

Production of Nitrite from the Photodegradation of Dissolved Organic Matter in Natural Waters

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Significant concentrations of nitrite NO_2^- were produced from the photodegradation of humic substances (HS) isolated from a variety of natural waters in coastal North Carolina. Nitrite concentrations were 40–118% higher after light exposure relative to initial levels, while no statistical differences were observed in dark controls before and after irradiation. The amount of nitrite produced upon irradiation was positively correlated to the concentration of HS added ($r = 0.97$, $p < 0.001$) and to the length of irradiation. The average production rate, normalized to both humic substance concentration and time of light exposure, for all HS studied ranged from 9.7 to 17 [$\text{nM (W-h)}^{-1} \text{m}^{-2} \text{mg}^{-1}$] $\times 10^5$. This translates into an average yearly nitrite production of $2 \times 10^6 \text{ mol yr}^{-1}$ in the top one meter of Onslow Bay. When natural waters were irradiated, changes in nitrite were influenced by both initial nitrite and humic substance concentrations. The rate of nitrite photochemical formation in typical coastal waters (4 nM h^{-1}) was smaller than its direct photolysis rate (23 nM h^{-1}) and smaller than reported rates of ammonium generation via DOM photodegradation (50 nM h^{-1}) and of the same order of magnitude as NH_4^+ incorporation during humification (1 nM h^{-1}). The photochemical release of biologically available nitrite from biologically refractory humic substances has significant implications with respect to nitrite biogeochemistry and N cycling in natural waters and suggests HS bound N is more biogeochemically labile than previously thought.

Introduction

Nitrite plays a crucial role in many significant chemical and biological processes occurring within natural waters and is a general indicator of events which drive the nitrogen cycle. It is an obligatory and stable intermediate in the assimilation of nitrate (NO_3^-) by phytoplankton and in the recycling back to the euphotic zone of new nitrogen from the deep ocean through bacterial nitrification of dissolved and particulate organic nitrogen. Trace amounts of nitrite in environmental samples can also indicate the extent of pollution and eutrophication of many natural waters.

Despite the direct involvement of nitrite in several biogeochemically significant processes, important questions still remain regarding the mechanisms responsible for its occurrence in natural waters. One potentially significant input of nitrite, which has not been studied, involves aquatic humic substances (HS), which make up a general class of biogenic,

high molecular weight organic matter containing between 3 and 6% nitrogen. Recent evidence has shown that the N within HS may undergo recycling and act as both a source and sink for ammonium in natural waters (1–4). We suggest that HS may also act as an important, unquantified source of nitrite in a manner similar to what has been observed for ammonium. This would represent a potentially important nonbiological input of nitrite, which would significantly impact our understanding of NO_2^- biogeochemistry in natural waters. Because this process represents the formation of a biologically available substrate (nitrite) from biologically unavailable HS, it also represents an important mechanism for the turnover of dissolved organic matter (DOM) in natural waters and may explain some of the enhancement of primary productivity caused by HS additions reported earlier (5–9). This mechanism would also suggest that humic substance bound nitrogen is more labile than previously reported and that these earlier studies underestimated the importance of HS as a photochemical precursor of inorganic nitrogen in natural waters.

Experimental Section

Sampling. Samples of coastal, estuarine, and freshwater were collected from various locations in eastern North Carolina. The following three sampling locations were within approximately 5 miles of Wilmington, NC ($33^\circ 9' \text{ N}$, $77^\circ 9' \text{ W}$); Cape Fear River, UNC–Wilmington Center for Marine Science Research, Myrtle Grove Laboratory, and a pocosin located on the UNC–Wilmington campus. Water was also collected from the Albemarle Estuarine System, NC ($35^\circ 9' \text{ N}$, $76^\circ 0' \text{ W}$). Water was collected in 4 L Teflon capped glass bottles prerinsed with sample at least three times prior to collection. Samples were immediately filtered through $0.7 \mu\text{m}$ glass fiber followed by filtration through $0.2 \mu\text{m}$ membrane filters under gentle vacuum ($< 200 \text{ mmHg}$). Humic substances were extracted from filtrates within 1 h of collection. Photolysis experiments were also conducted within 1 h of sample filtration. Water samples were characterized at the time of collection by temperature, salinity, and pH following standard techniques.

