Chemosphere 183 (2017) 197–203

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Graphene oxide in the water environment could affect tetracycline-antibiotic resistance

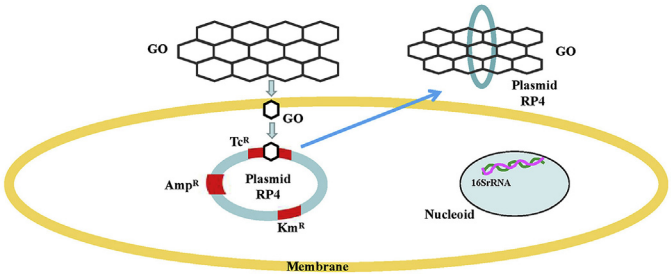
Mei-Ting Guo*, Guo-Sheng Zhang

State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, 200092, Shanghai, China

HIGHLIGHTS

- GO lowered tetracycline-antibiotic resistance.
- GO damaged drug resistance plasmids.
- GO had a great influence on conjugative transfer of ARGs.
- GO caused the oxidative stress in cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 February 2017
Received in revised form
26 April 2017
Accepted 27 April 2017
Available online 3 May 2017

Handling Editor: Tamara S. Galloway

Keywords:
Graphene oxide
Antibiotic resistant bacteria
Antibiotic resistance genes
Tetracycline
Conjugative transfer

ABSTRACT

In recent years, the influence of new materials like nanoparticles in the water environment on biological substances has been widely studied. Antibiotic resistance genes (ARGs) represent a new type of pollutant in the environment. Graphene oxide (GO), as a nano material, because of its unique structure, may have an impact on antibiotic resistance bacteria (ARB) and ARGs; however the research in this area is rarely reported. Therefore, this study mainly investigated the effects of GO on bacterial antibiotic resistance. The results showed that GO had a limited effect on ARB inactivation. A high concentration of GO (>10 mg/L) can damage resistant plasmids to reduce bacterial resistance to antibiotics, but low concentrations of GO (<1 mg/L) led to almost no damage to the plasmid. However, all tested concentrations of GO promoted the conjugative transfer from 1to over 3 folds, with low concentrations and high concentration (1–10 and 100 mg/L) of GO samples the least promoted. The overall effect of GO on antibiotic resistance needs further investigation.

1. Introduction

Graphene oxide (GO), as a new type of nano material, because of its excellent physical and chemical properties, has been applied in many fields. In water treatment, Because of its good adsorption and photocatalytic properties GO can remove pollutants such as microcystin (Pavagadhi et al., 2013) and methylene blue (Thuy-Duong et al., 2011), hence the use of GO may cause the presence of high concentrations of GO in water; at the same time, along with the extensive use of GO, the emissions of waste containing GO inevitably may release GO into the water environment and makes the water environment subject to low concentrations of GO. Therefore, an evaluation of the impact of GO on the water environment needs to be conducted to ensure the safety of water

* Corresponding author.
E-mail address: guomeiting@tongji.edu.cn (M.-T. Guo).

http://dx.doi.org/10.1016/j.chemosphere.2017.04.145
0045-6535/

environment.

With the extensive use of antibiotics, bacterial antibiotic resistance is gradually increasing, and antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) have become a serious threat to human health and safety (Pruden et al., 2006). ARGs are widely distributed in the water environment. Aquaculture wastewater (Zhang et al., 2013), medical wastewater (Guendogdu et al., 2013), sewage treatment plants (Gao et al., 2012), surface water (Proia et al., 2016) and groundwater (Li et al., 2015) are all important reservoirs of ARGs. ARGs can be transmitted through a horizontal transfer in the water environment, mainly including conjugative transfer, natural transformation, and transduction (Guo et al., 2015a), with conjugative transfer being the most studied. By conjugative transfer, ARGs are transferred from the donor bacteria to the recipient bacteria, along with the mobile genetic factors, such as plasmid, transposons, integration, etc. In recent years, studies have found some new materials, such as nano materials [e.g., Al_2O_3 (Qiu et al., 2012)] and ionic liquids [e.g., [BMIm][PF6] (Wang et al., 2015)], can promote the conjugative transfer of ARGs in water environments, and hence promote the spread of ARGs. ARBIn 2010, Hu et al. (2010) first discovered the inhibitory effect of GO on *Escherichia coli*; subsequent studies (Akhavan and Ghaderi, 2010; Liu et al., 2011; He et al., 2015) showed that many common bacteria, such as *Staphylococcus aureus* and *Escherichia coli*, can be inactivated by GO; Qiao et al. (2013) found that under the same conditions, compared with nano TiO_2 and nano ZnO, GO could cause a greater damage to the intracellular DNA; Castrillon et al. (2015) reported that GO would make cells produce oxidative stress and the oxidative stress might change cell membrane fluidity, and then promote the conjugative transfer of resistance genes. From the above analysis we can see that in the water environment, GO may have an inactivation effect on ARB, and is also likely to damage the ARGs and change the drug resistance of the bacteria, and then reduce the risk of drug resistance; but at the same time GO may promote the conjugative transfer of ARGs to increase the risk of the spread of resistance genes. Therefore, one needs to study the effects of GO on the ARB and ARGs, thereby better understand the risk of bacterial drug resistance. But at present there is little research in this area.

