https://doi.org/10.3311/PPch.36537

Periodica Polytechnica Chemical Engineering, 68(3), pp. 297–310, 2024

Biodegradation of Plastic Wastes in Soil: A Review on Testing and Evaluation Procedures

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Received: 25 January 2024, Accepted: 23 May 2024, Published online: 09 July 2024

Abstract

The recalcitrant behaviour of plastic creates an acute pollution on soil and aquatic biota. The plastic polymer synthesised from petroleum takes several hundred years to degrade. During excavation activities on the lands within or at the outskirts of urban limits, single use plastic wrappers and bags that were buried long ago, can be found in large quantities. Researchers have already identified the potentiality of microorganisms to biodegrade the plastic polymers. These microorganisms utilise plastic as a sole source of carbon and mineralise them into carbon dioxide under optimum environmental conditions. This paper provides a brief review on the biodegradation studies carried out by various researchers, spectra of microorganisms identified with the potential to degrade various polymeric substances, methods of testing and evaluations in order to quantify the effectiveness of degradation, their shortfalls and risks involved in implementing bioremediation technique in the field. A review on other related aspects that are deemed to be relevant to this topic are also discussed.

Keywords

biodegradation, single-use plastic, microorganisms, soil, laboratory testing

1 Introduction

In India, allocated landfill sites are overflowing as uncontrolled dump yard due to fast-growing industrial sector and urbanization. A statistical data on global production of plastic estimated that the expected production by the year 2050 to be around 8300 metric tons [1]. The increase in volume of municipal solid waste landfills is mainly attributed to the slower degradation of waste dumped compared to the pace with which they are produced. Around 43% of the plastic produced in the nation are utilized for packaging purposes that are mostly of single use only [2]. Littering of single use plastic all over the earth surface has resulted in large amount of ground pollution. Keller et al. [3] have reported the transportation and contamination of micro and nano plastic particles on land surface from the sewage sludge. Plastics are polymeric products manufactured from petroleum hydrocarbons. Hydrophobicity and high molecular weight hinder the degradation of plastic polymers. The process of degradation of plastic present on the soil surface could be achieved by microbial biodegradation. Plastic polymers act as a source of carbon for microbial assimilation. It has been reported by the researchers that extracellular enzyme secreted by microorganisms could facilitate the breakage of long chain polymers [4]. During the process of degradation many oxidized intermediate compounds such as aldehydes, ketones, alcohols, ester and acids are formed. Complete aerobic biodegradation of plastic ends up in the formation of carbon dioxide and water due to assimilation of hydrocarbons of plastic polymeric chains by microbes. The concept of microbial degradation of plastic waste has been so far approached from the biotechnological perspective. The feasibility of the technique for large scale field application and environmental aspects has to be analyzed. The main objective of this study is to analyze the effectiveness of various testing methods adopted by different researchers to assess the process of biodegradation. The biodegradation of different types of polymers, role of soil in the polymer degradation process, methods to estimate the rate and amount of degradation followed by various researchers are summarized.

Cite this article as: Sundar S., Padavala, H. K. "Biodegradation of Plastic Wastes in Soil: A Review on Testing and Evaluation Procedures", Periodica Polytechnica Chemical Engineering, 68(3), pp. 297–310, 2024. https://doi.org/10.3311/PPch.36537

2 Process of degradation

The process of biodegradation involves invasion of plastic polymers that contains hydrocarbon and mineralisation of the same through chain of enzymatic reactions. Simplified procedure of biodegradation study is given in Fig. 1.

2.1 Potential microbes

Monomers of commercially available plastic are held together by covalent bonds resulting in the formation of long chain polymer. These bonds make the carbon present in the plastic products not readily available for microbes for consumption and thus the degradation is reported to be a slow process. Presence of antioxidants such as butylated hydroxytoluene retards the degradation of polyethylene [5]. Microorganisms in the absence of other nutrients utilise polymers derived out of hydrocarbons as their carbon source. Biodegradation is preceded by abiotic deterioration or biodeterioration. Abiotic deterioration is initiated by UV-light and biodeterioration is initiated by some microorganisms through the process of hydro peroxidation [6]. Chemical pre-treatment using nitric acid makes the procedure uneconomical during field application. Many researchers reported the degradation of different synthetic polymeric products by various microbial strains isolated from the environment since the invention of biodegradation procedures. A typical methodology adopted in these works is represented in Fig. 2. These research works involved collection of soil and water from the polluted area and isolation of potential microbial strains through serial dilutions and biodegradation of polymeric material in the minimal salt media containing polymer as sole source carbon. The effectiveness of the microbial activity is qualitatively reported through advanced analytical instruments such as DSC, SEM, FTIR, GCMS, TGA [7–10] and other similar methods. Potential microbial species that have degraded different plastic polymers are tabulated in the Table 1.

