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Determination of [^{13}C]Pyrene Sequestration in Sediment Microcosms Using Flash Pyrolysis−GC−MS and ^{13}C NMR

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In this study, the use of a ^{13}C-labeled pollutant probe, [^{13}C]pyrene, and the application of flash pyrolysis−GC−MS and CPMAS ^{13}C NMR provided analytical capabilities to study pyrene interactions with soluble and insoluble compartments of sedimentary organic matter (S_DOM) during whole sediments incubations in aerated microcosms. Surface sediments were collected from a site of previous hydrocarbon contamination in New Orleans, LA. Over a period of 60 days, humic acid and humin fractions of S_DOM accumulated increasing amounts of pyrene that were resistant to exhaustive extraction with organic solvents. The sequestered pyrene was evident in CPMAS ^{13}C NMR spectra of humin fractions. The amount of sequestered pyrene in humic materials was quantified by flash pyrolysis−GC−MS, a technique that destroys the three-dimensional structure of macromolecular S_DOM. Noncovalent binding of pyrene to humic materials in S_DOM was greater in sediments incubated with biological activity than biocide-treated sediments. The combined analytical approaches demonstrate that the sequestered pyrene, or bound residue, is noncovalently associated with S_DOM and has not undergone structural alteration. Implications of these data are discussed in reference to S_DOM diagenesis and long-term availability of bound pollutant residues in sediments.

Introduction

Pollutant aging is a phenomenon that describes the time-dependent slow desorption or nonextractability of hydrophobic organic contaminants from sedimentary organic matter (S_DOM) (*1, 2*). Pollutant sequestration is believed to render contaminants less available for interactions with biological systems (*3*). Recent studies have proposed that sequestration of PAHs in sedimentary organic matter (S_DOM) is dependent on S_DOM diagenesis (*4*); the degree of chemical reduction or physical condensation in S_DOM (*5*); the presence of deposited combustion particles (*6, 7*); and the polarity and aromatic fraction content of S_DOM (*8, 9*). Suggested mechanisms for PAH immobilization in S_DOM include chemical sorption and physical micropore entrapment phenomena that are not well understood (*2, 10*). Limited information about the chemical composition of S_DOM continues to hinder prediction of pollutant fate and behavior in soil and sedimentary systems.

Bound pollutant residues are often defined as that fraction of the added parent material that is resistant to exhaustive solvent extraction and not removable from the S_DOM matrix (*3, 11*). Generally, bound residues are assumed to be more reactive byproducts of parent material that are covalently bound to S_DOM by either addition or oxidative coupling reactions (*12−14*). The formation of bound residues may occur via either biological or chemical processes.

Column adsorption, batch sorption, and gas-purge desorption are important techniques used to determine noncovalent associations of organic pollutants with organic matter. These techniques provide important quantitative information about concentrations of bound probe, but they do not provide information specific to bond formation or "bound" probe structure. On a similar note, radiolabeled studies can provide quantitative information about desorbed or extractable probe fractions but not on the binding mechanism or structure of the probe if it remains irreversibly bound to the S_DOM matrix.

Prior research has noted accumulation of nonextractable ^{14}C label from various radiolabeled organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), that were incubated in soils and sediments (*14−18*). To date, there is limited information regarding the actual structure of nonextractable or "bound" label associated with S_DOM. One way to determine the type of binding interactions between the pollutant of interest and both soluble and insoluble organic matter is to label pollutants with ^{13}C at specific carbon sites and to analyze their interactions with organic matter by solid and liquid nuclear magnetic resonance spectroscopy (NMR) (*13, 19*). A more detailed discussion and demonstration of NMR applications in environmental chemistry has been recently reviewed by Nanny et al. (*20*).

This study was designed to determine structural composition and molecular interactions of [^{13}C]pyrene, a ^{13}C-labeled organic pollutant probe, in soluble and insoluble organic matter fractions of sediments. Sediments treated with a biocide and untreated sediments were incubated with [^{13}C]-pyrene in aerated microcosms over a 60-day period. At several time points during incubation, sediments were chemically fractionated and then solvent-extracted to determine extractable [^{13}C]pyrene. To determine the structure of residual nonextractable label, its binding interactions with insoluble S_DOM, and S_DOM composition, humic acid and humin fractions of S_DOM were analyzed by flash pyrolysis−gas chromatography−mass spectrometry (flash pyrolysis−GC−MS) and cross-polarization magic-angle spinning (CPMAS) ^{13}C NMR. Both flash pyrolysis−GC−MS (*21, 22*) and CPMAS ^{13}C NMR (*23, 24*) have been used extensively to characterize macromolecular S_DOM.

Experimental Section

Materials. Sediments (0−10 cm) were collected by gravity core sampling from Bayou Trepagnier, a site of previous hydrocarbon contamination (New Orleans, LA), using a steel sediment bore. Sediments were transferred into autoclaved

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TABLE 1. Sediment Characteristics

	surface sediment (0−10 cm)
pH	7.18,[a] 7.20[b]
H[c]	1.83
N[c]	0.85
O[c]	13.7
TOC[c,e]	8.46
CEC[c,f]	42.7
sand[d]	32
silt[d]	63
clay[d]	4.7
type[d]	silty clay loam

[a] On site analysis by Corey Lambert, Tulane University. [b] Measured at PSU (10 g of soil/10 mL of water). [c] Analyzed by Huffman Labs, Golden, CO. [d] Analyzed by Hazen Research, Inc., Golden, CO. [e] Total organic carbon (TOC) in soils (SW-846 Method 9060). [f] Cation exchange capacity, (meq/100 cm^3).

