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Concentration-dependent effect of photoluminescent carbon dots on the microbial activity of the soil studied by combination methods

Wenjuan Liu[a], *Jun Yao*[a,b,*], *Hankuai Chai*[a], *Zhibo Zhao*[a], *Chi Zhang*[a], *Jingnan Jin*[a], *Martin M.F. Choi*[c,**]

[a] *School of Civil and Environmental Engineering, and National International Cooperation Based on Environment and Energy, University of Science and Technology Beijing, 30 Xueyuan Road, Haidian District, Beijing 100083, China*

[b] *Key Laboratory of Biogeology and Environmental Geology of Chinese Ministry of Education, and Sino-Hungarian Joint Laboratory of Environmental Science and Health, China University of Geoscience, Wuhan 430074, China*

[c] *Partner State Key Laboratory of Environmental and Biological Analysis, and Department of Chemistry, Hong Kong Baptist University, 224 Waterloo Road, Kowloon Tong, Hong Kong, China*

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ABSTRACT

Carbon dots (Cdots) have a great potential for their widespread biological applications. However, there are a few studies on the biosafety of Cdots. In this work, the biological effect of Cdots on soil microorganism was analyzed by microcalorimetry, soil enzymatic activities, and denaturing gradient gel electrophoresis (DGGE). The addition of Cdots causes a gradual increase of the maximum heat power (P_{peak}) and the growth rate (k) at low concentration of Cdots (0.0–50.00 μg/g). But there is no significant effect of Cdots on the total heat output (Q_{total}). The urease and fluorescein diacetate esterase activities demonstrate that introduction of Cdots to soil has almost no impact on the structure and function of the soil microbial community and microbial processes. The DGGE results exhibit that the control and the Cdots-treated soils display similar patterns, indicating that Cdots have little effect on the microbial community structure.

1. Introduction

Quantum dots (QDs) are one of most attractive semiconductor nanomaterials with excellent optical properties. Particularly, water-soluble and bio-functionalized QDs have played important roles in the biological, biomedical, and pharmaceutical application (Cui et al., 2001; Taton et al., 2000). However, most QDs are synthesized with heavy metal elements which possess some adverse effects on biological systems (Mei et al., 2014). In the past years, some researchers devoted lots of commitment to develop heavy-metal-free photoluminescence nanomaterials.

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[*] *Corresponding author at*: School of Civil and Environmental Engineering, and National International Cooperation Based on Environment and Energy, University of Science and Technology Beijing, 30 Xueyuan Road, Haidian District, Beijing 100083, China. Tel.: +86 10 62333305; fax: +86 10 62333305.

[**] *Corresponding author*. Tel.: +852 34117839; fax: +852 34117348.

E-mail addresses: yaojun@ustb.edu.cn (J. Yao), mfchoi@hkbu.edu.hk (M.M.F. Choi).

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Carbon dots (Cdots) as a fascinating class of "zero-dimensional" carbon nanomaterials exhibit some of the same major characteristics of QDs, such as high photostability, tunable emission, and broad UV excitation (Larson et al., 2003; Wang et al., 2010; Hu et al., 2014). Besides these advantages, compared with semiconductor, Cdots possess non-blinking fluorescence, excellent biocompatibility, and ease of preparation (Peng and Travas-Sejdic, 2009; Zhu et al., 2009). The most important criteria of Cdots are that they are free of heavy metals and more environmental friendly, inferring that they are much safer for biological applications (Tao et al., 2012). As a result, Cdots have attracted considerable attention in biological fields on account of their unique fluorescent performance. Cdots have a great potential in some novel applications through functionalizing Cdots surface with exotic functional chemistries. Despite the novel and potential applications such as drug targeting and in vivo biomedical imaging, it is necessary to thorough understanding of the effect on human health and environment. So far there are few studies on the impact of carbon nanomaterials including single-walled carbon nanotubes (Kang et al., 2008), fullerene (Tong et al., 2007), and functionalized carbon nanomaterials (Qin et al., 2014; Rodrigues et al., 2012) on microorganism or microbial community. To our knowledge, much work has not been done on assessing the dose-dependent effect of Cdots on microbial communities in soil.

