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Effects of Solution Chemistry on the Oxidative Transformation of 1-Naphthol and Its Complexation with Humic Acid

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The extent of 1-naphthol (1-hydroxynaphthalene) complexation with humic acid (HA) was studied as a function of solution chemistry [pH (4−11), ionic strength, *I* (0.001 and 0.1 M LiCl), and dissolved O_2 (DO) concentration (0 and 8 mg L^{-1})] using spectroscopic (fluorescence, UV absorbance) and macroscopic (equilibrium dialysis) techniques. 1-Naphthol is transformed by DO in aqueous solutions; oxidation increases with pH and *I*, producing (hydroxy)naphthoquinones and coupled reaction products. Quenching of 1-naphthol fluorescence by HA increased with equilibration time from 1 to 7 d. This time-dependent relationship was found to result from (a) weak complexation of 1-naphthol by HA and (b) oxidative transformation of 1-naphthol (slow reaction) resulting in the formation of secondary products that are more reactive with HA. Sorption of ^{14}C-labeled compound (as measured by equilibrium dialysis) followed a pH-dependent trend with minimal removal below pH 7.0, a sharp increase over a narrow pH range, and maximum retention around pH 9.0. Effects of *I* were observed only between pH 8 and pH 10 where a 100-fold increase in Li^+ concentration doubled the extent of sorption. Good agreement between fluorescence quenching and dialysis methods was obtained. Exclusion of DO from the reaction vessel resulted in only a moderate decrease in the amount of ^{14}C sorption, which suggests that functional groups in HA may participate directly in electron-transfer reactions with 1-naphthol.

Introduction

The transport and bioavailability of polycyclic aromatic compounds (PACs) in soils are regulated by interactions with dissolved and solid-phase humic substances (HS). Although the affinity of PACs for soil inorganic colloids may be increased or decreased by the formation of mineral−HS complexes (*1*−*3*), the presence of HS in soil solution can *decrease* this affinity through aqueous-phase binding interactions (*4*). In addition, the affinity of both dissolved and solid-phase HS for PACs may be highly dependent on solution chemistry (e.g., pH and ionic strength). Therefore, to predict the fate of PACs in soil systems, it is critical to isolate the reactivity of HS and to determine its dependence on solution chemistry. This effort is hindered, in part, by the availability of few methods capable of elucidating PAC−HS interactions in aqueous systems.

Most prior research on PAC−HS interactions has focused on nonpolar compounds. Fewer studies have been conducted on the sorption of polar and ionizable PACs despite the fact that many nonpolar PACs are degraded to polar intermediates whose interaction with soil surfaces affect pollutant fate (*5*). The present study addresses PAC−HS interactions of 1-naphthol, a primary metabolite of both naphthalene and the pesticide carbaryl. 1-Naphthol, an hydroxylated compound with $pK_a = 9.34$ (*6*), exhibits a mode of toxic action that is comparable to its parent compounds, but it is more water-soluble than naphthalene. As a result of hydroxylation, 1-naphthol may interact with soil particles through a variety of mechanisms that are likely to be impacted by solution chemistry.

At alkaline pH and in the presence of dissolved O_2, 1-naphthol is oxidized abiotically within a few days to produce naphthoquinones, hydroxynaphthoquinones, and coupled products (*7*, *8*). These compounds sorb strongly to poorly crystalline aluminum hydroxide [$Al(OH)_3$], whereas 1-naphthol itself exhibits a very low mineral−surface affinity (*8*). These findings indicate that abiotic transformation may control sorption behavior of 1-naphthol in soil environments. However, the effect of such transformation on binding to natural organic matter is unknown. Furthermore, electron-poor quinone moieties in humic matter may serve as electron acceptors to promote directly the oxidation of phenolic contaminant compounds, obviating the need for dissolved O_2 (*9*, *10*).

Previous studies of 1-naphthol ↔ HS interactions have been restricted to fluorescence quenching measurements conducted at short reaction times (minutes to hours) and a limited range in solution chemistry. Chen et al. (*11*) observed that 1-naphthol fluorescence decreased with increasing pH in the presence of humic acid. They attributed their results to cation bridging between the anionic form of 1-naphthol and negatively charged sites on HA. The fluorescence quenching results were unaffected by *I* between 0.001 and 0.5 M KCl. Fluorescence lifetime measurements and a lack of temperature effects led the authors to conclude that static quenching (i.e., ground-state complex formation) was responsible for diminished fluorescence (*12*). Morra et al. (*13*) concluded that a combined static and dynamic mode of quenching (caused by a close physical association between 1-naphthol and pseudomicellar entities of HA resulting in increased frequency of collisions) was operative. Due to the short-term nature of these studies, interactions involving transformation products of 1-naphthol could not be evaluated.

Fluorescence spectroscopy is a simple and noninvasive method for studying PAC sorption to HA (*14*, *15*). However, as indicated above, the dominant mode of quenching may be ambiguous and the quantification of absorption of incident radiation by humic substances (i.e., the "inner-filter effect") is uncertain (*13*). To avoid the misinterpretation of experimental artifacts in the present study, we measured humic complexation of 1-naphthol using independent fluorescence spectroscopy and equilibrium dialysis methods. Equilibrium dialysis has been used to obtain quantitative estimates of sorption to HS of a wide variety of organic compounds [e.g., PACs (*3*, *16*, *17*), herbicides (*18*), and pesticides (*19*, *20*)]. By combining these techniques, we have measured the extent of 1-naphthol interaction with humic acid as a function of time (up to 7 d) and dissolved O_2 concentration over a wide range of pH (4−11) and ionic strength (0.001−0.1 M LiCl).

The principal objective of the present study was to determine the effects of solution chemistry on the extent

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and mechanism of 1-naphthol binding to humic acid. Experiments were designed to elucidate whether humic acid (a) complexes 1-naphthol directly, (b) catalyzes its oxidative transformation, and/or (c) serves as a scavenger for transformation products. Therefore, a secondary objective was to assess the time, pH, and *I* dependence of 1-naphthol transformation.

