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IMPACT OF PHOTOOXIDATION AND BIODEGRADATION ON THE FATE OF THE OIL SPILLED DURING THE DEEPWATER HORIZON INCIDENT: THE ADVANCED STAGES OF WEATHERING

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BRIAN HEATH HARRIMAN
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IMPACT OF PHOTOOXIDATION AND BIODEGRADATION ON THE FATE OF THE OIL SPILLED DURING THE DEEPWATER HORIZON INCIDENT: THE ADVANCED STAGES OF WEATHERING

A THESIS APPROVED FOR THE
DEPARTMENT OF MICROBIOLOGY AND PLANT BIOLOGY

BY

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Dr. Joseph M. Suflita, Chair

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Dr. Amy V. Callaghan

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Dr. Kathleen E. Duncan

Dedication

I dedicate this thesis to my parents, Leigh and Howard Harriman. I would not be the man I am today without your constant support, no matter my endeavor.

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I would like to thank Dr. Joseph Sulfita, my advisor. You have been instrumental in my growth in the lab and at a keyboard. I have achieved more than I once thought I could, and strive to achieve more every day. I have been afforded opportunities that I never dreamed of, nor would have imagined had I not persisted in you taking me into your lab. I am amazed and honored to be able to claim the title of "Last Suflita Student."

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Table of Contents

List of Tables

List of Figures

Chapter 1.

Appendix A.

Abstract

The major biogeochemical forces influencing the environmental fate of spilled oil have been intensively investigated for many decades. The sinking and burying of spilled residual oil to form large mats that subsequently get broken up and washed onshore as sand patties has been well documented. While recognized as an integral part of the weathering process, the environmental fate and effects of sand patties and their component chemicals are not well known. We collected sand patties that were deposited in the swash zone on Gulf of Mexico beaches following the Deepwater Horizon oil spill. The exposure of sand patties to sunlight, seawater, and the indigenous microbial communities suggested that photooxidation and biodegradation might be important advanced weathering processes for these materials. When sand patties were exposed to simulated sunlight, a larger amount of dissolved organic matter (DOM) with greater UV absorption characteristics was photo-solubilized into seawater than the corresponding dark controls. This is consistent with the general ease of movement of seawater through the sand patties. High-resolution mass spectrometry as well as a variety of absorbance and fluorescence measurements revealed that the chemical nature of the DOM leached from the sand patties under dark and irradiated conditions was substantially different. In both cases, the DOM did not have a significant inhibitory influence on the endogenous rate of aerobic or anaerobic microbial respiratory processes. In addition, the DOM represented suitable electron donors to support at least short-term (3 d) aerobic microbial respiration activity. The water-soluble photooxidation products stimulated significantly more oxygen respiration (113 μM)

than either the dark (78 μM) or the endogenous (38 μM) forms of DOM. The residual DOM analyzed at the end of the incubation was consistent with biodegradation as an explanation for the various changes in DOM quality and quantity. Thus, these results confirm that sand patties are not recalcitrant and their deposition on beaches does not represent an end point in oil weathering processes. Rather, sand patties undergo a gradual dissolution of DOM in both the dark and in the light, but photooxidation accelerates the production of water-soluble polar organic compounds that are relatively more amenable to aerobic biodegradation. As such, these processes represent previously unrecognized advanced weathering stages that are important in the ultimate transformation of spilled crude oil.

Preface

Although researchers have studied the fate and effects of petroleum spilled in the environment for well over a century, no investigation has ever documented the complete mineralization of all oil constituents. An enormous literature exists that has helped clarify the integrated transformation of petroleum-derived hydrocarbons by a variety of physical, chemical and biological weathering processes. However, the advanced stages of oil weathering are still poorly understood. This is, in part, due to the increased complexity of even recognizing the component hydrocarbons in traditional analytical efforts. Rather than exploring oil per se, this thesis focuses on the environmental fate of highly weathered oil that could be readily collected from the field. As such, this work attempts to fill in some of the knowledge gaps as it relates to the further transformation of spilt oil and its effects on the resident microflora in coastal marine ecosystems.

In the wake of the Deepwater Horizon oil spill, highly weathered oil combined with Gulf of Mexico sediments to form sand patties that washed ashore and deposited on northern beaches. This thesis specifically details the effects of the combination of photooxidation and biodegradation processes on sand patties and sand patty-derived organic matter. During the study, it became clear that photooxidation of sand patties was capable of generating quantitatively more water-soluble and qualitatively more oxidized organic material than corresponding control experiments kept in the dark. While both the dark and the irradiated organic matter were amenable to aerobic biodegradation processes, more oxygen respiration was noted with the photo-solubilized components.

This thesis is comprised of a single chapter, supplemental material, and one appendix that collectively describe the impact of photooxidation and biodegradation on the environmental fate of sand patties formed following the Deepwater Horizon incident. The chapter and supplemental is written in the style that is consistent with the journal *Environmental Science & Technology*.

In Chapter 1, I collected sand patties and sediment from Fort Morgan and Gulf Shores, Alabama. These sand patties and sediment were utilized to perform aerobic and anaerobic toxicity evaluations on endogenous microbial communities, as well as to establish aerobic and anaerobic biodegradation experiments. The largest sand patties I collected were interrogated for their relative hydrophobicity via the use of a $^{35}SO_4$ tracer.

Additional sand patties were irradiated via the use of a solar simulator and the organic content was monitored via absorbance spectroscopy. This generated photooxidized water-soluble organic material that was subsequently used for toxicity and biodegradation experiments. Since aerobic experiments showed the potential for aerobic biodegradation, T_0 and T_{Final} samples were sent to collaborators at the National High Magnetic Field Laboratory at Florida State University in Tallahassee, Florida for further chemical characterization. Our collaborators extracted the samples and were able to run the samples on their Fourier transform ion cyclotron resonance mass spectrometer and fluorometer. Analysis of the mass spectrometry and fluorometry data was performed in Tallahassee. Interpretation of the mass spectrometry data was performed by myself and Dr. Suflita with the input of our collaborators at Florida State University, and at the University of New Orleans, in New Orleans, Louisiana.

Interpretation of the spectral data was performed by our collaborators at Florida State University and at The University of New Orleans.

In contrast to the main chapter and supplemental portion of the thesis, the Appendix details my efforts at characterizing the susceptibility of sand patty-derived organic matter to anaerobic biodegradation processes. This facet of the study documented that such organic matter is likely to be fairly recalcitrant, at least when exposed to microorganisms in coastal marine environments. I considered the prospect that my initial findings may have been too subtle to allow me to detect electron acceptor consumption against a large background signal. I therefore repeated the experiment by periodically interrogating the experiment with a sensitive radiotracer. The latter experiment reinforced the conclusion that the sand patty organic matter tended to resist anaerobic biodegradation processes.

Collectively, the findings argue that sand patty deposition does not represent an endpoint in the environmental fate of sand patties deposited on beaches following a major oil spill. Rather, photooxidation, water washing, and biodegradation are each important processes governing the ultimate fate of these highly weathered oil-derived structures.

Chapter 1

Introduction

On April 20, 2010, the catastrophic blowout of the Deepwater Horizon drilling rig resulted in the deaths of 11 workers and injury of another 17 individuals. Over the next 87 d, the Macondo wellhead spewed oil from approximately 1500 meters below the surface of the Gulf of Mexico (GoM), 68 kilometers southeast of the Louisiana coast. It has been estimated that between 4.2 and 4.9 million barrels of oil were released making it the largest accidental oil spill in history.[1–3]

Numerous studies have examined the fate and impact of released Macondo oil within the plume[4–8], on the surface of the Gulf [5,9,10], buried in ocean sediments (either directly or as marine snow) [6,10], in marshes [11–14], and on beaches.[10,12,14–19] Not surprisingly, the environmental fate of the oil was influenced by many well documented weathering processes.[20–25] These processes resulted in the massive transformation of the oil components and a chemical footprint that differed substantively from the crude oil released at the wellhead. Most notably, the transformation of the crude Macondo 252 oil resulted in the formation of a group of partially oxidized organic compounds collectively termed 'oxyhydrocarbons.'[20] The formation of the oxyhydrocarbons was coincident with a decrease in the fraction of *n*-alkanes and aromatic compounds in the residue. These oxyhydrocarbons are most obviously found in sand patties that wash up in the swash zone of northern GoM beaches due to prevailing winds and currents. That is, sand patties are thought to be highly weathered oil deposits that combine with sediment particulates and migrate along the seafloor after a spill and eventually reach

coastal beaches.[15,18,20] The oxyhydrocarbons constitute over 50% of the organic compounds in sand patties and of the residuum on 'oiled' rocks.[20,21]

Sand patties derived from oil are likely exposed to both anaerobic conditions during their migration along the seafloor and aerobic conditions once they reach the beachfront. Additionally, due to the nature of sand patties, it is possible that the patties themselves may develop an oxygen gradient within pores where diffusion may be limited. Once on beaches, sand patties are exposed to direct sunlight during the day and are constantly wetted from the action of the surf. Sunlight results in oil weathering by photooxidation that likely further transforms the constituents in sand patties.[24] Continuous exposure to weathering processes results in the loss of saturated and aromatic compounds in sand patties and the formation of oxygenated resins and polar components that are difficult to resolve by standard chromatographic methodologies.[20,23,26] While it is known that UV irradiation and biodegradation transform crude oil components, the long-term fate of many high molecular weight polar molecules resulting from the aforementioned combination of oil weathering processes is far from clear.

Hydrocarbons are known to inhibit and can be potentially toxic to individual microorganisms.[27] However, we sought to assess the impact of sand patties and derived organic matter on the overall functioning of marine microbial communities. This was necessary to ensure that subsequent biodegradation experiments were not predisposed to failure. To this end, the endogenous rate of electron acceptor utilization was used as an integrated measure of the baseline functioning of the active microflora in marine samples. The overall rate of both oxygen consumption and sulfide formation in the

presence and absence of sand patty carbon was evaluated. Additionally, this study sought to examine the combined effects of photooxidation and biodegradation on the environmental fate of oil-derived components found in sand patties recovered from impacted beaches on the northern GoM shores. We propose that these processes represent the near final stages in the mineralization of the contaminating Macondo oil.