Many researchers have also provided a list of polymers degrading microorganisms in their review works. [4], [11–16]. The authors summarized that the biodegradation

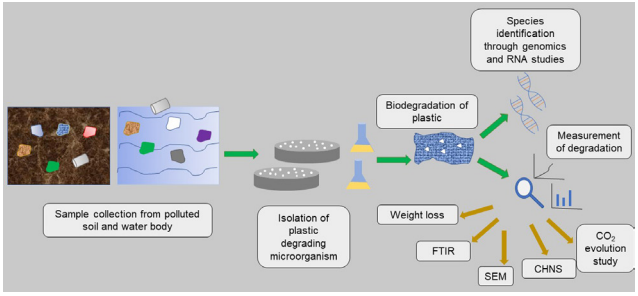


Fig. 1 Simplified biodegradation procedure

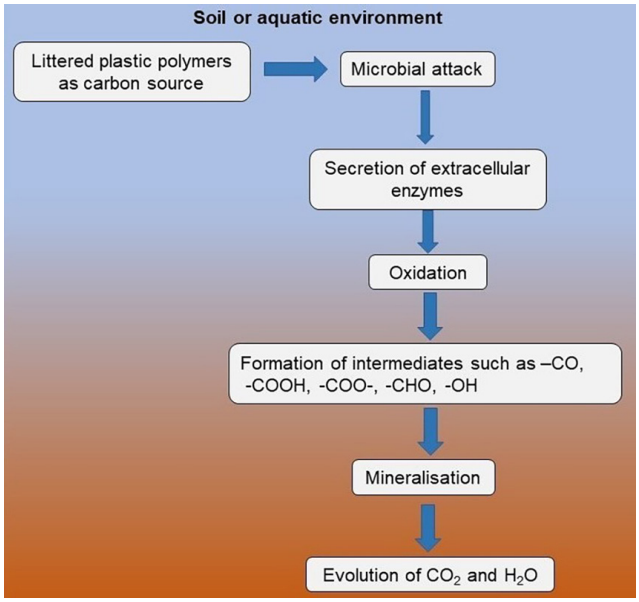


Fig. 2 Typical experimental methodology adopted in various biodegradation studies

studies were so far performed under three environmental conditions such as marine, compost and soil burial condition. Brandl and Püchner [17] attempted to study biodegradation of biodegradable plastic bottles suspended in aquatic ecosystem in-situ.

Low density polyethylene (LDPE) accounts for 64% of the single-use plastic used in the world [6]. The survival of the chosen microorganism for biodegradation technique in the prevailing soil condition must be ensured.

Odusanya et al. [18] identified *Serratia marcescens* has the capability to degrade polyethylene. The fact was proved using SEM micrographs, DSC study and weight loss experiment. Vimala and Mathew [19] studied the influence of biosurfactant in polythene degradation using *Bacillus subtilis* species. The authors extracted biosurfactant from the *Bacillus subtilis* cultures and compared microbial biodegradation with and without biosurfactant. Physical treatment using UV radiation was also found to enhance assimilation of polythene film by bacteria.

Azeko et al. [20] also isolated *Serratia marcescens* from soil and observed degradation of powdered polyethylene by the cultured species and its supernatant. Carbonless medium was prepared for the culturing of isolated species. Differential scanning calorimetry, mass loss experiment and scanning electron microscopy were deployed to study the rate of degradation. Degradation with the supernatant was reported to be greater than that with the species itself. But the enzymes present in the supernatant responsible for faster rate of degradation was not identified by the authors.

Table 1 Polymers and polymers degrading microbes

S. No.	Synthetic polymer	Microorganism/ Degrading medium	Literatures	Measurement of degradation
1.	Degradable polyethylene	*Rhodococcus rhodochrous* and *Cladosporium cladosporoides*	[21]	SEM and FTIR analysis
2.	Polythene bags and plastic cups	*Aspergillus glaucus* and *Pseudomonas sp.*	[22]	Weight loss
3.	Polyester Polyurethane	*Pseudomonas chlororaphis ATCC55729*	[23]	Measurement of pH, ammonia, diethylene glycol and SEM analysis
4.	Montmorillonite filled oxo-biodegradable polyethylene	*Pseudomonas aeruginosa*	[24]	SEM and FTIR analysis
5.	Polybutylene succinate	*Fusarium solani*	[25]	CO_2 evolution test
6.	HDPE	*Stenotrophomonas maltophilia* (*co-culturing*)	[26]	Weight loss method, FTIR analysis
7.	Polypropylene grafted with gelatin (PP-*g*-gelatin)		[27]	Weight loss test, hydrolysis test, SEM, XRD and TGA for physical characterisation.
8.	Polypropylene	*Bacillus circulans, Kurthia gibsonii,* and *Flavobacterium sp*	[28]	Baclight test, surface energy measurement, FTIR analyses, weight loss test, SEM analysis
9.	Modified LDPE	*Pseudomonas azotoformans, Pseudomonas stutzeri, Bacillus subtilis* and *Bacillus flexus*	[29]	Mechanical property and weight loss
10.	LDPE	Waste coal, forest soil and volcanic crater	[30]	Weight loss and SEM analysis, reduction of melting point
11.	LDPE	*Aspergillus spp.*	[31]	CO_2 evolution test, FTIR, SEM, analysis
12.	LDPE	Two strains of *Bacillus amyloliquefaciens*	[32]	Weight loss, SEM, AFM analysis, CO_2 evolution test, spectroscopy, FTIR analysis
13.	LDPE	*Aspergillus clavatus*	[33]	SEM and FTIR analysis
14.	LDPE	Sandy loam	[10]	Weight loss

Studies were not made to find out the degradation of polythene directly in the soil after bioaugmenting the soil with large quantities of potential microorganisms.

Laccase is one of the enzymes identified to enhance biodegradation of plastic aided by R. Ruber C208 strain. Production of laccase enzyme could be increased with the addition of copper [34]. Montazer et al. [6] emphasized that biodegradation of polythene by fungal species are restricted to the surface, whereas bacterial strains were identified to penetrate into lower layers. The authors further summarized that colonization and biofilm formation on the polythene could not justify that the polymer is being degraded by the microbial species. Fragmentation followed by mineralization of polymer into carbon dioxide and biomass only could ensure complete biodegradation [35].

