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Review

Biodegradation of Carbon Nanotubes, Graphene, and Their Derivatives

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Carbon nanotubes (CNTs), graphene (GRA), and their derivatives are promising materials for a wide range of applications such as pollutant removal, enzyme immobilization, bioimaging, biosensors, and drug delivery and are rapidly increasing in use and increasingly mass produced. The biodegradation of carbon nanomaterials by microbes and enzymes is now of great importance for both reducing their toxicity to living organisms and removing them from the environment. Here we review recent progress in the biodegradation field from the point of view of the primary microbes and enzymes that can degrade these nanomaterials, along with experimental and molecular simulation methods for the exploration of nanomaterial degradation. Further efforts should primarily aim toward expanding the repertoire of microbes and enzymes and exploring optimal conditions for the degradation of nanomaterials.

Why Do We Need to Biodegrade CNTs, GRA, and Their Derivatives?

CNTs, GRA, and their derivatives have many attractive properties (Box 1) and are widely used in numerous products such as drug carriers, electronics, biosensors, sorbents, and fuel cells [1–8]. Their widespread application is increasing the possibility of them entering the environment. The physical and chemical nature of CNTs, GRA, and their derivatives make them inert, stable, recalcitrant, and difficult to degrade [5,9,10]; many studies have reported their presence in the environment. The fates of CNTs and GRA may be related to their specific properties, including their length, degree of oxidation, and functionalization [11–13]. There has been a wide consensus that they pose potential risks to living organisms and the ecosystem [8,14,15] due to their toxicity to various living organisms and cells (Box 2). For example, Zhang *et al.* [16] reported cytotoxic effects induced by single-walled CNTs (SWCNTs) and GRA that are associated with the shapes and concentrations of the nanomaterials.

Several reviews have demonstrated the toxicity and other adverse effects of CNTs, GRA, and their derivatives [8,15,17–21]. For example, Shvedova *et al.* [17] reviewed the toxic mechanism of CNTs from the point of view of oxidative stress and Zhao *et al.* [8] conducted a detailed review of the toxicity of GRA and its derivatives in aquatic environments. Thus, in this review we do not introduce their toxicity in detail. Instead, we focus on the microbial and enzymatic degradation of carbon nanomaterials and the techniques used to explore their degradation.

The toxic effects and other unknown risks of CNTs, GRA, and their derivatives have raised environmental and health concerns among scientists and the public, so there exists a need to identify a safe and effective technology to remove them from the environment. Biodegradation technology may be able to meet this need (Figure 1). Modugno *et al.* [11] advocated assessing the biodegradability potential of CNTs. Sureshbabu *et al.* [22] held a similar view that assessing

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Multiple types of microbes including bacteria and fungi have the ability to degrade carbon nanotubes (CNTs), graphene (GRA), and their derivatives and in the future more species with this ability will be found.

Current applications of numerous enzymes in the biodegradation of CNTs, GRA, and their derivatives provide crucial clues for the design and development of safe nanomaterials and are helpful in understanding their fates in the environment.

A variety of experimental and molecular simulation technologies have been used to jointly explore the biodegradation of CNTs, GRA, and their derivatives.

Biodegradation approaches for CNTs, GRA, and their derivatives are moving from theory to plausible remediation practice for the removal of these materials from the environment but still face many challenges.

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Box 1. Chemistry and Defects of GRA, CNTs, and Their Derivatives

GRA and CNTs are widely used carbon nanomaterials. GRA is a single-layer sheet comprising sp^2-hybridized carbon atoms with a honeycomb structure [68]. It is a 2D material of one-atom thickness and it possesses outstanding physical, electrical, mechanical, optical, and thermal properties [69]. It is one of the strongest and thinnest materials available [70]. CNTs are formed by rolling one layer or multiple layers of GRA sheets into nanoscale tubes [71]. CNTs with only one layer are called SWCNTs; CNTs with multiple layers are called MWCNTs. Figure 1 shows the structures of GRA and CNTs.

Derivatives of GRA or CNTs are produced by oxidation or functionalization. Many GRA derivatives have been created, including GO, GRA nanoribbons, fluorographene, graphyne, porous GRA, and graphdiyne [72]. SWCNTs functionalized with PEG, PEG and aminoanthracene, or PEG and aminofluorene are three well-studied CNT derivatives [73].

