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Comprehensive Analysis of Changes in Crude Oil Chemical Composition during Biosouring and Treatments

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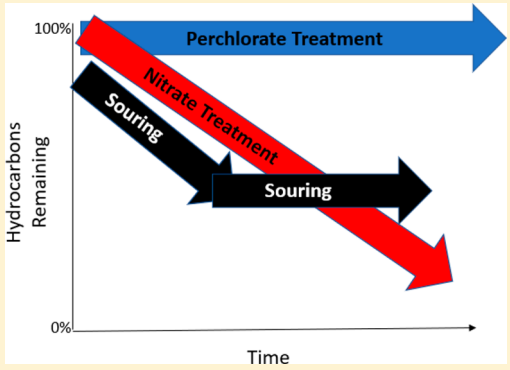
Supporting Information

ABSTRACT: Biosouring in crude oil reservoirs by sulfate-reducing microbial communities (SRCs) results in hydrogen sulfide production, precipitation of metal sulfide complexes, increased industrial costs of petroleum production, and exposure issues for personnel. Potential treatment strategies include nitrate or perchlorate injections into reservoirs. Gas chromatography with vacuum ultraviolet ionization and high-resolution time-of-flight mass spectrometry (GC-VUV-HTOF) and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) combined with electrospray ionization were applied in this study to identify hydrocarbon degradation patterns and product formations in crude oil samples from biosoured, nitrate-treated, and perchlorate-treated bioreactor column experiments. Crude oil hydrocarbons were selectively transformed based on molecular weight and compound class in the biosouring control environment. Both the nitrate and the perchlorate treatments significantly reduced sulfide production; however, the nitrate treatment enhanced crude oil biotransformation, while the perchlorate treatment inhibited crude oil biotransformation. Nitrogen- and oxygen-containing biodegradation products, particularly with chemical formulas consistent with monocarboxylic and dicarboxylic acids containing 10−60 carbon atoms, were observed in the oil samples from both the souring control and the nitrate-treated columns but were not observed in the oil samples from the perchlorate-treated column. These results demonstrate that hydrocarbon degradation and product formation of crude oil can span hydrocarbon isomers and molecular weights up to C_{60} and double-bond equivalent classes ranging from straight-chain alkanes to polycyclic aromatic hydrocarbons. Our results also strongly suggest that perchlorate injections may provide a preferred strategy to treat biosouring through inhibition of biotransformation.



INTRODUCTION

Secondary recovery of crude oil from an offshore reservoir is an enhanced oil recovery process which involves the injection of pressurized seawater and produced water into the reservoir's wellhead in order to displace oil toward a production well.[1] This industrial process increases the crude oil production lifetime of the offshore reservoir compared to that of a reservoir solely relying on natural subterranean pressure for oil recovery.[2,3] The seawater lowers the temperature of the oil reservoir in the area near the injection well. This decrease in temperature creates an optimal environment for the growth of mesophilic microbes such as sulfate-reducing microbial communities (SRCs).[4,5] These microbial communities in the injected seawater initiate biosouring, a metabolic process which reduces sulfate (SO_4^{2-}) to sulfide (S^{2-}) to form hydrogen sulfide (H_2S).[5] This compound is explosive, flammable, toxic, and potentially hazardous to personnel working near the reservoir.[6,7] Elevated sulfide concentrations initiated by sulfidogenesis lead to the precipitation of metal sulfide complexes that corrode pipelines and oil−water separators.[6,7] These precipitated complexes can often lead to facility failure with large associated costs.[7]

Strategies for controlling biosouring have included the applications of sulfate analogs and biocides.[8,9] These treatments have seen limited success due to their costs and restricted efficiencies, as they only inhibit specific strains of SRCs.[2,8,9] The most established current approach for souring control is the injection of nitrate (NO_3^-) anions into a crude oil reservoir. These anions serve as alternate electron acceptors for microbial communities in place of sulfate,[1,10] and the metabolic pathways of nitrate-reducing bacteria (NRB) are thermodynamically

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favorable when compared to the pathways of analogous sulfate reduction.[11] While nitrate injections have been tested in both laboratory[11,12] and field studies,[10] the overall effectiveness is poorly understood. As the concentration of nitrate depletes near the injection well of the reservoir, sulfate reduction may still be active deeper in the oil field.[10,13] Nitrite, a metabolic intermediate of nitrate reduction, can be toxic to SRCs but is also chemically and biologically labile and has a reduced half-life in a reservoir matrix.[13,14] These limitations require large nitrate injection concentrations to prevent depletion and to maximize nitrite production, a requirement which may not be economically or logistically feasible in a crude oil reservoir.

