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Immobilized Enzyme/Microorganism Complexes for Degradation of Microplastics: A Review of Recent Advances, Feasibility and Future Prospects

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Abstract

Environmental prevalence of microplastics has prompted the development of novel methods for their removal, one of which involves immobilization of microplastics-degrading enzymes. Various materials including nanomaterials have been studied for this purpose but there is currently a lack of review to present these studies in an organized manner to highlight the advances and feasibility. This article reviewed more than 100 peer-reviewed scholarly papers to elucidate the latest advances in the novel application of immobilized enzyme/microorganism complexes for microplastics degradation, its feasibility and future prospects. This review shows that metal nanoparticle-enzyme complexes improve biodegradation of microplastics in most studies through creating photogenerated radicals to facilitate polymer oxidation, accelerating growth of bacterial consortia for biodegradation, anchoring enzymes and improving their stability, and absorbing water for hydrolysis. In a study, the antimicrobial property of nanoparticles retarded the growth of microorganisms, hence biodegradation. Carbon particle-enzyme complexes enable enzymes to be immobilized on carbon-based support or matrix through covalent bonding, adsorption, entrapment, encapsulation, and a combination of the mechanisms, facilitated by formation of cross-links between enzymes. These complexes were shown to improve microplastics-degrading efficiency and recyclability of enzymes. Other emerging nanoparticles and/or enzymatic technologies are fusion of enzymes with hydrophobins, polymer binding module, peptide and novel nanoparticles. Nonetheless, the enzymes in the complexes present a limiting factor due

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to limited understanding of the degradation mechanisms. Besides, there is a lack of studies on the degradation of polypropylene and polyvinyl chloride. Genetic bioengineering and metagenomics could provide breakthrough in this area. This review highlights the optimism of using immobilized enzymes to increase the efficiency of microplastics degradation but optimization of enzymatic activities and synthesis of immobilized enzymes are crucial to overcome the barriers to their wide application.

Keywords: Enzymes, microplastics, nanoparticles, immobilization, complexes, synthesis

1. Introduction

Oil and gas are often used for energy production and heating as well as transportation purposes. Nevertheless, a small margin of natural gas or petroleum concentrates on the production of plastics globally. Apart from the 2008 global financial crisis and the recent COVID-19 pandemic in 2020, the global plastic production has been growing in an upward exponential trajectory since the 1950s (Ritchie and Roser, 2018) with annual increment rate of 8.7% (Rai et al., 2021). The application of plastics ranges from packaging to building constructions and textiles, among others, attributed to the ever-increasing demands from the consumers (Wong et al., 2020). In 2015, the plastic production for the building and construction sector amounted to 65 million tonnes while packaging sector contributed 146 million tonnes (Ritchie and Roser, 2018).

Furthermore, the use of plastics in textile industry is mostly seen in clothing and carpeting products surges as demands for synthetic fibers such as polyethylene terephthalate (PET) to replace natural fibers increases. This is largely ascribed to the significant farmland required to cultivate cotton and wool, hence shifting towards greater demand in synthetic fibers instead (Tang, 2020). At present, 60% of textiles are synthetic and derived from

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polyester, acrylic (polyacrylonitrile) and nylon (polyamide) (Sait et al., 2021). Studies have shown plastic fibers to shed during domestic washing, which results in subsequent release of microplastic fibers into waterways and wastewater treatment plants (Tang and Hadibarata, 2021). This has led to the discharge of microplastic fibers into aquatic environment which ranges between 1 x 10^5 and 1 x 10^7 particles daily (Freeman et al., 2020). As frequently defined, microplastics are plastic debris less than 5 mm in length (Wong et al., 2020).

Natural degradation of plastics in the environment consumes relatively long period of time ranging from 50 to more than 100 years (Choong et al., 2020). For comparison, PET requires approximately 450 years to degrade while low density polyethylene (LDPE) and high-density polyethylene (HDPE) require up to 600 years to degrade naturally (Ojeda, 2013). Plastics can be widely categorized into two groups – those solely made up of carbon-carbon backbone and plastics consisting of heteroatoms in the main chain (Mohanan et al., 2020). The latter is easily susceptible to hydrolytic attacks to the ester or amide bonds via enzymatic hydrolyzation (Müller et al., 2001). In recent years, research on biodegradation and depolymerization of plastic into monomers has been heavily focused and enzymatic degradation has proven to be a promising strategy.

Conventionally, biodegradation is the degradation of a specific material into products that are benign to the environment. As such, biological catalysts, namely enzymes, promote biodegradation phenomenon to derive the term of "enzymatic degradation". In this context, enzymatic degradation breaks down complex plastic polymers into carbon dioxide, water and biomass while potentially produce high value bio-products contemporaneously (Montazer et al., 2020). Nevertheless, biodegradation pathways are complex and depend extensively on a number of variables, e.g. availability of substrates, surface characteristics, morphology and physicochemical properties of the polymer structures. In addition, biodegradation mechanisms of plastics, especially petro-plastics, are dependent on the types of bonds in the

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aforementioned polymeric chains. The plastic-degrading enzymes can be divided into two main groups which are extracellular enzymes and intracellular enzymes (Gu, 2003). According to Glaser (2019), extracellular enzymes are the most commonly used plastic-degrading enzymes as they have a broader range of reactivity ranging from oxidative to hydrolytic functionality. Extracellular enzymes are primarily engaged in the depolymerization of long carbon chains in polymers to oligomers, dimers, and occasionally monomers. It is said that these various groups of enzymes demonstrate close behavior such as microbial laccases, peroxidases, lipases, esterases and cutinases (Gan and Zhang, 2019). Additionally, the extracellular enzymes are said to be engaged in heterogeneous reactions either in the solid or liquid boundary. This is associated to the macromolecules accessible at solid plastic's surface and exist in the liquid phase (Chinaglia et al., 2018). The surface functionalization of hydrophobic plastic surfaces, the breakdown of the plastic metabolic intermediates into monomeric units, and the ultimate mineralization of the final monomeric intermediates are all mediated by different enzyme groups. The aerobic and anaerobic processes required to convert intermediates to molecules that may be ingested by bacteria are carried out by a massive amount of intracellular enzymes (Pathak and Navneet, 2017). Amobonye et al. (2021) had conducted a critical review on the plastics-degrading enzymes acted on various polymers such as polyethylene (PE), polyurethane (PU), PET, polystyrene (PS) and nylon. The actinomycetal, bacterial and fungal sources (hydroxylases, laccases, peroxidases and reductases) are the examples for PE-degrading enzymes (Amobonye et al., 2021). For PU-degrading enzymes, they can be derived from bacterial and fungal sources, such as utinases, esterases, lipases, laccases, peroxidases, proteases and ureases (Amobonye et al., 2021). For PET-degrading enzymes, they can be from *Thermobifida fusca* hydrolases (Müller et al., 2005), *Streptomyces scabies* enzymes (cutinases, esterases) (Jabloune et al., 2020), lipases and carboxylesterases (Danso et al., 2019; Jabloune et al., 2020; Ru et al.,

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2020). For PS-degrading enzymes, it is still unclear on main enzymes involved in the beginning stage of the depolymerization. Nonetheless, Tahir et al. (2013) had successfully demonstrated the breakdown of PS via extracellular esterase from *Lentinus tigrinus*. Besides, it was also reported that *Bacillus and Pseudomonas* species could potentially degrade PS (Mohan et al., 2016). Lastly, for nylon-degrading enzyme, the manganese peroxidase from a white-rot fungus (Deguchi et al., 1998), and hydrolases in *Flavobacterium* and *Pseudomonas* strains (Negoro, 2000) had successfully broken down the nylon.

This article provides a systematic and didactic review of the most recent research on the unique use of immobilized enzyme/microorganism complexes for microplastics degradation, as well as its practicality and future prospects, spanning the years from 1998 to 2021. It is found that most studies focused on the effectiveness of the immobilized enzyme/microorganism complexes on the microplastics biodegradation via the formation of the photogenerated radicals to accelerate the process of the polymer oxidation, enhance the bacterial consortia growth for biodegradation, anchoring enzymes, promote stability and absorbing water for hydrolysis process. While we identified more than 100 peer-reviewed scholarly papers in our search, only one peer-reviewed scholarly paper discovered that the antimicrobial property of nanoparticles slowed the growth of microorganisms (Milošević et al., 2017). Thus, we believe that this review which presents on the recent advances and feasibility studies made in the degradation of synthetic microplastics via enzymatic complexes can provide useful information and guidance in driving industrial application decision specifically on the enzymatic technology for microplastic degradation. In addition, future perspectives based on the genetic bioengineering and metagenomics could potentially provide breakthrough to advance the microplastics degradation using novel nanoparticle-enzyme technology. Some essential recommendations have been suggested in order to unleash the potential of enzymatic technology for the breakdown of microplastics.

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2. Inorganic nanoparticle-enzyme/microorganism complexes

Inorganic nanoparticle has been used to form complexes with enzymes to enhance degradation of microplastics by functioning as a support via immobilization. Inorganic nanoparticle is an emerging material that is gaining large recognition and attention in this avenue due to its thermal, mechanical and chemical stability in comparison to their organic counterparts (Hartmann and Kostrov, 2013). Additionally, inorganic nanoparticle exhibits key advantages as support in comparison to other materials, which include a well-defined pore geometry and distribution, high surface area and large surface-to volume ratio, which vastly improve the loading capacity of enzyme (Zhou et al., 2013). Non-porous inorganic nanoparticles, such as silica, have been reported to experience no external diffusion issues, which promotes the competitive edge in commercial application that involves solid-liquid system (Vaghari et al., 2016). Moreover, inorganic nanoparticle with transition metal is easily functionalized, which serves as a good binding site for various catalytic species (Govan and Gun'ko, 2014). The presence of metallic ions in nanoparticles has also been reported to augment large conformational change in an enzyme, which reserves the substrate-binding grooves to remain in an open conformation condition while enhancing the enzyme to bind on the substrate (Samak et al., 2020). The outlined positive characteristics enable formation of inorganic nanoparticle-enzyme complexes with advantages in industrial application, which include stability towards harsh reaction conditions, convenience in handling, retaining or even improving the catalytic activity, reusability, specificity and selectivity of enzymes (Cao, 2005).

Another way in which enzymes can coexist with inorganic nanoparticles is through the presence of microorganisms, which secrete an abundant quantity of enzymes that participate in enzymatic degradation. It has been reported that nanosized metal particles and their oxides have positive, adverse and inhibitory effects on the growth of microorganisms

(Benckiser, 2019). The effects of metal nanoparticles and their different forms on the growth of a broad spectrum of microorganisms have been reported in detail (Benckiser, 2019). Simultaneously, enhanced microbial degradation of microplastics by enzymatic processes has been demonstrated (Othman et al., 2021). Hence, by properly supplementing microbial degradation with the correct inorganic nanoparticle-enzyme complexes, bacterial growth profile can be augmented, which further improves the biodegradation ability of microplastics. In addition, several possible proposed mechanisms through which inorganic nanoparticles can boost degradation of microplastics include enhancement of enzyme adsorption, neutralization of acidic by-products from hydrolysis that are detrimental to microbial growth, improvement in effective contact area, ease in functionalization that augments enzyme activity, creation of photogenerated radicals and introduction of hygroscopic characteristics. Figure 1 provides a schematic illustration of the different routes available for biodegradation of plastic monomers with the presence of inorganic nanoparticle.

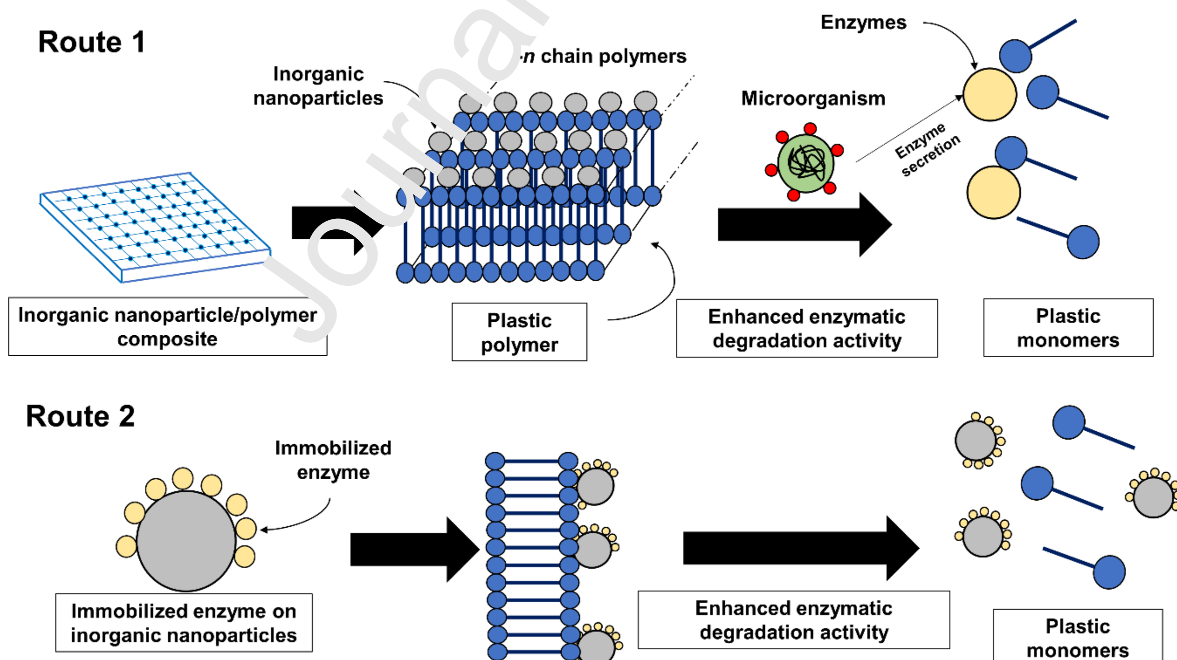

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Figure 1: A schematic illustration of the degradation of plastic polymers via inorganic nanoparticle/polymer composite (Route 1) and immobilized enzymes on inorganic nanoparticle (Route 2)

Recent advances of developing and elucidating the different inorganic nanoparticles-enzyme complexes at various operating conditions for biodegradation of microplastics have been reported and summarized in Table 1 with their respective efficiency, recyclability or stability and proposed mechanisms.