Defects such as lattice vacancies and the presence of impurity atoms, either naturally occurring or intentionally introduced, are often detected on CNTs, GRA, and their derivatives [74,75]. The presence of defects on these materials can alter their initial properties resulting in new and interesting properties. For example, the defects on CNTs make them more reactive and can act as attackable sites for biodegradation [9,76].

the biodegradability of CNTs, GRA, and other carbon-based nanomaterials is of great importance for their development and application in biomedical fields. It is widely recognized that studying the biodegradation of nanomaterials has become critically important for the exploration of the structural variations in the materials caused by enzymatic catalysis and for the design of degradable nanomaterials for practical applications [11], making it possible to meet future challenges related to nanomaterials released into the environment. However, studies investigating nanomaterial removal from the environment limited.

Which Microbes Can Degrade CNTs, GRA, and Their Derivatives?

Over past years, numerous studies have explored the biodegradation of CNTs, GRA, and their derivatives using various microbes (Table 1). Liu *et al.* [13] successfully isolated a naphthalene-degrading bacterium that could degrade graphitic materials including GRA oxide (GO), graphite, and reduced GO (RGO). Interestingly, the bacterium had different degrading effects on these materials. More defects were present in RGO, so RGO was more highly oxidized than graphite. Zhang *et al.* [23] oxidized graphite using *Acidithiobacillus ferrooxidans* CFMI-1 to produce graphite oxide. The size and height of the graphite oxide nanosheets formed by bacterial oxidation were 150–900 nm and 1.5–1.7 nm, respectively, and the bacterium-mediated oxidation of graphite was milder than chemical oxidation. Moreover, three bacteria (*Burkholderia kururiensis*, *Delftia acidovorans*, and *Stenotrophomonas maltophilia*) were reported to constitute a community of potential multiwalled CNT (MWCNT) degraders [24]. They decomposed MWCNTs into CO_2 with several intermediate products such as 2-methoxy naphthalene, 2-naphthol, cinnamaldehyde, and isophthalic acid. These bacteria are common microbes in the soil rhizosphere, surface water, and groundwater. Although individual bacteria in this community could only weakly degrade MWCNTs, they were much more efficient degraders

Box 2. Environmental Impacts of Carbon Nanomaterials

CNTs, GRA, and their derivatives are the most commercially relevant types of carbon nanomaterial [77]. They are used in a wide range of consumer products, such as sporting goods and rechargeable devices [71]. There is increasing evidence that CNTs, GRA, and their derivatives have adverse effects on human health and the natural environment.

CNTs are found to be very stable due to their structural features [24]. The environmental impacts of CNTs and their derivatives include, for example: (i) reproductive and developmental toxicity in mice, chicken, zebrafish, and other animal species [77]; (ii) phytotoxicity [78]; and (iii) modification of the structure or composition of soil microbial communities [79].

The main environmental impact of GRA and its derivatives are their toxic effects on a variety of living organisms (including bacteria, fungi, plants, and animals). On release into the environment, they can interact with living organisms and enter cells by penetration and endocytosis pathways [8]. Once inside they can cause cell membrane damage, induce oxidative stress, and attack DNA.

