An Overview of Aquatic Photochemistry as it Relates to Microbial Production

W. L. Miller

Department of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada

ABSTRACT

The field of natural water photochemistry has evolved over the last 20 years from a novelty to a necessity in the study of environmental chemistry in natural surface waters. Research on the significance of photochemistry to secondary biological production has recently expanded interest in both marine and freshwater systems. In a nutshell, we know that sunlight-induced alteration of chromophoric dissolved organic matter (CDOM) results in reduced average molecular weight, changes in water optical properties, and in the production of a complex mixture of reactive oxygen species, inorganic nutrients, and carbon photoproducts. The largest carbon product results from the direct photo-mineralization of dissolved organic carbon (DOC) to dissolved inorganic carbon (DIC), a bypass of the microbial web. Also produced are a suite of low molecular weight compounds which may affect microbial growth. Papers published in the last three years have noted; (1) growth enhancement of heterotrophic bacteria in natural samples exposed to sunlight; (2) lack of bacterial growth stimulation by photochemistry and; (3) a reduction in the ability of DOC to support bacterial growth after being exposed to UV-B radiation. In light of recent interest and conflicting results concerning the interaction between photochemical and biological processes in natural waters, this paper will attempt a review of some fundamental photochemistry and its potential impact on microbial processes in natural waters.

Introduction

Bacterial production has often been reported to depend, at least in part, on DOC concentrations and availability [8]. While a more complete model of microbial regulation also includes temperature, inorganic nutrients, and bacterial mortality, it is clear that any process influencing the fate and chemistry of DOC will provide a feedback to biological production through the microbial loop. Sunlight-induced organic photochemistry represents one such process.

Recent research has shown that the photochemical alteration of DOC in fresh and marine waters is a significant step in organic carbon cycles. It is well documented that natural photochemical processes result in a reduction of DOC average molecular weight, changes in water optical properties [10], and in the production of reactive oxygen [2] and carbon photoproducts, many serving as biological substrates [15]. Based on these reports of low molecular weight carbon compounds originating from biologically refractory DOC, there has recently been a great deal of interest in photochemistry as a significant chemical reaction relative to secondary biological production and carbon availability.

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Moran and Zepp [15] reviewed the literature on biologically available compounds generated by photochemical reactions involving CDOM. They list seven separate studies that noted growth enhancement of heterotrophic bacteria in natural samples exposed to both natural and artificial sunlight. Conversely, Amon and Benner [1] saw no significant stimulation of bacterial growth in irradiated samples from the Amazon even though sunlight treatment resulted in photochemical O_2 consumption and loss of DOC; both good indicators of photochemical reactivity. Naganuma et al. [16] showed a *reduction* in the ability of DOC (0.5% peptone solutions) to support bacterial growth after being exposed to UV-B radiation. It is not clear whether these results arise from differences in CDOM, the microbial community, or methodology, but the conflicting results suggest that an abbreviated review of some fundamental photochemistry and its potential impact on microbial growth may be useful. The focus here, as was the focus of the special symposium, is on the breakdown and alteration of DOC. A more lengthy discussion of photochemical fundamentals and their relation to experimental design can be found in Miller [11]. The direct photochemical effects on the microbial community (DNA damage, etc.) are not considered.

Some Photochemical Fundamentals

Absorption of radiation

The first law of photochemistry, attributed to Grotthus and Draper in the early 1800s, states that only absorbed radiation is effective in producing photochemical changes. This means that the absorption of incident radiation is *the* fundamental event behind all environmental photochemistry. If no light is absorbed, no photochemistry occurs. An additional requirement is that the light absorbed must provide enough energy to produce chemical reactions, either from direct bond breakage or electron activation leading to reactions with other chemicals in solution. For sunlight initiated photochemistry, this usually requires radiation contained in the UV-B (290-320 nm) and UV-A (320-400 nm) portions of the spectrum.

For most fresh and marine waters, the complex mixture of chromophoric (or coloured) dissolved organic matter dominates the absorption of solar radiation in the UV spectral region. While we have little direct knowledge of the molar concentration of specific chromophores, it is in this role as "light gatherer" that CDOM provides the pivotal physical event that drives environmental photochemistry. As a mixture of chromophores, CDOM absorbs light over a broad wavelength band and exhibits a featureless exponential decrease in absorbance from the UV to the visible portion of the spectrum. The radiation absorbed at any wavelength can usually be fit to an equation of the form

$$a_\lambda = a_r \, 10^{[S(r-\lambda)]} \qquad (1)$$

where a_λ and a_r are the absorptivities (m^{-1}) at wavelength λ (nm) and some reference wavelength (r). Absorptivity is related to the absorbance measured in a spectrophotometer (A_λ) using the relationship

$$a_\lambda = A_\lambda \, 2.303 \, l^{-1}, \qquad (2)$$

with spectrophotometric pathlength (l) expressed in meters. This convention eliminates confusion involving reports of A_λ using cells having various pathlengths and allows more direct integration into *in situ* optical models. The parameter S is the slope of the log-linearized spectrum, a measure of the rate at which absorptivity decreases at longer wavelengths.

