organic and inorganic particles, water and substances dissolved in the interstitial water (Flemming, 1998). At first, the matrix form coating surrounding the plastics and contaminate the adjacent media. Next, the additives present in the polymer leach out. Followed by, the depolymerization initiated by secretion of enzymes such as intracellular and extracellular depolymerases by the microbes (Goldberg, 1995; Doi, 1990; Gu *et al.,* 2000b). These enzymes break down the long molecular chain of polymer into shorter chain (monomers, dimers or oligomers) by oxidation or hydrolysis allowing the smaller plastics to enter the semi permeable outer membrane of microorganism (Aruna & Shanthi, 2015). Next, the microbes utilize the carbon and energy present in the plastics and convert them into metabolic end products ($H_2O$, $CO_2$, $CH_4$ and biomass) (Frazer, 1994; Hamilton *et al.,* 1995 David *et al.*, 1994; Chandra *et al.,* 1998). Two potential actions are involved in degradation of plastics are direct and indirect actions.

a) Direct action: Degradation of plastics by microorganism by consuming the nutrients in polymers for their growth or
b) Indirect action: The influence of metabolic products of the microorganisms, e.g., discoloration or further deterioration.

Gu *et al.* (2003) reported that the biodegradation mainly involved the change in physical structure or composition of polymers which resulted in a reduction in weight of both synthetic and natural polymers.

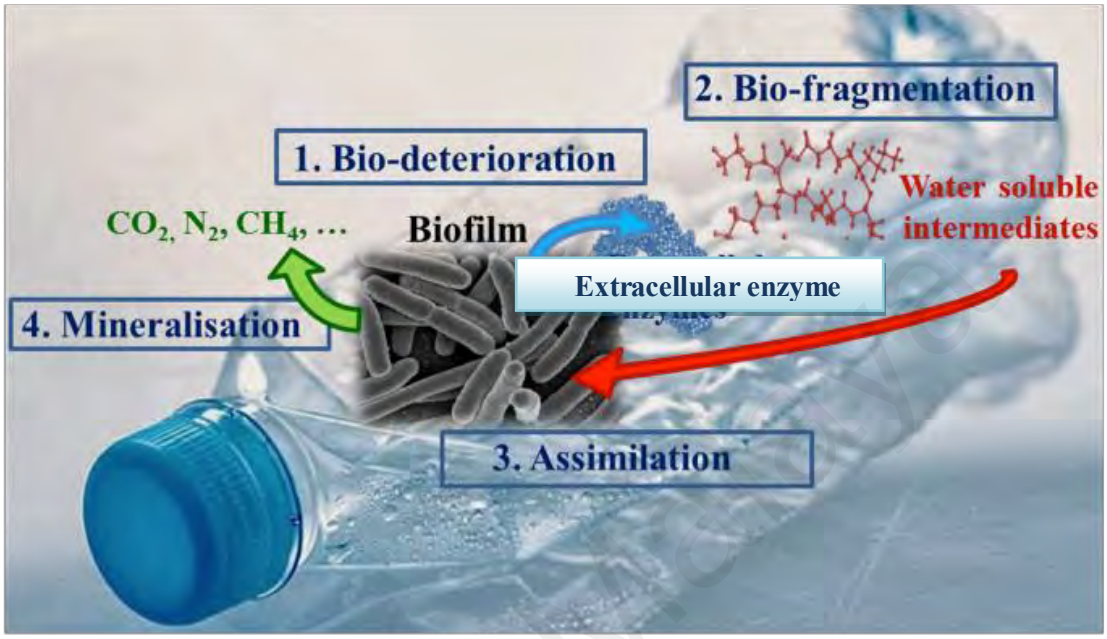



**Figure 2.11**: Mechanism of polymer biodegradation (Lucas *et al.,* 2008)

### 2.9.2 Potential plastics degrading microorganism

Microorganisms depolymerize the polymer into monomer through secretion of both endoenzymes and exoenzymes (Albinas *et al.,* 2003; Huang *et al.,* 1990). The secreted enzymes are proteins with high molecular weight and consist of hydrophilic groups (-COOH, -OH, and $-NH_2$) (Potts, 1978) which attach to the surface of the polymer and demineralize them. The degradation capability of microorganism differs from each other depending on the environment (soil, sea, etc.) as they have their own optimal growth conditions. Several factors such as availability of nutrient, water, temperature and redox potential influenced the growth of microorganism and played key role in biodegradation. Countless studies have documented plastic degrading bacteria and fungi as listed in Table 2.4.

**Table 2.4**: List of plastic degrading bacteria and fungi

| Type of plastics | Microorganisms | Degradation efficiency | References |
|---|---|---|---|
| PVC powder | *Aureo-basidium pullulans* | Weight reduction with 4.9%. | Peciulyte, 2002 |
| Polythene and plastic | *Aspergillus glaucus* | PE and plastics was degraded about 20.80% and 7.26% respectively. | Kathiresan, 2003 |
| Natural and synthetic | *Pseudomonas sp* | 39.7% and 19.6% of weight loss in natural and synthetic plastics respectively. | Nanda *et al*. 2010 |
| Polythene carry bags | *Phanerochaete chrysosporium* and *Pseudomonas aeruginosa* | 50% and 35% respectively. | Aswale, 2010 |
| High density polyethylene films | *Aspergillus oryzae* | *Aspergillus oryzae* degraded HDPE with 72%. | Konduri *et al*. 2010 |
| Polythene | *Pseudomonas, Brevibacillus* and *Rhodococcus* | *Pseudomonas*, *Brevibacillus* and *Rhodococcus* degraded polythene to 40.5%, 37.5%, and 33% respectively in terms of weight loss. | Nanda *et al*. 2010 |

### 2.9.3 Factors affecting biodegradation

Biodegradation process takes place with help of several factors such as properties of polymer, type of organism, and nature of pretreatment (Holmes, 1988; Sand, 2003). The physical parameters of polymer which influenced the biodegradation activity are surface area, hydrophilic and hydrophobic nature of polymer, molecular weight, chemical structure and crystallinity (Artham & Doble, 2008; Gu *et al.,* 2000; Tokiwa *et al.,* 2009). In many cases, molecular weight of plastics ranging from 400-500 da need to go through mechanical action before chemical or photodegradation or biological degradation (Lucas *et al*., 2008). It is very crucial for plastic wastes to undergo mechanical tear prior to biodegradation (Lucas *et al*., 2008). The pre-biodegradation steps allow reduction in molecular weight of plastics since increase in molecular weight of polymer does not favor biodegradation process by microorganism (Aruna & Shanthi, 2015). In another term, degradation activity reduced with increase in molecular weight.

Crystallinity is another vital factor that plays an important role in biodegradation. Increase in crystallinity resist biodegradation rate causing inaccessible to enzymes. Thus, enzymes mainly attack the amorphous domains of a polymer (Iwata & Doi, 1998; Tsuji *et al.,* 2002). Polymers with hydrophobicity group inhibit the degradation performance as they are unable to access into the cell membranes (Aruna & Shanthi, 2015). Hence, fragmentation of the polymers is necessary to allow the diffusion of the monomers into the cellular membrane. In contrast, polymers with hydrophilic nature ease the biodegrading activity with the support of certain humidity.

Environmental conditions also affect the activity of polymer degradation. It is obligatory for microorganism to have adequate water for their growth as the polymer can be biodegraded under certain humidity. Similarly, temperature alters the performance of microorganism since vigorous performance of microbes notable at

optimal conditions. Some microbes require higher temperature to speed up their metabolic activities or vice versa to promote rapid biodegradation (Margesin & Schinner, 2001). Temperature also has great influence on protein and enzymes activity. On the other hand, environment with optimal pH equally important to increase the microorganism metabolism along with biodegradation rate (Margesin & Schinner, 2001).

# CHAPTER 3: METHODOLOGY

This chapter incorporates detailed explanation on the methods adapted, including sampling, screening and post-screening techniques.

## 3.1 Sample collection

The polymer wastes namely PET, LDPE and HDPE were collected from Jeram Sanitary Landfill in an area that was closed for more than one year. The plastic waste samples were collected from a depth of 10 -20 cm as shown in Plate 3.1. The collected samples were immediately sealed in sterile plastic bags and brought to the laboratory for fungal isolation.

**Plate 3.1**: Plastic wastes collection at Jeram Sanitary landfill

### 3.2 Isolation of fungus

One gram of plastic waste was cut into smaller pieces with a pair of sterile scissor prior suspending in a test tube containing nine ml of sterile distilled water. The mixture was shaken well and one ml of the suspension was transferred to another test tube containing nine ml of sterile distilled water. The suspension was serially diluted from $10^{-1}$ to $10^{-5}$ and 0.1 ml of each mixture was transferred to Potato Dextrose Agar (PDA). The plates were incubated at 28°C for 5 days. The fungus colonies grown on the plates were observed and sub-cultured to fresh PDA to obtain pure culture.

### 3.3 Molecular identification

#### 3.3.1 Genomic DNA extraction

Once a pure fungal strain was obtained, 1.5 ml fungi culture grown in PDA broth was transferred into sterile 2 ml RNase-free centrifuge tube. The mixture was subjected for centrifugation at 10 000 rpm for 15 minutes. After carefully discarding the supernatant using a pipette tip, 100 µl of lysis solution contained 50 mmol l−1 sodium phosphate at pH 7.4, 1 mmol l−1 EDTA and 5% glycerol were added to the microcentrifuge tube. The mixture was finally incubated at 85°C in a water bath for 20–30 min. The crude extract contained genomic DNA and was stored at −20°C for further analysis.

#### 3.3.2 DNA amplification and sequencing

The multi-copy ITS-rDNA gene amplification was performed with universal fungal primer pairs ITS4/ ITS5 (White *et al*., 1990). The PCRs were performed in a 25 µl reaction volume containing 16 µl PCR grade water (Sigma), 2.5 µl PCR buffer (10×), 2.5 µl of 10mM dNTPs mix (Sigma-Aldrich), 1 µl of each primer (20 pmol/µl), 1 µl (5 U/µl) of Taq polymerase (SigmaAldrich) along with 20–50 ng of template DNA. PCR

was performed in an Eppendorf Master Cycler (Eppendorf, Hamburg). The amplification program consisted of an initial denaturation step at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing for 30 seconds at 55 °C and extension for 1 min at 72°C. A final extension step at 72°C for 7 min was included at the end of the amplification. All PCR products were electrophoresed, imaged and analyzed in a Gel Documentation System (Syngene Inc. Cambridge). For identification of the strains, the obtained nucleotide sequences were compared to those already stored in the National Center for Biotechnology and Information (NCBI) sequence database, using a research tool, BLAST.

### 3.4 Fungal growth curve

One loopful of pure culture was transferred from solid agar media to fresh 100 ml Potato Dextrose Broth (PDB) and incubated at $28^0$C for 6 days with rotation 120 rpm. Every 24 hours, the growth of the fungi cell was analyzed for optical density and dry cell weight. The 100ml of culture media was filtered using pre-weighted Whatman No. 1 filter paper to obtain filtrate and biomass. The filtrate was subjected to spectrophotometer at 600nm to obtain the optical density which represents the logarithm of the number of microorganisms. Meanwhile, the biomass obtained was dried overnight at 60 $^0$C and the results were recorded by deducting the initial weight of the filter paper as shown in the formula below:

Dry weight = [weight of filter paper + biomass] – [weight of filter paper]

### 3.5 Screening for potential fungus

Two methods were adapted to identify the ability of fungi isolates to degrade the plastic films. Radial diameter method was used to evaluate the fungus degradation capability in solid media while shake flask method was used to analyse the degradation

potential in broth media. To compare the degradation capability of the fungi isolates, two liquid media (BOD and Bushnell Haas) were used to determine the most desirable broth media by the fungus to perform their degradation activity.

### 3.5.1 Radial diameter method

PET, LDPE and HDPE powder with 0.1% (w/v) was added in Bushnell Haas agar media [1 g/L $KH_2PO_4$, 1 g/L $K_2HPO_4$, 1 g/L $NH_4CI$, 0.02 g/L $CaCI_2$, 0.05 g/L $FeCI_2$, 0.2 g/L $MgSO_4.7H_2O$ and 20 g/L agar] and homogenized for 15 minute. The mixture was then sterilized by autoclaving at 121°C for 15 minutes. The pure culture of isolates were prepared by transferring one loopful of pure fungi mycelium from solid agar media into 100 ml Potato Dextrose Broth (PDB) and incubated at $28^0$ C for 6 days with rotation 120 rpm. The potential fungus degradation was observed by inoculating the plates containing PET, LDPE and HDPE individually as carbon source with 0.1 ml of the isolated culture. The plates were incubated at $28^0$ C for 21 days and the radial diameter of fungi colonies were recorded for further study (Manna *et al.,* 1999).

### 3.5.2 Biodegradation of PET, LDPE and HDPE films using shake flask method

The PET bottles, LDPE and HDPE bags were cut into 2cm X 2cm strips and sterilized under UV light for one hour. The pre-weighted six strips were then transferred to fresh 100 ml Biochemical Oxygen Demand (BOD) media consisted phosphate buffer (0.85 g/L KH2PO4, 2.175 g/L K2HPO4, 3.34 g/L Na2HPO4.7H2O and 0.17 g/L NH4CI), calcium chloride solution (2.75 g/L CaCI2), ferric chloride solution (0.025 g/L FeCI2) and magnesium sulfate solution (2.25 g/L MgSO4.7H2O). Basically, the BOD water contains necessary nutrients required for fungi to grow. Since BOD was used as culture media to promote the fungus growth, thus it was referred as BOD media in this study.

To identify potential degrading fungus, the isolates pure culture were prepared by transferring one loopful of pure fungi mycelium from solid agar media to fresh 100 ml BOD media and incubated at $28^0$C for 6 days with rotation 120 rpm. The individual pure culture was inoculated to BOD media containing mixed plastics (PET, LDPE and HDPE films) as carbon source. The triplicate flasks containing BOD media with the individual isolate and mixed plastics were incubated for 30 days at $28^0$C with agitation 120 rpm. Control set was maintained without inoculation of fungus and the whole set was prepared in triplicate. After 30 days of incubation, the plastic samples were removed from the culture and were washed with 70% ethanol and distilled water to remove the biofilms. Subsequently, the samples were dried overnight in oven at $70^0$C and the percentage loss in weight was calculated. The formula used to calculate the percentage loss in weight is as shown below:

$$\text{Percentage weight loss of films (\%)} = \frac{[\text{Initial weight film- Final weight}] \text{ X } 100}{\text{Initial weight film}}$$

Same procedure was repeated using Bushnell Haas broth media which contain constituents 1 g/L $KH_2PO_4$, 1 g/L $K_2HPO_4$, 1 g/L $NH_4CI$, 0.02 g/L $CaCI_2$, 0.05 g/L $FeCI_2$ and 0.2 g/L $MgSO_4.7H_2O$. At the end of the experiment, the potential fungi and suitable broth media for degradation performance was determined.