2.2 Quantity of polymer used for degradation study

Most of the laboratory testing methods for degradation study have taken 1% (w/w) polymer in powder form with respect to the weight of soil [36, 37]. For the measurement of tensile strength and other properties, polymer film of 1 cm × 1.5 cm was used. Vijaya and Reddy [38] used 5 cm × 20 cm polythene in the compost soil for degradation. Gilan et al. [39] used 3 cm × 3 cm film to study the biodegradation phenomena of photo oxidized polythene. Dussud et al. [40] used circular film of 9 mm diameter and Boonmee et al. [41] prepared 2 cm × 2 cm pieces different biodegradable plastics as per ISO 15985 in their anaerobic biodegradation study. In all these studies weight of the recovered film after a period of time is investigated.

2.3 Pretreatment

Degradation phenomena of polythene could be initiated abiotically or biotically [4]. Researchers claimed that photooxidation of polythene film induced degradation phenomena by microorganisms [4], [39], [42–47]. Some of the researchers attempted to study the effect of non-ionic surfactants to enhance biodegradation phenomena [39], [26], [48, 49]. Studies remarked that pretreatment with mineral oil has no significant influence on rate of biodegradation high

density polyethylene (HDPE) film using *Arthobacter sp*. and *Pseudomonas sp*. Byrne et al. [50] subjected HDPE to an elaborate pyrolysis treatment before biodegradation. The authors demonstrated six different natural media are capable of degrading pyrolyzed HDPE products. The process of pyrolysis was done at a temperature of 575 ˚C. The economy of the process was not considered within the scope of their study. The phenomena of generating such a high temperature in natural condition and bringing down the temperature conducive for microbial growth was not done in the investigation. The method could be feasible by subjecting the segregated plastic in a huge pyrolytic reactor and then condensing the products before disposing them on naturally biodegrading media.

2.4 Optimal condition and field uncertainty

Dibble and Bartha [51] emphasized 7.5 as optimal pH for hydrocarbon biodegradation. Under field condition and especially in landfill environment pH dynamically varies as per the composition of wastes being deposited and also due to the chemical reaction between the reactive waste materials. At pH 3–5 microbes have shorter survival time compared to alkaline range [52]. Pischedda et al. [33] suggested that 20–28˚C (preferably at 25 ˚C) favors the growth of mesophilic bacteria for performing biodegradation of biodegradable mulch films. Ardali et al. 2004 [53] reported a drop in pH from 6 to 6.5 and 3.5 to 4.5 during fungal biodegradation process of starch blend polyethylene and low density polyethylene (LDPE) film.

3 International standards for biodegradation study

ASTM D5988 – 18 [54] suggests methods for measurement of aerobic biodegradation of plastic materials in soil based on carbon dioxide evolution. Twelve vessels similar to the one shown in Fig. 3 are used for the test (Blank, positive, negative, test – in triplicate). During degradation, $Ba(OH)_2$ or KOH traps carbon dioxide evolved.

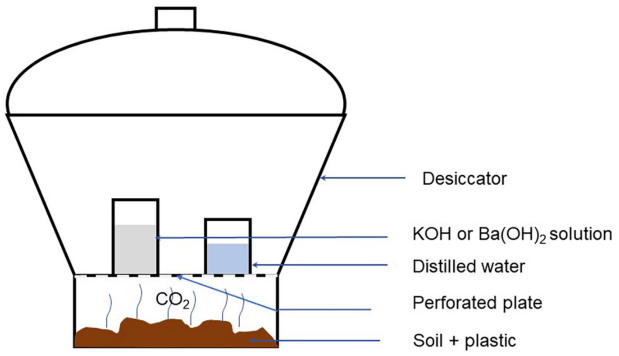


Fig. 3 Desiccator study adapted after ASTM D5988 – 18 [54]

Excess $Ba(OH)_2$ or KOH are titrated with 0.05 M HCl when 0.050 M $Ba(OH)_2$ is used or with 0.25 M HCl when 0.5 M KOH is used. Gajendiran et al. [32] used CO_2 evolution test which is otherwise known as Strum test to measure metabolic carbon dioxide.

ASTM D882 – 02 [55] provides testing procedure for measuring tensile strength of thin plastic sheeting. The test can be performed on the virgin plastic film and on the plastic film that has under gone certain amount of biodegradation in order to measure the reduction in tensile strength.

ISO – 17556:2019(E) [56] recommends to measure aerobic biodegradation of plastic in soil by measuring oxygen consumed and carbon dioxide evolved. Biochemical oxygen demand (BOD) measuring technique can be used to measure oxygen consumed. Theoretical oxygen demand (ThOD) can be measured from the molecular formula. For polyethylene, ThOD is around 3400 mg/g of substance. ISO – 17556:2019(E) [56] also recommends ASTM D5988 [54] procedure for measuring CO_2 evolved during aerobic biodegradation. Das and Kumar [31] attempted to calculate the theoretical amount of mineralised carbon dioxide from LDPE after two months of degradation through weight loss study. The amount of CO_2 evolved from the degrading of LDPE during CO_2 evolution test indicated that almost an equivalent amount of CO_2 as calculated got evolved. Pischedda [33] also investigated the effect of temperature on biodegradation of biodegradable polymer pellets using CO_2 evolution test.

