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Short Communication

Biodegradation of 1-Naphthol in the Presence of Humic Acid

William D. Burgos,[1]* Nipon Pisutpaisal,[1] Munruk Tuntoolavest,[1] Jon Chorover,[2] and Richard F. Unz[1]

[1]Department of Civil and Environmental Engineering
The Pennsylvania State University
University Park, PA 16802
[2]Soil Science Program
Department of Agronomy
The Pennsylvania State University
University Park, PA 16802

ABSTRACT

Biodegradation of 1-naphthol by a pure culture was measured in the presence (65 mg C L^{-1}) and absence of a forest soil humic acid (HA). Experiments were performed under nongrowth conditions and controlled ionic strength (I equivalent to 10 mM LiCl) at pH 8.0. 1-Naphthol was allowed to react with HA under aerobic conditions in the dark for 7 days to promote sorption prior to bacterial inoculation. During this abiotic sorption period, 3.7–12% of 1-naphthol was transformed into oxidative products (naphthoquinones). At low initial 1-naphthol concentrations (<2.7 mg L^{-1}), 1-naphthol mineralization (measured by $^{14}CO_2$ production) decreased from 25–35% to 9–16% with HA. 1-Naphthol was biodegraded to concentrations below HPLC-PDA detection limit (0.10 mg L^{-1}) except at the highest 1-naphthol concentrations tested (>9.0 mg L^{-1}) with or without HA. Both abiotic and biotic 1-naphthol transformation pathways accumulated similar products, and these products were more recalcitrant than 1-naphthol. The sorption of 1-naphthol abiotic transformation products and bacterial metabolites to HA (not 1-naphthol) reduced 1-naphthol mineralization.

Key words: bioavailability; biodegradation; humic acid; 1-naphthol; naphthoquinones

INTRODUCTION

PREVIOUS RESEARCH on the impact of dissolved humic substances (HS) on the biodegradation of organic contaminants has produced contradictory results. The presence of HS has been shown to increase (Liu *et al.*, 1983; Tranvik and Hofle, 1987; Larsson *et al.*, 1988; Ortega-Calvo and Siaz-Jimenez, 1998), decrease (Shimp and Pfaender, 1985), or have no effect (Amador and Alexander, 1988) on the rate and extent of degradation of aromatic contaminants. The difficulty in establishing a clear effect is caused by several factors including aqueous phase contaminant–HS interactions; contaminant metabolite–HS interactions; micro-organism–HS interactions; micro-organism enrichment techniques; and pure vs. mixed microbial culture. In addition, differences in experimental protocols regarding pH, ionic strength, presence or absence of media constituents to support

*Corresponding author: Department of Civil and Environmental Engineering, The Pennsylvania State University, 212 Sackett Building, University Park, PA 16802. *Phone:* 814-863-0578; *Fax:* 814-863-7304; *E-mail:* bburgos@psu.edu

growth, and contaminant–HS adsorption contact time can also affect experimental results and their interpretation.

The objectives of this research were to study the impact of humic acid (HA) on the biodegradation of 1-naphthol under conditions of controlled pH, ionic strength, and bacterial numbers to better understand these processes and shed light on the existing contradictory results reported in the literature. Experimental pH, ionic strength, and contact time were set based on previous measurements of 1-naphthol sorption to a purified forest soil HA (Karthikeyan and Chorover, 2000). Constant bacterial numbers and viability were measured and confirmed with the Live/Dead Baclight Bacterial Viability Kit. 1-Naphthol and its transformation products were measured and tentatively identified by high-performance liquid chromatography with photodiode array (HPLC-PDA) detection, and mineralization was measured using ^{14}C-1-naphthol. This complete data set overcame some of the interpretative difficulties listed above, and provided a more accurate, less speculative explanation of the results.

