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Efficient removal mechanism for antibiotic resistance genes from aquatic environments by graphene oxide nanosheet

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HIGHLIGHTS

- GO nanosheets exhibited excellent removal capacity against ARGs in water.
- Various forms or classes ARGs could be eliminated rapidly.
- The adsorption of ARGs is monolayer rather than multilayers.
- There are abundant oxygen containing groups, showing strong electron-transfer ability.

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ABSTRACT

In this study, removal efficiency and mechanism of four typical ARGs with two different molecular structures (i.e., cyclic (c)- and double-stranded (ds)-ARGs) by graphene oxide (GO) nanosheet were systematically investigated. The average removal of four ARGs was as high as 3.11 logs toward c-ARGs and 2.88 logs toward ds-ARGs at 300 μg/mL GO solution. The data of adsorption were fitted well with Freundlich isotherm and pseudo-second-order kinetic model. The apparent adsorption equilibrium can be obtained within 15 mins for both c-ARGs and ds-ARGs, indicating the effective removal by GO. The free-energy parameters demonstrated that the removal processes were exothermic and spontaneous. The structural differences of genetic molecular structures can be responsible for the removal discrepancy. Moreover, several removal factors containing initial ARGs concentration, pH and ion species were also investigated. The results of Raman spectra, Diffuse Reflectance Infrared Fourier Transform spectroscopy (DRIFTs) and electrochemical analysis indicated that the adsorption of ARGs by GO was mainly attributed to the oxygen containing groups and π-bonding system of GO nanosheet, which resulted in chemical binding with aromatic nucleic acid and strongly π-stacking interactions. Furthermore, a detailed verification test of real water samples was conducted and 80% of the ARGs can be removed from a natural water sample. As a result, it would be great potential to apply GO nanosheet as a novel adsorbent for effective treatment of ARG-contaminated waters.

1. Introduction

As one of the most important medical discoveries in the 20th century, antibiotics are indispensable for both treating and preventing infectious diseases in clinical medicine and are used broadly in animal husbandry to obtain high-yield and healthy livestock products [1–4]. Excessive use of antibiotics has led to the emergency and spread of antibiotic resistance genes (ARGs) in the environment [1,5]. ARGs have been widely detected in various environmental contexts such as surface [6] and ground water [7], sewage or municipal wastewater [8,9], and even drinking water [10]. Once present in the environment, ARGs may persist over many generations, even in the absence of selective pressure [11], which could enable dissemination of antibiotic resistance traits throughout non-resistant bacteria via transfer [12] (i.e. by cell division) as well as horizontal gene transfer (HGT including conjugation, transduction, and natural transformation) [4]. Intact DNA molecules may be either integrated into genome or stay extrachromosomal elements in the form of plasmid during the

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transformation process in aquatic environment [13]. Extracellular ARGs (eARGs) with genetic mobile platforms such as plasmid or chromosomal DNA in water have gained increasing attention for their durability and contribution to the spread of antibiotic resistance [11–13]. Recent evidence suggests that natural bacterial transformations by short DNA fragments are more frequent in the environment [14], suggesting free DNA in water would pose a serious threat to health care for human and ecosystem. Compared with linear chromosomal, plasmid DNA with a stiffer conformation is more readily adsorbed to soil particles in the presence of divalent cations [15]. However, very little is known about the adsorption behavior and removal performance of nanomaterials as effective adsorbents toward eARGs including plasmid or chromosomal DNA in water.

Compared with traditional contaminants, ARGs are very difficult to be effectively eliminated [16–18]. It is known that wastewater treatment plants (WWTPs) play a crucial role in removal of many common contaminants, such as heavy metals, organic matter, antibiotics and pathogenic microorganisms [9,13,14]. For ARGs, it is recently found that although the absolute abundances of ARGs (89.0–99.8%) were reduced, however, considerable ARGs levels [$(1.0 \pm 0.2) \times 10^3$ to $(9.5 \pm 1.8) \times 10^5$ copies/mL] were still detected in WWTP effluent [19]. Du and his coworkers confirmed that biological treatment units in WWTPs could promote bacterial growth and genetic exchange, which may lead to further ARGs proliferation [20]. Some efforts have been paid to examine the performance of traditional treatment technologies (chlorination and ultraviolet irradiation (UV)) on the inactivation and removal of ARGs, which seems to be unsatisfactory and inefficient. For example, Yuan et al. found that the removal level of erythromycin and tetracycline resistance genes by chlorination was only 0.42 ± 0.12 log and 0.10 ± 0.02 log, respectively [18]. Additionally, chlorination is not environmental friendly and sustainable due to the production of carcinogenic disinfection by-products (THMs, HAAs, HANs) [21]. Zhang demonstrated that the maximum log reduction of tetX and 16S rRNA genes were 0.58 and 0.60 in the UV irradiation experiment (249.5 mJ/cm^2 UV dosage), indicating the low removal efficiency of UV irradiation for removing ARGs [22]. Consequently, it is urgent and interesting to explore novel and effective technologies to remove ARGs from aquatic environments.