Diverse natural surface waters show a range of S values [20, 3, 6]. An apparent gradient of S from marine to freshwaters [$S_{(marine)} > S_{(fresh)}$] suggests that variations in S might be explained as the mixture of specific end members with different values for S. Recent results, however, indicate this relationship is likely to be more complicated, with photochemical conditioning also contributing to variations in S [19].

Rates

Much of the interest in photochemical reactions associated with aquatic DOC concerns the rate at which biologically labile carbon products are formed and/or the rate of change for the chemical characteristics of the remaining organic molecules. Consequently, an understanding of the rate at which the microbial community oxidizes carbon in sunlit waters may require direct knowledge of photochemical kinetics.

In simplest terms, the rate of any photochemical reaction can be expressed by

$$\frac{\mathrm{d[P]}}{\mathrm{d\,t}} = \sum_{\lambda} \Phi_\lambda E_{a\lambda} \qquad (3)$$

where [P] is the measured molar concentration of the reactant or product, t is time in seconds, Φ_λ is the quantum efficiency of the photochemical reaction in question (moles product per mole photons absorbed), and $E_{a\lambda}$ is the average photon absorption rate (moles photons absorbed l^{-1} s^{-1}). Note that the quantity E_a is not equivalent to incident light measured with a radiometer, E_o, which is usually reported in units of energy flux per area (e.g. W cm^{-2}). Conversion of E_o to E_a requires knowledge of the absorptivity (a_λ, defined above) and irradiation geometry (surface area, S, and volume irradiated, V). While other components (particles, non-reactive molecules, and the water itself) are capable of competing for the radiation present in solution, we can assume that CDOM absorbs essentially all UV radiation responsible for photochemistry. This allows the relationship between E_a and E_o to be described using the equation

$$E_{a\lambda} = E_{o\lambda}\left(1 - 10^{-\left(\frac{a\lambda l}{2.303}\right)}\right)\frac{S}{V}. \qquad (4)$$

Strictly speaking, this equation applies only to homogenous reactions and requires that a_λ reflect non-particulate absorptivity (i.e. 0.2 μm filtration). Extrapolation to *in situ* photochemical calculations requires additional information on the absorption of CDOM relative to other components that contribute to total attenuation measurements.

Equations (3) and (4) show clearly that photochemical rates are described predominantly by only three parameters: (1) the light present; (2) the portion of light

absorbed by the chromophore giving the product and; (3) the efficiency with which absorbed light results in a product, Φ. Changing any one of these parameters will change the production rate by an equivalent factor. When interpreting experimental results or making calculations for *in situ* photoproduction rates, it is critical to explicitly quantify each of these three parameters.

DOM Transformation and Feedbacks

Much of the DOM pool in natural waters is not available to the microbial community over time scales from hours to weeks [14]. These "refractory" compounds, often referred to as humic substances, usually make up a large part of the CDOM. Photochemistry can change the rate at which the microbial community consumes these refractory organic substances either through the production of fresh, consumable carbon compounds or by alteration of the refractory nature of the remaining DOC.

As might be expected for a complex organic mixture such as CDOM, numerous carbon compounds are produced during sunlight-induced photodegradation. Moran and Zepp [15] have reviewed the photochemical production of potential biological substrates in marine and freshwater environments. Identified products include 14 different low molecular weight carbonyl compounds (~ 50% by carbon content) and carbon monoxide (~ 50%). Using published photochemical production rates for these compounds, they estimate that more than 20% of the bacterial carbon demand in a continental shelf system might be supplied by sunlight initiated breakdown of CDOM. This may be an overestimate since some products such as oxalate and pyruvate can in turn be broken down photochemically. An additional photochemical breakdown product of CDOM is DIC (carbonate, bicarbonate, and CO_2), representing direct mineralization of DOC [17, 12, 5]. In fact, DIC formation rates are 15 to 20 times higher than those for CO [12, 13]. While not a microbial substrate, DIC is the largest identified carbon product from photochemical breakdown of CDOM and may represent a significant bypass of the microbial loop for this portion of the organic carbon pool.

As mentioned above, the other possible result of the photochemical oxidation of CDOM is a fundamental change in the refractory nature of the larger carbon molecules that cannot be measured as "identifiable" carbon products. In fact, this process may provide a larger source of consumable DOC than the sum of all the identified products listed by Moran and Zepp [15] taken together. Miller and Moran [13] used a mass balance approach to examine the relationship between photochemical carbon release and microbial growth. The two largest carbon photoproducts, DIC and CO, where quantified directly in filtered, irradiated samples from a coastal saltmarsh. The carbon contained in these products was compared to the carbon converted to biomass and respired in these same samples after receiving an inoculation of the resident microbial community. Using the measured CO photoproduction with the 50:50 production ratio from the compilation of Moran and Zepp [15], this study concluded that increased consumption of carbon from the larger, remaining molecules accounts for about 90% of the observed increase in microbial biomass compared to an estimated 10% from the suite of "identified" microbially labile compounds. Extending the modeling logic of Moran and Zepp [15], it is possible that essentially all of the bacterial carbon demand in a continental shelf system could be supplied by sunlight initiated breakdown and modification of CDOM. Since the direct production of consumable detrital

material and DOM by plankton is a known source of carbon to the microbial community, it is not reasonable to attribute the entire carbon supply to photochemical breakdown and conditioning of refractory molecules. This estimate demonstrates, however, that photochemical "conditioning" may be the rate-limiting step in the eventual biological breakdown of dissolved refractory carbon.

