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A comparative study on the enzymatic biodegradability of covalently functionalized double- and multi-walled carbon nanotubes

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A B S T R A C T

The assessment of the biodegradability potential of carbon nanotubes (CNTs) is a fundamental point towards their applications in materials science and biomedicine. Due to the continuous concerns about the fate of such type of nanomaterials, it is very important to understand if they can undergo degradation under certain conditions and if the morphology and structure of the nanotubes play a role in this process. For this purpose we have decided to undertake a comparative study on the enzymatic degradation of CNTs with concentric multilayers. Double-walled (DW) and multi-walled (MW) CNTs of various lengths, degrees of oxidation and functionalizations using different methods were treated with horseradish peroxidase (HRP). While all tested DWCNTs resulted resistant to the biodegradation, some of the MWCNTs were partially degraded by the enzyme. We have found that short oxidized multi-walled CNTs functionalized by amidation were reduced in length and presented a high amount of defects at the end of the period of treatment with HRP. This comparative study holds its importance in the understanding of the structural changes of different types of nanotubes towards the catalytic enzymatic degradation and will help to design safer CNTs for future applications.

1. Introduction

Due to their unique physicochemical, optical, and mechanical properties, carbon nanotubes (CNTs) are promising nanomaterials for a wide range of applications both in materials science [1] and biomedicine [2–4]. In particular, since CNTs present a high surface area, the chemistry of functionalized CNTs (*f*-CNTs) has been extensively investigated through the derivatization of their external tips and sidewalls [5]. Today, the increasing production of CNTs, together with their incorporation into new types of

composites and/or into biological systems have raised fundamental issues on their impact on human health and environment [6–8]. However, studies on the biopersistence and the fate of the CNTs, once released into the natural environments, are still limited. Particularly, the biodegradation of CNTs could help to avoid possible risk of contamination, pollution and damages to the living systems. Generally, the fate of CNTs in the environment may depend on specific properties such as functionalization, length and aggregation state [9].

Recently, few groups have reported the degradation of CNTs catalyzed by naturally occurring enzymes from plants and humans or by action of microorganisms [10–22]. Most of the degradation studies reported concern oxidized CNTs (*ox*-CNTs). In fact, the process of oxidation is often used to shorten pristine CNTs and to improve their dispersibility, by introducing carboxylic functions at the tips and the defect sites. Recent studies have shown the

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capacity of carboxylated single-walled CNTs (SWCNTs) to be abiotically degraded, within 10 days, by horseradish peroxidase (HRP) in the presence of low concentration of hydrogen peroxide [17,20]. The defect sites, introduced by the oxidation, clearly enhance the biodegradation rate, under the enzymatic oxidative conditions. Carboxylated SWCNTs can be also degraded inside the cells such as neutrophils and macrophages by the action of myeloperoxidase [16]. Other enzymes of potential interest for CNT biodegradation include ligninolytic heme peroxidases, such as manganese peroxidase (MnP) [21] and lactoperoxidase (LiP) [22]. These enzymes have shown oxidizing capacity against pristine and carboxylated SWCNTs.

While most of the degradation studies in the literature focus on SWCNTs, few studies concerned the possible degradation of CNTs with multiple concentric layers [i.e. multi-walled (MW) and double-walled (DW) CNTs]. Due to their more complex structure, these types of tubes are expected to be less sensitive to enzymatic degradation. Cellular degradation of DWCNTs was observed by using Raman spectroscopy to monitor changes in the carbon lattice [23]. Defects seemed to accumulate on the external graphene sheet of the nanotubes after internalization by human prostate adenocarcinoma cells. Moreover, carboxylated MWCNTs exposed to HRP/H₂O₂ treatment, over 60 days, were observed to undergo a degradation process, leading to shortened nanotubes and carbonaceous fragments [13]. The concurrent reduction in diameter of the tubes could be explained by exfoliation of defect-containing outer carboxylated walls, which were unzipped *via* the enzymatic oxidation, yielding bare CNTs with smaller diameters and possessing defectless surfaces. The exposure of the novel layers coincided with a reduction in the rate of degradation, highlighting that the number of walls is a crucial parameter that affects the biodegradability of CNTs. Independently, we also demonstrated that carboxylated MWCNTs underwent morphological changes (i.e. shortening and deformation) when incubated with HRP and H₂O₂ [14]. Contrary to the previous report, the results suggested that the biodegradation reduce the length of the tubes, creating a higher amount of defects on their walls, rather than exfoliating their external surface. In addition, Zhang et al. demonstrated the ability of some bacteria to degrade acid-treated MWCNTs under environmentally relevant conditions, leading to a decrease of their environmental persistence [18]. Very recently, we have demonstrated that specific functional molecules linked to CNTs can enhance the catalytic activity of HRP towards the degradation of multi-walled CNTs [24]. Coumarin derivatives and catechol were covalently conjugated to oxidized MWCNTs (*ox*-MWCNTs). These molecules are efficient reducing substrates for HRP. In addition, catechol is also a strong redox mediator of HRP as it favors electron transfer and prevents the enzyme inactivation. Other studies have evidenced the persistence and degradation of CNTs *in vitro* and *in vivo*. Sato et al. have shown the long-term biopersistence of tangled *ox*-MWCNTs *in vivo*, demonstrating their potential use as biological implantable materials [25]. In that study, *ox*-MWCNTs were implanted into rat subcutaneous tissues. Two years after the implantation, high resolution transmission electron microscopy (HRTEM) and Raman spectroscopy were used to assess if the nanotubes did undergo structural changes. Large agglomerates of *ox*-MWCNTs were present in the intercellular space and were not degraded. On the contrary, small agglomerates were engulfed by macrophages, where they were gradually degraded in lysosomes. No early stages of severe changes, such as necrosis and carcinogenesis, were observed in the tissues. More recently, another work also reported superior *in vivo* biodegradability of MWCNTs functionalized by Diels–Alder reaction and further oxidized to generate COOH groups, in comparison to pristine nanotubes [26]. The group of A. E. Porter has shown the effects of the functionalization and

