Photochemical Transformations of Carbon Dots in Aqueous Environments

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16 Pages, 13 Figures, 1 Table
Supporting Information

Synthesis of CDs

Carbon dots (CDs) were synthesized via bottom up microwave-assisted pyrolysis adapted from a previously reported method. 2 mL of a 4 M citric or malic acid solution and 540 µL of ethylenediamine were combined in a beaker, allowed to react (exothermic), and subsequently cooled at room temperature for 30 min. The mixture was then heated in a microwave oven at 700 W for 2 min. Approximately 10 mL of deionized water was added to the resultant solid and the mixture was magnetically stirred for 30 min, or until it completely dissolved. Dialysis tubing (0.1-1.0 kDa pore size) was prepared by soaking in deionized water for 30 min, before adding the dissolved CDs and dialyzing against deionized water for 24 h. The solution was transferred to an oven and dried at 40 °C until all water was evaporated and a red-brown solid remained.

Characterization of CDs

Transmission Electron Microscopy (TEM): TEM images of CACDs and MACDs were obtained using a FEI Tecnai F30 TEM with a 300 kV electron beam.

UV-Visible Spectroscopy (UV-Vis): UV-visible extinction spectra were measured from 200 to 800 nm using a Thermo Scientific Evolution 220 UV-Visible spectrometer (Waltham, MA). CDs were analyzed at 15 mg/L.

Photoluminescence (PL) Tests: Fluorescence (PL) spectra were obtained using a Thermo Scientific Lumina fluorescence spectrometer (Waltham, MA). An excitation wavelength of 350 nm was used and emission spectra were recorded from 360 to 650 nm with a 0.1 nm interval, a photomultiplier tube voltage of 700 V, and a 20 ms integration time. Excitation and emission slit widths of 2.5 nm were used for CACD solutions and slit widths of 5 nm were used for MACD solutions. To avoid interference from inner filter effects, all CD solutions used in PL spectroscopy had absorbances of ≤ 0.1 at the characteristic peak at approximately 350 nm. CDs were analyzed at 15 mg/L.
Total carbon (TC)/Total Nitrogen (TN)/Ion Chromatography (IC) analysis: Solutions of 100 mg/L CACDs and MACDs were exposed to natural sunlight or \( \cdot \)OH. Test tubes wrapped in aluminum foil to prevent light exposure were used as dark controls, and Milli-Q water samples were used as blank controls. CD samples were diluted with Milli-Q water after photolysis to a volume of 20 or 40 mL for TC analysis. Diluted samples were filtered through a 0.20 \( \mu \)m PES syringe filter into acid-washed TC vials before injection into a Shimadzu TOC-L total organic carbon analyzer equipped with a Shimadzu TNM-L total nitrogen measuring unit and an ASI-L Shimadzu auto sampler (Kyoto, Japan). TC and TN signals were converted into CD concentrations (mg/L) via reference to calibration curves prepared with CDs. For TN, the CD concentration was converted into mg N/L using a standard of sodium nitrate solution as a reference. For IC, filtered samples prepared for TC/TN analysis were transferred into 0.5 mL vials and analyzed with no further sample pre-treatment using Dionex ICS-2100 system (Thermo Fisher, Waltham, MA) equipped with an AS18 ion exchange column. Samples were run using a 67 mA suppressor current and 30 mM KOH eluent flow at a rate of 1 mL/min. A nitrate calibration curve was prepared from a combined seven anion standard (Thermo Fisher, Waltham, MA).

Attenuated Total Reflectance Infrared (ATR-FTIR) spectra: Solutions of CACDs and MACDs were exposed to natural sunlight (at 2000 mg/L) or \( \cdot \)OH (at 350 mg/L) and lyophilized (LabConco FreeZone 2.5 Plus, 2.5L, Kansas City, MO) into powders. ATR-FTIR of lyophilized CD samples were obtained using a Nicolet iS5 (Thermo Fisher, Waltham, MA) spectrometer with an iD5 ATR attachment using a scan range from 4000-525 cm\(^{-1}\) with 64 scans at 0.964 cm\(^{-1}\) resolution.

X-ray Photoelectron Spectroscopy (XPS): Solutions of CACDs and MACDs were exposed to natural sunlight (at 2000 mg/L) or \( \cdot \)OH (at 350 mg/L) and lyophilized into powders. XPS of lyophilized CDs were obtained using a PHI (Chanhassen, MN) 5600 XPS equipped with a Mg K\( \alpha \) flood source (1253.6 eV) and a hemispherical energy analyzer. High-resolution multiplex scans were collected at ultra-high vacuum (8 \( \times \) 10\(^{-8}\) torr) with a source power of 300 W, a pass energy of 29.35 eV, 10 sweeps per spectrum, and 0.125 eV/step. Survey scans (1200-0 eV binding energy) were collected at the same ultra-high
vacuum with a pass energy of 58.7 eV, 2 sweeps per spectrum, and 1.0 eV/step. Spectra were analyzed using CASA XPS software.

