Enzymatic Degradation of Multi-Walled Carbon Nanotubes

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Supporting Information

Table of Contents

Supplemental Experimental procedures:

Methods for Boehm's Titration: Boehm's Titration is an acid–base titration method for determination of functional groups on the surface of carbon materials. Approximately, 5 mg of p-MWNT, o-MWNT (5hr), and o-MWNT (8hr) samples were respectively immersed in sample vials with 5 mL of 10 mM NaOH aqueous solution. Then each vial was sonicated under vacuum for 2.5 min in order to disperse the sample and degas CO_2 from the solution. All three vials were then sealed with septum stoppers and parafilm and placed on a rotary shaker with continuous shaking (220 rpm) at room temperature for 72 hr. After the incubation process, the sample solution was filtered through a 0.22 μm Teflon membrane, and 1 mL of filtrate was taken and added with 2 drops of 0.1% Bromocresol Green and Methyl Red mixture (indicator, v/v = 3:2). The filtrate was titrated with approximately 1 mM HCl aqueous solution using a 10 mL buret. Three parallel titrations were performed on each sample to obtain reproducible results, and a reference sample with 5 mL of 10 mM NaOH was analysed the same way to give the accurate concentration of NaOH. The surface acidic group loading (mM / gram of MWNTs) was estimated by the following equation:

$$Loading = \frac{(c_{ref} - c) \times 5 \times 10^{-3} L}{m_{MWNT}} \quad (1)$$

where:

c_{ref}: Concentration of NaOH solution (mM);

c: Concentration of the NaOH fitrate after incubation with MWNTs (mM);

m_{MWNT}: Weight of each MWNT sample.

Atomic Force Microscopy (AFM): AFM imaging and height analysis was performed on a Multimode scanning probe microscope (Veeco). Sample was prepared on freshly cleaved mica substrate spin-coated with 20 μL of 0.1% (w/w) poly-L-lysine (aq) at 1400 rpm. 10 μL of sample solution (aq) was then spin-coated on the substrate and dried in ambient. AFM imaging was performed using a "Supersharp" Si tip (AppNano) in tapping mode, with a drive frequency of 193.023 Hz, an amplitude set point of 0.6066 V, and a drive amplitude of 261 mV. The cross-

sectional height of samples was quantified using sectional analysis.

Thermogravimetric Analysis (TGA): Three samples of p-MWNTs, o-MWNTs (5hr) and o-MWNTs (8hr) before enzymatic degradation were dried in oven overnight. From each sample about 4 mg of materials were transferred into a platinum boat on which TGA were performed (TA instrument, Q50). The temperature ramping was set from room temperature to 850°C at 5°C per min at N_2 atmosphere. Other samples were analyzed in air atmosphere with the temperature ramping to 800°C at 5°C per min.

Fourier transform infrared spectroscopy (FTIR): Samples of p-MWNTs, o-MWNTs (5hr) and o-MWNTs (8hr) were synthesized and dried in oven overnight. FTIR spectra were taken using a Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) accessory using dry SiC powder as matrix.

Amplex Red assay for monitoring HRP activity: HRP activity throughout the degradation process was monitored by Amplex Red reagent. A 10 mM stock solution was prepared by dissolving 5 mg of lyophilized Amplex Red in 1.94 mL dimethyl sulfoxide (DMSO) and kept in the dark at −20 °C. To test the enzymatic activity, 250 μL of sample suspensions before and during degradation were taken out and diluted with 235 μL double-distilled water, followed by adding 1 μL Amplex Red stock solution and 15 μL of 800 μM H_2O_2. The mixture was then analyzed using visible spectroscopy on a Lambda 900 spectrometer (Perkin-Elmer) with double-distilled water as the background.

The Amplex Red is often associated with HRP which is activated by H_2O_2. The Amplex Red is assumed to experience a one-electron transfer to active HRP and form phenoxy radicals which undergo a dismutation reaction forming resorufin, a colored compound with distinct absorption band at 570 nm. The reaction is quantitative with 1:1 ratio of Amplex Red to H_2O_2 and the presence of active HRP enzyme. Therefore a colorimetric assay can be performed for either testing the HRP activity or measuring the H_2O_2 concentration.