aggregation state of MWCNTs on internalization and biopersistence in microglia [27]. Dynamic processes of disentanglement of aggregates of *ox*-MWCNTs by microglia were observed by high resolution and dynamic imaging, triggering their internalization. This process of disentanglement did not occur with pristine MWCNTs. Some indications of damages to the structure of *ox*-MWCNTs suggested possible biodegradation.

The results discussed above clearly evidence that the defects or the sites of functionalization are important promoters of enzymatic action. As a consequence, we have chosen to extend the degradation studies to different types of functionalized CNTs with multiple concentric walls, not only bearing simple carboxylic functions. Particularly, chemical derivatization of CNTs is also of fundamental importance in order to reduce the undesired effects, enabling these materials to be used more widely in a safe manner [28]. Indeed, organic functionalization of CNTs is often used to increase their dispersibility in water, control their length and enhance their biocompatibility [5]. Low toxicity of CNTs has been also correlated to the presence of functional groups on the external surface of the tubes [29]. Thus, with the aim to render the nanotubes more biodegradable, MWCNTs and DWCNTs have been submitted to different chemical acid treatments, yielding oxidized tubes with different length and morphology. Moreover, we have introduced amino groups both onto the sidewalls and at the tips of oxidized CNTs using amidation and 1,3-dipolar cycloaddition reactions. Finally, we have investigated and compared the catalytic biodegradation of the different functionalized CNTs by incubation with HRP in test tubes. To enable the enzymatic activity, hydrogen peroxide was added daily (i.e. 6 times/day). The changes in nanotube morphology were observed by transmission electron microscopy (TEM) at different time points up to 20 days. Finally, their structures were further analyzed by Raman spectroscopy.

2. Experimental

Details on the materials, the instruments, the synthesis of DWCNTs and MWCNTs and the functionalized nanotubes, and the procedure for the enzymatic degradations are reported in the Supporting Information.

3. Results and discussion

3.1. Oxidation and organic functionalization of multi- and double-walled CNTs

In this work, we have compared the functionalization degree and the biodegradation properties of oxidized and functionalized CNTs with multiple concentric layers. Initially, different chemical treatments allowed to obtain a library of precursor CNTs differing in (i) length, (ii) number of layers (MW or DW) and (iii) amount of COOH functionalities. Table 1 displays the characteristics of the nanotubes, following the chemical treatments. Pristine MWCNTs exhibited an average length of 500 μm and a mean external diameter of 24 nm [30]. The outer and inner average diameters of

Table 1
Characteristics of the precursor CNTs used for the functionalization reactions.

Entry	Oxidized CNTs	Average length (μm)	O/C atomic ratio (%) (XPS)
1	L-MW 1	5.4 \pm 4.4	0.7
2	S-MW 2	1.2 \pm 0.6	0.7
3	L-MW 3	1.1 \pm 0.7	5.6
4	S-MW 4	1.3 \pm 1.0	4.5
5	DW 5	ND ^a	1.0

^a (ND: non-determined).

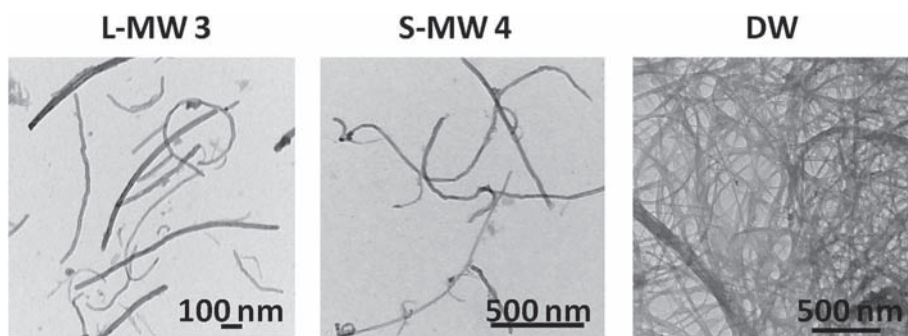


Fig. 1. TEM images of oxidized (long and short) multi (MW)- and double (DW)-walled CNTs.

the MWCNTs were 20–30 nm and 5–10 nm, respectively. We estimated the number of walls (~20–30) based on the sheet interlayer distance (0.34 nm).

