



A LABORATORY MICROCOSM STUDY OF MACONDO OIL DEGRADATION IN A COASTAL SALT MARSH

Daniel J. Fields, YueHan Lu, and Rona J. Donahoe

Department of Geological Sciences, University of Alabama, 201 7th Ave., Tuscaloosa, Alabama 35487-0338

ABSTRACT

Although Alabama shores were impacted by the *Deepwater Horizon* oil spill, few data are available for a reliable assessment of the fate of Macondo oil-derived pollutants in Alabama's coastal environments. Laboratory microcosm incubations were conducted to evaluate the degradation of hydrocarbons from Macondo oil in salt marsh sediments collected from Bayou La Batre, Alabama. Each microcosm containing salt marsh sediment and in situ seawater was spiked with Macondo oil and then incubated for 14 d in the dark. Total alkanes in the sediment decreased rapidly within the first 12 hr and thereafter remained relatively stable. At the end of the experiment, total alkanes in sediments decreased by $71.4 \pm 32.3\%$. The high degradation rate of alkanes in the first 12 hr of the incubations was attributed to aerobic microbial degradation, and the subsequent decrease in the degradation rate was likely due to oxygen depletion in the microcosms. The concentrations of dissolved inorganic nutrients (nitrate, nitrite, and phosphate) did not show evident patterns over the course of the incubation, indicating that nutrient availability was not the factor responsible for the decrease in the alkane degradation rate. No preferential degradation was observed between normal versus branched alkanes or between short-chain versus long-chain alkanes. Presumably, this may be due to adaptation of in situ microbes to degrade various types of alkane compounds because of their previous exposure to oil pollutants from the *Deepwater Horizon* oil spill as well as from prevalent natural oil seeps and oil drilling activities in the study area. Polycyclic aromatic hydrocarbons (PAHs) detected in microcosm sediment included naphthalene, acenaphthylene, fluorene, fluoranthene, and pyrene. The PAHs showed variable concentrations and demonstrated no consistent loss over time, indicating the resistance of these compounds to short-term biodegradation.

INTRODUCTION

The *Deepwater Horizon* oil spill of 2010 is the second largest marine oil spill in human history. During this spill, approximately 4.9 million barrels of oil were estimated to have been released into the Gulf of Mexico over approximately 3 mo (OSAT, 2010). The Oil Budget calculator estimated that 50% of the oil was removed via skimming, burning or capturing, or evaporation (Atlas and Hazen, 2011). Of the remaining oil, 24% was physically or chemically dispersed, and 26% is unaccounted for (Atlas and Hazen, 2011). Macondo oil is composed primarily of hydrocarbons having various molecular weights and structures. In isolated oil fractions of a sample of fluid exiting the Macondo well collected on June 21, 2010, Reddy et al. (2011) found saturated alkanes with a carbon number range of C_4 to C_{42} and polycyclic aromatic hydrocarbons (PAHs).

Although Macondo oil from the *Deepwater Horizon* oil spill reached Alabama coastal waters and beaches, including Bon

Secour (OSAT-2, 2011), Walker Island (Natter et al., 2012), and Mobile Bay (Atlas and Hazen, 2011; Beazley et al., 2012), much remains unknown about the fate of the oil in Alabama coastal environments. One of areas of greatest concern is salt marshes, which are often characterized by low tidal wave energy and low dissolved oxygen content and are thus susceptible to oil pollutant accumulation. The presence of marsh grasses further allows pollutants to remain sequestered in coastal sediments for years. After an oil spill, it is not unusual that the associated hydrocarbons persist and accumulate in salt marshes over a long time period (Burns et al., 1993; Oudot and Chaillan, 2010; Boopathy et al., 2012; Natter et al., 2012). For example, Oudot and Chaillan (2010) found that hydrocarbon compounds from the Amoco-Cadiz spill can still be found in salt marshes of Ile Grande on the French Coast 23 yr after the spill.

Salt marshes are both ecologically and economically important in that they serve as homes to many species of plants and animals and provide a major source of business revenue (King and Lester, 1995). Therefore, it is important to assess the fate (i.e., persistence, transformation, degradation) of hydrocarbon compounds from Macondo oil in Gulf Coast salt marshes. In the present study, laboratory microcosm incubations were conducted to assess short-term degradation of Macondo oil in Alabama salt marsh sediment by natural, in situ microbes. Both concentrations and compositions of gas chromatography (GC)-amenable hydro-

carbons were characterized to assess how degradation rates vary with molecular weight and structure, i.e., whether hydrocarbons with higher molecular weights and more complex structures will be degraded at lower rates than their counterparts with lower molecular weights and less complex structures. A suite of aqueous geochemical parameters were measured to understand the influence of environmental factors on hydrocarbon degradation. The results of this research provide insights into the short-term microbial degradation of Macondo oil in a representative Gulf Coast salt marsh system.

METHODS

Sample Collection

On March 17, 2011, seawater and sediment samples were collected just seaward of a salt marsh on the coastline southwest of Bayou La Batre, Alabama (30.38°N, 88.30°W), which is located in the Gulf Coastal Plain and approximately 16 km west of the Mobile Bay (Fig. 1). The sediments collected are typical of many areas along the Gulf Coast, consisting of inorganic clays, poorly-sorted sands, silty-sand, and sandy-clay (Rentschler, 2013). The seawater had a pH of 7.70 and total alkalinity of 83.07 mg CaCO₃/L (Rentschler, 2013).

All containers and sampling equipment to be in direct contact with water samples were either combusted at 450°C for 5 hr (glass materials), or acid soaked (10% HCl or 10% HNO₃) and thoroughly rinsed with Milli-Q water (plastic materials). Seawater was collected using polypropylene bottles which were rinsed with seawater, then filled and capped under the water surface. The upper 15–30 cm of sediment was collected into a 5 gal plastic bucket. All samples were transported on ice to the lab, where they were refrigerated in the dark until the microcosm experiment was conducted.

Microcosm Incubations

Each microcosm consisted of 326.94 g wet sediment (mass equivalent to 200 g dry sediment) and 173.06 g seawater in a 500 mL glass jar with Teflon-lined lids. Samples were thoroughly mixed on a shaker table set to 100 rpm for 24 hr before they were spiked with 10 g Macondo oil (MC 252 oil that was collected from the well head by the British Petroleum). The incubations then started and lasted 14 d in the dark. All jars were kept closed

and consistently shaken to ensure a thorough mixture of sediments and water throughout the experiment. Two jars were sacrificed at 0, 6, 12, 24, 48, 168, and 336 hr. One control sample that was not treated with oil was sacrificed at hr 336. Over the course of the incubation, the concentrations and compositions of alkanes and PAHs in the sediments, as well as the concentrations of dissolved organic carbon (DOC) and nutrients in aqueous solution were determined.

