Structural and proactive safety aspects of oxidation debris from multiwalled carbon nanotubes

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ARTICLE INFO

ABSTRACT

The removal of oxidation debris from the oxidized carbon nanotube surface with a NaOH treatment is a key step for an effective functionalization and quality improvement of the carbon nanotube samples. In this work, we show via infrared spectroscopy and ultrahigh resolution and accuracy mass spectrometry that oxidation debris obtained from HNO_3-treated multiwalled carbon nanotubes is a complex mixture of highly condensed aromatic oxygenated carbonaceous fragments. We have also evaluated their cytotoxicity by using BALB/c 3T3 mouse fibroblasts and HaCaT human keratinocytes as models. By knowing the negative aspects of dissolved organic carbon (DOC) to the water quality, we have demonstrated the removal of these carbon nanotube residues from the NaOH solution (wastewater) by using aluminium sulphate, which is a standard coagulant agent used in conventional drinking water purification and wastewater treatment plants. Our results contribute to elucidate the structural and proactive safety aspects of oxidation debris from oxidized carbon nanotubes towards a greener nanotechnology.

1. Introduction

Carbon nanotubes (CNTs) have many applications in electronics, the materials industry and bionanotechnology applied to medicine, agriculture and the environment. Most of these applications require a chemical processing of these nanomaterials during their purification and/or functionalization steps. The oxidation of CNTs with nitric acid (HNO_3) remains one of the most used methods for chemical modification, purification, functionalization and shortening of carbon nanotubes [1–5]. The side effect of this methodology is the generation of byproducts, commonly referred as carboxylated carbonaceous fragments (CCFs) or oxidation debris, which have been identified as polycyclic aromatic sheets with oxidized edges [6–9]. Due to the strong van der Waals interactions between these fragments and the carbon nanotube sidewalls, their removal is dependent on a treatment with a sodium hydroxide (NaOH) solution, which promotes the desorption of oxidation debris from the

nanotube surface. The oxidized CNTs free of these carbonaceous fragments can therefore be easily separated from the alkaline solution either by filtration or centrifugation [10–12].

It has recently been reported the influence of oxidation debris on the carbon nanotube behavior in chemical and biological environments [13]. This report has pointed out that oxidation debris might promote a bundling state of oxidized single walled carbon nanotubes by gluing nanotubes and consequently influencing on their colloidal properties, such as mean agglomerate size, surface charge and water dispersion stability. Indeed, it has been proposed that oxidation debris tends to stabilize oxidized carbon nanotube dispersions in water by acting as a pseudosurfactant [13].

By considering the scenario where many applications would demand oxidized CNTs, it is expected that the HNO_3 oxidation and NaOH washing processes will be widely used. Despite the impressive growth of the CNT industry and their chemical processing, the detailed structural properties of oxidation debris have been minimally studied so far. Moreover, their toxicity and waste management have not been addressed.

In this work, we have focused on the structural study of oxidation debris obtained from HNO_3-treated multiwalled carbon nanotubes by Fourier transform infrared spectroscopy (FT-IR) and

ultrahigh resolution and accuracy Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS). We have also evaluated their cytotoxicity in BALB/c 3T3 mouse fibroblasts and HaCaT human keratinocytes. By considering the similarities between oxidation debris and fulvic substances [12] and their potential negative impact on water quality and wastewater treatments [14–16], we have demonstrated that oxidation debris can be removed by using aluminium sulphate, a standard coagulant agent used in conventional drinking water purification and wastewater treatment plants [17]. This work calls attention to a proactive safety approach towards suitable strategies for carbon nanotube chemical processing.

2. Experimental

Multiwalled carbon nanotubes (MWCNTs) synthesized by a chemical vapor deposition (CVD) (diameter: ~10–40 nm and length: ~1–25 μm) were purchased from CNT Co. Ltd., batch number: 5029010C (Incheon, Korea). The BALB/c 3T3 mouse embryo fibroblast cell line was obtained from the National Institutes of Health (Baltimore, MD, USA). The HaCaT human keratinocyte cell line was kindly provided by Dr. Liudmila Kodach (Academic Medical Center, Amsterdam University, The Netherlands). Cells were maintained in Dulbecco's Modified Eagle's Medium [DMEM; Sigma (St. Louis, MO, USA)] supplemented with 100 U/mL of penicillin, 100 μg/mL of streptomycin and 10% of fetal bovine serum [(FBS; Gibco (Grand Island, NY, USA)]. Cells were grown in monolayers at 37 °C in a humidified atmosphere containing 5.0% of CO_2. The aluminium sulphate [$(Al_2(SO_4)_3 \cdot 14H_2O$] was obtained from Bauminas Ltd. (Cataguases, MG, Brazil).

2.1. HNO_3-treated multiwalled carbon nanotubes

The as-prepared MWCNTs (1.0 g) were refluxed in a 9.0 mol/L HNO_3 solution (200 mL) for 24 h at 150 °C. After cooling down to room temperature, they were filtered through a 0.2 μm PTFE membrane and washed with deionized water until a neutral pH was reached for the filtrate. The HNO_3-treated MWCNTs were dried under vacuum for 24 h.

2.2. Extraction of oxidation debris from HNO_3-treated MWCNTs

The extraction of oxidation debris from HNO_3-treated MWCNTs was performed according to Verdejo [10] with modifications. Briefly, HNO_3-treated MWCNTs (500 mg) were homogenized in a 0.1 mol/L NaOH solution (200 mL) and sonicated in an ultrasound bath (Cole-Parmer 8891) for 15 min. Afterwards, they were submitted to a 300 rpm magnetic stirring for 1 h at room temperature. A colored filtrate (orange-brown) was obtained by using a standard vacuum filtration system with a 0.2 μm PVDF membrane. This filtrate has been considered to contain CCFs or oxidation debris compounds (see supplementary material, Fig. S-1.). The resultant filtrate was dialyzed against deionized water for 3 days. After that, the dialyzed solution was lyophilized to obtain oxidation debris in the solid state. This solid sample was weighted by using an analytical microbalance (Perkin-Elmer AD-6) and is referred to as DEBRIS in this work.