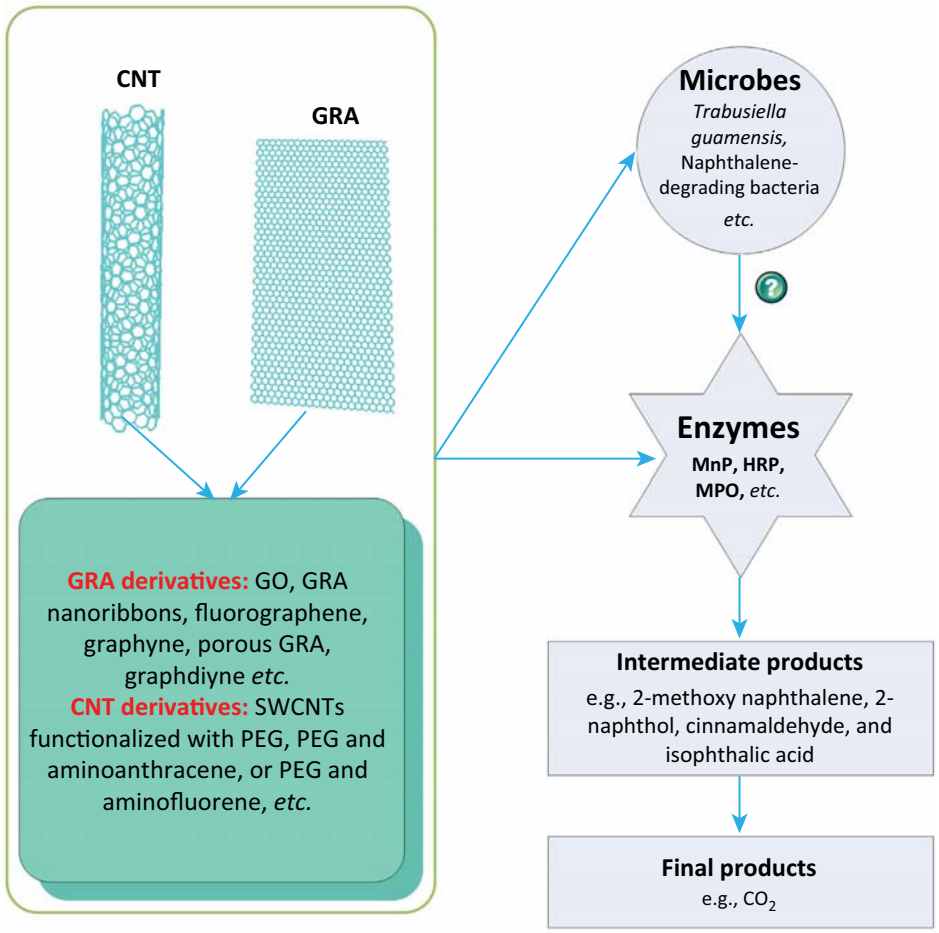

Figure 1. Microbial and Enzymatic Degradation of Carbon Nanotubes (CNTs), Graphene (GRA), and Their Derivatives. The derivatives of CNTs and GRA, microbes, enzymes, intermediate products, and final products are illustrated using some typical examples. '?' illustrates a research gap that links microbial degradation to enzymatic degradation in many previous studies. MnP, manganese peroxidase; HRP, horseradish peroxidase; MPO, myeloperoxidase.

Table 1. Microbes Capable of Degrading CNTs, GRA, and Their Derivatives

Microorganism	Taxonomy	Material	Refs
Naphthalene-degrading bacteria	Bacteria	GO, graphite, and RGO	[13]
A bacterial community comprising *Burkholderia kururiensis*, *Delftia acidovorans*, and *Stenotrophomonas maltophilia*	Bacteria	MWCNTs	[24]
Trabusiella guamensis	Bacteria	MWCNTs	[2]
Sparassis latifolia	Fungi	SWCNTs	[26]
White-rot fungi (*Phanerochaete chrysosporium*)	Fungi	SWCNTs, oxidized and reduced GRA nanoribbons	[3,33]
Trametes versicolor and natural microbial cultures	Fungi	SWCNTs	[25]

in combination [24]. In addition, *Trametes versicolor* and natural microbial cultures were studied for the biotransformation of SWCNTs, showing a weak degrading ability [25]. Recently, Chouhan *et al.* [2] obtained soil bacteria (*Trabusiella guamensis*) from a goldsmith site contaminated with nanomaterials and showed that the bacteria were adaptive and tolerant to the nanomaterials and thus could survive well in the contaminated soil. The bacteria were observed to be able to biotransform MWCNTs by an oxidation process.

In addition to bacteria, fungi have also been observed to decompose nanomaterials. For example, the *Sparassis latifolia* mushroom can secrete lignin peroxidase (LiP) to degrade both thermally treated and raw-grade carboxylated SWCNTs [26]. In addition, white-rot fungus (*Phanerochaete chrysosporium*) has been widely applied to degrade lignin [27,28], polycyclic aromatic hydrocarbons (PAHs) [29,30], dyes [31,32], and other pollutants. LiP secreted by white-rot fungus was reported to degrade oxidized and reduced GRA nanoribbons [3] and manganese peroxidase (MnP) from *P. chrysosporium* was reported to decompose pristine SWCNTs [33]. Recently, the toxicity of GO to *P. chrysosporium* was assessed [34]. Low concentrations of GO stimulated growth of *P. chrysosporium* while high concentrations of GO induced a negative effect on its growth and activity.

Which Enzymes Can Degrade CNTs, GRA, and Their Derivatives?