Chemical Analyses

Microcosm Sample Collection

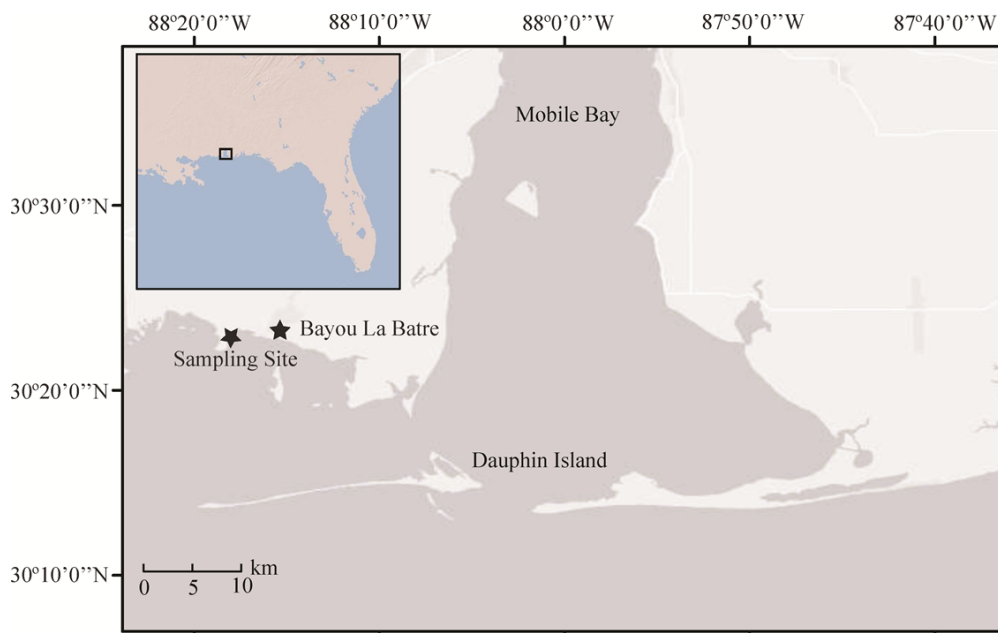
After the duplicate jars were sacrificed at their respective time points, the aqueous (seawater) and solid (sediment) phase samples within each glass jar were thoroughly mixed before they were sampled. Aqueous samples were collected by siphoning the upper liquid layer before and after centrifuging sediments at 750 rpm for 20 min. The aqueous samples were then filtered through 0.45 µm filters and stored frozen until analysis for DOC and nutrients (nitrate + nitrite and phosphate) concentrations.

Hydrocarbon Determination in Sediments

Solid phase samples were homogenized and then stored frozen in glass jars prior to hydrocarbon analysis. Hydrocarbon extraction followed the method described in Risdon et al. (2008) with modifications. Approximately 6 g of incubated sediments were mixed with Hydromatrix (Agilent Technologies) to remove water. Normal hexadecane-d34 (C₁₆D₃₄) and phenanthrene-d10 were added as surrogates to the samples prior to the extraction. Samples were then ultrasonically extracted with 4 mL acetone for 2 min at 20°C to ensure thorough mixing between the sediments and the surrogates. Twenty mL of acetone:hexane (1:1, vol:vol) was then added to the samples, ultrasonically extracted for 10 min at 20°C, and stored at 4°C overnight. Short copper ribbon was used to remove sulfur during the extraction process.

Samples were extracted again ultrasonically for 20 min at 20°C before the liquid portion of the samples was collected with disposable pipettes and concentrated with an ultra high purity (UHP) nitrogen stream. Three mL of acetone, 5 mL of hexane, 4 mL of Milli-Q water and a spatula of sodium chloride were added to the extracts, which were then manually shaken for 30 sec and allowed to settle for 20 min. The top hexane layer was

Figure 1. Map showing the greater Mobile Bay study area and the sampling site.



siphoned via disposable glass pipette as the hydrocarbon fraction and the more polar compounds dissolved in the lower acetone and water layer were discarded. The hydrocarbon fraction was then separated by silica column chromatography into aliphatic and aromatic hydrocarbon fractions, which were eluted by 10 mL of hexane and 12 mL of dichloromethane, respectively.

Aliphatic fractions were quantified and identified using gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS), respectively. The initial temperature for the GC oven was 50°C, held for one minute, followed by a 6°C/min increase until 310°C was reached, then holding for 15 min. UHP helium was the carrier gas used at a flow rate of 1.48 mL/min. The quantification of objective compounds was done by comparing their peak areas to the peak areas of n-Hexadecane-d34. Different instrumental responses for various compounds were corrected by regularly running a standard containing a series of alkane compounds (C₇–C₄₀ normal alkanes) and n-Hexadecane-d34.

Aromatic components were identified using GC–MS by comparing their relative retention times and mass spectra to those of multiple known PAHs in a standard mixture. They were quantified by comparing their peak areas to Phenanthrene-d10. The initial temperature of the GC oven was 50°C, held for 1 min, followed by a 20°C/min increase until 140°C was reached, and then a 6°C/min increase until 310°C was reached, holding for 15 min for a total run time of 47 min. The carrier gas was UHP helium with a flow rate of 1.48 mL/min.

DOC Concentration

The DOC concentration of aqueous samples was determined using a Shimadzu TOC–VCPN Total Organic Carbon Analyzer, with a potassium hydrogen phthalate (KHP) calibration standard solution and a Consensus Reference Material deep seawater DOC check standard (Hansell Laboratory, <http://yyy.rsmas.miami.edu/groups/biogechem/CRM.html>).

Dissolved Nutrients

Water samples were submitted to the Dauphin Island Sea Laboratory (Alabama, USA) for nutrient (nitrate + nitrite and phosphate) analyses using a Skalar San and continuous flow autoanalyser with wet chemistry colorimetric modules designed for the individual analytes (nitrate + nitrite consistent with Environmental Protection Agency [EPA] method 353.2, and phosphate consistent with EPA method 365.3). Sample absorbance was compared with regression statistics based on a five-point standard curve for each analyte, and the results were baseline- and drift-corrected throughout the sample run.