Materials and Methods

Sample Collection, Biomarker Analysis and Anion Analysis

Sand patties were collected from the swash zone of beaches in Gulf Shores and Fort Morgan, Alabama (30.24° N x 87.74° W and 30.22° N x 88.01° W, respectively) on January 20, 2014 and March 18, 2014. Initial forays for the collection of the morphologically recognizable sand patties were conducted in conjunction with Dr. Christoph Aeppli, who subsequently analyzed a subset of the collected specimens for the presence of biomarker compounds. Based on the identity of hopanoids with up to 31 carbons, as well as steranes and diasteranes that were in the original oil but not systematically altered by weathering processes, the sand patties were identified as originating from the Macondo oil.[21] Sand patties were placed in clean sealed glass containers, cooled with ice packs, and shipped overnight to the laboratory whereupon they were stored at -80^0C until use. Sediment from just below the water surface on the same beaches was collected using a wide mouth neoprene jar to scoop material from a depth of 5-10 cm. The jars were nearly filled, topped off with seawater to eliminate headspace, sealed, and also shipped to the laboratory, whereupon they were stored at 4^0C prior to use. Anion analysis (Cl^-, NO_3^- and SO_4^{-2}) of the aqueous samples was

performed with a Dionex Series 3000 Ion Chromatography System as previously described (Thermo Fisher, Waltham, MA).[28] The sulfate analysis was used for assessing the rates of sulfate reduction using a radiotracer method (below).

Impact Of Sand Patties On GoM Microbial Communities

For aerobic microbial metabolism, the rate of oxygen consumption was measured with a 10-channel Micro-Oxymax Respirometer outfitted with an electrochemical oxygen sensor (Columbus Instruments, Columbus, OH). Samples were incubated at room temperature in 250 mL bottles containing 10 g of sediment inoculum and 10 mL of filter-sterilized seawater. The headspace was automatically purged every 4 h and analyzed for O_2. The incubations were conducted in triplicate and results averaged for each experimental condition.

To assess the impact of sand patty organic matter on anaerobic respiration, we employed $^{35}SO_4^{-2}$ as a radiotracer and measured its conversion to ^{35}S-sulfide using a previously described procedure.[29] Briefly, 10 mL of seawater was combined with varying amounts of sediment and sand patty material to total 10 g. Then, 100 μL of a 5 μCi $^{35}SO_4^{-2}$ stock solution was added to each of the bottles to reach an initial dose of 50 nCi tracer. The bottles were then incubated for 7 d at room temperature. After incubation, 4 mL Cr(II)Cl and 4 mL HCl were added to volatilize biogenically produced $H_2{}^{35}S$ which was subsequently trapped in a zinc acetate solution. Radioactivity was measured with a scintillation counter (Triathler LSC, Hidex, Turku, Finland) by removing a 1 ml portion of the trap and adding it to 5 mL of Ultima Gold Liquid Scintillation Cocktail (Sigma Aldrich, St Louis, MO). The rates of sulfate reduction in sand patty-amended incubations were compared to positive control incubations that

received lactate (20mM), a substrate-unamended control with only endogenous substrates and an autoclaved negative control (without sand patty amendment) using a paired t-test.

Seawater Transport in Sand Patties

Since sand patties originate from spilled oil and contain a variety of hydrophobic compounds[15,18,21,23] there is a tendency to assume that these structures resist the penetration of seawater to their interiors. To test this prospect, several sand patties were placed on the surface of sediment that was overlain with seawater in the bottom of a standard petri dish. Approximately 5 μCi of $^{35}SO_4^{-2}$ was added to the seawater and the mixture was gently agitated at 45 rpm for 7 d at room temperature under a headspace of N_2:CO_2 (80:20). The sand patties were recovered from the petri dish and dissected into approximately 1.5 mm segments with a clean razor blade for each cut. The resulting sections were then laid flat and the distribution of the radioactivity was directly imaged using a Packard Instant Imager (Packard Instruments, Meriden, CT). Total β-emissions from ^{35}S-SO_4 and heat maps were generated using the associated imaging software.

Sand Patty Irradiation and Seawater Absorbance

To examine the impact of solar radiation on the environmental fate of sand patty constituents, approximately 12-15 g of sand patties were crumbled into 100 mL of filter sterilized seawater (0.2 μm). Sand patties were then irradiated in borosilicate glass jacketed beakers with quartz lids for 3 h or 12 h (equivalent to 3 days of average northern GoM sunlight) using a solar simulator (Atlas CPS+, Mount Prospect, IL) as previously described.[24] After each irradiation increment, seawater with associated solubilized organic matter was then removed, the beaker refilled with fresh sterile

seawater, and irradiated for another time increment. The irradiation and water replacement was repeated for up to 84 h. Sand patties incubated in sterile seawater and kept in the dark served as controls. Water-soluble and UV absorbing constituents present in the seawater for irradiation and dark control treatments were initially characterized using a scanning spectrophotometer (from 200 – 600 nm), but routinely monitored at 254 nm.

Biodegradation of Sand Patty DOM

The biodegradation of sand patty-derived and endogenous DOM was compared using GoM sediment (10 g) as an inoculum. Evidence for biodegradation included an assessment of the rate of electron acceptor utilization, in this case oxygen, using the aforementioned respirometer. The endogenous control incubation received the inoculum and 10 mL of filter-sterilized seawater. In other replicates, the seawater was replaced with the same volume of sand patty-derived DOM from either the irradiation procedure or dark control as described above. The oxygen utilization rates in the incubations were compared to each other and to a sterile negative control containing seawater and sediment that was autoclaved (20 min). All data are reported as the average of triplicate room temperature incubations that were monitored for oxygen content every 4 h. In addition to oxygen uptake, the change in the quality of the DOM as a result of biodegradation was also assessed by sampling at the beginning (T_0) and end (T_{Final}) of the aerobic incubation using the analytical procedures described below.

Dissolved Organic Matter (DOM) Characterization

Sample DOM concentration was determined by the high temperature catalytic oxidation method using a Shimadzu TOC-L_{CPH} analyzer (Shimadzu Corp., Japan).[30]

Each sample was acidified to pH 2 and sparged for 5 min at 75 ml min^{-1} with either ultra-pure air or ultra-pure oxygen to remove inorganic carbon prior to the measurement. The mean value of three to five 25 μL replicate samples is reported. The coefficient of variance (precision) was < 2% for replicate determinations.

Other Spectral Measurements

The sample pH was adjusted to 8 for spectral measurements as previously described.[31–33] Absorbance and fluorescence spectra were collected with an Aqualog® fluorometer (Horiba Scientific, Kyoto, Japan) in a 10 mm quartz cell at a constant room temperature of 20 °C. Sealed water cell blanks were analyzed initially to test instrument stability using the Raman peak of water at excitation 350 nm and emission 340-420 nm. Excitation and absorbance scans were collected from 240 to 800 nm at 5 nm increments with an integration time of 0.5 s. Emission spectra were collected every 5 nm from 245 to 800 nm with a charge-coupled device at 1.64 nm resolution. All samples were dilution corrected (absorbance at 254 = 0.1) with MilliQ water to reduce inner-filter effects.[34] Excitation-emission matrix fluorescence intensities were corrected for Rayleigh and Raman scattering by using a Milli-Q water blank-subtracted and instrument bias in excitation and emission prior to correction for inner-filter effects.[35] Fluorescence intensity was normalized to quinine sulfate units as previously described.[36]

Mass Spectrometry

Dissolved organic components were obtained by solid-phase extraction (SPE) using the SPE-DOM method described elsewhere.[37] Briefly, each sample was passed through a precombusted 0.27 μm glass-fiber filter and acidified to pH 2 prior to loading

onto an a Bond Elut PPL (Agilent Technologies) stationary phase cartridge. Each sample was then desalted with pH 2 MilliQ water and eluted with methanol at a final concentration of 100 μgC mL^{-1}. The extracts were stored in the dark at -4 °C in pre-combusted glass vials until analysis by negative-ion electrospray ionization coupled with a custom-built 9.4 tesla Fourier transform ion cyclotron resonance mass spectrometer (National High Magnetic Field Laboratory (NHMFL), Florida State University, Tallahassee).[38,39] Each mass spectrum was internally calibrated with a "walking" calibration equation followed by molecular formula assignment with internally developed software provided by the NHMFL.[40]

Results

Toxicity Screening

Many toxicity evaluations employ a single organism to assess the potential impact of a given toxicant.[41] Such evaluations are subject to a multitude of interpretational constraints when extrapolating the resulting information to other organisms or to larger community effects. It is arguably more environmentally relevant to examine the response to perturbations by monitoring overall community respiration. In marine habitats, the two most quantitatively important electron acceptors available to the requisite microbial communities are oxygen and sulfate.[42] The heterotrophic respiration of aerobic microbial communities was monitored as the impact of sand patties on the endogenous rate of oxygen utilization, while the corresponding anaerobic respiration activity was measured as a rate of sulfide formation (Table 1).

The sulfate reduction assays containing sediment, seawater, and varying amounts of sand patty material or lactate are shown in Table 1. The lactate-amended positive control reduced more sulfate than the endogenous control, confirming that the resident microflora included anaerobes that could respond to the introduction of a labile carbon source. Community respiration also generally increased as a function of the sand patty amendment with the most statistically significant amount of reduced sulfide evident in incubations receiving the highest amount of sand patty material. Thus, it can be concluded that sand patty organic material is not inherently detrimental to the native sulfate-reducing microflora. Rather, the maximal 39% increase in rate of sulfate reduction over the endogenous rate suggests that sediment microbes are capable of utilizing at least some components in sand patties as electron donors to support anaerobic respiratory activity.

Similarly, oxygen respiration was used as an indicator of the relative toxicity of sand patty organic matter on aerobic microbial communities. Table 1 shows the rate of oxygen consumption in nearly identical seawater-sediment incubations with or without an amendment of whole sand patty material. If sand patties were an inherent detriment to aerobic microbial communities, a decrease in the oxygen respiration rate would be evident upon sand patty addition. However, when aerobic incubations were amended with 1-2g of sand patty material, the oxygen respiration rate remained unaltered. Indeed, like the anaerobic incubations, larger sand patty amendments (5g) significantly stimulated aerobic microbial community respiration. These results suggest that the GoM microflora was largely impervious to the potential inhibitory effect of sand patties, but some associated components likely stimulated biodegradation activity.

