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The toxicity of graphene and its impacting on bioleaching of metal ions from sewages sludge by *Acidithiobacillus sp.*

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HIGHLIGHTS

- Graphene showed negative effects on the growth of *A.ferrooxidans.*
- Graphene reduced the bioleaching Cu and Zn from sewage sludge.
- These effects were dose-dependent, especially in smaller concentrations.
- SEMand AFM shows bacteria was effectively damaged.

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ABSTRACT

The increasing production of graphene raised concerns about their releasing into sewage sludge, however, there is little information about graphene impacting on the growth of bacteria and hence their bioleaching of metal ions from sewages sludge. In this study, we reported that *Acidithiobacillus sp.*, isolated from sewages, were used to bioleach Cu^{2+} and Zn^{2+} from sewages sludge in the presence of graphene. The negative effect on the growth of *Acidithiobacillus sp.* and dose-dependent were observed in presence of graphene, where the optical density (OD_{420}) of the culture decreased from 0.163 to 0.045, while the bioleaching efficiency of Cu^{2+} (70%–16%) and Zn^{2+} (80%–48%) were also reduced when the graphene dose decreased from 50 mg L^{-1} to 1 mg L^{-1}. Furthermore, scanning electron microscopy (SEM) and atomic force microscopy (AFM) confirmed that the direct contacts between graphene and cell at 1 mg L^{-1} graphene caused cell membrane disruption, while *Acidithiobacillus sp.* grew better by forming dense biofilms around the suspended graphene at a 50 mg L^{-1}. LIVE/DEAD staining further demonstrated that almost no live cells were detected at 1 mg L^{-1} graphene. The toxicity of graphene could generally be explained by depending on the concentration of graphene. The new findings provide an insight into dose dependence, which impacted on the growth of *Acidithiobacillus sp.* and their bioleaching of metal ion from sludge.

1. Introduction

Graphene, a novel carbon-based nanomaterial has attracted the attention due to its excellent physical, chemical, and biological characteristics (Geim and Novoselov, 2007). Therefore, graphene and graphene-based materials have great potential applications (Pumera et al., 2010; Tu et al., 2013a,b). As the production of graphene increases, it concerns about their potential health and ecological risks (Arvidsson et al., 2013; Gottschalk et al., 2013). For example, since they are many application of commercialized graphene, it leads to the graphene to be released into sewages (Suárez-Iglesias et al., 2016). The key issues need to be investigated in the full implementation of such nanomaterials in biological applications, and their relatively unknown cytotoxicity and biological activity. Such studies generate useful insights into the interaction between atomically flat graphitic structures and various biological systems (Duch et al., 2011).

Many studies demonstrated strong antibacterial activity of pristine graphene, graphene oxide (GO) and reduced graphene oxide (rGO), with severe cytotoxicity evident in bacteria (Akhavan and Ghaderi, 2012; Guo et al., 2017). Akhavan and Ghaderi (2010)

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found that the antibacterial activity of rGO which can inhibit 87% of *Escherichia coli* in 1 h exposure was more toxic to the bacteria than the GO. Guo et al. (2017) revealed that GO significantly enhanced the cell growth, biofilm formation, and biofilm development even up to a concentration of 500 mg L^{-1}, while rGO (≥50 mg L^{-1}) strongly inhibited cell growth and biofilm formation. In addition, many studies have shown that the cytotoxic effects of nanomaterials are dependent on dose, size and contact time (Akhavan et al., 2012; Smith and Rodrigues, 2015). The antimicrobial mechanism proposed by researchers includes physical disruption and chemical oxidation. Zucker et al. (2017) found that graphene nanosheet-driven membrane disruption stemmed from a physical mechanism rather than chemical oxidation. Graphene nanosheets can slice into the cells with sharp edges, cutting as well as destructively extracting lipid molecules, which eventually led to cell death (Zou et al., 2016). Additionally, physical trapping is one of the important mechanisms describing the cytotoxicity of graphene, which the aggregation of graphene sheets could capture the bacteria and inhibits their proliferation (Akhavan et al., 2011). Overall, many studies have reported that graphene has cytotoxic properties, whereas the toxic mechanisms still not being well understood.

Graphene based materials have been shown to be a promising solution for the removal of various biological and organic/inorganic contaminants from water (Smith and Rodrigues, 2015). It is expected that wastes containing graphene is generated and end up in the sludge of wastewater treatment plants (Qu et al., 2015; Nguyen et al., 2017). Bioleaching has been demonstrated as a feasible and effective technology for removing heavy metals from sludge (Ma et al., 2017). Chemolithotrophic bacteria such as *Acidithiobacillus ferrooxidans*, are frequently used to primary ores and sludge bioleaching (Rozas et al., 2017; Wen et al., 2012). In our previous study, the bioleaching of meal ions such as Cr, Cu, Pb and Zn from sewage sludge by *Acidithiobacillus sp.* was observed (Wen et al., 2012). However, to date, there are limited studies that report the effect of graphene on the response of microbes in the sewage sludge.