Photochemical Studies. A variety of photolysis experiments were performed on filtered sterilized natural water samples to measure production of NO_2^- from the photodegradation of humic substances. All experiments were conducted for 6 h, on sunny days between 10 and 4 Eastern Standard Time. In the first, humic substances were extracted from four separate aquatic ecosystems in southeastern North Carolina, spiked to a final concentration of 40 mg L^{-1} in Gulf Stream seawater and photolyzed under ambient sunlight for 6 h. A second set of identically prepared flasks were covered in aluminum foil to serve as photochemical controls and irradiated in the same manner as the light exposed flasks. The biota were purposefully removed from the sample matrix to make interpretation of results more clear. Each flask was sampled in quadruplicate, initially and after irradiation for NO_2^- concentration. All samples were thermally controlled by placing them in a constant temperature water bath ($\sim 28^\circ \text{C}$). Light doses integrated over the course of irradiations were measured using a radiometer with a broad band silicon detector equipped with a quartz eye diffuser head giving a response window of 250–1000 nm (Model IL 1700, International Light, Newburyport, MA). In addition to HS spiked samples, a variety of filter sterilized ($0.2 \mu\text{m}$) natural water samples were also photolyzed. Flasks were again sampled for initial and 6 h NO_2^- concentrations in an analogous manner to the HS spiked flasks. Deoxygenated samples were

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prepared by bubbling the water in the flasks with nitrogen gas for 2 h.

Humic Substances Extraction. Humic substances were extracted from natural water samples following the method of Amador et al. (12), using C₁₈ cartridges (Waters Chromatography, Milford, MA). Filtered water samples were acidified to pH 2.2–2.5 with 0.3 M HCl and loaded onto C₁₈ cartridges which had been previously washed with 20 mL of methanol followed by 20 mL deionized water. Samples were pulled through C₁₈ cartridges by a dialysis pump (Model 7553-30, Cole-Parmer, Chicago, IL) at a flow rate of 3.5 mL min⁻¹. Salts and any ambient nitrite adhering to the cartridge were washed off with extensive rinsing with DI water (40 mL). Humics were eluted with 5 mL of methanol into a 100 mL conical flask and concentrated to dryness under reduced pressure (Buchi Rotavapor, Model RE 111, Switzerland). Traces of water were removed under vacuum (Sargent-Welch Model 1400, Skokie, IL), and the resulting humic powder was stored in 7 mL Teflon vials at -20 °C.

The C₁₈ humic substance extraction technique was chosen over more conventional methods using XAD resins because C₁₈ is more efficient at removing the chromophoric humic substances from seawater which would be of most interest in our photochemical studies. Extraction of humic substances using C₁₈ cartridges has been reported to be between 22 and 84% more effective relative to XAD-2 (12). Recovery of aquatic fulvic acids using C₁₈ solid-phase extraction was also reported to be 83% (13). In addition, C₁₈ extraction is better able to retain the UV-visible and fluorescence characteristics of the isolated humic material relative to XAD (12). Since these were the characteristics of most interest in this study, C₁₈ extraction was the most logical choice for an extraction procedure. A second advantage of using C₁₈ extraction is that methanol is the eluant rather than 0.1 M NaOH. Therefore any degradation due to high pH is avoided. In addition, the potential for N addition to HS by NH₄OH during extraction is not an issue with the C₁₈ cartridge since ammonium hydroxide is not used in the extraction procedure but is occasionally used for elution of humic substances from XAD (12). In our studies, for XAD extraction, filtered water samples were acidified to pH 2.0 with 0.3 M HCl and loaded onto preconditioned XAD-2 columns. Humic substances were eluted with 60 mL of 0.1 M NaOH, neutralized with 0.3 M HCl, and concentrated to dryness.

Nitrite Analysis. Nitrite concentrations in water samples were determined by reacting the analyte with 2,4-dinitrophenylhydrazine reagent followed by HPLC analysis (10). In this rapid and extremely sensitive method, filtered samples and standards (5 mL transferred to 7 mL Teflon vials) are reacted with 2,4-dinitrophenylhydrazine reagent (50 μL) for at least 20 min in the dark to form an azide, which is separated from interfering substances by HPLC and quantified by UV detection at 308 nm. This method was chosen over the standard colorometric technique, formation of a diazo dye from the reaction of NO₂⁻ with *N*-1-naphthylethylenediamine and sulfanilamide (11), because of its lower limit of detection (0.1 nM) and better average precision of 4% RSD. Also, it has undetectable blanks, and the derivatized samples are stable for at least 4 weeks when kept cold in the dark. In addition, there are no known interferences in the analytical method in natural waters. This is important because earlier research has shown carbonyl compounds are photochemically produced from humic substances (17). These analytes, however, elute several minutes after NO₂⁻ and are therefore not affecting the quantification of the nitrite signal (10).