With the aim to obtain tubes with two different lengths, pristine MWCNTs were extensively sonicated in isopropyl alcohol (*i*PrOH). Following this treatment, the average length of the long tubes (nominated **L-MW**) was 5.4 μm after 12 h sonication (entry 1). MWCNTs, submitted to longer sonication treatment in *i*PrOH (24 h), and indicated as short MWCNTs (nominated **S-MW**, entry 2) exhibited a mean length of 1.2 μm . Moreover, as biodegradation increased with the introduction of functional groups, we submitted both long and short nanotubes to chemical oxidative treatments, using strong acidic conditions ($\text{H}_2\text{SO}_4/\text{HNO}_3$, 3:4) (entry 3–4). The oxidation procedure allowed also to obtain even shorter CNTs (Fig. 1), mainly endowed of carboxylic groups at their tips and sidewalls (Fig. 2).

X-ray photoelectron spectroscopy (XPS) indicated that the O/C atomic ratio increased from 0.7% to 5.6% and 4.5% after oxidation for

L-MW 3 and **S-MW 4**, respectively (Table 1, Fig. 2 and Figure S1 in Supporting Information). TGA performed under nitrogen showed that the pristine long MWCNTs **L-MW 1** and short MWCNTs **S-MW 2** were stable in inert atmosphere, while a significant weight loss attributed to oxygenated species was observed after oxidation in both cases (Fig. 3). The weight loss of **S-MW 4** was drastically higher than that observed for **L-MW 3**, which is indicative of a higher content of oxygenated species. The O/C atomic ratio for **S-MW 4** assessed by XPS was instead slightly lower compared to the value for **L-MW 3**. The TGA results are indicative of a high oxidation level for the short MWCNTs, which suggests that oxygenated species are not only localized at the nanotube surface, but also in CNT bulk as indicated by HRTEM analysis, showing defects even in the inner CNT walls (see Figure S2). XPS is a surface analysis with limited probing depth (estimated to 10–15 nm in carbon materials), while TGA is a bulk analysis. Therefore, the O/C ratio obtained by XPS is likely underestimated. Oxidized DWCNTs were obtained from pristine nanotubes by treatment using the following conditions: i)