The objective of this study was to test the cytotoxicity and toxicity mechanisms of graphene. *Acidithiobacillus sp.* was selected as a model organism, in order to investigate bioleaching of heavy metals in sludge (Wen et al., 2012). This study includes (1) exploring the effects of graphene dose on the growth of *Acidithiobacillus sp.*; (2) discussing the bioleaching of Cu^{2+} and Zn^{2+} by *Acidithiobacillus sp. from* sewage sludge in the presence of graphene; and 3) investigating the toxicity mechanisms using scanning electronic microscopy (SEM), atomic force microscopy (AFM) and LIVE/DEAD staining. This study may fill the knowledge gap between the toxicity of graphene and microbes in the sewage sludge.

2. Materials and methods

2.1. Graphene

Graphene was purchased from Beijing DK Nano Technology Company (Beijing, China). The length specified by the manufacturer was 1–5 μm. The purity of graphene was 99% and its thickness was 0.9–1.2 nm. The X-ray diffraction (XRD), Fourier Transform infrared spectroscopy (FTIR), and Atomic Force Microscope (AFM) were used to characterize graphene as shown in Fig. 1, which provides the basic property for graphene.

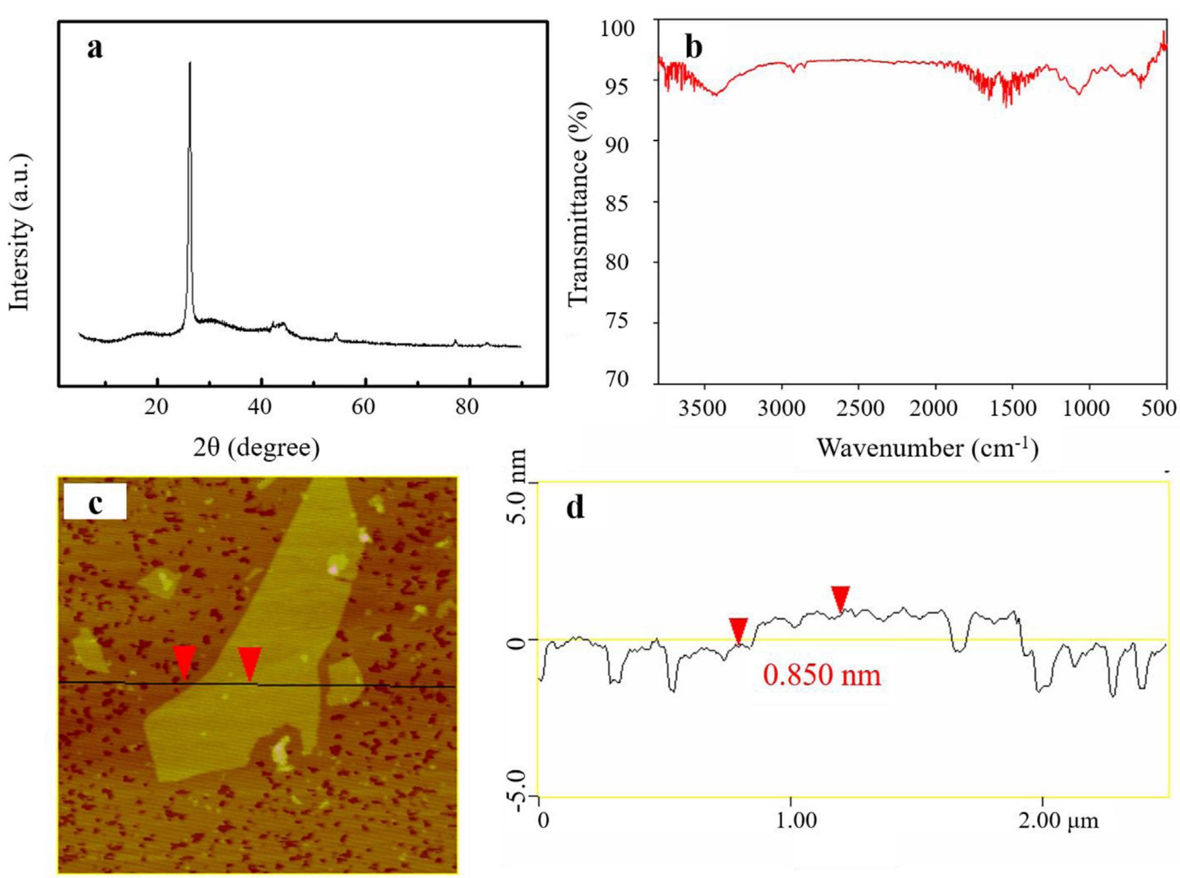

Fig. 1. The (a) XRD, (b) FTIR, (c) (d) AFM of graphene.