Chemical Engineering Journal 298 (2016) 1–9

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Biotransformation of multi-walled carbon nanotubes mediated by nanomaterial resistant soil bacteria

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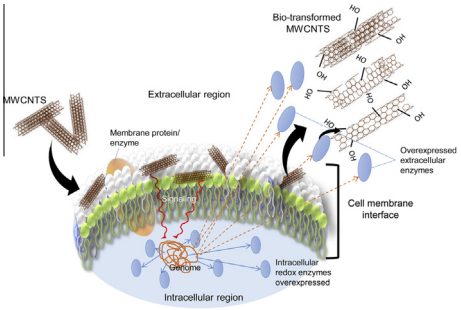
HIGHLIGHTS

- Isolated nanomaterial-resistant soil bacteria identified as *Trabusiella guamensis*.
- Bacteria bio-transformed multiwalled-carbon nanotubes (MWCNTs) by surface oxidation.
- Bacterial peroxidase lowered its affinity constant (K_m) due to MWCNT-adaptive-drift.
- Eco-friendly, cost-effective and green approach for oxidation of MWCNT is proposed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form 3 April 2016
Accepted 4 April 2016
Available online 9 April 2016

Keywords:
Bacteria
Biotransformation
Nanomaterial
Oxidation
Nanotubes

ABSTRACT

In this study, soil bacteria were isolated from nanomaterials (NMs) contaminated goldsmith site and enriched in the presence of multi-walled carbon nanotubes (MWCNTs) in order to obtain resistant bacteria. The isolated resistant bacteria were biochemically and genetically identified as *Trabusiella guamensis*. Redox-enzyme activity and cell viability assay showed molecular adaptation and no membrane damage in resistant bacteria under MWCNTs stress. The resistant bacteria were allowed to interact with engineered MWCNTs in order to study the bio-transformation in their structure. Raman spectra of bio-transformed MWCNTs revealed increased intensity ratio of I_D/I_G with subsequent formation of C=O and COOH groups on the outer walls of nanotubes that were also confirmed by Fourier transform infrared (FTIR) results. X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD) and ultraviolet–visible spectroscopy (UV–vis) analysis of bio-transformed MWCNTs revealed surface oxidation of CNTs. The structural changes in concentric walls were also evident from transmission electron microscopy (TEM) images. Our results demonstrated that the biotransformation of MWCNTs was mediated by resistant bacteria through oxidation process. The presented study showed an effective methodology that utilizes NMs resistant microbes for bio-transformation of MWCNTs in different biological settings which will have impact on "green nanotechnology".

1. Introduction

Carbon nanotubes (CNTs) gained importance in many applied fields such as composites, conductive materials, sensors, drug delivery vehicles and sorbents [1]. Widespread commercial

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http://dx.doi.org/10.1016/j.cej.2016.04.019
1385-8947/

applications and use of CNTs has been linked to their production in large-scale [2]. This may eventually lead to their introduction into the environmental system. In recent years, studies on aggregation and transportation of CNTs have been conducted to understand their possible impact and fate in the environment [3–5]. Most of studies have focused on biocompatibility of CNTs and very few exploring the possibility of their biodegradation [6,7]. A few reports suggest that biocompatibility of CNTs may be attributed to the availability of functional groups on the side walls of nanotubes [8–10]. However, enzymatic catalysis has shown to partially degrade CNTs through biocatalytic oxidations [11–14]. Enzymes that are found to degrade CNTs are horseradish peroxidase (HRP) [11,12,15,16] and neutrophil myeloperoxidase (nMPO) in the presence of H_2O_2 [13].

It is imperative to explore the possibility of CNTs biodegradation mainly by the action of microbes. Recent reports on biodegradation of graphene oxide (GO) by bacteria and the extent to which MWCNTs can be degraded by different bacteria, such as *Burkholderia kururiensis*, *Delftia acidovorans*, and *Stenotrophomonas maltophilia* are given more importance [17,18]. Bacterial community is capable of degrading ^{14}C-labeled MWCNTs into $^{14}CO_2$ in the presence of an external carbon source via co-metabolism. This degradation required external carbon source involving co-metabolism and the cooperation of several microbial consortia [18]. However, structural transformation in MWCNTs during the bacterial degradation process is still unclear. Therefore, degradation or biotransformation of carbon based NMs through different microbial pathways needs to be explored. So far, there are no direct studies reporting on possible biotransformation of CNTs through nanomaterials (NMs) resistant living microorganisms. Natural soil microbial flora requires evolutionary adaptation to the new man-made carbon nanostructures. This can be achieved in the laboratory at a relatively short time if soil bacteria are subjected to selective and forced evolutionary adaptation process.

