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The Classification of Micro-plastics and Biodegradation of Plastics/Micro-plastics

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ABSTRACT. *Plastic wastes have been accumulated in the environment, and these plastics finally fragmented into smaller debris. There are three common standards used to classify micro-plastics, which are diameters, origins (primary or secondary), and monomers. In addition, various techniques and methods are applied to examine the changes before and after biodegradation. This paper also introduces several methods used to improve the efficiency of biodegradation. In the end, there is a summary about some progresses of biodegradation of plastics/micro-plastics.*

KEYWORDS: *Micro-plastics, Biodegradation*

1. Introduction

Plastics, which are synthetic organic polymers manufactured by polymerization of various monomers, have increased significantly in past few decades [1]. Because of their low cost, lightweight, good mechanical performance, and stability, plastic materials have been used in a wide variety of applications and substituted natural materials in almost every aspect of life. In 2018, about 359 million tons of plastic were produced [2]. However, such man-made materials have accumulated in the environment due to their persistence and stable chemical structures [1]. The sources of plastic wastes are mainly bags, disposable containers, footwear, textile fiber, furniture, industrial wastes, various electronic products, and so on [3-4]. Plastic wastes produced in industrial production and people's life (e.g. such as food packaging bags, cosmetics, and tiny fibers discharged through sewage pipes of washing machines) finally enter the terrestrial and marine ecosystems through a series of paths. As per literature, plastic shares about 60–80% out of total marine floating garbage in the oceans and seas [5]. According to the previous

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studies and researches, plastics can be transported by ocean currents, which result in their world-wide distributions [6]. Therefore, how to degrade such persistent organic pollutant has become a hot spot in scientific researches. Microbes, due to their vigorous metabolic activities and eco-friendliness, are viewed as an effective mediate to degrade plastic materials, especially as a solution to treat micro-plastics spread in lands, rivers, seas, and deep oceans. Because of this, scientists have operated a lot of experiments to investigate the biodegradation of plastics/micro-plastics.

This review will be divided into three parts. The first part, focusing on "micro-plastics", introduces (a) the classification of micro-plastic wastes based on the diameters, the origins (primary- or secondary-) and the monomers. The second part explains how they are introduced and transported in the ecosystem. The third part, focusing on techniques used in experiments of biodegrading plastics/micro-plastics, introduces (a) several widely used devices and methods for examining the biodegradation effects, and (b) commonly used methods for promoting microbial degradation and their general principles.

2. The classification of micro-plastics (MPs)

Three classification standards for MPs are used in the existing researches and literature: the diameters of plastic debris, the origins of MPs (primary- or secondary-), and the sorts of monomers of MPs.

2.1 The diameters of plastic debris

Table 1 The ranges of diameters of plastic debris as reported in the literature

Plastics	Diameter
Nano-plastics	< 0.1 μm
Micro-plastics	$0.1 - 5$ μm
Meso-plastics	$5 - 25$ μm
Macro-plastics	$25 - 100$ μm
Mega-plastics	> 100 μm

Note: the boundary between meso-plastics and macro-plastics varies in pre papers. For example, 20 μm can be used as the criterion to identity whether a given piece of plastic is a meso-plastic or a marco-plastic [7].

In almost all available literature, it is generally admitted that plastics with particle size less than or equal to 5 μm are MPs [8]. Depending on different researching

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objectives or purposes, the sizes of plastics to be studied are different. For example, when studying the composition of coastal wastes, most of the floating plastic wastes observed are visible and large-scale plastics such as bottles, footwear, food or commodity packaging bags, clothing, etc., which belong to mega-plastics[9]. When researching on the plastic content in domestic sewage, water from sewage treatment plants, or polluted rivers and seas, the sizes of plastic are mostly in the range of micro-plastics [10]. In the study of the toxicological effects of MPs on plankton, plants' roots and growth of farm plants, the particle size of the plastics involved is in the range of micro-plastics, and there are abundant nano-plastics [11].