Perchlorate injections have recently been reported as an effective solution for souring control.[10,15] Like nitrate reduction, perchlorate reduction is thermodynamically favorable relative to sulfate reduction (E^o = +797 mV for ClO_4^-/Cl^- and E^o = −217 mV for SO_4^{2-}/S^{2-}). Previous studies have demonstrated that perchlorate is specifically inhibitory to sulfate reduction[16] and that dissimilatory perchlorate-reducing bacteria (DPRB) can outcompete SRCs to directly oxidize sulfide to sulfur as a metabolic end product.[17,18]

The importance of microbial biotransformation of crude oil hydrocarbons has been increasingly realized in the context of industrial petroleum production,[19] environmental oil spills,[20] and enhanced oil recovery from subsurface oil reservoirs.[21] Evidence of hydrocarbon biodegradation has previously been observed in metabolite profiling of subsurface oil reservoirs,[21] incubation studies of crude oil with known microbial inocula,[22] and gasoline amended aquifers.[23] Previous studies have investigated thermochemical sulfate reduction coupled to transformation of Gulf coast oils.[24] Microbiological sulfate, nitrate, and perchlorate reduction in conjunction with crude oil biotransformation remain poorly understood. Furthermore, biodegradation studies typically incorporate select compounds to serve as biotransformation markers, such as C_4−C_{10} straight-chain alkanes and monoaromatic compounds,[21−23] rather than tracking complete suites of hydrocarbon isomers of varying chemical sizes or compound classes. In this study, we apply comprehensive chemical analyses to provide insight into crude oil biotransformation, including hydrocarbon degradation and product formation, to understand the impact of biosouring and its potential treatments on crude oil's chemical composition.

The traditional gas chromatographic (GC) techniques used in crude oil analysis typically couple a volatility separation of the oil's components with a flame ionization detector (FID) or electronic ionization mass spectrometer (EI MS).[25,26] Due to crude oil's chemical complexity, much of the organic mass coelutes as an unresolved complex mixture (UCM),[27] a phenomenon which complicates mass spectra interpretation and compound identification. This study incorporates two complementary techniques to resolve UCM and understand biotransformation of soured, nitrate-treated, and perchlorate-treated crude oil from a 70 day experiment representative of the in situ crude oil reservoir. Gas chromatography combined with vacuum ultraviolet ionization and time-of-flight mass spectrometry (GC-VUV-HTOF) can comprehensively summarize the chemical composition of hydrocarbon isomers of complex organic mixtures, such as crude oil, up to the molecular volatility limit of the GC columns (~320 °C).[28] Ultra-high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) combined with atmospheric pressure ionization (such as electrospray ionization or atmospheric pressure photoionization, ESI and APPI, respectively) can selectively characterize polar and nonpolar crude oil components without boiling point limitations.[29−31] This technique routinely achieves resolving power sufficient to separate compounds that differ in mass by less than the mass of an electron in a single mass spectrum ($m/\Delta m_{50\%} \approx 1\,000\,000$, in which $\Delta m_{50\%}$ is the mass spectral peak width at half-maximum peak height at m/z 500).[32−34] Ultra-high-resolution FT-ICR MS can be used to identify compositional changes to biotransformed crude oil through comparison of molecular differences in oil samples from different stages of souring or treatment. Details of the GC-VUV-HTOF and FT-ICR MS techniques are described in the Supporting Information.

MATERIALS AND METHODS

Experimental Protocol. Bioreactor columns were established as described in detail in Shrestha et al.[35] Briefly, custom-designed 1 m × 10 cm glass columns were packed with a slurry of ~6 kg of pure sand (Iota Quartz, New Canaan, CT, USA) and 4.5 L of North Sea crude oil. The columns were maintained at 40 °C, mimicking the temperature of an offshore oil reservoir. Seawater and produced water, obtained directly from a North Sea oil field in a 60:40 ratio, were injected through the columns at 189 μL/min to create a 2-week residence time, mimicking the residence time of the seawater injected into an offshore oil reservoir. A volatile fatty acid compound mixture based on the empirically determined content of the North Sea oil reservoir's produced waters (acetate 565 μM; butyrate 19 μM; propionate 23 μM; formate 74 μM) was continuously added to all of the columns. As the experiment progressed, >70% of the initial oil in all of the columns was washed out by the bioreactor flow system. The observed transformations represent those of the residual oil rather than the initial bulk oil.

