Oxidative enzymatic response of white-rot fungi to single-walled carbon nanotubes

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Although carbon nanomaterials such as single-walled carbon nanotubes (SWCNT) are becoming increasingly prevalent in manufacturing, there is little knowledge on the environmental fate of these materials. Environmental degradation of SWCNT is hindered by their highly condensed aromatic structure as well as the size and aspect ratio, which prevents intracellular degradation and limits microbial decomposition to extracellular processes such as those catalyzed by oxidative enzymes. This study investigates the peroxidase and laccase enzymatic response of the saprotrophic white-rot fungi Trametes versicolor and Phlebia tremellosa when exposed to SWCNTs of different purity and surface chemistry under different growth conditions. Both unpurified, metal catalyst-rich SWCNT and purified, carboxylated SWCNTs promoted significant changes in the oxidative enzyme activity of the fungi while pristine SWCNT did not. These results suggest that functionalization of purified SWCNT is essential to upregulate enzymes that may be capable of decomposing CNT in the environment.

1. Introduction

Single-walled carbon nanotubes (SWCNTs), formed from single-atom thick sheets of carbon wound into nanometer scale tubes (Iijima, 1991), display a number of remarkable properties ranging from superior tensile strength, thermal and electrical conductivity, and relative ease of chemical modification (Georgakilas et al., 2002; Collins et al., 1997). These properties make CNTs promising components in next-generation thermopolymers, electronics, and drug delivery systems (Bianco et al., 2005; Trojanowicz, 2006). As a result of their wide range of uses and the rapidly advancing production methods, carbon nanotubes are increasingly prevalent in manufactured products. Despite the increase in CNT production, very little is known about the eventual fate of CNTs once introduced into the environment through accidental release, dispersal in landfills, and as a part of biosolid waste for land application (Wiesner et al., 2009; Turco et al., 2011; Holden et al., 2013).

As with the vast majority of industrial products, the environmental fate of CNTs is partially dependent on degradation by microorganisms found in soils, sediments, and landfills (Klaine et al., 2008). Commercially produced CNTs have lengths on par, or much larger than, many biological cells with aspect ratios of up to 1,000,000 to 1 making intracellular degradation unlikely. Instead, the most likely mechanism by which environmental CNTs are microbially transformed and degraded are via extracellular redox processes, such as those catalyzed by oxidative enzymes. This study investigates the peroxidase and laccase enzymatic response of the saprotrophic white-rot fungi Trametes versicolor and Phlebia tremellosa when exposed to SWCNTs of different purity and surface chemistry under different growth conditions. Both unpurified, metal-catalyst-rich SWCNT and purified, carboxylated SWCNTs promoted significant changes in the oxidative enzyme activity of the fungi while pristine SWCNT did not. These results suggest that functionalization of purified SWCNT is essential to upregulate enzymes that may be capable of decomposing CNT in the environment.

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compounds by laccase are able to serve as substrates for versatile peroxidases; this synergy allows lignin-modifying enzymes to function as powerful degraders of highly condensed compounds (Leonowicz et al., 2001), including functionalized SWCNT (Allen et al., 2008).

As a result of their repertoire of degradative enzymes, saprotrophic fungi, such as the wood-rotting Basidiomycetes Trametes versicolor and Phlebia tremellosa, are considered excellent candidates for the degradation of a wide range of industrially-produced xenobiotics (Rabinovich et al., 2004; Riva, 2006). However, there is limited research demonstrating enzymatic or direct microbial decomposition of manufactured carbon-based nanomaterials. Allen et al. (Allen et al., 2008) demonstrated that pristine CNT where unreactive toward purified solutions of horse radish peroxidase, while mildly carboxylated, analogs exhibited chain shortening and oxidation under the same conditions. Previous research by our group has demonstrated that lab-cultured white rot fungi are able to successfully degrade partially hydroxylated C60 fullerenes, i.e., C60 fullerols (Scheiner et al., 2009). The distinction between pristine and functionalized, hydroxylated or carboxylated, may be crucial when estimating the potential for microbial decay of CNT’s as the highly condensed nature of unfunctionalized CNTs, may dramatically impede microbial decomposition and increase the potential for long-term environmental accrual as has been seen for pristine fullerenes.