Soil is an open biogeochemical system where various biochemical reactions may occur. The soil ecosystem is likely to be the ultimate recipient for nanomaterials (Nowack and Bucheli, 2007). Therefore, the soil microbial activity is considered as one of the most significant parameters of soil microorganisms and has been suggested as an indicator of soil health. Microorganisms are an indispensable part in global biogeochemical cycles and especially sensitive indicator of soil's response to the environmental changes (Riding et al., 2012). The metabolic processes in soil are related with the measurements for evaluating toxicity in ecological studies. Since Cdots are small with diameters less than 10 nm, they can enter almost all areas of microorganisms in soil. And soil characteristics (soil type, pH and organic matter content) and environment factors (temperature and humidity) also affect the abundance of soil microorganisms and their activity. In this case, it will be worthwhile to exploit some accurate methods for assessing the metabolic processes of soil microorganisms in Cdots contaminated soil.

Techniques selected for investigating metabolic processes depend on the specific characteristics of system. Each research method for detecting the microbial activity has its own limitation. It is well known that microcalorimetric method is an alternative, non-destructive and effective technique to monitor metabolic processes of microorganism. As a sensitive monitor, this method continuously quantifies the entire metabolism of microbial biomass in soil, with the incubation time being the same as in actual condition (Wadsö et al., 2011). In this regard, microcalorimetry has proved to be an important technique for accurately evaluating the metabolism and microbial growth of microorganism in soil and requires only the observable difference between the treated and control incubations about the power production (Masakorala et al., 2014). In order to obtain more comprehensive and accurate results, the studies are usually combined with other methods. Soil enzymes can act as excellent indicators in the evaluation of soil quality and nutrient cycling in natural environment. The enzymes can easily respond to the external influences induced by both natural and human factors. Urease is considered as an important extracellular enzyme because of its role in the hydrolysis of urea and its application in nitrogen uptake and cycling (Chen et al., 2014). Moreover, fluorescein diacetate esterase (FDAE) is an ubiquitous enzyme present in soil microorganism. The hydrolytic activity of fluorescein diacetate is commonly used to evaluate the overall activity of soil microorganism. Additional information on the impact to the structure of microbial communities was obtained from PCR-denaturing gradient gel electrophoresis (PCR-DGGE) based on the universal bacterial biomarker for the bacterial variable V3 region following the extraction of total soil genomic DNA.

In this work, a simple one-step synthesis of water-soluble Cdots from multiwalled carbon nanotubes (MWCNTs) as the carbon source was adopted. The size, structure and morphology of Cdots were characterized by transmission electron microscopy (TEM) and Fourier transform infrared (FTIR) spectroscopy. The optical properties of Cdots including absorption and photoluminescence were also evaluated. Finally, the dose–toxicity relationship between thermodynamic parameters, enzyme activities, DGGE and doses of Cdots were applied to evaluate the toxic effect of Cdots to soil microbial activity. To our knowledge, this is the first report on applying microcalorimetry in conjunction with enzyme activity assay and PCR-DGGE method to assess the toxicity of Cdots to soil microbes.

2. Materials and methods

2.1. *Materials*

MWCNTs (inner diameter 20 nm, external diameter 40 nm) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). Nitric acid (65% HNO_3), sulfuric acid (98% H_2SO_4) and other reagents were obtained from Tianjin Reagent Factory (Tianjin, China). All reagents of analytical grade were used without prior purification. The solutions used in this work were prepared with deionized distilled water.

2.2. *Synthesis of Cdots*

Typical fluorescent Cdots sample was synthesized according to a literature method (Tao et al., 2012). In brief, 100 mg of MWCNTs were blended with 15 mL HNO_3 and 5.0 mL H_2SO_4 in a 50 mL flask and sonicated for 30 min. Then, the mixture was refluxed for 24 h at 80 °C. The reaction mixture was dispersed in 100 mL redistilled water after cooling down to ambient temperature. Then the dark-brown solution was filtered with a 0.1 μm microfilm filter. The purification was achieved by dialysis with a dialysis bag with molecular-weight cut-off of 14 kDa in 2 L deionized distilled water to remove the superfluous acid until the pH was almost neutral.