Briassoulis et al. [57] performed inter-laboratory study on the aerobic biodegradation testing procedure of plastic waste in soil as per ASTM and ISO standards. The study confirmed the repeatability of test procedures by performing degradation test on bio-based, conventional plastic and lubricants. It has also agreed the extrapolation of similar degradation phenomena on laboratory and field condition. Polymer strip has shown lesser rate of degradation in comparison to powdered polymer sample. The carbon: nitrogen (C:N) ratio of the test soils were maintained at 10:1 after studying the natural C:N ratio of soil. C:N insufficiency of the soil was rectified to codal standards by adding nutrient supplements such as nitric or ammonium phosphate fertilizer. The test was performed on standard soil in one of the participated laboratories. LDPE degradation was studied in one of the two participated laboratories. Even after 360 days of observation LDPE film does not show any degradation in soil. The reason may be due to the lack of LDPE degrading microorganism in the soil taken for testing. The study also emphasised that

the test must be terminated after observing no significant variations in the observed BOD values or carbon dioxide evolved values as per the ASTM recommendation for terminating the test once reaching the stable state.

The major drawback of carbon dioxide evolution test is that Potassium carbonate formed as a result of reaction between KOH and evolved carbon dioxide may react with HCl during titration. The ASTM code does not account for this reaction.

4 Testing methods for measurement of biodegradation

The following testing methods are generally recommended and adapted in various literatures to measure rate of biodegradation of polymers [58]

- Determination of molecular weight
- Determination of weight loss
- CO_2 evolution test
- Scanning electron microscopic analysis
- FTIR analysis
- Hydrolysis test
- Measurement of melting point
- Measurement of tensile strength of polymeric strip.

4.1 Studies based on weight loss technique

Weight loss technique is based on the initial and final weights of plastic materials subjected to biodegradation. It is easy to perform. But the technique could not distinguish whether the loss is due to simple disintegration or degradation. Deepika and Madhuri [59] compared the biodegradability bacterial and fungal species isolated from garbage soil. The isolated microorganisms were incubated to degrade polythene granules over a period of 6 months. The degradation phenomena were observed using weight loss measurement. Streptomyces species were reported to have higher rate of degradation compared to *Pseudomonas* species and two types of *Aspergillus* species. Performance of the isolated microbes under field condition on polythene degradation was not reported.

The weight loss method conducted by Boonmee et al. [41] proved that among biodegradable polymers bio-based polymer degrades better than the petroleum based degradable polymer. Polybutylene adipate-co-terephthalate considered in their study did not show any degradation within the test period under anaerobic condition.

4.2 Electrical method

Apart from the methods stated above, biodegradation was studied by measuring capacitance and conductance across PE film buried in soil. An electrode is placed inside the small polythene bag filled with soil and another electrode is placed in the soil surrounding the buried bag. The method is based on the fact that development of cracks or holes or reduction in thickness of the polyethylene foil increases conductance of electricity across soil. But the results demonstrated that only biodegradable polymer has undergone degradation and not the virgin polyethylene during the study period of over one year [60, 61].

4.3 FTIR method

Brandes and Brandl [62] used FTIR analysis for distinguishing bacterial spores of different origin. Rajandas et al. [63] reported that the presence of LDPE is indicated by the peak at 2920 cm^{-1}. FTIR method was used for the quantification of LDPE biodegradation on the basis of carbonyl index. Biodegradation was observed by the formation of new peaks at 630–1636 cm^{-1} and another at 825 cm^{-1} that corresponds to amide and nitrate ions. Su et al. [64] used FTIR analysis to study the amount of micro plastic in landfill refuse and leachate from landfill at different ages. The absorbance spectrum was compared with that of virgin plastic. The presence of peaks at 1780–1600 cm^{-1} that corresponds to carbonyl group in the absorbance spectrum of samples at different ages from landfill indicated oxidative degradation of polyethylene. The peak corresponding to methylene group is also found to decrease with age of the landfill. The authors attributed decrease in concentration of methylene group in the aged sample might be due to lesser consumption of polyethylene products in the past or due to oxidative degradation of polyethylene in soil. Vimala and Mathew [19] reported the formation of oxidized compounds of polyolefins through FTIR analysis after microbial biodegradation process. Montazer et al. [6] also observed significant peaks related to oxidative degradation of polythene observed in FTIR analysis. Rouillon et al. [65] recommended the usage of decrease of absorbance spectra at 1456 cm^{-1} corresponding to CH_3 to study the degradation of polypropylene instead of studying increase in absorbance at wave number 1710 cm^{-1} corresponding to carbonyl group. This is due to the fact that all the carbonyl products of degradation could not be detected in IR spectroscopy. The FTIR spectra of the undegraded and degraded LDPE film obtained Bhatia et al. [66] is shown in Fig. 4a and Fig. 4b. The authors affirmed the bacterial biodegradation of LDPE film by observing the absence of peak at 1166 cm^{-1} in the degraded sample which was clearly observed in the control LDPE. The process of biodegradation can be accounted from presence or absence of certain peaks, shifting of peaks and formation of oxidised products.