Research on the impacts of carbon nanomaterials on microorganisms has been largely focused on the impact of nanomaterials on bacterial monocultures where both CNT and fullerene returns have demonstrated antimicrobial properties (Lyon et al., 2006; Arias and Yang, 2009; Kang et al., 2007, 2009). In a study using a variety of different fullerene suspensions, the nanomaterials were found to function as potent antibacterial agents against the gram-positive bacteria Bacillus subtilis (Lyon et al., 2006), while unfunctionalized multiple-walled carbon nanotubes (MWCNT) were found to significantly decrease sporulation of the fungus Paecilomyces fumosoroseus in pure culture but had no effect on hyphal growth (Gorczyca et al., 2009). Kang et al. (2007, 2009) found that SWCNT were effective in inactivating pure cultures of Escheria coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus epidermis in defined media, though the authors found that antimicrobial activity against pure cultures was a poor indicator of microbial deactivation in more complex environmental samples (Kang et al., 2007, 2009).

It has been suggested that in more complex environmental systems, natural organic matter aids in the sorption of nanoparticles, diminishing their apparent toxicity (Li et al., 2008; Navarro et al., 2008). Studies of fullerene toxicity in soil have found limited impact on soil respiration or on bacterial and fungal communities (Tong et al., 2007). Additionally, a recent study found that unpurified CNTs that contain residual amorphous carbon and catalysts from synthesis are able to influence microbial community composition in soils with low organic matter content, diminishing certain fungal and bacteria groups, while having less impact in soils with higher organic matter content (Tong et al., 2012). Such findings are also consistent with reports of the microbial toxicity of carboxylated SWCNT in soils with low organic content (Rodrigues et al., 2013).

The surface chemistry of CNT may not just control their chemical “lability” with respect to microbial enzyme decay, but may also play an important role controlling cytotoxic interactions of CNTs with soil microbial communities (Karakoti et al., 2006; Rodriguez-Yanez et al., 2013). For example, the cytotoxicity of both SWCNTs and MWCNTs has been reported to significantly increase once their surface has been oxidized (Fenoglio et al., 2008; Bottini et al., 2006). Other studies, however, have demonstrated that increased density of surface functionalization of SWCNTs may actually decrease cytotoxicity (Sayes et al., 2006). These seemingly contradictory responses are most likely a function of the propensity of functionalized CNTs under certain conditions to either bind to cells, or to homo and heteroaggregate and thus self-mitigate certain toxicological effects (Arias and Yang, 2009; Pasquini et al., 2012; Handy et al., 2008).

Impurities in CNTs derived from the manufacturing process, e.g. amorphous carbon and metal catalysts, must also be considered as potential influences on soil microbial activity. Although a broad range of synthesis methods exist for commercially available CNTs, many rely on formation of the nanomaterials around metallic nanoparticles catalysts (Melechko et al., 2005). As a result, the majority of CNTs contain metal impurities (Ge et al., 2011). For example, one popular method of large-scale SWCNT synthesis, electric arc discharge, uses nickel and yttrium catalysts (Journet et al., 1997). Liu et al. (2007) reports that catalytic nickel from a variety of SWCNT synthesized by this method is readily bioavailable, more so than the nickel salt NiCl2 used as a reference (Liu et al., 2007). As is the case with the carbon nanomaterials themselves, bioavailability of the metal nanoparticles is highly dependent on environmental factors such as presence of soil organic matter and local redox conditions (Degryse et al., 2009; Auffan et al., 2010). One recent study in agricultural systems found that dissolution of natural organic matter by surfactants greatly increased mobility of heavy metals (Hernandez-Soriano and Jimenez-Lopez, 2012). Mitigation of metal toxicity by organic matter has previously been thought to render the catalyst’s toxicity negligible in short-term soil incubations, although the authors acknowledge that some evidence suggests CNTs act to compound the toxic effects of metals by increasing the mobility of catalytic metals (Tong et al., 2012; Liu et al., 2007).

To improve our understanding of the interaction between growth environment, in this case the nutrient richness of growth media, and fungal response to SWCNT of different purity and surface chemistry we compare the measured changes in oxidative enzymatic activity of the saprotrophic white-rot basidiomycetes Trametes versicolor and Phlebia tremellosa in inoculation experiments on both a simple, defined minimal media and a complex malt media high in organic and phenolic compounds. To minimize variables we use one commercial source of SWCNT, all prepared using the same electric arc process and an yttrium nickel catalyst, but treated to different levels of purity and surface functionalization.

