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REVIEW

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Stability, transport and ecosystem effects of graphene in water and soil environments

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Graphene nanomaterials (GMs), such as graphene oxide (GO) and reduced graphene oxide (rGO), have been widely applied in various fields. Due to the rapid increase in production and application, the inevitable release of GMs into water and soil environments poses potential health and ecosystem risks. Upon exposure, the behavior, transport, and fate of GMs may be altered after interacting with the relevant environmental conditions. GMs can affect the microbial communities as well. Thus, it is imperative to understand the interaction between the GMs and the environmental systems for predicting their risks. For this purpose, this review highlights the influence of the most relevant environmental factors on the stability, aggregation, and transformation of GMs in aquatic environments. Moreover, the transport of GMs and microbial communities changes have also been presented based on the recent findings. To the best of our knowledge, this review covered most of the recent related studies and will allow for accurate predictions of the fate and risks associated with GMs. In consideration of the diversity of GMs and the complexity of environmental factors, further studies should be focused on their inherent properties and amicable development.

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1. Introduction

Graphene, a single layer of sp²-bonded carbon atoms closely packed into a honeycomb lattice, is the first two-dimensional

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(2D) atomic crystal available to us. It has been considered as one of the most outstanding achievements in the 21st century.^{1,2} Since its discovery in 2004,³ graphene has been receiving intense research interest from the scientific and industrial communities. Due to its unique physicochemical properties such as high electron conductivity, superior thermal conductivity, excellent mechanical strength, and large specific surface area, it has been widely applied in various fields.^{4–6} Currently, the most common forms of graphene nanomaterials (GMs) used and studied are graphene oxide (GO),



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Nowadays, GMs have been widely used as industrial and commercial products worldwide. Due to the growing commercial interests and the potentially increasing market for graphene products,¹³ it is inevitable that GMs will be released into the environment during their manufacturing, transportation, use, and disposal. Upon being released into waters, sediments, and soils, they interact with a variety of physicochemical and biological factors, thus they possibly can cause significant adverse effects on environments and ecosystems. Consequently, serious concerns have been raised about the environmental behavior, fate, and risks of GMs.¹⁴⁻¹⁷ Waters and soils are the major environmental receptors of nanomaterials. Singh¹⁸ discussed how the fate of nanoparticles in the environment is not only determined by the intrinsic physicochemical properties of these nanoparticles, but also controlled by the environmental conditions. Meanwhile, an alarming problem of nanoparticles is their potential ecotoxicity. This report provides a basic study focus for investigating the fate and risk of GMs. Dale et al.¹⁹ discussed suitable models for predicting the environmental behavior of nanoparticles. Currently, the reviews regarding the behaviors of GMs are focused mainly on the aquatic environment,¹⁵ and the toxicity of GMs is mainly discussed based on in vitro and in vivo studies.20,21 Nadres et al.22 discussed the toxicity



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mechanisms (physical and chemical mechanisms) and potential environmental impacts of GMs. However, comprehensive information on the environmental behaviors and ecosystem effects of GMs within different environmental media is still lacking. To avoid any overlaps and to reduce the knowledge gaps, we will further enrich the information from three aspects: the behavior, transport, and ecosystem effects of GMs in the environment.

Owing to the miscellaneous chemical, physical, and biological factors in the environment, after entering the water and soil media, the possible behaviors of GMs will vary depending on the different environmental conditions. For example, GO and/or rGO may stabilize and be transported within a water flow in aqueous solutions with the presence of natural organic matter (NOM) and low concentrations of salt ions, whereas they may aggregate and precipitate to sediments in waters with high ionic strength.^{23,24} In soil and porous media, the transport of GO and/or rGO is inhibited in soil with high ionic strength, whereas it is enhanced in the presence of NOM.^{25,26} In addition, the properties and structures of GMs will be transformed due to the interactions with environmental media and microbes. On the other hand, they can also affect the survival of microbes, thereby causing a change in microbial communities.²⁷ Overall, this thorough review will focus mainly on the behavior of GMs under various aquatic environmental factors (such as pH, ionic strength, NOM, sunlight, minerals, and microbes), and the transport of GMs in soil and porous media, as well as the effects on microbial communities. Herein, we deem that this review will provide valuable insights into the risk assessment, their potential applications, and the amicable development of GMs.

2. Structural characteristics of graphene materials

GO, a classical type of GM, was called graphite oxide or graphite acid in the early days. It has a history going back nearly 160 years.^{28,29} The discovery of graphene intrigued researchers to study GO again to explore a preferable and more scalable method for producing graphene.^{30,31} Although a large number of studies have been conducted to attempt to explain the structure model of GO,³²⁻³⁴ it still remains elusive. To date, the most widely accepted model of GO is the one explained by Lerf and co-workers in 1998,34 who suggested that GO consists of a carbon backbone and oxygen-containing functional groups on their surfaces. However, their model still leaves some unanswered questions and cannot provide explanations for all the experimental observations. Based on several excellent experimental studies, a more recent view on the chemical structure of GO was summarized and described by Dimiev, who presented a more modern, complete perspective of the chemistry of GO (Fig. 1).³⁵ Generally, the obtained GO is a metastable material, whose structural and chemical transformations can be driven by external stimuli, such as temperature and light.^{36,37} Understanding the chemical transformations can



Fig. 1 The complete version of the Dimiev–Tour GO structural model (DT model), featuring all the proposed functionalities. A GO fragment contains a hole situated on the border between a graphenic domain (lower right corner) and an oxidized domain (upper left corner). Different structural features are represented by different numbers and colors. 1 Ketone and enol groups are formed at the point of C–C bond cleavage. 2 By hydration, ketones can turn into *gem*-diols, and further into hemiacetals. 3 Here, a *gem*-diol is in an α -position to ketone; this favors the stability of the *gem*-diol in aqueous solutions. 4–6 These are the vinylogous carboxylic acid. Conjugation in the carboxylic acid 4 is limited by two oxygen atoms. The conjugation of the vinylogous acids 5 and 6 extends to the entire graphitic domain; whereby acids 5 and 6 are stronger acids than acid 4. Organic sulfates are present in GO samples prepared in sulfuric acid medium. Reprinted with permission from ref. 35. Copyright © 2017, John Wiley & Sons, Ltd.

provide novel opportunities for controlling the properties of GO, which favor their various applications. GO is a nonstoichiometric compound with variations in the elemental content resulting from different degrees of oxidation, which is attributed to the oxidants used, the graphite source, and the reaction conditions.^{10,38} However, regardless of the synthesis method selected,^{28,29,39} all of the prepared GO products possess the same types of oxygen-containing functional groups, and the ratio of the composition content remains constant (typically presenting O: C ratios between 0.3 and 0.5) after the successive oxidative processes.⁴⁰⁻⁴² Because of the abundance of hydrophilic functional groups, such as hydroxyl and epoxide on the basal planes, and carboxylic groups on the periphery of the planes, GO can uniformly disperse in aqueous solutions.⁴³ Currently, the applications of GO in a wide variety of areas are potentially huge,44-47 thus making it necessary to survey the potential negative impacts to the environment and ecosystems.