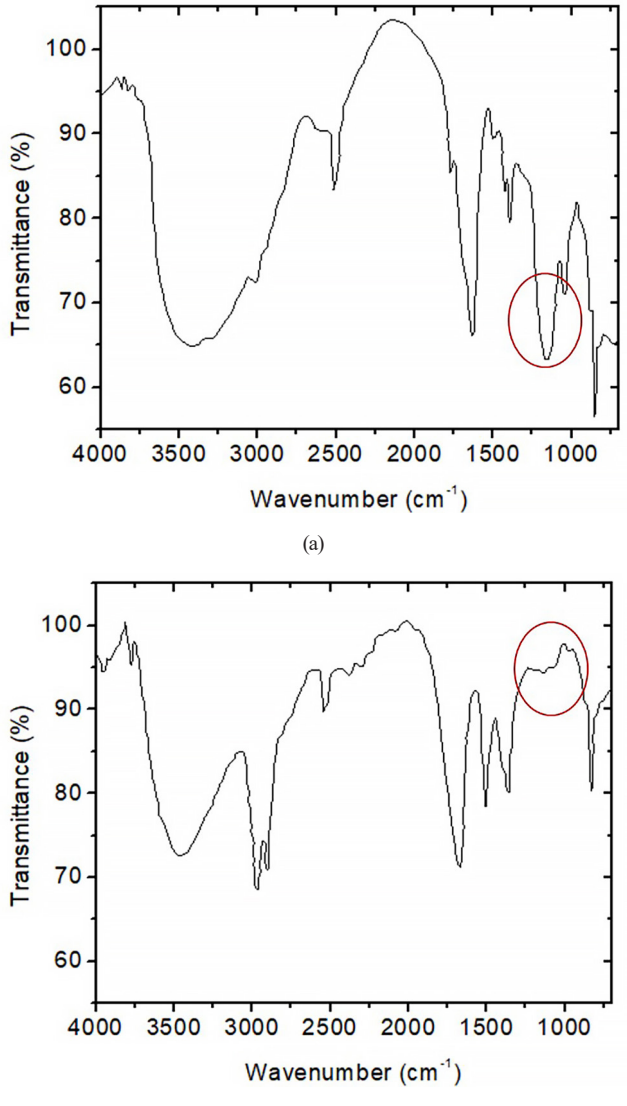


Fig. 4 FTIR spectral comparison of undegraded and degraded LDPE [66] a) Undegraded; b) Degraded

Watanabe et al. [67] accounted for biodegradation of LDPE mulch film by observing new peaks at 3400 cm^{-1} for OH stretching, 1640 cm^{-1} carbon double bond, OH deformation at 1080 cm^{-1}. The absorbance value at 1080 cm^{-1} is found to increase as a result of degradation. It has to be noted that the overall absorbance spectra of degraded sample are found to be higher than that of undegraded sample both in the field as well as in laboratory incubation. The authors did not give any inference for the overall increment of absorbance spectra. Balasubramanian et al. [26] reported that increase of carbonyl index of keto (1715 cm^{-1} vs 1465 cm^{-1}), ester (1740 cm^{-1} vs 1465 cm^{-1}) and vinyl group (1650 cm^{-1} vs 1465 cm^{-1}) as a result of microbial degradation of HDPE. Similar bands were analyzed by Devi et al. [9] in their study. But the indices were initially found to increase and later observed to decrease. The increment is due to the formation of acids by dissolved oxygen and the reduction has happened due to microbial activity.

Dussud et al. [40] considered oxidation of modified polyethylene subjected to mechanical degradation by observing increase of peak at 1712 cm^{-1}. Boonmee et al. [7] used FTIR characterization to study biodegradation of biodegradable plastic and confirmed the degradation activity by the observation of new peaks around 1600 cm^{-1} which was absent initially.

Park and Kim [89] reported different sets of spectral bands for polyethylene microplastics. The peaks are reported to decrease after 20 days of incubation and increased at 40 and 60 days of incubation. The research reports of Richardson [69], Elena et al. [70], Nandiyanto et al. [71] help to read FTIR spectra of various hydrocarbons.

The peaks observed and reported for the degradation of polymers by different authors are not consistent and varying. Standards must be framed pertaining to the degradation study based on FTIR analyses.

4.4 Carbon dioxide evolution test

Carbon dioxide evolution test using biometer flask was identified as an effective screening method for biodegradation [90]. Carbon dioxide evolution test provides quantitative confirmation of complete biodegradation of hydrocarbons. Strotmann et al. [91] attempted to develop modified carbon dioxide evolution test based on the variation of conductivity of absorbing material such as KOH and NaOH. The test offered continuous measurement of degradation activity and eliminated the difficulty of periodic titration. The mechanism involves entrapping carbon dioxide evolved as a complete degradation organic material with KOH and NaOH absorbing material. The electrode inserted into the absorbing material measures the conductivity of KOH or NaOH used. The evolved carbon dioxide precipitated KOH to K_2CO_3. The formation of carbonate has resulted in the decrement of conductivity. The reduction in conductivity is calibrated against evolved carbon dioxide. The testing was observed for the biodegradation of aniline which is the simplest aromatic amine. The effectiveness of the test for synthetic non-degradable material has to be investigated. The change in concentration of KOH solution due to evaporation was not observed in 28-day observation. This may be expected in long term degradation of synthetic non-degradable material and may affect the conductivity observed. Castellani et al. [72] developed sophisticated instrument to measure CO_2 evolved from the biodegradation of biodegradable polymer using infrared gas detector and the results were verified with conventional

titration procedure. The result confirmed the efficiency of continuous monitoring by infra-red gas detectors over the routine procedure. The technique may be suitable for measuring synthetic polymer biodegradation as the rate of emission of carbon dioxide is very low.