Reagents and Standards. All chemicals were obtained from Fisher Scientific (Fair Lawn, NJ) or Aldrich Chemical (Milwaukee, WI) and were HPLC grade or certified A.C.S., unless otherwise noted. 2,4-Dinitrophenylhydrazine (2,4-DNPH) was recrystallized twice from a 70:30 mixture of

acetonitrile and water (vol:vol) followed by a final recrystallization from pure acetonitrile. The reagent was dried under vacuum and stored in the dark in airtight 30 mL Teflon vials. The derivatizing agent was prepared in 30 mL Teflon vials by dissolving 20 mg of recrystallized 2,4-DNPH in 15 mL of concentrated HCl (~12 M), water, and acetonitrile in a ratio of 2.5:1 (vol:vol:vol). Just prior to use, nitrite contamination contained within the 2,4-DNPH reagent solution was removed with successive extractions with distilled (in house) carbon tetrachloride (CCl₄). CCl₄ (2 mL) was added to the reagent solution and shaken on a wrist action shaker for 5 min followed by centrifugation at 2000 rpm for 5 min in order to separate the phases. The organic layer was removed, and the reagent solution re-extracted twice more as described. The reagent solution was stable for one week after initial preparation. On subsequent days after initial preparation, the purified reagent was re-extracted once just prior to use. Stock solutions of nitrite (1000 mg/L N) were serially diluted to concentrations typically found in natural waters and were prepared immediately prior to use.

HPLC Instrumentation. Derivatized samples, injected into a Rheodyne six-port injector (Model 7125, Rheodyne Inc.) with a 2000 μL sample loop, were separated isocratically (40% aqueous acetonitrile, 2 mL/min) at ambient temperatures on an 8 mm i.d. × 20 cm C₁₈ reversed phase Radial PAK cartridge housed in a Radial compression module (Waters Chromatography Division, Model 8NVC184, Milford, MA). The mobile phase was generated by a Waters Association chromatography pump (Model 6000A) with samples detected by a Waters AZ tunable absorbance detector (Model 481) at 308 nm. Signals were integrated on a Model SP4270 Spectra-Physics integrator (Spectra-Physics, San Jose, CA).

Results

To determine if humic substances could act as a source of nitrite to natural waters, a series of photolysis experiments were conducted. Significant concentrations of nitrite were produced from the photodegradation of all HS studied (Table 1). Nitrite concentrations were 40–118% higher after light exposure relative to initial levels, while no statistical differences were observed in dark controls before and after irradiation (*t*-test, *p* < 0.001). The largest increases of NO₂⁻, both in terms of total nM produced and percent increase over initial levels were from estuarine humic substances collected from the Albemarle Sound and the Cape Fear estuary, while relatively lesser amounts were produced from coastal seawater HS collected from Wrightsville Beach, NC and freshwater HS of a marsh found on the University of NC at Wilmington campus. The average rate of production in all waters studied was 4 nM h⁻¹. The Gulf Stream seawater used to prepare the humic substance samples was also photolyzed as a matrix control. There was no statistically significant difference between the light and dark Gulf Stream samples before and after irradiation, indicating the observed changes were a result of the added HS and not an artifact of the sample matrix.

To further investigate the mechanism of photochemical production of nitrite from HS and to determine if production is dependent on the amount of humic substance added, a second photochemical experiment was performed with HS isolated from the Cape Fear estuary. In this study the amount of HS spiked into the Gulf Stream seawater was varied in four separate flasks during a 6 h irradiation. Two additional flasks were wrapped in Al foil, which again acted as a photochemical control. The results of this experiment are shown in Figure 1, where the difference in nitrite concentration before and after irradiation is represented on the *y* axis as NO₂⁻, with positive numbers representing net increases after photolysis relative to initial levels. The amount of nitrite produced in the light exposed flasks was positively correlated