Reduced graphene oxide (rGO) is another one of the most common forms of GMs. Likewise, rGO has a wide range of applications in many fields due to its exceptional properties. The production of chemical rGO usually uses reducing agents, including hydrazine,^{48,49} sodium borohydride,⁵⁰ vitamin C,⁵¹ and ascorbic acid.⁵² The chemical reduction process is proposed in Fig. 2. After reduction, a majority of the oxygencontaining groups are removed from the hydrophobic structural



Fig. 2 Schematic of the synthesis of chemical converted graphene by the reduction of graphene oxide. Reprinted with permission from ref. 11. Copyright © 2011 WILEY-VCH.

surfaces. It is worth noting that rGO can also be formed from the spontaneous reduction of GO in water bodies under certain factors emphasized in the following section. In addition, thermal treatment is another widely used method for the reduction of GO.⁵³ Similar to chemical reduction, the oxygen functionalities attached at the edges of the aromatic domains are difficult to remove, even when treated with thermal annealing at high temperature.⁵⁴ Thus, the residual groups are still present in rGO. It is clear that the dispersibility of rGO in aqueous solutions is inferior to that of GO due to a significant decrease in the oxygen-containing groups, and the dispersibility of graphene in water is the lowest.^{55–57} Generally, in view of the incomplete deoxygenation by reduction,^{58–60} it is still feasible to obtain stable rGO dispersion over a certain pH range.

3. Stability and aggregation of graphene materials in aquatic environments

Several studies have demonstrated that the colloidal stability of carbon nanomaterials (CNMs) plays a dominant role in controlling their environmental fate and bioavailability in natural aquatic systems.^{61–63} Nevertheless, comprehensive information about how the main environmental factors control the stability and aggregation of GMs is limited. Due to the uniform dispersibility of GO in aqueous solutions, the majority of investigations are related to the stability of GO under various environmental conditions.

Generally, the colloidal stability of charged particles is the result of the presence of various surface forces. These interaction forces have been described on the basis of Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, which assumes

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that the interfacial forces experienced between colloidal particles are dominated by van der Waalss (vdW) and electric double-layer (EL) forces.^{64,65} It is known that the vdW and EL interactions are always attractive and repulsive, respectively. Thus, what is required to predict the aggregation behavior of colloidal particles is to determine the sum of the vdW and EL interactions.^{65,66} Importantly, the aggregation and stability studies on various CNMs, such as fullerenes (C₆₀), carbon nanotubes (CNTs), and GMs, have shown that their aggregation follows the DLVO theory.^{66–70} Chen *et al.* (2010)⁶¹ provided some important information on the assessment of the colloid properties of C₆₀ nanoparticles and CNTs. The special physicochemical properties of CNMs (such as surface oxygen contents and particle size) have strong effects on their stability.^{61,70} In the case of GO and rGO, the stability of rGO is lower than that of GO in aqueous solutions. The reduction of GO decreases the surface charge density due to the significant decrease in the number of charged groups upon reduction, thereby significantly decreasing the strength of EL repulsion. Furthermore, the restoring of the conjugated graphene structure strengthens the vdW attraction.⁶⁶ Therefore, these driving forces are usually accepted to explain the origin of the stability (instability) of GO (rGO). Furthermore, the conditions in an aquatic environment (pH, divalent cations, NOM, etc.) can impact the colloidal properties of GO and rGO, thus changing their stability. Therefore, this section mainly covers the behavior of GO and partly rGO in various aquatic environment conditions.

3.1 Effect of pH

Solution pH is a major factor determining the stability of a graphene suspension.⁷¹ Generally, the zeta potential, an index of the magnitude of the electrostatic interaction between colloidal particles, is measured from the electrophoretic mobility at various solution conditions. Negatively charged colloids with zeta potentials lower than -30 mV are commonly considered to be electrostatically stable.⁵⁹ A highly negative zeta potential suggests better colloidal stability due to the stronger electrostatic repulsion between the dispersed nanoparticles.⁷² Because the stability of colloidal solutions depends on the balance between the EL and vdW forces, it provides an important criterion for describing the stability of colloidal particles.^{59,73} Theoretically, GO could be viewed as an amphiphilic substance due to its unique structure containing both hydrophilic edges and hydrophobic basal planes.74,75 Conceivably, its amphiphilicity could be altered by adjusting the pH, because the state of ionizable oxygen-containing groups present on GO will be varied at different pH values. After reduction, most of the oxygen-containing functional groups are removed from the rGO surface, resulting in a less stable rGO dispersion than the dispersion of GO in aqueous solution.

The influence of pH on the stability and aggregation behaviors of GO and rGO have been investigated by several researchers. To illuminate the impact of pH on the behaviors of GO aqueous solutions, Shih's group⁷⁶ conducted a series of

comparative experimental and molecular dynamics (MD) simulation studies under the desired pH conditions (pH 1 and 14). Their results showed that GO became less hydrophilic and easily formed aggregates at low pH solutions (zeta potential -4.25 ± 0.35 mV), but exhibited good dispersibility in bulk water at a high pH (zeta potential -44.73 ± 1.54 mV). In particular, they observed an interesting appearance that the formed aggregates, which are surface-active at low pH, were stable in the solution instead of settling down as a precipitate. This phenomenon was explained by calculating the potential of mean force (PMF) between two parallel GO sheets in a MD simulations study. This further clarified the pHdependent aggregation of GO from a microscopic perspective, suggesting that aggregates exhibit a GO/water/GO sandwichlike structure (Fig. 3).⁷⁶ However, the chosen pH values in their trials are nearly impossible to find in common aquatic environments, and could not suitably explain the variation in the aggregation/stability behavior of GO over the entire solution pH range well vet. Various studies have suggested that the negative charges of GO should depend on the degree of the ionization of the ionizable groups (carboxylic and/or hydroxyl group).⁷⁷⁻⁷⁹ Chowdhury et al.⁸⁰ verified that the surface charge of GO is pH sensitive. Moreover, measurements for the hydrodynamic characterization were used to analyze the aggregation process. They found that the hydrodynamic diameter (Dh) of GO remained almost constant (250 nm) from pH 4 to 10, while the colloidal size significantly increased at lower pH. Their results are consistent with the findings of Wu's group that the solution pH induces variations in the GO nanoparticle surface charges.⁸¹ For the stability of rGO in an aquatic environment, Li's group⁵⁹ demonstrated that stable rGO aqueous dispersion under certain conditions can be obtained due to the electrostatic stabilization. Konkena and Vasudevan⁸² measured the zeta potentials of rGO at different pH conditions. They found that the zeta potential of rGO dispersion was pH sensitive and that the stable rGO dispersion was merely observed at a higher pH range (8-11.5) with negative zeta potentials below -30 mV. Kashyap et al.⁸³ also found that rGO colloids gained stability at a relatively narrower pH range of 7-10, slightly different from the results reported by Konkena and Vasudevan.⁸² Qi et al.⁸⁴ found that the electrophoretic mobility (EPM) of rGO with a higher carboxyl content decreased significantly when the pH increased from 3 to 5.5. These studies suggest that the ionization of carboxylic groups should be the main explanation for the surface charge.

