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Response of anaerobic granular sludge to single-wall carbon nanotube exposure

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ARTICLE INFO

Article history:
Received 10 September 2014
Received in revised form
18 November 2014
Accepted 25 November 2014
Available online 3 December 2014

Keywords:
SWCNTs
Anaerobic digestion
Granular sludge
Methanogens
Toxicity

ABSTRACT

Rapid development and application of nanotechnology have introduced various nanopaticles, such as single-walled carbon nanotubes (SWCNTs), whose negative effects on aquatic organisms and cultured cells have been reported, into anaerobic wastewater treatment systems. In this study, the response of methanogenic sludge exposed to SWCNTs in anaerobic digestion process was investigated. Results show that SWCNTs, at a concentration up to 1000 mg/L, had no significant impact on the maximum methane yield. In contrast, they induced much faster substrate utilization and methane production rates. Scanning electron microscopy examination shows that more extracellular polymeric substances (EPS) were excreted from the anaerobic sludge and closely interacted with SWCNTs. Such an interaction prevented nanoparticles from piercing into cells, and thus reduced their cytotoxicity. In the compact anaerobic granule structure, SWCNTs exposure enhanced the electrical conductance of the sludge, which might promote direct interspecies electron transfer among anaerobic fermentative bacteria and methanogens in the anaerobic digestion process. Our results provide useful information to understand the response of anaerobic microorganisms to CNTs in complex environmental matrix.

1. Introduction

Carbon nanotubes (CNTs) have attracted great attention because of their small dimensions, high strength and remarkable physical properties (Odom et al., 1998). They have been integrated in diverse commercial products, ranging from coating materials and microelectronics to filtration membranes (De Volder et al., 2013). By 2011, the worldwide CNT production has jumped to about 4.6 kilotons per year (De Volder et al., 2013). With their expanding industrial applications, CNTs have entered the environment in the unintentional release from CNT-containing products (Petersen et al., 2011), and this has raised concerns over their environmental safety. So far most of investigations have been focused on the potential adverse effects of CNTs on human health and the environment (Helland et al., 2007). CNTs have shown toxic effects on aquatic organisms and cultured cells (Roberts et al., 2007; Mwangi et al., 2012). Antibacterial action of CNTs has also been observed (Simon-Deckers et al., 2009), suggesting that microbial process in natural and engineered systems might be affected.

Wastewater treatment plants (WWTPs) are designed for municipal sewage and industrial wastewater treatments, in which microorganisms play a crucial role in breaking down and converting pollutants (Hammer and Hammer, 2007).

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http://dx.doi.org/10.1016/j.watres.2014.11.042

While release of CNTs from CNT-containing products has been demonstrated (Bello et al., 2009; Nowack et al., 2013), these nanoparticles released in their production and applications will find their way into the WWTPs through various ways like waste disposal (Brar et al., 2010; Petersen et al., 2011). Although a relatively low fraction of CNTs is thought to be released into a WWTP (Gottschalk et al., 2009), the CNT nanoparticles can agglomerate with the sludge due to their high hydrophobic nature (Brar et al., 2010). Thus, they are likely to accumulate in the sludge.

The effects of CNTs on activated sludge under aerobic conditions have been investigated in several studies. It was reported that 270 mg/L of 90% purified SWCNTs had no negative impact on the performance of activated sludge, but improved the sludge settleability and dewaterability (Yin and Zhang, 2008). However, it was found that SWCNTs applied at the same dose affected the microbial community of activated sludge (Goyal et al., 2010). A dose-dependent respiration inhibition was observed for activated sludge exposed to multi-walled CNTs (Luongo and Zhang, 2010). In such a complex system, the effects of CNTs could be altered by their surface properties and environmental factors. Nevertheless, to date, the impacts of SWCNTs on anaerobic process for wastewater treatment are largely unknown yet.

Anaerobic digestion has been widely used for high-strength wastewater treatment and sludge stabilization as well as biogas production because of reduced sludge handling costs, substantial higher energy efficiency, and lower chemical usage compared to aerobic treatment (Latif et al., 2011; Abbasi and Abbasi, 2012). Methanogens play a key role in anaerobic digestion, but they are sensitive to various inorganic and organic substances, such as ammonia, organics, heavy metals and nanoparticles, etc. (Chen et al., 2008; Mu et al., 2012). Therefore, in this study, the response of anaerobic sludge to the dose of SWCNTs was examined, and substrate utilization and biogas production were monitored. Combined microbial community analysis with the concentration changes of intermediate products (H_2 and volatile fatty acids-VFAs) in anaerobic digestion, the impact of SWCNTs on anaerobic digestion was elucidated and their possible involvement in the interspecies electron transfer among anaerobic fermentative bacteria and methanogens in the anaerobic digestion process was explored. With these results, we could achieve a new insight into the response of anaerobic microorganisms to CNTs in complex environmental matrix.