TABLE 1. Initial and Final Concentration of Nitrite (nM), Light Intensity, and Date of Exposure in Irradiated and Dark Control Flasks during a 6 h Photolysis Study of 40 mg L⁻¹ Extracted HS^a

	coastal seawater		Albemarle sound		marsh		Cape Fear Estuary		Gulf Stream blank	
	light	dark	light	dark	light	dark	light	dark	light	dark
initial NO ₂ ⁻ (nM)	21.3 ± 2	20.9 ± 2	34.1 ± 1	38.2 ± 4	29.1 ± 2	28.1 ± 0.1	29.4 ± 0.7	30.1 ± 0.8	22.4 ± 7	26.6 ± 0.6
final NO ₂ ⁻ (nM)	29.9 ± 0.3	21.2 ± 0.2	74.2 ± 1	38.3 ± 1	40.8 ± 3	31.3 ± 3	58.8 ± 3	30.8 ± 0.9	21.6 ± 1	27.0 ± 2
% increase	40.00	1.40	118.00	0.30	40.00	11.00	100.00	2.30	<0	1.50
light dosage (watts-h m ⁻²)	1.16E4		1.54E4		1.54E4		1.16E4		9.4E3	
rate nM h ⁻¹	1.40		6.70		1.90		4.90			
date	May 23		May 31		May 31		May 23		Sept 24	

^a Each data point represents the average of four samples taken from an individual quartz flask. The percent increase in the light exposed flask after irradiation is relative to initial levels.

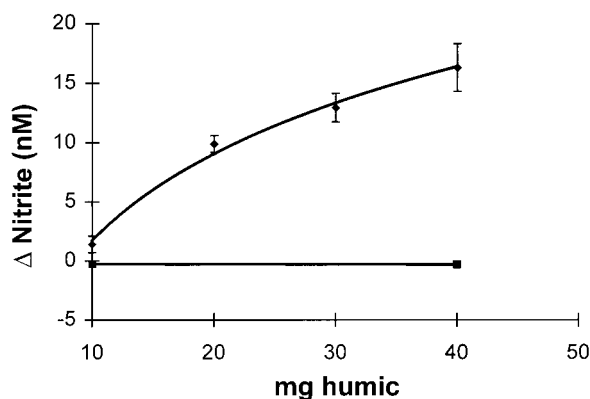


FIGURE 1. Changes in nitrite (nM) as a function of humic substance added (mg humic) in Gulf Stream seawater after a 6 h irradiation. Each data point represents the average of four replicate with Δ representing the difference between initial and 6 h irradiated values. Filled diamonds represent light exposed samples, while filled squares represent dark control samples. Error bars represent one standard deviation based on the replicates.

to the concentration of added humic substance ($r = 0.97$, $p < 0.001$). No statistical difference between initial and final levels was observed in the dark controls. These results suggest that as the amount of HS precursor is increased, up to at least 40 mg L⁻¹, the quantity of nitrite produced also increases. The downward curvature in this relationship most likely results from light attenuation in the more highly colored (30 and 40 mg L⁻¹) humic substance additions as samples became more optically thick.

In another series of photochemical studies, the impact of irradiation time on nitrite production was investigated. Cape Fear HS were again isolated and added to Gulf Stream seawater to a final concentration of 30 mg L⁻¹. In this study, an aliquot from each of four flasks was withdrawn initially and at 2 h time intervals over a 6 h irradiation period. The concentration of nitrite increased, relative to initial levels, as a function of irradiation time, with the majority (80%) of the increase occurring within the first 2 h (Figure 2). No statistically different changes were observed in dark controls.

To evaluate the role of dissolved O₂ on photolysis, samples of HS spiked Gulf Streamwater were prepared as O₂ saturated and deoxygenated and irradiated for 6 h in natural sunlight. The NO₂⁻ changes were statistically equivalent ($t = 2.66$, $d_f = 4$, $p < 0.01$) in the deaerated and O₂ exposed flasks, suggesting O₂ or oxygen generated radicals are not critical reactants in these photolysis experiments.

The results presented in Figures 1 and 2 suggest that photoproduction of nitrite is related both to the concentration of humic substances and irradiation exposure time. Additional samples of coastal, estuarine, and freshwater HS were isolated from the same locations presented in Table 1,

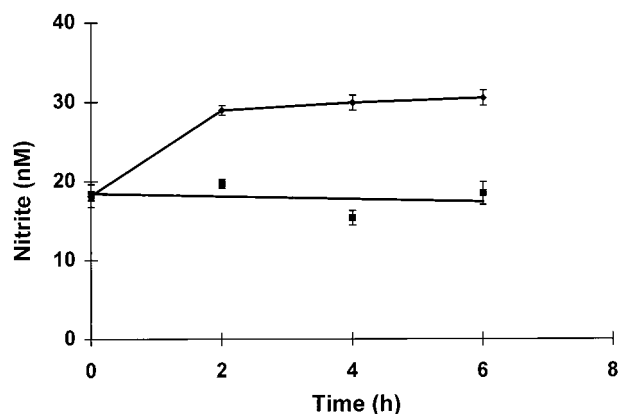


FIGURE 2. Changes in nitrite (nM) as a function of irradiation time with each data point representing the average of four aliquots. Filled diamonds represent light exposed samples, while filled squares represent dark control samples. Error bars represent one standard deviation based on the replicates.