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Table 1: Inorganic nanoparticle-enzyme/microorganism complexes for degradation of microplastics

Inorganic Nanoparticle	Enzyme/ Microorganism	Microplastics	Complexes	Mechanism	Biodegradation Operating Conditions	Efficiency	Retention	Recyclability	Reference
Titanium dioxide (TiO_2)	Lipase	PBS	Polymer/nanoparticle composite incubated with enzyme	Creation of photogenerated radicals to oxidize polymer by photocatalytic oxidation during enzymatic reaction.	• Nanoparticle concentration: 5 wt. % • Nanoparticle size: 7.0 (ST-01) and 30 nm (P-25) • Media: phosphate buffer saline • Temperature: 27 °C • pH: [illegible]8 RPM: [illegible]000 • Light source: UV radiation	Positive: Improvement in biodegradation from ~0.6 to 0.85 mg/cm[illegible] weight lost with presence of TiO_2 under simultaneous treatment of enzyme and UV irradiation	Not available (NA)	NA	(Miyauchi et al., 2008)
Nanobarium titanate (NBT)	*Microbacterium* species strain *MK3*, *Pseudomonas putida* strain *MK4* and *Bacterium Te68R* strain *PN12*	LDPE	Nanoparticle incubated with polymer and microorganism	• Accelerated growth of bacterial consortia used in biodegradation. • Inclusion of hydroxyl residues into hydrocarbon chain that increases solubility.	• Nanoparticle concentration: 0.01% • Nanoparticle size: 38.0 nm • Media: Davis minimal broth • Temperature: 37 °C • RPM: 150	Positive: Enhancement in growth of biodegradation assays from 4 to 3 days incubation via λ-max shifts, supported with shift in FT-IR and TG-DTG-DTA spectra that confirms augmented biodegradation.	NA	NA	(Kapri et al., 2009) (Kapri et al., 2010a)
Superparamagnetic iron oxide nanoparticles (SPION)	*Microbacterium* species strain *MK3*, *Pseudomonas putida* strain *MK4* and *Bacterium Te68R* strain *PN12*	LDPE	Nanoparticle incubated with polymer and microorganism	• Accelerated growth of bacterial consortia used in biodegradation. • Inclusion of hydroxyl residues into hydrocarbon	• Nanoparticle concentration: 0.01% • Nanoparticle size: 10.6, 20.0 and 37.8 nm • Media: Davis minimal broth • Temperature: 37 °C • RPM: 150	Positive: Enhancement in growth of biodegradation assays from 4 to 2 days incubation via λ-max shifts with 10.6 nm particle size, supported with shift in FT-IR and	NA	NA	(Kapri et al., 2010a) (Kapri et al., 2010b)

				chain that increases solubility.		TG-DTG-DTA spectra that confirms augmented biodegradation.			
Functionalized mesostructured silica (SBA-15)	Laccases from *T. versicolor sp.*	Naphthalene used in manufacture of plastics	Immobilization via covalent bonding	Heterogenous catalyst has lower activity than free enzyme as homogeneous catalysis system	• Media: acetate buffer • Temperature: 27 °C • pH: 4.5	Negative: Covalently attached laccase to aminopropyl (AP1) and aminobutyl (AB1) functionalized SBA-15 exhibit reduced activity for the degradation of naphthalene with 35 and 39% of removal in 5 hrs as compared to the control with 65%.	NA	AP1/Laccase keeps its initial activity for a second catalytic run but shows a decrease over 30% in the third cycle while AB1/Laccase experiences a dramatic decay of activity in the second use and almost completely losing it in the third reutilization.	(Bautista et al., 2010)
Titanium dioxide (TiO_2)	α-amylase from *Bacillus subtilis*	PLA	Polymer/nanoparticle composite incubated with enzyme	Presence of nanoparticles hinder diffusion of enzyme to the polymer for effective biodegradation.	• Nanoparticle concentration: 0.5, 1, 2, 5 and 10 wt.% • Nanoparticle size: 21 nm • Media: α-amylase solution • Temperature: 37 °C	Negative: Slight improvement in polymer weight loss from approximately 6.9 wt.% to 7.5 and 7.1 wt.% during the first 80 hrs in 0.5 and 1 wt.% TiO_2-polymer in enzyme. At 120 hrs, the percentage weight loss is highest (~10 wt.%) for pristine polymer and comparable with 0.5 wt. % TiO_2.	NA	NA	(Buzarovska and Grozdanov, 2012)

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Stearate-zinc aluminium lactate dehydrogenase (Zn_3Al LDH)	*Aspergillus niger* and *Aspergillus flavus*	PLA	Polymer/nanoparticle composite incubated with enzyme	• Presence of hydroxyl groups that absorbs water to initiate hydrolysis of polymer. • Inherent faster degradation rate of stearate groups in the nanocomposite as compared to pristine matrix.	• Nanoparticle concentration: 1, 3, 5, 7 and 10 wt.% • Nanoparticle size: 40.1 Å • Media: soil • Temperature: 20 - 30 °C • RH: 80%	Positive: Enhancement in polymer weight loss from 5 to 40% with 10wt% Stearate-Zn_3Al LDH	NA	NA	(Eili et al., 2012)
Titanium dioxide (TiO_2) sol-gel coated polyvinylidene fluoride (PVDF) membrane	Laccase from *Trametesversicolour (EC 1.10.3.2)*	BPA used in the production of PC	Immobilization via physical adsorption for the uncoated bio-catalytic membrane and covalent bonding for the coated counterpart	• Enhancement in enzyme stability required for biodegradation • Carriers for enzyme as active site for polymer degradation via large specific surface area and low steric hindrance.	• Membrane pore size: 0.1 and 0.45 µm • Nanoparticle concentration: ○ 0.1 µm membrane: 1.9 (cycle 1), 3.4 (cycle 2), 5.9 (cycle 3) and 7.4 wt.% (cycle 4) ○ 0.45 µm membrane: 1.3 (cycle 1), 2.0 (cycle 2), 5.0 (cycle 3) and 4.7 wt.% (cycle 4) • Media: acetate buffer • Temperature: room • pH: 5.5	Positive: Increment in biodegradation. In the case of 0.1 µm membrane, processing with 3 coatings exhibit highest BPA removal of ~90%, while with respect of 0.45 µm membrane, highest BPA removal of ~90% is achieved in structure with 4 coating cycles. Regardless of the membrane pore size, both exhibit higher removal as compared to the control (membrane with 1 nanoparticle coating cycle) by reduction of only 8% after 24 hrs.	NA	90% to 85% after 4 degradation runs for 0.1 µm membrane processing with 3 coatings but 90% to 40% for 0.45 µm membrane with 4 coating cycles.	(Hou et al., 2014b)

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Cu (II) and Mn (II) chelated magnetic microspheres	Laccase from *Trametes versicolor*	BPA used in the production of PC	Immobilization via physical adsorption	• Enhancement in enzyme stability required for biodegradation • Carriers for enzyme as active site for polymer degradation via large specific surface area and low steric hindrance.	• Media: phosphate buffer • Temperature: 5, 20, 35, 50 and 65 °C • pH: 3-8.0	Positive: > 85% removal efficiency in 12 hrs. Optimal operating conditions at pH = 5 and 35 ℃	• Higher thermal retention of 60% and 38% of initial activity as compared to free enzyme with only 30% and 0% after 55 and 65 ℃ heat treatment. • Higher storage retention of 62% of initial activity after 14 days as compared to free enzyme with 17%.	Activity does not significantly change during the adsorption–desorption cycles with only loss of approximately 10% of its original capacity after five usages.	(Lin et al., 2016)
3-aminopropyltriethoxysilane (APTES) and glutaraldehyde (GLU) functionalized titania (TiO_2)	Laccase from *P. ostreatus*	BPA used in the production of PC	Immobilization via covalent bonding	• Enhancement in enzyme stability required for biodegradation • Carriers for enzyme as active site for polymer degradation via large specific surface area and low steric hindrance.	• Media: citric acid-phosphate buffer (pH 2.5-5) or phosphate buffer (pH 6-7) • Temperature: 25-70 ℃ • pH: 2.5-7	Positive: > 90% removal efficiency at pH = 5 and 25 ℃ with 0.4 U/mL immobilized enzyme after 6 hrs.	• Higher thermal retention of 85% and 20% of initial activity as compared to free enzyme with only 50% and 0% after 50 and 70 ℃ heat treatment for 2 hrs. • Higher storage stability of 75% initial activity after 30 days as compared to free enzyme with 0%	50% of the initial enzymatic activity after 5 cycles in comparison to the control with >75 % lost.	(Ji et al., 2017)

							after 23 days. • Higher retentate with changing pH of almost 99% of initial activity as compared to free enzyme with 82% at pH = 5.		
Silver (Ag)/ Titanium dioxide (TiO_2)	Cellulase from *Trichoderma reesei*	Cotton (Co) /PET	Co/PET impregnated with composite incubated in enzyme	• Antimicrobial property of Ag and TiO_2 hinders colonizing of microorganisms and hence, decomposition of the material.	• Nanoparticle size: 60 Å (TiO_2) • Media: acetate buffer • Temperature: 50 °C • pH: 7.4	Negative: Although slight discrepancies have been obtained by the different test methods, the presence of Ag/TiO_2 is found to hamper degradation of cotton/PET (E.g., weight loss of Co + Ag/TiO_2 fabric is 22% lower than control Co; weight loss of Co/PET + Ag/TiO_2 fabric is 17.6% lower than control Co/PET with around 27% hydrolysis.	NA	NA	(Milošević et al., 2017)
Superparamagnetic Iron Oxide Nanoparticles (SPION)	Bacteria consortia, fungal consortia and mixed consortia	LDPE	Nanoparticle incubated with polymer and microorganism	• Accelerated growth of microorganism consortia used in biodegradation.	• Nanoparticle concentration: 0.1, 0.25, 0.5, 0.75 and 1% • Nanoparticle size: 10.6 nm • Media: Luria-Bertani (LB) broth and agar (bacteria),	Positive: Enhancement in polymer weight loss from 45 to 55% with mixed consortium. Optimum metal nanoparticle concentration has	NA	NA	(Patel et al., 2020)

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					Potato dextrose broth (PDB) and potato dextrose agar (PDA) as suspension and platting and striking culture (fungal), nutrient agar and LB broth (mixed) • Temperature: 37 °C (bacteria), 32 °C (mixed) and 25 °C (fungal) • RPM: 120	not been reported.			
Silver (Ag)	*Aspergillus oryzae*	LDPE and HDPE	Nanoparticle incubated with polymer and microorganism	• Antibacterial potency and reduced toxicity on higher organism to assist fungal growth. • Creation of photogenerated radicals to oxidize polymer by photocatalytic oxidation during enzymatic reaction. • Enhancement in biodegradable area of polymer.	• Nanoparticle concentration: 1 % • Media: KH_2PO_4-7.0 g/l; K_2HPO_4-2.0 g/l; $MgSO_4.7H_2O$-0.1 g/l; $(NH_4)_2SO_4$-1.0 g/l; yeast extract-0.6 g/l; glucose-10.0 g/l • Temperature: room • RPM: 150 • Light source: Visible light	Positive: Enhanced degradation of ~11% in week 1 to 64% of LDPE and 44% of HDPE in 5 weeks	NA	NA	(Jayaprakash and Palempalli, 2019)
Iron oxide (Fe_3O_4)	Trypsin, cathepsin G (CAT-G), lactate dehydrogenase (LDH), aminotransferase (AST), acetylcholinesterase	PMA	Polymer shells around nanoparticle incubated with enzyme	• Detailed mechanism has not been fully understood since different enzymes	• Nanoparticle size: 4.4 nm • Media: phosphate buffer saline • Temperature: room	Positive: Increment in release of dye, nanoparticles, and polymer fragments as a result of biodegradation	NA	NA	(Zhu et al., 2019b)

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	(ACHE), and proteinase K			exhibit reaction specificities with varying enzymes-substrates. • Proposal that LDH may cleave the bond that only exists in backbone of Fe_3O_4 PMA-Prop-Coumarin, while AST may catalyse the release of dyes in Fe_3O_4 PMA-Dy605 and Fe_3O_4 PMA-Fur-Dy5.5	• pH: 7.4 • RPM: 700	observed via higher emission intensity. The highest difference as compared to control without nanoparticles has been observed using AST and FBS, while trypsin has demonstrated moderate efficiency.			
Mesoporous silica (MSU-F)	Stabilized laccase from *Trametes versicolor*	BPA used in the production of PC	Immobilization via cross-linking	• Enhancement in enzyme stability required for biodegradation • Carriers for enzyme as active site for polymer degradation via large specific surface area and low steric hindrance.	• Media: citric-phosphate buffer solution • Temperature: 5-40 °C • pH: 3, 5, 7, 8 • RPM: 150	Positive: Increment in biotransformation of 90.1% over adsorbed and free enzyme at 75.4% and 69.9% after 8-hr reaction. Optimal pH = 5.	• Higher thermal retention of 92.5%, 84.9% and 75.7% of initial activity as compared to free enzyme with only 86.9%, 75% and 51.2% at 5, 10 and 25 ℃. • Higher retentate with changing pH of 68.6%, 87.9%, 87.7% and 87.9% initial activity as compared to	Enzymatic activity at 100%, 100%, 64.3%, 52.9% after two to five times use and 50% after 6 times.	(Piao et al., 2019)

