Time-Resolved Photo-Degradation of Natural Colored Dissolved Organic Matter (CDOM) and Contaminants in Fresh- and Marine Waters using a Custom-Designed Photo-Degradation System

淡水および海水中の溶存有機物および汚染物質の光分解性の半連続的評価

Michael GONSIOR
マイケル ゴンジオール

Photochemistry plays a key role in the environmental fate of organic compounds regardless if they originate from natural or anthropogenic sources. For example, photochemical degradation of colored dissolved organic matter (CDOM) or photobleaching in aquatic system is the most important mechanisms in degrading light-absorbing molecules in natural systems. This naturally occurring CDOM can also facilitate the photo-degradation of contaminants of emerging concern (CECs), even when CECs are not directly susceptible to direct photolysis. We developed a custom-designed photo- degradation system that can precisely measure photo-degradation of organic compounds semi-continuously using time-resolved changes in optical properties. We can precisely measure photo-degradation kinetics, changes in apparent fluorescent quantum yields and the time-resolved production of optically active photo-metabolites.

光化学は環境中の自然由来・人為由来の有機化合物の挙動に対して重要な役割を果たしている。例えば，水系における蛍光性溶存有機物（CDOM）の光化学分解や光退色は，自然環境中における分子の光吸収による化学分解の最も重要な機構である。これらの自然界に存在するCDOMは，直接光分解を受けない新たな汚染物質（CEC：Contaminants of Emerging Concern）の光分解にさえ関与している。我々はCDOM（organic compounds）の光分解特性を正確に捉えるために，水中の溶存有機物に対して，光分解特性の継時的変化を半連続的に測定するシステムを開発した。我々は，独自設計した光分解システムを用いることで，光分解反応速度，みかけの蛍光量子収率の変化，および，光代謝生成物の時間変化を精度よく測定することができる。

Introduction

Chromophoric or colored dissolved organic matter (CDOM) is a substantial component of the dissolved organic matter (DOM) present in all aquatic and engineered systems. It has also been recognized as the major precursor pool to from disinfection by-products in drinking water and treated effluent. However, we still lack a fundamental understanding of the molecular composition of presumably hundreds, if not thousands of compounds that absorb in the ultraviolet (UV) and visible spectrum of sunlight. The absorption of light in most chromophores also leads to the formation of reactive oxygen species (ROS) and excited state DOM species that in turn can degrade the chromophore itself, but also can directly react or transfer energy to other compounds that are not directly susceptible to photolysis. It has been known for decades that solar irradiation is one of the most efficient pathways to degrade natural CDOM. It is now also acknowledged that high energy UV radiation can be used to degrade CECs more effectively when CDOM is present. However, it is not well understood what the most important photosensitizers are in CDOM.

Controlled photo-degradation experiments under natural sunlight have been previously limited due to the fact that samples needed to be optically thin to be able to determine a photon dose and hence to quantify photo-degradation. To further complicate such experiments, oxygen needs to be maintained at steady concentrations to form ROS that are integral in degradation pathways. The pH of a sample also strongly affects the absorbance and hence photo-degradation needs to be conducted under constant pH to achieve reproducible results. To overcome