TABLE 2. Average Photochemical Production Rates of Nitrite Normalized to Exposure Time, Light Intensity, and mg Humic Substance Added during 6 h Irradiations^a

humic sample	prodn rate ^b [nM (W h) ⁻¹ m ⁻² mg ⁻¹] × 10 ⁵	pH	salinity	UV at 300 nm (AU)
Albemarle ($n = 2$)	17.00	5.60	1.00	1.02
Cape Fear ($n = 5$)	6.70	7.40	13.00	0.21
coastal seawater ($n = 5$)	9.70	7.90	26.00	0.08

^a Also presented are the physical parameters of the water at time of collection with (n) representing a separate humic substance extraction and photolysis study for each sample site. ^b Production rates were calculated by taking the nM nitrite produced in 6 h and dividing by the amount of HS added, the time, and the total solar irradiance between 200 and 1000 nm.

added to Gulf Stream seawater, and irradiated for 6 h (Table 2). Average rates of nitrite production over 6 h were calculated for three natural HS and were normalized both to exposure time, irradiation dose, and amount of added humic substance. Rates of nitrite production ranged from 9.7 to 17 [nM (W-h)⁻¹ m⁻² mg⁻¹] × 10⁵ in the samples tested. The two estuarine humic substances from the Cape Fear and coastal seawater had very similar production rates, while HS samples collected from the lower pH more optically thick Albemarle sound, had a somewhat higher production rate. It is possible that there are more terrestrial derived humic substances in the very low salinity Albemarle which may be more photoactive toward nitrite production, relative to the mix of marine and terrestrial HS likely to be found in the higher salinity samples, although more research is required to test this hypothesis.

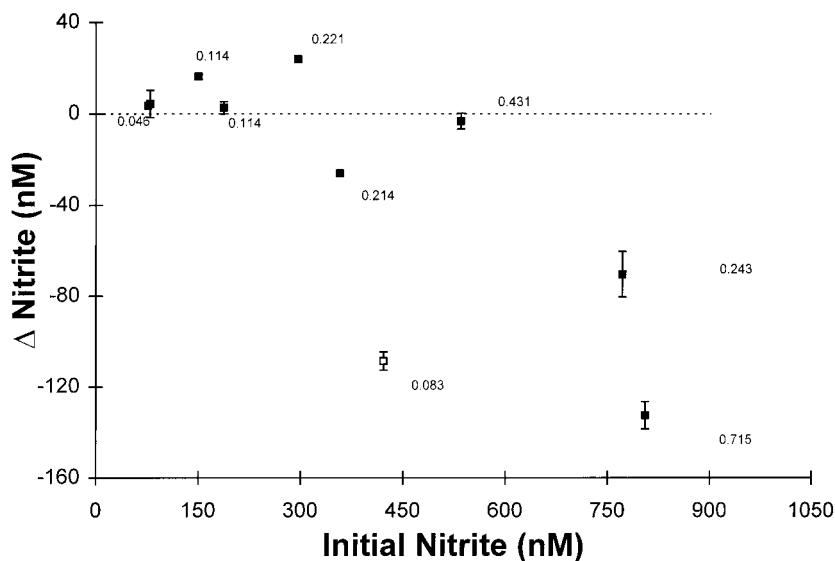


FIGURE 3. Change in nitrite (nM) as a function of initial nitrite (nM) concentration in 6 h natural sunlight photolysis of unspiked, natural coastal, and estuarine water. Corresponding absorbances (taken in a 10 cm path length cell) at 300 nm of samples before photolysis are noted on graph. Error bars represent one standard deviation based on the replicates. Total light dosage received 1.2E4 (watts-h m⁻²).