EXPERIMENTAL PROTOCOLS

Radiolabeled 1-naphthol (1-^{14}C) (Sigma Chemical, St. Louis, MO, specific activity of 7.7 mCi mmol^{-1}) was dissolved in methanol and purity was verified at >98% by HPLC-PDA. Unlabeled 1-naphthol solutions were prepared in a phosphate buffered background electrolyte (BE) that contained (g per liter of MilliQ H_2O): $K_2HOP_4 \cdot 3H_2O$, 0.36; KH_2PO_4, 0.05; Na_2SO_4, 0.04; $CaCl_2$, 0.04; $MgCl_2$, 0.02; pH 8.0, ionic strength (*I*) equivalent to 10 mM LiCl. 1-Naphthol concentrations were measured with an HPLC (Waters 2690, Milford, MA), equipped with a 150 × 4.6 mm reverse-phase Pinnacle ODS column (Restek, Bellefonte, PA), and a PDA (Waters 996) detector. The mobile phase consisted of 75% (v/v) methanol and 25% MilliQ H_2O at 1 mL min^{-1}. 1-Naphthol was quantified by integrating the 2.90 min peak at 280 nm wavelength. 1,2-Naphthoquinone and 2-hydroxy-1,4-naphthoquinone (Sigma Chemical) standards were prepared in methanol for comparison to 1-naphthol products detected in this study, and eluted at 2.18 and 1.56 min, respectively. HA sorbed to the guard column of the HPLC and reduced the measured 1-naphthol concentration but not in a reproducible manner; however, HA did not effect elution time or UV/Vis spectra of any analyte. Stock 1-naphthol solutions were prepared by premixing ^{14}C-labeled and unlabeled 1-naphthol, and bioreactors contained ca. 1.0 nCi mL^{-1}. ^{14}C-activity was measured by liquid scintillation counting (LSC; Rack-Beta, Wallac, Gaithersburg, MD).

HA used in this study was extracted from the A horizon of a northern hardwood Typic Dystrochrept (forest soil) collected from Nittany Ridge, Centre County, PA, and purified according to standard methods (Chorover *et al.*, 1999). Stock HA solutions (ca. 500 mg C L^{-1}) were prepared in MilliQ H_2O maintained at pH 7 with 0.10 M NaOH in an amber glass bottle. Dissolved organic carbon concentration of the stock solution was measured with a Shimadzu TOC-5000A analyzer (Columbia, MD).

A 1-naphthol-degrading pure culture (Gram-negative, motile rod) was isolated and purified from municipal wastewater activated sludge by conventional spread and streak plating, and then cryogenically preserved. Cryopreserved cultures were grown to high density in Tryptic Soy Broth at 28°C for 24 h, concentrated by centrifugation (10,000 × *g* for 10 min), washed twice with the BE, and allowed to rest at 4°C for 24 h prior to use. Nutrient forms of nitrogen were excluded from the BE to maintain nongrowth conditions. Cell suspension concentration was determined by acridine orange direct counts (AODC; Kepner and Pratt, 1994) and total volatile solids (TVS; APHA, 1995). Typical inocula densities (final concentrations) were ca. 10^8 counts mL^{-1} and ca. 15 mg TVS L^{-1}. Uninoculated abiotic controls and killed controls (inoculum first autoclaved at 15 psi, 121°C for 15 min), which contained 1-naphthol with and without HA, were also prepared.

Experiments were conducted in 125-mL glass serum bottles (wrapped in aluminum foil) fitted with a Teflon® adapter that held a 5-mL alkali trap (with 50% KOH) over the solution. Variable quantities of HA and 1-naphthol stock solutions were combined with BE for a total volume of 80 mL. All times herein are reported with respect to the biodegradation period (t = 0 h occurred at bacterial inoculation). Bottles were sealed with Teflon-faced rubber septa and allowed to preequilibrate (magnetic stirring) under aerobic conditions in the dark for 7 days prior to inoculation (set up at t = −7 days). Moments before inoculation (t = 0 h), aqueous and KOH samples were removed for HPLC and/or LSC analyses. Preliminary experiments revealed little 1-naphthol mineralization after 72 h, so all experiments were terminated at t = 72 h. After collection of the t = 72 h aqueous and KOH samples, 1 mL of 1 M HCl was added to the reactor, and the suspension (ca. pH 2) was stirred overnight to strip $^{14}CO_2$(g) out of the solution. The suspension was then filtered (0.45 μm) and the filter with retentate was combusted in a ^{14}C oxidizer (OX500, R.J. Harvey, Hillsdale, NJ). The filtrate was analyzed for 1-naphthol by HPLC and ^{14}C-activity by LSC, and final $^{14}CO_2$ in the KOH trap was measured by LSC. The filtration step was used to remove solids prior to HPLC analysis, and to measure the final distribution of ^{14}C radioactivity in so-

lution, associated with the biomass or HA (precipitated at pH 2), and evolved as $^{14}CO_2$. A ^{14}C mass balance was obtained by summing the total radioactivity recovered in these three fractions (aqueous phase, filter retentate, and KOH).