Graphene oxide (GO) nanosheet with abundant oxygen-containing groups including hydroxyl, epoxide and carboxyl groups is a highly oxidative form of graphene achieved by chemical exfoliation of graphite [23]. GO maintains superior properties of the 2D structure, aromatic plane and large surface area comparing with graphene [24]. It is an effective adsorbent for various environmental contaminants, such as organic pollutants [25–27], heavy metals (Pb^{2+}, Hg^{2+}, Cd^{2+} and Co^{2+}) [28], and pathogens such as *E. coli* and *S. aureus* [23]. Although GO nanosheet as a desirable adsorbent has been extensively discussed previously, to our best knowledge, the feasibility of GO for ARGs contaminated water treatment has not been investigated.

The overarching objective of this study was to improve the understanding of GO as a potential adsorbent and the removing mechanisms of GO for ARGs in aqueous solution. Four antibiotic resistance genes (*tet*A, *sul*2, *erm*B and *amp*C) were selected as target genes due to their high concentrations in aquatic environment compartments [29,30]. Specifically, survey such as that conducted by Guillaume have shown that *tet*A (against tetracycline) is commonly detected in the microbial community originating from activated sludge of hospital and urban wastewater treatment facilities [31]. The gene *sul*2 and *amp*C, against sulfonamide and β-lactams, are plentiful in many metagenomes from human and natural microbiomes [30]. The gene *erm*B (against macrolides), which is one of the most common erythromycin resistance genes in the environment, has been recently found on a conjugative plasmid [32]. Meanwhile, two basic types of ARGs: cyclic-ARGs (c-ARGs) and double-stranded-ARGs (ds-DNA) were applied in a comparative analysis of removal efficiency. The primary mechanism of GO removing ARGs based on the π-stacking interactions and chemical binding of functional groups between ARGs and GO by adsorption kinetics, isotherms models, electrochemical, and spectroscopy (Raman and DRIFTs) analysis. The effect of conditions of the procedure (i.e., solution pH, initial concentration of adsorbates, incubation time, temperature, and cation species) on the removal performance was also investigated. Furthermore, natural water sample was explored to validate the applicability and efficiency of GO nanosheet, suggesting excellent removal efficiency toward ARGs.

2. Materials and methods

2.1. Synthesis and characterization of GO nanosheet

GO nanosheet were synthesized based on the modified Hummers method described previously [23]. The detailed process and reagents used are presents in the SI-Section (S) 4. The GO surface morphology is measured by transmission electron microscopy (TEM). The surface elements, functionalities and oxygen content of GO were detected by X-ray diffraction (XRD), Fourier Transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). The specific processes of the structural characterization are summarized in the SI (Figs. S3 and S4).

2.2. Sample collection and ARGs extraction

Water samples were collected from the estuary of Haihe river (38° 59′9.78″N, 117° 42′42.40″E) at August 2015. There are many urban and agriculturally influenced regions of potential ARGs sources near the Haihe River, such as feedlots, fishponds and WWTPs as shown in Fig. S1 in Supporting Information (SI). The samples (1 L) were collected from the top 0.5 m of the water surface along the river and then they were delivered to laboratory on ice within 2 h. The water samples were vacuum-filtered through 0.45 μm membranes at room temperature and the membranes were incubated in 10 mL Luria-Bertani broth (LB) medium at 37 °C. Four antibiotic resistance bacteria (ARB) were cultured in LB medium with four antibiotics including 7.5 μg/mL tetracycline, 30 μg/mL sulfadimidine sodium, 30 μg/mL erythromycin, and 100 μg/mL ampicillin, respectively. According to the manufacturer's instructions, genome DNA (the model of ds-DNA) of ARB was extracted with Bacterial Genome DNA Extraction Kit (TIANGEN, Beijing, China) and plasmid (PMD-19, the model of c-DNA) was extracted with TIAN pure Mini Plasmid Kit (TIANGEN, Beijing, China). The purity and concentration of the DNA were determined by the GeneQuant 1300 spectrophotometer (GE Healthcare, Uppsala, Sweden). The purity of DNA in A_{260}/A_{280} = 1.8–2.0 were used further. The details of extracted DNA were displayed in SI-S1. Qualitative PCR assays were used to assess the presence of tetracycline, sulfonamide, β-lactam, and macrolide resistance genes in water samples. The PCR mixture (20 μL total volume) consisted of 0.5 μL primers (10 μmol/L), 10 μL 2 × Taq PCR MasterMix, 2 μL DNA template, and 7 μL sterile deionized water (for details, see SI-Section 2). PCR product sizes were specified in the range of 180–300 bp for q-PCR suitability. Purified PCR products from DNA extracts were cloned and sequenced to be further confirmed specificity and analyzed diversity of removal process. The details of primers and DNA sequence were displayed in SI (Table S1 and Fig. S2).