Seawater Transport in Sand Patties

Sand patties were incubated for 7 days in seawater containing $^{35}SO_4^{-2}$ and subsequently dissected and analyzed. The autoradiographic images clearly show that the water-soluble tracer was transported to the interior of the structures (Figure 1). The amount of radioactivity in various subsections of the dissected sand patty was roughly equivalent. However, the distribution of the label was more even in one distal end of the sand patty, presumably reflecting variations in thickness. Ignoring the surface radioactivity, the tracer that reached to the interior of the sand patty was largely normally distributed in all subsections (only representative data shown in Figure 1). However, several subsections had local hot spots of accumulated radioactivity for unknown reasons. Collectively, these findings argue that seawater was readily able to infiltrate sand patties and that the chemical nature of these structures does not represent a substantive barrier to water transport.

Absorption Characteristics of Sand Patty DOM

Sand patties were broken into pieces, placed in sterile seawater and exposed to simulated sunlight to examine the impact of irradiation on the weathering of these oil residue structures. The absorption characteristics of compounds photo-solubilized by this procedure were measured from 200 – 600 nm and compared with dark controls and the seawater alone. Routine monitoring at 254 nm of irradiated and dark samples as well as background seawater measurements revealed an increase in UV-absorbing material leached from the sand patties under both conditions (Figure 2). However, consistently more UV_{254} -absorbing material was produced upon irradiation, particularly over the first 12 h. The increase in UV absorbance at 254 is noteworthy since this value

is known to correlate with DOM content.[31] Near linear increase in DOM as evidenced by absorbance was observed in the first 12 h of irradiation. After the initial 12 h of irradiation, the release of UV-absorbing material then decreased to a slower, but also linear rate. Both irradiated and dark control samples showed similar behavior, except the rate of increase in absorbance was faster for the irradiated samples in both the initial 12 h period and the subsequent time periods. UV-absorbing material continued to solubilize from even the dark samples relative to seawater throughout the 84 h of the experiment. These findings indicate that sand patties represent a source of UV-absorbing material to the surrounding environment and that more photo-solubilized material is released upon exposure to sunlight. It is likely that these processes occur in the swash zone of GoM beaches as sand patties are exposed to natural sunlight and constantly impacted by wave action.

Biodegradation of Sand Patty-Derived DOM

An inoculum from the GoM was used to determine if the indigenous microflora were capable of metabolizing DOM released under dark and irradiated conditions. Figure 3 shows the total amount of oxygen consumed by aerobic GoM sediment communities over the course of a 3 d incubation when amended with irradiated, dark, or whole sand patty material. The aerobic toxicity screening (above) revealed that at least some components in the sand patties alone were amenable to biodegradation as evidenced by the increased rate of oxygen consumption relative to the endogenous control (no added organic matter) (Fig. 3). A very similar rate of oxygen consumption was observed when the DOM from the dark control incubations were similarly assayed. We presume that the same or similar suite of seawater-soluble DOM components

leached from the whole sand patties in the experimental systems and served as suitable electron donors for the resident aerobic microflora. Since seawater could readily penetrate the sand patties (Fig. 1), the DOM components in both the dark controls and whole sand patty treatments was likely comparable.

When photo-solubilized DOM from sand patties was utilized as an amendment for the GoM inoculum, the rate of oxygen consumption was clearly increased relative to the dark DOM treatment or the endogenous level of respiration (Fig. 3). Over the course of a 3 d incubation, the endogenous microflora respired 38 μM O_2 with natural seawater/sediment organic matter serving as electron donors, while 64 and 67 μM O_2 were utilized when the same inoculum used whole sand patty organic matter or the DOM from dark controls, respectively. However, when photo-solubilized DOM from sand patties was similarly assayed, a total of 114 μM O_2 was utilized. These findings attest to the increased susceptibility to aerobic biodegradation of the soluble organic matter formed upon exposure to solar radiation.

Chemical Characterization of DOM Biodegradation

The nature of material leached from sand patties under both irradiated and dark conditions were furthered characterized by ultrahigh-resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry as well as absorbance and fluorescence measurements commonly used to characterize DOM.[38,39,43] The latter procedures are typically applied to soil, plant or algal organic matter, but were used here to characterize the sand patty-derived DOM. Clearly, the organic matter in sand patties differs markedly from typical sources of DOM, so the values derived from fluorescence and optical measurements are likely to be somewhat unconventional; however, the

relative changes in the values following phototransformation and biodegradation can be interpreted in a comparable manner as for other types of DOM.

Mass Spectrometry: Van Krevelen plots of the mass spectral data (Supplemental Figure S1) show a vast array of chemical features representing DOM with a diverse range of oxygen to carbon (O/C) and hydrogen to carbon (H/C) ratios leached from the sand patties under both irradiated and dark conditions. However, this analysis does not allow for ready comparison of the qualitative (or quantitative) nature of the leached DOM. More revealing are Van Krevelen difference plots that remove chemical features that were common to and persisted over the course of the biodegradation experiment (Figure 4). That is, Fig. 4A and 4C emphasize the chemical features that were altered during the aerobic biodegradation experiment using the DOM released to the seawater dark and irradiated conditions, respectively. Similarly, Fig. 4B and 4D highlight chemical features that were newly formed during the course of the biodegradation experiment.

The comparison of Figures 4A and 4C reveal that prior to biodegradation, the DOM in the irradiated samples had more chemical features and more diverse chemical constituents that exhibited a wider range of both H/C and O/C ratios. Chemical features with low H/C tend to be removed through photochemical processes, while microbial transformation activities remove features with high H/C. In both cases, the general trend is for the transformation products to be shifted toward the center of the plot and relatively high O/C values.

However, there is only a 24% degree of similarity between the plots depicted in Figure 4A and 4C. Thus, the DOM leached from the sand patty into seawater in the

dark and the light are fundamentally different. The greater tendency toward higher O/C ratios in the irradiated samples indicates that photooxidation results in the generation of more oxidized compounds from the sand patties that may then partition to the seawater. This observation is in agreement with previous reports.[24]

The relative differences in the DOM formed in both the dark and irradiated samples are also evident at the end of the biodegradation experiment (Fig. 4). A comparison of Figure 4B and 4D reveal that a greater number and diversity of chemical features were formed in incubations receiving dark DOM. These intermediates exhibited a wide range in both the H/C and O/C ratios, but a greater tendency for the production of less oxidized and more saturated constituents. In contrast, the intermediate constituents formed in the biodegradation experiment amended with irradiated DOM was less diverse and relatively more oxidized. Presumably the fewer and more oxidized constituents reflect that fact that more of the DOM was in a relatively more advanced state of decomposition and/or completely mineralized. These findings are consistent with the oxygen respiration data and further suggest that the irradiated DOM was more amenable to biodegradation and complete mineralization.

Characterization of DOM Optical Properties: The DOM concentration, slope ratio, humification index, and freshness index was determined for each sample at the beginning (T_0) and end (T_{Final}) of the biodegradation experiment is shown in Figure 5. A marked increase in DOM material leached from the sand patties after exposure to simulated sunlight was observed relative to those kept in the dark; an indication that photooxidation enhances the formation of water-soluble organic components from the weathered oil residuum as previously suggested.[24] The fact that the DOM content was

significantly reduced by the end of the incubation for the dark and irradiated samples by 67% and 56%, respectively strongly suggests that the water-soluble organic material leached from sand patties was available to the resident microflora and amenable to biodegradation.

The amount of DOM in the endogenous incubation at either T_0 or T_{Final} was only about a third and a fifth of that initially formed in the dark and irradiated samples, respectively. This is not surprising since the endogenous sample did not contain sand patty material. The lack of a substantive change in DOM at such low concentrations over the course of the experiment suggests that the determination is too insensitive an indicator when losses due to aerobic respiration (Fig. 5A) are possibly offset by increases in microbial growth. The DOM in the sterile incubation increased over the endogenous control following the intense heating during sterilization. The release of DOM from marine sediments following heating is not without precedence.[44] However, such extreme heating has no substantive environmental relevance in coastal marine sediments and is included here for comparative purposes, but this treatment is only interpretationally useful for other DOM characterization efforts. Collectively, the findings are significant in the context of the ultimate fate of petroleum hydrocarbons in the environment. They suggest that the already partially oxidized sand patty constituents can continuously release water-soluble and biodegradable organic constituents to the surrounding environment by wave action during the day and at night. However, solar irradiation further oxidizes the sand patty constituents, alters the quality of the organic matter leached to the surroundings and increases its propensity to undergo aerobic attack.

The nature of DOM at the beginning and end of the biodegradation experiment were further characterized by the changes in spectral properties as previously detailed by Hansen et. al., 2016.[43] The spectral slope ($S_{275-295}$; $S_{350-400}$) was calculated by applying a nonlinear fit of an exponential function to an absorbance spectrum in the range of 275-295 nm (Fig. 5B) and 350-400 nm (Fig. 5C) and the spectral slope ratio (S_R) was calculated as the ratio of $S_{275-295}$ to $S_{350-400}$ (Fig 5D).[45] The three parameters were shown to correlate with the molecular weight, aromaticity and source of DOM and relative changes in these values can be attributed to photochemical and microbial degradation processes.[45] Basically, the increase in the slope ratio from 1.4 to 1.6 in the dark and irradiated samples, respectively indicates that more lower molecular weight constituents were photosolubilized prior to the biodegradation experiment upon irradiation (Fig. 5D). Figure 5 also shows that mean $S_{275-295}$ and S_R values increase for the DOM produced from sand patty-amended incubations relative to the endogenous or sterile controls, particularly in replicates that were exposed to sunlight. Relatively steep $S_{275-295}$ and high S_R are related to a decrease in DOM molecular weight and aromaticity.[45–48] The compounds leached from the sand patties were utilized by the resident microflora during the course of the biodegradation experiment as evidenced by an increase in $S_{350-400}$ and decrease in S_R (Fig. 5C and 5D). The significant decrease in S_R after biodegradation was a result of a corresponding increase in $S_{350-400}$ while $S_{275-295}$ remained constant. This decrease in S_R was likely a result of microbial metabolism and/or selective preservation of relatively large, aromatic compounds.