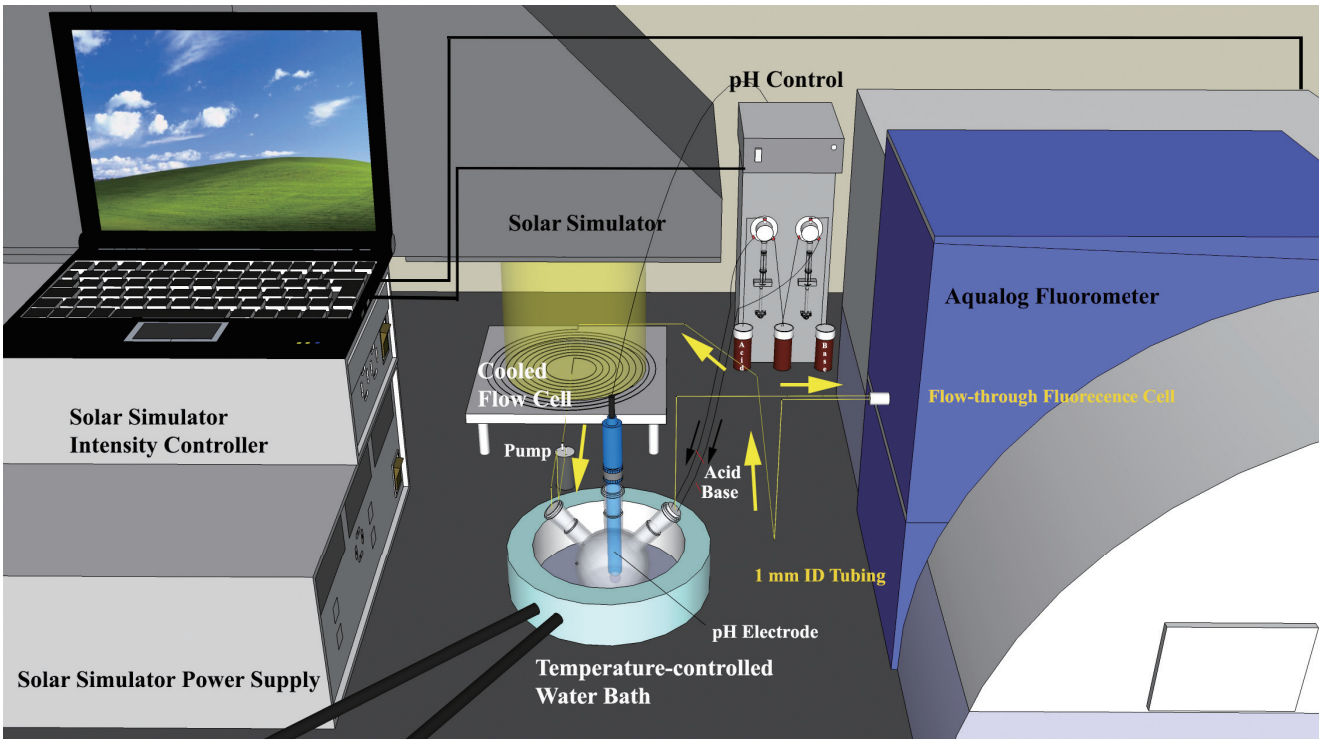

Figure 1 Custom-designed photo-degradation system.

the aforementioned constraints, we developed a photo-degradation system that addresses previous limitations.

Design of the custom-built photo-degradation system

The custom-built photo-degradation system was designed to avoid previous problems such as inner filtering effects also called self-shading, which would prevent the determination of reliable and dose-dependent photo-kinetic data. Another problem to overcome was the potential depletion of oxygen in closed systems. Hence, we developed a flow-through system that integrated an equilibrator, where equilibrium between air and solution is effectively achieved. Photochemical reactions are likely not effected strongly by small temperature changes, but we still needed to control the temperature in the system to undertake accurate measurements of optical properties. Furthermore, optical properties and as a result photochemical reactivity are highly pH dependent and hence pH also needed to be precisely controlled during light exposure experiments. Another challenge was the design of a flow-cell that holds sufficient volume but has a very small pathlength (1 mm) to avoid inner filtering effects over a large concentration range. To be able to simultaneously and fast undertake ultraviolet and visible (UV-Vis) absorbance and fluorescence measurements, we used the Horiba Aqualog fluorometer with CCD detector, which is to our knowledge the only instrument which is sufficiently fast and can simultaneously measure these optical properties. We chose to use a solar simulator that has a consistent illumination within its collimated beam (Oriel ABA solar simulator) and an intensity of 1 sun at 45°, at solar noon in summer. The solar simulator has an integrated air mass filter to match closely the irradiation reaching the surface of Earth. To be able to undertake long-term irradiation experiments, any shifts in light intensities were measured and adjusted in real time using a controller unit with a light sensor directly adjacent to the collimated beam. Absorbance and consequently fluorescence are highly pH dependent and hence pH would need to be controlled to achieve good reproducibility and to be able to compare samples with a different initial pH. The pH control was achieved using a J-Kem Scientific Infinity II pH controller attached to a J-Kem dual syringe pump (Series 2000). The pH was continuously measured with a pH probe and microinjections of acid or base were used to hold the pH within 0.1 pH units during the duration of experiments. Finally, the sample needed to be pumped continuously through the system to equilibrate with air, to be irradiated with a known dose and to measure the optical properties. Precise pumping was achieved using an micro annular gear pump manufactured by HNP Mikrosystems (HNPM, pump model mzr-4665). The layout of the instrumental setup is demonstrated in Figure 1.