Nalgene bottles and stored at 32 °C until used in experiments. Analysis of surface sediments in our laboratory did not indicate PAH contamination. Sediment characteristics are shown in Table 1.

Deionized, carbon filtered, sterile-filtered water (Elgastat UHQ II F/TAP Water Feed RO, Bucks, England) was used for all solutions and soil rinses. Solvents, methylene chloride (DCM), methanol (MeOH), and acetonitrile (ACN), were of the highest quality available. The [4,9-^{13}C]-labeled pyrene was synthesized in our laboratory by Jack Richman (University of Minnesota).

Aerated Microcosm Systems for Soil Incubations. Aliquots of wet sediment (10 g) were mixed with 40 mL of sterile, distilled, and deionized water in 250-mL amber glass jars (Scientific Specialties Service, Inc, Randallstown, MD). Sediment slurries were divided into three groups for incubation in aerated chambers. The first group was active sediment amended with 3.5 mg of [4,9-^{13}C]pyrene via a DCM carrier (74 μL) using a glass Hamilton microliter syringe to give a concentration of 350 μg PAH/g sediment. The second group of sediments was treated with a biocide by adding 2 g of NaN_3 to the slurry before the [^{13}C]pyrene addition. The last group consisted of sediment slurries without [^{13}C]pyrene or biocide treatment.

The 250-mL amber glass jars were fitted with Teflon/silicone septa lined polypropylene caps and 0.015-mm Teflon tubing for connections to pumps and volatile trapping solutions (Scientific Specialties Service, Inc, Randallstown, MD). The slurries were continuously aerated with a Whisper 400 aquarium pump (Oakland, NJ) at 25 mL/min, representing approximately 10 air exchanges per hour. Air was filtered through activated charcoal, glass wool, and 1 N KOH prior to introduction to chambers. Evolved carbon dioxide was trapped by bubbling air through two sequential solutions of 1 N KOH. Trapping solutions were removed and replaced at day 30, 45, and 60.

Fractionation of Sediment Organic Matter. Three 250-mL glass jars were removed at 0, 30, 45, and 60 days from each treatment group for fractionation. Sediments were fractionated into water-soluble extracts, bulk sediment, humic and fulvic acids, and humin. Individual fractions were lipid extracted by adding DCM/MeOH (2:1 v/v, 15 mL) to sediments, allowing the mixture to sit for 12−24 h, sonicating solvent and sediment with ultrasonication (pulse mode, 20 s; Branson Sonifier 250), and allowing solvent and sediments to stand for another 12−24 h prior to decanting the solvent supernatant.

The S_DOM fractionation protocol involved acidification of sediment slurry with a 20% H_3PO_4 (v/v) solution for 24 h. Acidified sediment slurries were decanted into 50-mL Oak Ridge Teflon centrifuge tubes (Fisher Scientific, Pittsburgh PA), and centrifuged (Sorvall RC 5C Plus Superspeed centrifuge) at 10000*g* for 20 min. The aqueous layer was decanted into 40-mL amber EPA vials and lipid extracted. A mixture of DCM/MeOH (2:1 v/v; 15 mL) was added to the remaining bulk sediment. The sediment/organic solvent mixture was lipid extracted, as described above, before removing the organic solvents and repeating the lipid extraction procedure. Lipid extracts from bulk sediment were combined together for analysis.

The remaining bulk sediment was then extracted with a 0.1 M NaOH (25−35 mL) solution to remove humic and fulvic acids and to liberate sorbed pyrene. Centrifuge tubes, containing 0.1 M NaOH and sediments, were purged with nitrogen, closed, and shaken in the dark for 24 h. Afterward, dissolved humic and fulvic acids were decanted into 40-mL amber EPA glass vials. Both humic acids and the remaining humin material were lipid extracted separately. A 10% HF/HCl solution was added to the remaining humin in centrifuge tubes, purged again with N_2, and allowed to stand in the dark for a week. HF/HCl treatment dissolves mineral components of humin, reduces paramagnetic interference with ^{13}C NMR, improves resolution of specific carbon compounds (*25*), and may further liberate residual pyrene sorbed to mineral and organic surfaces. After a week, HF/HCl-treated humin was lipid extracted in toto before decanting the HF/HCl supernatant. Lipid extracts from different sediment fractions were reduced with a gentle stream of N_2 prior to analysis by HPLC. Humic acids and HF-treated humin were freeze-dried for 1−3 days (Benchtop 3.3/Vacu-Freeze) (VirTis Co., Gardinar, NY).