Here, we report on isolation of soil bacteria from goldsmith contaminated site that are resistant to MWCNTs and identified as *Trabusiella guamensis* which belongs to *Enterobacteriaceae* family and resembles *Salmonella* subgroups. The *T. guamensis* finds niche in a wide variety of environments including marine sediments, various fish species, ocean water and spoiled foods [19,20]. The isolated bacteria were enriched with MWCNTs to induce their strong NMs resistance property. These NM resistant bacteria were further utilized for studying bio-transformation of engineered MWCNTs. Biochemical and physico-chemical methods were employed to identify the pathways and mechanisms for biotransformation of MWCNTs.

2. Materials and methods

2.1. Materials

MWCNTs used in this study had O.D. × L 7–15 nm × 0.5–10 μm (Arry, Hong Kong). Almar blue, Amplex Red (N-acetyl-3,7-dihydroxyphenoxazine) and Lactate dehydrogenase (LDH) was purchased from Pierce Biotech., Inc. USA. Triton-X 100 was procured from Merck, Germany. Dimethyl Sulfoxide (DMSO) was purchased from Sigma Aldrich, USA. All other reagents used in this study were of analytical grade and filtered through 0.22 μm sterile filters. Growth studies and enzyme assays were carried out in 96-well NUNC clear microtiter plates (Thermo Scientific, USA). All bacterial studies and related work were carried out under sterile conditions.

2.2. Collection of soil sample and storage

Soil samples were collected from the gold processing industrial wastes from central part of India. The collected soil samples were appropriately labeled and transported to the laboratory using Standard Operating Procedures (SOPs). The collected soil samples were homogenized in a container constructed of inert material and stored at 4 °C or transferred to appropriate growth medium for the propagation of soil bacteria.

2.3. Isolation and enrichment of NM resistant bacteria

Soil samples (25 g each) were suspended in basal mineral medium (MM) separately, diluted appropriately and filtered through a column with a glass-wool plug to remove undesirable suspended particles from the soil. The basal MM (per liter) contained 3 g KH_2PO_4, 12.8 g $Na_2HPO_4{\cdot}7H_2O$, 1 g NH_4Cl, 0.5 g NaCl and supplement sources such as $MgSO_4$ (2 mM), $CaCl_2$ (0.1 mM) and glucose (varying from 0.3% to 0.4%). The pH (6.5–7.5) of basal MM and the temperature (25–37 °C) were varied depending upon the growth requirements of the soil microbial flora and modified by removing specific salts accompanied by supplementing with different carbon/nitrogen sources (eg., NH_4NO_3 in place of NH_4Cl). Alternatively, resistant bacteria were also grown in TSB (Tryptic say broth) medium for rapid screening of mixed bacterial colony characteristics. The TSB medium contained (per liter) 17 g Tryptone (pancreatic digest of casein), 3 g Soytone, 2.5 g dextrose, 5 g Sodium chloride and 2.5 g K_2HPO_4, pH 7.3. Cultures grown on basal MM were only considered for enrichment while cultures grown in nutrient rich TSB medium were used only for screening colony characteristics because of the possibilities of losing the ability of bacterial adaptation to MWCNTs.

Culture flasks that showed good growth in MM were amended with MWCNTs. MWCNTs suspension was prepared in PBS pH-7.4 containing 0.01% Triton-X 100. The mixture was homogenized using probe ultrasonicator for 15 min. The homogeneous stock suspension of MWCNTs (5–15 μg/mL) was prepared freshly prior to the start of the experiment. To avoid the undesirable growth of contaminating fungi in cultures, >0.5 μg/mL of cycloheximide (an anti-fungal agent) was added into the medium before inoculation for three subsequent subcultures and withdrawn when all of the fungi were eliminated. Initially, 5 μg/mL MWCNTs were amended in the media to interact with the soil bacteria and incubated the flasks at 25–37 °C at 125 rpm and replenished with fresh medium every 30 days interval for over 14 months. Depending upon the resistance of bacteria, MWCNTs concentration was increased to 15 μg/mL and this concentration was maintained throughout the enrichment process. Thus obtained resistant bacteria were isolated and utilized for characterization.

2.4. Biochemical characteristics of isolated NMs resistant bacteria

In this study, standard biochemical tests that are commonly used (listed in Supporting Information, SI Table S1) for bacterial identification were applied for identification of isolated NM-resistant bacteria. Fresh colonies from all the isolated samples were selected and grown in MM agar containing 0.4% glucose for 48 h. Bacterial colonies from the MM agar plates were picked, suspended in sterile saline solution and vortexed. Each sample was assigned a unique identity to avoid confusion for interpretation of final results. A total of 21 biochemical tests were carried out for the identification of bacteria and the results were interpreted using an online chart (http://faculty.ivytech.edu/~bsipe/UNKN/ukkey.htm) according to Bergey's Manual of Systematic Bacteriology [21]. All biochemical tests that were carried out are listed in SI Table S2 using standard commercial biochemical kits (Analytab Products, Inc.).