In this study, the effects of GO on ARB and ARGs are explored from three aspects. First of all, the rules of the inactivation of GO on ARB are explored; secondly, the effects of GO on ARB' drug resistance characteristics are studied, and then the concentration of ARGs is quantified to explore the mechanism that GO damage drug resistance plasmids to change ARB' drug resistance characteristic; finally, the effects of GO on ARGs' transfer are studied, and the mechanism is explored with regard to increase the permeability of cell membrane to promote the conjugative transfer of ARGs caused by GO.

2. Materials and methods

2.1. GO and bacteria

Flake GO was obtained from XF NANO Co., Ltd., Nanjing China. GO was dispersed in deionized water (DI) via bath sonication at 300 W for 2 h and then diluted into different concentrations.

The donor strain was *E. coli* HB101, obtained from Tianjin Institute of Health and Environmental Medicine. The strain *E. coli* HB101 had the plasmid RP4 which carried ampicillin, kanamycin, and tetracycline resistance (Amp^R, Km^R, and Tc^R). The recipient strain was *E. coli* NK5449 (CGMCC NO.1.1437), obtained from China General Microbiological Culture Collection Center (CGMCC), which encodes high-level resistance to rifampicin and nalidixic acid.

2.2. Bacterial culture

E. coli HB101 and NK5449 glycerol tubes in −80 °C refrigerator were melted, then 50 μL *E. coli* HB101 bacterial suspension was added to LB liquid culture medium which contained 16 *mg/L* tetracycline, 50 *mg*/L kanamycin, 100 *mg*/L ampicillin, and 50 μL *E. coli* NK5449 bacterial suspension was added to LB liquid culture medium which contained 160 *mg/L* rifampicin. After shaking for 16 h on a shaker incubator (160 r/min) at 37 °C, the mixture was centrifuged at 8000 r/min for 10 min. The cells were washed twice to remove residual growth medium constituents and then resuspended in phosphate buffer solution (PBS, pH = 7.0), made of different concentrations of bacterial suspension.

2.3. Cell viability test

GO was added to the HB101 and NK5449 bacterial suspension (concentration of about 10^7 CFU/mL, pH = 7.0) to make the final solution contain 0, 0.1, 1, 10, 50, 80, 100 mg/L GO, respectively. After shaking for 4 h on a shaker incubator (160 r/min) at 37 °C, the mixture before and after reaction was properly diluted by gradient, and the diluted mixture was evenly coated on LB agar plates. Next the LB agar plates were put into a constant temperature incubator (37 °C) for 24 h. Finally the number of colonies on the plates was counted. The viable cell number was counted as colony forming units per milliliter (CFU/mL).

2.4. Detection antibiotic resistance characteristics

GO was added to the HB101 bacterial suspension (concentration of about 10^7 CFU/mL, pH = 7.0) to make the final solution contain 0, 1, 10, 80 and 100 mg/L GO, respectively. After shaking for 4 h on a shaker incubator (160 r/min) at 37 °C, the mixture before and after reaction was properly diluted, and then the diluted mixture was evenly coated on LB agar plates which contained 0, 25, 50, 100, 150, 200 *mg/L* tetracycline, respectively. Finally the LB agar plates were put into a constant temperature incubator (37 °C) for 24 h and the number of colonies on the plates was counted.

2.5. Detection of ARGs' copies by real-time PCR

16SrRNA, *tra*F which was a specific gene on RP4 plasmid, tetracycline resistance gene (*tet*A) and kanamycin resistance gene (*aph*A) on RP4 plasmid were quantitatively measured by Real-time PCR. PCR primers and PCR conditions were showed in Table S1 (Supplementary material) and the details of qPCR process have been described in Supplementary material.

GO was added to the HB101 bacteria suspension (concentration of about 10^7 CFU/mL, pH = 7.0) to make the final solution contain 0, 0.1, 1, 10, 50, 80, 100 mg/L GO, respectively. After shaking for 4 h on a shaker incubator (160 r/min) at 37 °**C**, the DNA in the mixture was extracted by using Rapid Bacterial Genomic DNA Isolation Kit (Sangon Biotech, China) and finally the four standard plasmids were prepared. The concentration of standard plasmids were measured by micro-spectrophotometer (NanoDrop 2000C, NanDrop Technologies, Willmington, DE) and the copies of standard plasmids were calculated. Then the standard plasmids were diluted by 10 times and the standard curves were drawn by real time fluorescence quantitative PCR instrument (ABI7500, Applied Biosystems,USA). Finally, based on the standard curves, the copies of genes were calculated by using the CT value of test samples.

2.6. Conjugation experiment treated with GO

Four single-factors (mating time, bacterial concentration, donor/