HPLC. Lipid extracts were analyzed by HPLC chromatography on a Waters 600C System controller and a Supelco LC−PAH column (250 mm × 4.6 mm, i.d., Bellefonte, PA) using an elution gradient from 60% acetonitrile/40% water to 100% acetonitrile at 18 min with a flow of 1 mL/min. [^{13}C]-Pyrene was detected with a 991 photodiode array detector (Millipore Corporation, Milford, MA). HPLC data was analyzed with Millennium 2010 software (Millipore Corporation, Milford, MA).

Flash Pyrolysis−GC−MS. Freeze-dried humic acids and humin were analyzed by flash pyrolysis−GC−MS using a Chemical Data System Pyroprobe 1000, a Varian 2700 gas chromatograph, and a Kratos MS-25 RFA mass spectrometer fitted with a Kratos Mach 3-Dart data system. Approximately 0.5 mg of humic acid or humin was placed in a fire polished quartz pyrolysis tube (CDS Analytical Inc.) that was fitted between the coils of the pyroprobe prior to insertion into the injection port of the gas chromatograph. The injection port temperature was maintained at 220 °C. The pyroprobe temperature was then ramped at 5 °C/ms to 610 °C and held for 10 s. Volatiles were swept into a 25-m fused silica capillary column coated with chemically bound J&W DB-1701 (0.25 mm i.d., a film thickness 0.25 μm) (VWR, Bridgeport, NJ). The GC oven was programmed from an initial temperature of 35−290 °C at a heating rate of 7 °C/min and held at 290 °C for 10 min.

CPMAS ^{13}C NMR. Solid-state ^{13}C NMR of humic acids and humin utilized cross-polarization magic angle spinning (CPMAS) and was performed using a Chemagnetics, Inc. M-100 instrument with a ^{13}C resonance frequency of 25.2 MHz. The spectra were obtained from 100 000 to 400 000 accumulated scans using a recycle time of 1s, a contact time of 1 ms, and a spinning speed of 3.2 kHz. The free induction decays were defined by exactly 512 data points and the data was zero filled to 4096 data points prior to Fourier transformation.

Statistical Analyses. Statistical significance was determined by Student's *t*-test ($p < 0.05$) when two data sets were compared. Unless otherwise noted, triplicate samples were used to determine the averaged recoveries of pyrene in

TABLE 2. Mass Amounts of Pyrene (μg) in Lipid Extracts of Sediment Fractions[a,b]

SOM	day 0	day 30	day 45	day 60	day 60 control[c]
total pyrene added	3433 ± 101	3433 ± 101	3433 ± 101	3433 ± 101	2682 ± 597
water	97 ± 30	5 ± 2.2	6 ± 0.1	5 ± 0.3	256 ± 16
bulk sediment	2968 ± 253	1564 ± 137	1068 ± 279	1321 ± 168	1750 ± 186
humic acids	ND	6.4 ± 2.9	26 ± 2.6	28 ± 6.4	10 ± 1.4
bulk humin	14 ± 9.2	376 ± 56.4	189 ± 15.9	89 ± 61	62 ± 13
HF/HCl-treated humin	ND	74 ± 20	99 ± 16	306 ± 51.6	55 ± 6.4
total extractable pyrene	3104 ± 296	2025 ± 195	1382 ± 295	1828 ± 138	2074 ± 238
recovery of pyrene (%)	90 ± 8.6	59 ± 5.7	40 ± 8.6	53 ± 4.0	77 ± 8.9

[a] Sediment fractions were extracted by sonification with DCM/MeOH (8:2 v/v). [b] Values are means ± one standard deviation. ND = not detected. [c] Sediment was treated periodically with a biocide.