Hydrogen sulfide was measured as a proxy for SRC activity. When the columns were soured and the sulfide concentrations were stable (>0.68 mM), pulsed doses of nitrate (weekly doses of ~17.5 mM) and perchlorate (weekly doses of ~17 mM) began in separate duplicate columns. One column was left untreated to maintain the biosouring environment as a control column. Column liquid containing crude oil was extracted from a port on each column using a 1 mL glass syringe (Hamilton, Reno, NV, USA). The liquid was then placed into a vial containing 1 mL of chloroform (HPLC grade, Sigma-Aldrich, St. Louis, MO, USA). Oil samples from the nitrate- and perchlorate-treated columns were extracted at days 0, 21, 42, and 70 of treatment. Control oil samples from the biosouring column were extracted at the corresponding days 0, 28, 43, and 70. Water samples were continuously extracted from the columns for sulfide analysis. The Cline assay was used to determine sulfide concentrations in these samples.[36]

Oil samples were analyzed via GC-VUV-HTOF (Figure S1), which extends the analytical capabilities of GC-MS and two-dimensional gas chromatography−mass spectrometry (GC×GC-MS) to allow a complete characterization of C_9−C_{30} crude oil hydrocarbon isomers as a function of both carbon number and structural class. Oil was directly injected via a septumless head into a liquid nitrogen-cooled inlet (−40 °C) on a fused silica liner (CIS4, Gerstel Inc., Linthicum, MD, USA). The sample was transferred to the gas chromatograph (GC, Agilent 7890, Santa Clara, CA, USA) by heating the liner to 320 °C at 10 °C/s. The chemical components of the oil (hereafter referred to as "analyte") were separated based on volatility using a nonpolar column (60 m × 0.25 mm × 0.25 μm Rxi-5Sil-MS; Restek, Bellefonte, PA, USA) with a helium

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gas flow rate of 2 mL/min. The GC program began at 40 °C, and the ramp rate was 3.5 °C/min. The program ended at a maximum temperature of 320 °C with a 10 min final hold time. The analyte was transferred from the GC to a 170 °C ion source via a 270 °C transfer line and ionized with 10.5 ± 0.2 eV VUV photoionization. The ions were detected using a high-resolution ($m/\Delta m_{50\%} \approx 4000$) time-of-flight mass spectrometer (HTOF, Tofwerk, Thun, Switzerland). The VUV beam was from the Chemical Dynamics Beamline 9.0.2 of the Advanced Light Source at the Lawrence Berkeley National Laboratory. An ionization energy of 10.5 eV was chosen to detect all parent ions and to minimize contributions from fragment ions that could create UCM. Mass spectra were collected at 100 Hz and then averaged to 0.5 Hz to deconvolute mass spectral peaks in high-resolution postprocessing peak fitting. High-mass resolution data processing was done using custom code written in Igor 6.3.7 (Wavemetrics).[37] Measurement calibrations followed previously established methods described in Chan et al.[38] and Worton et al.[37] These methods are described in detail in the Supporting Information.

Ultra-high-resolution FT-ICR MS analysis was done at the National High Magnetic Field Laboratory at Florida State University to expand the analytical window beyond those hydrocarbons measurable using GC-VUV-HTOF in order to observe compounds > C_{30} and to identify heteroatom-containing chemical formulas.[39] All solvents used in this study were HPLC grade (Sigma-Aldrich, St Louis, MO, USA). During sample preparation, 1 g of oil was diluted with 1 mL of toluene that was further diluted with equal parts methanol spiked with either 0.25% by volume tetramethylammonium hydroxide (TMAH) for negative electrospray ionization (-ESI) FT-ICR MS[40] or 8% by volume formic acid for positive electrospray ionization (+ESI) FT-ICR MS analysis.[41]

Positive ion electrospray ionization selectively ionizes basic compounds through protonation via formic acid to form quasimolecular ions $[M + H]^+$.[41] Negative ion electrospray ionization selectively ionizes acidic species through deprotonation via TMAH to form quasimolecular ions $[M - H]^-$.[40] When combined with ultra-high-resolution FT-ICR MS and Kendrick mass sorting,[31,33] these ionization techniques provide subppm mass accuracy necessary for elemental composition assignments to polar compounds in transformed crude oil.[42] Molecular formulas are grouped by heteroatom class (species with the same $C_cH_hN_nO_oS_s$ elemental composition differing only by the degree of alkylation) based on relative abundance in the mass spectrum in order to identify products in biotransformed crude oil when compared to the parent crude oil. Details regarding the ionization sources, FT-ICR MS instrumentation, broad-band phase correction, and frequency-to-mass conversion are provided in the Supporting Information.

