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Modifications in physiochemical property of engineered graphene oxide by nanomaterials resistant bacteria

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Abstract

In this study, soil bacteria were isolated from nanomaterials (NMs) contaminated site and enriched in the presence of graphene oxide (GO). SEM images of isolated GO-resistant bacteria showed adaptation with a high degree of survival and tolerance to GO. The GO-resistant bacteria were interacted with engineered GO and changes in their structural functionality were studied by Fourier transform infrared (FTIR). The results showed exfoliation and reduction of oxygen species in GO structure occurred upon their interaction with GO resistant bacteria. The presented approach has potential in biological modification of NMs to obtain non-toxic intermediates that are safe for industrial applications.

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1. Introduction

The unique physiochemical properties of carbon nanostructures such as graphene showed high impact applications in materials [1, 2] and biomedical sciences [3-6]. GO basically possesses suboptimal electrical conductivity (because of sp² bonds), and has been subjected to reduction by physical and chemical methods [7]. There have been few contradictory reports on their short-term biocompatibility and impacts on environmental toxicology [8, 9]. Therefore, prolonged impact of graphene, graphene oxide (GO), and reduced graphene oxide (rGO) on the environment and human health remains unknown. Several groups have reported structural degradation of carbon nanotubes and graphene upon interaction with strong oxidants and acids [10, 11]. The use of strong acids and oxidants not only add up to the increased cost, but also have harmful unpredicted effects on vegetative flora and

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fauna present in the environment. Therefore, these approaches find limitations to biotransform carbon based nanomaterials (NMs). Recently, a study on degradation of GO using the peroxidase family of enzymes, such as horseradish peroxidases (HRP) and myeloperoxidases (MPO) were reported [12]. However, this method are based on green technology approach, but finds several limitations, such as high cost of enzymes, low degradation efficiency and complex enzyme substrate chemistry. Due to these limitations, development of cost-effective and eco-friendly green strategies are required where biotransformation/degradation of carbon based NMs are the better alternatives. Here, we utilized soil microbial flora found in natural NMs contaminated site and these were enriched after repeated exposure to GO and obtained GO-resistant soil bacteria. These GO-resistant bacteria modified the physicochemical properties of engineered GO.

2. Experimental

2.1. Materials

Nano platelets of GO used in this study had thickness of ~ 0.8 nm with diameter of $0.5\text{--}2$ μm (Carbon solutions Inc., USA). Triton-X 100 was procured from Merck, Germany. Dimethyl Sulfoxide (DMSO) was purchased from Sigma-Aldrich, USA. All other reagents used in this study were of analytical grade and filtered through 0.22 μm sterile filters. All bacterial studies and related work were carried out under sterile conditions.

2.2. Isolation and enrichment of GO-resistant bacteria

Soil samples were collected from NMs contaminated site. The bacteria from soil samples were isolated and enriched with GO by using standard microbiological methods in mineral medium (MM) containing glucose as the only carbon source. The MM contained (per liter) 3 g KH_2PO_4 , 12.8 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 1 g NH_4Cl , 0.5 g NaCl and supplement sources such as MgSO_4 (2 mM), CaCl_2 (0.1 mM) and glucose (varying from 0.3–0.4%) pH (6.5–7.5). For repeated enrichment and adaptation of bacteria, the MM was amended with 1 mg/mL GO. The bacteria were allowed to grow at 37 $^\circ\text{C}$ under constant shaking in an orbital shaker.

2.3. Interaction of engineered GO with GO resistant bacteria and extraction

A 30-days old GO resistant-bacteria culture was harvested by centrifugation at 9000 rpm for 10 min. The pellet containing GO, cells and cell-debris were washed thrice with pure ethanol, sonicated and centrifuged to separate the cells and cell-debris. The brownish GO pellet was later phase separated in a mixture of 1:1 *n*-hexane and deionized water. A black colored ring of purified GO appeared at the interface of two solvent phases which was finally collected in a separate glass vial and dried in a vacuum desiccator. Appropriate control samples were also treated the same ways as the test samples for comparison that were utilized for further characterization.