Materials and Methods

Preparation of Humic Acid. Humic acid (HA) was extracted from the *A* horizon of a northern hardwood Typic Dystrochrept (forest soil) collected from Nittany Ridge, Centre County, PA. Extraction of the Nittany Ridge humic acid in NaOH under N_2(g) and purification in HF/HCl were performed using standard procedures (*21*). Following acid treatment, NRHA was dialyzed (SpectraPor 3500 MWCO) against MilliQ H_2O until Cl^- was not detected in the dialysate. Ash content was <2%. The HA was then freeze-dried prior to use. Carbon content of the HA was measured on Shimadzu TOC 5000A equipped with a solid-sample module. Functional group composition of the HA was measured by ^{13}C cross-polarization magic angle spinning nuclear magnetic resonance spectroscopy (CPMAS-NMR). Carboxylic and phenolic group acidity were measured by alkalimetric titration (*22*). High-performance size exclusion chromatographic analysis of the HA revealed a bimodal size distribution with weight average molecular masses (M_w) of 7.1 kDa (86.3% of the integrated chromatogram area) and 61.4 kDa (13.7% of the total area).

Fluorescence Quenching. Variable proportions of LiCl, LiOH, and HCl at the appropriate *I* (0.001 and 0.1 mol L^{-1}) were added to 40-mL amber vials to obtain a range of final pH values. Sodium azide (NaN_3, 5 mg L^{-1}) was added to prevent microbial growth. An aliquot of concentrated HA (1.0 g L^{-1}) was added to yield a final DOC concentration of 11 mg L^{-1}. Stock 1-naphthol solution was added to give a total concentration of 8 μmol L^{-1}. The suspensions were equilibrated in the dark at 25 °C in a platform orbital shaker (65 rpm) for the desired reaction time. To obtain the fluorescence intensity (F_o) of the target compound in the absence of the quencher, reaction vessels (1-naphthol blanks) were prepared by following the procedure outlined above except that no HA was added. HA blanks (no 1-naphthol) were also prepared. Since the fluorescence intensities of 1-naphthol and HA vary with pH, we ensured that all experimental systems (1-naphthol:HA; 1-naphthol blank; HA blank) bracketed the same pH range. Fluorescence measurements were performed using a Photon Technology Inc. spectrometer. Full spectrum preliminary experiments were conducted to identify excitation (λ_{ex}) and emission (λ_{em}) wavelengths at 294 and 460 nm, respectively, with a bandpass of 1 nm. These values are consistent with prior studies of 1-naphthol fluorescence (*11*).

For the 1-naphthol−HA suspensions, fluorescence of HA alone (measured on HA blanks) was subtracted from the total prior to corrections for inner-filter effects (IFE) using the method of Gauthier et al. (*14*). Absorbance measurements for IFE correction were performed using a Shimadzu PC 3101 UV−vis−NIR spectrometer at the emission and excitation wavelengths. The IFE correction factors ranged from 1.95 to 2.18 for 1-naphthol−HA suspensions and from 1.03 to 1.06 for 1-naphthol blanks, which are well below the recommended maximum value of 3.0 (*23*). Since UV absorbance by HA increased with pH, the IFE correction factors exhibited a similar trend.

Dialysis Experiments. Equilibrium dialysis was used to quantify sorption of 1-^{14}C-1-naphthol to HA. Spectra/Por 6 dialysis membrane (1000 MWCO) was washed thoroughly with deionized water prior to its placement in a 250-mL amber glass bottle filled with LiCl/LiOH or LiCl/HCl solution to give equilibrium pH values ranging from pH 4 to pH 11 at 0.001 or 0.1 mol L^{-1} ionic strength. The dialysis cell was filled with external solution, and a volume of concentrated stock (1.0 g L^{-1}, pH 7) HA solution was added to provide a dissolved organic carbon (DOC) concentration of 11 mg L^{-1}. The cell was then sealed. Stock radiolabeled solution comprising 1-^{14}C-1-naphthol (specific activity = 7.71 mCi mmol^{-1}, Sigma Chemical) was added to the external solution of each reaction vessel to give a total system 1-naphthol concentration of 8 μmol L^{-1} with ≈13% of the total 1-naphthol ^{14}C-labeled. Fresh stock solutions were prepared for each experiment. Sodium azide (5 mg L^{-1}) was added to prevent microbial growth. Replicate assays without NaN_3 indicated that it had no detectable effect on sorption reactions. All the experimental glassware was autoclaved before use, and solutions were prepared using distilled water that was passed through a MilliQ UV-plus water purification system.

Humic−naphthol suspensions were agitated in a platform orbital shaker in the dark at 65 rpm for 7 d. Concentrations of ^{14}C and 1-naphthol inside and outside the dialysis cell were determined, respectively, by liquid scintillation counting (LSC, Beckman LS 8100) and high-performance liquid chromatography (HPLC, Waters Inc., with a reverse-phase Beta-basic 18, Keystone Scientific column followed by photodiode array detection) within 4 h of terminating an experiment. The mobile phase consisted of 80% (v/v) methanol with a run time of 5 min and injection volume of 20 μL. 1-Naphthol was quantified by integrating the 2.95 min peak at 280 nm wavelength. The quantity of ^{14}C label removed from solution after interaction with HA was determined from the difference between inside (free and HA-bound) and outside (free) ^{14}C concentrations. Selected samples were analyzed by HPLC-mass spectrometry (HPLC-MS) for determination of reaction products on a Hewlett-Packard 1100 HPLC interfaced to a Perseptive Mariner mass spectrometer (PE Biosystems, Framingham, MA) using atmospheric pressure chemical ionization in negative ion mode. A series of controls was established to measure 1-naphthol sorption to reaction vessels and membrane surfaces.

To examine the effects of dissolved oxygen (DO) on the HA−naphthol reaction, we performed a series of dialysis experiments with O_2 excluded. These experiments required the use of serum bottles with Teflon-lined rubber septa as reaction vessels to achieve complete DO elimination. The experimental procedure was similar to that described above, with the following modifications. After the addition of all solutions and HA, the contents of the serum bottle were purged with N_2(g) for 1 h and then sealed. The bottle headspace was then purged for an additional 30 min. This purge had a negligible effect on volatilization of 1-naphthol because ^{14}C recoveries were consistently greater than 95%. At the end of the reaction period, DO was measured immediately after the removal of the septum using an Orion DO meter (range 0−8.5 mg L^{-1}).