RESULTS AND DISCUSSION

Crude Oil Chemical Composition and Sulfide Production. The distribution of the parent crude oil's GC-amendable measured hydrocarbon mass fraction (milligrams per kilogram injected oil) as a function of both carbon number and double-bond equivalent (N_{DBE})[43] is shown in Figure 1a, where N_{DBE} for a pure hydrocarbon is determined by eq 1

$$N_{DBE}(C_xH_y) = X - Y/2 + 1 \quad (1)$$

When using N_{DBE} to classify crude oil hydrocarbons, N_{DBE} = 0 represents normal and branched alkanes, N_{DBE} = 1 represents

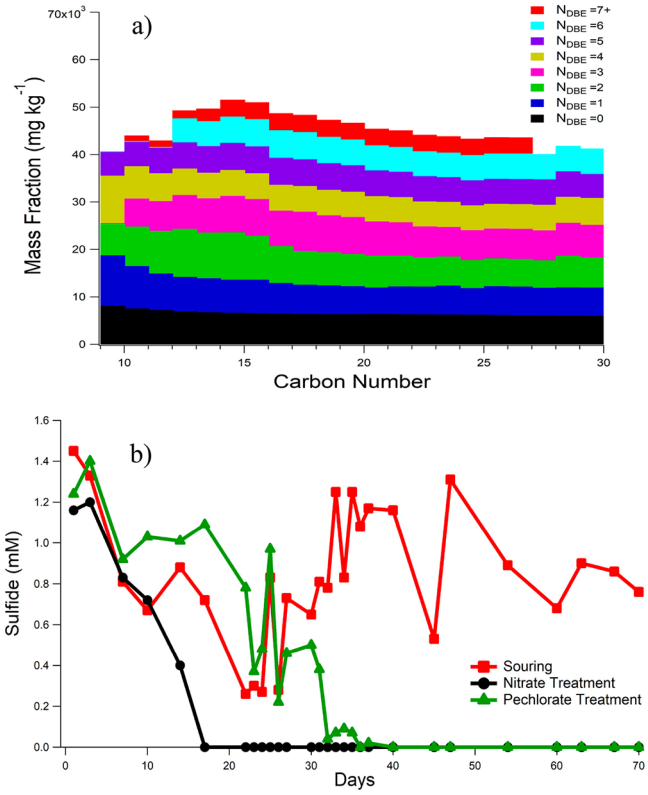

Figure 1. (a) Chemical composition of the original parent North Sea crude oil as a function of both carbon number (C_9–C_{30}) and double-bond equivalent (N_{DBE}) chemical class. (b) Sulfide concentrations as a function of time in the souring control column, nitrate-treated column, and perchlorate-treated column.

monocycloalkanes, N_{DBE} = 4 represents monoaromatic compounds and four alkyl ringed steranes, and N_{DBE} = 7 represents polycyclic aromatic hydrocarbons (PAHs). Crude oil contains little to no olefinic compounds due to the compounds' instability and reactivity.[44,45] Crude oil N_{DBE} hydrocarbon classes can therefore be assumed to represent the number of cyclic rings and aromaticity rather than the number of nonaromatic double bonds.

A ~50% split of the mass fractions in the parent crude oil between GC-amendable C_9–C_{19} and C_{20}–C_{30} species is illustrated in Figure 1a. Approximately 60% of the total measured hydrocarbon mass fraction represents purely aliphatic compound classes (N_{DBE} = 0–3), and 40% represents classes with a $N_{DBE} \geq 4$ that can contain aromatic rings.

The differences in sulfide production among the untreated souring control column, the nitrate-treated column, and the perchlorate-treated column are illustrated in Figure 1b. Sulfide concentrations in the biosouring control column fluctuated between 0.3 and 1.4 mM over the 70-day experiment. These levels were indicative of sulfate reduction by SRCs throughout the experiment. After 18 days of nitrate treatment and 40 days of perchlorate treatment in the respective treated columns, sulfide concentrations dropped to 0 mM, indicating effective biosouring control. The decrease in sulfide concentrations correlated with an increase in sulfate concentrations in the treated columns. The companion manuscript Shrestha et al.[35] contains further details on sulfide and sulfate temporal concentration changes.