Cell viability and numbers were measured with the Live/Dead Baclight Bacteria Viability Kit (Molecular Probes, Eugene, OR) for select experiments. Bacteria suspension was treated with dyes and filtered (0.2 μm) through a low-autofluorescence membrane filter (Corning, Corning, NY). The numbers of green (live) and red (dead) bacteria within a 5 × 5-μm field were counted for 20 random fields from one suspension aliquot, and results were averaged. To determine if 1-naphthol was toxic to the pure culture, Baclight measurements were made at t = 0 h and t = 72 h for the highest 1-naphthol concentration (20 mg L^{-1}) tested in this study. To determine if HA was toxic to the pure culture, Baclight measurements

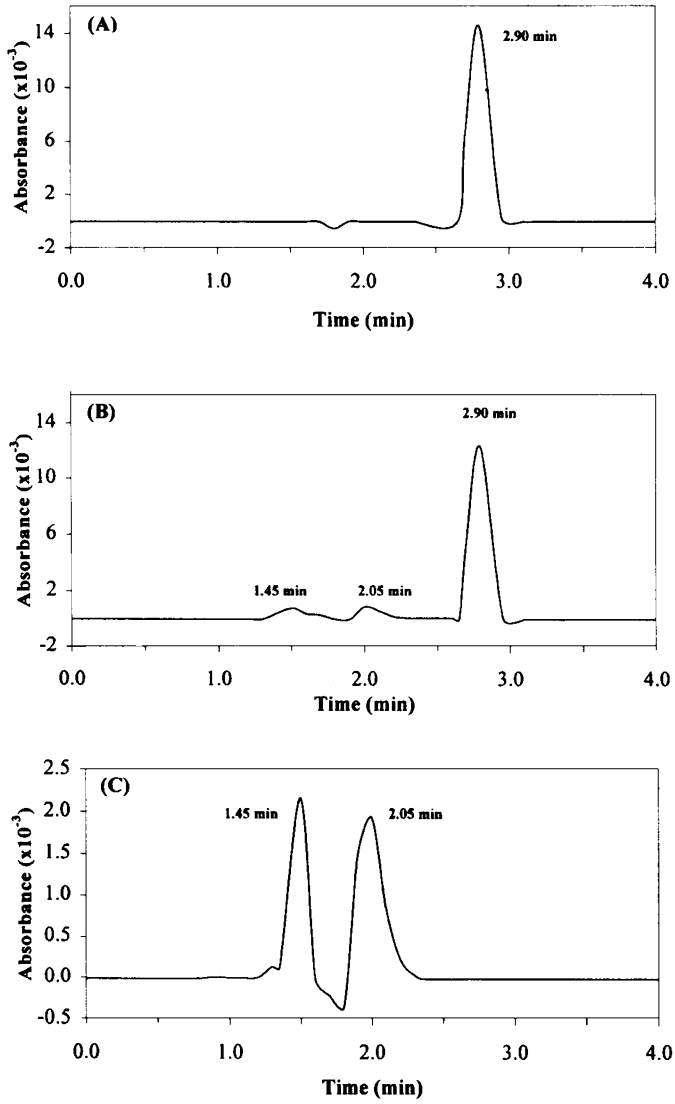

Figure 1. HPLC chromatograms of bioreactor contents during the experiment for $[1\text{-naphthol}]_{t=0h}$ = 7.84 mg L^{-1} and [HA] = 0 mg C L^{-1}. (**A**) At the start of the 7-day aerobic, abiotic preequilibration period, $[1\text{-naphthol}]_{t=-7d}$ = 8.91 mg L^{-1} (2.90 min elution time). (**B**) After the 7-day preequilibration and before inoculation, $[1\text{-naphthol}]_{t=0h}$ = 7.84 mg L^{-1} and abiotic oxidative transformation products were detected at elution times of 2.05 and 1.45 min. (**C**) After the 72-h biodegradation period, $[1\text{-naphthol}]_{t=72h}$ was below HPLC-PDA detection limit (0.10 mg L^{-1}) but concentrations of transformation products increased (note scales in **B** vs. **C**).

were made at t = 0 h and t = 72 h in the absence and presence of HA (65 mg C L^{-1}) at a constant 1-naphthol concentration (5 mg L^{-1}).