The results presented in Tables 1 and 2 and Figures 1 and 2 should be viewed with caution since only DOM hydrophobic enough to be retained by the C₁₈ cartridge were photolyzed. This represents approximately 50% of the total dissolved organic matter on average in these natural waters (14). To investigate potential artifacts from the extraction procedure on photoproduction of NO₂⁻, a comparison study was performed using HS isolated both by C₁₈ and XAD-2 resins (12). In the XAD humic substance, samples were acidified to pH 2.0 and loaded onto preconditioned XAD-2 columns. Humic substances were eluted with 0.1 M NaOH into three 30 mL Teflon vials, neutralized, and concentrated to dryness. Extracted HS from both resins were added to Gulf Stream seawater to a final concentration of 3 mg L⁻¹ resulting in optically similar solutions with corresponding absorbances at 300 nm for each of 0.03 ASU. Each sample was then photolyzed for 6 h. There was no statistical difference between the amount of nitrite produced upon photolysis of the C₁₈ and XAD HS extracts with 3 nM produced from each humic substance type after 6 h (t-test, *p* < 0.001). Consistent with the earlier results, there was also no statistical change in the dark controls after photolysis relative to initial levels.

The results presented in the preceding paragraph indicate that different extraction procedures do not significantly impact photoproduction of nitrite from humic substances. Using C₁₈ extracted HS was extremely useful because it allowed for comparison of the photolability of nitrogen bound within various HS samples isolated from different natural waters (Tables 1 and 2). However, both extraction procedures suffer from the same limitation regarding their inability to isolate all the ambient DOM. To extend these results to more realistic environmental conditions, these same natural waters were simply filtered and photolyzed without the addition of powdered humic substance extracts. In contrast to HS spiked samples, the final concentration of nitrite in these natural waters was in some instances higher after photolysis and sometimes lower after photolysis relative to initial levels. The change in nitrite, relative to initial levels, is presented on the y axis of Figure 3, where positive numbers reflect net increases and negative numbers represent net loss in NO₂⁻ upon photolysis. To indicate the optical characteristics of each water sample corresponding UV absorbances at 300 nm (to approximate the HS concentrations) are listed below each data point in Figure 3. At initial concentrations of less than 300 nM NO₂⁻ in the water sample, net production of

NO₂⁻ was observed, while at concentrations greater than 300 nM NO₂⁻, a net decrease in NO₂⁻ concentration was observed. Nitrite in dark controls showed no statistical change relative to initial levels.

Although the pattern is somewhat noisy, the results presented in Figure 3 suggest the observed increase or decrease in NO₂⁻ concentrations after irradiation is a function of initial nitrite concentration of the sample. To test this hypothesis more rigorously, a more controlled photolysis study was performed using water collected from the Cape Fear estuary. Varying concentrations of NO₂⁻, ranging from 50 to 500 nM, were added to Cape Fear Estuary water. The water was irradiated for 6 h (6 h was chosen in order to compare photochemical results to the earlier studies) in natural sunlight and sampled (*n* = 4) initially and after 6 h. At concentrations less than 150 nM NO₂⁻, net production of NO₂⁻ was observed, while at concentrations greater than 150 nM NO₂⁻ a net decrease in NO₂⁻ concentration was observed (Figure 4). The dark controls showed little or no net change in NO₂⁻ concentration relative to initial levels. These results are consistent with those presented in Figure 3 and suggest that net changes in nitrite concentrations upon photolysis of natural samples are influenced by the initial level of nitrite.

To further investigate the relationship among HS, light, and nitrite and to evaluate the role of nitrate, an additional controlled photolysis experiment was conducted. In this study, Cape Fear humic substances were added to Gulf Stream seawater to a final concentration of 30 mg L⁻¹, as was done earlier. A separate set of flasks contained the estuarine HS as well as typical ambient level spikes of both nitrite (1 μM) and nitrate (20 μM). A final set of flasks containing only the nitrite and nitrate spikes without added humic substances was also photolyzed. When estuarine HS alone were photolyzed, there was a net increase in nitrite of almost 30 nM relative to initial levels (Table 3), consistent with the results presented earlier. This production was accompanied by photodegradation of humic substances, seen as a net loss in absorbance at 300 nm in the light exposed flasks. This photobleaching of absorbance is consistent with earlier studies which found humic substance absorbance decreased after exposure to sunlight (2, 15–17).

When the nitrite/nitrate spiked seawater without Cape Fear HS was photolyzed, there was a large net decrease in nitrite of 139 nM, corresponding to direct photolysis of nitrite.

