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Aging of fullerene C_{60} nanoparticle suspensions in the presence of microbes

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ABSTRACT

Despite the growing use of carbon nanomaterials in commercial applications, very little is known about the fate of these nanomaterials once they are released into the environment. The carbon–carbon bonding of spherical sp^2 hybridized fullerene (C_{60}) forms a strong and resilient material that resists biodegradation. Moreover, C_{60} is widely reported to be bactericidal. Here however, we observe the changing properties of fullerene nanoparticle aggregates aged in the presence of microbes. C_{60} aggregates were observed to decrease in size with aging, while hydroxylation and photosensitized reactivity measured by the production of reactive oxygen species (ROS) increased, suggesting that chemically and/or biologically-mediated activity is capable of partially transforming fullerene structure and reactivity in the environment. However, stable-isotope-labeling C_{60} aggregates incubated with microbial cultures from aged suspensions for 203 days did not produce significant labeled carbon dioxide, despite significant reduction in aggregate radius for biological samples. These results suggest that either the rate of biodegradation of these particles is too slow to quantify or that the biologically-enhanced transformation of these particles does not occur through microbial biodegradation to carbon dioxide.

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1. Introduction

There are growing commercial and consumer markets for engineered nanomaterials with applications including cosmetics, tires, pharmaceuticals, alternative energy, and paints. However, the potential human health and environmental impacts of nanomaterials are of increasing concern (Wiesner et al., 2006). Moreover, the very properties that are selected for in manufacturing nanomaterials such as their stability, as well as electronic, optical, and thermal properties (Auffan et al., 2009) may impede their degradation in the environment, enhancing their persistence and, perhaps, impart toxicity.

Carbon-based nanomaterials such as fullerenes and carbon nanotubes (CNT) have proven to be particularly resistant to biodegradation, likely due to the latticework structure of sp^2 hybridized carbon–carbon bonds and demonstrated toxicity for bacteria (Tsao et al., 2002). There are no known reports of microbial degradation of fullerene C_{60} and the molecule has proven to be highly resistant to chemical attack (Cataldo, 2002). However, there is recent evidence of fungal co-metabolism of hydroxylated fullerene, (*i.e.*, fullerol, $C_{60}(OH)_{24}$) (Schreiner et al., 2009). And addition of strong peroxidases was shown to degrade single-wall (SW) CNTs (Allen et al., 2008; Kagan et al., 2010) yielding products that appeared to be less toxic than the original SWCNTs (Kagan et al., 2010).

Although the discovery of fullerene nanoparticles and the ability to produce them in large quantities has occurred over the last two decades, similar compounds occur naturally or incidental to human activities. Documented environmental sources for fullerenes include lightning strikes, coal, meteorite impact craters, and certain rock formations (Buseck, 2002; Buseck et al., 1992). Incomplete combustion of organic matter in forest fires or internal combustion engines might also produce fullerene-like materials (Murr et al., 2004) as well as carbon black and soot (Middelburg et al., 1999). However, fullerenes do not appear to accumulate in the environment to high levels (Buseck, 2002), suggesting that there may be a pathway for natural degradation of these carbon compounds (Jaffe et al., 2013).

The question of whether or not microbes are able to alter underivatized fullerenes released to the environment is pivotal in determining the potential for environmental persistence and associated risks of these manufactured nanomaterials. The question is equally pertinent in deciphering the significance of fullerenes in geologic deposits and evaluating the cycling of fullerene-bound carbon in ecosystems. Here, we explore the potential for C_{60} fullerene nanoparticles present as colloidal aggregates (nC_{60}) in water to be transformed or degraded and report on the subsequent changes in biologically aged fullerene nanomaterials.

2. Materials and methods

2.1. Preparation and characterization of fullerene and fullerol suspensions

Fullerene (C_{60}, 99.9%) and poly-hydroxylated fullerene ($C_{60}(OH)_{24}$, purity is unknown but the manufacturer claims the material contains small amounts of sodium and water) (M.E.R, Tucson, AZ) colloidal suspensions were prepared using a sonication method as described previously (Chae et al., 2009b). Both fullerene and fullerol suspensions were prepared at various concentrations depending on the experimental design and stored in a refrigerator at 4 °C without further sterilization and maintained under clean but not aseptic conditions. Total carbon (TC) and non-purgeable organic carbon (NPOC) concentrations of the fullerene and fullerol suspensions that were produced at and stored for different amounts of time were measured by a total organic carbon (TOC) analyzer (TOC-5050A, Shimadzu). Hydrodynamic diameter (d_h) of the fullerene and fullerol aggregates was measured by dynamic light scattering (DLS) at 173° by Zetasizer Nano (Malvern Instrument, Bedford, MA). Total suspended solid (TSS) of the fullerene and fullerol suspensions was measured by filtering 10 mL of a sample through a membrane having nominal pore size of 1.2 μm (GF/C, Whatman) and pH of the suspensions was measured using a bench-top pH meter (Accumet Excel XL20, Fisher Scientific, Pittsburgh, PA). Absorbance wavelength of the fullerene suspensions was measured by using an UV/Visible spectrophotometer (U-2000, Hitachi).

Extracellular polymeric substances (EPS) was classified as proteins and carbohydrates, which are the dominant components typically found in EPS (Bura et al., 1998). EPS was extracted using a cation exchange resin (Dowex® C-211, J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg NJ) (Tourney et al., 2008). Carbohydrate EPS was determined according to the phenol-sulfuric acid method (Dubois et al., 1956) with a glucose as a standard material at 488 nm and protein EPS was measured using a bicinchoninic acid (BCA) (Brown and Lester, 1980) protein assay kit (Thermo Scientific, Waltham, MA) with a BSA as a standard material at 562 nm. Raman spectra of the samples were measured by Raman microscopy (Horiba Jobin Yvon LabRam ARAMIS). All measurements were performed in triplicate and standard deviations are included. For all studies, the Student *t*-test was used to assess the significance of the results with a 95% confidence interval.

2.2. Electron microscopy

High magnification images of the suspensions were obtained by a variable pressure scanning electron microscope (SEM) (FEI XL30 ESEM, Hillsboro, OR) and transmission electron microscopy (TEM) (FEI Tecnai G2 Twin, Hillsboro, OR). Ten microliters of each sample was dropped on a lacey carbon/Cu grid (300 mesh, Electron Microscopy Sciences, Hatfield, PA) and dried in air before TEM measurement. Image analysis of diameter (d_{TEM}) of the nC_{60} on the TEM pictures obtained was performed by using an Image-Pro version 4.5 (Media Cybernetics, Inc. Bethesda, MD).

2.3. Identification of microbial community structure by rRNA gene sequencing

Microbial isolates from the two-year old fullerene mixed community were obtained by plating on low nutrient media (0.05 g/L tryptone, 0.01 g/L yeast extract) and streaking colonies 3 times to isolation. DNA was extracted from isolates and the mixed microbial community in the two-year old C_{60}