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							free enzyme with 22.5%, 37.5%, 60.1% and 19.7% at pH = 3, 5, 7and 8.		
Silica (SiO_2)	Laccase from *Trametes versicolor*	BPA used in the production of PC	Immobilization via covalent bonding	• Enhancement in enzyme stability required for biodegradation • Carriers for enzyme as active site for polymer degradation via large specific surface area and low steric hindrance.	• Media: sodium citrate buffer • Temperature: 50 °C • pH: 5 • Additive (e.g., Triton X-100 SDS, Tween 80, CTAB, [BMIM]Cl, [AMIM]Cl concentration: 0.1, 0.[illegible] and 1 mM	Positive: 85% and 100% removal after 1 hr and 5 hrs, respectively, as compared to the control of [illegible]0% and 80% in the presence of TX-100 additive.	Storage stability >75% for immobilized enzyme as compared to free enzyme with 8% for storage of 2 months period at 4℃and pH = 7.	Enzymatic activity at 92% 89% and 83% after 10, 20 and 30 cycles, respectively.	(Chang et al., 2019)
Cu-MOF (HKUST-1)	Laccase from *Trametes versicolor*	BPA used in the production of PC	Immobilization via one-pot encapsulation	• Enhancement in enzyme stability required for biodegradation • Carriers for enzyme as active site for polymer degradation via large specific surface area and low steric hindrance.	• Temperature: 30-70 ℃ • pH: 3-6.5	Positive: 100% degradation after 4 hrs at optimum pH = 6.5 and temperature 40 ℃in comparison to only 35.5% efficiency in control with free enzyme.	Higher storage retention with 70% of initial activity after 30 days storage as compared to free enzyme with only 10%.	88% and 75.9% of the initial enzymatic activity after 8 and 10 cycles, respectively.	(Zhang et al., 2020)
Magnetic iron oxide (MIO)	PETaseS238F/W159H from *E. coli BL21*	PET	Immobilization via His-tag	• Enhancement in enzyme stability required for biodegradatio	• Nanoparticle size: 10 nm • Media: 4-nitrophenyl-acetate (pNP-	Positive: Enhancement in biodegradation via higher monomers terephthalic acid	NA	• Minimal activity changes up to two cycles.	(Schwaminger et al., 2021)

				n • Carriers for enzyme as active site for polymer degradation via large specific surface area and low steric hindrance.	acetate) in 50 mM Tris buffer • Temperature: 30 °C • pH: 7.5 • RPM: 700	(TPA) concentration of 27 µM as compared to 15 µM in free enzyme control.		• 50% of the initial enzymatic activity after 10 cycles	
Silica (SiO_2) and SiO_2 coated magnetite ($Fe_3O_4@SiO_2$)	Lipase and cutinase	PCL	Immobilization via covalent bonding	• Enhancement in enzyme stability required for biodegradation • Carriers for enzyme as active site for polymer degradation via large specific surface area and low steric hindrance.	• Media: phosphate buffer saline • Temperature: ambient	Positive: Improvement of biodegradation with highest mass loss of 75.6% achieved in polymer incubated with 2 mg/mL $Fe_3O_4@SiO_2$-lipase as compared to 59.9% in SiO_2-lipase particles. Similarly, combination of both enzymes led to mass loss of 38% for $Fe_3O_4@SiO_2$ particles as compared to 18% for SiO_2.	80%, 35% and only 12% of initial enzymatic efficiency after 132, 144 and 132 hrs for 2 mg/mL $Fe_3O_4@SiO_2$-lipase, SiO_2-cutinase and $Fe_3O_4@SiO_2$-cutinase, respectively.	NA	(Krakor et al., 2021)
Cobalt phosphate ($Co_3(PO_4)_2$)	PET hydrolase (PETase) from *Ideonella sakaiensis*	PET	Immobilization via His-tag	• Enhancement in enzyme stability required for biodegradation • Carriers for enzyme as active site for polymer degradation via large specific surface area and low steric hindrance.	• Media: Acetate buffer (pH 4-6), phosphate buffer (pH 7-10) and borate buffer (pH 10-12) • Temperature: 20-60 °C • pH: 4-12	Positive: Degradation of 25.8% and 21.8% as compared to control with only 7.4% and 7,7% for plastic particles and film, respectively. Optimal operating conditions at pH = 7 and 45 ℃	• Higher thermal retention of 94.4% and 82.8% initial activity as compared to free enzyme with 57.3% and 17.2% after 40 and 45 ℃ heat treatment for 3 hrs. • Higher storage	• 70.2% of the initial enzymatic activity after 10 cycles.	(Jia et al., 2021)

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							stability of 75% of initial activity after 12 days at room temperature as compared to inactive free enzyme. • Higher retentate with changing pH of 67.6% and 62.3% of initial activity as compared inactive free enzyme at pH 10 and 4.		

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2.1. Synthesis of inorganic nanoparticle-enzyme/microorganism complexes

Majority of the works have synthesized inorganic-nanoparticle/plastic composites to enhance adhesion while incubating with enzymes or microorganisms. Titanium dioxide (TiO_2) has been added into poly (butylene succinate) (PBS) for enzymatic degradation with and without the presence of UV radiation (Miyauchi et al., 2008). Sheets of TiO_2/PBS composite prepared through melt-blending, high-speed rotation, extrusion and hot pressing were immersed in lipase-PS solution to decompose PBS with and without presence of simultaneous UV irradiation treatment. In a separate study, nano barium titanate (NBT) had been incorporated to alter the growth profiles of low-density polyethylene (LDPE) degrading consortia using *Microbacterium* species (Kapri et al., 2009; Kapri et al., 2010a). The NBT nanoparticle was pre-treated with sonification prior to incubation with LDPE and the consortia at controlled operating conditions. Furthermore, superparamagnetic iron oxide nanoparticles (SPION) had been prepared via coprecipitation of Fe(II) and Fe(III) chloride solutions to evaluate the feasibility in enhancing biodegradation of microplastic in a comparative analysis (Kapri et al., 2010a; Kapri et al., 2010b). In another similar work by Patel et al. (2017), they extended the study to elucidate the effect of altering the SPION nanoparticle concentration (e.g., 0.1, 0.25, 0.5, 0.75 and 1%) and using different consortia (e.g., bacteria, fungal and mixed) on the biodegradation of LDPE (Patel et al., 2020).

The biodegradability of poly-L-lactic acid (PLLA) has been elucidated using α-amylase enzyme with different weight percentages of TiO_2 as nanoparticles (Buzarovska and Grozdanov, 2012). The polymer and TiO_2 had been treated with ultrasonic radiation before being processed to nanocomposites using solvent casting method. Finally, the PLLA/TiO_2 composites had been successfully degraded with α-amylase solutions at 37 °C and 5 U mg^{-1} polymer. Eili et al. (2012) prepared stearate-zinc aluminium lactate dehydrogenase (stearate-Zn_3Al LDH) via co-precipitation method at pH 7.0 and ion exchange reaction, which was

incorporated into PLA to prepare the PLA/LDH nanocomposites using solution casting method. To study the biodegradability performance, samples were buried in soil with naturally present microorganisms placed in an uncovered gazebo with the temperature varying from 20 to 30 °C and a humidity above 80% to monitor the polymer weight loss. In another study, TiO_2 nanoparticles had been synthesized via acidic hydrolysis of titanium tetrachloride ($TiCl_4$) followed by impregnation into Co/ PET and in situ photoreduction of silver cations (Ag^+) (Milošević et al., 2017). Cellulase was used for elucidation of enzymatic hydrolysis via incubation at 50 ℃ and pH 5.0 while monitoring the PET weight loss. Ag was synthesized by Jayaprakash and Palempalli (2019) from reduction of silver nitrate ($AgNO_3$) using fungal at pH 6.1, room temperature and dark condition to avoid photosensitivity issue. LDPE and HDPE from plastic bags were used to evaluate efficiency of the 1% bio-synthesized Ag incubation for biodegradation study with a duration of 5 weeks. In the work of Zhu et al. (2019b), they incorporated iron oxide (Fe_3O_4) with many different enzymes to examine their impact on biodegradation of dodecylamine modified poly (isobutylene-alt-maleic anhydride) (PMA). Thermal decomposition reaction was performed to synthesize Fe_3O_4, while PMA had been obtained from solvent evaporation method, which was further treated for surface functionalization with furfurylamine and propagylamine. Subsequently, the Fe_3O_4 nanoparticles were coated with the modified PMA by hydrophobic interaction between the alkyl chains of the polymer and the surfactant molecules on the nanoparticles to induce hydrophilicity. In order to evaluate enzymatic degradation of the polymer coating, the Fe_3O_4 complexes have been incubated with the different aforementioned enzymes.

Recent advancements have also emerged to study enzyme immobilization on inorganic nanomaterials using varying methodologies to form a stable and reusable complex with enhanced performances, such as activity and specificity. Typical approaches for enzyme immobilization on inorganic nanomaterials to biodegrade microplastics are shown in Figure 2.

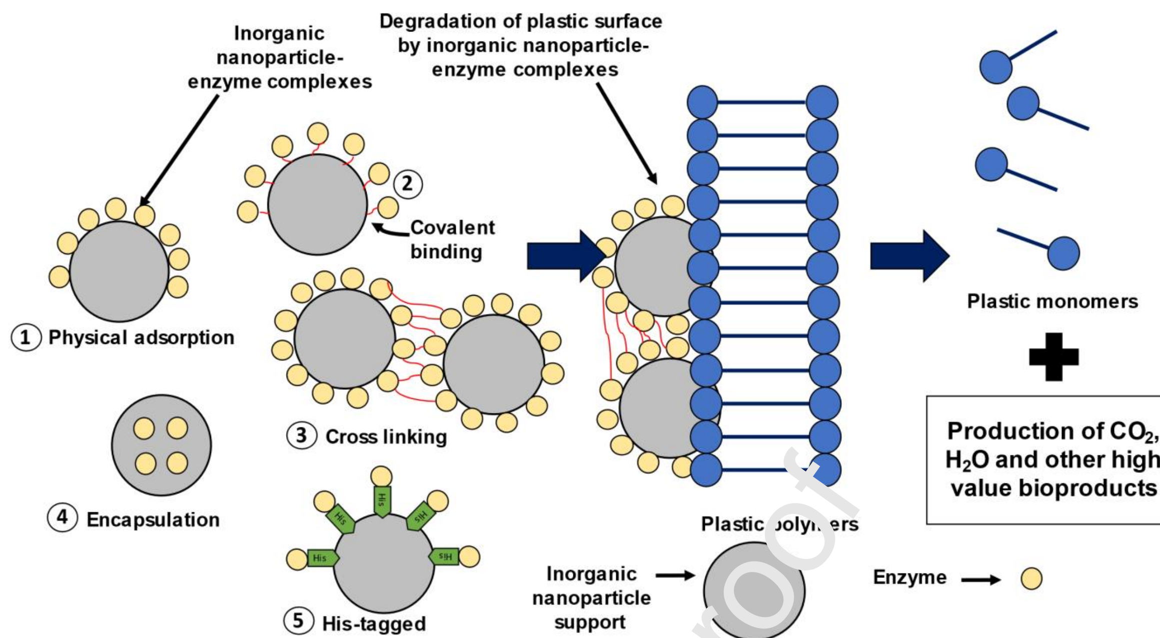

Figure 2: Different approaches for immobilizing enzymes on inorganic nanoparticles for biodegradation of microplastics

Persistent chemicals released from microplastic particles, such as naphthalene and bisphenol A (BPA) were subject of interest in this avenue due to its ease of preparation and testing. In an initial study by Bautista et al. (2010), siliceous SBA-15 was functionalized via co-condensation method (direct synthesis) to incorporate amino functional group with varying degree of organic functionalization into the inorganic material. Subsequently, immobilization had been achieved via covalent grafting of the enzyme to the silica surface with presence of grafted alkyl-amine moieties that act as anchoring sites. Efficiency of the immobilized enzyme on SBA-15 in terms of degradation of naphthalene that is applied in the manufacturing of polyvinyl chloride (PVC) plastics and reusability in different cycles had been elucidated. The enhancement effect of incorporating TiO_2 nanoparticle had been reaffirmed Hou et al. (2014) in which they demonstrated the bio-

Degradation with laccase immobilized on the TiO_2 functionalized polyvinylidene fluoride (PVDF) with pore sizes of 0.1 and 0.45 µm using sol–gel coating methodology on

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the membrane via a series of dip-coating cycles (Hou et al., 2014b). Then, bio-catalytic membrane had been fabricated by immobilizing laccase onto the uncoated PVDF via adsorption and coated membrane through 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde (GLU) sequential immobilization process to form covalent binding. BPA, which is a chemical commonly found in polycarbonate (PC) microplastic, had been used for degradation study by attaching the bio-catalytic membrane on a cell with observation of trans-membrane pressure and flux at room temperature and pH 5.5 while also elucidating its recyclability in degradation study. Magnetic (Fe_3O_4) particles had been produced from solvothermal reduction proceeded with chitosan (CS) coating via suspension cross-linking technique prior to chelating of Cu (II) and Mn (II) cations (Lin et al., 2016). Subsequently, laccase enzyme was reversibly immobilized onto the chelated Fe_3O_4 microspheres via adsorption. BPA degradation studies had been conducted using free and immobilized enzymes for comparison at varying operating conditions, such as pH and operating temperature, followed by interrogation of thermal retention, storage stability and reusability characteristics (Lin et al., 2016). Ji et al. (2017) in a similar study immobilized laccase on titania (TiO_2) nanoparticles via a sequential technique introduced earlier by Hou et al. (2014c). Briefly, TiO_2 nanoparticles were functionalized by APTES and GLU to induce strong covalent binding on the nanoparticle-enzyme complexes. Other than the thermal and storage stability with recyclability, they also extended the study to investigate effect of changing pH on retention of the enzyme activity. Piao et al. (2019) developed a high efficiency system for BPA removal in a fluidized bed reactor (FBR) through employment of stabilized laccase immobilized on mesoporous silica (MSU-F), which had been prepared via cross-linking of enzyme inside the porous inorganic material using GLU, similar to the study of Ji et al. (2017). On the other hand, Chang et al. (2019) immobilized laccase on modified silica (SiO_2) that was salinized with APTES and treated with GLU to form covalent binding,

which had been proceeded with reactivity study to degrade BPA, followed by conducting storage stability and recyclability test. Zhang et al. (2020) also immobilized laccase but on Cu-metal organic framework (MOF) (HKUST-1) using a one-pot encapsulation procedure for biodegradation study of BPA.