**NMR:** For $^1$H-NMR analysis, 600 μL of a 5 mg/mL solution of CACDs was exposed to natural sunlight and analyzed in a NMR tube using a 400 MHz NMR (Bruker UltraShield, Billerica, MA). For $^{13}$C-NMR, 600 μL of a $1.7 \times 10^4$ mg/L solution of photobleached CACDs exposed to ‘OH was analyzed using the same spectrometer, run for 100 scans and 160 loops. Due to the higher concentration of CDs needed for NMR ($1.7 \times 10^4$ mg/L vs. 350 mg/L for ATR-FTIR/XPS data), a higher ‘OH dose was required to drop the TC in solution to 40% of the initial concentration, allowing for the direct comparison between the XPS/ATR-FTIR and NMR of the CDs. This amount of carbon loss required a ‘OH dose of $5.7 \times 10^{-8}$ M*min and corresponded to the point in the carbon loss profile at the end of the first phase of degradation by ‘OH (see Figure 3).

**Natural sunlight**

**Photobleaching:** CACD and MACD solutions with concentrations of 15 mg/L were prepared; solutions wrapped in aluminum foil to prevent light exposure were used as dark controls. Samples were exposed to natural sunlight and at time intervals of 0 min, 30 min, 1 h, 2 h, and 12 h, two irradiated CD samples (light replicates) and a single dark control were removed. After removal, undiluted samples were analyzed using UV-vis and PL spectroscopy.

**Photobleaching Images:** CACD and MACD solutions with concentrations of 100 mg/L were prepared and exposed to natural sunlight for 0, 30 min, and 1, 4, 8, 12, 24, and 48 h. After exposure, samples were analyzed with UV-Vis and PL spectroscopy and images were taken under indoor or 350 nm light.

**Photobleaching in Lab Light:** CACD and MACD solutions with concentrations of 100 mg/L were prepared and exposed to laboratory fluorescent lighting (emission spectrum in Figure S9b) for 10 days. After exposure, samples were analyzed with PL spectroscopy.
Effect of O\textsubscript{2} and N\textsubscript{2} Sparging: CACD and MACD solutions with concentrations of 15 mg/L were prepared and either left untreated or were treated by bubbling with N\textsubscript{2} or O\textsubscript{2} for 15 minutes. Samples wrapped in aluminum foil to prevent light exposure were used as dark controls. Samples were exposed to natural sunlight at time intervals of 2 min, 4 min, 6 min, 10 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h. For each time interval, two irradiated CD samples (light replicates) and a single dark control were removed. After exposure, undiluted samples were analyzed using UV-visible and PL spectroscopy.

Aggregation/Settling Test: CACD and MACD solutions with CD concentrations of 15 mg/L were prepared and the pH was adjusted to 7 or 8 using 1M HCl or 4M NaOH. For each type of CD at each pH, four samples containing 8 mL each of CD solution were prepared. One irradiated sample and one dark sample had NaCl, KCl, and CaCl\textsubscript{2} added to produce final salt concentrations of 15 mg/L NaCl, 400 mg/L KCl, and 400 mg/L CaCl\textsubscript{2} (4 mM Ca\textsuperscript{2+}, 13 mM Cl\textsuperscript{-}, 0.3 mM Na\textsuperscript{+}, 5 mM K\textsuperscript{+}). Blanks containing Milli-Q water with or without salts were also prepared. All samples were analyzed after 1 week of settling using PL spectroscopy.

TC and TN: Solutions of 100 mg/L CACDs and MACDs were exposed to natural sunlight for 0, 1, 2, 3, 4, 5, and 6 weeks. Samples were analyzed for TC and TN. Samples wrapped in aluminum foil were used as dark controls and Milli-Q water was used for blank controls.

ATR-FTIR and XPS: Solutions of 2000 mg/L CACDs and MACDs were exposed to natural sunlight for 2, 4, and 6 weeks and lyophilized (LabConco FreeZone 2.5 Plus, 2.5L, Kansas City, MO) into powders. ATR-FTIR and XPS were run on the recovered CD powder from each time point.

\textsuperscript{1}H-NMR: A 900 \mu L sample of CACDs at 5 mg/mL in D\textsubscript{2}O was exposed to natural sunlight alongside an identical sample wrapped in aluminum foil to serve as a dark control. After 5 d of exposure, samples were retrieved from the roof and immediately transferred into an NMR tube for analysis.