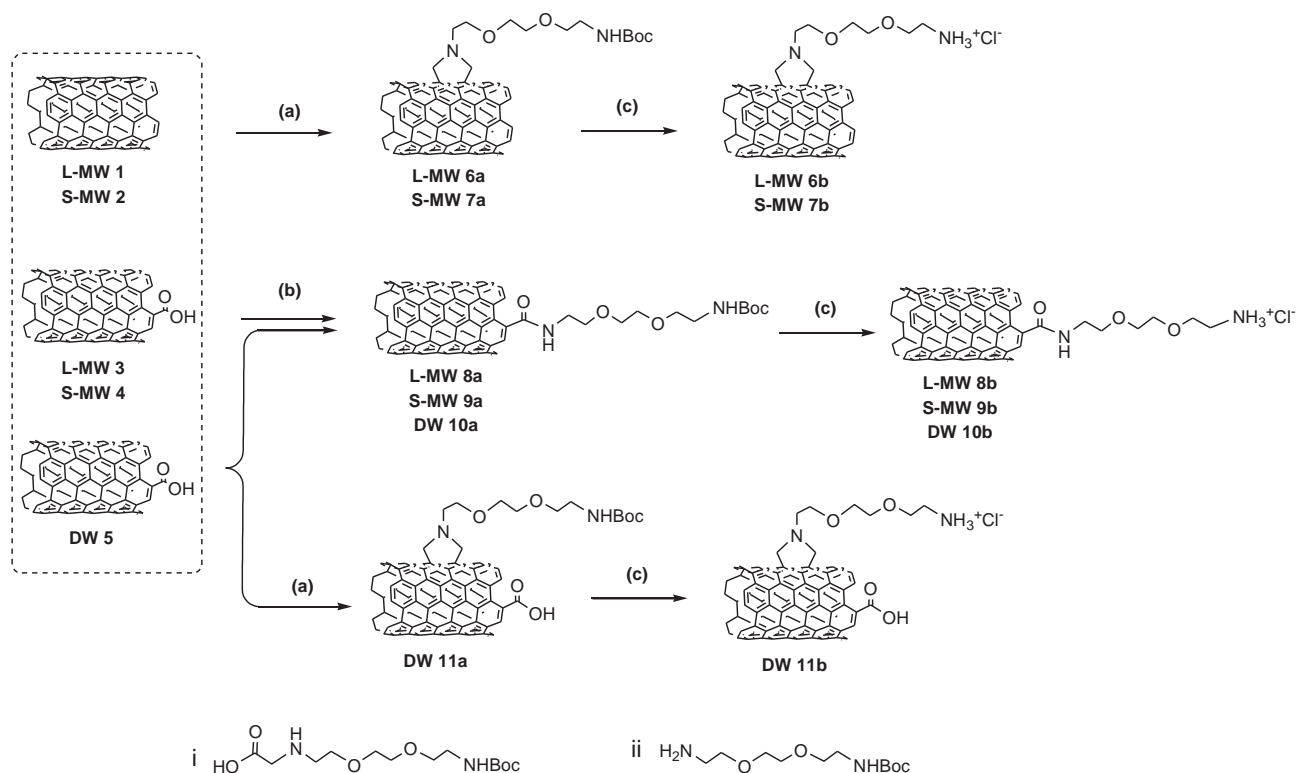


Fig. 2. Synthetic routes for the functionalization of the precursor CNTs. (a) 1,3-Dipolar cycloaddition reaction: i, $(\text{CH}_2\text{O})_n$, DMF, 125 $^\circ\text{C}$, 24 h or 72 h; (b) amidation reaction: 1) SOCl_2 or COCl_2 , reflux, 24 h; 2) ii, THF, reflux, 48 h; (c) deprotection: HCl in 1,4-dioxane, rt, overnight.

HNO₃ (3 M, 130 °C, 24 h), ii) H₂SO₄/HNO₃ (3:1, 70 °C, 5 h), and iii) NaOH washing (4 M) (Table 1, entry 5). As it is evident from TEM images reported in Fig. 1, oxidized DWCNTs show a different behavior respect to oxidized MWCNTs. Since nanotubes with a double wall are more prone to form strong aggregates, their

dispersibility in both organic solvents and water resulted much lower [31]. The CNTs described in Table 1 (entry 1–5) were used as precursor CNTs for further functionalization reactions. Indeed, the oxidative treatments not only contribute to shorten the CNTs, but offers also an anchor point for further covalent derivatization of the nanotubes (Fig. 2).

Initially, L-MW 1, S-MW 2 and DW 5, were functionalized by 1,3-dipolar cycloaddition of azomethine ylides, using a triethylene glycol *N*-modified amino acid with the terminal amine group protected with Boc, to afford L-MW 6a, S-MW 7a and DW 11a, respectively [Fig. 2, route (a)]. In parallel, the carboxylic groups of the oxidized CNTs (i.e. L-MW 3, S-MW 4 and DW 5) were directly converted into the corresponding amides L-MW 8a, S-MW 9a and DW 10a respectively, through condensation with Boc-protected diaminotriethylene glycol [Fig. 2, route (b)]. Boc protecting groups were then cleaved using a solution of HCl in 1,4-dioxane, affording ammonium-functionalized L-MW 6b, S-MW 7b, L-MW 8b, S-MW 9b, DW 10b and DW 11b [Fig. 2, route (c)].

TGA was again used to confirm the successful functionalization of both long and short MWCNTs (Fig. 3). A significant weight loss was observed after functionalization by 1,3-dipolar cycloaddition compared to the pristine long MWCNTs L-MW 1 (Fig. 3, top). The weight loss of the amidated long MWCNTs L-MW 8b was higher compared to the oxidized long MWCNTs L-MW 3, confirming that the COOH groups have been derivatized with the diaminotriethylene glycol chain. In the case of the short MWCNTs, the thermal profile of the nanotubes functionalized by cycloaddition and amidation is rather similar to that of the functionalized long MWCNTs, respectively (Fig. 3, middle).

Surprisingly, the weight loss after amidation was lower in comparison to that of the short oxidized MWCNTs S-MW 4. We tried to hypothesize that this unexpected behavior might be due to the removal of some labile oxygenated groups from the nanotube surface in the conditions used to perform the amidation. Finally, in the case of the DWCNTs, the weight loss is minimal for the pristine DWCNTs, while there is an increased weight loss after oxidation and further functionalization by 1,3-dipolar cycloaddition and amidation (Fig. 3, bottom). The level of functionalization was higher for DWCNTs obtained by amidation reaction (DW 10a) compared to those functionalized by cycloaddition (DW 11a).

After deprotection of the Boc groups, the amount of free amino groups on the different conjugates was assessed using the Kaiser test. The level of functionalization was similar for long and short oxidized MWCNTs functionalized by amidation: 221 (L-MW 8b) and 204 μmol (S-MW 9b) of amino functions per gram of *f*-CNTs, respectively (Table 2). The degree of functionalization was lower for long and short oxidized MWCNTs functionalized by 1,3-dipolar cycloaddition: 14 (L-MW 6b) and 23 μmol/g (S-MW 7b), respectively. In the case of functionalized DWCNTs, we obtained a loading of 82 (DW 11b) and 275 μmol/g (DW 10b) for the cycloaddition and the amidation, respectively.