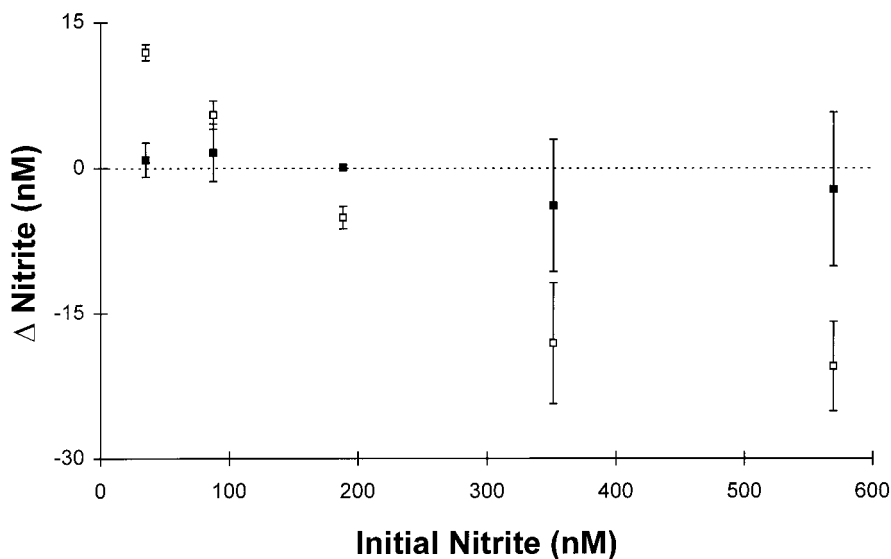


FIGURE 4. Change in nitrite (nM) as a function of initial nitrite (nM) concentration in 6 h natural sunlight photolysis of Cape Fear HS spiked Gulf Stream (30 mg L⁻¹) seawater with added NO₂⁻/NO₃⁻. Error bars represent one standard deviation based on the replicates. Open symbols represent light exposed flasks, and closed symbols are dark controls. Total light dosage received 1.1E4 (watts-h m⁻²).

TABLE 3. Initial and Final Nitrite Concentration (nM) in 30 mg L⁻¹ Cape Fear HS Spiked Seawater, Cape Fear Humic Substance and NO₂⁻/NO₃⁻ Spiked (1 μM/20 μM) Seawater, and NO₂⁻/NO₃⁻ Spiked Seawater^a

	Cape Fear		Cape Fear + NO ₂ ⁻ /NO ₃ ⁻		NO ₂ ⁻ /NO ₃ ⁻	
	light	dark	light	dark	light	dark
initial NO ₂ ⁻ (nM)	29.4 ± 0.7	30.1 ± 0.8	1062 ± 6	1217 ± 7	1194 ± 7	1135 ± 0.2
final NO ₂ ⁻ (nM)	58.8 ± 2	30.8 ± 0.9	942 ± 2	1211 ± 40	1055 ± 18	1129 ± 48
NO ₂ ⁻ change	+29.4 ± 2	+0.7 ± 1	-120 ± 6	-6 ± 40	-139 ± 19	-6 ± 48
absorbance change ₃₀₀	-0.015	ND ^b	-0.018	ND ^b	ND ^b	ND ^b

^a Also presented are the change in nitrite and absorbance after photolysis. ^b ND = none detected.

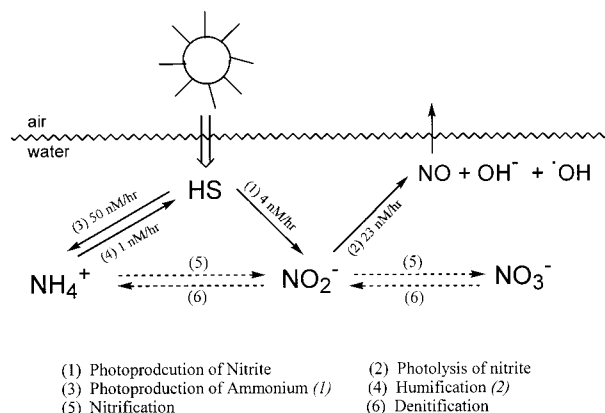


FIGURE 5. Conceptual model depicting the photochemically driven processes involved in the interconversion of humic substances and nitrite in natural waters.

Photolysis of the water containing both humic substances and nitrite/nitrate spikes produced a smaller net loss in nitrite upon photolysis (120 nM) relative to the sample without the HS addition (139 nM). The difference is approximately what would be predicted based on the concurrent production of nitrite from HS (29 nM).

