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Combined effects of graphene oxide and Cd on the photosynthetic capacity and survival of *Microcystis aeruginosa*

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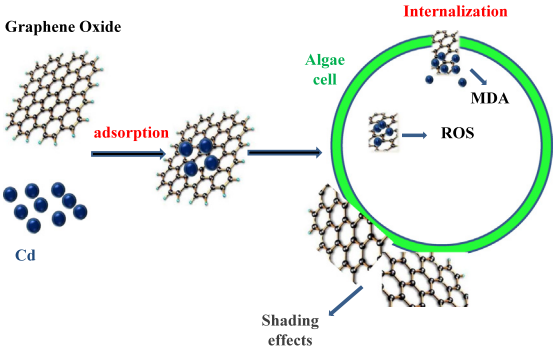
HIGHLIGHTS

- Combined effects of graphene oxide and Cd^{2+} to *M. aeruginosa* were investigated.
- Chlorophyll fluorescence parameters were obtained by PAM.
- OS and MDA were measured to evaluate algae toxicity.
- GO at low concentration enhanced Cd^{2+} toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, the combined effects of graphene oxide (GO) and Cd^{2+} solution on *Microcystis aeruginosa* were investigated. Chlorophyll fluorescence parameters were measured by a pulse-amplitude modulated fluorometer. GO at low concentrations exhibited no significant toxicity. The presence of GO at low concentrations significantly enhanced Cd^{2+} toxicity as the 96 h half maximal effective concentration of the Cd^{2+} reduced from 0.51 ± 0.01 to 0.474 ± 0.01 mg/L. However, concentrations of GO above 5 mg/L did not significantly increase the toxicity of the Cd^{2+}/GO system. Observations through scanning and transmission electron microscopy revealed that GO, with Cd^{2+}, easily attached to and entered into the algae. Reactive oxygen species and malondialdehyde were measured to explain the toxicity mechanism. The photosynthetic parameters were useful in measuring the combined toxicity of the nanoparticles and heavy metals.

1. Introduction

Graphene is a 2D carbon nanomaterial with a honeycomb-like structure. It has unique properties, such as ballistic electron transport (Rosenstein et al., 2010) at room temperature, tunable band-gap, high chemical and mechanical abilities, low electrical noise, high thermal conductivity, and biocompatibility. This nanomaterial has been used in numerous advanced devices ranging from ultracapacitors to spintronic devices (Erickson et al., 2010; Geim and Novoselov, 2007; Mazzamuto et al., 2011; Yan et al., 2012b). Graphene oxide (GO), which is the functionalized form of graphene, contains epoxy, hydroxyl and carboxyl groups (Boukhvalov and Katsnelson, 2008). GO, in both pure and nanocomposite forms, has wide applications in electronics, biosensors, pipes, semiconductors, and packaging because of its excellent electrochemical properties (Arvidsson et al., 2013; Brody, 2006; Hu and Zhou, 2013).

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The market for graphene-based products is estimated to reach nearly $675 million by 2020. As a result, large quantities of graphene-based wastes are predicted to be generated by that time (Ahmed and Rodrigues, 2013).

The increased use of GO in industrial applications has also prompted significant concerns regarding its effects on human health and causing ecological risks. Several studies have investigated the *in vivo* cytotoxicity of GO on bacteria, plant, mammalian, or human cells (Akhavan and Ghaderi, 2010; Chang et al., 2011; Krishnamoorthy et al., 2012; Nair et al., 2012; Yan et al., 2012a). Different results and mechanisms have been reported for the toxicity of GO. GO and reduced graphene oxide (rGO) tend to inhibit bacterial growth with minimal toxicity to human alveolar epithelial A549 cells (Hu et al., 2010). Akhaven et al. found that GO and rGO can damage the membranes of *Escherichia* and *Staphylococcus* and that hydrazine-rGO is more toxic than untreated GO (Akhavan and Ghaderi, 2010). In contrast, GO does not display intrinsic antibacterial, bacteriostatic, or cytotoxic properties in either bacterial or mammalian cells (Ruiz et al., 2011). The toxic action of GO potentially involves at least three distinct mechanisms. First, the sharp edges of rGO can physically break the membranes of cells, bacteria, and viruses, thereby causing physical damage to living organisms (Akhavan and Ghaderi, 2010; Liu et al., 2011). Second, oxidative stress is a ubiquitous mechanism of cytotoxicity, and GO can deplete the mitochondrial membrane potential, increase the intracellular reactive oxygen species (ROS), and trigger apoptosis by activating the mitochondrial pathway (Wang et al., 2013a). Third, GO can envelop cells, affect cell signal transformation and result in noninvasive damage (Rosenstein et al., 2010). Most studies have focused on only the *in vivo* toxicity of GO in bacterial, adherent mammalian, or cancerous cells. No study has investigated and compared the toxicity of GO to algal cells.

Most heavy metals are toxic and pose a serious threat to the environment and public health, even at very low concentrations. Due to its large surface area and high adsorption capacity, GO is considered to be a promising nanomaterial for the removal of heavy metals and organic pollutants in water treatment (Deng et al., 2010). However, the mechanisms for how GO influences the toxicity of heavy metals have not been investigated. *Microcystis aeruginosa* (*M. aeruginosa*) has been used as a model to explore the mechanism of the combined toxicity of GO and heavy metals on algae. Cd is a highly toxic metal, and its most toxic form, Cd^{2+}, can exert serious toxicity on algae (Zeng et al., 2009).