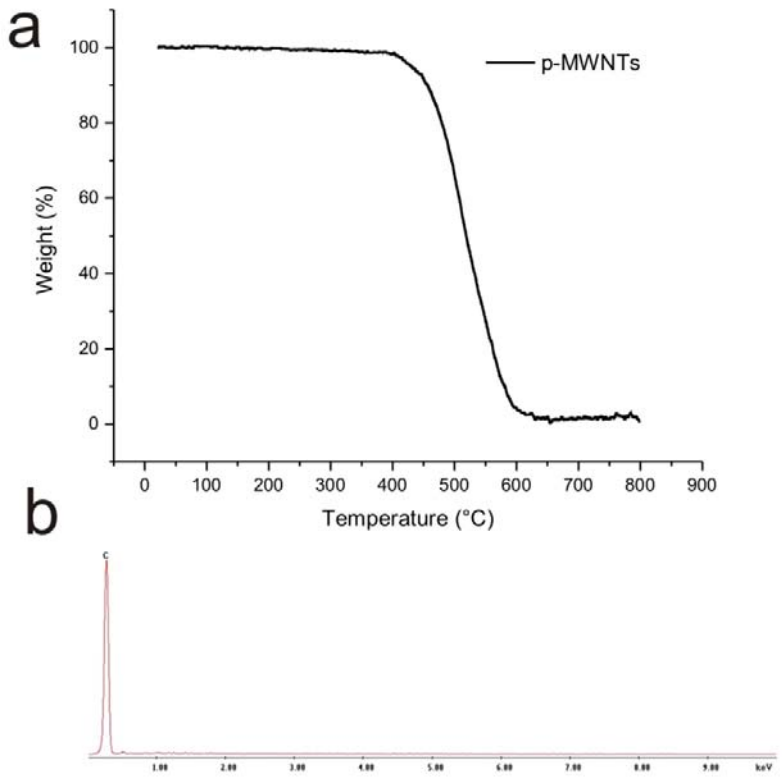

Figure S1. (a) TGA curve of p-MWNTs in air. There was almost no material left after burning in air above 600°C. (b) Energy-dispersive X-ray spectroscopy (EDS) elemental analysis of p-MWNTs. No iron peaks (6 – 8 keV) were observed.

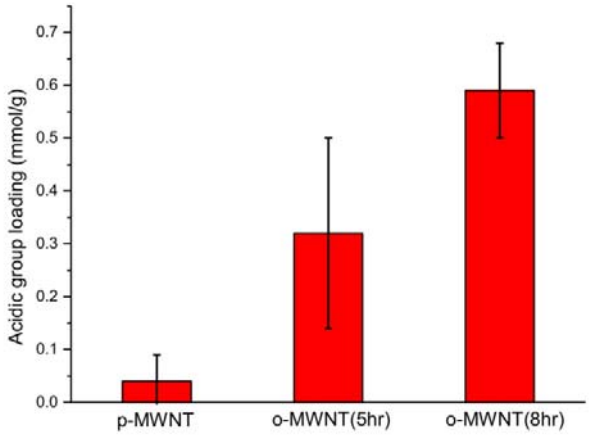

Figure S2. Histograph showing increasing acidic group loadings on the surface of MWNTs along with 0, 5 and 8 hr carboxylation determined by Boehm's Titration.

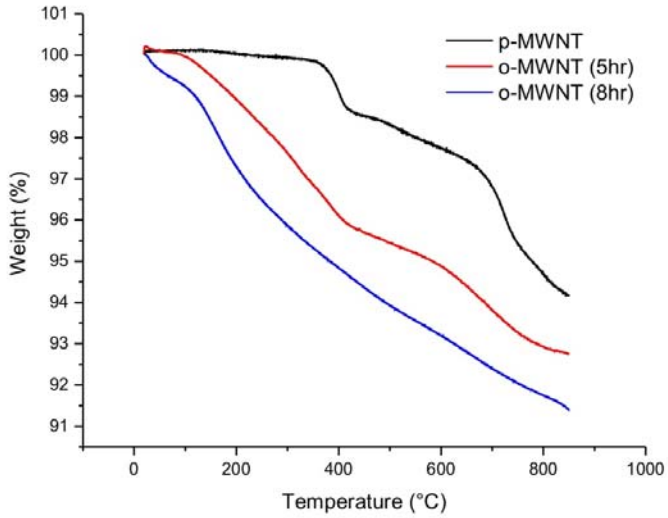

Figure S3. TGA curves for p-MWNTs, o-MWNTs (5hr) and o-MWNTs (8hr) in N_2 atmosphere. The TGA curves showed a progressively increasing weight loss along with the increasing carboxylation time, indicating more functional groups grafted on the nanotubes.