### 3.6 Optimization for plastic degradation using free fungus cell

To optimize the plastics degradation by fungus, the concentration parameter was selected to evaluate the optimal concentration suitable to perform their activity. The selection of inoculum concentrations for plastic degradation was based on previous reported studies. Phatake *et al*. (2015) reported that the optimum concentration of inoculum of *Aspergillus spp* effective for decolorizing Bromocresol Purple is 1.0%

which showed highest degradation (65.85%). Another study by Kumar Praveen and Bhat (2012) found ideal volume of inoculum for degradation of azo dye-Red 3BN by *A. niger* was 10%. Similar results were reported by Liu *et al.* (2011), in which the highest weight loss of petroleum hydrocarbons was achieved by 10% (v/v) inoculum concentration and further increase in inoculum concentration beyond 10% (v/v) resulted in decrease in biodegradation. Thus, three different wet mycelium concentrations (1%, 5% and 10%) were used in this experiment. The culture was prepared similar to section 3.5.2 and at the end of 6 days of incubation period, the wet mycelium was pre-weighted using weighing balance under sterile condition. 1% inoculum concentration was obtained by weighing 1g of wet mycelium and transferred into 99 ml fresh BOD broth. While 5% and 10% inoculum concentrations were prepared by weighing 5g and 10g of wet mycelium and transferred into 95 ml and 90 ml fresh BOD broth respectively. The purpose of this experiment is to analyse the suitable concentration of the selected fungi to degrade three different plastics (PET, LDPE and HDPE) and to study the type of plastic which undergo rapid biodegradation. Similar to Section 3.5.2, 2cm X 2cm strips of pre-disinfected PET, LDPE and HDPE plastic were transferred to selected broth media which was preferred by fungi to degrade the plastic films in the screening experiment. The set up was conducted in triplicates in shake flasks and incubated for 30 days at $28^0$C with agitation at 120 rpm. Monitoring activities were conducted for every six days whereby 6 ml of the cultured broth was removed from shake flasks for analysis as below:

### 3.6.1 Microbial count

The microbial count measurement was employed to monitor growth of fungi using different plastics as carbon source in BOD media during the optimization experiment. One ml of the broth culture incubated during the optimization experiment was

proceeded with serial dilution to obtain dilution factor of $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$. The PDA solution contained 200 g/L potato infusion, 20 g/L dextrose and 15 g/L agar was diluted in distilled water before autoclaving. The following diluted suspensions were then plated on PDA solid media using pour plate method by way of 0.1 ml of the suspension was place on the petri dish and PDA solution was poured approximately 20 ml to 30 ml. The mixtures were swirled slowly and allowed to solidify at room temperature. The plates were then incubated at 28°C and the fungal colonies were counted on the fifth day of incubation period. The total microbial count was conducted using the formula below:

$$\text{C.F.U. /g} = \frac{\text{Number of colonies X dilution factor}}{\text{Inoculum size (ml)}}$$

### 3.6.2 Physical changes

Multi probe meter (YSI Professional Plus, USA) was used to monitor changes in pH for every six days while the results for dissolved oxygen were recorded on the final day of incubation. The multi probe was disinfected in 95% alcohol prior to use. The physical parameters monitoring were performed under sterilize condition to avoid contamination.

### 3.6.3 Carbon dioxide evolved

The fungi enzymatically breakdown polymer chains of different sizes, known as mineralization generates low mass fractions, $CO_2$ and/or $H_2O$ with presence of oxygen. According to Webb *et al*. (2000), the release of $CO_2$ during co-cultivation of fungi and plastics in broth is considered a reliable indicator of biodegradation. Thus, in this study, the level of $CO_2$ was calculated using volumetric method.

After 30 days of incubation, the 100 ml of culture media was filtered using pre-weighted Whatman No. 1 filter paper to obtain the filtrate. To determine the dissolved carbon dioxide in the broth culture, two drops of phenolphthalein were added into the filtrate until the mixture changed into pink color. The mixture was then titrated with NaOH solution until the solution turn into yellow color. The average NaOH used was recorded and the amount of dissolved carbon dioxide was calculated using the formula below:

$$\text{Dissolved Carbon dioxide} = \frac{V_2 \text{ X N X } 50 \text{ X } 100}{V_1}$$

Where:

$V_1$ = Volume of water sample in ml,

$V_2$ = Volume of NaOH in ml and

N = Normality of the NaOH solution.

### 3.6.4 Plastic weight reduction

Similar to Section 3.5.2, at the end of the incubation period, the plastic films were isolated from the broth culture and then soaked in 70% ethanol for one hour to remove the biofilm. Next the plastics were rinsed in distilled water and dried in oven overnight at 70$^{o}$C. The plastic weight reduction was calculated accordingly.

## 3.7 FTIR analysis

The structural analysis is the important parameter to identify the structural changes which appear during degradation responsible for weight loss. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) at the frequency range of 4000-450 cm$^{-1}$ was used to characterize and study the bonding mechanism of the

degraded plastics. The ATR diamond crystal was cleaned with 70% 2-propanol. The degraded plastic (PET, LDPE and HDPE) samples were placed in a transmission cell fitted to a Nicolet 510 FTIR spectrophotometer (DTGS detector) with air purge. The spectra were made up a 50 scans with a resolution of 4 $cm^{-1}$. Each sample was compressed against the diamond with a force of at least 80N to ensure good contact between and sample and ATR crystal. Absorption bands identified using a peak height algorithm within the Perkin Elmer software were recorded. The FTIR spectra was superimposed against the control samples of the PET, LDPE and HDPE sheets incubated without inoculum.

### 3.8 Statistical Analysis

All plastic degradation experiments were performed in triplicate and the standard error indicated by an error bar. All data obtained were subjected to Statistical Analysis in the Excel with p-value =0.05.

# CHAPTER 4: RESULTS AND DISCUSSION

## 4.1 Isolation of potential plastic-degrading fungi

A total of eight fungal strains were isolated from plastic wastes dumped in Jeram Sanitary Landfill. The strains were successfully isolated through serial dilution of plastic waste stock. The isolated strains were inoculated onto PDA plates for seven days. At the end of the seven incubation days at 28℃, the plates were fully covered with fungal mycelium and fungal spores. From the physical observation, fungi formed a network of mycelium, covered the agar plates and also penetrated the medium, forming branches of mycelium. According to Mishra and Kumar (2007), fungi have the ability to grow away from the initial point, such that hyphae at the edge of a colony are constantly exposed to fresh nutrients. Plate 4.1 represents the luxuriant growth of microbes on Potato Dextrose Agar (PDA) media and their microscopic morphology.

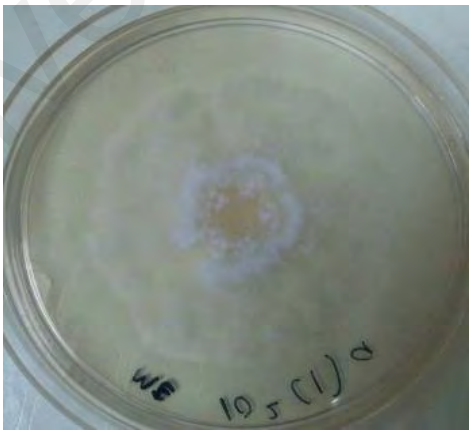
| Codes of Fungi | Fungi colony | Microscopic Morphology (40X) |
|---|---|---|
| FI 1 |  |  |

**Plate 4.1**: Eight types of isolated fungi grown on PDA and their microscopic morphology (40X)

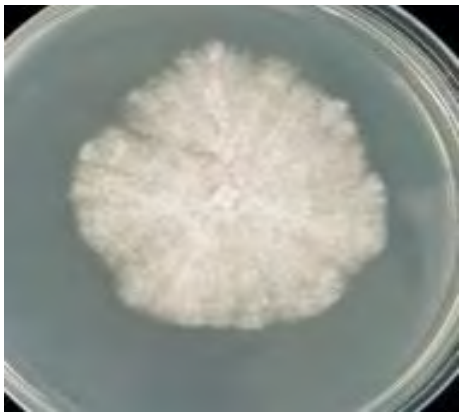
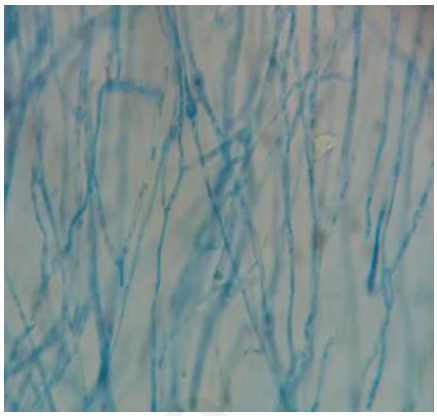
| Codes of Fungi | Fungi colony | Microscopic Morphology (40X) |
|---|---|---|
| FI 2 |  |  |
| FI 3 |  |  |
| FI 4 |  |  |
| FI 5 |  |  |

**Plate 4.1,** continued.

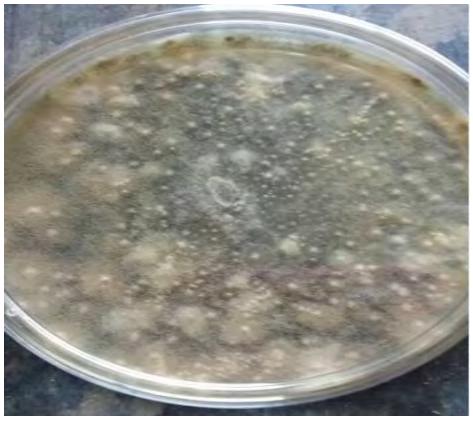
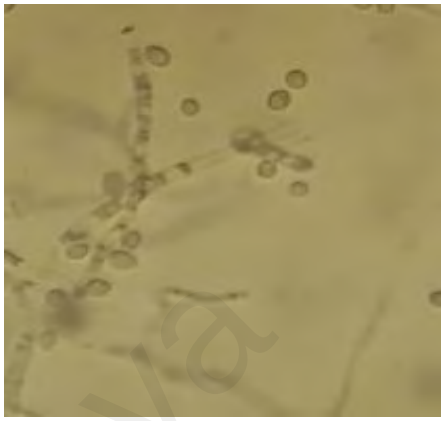
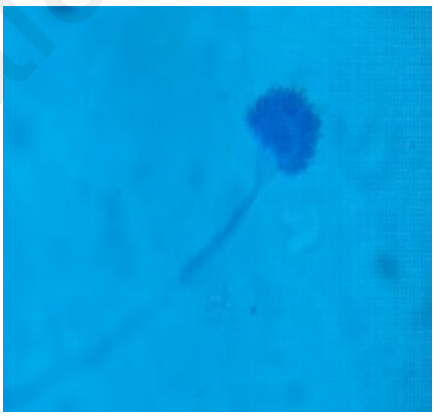
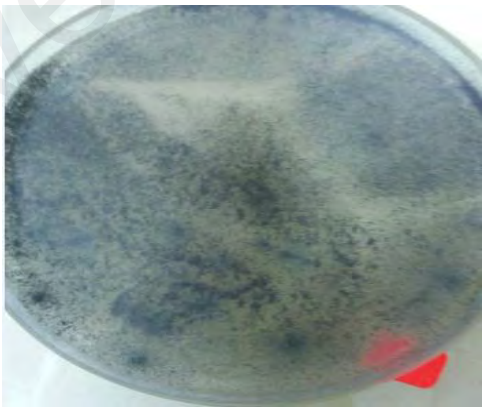
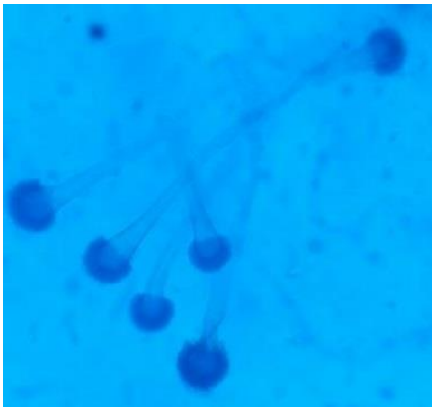
| Codes of Fungi | Fungi colony | Microscopic Morphology (40X) |
|---|---|---|
| FI 6 |  |  |
| FI 7 |  |  |
| FI 8 |  |  |

**Plate 4.1,** continued.

## 4.2 Growth curve of isolated fungal strains

Figure 4.1 displays the changes of dry weight of mycelium over a period of time.

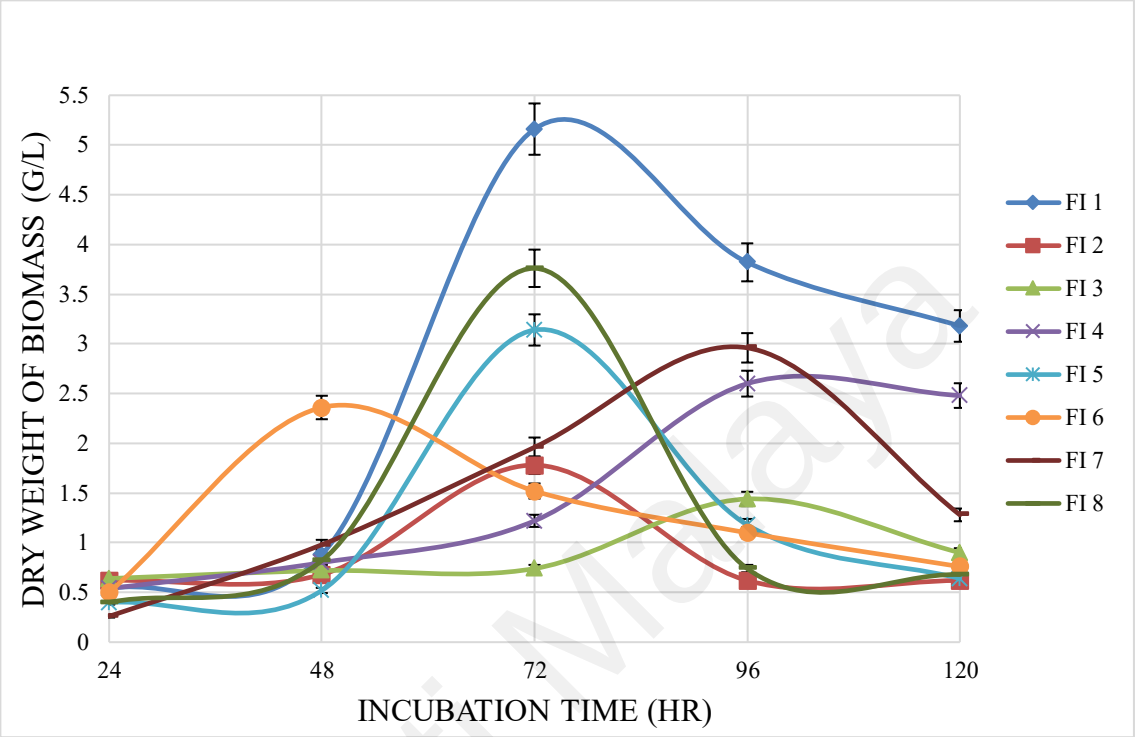



**Figure 4.1**: Growth curve of fungi isolates

The patterns of the growth were different depending on the type of fungi species tested. For isolates FI 1, FI 2, FI 5 and FI 8, the growth remained within the lag phase which represents the earliest and temporary period of non-replication seen in fungi was 24 h to 48 h. It may be possible due to the adaptation of fungal strains to the new nutrient medium as the fungi strains were transferred from solid PDA to fresh PDB. The biomass production was the highest at 72 hours which was characterized by significant growth that indicates increase in microbial cell load. The maximum mycelial mass obtained by FI 1, FI 2, FI 5 and FI 8 were average of triplicate of 5.16 ± 0.3 g/L, 1.78 ± 0.1 g/L, 3.14 ± 0.1 g/L and 3.76 ± 0.2 g/L, respectively. Kaur and Aggarwal (2015) reported maximum dry weight of *A. macrospora* (0.52 g/L) was recovered after five days of incubation. Another study by Singh and Chauhan (2013) recorded maximum

mycelia mass of *Penicillium chrysogenum* (0.39 3± 0.15 g) at 30°C. Next, an accentuated fall in the mycelial mass until 120 h was observed, possibly because of lack of nutrients, accumulation of acid and other metabolic waste products that lead to the death phase. Unlike for FI 6 isolate, the exponential phase continued to a maximum mass at 48h (2.36 ± 0.15 g/L). After that, the growth decreased in mass of the mycelium until 120 h (0.46 ± 0.15 g/L). For strains FI 3, FI 4 and FI 7, the growth curve showed the longest lag phase until 72 h, where the highest mycelia growth were (1.44 ± 0.12 g/L, 2.6 ± 0.08 g/L and 2.96 ± 0.25 g/L correspondingly), observed at 96 h. Following that, there was a decrease in mass of the mycelium. This is agreeable to findings by Melgal *et al.* (2013), *Rhizopus sp.* showed maximum mycelia mass at 96h (3.6 g/L) and a decrease in mass of the mycelium until 144h.