2.4. Physicochemical characterization

SEM images of isolated and enriched GO-resistant bacteria were acquired using a LEO Supra 35VP scanning electron microscope operated at 3 kV. Samples were mounted on a silicon wafer and sputter coated with a thin layer of Pd–Au before taking SEM images. Attenuated Total Reflectance-IR (ATR-IR) spectra of engineered GO before and after the interactions with GO-resistant bacteria were characterized for functional groups present on the biotransformed GO structure. Spectra were acquired with a Nicolet iS10 FT-IR Spectrometer (Thermo Scientific, USA) with mercury cadmium telluride detector (4 cm^{-1} resolution) equipped with a diamond crystal in single reflection mode. IR spectra of sample mounted on silicon wafer were collected with an average of 100 scans with a wide spectral range from 300 – 4000 cm^{-1} .

3. Results and discussion

3.1. SEM characterization isolated and enriched NMs resistant bacteria

The soil bacteria were isolated from NMs contaminated site and enriched in presence of GO. The GO-resistant bacteria showed adaptation with a high degree of survival and tolerance to GO. SEM images of isolated GO-resistant bacteria showed that they are physically attached with few layer of nanoribbons (Fig. 1a-b).

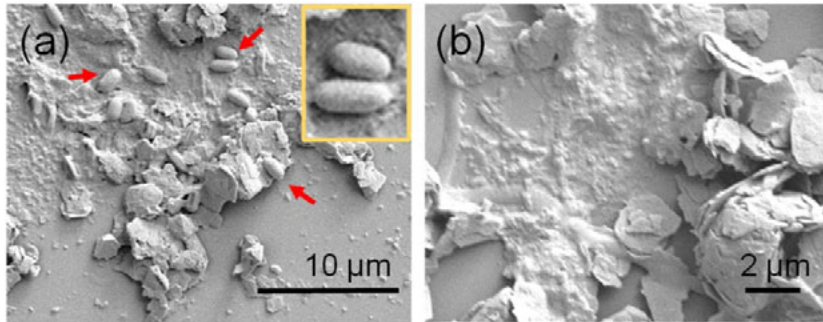


Fig. 1. SEM images of (a) GO-resistant bacteria and GO nanoribbons aggregates and (b) GO nano-sheets forming a thick blanket-like structure round GO-resistant bacteria.

The morphology of GO-resistant bacteria was persistent during the incubation and interaction with GO nanosheets (Fig. 1a insert). The GO-resistant bacteria and GO aggregates formed a blanket-like appearance (Fig. 1b). Dispersed GO-resistant bacteria formed the aggregates and GO sheets were entrapped with the cells, where extracellular enzymes of cells are likely to be interacting that induce structural artifacts on GO nanostructures.

3.2. FTIR results

FTIR spectra of engineered GO with (test) and without (control) interaction with GO-resistant bacteria are shown in Fig. 2.

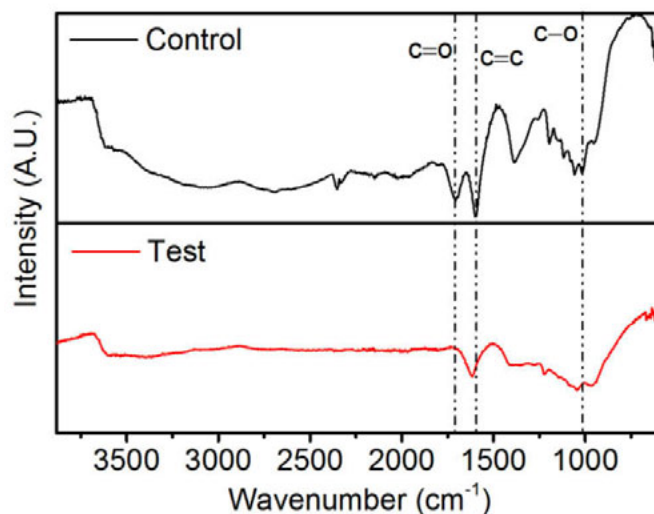


Fig. 2. FTIR spectra of engineered GO with and without interaction of GO-resistant bacteria.