Experiments

Photochemical experiments can be carried out with a sample volume of about 20 mL and sample is pumped inline continuously through the Peltier-cooled Hellma

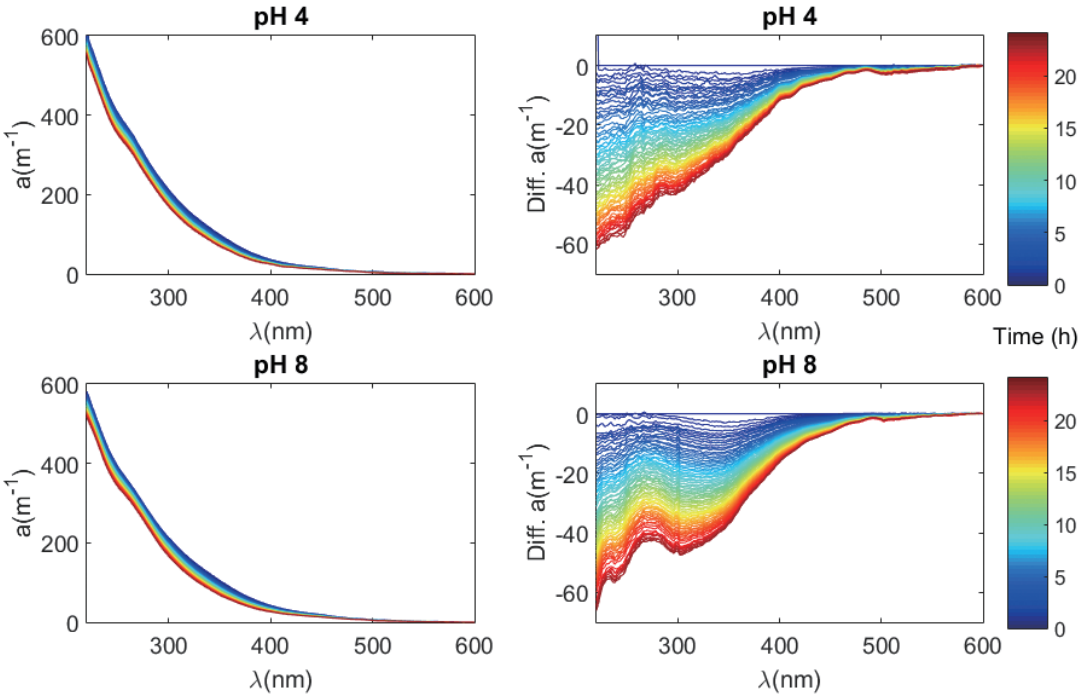

Figure 2 Photochemical pH dependency of CDOM in Suwannee River NOM Reference material (IHSS) during simulated sunlight exposure.

Analytics irradiation flow cell and through a fluorescence flow cell within the Horiba Aqualog Fluorometer. UV-Vis and excitation emission matrix fluorescence (EEM) spectra were recorded every 20 minutes during 24h irradiation experiments. The fastest recording settings would be every 2 minutes and no limit of exposure time. To showcase the effect of pH on the photo-degradation, we used an International Humic Substances Society (IHSS) reference material, Suwannee River Natural Organic Matter (SRNOM), and carried out experiments at pH 4 and 8 (Figure 2).

To demonstrate the benefits of time-resolved photo-degradation data, we also conducted an photo-degradation experiment of Phycocyanobilin, which is a phytochrome present in picocyanobacteria (*Synechococcus*). The photo-degradation of this chromophore produced strongly fluorescent photo-metabolites (Figure 3).