Recent work had also emerged to study the biodegradation of microplastics in film, powder or pellet forms to be more relatable to real-life application. PETase enzymes had been immobilized on magnetic iron oxide (MIO) nanoparticles that were synthesized using co-precipitation from ferric and ferrous chloride and alkaline ammonia for the decomposition of powder-like microplastic PET (Schwaminger et al., 2021). The immobilization was achieved through a new selective (His-Arg)$_4$ peptide-tag proposed recently by Zanker et al. (2021) prior to proceeding with incubation. Other than determining biodegradability of PET, the authors had also evaluated the reuse of the immobilized enzymes for different storage times up to two weeks at 4 °C. Recently, Krakor et al. (2021) studied the feasibility of immobilizing lipase and cutinase enzymes on Silica Coated Magnetite (Fe_3O_4@SiO_2) towards degradation of polycaprolactone (PCL) fiber mats. With regards to preparation of the nanoparticles, SiO_2 particles had been initially synthesized using a modified Stöber method, followed by fabrication of Fe_3O_4 via solvothermal approach and finally coating of Fe_3O_4 with SiO_2. Subsequently, cutinase and lipase enzymes were immobilized on the nanoparticles through surface modification with APTMS and GLU linkers. It had been reported that a bifunctional linker was successfully covalently grafted onto the surface of the inorganic nanoparticles for attachment of enzymes. The nanoparticle-enzyme complexes were incubated with PCL for successful degradation study by observing the mass loss while proceeded with stability study. Immobilization of His-tagged PETase on Cobalt phosphate ($Co_3(PO_4)_2$) was achieved by synthesizing enzyme inorganic nanoflowers, PETase@$Co_3(PO_4)_2$, which had been designed based on the principle of biomimetic

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mineralization in work by Jia et al. (2021). Performance of the enzyme immobilized complexes in degrading PET particles and amorphous film forms had been evaluated at varying pH and temperatures.

2.2. Efficiency of inorganic nanoparticle-enzyme/microorganism complexes

The presence of NPT was able to alter the growth-profiles of LDPE degrading consortia, which demonstrated acceleration in the plastic biodegradation capacity by 1.3 times faster as compared to the control without presence of the nanoparticle (Kapri et al., 2009; Kapri et al., 2010a). In this regard, the degradation of LDPE without NPT had merely shown a two-step thermal decomposition at 61 ℃ and 200 ℃ with weight losses of 5.33% and 9.74%, respectively, while the presence of NBT enhanced decomposition of LDPE into four steps, such as 3.88%, 9.01%, 11.32% and 14.34%, respectively at 60 ℃, 200 ℃, 274 ℃ and 380 ℃. The LDPE biodegradation assay using SPION under the same consortia and operating conditions was found to be further accelerated by 2 times as compared to the sample with only polymer and bacteria. In a similar manner, LDPE in the presence of SPION induced an enhanced three-steps decomposition with weight loss of 3.05%, 4.95% and 10.89% at 62 ℃, 181 ℃ and 350 ℃. The enhancement could be rationalized via increment in the growth of bacteria required in biodegradation with presence of NPT and SPION as supplement and insertion of hydroxyl residues that improved polymer solubility. In another similar work, LDPE degradation was reported in 50 days, which demonstrated improved and highest efficiency of 55% with presence of SPION and mixed bacterial and fungal consortia culture when compared to the control due to improved growth of microorganisms (Patel et al., 2020). Similarly, it was reported by Eili et al. (2012) that PLA degradation had been enhanced with presence of 10 wt.% of Stearate-Zn_3Al LDH, in which an additional weight loss of 40% had been achieved as compared to the neat sample. The improvement could be rationalized via faster degradation rate of stearate groups and presence of hydrophilic hydroxyl groups in the

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nanoparticle that enhances polymer hydrolysis with absorption of water. Furthermore, the biosynthesized Ag by Jayaprakash and Palempalli (2019) had also been reported to be capable of degrading 64% of LDPE and 44% of HDPE. Nonetheless, the biodegradation capabilities of neat LDPE and HDPE were not reported for comparison analysis in the study. The green-synthesized Ag was proposed to exhibit antibacterial potency and reduced toxicity on higher organisms, which promoted the growth of *Aspergillus oryzae* required for biodegradation. In addition, the presence of Ag nanoparticle was found to support photocatalytic degradation of polyethylene with simultaneous presence of fungus and photocatalysis under visible light. Ag also aided in increasing the biodegradable area of the polymer. In another study, the presence of Fe_3O_4 nanoparticle-enzyme complexes had been proven to consistently enhance biodegradation demonstrated through higher emission intensities that depicted greater dye release as compared to the control experiments, typically for AST and FBS (Zhu et al., 2019b). Nonetheless, a specific mechanism could not be specifically highlighted in the work to suggest the role of the nanoparticle-enzyme complexes in promoting biodegradation since a variety of enzymes were employed.

Other than biodegradability efficiency, recyclability and stability of the metal nanoparticles-enzyme/microorganism complexes are also important aspects to be considered, especially to ensure sustainability and economic feasibility in industrial scale application. It had been reported that the biodegradation efficiency was enhanced with enzyme laccase immobilized TiO_2 functionalized PVDF membrane, with highest BPA removal of approximately 90% achieved in 0.1 μm pore sized structure processed via 3 coating cycles and 0.45 μm membrane with 4 coating cycles in comparison to the control with only 8% efficiency (Hou et al., 2014b). The enhancement was rationalized via improvement in enzyme stability immobilized on nanoparticle with stronger covalent bonding for the coated samples, which augmented biodegradation activity. In addition, activities were reported to be

0.42 and 0.41 U/cm^2, respectively, for the 0.1 μm and 0.45 μm membranes with 3 coating cycles, which surpassed performance of reported bio-catalytic membrane from literatures due to higher enzyme loading. Nonetheless, the good recyclability was only observed in the 0.1 μm pore sized membrane structure processed via 3 coating cycles with a drop of BPA removal from 90% to 85%, while a substantial decrement from 90% to 40% had been observed in the 0.45 μm membrane with 4 coating cycles. In addition, reversible immobilization of laccase onto metal-ion-chelated magnetic microspheres for BPA removal had been proven promising. 85% and 88% removal efficiencies were achieved by the Cu (II)- and Mn (II)-immobilized enzymes at optimum pH = 5 and temperature = 35 ℃, which surpassed the experiment utilizing free enzymes with only 55% by Lin et al. (2016). The great enhancement in removal efficiency had been attributed to high loadings of the immobilized enzymes on the metal ion-chelated magnetic microsphere with 100 and 105 mg/g for Cu (II)- and Mn (II) system, respectively. Other than the higher activity that contributed to improved BPA removal, the immobilized enzymes also demonstrated higher thermal retention (e.g., 60% vs 30% at 55 ℃ and 38% vs 0% at 65 ℃) and 14 days storage stability (e.g., 62% vs 17%) as compared to the free counterpart, while exhibiting only 10% loss in its enzyme activity after several adsorption–desorption cycles. Ji et al. (2017) reported feasible application in BPA removal employing immobilization of laccase on APTES and GLU functionalized TiO_2. Loading and activity of the immobilized enzyme from purified commercial laccase was reported to be 7.4 μg/mg and 0.15 U/mg of nanoparticle, respectively, and was able to achieve > 90% removal efficiency at pH = 5 and 25 ℃ with 0.4 U/mL immobilized enzyme after 6 hrs. Advancement in thermal retention after 2 hrs heat treatments (e.g., 85% vs 50% at 50 ℃ and 20% vs 0% at 70 ℃), storage stability (75% after 30 days vs 0% after 23 days) and resistance to pH change (99% vs 82% at pH = 5), recyclability (50% vs 25% after 5 cycles) suggested that the developed immobilized enzyme

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can be potentially applied in harsh industrial environment while being sustainable. In a similar study by Piao et al. (2019), improvement in biotransformation of 90.1% in BFA degradation had been observed as compared to adsorbed and free enzyme with merely 75.4% and 69.9% after 8-hr reaction at optimal pH = 5. Desirable characteristics, such as thermal retention (92.5% vs 86.9% at 5 ℃, 84.9% vs 75% at 10 ℃ and 75.4% vs 51.2% at 25 ℃) and resistance to changing pH (e.g., 68.6% vs 22.5% at pH = 3, 87.9% vs 37.5% at pH = 5, 87.7% vs 60.1% at pH = 7 and 87.9% vs 19.7% at pH = 8) were demonstrated as compared to the control, which was further supplemented with high reusability in which enzymatic activity was at 100%, 100%, 64.3% and 52.9% after two to five times use and 50% after 6 times. Laccase immobilized on SiO_2 had been similarly studied by Chang et al. (2019) for BPA removal using presence of different additives at varying concentrations. In an overall, TX-100 was the most efficient surfactant in promoting enzyme with 43% increment of relative activity in comparison to the control at 0.1 mM concentration, while higher concentration was found to be detrimental to enzyme reactivity. Moreover, ionic liquid, e.g., [AMIM]Cl, was found to increase relative activity by 23% at high concentration of 1.0 mM. The study proceeded with TX-100 as additive had been found to be beneficial to the removal of BPA with 100% and 80% for system with immobilized and free enzyme, respectively, which was consistently higher than their counterpart without the presence of TX-100. Laccase immobilized on SiO_2 was also found to be stable by retaining its activity by 75% as compared to the free enzyme with only 8% after 2 months storage at 4 ℃ and neutral condition. Additionally, it has high potential to be reused with 92%, 89% and 83% activity after 10, 20 and 30 cycles. In another study by Zhang et al. (2020), laccase immobilized on HKUST-1 was found to exhibit positive features since the inorganic nanomaterial with its flower-like structure and a large exposed surface area demonstrated complete degradation of BPA after 4 hrs at optimum pH = 6.5 and temperature 40 ^{o}C in comparison to only 35.5% efficiency in

control with free enzyme. The immobilized structure also enabled long-term storage with 70% of initial activity after 30 days as compared to free enzyme with only 10% and high reusability of 88% and 75.9% of the initial enzymatic activity after 8 and 10 cycles.

In terms of biodegradability, Schwaminger et al. (2021) monitored concentration of terephthalic acid (TPA) as a monomer of PET after 42 hrs. The nanoparticle-enzyme structure demonstrated higher concentration of 27 µM terephthalic acid (TPA) in comparison to 15 µM in free enzyme control, which could be explained through enhancement in enzyme =stability and active site for polymer degradation. The advantages of these nano-biocatalysts also enable feasibility study in magnetic recycling, which had been reported to maintain around 50% of their initial enzymatic activity even after 10 cycles. Additionally, the effect of immobilizing different enzymes (e.g., lipase, cutinase and a combination of lipase-cutinase) with SiO_2 and $Fe_3O_4@SiO_2$, particularly the stability and activity, had been studied by Krakor et al. (2021). It was demonstrated in the study that optimal mass loss of 76.6% was achieved in PCL incubated with 2 mg/mL $Fe_3O_4@SiO_2$-lipase in comparison to only 59.9% in SiO_2-lipase particles, followed by PCL fiber mats incubated with SiO_2-cutinase or $Fe_3O_4@SiO_2$-cutinase with minimal efficiency. Additionally, it was observed that for system in which both lipase and cutinase enzymes were used simultaneously, $Fe_3O_4@SiO_2$ nanoparticles also exhibited enhanced activity as compared to only SiO_2 with 38% and 18% mass loss observed, respectively. For the stability test, $Fe_3O_4@SiO_2$ again demonstrated the best performance by maintaining 80% of initial enzymatic activity after 132 hrs, followed by SiO_2-cutinase and $Fe_3O_4@SiO_2$-cutinase with 35% after 144 hrs and 12% after 132 hrs. Jia et al. (2021) elucidated the degradation of PET with PETase immobilized via His-tag on $Co_3(PO_4)_2$, which was enhanced to 25.8% and 21.8% as compared to control with only 7.4% and 7,7% for plastic particles and film, respectively. Additionally, they had reported the optimal operating conditions for PET degradation to be at pH = 7 and operating temperature of 45 ℃. They also

reported higher thermal retention after 3 hrs heat treatment (e.g., 94.4% vs 57.3% at 40 ℃ and 82.8% vs 17.2% at 45 ℃), storage stability (75% vs 0%) after 12 days at room temperature and higher resistivity to changing pH with 67.6% and 62.3% of initial activity as compared inactive free enzyme at pH 10 and 4, respectively. With respect to the reusability, the enzyme was depicted to be feasible in industrial use with 70.2% of the initial enzymatic activity being maintained even after 10 cycles.