This study investigated the combined toxicity of GO and Cd^{2+} on *M. aeruginosa* with a quick and efficient photosynthetic tool using a pulse-amplitude modulated (PAM) fluorometer. The correlation between *M. aeruginosa* cell growth and photosynthetic fluorescence in the presence of solutionized Cd^{2+} or pure GO was also investigated. Intracellular ROS and malondialdehyde (MDA) were measured to evaluate the toxicity mechanism. The results of this study provided insights into the possible risks of GO in aquatic conditions.

2. Materials and methods

2.1. Characterization of GO in culture medium

Graphene oxide was purchased from XF NANO Co., Ltd., Nanjing, China. GO particles were dispersed in deionized (DI) water purified by Millipore to prepare the stock solution. The stock solution was sonicated for 30 min before being diluted to different exposure concentrations. Zeta potentials for GO were determined by a dynamic light scattering (DLS) size analyzer (Zetasizer Nano-ZS, Malvern, U.K.).

2.2. Culture of M. aeruginosa

A strain of *M. aeruginosa*, obtained from the Institute of Wuhan Hydrobiology (China), was incubated in the BG11 medium (Zhou et al., 2014) at 25 ± 1 °C. DI water was used to prepare reagents and culture medium. The initial pH of the medium was approximately 7.0. The culturing of *M. aeruginosa* was performed inside an incubator with a cycle of 12 h of natural light and 12 h of darkness. The growth curves were recorded daily. The strain was harvested according to their logarithmic growth phase and diluted in the BG11 medium. The cell concentrations of the experimental samples were determined by a spectrophotometer. The optical density (OD) values were linearly proportional to the algal concentration. The OD at 680 nm of the culture was between 0.11 and 0.12, which corresponded to an algal concentration of 2.32×10^9 cells/L (Wang et al., 2013b).

2.3. Sorption of Cd^{2+} on GO

The sorption behavior of Cd^{2+} on GO was investigated by performing a traditional batch sorption experiment. $CdCl_2 \cdot 2.5H_2O$ was dissolved in DI water to prepare a stock solution of Cd^{2+}. The solution was diluted with the culture medium to different concentrations: 0.1, 0.3, 0.5, 1.0, 2.0, and 5.0 mg/L. The pH of each solution was adjusted to 7.0 ± 0.1 with 0.01 mM HCl or NaOH. GO was added to each vessel at a concentration of 10.0 ± 0.1 mg/L. The mixed suspensions were then shaken for 1 h to achieve sorption equilibrium. After 10 min of centrifugation at 5000 rpm, the supernatant was removed and the remaining Cd^{2+} concentration was determined by inductively coupled plasma atomic emission spectroscopy (ICP-optima 2001DV, Perkin-Elmer, USA).

2.4. Toxicity tests

The toxicity was evaluated by measuring the culture yield and chlorophyll fluorescence parameters in relation to the control experiment. The growth density of *M. aeruginosa* was measured daily for 96 h and assessed by the initial and the final OD at 680 nm. All of the chlorophyll fluorescence parameters were measured with a Phyto-PAM fluorometer (Walz, Germany). Phyto-PAM is used to determine the chlorophyll content and assess the photosynthetic activity of planktonic algae. The variables, Chl-*a* fluorescence (F_v) and maximal fluorescence (F_M), were measured. Photosystem II activity was determined using the ΔF mode ($\Delta F = F_M - F_v$). The yield is a frequently used parameter for the assessment of the maximal quantum conversion efficiency of Photosystem II. It is calculated as $Y = F_v / F_M$. Photosynthetic yield, photosynthetic efficiency (α), and the maximum electron transport rate ($r\mathrm{ETR}_{max}$) of the algae were immediately measured after different treatments. The photosynthetic parameter α represents the maximum rate of increase of light-limited photosynthesis with units of μmol electrons m^{-2} s^{-1}/μmol photons m^{-2} s^{-1}. Meanwhile, *r*ETRmax indicates the maximum photosynthetic capacity with units of μmol m^{-2} s^{-1} (Ihnken et al., 2010). *M. aeruginosa* solutions, with a series of Cd^{2+} concentrations, were observed and cultured in an incubator. The toxicity of GO alone was tested by following the same methods as for the Cd^{2+} toxicity test. Two sets of experiments were conducted to investigate the combined effects of Cd^{2+} and GO. The first set studied the toxic effects of Cd^{2+} with fixed GO concentrations. The second set examined the toxic effects of GO with fixed Cd^{2+} concentrations. The final and initial algal densities and chlorophyll fluorescence parameters were measured for each test.

2.5. Detection of ROS production

M. aeruginosa was incubated for 24 h in the culture medium with different levels of GO or the GO/Cd^{2+} system. The total ROS production was determined using the cell permeable probe, 2′7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) (Cherchi et al., 2011). The specific method of operation was as follows: 1.0 mL of algal cells was cultivated for 48 h, centrifuged (10,000 rpm for 10 min), placed in phosphate buffer solution (PBS) and washed twice, and then transferred immediately (with 10 μM H_2DCFDA) to the cell pellet. Next, it was cultured in a water bath at 37 °C for 2 h and washed with PBS again. Finally, the fluorescence values were measured by a microplate reader