The spectrum of control showed a strong and broad absorption at 3400 cm^{-1} due to the O–H stretching vibration. The C=O stretching peak is observed at 1730 cm^{-1} and the peak at 1620 cm^{-1} may be attributed to C=C stretching vibration of carbon skeleton vibrations of GO. The peak at 1400 cm^{-1} may be assigned to tertiary C–OH groups and peak at 1216 cm^{-1} represents stretching of C–O–C and the peak at 1082 cm^{-1} corresponds to C–O groups in GO control structure [13, 14]. The spectrum of test GO sample showed changes in functional groups as compared to control GO. For test GO, C=O peak at 1730 cm^{-1} disappeared. These changes suggest that most of the hydroxyls and carbonyls tend to diminish from the GO after their interaction with GO-resistant bacteria. It was reported that the C=O removal from the graphitic nanoribbons was mainly because of removal of oxygen-containing groups [15]. Therefore, the exfoliation and reduction of oxygen in GO structure were observed upon interaction with GO resistant bacteria. Thus, FTIR results suggested that the biologically transformed GO structure seemed to have modified physicochemical property that was induced by the GO-resistant bacteria. It is postulated that these changes could be due to biocatalytic action of bacterial enzymes at the close proximity during their interaction with engineered GO.

4. Conclusions

In this study, we reported an approach that utilized enriched natural soil NMs resistant bacteria for modifying the physicochemical property of engineered GO. The bacteria were initially isolated after their adaptation to GO by enrichment process in the laboratory. The GO resistant bacteria showed adaptation with a high degree of survival and tolerance to GO. The GO-resistant bacteria formed aggregates with GO by blanketing GO nanoribbons around cells during the enrichment process. Structural attributes in biotransformed GO nanostructures showed the native hydroxyl and carbonyl groups tend to diminish upon their interaction with GO-resistant bacteria. These structural changes seemed to have occurred due to biocatalytic action of enzymes secreted by the GO-resistant bacteria at close proximities and at the interface of bacteria and engineered GO.

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References

- [1] H.-P. Boehm, *Angewandte Chemie International Edition*, 49 (2010) 9332-9335.
- [2] A.K. Geim, K.S. Novoselov, *Nature Materials*, 6 (2007) 183-191.
- [3] K. Yang, S. Zhang, G. Zhang, X. Sun, S.-T. Lee, Z. Liu, *Nano Letters*, 10 (2010) 3318-3323.
- [4] G. Lalwani, A.M. Henslee, B. Farshid, L. Lin, F.K. Kasper, Y.-X. Qin, A.G. Mikos, B. Sitharaman, *Biomacromolecules*, 14 (2013) 900-909.
- [5] J.T. Robinson, F.K. Perkins, E.S. Snow, Z. Wei, P.E. Sheehan, *Nano Letters*, 8 (2008) 3137-3140.
- [6] Y. Talukdar, J.T. Rashkow, G. Lalwani, S. Kanakia, B. Sitharaman, *Biomaterials*, 35 (2014) 4863-4877.
- [7] S. Pei, H.-M. Cheng, *Carbon*, 50 (2012) 3210-3228.
- [8] Y. Chang, S.-T. Yang, J.-H. Liu, E. Dong, Y. Wang, A. Cao, Y. Liu, H. Wang, *Toxicology Letters*, 200 (2011) 201-210.
- [9] S.J. Klaine, P.J.J. Alvarez, G.E. Batley, T.F. Fernandes, R.D. Handy, D.Y. Lyon, S. Mahendra, M.J. McLaughlin, J.R. Lead, *Environmental Toxicology and Chemistry*, 27 (2008) 1825.
- [10] I.I. Vlasova, T.V. Vakhrusheva, A.V. Sokolov, V.A. Kostevich, A.A. Ragimov, *J. Phys.: Conf. Ser.*, 291 (2011) 012056.
- [11] W. Xing, G. Lalwani, I. Rusakova, B. Sitharaman, *Part. Part. Syst. Charact.*, 31 (2014) 745-750.
- [12] B.L. Allen, P.D. Kichambare, P. Gou, I.I. Vlasova, A.A. Kapralov, N. Konduru, V.E. Kagan, A. Star, *Nano Letters*, 8 (2008) 3899-3903.
- [13] T. Szabo, O. Berkesi, P. Forgo, K. Josepovits, Y. Sanakis, D. Petridis, I. Dekany, *Chem Mater*, 18 (2006) 2740-2749.
- [14] C.D. Zangmeister, *Chem Mater*, 22 (2010) 5625-5629.
- [15] H.T. Liu, L. Zhang, Y.L. Guo, C. Cheng, L.J. Yang, L. Jiang, G. Yu, W.P. Hu, Y.Q. Liu, D.B. Zhu, *J Mater Chem C*, 1 (2013) 3104-3109.