2.3. Characterization methods

The Fourier transform infrared spectroscopy (FT-IR) of DEBRIS was recorded using an ATR accessory on a Bomem MB spectrometer in the 400–4000 cm^{-1} frequency range. A total of 34 scans and resolution of 4 cm^{-1} were employed to obtain the spectrum. Absorption spectra of the solutions were obtained by using a UV–vis spectrophotometer (Shimadzu UV-1650PC). The Raman

spectroscopy analysis with a laser wavelength of 532 nm and an excitation power of 4 mW (WITec TS-150 spectrometer) showed that DEBRIS is a highly disordered carbonaceous material (see supplementary material, Fig. S-2).The mass spectrometry analysis was performed on a LTQ FT Ultra mass spectrometer (ThermoScientific). The electrospray ionization (ESI) was performed followed by a Fourier transform ion cyclotron resonance (FT-ICR) analysis (ESI FT-ICR MS). For the ESI(−) experiment in the negative ion mode, a solution was prepared by dissolving 1.0 mg of DEBRIS in 1.0 mL of a mixture of methanol and ultrapure Milli-Q water (50:50). This solution was was diluted with isopropyl alcohol prior to analysis. A direct infusion was performed by using an automated chip-based nano-ESI-MS Triversa NanoMate 100 system (Advion BioSciences). The sample was loaded in 96-well plates (total volume of 100 μL). General ESI conditions were: gas pressure of 0.3 psi, capillary voltage of 1.55 kV and a flow rate of 250 nL/min. The mass spectrum was the result of the combination of 100 microscans processed via the Xcalibur 2.0 software (ThermoScientific).

The Zeta-potential (ζ) of DEBRIS (100 μg/mL) was measured on a Zetasizer-Nano (Malvern Instruments) by using Milli-Q water as dispersant.

The morphology and thermal behavior of the MWCNTs were analyzed by transmission electron microscopy (TEM; Zeiss CEM-902 analyzer with EELS) and thermogravimetric analyses (TGA; SDTQ600 TA Instruments), respectively. TEM and TGA analyses were performed on the as-prepared MWCNTs aiming to visualize and quantify the total content of amorphous carbon in our starting sample (see supplementary material, Figs. S-3(a) and S-4, respectively). The as-prepared MWCNTs showed low content of amorphous carbon (<0.2%) compared to the total MWCNTs weight. TGA analyses were performed by using 5.0 mg of the dried sample placed in a platinum crucible. The heating rate was maintained at 5.0 °C/min and air flow rate at 100 mL/min. The as-prepared MWCNTs showed a specific surface area of 190 m^2/g, evaluated by the Brunauer–Emmett–Teller method (ASAP 2010 Micromeritics Instruments). The HNO_3-treated MWCNTs were also analyzed by TEM, TGA and Raman spectroscopy to demonstrate the effect of the oxidative treatment (see supplementary material, Figs. S-3(b), S-4 and S-5).

2.4. Cytotoxicity assay

The DEBRIS cytotoxicity was assessed by the enzymatic reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma), as previously described by Mosmann [18]. HaCaT (7×10^3/well) and 3T3 (1×10^4/well) cells were incubated in 96-well plates until semiconfluence was reached and then treated with different DEBRIS solutions (10–100 μg/mL) for 24, 48 and 72 h. Afterwards, the culture medium containing DEBRIS was removed and the cells were washed with a 0.1 mol/L phosphate-buffered saline solution (PBS; pH 7.4), followed by the addition of 100 μL of a MTT solution (5.0 mg mL^{-1} in culture medium). After incubation with the MTT solution for 4 h at 37 °C, the medium was discarded and the formazan crystals were solubilized in dimethyl sulfoxide. The plates were shaken for 15 min on a plate shaker and the absorbance measured at 570 nm in a microplate reader (ELx800 BioTek Instruments). Three independent experiments in six replicates were performed to ensure the reproducibility. The results were expressed as percentage of untreated controls (100%). The results shown in the graphs represent the means ± standard deviations (S.D.).

2.5. Removal process by using aluminium sulphate

Coagulation experiments were performed by adding aluminium sulphate to the DEBRIS wastewater stock solution. Briefly, HNO_3-
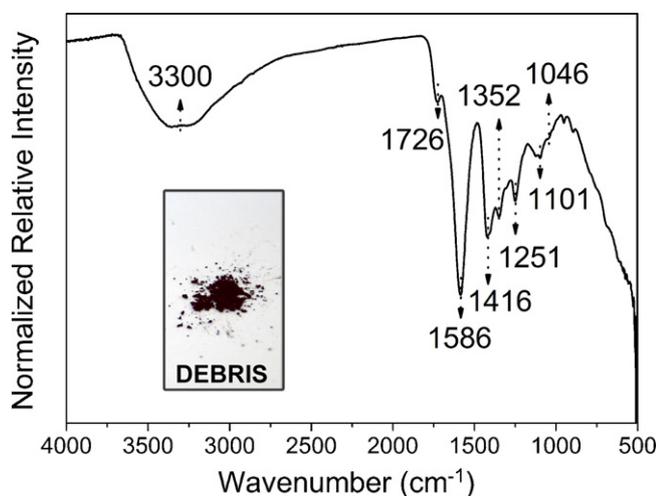
Fig. 1. FT-IR spectrum of DEBRIS obtained from HNO₃-treated multiwalled carbon nanotubes. Inset: picture of DEBRIS (dark brown color) in the solid state after lyophilization. (For interpretation of the references to color in this sentence, the reader is referred to the web version of the article.)