Nonetheless, the enhancement of biodegradability has not been consistently observed. Miyauchi et al. (2008) reported that the decomposition rate of TiO_2/PBS under enzymatic treatment was less than pure PBS since the particles inhibit the diffusion and adsorption of enzyme molecules onto the polymer surface. On the contrary, a simultaneous treatment of the enzyme and UV irradiation onto the TiO_2/PBS exhibited enhancement of degradation activity observed via increment in weight lost from 0.6 from 0.85 mg/cm^2 in comparison to neat sample without TiO_2. The presence of TiO_2 was rationalized to induce photogenerated radicals to oxidize PBS, while the esterase reaction proceeded in the polymer at the same time. In another work by Buzarovska and Grozdanov (2012), although the catalytic effect of TiO_2 nanoparticles on the degradation process of PLLA had been affirmed via hydrolytic degradation with exposure to UV light, the same enhancement was not observed on enzymatic biodegradation. It had been proposed in their study that the TiO_2 nanoparticles hampered the transport of enzyme to polymer surface for required degradation to occur. It was also reported in a subsequent study that the presence of Ag/TiO_2 nanoparticles actually caused retardation in biodegradation of cotton (Co) and Co/ polyethylene terephthalate (PET) fibers (Milošević et al., 2017). The weight loss for samples with Co + Ag/TiO_2 nanoparticles and Co/PET + Ag/TiO_2 were reported to be lower, e.g., 22% and 17.6%, than the control with mere Co/PET with approximately 27% degradation. The retardation was probably due to antimicrobial attributes of the metals that slow down the growth of microorganisms for

biodegradation. In earlier work by Bautista et al. (2010), laccase enzyme immobilized on the functionalized mesostructured silica (SBA-15), which was covalently attached to aminopropyl (AP1) and aminobutyl (AB1) at 1% and 10% total silicon., had unfortunately demonstrated reduced activity, e.g., 3473, 2790, 3798 and 2828, polyphenol oxidase units (POU)/g in AP1, AP10, AB1 and AB10 materials, as compared to free enzyme with 11550 (POU)/g. With the decline in activity, enzyme immobilized on AP1 and AB1 were only able to remove 35 and 39% of naphthalene in comparison to the control that achieved 65% reduction. The reduction was due to their nature of being heterogeneous catalysts as compared to the homogenous counterpart. With regards to reusability, AP1/laccase complex retained majority of its enzyme activity during the second degradation run and a reduction of only 30% during the third, while AB1/laccase demonstrated low sustainability with almost complete loss of enzyme activity during the third run.

2.3. Implications of inorganic nanoparticle-enzyme/microorganism complexes

While the studies of synthesizing suitable immobilized-enzyme nanomaterials have emerged over the last decades, they have been mostly confined to interrogation of the kinetics and activities parameters, while their application in degrading microplastics has received less scrutiny. Although emerging works have been reported in this avenue, they have been majorly devoted to degradation of chemicals from microplastics typically BPA using laccase enzyme. Only few research works have tested the immobilized enzymes in biodegradation of microplastics in film, powder or pellet forms to be applicable in actual commercial scale application. Study by Krakor et al. (2021) highlights the importance of selecting the correct enzyme-inorganic nanoparticle combination in enhancing the activity.

The study involving selected immobilization via His-tag methodology has caught emerging attention recently and proven to be successful in degrading microplastics in film,

particle and pellet forms with their accompanied advantages, which include ultrahigh enzyme activity recovery rate, mild reaction conditions and enhanced stability (Cao et al., 2018). In this context, a tag that involves introduction of affinity tag at the N or C-terminal of the enzyme is realized to enable its purification and immobilization, followed by inorganic salt precipitation to form the carrier (Jia et al., 2021). Additionally, another immobilization approach is fabrication of bio-catalytic membrane system, which has been reported to possess benefits, which include higher stability and flexibility, larger surface area and controllable morphology. As a result, biodegradation was achieved by incorporating enzyme immobilized inorganic nanomaterial into polymeric matrix (Hou et al., 2014a). However, a limited number of studies are available for degradation of microplastics, which necessitates further investigation of the bio-catalytic membrane system in the actual wastewater treatment processes with prolonged operation and presence of a wide range of physicochemical conditions or contaminants.

Interestingly, it has been reported that the presence of additives, which include surfactants and inhibitors, is able to increase the enzyme activity with proper selection of species and its concentration (Chang et al., 2019; Hou et al., 2014a). The potential of incorporating ionic liquid has been preliminarily studied and it demonstrates promising potential to be utilized for improvement of enzyme activity (Chang et al., 2019). Hence, it is recommended to explore the presence of any potential inhibitors or activators and their concentration on the activity, stability and reusability of enzyme for improved industrial application in a long run.

In addition, from the review, it is highlighted that magnetic Fe_2O_3 nanoparticle is a popular inorganic nanomaterial to be explored due to its interesting characteristics. Other than being available as a supplement for microorganism to feed upon that enhances microbial growth (Kapri et al., 2010a; Patel et al., 2020), it also inherits superparamagnetic properties,

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which enable separation from their surrounding medium by inducing a magnetic field, higher specific surface area, lower steric hindrance in comparison to porous materials, as well as being cost efficient and safe (Schwaminger et al., 2021). Hence, they are suitable solid carrier candidate for enzymatic immobilization purposes to generate advantages, such as increasing durability and stability, while allowing a high separability and recyclability grade for a straightforward process handling due to high magnetic separation feature.

It is also found that not all studies have demonstrated positive outcomes, in which some have reduced activities as compared to the free enzyme control, either due to inhibitory effect towards microorganism growth or issues deduced by the nanomaterial, which include but are not limited to diffusional limits, steric effects, structural changes to the active sites by the support, or loss of enzyme flexibility that is necessary for optimal substrate binding. Hence, it is important to ensure that proper combination between enzymes and inorganic nanomaterials with suitable microplastic substrate is unraveled to ensure good biodegradation.

3. Carbon-based particle/polymer-enzyme complexes

Carbon-based nanoparticles have been increasingly employed for immobilization of enzymes. While inorganic nanoparticles such as those of gold, silver and silica are gaining popularity in recent years, for carbon-based nanoparticles, carbon nanotube is still very commonly used to immobilize enzymes due to its modifiable surface, large surface to volume ratio as well as chemical, thermal, and mechanical stability (Cacicedo et al., 2019). There are two types of carbon nanotubes, i.e., single-walled and multiwalled. The former is fundamentally a single graphite layer with a central tubule while the latter has multiple graphite layers around the central tubule (Feng and Ji, 2011). Other carbon-based materials which have been used for immobilization of plastics-degrading enzymes include chitosan,

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macroporous cross-linked polymers and resin but they are not of nano-scale (Hegde and Veeranki, 2014)(Su et al., 2018). While immobilization of enzymes on carbon-based nanoparticles significantly enhances their degradative activities, the efficiency often relies on careful synthesis of the immobilized enzyme complexes to ensure successful immobilization of the enzymes and optimization of their activities under wider experimental or environmental conditions. Besides, it is desirable that the immobilized enzymes could be recycled multiple times to make them feasible for real-life applications (Chen et al., 2017).

3.1. Synthesis of carbon-based particle/polymer-enzyme complexes

Immobilization of plastics-degrading enzymes on suitable materials could enhance catalytic activity of the enzymes while conferring them thermal stability and reusability. Enzyme immobilization on carbon-based particles can be achieved through covalent bonding, physical adsorption, entrapment and encapsulation, similar to that on inorganic nanoparticles (Figure 2).

T. fusca cutinases have been successfully immobilized on GA-activated chitosan beads, giving a yield of more than 70%. Enzyme immobilization was achieved by incubating chitosan beads activated with GA at concentrations between 1.5% to 4%, with purified cutinase at 4 ^{o}C over a period of 24 hrs (Table 2) (Hegde and Veeranki, 2014). During the incubation, *T. fusca* cutinases adsorbed onto the surface of chitosan beads through the formation of covalent bonds with the functional groups conferred by GA (Figure 2). The previous success in the immobilization of enzymes on commercial resins, for instance the immobilization of an engineered *Humicola insolens* cutinase (HiC) on Lewatit VP OC 1600 by Mo et al. (2008), has prompted Su et al. (2018) to immobilize cutinase derived from *Thielavia terrestris* physically on Lewatit VP OC 1600, yielding a complex with thermal stability as high as 80 ^{o}C and was 64% active even at 90 ^{o}C (Table 2). It is noteworthy that

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the immobilized cutinase was used on bio-additives of plastics. This study was instrumental in elucidating the potential of synthesizing cutinase-resin complex for degradation of microplastics, particularly when the cutinase of *Thielavia terrestris* had been reported to degrade multiple ester polymers comprising PET, polycaprolactone (PCL) and poly(butylene succinate) (PBS) (Yang et al., 2013). Su et al. (2018) also successfully adsorbed the cutinases of *Humicola insolens* and *Aspergillus oryzae* respectively on Lewatit VP OC 1600, and both cutinases had been shown in separate studies to demonstrate the potential to degrade plastics, especially PET and PCL (Carniel et al., 2017; Liu et al., 2009). In synthesizing the enzyme-resin complexes, cutinases were first expressed in *Komagataella pastoris* (ATCC 76273), purified, lyophilized and dissolved in an immobilization buffer. The enzymes were then centrifuged with Lewatit VP OC 1600 as the immobilization matrix and the mixture was filtered to remove the free enzymes. Butyl laurate assay (BL-assay) was conducted to determine the activity of the immobilized enzymes (Su et al., 2018). Unlike the synthesis conducted by Hegde and Veeranki (2014) involving covalent immobilization which has the advantage having lower enzyme desorption, enzyme immobilization by Su et al. (2018) was accomplished through hydrophobic interaction to preserve enzyme activity which could be lost through chemical bonding (see Figure 2 for adsorption on a support). Similar to Su et al's immobilization on enzymes on a commercial resin, Barth et al. (2016) synthesized a resin-immobilized carboxylesterase TfCa from *Thermobifida fusca* KW3 which was subsequently used in a dual-enzyme system with free LC-cutinase to degrade PET films at 60 ^{o}C (Table 2). In their work, the immobilized enzyme was employed together with free cutinase, thus constituting the dual-enzyme system. The enzyme immobilization was attained with covalent-bonding predominantly, instead of hydrophobic interaction.

Hidayat and Tachibana (2012) entrapped the mycelia of *Pleurotus ostreatus* in immobilized Ca-alginate beads (Table 2). Distinct from covalent and physical immobilization,

entrapment in this study involves embedding an organism or parts of an organism that could produce plastics-degrading enzymes in a matrix. In the synthesis, Hidayat and Tachibana, (2012) mixed the homogenized mixture of a liquid culture and mycelia with 1.5% Na-alginate. The mixture was then added to a 0.1 M $CaCl_2$ solution to form Ca-alginate beads containing immobilized mycelia, which were filtered and washed. The beads were sandwiched between two pieces of PLA/kenaf samples pre-treated by immersion with 2% glucose and addition of 2% Na-alginate and a nutrient on one of the respective surfaces, prior to incubation at 25 ^{o}C. While entrapment can be performed on an external medium such as Ca-alginate bead using living enzyme-producing parts, it can also be conducted on biodegradable plastics using enzymes or catalysts with the plastics acting as the medium. Huang et al. (2020) experimented on the facilitated self-degradation of biodegradable plastics by embedding immobilized or free Proteinase K extracted from *Tritirachium album* in PLLA (Figure 3). Proteinase K was immobilized in a Micro Bio-Spin column with Bio-Gel P-30 (polyacrylamide gel) through centrifugation. Immobilized Proteinase K or Proteinase K was added to PLLA polymer solution to produce solution-cast films with the respective enzymes embedded. PLLA extruded films with immobilized Proteinase K or Proteinase K were also produced. The immobilization of Proteinase K in this study is akin to that of Su et al. (2018) which involves the retention of the enzyme within the cross-linkages of the polymeric matrix through hydrophobic interaction rather than covalent bonding.