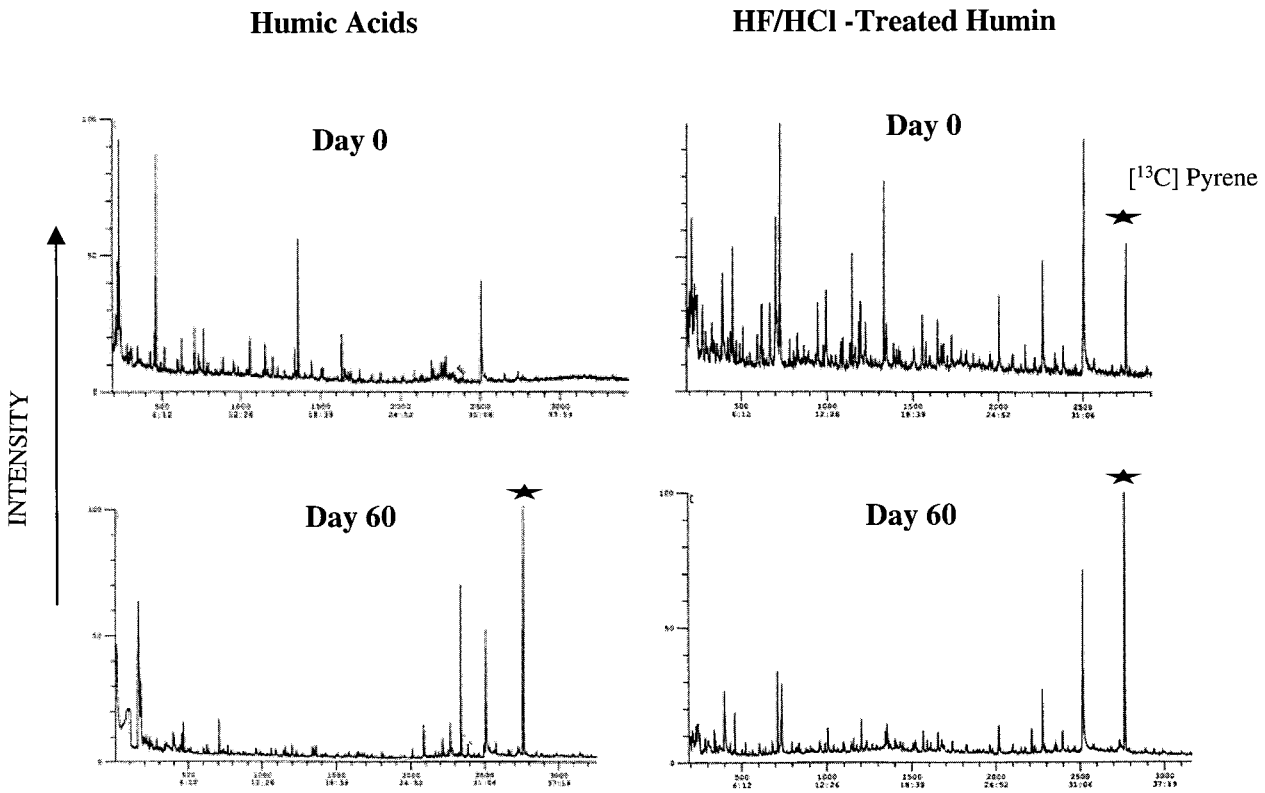

FIGURE 1. Flash pyrolysis−GC−MS of exhaustively extracted humic acid and humin fractions from sediments before and after incubation with biological activity in aerated microcosms. Starred peaks (★) denote [^{13}C]pyrene, MW = 204.

sediment extracts. Values in tables represent the mean ± one standard deviation.

Results and Discussion

There are several advantages to using combined techniques of ^{13}C-labeled organic contaminants, ^{13}C NMR, and flash pyrolysis−GC−MS to study organic pollutant fate in sedimentary systems. Direct identification of and structural changes to the labeled, stable isotope probe retained in insoluble sediment organic matrixes (S_DOM) can be determined by ^{13}C NMR (*19, 26*) and flash pyrolysis−GC−MS (*21*). Noncovalent and covalent interactions between the probe and S_DOM can be evaluated by liquid- and solid-state ^{13}C NMR if probe concentrations are adequate (*13, 19, 26*). The use of labeled materials in controlled systems and the aforementioned analytical techniques also provide a complete mass balance of both degraded and nondegraded labeled material.

The same techniques used to study the fate of [^{13}C] sorbate can also evaluate modifications to the structural composition of the soluble or insoluble sorbent. The data presented here provides information about the association of [^{13}C]pyrene with S_DOM in the presence and absence of biological activity. The primary objective of this study was to evaluate the type of binding interactions influenced by changes in S_DOM composition. For clarity, bulk sediment is an acidified sediment extracted with organic solvents, humic acid is that fraction of bulk sediment that is soluble in dilute alkali, and humin is that fraction of bulk sediment that is insoluble in organic solvents, dilute alkali, and dilute acid. Bulk humin is humin prior to HF/HCl treatment. Only ^{13}C-labeled pyrene was used in microcosm experiments.

Association of [^{13}C]Pyrene with S_DOM. The association of pyrene with S_DOM over a 60-day period was first evaluated by traditional solvent extraction techniques. The amounts of pyrene extracted from bulk sediment, humic and fulvic acids, and humin were quantified by HPLC. The amounts of pyrene in lipid extracts of soil fractions and total extractable pyrene are shown in Table 2. The percent recovery of pyrene was determined by dividing the total extractable pyrene by the amount originally added to each microcosm in the sample set (n = 3). Total percent recoveries of pyrene for days 30,

TABLE 3. Mass Amounts of Pyrene (μg) Released by Pyrolysis−GC−MS[a,b] of Humic Acid and Humin

	humic acid	humin
day 0	4.9 ± 2.1	82 ± 23
day 30	6.0 ± 2.3	629 ± 78.2
day 45	19 ± 3.3	1842 ± 68.10
day 60	107 ± 52.8	1316 ± 11.00
day 60 control[c]	68 ± 7.2	377 ± 148

[a] Soil fractions were extracted by sonification with DCM/MeOH (8:2 v/v). [b] Values are means ± one standard deviation ($n = 2$). [c] Sediment was treated periodically with a biocide.

45, and 60 were significantly less in biologically active sediments (40−59%) than either day 0 or biocide-treated sediments (90% and 77%, respectively) ($p \leq 0.05$). By day 60, significantly greater amounts of pyrene were present in solvent extracts of humic acids and humin of active sediments than day 0 and biocide-treated sediments ($p \leq 0.05$) (Table 2). For humin fractions, pyrene concentrations continued to increase in HF/HCl treated humin while concentrations in bulk humin decreased over time except for an initial increase at day 30 (Table 2).