treated MWCNTs (0.25 g) were homogenized in a 0.1 mol/L NaOH solution (100 mL) and sonicated in an ultrasound bath (Cole-palmer 8891) for 15 min. Afterwards, the suspension was submitted to magnetic stirring (300 rpm) for 1 h at room temperature. A colored filtrate (orange-brown) was obtained by using a standard vacuum filtration system with a 0.2 μm PVDF membrane. This filtrate containing dissolved DEBRIS fragments has been considered the wastewater stock solution. The coagulation experiments were carried out in centrifuge tubes containing 5.0 mL of the DEBRIS wastewater stock solution and several amounts of aluminium sulphate (from 2.0 to 30 mg/mL). These tubes were submitted to magnetic stirring (250 rpm) for 90 min at room temperature, kept static for 24 h aiming to the particles sedimentation, and then the supernatant was measured (at 400 nm) by using a UV–vis spectrometer.

3. Results and discussion

Oxidation debris emerges as an important byproducts to be considered in carbon nanotube surface chemistry [19–22]. The generation of DEBRIS in our HNO₃-treated MWCNT sample was approximately 2.6%-weight. This byproduct shows a dark brown coloration, rather distinct from the black appearance of multiwalled carbon nanotubes (see supplementary material, Fig. S-6.). Its Fourier transform infrared spectrum (FT-IR) presents bands at 3300, 1726 and 1586 cm⁻¹, related to the O–H stretching [υ(O–H)], C=O stretching in carbonyl and carboxyl groups [υ(C=O)], and C–C (aromatic) stretching vibrational [υ(C=C)] modes, respectively (Fig. 1.). The large intensity of the υ(C=C) mode is explained by the dipole moment created in carbon double bonds (or conjugated double bonds) by the presence of adjacent oxygenated groups. The broad band located from 1000 to 1450 cm⁻¹ could be related to several possible modes: the C–H asymmetric and symmetric bending [δ(CH₂) + δₐₛ(CH₃) + δ(CH₃); 1300–1450 cm⁻¹], C–(CO)–C stretching and bending [υ(C–CO–C) + δ(C–CO–C): 1100–1300 cm⁻¹ for ketones], C–O–C asymmetric and symmetric stretching [υₐₛ(C–O–C) + υ(C–O–C): 1040–1275 cm⁻¹ for ethers] and C–O stretching [υ(C–O): 1000–1250 cm⁻¹ for alcohols and phenols] modes. The individual assignment of these modes becomes very complex to be done because they are not resolved.

Considering that the DEBRIS fragments are originated from the nanotube surface, it is reasonable to expect similarities between the oxygenated groups on the nanotube sidewall and DEBRIS frag-
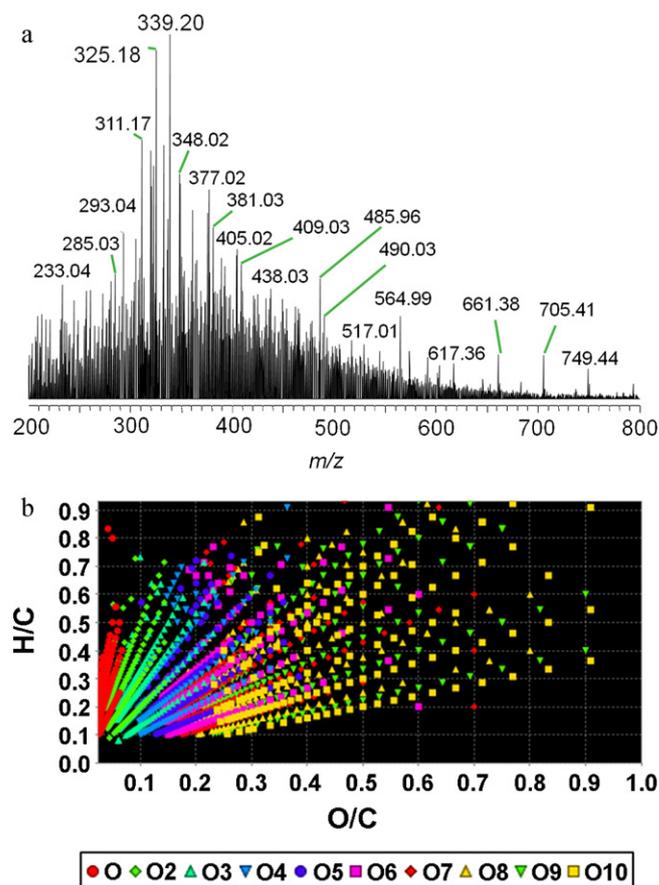
Fig. 2. (a) ESI(−) FT-ICR mass spectrum of DEBRIS obtained from HNO₃-treated multiwall carbon nanotubes; (b) the van Krevelen diagram for elemental data calculated from the mass spectrum.

ments. In this way, depending on the parameters related to the oxidation process (e.g. CNT structure and morphology, time of reaction, acid concentration and temperature), the presence of some groups such as ketones, ethers, alcohols, phenols, carboxylic acids, quinones, lactones and anhydrides, which were already identified for oxidized carbon nanotubes [23,24], could be also expected to be present on DEBRIS fragments.

The electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) technique has been shown to provide a comprehensive overview, at the molecular level, of complex mixtures such as natural organic matter and crude oils [25,26]. Therefore, we decided to study DEBRIS obtained from HNO₃-treated MWCNTs by the ESI FT-ICR MS technique. In consistence to previous results [12], the analysis showed a huge amount of components, thus confirming the very complex chemical nature of the sample. However, the high mass resolving power ($m/\Delta m_{50\%} > 350,000$ for $200 < m/z < 1000$) and high mass accuracy (<1 ppm) of ESI FT-ICR MS, allowed for the unambiguous assignment of the elemental composition for thousands of species found in this sample. Over 826 ions with >4% relative abundance were detected in the m/z range from 200 to 800 [Fig. 2(a)]. In this way, for the first time, the ESI FT-ICR MS technique is used in the carbon nanotube research, yielding molecular information on hundreds of compounds presented in oxidation debris.

The remaining issue was to find a proper way to display this complex chemical composition in terms of the several classes of compounds. For that purpose, we used the well-known van Krevelen diagram [27–29]. This diagram displays oxygen classes on the x-axis (O/C molar ratio) and double-bond equivalent (DBE) compounds along the y-axis (H/C molar ratio). As seen

in Fig. 2(b), as the H/C ratio increases and the number of rings plus double bonds decreases, the van Krevelen diagram allows for a convenient visual separation of the oxygen classes and DBE types.

The aqueous solubility of oxidation debris under all pH conditions, fluorescence properties and mass spectrometry studies induced authors to point out their similarity with fulvic acids [12]. However, according to our van Krevelen diagram [Fig. 2(b)], the H/C (from 0.1 to 0.7) and O/C (from 0.0 to 0.5) ranges correspond to a typical region of highly condensed aromatic structures having sufficient oxygen functional groups, which render them soluble in polar solvents [28,30,31]. This result contrasts to previous ESI FT-ICR MS data of fulvic acids, which have indicated a predominance of ions with an H/C range greater than 0.75 and O/C range greater than 0.25 [32,33]. This contrast in the van Krevelen diagrams for DEBRIS and fulvic acids could be associated to differences in the chemical composition, considering that fulvic acids are originated from the humification process having proteins, carbohydrates, lipids and secondary metabolites as precursors.

As the carbon nanotube industry expands at high rates [34,35], it becomes necessary that suitable wastewater treatments and recycling technologies be implemented in their industrial processing [36–39]. Currently, however, the efforts involving the carbon nanotube safety have focused on evaluating their biomedical and environmental applications, and their effects on living organisms as well as soil, water and air environments [40–44]. Less attention has been paid for evaluating, mitigating and eliminating the byproducts and wastes from the carbon nanotube chemical processing [45–47]. Thus, it has been recently reported that over 45 byproducts are formed in the air effluent from the main synthetic process of carbon nanotubes (i.e. catalytic chemical vapor deposition), including volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PHAs). This finding may lead to an important occupational health problem in the next years [48]. To the best of our knowledge, there are no studies describing the safety aspects of oxidation debris originated from carbon nanotubes. In order to achieve a proactive approach, we evaluated their cytotoxicity in BALB/c 3T3 mouse fibroblasts and HaCaT human keratinocytes, considering that the skin is the primary barrier to the chemical adsorption and an important route to the occupational, environmental and consumer exposure. As indicated in Fig. 3, the treatment of the two cell lines as a function of increasing DEBRIS concentrations of up to 100 μg/mL did not affect the cellular viability, thus suggesting a lower dermal toxicity risk. Cytotoxicity reflects the cell response to particular kinds of damage, such as the mitochondria insult and/or oxidative stress. The reduction of MTT to formazan in isolated cells is an indicative of the cell redox activity, and the reaction is attributed mainly to mitochondrial enzymes and electron carriers [49]. The MTT assay is considered a standard colorimetric viability test. However, other biochemical toxicity biomarkers must be considered and evaluated, such as lysosomes and the membrane integrity.

Despite DEBRIS did not show cytotoxic effects on 3T3 fibroblasts and HaCaT keratinocytes in our experimental conditions, their influence and implications when adsorbed on the carbon nanotube surface on biomolecular and cellular interfaces cannot be ignored [50,51], once their synergic effects were minimally studied so far. Besides, the generation of these byproducts is directly related to the CNT morphology as well as the oxidative acid conditions employed, which increases the effects possibilities [9,11,23]. In this study, we do not have evaluated the cytotoxicity of HNO$_3$-treated MWCNTs and HNO$_3$-treated MWCNTs without DEBRIS (after the NaOH washing step), because it has been reported that cell viability assays, including MTT, may lead to false positive results due to the assay reagent adsorption on the CNT surface [52]. Clearly, further studies are necessary to address this issue.
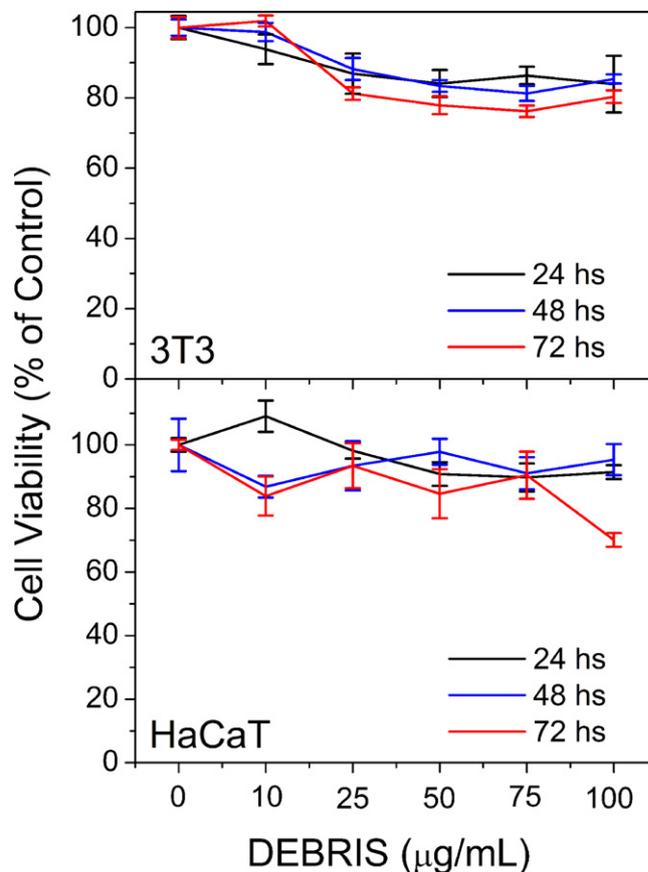
Fig. 3. Cytotoxicity of DEBRIS on 3T3 mouse fibroblasts and HaCaT human keratinocytes cells for 24, 48 and 72 h using the MTT assay. The percent of cell viability was calculated relative to the untreated control (100%). The results represent means ± S.D.