Microbial transformation of low molecular weight organic compounds into relatively condensed, high molecular weight macromolecules is often assessed through

changes in the humification index. The process of humification results in a red shift in emission spectra that corresponds to a decrease in the hydrogen to carbon ratio. The humification index is calculated by dividing the area under Em. 435-480 by the peak area 300-435 nm + 435-480 nm, with excitation at 254 nm.[34] The humification index of the DOM, was nearly equivalent at the beginning of the biodegradation experiment in both the endogenous and sterile incubations that were not amended with a sand patty (Fig 5E). The same index of the DOM leached form the sand patties were significantly lower and different at T_0 between the dark and irradiated treatments. That is, the humification index decreased from 1.5 in dark samples to 1.0 in irradiated incubations. This result suggests that the DOM photosolubilized from sand patties had a higher hydrogen to carbon ratio relative to material leached in the dark and was presumably more bioavailable. At the end of the biodegradation experiment, the humification index rose in all incubations except the sterile control. Presumably, the more labile DOM constituents were preferentially metabolized by the GoM microflora resulting in a relatively higher humification index in each case. The increase in the T_{Final} humification index for the sand patty-derived DOM (83%) leached in the dark was higher than the comparable measure for DOM leached in the presence of sunlight (62%). This difference may reflect an increase in the quantity of relatively aliphatic, small, bioavailable DOM formed after the sand patties were exposed to sunlight. An increase in the pool of labile DOM may result in a slower rate of change in the humification index values relative to a small pool that was rapidly depleted by microbes. The residual DOM at T_{Final} in the endogenous incubations had the highest humification index than either of the sand patty incubations. However, the humification

index in the sterile incubation decreased with time, probably reflecting an unknown abiotic change in DOM quality.

Freshness index *(β:α)* describes the ratio of "fresh-like" (aliphatic) to "humic-like" (aromatic) DOM.[45] The value is obtained from the emission intensity at 380 nm divided by the Em_{max} between 420 and 435 nm, with excitation at 310 nm.[49] Relatively high *β:α* values are indicative of labile, bioavailable DOM.[43] Microbial utilization of the labile DOM pool results in a decrease in *β:α* as the material is converted to more persistent DOM. Thus, the increase from 1.1 in dark samples to 1.4 in irradiated samples indicates an increase in smaller, less conjugated compounds due to photooxidation prior to microbial treatment (Fig. 5F). Similarly, the freshness index of the endogenous DOM at the beginning of the experiment (0.9) was lowest (more recalcitrant) relative to either the dark or irradiated samples. This is yet another indication that the DOM produced in sand patty-amended incubations is substantially different from the endogenous forms of organic matter in the GoM. At the end of the biodegradation experiment (T_{Final}; Fig 5F), the same relative increasing trend in the DOM freshness index is apparent when comparing the endogenous, dark and irradiated samples. However in each case, the index decreased relative to the T_0 determinations. This result suggests that the conjugated DOM is at least partially amenable to microbial decay, resulting in the residual pool of organic matter increasing in relative recalcitrance.

Discussion

The formation of sand patties as a residual form of the Deepwater Horizon oil and their and deposition on northern GoM shores has been previously reported.[15,18,20,21] Aeppli and colleagues noted the apparent recalcitrance of sand patties and characterized their chemical composition.[20] They reported that >50% of the mass of the material in these structures is not hydrocarbon at all, but oxygenated hydrocarbon-derived chemical features produced through a combination of weathering processes. We questioned the environmental fate and impact of these residual oil structures, and considering their deposition in the beach swash zone, explored the susceptibility of sand patties to the most likely forms of advanced decomposition – photoxidation and biodegradation.

Even though we retrieved sand patty samples from beaches, previous work demonstrated that such residual oil components could be buried in sediments where anaerobic conditions might prevail.[50–52] Therefore, the potential toxicological impact of sand patties to the resident microflora was assessed under the predominant electron accepting conditions in such marine coastal areas. Whole sand patty amendments were found to have no substantive negative impact on the endogenous rate of either aerobic or anaerobic metabolism as evidenced by the rates of oxygen consumption or sulfide formation, respectively (Table 1). In fact, with increasing amendment, whole sand patties stimulated respiratory processes, confirming that at least some of the chemical constituents represented suitable electron donors for the indigenous microflora. Such findings have two important implications. First, the subsequent biodegradation experiments were unlikely to be predisposed to failure due to the inherent inhibitory nature of the constituent chemicals in sand patties. Secondly, given the lack of

substantive impact on the overall rate of microbial community respiration, toxicological concerns associated with residual oil deposition should probably be targeted at other trophic levels.

The presumed hydrophobic nature of sand patties was also evaluated with a seawater-soluble radiotracer. We found that $^{35}SO_4^{-2}$ readily penetrated into the interior of the sand patties. The transport of seawater through sand patties has important inferences for the potential transformation of the constituent chemicals. Seawater is rich with nutrients, microbes and a variety of potential electron acceptors and donors that may be carried to the interior of sand patties.[42] Conversely, metabolic end products and partially transformed organic molecules can readily be leached from the sand patty interiors. Thus, sand patties likely represent a suitable habitat for the enrichment and proliferation of microorganisms with the ability to metabolize the oil-derived organic matter. Moreover, the relocation of potentially photosolubilized organic material or microbial transformation products from the interior of sand patties to the surrounding environment would likely be greatly facilitated by the penetration of seawater through these structures.

Indeed, the UV absorbance characteristics of irradiated and dark sand patty material that partitioned to seawater showed increases in absorbance between 250-280 nm (Supplemental Figure S2) relative to the endogenous organic matter. This spectral region is where aromatic ring structures absorb light and is consistent with the notion that the resulting DOM is likely oil-derived. Similarly, the DOM in both the irradiated and dark samples were quantitatively far more important than the measured endogenous DOM levels (Fig 5A). The general absorbance features in this range of wavelengths of

the sand patty-derived DOM were reminiscent of other reports of DOM emanating from the weathering of the Deepwater Horizon oil.[53] Most notably, the quantitative increase in aromatic DOM constituents formed as a result of natural weathering processes seems to be a generalizing feature of oil decomposition.[24]

An assessment of the aerobic biodegradability of sand patty-derived DOM was examined by comparing the rate of oxygen consumption in sediment samples amended with dark or irradiated DOM, whole sand patty material or only endogenous organic matter. The rate of oxygen consumption and thus the amount of biodegradable organic matter was lowest in samples amended with endogenous organic matter (after 24 h), intermediate with dark DOM or whole sand patty material and highest with irradiated samples (Fig. 3). Presumably the potential electron donors emanating from the whole sand patty and the dark DOM are somewhat comparable as they gave overlapping rates of oxygen consumption. Of course, the significant increase in rate of oxygen consumption observed with the irradiated DOM suggests that this material is more susceptible to aerobic decay processes. However, there is also no doubt that both the quality (Fig 2) and quantity (Fig 5) of the DOM in this sample were far different from either the dark or endogenous DOM. Thus, a comparison of the changes in DOM both before and after the biodegradation experiment was conducted.

There were clear qualitative differences in DOM at the start of the biodegradation experiment between the sand patty-derived organic matter formed by the dark and irradiation procedures. In both cases, the Van Krevelen difference plots (Fig.4) revealed that both sources of DOM represented complex molecular mixtures. These plots confirm that there were a greater number of molecular features in the DOM

produced through irradiation than the corresponding dark samples. In addition, the DOM formed by irradiation tended to have lower H/C and higher O/C ratios, confirming the more oxidized nature of the starting substrates for microbial attack.

Following the biodegradation experiment, the major chemical difference in DOM produced in the presence of artificial sunlight is the removal of an abundant group of molecular features with an O/C value of ~0.4 and H/C of about ~1.25. A plausible explanation is that photochemical processes result in the disaggregation of relatively small, but highly alkylated aromatic compounds (1-2 ring), that are then readily available to the indigenous microflora. This contention is supported by the relatively high slope ratio (small molecules), low humification index (unconjugated), and high freshness index (aliphatic) observed for the molecular features produced at the end of the biodegradation experiment. In contrast, the lower spectral slope and freshness index as well as the higher humification index collectively argue that the dark DOM, at both T_0 and T_{Final}, had more complex character than the comparable determinations on the organic matter formed in the presence of the sunlight. The reduced complex character likely accounts for the greater susceptibility of the irradiated DOM to aerobic biodegradation.

In fact, the dark and irradiated DOM exhibited a classical biodegradation trend of the removal of predominately high H/C molecular features and production of molecular features with relatively low H/C (Fig 4). The molecular features that were manifest at the end of the biodegradation experiment exhibited a higher O/C ratio suggesting that the more highly oxidized nature of the intermediates or end products that were formed. These results corroborate and support the optical measurement trends

that are indicative of biodegradation. That is, there was an increase in the humification index, as well as a decrease in both the freshness index and the slope ratio associated with the biodegradation of the endogenous, dark and irradiated DOM.

The long-term effects of the Deepwater Horizon oil spill are still under intense scientific scrutiny. Numerous studies examined the fate of the spilled oil and its transformation by a variety of weathering processes. This study found that both sunlight and aerobic microbial metabolism could further transform oil-derived sand patties and represent a major form of advanced oil weathering processes. Hayworth et al. detailed a conceptual model wherein sand patties that washed onto beaches may be biochemically degraded and shrink in size until they become non-recoverable.[18] Our study helps provide a mechanistic basis for how sand patties might undergo this reduction in mass. Thus, sand patties get deposited onto GoM beaches and are exposed to sunlight during daylight hours and wave action throughout the day. The sand patties then represent a source of DOM to the surrounding environment as seawater readily penetrates these structures. Upon exposure to sunlight and seawater, a complex suite of oxidized organic material is photosolubilized from the sand patties. Under dark conditions, a different suite of complex molecular features is also leached from the sand patties. In either the dark or the light, the water-soluble DOM is not inhibitory to the resident microflora and at least partially amenable to aerobic biodegradation processes. However, the photooxidized DOM is quantitatively more important than the dark DOM and represents a better source of electron donors supporting aerobic microbial respiration. These experiments clearly indicate the importance of sunlight in controlling the fate of highly weathered oil residue as well as the complex interplay between

photochemical and biological transformation processes. Finally, a comparable set of experiments designed to evaluate the anaerobic biodegradation of the sand patty-derived DOM suggests that this organic material tends to be recalcitrant under sulfate reducing conditions (Appendix A).