The increased association of pyrene with humic materials was confirmed by both flash pyrolysis−GC−MS and CPMAS ^{13}C NMR. After HPLC analysis of solvent extracts of humic acids and humin, the remaining humic acids and humin solutions were freeze-dried for pyrolysis. Representative chromatograms for flash pyrolysis−GC−MS are shown in Figure 1, and quantified recoveries of pyrene are shown for all days in Table 3. It is important to note that humic materials were pyrolyzed after various chemical treatments and solvent extraction; thus, pyrene released by pyrolysis represents pyrene that appears to be sequestered within the macromolecular structure of humic materials and is resistant to solvent extraction. Released pyrene is not a product derived from pyrolysis of S_DOM because the molecular weight of eluted [^{13}C]pyrene was 204; whereas the molecular weight of nonlabeled pyrene is 202.

The amounts of pyrolysis-released pyrene increased with time for all humic samples, but the amounts of pyrene in humic materials from biologically active sediments were significantly greater than day 0 or biocide-treated sediments (Table 3). When total recoveries of pyrene from flash pyrolysis−GC−MS were added to HPLC recoveries, averaged recoveries of pyrene for all treatment groups averaged 87% ± 8.9. These findings suggest that pyrene was not significantly degraded in any treatment group during the incubation period. Together, HPLC extraction data and pyrolysis−GC−MS data suggest a relationship between biological activity and (i) increased resistance of pyrene to lipid extraction over time and (ii) movement of pyrene into refractory organic matrixes such as humic acids and humin.

CPMAS ^{13}C NMR spectra of freeze-dried humin samples confirmed the influence of biological activity on increased pyrene association with humic materials. As shown in Figure 2, the increased intensity of the signal for [^{13}C]pyrene (127 ppm) is apparent in humin samples for both active sediments and biocide-treated sediments. Biocide-treated sediments were repeatedly treated with NaN_3 to maintain biological inhibition during the incubation period. Thus, accumulation of pyrene in biocide-treated sediments is considered the result of abiotic sorption processes such as partitioning or micropore diffusion.

CPMAS ^{13}C NMR can determine major organic carbon functionalities without alteration of sample composition or structure. When used in tandem with ^{13}C-labeled compounds, CPMAS ^{13}C NMR allows for in situ observations of the labeled probe interactions with S_DOM. The increased peak intensity

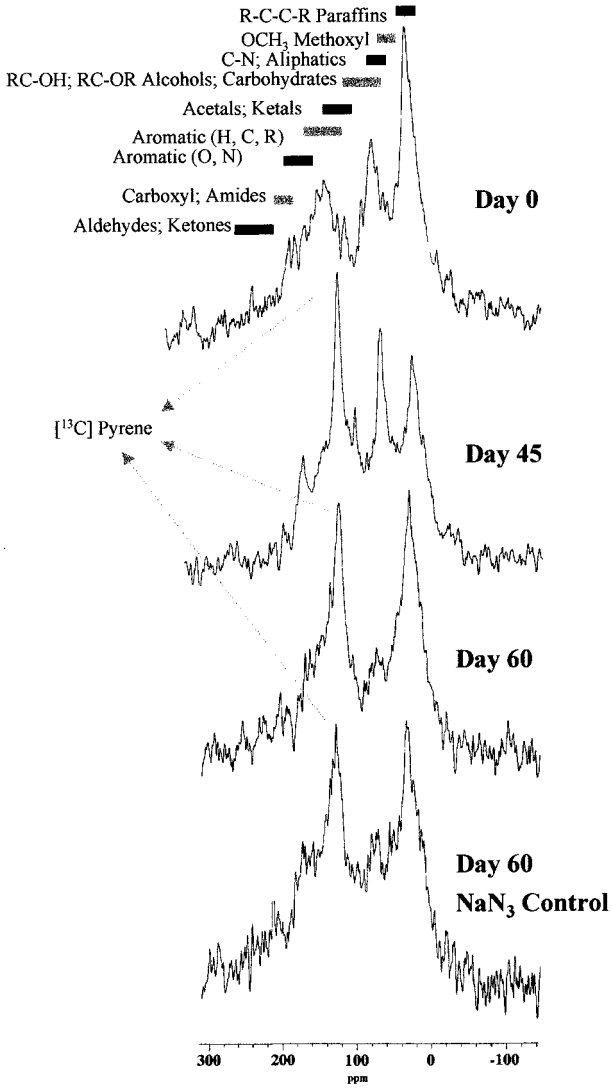

FIGURE 2. CPMAS ^{13}C NMR spectra of exhaustively extracted humin fractions from sediments incubated with [^{13}C]pyrene and from sediments incubated with [^{13}C]pyrene and a biocide (NaN_3). The chemical shift signal for [^{13}C]pyrene is 127 ppm. The chemical shifts for other carbon functional groups are shown with bars.

of the signal at 127 ppm and the absence of additional peaks in spectra, other than those attributed to humin, suggest that the persistent labeled material associated with humin is pyrene (Figure 2). Chemical shifts indicating reactions at or near the labeled carbon in pyrene are absent in the spectra. These findings further support thermal desorption of pyrene by flash pyrolysis−GC−MS as the primary mechanism of pyrene release and demonstrate that released pyrene is not a pyrolytic product of larger pyrene-bound compounds.