RESULTS

Temporal Changes in Hydrocarbons in the Sediment

Sediments from oiled-microcosms showed alkanes ranging in carbon number from 9 through 33, including both normal and isoprenoid alkanes. At the end of the 14-d incubation, total alkanes in the sediment decreased by $71.4 \pm 32.3\%$ (mean \pm standard deviation; the standard deviation (SD) was calculated from propagation of the SD of duplicate jars sacrificed at hr 0 and hr 336) (Fig. 2). Over the course of the experiment, total alkanes in the sediment showed a rapid decrease during the first 12 hr and remained relatively stable after 12 hr (Fig. 3a). This pattern of alkane loss agrees with the general pattern of natural organic matter degradation observed in seawater and sediment systems (Westrich and Berner, 1984), and it may be described using the multi-G model of Berner (1964):

$$-dG_i/dt = \sum k_i G_i \quad (1)$$

$$G_i = \sum G_j = (G_1)_0 \exp(-k_1 t) + (G_2)_0 \exp(-k_2 t) + (G_3)_0 \exp(-k_3 t) + \dots \quad (2)$$

where k_i is the first-order degradation rate constant of fraction i ; G_i is the concentration of fraction i and $-dG_i/dt$ is the degradation rate of all fractions. On average, 87% of total alkanes in the microcosm sediment degraded within the first 48 hr (Fig. 3a), accounting for the initial, reactive fraction of the oil-derived alkanes (G_1) and yielding k_1 as 0.0373 hr^{-1} (coefficient of determination [R^2] = 0.62 for the fitting curve of $\ln[\text{total alkanes}]$ versus time). Because the degradation rate decreased rapidly after hr 48, the total alkanes between hr 48–336 can be treated as a more resistant fraction (i.e., G_2). However, as only three time points were sampled after hr 48, a reliable value for k_2 cannot be obtained from our experiment.

At the end of the incubation experiment, short-chain ($C < 17$) alkanes and long-chain ($C \geq 17$) alkanes in the sediment decreased by an average of $74.6 \pm 31.7\%$ and $67.8 \pm 32.3\%$, respectively (Fig. 2). The loss of these two compound groups followed a pattern similar to that of the total alkanes, i.e., rapid degradation within the first 12 hr and remaining relatively stable thereafter (Figs. 3b and 3c). Based on Equations 1 and 2, k_1 values for short-chain and long-chain alkanes during the first 48 hr were 0.0389 hr^{-1} ($R^2 = 0.73$) and 0.0384 hr^{-1} ($R^2 = 0.50$), respectively. Although the percentage and rate of loss of the short-chain alkanes were slightly higher than corresponding values for the long-chain alkanes, the ratio of short-chain to long-chain alkanes did not show an evident pattern throughout the experiment (Fig. 4a). Similarly, the degradation rates of normal and

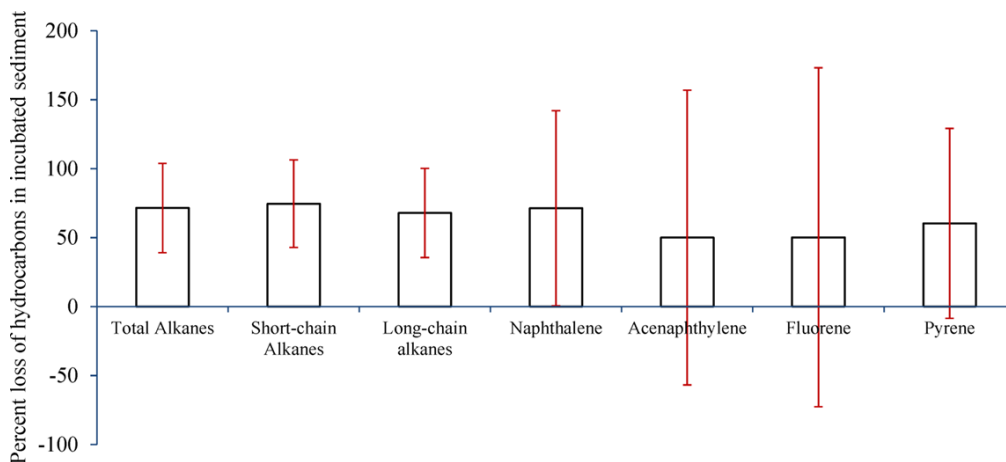


Figure 2. Percent loss of different hydrocarbon compounds/groups in incubated sediments at the end of experiment (i.e., hr 336) relative to the initial time point of the experiment (hr 0). Error bars represent standard deviations calculated from propagating the standard deviations of duplicate jars sacrificed at hr 0 and hr 336.

Figure 3. Changes in the concentrations of (a) total alkanes, (b) short-chain alkanes, and (c) long-chain alkanes in incubated sediments over the course of the experiment.