Mohee and Unmar [73] studied the biodegradability of two commercially available plastics based on CO_2 evolution test. Schöpfer et al. [74] also evaluated biodegradability of LDPE by CO_2 evolution Test. The amount of CO_2 evolved out of from soil amended with LDPE was reported to be very less even after 230 days of treatment. Ghatge et al. [75] summarized that instead of weight loss and surface structural changes after microbial biodegradation, quantification of CO_2 evolved using gas chromatography provided reliable information on mineralization of polymers.

Al-Salem et al. [76] followed ASTM D 5988-12 to study biodegradability of biodegradable plastic by adapting Barium Hydroxide titration procedure. The results confirmed the suitability of the method for studying rate of biodegradation of biodegradable plastic.

Completion of biodegradation in case of biodegradable plastic is standardized as 90% mineralization considering 10% for the biomass and the by-products formed which are not measurable. Ecotoxicity tests based on seed germination, plant growth, survival of earthworm and aquatic organisms are also recommended to test the soil in which biodegradable plastics were once buried [37]. There are no evidences in the available literatures for 90% mineralization of conventional synthetic plastic polymer and ecotoxicity test on soil after biodegradation process.

4.5 Other methods

Differential scanning calorimetry, carbon, hydrogen, nitrogen and sulphur (CHNS) analysis were also performed on the treated and control samples in order to study rate of biodegradation [77]. Boonmee et al. [41] utilized CHNS analysis only for initial characterization of their prepared soil-sludge medium. Zumstein et al. [78] introduced carbon isotopes in the synthesis of biodegradable polymers and studied the biodegradation phenomena in soil environment. Feasibility of the technique to quantify biodegradation of non-degradable polymer in soil environment has yet to be investigated.

5 Major findings

5.1 Scanning Electron Microscopy (SEM) qualitative report

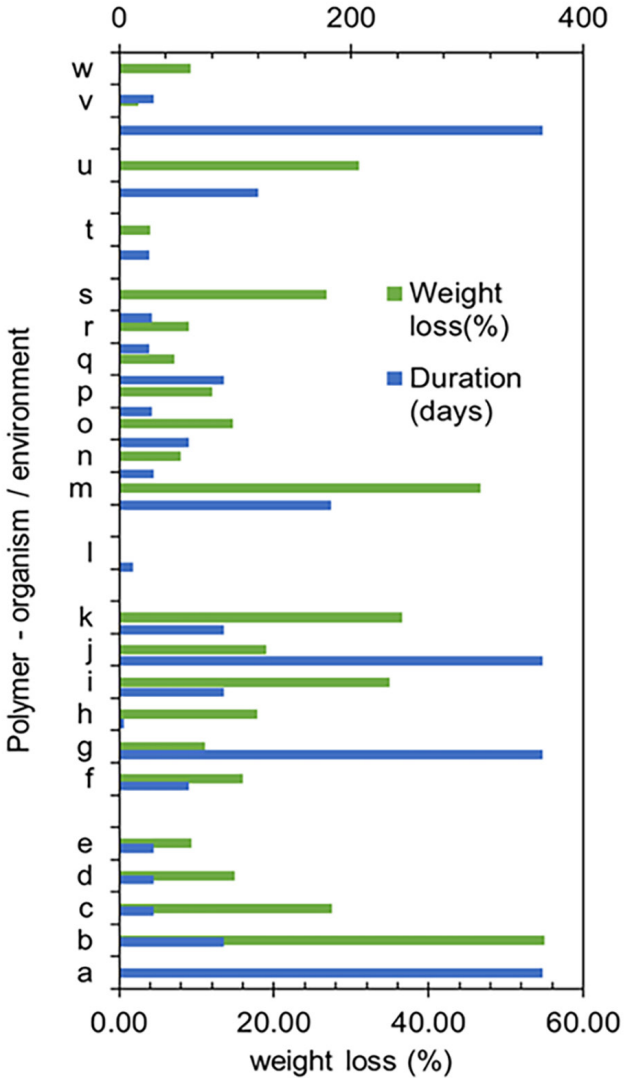
SEM image analysis provided by various researchers across the globe provide only qualitative confirmation of biodegradation phenomena. The analyses were based on the observation of modification on the surface morphology of polymer film subjected to biodegradation.

The change in surface morphology could not be distinguished whether due to biodegradation or due to physical disintegration due to aging. SEM micrographs obtained by Govindan et al. [77] indicated the formation of groove by the microorganisms on the surface of the polymer. SEM analysis has failed to provide quantitative accountability of degradation procedure and provided no information regarding products formed during degradation. Surface rupture, crack formation, presence of body marks due to microbial degradation on polymeric surface are reported by various researchers on different scale of magnification [9], [67], [79–82]. There are no evidences of quantitatively estimating the amount of polymer biodegradation using SEM analysis. SEM micrographs of LDPE film biodegraded using *Spingobacterium moltivorum* revealed that biodegradation follows colonization by microorganisms, biofilm formation and development of cracks and holes on the surface of LDPE sheets [13]. Similar SEM images are reported by various researchers in their studies. In case of natural degradation only minor cracks have developed on the surface of LDPE.