### 4.3 Determining the plastic degrading capability of the isolated fungi strains

In this section, the plastic films mainly PET, LDPE and HDPE were treated with the fungal isolates in the solid media (Bushnell Haas agar) and the potential of strain to use plastic films as sole carbon were recorded. Table 4.1 displays the radial diameter of the isolated fungi in the Bushnell Haas agar medium containing plastic films.

**Table 4.1**: Radial diameter of fungi colonies in the Bushnell Haas agar medium contained plastic films as sole carbon source after 21 days of incubation at 28°C

| | Radial diameter (cm) | | |
|---|---|---|---|
| | Type of plastic films | | |
| Codes of fungi | PET | LDPE | HDPE |
| Control (C) | No growth | No growth | No growth |
| FI 1 | 8.3 ± 0.07 | 8.3 ± 0.14 | 8.2 ±0.28 |
| FI 2 | 8.0 ± 0.28 | 8.3 ± 0.14 | 8.3 ± 0.14 |
| FI 3 | 2.2 ±0.14 | 2.1 ± 0.35 | 1.9 ± 0.07 |
| FI 4 | 8.2 ± 0.07 | 8.3± 0.07 | 8.0 ± 0.07 |
| FI 5 | 8.4 ± 0.07 | 8.3 ± 0.0 | 8.4 ± 0.07 |
| FI 6 | 8.3 ± 0.07 | 8.3 ± 0.07 | 8.3 ± 0.0 |
| FI 7 | 8.4 ± 0.0 | 8.5 ± 0.07 | 8.5 ± 0.07 |
| FI 8 | 8.4 ± 0.21 | 8.3 ± 0.07 | 8.1± 0.35 |

Results are the means of 3 triplicates, represented as: mean ± standard deviation (M ± SD)

Most of the isolated strains presented radial diameters were between 8.0 cm to 8.5 cm. Though all fungal strains were able to grow, FI 7 showed the maximum radial diameter among the tested species. The radial diameter of FI 7 strain supplemented with PET, LDPE and HDPE films was 8.4 cm, 8.5± 0.07 cm and 8.5 ± 0.07 cm respectively. FI 7 strain showed maximum radial diameter supplied with polyethylene (8.5 ± 0.07) than PET films due to the high molecular weight of polyethylene which increases the degradation activities by the fungi (Sabrina *et al*., 2018). Comparing with other fungal strains, FI 3 demonstrated lower degradation with mean radial diameter for PET, LDPE and HDPE at 2.2 ±0.14 cm, 2.1 ± 0.35 cm, and 1.9 ± 0.07cm, correspondingly. Comparing the three results obtained from PET, LDPE and HDPE films, it is found that the FI 3 strain showed some potential in degrading PET compared with polyethylene films. One of the reasons might be that, the distinction within the ability of beings to biodegrade plastics depends on the active catalysts made by a specific microorganism. The distinction in degradation of plastics by the isolates is supported by Bhardwaj *et al*.

(2012) where microorganism possess different biological characteristics, and thus the degradation varies from one microbe to another. Overall, FI 7 strain had the best degrading activity as the radial diameters were higher than the formed diameter by the other isolated strains. This study revealed that all isolated organisms have the capacity to utilize plastics as their sole carbon source, and depolymerize the polymer, which is the first step of biodegradation. Thus, these fungal strains were selected for the liquid shaking culture methods.

## 4.4 Selection of potential fungal plastics degrading strain using shake flask method

These experiments were conducted to test the ability of the isolated fungi to cause weight loss of plastics in liquid shake flask. The biodegradation of plastic films, incubated with the isolated individual fungal strains were observed in BOD and Bushnell Haas broth media as shown in Figure 4.2. The results demonstrated that all isolated fungi were capable of biodegrading the plastics but at different rates. There was no weight reduction in the control. A study conducted by Sonil *et al*. (2010) revealed that microbial degradation of plastics is caused by protein activities resulting in cleavage of the compound into micromolecules.

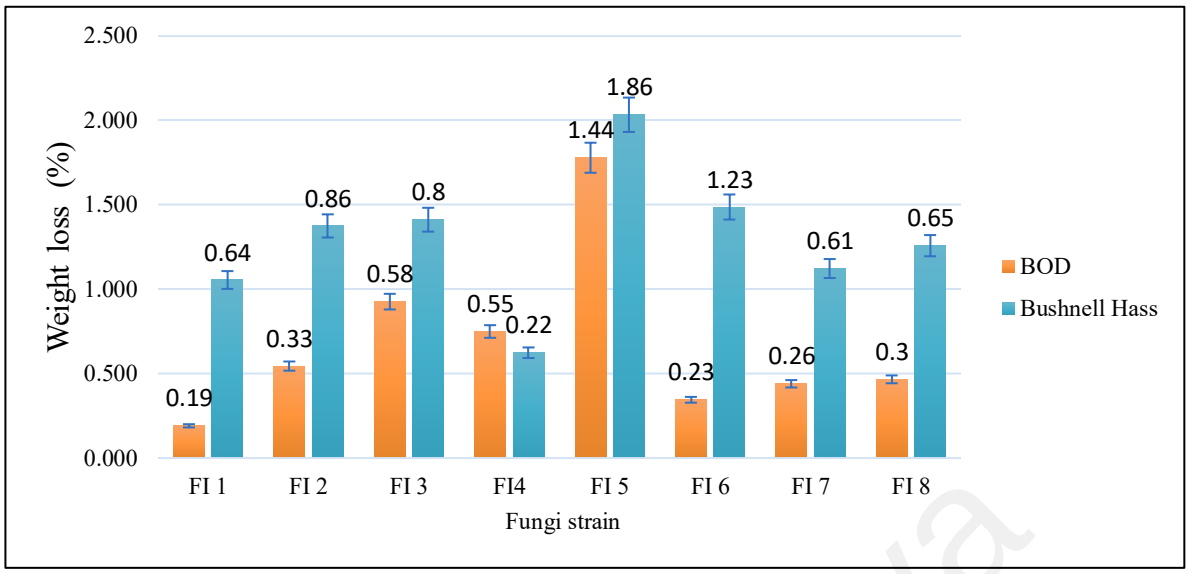



**Figure 4.2**: Biodegradation of mixed plastics (PET, LDPE and HDPE) by 1% of fungus inoculum

Among the fungi isolates tested in BOD media, FI 5 strain yielded highest percentage of mass loss of plastics (1.76%) whereas FI 1 species had the lowest weight loss of plastics. The degradation activity differed between FI 1 and FI 5 because of the presents of different lignocellulolytic enzymes in the fungi that are produced during fungal growth on the plastics sheets. While for the biodegradation activity by the fungi isolates in Bushnell Haas media, FI 5 recorded the highest weight loss of plastics (2.03%), and the least capable of biodegradation was attributed by fungi FI 4. Slow degradation of plastic films by FI 4 and FI 1, in Bushnell Haas and BOD media respectively indicates that the organisms were not well adapted to the available carbon source. The plastic weight loss recorded by FI 1 and FI 4 could probably mean that the microbes were less hydrophobic, and therefore, may not have been able to produce significant biofilm on plastics, and were therefore, less efficient in degrading plastics (Orr *et al.,* 2004). Different microorganisms were determined to own totally different abilities in degrading plastics (Bhardwaj *et al*., 2012). This was evidenced by their rate of degrading plastics as represented by the weight loss.

Overall, FI 5 displayed the greatest biodegradation ability with the plastic films compared with other isolates in both Bushnell Haas and BOD media. This indicates that plastic films can be degraded by microorganism if provided with suitable medium (Asmita *et al*., 2015). Statistical analysis showed that fungi degraded plastics was significantly higher for FI 5 strain ($p< 0.05$) in both the media. Hence, this strain was subjected for identification method. This study is the first report on biodegradation of plastics using BOD as a media. Numerous studies have investigated the biodegradation of plastics using Bushnell Haas media. Syamimi and Rosli (2018), reported PE was reduced by 27.9%, PET by 24%, PP by 19.5% and PS by 15% by potential degrading bacteria microbial consortium inoculated in Bushnell Haas broth. Kotwal Niloufer and Vaidya Rajnish (2017) in their study, recorded maximum degradation of LDPE by isolate PE-8 (*Pseudomonas stutzeri strain AT11*), that is 2% reduction in LDPE weight in 4 weeks of incubation in Bushnell Haas media. Fatime *et al.* (2001) also reported, *A.fumigatus* produced the greatest reduction in pH and surface tension and was able to degrade hydrocarbons in Bushnell Haas media with diesel oil more efficiently than other microorganisms tested (*Hormoconis resinae* and *Candida silvicola*).

Comparing the results from the Bushnell Haas agar media and broth media, strain FI 5 have greater potential in degrading the plastic films in the broth media whereas FI 7 showed maximum radial growth in the agar media. In the agar test, the radial diameter indicates the ability of the fungi to catabolize the plastics and use them as sole carbon source to promote their growth. On the contrary, the weight reduction method was used in the broth test to analyse the ability the fungi to use the plastics as carbon source. No weight reduction of plastics was seen in the control (un-inoculated) flask. The weight reduction of plastics after incubation could be an aftereffect of microbial action and demonstrated the rate decline in weight as well as the loss of specific properties, thus alluding to the physical breakdown and degradation of plastics by the microorganisms

(Board, 2006). On the whole, the performance of the isolated fungus to degrade the plastics films were slightly lower in BOD media (1.76%) compared with Bushnell Haas media (2.03%). Yet, consideration on the relative price of both media needs to be investigated to determine the cost effectiveness. Table 4.2 displays the chemical constituents present in the nutrient media and their respective costs.

**Table 4.2**: Price of chemical constituents for BOD and Bushnell Haas media

| Chemical formula | Amount of chemicals required per liter | |
|---|---|---|
| | BOD (gm/lit) | Bushnell Haas (gm/lit) |
| Magnesium sulphate | 0.2250 | 0.2 |
| Calcium Chloride | 0.2750 | 0.02 |
| Monopotassium phosphate | 0.0085 | 1 |
| Dipotassium phosphate | 0.2175 | 1 |
| Ammonium chloride | 0.0170 | 1 |
| Sodium phosphate dibasic heptahydrate | 0.3340 | none |
| Ferric chloride | 0.0025 | 0.05 |
| **Total Price in the market** | **RM 169.5 / 3 kg** | **RM 208.7 / 500 g** |
| **Price per gram** | **RM 0.06 / g** | **RM 0.42 / g** |

Both the media contained similar chemical constituents except for the absence of sodium phosphate dibasic heptahydrate in Bushnell Haas media. Thus, it was determine that BOD media incurred much cheaper cost at RM 0.06/g, compared to Bushnell Haas which costs RM 0.42/g. Thus, BOD media was used in the consecutive study to evaluate the optimal concentrations of FI 5 strain required to perform biodegradation activities.

## 4.5 Identification of potential plastic-degrading fungi FI 5 strain

The sequences of the partial 5.8S rDNA gene fragments cloned from the isolated strain were compared with similar data available at the GenBank by an online alignment search. After blasting the sequences with the NCBI database, the names of the closest species match were listed. The phylogenetic tree based on a comparison of the sequences is shown in Figure 4.3. The results indicated that the partial 5.8S rDNA sequence of FI 5 were 100% identical to that of *Aspergillus Fumigatus.* A good identification of fungal names is given when the nucleotide identity was equal or above 97% (Garnica *et al*., 2016).

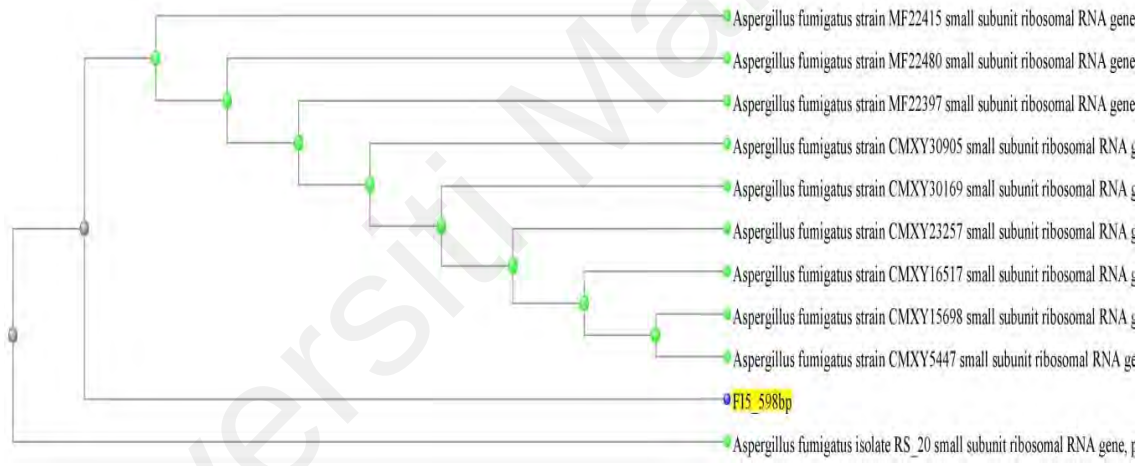



**Figure 4.3**: Phylogenetic dendrogram of the relationship between the 5.8S rDNA gene sequences retrieved from GenBank and the 5.8S rDNA of the best degradative *A. fumigatus* strain

Amplification of 5.8S rDNA, and flaking ITS1 and ITS4 for fungi produced PCR products with the size ranging between 300 and 800 bp (Figure 4.4). The DNA was of sufficient quality and quantity for DNA sequencing.