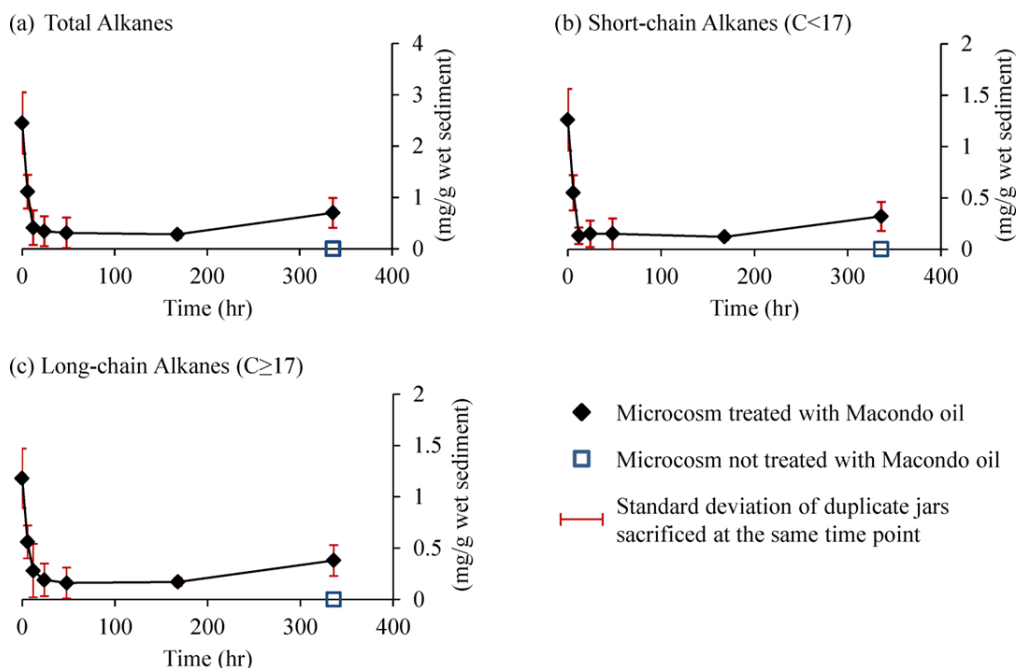
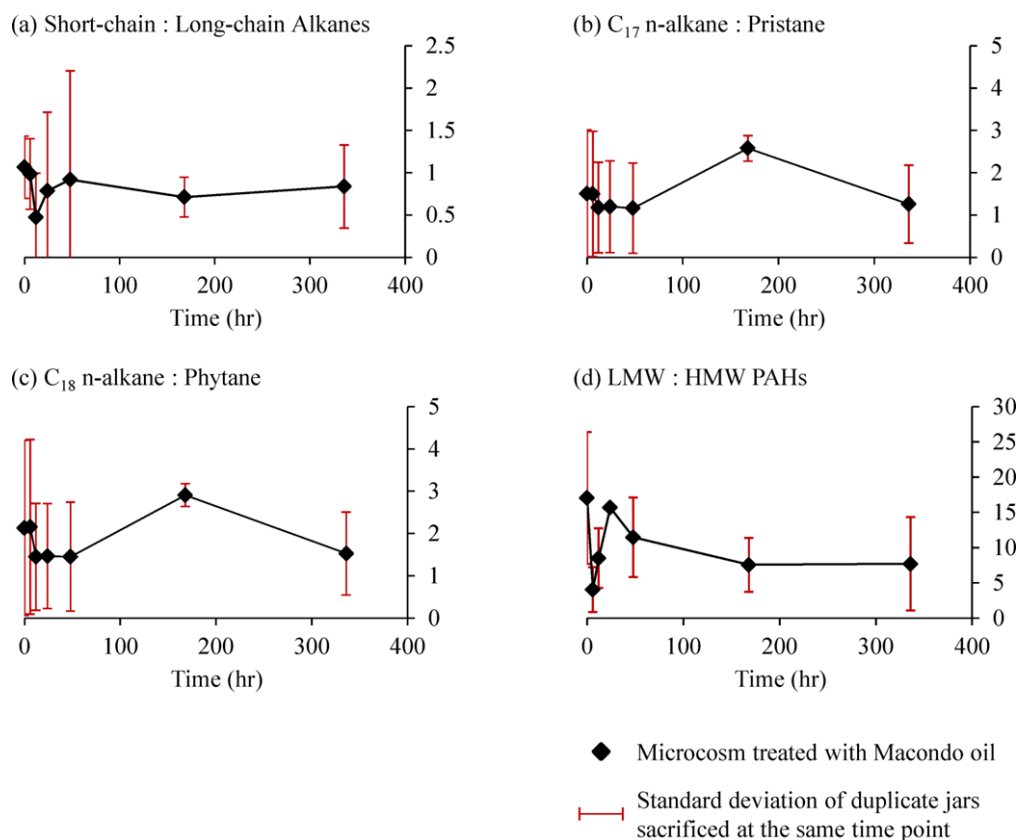


Figure 4. Temporal changes in the ratios of hydrocarbon compounds having different structures or molecular weights in microcosm sediments.



branched alkanes did not show evident differences, as shown by the ratio of C₁₇ normal alkane to pristane (n-C₁₇:Pr) and the ratio of C₁₈ normal alkane to phytane (n-C₁₈:Ph) not exhibiting any apparent trends over the course of the experiment (Figs. 4b and 4c).

For the aromatic fractions, naphthalene, acenaphthylene, fluorene, fluoranthene, and pyrene were identified in the sediment from oil-treated microcosms. However, these compounds

showed highly variable concentrations in duplicate microcosms, and did not show consistent loss during the two week incubation (Figs. 2 and 5). Likewise, the ratio of low molecular weight (LMW) PAHs (i.e., two to three ring compounds which included naphthalene, acenaphthylene, and fluorene detected in this study) to high molecular weight (HMW) PAHs (i.e., four-ringed fluoranthene and pyrene in the present study) displayed no evident trend over the course of the experiment (Fig. 4d).