3.2. Biodegradation of functionalized multi- and double-walled CNTs

All degradation studies have been carried out by incubating the different CNTs (10 μg/mL) with HRP (20 U/μg/mL) in phosphate buffer saline solution. To enable the enzymatic activity, a solution of H₂O₂ was added daily every 2 h for a total of 6 times per day. The solutions were kept in the dark and stirred at 25 °C for the entire duration of the experiment (20 days). In comparison to our previous procedure [14], this protocol resulted in an acceleration of the degradation process of CNTs. Aliquots were taken at time 0, 10 and 20 days, and stored at -20 °C in the dark until characterization by TEM and Raman spectroscopy. The latter technique is generally

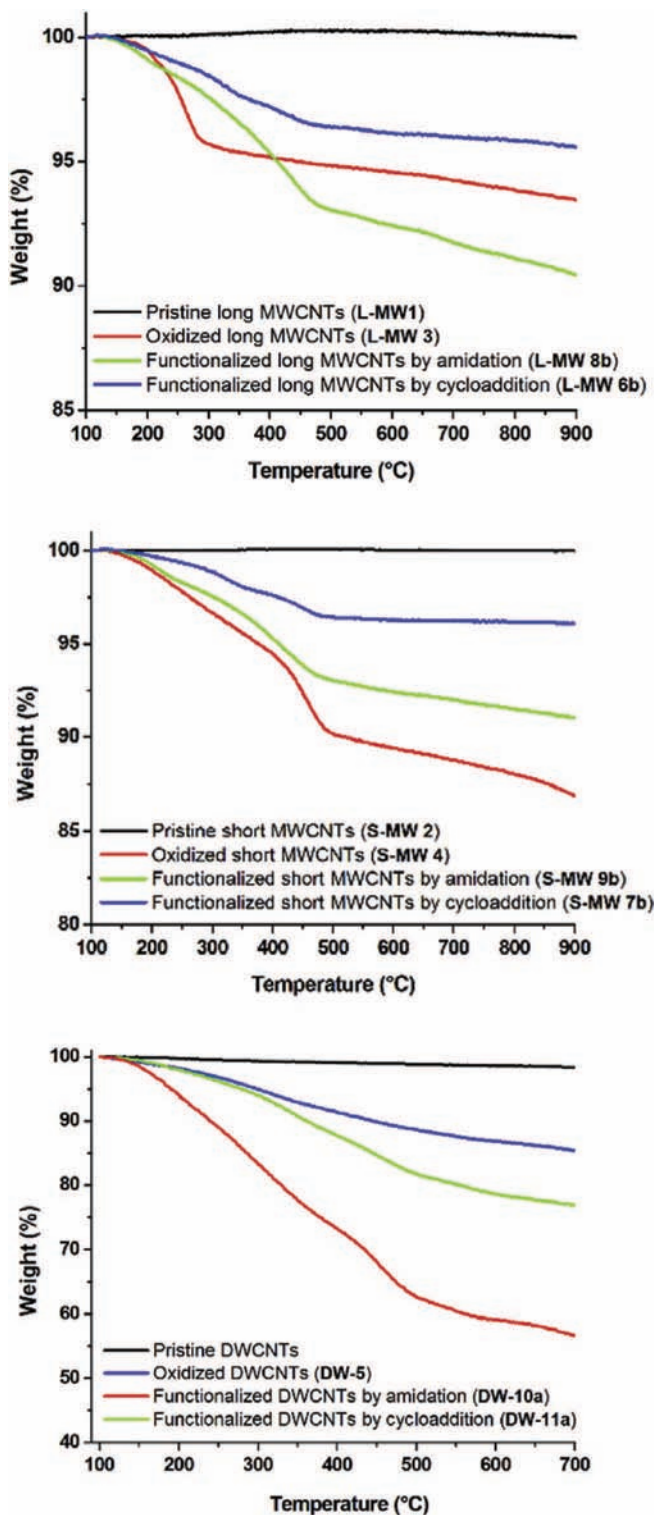


Fig. 3. Top: TGA of the pristine (L-MW 1), oxidized (L-MW 3), and long *f*-MWCNTs (L-MW 6b and L-MW 8b); Middle: TGA of the pristine (S-MW 2), oxidized (S-MW 4), and short *f*-MWCNTs (S-MW 7b and S-MW 9b); Bottom: TGA of the pristine, oxidized (DW 5), and Boc-protected *f*-DWCNTs (DW 10a and DW 11a). (A color version of this figure can be viewed online.)

Table 2
Changes of the structural parameters to monitor oxidized and *f*-CNT biodegradation.