The preservation of the [^{13}C]pyrene signal at 127 ppm in humin suggests that interactions between pyrene and humin are noncovalent in nature. Noncovalent interactions between pyrene and humin probably involve van der Waals forces and hydrogen bonding (*10, 27*). These forces are weak relative to covalent and ionic exchange interactions, but they are apparently sufficient in strength to prevent removal of pyrene by stringent solvent extraction methods. Recently, "bound" pesticide residues were shown to be noncovalently bound to soil by cation exchange and hydrophobic interactions (*11*). Results from this study suggest that "bound" residues for nonionic pollutants may also be primarily parent material and that the noncovalent binding of pollutants to S_DOM is enhanced by biological activity. How biological activity may

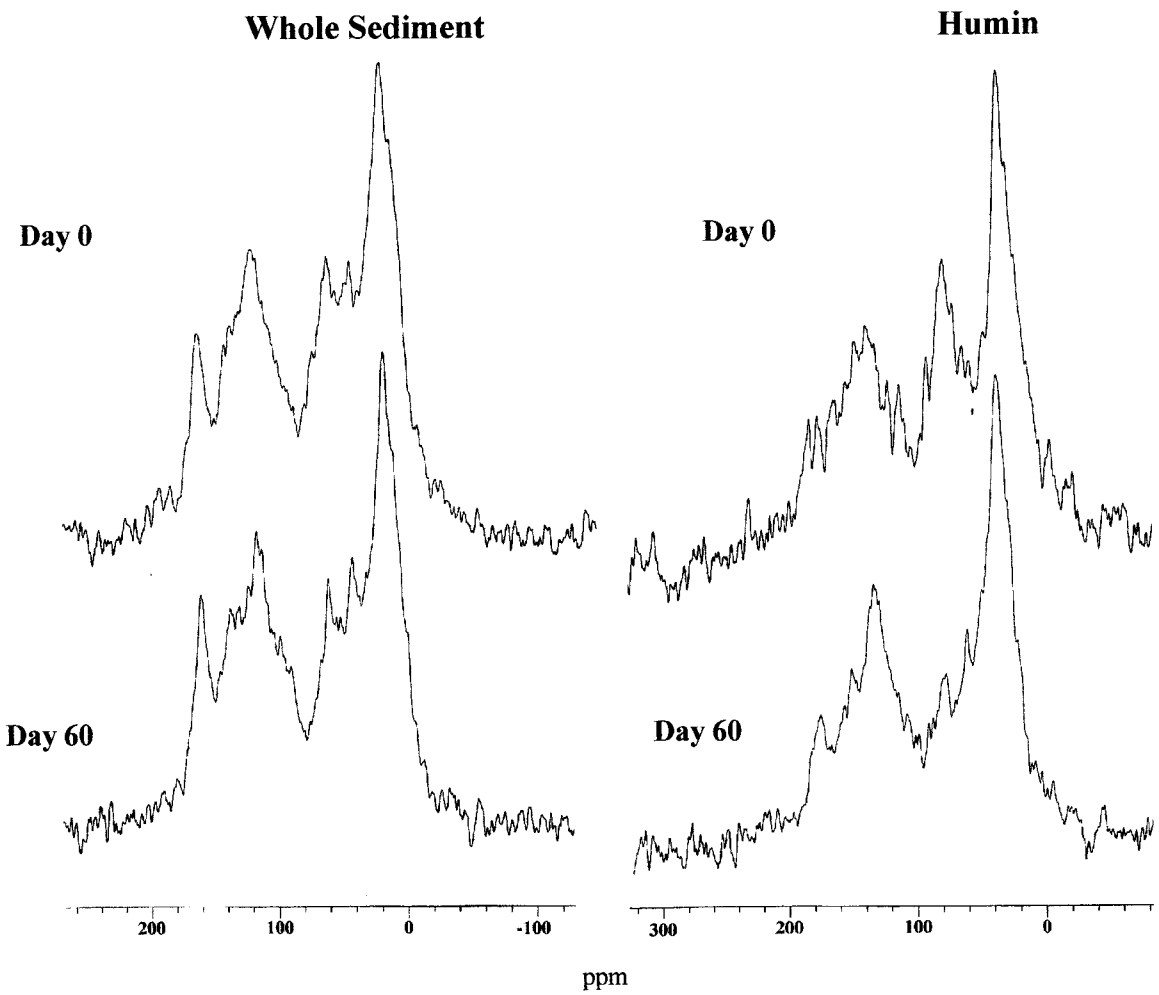

FIGURE 3. CPMAS ^{13}C NMR spectra of whole sediments and their humin fractions at day 0 and day 60. Sediments were incubated without [^{13}C]pyrene or biocide amendments.

enhance noncovalent interactions between pyrene and S_DOM was investigated by examining the structural composition of S_DOM before and after biological incubation.