The photo-degradation kinetics can also be determined at individual wavelengths for the absorbance data (Figure 4), and we chose 600 nm excitation to show the photo-degradation of the parent compound and 270 nm excitation to determine the time-resolved photo-production of a still unknown photo-metabolite.

We can also model the fluorescence data using parallel factor analysis[1] and then determine the time-resolved photo-kinetics of individual statistical components determined by the model. A typical EEM-PARAFAC output is given in Figure 5a. The different components Fmax1-4 described well (99.7%) the changes in the fluorescence data during the course of the experiment. Component Fmax4 was indicative of the parent compound phycocyanobilin and the fluorescence at long wavelength quickly decreased during solar simulated irradiation (Figure 5b). Components Fmax1-3 were indicative of intermediate and stable fluorescent photo-products

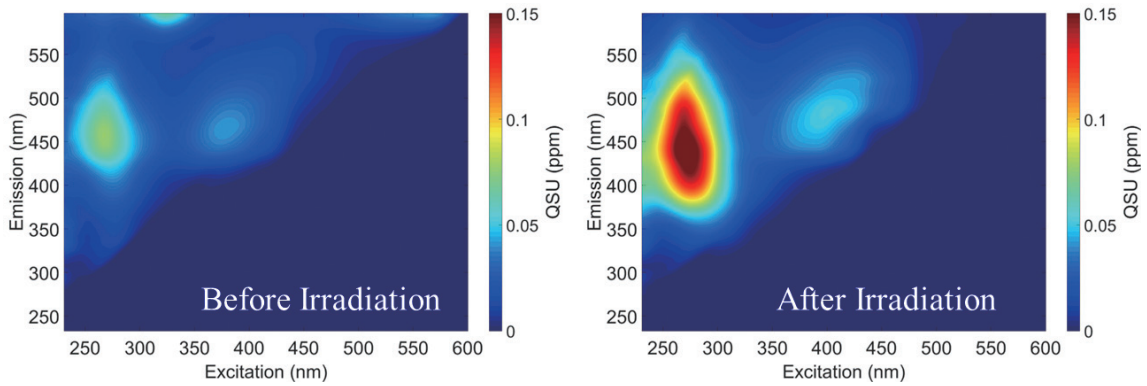

Figure 3 EEM spectra of phycocyanobilin before and after 24h solar-simulated irradiation.

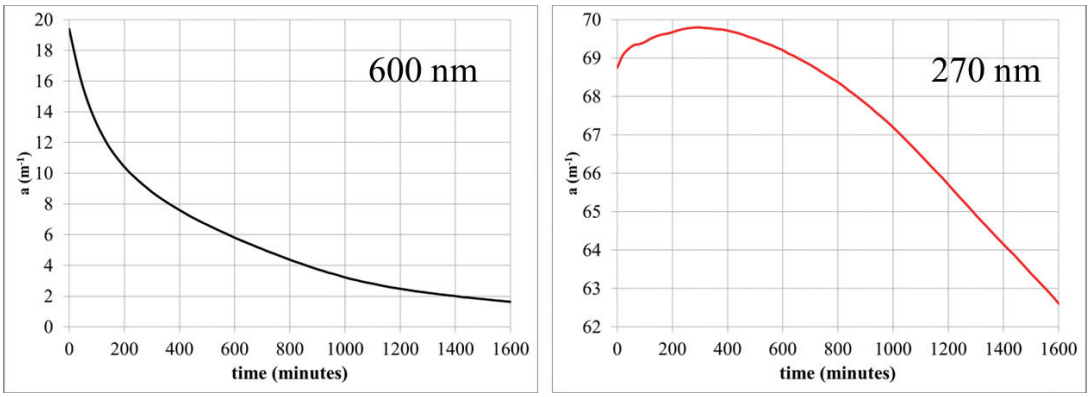

Figure 4 Photo-degradation of phycocyanobilin (monitored at 600 nm, left panel) and initial photo-production of unknown absorbing chromophores during first 300 min of irradiation (monitored at 270 nm, right panel).