Entry	CNT	Functionalization	Amount of functional groups ^a (μmol/g)	I_D/I_G^b		Average length ^c (nm)	
				(d0)	(d20)	(d0)	(d20)
1	L-MW 3	COOH	–	0.25	0.22	1200	660
2	L-MW 6b	cycloaddition	14	0.23	0.25	2830	1310
3	L-MW 8b	COOH/amidation	221	0.38	0.42	2020	1450
4	S-MW 4	COOH	–	0.52	0.46	1350	1230
5	S-MW 7b	cycloaddition	23	0.23	0.28	990	930
6	S-MW 9b	COOH/amidation	204	0.39	0.64	1380	900
7	DW 5	COOH	–	0.05	0.06	ND	ND
8	DW 11b	COOH/cycloaddition	82	0.05	0.08	ND	ND
9	DW 10b	COOH/amidation	275	0.05	0.05	ND	ND

ND: not determined.

^a Calculated by Kaiser test.

^b Calculated by Raman spectroscopy (I_D : D band intensity; I_G : G band intensity) for day 0 (d0) and 20 days (d20) after the treatment with HRP.

^c Measured by TEM.

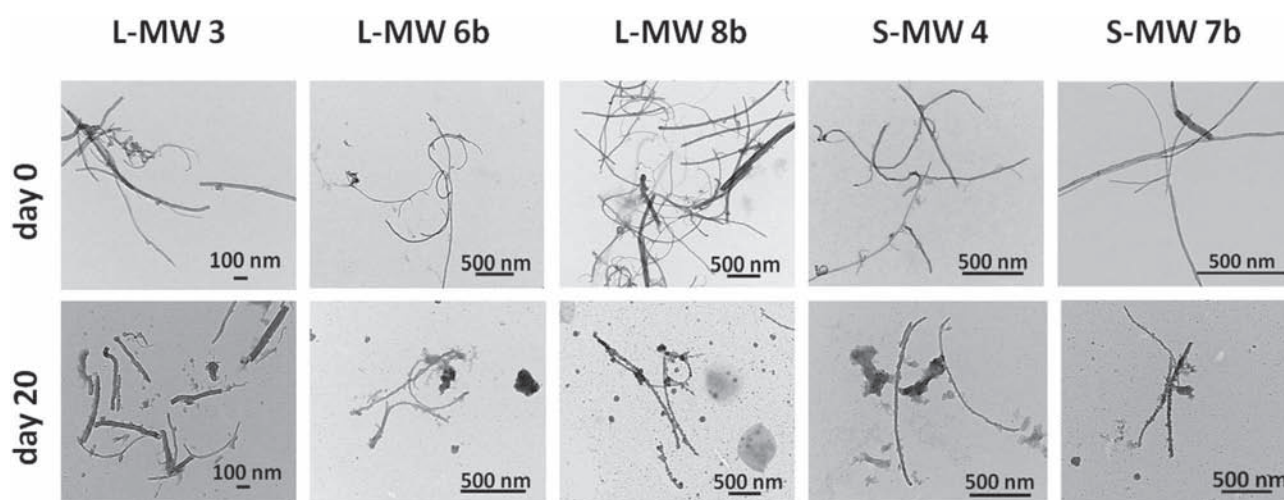


Fig. 4. TEM images of **L-MW 3**, **L-MW 6b**, **L-MW 8b**, **S-MW 4** and **S-MW 7b** (from the left) during the degradation process. The samples were observed after 20 days and compared to the starting material (day 0).

useful for the characterization of CNTs to evidence the variation of the intensity of the D band (frequency at $\sim 1350 \text{ cm}^{-1}$) to the G band (frequency at $\sim 1590 \text{ cm}^{-1}$), and therefore the defect density on the nanotubes. More precisely, the D band is associated to the functional groups and defects generated by both the initial acidic treatment and the functionalization. Hence, the progressive variation of this band can suggest that the tubes are undergoing morphological modifications, as a result of the biodegrading action [14]. In particular, in the case of MWCNTs, although they already exhibit an intense D band as compared to DWCNTs, the biodegradation process in the presence of hydrogen peroxide creates more defects on the nanotubes, giving rise to an increase of this characteristic band. We have reported Raman spectra normalized to the G band because absolute values should be normalized on the volume of homogenous samples, which are very difficult to obtain. Therefore, the Raman spectra allow the monitoring of the qualitative evolution of defects on the nanotubes up to complete disappearance of the signal if total CNT degradation is achieved (see Figures in Supporting Information) [14,17]. In Table 2 we have summarized the I_D/I_G values for the CNTs (entry 1–9), obtained from the Raman spectra of the samples before (d0) and 20 days (d20) after treatment with HRP. Moreover, TEM images collected before (d0) and at the end (d20) of the degradation experiment allowed to monitor the morphological and length variations of the CNTs. Table 2 shows a clear dependence of the CNT biodegradation