RESULTS

Herein, biodegradation refers to any transformation of 1-naphthol that is biologically mediated, while mineralization refers to the complete oxidation of 1-naphthol to CO_2. Biodegradation was measured by HPLC-PDA analysis of 1-naphthol (Figs. 1 and 2), while mineralization was measured by $^{14}CO_2$ evolution (Table 1). The normalized extent of biodegradation was calculated as {([1-Naphthol]$_{t=0h}$ − [1-Naphthol]$_{t=72h}$])/[1-Naphthol]$_{t=0h}$} × 100%, and the normalized extent of mineralization was calculated as ([$^{14}CO_2$ evolved over 72 h]/[initial ^{14}C-activity at t = 0 h]) × 100%. Prior to bacterial inoculation, 1-naphthol was abiotically oxidized to products tentatively identified as naphthoquinones and hydroxynaphthoquinones. Product identification was based on polarity (i.e., chromatographic separation, Fig. 1) and UV/Vis spectra

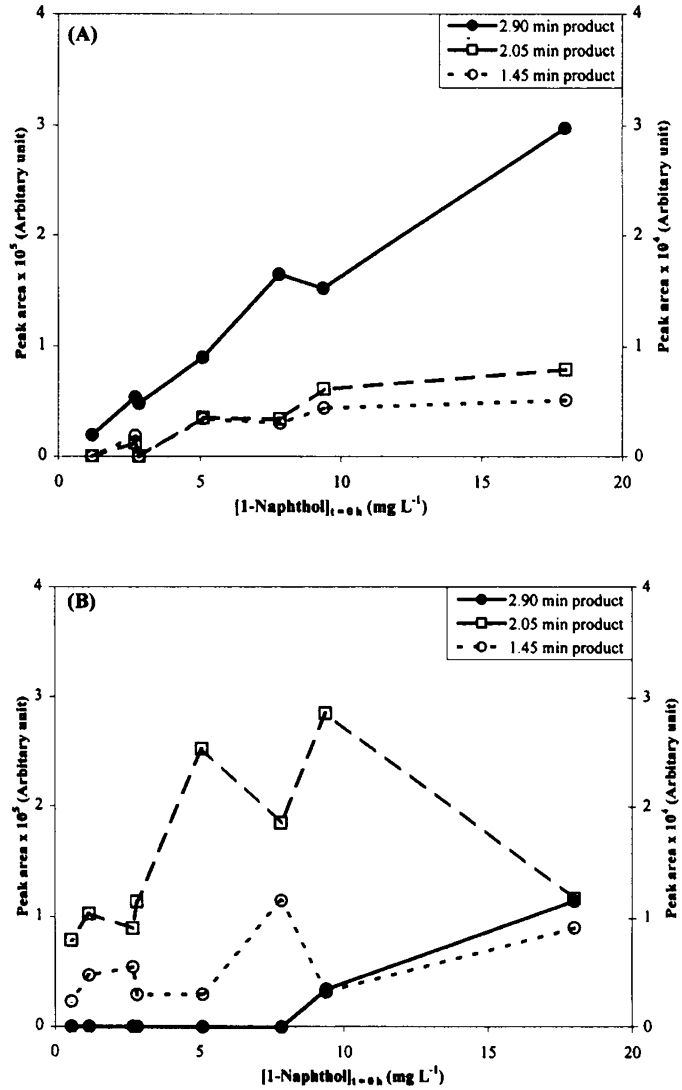

Figure 2. Relative concentrations of 1-naphthol and its transformation products during the experiment as function of [1-naphthol]$_{t\ =\ 0h}$. 1-Naphthol eluted at 2.90 min, while the transformation products eluted at 2.05 and 1.45 min, and all peak areas were integrated at 280 nm wavelength. 1-Naphthol peak areas are read from the primary y-axis, while peak areas of transformation products are read from the secondary y-axis. Results are presented for (**A**) t = 0 h, and (**B**) t = 72 h.

Table 1. Extent of 1-naphthol biodegradation (loss of 1-naphthol measured by HPLC-PDA) and mineralization (measured by $^{14}CO_2$ evolution), and ^{14}C-radioactivity recovered from the bioreactors after a 72 h aerobic incubation period with a 1-naphthol-degrading pure culture.