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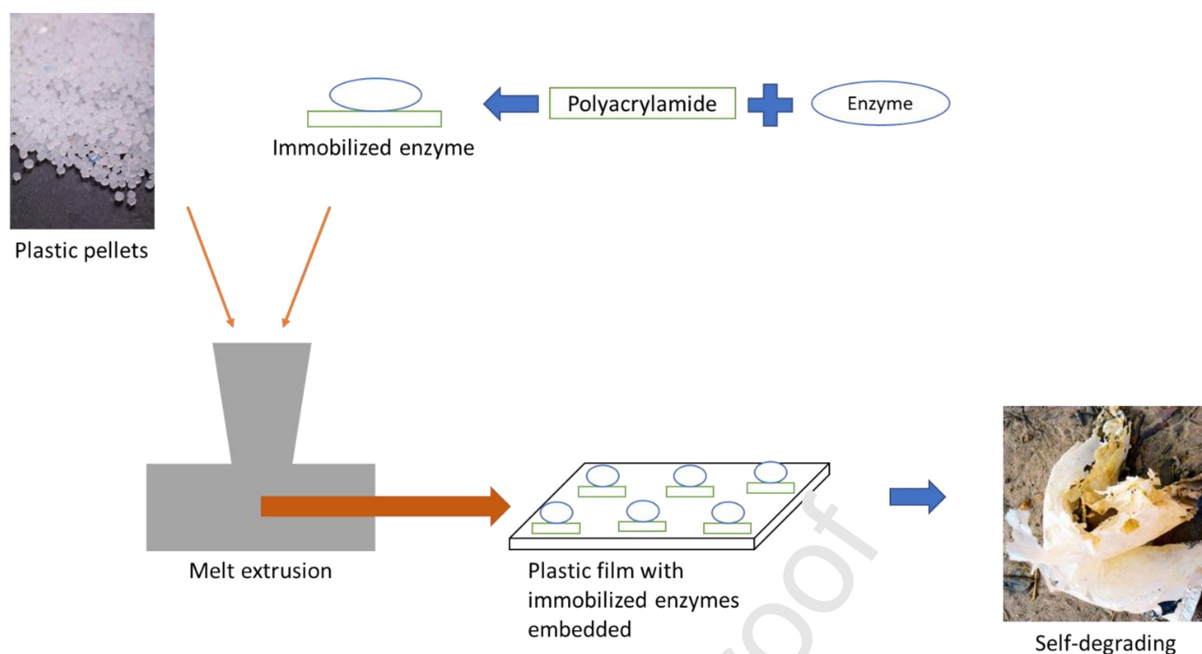

Figure 3: Synthesis of self-degrading plastics containing immobilized enzymes

In a study on laccase immobilization, Costa et al. (2019) functionalized multi-walled CNTs through a series of steps consisting of hydrothermal oxidation with nitric acid, treatment with GA (crosslinker), silanization with APTES, as well as a combination of silanization and crosslinker addition, followed by treatment with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as crosslinker and finally N-hydroxysuccinimide as stabilizer. The functionalized CNTs were incorporated into polysulfone (PSf) membranes through non-solvent induced phase separation. Laccase was immobilized directly on the functionalized CNTs or over the CNTs/PSf membranes. The synthesis is fundamentally the immobilization of enzyme through predominantly covalent bonding, as that of Hegde and Veeranki (2014), either on functionalized CNTs or CNTs/PSf membranes, though hydrophobic interaction occurs to a certain extent. While the laccase-CNT complexes yielded were intended for degradation of phenolic compounds, laccase has been reported to demonstrate plastics-degrading ability in other studies (Magnin et al.,

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2021)(Santacruz-Juárez et al., 2021), thus bringing into attention the potential of these complexes to degrade microplastics.

3.2. Efficiency of carbon-based particle/polymer-enzyme complexes

The cutinase-chitosan complexes synthesized by Hegde and Veeranki (2014) reacted optimally with PET at 55 ^oC with pH 8 and demonstrated operational stability at temperature between 45 ^oC and 70 ^oC with reusability up to ten cycles at 80% efficiency. After storing for 13 days at 4 ^oC , its catalytic activity reduced to 50%. Su et al. (2018), in their immobilization of cutinases on a commercial resin, reported that loading ratio defined as the ratio of enzyme molecules to the surface area of resin, is an important factor determining the activity of immobilized enzyme and overloading could lead to lower enzyme activity due to the loss of binding sites. They revealed that a loading ratio of 100 mg/g was optimal for the activity of immobilized *Aspergillus oryzae* cutinase. They also revealed that the buffer media for immobilization should have pH optimal to the activities of the respective cutinases used. In addition, they investigated how solvent polarity of the rection media affected the activities of immobilized enzymes and found that *Humicola insolens* cutinase could tolerate increased solvent polarity to the highest extent, followed by *Thielavia terrestris* cutinase and *Aspergillus oryzae* cutinase losing 49% of its activity with increased solvent polarity. The authors provided insight into the potential effects of the types of media, namely organic or aqueous on thermal stability of the enzymes where they pointed to the potential ability of organic media in enhancing enzyme conformational rigidity due to less water present in the media.

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Table 2: Carbon-based particle/polymer-enzyme complexes for degradation of microplastics

Enzyme	Support/ Immobilization matrix	Synthesis	Optimal operational condition	Substrate	Efficiency and Kinetic Stability	Recyclability	Reference
T. fusca cutinases	GA-activated chitosan beads	Covalent immobilization, with 74% and 71% immobilization for Cutinase 1 and Cutinase 2 respectively	pH = 8 Temperature = 55 ^oC	PET	Efficiency not stated; activity reduced to 50% after 13 days storage at 4^oC and 60% residual activity after 50-hr incubation at 40 to 60^oC	10 cycles at 80% efficiency at 4 ^oC	(Hegde and Veeranki, 2014)
Aspergillus oryzae cutinase	Lewatit VP OC. 1600	Physical adsorption with > 98% immobilization achieved	pH = 7 and 8 Temperature = 70 ^oC (with solvent) Temperature = 60 ^oC (solvent free)	Butanol-lauric acid used as bio-additive of plastics	Efficiency not stated; good stability at 70 ^oC with slight loss of activity with time; full activity for the first hour at 80 ^oC with significant decrease subsequently	NA	(Su et al., 2018)
Humicola insolens cutinase	Lewatit VP OC. 1600	Physical adsorption with > 98% immobilization achieved	pH = 5 – 8 Temperature = 75 ^oC (with solvent) Temperature = 60 ^oC (solvent free)	Butanol-lauric acid used as bio-additive of plastics	Efficiency not stated; good stability at 70 ^oC with slight loss of activity with time; residual activity of 76% and 50% at	NA	(Su et al., 2018)

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					the first and second hour respectively at 80 ^{o}C		
Thielavia terrestris cutinase	Lewatit VP OC. 1600	Physical adsorption with > 98% immobilization achieved	pH = 5 – 8 Temperature = 60 ^{o}C – 90 ^{o}C (with solvent) Temperature = 60 ^{o}C – 75 ^{o}C (solvent free)	Butanol-lauric acid used as bio-additive of plastics	Efficiency not stated; Good stability at 70 ^{o}C with slight loss of activity with time; residual activity of 80% at 80 ^{o}C for the first hour and 77% at 80 ^{o}C for the second hour	NA	(Su et al., 2018)
TfCa from *T. fusca* KW3 with free LC-cutinase	SulfoLink resin	Covalent immobilization, with a maximum of 5.7 ± 0.8 mg/mL TfCa immobilized at 4°C	pH = 8, temperature = 60 ^{o}C, using a dual-enzyme system (resin-immobilized TfCa and free LC cutinase) which was compared against free TfCut2 alone	PET	Dual enzyme system is 2.4 times more efficient than free TfCut2; 94% of the activity of immobilized TfCa retained at 60°C and the activity reduced to 77% after 8-hr incubation, while only a residual activity of 12% reported at 75 ^{o}C after 1-hr incubation	NA	(Barth et al., 2016)
Mycelia of *Pleurotus*	Ca-alginate	Entrapment	Weight loss of PLA/kenaf composite	PLA/kenaf	48% weight loss after 6 months;	NA	(Hidayat and

ostreatus	beads		fastest after 3 months incubation	composite	activity level of an enzyme involved in the degradation – manganese oxide was 0.26 U/mg after incubation for 6 months		Tachibana, 2012)
Proteinase K from *Tritirachium album*	PLLA	Immobilization of proteinase K on Bio-Gel P-20 with and without a subsequent embedding of the immobilized enzymes on solution-cast/ extruded PLLA films	pH = 8.5, rate of degradation accelerated by breaking up the films	PLLA	78% weight loss after 96 hrs; proteinase K retained its activity even after heat treatment up to 200 ^oC but extruded PLLA films with proteinase K embedded showed significantly lower degradation than solution-cast films	NA	(Huang et al., 2020)
Laccase	Functionalized multi-walled CNTs or CNTs/ PSf membranes	Covalent immobilization with up to 96% yield	CNTs oxidized with HNO_3 0.30 M, and treated with EDC and N-hydroxysuccinimide yielded higher immobilization efficiency and recovered activity	4-methoxyphenol used as a plastic additive	100% removal in 10 mins and 15 mins for Laccase-CNTs and Laccase-CNTs/PSf membranes respectively; residual activity decreased to 84% and 71% after	Progressive loss of enzyme activity for the second and third utilizations for Laccase-CNTs; lost removal efficiency after 3 cycles for Laccase-CNTs/PSf membranes; generally, laccase	(Costa et al., 2019)*

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					incubating for 4 hrs at 50 ^{o}C and 60 ^{o}C respectively	activity > 65% of initial value after 5 consecutive cycles of reuse	

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In fact, high water content has been reported to be undesirable for esterification and transesterification by cutinase. Hegde and Veeranki (2014) resorted to freeze-drying to remove the water content and found their chitosan-immobilized cutinase to remain active after 3 cycles of freeze-drying. Furthermore, immobilized enzymes exhibited better thermostability than the free enzymes (free enzymes underwent faster deactivation at 40-60 ^{o}C after 12 hrs). In line with the findings of Hegde and Veeranki (2014), pH and temperature are important performance determinants of immobilized enzymes which have distinct temperature ranges of thermostability. In addition, solvent polarity and types of reaction media also influenced immobilized enzyme performance (Su et al., 2018). The dual-enzyme system of Barth et al. (2016) was 2.4 times more efficient than free TfCut2 enzyme alone in hydrolyzing PET. The increased efficiency was achieved through hydrolysis of mono-(2-hydroxyethyl) terephthalate (MHET) by immobilized TfCa. MHET is an intermediate product of PET hydrolysis by LC cutinase or TfCut2 which has an inhibitory effect on the enzymes. Besides, immobilizing TfCa on SulfoLink resin enhanced its thermal stability through conferring conformational rigidity to prevent its breakdown at elevated temperature. At 60 ^{o}C, the immobilized TfCa retained 94% of its activity which was only reduced to 77% after 8-hr incubation. The importance of conformational rigidity of enzymes was also reported by Su et al. (2018). This study is instrumental in elucidating the potential of using complementary immobilized and free enzymes together to improve the efficiency of microplastics degradation.

Hidayat and Tachibana (2012) entrapped the mycelia of *Pleurotus ostreatus* in immobilized Ca-alginate beads and reported up to 48% degradation of polylactic acid (PLA)/kenaf composite in 6 months, characterized by shortening of fibers and a loss of 84% mechanical properties. The degradation was attained through an initial hydrolytic depolymerization of the composite and a faster subsequent oxidation likely to be catalyzed by

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manganese peroxidase secreted by *Pleurotus ostreatus*. As this study aimed to examine the application of a novel entrapment method in degrading PLA/kenaf composite, it did not investigate the factors that could affect the efficiency of degradation. However, the study involved pre-treatment of the substrate with glucose and a nutrient which may imply the need of certain nutrients for optimal performance of the entrapped fungal living parts. This may be translated to additional cost for degradation. In the study of Huang et al. (2020) on self-biodegradation of PLLA, the weight of PLLA solution-cast films with Proteinase K incorporated was reported to reduce by 78% after being incubated for 96 hrs. However, the rate of weight loss reduced significantly with increasing degradation time owing probably to the degradation of the films' surface contacting immobilized Proteinase K since Proteinase K acts directly on the surface on which it is adsorbed. This constitutes an important limitation to the entrapment method employed. The differences in the rates of degradation of extruded PLLA with and without enzyme immobilization indicated that the enzyme immobilization contributed significantly to prevent thermal denaturation of protein during the extrusion process at elevated temperature. Unlike on solution-cast films, the immobilized Proteinase K in the extruded films demonstrated sustained degradative activity until 504 hrs as the enzyme degraded the films from the inside, forming holes and cavities. The degradation rate of extruded films with Proteinase K embedded, however, was substantially slower attributed probably to denser surfaces and more difficult penetration of water required for the action of Proteinase K. Breaking up the films increased the rate of degradation, which tends to correlate with the sizes of the fragments. This implies that such self-degradation could be accelerated for microplastics formed from self-degrading plastics (Huang et al., 2020).

Costa et al. (2019) found modification of CNTs in the synthesis of laccase-CNTs complexes was crucial in determining the performance of the complexes, particularly their thermal stability. Entrapping laccase-CNTs complexes over PSf membrane was found to

enhance their recyclability and degradative activity, leading to the retention of more than 65% of laccase activity after 5 cycles of reuse in comparison to 3 cycles for laccase-CNTs alone. This study reveals recyclability as an important feature of immobilized enzymes. This review also points to a general enhancement of the recyclability of enzymes or catalysts immobilized on carbon-based matrix (Hegde and Veeranki, 2014; Costa et al., 2019).

The studies reviewed have invariably revealed the enhanced performance of immobilized enzymes, either in yielding higher efficiency in microplastics or microplastics-related degradation, or in exhibiting greater kinetic stability than free enzymes. Despite having better efficiency and stability, the immobilized enzymes tend to lose their activities upon prolonged incubation or storage even at optimal temperatures, except for the use of fungal mycelia which revealed higher activity level of a degradative enzyme with time (Hidayat and Tachibana, 2012). As mycelia secrete a mixture of enzymes, it is uncertain whether such increase in activity level was observed for other enzymes. The losses of enzymatic activities were even more significant with higher increase of temperature above the optima. Where recyclability was tested, immobilized enzymes showed better recyclability though at compromised activities. Besides, the studies also showed the methods of immobilization could affect enzymatic efficiency, stability and recyclability, for instance, a further entrapping of immobilized enzymes on membranes produced better performance. This implies the potential of combining different immobilization methods to optimize enzymatic degradation of microplastics. Also, a combination of enzymes and immobilized enzymes has been reported to give higher performance (Barth et al., 2016), and this could add to the possible permutations for optimization of enzymatic degradation of microplastics. Finally, embedding immobilized microplastics-degrading enzymes into plastics provides an innovative way of producing self-degrading plastics, but it is important to note that the

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synthesis of the plastics could affect the performance of the enzymes and in all studies, total degradation or mineralization of microplastics is rarely achieved.