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Table 1. The impact of sand patties on the rate of microbial community respiration in room temperature GoM seawater-sediment incubations. Sulfide production was monitored in 10 mL incubations over 7 d while oxygen consumption was measured in 10 mL incubations over 3 d. The positive and negative controls contained only seawater and sediment.

Sand Patty Amendment (g)	nM S • day^{-1} • g^{-1}	µM O_2 • day^{-1} • g^{-1}
0 (endogenous)	38.9 ± 0.4	1.76 ± 0.08
1	-[a]	2.1 ± 0.4
2	-	2.1 ± 0.5
2.5	57 ± 14	-
5	53 ± 10	2.7 ± .3 *
7.5	80 ± 7 *	-
10	99 ± 30 *	-
Positive Control [b]	65 ± 9 *	-
Negative Control [c]	3.3 ± 0.3 *	0.7 ± 0.2 *

*significant difference from the endogenous rate of respiration based on student T-test

[a] -: not determined
[b] Positive Control: Lactate (20 mM)
[c] Negative Control: Autoclaved (20 min)

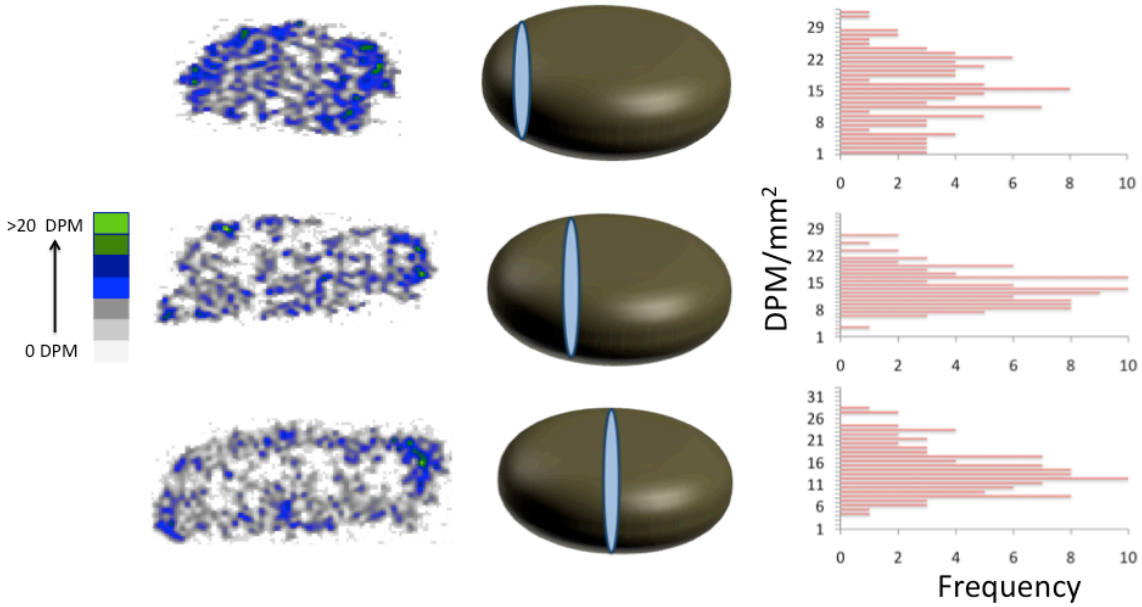


Figure 1. The Transport of radioactively labeled sulfate in seawater to the interior of a sand patty. False color autoradiographic images (left) illustrate the presence of the tracer in cross-sections of dissected portions of a sand patty. The idealized depictions (center) illustrate the approximate proximal to distal locations of the dissection areas in the irregularly shaped sand patties. The distribution of radioactivity (right) in a 10 x 10 mm^2 interior portion of the sand patty sections is also shown.

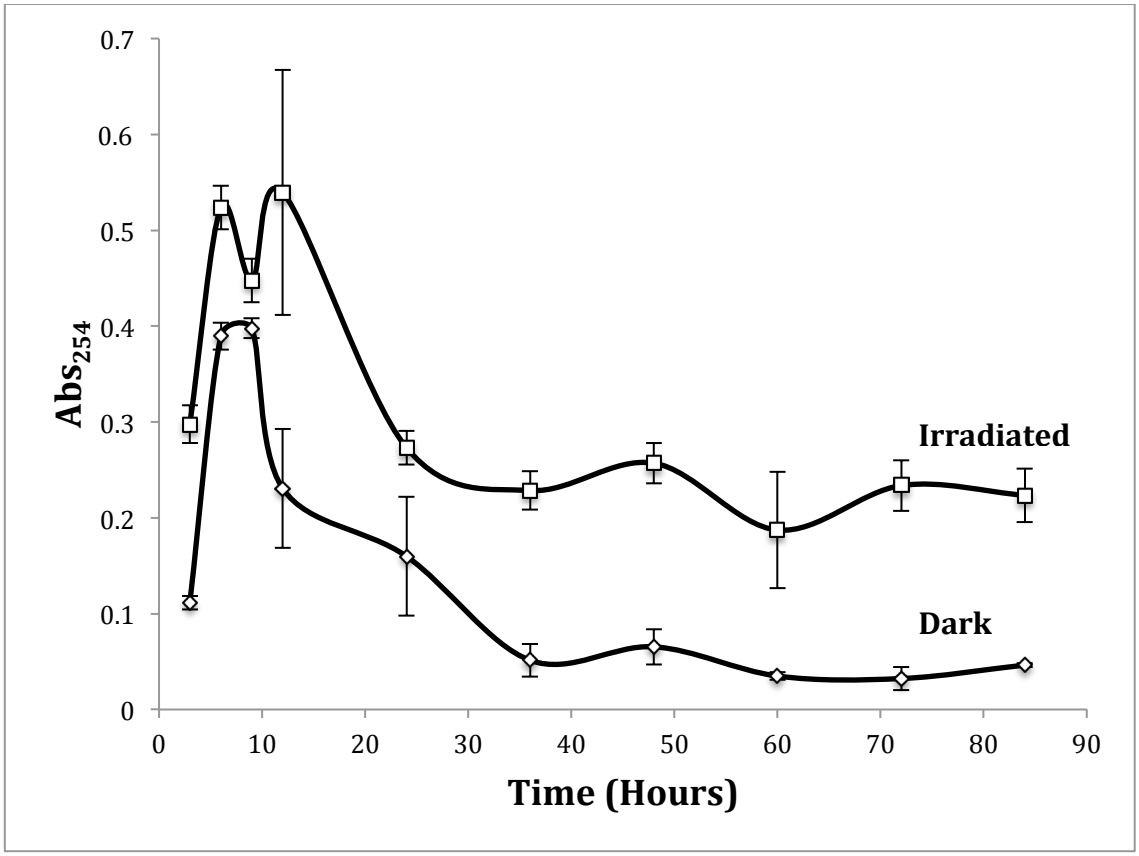


Figure 2. Time course absorbance measurements at 254 nM on irradiated and non-irradiated water-soluble sand patty extracts. Standard deviations are indicated for triplicate measurements. After each time point, all seawater was removed from the sample and replaced with fresh seawater.

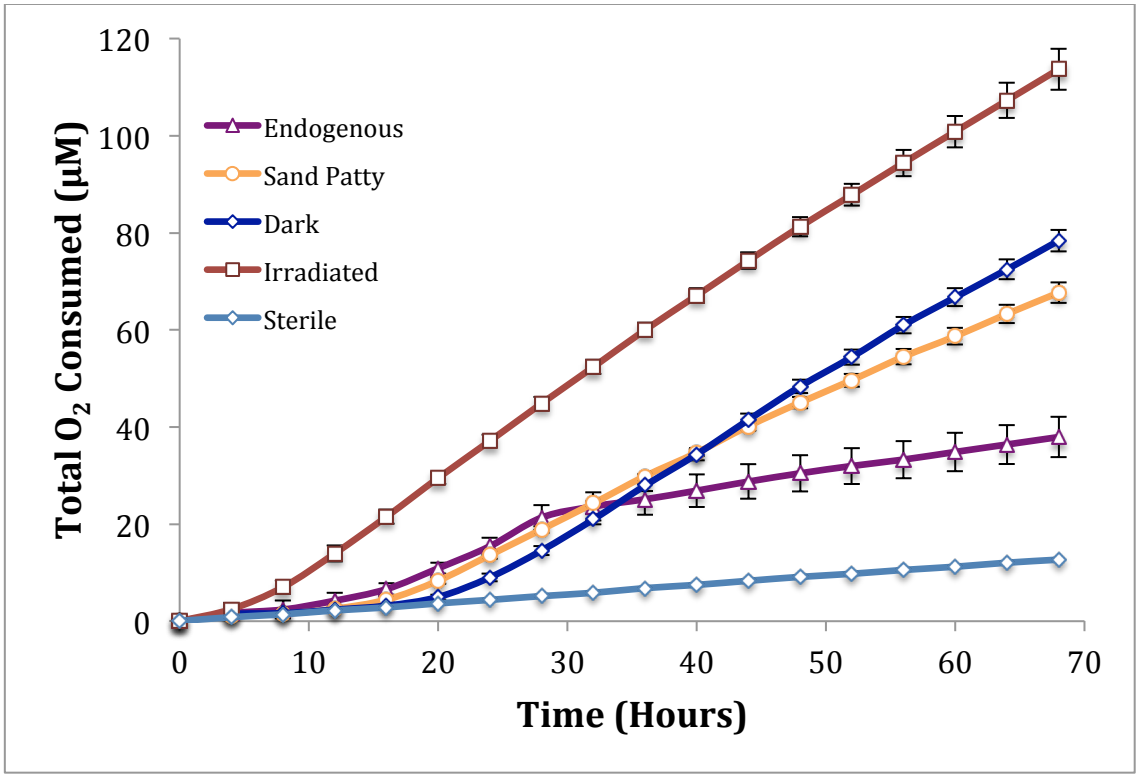


Figure 3. Total amount of O_2 consumed over the course of 68 hours in aerobic GoM sediment incubations. The standard deviation for triplicate measurements are indicated by the error bars. Sterile controls were conducted in duplicate due to channel constraints on the instrument. In this case, the error bars represent ranges and are generally less than the size of the symbol. All incubations contain 10 g GoM sediment and were amended with fluid as follows: endogenous incubations (Δ) contained 10 mL of filter-sterilized GoM seawater; dark incubations (◇) contain 10 mL of DOM material leached from sand patties in the dark; sand patty incubations (o) contain 10 mL of filter-sterilized GoM seawater and 1 g of crumbled whole sand patty material; irradiated incubations (□) contained 10 mL of DOM material leached from sand patties following irradiation; and sterile incubations (◇) contained 10 mL of GoM seawater that was autoclaved prior to the incubation.