Overall, the pH-dependent stability of GO and rGO is attributed to the protonation or deprotonation of surface functional groups. For example, at low pH, the ionizable groups on GO are protonated, but when the pH is shifted to alkaline, the ionizable groups are deprotonated ($-COOH(s) + OH^{-}(aq) \rightarrow -COO^{-}(s) + H_2O$) and the charged GO surface becomes much more negative, as shown in Fig. 4B. Consequently, the electrostatic repulsion among the negative charges is a major driving force to maintain the stability of GMs suspension by preventing their aggregation.



Fig. 3 Simulated (a) potential of mean force between two parallel, fixed GO sheets, and (b) the number of hydrogen bonds formed between the two sheets and the surrounding water molecules as a function of intersheet separation, *d*. Three forms of GO { $C_{10}(O)_1(OH)_1$, $C_{10}(O)_1(OH)_1(COOH)_{0.5}$, and $C_{10}(O)_1(OH)_1(COO)_{0.5}$ } were considered. The vertical dashed lines correspond to the energy-minimized configurations of the GO/single-layer water/GO and GO/two-layer water/GO sandwich structures. (c) Post-equilibrium molecular dynamics simulation snapshot of two parallel $C_{10}O_1(OH)_1(COOH)_{0.5}$ sheets solvated in water at *d* = 7.5 Å showing a single layer of water molecules confined between the two GO sheets. Color code: red, oxygen; white, hydrogen; and gray, carbon. At low pH, the protonated carboxyl groups at the GO edges (-COOH) tend to aggregate due to the lower potential mean force. Reprinted with permission from ref. 76. Copyright © 2012, American Chemical Society.

3.2 Effect of the ionic strength and salt types

Several studies have been carried out to research the influences of ionic strength (IS) and/or salt types on the stability of GO and rGO. As mentioned above, GO is highly negatively charged in deionized (DI) water, and the generated strong electrostatic repulsion can keep the GO suspension highly stable in aqueous solutions. However, both the ionic strength and salt types were found to have significant effects on their stability. Lanphere et al.¹⁶ found that GO colloids were stable in solution at a lower IS range $(10^{-3} \text{ and } 10^{-2} \text{ M KCl})$, while GO became unstable and began to aggregate when IS $\geq 10^{-1.5}$ M KCl. Chowdhury et al.⁸⁰ also observed a similar trend; whereby, with the concentration of NaCl (representing the IS) increased from 1 to 300 mM, the $D_{\rm h}$ of GO increased notably. Additionally, this trend has also been reported with other carbonaceous nanomaterials.^{67,68,85} This phenomenon can be explained as follows. The increasing IS can increase the surface charge density due to the adsorption of sufficient protons on the colloidal surface. However, the separation of the subsequent protons from the GO surface is resisted by the electric field created by "free" ions in the solution. Furthermore, due to electroneutrality, the charges on the diffuse layer and inner layer grow by the same amount, but the charges accumulate closer to the surface, which can result in a decrease in the surface potential.^{64,77} With the IS increasing, due to electrical double-layer (EDL) compression, the electrostatic repulsive forces between particles will be suppressed, thus the aggregation happens eventually. In addition, the attachment efficiency (α), which is defined as the initial aggregation rate constant normalized by the rate constant measured under favorable conditions, is usually used to quantify the aggregation kinetics of nanoparticles.68,86 A positive correlation between α and IS is observed in the reaction-limited regime, where, beyond a certain IS, α does not increase due to the complete suppression of EDL repulsion. Furthermore, the critical coagulation concentration (CCC) values of GO for a special background solution can be determined from the intersection of extrapolated lines through the diffusion and reaction limited regimes, indicating the total suppression of the EDL repulsion.80,81,87

For understanding the influence of the salt type (different cationic salts) on the aggregation kinetics and stability of GO, monovalent cation (Na⁺) and divalent cations (Ca²⁺, Mg²⁺) solutions were used by several researchers to carry out comparative experiments. Collectively, their work shows that divalent cations are more effective than monovalent cations in aggregating/ destabilizing GO suspension, which is attributed to the mechanisms of both charge screening and cross-linking interactions (stronger than other interactions, as shown in Fig. 4A). These interactions cause the aggregation of GO by divalent cations, while the monovalent cation (Na⁺) does not have specific interactions with the functional groups on GO surfaces.^{77,81} In addition, Ca²⁺ destabilized GO more easily than Mg²⁺. The reported CCC value of GO was determined to be 1.3 mM MgCl₂, which was higher than that of CaCl₂ (0.9 mM).⁸⁰ This result



Fig. 4 Aggregation mechanisms of GO: (A) cross-linking by divalent cations, (B) in solutions with different pH: deprotonation of the carboxyl groups at the edges at alkaline solutions and protonation in acidic solutions. (C) Summary of the aggregation modes of GO nanosheets. Reprinted with permission from ref. 81. Copyright © 2013, American Chemical Society.

could be explained by the cross-linking mechanism, whereby the Ca²⁺ ion has a higher binding capacity with carboxylated groups.^{88,89} Furthermore, Wu et al.⁸¹ suggested that the edge-toedge interaction model (Fig. 4C) should be the dominant GO aggregation model in the presence of divalent cation salts, as reported by Cote et al.⁹⁰ It is noteworthy that the aggregation of GO is more sensitive to heavy metal cations $(Cr^{3+}, Pb^{2+}, Cd^{2+},$ Ag^{+}) than common environmental cations. This is attributed to the complex interaction mechanisms, including the EDL suppression, surface coordination, and cation– π complex.⁸⁷ Therefore, it is important to realize the dominant cation types in the specific water bodies in order to predict the aggregation behavior of GO. For example, in most freshwater bodies (such as surface and ground waters), the concentration of monovalent ions (less than 10 mM)⁸⁰ is lower than the CCC values, which thus means they cannot change the stability of GO. Thus, one should mainly take the divalent ions into consideration in these water bodies.

Although the CCC of rGO was lower than that of GO in NaCl solutions, the trend observed was similar with the aggregation of GO under different salts solutions. The aggregation of rGO was much easier in the presence of divalent cations than monovalent cations, and a more obvious aggregation of rGO occurred in the presence of Ca²⁺ due to its stronger complexing capacity.⁸⁴ However, the aggregation behaviors of GMs in the waters are mainly focused on single-salt solutions. How GMs respond to mixed-salts solutions is unclear and needs to be explored in future work.

3.3 Effect of NOM

Natural organic matter (NOM) originates from the by-products of decaying plants and animals residues present in natural

waters and is mainly composed of humic substances, such as humic and fulvic acids.^{91,92} Generally, NOM molecules contain aromatic rings, carboxylic acid, and phenolic groups.⁹³ Their inherent physical and chemical characteristics can cause them to adsorb on most natural surfaces and particles in natural waters.^{94,95} Since NOM is ubiquitous in natural waters, a realistic investigation is necessary to observe the stability and aggregation behaviors of GMs in the presence of NOM.