Although the removal of oxidation debris from the carbon nanotube surface is a very important cleaning step [53–55], this process may introduce a potential environmental problem since they are viewed as a mixture of carbonaceous polyaromatic systems with similarities to fulvic substances and DOC. It is well known that fulvic substances and DOC play an important role in water quality and wastewater treatments (e.g. color, odor and mutagens formation during chlorination) [56,57], as well as in the interactions with environmental pollutants (e.g. heavy metals, PHAs and pesticides) [58–60]. Besides, it was verified that DEBRIS compounds, as well as fulvic substances, do not coagulate under low pH values, thus increasing the difficulty of their removal from the aqueous media (see supplementary material, Fig. S-7).

Hutchison [61] discussed the importance of implementing proactive practices and green chemistry principles in nanoscience and nanotechnology. Motivated by this highlight report, we have demonstrated that aluminium sulphate, a major coagulant agent used in drinking water purification and wastewater treatment plants [62], is capable of removing DEBRIS from the NaOH solution (wastewater) with a high efficiency (Fig. 4). In our experimental conditions, a maximum removal of 95% was observed for a saturation limiting point of 10 mg/mL of aluminium sulphate. By the qualitative and quantitative assessments presented in Fig. 4, it was demonstrated that aluminium sulphate is a very efficient coagulant agent for remediation of DEBRIS-based effluents.

We have also evaluated other materials used in wastewater treatments such as polymeric flocculants and hydrotalcites. Both materials evaluated were able to remove DEBRIS compounds from the NaOH solution. These results, as well as the optimization of
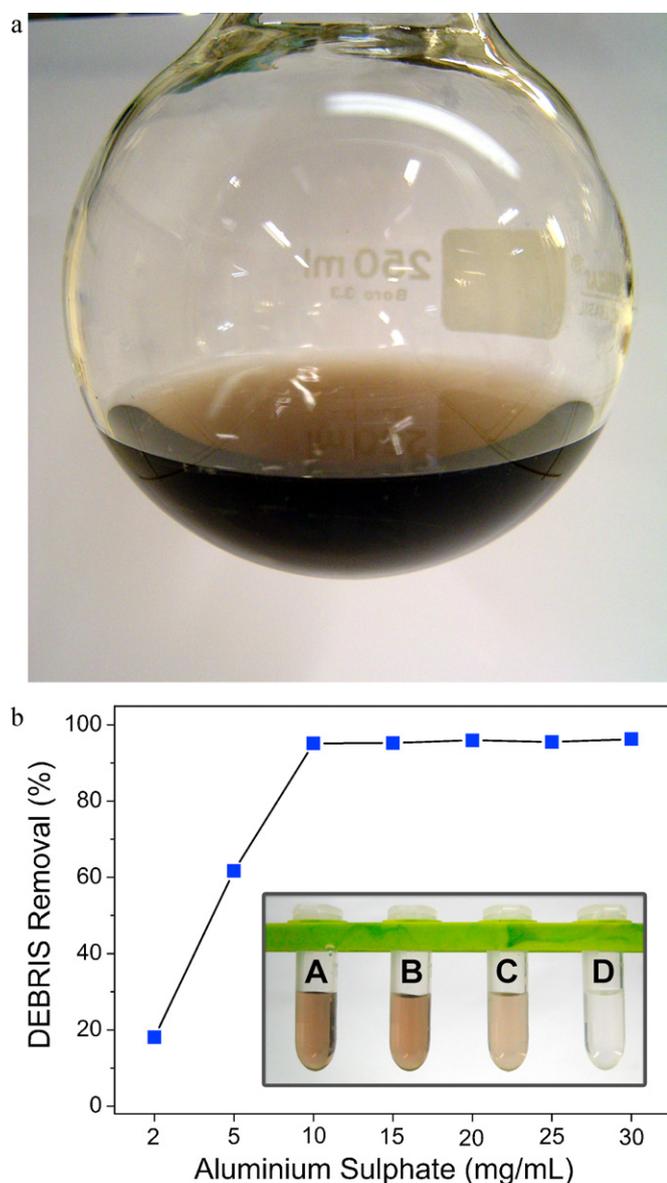
Fig. 4. (a) Picture of DEBRIS fragments in a 0.1 mol/L NaOH solution (wastewater stock solution) originated from HNO₃-treated multiwalled carbon nanotubes and (b) the percentage of the DEBRIS removal from the wastewater stock solution as a function of the aluminium sulphate content measured by the absorbance at 400 nm. Inset: pictures of the (A) DEBRIS wastewater stock solution (control); and resultant solutions after the remediation with (B) 2.0 mg/mL; (C) 5.0 mg/mL; (D) 10 mg/mL of aluminium sulphate, respectively.

the aluminium sulphate remediation process (e.g. coagulant dose, coagulant mixing time and stirring rate) will be published in a forthcoming paper. The Zeta-potential (ζ) analysis has indicated that DEBRIS compounds are negatively charged (-45 mV $\pm$ 5.0 S.D.), which explains their affinity for aluminium sulphate, polymeric flocculants and hydrotalcites, which are cationic materials commonly used in wastewater treatments.