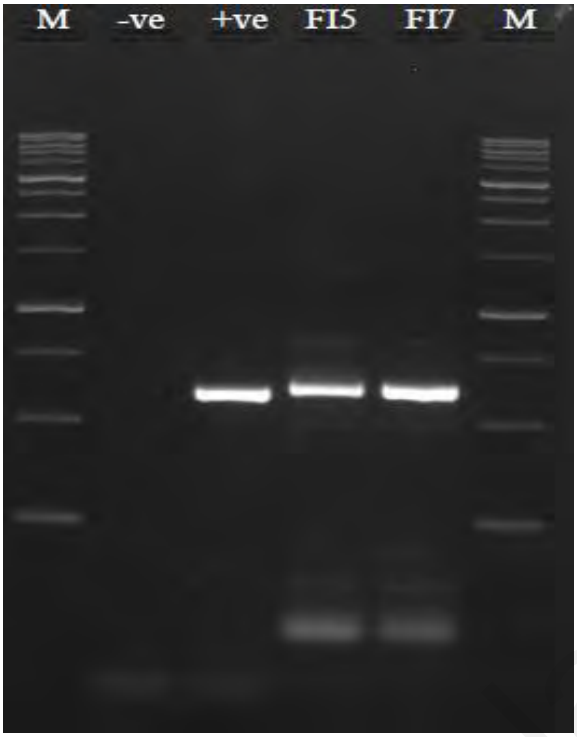



**Figure 4.4**: Agarose gel electrophoresis bands of PCR products from the microbial isolates

Following sequencing of the 5.8S rDNA, the fungi isolate which was capable of degrading the plastics was *A.fumigatus*. Various researches have also reported on the abilities of different genera/species of fungi in degrading different types of plastics. Previous studies by Zahra *et al.* (2010), observed high affinity in colonizing and rapid microbial degradation of plastic films by *Acremonium flavum, Candida rugosa, Arthrographis kalrae, Aspergillus sp, Lichtheimia sp, Aspergillus fumigatus, Emericella nidulans, Aspergillus terreus* and *Fusarium solanifrom*. Besides, Raaman *et al*. (2012) reported biodegradation of plastics by *Aspergillus spp* including *Aspergillus terreus*, isolated from polythene polluted sites around Chennai in India. Other studies have observed *Eupenicillium sp*, *Talaromyes sp,* and *Penicillium simplicissimum* to have the ability of degrading polyethylene (Sowmya *et al*., 2014).

## 4.6 Optimum growth parameters for isolates

Lodhi *et al*. (2011) stated that certain ranges of optimal conditions required by microbes may vary for different microorganisms as microbial activities are controlled by enzymes which work optimally at different conditions. Thus, in this study, concentrations of *A. fumigatus* strain were investigated to identify the optimal concentration required to yield maximum weight loss of plastics (PET, LDPE and HDPE).

### 4.6.1 Effect of inoculum concentrations on the biodegradation of PET plastics

#### i) Microbial count of *A. fumigatus* strain during biodegradation studies

The growth curve of fungal isolate with the presence of PET plastics as sole carbon was studied using the colony forming unit (CFU) count. The growth curve exhibited by *A. fumigatus* was evaluated and the results are presented in Figure 4.5.

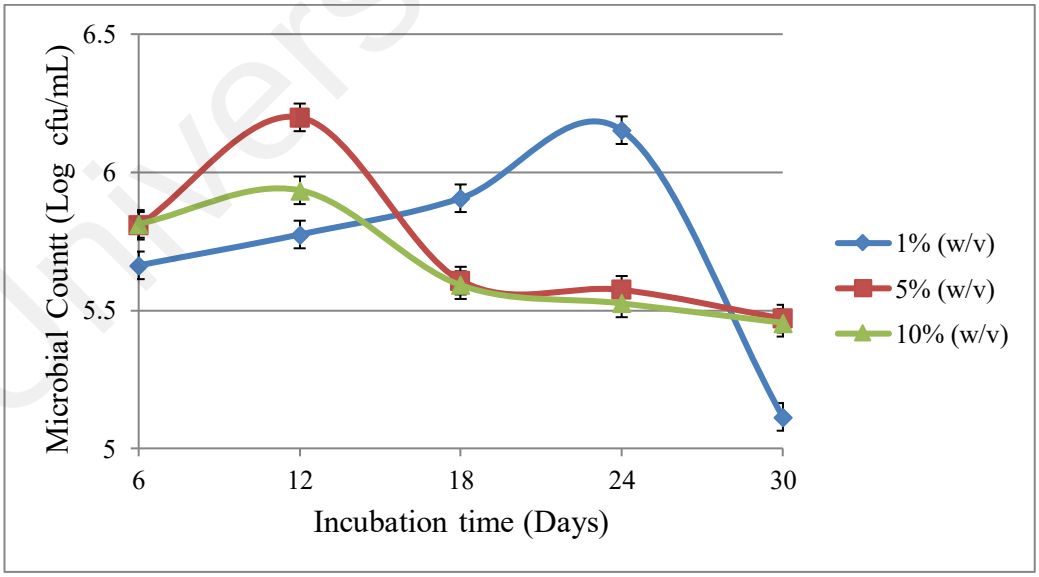



**Figure 4.5**: Changes in microbial count of different concentrations of *A. fumigatus* strain during PET biodegradation studies

The microbial growth test was conducted to study the growth pattern of the microorganism during the biodegradation studies. Through the growth pattern, the favorable period for the interaction between the fungus cell membrane and the plastics films can be observed. 1% (w/v) of *A. fumigatus* showed an exponential growth up until the 24$^{th}$ day on exposure to the PET plastics. The growth of the fungi accelerated towards a positive growth pattern from 5.66 log cfu/mL on the 6$^{th}$ day to 6.15 log cfu/mL on the 24th day, which was the highest of the isolates. The acceleration of the 1% (w/v) of the strain showed that the interaction between the isolated strain and PET plastics was favorable for rapid metabolism due to plastics utilization as the carbon and energy source. Increase in inoculum has been reported to have significant effect on degradation (Dada *et al.*, 2012). Kauselya *et al.* (2015) also reported increase in degradation of Pentachlorophenol (PCP) and benzene with increasing inoculum concentration, respectively. Afterward, the fungal cell counts showed a sharp decline in the growth up to the last 30 days of the experiment to 5.11 log cfu/mL. Lower microbial count of *A. fumigatus* than the initial reading may be possibly due to the lysis of cells, nutrient depletion or presence of inhibitory products in the culture media. Unlike 1% (w/v) of *A. fumigatus,* shorter positive growth pattern was observed for both the 5% (w/v) and 10% (w/v) of the isolated strain. The highest fungal cell counts recorded on the 12th day for both 5% (w/v) and 10% (w/v) of *A. fumigatus* were 6.19 log cfu/mL and 5.93 log cfu/mL, respectively. Since other carbon source is absent, growth by fungus might be indicating the utilization of PET films as the sole source of carbon via degradation of the polymer. The growth of the fungus decelerated for both 5% (w/v) (5.46 log cfu/mL) and 10% (w/v) (5.45 log cfu/mL) of *A. fumigatus.* Reduction in the number of cells might result from the inability of the microbe to completely adapt to the media or the presence of degradation metabolites that might have rendered the culture media unfavorable for growth and multiplication. This finding is supported by the study

of Vasquez-Murrieta *et al*. (2016) that when a population is introduced into a foreign environment, the population count tends to decrease with time due to biotic or abiotic factors. Therefore, 1% (w/v) strain exhibited a higher metabolic potential on the PET films than other concentrations based on the observed weight loss that was higher than that of 5% (w/v) and 10% (w/v). There was significant difference in the growth response of the microbes ($p < 0.05$).

### ii) Changes in pH of PET plastics-infused BOD media during biodegradation studies

According to Xu *et al.* (2011), pH factor plays a crucial role on microbial growth and the rate of degradation containing PET films as sole carbon. Figure 4.6 displays the changes in the pH of culture media before, during and after biodegradation studies with *A. fumigatus* strain.

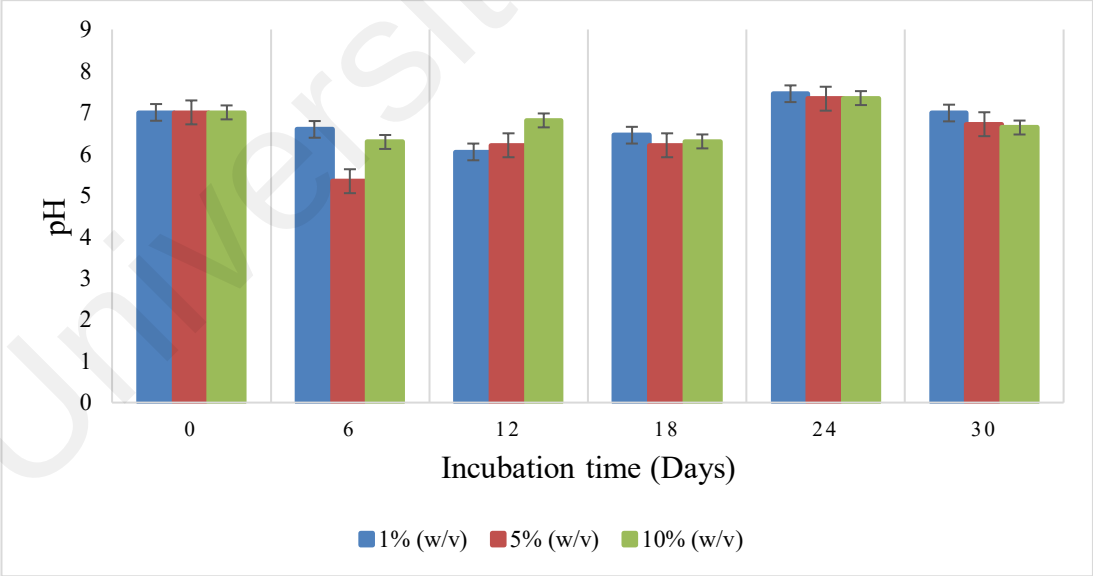



**Figure 4.6**: Changes in pH of different concentrations of *A. fumigatus* strain during PET biodegradation studies

As stated by Das and Kumar (2014), the pH of the media changes as the polymer degraded due to the presence of different monomer products. For all fungal broth culture, initial pH was 7.0. But after the incubation period, the pH value decline slightly

in 1% (w/V) of *A. fumigatus* strain until 12$^{th}$ day (pH 6.0) followed by an increased in pH on the 24$^{th}$ day (pH 7.5). The increase showed that the period was favorable as optimum growth of *A. fumigatus* (6.15 log cfu/mL) was achieved which consequently allowed rapid metabolism to take place. This indicates the possible increase in the number of hydroxyl radicals during PET films degradation which caused pH to turn alkaline (Oranusi & Ogugbue, 2005). Statistical analysis showed that the optimum growth was at pH 7.5, and it's significant at $p<0.05$. The pH variation was slightly different for 5% (w/v) and 10% (w/v) of *A. fumigatus* strain, as the pH values were lower than the initial with pH 5.3 and pH 6.3, respectively. During this period of time, both the 5% (w/v) and 10% (w/v) of *A. fumigatus* strain showed maximum growth with 6.19 log cfu/mL and 5.93 log cfu/mL correspondingly. The decrease in pH values observed could be attributed to the production of organics acids during plastic degradation. Ghorpade *et al.* (2001), also reported similar decrease in pH and recorded that lactic acid generation occur during PLA biodegradation which result in pH decrease. Thus, this implies that these pH values were the optimum pH for the growth of the isolates. Afterward, significant increase in the pH of the aqueous media towards alkalinity was observed for both the culture. For 5% (w/v) isolates, the pH increased continuously up to the 24th day (pH 7.3), followed by changes in pH values towards acidic condition. While, for 10% (w/v) of *A. fumigatus* strain, the pH of the aqueous media wasn't consistent as the value was increasing and declining simultaneously throughout the biodegradation studies. Increase in pH towards alkaline range can be attributed to the production and accumulation of basic aromatic compounds and other metabolites in the media. Rate of PET hydrolysis has been reported to be higher under acidic or basic conditions, and results in the formation of alcohol functional groups and carboxylic end groups (Gewert *et al*., 2015).

### iii) Changes in Dissolved Oxygen (DO) and Carbon Dioxide ($CO_2$) during biodegradation studies

Figure 4.7 displays the oxygen content and $CO_2$ evolution during the degradation of PET films by the *A. fumigatus* strain.

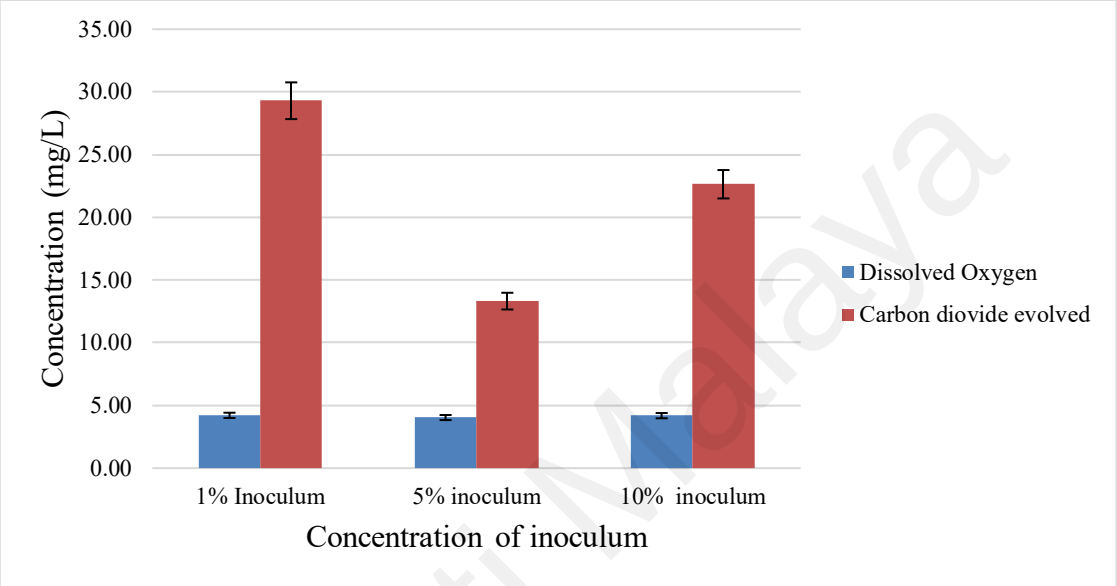



**Figure 4.7**: Concentration of oxygen content and dissolved carbon dioxide of different concentrations of *A. fumigatus* strain during PET biodegradation studies

After 4 weeks of biodegradation, the $O_2$ contents for 1% (w/v), 5% (w/v), and 10% (w/v) of *A. fumigatus* strain was 4.21 mg/L of $O_2$, 4.03 mg/L of $O_2$, and 4.18 mg/L of $O_2$, respectively. The oxygen content after 30 days incubation for all the three concentrations did not differ markedly from each other. However, 1% (w/v) of isolate exhibited the highest carbon dioxide content (29.30 mg/L of $CO_2$), followed by 10% (w/v) and 5% (w/v) of *A. fumigatus* inoculum. 10% (w/v) and 5% (w/v) of the isolates were found to evolve about 22.64 mg/L of $CO_2$, and 13.32 mg/L of $CO_2$, respectively. According to Mohan (2011), it was observed that biodegradation produces carbon dioxide into the medium, resulting in a decrease in the dissolved oxygen concentration.

Thus, 1% (w/v) of inoculum has demonstrated the capability to grow under low $O_2$ concentrations ($p$<0.05).

### iv) Weight loss of the PET films during biodegradation studies

Figure 4.8 illustrates the weight loss of the PET films treated with 1 % (w/v), 5 % (w/v) and 10 % (w/v) of *Aspergillus Fumigatus* in BOD media.