Results and Discussion

Standard spectral integration of the ^{13}C CPMAS-NMR spectrum of Nittany Ridge HA revealed that carbon is distributed dominantly among aliphatic (0−65 ppm, 35%), aromatic (100−160 ppm, 32%), carbohydrate (65−100 ppm, 12%), and carboxylic (160−200 ppm, 21%) functionalities. Assignment of chemical shifts in the NMR spectrum of HA was accomplished using the designations provided in Malcolm (*24*). Carbon accounts for approximately 54% of the total HA mass. Titration data indicate that the HA comprises 2.84 mol kg^{-1} of carboxylic acidity (functional groups dissociated between pH 3 and pH 8) and 1.36 mol kg^{-1} of phenolic group acidity (assuming functional groups 50% dissociated at pH 10) (*22*).

Oxidative Transformation of 1-Naphthol in the Absence of Humic Acid. DO promotes aqueous-phase oxidative

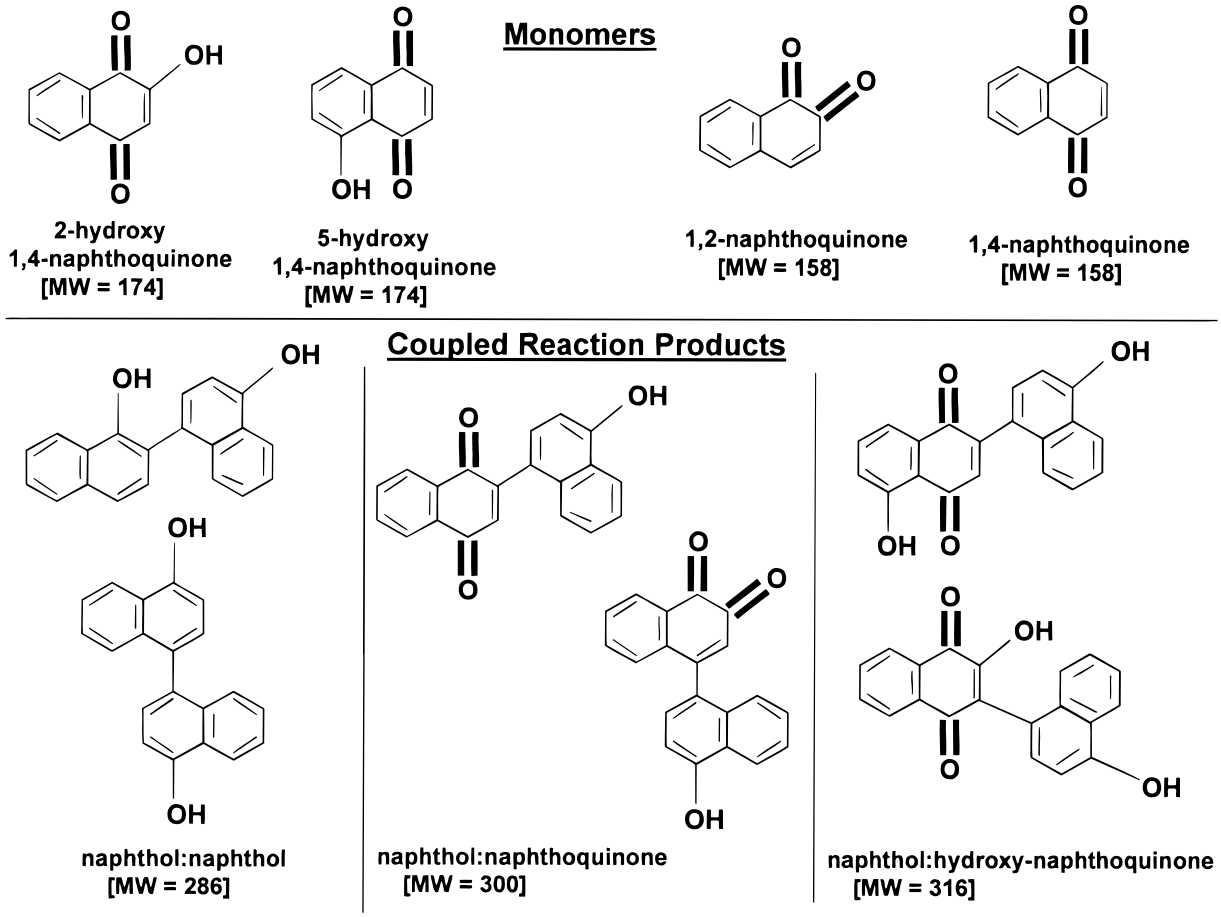

FIGURE 1. Reaction products produced by the oxidative transformation of 1-naphthol as identified by HPLC/MS.

transformation of 1-naphthol, and the extent of transformation is controlled by pH (*8*) and ionic strength (*I*). The fraction of 1-naphthol transformed (in the absence of HA) exhibits a pH-dependent trend: negligible below pH 6.5, increasing with pH above pH 7.0, and leveling off around pH 9.0 (*8*). In addition to this pH trend, transformation increases significantly with *I* (data not shown), and much greater values are obtained at the higher *I* of 0.1 M (70% maximum; pH 8.9) as compared to 0.01 M (13.5% maximum; pH 9.2). Transformation products were identified by HPLC-MS, and they comprise the naphthoquinone-type monomers and coupled reaction products that are shown in Figure 1. Photolytic transformation of 1-naphthol has been shown to result in the formation of 2-hydroxy-1,4-naphthoquinone (lawsone), 1,4-naphthoquinone, and 1,2-naphthoquinone (*25*). The byproducts of naphthalene biodegradation (with 1-naphthol as an intermediate) have been shown to include 1,4-naphthoquinone and 4-hydroxy-1-tetralone (*26*). However, it is important to note that in our study these oxidation products were produced abiotically in the dark and in the absence of humic substances. In the absence of DO and HA, 1-naphthol is stable at all solution conditions (pH and *I*) (*8*).