[HA] *mg C L^{-1}*	*[1-naphthol]$_{t=0\ h}$* *mg L^{-1}*	*[1-naphthol]$_{t=72\ h}$* *mg L^{-1}*	*% Biodegradation*[a]	*% Mineralization*[b]	*Total recovered*[c] *(n replicates)*
0	0.16	<0.10[d]	>37.5	28.0 ± 4.6, A	99.5 ± 8.6 (4)
65	0.16	<0.10	>37.5	9.6 ± 4.9, B	95.6 ± 4.8 (4)
0	0.56	<0.10	>82.1	35.1 ± 4.2, A	95.9 ± 3.4 (3)
65	0.56	<0.10	>82.1	9.30 ± 3.6, B	107 ± 4.3 (4)
0	0.57	<0.10	>82.5	25.3 ± 2.1, A	93.5 ± 3.4 (4)
65	0.57	<0.10	>82.5	15.8 ± 4.6, B	112 ± 2.5 (3)
0	2.70	<0.10	>92.3	31.6 ± 1.1, A	87.1 ± 1.2 (3)
65	2.70	<0.10	>92.3	16.0 ± 0.7, B	105 ± 1.6 (4)
0	5.94	<0.10	>98.3	34.6 ± 2.9, A	92.6 ± 4.0 (3)
65	5.94	<0.10	>98.3	29.1 ± 4.8, A	98.6 ± 5.4 (4)
0	7.84	<0.10	>98.7	35.4 ± 4.4, A	88.7 ± 4.2 (3)
65	7.84	<0.10	>98.7	19.5 ± 1.3, B	110 ± 2.5 (4)
0	21.3	NM	NC	33.6 ± 3.0, A	88.5 ± 4.0 (3)
65	21.3	NM	NC	33.9 ± 2.7, A	97.4 ± 5.4 (4)
0	7.84[e]	NM	NC	0.3 ± 0.1	99.0 ± 1.0 (2)
65	7.84[e]	NM	NC	0.7	105.9 (1)
0	9.00[e]	NM	NC	6.3 ± 0.03	79.0 ± 11.2 (2)
65	9.00[e]	NM	NC	0.4 ± 0.02 (2)	109 ± 4.1 (2)
0	9.00[f]	NM	NC	0.3 ± 0.03 (2)	97.5 ± 2.3 (2)
65	9.00[f]	NM	NC	0.6 (1)	112 (1)

All values are reported as mean ± standard deviation for (n) replicate reactors.

[a]% Biodegradation = {([1-naphthol]$_{t=0\ h}$ − [1-naphthol]$_{t=72\ h}$)/[1-naphthol]$_{t=72\ h}$} × 100%. For [1-naphthol]$_{t=72\ h}$ below HPLC-PDA detection limit, the value of 0.10 mg L^{21} was used. [b]% Mineralization = ([$^{14}CO_2$ evolved over 72 h]/[initial ^{14}C-activity at t = 0 h]) × 100%. Values with a common letter are not significantly different (α = 0.05). Statistical comparisons are limited to the same [1-napthol]$_{t=0\ h}$ (i.e., difference reflects effect of HA). [c]Calculated based on mean values of ^{14}C-radioactivity collected in the aqueous phase, filter retenate, and KOH trap. [d]1-Naphthol HPLC-PDA detection limit was 0.10 mg L^{-1}. [e]Sterile controls. [f]Killed controls. NM = Not measured. NC = Not able to be calculated.

(Fig. 3) compared to standards of 1,2-naphthoquinone and 2-hydroxy-1,4-naphthoquinone. The normalized extent of abiotic transformation (Table 1) was measured by HPLC-PDA and calculated as {([1-Naphthol]$_{t=7d}$ − [1-Naphthol]$_{t=0h}$/[1-Naphthol]$_{t=-7d}$} × 100%. At t = 0 h, 3.7–12.0% of 1-naphthol was transformed into oxidative products (Table 2), but no $^{14}CO_2$ was ever detected in the KOH trap at t = 0 h.

In the current study, mineralization was low (9 to 35%), while the biodegradation of 1-naphthol was high (usually 100%). 1-Naphthol was biodegraded to concentrations below HPLC-PDA detection limit (0.10 mg L^{-1}) except at the highest 1-naphthol concentrations tested (Fig. 2). Aqueous-phase HA exerted little effect on 1-naphthol biodegradation but a significant effect on mineralization (Table 1). At low initial 1-naphthol concentrations (<2.70 mg L^{-1}), 1-naphthol mineralization decreased from 25–35 to 9–16% with HA, while 1-naphthol biodegradation was essentially unchanged (Table 1). At higher initial 1-naphthol concentrations (ca. >5.0 mg L^{-1}), HA caused little differences in 1-naphthol mineralization or biodegradation.