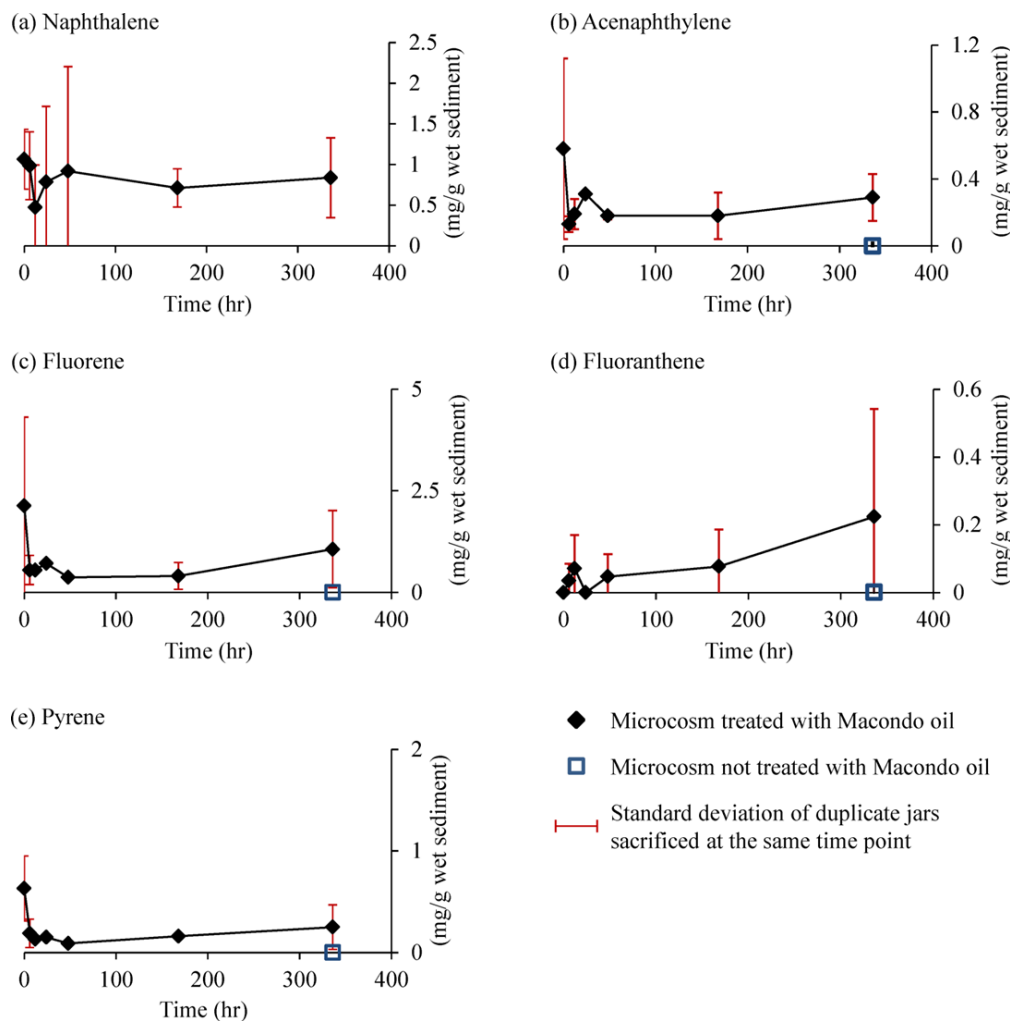


Figure 5. Changes in the concentrations of PAH compounds in incubated sediments over the course of the experiment.

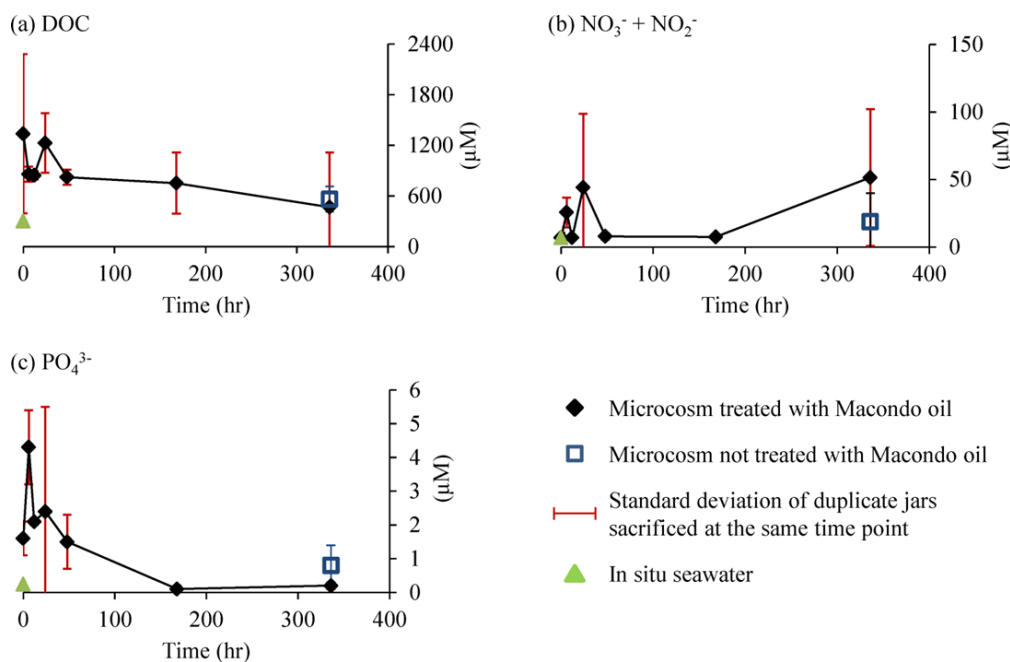


Figure 6. Changes in the concentrations of (a) dissolved organic carbon, (b) aqueous nitrate + nitrite, and (c) aqueous phosphate over the course of the experiment.

Changes in Aqueous Inorganic N and P and Organic C

Nitrate + nitrite concentrations ranged from 6.8–51.5 μM , and phosphate concentration varied from 0.1–4.3 μM . None of these inorganic species showed consistently increasing or decreasing trends over time (Fig. 6). DOC concentrations ranged from the initial concentration of 1336 μM to the final concentration of 466 μM , showing an overall decreasing trend after hr 48 (Fig. 6). The final DOC concentration was slightly higher than that of initial, in situ seawater.

DISCUSSION

Two pathways could account for the rapid loss of aliphatic alkanes in sediments within the first 12 hr (Fig. 3). The loss could be due to the release of hydrocarbons from sediments to overlying seawater, or could be caused by microbial degradation. The hydrocarbon compounds detected in the present study are largely non-polar and therefore preferentially partition towards organic matter in sediment particles, rather than towards water (Wang et al., 2001). Using tetradecane ($\text{C}_{14}\text{H}_{30}$) as an example, the ratio of its concentration in sediments to its concentration in seawater should be approximately 10^6 , calculated as $\% \text{organic carbon in the sediments} * K_{oc} = 1\% * 1.724 * K_{ow}$, where K_{oc} is the partitioning coefficient between solid organic matter and water and K_{ow} is the partitioning coefficient between octanol and water ($K_{ow} = 10^8$ from Sangester, 1989; $\% \text{organic carbon in our sediments}$ ranged between 0.88% and 1.45%). As such, the amount of hydrocarbons in seawater should be insignificant relative to that in the sediment. The release of hydrocarbons from sediment to overlying seawater was therefore unlikely the cause for the majority of loss of hydrocarbons in the sediment. This argument is further supported by the DOC concentrations in the microcosms. The DOC concentrations of oil-spiked microcosms were higher than the initial, in situ seawater and the non-oiled control (Fig. 6), indicating that Macondo oil increased the DOC concentration in the seawater due to soluble LMW aromatic hydrocarbons present in oil, such as naphthalene (Volkman et al., 1984; Zhou et al., 2013a). At hr 0, the DOC concentration in the oiled-microcosms was higher than the initial, in situ seawater by 1031 μM , which was equal to 12,372 μg carbon, accounting for only <2% of the total mass of extractable hydrocarbon in the sediments (i.e., 12,372 μg / [2.45 mg alkanes per g wet sediments * 326.94 g wet sediments] \approx 1.5%). Between 0 to 12 hr, when the alkane loss was most significant in the sediments, DOC concentrations did not show an apparent increase (Fig. 6), indicating that no significant amounts of alkanes moved from the sediment to the overlying water.