Fluorescence Spectroscopy. Fluorescence intensity of a fluorophore in the absence of a quencher (F_o) is directly proportional to its concentration in solution (*27*). Therefore, time-dependent changes in F_o can be used to assess the stability of 1-naphthol under different solution conditions (pH and *I*) as a function of time. The effects of pH, *I*, and reaction time on F_o values for 1-naphthol are shown in Figure 2a ($I = 0.1$ M) and b ($I = 0.001$ M). Initial measurements of F_o were obtained immediately after 1-naphthol addition to the reaction vessel and mixing for 1 min. These initial values at both *I* show a pH-dependent decrease in F_o close to the conditional dissociation constant at the respective *I* (p^cK_a) of 1-naphthol. Measured values of F_o were correlated ($r^2 > 0.95$) with the undissociated fraction of total 1-naphthol as calculated from the p^cK_a, which indicates that the neutral form is the predominant fluorescing species at $\lambda_{ex} = 294$ nm. It is worth noting that increasing λ_{ex} to the isosbestic point (i.e., the wavelength where both anion and neutral forms exhibit the same extent of absorbance) results in an *increase* in F_o with increasing pH, as also reported by Harris and Selinger (*28*). At this λ_{ex}, the anionic species fluoresces more intensely than the neutral form. Therefore, the choice of λ_{ex} can significantly impact observed pH trends in fluorescence.

Any decrease in F_o below the initial baseline value can be attributed to 1-naphthol loss by transformation as confirmed by simultaneous HPLC measurements. With increasing time, there is clearly a decrease in F_o above pH 8. For all solution conditions (pH and *I*) and reaction times, diminished F_o was well correlated with the decrease in HPLC-measured concentration of 1-naphthol (e.g., insert in Figure 2a). However, a larger decrease in F_o was observed at the higher *I*. For example, in 0.1 M LiCl, fluorescence intensity was reduced by as much as 83% after 15 d (pH 9), whereas a maximum decrease of 30% (pH 10.3) was observed for the 0.001 M LiCl case. In Figure 2b (insert), the measured F_o values after a contact time of 7 d are compared for both *I* values.

The correlation between 1-naphthol transformation and ionic strength suggests that complexation of Li^+ with the naphthol hydroxyl group may reduce 1-naphthol stability and catalyze its oxidation. Indeed, Li^+ is a hard Lewis acid [*Misono softness* parameter, $Y = 0.055$ nm (*29*)] that should favor complexes with hard Lewis bases, such as phenolic-OH groups (*30*). To test this hypothesis, identical measurements were made in 0.1 M CsCl solutions. Since Cs^+ is a borderline Lewis acid [$Y = 0.259$ nm (*29*)], it should form relatively weaker complexes with 1-naphthol as compared

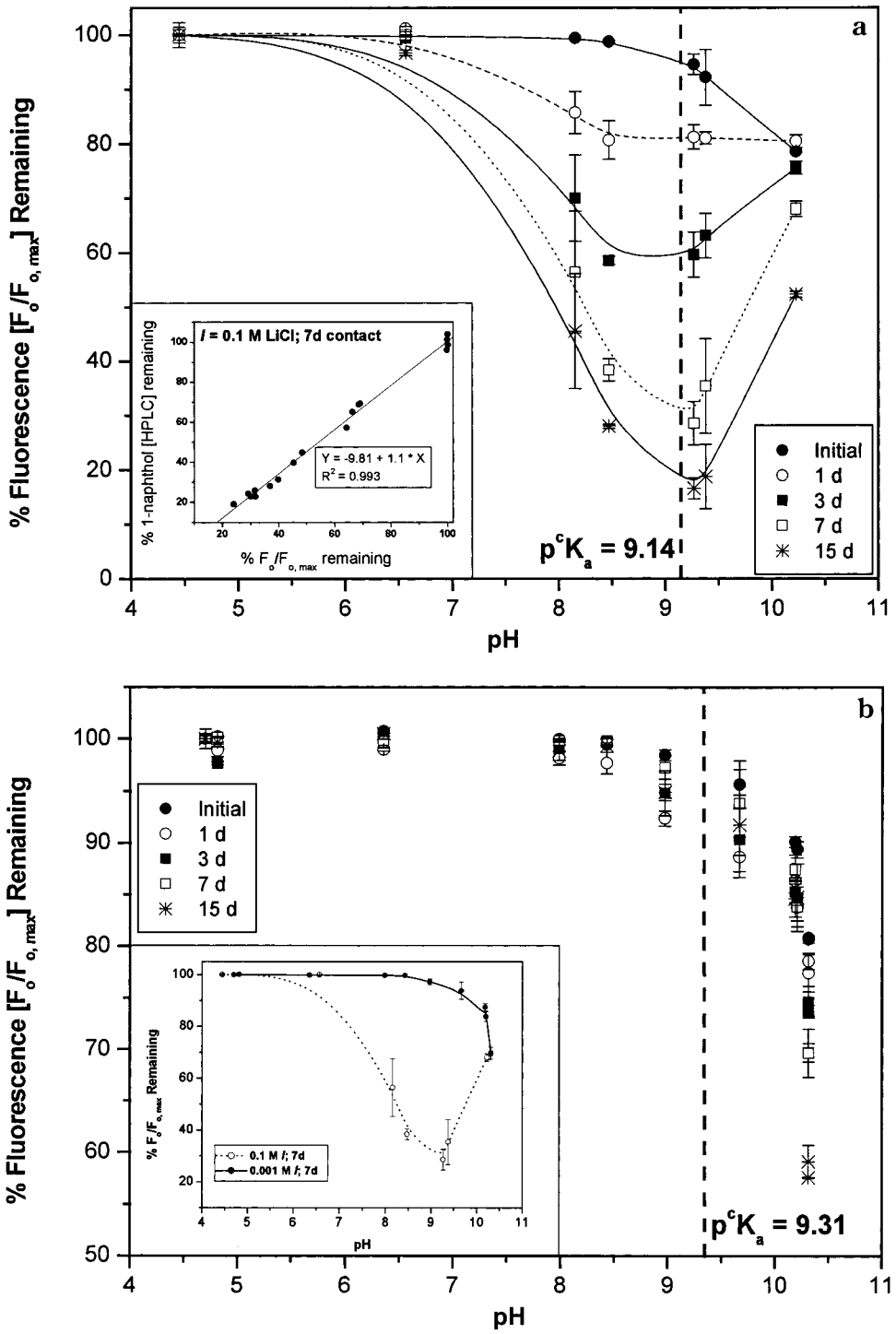

FIGURE 2. Percent fluorescence intensity of 1-naphthol (F_o, absence of HA) remaining as a function of pH and reaction time (1-naphthol = 8 μmol L^{-1}). (a) I = 0.1 M LiCl. A plot of % $F_o/F_{o,max}$ remaining versus % 1-naphthol (HPLC-determined) remaining in solution for a reaction time of 7 d is provided as an insert. $F_{o,max}$ is the value of F_o at pH 4.4 after each corresponding reaction time. (b) I = 0.001 M LiCl. A comparison of % $F_o/F_{o,max}$ remaining after 7 d contact time at 0.1 and 0.001 M LiCl background is provided as an insert. $F_{o,max}$ is the value of F_o at pH 4.7 after each corresponding reaction time.

