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Mitigation of bactericidal effect of carbon nanotubes by cell entrapment

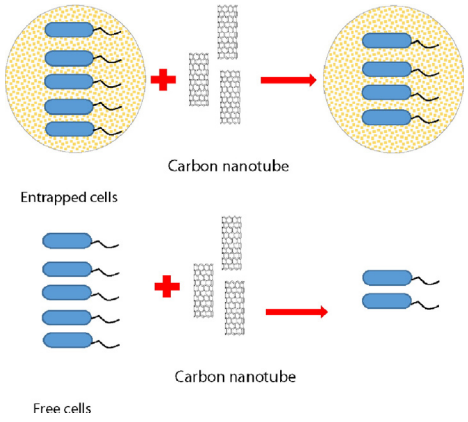
Tu Thi Anh Le [a,d], John McEvoy [b], Eakalak Khan [c,*]

[a] Environmental and Conservation Sciences Program, North Dakota State University, Fargo, ND 58108, USA
[b] Veterinary and Microbiological Sciences Department, North Dakota State University, Fargo, ND 58108, USA
[c] Civil and Environmental Engineering Department, North Dakota State University, Fargo, ND 58108, USA
[d] Biology Department, Da Lat University, Da Lat, Vietnam

HIGHLIGHTS

- Polymeric gel entrapment reduces toxicity of carbon nanotubes on *E. coli*.
- Polyvinyl alcohol and alginate similarly mitigate toxicity of nanotubes on *E. coli*.
- The benefit of gel entrapment is more obvious at high nanotube concentrations.
- Long carbon nanotubes have more negative effect on entrapped *E.coli* than short ones.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the effects of the alginate and polyvinyl alcohol (PVA) entrapment on the viability of *Escherichia coli* cells exposed to single wall carbon nanotubes (SWCNTs) with a diameter of 1–2 nm. Viability was examined using a galactosidase enzyme assay, LIVE/DEAD *Bac*Light assay, and total ribonucleic acid quantity. Variables studied included SWCNT concentration (5, 10, 20, 50, 100, 200, 500, and 1000 μg/ml), SWCNT length (0.5–2 μm for short SWCNTs and 5–30 μm for long SWCNTs), and initial bacterial concentration (6.5 $\log_{10}$ CFU and 9 $\log_{10}$ CFU per test). Results showed that both alginate and PVA entrapments mitigate the bactericidal effect of SWCNTs. At the highest SWCNT concentration tested (1000 μg/ml), the viability of the cells relative to controls (systems with only *E. coli*, no SWCNTs), was 0–60% for free cells and 60–90% for alginate and PVA entrapped cells. The bactericidal effect depended on SWCNT type and concentration, and bacterial concentration. In general, long SWCNTs (5–30 μm) caused significantly greater reductions in the viability of entrapped cells than the short SWCNTs except for the two highest SWCNT concentrations studied, 500 and 1000 μg/ml. Microscopy showed that the entrapment matrices prevented SWCNTs from entering the beads. This study shows that bacterial entrapment is effective at limiting the bactericidal effect of SWCNTs.

* Corresponding author at: Department of Civil and Environmental Engineering (# 2470), North Dakota State University, P.O. Box 6050, Fargo, ND 58108-6050, USA.
E-mail address: eakalak.khan@ndsu.edu (E. Khan).

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1. Introduction

Carbon nanotubes (CNTs) are one of the most widely used nanomaterials in consumer products due to their large surface area to volume ratio, electrical properties, high tensile strength, and ultra light weight. Common consumer products such as bicycle frames (MBC Swissland) and rims (ENVE composites and Zyvex technologies), tennis rackets (Babolat), hockey sticks (Easton), and plastic films (Unidym) incorporate CNTs as key components. Consequently, novel materials containing CNTs are entering the environment at an alarming rate, and research to understand the environmental and public health impact is struggling to keep pace. However, studies have shown that CNTs can negatively impact organisms, organs, cells, and biomacromolocules (Al-Hakami et al., 2013; Kang et al., 2007; Kang et al., 2008; Le et al., 2015; Li et al., 2013; Rodrigues et al., 2013; Zhao and Liu, 2012).

CNTs can cause dermal toxicity (following skin contact) and possibly lung cancer (following inhalational exposure) in humans. The pathology is due to oxidative stress and immune-mediated responses including inflammation, granuloma formation, fibrosis, and apoptosis (Crouzier et al., 2010; Shvedova et al., 2008; Wang et al., 2010). CNTs also negatively impact non-mammal animal species such as zebrafish (Cheng et al., 2009), earthworms (Li et al., 2013), plant species (Stampoulis et al., 2009), and bacteria (Al-Hakami et al., 2013; Kang et al., 2008; Le et al., 2015; Rodrigues et al., 2013; Vecitis et al., 2010).

The antibacterial effect of CNTs is dependent on factors such as solution buffering capacity, CNT concentration and length, species and concentration of bacteria, and exposure time (Le et al., 2015; Zarubina et al., 2010). Although the precise mechanism of CNT toxicity for bacteria is unclear, direct contact appears to be important. Both gram-negative and gram-positive species show altered cell morphology, membrane damage, cell wall disruption, and cytoplasm leakage following exposure to CNTs (Kang et al., 2007; Le et al., 2015; Liu et al., 2010; Obraztsova et al., 2009). It appears that damage to bacterial cells is caused by a mesh of interacting CNTs rather than individual CNTs (Liu et al., 2010).