1-Naphthol transformation products were formed both abiotically prior to bacterial inoculation [Figs. 1(B) and 2(A)] and biotically during biodegradation [Figs. 1(C) and 2(B)]. The primary 1-naphthol transformation products eluted from the HPLC column at 2.05 and 1.45 min, and were tentatively identified as 1,2-naphthoquinone and 2-hydroxy-1,4-naphthoquinone, respectively. Naphthoquinones have been shown to form abiotically via the oxidation of 1-naphthol (Karthikeyan *et al.*, 1999). Also, the biodegradation of naphthalene (with 1-naphthol as an intermediate) and 1-naphthol has been shown to produce 1,4-naphthoquinone and 4-hydroxy-1-tetralone (Bollag *et al.*, 1975; Gibson, 1978). The transformation product peaks produced abiotically [Fig. 1(B)] and biotically [Fig. 1(C)] were identified by HPLC-PDA as the same compounds.

Although UV/Vis extinction coefficients at 280 nm wavelength for 1-naphthol and these naphthoquinones are

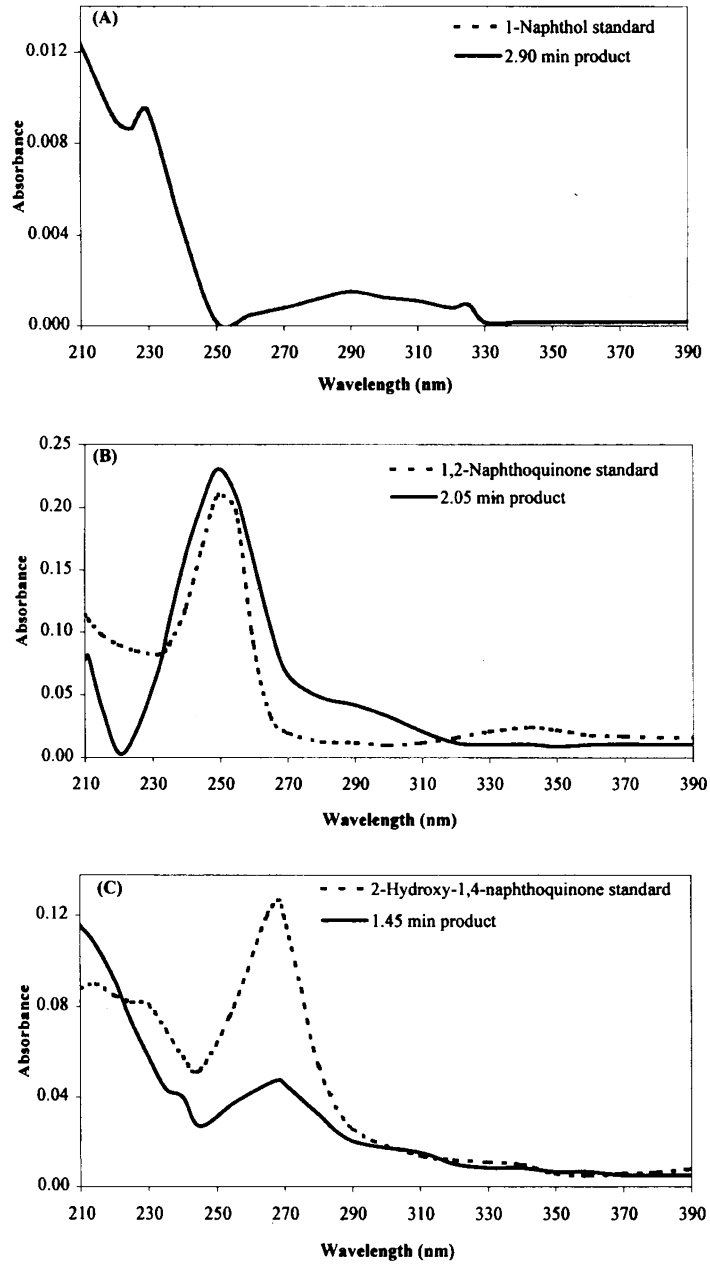

Figure 3. UV/Vis absorbance spectra for 1-naphthol, its transformation products, and naphthoquinone standards. (**A**) Spectra for the 2.90-min product peak measured after the 72 h biodegradation period (t = 72 h) compared to freshly prepared 1-naphthol standard. (**B**) Spectra for the 2.05-min product peak measured at t = 72 h compared to 1,2-naphthoquinone standard (eluted at 2.18 min). (**C**) spectra for the 1.45-min product peak measured at t = 72 h compared to 2-hydroxy-1,4-naphthoquinone standard (eluted at 1.56 min).