to Li^+. Table 1 lists the first-order rate coefficients for loss of 1-naphthol fluorescence as a function of cation composition (Li^+, Cs^+) and pH. The rate constants clearly show that transformation kinetics are slower in the presence of Cs^+ relative to Li^+. The r^2 values for the regression of $\ln(F_o/F_{o,\,initial})$ versus time indicate that a first-order expression is appropriate. Therefore, the effects of I appear to derive from the formation of soluble M^+−naphthol complexes, which promote oxidation and diminish F_o values.

Complexation with Humic Acid. The ratio of fluorescence intensity in the absence and presence of the quencher (HA) was used to measure 1-naphthol ↔ HA association. The Stern−Volmer equation (*27*) was used to calculate the proportion of 1-naphthol bound to HA:

$$F_o/F = 1 + K_{SV}(\mathrm{HA}) \qquad (1)$$

where F_o and F are the fluorescence intensities of 1-naphthol

TABLE 1. First-Order Rate Coefficients for Loss of 1-Naphthol Fluorescence as a Function of Cation Composition[a]

pH	k_{Li^+} [b] (h^{-1})	k_{Cs^+} [b] (h^{-1})	$r^2_{Li^+}$ [c]	$r^2_{Cs^+}$ [c]
9	4.25×10^{-3}	2.86×10^{-3}	0.96 ($p = 0.0037$)	0.99 ($p = 0.0003$)
10	3.21×10^{-3}	1.67×10^{-3}	0.99 ($p = 0.0004$)	0.96 ($p = 0.004$)

[a] Both solutions are at 0.1 M ionic strength. [b] First-order rate coefficients calculated from linear regression of $\ln(F_o/F_{o,initial})$ versus time (h). [c] Correlation coefficient of linear regression.

in the absence and presence of HA, respectively. K_{SV}, the Stern−Volmer constant, is equal to the conditional binding constant for 1-naphthol ↔ HA sorption if a static quenching mechanism is operative (*14*).

The Stern−Volmer equation may be rearranged to calculate the humic-bound fraction for direct comparison with the equilibrium dialysis results. At high ratios of HA to 1-naphthol, we can assume that the fraction of naphthol-bound HA is small and that $[HA]_{free} \approx [HA]_{initial}$. Equation 1 can then be written to obtain the fraction of sorbed contaminant as

$$\text{1-naphthol} + \text{HA} \leftrightarrow \text{1-naphthol:HA} \quad K_{sv} \qquad (2)$$

$$K_{sv} = \frac{[\text{1-naphthol:HA}]}{[\text{1-naphthol}]_{free}[\text{HA}]_{free}} \qquad (3)$$

where [1-naphthol:HA] corresponds to humic-bound 1-naphthol. Substituting for K_{sv} in eq 1 and assuming $[HA]_{free} \approx [HA]_{initial}$

$$\%\ \text{bound to HA} = [\text{1-naphthol:HA}]/[\text{1-naphthol}_{initial}] = (1 - F/F_o) \qquad (4)$$

where $\text{1-naphthol}_{initial} = ([\text{1-naphthol:HA}] + [\text{1-naphthol}]_{free})$.

Diminished 1-naphthol fluorescence could be brought about by (a) binding to HA or (b) oxidative transformation to a secondary product that may or may not sorb to HA. In an earlier study of 1-naphthol sorption to $Al(OH)_3$(s), we found very low levels of secondary products remaining in solution indicating that >80% of the oxidized compound was sorbed onto the mineral surface (*8*). Therefore, we postulated that these secondary products would also sorb strongly to humic acid.

The FQ results are illustrated in Figure 3a,b. We calculated F_o/F two ways: (a) F_o and F measured after the same reaction period and (b) using only initial measurements of F_o throughout. For the lower I of 0.001 M, both approaches provided similar results since there is only a moderate decrease in the time-dependent F_o measurements as compared to the initial value (Figure 2b). Conversely, for most cases at $I = 0.1$ M, simultaneous measurement of F_o and F at contact times ≥ 1 d resulted in lower values of F_o (absence of HA) than for F (presence of HA). Therefore, for these samples, approach (a) resulted in F_o/F values <1. Evidently, the presence of humic acid diminished the Li^+-mediated transformation of 1-naphthol that was observed over time in Figure 2a. Although the precise stabilization mechanism is not known, it is clear that the use of time-dependent changes in F_o would lead to erroneous results. Therefore, for both ionic strengths, we used initial measurements of F_o to calculate fluorescence quenching. Equation 4 was then used to obtain a measure of the humic-bound fraction (right axes in Figure 3a,b) that could be compared directly to equilibrium dialysis results (Figures 4 and 5).

The FQ data (Figure 3a,b) suggest a time-dependent increase (1−7 d) in the quenching of naphthol fluorescence by HA at both I values. These time-dependent trends provide indirect evidence for the operative reaction mechanism. Direct sorption/complexation of 1-naphthol to HA is a relatively fast reaction that should be completed within 24 h. However, oxidative transformation of 1-naphthol followed by sorption of the resulting product should be characterized by a longer contact time (see Figure 2a,b). Figure 3a,b indicates that sorption levels are moderate even after 3 d of equilibration. Therefore, we postulated that greater quenching at longer times (7 d) resulted from enhanced sorption of 1-naphthol transformation products to HA. The fact that the reduced fluorescence results from humic complexation, and not simply from 1-naphthol transformation, was confirmed by independent dialysis experiments (Figures 4 and 5) as discussed below.