Recent studies have elucidated that the presence of NOM could enhance the stability of GO in the aquatic environment. It has been reported that GO can adsorb NOM via hydrogen bonds, Lewis acid-base interactions, and π - π interactions.⁹⁶ However, the effects of NOM on the zeta potential of GO were found to be negligible, which indicated that the significant increase in GO stability in the presence of humic acid (HA) was mainly due to steric repulsion.⁸⁰ A similar result and mechanism have been manifested in other carbon-based materials.^{97,98} Lanphere et al.²³ also noted similar effects in which the GO dispersion was more stable in the presence of NOM (0.1–10 mg L^{-1} humic acid), even in the coexistence of cationic salts. To estimate the stability and propensity toward the formation of the aggregates, Hua et al.99 calculated the aggregation strength factors (SF) and fractal dimension (FD) of GO aggregates. Their findings show that the aggregate SF decreased visibly with lower FD in the presence of NOM, consequently enhancing the stability of GO.

3.4 Effect of natural solid particles

Natural solid particles are widely present in aquatic-terrestrial transition zones, and minerals are important components of sediments and soils that can enter into natural waters. Predictably, these natural solid particles may affect the

environmental behaviors of GMs. Unfortunately, an extremely limited number of papers have been published on their interactions. Ren et al.¹⁰⁰ conducted systematic research to investigate GO aggregation and its deposition on natural solid particles under different solution chemical properties. In their experiment, they indicated that the nature of solid particles was closely related to the colloid stability of GO in solution. The pH_{PZC} (point of zero charge) values and the solubility of solid particles exemplified this point of view. Compared to Al_2O_3 with a pH_{PZC} of ~8.7, SiO₂ has a lower pH_{PZC} of ~2. Consequently, the residual GO concentration in the supernatant was higher in the presence of SiO₂ due to the strong electrostatic repulsion between the negatively charged SiO₂ and GO. In addition, the aluminum species will be transformed at a diverse pH range because of the dissolution of Al₂O₃. For example, at a low pH (4.5–1.6), Al³⁺ ions are generated and act as cross-linkers to promote the formation of GO aggregates, although it was noted also that the pH itself affects GO aggregation; at high pH (8.7–11.0), even with the existence of strong electrostatic repulsion, the formed $Al(OH)_4^{-}$ in the aqueous solutions would create electron donor-acceptor complexes with GO nanosheets through the Lewis acid-base interactions, thereby contributing to the aggregation of GO. In the meantime, they found that the electrolytes in the aqueous solutions could cause changes to the surface charges of GO, as well as to the natural solid particles, thus influencing the GO stability. However, this experiment did not explain the interactions between complex minerals and GO. Zhao et al.¹⁰¹ selected three common minerals (montmorillonite, kaolinite, and goethite) to explore the heteroaggregation mechanisms with GO based on reported mineral-nanoparticle heteroaggregation. During their experiment, they found that GO significantly enhanced the dispersion of positively charged goethite to be absorbed onto goethite, but had no influence on the negatively charged minerals (montmorillonite and kaolinite). At the same time, they confirmed that electrostatic interaction was the dominant force in GO-mineral association. More importantly, the adsorbed GO was unable to redisperse or desorb from the goethite due to irreversible heteroaggregation with the formation of a stable multi-layered structure GOgoethite complex. Thus, the interactions of natural solid particles with GO, especially the irreversible interaction, would be inevitable to influence the behavior and fate of GO in aquatic environments.

3.5 Effect of sunlight

The sunlight factor is not often taken into consideration in a laboratory experimental design, but the stability and transformation of several carbonaceous nanomaterials in natural waters have been changed with exposure to sunlight.^{102–104} Thus, understanding how the sunlight affects the fate of GMs is necessary for estimating their environmental behaviors. Chowdhury *et al.*¹⁰⁴ investigated exclusively the effects of simulated sunlight on the aggregation and deposition of GO in an aquatic environment. Their findings were crucial to manifesting the role of sunlight on the behavior of GO in natural

waters. After irradiation, GO was broken into smaller fragments due to the concurrent oxidation and reduction; the mechanism (Fig. 5), which was analyzed in a previous paper,¹⁰² demonstrated that it was likely driven by the potential creation of electron-hole pairs under sunlight conditions. Meanwhile, they found that the $D_{\rm h}$ of GO was reduced initially with the increasing irradiation time, but then plateaued after 200 h of irradiation.¹⁰⁴ However, the hydrodynamic property was somewhat different under UV light illumination. Andryushina's group¹⁰⁵ found that the hydrodynamic size (HDS) of GO increased sharply after UV light exposure for 30 min, which resulted from the elimination of certain functional groups of GO and the breakup of hydrogen bonds among the GO particles; then, HDS decreased gradually due to the reverse crumpling or folding of the partially reduced GO sheets. Regardless of the variation process, their results both suggested that the size of GO was decreased after a period of irradiation. Meanwhile, a longer exposure to sunlight promoted the aggregation of GO owing to the successive reduction



Fig. 5 Schematic of the pathways of GO phototransformation in sunlight. Irradiation mainly removes the C–O functional groups present on the basal plane of GO and along with the formation of CO₂ breaks GO into smaller fragments. Reprinted with permission from ref. 102. Copyright © 2015, American Chemical Society.

as well as the smaller splitting fragments that conduced to form larger aggregates, indicating that sunlight can transform and destabilize this nanomaterial in the aquatic environment. More importantly, direct photolysis will contribute to the partial removal of GO by converting it to CO_2 under sunlight conditions.¹⁰²

3.6 Effect of microbes

Some microbes are able to reduce and degrade GO materials, leading to changes in the fate of GO in aquatic environments. Several studies have focused on the interaction of GO with the genus Shewanella, an important model environmental microbe with a worldwide distribution.¹⁰⁶ For example, in 2010, Salas et al.107 first chose five strains of this genus to test the reduction of GO under strictly anaerobic conditions. After incubation for 24 h with GO serving as the electron acceptor, they observed apparent graphene precipitation in the solution, which indicated that the reduction process occurred. Additionally, they proposed that extracellular electron transfer (EET) pathways played a key role in the reduction of GO. In 2011, Wang et al.¹⁰⁸ demonstrated the results explained by Salas's group,¹⁰⁷ and they systematically investigated the EET pathways in GO reduction further. Their experiment demonstrated that the anaerobic conditions were not necessary for Shewanella to reduce GO. They also observed that the GO solution color turned dark-brown within 48 h in the Shewanella growth culture, which indicated that self-secreted electron mediators (redox active compounds) functioned partly in the microbial reduction of GO. In addition, they found that outer membrane c-type cytochromes are also involved in the mediation of EET during the reduction of GO, suggesting that the direct charge transfer was also involved in the reduction of GO. In the same year, Jiao et al.¹⁰⁹ deciphered the electron transport pathway for GO reduction and confirmed that electron shuttles, such as riboflavin and 9,10-anthraquinone-2,6disulfonic acid (AQDS), were important for accelerating the GO reduction. According to the above reports, the proposed reduction mechanisms mainly include direct contact and electron shuttling. In addition to the genus Shewanella strains used, Akhavan and Ghaderi¹¹⁰ found *Escherichia coli* bacteria also have the ability to reduce GO under a mixed-acid fermentation under anaerobic conditions. They suggested that the proliferation of bacteria causes the deoxygenation of GO surface, while the glycolysis process is responsible for the reduction of GO. Apparently, the transformation of GO by some special microbes in waters will change their inherent properties and change their stability and fate in complex aquatic environments. Excitingly, from another perspective, the reduction of GO by environmental microbes may provide an effective and green approach for the production of rGO. Currently, the physical and chemical approaches are the main route to prepare rGO,¹¹¹⁻¹¹⁵ even though these approaches have some drawbacks, such as being energy consuming, costly, and toxic. Therefore, understanding the abilities and mechanisms of bioreduction of GO by microbes will broaden our knowledge of the microbe-mediated green synthesis of highperformance nanomaterials.