Enzymatic Degradation of CNTs and Their Derivatives

Myeloperoxidase has been shown to oxidize SWCNTs [35]. Vlasoval *et al.* [36] further investigated the CNT degradation mechanism of this enzyme and observed that the degradation relied on the production of hypochlorite by myeloperoxidase *in vivo*. It has been shown that the binding of SWCNTs to human serum albumin by electrostatic interactions between SWCNT carboxyl groups and the Arg residues of the protein and π–π stacking interactions of SWCNTs with the protein's Tyr residues significantly enhanced SWCNT biodegradation [37] as the interactions accelerated the release of myeloperoxidase and the production of hypochlorite. Another study, performed by Bhattacharya *et al.* [38], observed that myeloperoxidase was capable of degrading SWCNTs that were modified by poly(ethylene glycol) (PEG) molecules with various molecular weights. Finally, the activity of myeloperoxidase for CNT degradation can be inhibited by the presence of antioxidants such as glutathione and ascorbic acid [39].

In addition to myeloperoxidase, SWCNT biodegradation on incubation with human eosinophil peroxidase and H_2O_2 has been reported in a study by Andón *et al.* [40]. The incorporation of NaBr enhanced SWCNT biodegradation because NaBr can prevent the decrease of enzyme activity with time and activate the enzyme. Lactoperoxidase, a secreted peroxidase enzyme found in airways, was also reported to be capable of degrading oxidized SWCNTs, with and without a pulmonary surfactant [41]. In this study the authors first oxidized SWCNTs and then confirmed the formation of oxidized SWCNTs by X-ray photoelectron spectroscopy. Afterward, they performed biodegradation experiments and investigated the biodegradation chemistry of the oxidized SWCNTs using UV–visible light (Vis)–near-IR (NIR) spectroscopy, Raman spectroscopy, scanning electron microscopy (SEM), and atomic force microscopy. A widely held viewpoint in the nanotechnology field is that surface modification (e.g., the incorporation of carboxyl groups) is a prerequisite for CNT biodegradation. However, a study performed by Zhang *et al.* [33] has challenged this viewpoint. Using transmission electron microscopy (TEM), NIR spectroscopy, and Raman spectroscopy, their study showed that MnP from *P. chrysosporium* could degrade pristine CNTs. Interestingly, MnP is incapable of attacking surface-oxidized SWCNTs because the carboxyl groups of oxidized SWCNTs disturb the catalytic cycle between Mn^{2+} and Mn^{3+}, which is important for MnP activity, by binding to Mn^{2+} at the binding site for MnP.

Several studies have reported the ability of horseradish peroxidase to degrade CNTs. The biodegradation of carboxylated CNTs by horseradish peroxidase and H_2O_2 has been analyzed

previously [42,43]. These two studies focused on the interaction of horseradish peroxidase with carboxylated SWCNTs by various methods. Carboxylated SWCNTs, rather than pristine SWCNTs, were degraded. Allen *et al.* [42] believed that hydrophobic interactions were the factor that prevented the biodegradation of pristine SWCNTs. Notably, incubation with hemin or $FeCl_3$ caused significant degradation of these two types of SWCNT. The degradation of nitrogen-doped and carboxylated MWCNTs by treatment with horseradish peroxidase and H_2O_2 was explored [44]. The degradation rate of MWCNTs was related to the extent of carboxylation. MWCNTs were more difficult to degrade than SWCNTs, as MWCNTs comprise multiple layers that would cause more resistance to decomposition mediated by horseradish peroxidase. MWCNTs required a longer time to degrade than SWCNTs by horseradish peroxidase. The degradation generally started at the defective sites on the MWCNTs [44].