Mass Recovery Experiments: A known mass of CACDs (~15 mg) was added to 6 mL of DI water and exposed in triplicate to natural sunlight for 21 d on a rooftop. Controls included an identical triplicate set
of tubes wrapped in aluminum foil to serve as dark controls, and triplicate vials of pure DI water to serve
as blanks. After 21 d of irradiation, the samples were lyophilized in pre-weighed centrifuge tubes and
weighed using an analytical balance to determine the % recovery of CDs after natural sunlight exposure.
It was determined that CACDs exposed to sunlight for 21 d yielded 101.4 % +/- 2.1 % recovery,
comparable to the dark control samples which were recovered at 95.8 % +/- 3.5 %, while, as expected, no
mass was recovered from the blank controls. pH of these solutions was measured to be ~7 using litmus
paper both before and after 21 days of exposure to natural sunlight.

Exposure to ‘OH Radicals

CD solutions at 100 mg/L were placed into a Rayonet reactor and irradiated with 300 nm UV light (16
bulbs, RPR-300). Samples were dosed daily with 100 mM H$_2$O$_2$. UV-Vis was used to measure the loss of
H$_2$O$_2$ over time and ensure that each dose of H$_2$O$_2$ was fully reacted before the next H$_2$O$_2$ dose was added.

TC/TN/IC: 10 mL samples at 100 mg/L CACD and MACD were removed from the Rayonet after ‘OH
doses up to 4.0×10$^{-8}$ M*min and 2.8×10$^{-8}$ M*min, respectively. Samples wrapped in aluminum foil were
used as dark controls and Milli-Q water was used as a blank control. Samples were analyzed using TC,
TN, and IC.

ATR-FTIR and XPS: Samples for spectroscopic analysis were prepared at 350 mg/L CACDs or MACDs
and exposed to ‘OH doses up to 1.1×10$^{-8}$ M*min before being lyophilized and compared to the unexposed
parent CDs. Samples were analyzed using ATR-FTIR and XPS.

$^{13}$C-NMR: CD samples for $^{13}$C-NMR were first photobleached at 1.7×10$^4$ mg/L and then reacted with an
‘OH dose of 5.7×10$^{-8}$ M*min ‘OH. A $^{13}$C-NMR was then prepared at 1.7×10$^4$ mg/L CACD/L D$_2$O
solution.

Quantification of ‘OH Radical Dose
Procedures used here have been reported in greater detail by Lankone et al.\textsuperscript{,2} In brief, salicylic acid (SA) was used at 0.07 mM as a probe molecule that could be quantified using UV-Vis spectroscopy. In the presence of •OH radicals, SA reacts to form 2,3-dihydroxybenzoic acid and 2,5-dihydroxybenzoic acid. Under irradiance with 300 nm light (in absence of •OH radicals), SA is photostable and decreases in concentration by less than 4% over the 30 s necessary to perform the outlined measurements. To enable deconvolution of SA from the two product species (2,3-dihydroxybenzoic acid and 2,5-dihydroxybenzoic acid), reference solutions of each compound at a known concentration were prepared. UV-Vis spectra were collected of these reference solutions and later used to determine the concentration of SA during •OH exposure in solutions containing a mixture of SA, 2,3-dihydroxybenzoic acid and 2,5-dihydroxybenzoic acid. Since the rate constant for the reaction of SA with •OH radicals is known (5x10\textsuperscript{9} M\textsuperscript{-1} s\textsuperscript{-1}), measuring the rate of SA degradation enabled the steady state •OH concentration to be determined for a given dose of H\textsubscript{2}O\textsubscript{2}. Crucially, CD exposure to H\textsubscript{2}O\textsubscript{2} in the present study was performed in the absence of SA, necessitating a correction factor be applied to the •OH radical concentration measured with SA (from Lankone et al.) to determine the actual •OH radical concentration present in solutions used to photolyze CDs in these experiments. As part of the present study, we evaluated the [OH]\textsubscript{ss} in the presence of CDs to show that for the concentrations used (1 - 319 mg/L), the presence of CDs did not have an impact on [•OH]\textsubscript{ss} (Figure S1).

H\textsubscript{2}O\textsubscript{2} concentration in the quartz vessels was tracked over time using UV-Vis spectroscopy, which, along with knowledge of the [•OH]\textsubscript{ss} throughout the decomposition of H\textsubscript{2}O\textsubscript{2}, allowed for the dose of •OH radicals for each experiment to be determined. Moreover, this dose could also be related to the timescale in the environment which would deliver the equivalent •OH dose. For example, a single 100 mM dose of H\textsubscript{2}O\textsubscript{2} results in an average steady state •OH radical concentration of 5.79×10\textsuperscript{-12} M*min for a total exposure time of approximately 8 h. Environmental [•OH]\textsubscript{ss} values in surface waters have been reported on the order of 10\textsuperscript{-15}-10\textsuperscript{-17} M.\textsuperscript{3-5} In a given month, then (assuming a maximum exposure to •OH at 10\textsuperscript{-15} M), we can estimate the dose of •OH radicals delivered in the natural environment is the product of
10^{-15} \text{ M} \text{ and } 21,600 \text{ min (i.e., minutes in a 30 day month, assuming 12 h of sunlight per day). Therefore, cumulative exposure to \textsuperscript{\textcircled{•}}\text{OH from a single 100 mM H}_2\text{O}_2 \text{ dose across its degradation period (i.e., 8 hours of 300 nm irradiation) can be estimated to be roughly 129 months of environmental \textsuperscript{•}{\text{OH exposure. Table S1 summarizes the relationship between H}_2\text{O}_2 \text{ dose and environmental timescale.}}}