Actually, various influencing factors usually coexist and are associated with each other in aquatic environment,¹¹⁶ such as the effects of complex solution chemistry conditions on GO stability (Table 1), making it difficult to predict the fate and transport of GMs. The stability of GMs plays a dominant role

Table 1	Effects of the chemical	properties of	a solution on	the hydrodynamic	c characterizatior	n and on the c	ritical coagul	ation cor	ncentration	(CCC)
for grapl	nene oxide (GO)									

Concentration of GO (mg L^{-1})	Cation type	Ionic strength (mM)	pН	Humic acid (mg L ⁻¹)	Hydrodynamic diameter (nm)	CCC (mM)	References
25	KCl	1	9		~241		Lanphere <i>et al.</i> , $(2013)^{16}$
			5		~235		1 ,()
		10			~ 257		
		100			~5600		
25	KCl	31.6		0	~1598.1		Lanphere <i>et al.</i> , $(2014)^{23}$
				0.1	~1159		I I I I I I I I I I I I I I I I I I I
				1	~573.7		
			_	10	~ 248.8		
	CaCl ₂	1.0		0	~2797.8		
	-			0.1	~ 2453.5		
				1	~ 1556.4		
				10	~273.6		
10	NaCl		2			24	Wu <i>et al.</i> , $(2013)^{81}$
			5			188	
			12			482	
40	NaCl			0		44	Chowdhury <i>et al.</i> , $(2013)^{80}$
				5		125	, (· · ·)
	MgCl ₂		_	0		1.3	
	0-2			5		3.9	
	CaCl ₂			0		0.9	
	-			5		2.2	

in their migration and fate in aquatic environments. Although the effects of various factors on the stability of GMs are expounded, it is necessary to investigate the stability of GMs response to different water bodies for understanding the actual behavior. Unlike the well-controlled single factor or double factor solution chemistry analyses, understanding the mechanisms that affect the stability of GMs in real waters is highly complex. The efforts of several groups have contributed to our understanding of this question. For instance, Chowdhury's group⁸⁰ investigated the stability of GO in natural and synthetic waters for about one month. They found that more than 30% of GO was settled immediately, while the rapid complete aggregation was observed within one day in the wastewater collected from a water reclamation facility. Only less than 10% of GO was destabilized in natural surface water from a stream over a month. The difference indicates that the water type plays a crucial role in the stability of GO. For the stability of GO in synthetic surface water and groundwater, they found that the presence of HA could extend the time of GO stability in surface water, albeit it was still eventually deposited completely. However, the aggregation of GO occurred more easily in groundwater within a day regardless of the presence of HA, due to a higher concentration of divalent ions. In addition, most of GO (>90%) remained stable in synthetic wastewater for about a month, which differs from the results of the real wastewater test, which may be ascribed to the lack of a biological treatment process. In 2015, their group explored the long-term stability of rGO as well. They found that a minority of rGO (partially, intermediate, fully reduced GO) was still suspended in natural surface water after approximately one month. In synthetic surface water, they found that NOM was capable of retaining the stability of partially and intermediate reduced GO after 28 days due to steric repulsion; however, this is not the case for fully reduced GO. Similar to GO, rGO also deposits in synthetic groundwater.²⁴ In 2014, Lanphere's group²³ investigated the stability of GO in groundwater and surface water, and evaluated the roles of NOM and divalent ions. Similarly, they also found different behaviors of GO in the two different water systems, which is consistent with the above study that demonstrated GO stability in surface water due to the presence of greater NOM and GO instability in groundwater as a result of the higher hardness content. Their research suggests that NOM and divalent ions hold considerable sway in the stability of GO and rGO in various waters. Nevertheless, considerable efforts should be paid to understanding the aggregation mechanisms in natural aquatic environments.

4. Transport of graphene materials in soil and porous media

Soil is an important sink for nanomaterials after their release into the environment.¹¹⁷ Nanoparticles with high mobility may penetrate the soil layers and find their way into groundwater systems, thereby posing potential risks to living organisms through the food chains eventually.¹¹⁸⁻¹²⁰ Many studies have shown that the solution chemistry (such as pH, NOM, salts ions, etc.) and soil properties (such as size, surface area, charge behavior, etc.) impact the aggregation and transport of nanoparticles in soil.^{118,121,122} The relevant factors (such as particle shape, surface properties, grain size, moisture content, solution chemistry, etc.) controlling the transport of engineered nanoparticles in porous media or soil media have been presented in several reviews.^{122,123} However, the physical shape and surface chemistry of GO are remarkably different from those of other nanoparticles, thus the transport properties of GO may be different from the available findings.¹²⁴ Therefore, it is of great importance to have good knowledge about the key factors controlling the retention and transport of GMs in soil and porous media. Based on the best of our knowledge, the transport and retention experiments, in most cases, were performed in columns packed with quartz sand as the porous media, which is essential to understanding the behavior of GMs in soil systems, thus minimizing their potential adverse impacts. We have tabulated some factors that control the transport of GO in porous media (Table 2). Furthermore, in this section, the transport and retention of GO in porous media and soils under various conditions are summarized.