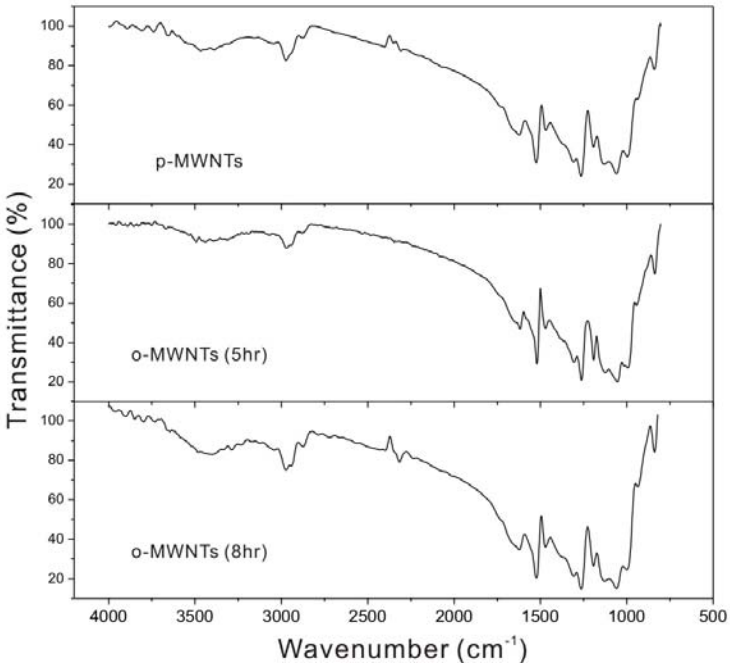

Figure S4. FTIR spectra for p-MWNTs, o-MWNTs (5hr) and o-MWNTs (8hr) before enzymatic degradation. The spectra show different vibrational modes in each sample including C=O stretching (1640 cm^{-1}), C–O stretching (1100 cm^{-1}), C–H stretching (2980 cm^{-1}), O–H stretching (3440 cm^{-1}) and so on, which indicate the existence of oxygen-containing defects such as carboxylic groups on MWNTs.

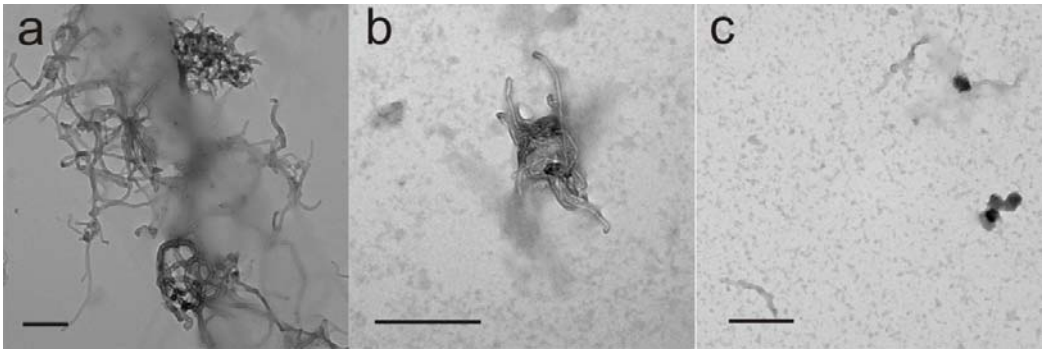

Figure S5. TEM images for (a) p-MWNT, (b) o-MWNT (5hr), and (c) o-MWNT (8hr) samples incubated with HRP under daily H_2O_2 additions for 80 days. The morphology of nanotubes remained similar to samples observed at Day 60, however, the carbonaceous "flakes" were seen to be continuously degraded into even smaller pieces. All scale bars are 200 nm.