Time Course Biological Changes of S_DOM. Adsorption (*28, 29*) and partitioning (*10, 30*) within S_DOM or micropores are considered primary mechanisms for PAH association with organic matter. These associations involve noncovalent interactions between pollutant and hydrophobic regions in humic materials (*26, 27*). The extent of PAH sorption to S_DOM is influenced by S_DOM origin (*5, 31*), polarity (*8, 9*), aromatic content (*6, 30*), and organic contamination (*7−9*). The association of hydrophobic pollutants to humic materials, in particular, is dependent on structural and chemical properties of the contaminant (*32−34*), the humic material (*35−39*), and environmental conditions, such as pH or ionic strength (*38, 41, 42*). In this study, both destructive (pyrolysis) and nondestructive analyses (NMR) were undertaken to determine compositional changes to sorbent matrixes, specifically, whole sediments and humic fractions.

Freeze-dried samples of whole sediments that were incubated for 60 days without pyrene were analyzed by CPMAS ^{13}C NMR to compare relative changes to S_DOM structure during biological incubation. Significant changes to whole sediments were not apparent in the CPMAS ^{13}C NMR spectra over the incubation period (Figure 3). Because pyrene remained primarily associated with humin fractions of sediments, freeze-dried humin fractions from these sediments were examined by CPMAS ^{13}C NMR to evaluate the effect of biological activity on humin composition (Figure 3). NMR spectra indicated a loss of observed signals in regions assigned to alcohols (60−95 ppm) and carbohydrates (60−110 ppm) from day 0 to day 60; while paraffinic (0−45 ppm) and aromatic (110−160 ppm) functional groups were conserved over the incubation period.

Humin samples were also analyzed by flash pyrolysis−GC−MS to further assess changes to S_DOM composition. Chromatograms for pyrolyzed samples from active and biocide-treated sediments at day 60 are shown in Figure 4 (gray box insert). As already noted, humin from active sediments contained more nonextractable pyrene than humin from biocide-treated sediments (1316 and 377 μg, respectively). A comparison of the expanded chromatographic regions prior to elution of pyrene indicates loss of fatty acids, proteins, and polysaccharides in humin fractions of biodegraded sediments versus biocide-treated sediments. Thus, humin from biologically active sediments was less polar and predominantly more hydrocarbon-rich, i.e., aromatic and paraffinic, than original humin or humin from biocide-treated sediments. These findings are in general agreement with NMR data presented in Figure 3 and suggest that pyrene accumulation in biodegraded sediments can be correlated with biodegraded humin.

Humic materials are considered precursors to mature geosorbents and have been shown to have a higher sorptive capacity for PAHs than bulk sediment or soil materials (*35*). The degree of pyrene sorption to humic materials increases with greater aromaticity (*27, 34, 35, 38, 42*) or reduced polarity (*9*) of the humic materials. However, as shown in Figure 3,

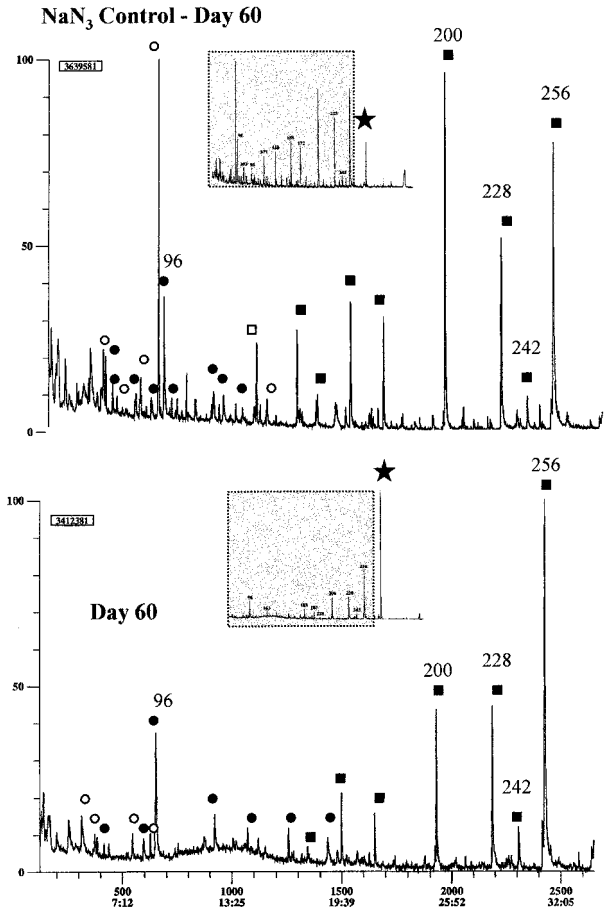

FIGURE 4. Flash pyrolysis−GC−MS of exhaustively extracted humin fractions from sediments incubated with [^{13}C]pyrene and sediments incubated with [^{13}C]pyrene and a biocide (NaN_3). Gray boxes indicate the regions of the chromatograms that are enlarged. Symbols represent [^{13}C]pyrene (★); fatty acids (■); pyrolysis products of polysaacharides (●); and pyrolysis products of protein (○). Numbers above the peaks indicate the molecular masses of the compounds representing the peaks.

paraffinic structures constitute a significant portion of carbon in humin samples. In fact, paraffinic structures are common to hydrolyzed humin from aerobic soils, sediments, and peats, and are believed to be derived from primarily microbial sources (*43*). Because CPMAS ^{13}C NMR spectra cannot resolve if pyrene resides in aliphatic or aromatic components of the humin matrix, pyrene may be "noncovalently" associated with the polymeric matrix of paraffinic compounds in these humin samples.