In this work, we have decided to study oxidation debris from multiwalled carbon nanotubes because their industrial mass production is much higher than that of single walled carbon nanotubes. Nevertheless, the approach presented here can be useful to further studies involving oxidation debris generated from other types of carbon nanomaterials, such as ultra-short carbon nanotubes [63], unzipping carbon nanotubes [64] and graphene nanoflakes [65], since all of them are faced as emerging materials.

4. Conclusions

Via infrared spectroscopy (FT-IR) and ultra-high resolution and accuracy mass spectrometry (ESI FT-ICR MS) we have shown that oxidation debris obtained from HNO₃-treated multiwalled carbon nanotubes is a complex mixture of highly condensed aromatic oxygenated carbonaceous fragments with ion mass distribution between 200 and 800 *m/z*. Despite their similarity to fulvic acids, the van Krevelen diagram has demonstrated that these carbonaceous fragments display a molecular profile distinct from fulvic acids originated from natural organic matter. Towards the development of proactive safety approaches, the cytotoxicity evaluation of oxidation debris in mouse fibroblasts and human keratinocytes was done, resulting in the absence of cytotoxic effects. In addition, by knowing the negative aspects of fulvic substances and dissolved organic carbon (DOC) to the water quality, processes for the removal of oxidation debris from aqueous solutions were also studied. Aluminium sulphate, a conventional coagulant agent used in water purification and wastewater treatment, was highly efficient for removing the oxidation debris fragments from the NaOH solution (wastewater). Considering that carbon nanotube mass production is increasing at high rates, the results presented here are important for future discussions regarding surface, toxicology and environmental chemistry aspects of oxidized carbon nanotubes.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.02.050.

References

[1] Z.J. Jia, Z.Y. Wang, J. Liang, B.Q. Wei, D.H. Wu, Production of short multi-walled carbon nanotubes, Carbon 37 (1999) 903–906.
[2] Y.P. Sun, K.F. Fu, Y. Lin, W.J. Huang, Functionalized carbon nanotubes: properties and applications, Acc. Chem. Res. 35 (2002) 1096–1104.
[3] V. Datsyuk, M. Kalyva, K. Papagelis, J. Parthenios, D. Tasis, A. Siokou, I. Kallitsis, C. Galiotis, Chemical oxidation of multiwalled carbon nanotubes, Carbon 46 (2008) 833–840.
[4] J. Hu, C.L. Chen, X.X. Zhu, X.K. Wang, Removal of chromium from aqueous solution by using oxidized multiwalled carbon nanotubes, J. Hazard. Mater. 162 (2009) 1542–1550.
[5] S.T. Yang, J.X. Li, D.D. Shao, J. Hu, X.K. Wang, Adsorption of Ni(II) on oxidized multi-walled carbon nanotubes: effect of contact time, pH, foreign ions and PAA, J. Hazard. Mater. 166 (2009) 109–116.
[6] I.D. Rosca, F. Watari, M. Uo, T. Akaska, Oxidation of multiwalled carbon nanotubes by nitric acid, Carbon 43 (2005) 3124–3131.
[7] M.N. Tchoul, W.T. Ford, G. Lolli, D.E. Resasco, S. Arepalli, Effect of mild nitric acid oxidation on dispersability, size, and structure of single-walled carbon nanotubes, Chem. Mater. 19 (2007) 5765–5772.
[8] C.G. Salzmann, S.A. Llewellyn, G. Tobias, M.A.H. Ward, Y. Huh, M.L.H. Green, The role of carboxylated carbonaceous fragments in the functionalization and spectroscopy of a single-walled carbon-nanotube material, Adv. Mater. 19 (2007) 883–887.
[9] H. Yu, Y.G. Jin, F. Peng, H.J. Wang, J. Yang, Kinetically controlled side-wall functionalization of carbon nanotubes by nitric acid oxidation, J. Phys. Chem. C 112 (2008) 6758–6763.

[10] R. Verdejo, S. Lamoriniere, B. Cottam, A. Bismarck, M. Shaffer, Removal of oxidation debris from multi-walled carbon nanotubes, Chem. Commun. (2007) 513–515.

[11] S. Fogden, R. Verdejo, B. Cottam, M. Shaffer, Purification of single walled carbon nanotubes: the problem with oxidation debris, Chem. Phys. Lett. 460 (2008) 162–167.

[12] Z.W. Wang, M.D. Shirley, S.T. Meikle, R.L.D. Whitby, S.V. Mikhalovsky, The surface acidity of acid oxidised multi-walled carbon nanotubes and the influence of in-situ generated fulvic acids on their stability in aqueous dispersions, Carbon 47 (2009) 73–79.

[13] E. Heister, C. Lamprecht, V. Neves, C. Tilmaciu, L. Datas, E. Flahaut, B. Soula, P. Hinterdorfer, H.M. Coley, S.R.P. Silva, J. McFadden, Higher dispersion efficacy of functionalized carbon nanotubes in chemical and biological environments, ACS Nano 4 (2010) 2615–2626.

[14] J.F. Fu, Y.Q. Zhao, Q.L. Wu, Optimising photoelectrocatalytic oxidation of fulvic acid using response surface methodology, J. Hazard. Mater. 144 (2007) 499–505.

[15] S.W. Krasner, P. Westerhoff, B.Y. Chen, B.E. Rittmann, S.N. Nam, G. Amy, Impact of wastewater treatment processes on organic carbon, organic nitrogen, and dbp precursors in effluent organic matter, Environ. Sci. Technol. 43 (2009) 2911–2918.