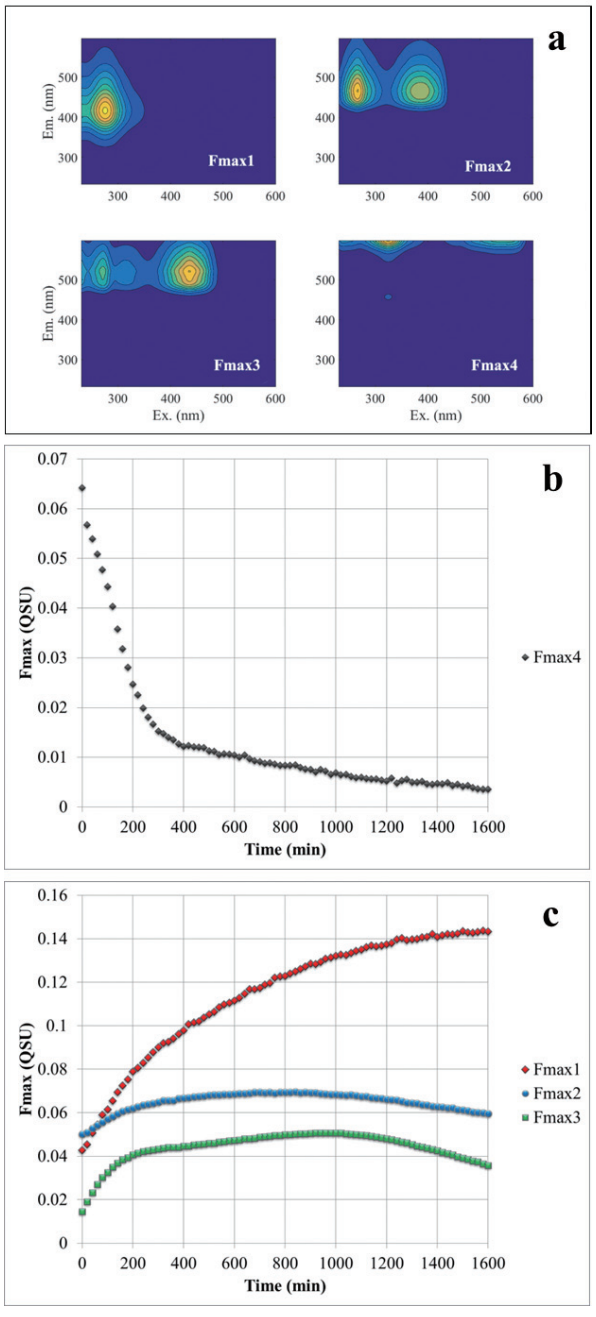

Figure 5 EEM-PARAFAC model and time-resolved changes in PARAFAC components Fmax1-4 during a 27 h photo-degradation experiment.

(Figure 5c). Such analysis of intermediate changes of chromophores would not have been possible without the custom-designed photo-degradation system and a fast scanning fluorometer.

Conclusion

The custom-designed photo-degradation system generated highly reproducible data while avoiding inner filtering effects and oxygen depletion and shifts in pH. The benefits of such a system were demonstrated using a known phytochrome, phycocyanobilin to demonstrate the advantages of time-resolved photo-degradation studies and the simultaneously measurements of absorbance and fluorescence properties of the analytes. The system can accurately determine photo kinetic data of natural CDOM and other chromophores or can be used to monitor the degradation of CECs, when combined with quantification of target compounds during photo-degradation experiments. Future work will include the determination of photochemical fate of disinfection by-products, the discovery of photo-products arising from phytochromes and the photo-ammonification efficiency of dissolved organic nitrogen in natural waters and wastewaters.

References

[1] Murphy, K. R.; Stedmon, C. A.; Graeber, D.; Bro, R. "Fluorescence spectroscopy and multi-way techniques. PARAFAC", *Analytical Methods*, 2013, **5** (23), 6557-6566.

Michael GONSIOR
マイケル ゴンジオール
Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science
Assistant Professor
Ph.D.