We obtained a value of 1.07 for F_o/F at pH 7.0 ($I = 0.001$ M LiCl) after 1 d equilibration (Figure 3a). This is slightly lower than the value of 1.2 reported by Chen et al. (*11*) and Morra et al. (*13*) for similar conditions. The ratio of F_o/F is highly dependent on the methods used to correct IFE, equilibration time, and the functional group chemistry of humic acid. Chen et al. (*11*) also observed an increase in F_o/F with increasing pH [i.e., $(F_o/F)_{pH10}/(F_o/F)_{pH7} = 1.1$]. They attributed this increase to a cation-bridging type mechanism involving the naphtholate anion, which appears plausible since transformation of 1-naphthol requires longer times than the short-term duration (12 h) of their study. In our study, at $I = 0.001$ M, the ratio of $(F_o/F)_{pH10}$ to $(F_o/F)_{pH7}$ after 1 d is 1.04 (Figure 3a). However, this ratio increases to 1.2 after 7 d reaction, and this time-dependent increase cannot be explained solely on the basis of a cation-bridging interaction. At $I = 0.1$ M, the effect of contact time is more pronounced, with $(F_o/F)_{pH10}/(F_o/F)_{pH7}$ increasing from 1.07 after 1 d to 1.51 for a 7-d equilibration period (Figure 3b).

Equilibrium Dialysis. We used equilibrium dialysis to assess independently whether the decrease in fluorescence with time is an artifact of naphthol transformation alone (Figure 2a,b) or due to progressive complexation of transformation products with humics. Preliminary experiments indicated that over the entire pH range (a) the equilibrium in 1-naphthol_{free} distribution was achieved in < 24 h, (b) the membrane was impermeable to HA, and (c) the sorption of ^{14}C-labeled compound and HA to the membrane was negligible.

Sorption of ^{14}C-labeled compound to HA (dialysis results) as a function of pH, I, and DO concentration is illustrated in Figures 4 (0.001 M) and 5 (0.1 M). In these figures, the extent of sorption calculated from fluorescence quenching (FQ) is provided for comparison. There is a good correspondence in the amount of contaminant sorbed and the shape of the pH-edge obtained using these two independent methods. For all conditions, the extent of sorption was low (<5%) and unaffected by I between pH 4 and pH 7 after a 7-d equilibration period. Evidently, at pH < 7.0 there is no strong interaction between 1-naphthol and HA. In contrast, a significant increase in sorption of the ^{14}C-labeled compound (dialysis) occurs with increasing pH above pH 7 and effects of I become apparent (compare Figures 4 and 5). At the higher I (Figure 5), loss of ^{14}C label sharply increases over a narrow pH range, achieves a maximum around pH 9.5 (40 mg g^{-1} C; 37.4% removal), and then decreases. The extent of complexation is lower (Figure 4) at lower I (maximum = 20 mg g^{-1} C) and it stays relatively constant above pH 9.0. A similar effect of pH on 1-naphthol removal from solution in the presence of $Al(OH)_3$(s) was observed previously and was found to result from oxidative transformation of 1-naphthol at alkaline pH (*8*). The combination of dialysis and fluorescence results in the present study indicate that 1-naphthol transformation products (Figure 1) exhibit a high affinity for HA at pH > 7.

The influence of I might be attributed to its effect on humic conformation. Increased electrolyte concentration has