[16] K. Yang, B.S. Xing, Adsorption of fulvic acid by carbon nanotubes from water, Environ. Pollut. 157 (2009) 1095–1100.

[17] J.R. Dominguez, T. Gonzalez, H.M. Garcia, F. Sanchez-Lavado, J. de Heredia, Aluminium sulfate as coagulant for highly polluted cork processing wastewaters: Removal of organic matter, J. Hazard. Mater. 148 (2007) 15–21.

[18] T. Mosmann, Rapid colorimetric assay for cellular growth and survival – application to proliferation and cyto-toxicity assays, J. Immunol. Methods 65 (1983) 55–63.

[19] K.A. Worsley, I. Kalinina, E. Bekyarova, R.C. Haddon, Functionalization and dissolution of nitric acid treated single-walled carbon nanotubes, J. Am. Chem. Soc. 131 (2009) 18153–18158.

[20] J.P. Tessonnier, D. Rosenthal, F. Girgsdies, J. Amadou, D. Begin, C. Pham-Huu, D.S. Su, R. Schlogl, Influence of the graphitisation of hollow carbon nanofibers on their functionalisation and subsequent filling with metal nanoparticles, Chem. Commun. (2009) 7158–7160.

[21] L. Minati, G. Speranza, I. Bernagozzi, S. Torrengo, L. Toniutti, B. Rossi, M. Ferrari, A. Chiasera, Investigation on the electronic and optical properties of short oxidized multiwalled carbon nanotubes, J. Phys. Chem. C 114 (2010) 11068–11073.

[22] Z. Wang, A. Korobeinyk, R.L.D. Whitby, S.T. Meikle, S.V. Mikhalovsky, S.F.A. Acquah, H.W. Kroto, Direct confirmation that carbon nanotubes still react covalently after removal of acid-oxidative lattice fragments, Carbon 48 (2010) 916–918.

[23] R.R.N. Marques, B.F. Machado, J.L. Faria, A.M.T. Silva, Controlled generation of oxygen functionalities on the surface of single-walled carbon nanotubes by HNO_3 hydrothermal oxidation, Carbon 48 (2010) 1515–1523.

[24] S. Kundu, Y.M. Wang, W. Xia, M. Muhler, Thermal stability and reducibility of oxygen-containing functional groups on multiwalled carbon nanotube surfaces: a quantitative high-resolution XPS and TPD/TPR study, J. Phys. Chem. C 112 (2008) 16869–16878.

[25] N. Hertkorn, M. Frommberger, M. Witt, B.P. Koch, P. Schmitt-Kopplin, E.M. Perdue, Natural organic matter and the event horizon of mass spectrometry, Anal. Chem. 80 (2008) 8908–8919.

[26] Y.E. Corilo, B.G. Vaz, R.C. Simas, H.D.L. Nascimento, C.F. Klitzke, R.C.L. Pereira, W.L. Bastos, E.V.S. Neto, R.P. Rodgers, M.N. Eberlin, Petroleomics by EASI(+/−) FT–ICR MS, Anal. Chem. 82 (2010) 3990–3996.

[27] D.W. Van Krevelen, Graphical-statistical method for the study of structure and reaction process of coal, Fuel 29 (1950) 269–284.

[28] S. Kim, R.W. Kramer, P.G. Hatcher, Graphical method for analysis of ultrahigh-resolution broadband mass spectra of natural organic matter, the van Krevelen diagram, Anal. Chem. 75 (2003) 5336–5344.

[29] S.A. Visser, Application of Van Krevelens graphical statistical-method for the study of aquatic humic material, Environ. Sci. Technol. 17 (1983) 412–417.

[30] R.W. Kramer, E.B. Kujawinski, P.G. Hatcher, Identification of black carbon derived structures in a volcanic ash soil humic acid by Fourier transform ion cyclotron resonance mass spectrometry, Environ. Sci. Technol. 38 (2004) 3387–3395.

[31] R.L. Sleighter, P.G. Hatcher, The application of electrospray ionization coupled to ultrahigh resolution mass spectrometry for the molecular characterization of natural organic matter, J. Mass Spectrom. 42 (2007) 559–574.

[32] R. Khanna, M. Witt, M.K. Anwer, S.P. Agarwal, B.P. Koch, Spectroscopic characterization of fulvic acids extracted from the rock exudate Shilajit, Org. Geochem. 39 (2008) 1719–1724.

[33] B.P. Koch, T. Dittmar, From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter, Rapid Commun. Mass Spectrom. 20 (2006) 926–932.

[34] A.M. Thayer, Carbon nanotubes by the metric ton, Chem. Eng. News 85 (2007) 29–30.

[35] D. Hwang, J. Bradley, MWNTs: Leaders move to dominate market with massive scale-up (Online), in ELECTROIQ (2010).

[36] M.A. Albrecht, C.W. Evans, C.L. Raston, Green chemistry and the health implications of nanoparticles, Green Chem. 8 (2006) 417–432.

[37] S.K. Brar, M. Verma, R.D. Tyagi, R.Y. Surampalli, Engineered nanoparticles in wastewater and wastewater sludge—evidence and impacts, Waste Manage. 30 (2010) 504–520.

[38] L.A. Luongo, X.Q. Zhang, Toxicity of carbon nanotubes to the activated sludge process, J. Hazard. Mater. 178 (2010) 356–362.

[39] S. Kang, M.S. Mauter, M. Elimelech, Microbial cytotoxicity of carbon-based nanomaterials: implications for river water and wastewater effluent, Environ. Sci. Technol. 43 (2009) 2648–2653.