We expect that purified SWCNT will induce a minimal enzymatic response in either media treatment given its lack of chemical functionality, low metal content and large aspect ratio. However, SWCNT with either high metal content or surface functionalization will induce oxidative enzymes activity, either as a detoxification response or an induction of aromatic/lignin-like decay processes. Growth media nutrient content, which may be considered as a proxy for soil nutrient status available to microbes in a natural system, should modulate the ability of fungi to produce oxidative enzymes and thus we expect fungal on minimal media to have significantly reduced responses to metals or functionalized SWCNT.

2. Materials and methods
2.1. Carbon nanomaterials
Carbon nanotubes used in this study were purchased from Carbon Solutions Inc. (Riverside, CA, USA). Carbon Solutions uses an electric arc discharge method with an yttrium/nickel catalyst to produce the nanotubes (Niyogi et al., 2002). Transmission electron microscopy (TEM), using a FEI/Philips CM-100 Transmission Electron Microscope (FEI Company, Hillsboro, Oregon, USA) was employed to investigate the physical form, relative proportion of amorphous materials, and bundling of the SWCNT and compared with the manufacturer specifications to ensure conformity of the products. A summary of the chemical and physical characteristics of the
SWCNT is provided in Supporting Information of this manuscript. The unpurified product, AP-SWCNT, contains single-walled carbon nanotubes, amorphous carbon, and metal catalyst. To produce P2-SWCNT, an unfunctionalized but high-purity SWCNT, and the carboxyl functionalized and purified analog, P3-SWCNT, the AP-SWCNT stock is oxidized by reflux in concentrated nitric acid (Hu et al., 2003) and purified by cross-flow filtration. P2-SWCNT and P3-SWCNT can then be separated by differences in charge (Rinzler et al., 1998). P3-SWCNT have a surface functionalization of 1.0–3.0% carboxylic acid which is the only significant difference between it and the purified but unfunctionalized P2-SWCNT. Catalytic metal content and carbonaceous purity are determined by the manufacturer through thermogravimetric analysis and near-IR spectroscopy respectively (Itkis et al., 2003).

2.2. Media and plating

A two-layer media plating strategy with a denser base-layer was used to prevent carbon nanomaterials from settling to the bottom of the plate, away from the fungi which predominantly occupy the surface of the media. Specifically, each plate contained a 7.5 mm deep base layer of 3% agar (w/w) with a second 2.5 mm layer of malt extract or minimal media agar poured on the surface of this layer. The malt extract media contained barley malt extract (21 g/w) (VWR LLC., Randor, PE, USA) and minimal media contains glucose (2% w/w) and 1 mL yeast nitrogen base without amino acids made to manufacturer’s recommendations (Sigma–Aldrich, St. Louis, MO, USA) in addition to 2% agar (w/w). It is important to note that malt extract agar is a relatively complex chemical media as compared to the minimal agar media as it contains an array of plant-derived aromatic compounds and micronutrients (Zhao et al., 2008; Bell et al., 1991). Treatments containing SWCNT were prepared by adding 50 mg of the desired nanomaterials (AP-SWCNT, P2-SWCNT, or P3-SWCNT) to 80 mL of media prior to autoclaving. Cultures of Trametes versicolor (strain MAD697-R) and Phellinus tremelloides (strain PRL 2845) were maintained on malt extract media (mean ± SD) reported in mg metal/L digest after reflux of media plug or 10 mg mycelia in 15 mL of nitric acid (see methods section).