In sediment, humin is considered a primary precursor to coals and kerogens, aged geosorbents that have a high sorptive capacity for PAHs (*5, 9, 31*). Kerogen formation from humin requires alteration of S_DOM composition followed by heat and pressure. Initial humin transformation to kerogen, or early diagenesis, may occur by (i) condensation reactions of humic substances released by humin degradation or by (ii) selective preservation of aromatic and aliphatic structures present in humin after degradation of more labile humin constituents, such as polysaccharides, proteins, and fatty acids (*43*).

In most contaminated sediments, the physical processes, i.e., pressure and heat, that lead to complete kerogen diagenesis are slow and not likely to have occurred over recent pollutant weathering events of 50−100 years. Thus, many contaminated sediments are in a stage of early diagenesis. Key biologically mediated processes present in early diagenesis are oxidation, sulfate reduction, methanogenesis, and acetate oxidation of the sediment matrix. These processes appear to rapidly decompose oxygenated organic compounds (i.e., polysaccharides and proteins) at equivalent rates in both oxic and anoxic zones (*44*) although these organic compounds may also be "encapsulated" in the S_DOM matrix (*45*). Carbon-rich polymers, such as lignins, tannins, algaenans, and humic materials, degrade more slowly, and often constitute the bulk of preserved S_DOM (*43−45*). Both NMR and flash pyrolysis−GC−MS data from this study demonstrated the loss of proteinaceous and carbohydrate materials from humin in biologically active samples while more reduced carbon materials, such as humic materials and paraffins, were preserved.

Lignins, tannins, and other refractory organic materials are eventually degraded in the sediments by oxidation reactions or metal-coupled redox reactions (*47*). Whether these reactions are biologically mediated or spontaneous is not known; however, their intrinsic rates of degradation are important to understanding the ultimate fate of aged pollutants residing in refractory organic materials. What is clear is that the formation of humin, and eventually kerogen, includes microbial decomposition of the S_DOM matrix; thus, there is precedence for biologically mediated mechanisms leading to pollutant encapulsation and observed pollutant hysteresis in refractory organic matter. In this study, more pyrene was "sequestered" and resistant to exhaustive extraction in biologically active sediments than biocide-treated sediments.

Kan et al. (*30*) have suggested that pollutants may irreversibly bind to S_DOM via noncovalent interactions if the organic matrix is physically altered. Some of the suggested mechanisms for physical alteration of S_DOM include changes in ionic strength, pH, or coagulation of mineral and/or organic particles. These conditions can be influenced by early diagenetic processes. Other physical processes, such as bioturbation of refractory organic macromolecules and the physical deposition of atmospheric combusted particles, could also influence S_DOM composition (*7, 47*). Current kinetic models describing early S_DOM diagenesis are biased toward describing rapid organic carbon decay but not the decay of preserved carbon with sediment depth. The proposed mechanisms that preserve refractory organic materials (*47*) are similar to sorptive mechanisms proposed for reduced availability of aged hydrophobic pollutants (*2*). Understanding the chemical and biological processes present in early S_DOM diagenesis is a certain prerequisite to understanding the formation and eventual diagenesis of aged organic pollutant residues.

The findings presented here suggest that nonextractable fractions of "bound" residues from [^{13}C]pyrene incubations in sediments cannot be assumed to be covalently bound intermediate products from pyrene degradation, but are adsorbed or encapsulated pyrene in sediment humin. Nonspecific biological activity, that is, biological activity that altered S_DOM composition but did not degrade pyrene, increased the amount of adsorbed or encapsulated pyrene. In light of this research, we propose a model whereby early biologically mediated diagenesis changes S_DOM composition and increases the availability of hydrophobic sites for noncovalent interactions with [^{13}C]pyrene. These hydrophobic sites are present in both humic acid and humin, but as reflected by the data in this study, humin has a greater propensity to sequester pyrene. The actual phenomenon of pollutant binding remains a physical process of sorption, but the sorbent, the organic matrix of association, is biologically processed, and thus, biologically mediated. As a postulated example, pollutant sorbed to aromatic or paraffinic material on sediment surfaces or the surfaces of sediment micropores may be "sequestered" as biological activity (i) deposits additional biomass and microbially

processed S_DOM or (ii) reduces surface carbon to hydrophobic, nonlabile fractions of S_DOM, i.e., lignins or humic materials.

In summary, the use of ^{13}C-labeled pollutant probes provides more extensive, molecular information about the fate and interactions of labeled probes with insoluble sorbents than similar radiolabeled tracer studies. Furthermore, analytical techniques used to evaluate the fate of ^{13}C-labeled probes are also useful for characterizing the compositional structure of the sorbent matrix during experimentation. If mechanisms of PAH persistence in sediments are related to biologically mediated transformation of S_DOM and are enhanced by nonspecific biological activity, then acceptable endpoints of both intrinsic remediation and bioremediation treatments will require additional assessment. Further studies are required to examine the stability of these noncovalently "bound" residues in relation to S_DOM diagenesis or turbation and to determine if similar results are obtained for soils and sediments from more oxidized or reduced environments.

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