not equal (Kamlet, 1960), the integrated peak areas for 1-naphthol and both products were all measured at 280 nm (Fig. 2). Therefore, the integrated peak area values for each compound in Fig. 2 reflect the relative change in concentration for each compound, and do not reflect concentrations of one compound vs. another. At the end of the experiment (t = 72 h), the products did not change [Figs. 1(B) vs. 1(C)], and their concentrations increased [Figs. 2(A) vs. 2(B)]. These results demonstrate that both abiotic and biotic 1-naphthol transformation pathways ac-

Table 2. Extent of 1-naphthol abiotic transformation (loss of 1-naphthol measured by HPLC-PDA during 7-day abiotic sorption period) and biodegradation (loss of 1-naphthol measured by HPLC-PDA during 72-h biodegradation period) for a set of experiments without HA.

$[1\text{-naphthol}]_{t=-7\,d}$ mg L^{-1}	$[1\text{-naphthol}]_{t=0\,h}$ mg L^{-1}	$[1\text{-naphthol}]_{t=72\,h}$ mg L^{-1}	% Abiotic transformation[a]	% Biodegradation[b]
1.19 ± 0.02 (2)	1.07 ± 0.02 (2)	<0.10[c]	10.1	>90.7
2.86 ± 0.02 (2)	2.72 ± 0.05 (2)	<0.10	4.90	>96.3
5.41 (1)	5.21 (1)	<0.10	3.70	>98.1
8.91 ± 0.05 (3)	7.84 ± 0.05 (3)	<0.10	12.0	>98.7
9.45 ± 0.09 (2)	9.02 ± 0.25 (2)	2.01 (1)	4.55	77.7
18.2 ± 0.09 (2)	17.3 ± 0.11 (2)	6.72 (1)	4.95	61.2

All values are reported as mean ± standard deviation for (n) replicate reactors.

[a]% Abiotic Transformation = $\{([1\text{-naphthol}]_{t=-7\,d} - [1\text{-naphthol}]_{t=0\,h})/[1\text{-naphthol}]_{t=-7\,d}\} \times 100\%$, based on mean concentration values. [b]% Biodegradation = $\{([1\text{-naphthol}]_{t=0\,h} - [1\text{-naphthol}]_{t=72\,h})/[1\text{-naphthol}]_{t=72\,h}\} \times 100\%$, based on mean concentration values. For $[1\text{-naphthol}]_{t=72\,h}$ below HPLC-PDA detection limit, the value of 0.10 mg L^{-1} was used. [c]1-Naphthol HPLC-PDA detection limit was 0.10 mg L^{-1}.

cumulated similar products (naphthoquinones), and that these transformation products were more recalcitrant than 1-naphthol.

HA was not shown to be toxic to the pure culture based on Baclight Viability measurements (measure of cell wall integrity). Viable cell numbers remained constant (10^8 cells mL^{-1}) between t = 0 and 72 h, and no significant numbers of dead cells were detected with or without HA. 1-Naphthol also was not shown to be toxic to the pure culture based on Baclight Viability measurements. Viable cell numbers remained constant between t = 0 and 72 h, and no significant numbers of dead cells were detected with the highest 1-napthol concentration tested (20 mg L^{-1}).

DISCUSSION

Karthikeyan and Chorover (2000) found the interaction of 1-naphthol with the same HA used in this study to be pH dependent, with minimal sorption below pH 7.0, a sharp increase between pH 7.5 to 8.5, and maximum sorption around pH 9.0. The sorption of 1-naphthol itself was a minor process caused by the complexion of 1-naphthol with HA, while the abiotic oxidation of 1-naphthol produced transformation products (naphthoquinones) that sorbed more strongly and to a greater extent than 1-naphthol. The formation of naphthoquinones was time dependent even beyond 7 days, and directly affected the sorption of ^{14}C-labeled compound (i.e., 1-naphthol and its products) with this HA (Karthikeyan and Chorover, 2000). These previous findings indicated that abiotic transformations of 1-naphthol controlled its sorption behavior to HA.