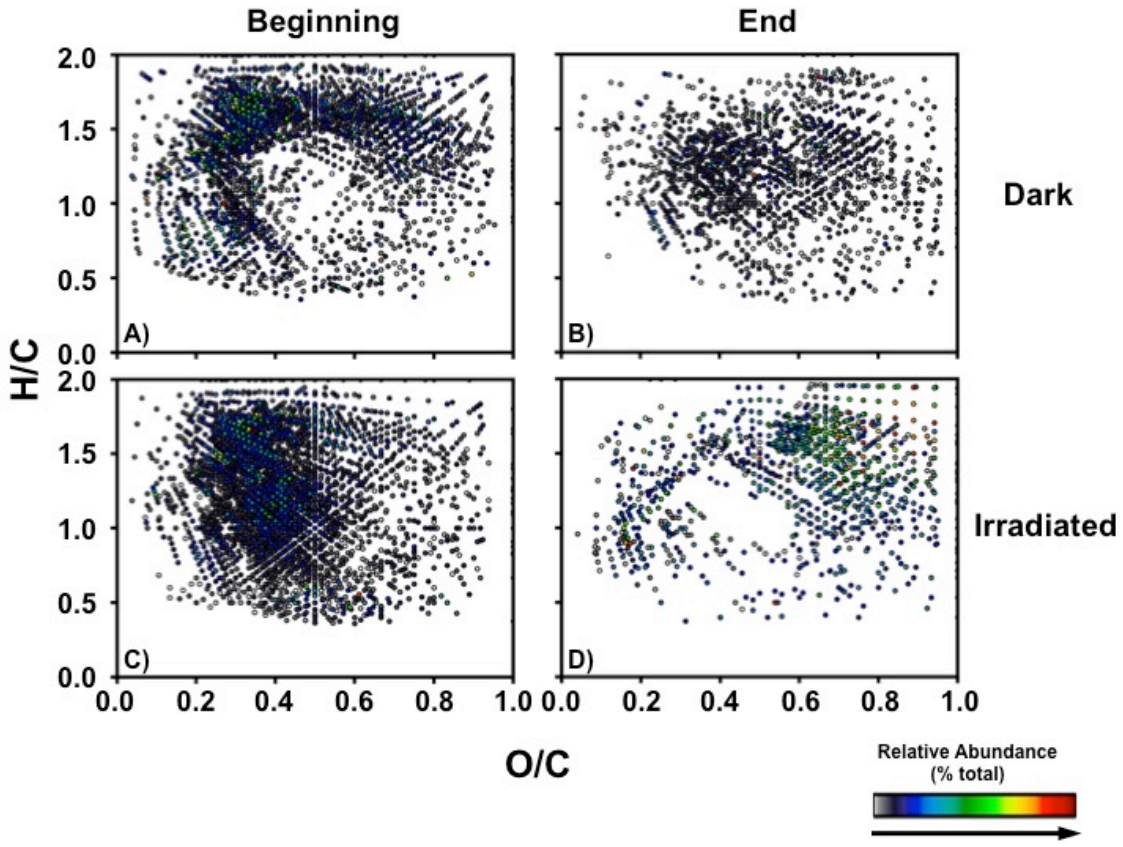


Figure 4. Subtracted Van Krevelen plots for aerobic biodegradation incubations. Subtracted Van Krevelen plots were generated by removing molecular formulas common to both T_0 and T_{Final}, revealing only those unique molecular formulas present prior to microbial processing (A and C) or those newly formed after microbial processing (B and D).

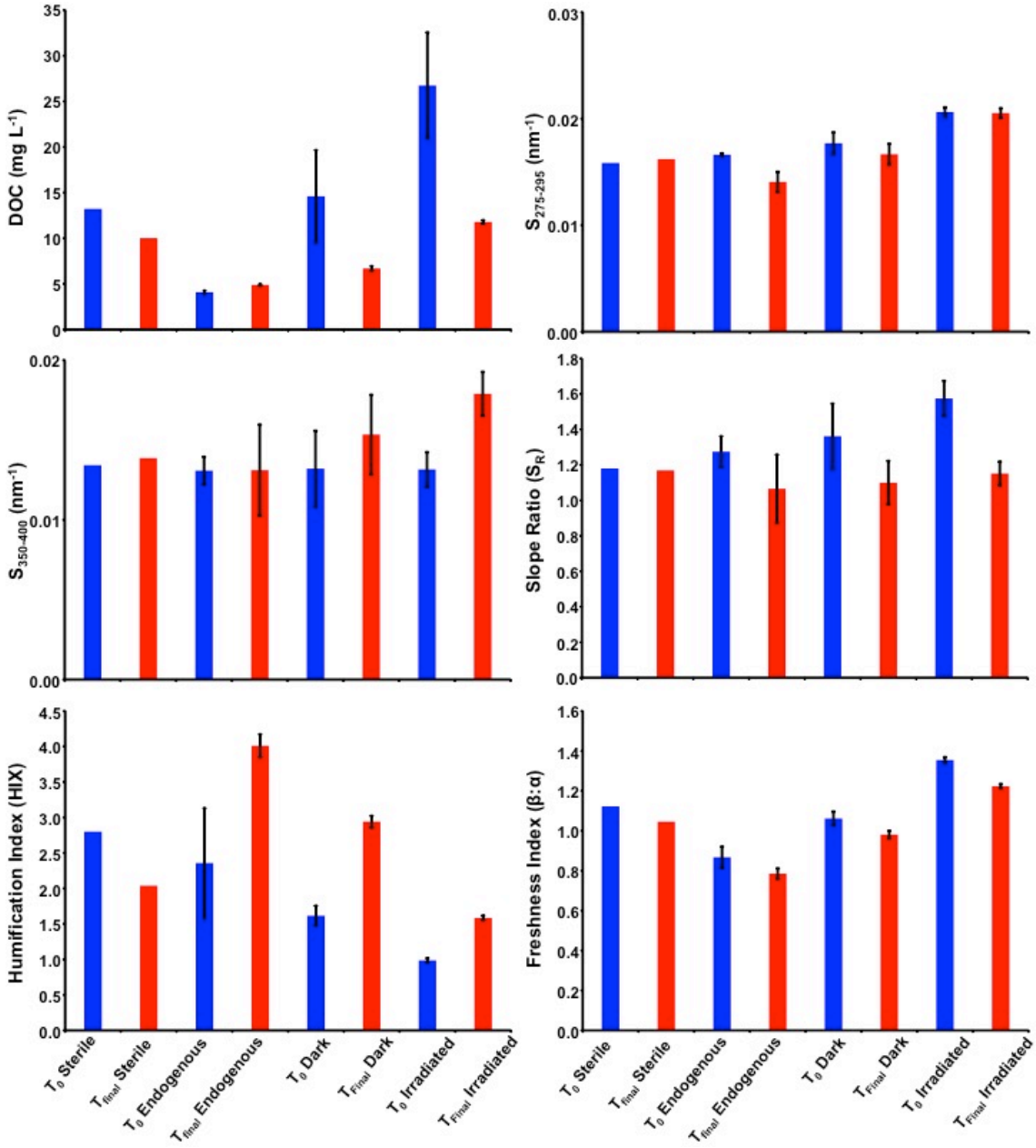
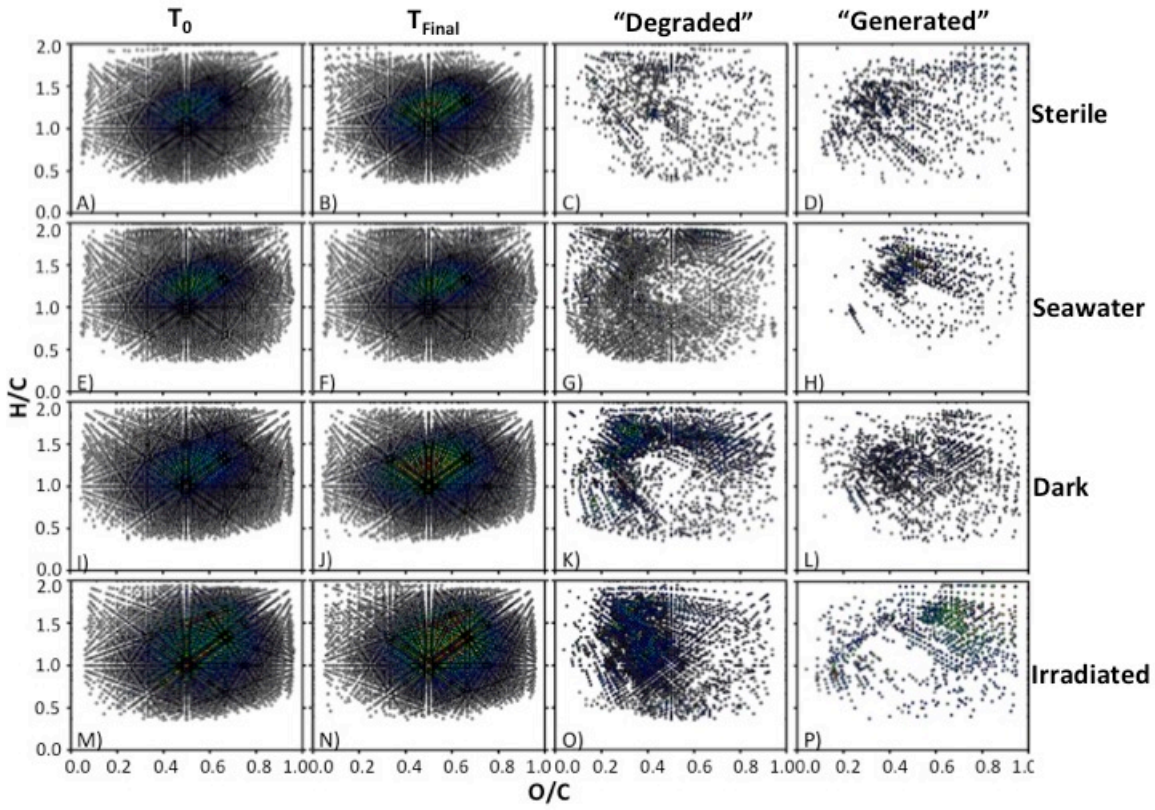
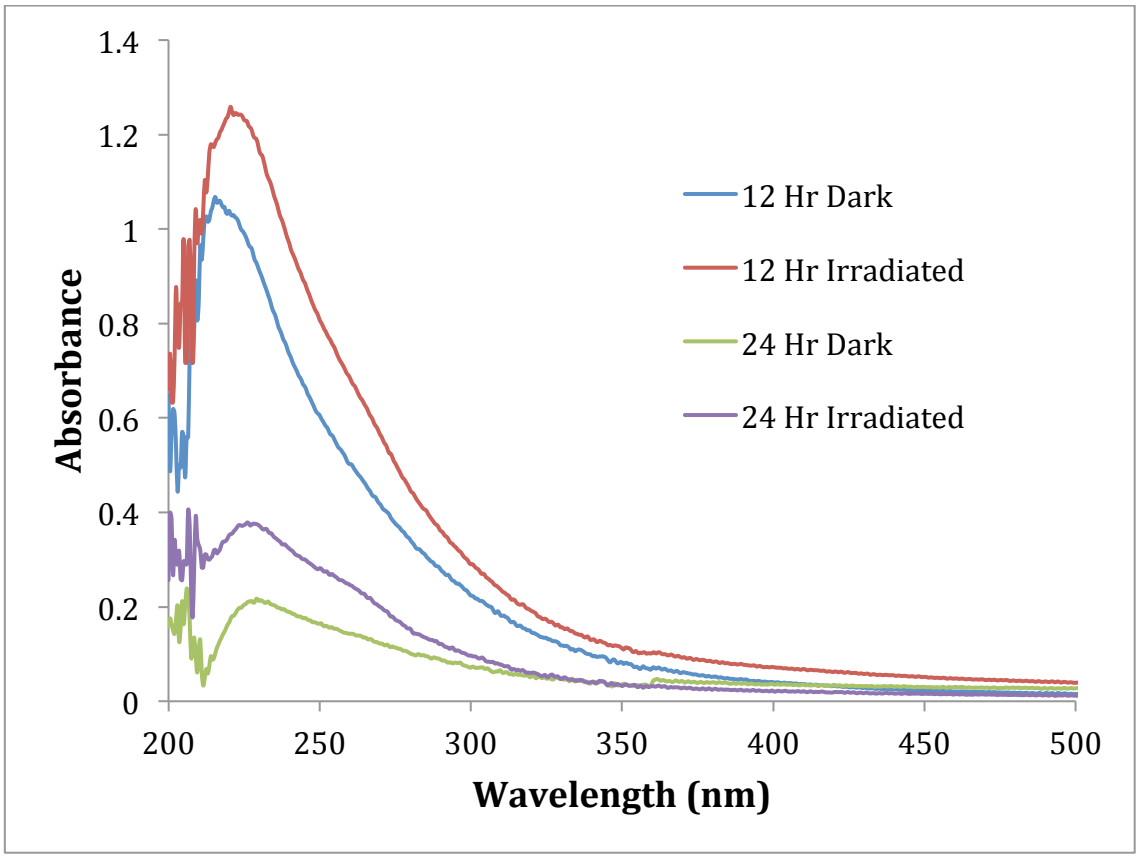