[40] K. Kostarelos, A. Bianco, M. Prato, Promises, facts and challenges for carbon nanotubes in imaging and therapeutics, Nat. Nanotechnol. 4 (2009) 627–633.

[41] Z. Liu, S. Tabakman, K. Welsher, H.J. Dai, Carbon nanotubes in biology and medicine: in vitro and in vivo detection, imaging and drug delivery, Nano Res. 2 (2009) 85–120.

[42] S. Perez, M. Farre, D. Barcelo, Analysis, behavior and ecotoxicity of carbon-based nanomaterials in the aquatic environment, TrAc, Trends Anal. Chem. 28 (2009) 820–832.

[43] G.D. Sheng, D.D. Shao, X.M. Ren, X.Q. Wang, J.X. Li, Y.X. Chen, X.K. Wang, Kinetics and thermodynamics of adsorption of ionizable aromatic compounds from aqueous solutions by as-prepared and oxidized multiwalled carbon nanotubes, J. Hazard. Mater. 178 (2010) 505–516.

[44] C.L. Chen, X.K. Wang, M. Nagatsu, Europium adsorption on multiwall carbon nanotube/iron oxide magnetic composite in the presence of polyacrylic acid, Environ. Sci. Technol. 43 (2009) 2362–2367.

[45] S.F. Hansen, A. Maynard, A. Baun, J.A. Tickner, Late lessons from early warnings for nanotechnology, Nat. Nanotechnol. 3 (2008) 444–447.

[46] L.K. Limbach, R. Bereiter, E. Mueller, R. Krebs, R. Gaelli, W.J. Stark, Removal of oxide nanoparticles in a model wastewater treatment plant: influence of agglomeration and surfactants on clearing efficiency, Environ. Sci. Technol. 42 (2008) 5828–5833.

[47] A.L. Chun, Carbon nanotubes: safe production? Nat. Nanotechnol. (2009).

[48] D.L. Plata, A.J. Hart, C.M. Reddy, P.M. Gschwend, Early evaluation of potential environmental impacts of carbon nanotube synthesis by chemical vapor deposition, Environ. Sci. Technol. 43 (2009) 8367–8373.

[49] T. Bernas, J. Dobrucki, Mitochondrial and nonmitochondrial reduction of MTT: interaction of MTT with TMRE, JC-1, and NAO mitochondrial fluorescent probes, Cytometry 47 (2002) 236–242.

[50] A.E. Nel, L. Madler, D. Velegol, T. Xia, E.M.V. Hoek, P. Somasundaran, F. Klaessig, V. Castranova, M. Thompson, Understanding biophysicochemical interactions at the nano-bio interface, Nat. Mater. 8 (2009) 543–557.

[51] D. Walczyk, F.B. Bombelli, M.P. Monopoli, I. Lynch, K.A. Dawson, What the cell "sees" in bionanoscience, J. Am. Chem. Soc. 132 (2010) 5761–5768.

[52] J.M. Worle-Knirsch, K. Pulskamp, H.F. Krug, Oops they did it again! Carbon nanotubes hoax scientists in viability assays, Nano Lett. 6 (2006) 1261–1268.

[53] N. Grobert, Carbon nanotubes – becoming clean, Mater. Today 10 (2006) 28–35.

[54] G. Tobias, L.D. Shao, B. Ballesteros, M.L.H. Green, Enhanced sidewall functionalization of single-wall carbon nanotubes using nitric acid, J. Nanosci. Nanotechnol. 9 (2009) 6072–6077.

[55] M. Prato, Materials chemistry: controlled nanotube reactions, Nature 465 (2010) 172–173.

[56] L. Liang, P.C. Singer, Factors influencing the formation and relative distribution of haloacetic acids and trihalomethanes in drinking water, Environ. Sci. Technol. 37 (2003) 2920–2928.

[57] J. Yang, J.P. Jia, J. Liao, Y.L. Wang, Removal of fulvic acid from water electrochemically using active carbon fiber electrode, Water Res. 38 (2004) 4353–4360.

[58] S.W.C. Chien, M.C. Wang, C.C. Huang, Reactions of compost-derived humic substances with lead, copper, cadmium, and zinc, Chemosphere 64 (2006) 1353–1361.

[59] J.C.G.E. da Silva, M.C.P.O. Marques, Pentachlorophenol association with fulvic acids from recycled wastes, Environ. Pollut. 146 (2007) 174–179.

[60] G.D. Sheng, J.X. Li, D.D. Shao, J. Hu, C.L. Chen, Y.X. Chen, X.K. Wang, Adsorption of copper(II) on multiwalled carbon nanotubes in the absence and presence of humic or fulvic acids, J. Hazard. Mater. 178 (2010) 333–340.

[61] J.E. Hutchison, Greener nanoscience: a proactive approach to advancing applications and reducing implications of nanotechnology, ACS Nano 2 (2008) 395–402.

[62] C.P. Huang, H.L. Shiu, Interactions between alum and organics in coagulation, Colloid Surf. A 113 (1996) 155–163.

[63] B.K. Price, J.R. Lomeda, J.M. Tour, Aggressively oxidized ultra-short single-walled carbon nanotubes having oxidized sidewalls, Chem. Mater. 21 (2009) 3917–3923.

[64] D.V. Kosynkin, A.L. Higginbotham, A. Sinitskii, J.R. Lomeda, A. Dimiev, B.K. Price, J.M. Tour, Longitudinal unzipping of carbon nanotubes to form graphene nanoribbons, Nature 458 (2009) 872–875.

[65] C.G. Salzmann, V. Nicolosi, M.L.H. Green, Edge-carboxylated graphene nanoflakes from nitric acid oxidised arc-discharge material, J. Mater. Chem. 20 (2010) 314–319.