Most of these previous studies of biodegradation were based on qualitative results rather than the biotransformation rate. However, Flores-Cervantes *et al.* [9] determined the CNT biotransformability of horseradish peroxidase by incubating 13 different classes of CNT. These CNTs differed in length, outer diameter, or structure (SWCNTs and MWCNTs), with and without functional groups. The purpose of this study was to observe the effects of CNT features (shape, size, and functionalization extent) on CNT biodegradation. Ultimately, the authors found that the rate of transformation by horseradish peroxidase is very low for all types of CNT. Furthermore, the authors concluded that SEM and TEM were not good options for the assessment of biodegradation and biotransformation due to their limitations in qualitative analyses. Recently, Modugno *et al.* [11] investigated the biodegradability of covalently oxidized double-walled CNTs and MWCNTs of differing lengths and oxidation extent by horseradish peroxidase. Double-walled CNTs were resistant to degradation by horseradish peroxidase while MWCNTs could be partly biodegraded. Treatment with horseradish peroxidase and H_2O_2 resulted in the formation of many defects on the MWCNTs. In addition, the functional groups on the MWCNTs were helpful in their biodegradation. It has been demonstrated that horseradish peroxidase and xanthine oxidase are able of degrading functionalized CNTs [22]. Coumarins and cathecol derivative were used to functionalize the surfaces of MWCNTs leading to enhanced catalytic activity of horseradish peroxidase. However, functionalization by purine failed to improve the catalytic activity of xanthine oxidase.

Recently, Chen *et al.* [45] investigated the enzyme-catalyzed molecular basis of SWCNT biodegradation or lack of biodegradation with two enzymes: a CNT-degrading enzyme (*P. chrysosporium* MnP) and a CNT-non-degrading enzyme (*P. chrysosporium* LiP). Transitions in the native conformations were found to be necessary for SWCNT biodegradation by enzymes. Pristine SWCNT bound to the loop region of *P. chrysosporium* LiP inhibited its native conformational changes making it unable to degrade SWCNTs. By contrast, pristine SWCNTs bound to the loop and helical region of *P. chrysosporium* MnP and did not prevent conformational changes.

Enzymatic Degradation of GRA and Its Derivatives

Compared with CNTs, enzymatic degradation of GRA has been less studied. The widespread application of GRA and its derivatives has caused many environmental issues (Box 2), which has increased research interest in their biodegradation by enzymes. Several studies have reported the enzymatic degradation of GRA and its derivatives. The most frequently used enzymes for CNT biodegradation, such as myeloperoxidase and horseradish peroxidase, were also tested for their ability to degrade GRA and its derivatives. The potential for the biodegradation of GO by myeloperoxidase was investigated in the presence of H_2O_2, and myeloperoxidase-mediated degradation was shown to strongly depend on the dispersibility of GO [5]. Highly dispersed GO was completely degraded but almost no structural changes occurred in the most aggregated GO. High dispersibility means that the nanomaterials do not aggregate and disperse well in aqueous solutions, thus facilitating enzymatic attack against the nanomaterials. White GRA, also known as

hexagonal boron nitride nanosheets, was observed to be partially degraded by myeloperoxidase after 35 h but was not decomposed by horseradish peroxidase within 60 days. This degradation pattern was inconsistent with that of GO or GRA [46]. A previous study found that GO could be degraded by low concentrations of horseradish peroxidase leading to the appearance of holes on its surface [47]. However, it was unable to degrade chemically reduced GO.

Functionalization is believed to be helpful in mitigating the toxicity of nanomaterials. However, functionalization also may make the nanomaterials difficult to biodegrade. For example, GO coated with bovine serum albumin or PEG reduced its cytotoxicity but inhibited the activity of horseradish peroxidase [48]. The authors of this study further explained that these coating molecules might interfere with interactions between the GO sheet and the enzyme by spatial hindrance. Another study from Zhang *et al.* [49] examined the impact of GRA, GO, and RGO on the stability and activity of horseradish peroxidase. The enzyme's stability was significantly decreased by GRA and GO but was increased sevenfold by RGO, which is capable of acting as a redox mediator and radical quencher. Complete oxidation of oxidized GRA nanoribbons and partial degradation of RGO nanoribbons by LiP from white-rot fungus occurred within 96 h in the presence of H_2O_2 and veratryl alcohol [3]. Veratryl alcohol was implied to play an important role in aiding the LiP-mediated degradation of these GRA derivatives.

In addition to the enzymes mentioned above, some enzymes were also tested for their ability to biodegrade CNTs or GRA but were found to be unable to efficiently degrade CNTs or GRA. Tyrosinase and laccase obtained from Sigma-Aldrich were observed to be incapable of decomposing MWCNTs into CO_2 [24].

Clearly, most of these previous studies focused on the degradation of oxidized CNTs and GRA, which may be because oxidization and functionalization often make them more biocompatible and reactive [41,50–54]. Oxidation can create defect sites, leading to an increase in the biodegradation rate of CNTs and GRA [11,13]. However, adding too many functional groups on these nanomaterials may not achieve the goal of enhancing their biodegradation [24].