Figure 5. Dissolved organic carbon and fluorescence indices for T_0 and T_{Final} aerobic incubations. Bar charts were generated by measuring DOC (A) and fluorescence indices for Spectral Slopes ($S_{275-285}$) (B), and ($S_{350-400}$) (C), Slope Ratio (S_R) (D), Humification Index (HIX) (E), and Freshness Index (β:α) (F). Standard deviations are indicated for triplicate native, dark, and irradiated incubations, and sterile samples represent single replicates.



Supplemental 1. Van Krevelen and subtracted Van Krevelen plots for aerobic biodegradation incubations. Subtracted Van Krevelen plots representing the oxygen/carbon ratio on the x-axis (O/C) and the hydrogen/carbon ratio on the y-axis (H/C) were generated by removing compounds common to both T-0 and T-Final plots, leaving only those present in T-0 samples or those present only in T-Final samples. Panels K, L, O, and P are the same as the data in Figure 4, but are included here for easy comparison.



Supplemental 2. Absorbance scans on photooxidized and control sand patty organic matter at 12 and 24 hours.

Appendix A

Impact of Photooxidation and Biodegradation on the Environmental Fate of Sand Patties Formed From Oil Spilled During the Deepwater Horizon Incident: Anaerobic Conditions

Introduction

The Deepwater Horizon drilling rig blowout resulted in the release of an estimated 4.2 - 4.9 million barrels of Macondo 252 crude oil.[1–3] This crude oil was transformed by a number of weathering processes and eventually converted into sand patties that are largely composed of partially oxidized organic compounds termed 'oxyhydrocarbons'.[20,21,25,54] The weathered sand patties may remain on the seafloor or be transported to beaches where they can settle in the swash zone.[15,18,55,56] where they are exposed to direct sunlight, continual wave action and the ambient microbial communities. In addition, the sand patties themselves likely represent a suitable habitat for the proliferation of microorganisms (see Chapter 1). The most quantitatively important and likely electron acceptors available to the resident microflora in such marine habitats are oxygen and sulfate.[42] Chapter 1 of this thesis focused on the advanced stages of sand patty weathering as influenced by photooxidation and aerobic biodegradation. This appendix provides a brief description of how anaerobic microorganisms transform the same dissolved organic matter (DOM) fractions released from sand patties derived from the Deepwater Horizon oil spill. Two separate experiments using different assay procedures confirm that the DOM released by sand

patties tend to resist decomposition under sulfate reducing conditions. This finding contrasted with the greater propensity for aerobic decomposition of the DOM fractions that were documented using the same inoculum under aerobic conditions (Chapter 1). Thus, while the susceptibility of sand patty DOM to anaerobic biodegradation is limited when using a shallow coastal marine sedimentary microbial community as an inoculum, it remains an open question whether such information can be extrapolated to other anaerobic environments.

Materials and Methods

Sample Collection and Anion Analysis

Sand patties, sediments, and seawater were collected from the swash zone of beaches in Gulf Shores and Fort Morgan, Alabama as described in Chapter 1. Anion analysis (Cl^-, NO_3^-, and SO_4^{2-}) of the aqueous samples was performed with a Dionex Series 3000 Ion Chromatography System as previously described (Thermo Fisher, Waltham, MA).[28]

Generation of Sand Patty-Derived Dissolved Organic Matter

Sand patties were irradiated in sterile seawater via the use of a solar simulator to generate photooxidized water-soluble organic matter as described in Chapter 1. Irradiation and water replacement was repeated for up to 84 hours. Sand patties incubated in sterile seawater and kept in the dark served as controls.

Biodegradation of Sand Patty Organic Matter

The biodegradation of sand patty-derived and endogenous DOM was compared using surficial coastal marine sediment collected from the swash zone of a northern Gulf of Mexico beach as an inoculum. Initially, evidence for biodegradation included monitoring sulfate disappearance in incubations amended with the various DOM fractions relative to endogenous, and appropriate positive (lactate-amended; 25 mM) and negative (autoclaved) controls. As this procedure may have been too insensitive to detect biodegradation, subsequent experiments monitored the rate of ^{35}S-sulfide formation in the same incubations at various time intervals and adding $^{35}SO_4^{-2}$ as a sensitive radiotracer. Seawater and sediment mixtures were flushed with N_2:CO_2 (80:20) prior to being used as inoculum for all experimental conditions. The endogenous incubation received the sediment inoculum and 10 mL of filter-sterilized seawater. In other incubations, the seawater was replaced with the same volume of sand patty-derived DOM from either the irradiation procedure or dark control as described in Chapter 1. Sulfate depletion was compared to triplicate incubations as well as to sterile negative controls that were autoclaved (20 min). At each time point, a set of replicates was sacrificed and a radioactive sulfate reduction assay was performed in which approximately 50 nCI $^{35}SO_4^{-2}$ was added to each of the bottles. These bottles were extracted and radioactivity was counted as described in Chapter 1.

Results

A sediment inoculum from the GoM was utilized to determine if the endogenous microflora was capable of metabolizing either whole sand patties or sand patty-derived DOM produced under irradiated or dark conditions. The first such determination contained a GoM sediment inoculum that was amended with filter sterilized seawater and 10 g of sand patty material (Figure A1). The loss of sulfate from these incubations was marginal from all incubations except the replicates amended with lactate as a positive control substrate. In lactate-amended incubations, the amount of sulfate utilized represented approximately 60% of the amount necessary to fully mineralize the amended lactate. After 300 d, the amount of sulfate utilized in incubations amended with whole sand patties was slightly increased relative to the other treatments. This trend continued with time, but only approximately 10% of the available sulfate pool was consumed in sand patty-amended incubations over the course of the next 200 days (Fig. A1).