4. Emerging nanoparticles/ enzymatic technologies for microplastics degradation

The efficacy and rate of microplastic degradation/hydrolysis by enzymes is often limited by the high degree of crystallinity and hydrophobicity of microplastics' surfaces. Hence, the key to debottleneck this limitation lies in the ability and ease of enzymes to approach and attach themselves to the specific hydrolytic sites or surface of microplastic. Aside from metal- and carbon-based nanoparticle-enzyme complexes, one of the emerging techniques for microplastic degradation is via the enzyme immobilized on material binding agents or adhesion promoters of bio-based. These agents will enhance the adsorption of enzymes on the surface of microplastics and thus improve the degradation rate and efficiency of microplastics. Besides, hybrid treatment of nanoparticle-enzyme complexes with photocatalysis is also gaining research momentum. Table 3 shows a summary of relevant studies with regards to the emerging nanoparticle/ enzymatic techniques for microplastic degradation.

Ribitsch et al. (2015) reported enhanced cutinase hydrolysis of PET when the enzyme was covalently fused with hydrophobins. A remarkable >16-fold enhancement was observed when using hydrophobins species HFB4 and HFB7. In another study by Gamerith et al., (2016), polyamidase (PA) fused with a polymer binding module (PBM) via a linker was employed to study the hydrolysis of a water-soluble PU model substrate and PU pellets. As compared to the commercial enzyme Penicillin G Amidase which recorded low activity (0.015 U/mg) on soluble PU model substrate, the fusion of PA and PBM (PA_PBM) enhanced the adsorption of the enzyme complex on the surface of PU, as evident from a

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significant improvement in the enzyme activity (1.13 U/mg). When tested on PU pellets, the fusion enzyme was able to boost the PU hydrolysis by 4 times, indicating that the poor enzyme adsorption/binding on the surface of PU could be overcome by grafting and engineering fusion enzymes (Zhu et al., 2021).

Islam et al. (2019) investigated the fusion of anchor peptide (Tachystatin A2) with cutinase (Tcur1278) derived from *Thermomonospora curvata* in the degradation of PE-PU dispersion. It was found that the anchor peptide improved the degradation kinetic of microplastics by 6.6-fold, as well as the degradation half-life of the microplastic suspension shortened significantly from 41.8 hrs to 6.2 hrs. The hydrolytic cleavage of ester bonds was greatly enhanced, possibly due to the intensified enzymatic reaction as a result of higher biocatalyst concentration at the proximity of microplastic particles. Similar observation was reported by Dai et al. (2021), where *Is*PETase^{EHA} bonded to cellulose-binding module (CBM) derived from cellobiohydrolase I was used to hydrolyze PET at different temperatures and enzyme concentrations. Analysis of PET hydrolysis products (terephthalic acid, TPA and mono(2-hydroxyethyl) terephthalate, MHET) showed that the synergy in the fused enzyme *Is*PETase^{EHA}_CBM increased its enzymatic activity by 44.5 – 71.5% (at fixed enzyme concentration of 10 μg/mL), while increasing the enzyme concentration to 20 μg/mL further resulted in 86% increase in catalytic activity as compared to that of parental enzyme. It was deduced that CBM constituted several aromatic amino acids at the flat side, which was having a higher degree of hydrophobicity and thus facilitated its adhesion to the PET surface and subsequently promoted the hydrolysis efficiency.

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Table 3: Summary of emerging nanoparticle/enzymatic technologies for microplastics degradation

Enzymatic system	Type of microplastic	Treatment conditions	Degradation efficiency / performance	Major findings	Reference
Cutinase (Thc_Cut1) covalently fused with hydrophobins (HFB4, HFB7 and HFB9b)	PET	Plastic concentration: PET films in 0.1 M buffer (K_3PO_4) Enzyme concentration: 5 µM pH: 7.0 Temperature: 50 °C Stirring rate: 100 rpm Duration: 24 hrs	• Hydrolysis of PET enhanced by >16-fold for Thc_Cut1_ HFB4 and Thc_Cut1_ HFB7.	• Effect of HFB9b was most prominent when added separately to Thc_Cut1, while its fusion with Thc_Cut1 did not show any stimulatory effect on cutinase activity. • Stimulatory effects of individual hydrophobins were different for different scenarios (physically mixed with cutinase, covalently fused with cutinase or preincubated with PET).	(Ribitsch et al., 2015)
Polyamidase fused with polymer binding module (PA_PBM)	PU	Plastic concentration: 200 mg in buffer (100 mM Tris-HCl) Enzyme concentration: 2.5 µM pH: 7.0 Temperature: 50 °C Stirring rate: 100 rpm Duration: 7 days	• PA_PBM fusion enzyme showed higher hydrolysis activity on polyurethane pellets (4 times of native enzyme PA).	• Compared to commercial Penicillin G Amidase enzyme with very low activity on soluble polyurethane substrate (0.015 U/mg), PA_PBM fusion enzyme had a much higher activity of 1.13 U/mg. • Lower catalytic efficiency of PA_PBM (1.13 U/mg) on soluble polyurethane substrate as compared to that of PA (10.5 U/mg) might be due to potential steric hindrance and solubility issues.	(Gamerith et al., 2016)

Cutinase (Tcur1278) fused with anchor peptide (Tachystatin A2)	PE-PU	Plastic concentration: 130 μL (Polyester:polyurethane of 0.033 – 0.4 w/v%) dispersion in buffer (100 mM Tris-HCl) Enzyme concentration: 0.6 nM pH: 8.0 Duration: 24 hrs	• Degradation kinetics: (-1)*5.85*10^{-3} – (-1)*8.58*10^{-3} A.U./hr • Half-life: ~ 5 – 35 hrs	• Degradation kinetics of microplastics in the presence of anchor peptide were improved by 2.59 – 6.62 times. • Optimum reduction in half-life of 6.7-fold (from 41.8 hrs to 6.2 hrs) was achieved at a dilution of 1:600. • Generally, higher dilution resulted in lower half-life of enzymatic degradation. • Smaller nanoparticles were obtained upon enzymatic degradation with anchor peptide (0.08 nm) as compared to that of enzyme alone (0.33 nm).	(Islam et al., 2019)
*Is*PETase^{EHA} fused with cellulose binding module (*Is*PETase^{EHA}_CBM)	PET	Plastic concentration: 3 mg/mL PET in 50 mM glycine–NaOH Enzyme concentration: 2.5–25 μg/mL pH: 9.0 Temperature: 30, 40 °C Stirring rate: 800 rpm Duration: 18 hrs	• Degradation products: - 30 °C, 10 μg/mL enzyme: 17.4 μM (TPA) and 13.3 μM (MHET) - 40 °C, 10 μg/mL enzyme: 87.0 μM (TPA) and 164.5 μM (MHET) - 40 °C, 20 μg/mL enzyme: 89.3 μM (TPA) and 115.7 μM (MHET)	• The performance of *Is*PETase^{EHA}_CBM was superior compared to other fusion enzymes (*Is*PETase^{EHA}_PBM and *Is*PETase^{EHA}_HFB4). • *Is*PETase^{EHA}_CBM demonstrated significantly enhanced hydrolytic activity (71.5% and 44.5% at 30 °C and 40 °C, respectively) as compared to the pristine parental enzyme.	(Dai et al., 2021)
Non enzymatic system – Bio based synthesis of gold nanoparticles using Ananas comosus aqueous	LDPE	Reaction conditions: Ambient Type of irradiation: Solar light	• The LDPE degradation performance over Au nanoparticles in the presence of solar	• The degradation efficiency of Au nanoparticles exhibited excellent reusability up to five cycles with degradation	(Olajire and Mohammed, 2021)

leaf extract		Duration: 240 hrs (30 days)	irradiation (90.8%) was significantly higher than the control run (<10%, without the photocatalytic materials) after 240 hrs.	efficiency of 82.3% in the fifth cycle.	
Protein-coated titania nanoparticles and *Lactobacillus plantarum*	LDPE	Temperature: Ambient and 50˚C Size of LDPE: 30 microns Type of irradiation: UV irradiation, visible light, high temperature (50˚C) Duration: 21 days	• The degradation rate and elongation were improved up to 59% and 51%, respectively after 21 days of visible irradiation exposure. • Tensile strength of LDPE was reduced by 21% after 21 days.	• FTIR analysis of the degraded plastic revealed strong absorptions band of the carbonyl group (C=O) and breaking/weaking of existing absorption bands with formation of new carbonyl function groups.	Dave et al. (2021)

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Lately, photocatalytic degradation of LDPE film using photosensitive catalytic materials has slowly become an active research topic in the field of microplastic degradation. Olajire and Mohammed (2021) have successfully synthesized gold nanoparticles via reduction of hydrogen tetrachloroaurate solution by using aqueous leaf extract of *Ananas comosus* (Figure 4). The resultant gold nanoparticles showed excellent photocatalytic degradation efficiency of 90.8% for LDPE film after 240 hrs of solar irradiation. The bio-based gold nanoparticles also exhibited satisfactory reusability and sustainability in photocatalytic degradation up to five consecutive cycles without substantial loss in catalytic activity. Along the same line, Dave et al. (2021) also developed protein-coated titania nanoparticles and incorporated *Lactobacillus plantarum* as the enzyme for photocatalytic degradation of LDPE and the protein coated catalytic material has shown high photocatalytic degradation performance with reduction in tensile strength up to 20% after 21 days of visible light irradiation. From this review, the authors envisage that this could be one of the promising and emerging microplastic degradation technologies that allows the LDPE materials to be degraded photocatalytically into smaller fragments before being decomposed by the enzymes or bacteria.

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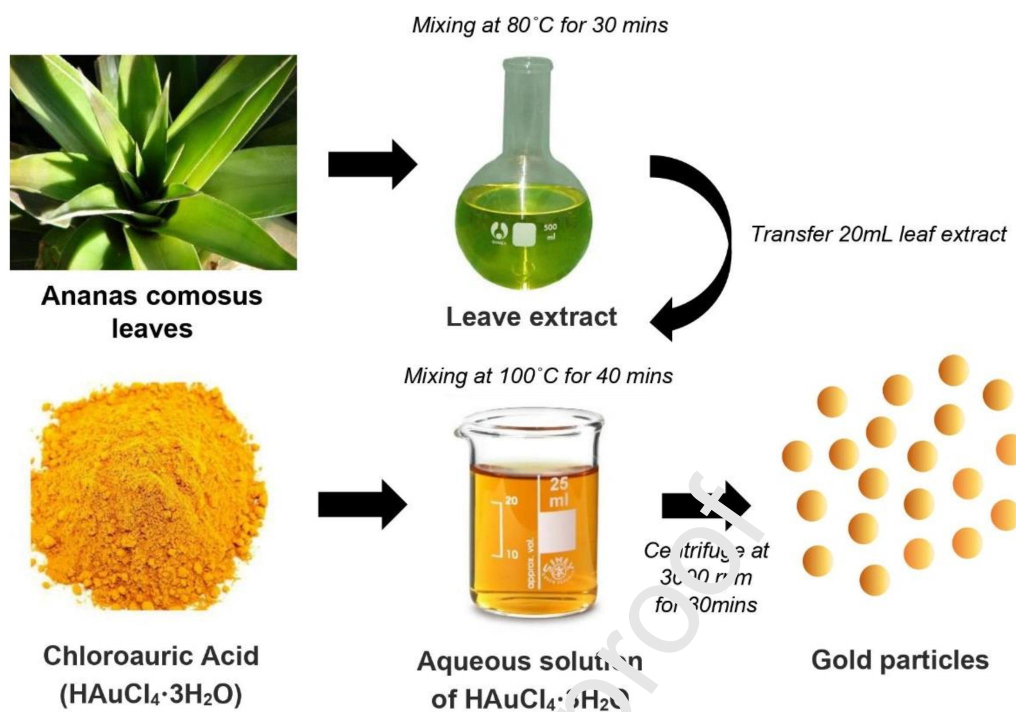

Figure 4: Synthesis of gold nanoparticles from aqueous lead extract of Ananas comosus

Overall, these studies have evaluated the performance of various bio-based enzyme binding agents and hybrid photocatalytic-enzymatic hydrolysis in the degradation of microplastics, and generally yielded superior results. However, the lack of reusability/recyclability data from these studies warrants further scrutinization in order to assess the operability of these techniques in the commercial scales. Furthermore, complementary studies employing computational enzymology would be essential to understand the fundamental behavior of enzymes and to assist in enzyme engineering and modification for enhanced microplastic degradation.

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5. Feasibility and limitations

According to Othman et al. (2021), microplastics can be classified in accordance with their respective monomer types. The feasibility and practicability of the enzyme complexes which are accountable for the degradation of microplastics have been reviewed comprehensively in this section. PE can be categorized into two different groups suchlike the HDPE and LDPE as reported by Patel et al. (2020), Restrepo-Flórez et al. (2014) as well as Wu and Montalvo (2021). Generally, the widespread pollution in the natural environment has aroused the attention of researchers to study on the biodegradation mechanisms of PE via enzymes activities. For instance, laccase, alkane hydrolase, manganese peroxidase and soybean peroxidase are among the enzymes which are accountable for the degradation of PE. Laccase is reported to be able to initiate the depolymerization of polymers via the oxidative cleavage of the HDPE amorphous region. Resultantly, this brings about an easily accessible carbonyl region in the interior of the polymer chain (Ghatge et al., 2020; Kang et al., 2019; Kumar Sen and Raut, 2015; Montazer et al., 2020). In addition to that, manganese peroxidase is capable of decreasing the tensile strength and total molecular weight of PE whereas soybean peroxidase is able to reduce the hydrophobicity of PE (Montazer et al., 2020).