The number of microbes and enzymes that have been found to be involved in nanomaterial biodegradation remains limited (Tables 1 and 2) and more biodegradation studies are needed to characterize them. Moreover, it may be possible to improve the efficiency of the known microbes

Table 2. Enzymatic Biodegradation of CNTs, GRA, and Their Derivatives

Enzyme	Substrate	Refs
Enzymes found to be able to degrade CNTs, GRA, or their derivatives		
Lactoperoxidase	SWCNTs	[41]
Horseradish peroxidase	SWCNTs, MWCNTs, and GO	[9,11,22,42,44,48]
Myeloperoxidase	SWCNTs and GO	[5,35,37–39]
Xanthine oxidase	MWCNTs	[22]
Eosinophil peroxidase	SWCNTs	[40]
LiP	SWCNTs, oxidized and reduced GRA nanoribbons	[3,26]
MnP	SWCNTs	[33]
Enzymes found to be unable to degrade CNTs, GRA, or their derivatives		
Tyrosinase	MWCNTs	[24]
Laccase	SWCNTs, MWCNTs	[24,33]

and enzymes in degrading nanomaterials. In particular, there remain very few studies of their practical application in the field, probably because field environmental conditions are more complex than laboratory conditions, which strongly affects microbe growth and enzymatic activity.

What Are the Main Experimental and Theoretical Technologies Used to Investigate the Biodegradation of CNTs, GRA, and Their Derivatives?

Various experimental and molecular simulation technologies have been used to explore the biodegradation of CNTs, GRA, and their derivatives. Among these, TEM, Raman spectroscopy, and Vis–NIR spectroscopy are most often used. TEM has been used to observe the morphological changes in SWCNTs, double-walled CNTs, MWCNTs, GO, and other derivatives

Table 3. Main Experimental and Theoretical Technologies Used to Investigate Biodegradation

Method	Main function for biodegradation studies	Usage
Experimental methods		
SEM	Observing the morphological changes in nanomaterials caused by enzymatic or microbial degradation; investigating whether CNTs or GRA were successfully functionalized	[2,24,26,37]
TEM	Observing the morphological changes in nanomaterials caused by enzymatic or microbial degradation	[2,11,22,24, 26,38–40,48]
Raman spectroscopy/microscopy	Observing the degradation process of nanomaterials by visualizing variations in G- and D-band intensities	[2,11,22,26, 35,37–40]
Vis–NIR spectroscopy/UV–Vis–NIR spectra	Monitoring the degradation of GRA or CNTs, e.g., by observing changes in the M1 and S2 bands	[11,26,35, 37–40]
Electron spin resonance (ESR) spectroscopy	Identifying the formation of radicals and describing enzyme activity during the biodegradation of CNTs or GRA	[26]
Atomic force microscopy	Characterizing the oxidation and conformation of CNTs or GRA	[37,38]
^{14}C labeling	Tracing the end products of CNT degradation	[24]
Liquid chromatography–tandem mass spectrometry (LC-MS/MS)	Identifying the intermediates and/or final products of CNT biodegradation	[24,26]
Gas chromatography–mass spectrometry (GC-MS)	Identifying the intermediates and/or products of CNT biodegradation	[24,26]
Electron paramagnetic resonance (EPR) spectroscopy	Qualitatively confirming the occurrence of oxidation	[39]
Electrospray ionization–mass spectrometry (ESI-MS)	Qualitatively confirming the occurrence of oxidation	[39]
Circular dichroism spectroscopy	Analyzing the interactions between CNTs and proteins	[37]
Attenuated total reflectance–IR (ATR-IR) spectra	Studying the functional groups on MWCNTs during microbial biodegradation	[2]
X-ray diffraction	Observing the biodegradation process of CNTs	[2]
Molecular simulation methods		
Molecular docking	Detecting the binding sites and modes of CNT/GRA interaction with enzymes	[35,37,40, 42,45,47]
Molecular dynamics simulation	Tracing the interactional dynamics between CNTs/GRA and enzymes	[45]
Homology modeling	Generating 3D structural models of enzymes using known templates	[40]

[9,11,26,48] caused by enzymatic or microbial degradation. Raman spectroscopy is often used to monitor the biodegradation progress of CNTs, GRA, and their derivatives by visualizing variations in G- and D-band intensities, where the G band is used to assess C–C bond stretching and the D band corresponds to sp^2-hybridized carbon systems [11,47,55]. In addition, Vis–NIR spectroscopy is usually employed to characterize the biodegradation of nanomaterials on the basis of the S_2 and M_1 bands [26]. An overview of the main experimental technologies for biodegradation studies of CNTs, GRA, and their derivatives is provided in Table 3.