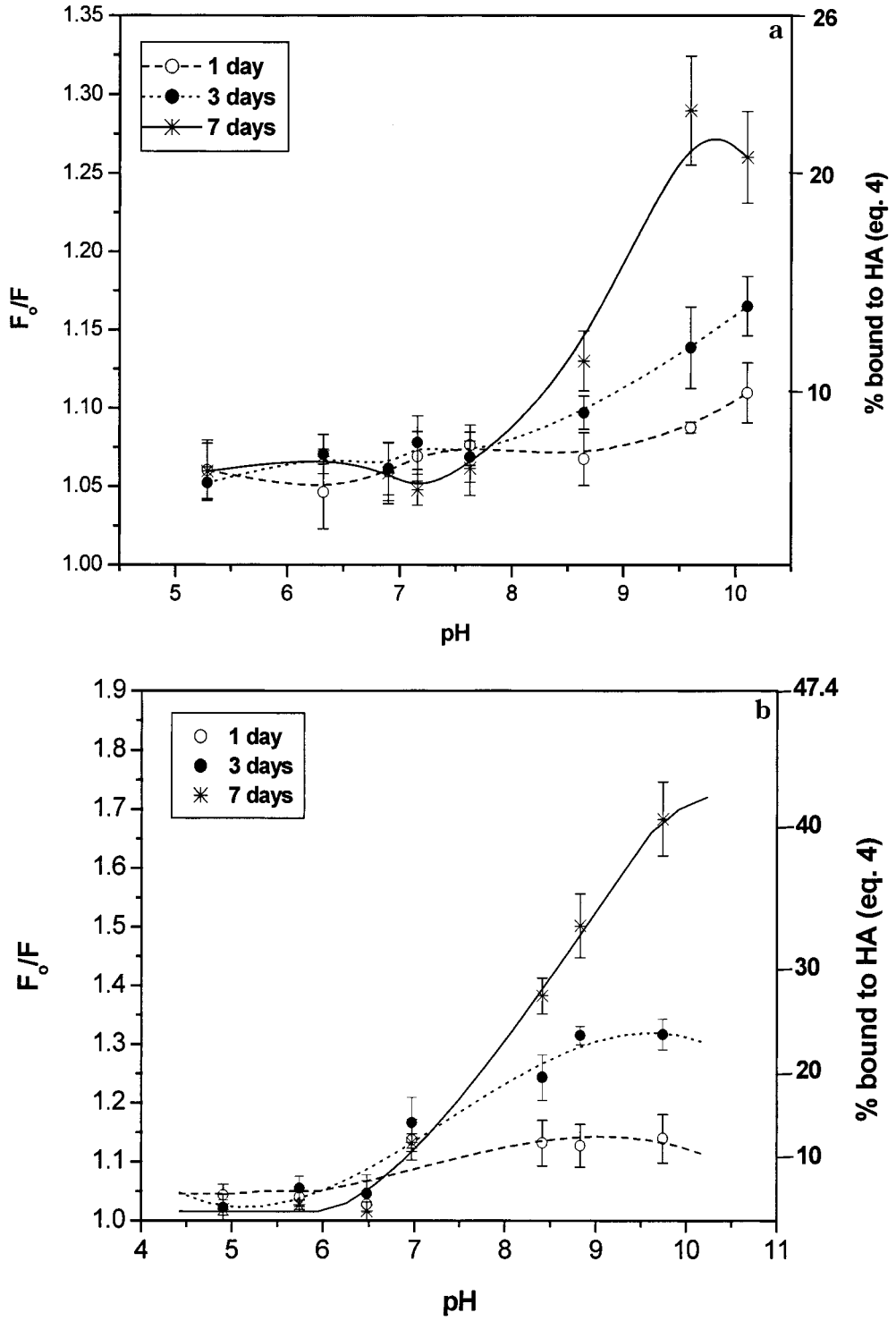

FIGURE 3. Fluorescence quenching (FQ) of 1-naphthol in the presence of HA as a function of pH and reaction time (1-naphthol = 8 μmol L^{-1}; HA = 11 ppm C). The humic-bound fraction (%) is provided in the right *Y*-axis as obtained using eq 4. (a) *I* = 0.001 M LiCl. (b) *I* = 0.1 M LiCl.

been shown to result in increased coiling and co-association of the macromolecules (*31, 32*). Prior work has yielded conflicting results regarding the effects of conformation on sorption of *nonpolar* PACs. Increasing *I* has led to both increased (*19*) and decreased (*23*) sorption. However, the effect of *I* in the present case was observed only in the pH range of 8−10, where a 100-fold increase in Li^+ concentration doubled retention of ^{14}C label by HA (compare Figures 4 and 5). If humic conformation was the primary cause of increased sorption with *I*, such an effect would also be observed at intermediate pH values (e.g., pH 5−7) where the degree of carboxyl group dissociation is still high and the concentration of H^+ is small relative to that of Li^+ concentration. Therefore, a more plausible explanation is that the aqueous stability of 1-naphthol is decreased at higher *I*. There is a good agreement between the pH-dependent trend observed for sorption (Figures 4 and 5) and 1-naphthol transformation as a function of *I* (Figure 2b, insert).

Our prior work indicated that exclusion of dissolved O_2 completely suppressed 1-naphthol transformation and, therefore, sorption of oxidation products to the surface of $Al(OH)_3$ (*8*). 1-Naphthol itself exhibited very low affinity for

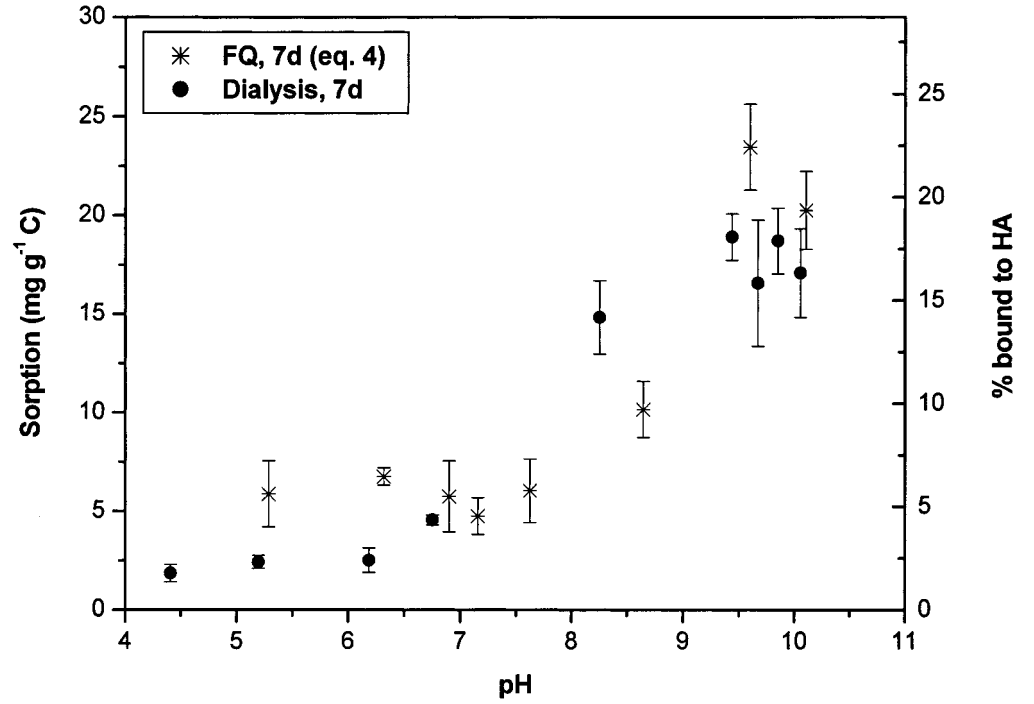

FIGURE 4. Sorption of ^{14}C-labeled compound (measured using dialysis technique as the difference between internal and external ^{14}C activities) as a function of pH (1-naphthol = 8 μmol L^{-1}; HA = 11 ppm C; I = 0.001 M LiCl; 7 d reaction). The humic-bound fraction as determined by FQ (eq 4) for a 7-d reaction time is also provided.