2. Materials and methods

2.1. Preparation of SWCNT suspension

Commercially available SWCNTs were purchased from Alpha Nano Technology Co. (Chengdu, China). SWCNTs were more than 90% pure by dry weight, with a specific surface area greater than 400 m^2/g, an average diameter of 1–2 nm, and 5–20 μm in the length. SWCNT stock suspension was prepared by mixing SWCNTs in distilled water for 30 min in an ultrasonic bath, followed by further sonication with an ultrasonic cell disruptor (Ningbo Scientz Biotechnology Co., China) for 1 h at a power output of 80 w. The suspension was sonicated in the ultrasonic bath for 30 min before use to ensure a homogeneous mixture.

2.2. Anaerobic reactor set-up and SWCNT exposure

The seed sludge was anaerobic granular sludge (AGS) collected from an upflow anaerobic sludge blanket (UASB) reactor at our laboratory and acclimated in a mesophilic UASB reactor, which was continuously operated at 35 ± 1 °C with a hydraulic retention time of 12 h, a volumetric loading rate of 10.28 kg chemical oxygen demand (COD)/($m^3 \cdot d$). Sucrose was used as a substrate for the UASB reactor, which exhibited a COD removal efficiency higher than 95% under the steady-state condition. The compositions of basal medium were as follows (mg/L): $NaHCO_3$, 1500; NH_4HCO_3, 410; $K_2HPO_4 \cdot 3H_2O$, 210; $CaCl_2$, 50; $MgCl_2 \cdot 6H_2O$, 100; NaCl, 10; $FeCl_2 \cdot 4H_2O$, 39; $MnCl_2 \cdot 4H_2O$, 5; $CoCl_2 \cdot 6H_2O$, 5; $AlCl_3 \cdot 6H_2O$, 4.5; H_3BO_3, 5; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 5; $NiCl_2 \cdot 6H_2O$, 5; $ZnCl_2$, 5; and $CuSO_4 \cdot 5H_2O$, 5.

Activities of anaerobic sludge exposed to SWCNTs were measured in 160-mL glass serum bottles with a working volume of 80 mL. Firstly, the AGS from the mesophilic UASB reactor were grinded and filtered through a 0.9-mm stainless steel mesh to remove large particles before exposure (Yang et al., 2012). Each serum bottle was fed with filtered sludge to a total suspended solids (TSS) concentration of 500 mg/L. To minimize the adsorption of COD by the SWCNT in our study, SWCNT stock suspension was equilibrated in basal medium with sucrose (600 mg COD/L) for 30 min. Then, the suspension was centrifuged to be replaced with new medium before the sludge was added. The SWCNTs suspension was set at 1000 mg/L, and a serum bottle without dose of SWCNTs was used as the control. Oxygen was removed by sparging the medium with nitrogen gas for at least 15 min to ensure anaerobic conditions. After sealing the bottles with rubber stoppers and aluminum caps, all glass serum bottles were incubated at 35 ± 1 °C in an air bath shaker. Changes of basal medium COD, production of headspace H_2 and CH_4 gases, VFAs were measured at given time intervals. The tests were conducted in triplicate. Results were expressed as mean ± standard error. Analysis of variance (ANOVA) was used to compare the results, and $p < 0.05$ was considered statistically significant (Mu et al., 2007).

After the depletion of substrate, the archaeal and bacterial community compositions of the anaerobic sludge were analyzed. Distribution of extracellular polymeric substances (EPS) in the sludge samples was observed with multiple fluorescent staining. The interactions between SWCNTs and the granular sludge were observed with a scanning electron microscope (SEM, JSM-6700F, JEOL Co., Japan).

2.3. Analytical methods

The headspace was sampled 0.6 mL at given time intervals using a gas-tight syringe. The H_2 and CH_4 contents in gas samples were analyzed using a gas chromatograph (GC, Model SP-6800A, Lunan Co., China) according to Sheng and Yu (2007). The following model was used to estimate the cumulative CH_4 production process and facilitate comparison (Borja et al., 1995):