Discussion

A simple conceptual model summarizing the interactions between light, humic substances, and nitrite is depicted in Figure 5. One important feature of Figure 5 is that changes observed in NO₂⁻ when natural water samples are photolyzed are complex. The rates presented in Figure 5 for processes

1, 2, and 4 apply to 35°N during summer for near surface conditions. Changes are a function of both initial HS and NO₂⁻ concentrations and are the result of both photochemical production from humic substances and direct photochemical decay of NO₂⁻. Our experimentally determined rate of nitrite photolysis (23 nM h⁻¹) was larger than the rate determined for photochemical production (4 nM h⁻¹). Therefore samples with high initial nitrite and low HS would result in a net loss in NO₂⁻ with time. This point is illustrated in Figure 3 by the open data point at 400 nM initial NO₂⁻, which had low humic substance concentration (*A*₃₀₀ = 0.083) relative to its initial NO₂⁻ (421 nM). At such low HS levels, the production of NO₂⁻ from HS was too small to offset the loss of NO₂⁻ by photochemical decay. Therefore, the net loss of NO₂⁻ was large (ΔNO₂⁻ = -110 nM) compared to other waters with higher HS concentrations but similar initial nitrite levels.

The rate of nitrite photochemical production was smaller than the rate of ammonium formation (1) via DOM photodegradation when Satilla River estuary fulvic acid was exposed to natural irradiation in Athens, GA (4 nM h⁻¹ vs 50 nM h⁻¹). However, nitrite photoproduction is of the same order of magnitude as NH₄⁺ incorporation (1 nM h⁻¹) during model humification experiments (2). In these latter studies, triglycerides were photolyzed and allowed to humify in the presence of ¹⁵NH₄⁺. Subsequent ¹⁵N NMR analysis of the isolated humics showed an ¹⁵N signal in the region of organically bound nitro groups (18). Thus, oxidation of imines, resulting from the reaction of ammonia with photooxidatively generated aldehyde or keto groups or amines within the humic substance, could generate new nitro groups. Nitroalkanes can be photooxidatively cleaved through reac-

tion with singlet oxygen (19), providing a source of photolabile nitrite.

The formation of nitrite by HS represented in Figure 5 may explain the wide range of direct nitrite photolysis rates reported earlier in coastal and oligotrophic waters, since those rates did not account for this additional production mechanism (20). It may also shed insight into an unexplained increase in nitrite observed upon exposure of a coastal seawater sample to sunlight and why nitrite photolysis rates were much faster in DI water relative to natural samples (21). The abiotic nitrite photochemical loss in seawater estimated by Zafiriou and True (21) was approximately 10% per day, which is in good agreement with the loss reported here (Figure 5). One additional process which may influence nitrite cycling in natural waters is production via photodegradation of nitrate. Zafiriou and True (21) suggested, however, that nitrite production from nitrate photolysis is insignificant and that the anions behaved independently when photolyzed together (21). This is consistent with the results presented in Table 3 where a large loss in nitrite is observed even in the presence of high nitrate. Nitrite production from humic substances is also important because NO_2^- , when photolyzed, generates hydroxyl radicals and NO in natural waters via process (2). The fate of NO not recycled back to nitrite is not well understood, but it has been suggested this process may represent a net flux of NO from the sea to the air and the marine boundary layer resulting in a loss of N from the system (21, 22).

In summary, photochemical experiments with humic substance spiked Gulf Stream seawater provide strong evidence for the production of NO_2^- from the photodegradation of HS at a rate of approximately 4 nM h^{-1} in coastal seawater. The net nitrite change observed in natural waters will, however, depend on the relative concentrations of nitrite and humic substances in the samples, given the competing process of direct photolysis of NO_2^- (23 nM h^{-1}). The release of a biologically utilizable nitrogen species after exposure of biologically refractory HS to UV light presents an important mechanism in the interconversion of N species and dissolved organic matter in aquatic ecosystems. The significance of this process on a regional scale can be calculated for Onslow Bay in the Atlantic off the southeastern United States coastline. In the top 1 m of Onslow Bay, given its volume is $1 \times 10^{13} \text{ L}$ and an average HS concentration of 5 mg L^{-1} with 6 h of irradiation per day, $2 \times 10^6 \text{ mol}$ of nitrite per year is produced. This should be viewed as an upper limit because the effects of HS photobleaching on the nitrite production rates reported in Table 1 is not known nor is the quantum efficiency of the production known.

Acknowledgments

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