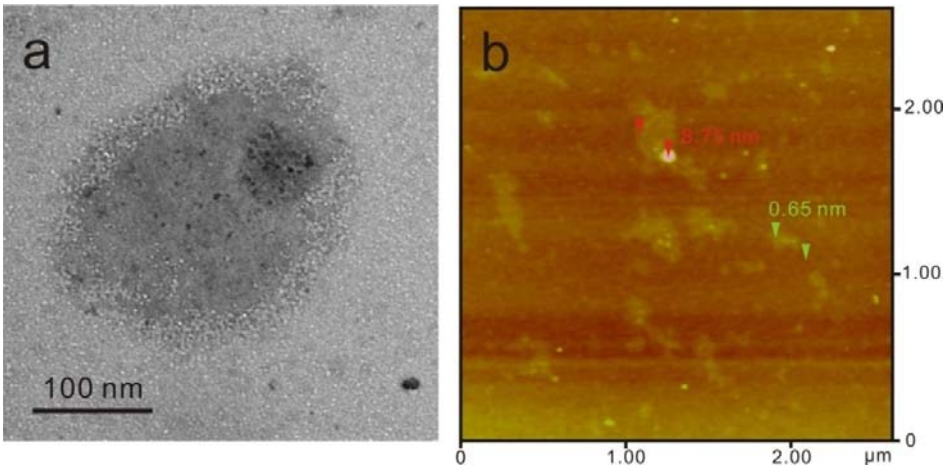

Figure S6. (a) TEM image of magnified carbonaceous flakes for o-MWNT (8hr) enzymatically degraded with constant H_2O_2 addition at Day 2. The black dots with diameters around 5 nm adsorbed on the flakes are presumably HRP particles. (b) AFM image for the sample at Day 4. Large amounts of carbonaceous flakes were seen with thickness of about 0.65 nm.

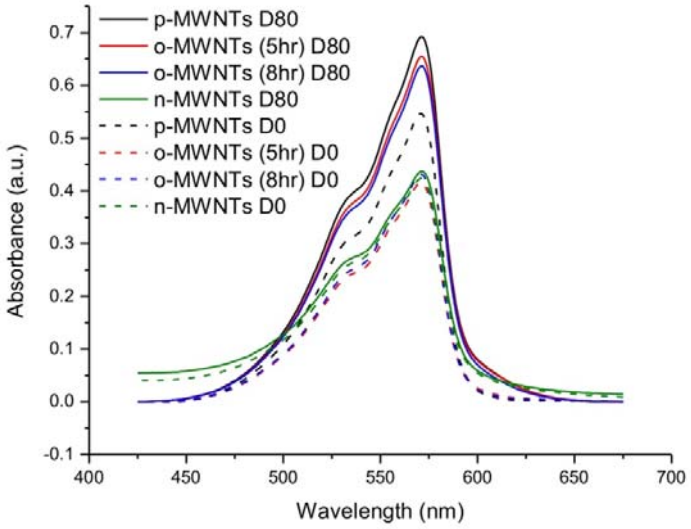

Figure S7. Visible absorption spectra of HRP activity tested by Amplex Red and H_2O_2 for p-MWNT, o-MWNT (5hr), o-MWNT (8hr) and n-MWNT samples before (dash lines) and after (solid lines) 80 days of degradation process.

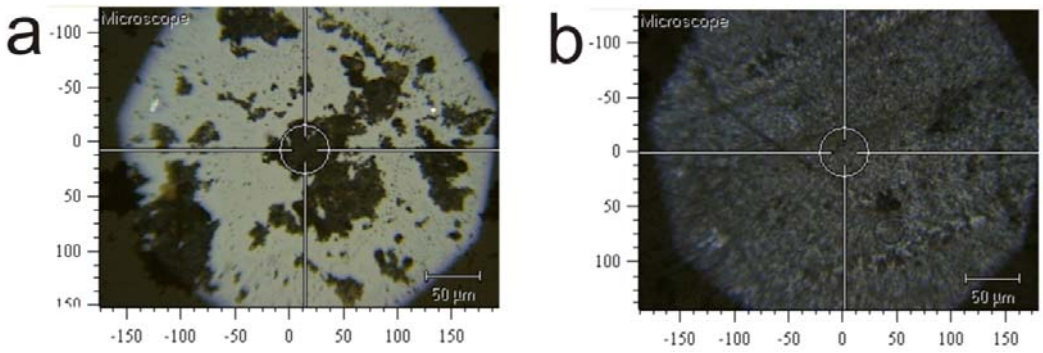

Figure S8. Images from the optical microscope on the Raman sample stage. (a) Image of o-MWNTs (8hr) before enzymatic degradation, and (b) after enzymatic degradation. The sample before enzymatic degradation tended to aggregate into discrete spots after dried on a sample glass slide, while the sample after degradation formed an even, continuous film. Both samples appeared to be homogeneous as visually observed. 5 spectra were collected and averaged from different spots for both samples.