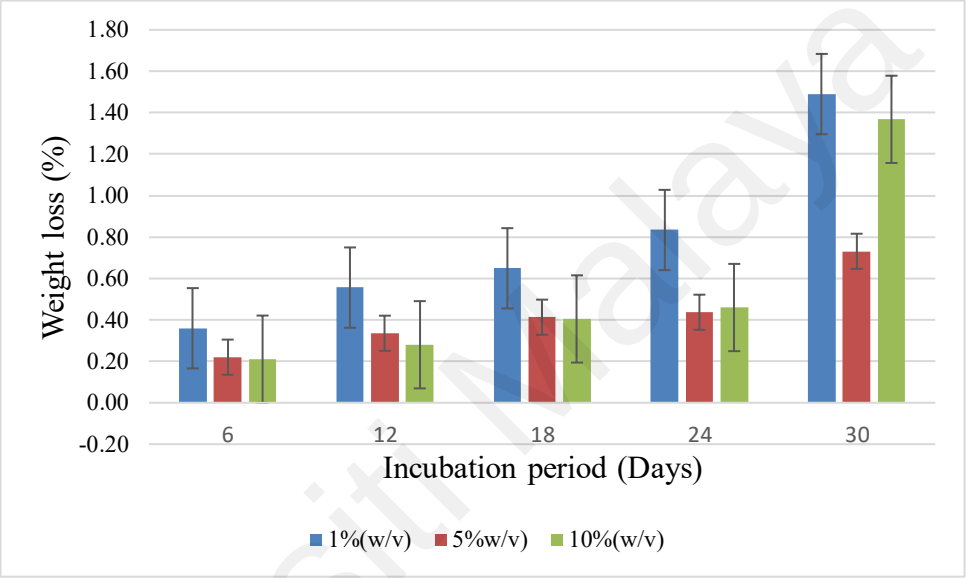



**Figure 4.8**: Weight loss of PET films using different concentrations of *A. fumigatus* strain

The control film showed no weight loss. The treated PET films after 30 days in different concentrations showed considerable weight loss in all. Therefore, this result indicates that the weight loss of PET films during the incubation with the respective isolates was due to the utilization of the films as the sole carbon source. The highest fungal degradation activity was a weight reduction of 1.5% attributed to 1% (w/v) of the isolated strain followed by (10% (w/v) and 5% (w/v) inoculum with degradation of PET films by 1.4% and 0.7% respectively. The results depict that 1 % (w/v) inoculum concentration was the optimal concentration for *A. fumigatus* strain to exhibited greater ability to degrade the PET films. This finding may not be possible without a firm attachment between the fungal cells and the substrate surface. The higher degradation

attributed by 1% (w/v) can be possible by rapid metabolic reactions that contributed adsorption, desorption, and breakdown of the PET plastics. At the end of the experiment, the surface of plastic materials has turned from smooth to rough with cracking. This may be due to the extracellular compounds secreted by the microbes that may break the complex molecular structure of plastics. No weight loss was observed in uninoculated (control) PET films. It can therefore, be stated that the percentage weight loss of PET films observed when inoculated with the isolates could have been as a result of biological process and not as a result of the chemicals in the BOD medium. Similarly, Marques-Calvo *et al.* (2006), in their study of biodegradation of PET, recorded no weight loss in PET when subjected to hydrolytic degradation. A few studies on PET biodegradation by enzymatic and microbial methods have been observed. The weight loss depicted by the different inoculum concentrations differed statistically ($p < 00.5$).

### vi) FTIR analysis of degraded PET films after 30 days of biodegradation studies

The 1 % (w/v) of *A. fumigatus* strain which gave highest degradation of PET films (1.5%) compared with other concentrations was subjected to FTIR analysis.

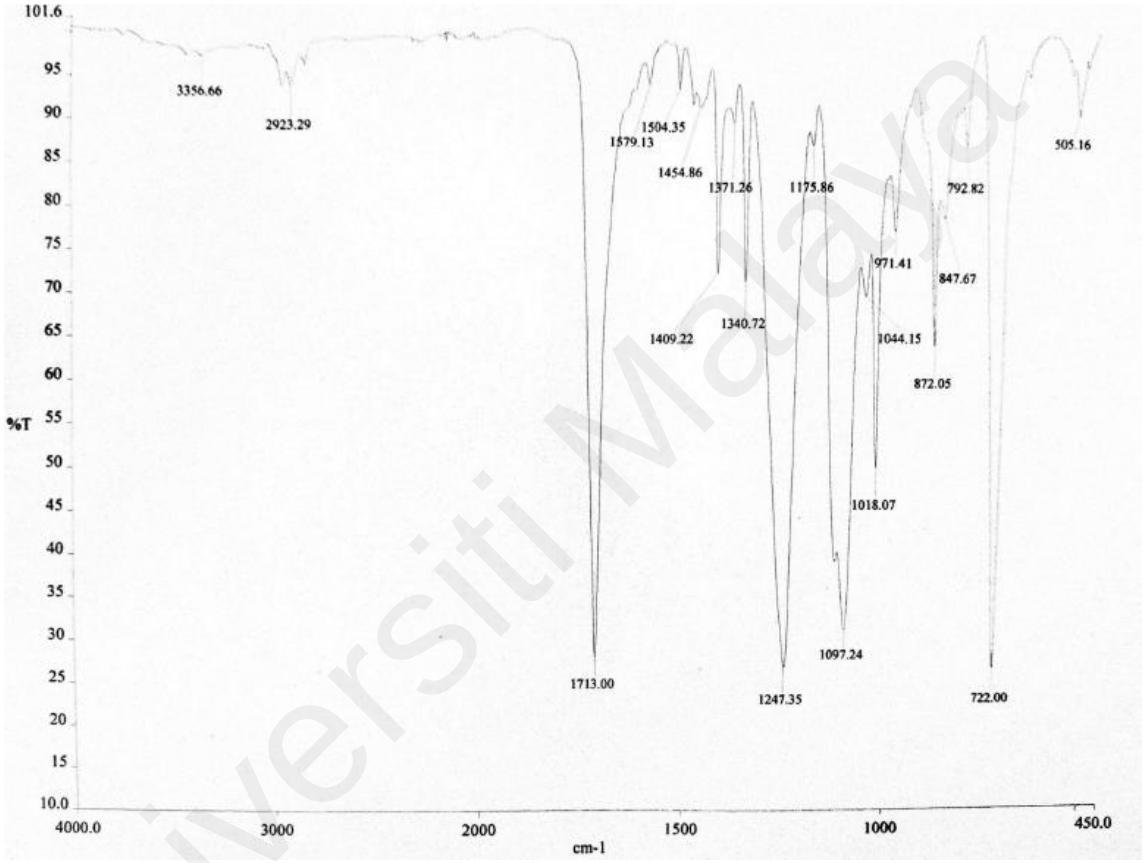



**Figure 4.9**: FTIR spectrum of control (uninoculated) PET film

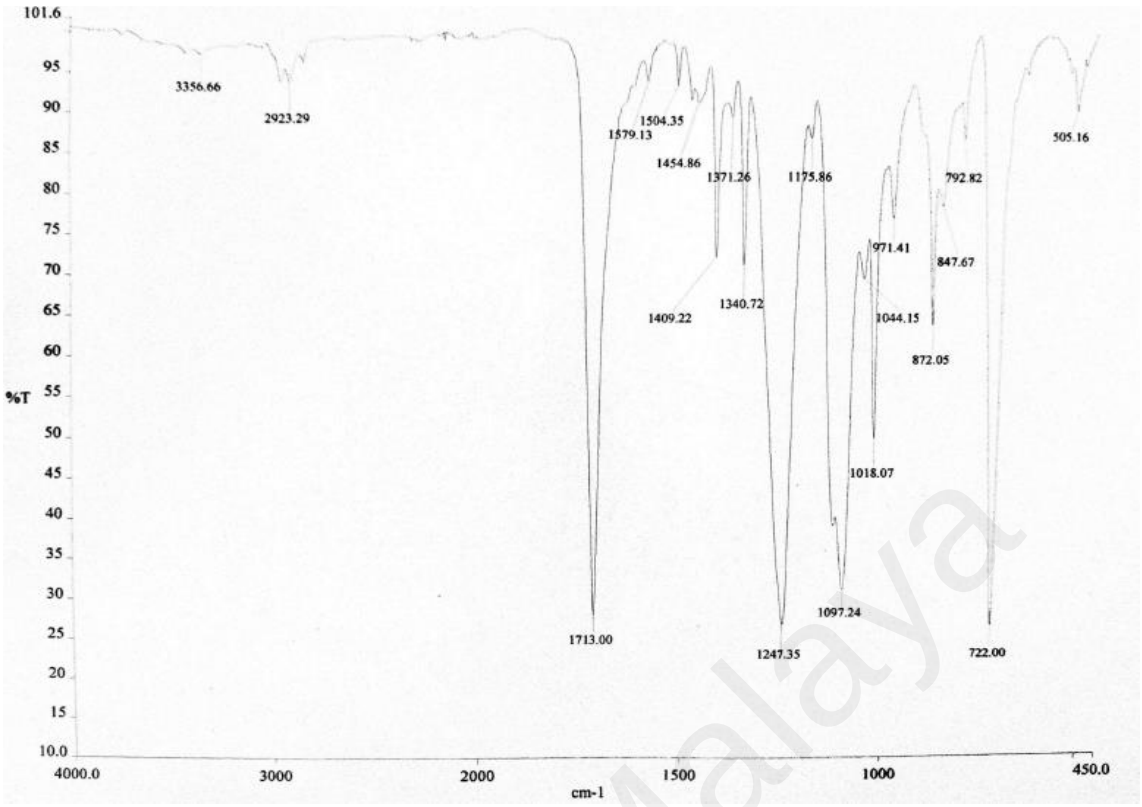



**Figure 4.10**: FTIR spectrum of PET film inoculated with *A. Fumigatus*

**Table 4.3**: FTIR peaks of uninoculated and inoculated PET film

| FT-IR Peaks ($cm^{-1}$) | | Functional groups |
|---|---|---|
| Control PET film | Inoculated PET film | |
| 3428.61 | 3356.66 | N–H stretching of C=O group |
| 2965.06 | 2923.29 | Aliphatic C-H stretching |
| 1713.61 | 1713 | C=O stretching |
| 1578.64 | 1579.13 | C–C=C symmetric of aromatic ring |
| 1503.91 | 1504.35 | C–C=C symmetric of aromatic ring |
| 1452.63 | 1454.86 | C-H bending of the ($CH_2$) group |
| 1408.89 | 1409.22 | C-H bending of the ($CH_3$) group |
| 1371.50 | 1371.26 | O-H bending |
| 1339.43 | 1340.72 | O-H bending |
| 1246.27 | 1247.35 | O-H bending |
| 1175.86 | 1175.86 | O-H bending |
| 1096.12 | 1097.24 | C–O stretching of ether group |
| 1046.15 | 1044.15 | O-H bending |
| 1017.61 | 1018.07 | O-H bending |
| 970.22 | 971.41 | O-H bending |
| 872.28 | 872.05 | O-H bending |
| 847.20 | 847.67 | C-H alkyl bending |
| 792.80 | 792.82 | C-H alkyl bending |
| 722.19 | 722 | Deformation of C–C=C symmetric |
| 632.10 | NA | C-CI alkyl halide stretching |
| 502.24 | 505.16 | O-H stretching |
| 457.86 | NA | C-I stretching |

NA= Not available

Compared with the corresponding control, some changes in the spectra of the PET film were observed after 30 days incubation (Table 4.3). FTIR analysis of the degraded PET films gives a close view of N–H stretching of aldehyde group at 3356 $cm^{-1}$, C–C=C symmetric of aromatic ring at 1504 $cm^{-1}$ and 1579 $cm^{-1}$, and C–O stretching of ether group at 1097 $cm^{-1}$ (Figure 4.10). The FTIR analysis of PET after 30-day incubation with *A. fumigatus* revealed an increase in intensity of the band at 3356 $cm^{-1}$ assigned to the stretching of O-H of diethylene end-group. The increased in intensity of the bands between 1000–1700 $cm^{-1}$ range (1018 $cm^{-1}$, 1097 $cm^{-1}$, 1247 $cm^{-1}$, 1340 $cm^{-1}$, 1371 $cm^{-1}$, 1409 $cm^{-1}$, 1454 $cm^{-1}$, 1504 $cm^{-1}$ and 1579 $cm^{-1}$) were attributed to the oxidized fractions such as moieties containing O-H groups because of the action of the selected strain (Esmaeili *et al*., 2013). The peaks at 1454 $cm^{-1}$ and 1409 $cm^{-1}$ were attributed to the C-H bend of the methylene ($CH_2$) group and C-H bend of the methyl group ($CH_3$), respectively. In addition, the increased in the intensity at 847 $cm^{-1}$ and 792 $cm^{-1}$ peaks were attributed to the C-H alkyl bend. On the other hand, decrease in the intensity of peak was observed at 2923 $cm^{-1}$, 1713 $cm^{-1}$, 1044 $cm^{-1}$, 872 $cm^{-1}$, and 722 $cm^{-1}$ when compared with the control. Enzymatic degradation of PET had an influence on shifts in wavenumbers of bands correlated with aliphatic C-H stretching from 2965 $cm^{-1}$ to 2923 $cm^{-1}$, which were caused by oxygen building into the aliphatic chain. Besides, the intensity of the carbonyl band at 1713 $cm^{-1}$ reduced during the process with the selected concentration of the stain 1% (w/v). These outcomes are agreeable with the findings of Gulmine *et al*. (2003) who confirmed appearance of an absorption band around 1713 $cm^{-1}$, which could be assigned to the C=O stretching vibration of a ketone group and which grew in intensity with extended aging. Decreased of a peak at 722 $cm^{-1}$ was observed in the study, and the presence of band at 720–724 $cm^{-1}$ indicates a rocking deformation (Ibiene *et al*., 2013) accompanied with change in its shape bound with out of plane deformation of the two carbonyl

substituents in the aromatic ring. In addition, C-CI alkyl halide band at 632 $cm^{-1}$ disappeared in PET films treated with 1 % (w/v) of *A. fumigatus*. In overall, this study is supported by Umeshwari *et al.* (2013), revealed that PET can be degraded by fungi by cleaving bonds of PET polymer which showed stretching between the constituent bonds like C=C, C-H, O-H, C-O and C=O of polymer.

### 4.6.2 Effect of inoculum concentrations on the biodegradation of LDPE plastics

#### i) Microbial count of *A. fumigatus* strain during biodegradation studies

The fungal population counts of *A. fumigatus* strain with different concentrations (1% (w/v), 5% (w/v) and 10% (w/v)) were measured during the biodegradation period (Figure 4.11).