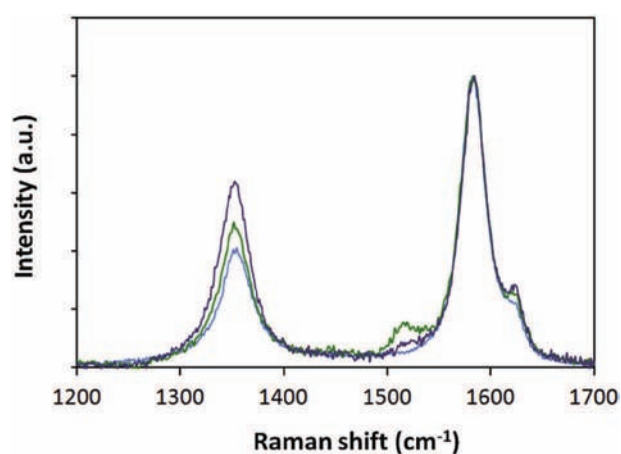


Fig. 5. Raman spectra of short *ox*-MWCNTs functionalized by amidation (**S-MW 9b**) during the degradation process. The sample was observed after 10 (green) and 20 days (violet). Blue line corresponds to the spectrum of the starting material. All spectra have been normalized on the G band. (A color version of this figure can be viewed online.)

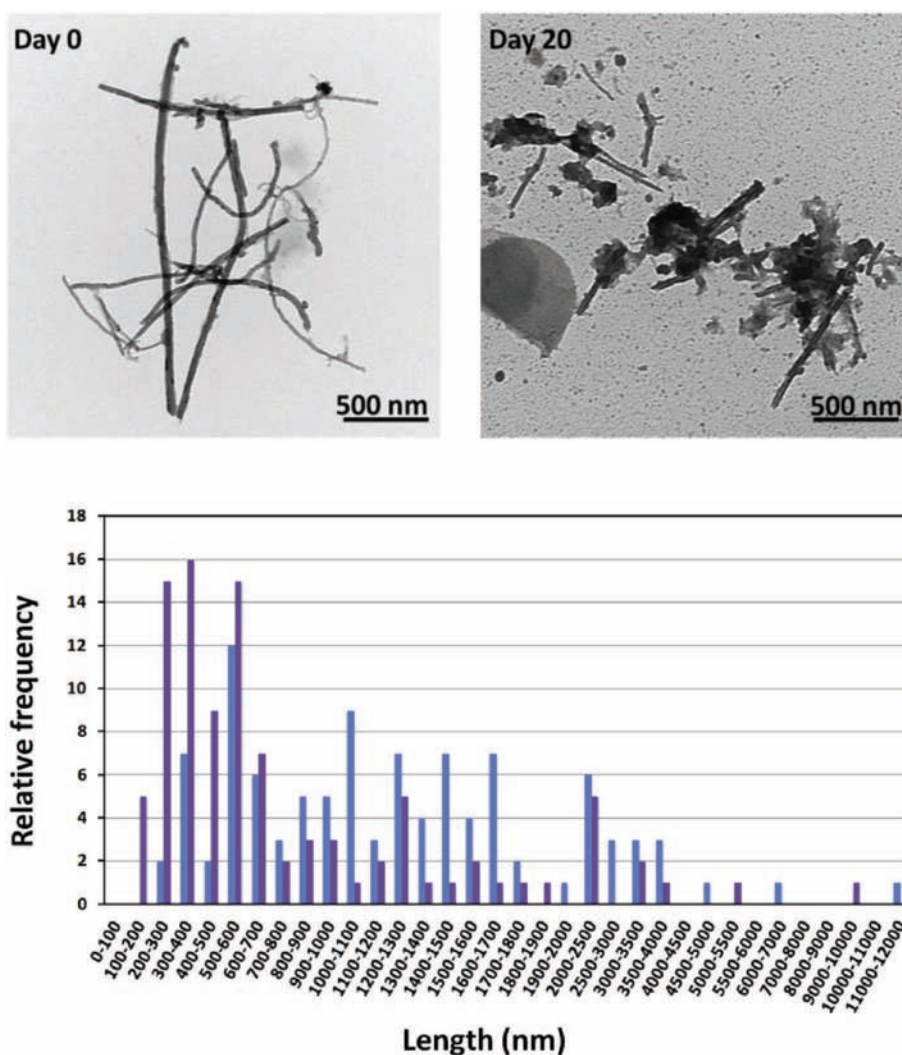


Fig. 6. Top: TEM images of short *ox*-MWCNTs functionalized by amidation (**S-MW 9b**) during the degradation process. The sample was observed before (day 0) and at the end of the degradation process (day 20). Bottom: statistical distribution of the length of the short *ox*-MWCNTs functionalized by amidation (**S-MW 9b**) before (blue bars) and at the end of the HRP treatment (violet bars). (A color version of this figure can be viewed online.)

on the type of functionalization. Indeed, functionalized MWCNTs showed a low variation in the D band intensity (Table 2, entry 1–5) under HRP treatment, suggesting a scarce ability of this type of *f*-CNTs to undergo degradation (*vide infra*). Nevertheless, in the case of long *f*-MWCNTs (entry 1–3), although Raman analysis did not display any significant structural change, TEM analysis revealed a clear reduction of the length of the tubes, as a result of the action of the enzymatic process (see also Figures in Supporting Information). These data suggest that the HRP can induce the cleavage of long *f*-CNTs into shorter tubes starting from the defect sites of the external layer created by the functionalization [19]. Fig. 4 shows the TEM images of functionalized long and short MWCNTs (entry 1–5) collected during the enzymatic process.