Furthermore, there are two types of enzymes known as PETase and MHETase which are highly responsible for the degradation of polyethylene terephthalate (PET). First, PETase tends to convert the PET polymer into mono(2-hydroxyethyl) terephthalic acid (MHET). Subsequently, MHETase as the secondary enzyme further converts the MHET into terephthalic acid and ethylene glycol. Consequently, the mechanisms of PETase and MHETase are reported to be highly feasible (Chen et al., 2018; Salvador et al., 2019; Shosuke et al., 2016). Nevertheless, the mechanisms of other similar enzymes such as lipase and esterase which can just as well aid in the degradation of PET are still undiscovered and unascertained (Othman et al., 2021). Barth et al. (2015) demonstrated the conversion of

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amorphous PET into the respective monomers by utilizing an enzyme reactor. Though the enzyme (TfCut2 from *Thermobifida fusca* KW3 and the metagenome-derived LC-cutinase) is capable of degrading the crystalline parts of PET, the process is deemed slow.

Numerous literatures have been reported on the degradation of PS via various enzymes such as the styrene monooxygenase, styrene oxide isomerase, phenylacetaldehyde dehydrogenase as well as phenylacetyl coenzyme A ligase. Resultantly, acetyl-CoA can be regarded as the final monomer from the degradation process. However, there is insufficient research with regards to the mechanisms of hydrolase on the degradation of PS (Othman et al., 2021). Padervand et al. (2020) reported on the practicability of the degradation of microplastics via zooplankton. It was stated that a high zooplankton concentration in the environment would initiate PS removal. Though multitudinous literatures have been widely reported on the degradation of PS via microorganisms, nevertheless, there are still inadequate findings with respect to the data on the respective enzymatic degradation mechanism of microplastic (Atiq et al., 2010; Bhardwaj et al., 2013).

On top of that, Uheida et al. (2021) stated in their research that the essential polypropylene (PP) microplastic typically originates from cosmetics and various personal care products. Bikker et al. (2020) and Huang et al. (2019) claimed that PP is the most substantial microplastic found in the South China Sea and USA regions. Literatures on the microbial degradation of PP have been comprehensively reviewed in which the degradation of PP is mainly targeted on the backbone of the polymer as well as plasticizers within the surface of the polymer (Ru et al., 2020). Generally, *Rhodococcus sp.* strain 36, *Bacillus sp.* strain 27 as well as *Bacillus gottheilii* are among the bacteria that are reported to be accountable for PP degradation in the environment (Auta et al., 2017). In spite of the fact that bacteria species are deemed feasible in degrading PP, there is still a lack of research and explanation in relation to the mechanisms of enzymatic degradation of PP (Ganesh Kumar et

al., 2020). Pires et al. (2019) has successfully proven the practicability of enzymatic degradation of PP. However, there is a scarcity of reference with respect to the actual enzymes and the respective characteristics. Predominantly, in comparison to some other microplastics, there is indeed an insufficient information with regards to PP removal and degradation. Several literatures have been disclosed on the capability of various microorganisms to degrade PP. Therefore, in consideration of the foregoing statement, it is utterly crucial to expand the research area with respect to the enzymatic degradation of PP via microorganisms from the environment (Othman et al., 2021).

Apart from that, for polyvinyl chloride (PVC), there is lack of data regarding the enzymatic degradation of the respective polymer. Ru et al. (2020) claimed that there is no research on the feasibility of biological components involved in PVC degradation. Nevertheless, there are few publications reported on the practicability of PVC degradation via fungi. Although it is stated that fungi is favorable in PVC degradation, there is insufficient detail relating to the mechanisms of enzymes take place in the reaction (Ali et al., 2014a; Ali et al., 2014b; Sumathi et al., 2016). Hence, more in-depth researches particularly on PVC degradation via microorganisms would be necessary in the near future which are advantageous in microplastics treatment.

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According to (Zhu et al., 2019a), application of enzymes in large-scale reactions is not economical since the enzymes are hard to be recovered or reused with relatively short lifetimes. The enzyme systems need to be further improved in terms of their robustness, efficiency and recyclability for real-world applications. The issue of short enzyme lifetimes can be solved through engineering of whole-cell biocatalysts and microorganisms with the ability of degrading plastics naturally which further leads to production of practical plastic-degrading enzymes (Moog et al., 2019; Yan et al., 2021; Moog et al., 2019). Nonetheless, the activeness of microorganisms in industrial plastic degradation would be reduced significantly

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since degradation of plastics with a high glass transition temperature required extreme temperatures. Although thermophilic microorganisms can be applied to overcome the issue, genetic engineering toolboxes for these distinctive host microorganisms are yet to be explored.

In addition, it is essential to conduct ecological risk assessment for the real-world applications of genetically engineered microorganisms since the mechanisms of toxic chemicals adsorb/desorb onto/from microplastics such as the ageing of particles, hydrophobic interactions, polymer composition and pH variations are scarcely reported (Campanale et al., 2020). In this sense, application of enzyme biocatalysis has gained higher recognition. Immobilization approaches such as cell surface display and crosslinking should be considered for the enhancement of enzyme reusability and stability since both of these methods are able to maintain the accessibility of enzyme to solid plastic substrates (Chen et al., 2020).

Based on the preceding discussion, it can be concluded that the enzymatic degradation of particular microplastics such as PE, PET as well as PS have been extensively reported. Nevertheless, the information on the degradation of some other microplastics such as PP and PVC via enzymatic reaction has not been fully discovered (Othman et al., 2021). As such, further research would be necessary in order to truly comprehend the enzymatic degradation of the respective microplastics which include the purification and discovery of enzymes which are accountable for the degradation of both PP and PVC along with the interpretation of mechanisms involved in the degradation of microplastics via each enzyme. It is crucial to identify various approaches in improving the performance of the discovered enzymes via diverse advanced engineering knowledge (Othman et al., 2021). The understanding of enzymatic reactions in the degradation of microplastics is vitally significant as the successful findings in the particular research area tend to serve as a feasible solution in relation to the current pollution issues.

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6. Recommendation and future prospects

Based on above discussion, we can conclude that our fundamental understanding towards the interactions between incidental microplastics and the environment is still in its infancy. Owing to the emerging engineered technologies for degradation of microplastic, the mechanical principle concerning the synergy of immobilized enzyme/microorganism complexes on degradation microplastics still remains superficial, thus, unable to underpin the degradation fundamental mechanism behind it. Also, the high diversity of microorganisms across different natural habitats or environment has not been significantly explored, such as yeast as a plastic bio-degrader. Another main challenge is the chemical composition of plastics. Even though plastics are often thought of as chemically homogenous on a molecular level, commercial plastics are rarely a single component; more often blend with other small-molecule additives chemically which hinders the in-depth of microplastic degradation mechanism to be discovered. Most importantly, the understanding on the behavior of nanoparticle-enzymes complex in engineered design of plastic bio-degraders that eliminate the secondary environmental contamination is essential, enabling a holistic strategy to solve the global plastic issues. Even though the degradation of microplastics via nanoparticle enzymes can have significant results in reducing marine plastic waste pollution, the economic feasibility and the period of degradation via nano-enzyme still remains largely unknown or not fully being investigated. Many years of research have focused on the technical development, including physicochemical properties of microplastics and mechanisms of degradation of microplastics using nano-enzymes, but, to-date, there is still not any 3Es (Economic, Environmental and Energy) assessment available in literature, which hinders the industrial attractiveness in bulk production of nano-enzymes.

In order to create a circular economy for microplastic degradation, we have highlighted few key takeaways that researchers can consider in their future study. These

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recommendations are also aimed to provide a critical throughput in solving the global plastics waste problem.

1. Integrating both biological and chemical process to generate a self-degradation enzyme-microplastics over time, ideally with less energy input, environmentally friendly, and cheaper than that of the current plastic available in market.
2. More focus should be dedicated to immobilized enzyme/microorganism complexes for degradation of PP and PVC, since most studies in literature are specifically associated with depolymerization of PET and PE.
3. A novel genetic bioengineering approach of developing hybrids combination of enzymes with algae that can enhance biodegradation of microplastic at a shorter period of time with high efficiency.
4. Application of metagenomics to ensure both culturable and unculturable microbes are in good terms in enhancing the identification of microbes and bio-catalysts with potentials for microplastic biodegradation.
5. Application of additive manufacturing, such as 4D printing in developing of immobilized enzyme complexes with same optimum genes at bulk quantity, enhancing the possibility to create a long-term promising and sustainable results.
6. Application of metaverse technology in the development of immobilized enzyme complexes bio-catalysts and the degradation process, encapsulating the ability of the biocatalysts in the enzymatic-degradation system.
7. Application of “Omic tools” including genomics, transcriptomics, proteomics, and metabolomics which can aids the biological interactions that occur between genes, proteins, and metabolites during microplastic degradation.
8. Application of Artificial Intelligence in accelerating the design of immobilized enzyme complexes to yield a high rate of microplastics degradation.

9. A systematic orientation related to enforcement of microplastic regulation with specific emphasis on the new definitions by incorporating immobilized enzymes/microorganisms should be elucidated.
10. The technical, energy and environmental assessment (TEEA) of the degradation of microplastics via immobilized enzymes/microorganisms should be performed in future to enhance the credibility and feasibility of this technology.

7. Conclusion

In conclusion, this review shows that inorganic nanoparticle-enzyme/microorganism complexes, and carbon-based particle/polymer-enzyme complexes are feasible treatment technologies for microplastics degradation. This review also elucidates those emerging nanoparticles/enzymatic technologies for microplastics degradation have shown promising results and they merit more comprehensive investigation in the near future. The different performance results (i.e. degradation efficiency or degradation time) as revealed in the literature are mainly due to the differences in the types of microplastics, types of immobilized enzyme/microorganism complexes and the associated experimental conditions. It is noteworthy that regardless of the types of immobilized enzyme/microorganism complexes or immobilized emerging enzymatic technologies which are being employed to degrade microplastics, the optimization of experimental operating conditions is of paramount importance to yield the highest degradation efficiency for the microplastics. Particularly, the mechanisms of immobilization and the mechanisms of microplastics degradation should be investigated at a deeper level as they play a crucial role in governing the efficiency of microplastics degradation. It is acknowledged that the immobilized enzyme/microorganism complexes also have limitations in microplastics degradation. In order to systematically

address the current limitations and to propel the research breakthroughs to a higher level, there is an increasing need to conduct more in-depth and comprehensive investigation in this research area. Thus, this review has provided several pertinent recommendations to overcome the relevant shortcomings, and these recommendations also offer the research directions in order to bridge the current knowledge gap in this field. It is generally clear that this research field holds much promise for the future because the cutting-edge discoveries are anticipated to realize the wider and practical application of immobilized enzyme/microorganism complexes for microplastics degradation. Indeed, immobilized enzymatic or microbial-based technology is a sustainable approach which can contribute to climate change mitigation, accomplishment of carbon neutrality and sustainable development in the long term.

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Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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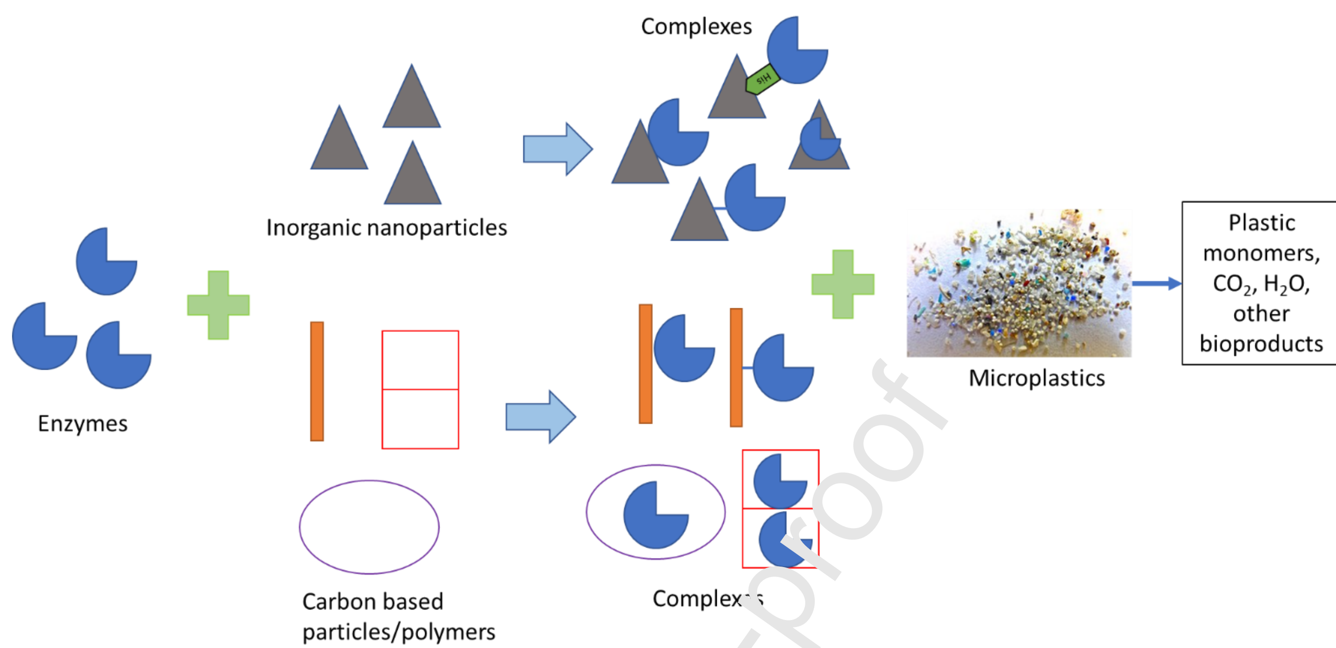
Graphical abstract



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Highlights:

1. Immobilized enzyme complexes are novel in microplastics degradation
2. Metal nanoparticles-enzyme complexes aid microplastics oxidation and hydrolysis
3. Antimicrobial metal nanoparticles might retard microplastics biodegradation
4. Carbon particle-enzyme complexes entrap, encapsulate, bond and adsorb enzymes
5. New complexes include enzymes-hydrophobins and novel nanoparticles

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