Table 1

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<td>Control</td>
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<tr>
<td>P2-SWCNT</td>
<td>1.490 ± 0.218</td>
<td>0.146 ± 0.044</td>
<td>0.588 ± 0.154</td>
<td>0.118 ± 0.064</td>
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<td>P3-SWCNT</td>
<td>0.079 ± 0.029</td>
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Elmer DRC-e ICP-MS (Perkin Elmer, Waltham, MA, USA). Fungal tissue was collected by using sterile forceps to peel the surface hyphae from the plates, taking care not to remove any non-fungal material on the media surface; to insure that media components didn’t contaminate fungal samples, only treatment conditions resulting in dense surface mycelium were sampled. Removal of T. versicolor hyphae from plates was facilitated by freezing overnight at −20 °C and thawing to separate media layers. As SWCNT are dispersed evenly throughout, two 8 mm plugs of media were taken from each plate and homogenized and considered one replicate. Metals present in media and fungal hyphae were extracted by dissolving the media or tissue in 10% nitric acid at 100 °C for 60 min. Following extraction and filtering to remove insoluble materials, solutions were diluted ten-fold to produce an extract in 1% nitric acid matrix for introduction into mass spectrometer by nebulizer. A low concentration of nitric acid was used in the extraction in order to insure that SWCNT in the media plugs were not readily oxidized to release encapsulated catalyst, limiting the metals measured to that which have diffused from the surface of the nanomaterials. Nickel and yttrium were quantified against external yttrium and nickel standards. An internal standard of 3.75 ppm gallium was used to correct for instrument drift. The aluminum, chromium, manganese, iron, copper, and zinc content of the initial supporting media, prior to SWCNT loading, was also determined by ICP-MS using the same extraction procedures.

2.5. Statistical analyses

Effects of SWCNT and media treatment on fungal enzyme activity were compared using two-way ANOVA with interaction using a standard least squares type model. Treatment effects were analyzed separately for each sampling zone. Significance of inclusion of catalytic metals in media and differences between enzyme activity of SWCNT containing plates and controls was determined using student’s t-test. All statistical analysis was performed with JMP 8 (v8.0.2 SAS Institute, Cary, NC, USA). Statistical significance was determined for α = 0.05 for all tests unless otherwise noted.

3. Results and discussion

3.1. Catalyst metal uptake into fungal tissue and dispersal in growth media

T. versicolor mycelia harvested from the AP-SWCNT/malt media treatment had a significantly higher concentration of both catalytic metals in comparison to mycelium grown on malt media control. Similarly, T. versicolor mycelium also had significantly increased metal concentrations when grown on P3-SWCNT supplemented media in comparison to control despite the manufacture’s chemical purification of the nanomaterials (see Table 1). Unfortunately, the methods used in this study are unable to determine whether
T. versicolor accumulated catalytic metals when plated on minimal media due to insufficient mycelia for harvest.

As expected, the inclusion of unpurified AP-SWCNT in growth media greatly increased concentrations of nickel and yttrium in each media type compared to controls. The purified P2 and P3-SWCNTs, however, contribute significantly lower but still measurable quantities of the catalyst metals to the media (Fig. 2). There was no significant difference in nickel concentrations between different media types when supplemented with the same nanomaterials, indicating that media type does not impact mobilization of this metal into media and that the extraction method used can be consistently applied with both minimal and enriched media. The differences in yttrium concentrations between malt extract media containing AP-SWCNT when plated with T. versicolor or P. tremellosa suggests that under nutrient rich conditions P. tremellosa may have a role in sequestration of yttrium but no significant effect on nickel concentration of the media.

The effect of heavy metals on fungi have been widely studied, particularly in the context of fungal-based bioremediation processes, as environmental metal contamination frequently coincides with xenobiotic and organic wastes (Baldrian, 2003). One such study of contaminated soil demonstrated that the white-rot fungi Phanerochaete chrysosporium and Pleurotus pulmonarius were able to colonize contaminated soil and degrade a variety of aromatic pollutants despite contamination with various heavy metals, including nickel (D’Annibale et al., 2005). In environments with significant concentrations of heavy metals, fungi often serve as major sinks of the metals (D’Annibale et al., 2005; Gast et al., 1988; Svoboda et al., 2006). The primary method of fungal accumulation of metal is sorption to fungal cell walls (Gabriel et al., 2001). With respect to the present study, sorption to fungal cell walls is the most likely mechanism of fungal uptake of catalytic metals, as the large physical size of SWCNT-bundles would prohibit the direct uptake of nanomaterials. Studies on the absorptive capacity of nickel by T. versicolor have found that the fungus is capable of significant nickel uptake and growth in a liquid media with nickel concentrations of up to 400 mg/L which is far below the concentrations of nickel found in the media of the present study (Dilek et al., 2002; Yetiş et al., 1998).