Therefore, microbial degradation of hydrocarbons becomes the most plausible pathway accounting for the loss of alkanes in the sediment. The rapid loss of hydrocarbons within the first 12 hr (Fig. 3) may be attributed to aerobic microbial degradation, beginning with dioxygenase enzymes inserting O_2 into the hydrocarbon structure and yielding CO_2 and H_2O (Atlas and Philp, 2005). Afterwards, the rapid decrease in the degradation rate (Fig. 3) can be explained by the depletion of oxygen within the sediments, where aerobes could no longer efficiently metabolize oil using oxygen as the electron acceptor. Rentschler (2013) measured the changes in aqueous iron concentrations over the course of this experiment, reporting that iron increased from <1 mg/L in the first 48 hr to >6 mg/L at hr 168. This pattern suggests that microbes released insoluble iron from sediments in order to use it as an electron acceptor for alkane degradation when oxygen became depleted.

Previous studies have suggested that N and P availability may also limit hydrocarbon degradation rates (Xu and Obbard, 2003; Das and Chandran, 2011). In the microcosm water samples, nitrate + nitrite and phosphate did not show apparent de-

creases after hr 12 (Fig. 6), indicating the availability of these nutrients were not the reason for the rapid decrease in the degradation rate after hr 12. Iron may also serve as a limiting nutrient for hydrocarbon degradation (Das and Chandran, 2011). For example, Bælum et al. (2012) observed that Fe addition led to short-term increases in hydrocarbon biodegradation rates in deep sea water samples from the Gulf of Mexico. In the present study, the high total dissolved iron concentrations in the microcosms (>6 mg/L) between 168–336 hr (Rentschler, 2013) indicate that iron availability was not the factor limiting the alkane degradation rate during the experiment.

The ratios of n-C₁₇:Pristane and n-C₁₈:Phytane are indicative of the degree of biodegradation (Wang et al., 1999). These ratios are based on the observation that bacteria generally metabolize straight-chain alkanes faster than branched alkanes, and therefore more degraded oil has overall lower ratios than less degraded, fresher oil (Wang et al., 1999). In the microcosm sediment samples, these two ratios showed no evident temporal pattern (Fig. 4), likely because in situ microbes have adapted to degrading a variety of alkanes. When microbial communities are exposed to hydrocarbons, genetic changes may occur and the proportion of oil-degrading bacteria often increases, resulting in an increase in the hydrocarbon-oxidizing potential of the community (Atlas and Atlas, 1991; Haritash and Kaushik, 2009). At our sampling site, Beazley et al. (2012) observed that total petroleum hydrocarbon concentrations in the marsh sediments increased during the *Deepwater Horizon* oil spill (i.e., in June and July of 2010). Additionally, there are a large number of natural oil seeps and oil drilling activities that take place within the Gulf of Mexico (MacDonald et al., 1993). As a result, in situ microbes may have adapted to be capable of efficient degradation of both normal and branched alkanes, thus showing no apparent preference between these groups of compounds. By comparison, in Prince William Sound, Alaska, where preferential biodegradation of normal alkanes over branched alkanes was observed after the *Exxon Valdez* oil spill, natural oil seeps are scarce due to highly metamorphosed sedimentary rock in the region (Bence et al., 1996).

Petroleum biodegradation may differ as a function of hydrocarbon chain length and molecular weights. Zhou et al. (2013b) conducted laboratory degradation of Macondo oil in Gulf of Mexico seawater, where they observed preferential degradation of LMW n-alkanes over HMW n-alkanes, as well as of naphthalene and phenanthrene over the other PAH compounds. Similarly, Ke et al. (2002) reported preferential biodegradation of LMW PAHs over HMW PAHs in sediments from a mangrove swamp in Hong Kong after an oil spill accident. In the microcosm sediment samples, short-chain alkanes demonstrated only slightly higher first-order degradation rates than long-chain alkanes during the first 48 hr (i.e., $k_1 = 0.0389 \text{ hr}^{-1}$ for LMW alkanes versus 0.0384 hr^{-1} for HMW alkanes), and thus showed a slightly higher %loss at the end of the incubation (Fig. 2). However, this preference for short-chain alkanes was not apparent based on the ratio of short-chain to long-chain alkanes, which did not show a pattern over the course of the incubation. This observation may be also attributed to the adaptation of in situ microbes to efficiently degrade various types of alkanes.

Previous laboratory microcosm studies aimed at assessing the degradation of hydrocarbons from diesel and oil pollutants in sediments and water have shown various percentages of biodegradable hydrocarbons (Table 1). Across-study comparisons highlighted two primary limiting factors for biodegradation, where the first is oxygen availability. Boopathy et al. (2012) incubated salt marsh sediment from southeastern Louisiana that was contaminated with 720 ppm Macondo oil. Under anaerobic conditions, they found that 19–78% of total petroleum hydrocarbons degraded over 80 d, depending on the availability of electron acceptors. In the present study, the majority of alkanes was observed to decrease within the first 12 hr, and is attributed most-

Table 1. Comparison of experimental conditions and results of microcosm incubations for assessment of biodegradation of oil/hydrocarbons across various studies.

Study system	Oil and hydrocarbon type	Incubation conditions	% Degraded hydrocarbons	Source
Gulf of Mexico seawater	Macondo oil	5 d with Gulf of Mexico deep-water natural microbes; aerobic conditions	25% loss of dissolved total petroleum hydrocarbons	Bælum et al. (2012)
Estuarine sediment Lima River Estuary, Portugal	Arabian light oil	15 d with natural microbes in sediments	Maximum of 32% of total petroleum hydrocarbons in sandy sediments	Almeida et al. (2013)
Salt marsh sediment, Louisiana, USA	Macondo oil	80 d with natural microbes in sediments; anaerobic conditions	Up to 78% of total petroleum hydrocarbons	Boopathy et al. (2012)
Oil contaminated soil in Chennai, India	diesel oil, naphthalene, and phenanthrene	7 d with petroleum degrading actinomycete isolated from soils	98.25% of diesel oil, 99.14% naphthalene, and 17.5% phenanthrene	Balachandran et al. (2012)
Lacustrine sediments (Arkansas, USA) and estuarine sediments (Texas, USA)	naphthalene	8 wk with natural microbes in sediments; aerobic conditions	60–70% of naphthalene	Heitkamp et al. (1987)
Oil-refining wastewater sludge (Fuzhou, China)	naphthalene	96 hr with bacterial strains isolated from oil refining wastewater sludge	More than 99.1% of naphthalene	Lin et al. (2010)
Salt marsh sediments, Alabama, USA	Macondo oil	14 d with natural microbes in sediments	71% of total alkanes; no consistent loss of PAHs	This study