Such a small amount of sulfate utilization suggested that the sand patties tended to resist transformation by anaerobic microorganisms. Presuming biodegradation was slow at best, monitoring losses in seawater sulfate concentrations against the large background level of this anion may render this assay too insensitive to detect the likely subtle rates of microbial metabolism. Thus, the aforementioned experiment was repeated with whole sand patty material as well as the various DOM fractions identified in Chapter 1 as potential electron donors. However, in this follow up experiment, a more sensitive radioactivity assay of the rate of ^{35}S-sulfide formation was used to monitor microbial respiratory activity in conjunction with sulfate depletion.

No substantive increase in the rate of sulfate depletion against the large background of this anion in the seawater was observed for any of the electron donor treatments, with the exception of the positive control incubation (Figure A2). This essentially mirrors the observations made previously with this approach.

However, when the rates of sulfide formation were assayed in the same incubations upon periodic amendment with a radiotracer ($^{35}SO_4^{-2}$), sulfate reduction rates increased relative to the sterile controls in all samples over the first 42 days of incubation. The lactate-amended positive control treatment exhibited the largest rates of microbial respiration that averaged 0.167 μmol S□mL^{-1} □day^{-1} over the first 56 d of incubation and declined thereafter (Fig. A2). This rate of sulfate reduction, if extended over the entire 216 d incubation period, could account for about 12 mM sulfate depletion. However, the rate decreased over time (Fig. A2) and correspondingly less sulfate removal was evident in the positive control incubation. The whole sand patty amended incubations as well as the treatments that received sand patty-derived DOM did show increases the rate of sulfide formation over sterile controls (Fig. 2A). However, the rates observed were not significantly different that the rates measured with the endogenous forms DOM. Thus, this experiment confirmed the sand patties themselves, as well as the sand patty derived DOM, tended to resist anaerobic decomposition under sulfate reducing conditions.

Discussion

The weathering of the Deepwater Horizon oil resulted in the formation and deposition of sand patties made up mostly of chemical constituents that have been termed 'oxyhydrocarbons'.[15,18,20,23] The environmental fate of the sand patties was explored and documented in Chapter 1. It was found that photooxidation and aerobic biodegradation were significant advanced weathering processes impacting the stability of these structures. There is also no doubt that sand patties can also be buried in sediments where anaerobic conditions might prevail.[16,18] Given this prospect, an assessment of the potential for anaerobic biodegradation of sand patties and sand patty-derived organic material under sulfate reducing conditions was also conducted. More specifically, the fate of the sand patty-derived organic carbon was evaluated under sulfate reducing conditions by monitoring both sulfate depletion and sulfide formation in separate experiments. In both cases, the ability of whole sand patties or sand patty-derived organic material to serve as suitable electron donors for the indigenous anaerobic microflora was marginal at best. In contrast, the same forms of organic matter significantly stimulated microbial respiratory processes when oxygen was available as an electron acceptor (Chapter 1).

It is important to recognize the interpretational limits of these findings. While tempting to conclude that sand patty-derived organic matter is recalcitrant under anaerobic conditions, the only source of inoculum evaluated in this study was a shallow surficial sediment in the northern Gulf of Mexico. These sediments likely represent a very oxidizing habitat given the constant impact of wind, wave and tidal actions. Weathered residual oil fractions, including sand patties, oil mats, and marine snow are

also known to be deposited in deeper sedimentary regions of the Gulf of Mexico where oxygen is essentially unavailable past the first few centimeters.[57] There is no *a priori* reason to suspect that the anaerobic metabolic potential available in other regions of the Gulf are necessarily as restricted as the marine coastal areas that were the source of inoculum used in these experiments. That is, the lessons learned relative to the toxicological impact of sand patty-derived organic matter and to the susceptibility of such highly weathered residual oil material to anaerobic biodegradation should not be extrapolated broadly.

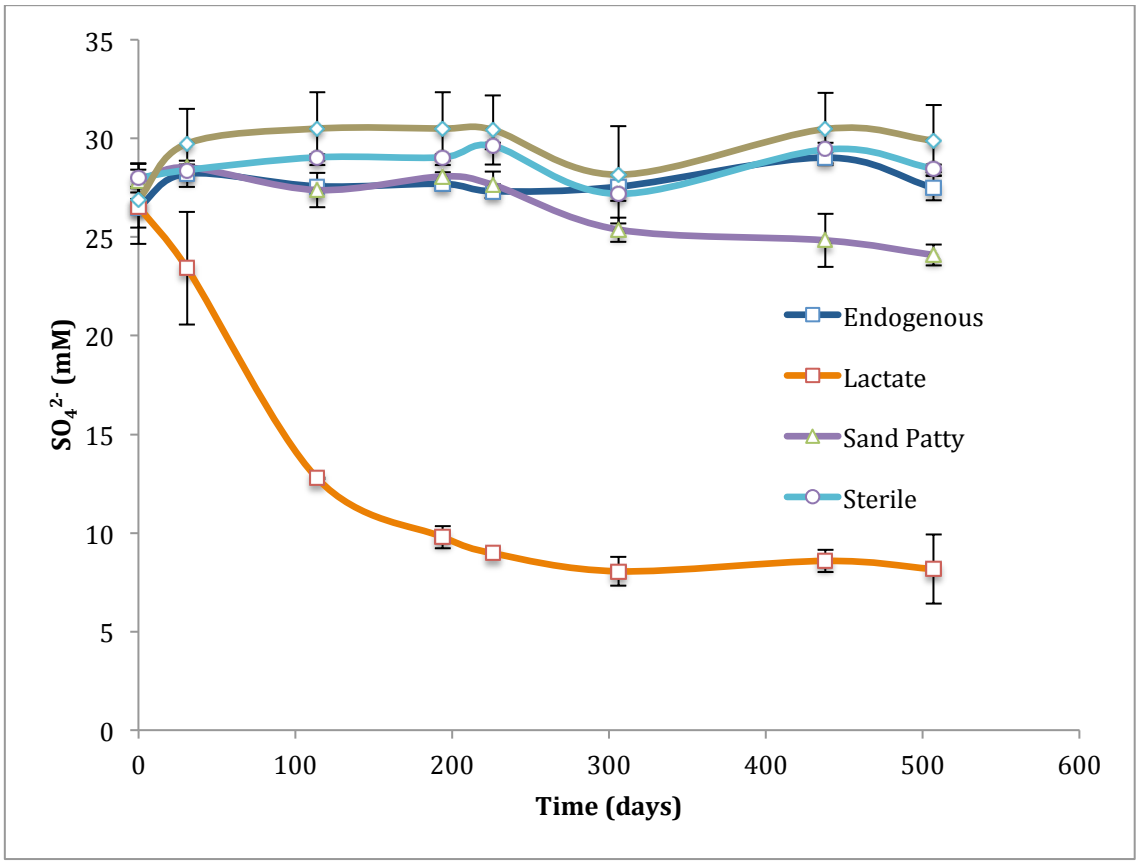


Figure A1. Total amount of SO_4^{2-} reduced over the course of 507 days in anaerobic GoM sediment incubations. Standard deviations for triplicate measurements are indicated.

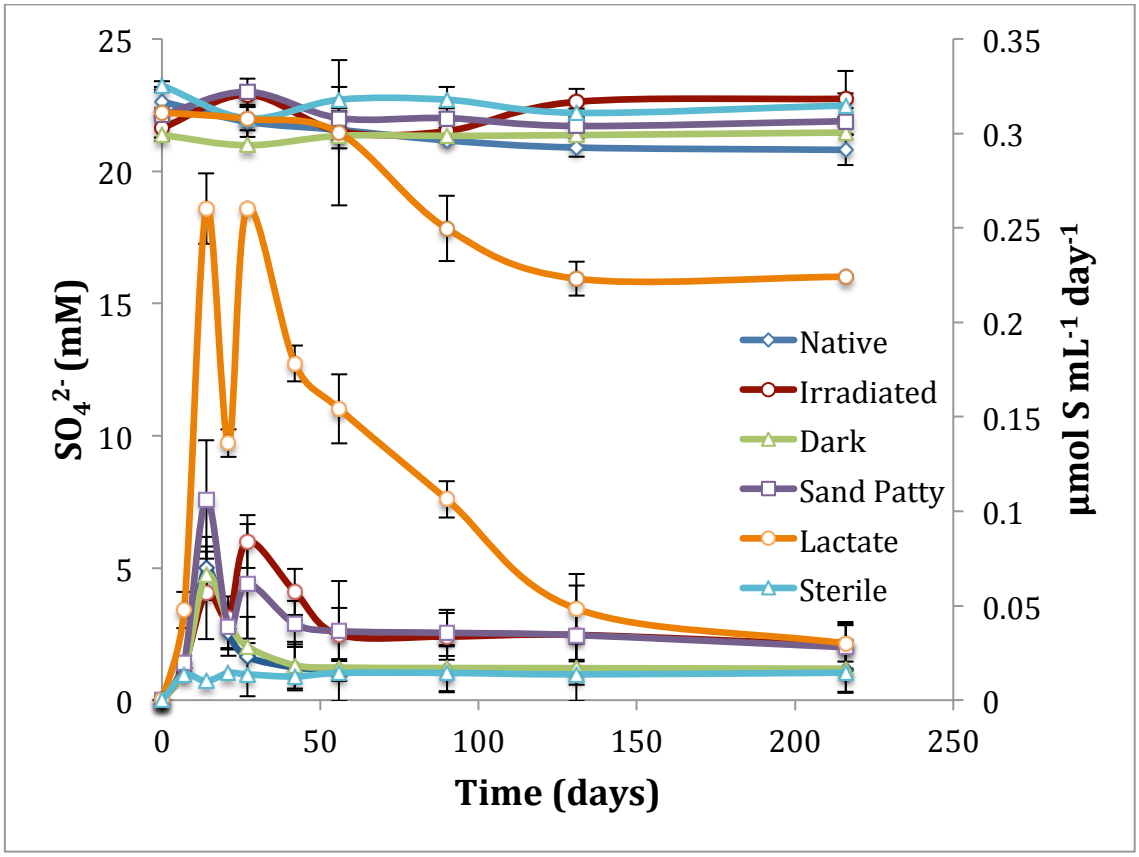


Figure A2. Total amount of SO_4 reduced and the associated sulfate reduction rate as measured by $^{35}SO_4^{2-}$ tracer. Standard deviations for triplicate measurements are indicated.