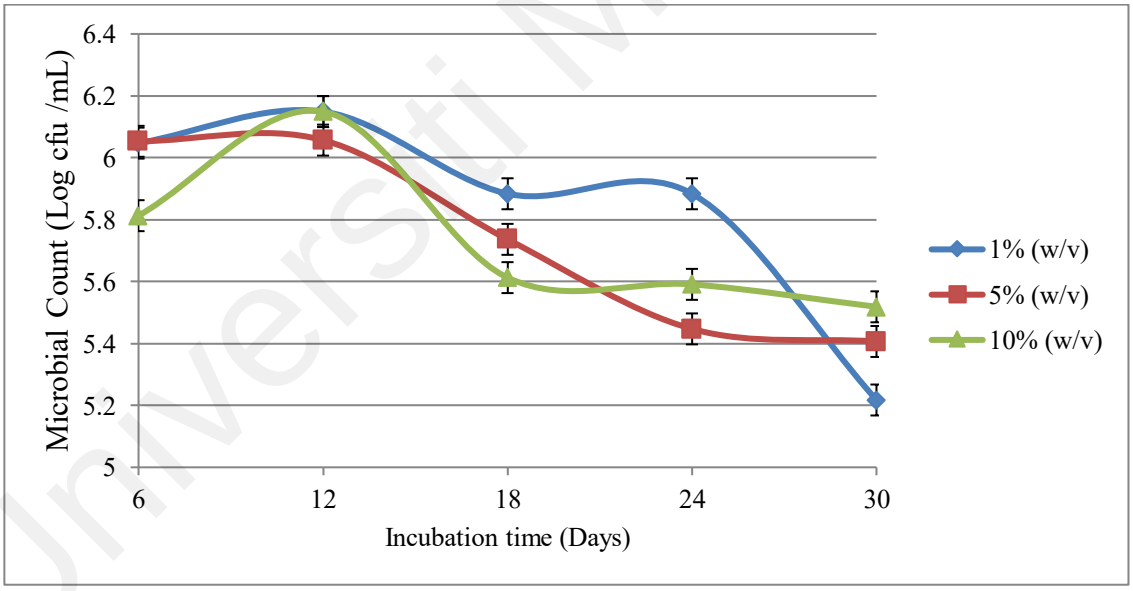



**Figure 4.11**: Changes in microbial count of different concentrations of *A. fumigatus* strain during LDPE biodegradation studies

The counts of 1% (w/v) of the isolated fungi increased from 6.04 log cfu/ mL at the start of the experiment to 6.14 log cfu/ mL on the 12th day. The increase in fungal population depicted the adaptation of the microbes in the culture media to utilize the LDPE films as a carbon source for growth. Imam *et al.* (1999) observed that significant

biodegradation of plastic can occurred only after colonization by resident microbial populations and he concludes that an increase in the microorganism load has correlation with degradation of the polymer. The counts of the strain, however, decreased to 5.21 log CFU/ml. Similar growth pattern was noticed for 5% (w/v) and 10% (w/v) of *A. fumigatus* strain which recorded the highest counts of 6.05 log cfu/ mL and 6.14 log cfu/ mL, respectively on the 12$^{th}$ day of biodegradation period. These findings indicate that during this period of time, the culture environment is favorable for the growth and proliferation of the isolate. Following that, the population of 5% (w/v) and 10% (w/v) strain continuously decreased until the last day of the experiment to 5.4 log cfu/ mL and 5.51 log cfu/ mL, respectively. This might be resulted from lack of nutrients in the culture media or the presence of degradation metabolites which create unfavorable environment for microbes to grow. Statistically, the growth of the isolate showed significant difference at $p< 0.05$.

**ii) Changes in pH of LDPE plastics-infused BOD media during biodegradation studies**

Figure 4.12 depicts the changes in the pH of the LDPE containing media incubated with *A. fumigatus* strain during the 30 days biodegradation studies.

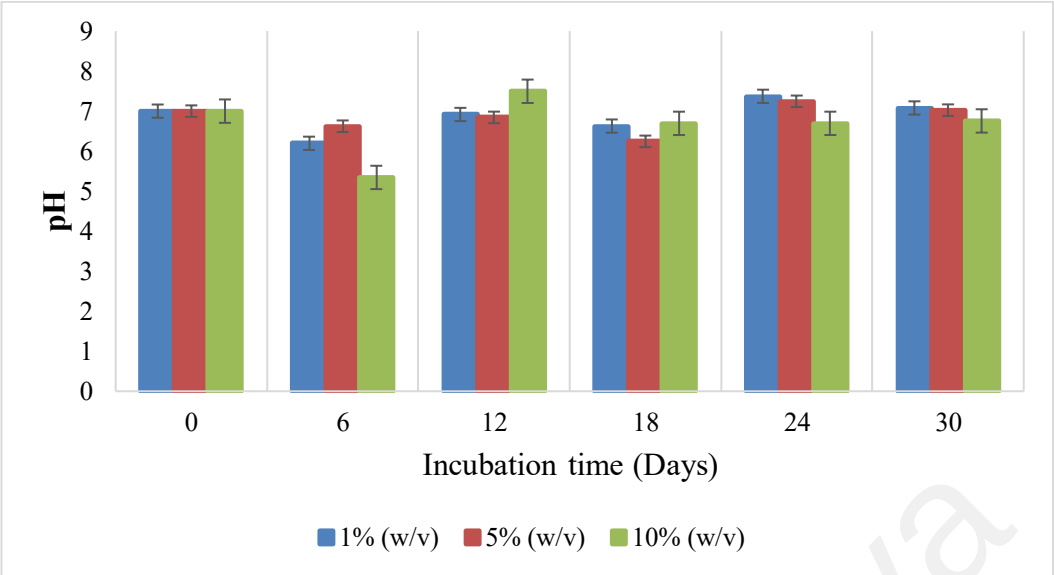



**Figure 4.12**: Changes in pH during LDPE biodegradation studies at different concentrations of *A. fumigatus* strain

The initial pH of the aqueous media was pH 7. However, the degradation of LDPE plastics cause the pH of the media change towards acidic on the 6$^{th}$ day and further towards neutral condition on the 12$^{th}$ day of experiment. The optimum growth of *A. fumigatus* strain with concentrations 1% (w/v) and 5% (w/v) were achieved on the 12th day when it reached pH 6.8 and pH 6.9 with cell count 6.14 log cfu/ mL and 6.05 log cfu/ mL, respectively. While, 10% (w/v) strain achieved optimum growth (6.14 log cfu/ mL) at pH 7.5. The increase in pH to alkaline level could have been due to ammonification of nitrogen components in the culture media (Zahra *et al.*, 2010). This outcome implied that these pH values were the optimum pH for the growth of the isolates. According to Mentzer and Ebere (1996), the optimum pH for biodegradation of hydrocarbons is around pH 6 to pH 8. Overall pH variations for the three different concentrations of inoculum weren't constant as continuous acceleration and deceleration in pH values were observed throughout the biodegradation studies.

### iii) Changes in Dissolved Oxygen (DO) and Carbon Dioxide ($CO_2$) during biodegradation studies

To determine the biodegradation of LDPE plastics, measurement of carbon dioxide evolution and oxygen during the biodegradation studies were recorded as shown in Figure 4.13.

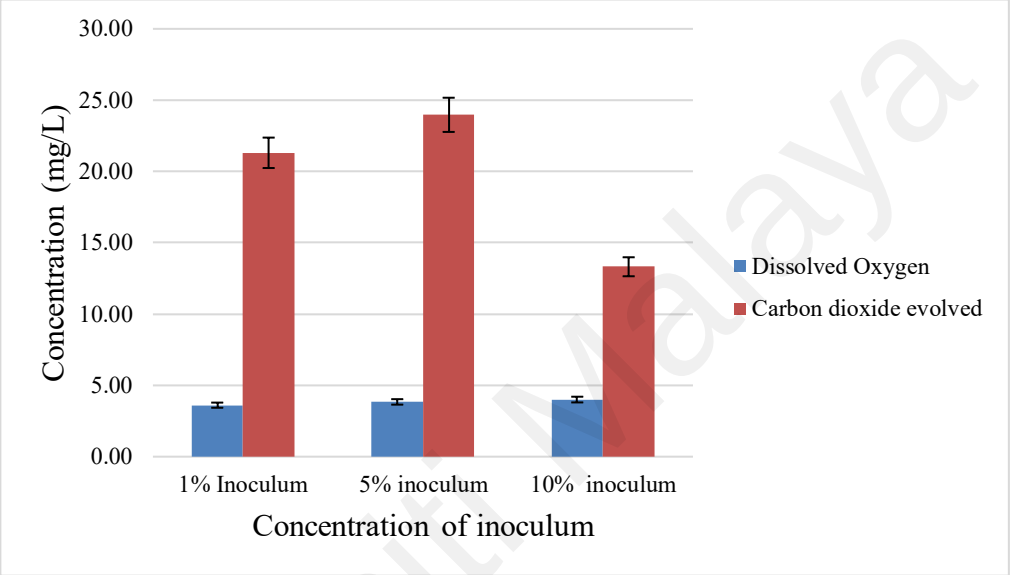



**Figure 4.13**: Concentration of oxygen content and dissolved carbon dioxide of different concentrations of A. fumigatus strain during LDPE biodegradation studies

The amount of oxygen contents on the 30$^{th}$ day of experiment doesn't varies much between concentrations at 1% (w/v), 5% (w/v) and 10% (w/v) of *A. fumigatus* strain which recorded 3.61 mg/L, 3.84 mg/L and 4.0 mg/L, respectively. However, the total amount of $CO_2$ evolved for the fungal strain on LDPE films after a 30 day period of growth were significantly higher (23.9 mg/L) for isolate with 5% (w/v) concentration. At concentration of 1% (w/v) and 10% (w/v), the level of $CO_2$ reached 21.3 mg/L and 13.32 mg/L, respectively. The maximum yield of $CO_2$ contents by 5% (w/v) may be due to the ability of the microorganisms to utilize the LDPE as carbon source. This trend closely matches with rapid biodegradation activity at 5% (w/v) concentration which degraded 21.9 % of LDPE in one month of incubation time which was

significantly higher than other concentrations of inoculum ($p$<0.05). These findings showed lower $CO_2$ evolution than the studies by Aamer *et al*. (2009) who reported $CO_2$ concentration of about 18500 mg/L after a 30 days growth of a fungal strain of *Fusarium sp*. on LDPE films. Work done by Gajendiran *et al.* (2009), also reported higher evolution of $CO_2$ (23200 mg/ L) after a 30 day period of incubation with fungal strain of *A. clavatus sp.* on LDPE films.

### iv) Weight loss of the LDPE films during biodegradation studies

Changes that occurred as a result of microbial degradation were assessed qualitatively by measuring the weight loss of the LDPE films after inoculation with *A. fumigatus* strain. Weight loss of LDPE is proportional to the surface area since biodegradation usually is initiated at the surface of the polymer. The reduction in weight was observed after the biodegradation of LDPE are shown in Figure 4.14.

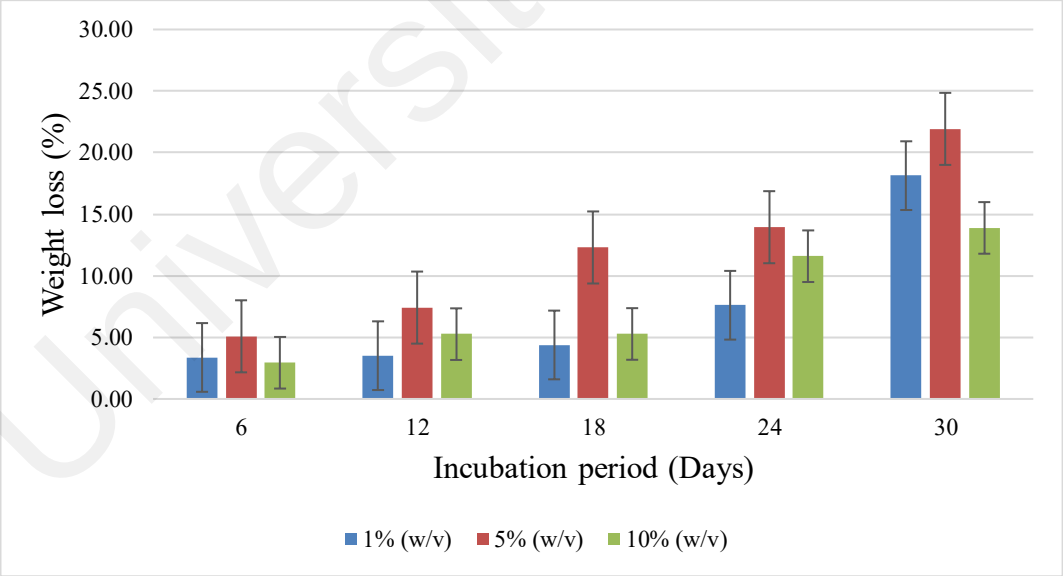



**Figure 4.14**: Weight loss of LDPE films using different concentrations of *A. fumigatus* strain during biodegradation studies

The weight loss of LDPE films was 18.1%, 21.9% and 13.9% after 30 days with 1% (w/v), 5% (w/v) and 10% (w/v) of *A. fumigatus* strain, respectively, while no weight

loss of LDPE films was observed in the control. The highest weight loss (21.9%) was recorded in the degradation of LDPE with 5% (w/v) inoculum, which was significantly different ($p$<0.05) from others. At 1% (w/v) inoculum, degradation capacity was 18.1%. This outcome is agreeable with a study conducted by Zahra *et al*. (2010), where *A. fumigatus* was the best degrader compared to *A. terreus*, and *F. solani* in degrading polyethylene. In contrast with the findings above, Singh *et al.* (2012) reported that *Penicillium sp*. was more active in reducing LDPE i.e up to 6.58% compared to *A. fumigatus* as it reduced the weight up to 4.65%. This is possible due to different extracellular enzymes release by the organisms which causes biodegradation of the plastic films (Vijaya & Reddy, 2008).

The high weight loss exhibited by isolate implied the ability of the fungal isolate to excrete specific enzymes that can putatively attack LDPE films and consequently cause partial biodegradation after treatment with different concentrations (1% (w/v), 5% (w/v) and 10% (w/v) of *A. fumigatus* strain. The isolates possibly catalyzed metabolic reactions that contributed to the adsorption, desorption, and breakdown of the LDPE plastics. During the incubation period, the formation of halos of discoloration in the plastic waste was also observed when compared to the control. These halos may be caused by the activity of the lignocellulolytic enzymes secreted by the fungus. Several authors have observed the activity of these enzymes in the decolorization of industrial dyes (Chattopadhyay & Madras, 2003; Harazono & Nakamura, 2005; and Heinfling *et al*., 1997). Related to the degradation of plastic waste, the observation of the formation of halos of discoloration is important because it is common plastic bags that are used in supermarkets in the presence of different dyes. Besides, the weight loss of LDPE films after incubation could be a result of microbial activity and indicated not only the percentage decrease in weight but also the loss of certain properties, hence resulted to physical breakdown, and degradation of the plastics by the isolated strain (Board, 2006).

### v) FTIR analysis of degraded LDPE films after 30 days of biodegradation studies

Figure 4.15 shows the FTIR spectrum of control LDPE film. For control spectrum, the characteristic absorption bands were assigned at 512 $cm^{-1}$ (C-Br stretch), 717 $cm^{-1}$, 730 $cm^{-1}$ and 875 $cm^{-1}$ (C = C stretching), 1423 $cm^{-1}$ (O-H bending), 1462 $cm^{-1}$ and 1472 $cm^{-1}$ (C-H bending) and 2848 $cm^{-1}$, 2915 $cm^{-1}$ (both due to C–H stretching) (Table 4.4). The FTIR spectroscopy of the LDPE structures inoculated with 5% (w/v) *A. fumigatus* strain is shown in Figure 4.16.