Among molecular simulation methods, the most commonly used method is molecular docking, followed by molecular dynamics simulations and homology modeling (Table 3 and Figure 2). The 3D structures of CNTs have often been produced by VMD [56] or Nanotube Modeler software, while enzymatic structures are generally retrieved from the Protein Data Bank (PDB) [57]. In

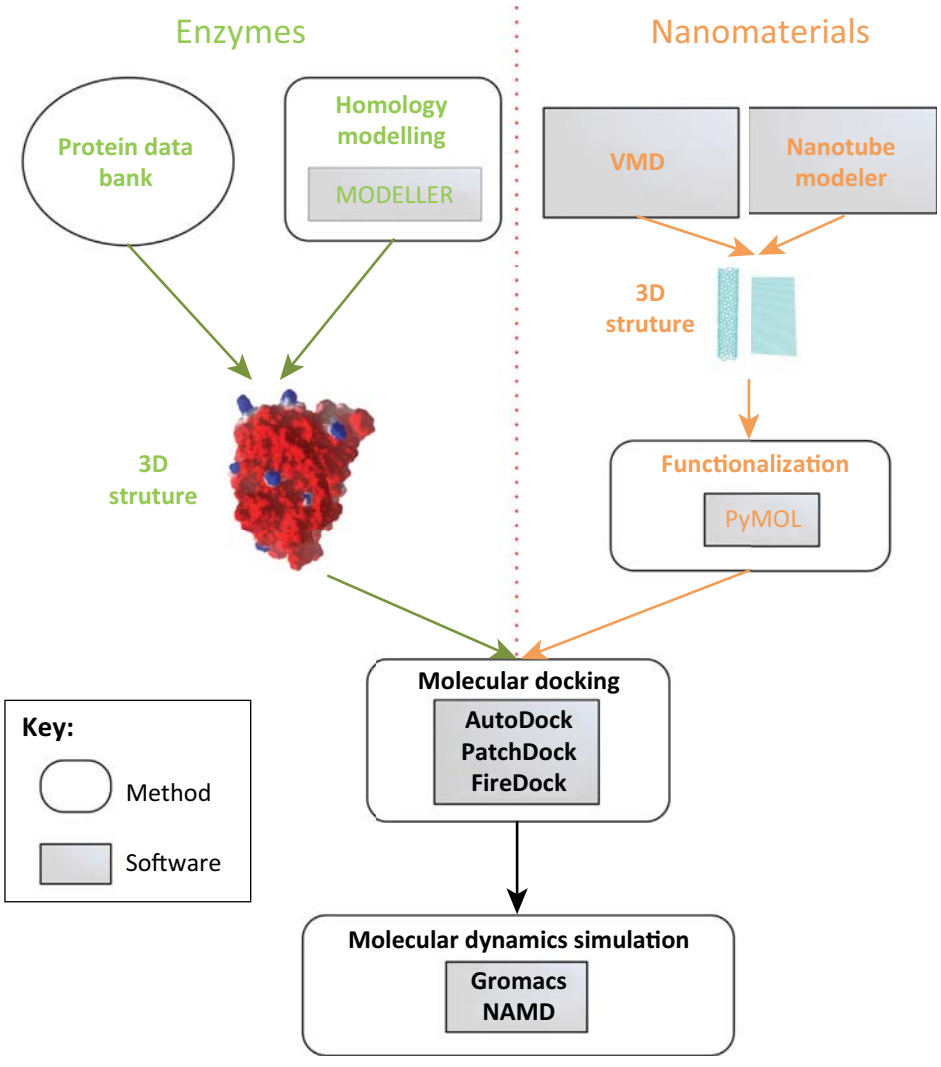

Figure 2. Molecular Simulation Studies on the Biodegradation of Carbon Nanotubes, Graphene, and Their Derivatives. The 3D structure of manganese peroxidase [Protein Data Bank (PDB) ID: 3M5Q] [80] is used as an example.