ly to the aerobic biodegradation of hydrocarbons; hydrocarbon degradation was undetectable afterwards, which is attributed to oxygen depletion. The second factor limiting degradation is the exposure history of microbes to oil. This was evidenced by microbes isolated from pre-contaminated sites being capable of degrading hydrocarbons more efficiently, with greater percentage loss in a shorter timeframe (e.g., Lin et al., 2010; Balachandran et al., 2012) (Table 1). In the present study, the non-selective degradation of alkanes is attributed to the previous exposure of microbes from the study site to oil from natural and anthropogenic sources, including Macondo oil. Lastly, it is important to point out that the microcosm experiment performed in this study, along with the studies shown in Table 1, assessed only GC-amenable hydrocarbons and did not assess those compounds that cannot be resolved by GC. As Macondo oil reaching the Alabama shoreline would have been highly weathered, the associated hydrocarbons likely comprise mostly compounds not amenable to GC detection. These compounds are generally more resistant to biodegradation than GC-amenable compounds, and thus have greater tendency to accumulate in coastal environments and to be transferred to food webs as a toxic threat to marine organisms (Culbertson et al., 2007; Scarlett et al., 2008).

CONCLUSIONS

The objective of the present study was to understand better the fate of hydrocarbons, derived from Macondo oil, in Alabama salt marsh sediments. The majority of alkanes were degraded within 12 hr, and the degradation rates were much lower or even below detection afterwards, suggesting that oxygen availability was the main limiting factor for the rate and extent of microbial degradation of the alkanes. No apparent preferential degradation of alkanes of different structures or molecular weights (i.e., normal versus branched, short-chain versus long-chain) was detected, which may indicate that in situ microbial communities have adapted to degrading various types of alkanes from their frequent exposure to a variety of hydrocarbon compounds from natural seeps and anthropogenic activities. In contrast, the concentrations of PAH compounds were highly variable throughout the experiment and showed no consistent degradation pattern. These

findings improve our understanding of the short-term fate of Macondo oil in Alabama salt marsh sediments, demonstrating that alkanes can be rapidly degraded (hours to days) by in situ, natural microbial communities when oxygen is readily available while the biodegradation of PAHs would require a longer time (>2 wk). More research is needed for a reliable assessment of the fate (i.e., degradation or persistence) of PAHs in the study system.

ACKNOWLEDGMENTS

This work was funded by the Marine Environmental Sciences Consortium / British Petroleum Gulf of Mexico Research Initiative (MESC / BP GRI). We also acknowledge student research support from the Johnson Fund (Department of Geological Sciences, University of Alabama) and an ExxonMobil Summer Internship to D. J. F.

REFERENCES CITED

- Almeida, R., A. Mucha, C. Teixeira, A. Bordalo, and C. Almeida, 2013, Biodegradation of petroleum hydrocarbons in estuarine sediments: Metal influence: *Biodegradation*, v. 24, p. 111–123.
- Atlas R. M., and M. C. Atlas, 1991, *Biodegradation of oil and bioremediation of oil spills: Current Opinion in Biotechnology*, v. 2, p. 440–443.
- Atlas, R. M., and T. C. Hazen, 2011, Oil biodegradation and bioremediation: a tale of the two worst spills in U.S. history: *Environmental Science & Technology*, v. 45, p. 6709–6715.
- Atlas, R. M., and J. Philp, 2005, *Bioremediation: Applied microbial solutions for real-world environmental cleanup*: American Society for Microbiology Press, Washington, D.C., 292 p.
- Balachandran, C., V. Duraipandiyam, K. Balakrishna, and S. Ignacimuthu, 2012, Petroleum and polycyclic aromatic hydrocarbons (PAHs) degradation and naphthalene metabolism in *Streptomyces* sp. (ERI-CPDA-1) isolated from oil contaminated soil: *Bioresource Technology*, v. 112, p. 83–90.
- Bælum, J., S. Borglin, R. Chakraborty, J. L. Fortney, R. Lamendella, O. U. Mason, M. Auer, M. Zemla, M. Bill, M. E. Conrad, S. A. Malfatti, S. G. Tringe, H. Holman, T. C. Hazen, J. K. Jansson, 2012, Deep-sea bacteria enriched by oil and dis-