On the contrary, a significant relative increase of the D band intensity, already after 10 days of enzymatic treatment, was observed for short MWCNTs functionalized by amidation (**S-MW 9b**, entry 6). In this specific case, the variation of the D band is a clear evidence that the tubes are undergoing structural modifications, *via* a biodegradation process (Fig. 5).

The degradation of these CNTs is also evident from the TEM images, collected at the end of the degradation and compared to day zero. Particularly, in the TEM images of **S-MW 9b**, we observed

many small fragments and carbonaceous debris of nanotubes that are indicative of an efficient oxidative enzymatic action (Fig. 6, top). The length of the nanotubes seems also significantly reduced. Fig. 6 (bottom) displays the statistical length distribution measured by TEM of short *ox*-MWCNTs functionalized by amidation before and after treatment with HRP. **S-MW 9b** became much shorter by action of HRP: the average length decreased from 1380 nm to 900 nm, with 36% of the tubes shorter than 400 nm (Table 2, entry 6 and Fig. 6, bottom). The comparison between the TEM images and the Raman analysis suggests that the biodegrading action in the presence of hydrogen peroxide creates more defects on the nanotubes rather than exfoliating their external surface [14].

S-MW 9b, being short nanotubes functionalized by amidation, if compared to the other *f*-CNTs, present the best characteristics for undergoing an efficient biodegradation. First, the higher dispersibility of short *f*-CNTs (**S-MW 7a/b** and **S-MW 9a/b**), in comparison with long *f*-CNTs (**L-MW 6a/b** and **L-MW 8a/b**), can facilitate the interaction between the tubes and the enzyme in solution. Moreover, as confirmed by TGA (Fig. 3) and Kaiser test (Table 2), *f*-CNTs obtained by amidation reaction (**L-MW 8a/b** and **S-MW 9a/b**) display a higher degree of functionalization compared with those functionalized by cycloaddition reaction (**L-MW 6a/b**

and **S-MW 7a/b**). A higher amount of functional groups is also correlated to the presence of more defects on the graphitic surface. This was confirmed by Raman spectroscopy: **L-MW 8a/b** and **S-MW 9a/b** display an I_D/I_G value in the range of 0.38–0.39 (Table 2, entry 3 and 6), while the I_D/I_G value for both **L-MW 6a/b** and **S-MW 7a/b** corresponds to 0.23 (Table 2, entry 2 and 5). As a consequence, in the case of **S-MW 9b**, a good dispersibility together with a significant presence of functional and defect sites, probably offer the best conditions to induce a favorable interaction of the CNTs with the oxidative agents responsible for the tube degradation. The design of biodegradable nanotubes, through their length reduction and covalent organic derivatization, is of fundamental importance in view of the development of this type of materials for nanotechnology and biomedical applications.

In the case of functionalized DWCNTs, Raman analysis showed that this type of CNTs is more resistant to the degradation action of HRP (Table 2, entry 7–9). Indeed, in all cases, no significant increase in the D band intensity was observed after the treatment (see also Figures in Supporting Information). Since these tubes failed to degrade when incubated with HRP and H_2O_2 , we conclude that defects and functionalized sites are not enough to promote any enzymatic action. Indeed, the Raman spectra of the *f*-DWCNTs already exhibit a really low intensity of the D band. Moreover, even after functionalization, the DWCNTs are prone to form strong aggregates that can hamper the accessibility of the enzyme active site to the defects of DWCNTs and slow down or prevent the degradation reaction. The presence of such aggregates made also difficult the analysis of the morphology of the nanotubes (i.e. possible shortening of the length), by means of TEM during the enzymatic treatment (see also Figures in Supporting Information). We expect that DWCNTs with a higher level of functionalization resulting in a higher dispersibility should be more degraded due to enhanced interactions with the enzyme. A similar behavior was recently evidenced on the degradation of graphene oxide [32]. Indeed, while the oxidative enzymes were able to completely degrade highly dispersed samples, it was not the case of a sample that was highly aggregated. Overall, these results provide further evidence that the presence of defects and/or functional groups, and the dispersibility of the material are key parameters that affect the biodegradability of CNTs.

4. Conclusion

The results reported in this work show the importance of the physico-chemical characteristics of the nanotubes, including the extent of existing structural defects on the carbon backbone, the dispersibility, and the role of chemical functionalization on the capacity of oxidative enzymes to degrade CNTs. We have demonstrated that the presence of functional groups on the CNTs can favor the biodegradation of MWCNTs, while DWCNTs are rather resistant to the enzymatic action, probably because of low dispersibility resulting in less interactions with the enzyme. The demonstration of biodegradability of *f*-CNTs has important implications in the biological impact of these materials, particularly related to any industrial and future biomedical applications. Further studies will be needed to address the possible risks associated with the degradation products of nanotubes rather than the intact nanomaterials.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.carbon.2016.01.023>.

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