The effects of the solution chemistry, such as ionic strength and pH, on the transport and retention of GO have been examined in porous media. In 2012, Feriancikova and Xu¹²⁵ investigated the deposition kinetics of GO within uniform and clean saturated quartz sands under various IS conditions (1-100 mM NaCl). They found that GO displayed high mobility under a low IS condition (1 mM NaCl) and that the retention of GO significantly increased with the increasing IS. The breakthrough concentrations of GO particles increased with time under all the IS conditions owing to the occupation of available deposition sites, thereby reducing the deposition rates. In general, an increase in IS alters the surface properties of the sands and GO, such as increasing the zeta potential, which could lead to the suppression of EDL. More specifically, the transport behavior of GO could be described using a Langmuir-type model. In the meantime, the extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory could explain the observed trend in the mobility of GO particles under various IS conditions. In addition, the reduction of IS could lead to the remobilization of retained GO particles. In 2013, Lanphere et al.¹⁶ reported that the hydrodynamic diameter and the electrophoretic mobility remained nearly constant within an environmentally relevant pH range from 5 to 9, whereas they were sensitive to IS. The increased retention trend of GO particles from 5% to 100% under the IS conditions ranging from 10^{-2} to 10^{-1} M KCl in the packed bed column was due to the combination of increased aggregation and more attractive forces between particles. Similarly, the release study also suggested that the deposition is reversible. The release of GO particles at high IS conditions after a dilution (such as a storm or rainfall) can lead to the remobilization of trapped GO, which can cause severe biological effects on soil life. Qi et al.¹²⁴ also reported that high IS conditions inhibited the transport of GO significantly due to

Table 2 Effects of various factors on the transport of GO in porous media

Concentration of GO	Column	Sand diameter	Background solution	Velocity	Breakthrough rate (%)	References	
5 mg L ⁻¹ 25.6 mg L ⁻¹ 12 mg L ⁻¹	Saturated clean silica sands column Ultrapure quartz sand column Bubble column	0.211-0.297 mm 250-300 μm 0.5-0.6 mm	1, 5, 20, 100 mM NaCl 10 ⁻³ , 10 ⁻² , 10 ^{-1.5} , 10 ⁻¹ M KCl 1, 10, 100 mM NaCl	0.31 cm min^{-1} 2 mL min^{-1} Air flow	93.5, 90.0, 34.8, 3.1 96, 95, 53, 0.3 ~100	Feriancikova and Xu (2012) ¹²⁵ Lanphere <i>et al.</i> , (2013) ¹⁶ Liu <i>et al.</i> , (2013) ¹²⁶	
	Unsaturated quartz sand column Saturated quartz sand column			5 mL min ⁻¹ 1 mL min ⁻¹	95.6, 75.6, 1.5 99.4, 85.9, 3.0		
	Saturated quartz sand column	0.21–0.30 mm	10 mM (Na ⁺ or K ⁺) 10 mM Cs ⁺ 20 mM Na ⁺ 20 mM K ⁺ 20 mM Cs ⁺ 0.5 mM Ba ²⁺ 0.5 mM Ca ²⁺		~90 (GO) ~80 (rGO) ~44 (GO) ~30 (rGO) ~83 (GO) ~40 (rGO) ~76 (GO) ~20 (rGO) ~0 (rGO) ~0 (GO, rGO) ~80 (GO) ~0 (rGO) ~85 (GO) ~70 (rGO)	Xia <i>et al.</i> , (2016) ¹²⁷	
5–25 mg L ⁻¹	Saturated quartz sand column	0.1–0.2 mm 0.5–0.6 mm 0.85–1.0 mm	20 mM NaCl	1 mL min ⁻¹	~0 16.4-33.2 26.6-56.9	Sun <i>et al.</i> , (2015) ¹³¹	

aggregation, which resulted in straining and clogging of the column near the inlet, while the pH had only a minor effect on transport. More importantly, Liu et al.¹²⁶ found that the effects of IS on the retention and transport of GO in unsaturated systems were similar to those in saturated systems. They described the transport of GO through both saturated and unsaturated porous media with the advection-dispersion-reaction model. However, the mobility of GO in unsaturated columns was reduced compared with that in saturated porous media. In addition to the secondary minimum deposition mechanism, an additional mechanism, such as film straining, was another possible explanation. Furthermore, the bubble column experiments suggested that GO particles could not be captured by air-water interfaces. Overall, the effects of IS on the transport of GO can be ascribed to the changes of the surface charges and particle sizes of the GO particles.

However, the above-mentioned experiments only consisted of one type of electrolyte solution (*e.g.*, KCl or NaCl), which does not reflect the influence of cation valence on GO transport. Compared with monovalent cations, divalent cations (Ca²⁺) exhibit more obvious inhibitory effects on GO transport in soil.^{26,127} Furthermore, Xia *et al.*¹²⁷ found that the transport-inhibition effects were hydrated radius-dependent, following the order of Na⁺ < K⁺ < Cs⁺ and Mg²⁺ < Ca²⁺ < Ba²⁺. Fan *et al.*¹²⁸ conducted a transport experiment in saturated porous media containing mixed Na–Ca electrolyte systems, and found that GO nanoparticles in the sand column were more sensitive to deposition in Ca²⁺ solutions than in Na⁺ solutions. In addition to the screening of surface charges similar with Na⁺, the Ca²⁺–GO cross-link interactions that led to the aggregation and the intensified straining effect also played key roles in the transport. Practically speaking, the reduction of GO particles occurs under certain environmental conditions, such as a low concentration of sulfide, which alter their transport behavior. Xia *et al.*²⁵ explored the transport and retention of sulfide-reduced GOs (RGOs) in porous media and found that the transport inhibition of RGOs was more remarkable than that of GO in both monovalent cation (Na⁺) and divalent cation (Ca²⁺) conditions. Meanwhile, they suggested that cation bridging is the most predominant retention mechanism in the presence of divalent cations and that deposition at the secondary minimum energy is the main retention mechanism of RGOs in the presence of monovalent cations.

Furthermore, soil organic matter and clay minerals are ubiquitous in natural subsurface environments and can interact with GMs, thereby influencing the transport of GMs in soil systems. Xia *et al.*²⁵ found that the transport of rGO was enhanced in the presence of HA due to the adsorption of HA both on sand grains and on nanoparticles. Similarly, Qi *et al.*¹²⁴ found that HA could enhance the transport of GO because of the steric repulsion between GO and quartz sand and the inhibition of GO flakes stacking. The flat shape of GO allows better contact with dissolved organic matter, which is conducive to the dispersion and mobility. However, the effect of HA on the transport of GO in soil was small due to the much smaller grain sizes and much more heterogeneous

nature of soil.²⁶ Recently, Lu *et al.*¹²⁹ investigated the effects of three typical clay minerals of porous media (kaolinite, montmorillonite, and illite) on the transport of GO and found that the presence of clay minerals inhibited the transport of GO significantly, with the inhibition effects following the order of kaolinite > montmorillonite > illite. The charges of amphoteric sites present in the clay minerals, such as octahedral Al–OH sites, were dependent on the pH conditions. Thus, they suggested that the existence of positively charged edge sites on these clay minerals are responsible for the transport-inhibition effects (a conceptual model is shown Fig. 6).

The flow velocity has noticeable effects on the transport of GMs in soil.²⁶ The transport of GO at high IS can be enhanced by various flow velocities. In particular, it has been suggested that a transport model of GO particles should take into account both the blocking-affected attachment process and the straining effects.¹²⁴ Furthermore, moisture content also plays an important role in the retention of GO in porous media.¹²⁶ However, the mechanisms remain unclear. Meanwhile, Liu's group¹³⁰ conducted another column experiment to determine the effects of surface hydroxides and solution pH on the deposition of carboxyl-functionalized graphene in saturated porous media. Compared to cleaned sand columns with high mobility and high recovery rates close to 100% at pH 5.6 and 8.3, the mobility and recovery rates (88.4% and 96.5% at pH 5.6 and 8.3, respectively) of functionalized graphene (GR) were reduced in the natural sand columns, which was attributed to the interaction between the carboxyl functional groups of the GR and the surface metal hydroxides on the sand grains. The findings indicate that the transport of nanoparticles in soil systems is fairly complex.