2.2 Primary- or secondary-MPs

Compared with the method of classifying plastic debris according to their diameters, it is more common to classify them depending on their initial sizes when they enter the terrestrial or aquatic ecosystems. MPs are divided into primary- and secondary-MPs [12]. Primary-MPs contain micro-beads in personal care products, tiny beads used for exfoliation, the abrasives in toothpastes, the plastic pellets used for grinding and polishing in industrial production, or the tiny debris originally produced in the manufacturing process [6, 12]. Secondary-MPs refer to MPs fragmented from plastic objects with large sizes [8, 10]. The large plastic objects receive solar radiation, hydraulic power in rivers and oceans, thermal oxidation, etc., and undergo cracking and fragmenting [8]. Finally, they become extremely small fragments with particle size less than 5 μm [13]. Secondary-MPs are mainly from the industrial and daily plastic goods discarded in the environment (e.g. bottles, packaging bags, boxes, clothing, various instruments, production wastes, etc.) [13-14].

2.3 Monomers of MPs

Compared with above two standards, classifying MPs based on their polymeric monomers comes to the molecular level. It is conducive to the study of the degradation mechanism of MPs and the change of chemical composition of plastic materials in the degradation process [15-17]. According to the different sources of monomers, MPs can be divided into petroleum-based plastics (e.g. polyethylene, polypropylene, polystyrene, polyvinyl chloride, polyamide, and polyethylene terephthalate) and bio-based plastics (e.g. Starch, PLA, and Cellulose) [8, 18]. Petroleum-based plastics mainly come from coal, natural gas and oil, and Bio-based plastics mainly originate from various polymers synthesized by organisms [8, 18].

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3. Transfer of micro-plastics in the ecosystem

The pollutants that are transported together with MPs cause harm to the ecosystem can be divided into two types: one is the additives added in the plastics which are used to improve the performance of plastic materials; the other is the pollutants (chemical substances or pathogens) absorbed and carried by MPs from the surroundings in the process of continuous transfer in the environment [4, 6, 8]. When the plastic ages in the environment, the absorption of pollutants (e.g. heavy metals, POPs, microbes) are promoted [4, 8, 19]. In addition, with the increasing abundance of marine MPs, the contact between MPs and marine organisms is more frequent [20]. In marine, MPs can be ingested or attached by organisms at various trophic levels and be transferred to organisms at higher trophic levels along the food chain, eventually leading to the flow of MPs in the food web [21]. The movements of MPs in the food web can cause physical and chemical damages to organisms. The pollutants or pathogens transmitted by plastic particles will also be carried in the food web and transported along the food chain [4].

4. Several methods for examining the biodegradation of micro-plastics/plastics

Most experiments were carried out to observe and record the changes of micro-plastic particles, plastic films or plastic foams before and after biodegradation (structural changes, mass loss and chemical composition changes). The techniques used in existing experiments can be divided into three categories: (1) observing the morphological and structural changes of plastics, (2) determining the degradation efficiency (mass loss), and (3) investigating the metabolic pathways of biodegradation.

4.1 Methods used to determine morphological and structural changes

Visual observation: Various techniques are adopted to estimate the effects of degradation, but the most direct and fundamental approach is visual observation. The change of color, appearance of cracks, fragmentation, and formation of biofilm can be determined by either direct watching or using scanning electron microscopy (SEM). In previous studies, researchers observed the roughing and cracking of plastic films [22]. In Ohura's study, P(3HB-co-14%3HV) monofilament fibers wrinkled and became porous after biodegradation for 21 days (in seawater, at 25°C), and this outcome was detected by SEM [23]. In addition, in Sangeetha Devi's test, the erosion of HDPE film (30 days incubation) was visualized under scanning electron microscopy [20].

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4.2 Methods used to determine weight loss /degradation efficiency

CO_2 evolution/O_2 consumption: The amount of plastics degraded is the most important index in microbial degradation experiment, because it indicates whether or not the microorganisms used in the test can be applied as the media for effective degradation of plastics. The formula (1) used to calculate percentage weight loss is given below:

$$\text{Weight loss }\% = \left(\frac{W_0 - W_1}{W_0}\right) \times 100 \quad (1)$$

Where W_0 and W_1 represent the weights of plastic before and after degradation [24].

In most tests, the efficiency of degradation was less than 10 percent, and some examples are listed in Table 2. The efficiency of degradation can be influenced by various factors which will be further explained in 4.4.