Quantitative evaluation of the significance of photochemical reactions involving CDOM in aquatic systems and the resulting effects on the microbial growth is a complex undertaking. As shown in Fig. 1, most of the CDOM molecular characteristics that control photochemical reactions are subject to modification by the photoreactions they control. For this reason, careful consideration is required for interpretation of photochemical results. Recall that the initial event in photochemistry is the absorption of a photon. Consequently, the quality and intensity of the radiation field along with the absorptive properties of CDOM are critically important. Quantifying these two parameters is difficult, however, since UV spectral irradiance is hard to measure and the optical characteristics of CDOM absorptivity change as photochemical reactions proceed.

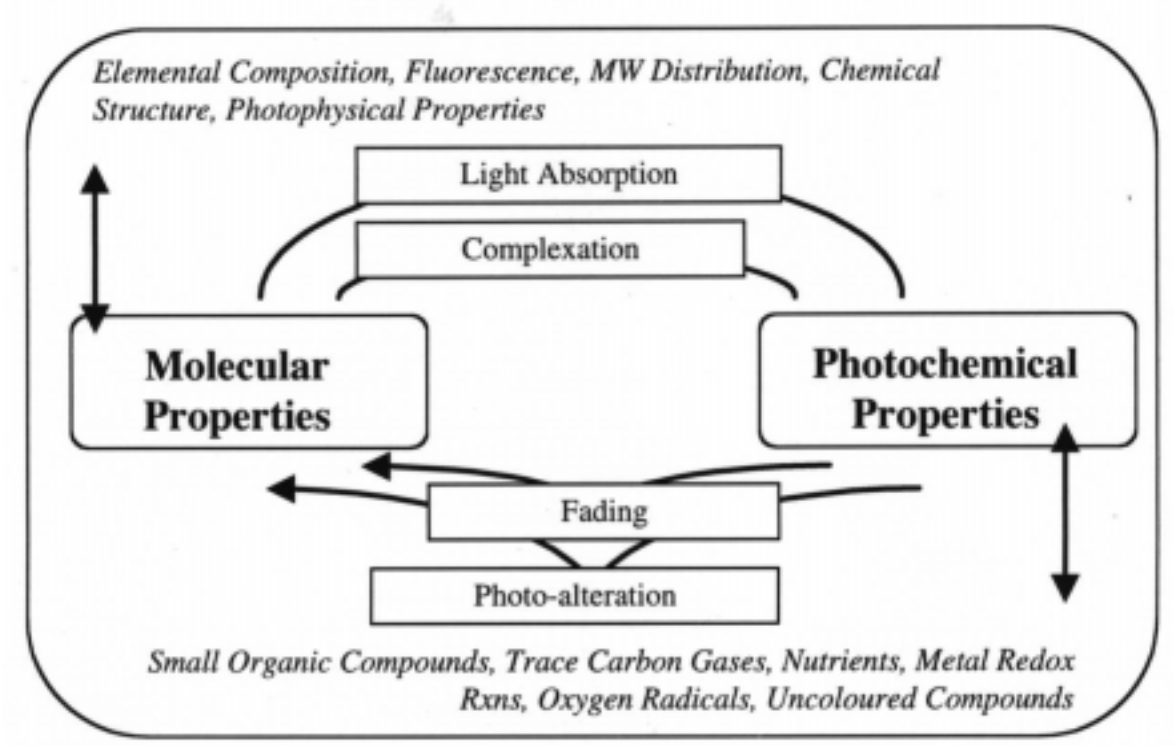

Fig. 1. Relationships and feedbacks for CDOM molecular properties and photoreactivity.

Controlled exposures show that sunlight causes an eventual loss of CDOM colour across the entire absorbance spectrum [9, 7], a process known as photochemical fading or photobleaching. This phenomenon has long been reported in laboratory exposures and recent field data suggest that the effect is directly observable in surface waters [4, 18, 19]. Since fading occurs at all wavelengths and at different rates for each wavelength depending on the spectral quality of absorbed radiation, it is difficult to model. Nonetheless, it is obvious that when fading does occur, it provides a negative feedback to photochemical rates in the environment. The fewer photons absorbed the less potential for

photochemistry. While the magnitude and rate of fading in open water is still under investigation, most studies indicate that fading proceeds faster than DOC loss. Study of DIC production [12] indicates that only about 15% of the DOC would be converted to DIC before the absorbance at 350 nm faded to essentially zero.

Clearly photochemical reactions involving CDOM are important to microbial processes in natural waters. Careful attention to fundamental relationships will provide future insight into the relationship. A clear, quantitative understanding of the association will require continued collaboration between microbial ecologists and physiologists, optical modelers and photochemists.

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