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<th>\textsuperscript{•}{\text{OH}} Dose (M*min)</th>
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**Table S1.** Relationship between initial H$_2$O$_2$ concentration and photolysis time to \textsuperscript{•}{\text{OH}} dose. The range of environmental timescale for equivalent \textsuperscript{•}{\text{OH}} exposure in natural surface waters is also indicated, with the minimum and maximum values determined assuming a steady state \textsuperscript{•}{\text{OH}} concentration of 10^{-15} \text{ M} and 10^{-17} \text{ M}, respectively. Hydrogen peroxide doses beyond (2x) 100 mM yielded a linear increase in both hydroxyl radical dose and equivalent environmental exposure.

**Figure S1.** Steady state concentration of \textsuperscript{•}{\text{OH}} ([\textsuperscript{•}{\text{OH}}]$_{ss}$) generated by photolysis of 100 mM H$_2$O$_2$ in the presence of 1-319 mg/L photobleached CACDs.
Figure S2. Characterization of parent CDs. Emission and absorption profiles of CACDs (a) and MACDs (b). Transmission electron microscopy of CACDs (c) and MACDs (d).

Figure S3. ATR-FTIR spectra of lyophilized as-synthesized CACDs (a) and MACDs (b).
Figure S4. Normalized C (1s), N (1s), O (1s), and Si (2p) XPS regions of lyophilized CACDs after exposure to 0 (black), 2 (red), 4 (blue), and 6 (green) weeks of natural sunlight. Silicon is from SiO$_2$, residual from CD synthesis.

Figure S5. Normalized C (1s), N (1s), O (1s), and Si (2p) XPS regions of lyophilized MACDs after exposure to 0 (black), 2 (red), 4 (blue), and 6 (green) weeks of natural sunlight. Silicon is from SiO$_2$, residual from CD synthesis.
**Figure S6.** a) Absorption profile of CACDs (red) overlaid with the solar irradiance spectrum (blue, obtained from [https://www.nrel.gov/grid/solar-resource/spectra-astm-e490.html](https://www.nrel.gov/grid/solar-resource/spectra-astm-e490.html)). b) Solar irradiance spectrum with units.

**Figure S7.** Photobleaching rates of 15 mg/L CACDs (red) or MACDs (black) after 240 min of natural sunlight exposure as measured by photoluminescence spectroscopy (PL). Fluorescence is shown in terms of the area of the emission curve at each time point normalized to the initial emission at t=0.
Figure S8. Photographs of 100 mg/L solutions of CACDs (a) and MACDs (b) after exposure to 0, 0.5, 1, 4, 8, 12, 24, or 48 hr (left to right) of sunlight. Samples are shown post-exposure under either lab light (top) or 350 nm light (bottom).

Figure S9. a) Photoluminescence spectra of CACDs before (black) and after (red) 10 days of laboratory fluorescent light exposure. b) Absorption profile of CACDs (red) overlaid with the emission spectrum of fluorescent indoor bulbs (black). CD concentration matched that used in natural sunlight exposures (i.e., 100 mg/L).
**Figure S10.** Atomic composition of lyophilized MACDs exposed to 0-6 weeks of natural sunlight as determined by XPS.

**Figure S11.** Photoluminescence spectroscopy of 15 mg/L CACD fluorescence before and after 1 week of settling at pH 7 or 8 in pure water or in a mixture of 15 mg/L NaCl, 400 mg/L KCl, and 400 mg/L CaCl$_2$ (4 mM Ca$^{2+}$, 13 mM Cl$^-$, 0.3 mM Na$^+$, 5 mM K$^+$).
Figure S12. Atomic % of carbon (black) and nitrogen (red) in lyophilized CACDs after exposure to a dose of $0-1.1 \times 10^{-8}$ M*min •OH as determined with XPS.

Figure S13. ATR-FTIR spectrum of glutaric acid (black) compared to lyophilized MACDs exposed to $5.5 \times 10^{-9}$ M*min •OH (red). Dotted line at 1680 cm$^{-1}$ marks the C=O stretch of a carboxylic acid group.
References