4.3 Methods used to trace the metabolic pathway

In fact, the comprehensive and complete metabolic pathways of bio-degradation is still under exploration [8]. However, in the existing papers, radioisotope labeling method was used to demonstrate the ingestion, mineralization, and assimilation of plastic. In Yang's experiment, α ^{13}C- or β ^{13}C-labeled PS were used as the sole carbon source, and ^{13}C was detected in both carbon dioxide and lipids [25]. This result was considered as the evidences of mineralization and assimilation, respectively. For invertebrates, the uptake and transfer of micro-plastic particles from Artemia nauplii (instar II) to zebrafish (Danio rerio) can be demonstrated by fluorescently labeled plastic beads [26]. In this study, the diameter (1-μm to 5-μm and 10-μm to 20-μm) and abundance of MPs inside organisms could be clearly observed. In many experiments, Fourier transform infrared spectroscopy (FTIR spectroscopy) can be used to detect the changes of composition of plastic. In Yang's study, the newly emerging resonance signals in the spectrum of the fecula indicated the occurrence of new chemical bonds and functional groups. These changes can be used as the basis to investigate the pathway of metabolism [25].

4.4 Commonly used methods for promoting microbial degradation

Because the efficiency of degradation is quite low in natural conditions, many potential approaches are examined to promote biodegradation of plastics. The general principle of existing methods is promoting the formation of oxygen-containing functional groups, which increases the ability of polymer to be oxidized. Pro-oxidants, thermal oxidation, and UV radiation are the most common approaches applied in pre studies [27]. In Vimala's test, 9.26% of PE sample was decomposed under pretreatments (bio-surfacant and UV treatment for 72 hours), and the efficiency of degradation was greatly improved [27]. In Chiellini's study, LDPE was pretreated by pro-oxidant

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additives and thermo-oxidation, and about 60% of the sample was degraded [28]. Some results are listed in Table 2.

Table 2 Recent studies of biodegradation of plastics/micro-plastics

Sample type	Microorganisms	Sources	Pretreatments			Duration of degradation (d)	Weight loss (%)	References
			pro-oxidant additives	Thermal oxidation	UV			
PE	*Zalerion maritimum*	Marine	-	-	-	14 days	56.7%	[29]
PE	mixed bacterial culture mainly consisting of *Bacillus sp.* and *Paenibacillus sp.*	Landfill site	-	-	-	60 days	14.7%	[22]
PE	*Bacillus subtilis*	Unknown	Biosurfactants	-	UV treatment for 72 hours	30 days	9.26%	[27]
LDPE	*Aspergillus niger* ATCC 9642	Unknown	-	(80°C, 15 days)	-	31 months	0.50 % and 0.57 % (with and without ethanol)	[30]
LDPE	Unknown	Soil, compost	TDPATM pro-oxidant additives from EPI Inc.	55°C	-	Unknown	above 60%	[28]
PET	*Bacillus cereus*	Mangrove sediment	-	-	25 days	40 days	6.6%	[31]
PS	*Bacillus species*	Soil	-	-	-	30 days	23%	[32]
PS	Unknown	Plastic-eating mealworms	-	-	-	16 days	47.7%	[25]
PP	*Bacillus sp.* Strain 27	Mangrove sediments	-	-	UV treatment (time unknown)	40 days	4.0%	[31]
PP	Co-culture of *Bacillus* and *Pseudomonas*	Soil samples	-	Thermally pretreated	-	12 months	1.95%	[33]
PVC	Unknown	Enriched from marine samples (litter and	-	-	-	7 months	11.1~12.3%	[2]

		water)						
PU	*Bacillus subtilis* strain MZA-75	Soil samples	-	-	-	-	-	[34]

5. Conclusions

This paper concludes the classification of MPs, a short description of ingestion and transfer of MPs, and main techniques adopted in bio-degradation to evaluate and promote the degradation of MPs. Specifically, the efficiency of natural bio-degradation is still quite low, so some approaches (pro-oxidant, thermal-treatment, and UV-radiation) are applied to promote the efficiency. Further researches can explore potential ways to promote degradation, as well as the use of isotopic atomic tracer and more methods to reveal the pathway of microbial metabolism of plastic materials.

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