The particle input concentration and the grain size of porous media can strongly affect the retention and transport of GO in the porous media. Sun *et al.*¹³¹ found that there was little to no GO breakthrough in a fine sand column, but that the retention of GO decreased with increasing sand size (*e.g.*, coarse < medium < fine), which can be explained by the colloid filtration and XDLVO theories. Meanwhile, the mobility



Fig. 6 Conceptual model showing the proposed mechanism by which clay minerals affect the transport of GO in saturated quartz sand. Reprinted with permission from ref. 129. Copyright © 2016 WILEY-VCH.

of GO in porous media increased at higher input concentrations in both the coarse and medium sand columns, where this reduced retention can be explained by a blocking mechanism. In addition, they observed that the recovery rates of GO in coarse sand were higher than those in medium sand columns at the same given input concentration, and that the GO size increased dramatically with the travel distance through the porous media. Excitingly, biofilms were found to play an important role in inhibiting the transport of GO, as reported by He's group.¹³² Their results showed that the increased retention of GO in biofilms-coated sand, regardless of the bacteria type (e.g., Bacillus subtilis (Gram-positive) and Pseudomonas putida (Gram-negative)) was mainly due to an increase in the surface roughness and physical straining. Generally speaking, the transport of GMs in soil is difficult to understand due to the complex and uncontrolled soil conditions. Considerable efforts should be paid to explore the transport behaviors and mechanisms of GMs in different environments.

5. Ecosystem effect of graphene materials

In general, the toxicity effects of GMs mainly focus on the typical organisms in culture studies. Understanding the mechanisms of the microbial toxicity is meaningful and essential for assessing the ecological risks of GMs. However, it is still difficult to predict their adverse effects on microorganisms in actual environment systems, which are much more complex and uncontrolled, especially in the case of the stability, mobility, and bioavailability of GMs affected by the environmental conditions. Furthermore, the valid information on the effects of graphene on the structure and function of microbial communities in the environment is lacking. Note that as soil and water systems are the potential recipients of GMs, determining whether and how GMs alter the structure and function of microbial communities is important. To fill the knowledge gaps, in this section we analyze the recent work regarding the change in microbial communities with exposure to GMs.

5.1 Environmental toxicity and toxicity mechanisms of GMs

To date, a number of studies have determined the potential toxicological effects of GMs on various organisms. For example, it has been reported that GO and/or rGO show strong cytotoxicity toward bacteria,^{133,134} fungi,^{135,136} algae,¹³⁷ plants,^{138,139} animals,^{140,141} *etc.* Compared with Gram-negative bacteria, GMs are more toxic to Gram-positive bacteria, as only 26% and 5% of the Gram-positive bacteria (or 41% and 16% of Gram-negative bacteria) survived after being exposed to GO nanowalls and rGO nanowalls for 1 h, respectively.¹³³ Liu *et al.*¹³⁴ further suggested that the toxicity of GMs is both time dependent and concentration dependent. The main toxicity mechanisms are commonly divided into two major categories, including physical interaction (cell membrane damage) and chemical reaction (reaction oxygen species (ROS) generation).



Fig. 7 (a) Toxicity mechanisms of graphene, including physical and chemical interaction, and (b) defense mechanisms against oxidative stress. Adapted from ref. 22.

Generally, physical membrane damage is caused by the direct contact between the microorganisms and the sharp edges of GMs, which can lead to the release of intracellular contents, and eventually cell death.^{133,134} Furthermore, graphene sheets can wrap or trap bacterial cells, thus inhibiting nutrient consumption and cell proliferation.^{22,134} The generation of ROS and the subsequent oxidative stress are frequently proposed to be the chemical mechanisms of GMsinduced toxicity. It is known that ROS is a collective definition including superoxide anions ($^{\circ}O_2^{-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ('OH). In normal biological systems, the generation and elimination of ROS maintain equilibrium during cellular homeostasis. However, harmful external stimuli can disturb the balance, causing an excess of ROS, which can damage the cellular components and alter cellular functions. To prevent oxidative damage, cells have developed defense mechanisms, including both indirect (physical defense and cell repair system) and direct (antioxidant enzymes and low molecular weight antioxidants) defense systems. Oxidative stress can be evaluated by the quantification of ROS, which is usually determined by measuring malondiadehyde (MDA), superoxide dismutase (SOD), and H₂O₂, etc.²² These mechanisms are illustrated in Fig. 7. Comprehensive information on the toxicity issues and mechanisms of GMs in biological systems have been summarized in several reviews.^{20,22,142,143}

5.2 Effects of GMs on the changes to microbial communities

Du *et al.*²⁷ investigated the changes of bacterial communities in soil after aging for 90 days and found, through highthroughput sequencing analysis based on the 16S rRNA gene library. That the richness and diversity of soil bacterial communities increased after the introduction of pristine GO (PGO). Interestingly, PGO could selectively enrich some nitrogen-fixing bacteria genus (such as *Azospirillum* and *Azoarcus*) and dissimilatory iron reducing bacteria genus (such as *Geobacter*). Meanwhile, a range of changes in PGO properties occurred simultaneously in the soil, including surface morphology, surface chemical groups, and surface charges, etc. However, the internal links among these changes are unclear. Chung et al.¹⁴⁴ reported that the change in soil microbial biomass in response to short-term GO exposure (59 days) was not obvious, thus suggesting that the GO toxicity may be transient in the short-term response. Moreover, the activities of soil enzymes (xylosidase, 1,4-β-glucosidase, cellobiohydrolase, 1,4-β-*N*-acetyl glucosaminidase, and phosphatase) that mediate C, N, and P cycling were lowered transiently, but the effects subsided afterwards. Ren et al.145 found that the effect of graphene on soil bacterial communities was time dependent. In addition, the fluorescein diacetate (FDA) esterase and soil dehydrogenase activities, indicators of the overall microbiological activity of soil, increased after 4 days with increasing graphene concentrations below 100 mg kg⁻¹. Then, no effect on FDA esterase was observed, whereas the dehydrogenase activity was inhibited strongly when exposed to the highest graphene concentration (1000 mg kg^{-1}). Likewise, promotion of the richness and diversity of bacterial communities was observed after 4 days of exposure to graphene concentrations below 100 mg kg⁻¹. However, no significant differences occurred at the highest graphene concentration after 21 days or the whole study term (60 days). The bacterial community structure analysis showed a significant shift after exposure for 4 days; then, the shift became weaker. More importantly, although a majority of bacterial phylotypes remained unaffected, the special bacterial populations, such as the genera involved in nitrogen biogeochemical cycles and in the degradation of organic compounds, can be affected. The above-mentioned reports suggest that the effect of graphene on soil microbial activity may be time dependent and may show little change in microbial communities under short-term exposure. Thus, it is of great importance to determine the long-term response of soil microorganisms to graphene. With respect to the longterm effects of graphene on microbial communities, Ge et al.¹⁴⁶ conducted a comparative experiment among several carbonaceous nanomaterials (CNMs) to address this issue. Herein, we merely utilize the findings relating to the graphene

studies. After an exposure of 1 year, graphene-reduced soil DNA, but did not affect soil fungal communities, whereas it did alter the bacterial communities. Obviously, GMs released into soil will cause certain effects on the microorganisms, especially with the initial exposure, and thus considerable efforts should be devoted to understanding the short-term and long-term exposure effects of graphene on the structure, function, and activity of soil microbial communities.