Although the inclusion of these SWCNTs did not necessarily introduce toxic concentrations of catalytic metals into growth media, their concentrations may still be physiologically relevant. For example, morphological changes to basidiomycetes upon exposure to heavy metals has included increased density of mycelium as a result of more frequent branching and looping of hyphae (Lilly et al., 1992; Darlington and Rauser, 1988). Additionally, a large number of fungi are found to produce both extracellular and cell-bound pigments in response to metal exposure (Baldrian, 2003; Gadd, 1993); these protective pigments include various melanins which are able to bind to and insolubilize metals (Fogarty and Tobin, 1996). In previous studies, T. versicolor and the related Trametes pubescens were observed to produce a dark brown melanin pigment in response to heavy metal exposure, these same studies reported greater changes in morphology (hypothesized to indicate increased toxicity) in less complex media than in corresponding cultures grown in enriched media (Baldrian and Gabriel, 1997; Galhaup and Haltrich, 2001). In the present study, T. versicolor grown on minimal media containing AP-SWCNT were found to produce dark melanin-like pigments, which were easily observed by visual inspection (see Fig. 4). These pigments were not produced by cultures grown with purified SWCNT suggesting a metal-induced response in this experiment, consistent with previous studies using this fungus. It is also worth noting that only the minimal media with AP-SWCNT treatment did not have confluent growth at the end of the study, all other treatments showed symmetric growth rings about the initial fungal plug. Melanin production and asymmetric growth highlight the high metabolic cost of mitigating environmental metal exposure (Baldrian, 2003; Gadd, 1993).

![Fig. 2. Concentrations of residual CNT catalyst metals in minimal and malt extract agar media containing AP, P2, or P3 SWCNTs for media plates inoculated with T. versicolor and P. tremellosa. Asterisks indicate significant difference between fungal treatments when grown on the same combination of SWCNT/media at α = 0.05; no significant difference was found between any SWCNT/fungi combination grown on different media.](image-url)
3.2. Enzyme activity

Differences in enzyme activity between cultures grown with and without SWCNT are presented in Fig. 3. Inclusion of unpurified or carboxylated SWCNTs has a significant impact on the activity of oxidative enzymes produced by both tested fungi whereas purified, unfunctionalized SWCNTs have little impact on enzyme activity in either the malt or minimal media used in this experiment. Additionally, the inclusion of unpurified AP-SWCNT to malt extract agar resulted in significant increases in laccase and peroxidase activity of T. versicolor cultures while only laccase activity is elevated when AP-SWCNT is included in minimal media. Cultures of both species exposed to AP-SWCNT in minimal media also had significantly increased laccase production, while only P. tremellosa demonstrated increased peroxidase activity under these conditions. These results are thought to reflect the differing nutrient requirements and substrate interactions important in controlling enzyme production by the two fungi. Similarly complex interactions between nutrient availability and the presence of aromatic compounds in media have been reported previously in white-rot fungi and were found to vary between fungal strains (Rogalski et al., 1991).

Melanin production by T. versicolor was also observed in the older hyphae of zone 1 when grown under nutrient limited conditions with AP-SWCNT (see Fig. 4). The significant increase in laccase activity in this growth zone is consistent with the role played by oxidative enzymes in the formation of extracellular melanin in white-rot fungi (Bell and Wheeler, 1986). Extracellular melanin can be produced by the oxidative degradation of phenolic compounds, either located in the environment or secreted by the fungus itself for that purpose. Similar responses have been demonstrated in other white-rot fungi in response to high metal concentrations; Galhaup et al. found that both laccase activity and melanin formation increased with copper concentration (Galhaup and Haltrich, 2001).

Exposure to the purified and unfunctionalized P2-SWCNTs to either growth media did not significantly affect the enzymatic activity of either fungus, while the effect of inclusion of P3-SWCNT on enzyme activity was dependent on a combination of hyphal location and media composition. Specifically, in the presence of P3-

![Fig. 3. Differences in laccase and peroxidase activity in growth zones 1 (top) and 2 (bottom) in response to SWCNT inclusion in minimal and malt extract media containing AP, P2 or P3 SWCNTs inoculated with T. versicolor or P. tremellosa. Activity is reported in mU/mL liquid extract from media. Asterisks indicate a significant difference between SWCNT treatment and control as determined by student's T-test with \( \alpha = 0.05 \).