The well-documented antibacterial properties of CNTs (Al-Hakami et al., 2013; Brady-Estevez et al., 2010; Kang et al., 2008; Rodrigues et al., 2013; Vecitis et al., 2010) have been exploited to enhance the effectiveness of products used for decontamination. For example, CNT embedded filters substantially reduce the levels of bacteria and viruses in water, and fungi and bacteria in air relative to standard filters (Brady-Estevez et al., 2010; Xu and Yao, 2011). Unfortunately, increasing levels of CNTs in environments such as soil and activated sludge can also cause reductions in populations of ecologically important bacterial species (Chung et al., 2011; Hai et al., 2014; Jin et al., 2013; Parise et al., 2014), upsetting the delicate balance of these ecosystems. Therefore, solutions are required to mitigate the potential negative effects of CNTs on beneficial bacteria in critical ecosystems. One proposed solution focused on the CNT; multiple walled CNTs were coated with a polystyrene-based polymer, which altered the surface property and reduced lung cell toxicity (Tabet et al., 2011). In contrast, the present study proposes a solution that focuses on protecting beneficial bacteria, such as those in wastewater treatment and bioremediation systems, from toxicity of CNTs by cell entrapment in porous polymeric matrices. In fact, cell entrapment is a known alternative process for wastewater treatment and bioremediation.

Entrapped microbial cells are widely used in food (e.g. alcohol fermentation) and pharmaceutical production (e.g. antibiotics) (Kumaravel and Gopal, 2010; Srinivasulu et al., 2003) because of their enhanced resistance to environmental stresses, such as shear forces in mixing vessels, relative to free cells. Cells are typically entrapped in a hydrogel, which may be natural (alginate, carrageenan, and chitosan) or synthetic (polyacrylamide, polyacrylate, and polyurethane, polyvinyl alcohol) (Aykut et al., 1988; Kumaravel and Gopal, 2010; Manohar et al., 2001; Pramanik et al., 2011). Polyvinyl alcohol (PVA) and alginate are frequently used for cell entrapment because they are economical, nontoxic polymers, physically and chemically stable, and have a low mass transfer resistance (Albert et al., 2011). In addition, alginate is known to be inert and has high water-holding capacity while advantages of PVA over other polymeric materials include durability and mechanical strength.

The goal of this study was to investigate the effect of purified single walled carbon nanotubes (SWCNTs) on the viability of bacterial cells entrapped in PVA and alginate. *Escherichia coli* ATCC 8739 was used as a model organism. The strain has been used in laboratory bactericidal studies (Le et al., 2015; Maria-Neto et al., 2012). A beta-D-galactosidase assay, the LIVE/DEAD *Bac*Light assay, and total RNA quantification were used as surrogates of *E. coli* viability during experiments. The advantages of these approaches are that they produce faster results compared to the standard plate count method and can account for viable but non-culturable cells. The galactosidase assay can be applied to entrapped cells in situ without a need to remove the bacterial cells from the matrix. A previous study on impact of SWCNTs on *E. coli* ATCC 8739 demonstrated that the three non-culture based methods used in this study provide similar cell viability results as the plate count (Le et al., 2015). Variables examined included SWCNT length and concentration, and initial concentration of entrapped *E. coli* cells. Free cells were included in the study for a comparative purpose. Light and electron microscopies were used to monitor changes in the entrapment bead structure following exposure to SWCNTs.

2. Materials and methods

2.1. Chemicals and cell culture

PVA, boric acid, sodium orthophosphate, sodium alginate, and calcium chloride were purchased from VWR international, Inc. (West Chester, Pennsylvania, USA). The PVA used in this study has a molecular weight of 77,000 to 79,000 and a degree of hydrolysis of 99.0% to 99.8%. The specific gravity and melting point of the PVA are 1.19–1.31 and 200 °C, respectively. The melting, boiling, and flash points of sodium alginate are 300 °C, 495.2 °C (at 760 mm Hg), and 211.1 °C, respectively.

4-methylumbelliferyl-β-D-galactoside, isopropyl-β-D-thiogalactopyranoside, and Xgal were purchased from Sigma Chemical Co. (MO, USA). *E. coli* ATCC 8739 was obtained from the Global Bioresource Center (American Type Culture Collection, VA, USA). The strain was plated onto nutrient agar (NA) and incubated overnight at 37 °C. A single colony from the overnight incubation was used to prepare a liquid culture for the experiments. This liquid culture was incubated overnight at 37 °C and shaken continuously at 1500 rpm (Lab-line® orbital shaker 3590, IL, USA). Cells were harvested during stationary growth, pelletized at 4500 × *g* for 15 min, and washed twice in phosphate buffer saline (PBS) before being entrapped or directly used in experiments (for free cells).

2.2. Single-walled carbon nanotubes

SWCNTs with a diameter of 1–2 nm, and a length of 0.5–2 μm (short SWCNTs) and 5–30 μm (long SWCNTs) were obtained from Cheap Tube Inc. (Brattlebro, VT, USA). They were purified as described in Le et al. (2015) to remove silica, cobalt, and amorphous carbon before being used in experiments. Characteristics of the purified CNTs including Raman spectra and purity are described in Le et al. (2015).

2.3. Cell entrapment

E. coli ATCC 8739 cells were entrapped in PVA according to Pramanik et al. (2011). For alginate entrapment, the procedure described by Konsoula and Liakopoulou-Kyriakides (2006) was modified. The modification involved hardening the alginate beads overnight.

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