5.2 Amount of polymer degraded

The amount polymer degraded by various microorganisms are quantitatively reported using weight loss measurement by different researchers. Balasubramanian et al. [26] quantified biodegradation of HDPE film by *Arthobacter sp*. and *Pseudomonas sp*. through weight loss method. The authors emphasized that on 30 days of observation, *Pseudomonas sp*. and *Arthobacter sp*. degraded plastics by 15% and 12% respectively. Arkatkar et al. [28] observed 2.5% weight loss of UV-pre-treated polypropylene films by B. flexus species after 12 months of degradation. Chamas et al. [83] predicted half-life of single-use plastic bottles as 58 years under natural condition considering various environmental factors. Bhatia et al. [66] have observed 17.8% of degradation of LDPE happened by *Pseudomonas citronellolis*. Vimala and Mathew [19] witnessed 9.26 % degradation of pretreated polyethene films in period of 30 days by *Bacillus subtilis*.

The amount of weight loss reported by various researchers observed during their respective duration of study is depicted in Fig. 5. Weight loss studies of HDPE, LDPE, PP, polyester polyurethane and pretreated materials are reported. As some of the researchers did not specify their polymer as HDPE or LDPE, they were mentioned as polyethylene. In order to estimate the highest rate of degradation, a parameter called percent weight



Note :

w	Pretreated PE-*Bacillus subtilis*
v	UV Pretreated PP-*Bacillus flexus*
u	PP-*g*- gelatin composite-Soil burial
t	PP- *Lysinibacillus* species JJY0216
s	PE-Acinetobacter pitti
r	PE- *Lysinibacillus* species JJY0217
q	PE-*Pseudomonas fluorescens*
p	PE-*Aspergillus Japonicus*
o	PE-*Bacillus* Species and *Paenibacillus* Species
n	PE-*Rhodococcus Ruber* C208
m	PE-*Streptomyces* species
l	Polyester Polyurethane-*Pseudomonas chlororaphis* ATCC55729
k	LDPE-(*Penicillium oxalicum* NS4 and *Penicillium chrysogenum* NS10
j	LDPE-*Bacillus sphericus*
i	LDPE-*Aspergillus Clavatus* Strain JASKI
h	LDPE-*Pseudomonas citronellolis* strain EMBS027
g	LDPE-Compost
f	LDPE-*Bacillus amyloliquefaciens* BSM 2
e	HDPE with mineral oil-*Aspergillus lavus* VRKPT2
d	HDPE-*Pseudomonas* species
c	HDPE-*Bacillus aryabhattai*
b	HDPE-*Penicillium oxalicum* NS4 and *Penicillium chrysogenum* NS10
a	HDPE-*Bacillus sphericus*

Fig. 5 Amount of weight loss (%) reported by various researchers

loss/day (Pwl / day) is calculated and mentioned in Table 2. The highest Pwl/day of 4.45 was observed in the study carried out by Bhatia et al. [66] on LDPE using *Pseudomonas citronellolis* strain EMBS027. The rate of degradation could not be decided only based on Pwl/day. It depends on the environmental condition and nature of polymer used in the study. Weight loss studies are effective only if the polymers are in film or strip form. In case of powdered samples, sample recovery itself is difficult. One of the major shortfalls of weight loss technique in biodegradation procedure is that losses due to fragmentation or disintegration are considered as biodegradation.

6 Role of soil

Soil encompasses enormous number of microbial species that are aggressive towards polymer degradation. Soil or soil with compost burial technique is widely used for biodegradation of plastic waste due to its similarity with actual field disposal [38]. Table 3 gives lists of plastic polymers that were being attempted by various researchers to degrade in the soil environment. Earlier studies conducted on polypropylene (PP), polyethylene (PE), plasticized PVC and other degradable food packaging materials in soil indicated that among nondegradable materials, plasticized PVC are more degradable in the soil medium compared to PP and PE materials [36]. Kathiresan [22] attempted to biodegrade polythene bags and plastic cups in different locations on mangrove soil. The degradation was measured in terms of weight loss. Similar amount of microbial community was reported from the degrading materials at either location. The degradation of polythene has just started after a period of 6 to 9 months and that of plastic cups has commenced after 9 months. The polymer degradation was observed only under natural existing field condition in mangrove soil.

Table 2 Polymers-microorganisms/environment and their Pwl/day ratio

S.No.	Literatures	Polymer-Organism/ Environment	Pwl/day
1	[83]	HDPE-*Bacillus sphericus*	0.02
2	[84]	HDPE-*Penicillium oxalicum* NS4 and *Penicillium chrysogenum* NS10	0.61
3	[9]	HDPE-*Bacillus aryabhattai*	0.92
4	[26]	HDPE-*Pseudomonas* species	0.50
5	[85]	HDPE with mineral oil-*Aspergillus* lavus VRKPT2	0.31
6	[31]	LDPE-*Bacillus amyloliquefaciens* BSM 2	0.27
7	[38]	LDPE-Compost	0.03
8	[66]	LDPE-*Pseudomonas citronellolis* strain EMBS027	4.45
9	[32]	LDPE-*Aspergillus Clavatus Strain* JASKI	0.39
10	[86]	LDPE-*Bacillus sphericus*	0.05
11	[84]	LDPE-(*Penicillium oxalicum* NS4 and *Penicillium chrysogenum* NS10	0.41
12	[23]	Polyester Polyurethane-*Pseudomonas chlororaphis* ATCC55729	0.00
13	[59]	PE-*Streptomyces* species	0.26
14	[39]	PE-*Rhodococcus Ruber* C208	0.27
15	[89]	PE-*Bacillus* species and *Paenibacillus* species	0.25
16	[30]	PE-*Aspergillus Japonicus*	0.43
17	[87]	PE-*Pseudomonas fluoresces*	0.08
18	[10]	PE-*Lysinibacillus species* JJY0217	0.35
19	[13]	PE-*Acinetobacter pitti*	0.96
20	[10]	PP-*Lysinibacillus* species JJY0216	0.15
21	[27]	PP-*g*-gelatin composite-Soil burial	0.26
22	[28]	UV pretreated PP-*Bacillus flexus*	0.01
23	[19]	Pretreated PP-*Bacillus subtilis*	0.31