**Figure 4.15**: FTIR spectrum of control (uninoculated) LDPE film

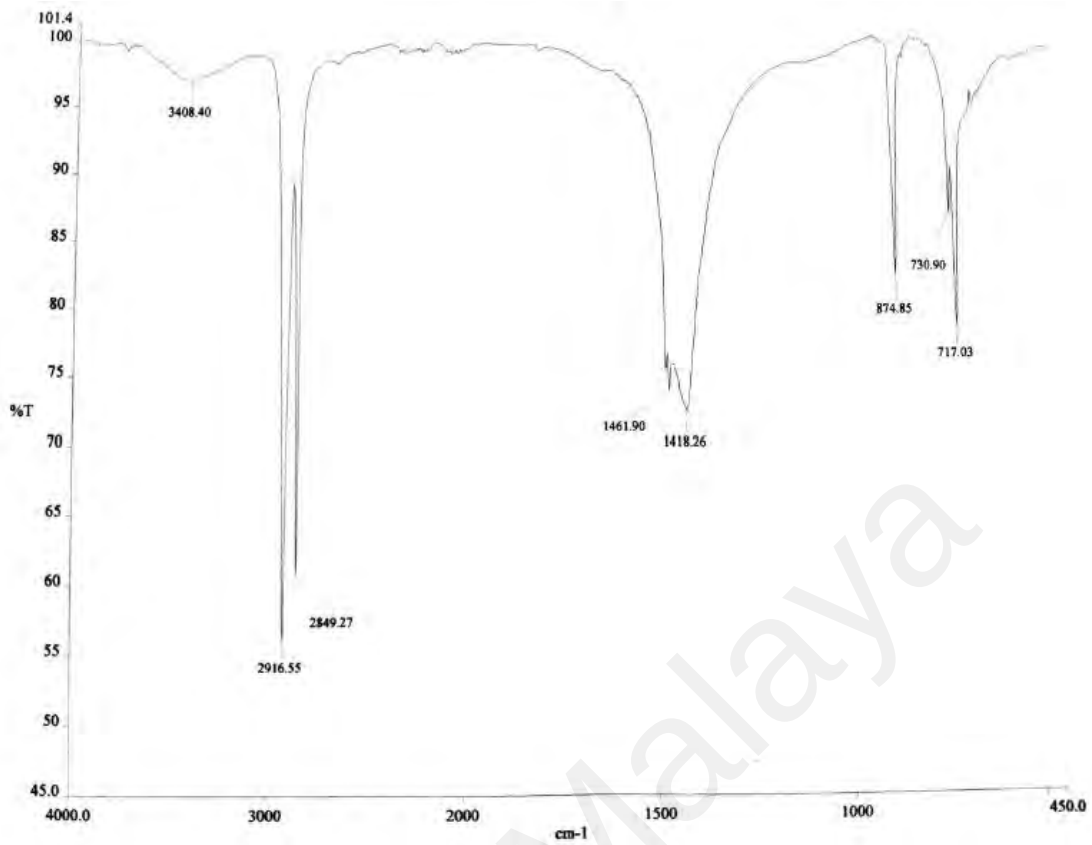



**Figure 4.16**: FTIR spectrum of LDPE film inoculated with *A. fumigatus*

**Table 4.4**: FTIR peaks of uninoculated and inoculated LDPE film

| FT-IR Peaks ($cm^{-1}$) | | Functional groups |
|---|---|---|
| Control LDPE film | Inoculated LDPE film | |
| NA | 3408.40 | O-H bond stretching |
| 2915.90 | 2916.55 | C–H stretching |
| 2848.94 | 2849.27 | C–H stretching |
| 1472 | NA | C-H bending |
| 1462.10 | 1461.90 | C-H bending |
| 1423.90 | 1418.26 | O-H bending |
| 875.30 | 874.85 | C = C stretching |
| 730.87 | 730.90 | C = C stretching |
| 717.66 | 717.03 | C = C stretching |
| 512.19 | NA | C-Br stretch |

NA= Not available

Analysis of the LDPE spectral showed formation of new peaks at 3408 $cm^{-1}$ indicating formation of more than one oxidation product. This peak was observed due to the vibrations in the stretching of the O-H bond in alcohols and phenols. Similarly Guadagno *et al*. (2001) found and agreed with the report of degradation of irradiated linear and low density polyethylene in which an increase in the -OH stretching region of hydroxyl group 3050 - 3570 $cm^{-1}$, due to formation of hydroxyperoxide and alcohol during photo-oxidation were observed. The increased in intensity at 2849 $cm^{-1}$ and 2916 $cm^{-1}$ peak represent C–H stretching vibration of functional group supporting the conformational changes on polymer surface (Das & Kumar, 2015). The peak at 1418 $cm^{-1}$ and 1461 $cm^{-1}$ which attributed to the C-H bend of the methylene ($CH_2$) group showed a decreased intensity. In addition, there was a decrease in the intensity of peaks that corresponded to C=C bending deformation (1461 $cm^{-1}$ and 1418 $cm^{-1}$) and the peak at 1423 $cm^{-1}$ disappeared from the surface of the films incubated with the isolates. Results showed a decrease in the C=C at peaks 874.85 $cm^{-1}$, 730.90 $cm^{-1}$, and 717.03 $cm^{-1}$ after microbial treatment. According to Esmaeili *et al*. (2014), the absorbance range of 700–900 $cm^{-1}$ corresponds to $-C=C-$ stretching, and the presence of alkene group. These findings were further supported by Mouallif *et al*. (2011) studies which reported the present of a band situated about 2900 $cm^{-1}$ assignable to $CH_2$ as an asymmetric stretching, a band around 1461 – 1466 $cm^{-1}$ revealing a bending deformation, and another band at 720 – 724 $cm^{-1}$ which indicates a rocking deformation in polyethylene films. The deformation of C- Br stretching alkyl halides group was observed at the range of 512 $cm^{-1}$. The changes in the peak values support the conformational changes on LDPE films.

### 4.6.3 Effect of inoculum concentrations on the biodegradation of HDPE plastics

#### i) Microbial count of *A. fumigatus* strain during biodegradation studies

The growth pattern exhibited by *A. fumigatus* strain during HDPE biodegradation studies was recorded and the results are displayed in Figure 4.17.

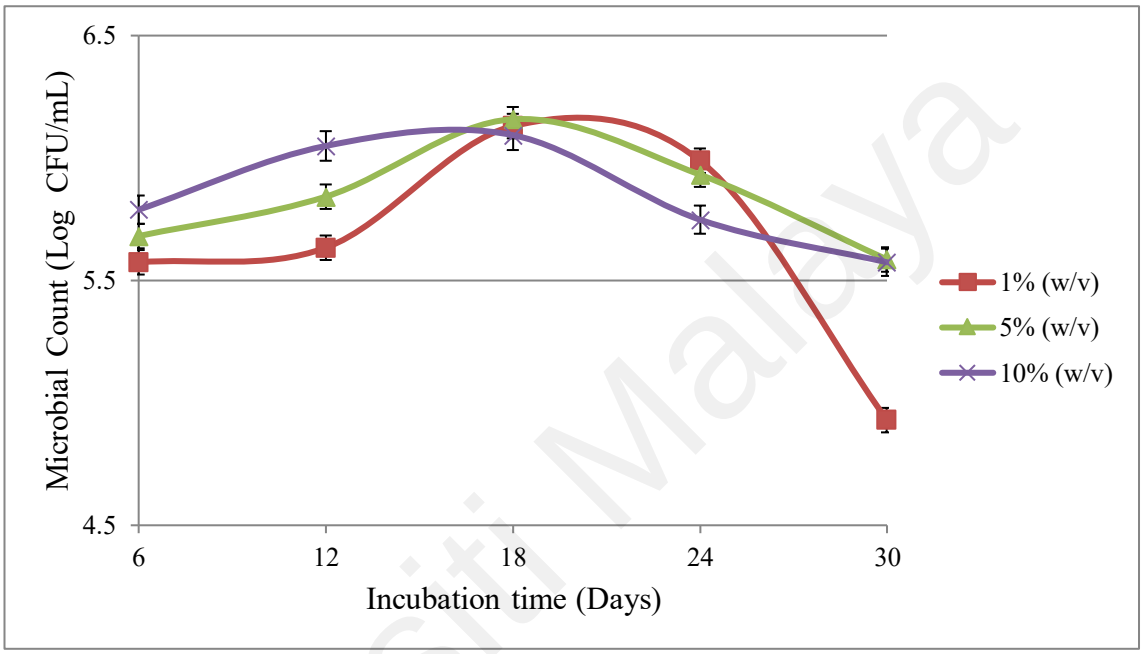



**Figure 4.17**: Changes in microbial count of different concentrations of *A. fumigatus* strain during HDPE biodegradation studies

Similar trend of the growth was observed for all three concentrations (1% (w/v), 5% (w/v) and 10% (w/v)) of inoculum upon exposure to HDPE films. A rapid exponential growth response was observed from 6$^{th}$ day to 18$^{th}$ day followed by decline in microbial population up to 30$^{th}$ day of experiment. The highest fungal cell counts for 1% (w/v), 5% (w/v) and 10% (w/v) inoculum were recorded on the 18th day with cell counts 6.13 log cfu/ mL, 6.15 log cfu/ mL and 6.09 log cfu/ mL, respectively. A swift acceleration in microbial population between 12$^{th}$ day to 18$^{th}$ day indicates that the environment was desirable for the strain to perform rapid metabolism by utilizing the HDPE films as carbon and energy source. A sharp decline in the growth of the isolate until the final day

of may possibly due to the lysis of cells and depletion of nutrient and the culture media. Statistically, the growth of the isolate showed significant difference at $p < 0.05$.

### ii) Changes in pH of HDPE plastics-infused BOD media during biodegradation studies

Figure 4.18 shows the variation in pH of the medium during biodegradation study by *A. fumigatus* strain.

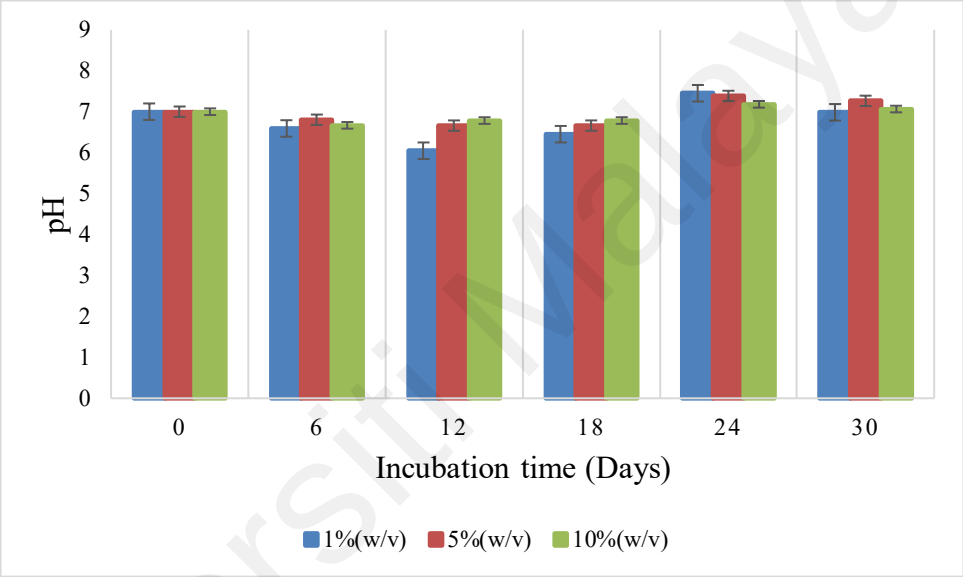



**Figure 4.18**: Changes in pH of different concentrations of *A. fumigatus* strain during HDPE biodegradation studies

The initial pH 7 of the culture media showed decline in the values on the 6$^{th}$ day for 1% (w/v), 5% (w/v) and 10% (w/v) of the strain to pH 6.7, pH 6.8 and pH 6.7, respectively. The reduction in pH validates that the culture was still metabolically active and HDPE films are utilized for its growth. The reduction in pH also affirms the consumption of the polyethylene film as their sole carbon source. Afterward, the pH increased continuously up to the 24th day, which recorded pH values of pH 7.5, pH 7.4 and pH 7.2 for 1 % (w/v), 5% (w/v) and 10% (w/v), respectively. Increasing in pH towards the alkaline range can be attributed to production of some enzymes or

metabolites supporting the metabolic activity of *A. fumigatus* on the HDPE substrate and further degrade the polymer. During this period of time, the maximum cell growth for 1 % (w/v) (6.13 log cfu/ mL), 5% (w/v) (6.15 log cfu/ mL ) and 10% (w/v) (6.09 log cfu/ mL) were recorded on the 18th day when the pH reached pH 7.1, pH 6.7 and pH 6.8, respectively. Statistical analysis showed significant difference at $p<0.05$. On the $30^{th}$ day of experiment, all the concentrations of inoculum showed slight reduction in pH values lead to a decline in the growth of the microbes. The shift in pH condition may be due to the increase in the degradation of HDPE films resulting in accumulation of acidic metabolites.

### iii) Changes in Dissolved Oxygen (DO) and Carbon Dioxide ($CO_2$) during biodegradation studies

Percentage of biodegradation is the evolution of $CO_2$ during depolymerization in which polymer is first converted to monomers by breaking the links and then to simpler compounds to be assimilated into the living cells (Merina & Santosh, 2014). Increased in the amount of dissolved $CO_2$ in the culture media has resulted in a decrease in the dissolved oxygen concentration. The total amount of $O_2$ remained in the culture media after 30 days of incubation for 1% (w/v), 5% (w/v) and 10% (w/v) of inoculum were 3.6 mg/L, 4.6 mg/L and 4.2 mg/L, respectively (Figure 4.19).

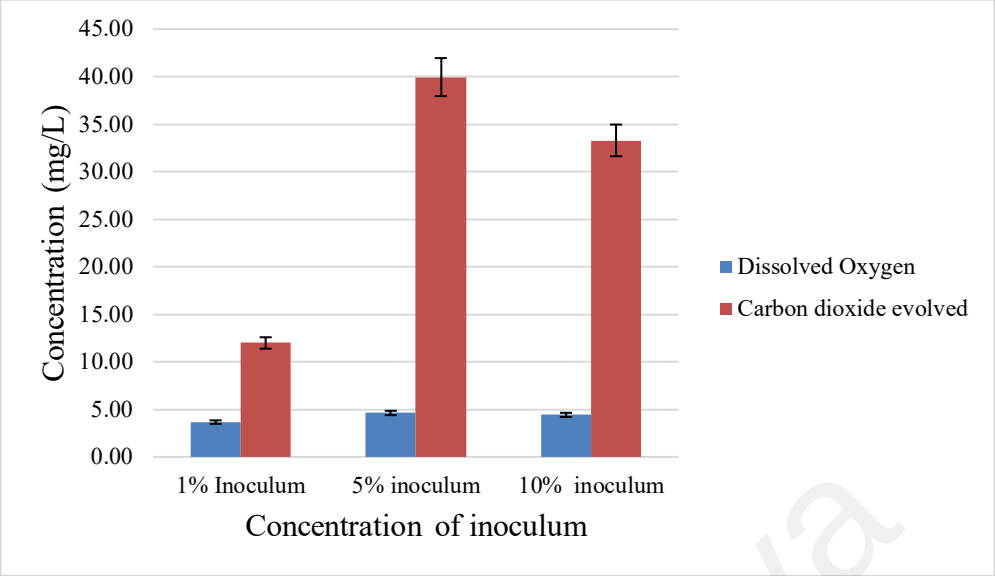



**Figure 4.19**: Concentration of oxygen content and dissolved carbon dioxide of different concentrations of A. fumigatus strain during HDPE biodegradation studies

At inoculum concentration of 1% (w/v), the $CO_2$ evolution was 11.9 mg/L. While increasing the concentration to 5% (w/v), highest value of $CO_2$ evolution of 39.9 mg/L was recorded. Further increase in inoculum concentration to 10% (w/v) resulted in decrease in $CO_2$ evolution (33.3 mg/L). Higher emission of $CO_2$ recorded by 5% (w/v) matches with the rapid metabolism of the isolate for the interaction between the fungal cell membrane and the HDPE films, and consequently allowed maximum weight loss of plastics (1.31%) ($p$<0.05). The result shows the potential of 5% (w/v) inoculum *to* support rapid biodegradation and biomineralization of this polymer as compared to other concentrations of inoculum.

### iv) Weight loss of the HDPE films during biodegradation studies

Fungi are considered to be highly suitable candidates for the biodegradation of plastic materials because of their ability to bind to the surface of the substrate (Volke *et al*.,(2002)) and their capacity to produce enzymes of diverse nature under hugely variable conditions during the biodegradation studies (Mancera *et al*., 2007). Figure 4.20 illustrates the weight loss of HDPE films at different concentration (1% (w/v), 5% (w/v) and 10% (w/v)) of *A. fumigatus* strain.