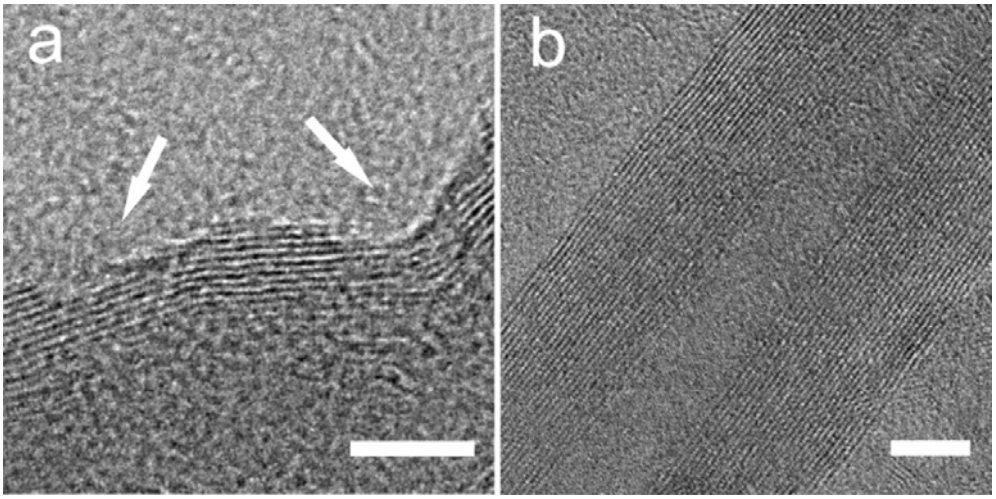

Figure S9. High-resolution TEM images for o-MWNTs (8hr) (a), and p-MWNTs (b) before enzymatic degradation. All scale bars are 5 nm. The defective sites are shown on the surface of o-MWNT samples as arrowed, in which about 5 – 8 graphitic walls were broken, while the p-MWNTs remain high graphitic integrity with well-defined walls.

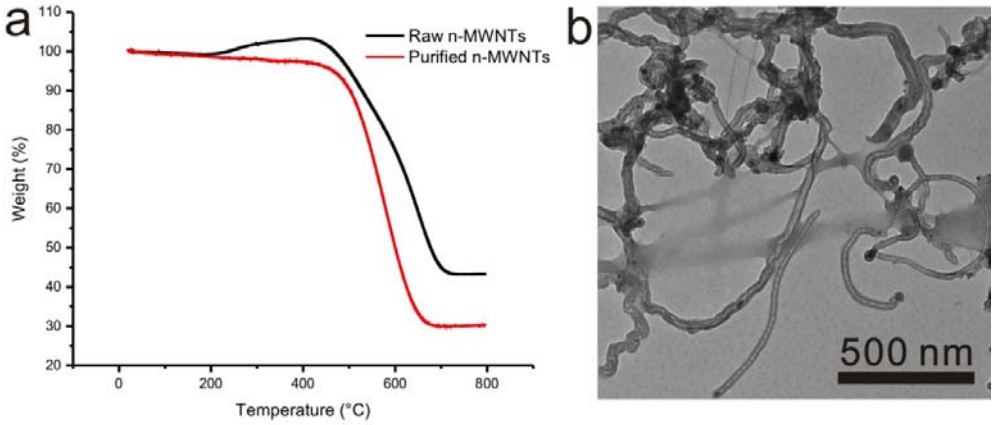

Figure S10. (a) TGA curves for n-MWNTs before (black) and after (red) purification process in air. (b) TEM image of purified n-MWNT samples after 80 days of incubation in Fenton oxidation environment. The extent of degradation was limited.