cases where the 3D structures of the enzymes are unavailable in the PDB, homology modeling could be employed to build 3D structural models [58,59]. For example, in a study of SWCNT biodegradation mediated by eosinophil peroxidase, whose 3D structure was unavailable [40], the authors built the 3D structure of the enzyme by homology modeling using the MODELLER program [60,61]. Molecular docking is a computational method to accurately predict the interaction between a ligand and a receptor [62]. In cases where enzymatic degradation of nanomaterials is studied by molecular docking, the ligand is the nanomaterial and the receptor is its degrading enzyme. AutoDock is docking software commonly used to model the docking between CNTs, GRA, or derivatives and degrading enzymes [35,37,40,42,47]. In a recent study by Chen *et al.* [45], the binding conformations between the enzymes and SWCNT were first produced using PatchDock [63]; then, FireDock [64] was employed to refine them to obtain the best structural model. All of these docking tools have been shown to be accurate and efficient in these studies.

Molecular simulation studies are also helpful in revealing the molecular basis and mechanisms for the biodegradation and oxidation of nanomaterials. For instance, Kotchey *et al.* [47] found that the molecular mechanism for the oxidation of GO was associated with the binding strength of horseradish peroxidase to GRA sheets as well as the GO structure and orientation, based on molecular docking results.

Other basic technologies related to biodegradation studies of nanomaterials, such as the synthesis and functionalization of CNTs and GRA, and the measurement of enzyme activity have been thoroughly discussed by previously published articles (e.g., [65]).

Concluding Remarks

We have reviewed the biodegradation of CNTs, GRA, and their derivatives by fungi, bacteria, and plant, animal, and microbial enzymes and provided an overview of the common experimental and molecular simulation methods to study the biodegradation of these nanomaterials. Microbial degradation appears to be the most promising for practical applications compared with enzymatic degradation because enzymatic degradation often strictly requires a suitable temperature and pH. If environmental conditions are not appropriate, the enzyme activity could be inhibited or disappear. The limits for microbial degradation are relatively lower because microorganisms can grow under a variety of conditions [29,66]. Despite successful applications in the environmental removal of nanomaterials, there remain many problems and obstacles in the field of biodegrading carbon nanomaterials (see Outstanding Questions). It has been demonstrated that microbial degradation is often related to secreted enzymes (http://eawag-bbd.ethz.ch/) [67]. However, which enzymes are secreted and which enzymes are responsible for the nanomaterial degradation in the studies of microbial degradation have not been reported [2,24,25]. In these studies, the authors focused on isolating and identifying nanomaterial-degrading microbes, structural changes in the nanomaterials, and metabolic products. This reflects a research gap linking microbial degradation to enzymatic degradation (Figure 1) that should be focused on in future studies.

Surface modification or functionalization can improve or hinder biodegradation largely related to the properties of the additives. The nanomaterial-degrading ability of individual microbes or pure microbe cultures is often weak [24], but a community comprising multiple nanomaterial microbial degraders can significantly enhance the degradation of nanomaterials. Thus, it is critical to determine which microbial combinations are more efficient than individual microbes. Then, the key microbial interactions and mechanisms that improve the degradation of CNTs or GRA can be further investigated. Several enzymes can degrade CNTs, GRA, and their derivatives (Table 2). However, their environmental applications in the removal of these nanomaterials still face many challenges. For example, slow biotransformation rates for some degrading enzymes,

Outstanding Questions

How can we overcome the limitations of current biodegradation strategies and technologies for CNTs, GRA, and their derivatives?

Are there more microbes and enzymes that can degrade CNTs, GRA, and their derivatives?

Can we find a safe, efficient, and reliable design procedure for the functionalization of CNTs and GRA?

Can the biodegradation of CNTs, GRA, and their derivatives be investigated simultaneously?

What are the rules for structural changes in GRA and CNTs during their microbial or enzymatic degradation?

How can we better explore the metabolic pathways of microbes related to the degradation of GRA and CNTs?

low substrate specificity, and poor performance under adverse environmental conditions [3,9] largely limit their practical use for environmental remediation.

In general, current studies focus separately on the biodegradation of CNTs or GRA. Few studies have explored their combined biodegradation effects. However, in actual applications, sites contaminated with CNTs also may be polluted by GRA and its derivatives. Therefore, it is critical to understand how CNTs and GRA interact and what effects this interaction may cause.

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