Our current findings suggest that the sorption of 1-naphthol and its transformation products by HA directly controlled their biodegradation behavior. In the case of nonsorbing 1-naphthol, biodegradation was not effected by HA. In the case of strongly sorbing naphthoquinones, biodegradation was significantly reduced by HA. Interestingly, naphthoquinones were recalcitrant, even in the absence of HA. However, the net effect is that 1-naphthol mineralization is reduced by HA.

The reduced mineralization of 1-naphthol (for $[1\text{-naphthol}]_{t=0h} < 2.70$ mg L^{-1}) with HA could be caused by (1) HA sorption of 1-naphthol transformation products (prior to bacterial inoculation), (2) HA sorption of 1-naphthol metabolites produced during the biodegradation period, or other unknown effects. However, reduced 1-naphthol mineralization was not caused by HA or 1-naphthol toxicity to the pure culture. HA sorption of 1-naphthol transformation products under conditions identical to the methods employed in the biodegradation experiments (I = 10 mM; pH = 8.0; 7-day aerobic, dark, abiotic reaction period) was measured by Karthikeyan and Chorover (2000). At a 1-naphthol concentration of 1.15 mg L^{-1} and HA concentrations of 11 and 65 mg C L^{-1}, ca. 22 and 32%, respectively, of 1-naphthol ^{14}C-activity were sorbed to HA. We will assume that with 65 mg C L^{-1} HA and 1-naphthol concentrations <2.70 mg L^{-1}, ca. 20–30% of 1-naphthol ^{14}C-activity would have been sorbed prior to bacterial inoculation. This sorbed quantity approximately equals the difference between the amount of 1-naphthol mineralization measured without (25–35%) and with HA (9–16%).

However, mineralization should be reduced only if the sorbed species were covalently bound to HA (i.e., weakly sorbed compounds eventually become bioavailable; e.g., Martin and Haider, 1976; Dec and Bollag, 1988). Karthikeyan and Chorover (2000) did not show or claim

that all of the sorbed ^{14}C-activity was covalently bound to HA, although high molecular weight-coupled reaction products (e.g., polynaphthoquinones) were identified. Thus, another process also likely contributed to decreased 1-naphthol mineralization in the presence of HA. 1-Naphthol metabolites such as salicylic acid and catechol (Eaton and Chapman, 1992) and 4-hydroxy-1-tetralone (Bollag *et al.*, 1975) could potentially sorb strongly to HA if the bacteria excreted these compounds into solution before complete mineralization. Several studies have reported that PAHs are biodegraded in a two-stage pattern where 1-hydroxy-2-carboxy aromatic metabolites accumulate in solution (external to cell) before further degradation occurs (e.g., Klausmeier and Strawinski, 1957; Guerin and Jones, 1988). In addition, the incomplete or partial biodegradation of PAHs is common (LeBlond *et al.*, 2000). Thus, we speculate that the binding of 1-naphthol metabolites to HA could also have contributed to decreased 1-naphthol mineralization.

In a related study, Amador and Alexander (1988) measured both the sorption of benzylamine, benzoic acid, and phenylacetic acid to a soil HA (by equilibrium dialysis), and the biodegradation of these pollutants by a soil mixed culture with HA. Benzylamine sorbed to HA, while benzoic and phenylacetic acid did not. For sorbing or nonsorbing contaminants, HA had little to no effect on the rate of degradation, but did tend to reduce the extent of mineralization. The authors suggested that benzylamine sorbed weakly to the HA but soluble metabolites of all contaminants may have become strongly bound, limiting their mineralization. Our results with 1-naphthol would support this interpretation.

This study measured the biodegradation and mineralization of 1-naphthol and its transformation products with (65 mg C L^{-1}) or without HA. Experimental conditions of controlled pH, ionic strength, and constant viable bacterial numbers were used to better interpret the effect of HA. Our results showed that the sorption of both 1-naphthol abiotic transformation products and bacterial metabolites to HA reduced 1-naphthol mineralization. Because 1-naphthol did not sorb to HA, HA had no effect on 1-naphthol biodegradation. These results demonstrate that the reactivity of transformation products is important in controlling the fate and bioavailability of organic contaminants.

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