Natural and engineered aquatic systems are complex, presenting the need for understanding the real impacts of GMs on microbial communities. It has been reported that the toxic effects of GO on the microbial communities in wastewater systems are dose dependent.¹⁴⁷ In this study, the metabolic activity and viability of the microbial community were significantly reduced in the presence of GO, which can be attributed to the production of ROS, which increases the toxicity, thus causing the inhibition of essential biological functions of bacteria, including biodegradation, nitrogen removal, and phosphorus accumulation, in the activated sludge process. Different with the inhibition of ammonia oxidizing bacteria (AOB) for nitrogen removal in this study, the presence of GO can enhance the activity of anammox bacteria for nitrogen removal, as reported by Wang et al.,148 signifying that different effects of GMs can be seen for different functional microorganisms. Furthermore, Ahmed and Rodrigues¹⁴⁷ reported that with increasing GO concentrations, the turbidity of the effluent, an indication of the effectiveness of the treatment process, increased steadily due to the presence of both suspended GO and attached organic matter onto GO surfaces, with a result that the dewatering capacity was significantly decreased. More importantly, the retention of GO nanosheets that accumulate inside the floc matrix in the wastewater treatment system, which could adsorb bacteria and other microorganisms, may pose chronic toxicity effects. Therefore, it is essential to investigate both acute and chronic microbial exposure to GMs for a complete understanding of the effects of graphene on wastewater treatment processes.

6. Challenges and perspectives

Graphene, as an advanced nanomaterial, has driven widespread development in various research and engineering fields. Research relating to GMs is increasing at an incredible rate in a variety of disciplines. During the process of GMs development, it is imperative to take the positive and negative impacts on humans and ecosystems into account. This is the first report that describes the environmental behaviors and risks of GMs in both water and soil environments. Nevertheless, a number of scientific "blind spots" and knowledge gaps in GMs research still exist.

It is evident that GMs will inevitably interact with environmental relevant conditions, thereby impacting their fate and transport in the environment. Understanding the environmental behaviors of GMs is critical for predicting their adverse impacts. Therefore, we have reviewed several critical factors controlling the stability and behavior of GMs in aquatic environments. It is a complex challenge to illuminate the action mechanisms of GMs due to the mutual effects in environmental relevant conditions. Generally speaking, NOM and bivalent ions have been certified as the main contributing factors for GMs stability/dispersion and transport, while sunlight and microbes are important for the transformation of GO. GMs can also impact the structure and function of microbial communities. However, research on those interactions is still in its infancy. There is a great deal of work needed to be done before we can understand the environmental behaviors of GMs. Herein, some important issues are highlighted to suggest areas for additional research to enhance knowledge.

(1) Investigate the environmental risks of solid GMs: It is well known that the main forms of GMs are prepared as liquids and solids. However, a large amount of research is focused on the environmental behaviors of GMs and their risks in solution form. Valid information on the impacts of solid GMs in terms of the environmental risks is lacking. Thus, further studies investigating solid GMs are needed to reduce the knowledge gap in this area.

(2) Research the behavior and fate of functionalized GMs on the environment: Functionalized GMs are usually synthesized to improve the dispersion of graphene or to enhance a specific special function. Theoretically, the characteristics of pristine GMs will be changed; for example, a polystyrene–poly-acrylamide (PS–PAM) copolymer covalently grafted on the graphene sheets can render graphene amphiphilic.¹⁴⁹ How the functionalized GMs will impact the environment is still an open question. Likewise, it is necessary to research the colloidal properties and ecosystem risks in the environment.

(3) Seek feasible methods to remove GMs from environmental media: Since the potential risks from GMs are presented in the environment and ecosystems, their removal from environmental media is important for reducing the adverse impacts. Duan *et al.*¹⁵⁰ reported the removal of GO from waters *via* coagulation; however, the removal efficiency was not ideal due to the complexity of the conditions. It is an arduous task to seek an efficient approach for removing GMs from the environment.

(4) Study the concentration-dependent stability of GMs in aquatic environments: To date, although GMs have been extensively applied for gas, pathogen, and biomolecule detection, there is little information regarding the concentration of GMs present in the environment. Based on the literature, the antibacterial activities of GMs were proved to be concentration dependent.¹³⁴ Thus, it is necessary to establish a suitable method and detection technique to determine their concentrations in the environment. In addition, the above-mentioned studies reported on the stability and aggregation behavior of GMs in aqueous solutions at a fixed concentration. There has been no effort made toward investigating how the different initial concentrations of GMs respond to various factors in pure aqueous solutions and waters. Such research is essential for building a concentration change mode, which will also be conducive to the risk evaluation.

(5) Understand the interactions between GMs and environmental pollutants: It is well known that GMs are excellent absorbents for removing heavy metals and organic pollutants. Apart from the ability to remove pollutants, the potential changes in the colloidal properties and the combined toxic effect also exist. For example, GO can be aggregated by heavy metal ions, such as Cu²⁺, in aqueous solution.¹⁵¹ The combined toxic effect of GO and heavy metals on algae has been investigated, signifying that it is critical to determine the environmental behaviors of GMs in wastewaters.¹⁵² Thus, understanding how the main pollutants interact with GMs in effluents can help us to better understand the probable toxicity effect.

(6) Study the effects of EPS on the stability of GO: Besides humic substances, extracellular polymeric substances (EPS), such as polysaccharides and proteins secreted by organisms, are also classified as NOM. To the best of our knowledge, the major research is focused on the effects of humic substances on the stability of GMs, and the effects of EPS are not clear. It has been reported that EPS not only impact the dissolution and species of copper-based nanoparticles, but also improve their stability in most conditions, according to Adeleye *et al.*¹⁵³ Therefore, understanding the effects of EPS is of great importance and will improve our comprehension on the dispersion/ aggregation of GMs in the presence of NOM.

(7) Explore the environmental behaviors of GMs in coexistence with other nanomaterials: With the development of nanotechnology, the applications of various nanomaterials are booming. It is likely that different nanomaterials will be present in waters simultaneously. Nanomaterials possess unique surface properties that can be affected by environmental parameters, for example, the pH of an aquatic system can tune the surface charge of nanomaterials,¹⁵⁴ thereby influencing the stability *via* their interactions. However, whether and how the presence of other nanomaterials will influence the state of GMs in waters remain poorly understood. Therefore, more research is needed to examine the practical dynamic changes in complex waters containing different nanomaterials.

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