Table 3 Soil and plastic polymer

Type of soil	Plastic Polymer	Literature
Sandy loam	Various commercial plastics	[36]
Mangrove soil	Various commercial plastics	[22]
Soil + 50% compost	HDPE, LDPE and starch blend polyethylene	[53]
Soil compost	HDPE and LDPE	[38]
Montmorillonite clay	Oxo degradable polyethylene	[24]
Mangrove sediment and beach sand	Commercially available plastic bag	[88]

Bioremediation techniques such as biostimulation and bioaugmentation were not performed in the mangrove soil in order to enhance microbial degradation further. Seven species comprising of bacteria and fungi were isolated from mangrove soil and separately allowed to degrade plastic and polythene material under laboratory condition in nutrient medium. The rate of degradation of polythene reported after a period of observation was as high 20.54% to 28.8% in case of *Pseudomonas* and *Aspergillus* species. Plastic degradation was comparatively lower even under laboratory condition. Researches performed by Ardali et al. [53] using soil burial technique demonstrated that HDPE films are less vulnerable to microbial attack than LDPE and starch blend polyethylene. The study was performed on soil enriched with 50% compost obtained from municipal solid waste. Vijaya and Reddy [38] reported 2.9 to 4.5 % weight loss of HDPE and 10.5 to 11.6% weight loss of LDPE films in the compost environment after 4 months of burial. Reddy et al. [24] introduced montmorillonite clay mineral in the polymeric chain of oxo degradable polyethylene and reported accelerated degradation in the biotic phase. Shovitri et al., [88] found out that in mangrove soil biodegradation is effective even without bioaugmentation which is contradictory to the observation of Kathiresan [22].

7 Risks due to microbial migration

Transformation of bioremediation technique to field also demands study on the effect of transportation of microbial population to ground and surface water. Pathogenicity of selected microbe has to be studied. Vertical movement of the microbes can be studied using undisturbed and disturbed

soil column qualitatively in the laboratory [52]. The authors also summarized the optimal conditions for microbial survival such as wet soil favors microbial growth than dry soil. Presence of organic matter increases the survival and possible growth of microbial community. Smaller particle size such as those of clay minerals favored the growth of microorganism. Microorganisms survived better at sorbed condition. Sterile soil supported the growth of new strains being introduced due to the absence of native species. Antibiotics and toxic substances rendered unsuitable condition for microbial growth. The best time of application for microbes on soil are winter and spring in tropical regions like India. Ionic concentration of soil is also found to influence the microbial population. In sand or gravel, the ratio of mean grain size to the size of microorganism is greater than 1000. Hence the microorganisms are not retained. Advection is one of the predominant mechanisms that influences microbial transport than dispersion or diffusion.

8 Research gap

In most of the literatures potential polymer degrading microorganisms (PPDMs) are identified and isolated from soil and grown under laboratory condition and rate of degradation is studied. In some of the literatures [22] polythene and other plastic waste are buried in the soil and the rate of degradation in the natural environment was measured. Biodegradation of polyethylene by potential species in landfill environment was one of the gaps identified in the literature for further study. Bio stimulation of landfill in order to support the micro-organism involved in degradation process, bioaugmentation of landfill soil with PPDMs, their survival and rate of degradation further are not yet studied. The role of soil that supports the degradation phenomena has to be studied. The study on geotechnical and geo-environmental aspects of soils polluted by plastics are limited. The behaviour of polymer polluted soil after bioremediation from geotechnical and geo environmental aspect has to studied.

9 Conclusions

The present study forms a technical review on the state-of-the-art developments and methods followed in testing procedures in assessing biodegradation of plastic by soil. Most of the identified PPDMs belong to *Pseudomonas*, *Bacillus* and *Aspergillus* genera of bacterial and fungal origin. Numerous laboratory investigations were used to confirm biodegradation of plastic polymer. The maximum amount of weight loss of plastic polymer observed by researchers is around 40–50% within their study period. The amount of disintegrated plastic can only be identified in weight loss technique and it may not account for complete mineralization of plastic waste. In case of advanced analytical instruments, a very small amount of 2–5 mg of sample has been used. If the plastic particles are subjected to any form of degradation in soil, the sample collected after certain period of time is highly heterogeneous in nature. Smaller quantity of samples considered in sophisticated analysis will not account for heterogeneity of sample. Homogenization of soil-plastic mixture may alter the true nature of sample.

FTIR analysis qualitatively indicated the oxidized intermediates formed during degradation. Morphological changes and microbial attacks could be qualitatively witnessed in SEM micrographs. The process of extraction of plastics from treated plastic polluted soil has to be regulated for quantification studies. Carbon dioxide evolution test proved to be a useful tool for quantifying biodegradability. But the amount of CO_2 evolved during biodegradation of petroleum-based polymer is very less. High precision infrared-based CO_2 gas sensor may be used for real time measurement of CO_2 evolved.

Acknowledgement

The review work presented in this article is supported by National Institute of Technology, Warangal, India and VNR Vignan Jyothi Institute of Technology, Hyderabad, India.

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