Oil samples were analyzed by GC-VUV-HTOF to document the transformation of crude oil hydrocarbons in all three

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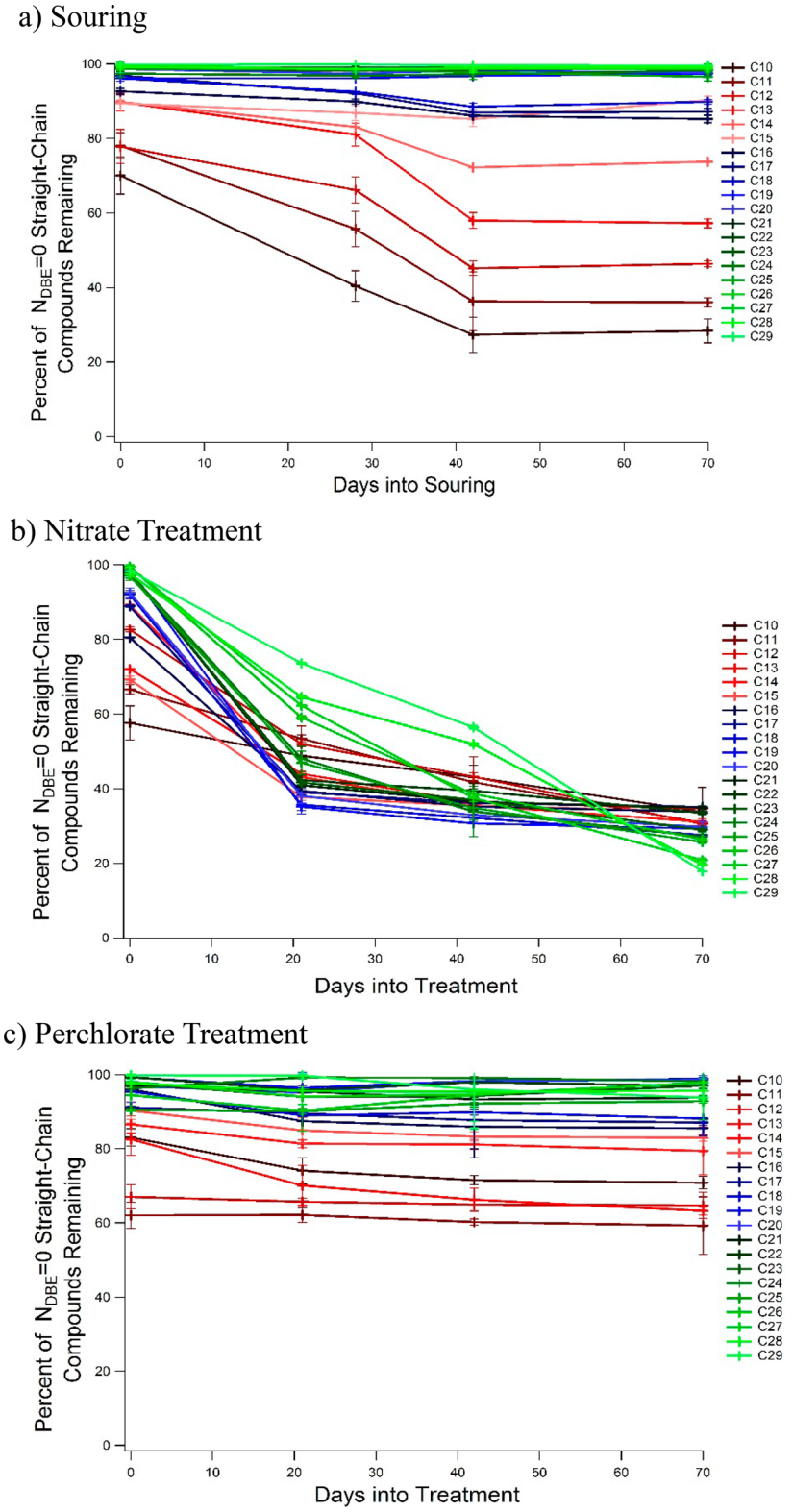

Figure 2. Transformations of straight-chain alkanes (N_{DBE} = 0) as a function of time in oils from the (a) souring control, (b) nitrate-treated, and (c) perchlorate-treated columns, measured by GC-VUV-HTOF. Bars indicate minimum and maximum percentages (n = 2).

columns as a function of time. All hydrocarbon concentrations reported were normalized to a $C_{30}H_{52}$ hopane recalcitrant biomarker.[46] We compared the normalized concentration of each compound in the biodegraded oil, based on carbon number and N_{DBE}, to its original concentration in the parent crude oil to determine the percent of the compound remaining in the transformed crude oil. Day zero in all timelines represents the time point when the biosouring treatments began in the soured columns.