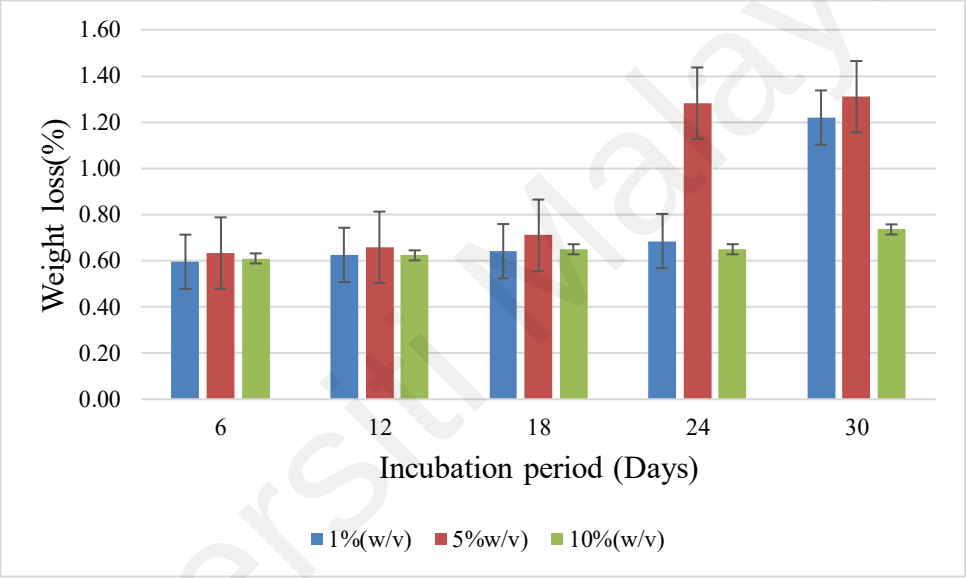



**Figure 4.20**: Weight loss of HDPE films using different concentrations of *A. fumigatus* strain during biodegradation studies

The highest fungal degradation activity was a weight reduction of 1.31% attributed to 5% (w/v) of the isolated strain followed by (1% (w/v) and 10% (w/v) inoculum with degradation of HDPE films by 1.2% and 0.7% respectively. The control film showed no weight loss. This result indicates that the weight loss of HDPE during the incubation with the respective isolate was due to the utilization of the films as sole carbon source. The results indicate that the 5% (w/v) concentrations of the isolate are more capable of degrading HDPE than that at 1% (w/v) and 10% (w/v). These results evident profuse growth of the active 5% (w/v) fungi around the film in the absence of any carbon source

in the broth indicates that it is an optimal concentration for the strain to consume the film more rapidly, which can be possible only after needful breakdown of the film material. Statistical analysis showed that there was no significant growth observed.

**v) FTIR analysis of degraded HDPE films after 30 days of biodegradation studies**

Figure 4.21 shows the FTIR spectrum of control LDPE film. For control spectrum, the characteristic absorption bands were assigned at 661 $cm^{-1}$ (C-Br stretch), 717 $cm^{-1}$ and 730 $cm^{-1}$ (C = C stretching), 1423 $cm^{-1}$ (O-H bending), 1462 $cm^{-1}$ and 1472 $cm^{-1}$ (C-H bending), 1367 (C-O stretching) 2161, 2848 $cm^{-1}$, 2915 $cm^{-1}$ (to C–H stretching) and 3419 (O-H stretching). During the degradation process, changes in functional groups and/or side chain modification occur due to the action of 5% (w/v) *A. fumigatus* strain over HDPE surface is demonstrated in Figure 4.22.

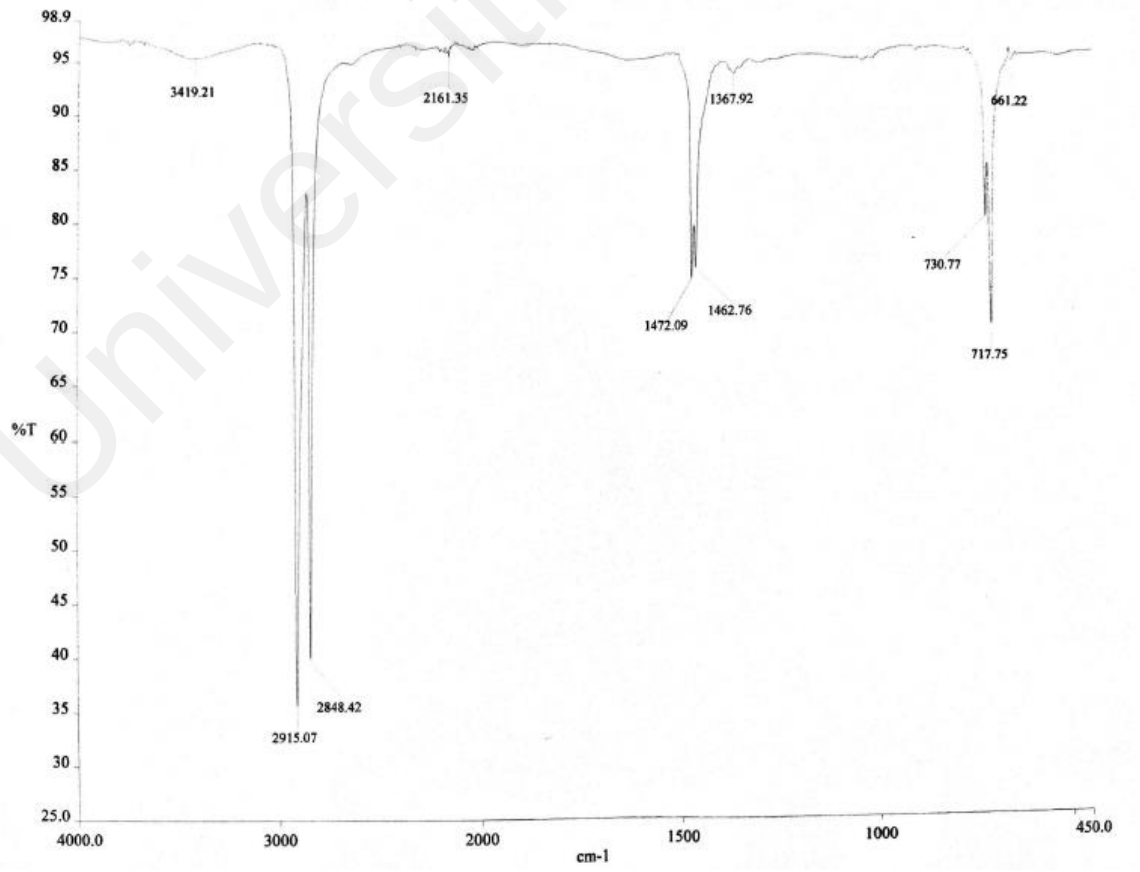



**Figure 4.21**: FTIR spectrum of control (uninoculated) HDPE film inoculated

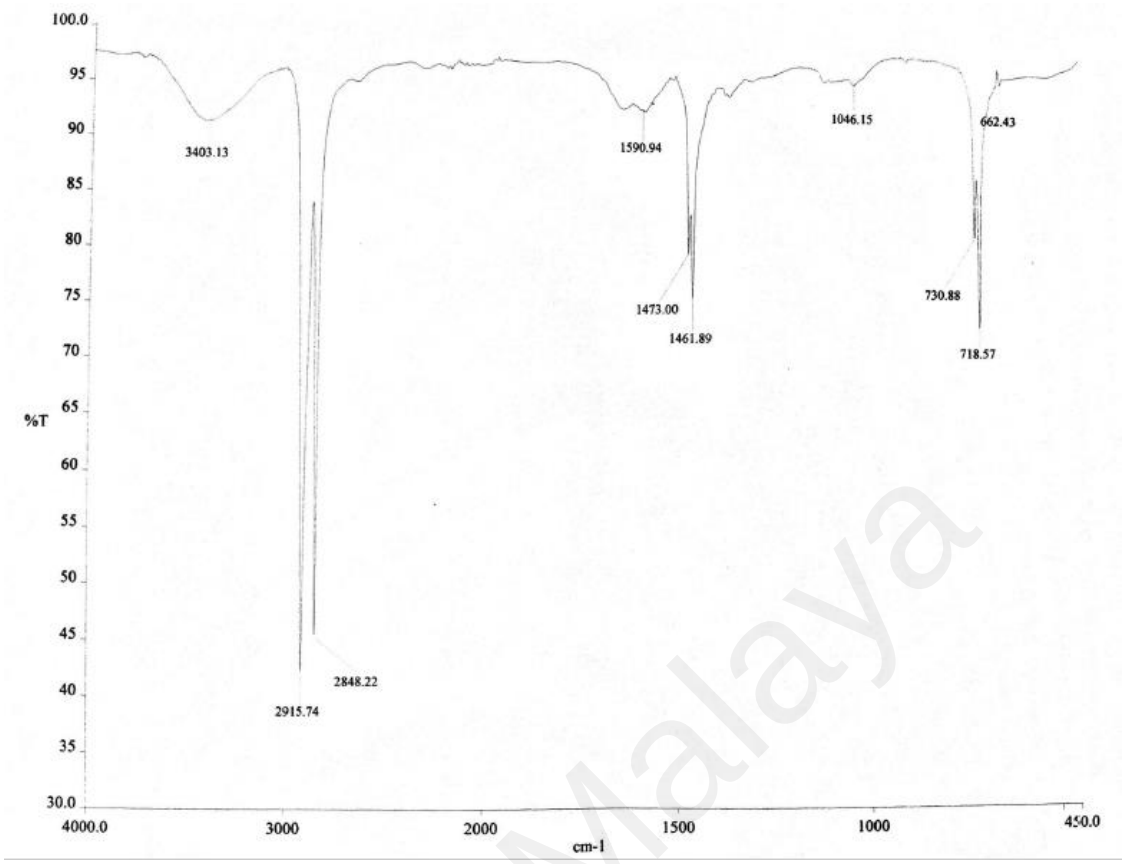



**Figure 4.22**: FTIR spectrum of HDPE film inoculated with *A. fumigatus*

**Table 4.5**: FTIR peaks of uninoculated and inoculated HDPE film

| FT-IR Peaks ($cm^{-1}$) | | Functional groups |
|---|---|---|
| Control HDPE film | Inoculated HDPE film | |
| 3419.21 | 3403.13 | O-H stretching |
| 2915.07 | 2915.74 | C–H stretching |
| 2848.42 | 2848.22 | C–H stretching |
| 2161.35 | NA | C–H stretching |
| NA | 1590.94 | CH2 stretching |
| 1472.09 | 1473 | C-H bending |
| 1462.76 | 1461.89 | C-H bending |
| 1367.92 | NA | C-O stretching |
| NA | 1046.15 | C-O stretching |
| 730.77 | 730.88 | C = C stretching |
| 717.75 | 718.57 | C = C stretching |
| 661.22 | 662.43 | C-Br stretch |

NA= Not available

After 30 days of exposure to *A. fumigatus*, some of the peaks disappeared from the FTIR spectra of HDPE at 2161 $cm^{-1}$ (C-H bond stretching) and 1367 $cm^{-1}$ (C-O bond stretching). In addition, the formation of new peaks in the 1000-1200 $cm^{-1}$ region of the FTIR spectrum correlates with primary and secondary alcohols at peak 1046 $cm^{-1}$, resulting from biodegradation by the selected microorganisms were observed. The formation of another new peak at 1590 $cm^{-1}$ corresponds to $-CH_2$ stretching, and presence of aromatics was also observed. Further, increase in the intensity of peak was observed at 2915 $cm^{-1}$, 1473 $cm^{-1}$, 730 $cm^{-1}$, 718 $cm^{-1}$, and 662 $cm^{-1}$. The peak at 2915 $cm^{-1}$ assigned to C-H aliphatic stretching became elongated compared to control. Peaks at 662 $cm^{-1}$, 730 $cm^{-1}$ and 718 $cm^{-1}$ were assigned to rocking deformation mode of $CH_2$ group with medium intensity similar to that of Krimm *et al*. (1956). On the other hand, decrease in the intensity of peak was evinced at 3403 $cm^{-1}$, 2848 $cm^{-1}$ and 1461 $cm^{-1}$ when compared to the control. The reducing in the length of peak at about 1461 $cm^{-1}$ in polyethylene is due mainly to the bending mode of the $CH_2$ group as reported by Gulmine *et al*. (2002). While, decrease in intensity at peak 3403 $cm^{-1}$ was attributed to a decrease in the carbonyl group after microbial treatment and removal of -OH bounded compounds (alcohol, hydroxyperoxide and carboxylic acids) in the 3100 – 3600 $cm^{-1}$ regions.

## 4.7 RECOMMENDATION

1. Plastic samples from soil, leachate and many other sources at the landfill sites can be thoroughly screened for potential fungus for their ability to promote biodegradation.

2. These fungi isolates can be used to carry out bioremediation of plastics in the landfill field (in situ). The fungi can be inoculated in the BOD media and introduced on the selected area of landfill. The potential of the fungi to degrade the plastics on laboratory scale and field scale can be compared based on the weight reduction of the plastics being introduced with the culture. Large-scale biodegradation of PET, LDPE and HDPE is required to address its massive accumulation in the environment. This work provides a comprehensive idea of how to accelerate the biodegradation of plastics at larger scales using fungi.

3. Plastics contaminated marine environments like mangrove sediment and coastal sites can be exploited for other fungal strains capable of degrading plastics. Fungus isolated from marine environments will possess different metabolic rates and growth requirements. This variation might lead to the isolation of more competent strains with higher degradation capabilities. Along with biodegradation, biofouling can be seen during biodegradation in the marine ecosystem (Flemming 1998), which may enhance the degradation effect.

# CHAPTER 5: CONCLUSION

## 5.1 CONCLUSION

Eight fungal strains were isolated from plastics in Jeram Sanitary landfill. The results revealed that all the isolates were capable of degrading plastics, which signifies the utilization of these films as their source of nutrients as well as energy. The efficiency of *A.fumigatus* (FI 5) to degrade plastics was higher (1.75%) than that of other isolates. The highest reduction, in terms of weight of the degraded plastic films signified that *A.fumigatus* has the potential to aid biodegradation of plastics. Considering the cost in this study, BOD media was selected over Bushnell Haas media to evaluate the suitability of the media for the fungus to perform their biodegradation. The study also showed that inoculum concentrations affected the rate of PET, LDPE and HDPE degradation by *A.fumigatus*. Experimental data of the biodegradation studies revealed that 1% (w/v) *A.fumigatus* was the optimal condition to obtain maximal degradation of PET plastics (1.5%) in term of weight loss. For LDPE and HDPE plastics, highest fungal degradation activity was a weight reduction of 21.92% and 1.31%, respectively, was achieved by 5% (w/v) *A.fumigatus.* The effectiveness of the strains to perform biodegradation activity can be clearly seen during the optimal condition. The concentrations of inoculum strongly suggest that the factor have a great effect on the degradation activity by the fungi. Analysis through FTIR, of the degraded PET, LDPE and HDPE films by the *A.fumigatus* showed surface chemical changes confirming that degradation had occurred by the action of the respective isolated fungi. *A.fumigatus* demonstrated the potential for the degradation of PET, HDPE and LDPE, and can therefore be used to reduce the quantity of plastic wastes in the landfills.

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