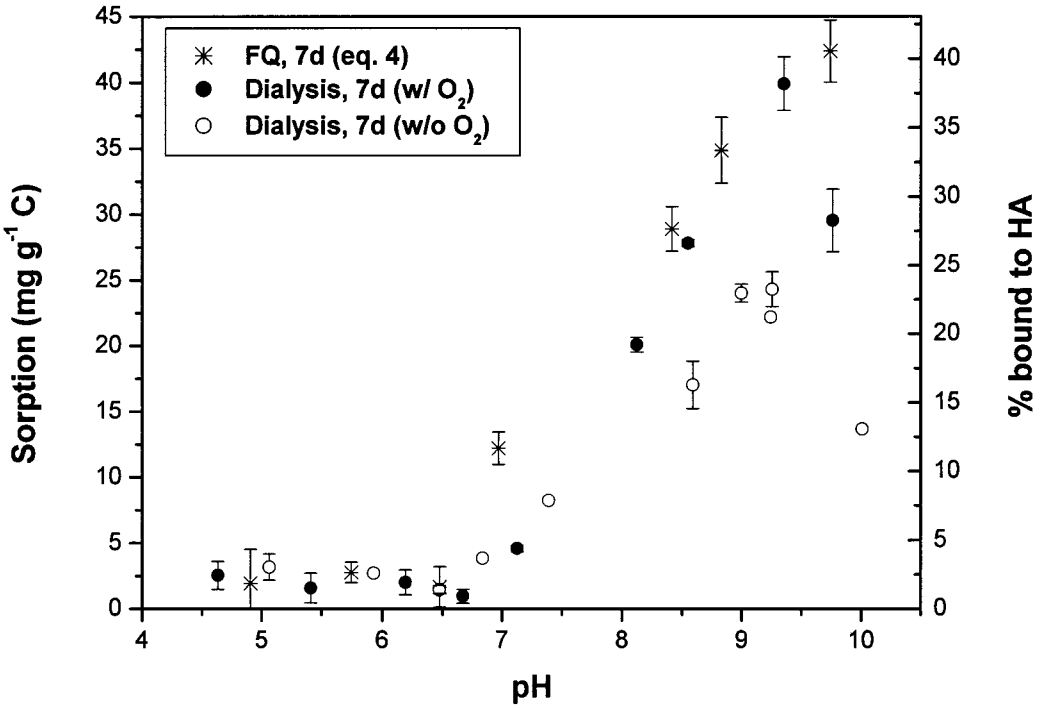

FIGURE 5. Sorption of ^{14}C-labeled compound (measured using dialysis technique as the difference between internal and external ^{14}C activities) as a function of pH and dissolved O_2 (DO) concentration (1-naphthol = 8 μmol L^{-1}; HA = 11 ppm C; I = 0.1 M LiCl; w/ $O_2 \approx$ 8 mg L^{-1}; w/o $O_2 \approx$ 0 mg L^{-1}; 7 d reaction). The humic-bound fraction as determined by FQ (eq 4) for a 7-d reaction time is also provided.

the mineral surface. However, humic substances are known to contain quinone moieties that act as electron acceptors under anaerobic conditions (*9, 10*). To assess the ability of HA to oxidize 1-naphthol directly, experiments were performed in the absence of O_2. Exclusion of DO from the reaction vessel resulted only in a partial decrease in the amount of sorbed compound (Figure 5). These results suggest that humic acid may facilitate the oxidative transformation of 1-naphthol directly and thereby promote binding under low dissolved O_2 conditions.

UV Absorbance Spectroscopy. Changes in UV−vis absorption spectra of 1-naphthol in the presence of HA can be caused by (a) ground-state association with HA (*13*) and/or (b) oxidative transformation reactions. Figure 6 shows the UV−vis spectra of products resulting from reaction of 1-naphthol with HA as a function of pH (obtained by subtracting the spectrum of HA from that of 1-naphthol−HA at the corresponding pH). The spectrum of 1-naphthol is also shown for comparison. At pH 4.6, the difference spectrum is not significantly different from that of 1-naphthol. However, at

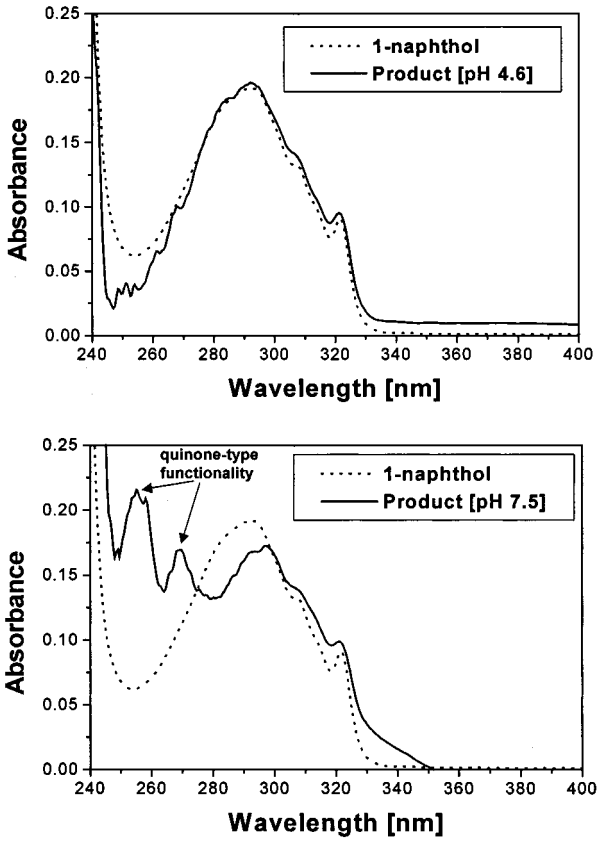

FIGURE 6. UV absorbance spectra of the product resulting from the reaction of 1-naphthol with HA. The spectrum of 1-naphthol is provided for comparison.

pH 7.5, new peaks emerge that are consistent with quinone absorbance around 270 nm (e.g., 2-hydroxy-1,4-naphthoquinone (*33*)) and 250 nm (e.g., 2-hydroxy-1,4-naphthoquinone; 5-hydroxy-1,4-naphthoquinone; 1,2-naphthoquinone, and 1,4-naphthoquinone (*33*)). Diminished absorbance at 290 nm is also consistent with oxidative transformation of 1-naphthol.

Our results indicate that interaction of 1-naphthol with dissolved HA is influenced by the formation of secondary products. Oxidative transformation of 1-naphthol is mediated by dissolved O_2. However, in the absence of DO, HA itself may promote 1-naphthol oxidation. These reactions are clearly affected by changes in solution chemistry (pH, *I*, and cation composition) and are reflected in the extent of binding to HA. Fluorescence quenching data indicate that complexation with HA, which continued to increase throughout the entire experimental period, was limited by time dependent 1-naphthol transformation reactions. Aqueous stability of 1-naphthol (in the absence of HA) seems to be strongly affected by the increase in Li^+ concentration as exemplified by the tremendous loss in fluorescence intensity (≈83%) at $I = 0.1$ M. This study contributes to mounting evidence that transformed secondary products control the reactivity of this organic contaminant toward diverse environmental sorbents.

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