- persant from the *Deepwater Horizon* spill: Environmental Microbiology, v. 14, p. 2405–2416.
- Beazley, M. J., R. J. Martinez, S. Rajan, J. Powell, Y. M. Piceno, L. M. Tom, G. L. Andersen, T. C. Hazen, J. D. Van Nostrand, J. Z. Zhou, B. Mortazavi, and P. A. Sobecky, 2012, Microbial community analysis of a coastal salt marsh affected by the *Deepwater Horizon* Oil Spill: PLoS ONE, v. 7, e41305, doi:10.1371/journal.pone.0041305.
- Bence, A. E., K. A. Kvenvolden, and M. C. Kennicutt II, 1996, Organic geochemistry applied to environmental assessments of Prince William Sound, Alaska, after the Exxon Valdez oil spill—A review: Organic Geochemistry, v. 24, p. 7–42.
- Berner, R. A., 1964, An idealized model of dissolved sulfate distribution in recent sediment: *Geochimica et Cosmochimica Acta*, v. 28, p. 1497–1503.
- Boopathy, R., S. Shields, and S. Nunna, 2012, Biodegradation of crude oil from the BP oil spill in the marsh sediments of Southeast Louisiana, USA: Applied Biochemistry and Biotechnology, v. 167, p. 1560–1568.
- Burns, K. A., S. Levings, and S. Garrity, 1993, How many years before mangrove ecosystems recover from catastrophic oil spills?: Marine Pollution Bulletin, v. 26, p. 239–364.
- Bælum, J., S. Borglin, R. Chakraborty, J. L. Fortney, R. Lamendella, O. U. Mason, M. Auer, M. Zemla, M. Bill, M. E. Conrad, S. A. Malfatti, S. G. Tringe, H. Holman, T. C. Hazen, and J. K. Jansson, 2012, Deep-sea bacteria enriched by oil and dispersant from the *Deepwater Horizon* spill: Environmental Microbiology, v. 14, p. 2405–2416.
- Culbertson, J. B., I. Valiela, E. E. Peacock, C. M. Reddy, A. Carter, and R. VanderKruik, 2007, Long-term biological effects of petroleum residues on fiddler crabs in salt marshes: Marine Pollution Bulletin, v. 54, p. 955–962.
- Das, N., and P. Chandran, 2011, Microbial degradation of petroleum hydrocarbon contaminants: An overview: Biotechnology Research International, v. 2011, Article 941810, doi:10.4061/2011/941810.
- Haritash, A. K., and C. P. Kaushik, 2009, Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review: Journal of Hazardous Materials, v. 169, p. 1–15.
- Heitkamp, M. A., J. P. Freeman, and C. E. Cerniglia, 1987, Naphthalene biodegradation in environmental microcosms: Estimates of degradation rates and characterization of metabolites: Applied Environmental Microbiology, v. 53, p. 129–136.
- Ke, L., T. W. Y. Wong, Y. S. Wong, and N. F. Y. Tam, 2002, Fate of polycyclic aromatic hydrocarbon (PAH) contamination in a mangrove swamp in Hong Kong following an oil spill: Marine Pollution Bulletin, v. 45, p. 339–347.
- King, S. E., and J. N. Lester, 1995, The value of salt marsh as a sea defense: Marine Pollution Bulletin, v. 30, p. 180–189.
- Lin, C., L. Gan, and Z. L. Chen, 2010, Biodegradation of naphthalene by strain *Bacillus fusiformis* (BFN): Journal of Hazardous Materials, v. 182, p. 771–777.
- MacDonald, I. R., N. L. Guinasso, S. G. Ackleson, J. F. Amos, R. Duckworth, R. Sassen, and J. M. Brooks, 1993, Natural oil-slicks in the Gulf of Mexico visible from space: Journal of Geophysical Research: Oceans, v. 98, p. 16351–16364.
- Natter, M., J. Keevan, Y. Wang, A. R. Keimowitz, B. C. Okeke, A. Son, and M. Lee, 2012, Level and degradation of *Deepwater Horizon* spilled oil in coastal marsh sediments and pore-water: Environmental Science and Technology, v. 46, p. 5744–5755.
- OSAT (Operational Science Advisory Team), 2010, Summary report for sub-sea and sub-surface oil and dispersant detection: Sampling and monitoring, <http://www.restorethegulf.gov/sites/default/files/documents/pdf/SAT_Report_FINAL_17DEC.pdf> Last accessed August 25, 2014.
- OSAT-2 (Operational Science Advisory Team), 2011, Summary report for fate and effects of remnant oil in the beach environment, <<http://www.restorethegulf.gov/sites/default/files/u316/OSAT-2%20Report%20no%20ltr.pdf>> Last accessed August 25, 2014.
- Oudot, J., and F. Chaillan, 2010, Pyrolysis of asphaltenes and biomarkers for the fingerprinting of the Amoco-Cadiz oil spill after 23 years: *Comptes Rendus Chimie*, v. 13, p. 548–552.
- Sangster, J., 1989, Octanol-water partition coefficients of simple organic compounds: Journal of Physics and Chemistry Reference Data, v. 18, p. 1111–1227.
- Reddy, C. M., J. S. Arey, J. S. Seewald, S. P. Sylva, K. L. Lemkau, R. K. Nelson, C. A. Carmichael, C. P. McIntyre, J. Fenwick, G. T. Ventura, B. A. S. Van Mooy, and R. Camilli, 2011, Composition and fate of gas and oil released to the water column during the *Deepwater Horizon* oil spill: Proceedings of the National Academy of Sciences of the United States of America, v. 109, p. 20229–20234.
- Rentschler, E. K., 2013, *Deepwater Horizon* oil spill: Using microcosms to study effects of crude oil in coastal sediments: M.S. thesis, University of Alabama, Tuscaloosa, 86 p.
- Risdon, G. C., S. J. T. Pollard, K. J. Brassington, J. N. McEwin, G. I. Paton, K. T. Semple, and F. Coulon, 2008, Development of an analytical procedure for weathered hydrocarbon contaminated soil within a UK risk-based framework: Analytical Chemistry, v. 80, p. 7090–7096.
- Scarlett, A., S. J. Rowland, T. S. Galloway, A. C. Lewis, and A. M. Booth, 2008, Chronic sublethal effects associated with branched alkylbenzenes bioaccumulated by mussels: Environmental Toxicology and Chemistry, v. 27, p. 561–567.
- Volkman, J. K., R. Alexander, R. I. Kagi, S. J. Rowland, and P. N. Sheppard, 1984, Biodegradation of aromatic hydrocarbons in crude oils from the Barrow sub-basin of Western Australia: Organic Geochemistry, v. 6, p. 619–632.
- Wang, X., Y. Zhang, and R. F. Chen, 2001, Distribution and partitioning of polycyclic aromatic hydrocarbons (PAHs) in different size fractions in sediments from Boston Harbor, United States: Marine Pollution Bulletin, v. 42, p. 1139–1149.
- Wang, Z., M. Fingas, and D. S. Page, 1999, Oil spill identification: Journal of Chromatography A, v. 843, p. 369–411.
- Westrich, J. T., and R. A. Berner, 1984, The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested: Limnology and Oceanography, v. 29, p. 236–249.
- Xu, R. and J. P. Obbard, 2003, Effect of nutrient amendments on indigenous hydrocarbon biodegradation in oil-contaminated beach sediments: Journal of Environmental Quality, v. 32, p. 1234–1243.
- Zhou, Z. Z., L. D. Guo, A. M. Shiller, S. E. Lohrenz, V. L. Asper, and C. L. Osburn, 2013a, Characterization of oil components from the *Deepwater Horizon* oil spill in the Gulf of Mexico using fluorescence EEM and PARAFAC techniques: Marine Chemistry, v. 148, p. 10–21.
- Zhou, Z., Z. Liu, and L. Guo, 2013b, Chemical evolution of Macondo crude oil during laboratory degradation as characterized by fluorescence EEMs and hydrocarbon composition: Marine Pollution Bulletin, v. 66, p. 164–175.