![Fig. 4. T. Versicolor colonies after one month growth on minimal media (A), minimal media with AP-SWCNT (B), and malt extract media with AP-SWCNT (C). Mycelia density is increased when grown on malt extract agar in comparison to minimal media; growth in the presence of AP-SWCNT is confluent on malt extract media (surface mycelia conceal SWCNT in media). T. versicolor cultured on minimal media with AP-SWCNT produced a dark pigment not found in cultures grown on other media types. Concentric circles indicate distance representing 25% (zone 1) and 75% (zone 2) of the distance between center of plate and plate wall.](image-url)
SWCNT, T. versicolor peroxidase activity was suppressed in minimal media when sampled in zone 1, near the original fungal plug, but was not significantly altered when sampled distally in zone 2. In contrast, growth in the presence of P3-SWCNT on malt agar led to a significant increase in peroxidase activity of hyphae in zone 2. T. versicolor laccase activity was elevated with P3-SWCNT exposure in zone 1 on both media types and dramatically increased under minimal conditions when sampled in zone 2. As P2 and P3-SWCNT contain similar quantities of residual catalyst, the presence of metal is not likely to be the source of the elevated enzyme activity—the primary distinction between two SWCNTs is the inclusion of carboxyl into the structure of P3-SWCNT as a result of carboxylic acid functionalization. This indicates that the carboxyl functional groups on P3-SWCNT directly induced oxidative enzyme activity and thus that functionalization may be a necessary condition for fungal-driven decomposition of CNT.

In contrast to T. versicolor peroxidase activity when grown on minimal media containing P3-SWCNT, P. tremellosa peroxidase activity was dramatically increased under these same conditions. Although P. tremellosa produces a compliment of manganese-independent peroxidases (Hatakka et al., 1992) which might be expected to limit the reliance of peroxidase activity on media factors (Table 2). Previous studies have demonstrated that the age of mycelia is important in determining enzyme activity; some species focus on enzymatic production during expansion while others have greater enzymatic activity once expansive growth has been completed and the fungi enters a secondary metabolic stage (Hatakka, 1994). Decreased age of mycelia (by proxy of sampling from zone 2 rather than zone 1; Fig. 4) was found to only interact with the variable of SWCNT addition on T. versicolor laccase activity and of media type on P. tremellosa peroxidase activity (compare zones 1 and 2 on Table 2). These findings suggest that the drivers of enzymatic response to SWCNT exposure may differ in less-mature mycelia between fungi, with T. versicolor enzyme production being controlled by media composition while P. tremellosa enzyme activity is more impacted by SWCNT characteristics.

3.3. Implications of study in fungal/nanomaterial interactions

The patterns of enzyme activity outlined in this study offer valuable insights on the interactions between SWCNT surface chemistry, nutrient growth conditions, and type of saprotrophic fungi. As purified, carboxylated SWCNT resulted in increased activity of oxidative enzymes while purified but unfunctionalized analogs exhibited no change in activity it can be concluded that surface chemistry of the nanomaterial has a direct influence on fungal response to SWCNT. Contrary to our original hypothesis that nutrient rich media would allow for a more significant change in enzyme activity in response to functionalized SWCNT, we found oxidative enzyme activity to be diminished when compared to nutrient poor growth conditions. This suppressed response may be a result of phenolic compounds in the malt extract media, which could bind to or help aggregate surface functionalized tubes, thus reducing effective exposure to the hyphae. Although some changes in enzyme activity in the AP-SWCNT treatments might be explained as an induction of enzyme production by metals and other impurities in the AP product, not all enzymatic responses can be considered a result of non-SWCNT factors. Specifically, the induction of oxidative enzyme activity by P3-SWCNT but not by the unfunctionalized and purified P2-SWCNT, nanomaterials which differ only in surface functionalization, indicates that the 1–3% carboxyl content of P3-SWCNT is the driver of the observed changes in enzyme activity.

The observed elevation of oxidative enzyme activity when fungi were exposed to unpurified SWCNT and functionalized SWCNT stands in contrast to the lack of significant enzymatic response to purified, unfunctionalized SWCNT. This finding suggests that pristine SWCNT released in the environment would not promote a degradative response by saprotrophic fungi in soils. Although this study provides no direct evidence of SWCNT transformation by fungal oxidative enzymes, we have demonstrated for the first time that activity of oxidative enzymes in the laccase and peroxidase family is significantly impacted by exposure to certain formulations of SWCNT in pure cultures and in doing so provide important context for future mechanistic studies of interactions between white-rot fungi and SWCNT.

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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.envpol.2014.06.013.

References