Straight-Chain Alkane Transformations. The patterns of straight-chain alkane (N_{DBE} = 0) biotransformations are different among oil samples from the biosouring control,

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nitrate-treated, and perchlorate-treated columns, implying that biotransformation patterns of the crude oil's chemical composition change if the microbial environment is sulfate reducing, nitrate reducing, or perchlorate reducing. Straight-chain alkanes, ranging in size from C_{10} to C_{14}, in all oil samples from the three columns were degraded to ~60−80% of the initial parent oil concentrations prior to the beginning of treatment at day 0. In the oil samples from the biosouring control column, the C_{10}−C_{14} straight-chain alkanes were transformed to ~25−70% of the parent oil concentrations in the first 43 days of the experiment but remained recalcitrant for the final 27 days (Figure 2a). This incomplete transformation of straight-chain alkanes in a sulfate-reducing environment has previously been observed in long-term microbial incubation studies with crude oil.[47] Straight-chain alkanes ranging from C_{15} to C_{18} in the oil from the souring control column were reduced to ~85−90% of the original concentrations after 70 days, and C_{19}−C_{29} straight-chain alkanes underwent minimal (less than 5%) transformation during souring. Previous studies have similarly shown crude oil degrading microbial communities transforming straight-chain C_4−C_{15} alkanes before degrading larger C_{16}−C_{30} alkanes under sulfate-reducing conditions, in agreement with our findings.[47,48]

The introduction of nitrate and perchlorate into the soured column led to large changes in crude oil biotransformations when compared to the transformations of the hydrocarbons in the souring control oil. Nitrate introduction led to a rapid increase in C_{12}−C_{29} straight-chain alkane transformations in the oil, without dependence on molecular weight. After 21 days, these compounds were depleted to ~40−60% of the original parent oil concentrations (Figure 2b). At the end of the 70-day experiment, all straight-chain alkanes in the oil samples from the nitrate-treated column were transformed to ~30−40% of the original concentrations in the parent crude oil. Introduction of perchlorate into the soured column rapidly inhibited the transformation of straight-chain alkanes in the oil (Figure 2c). Over the complete 70-day perchlorate treatment, straight-chain alkanes in the oil were transformed <10% when compared to the concentrations in the parent crude oil. The lack of straight-chain alkane transformations in the presence of perchlorate suggests that DPRB were unable to utilize crude oil as an electron donor or carbon source to drive metabolism.

Metabolic intermediates such as nitrite and chlorite were detected in the treatment environments, providing evidence that the anaerobic processes of nitrate and perchlorate reduction by microbial communities occurred. Furthermore, total microbial community analysis revealed an increase in the relative abundances of functional NRB and DPRB communities in the respective columns upon introduction of nitrate or perchlorate as the electron acceptor. The microbiological evidence further explained in Shrestha et al.[35] corroborates the biological origin of the crude oil hydrocarbon transformation products observed in this study, implying that these differences in hydrocarbon transformation patterns stem from differences in the relative abundances of microbial communities, their activities, and the availability of a dominant terminal electron acceptor.

Biotransformations of Monocycloalkanes. Transformations of isomerically summed monocycloalkanes in oil samples from the untreated biosouring control, nitrate-treated, and perchlorate-treated columns are shown in Figure S2. The C_{10}−C_{12} monocycloalkane concentrations in oil samples from the three columns were reduced to ~45−75% of the initial monocycloalkane concentrations in the parent crude oil at day 0, before biosouring treatment began. This depletion is similar to the depletions in the straight-chain alkanes observed during the same time period. After 43 days of souring, the C_{10}−C_{19} crude oil monocycloalkanes in the control column were transformed to ~25−85% of the original parent oil concentrations. These same compounds were only transformed an additional ~1−10% between 43 and 70 days. In contrast, the C_{20}−C_{29} monocycloalkanes in oil samples from the biosouring control column underwent little to no change in concentration throughout the timeline. Our observed hydrocarbon transformation pattern agrees with earlier studies, suggesting that microbial communities transform C_{10}−C_{15} aliphatic compounds (including straight-chain alkanes and monocycloalkanes) before transforming C_{20}−C_{30} aliphatic compounds.[22,47]

Transformations of isomerically summed monocycloalkanes in oil samples from the nitrate- and perchlorate-treated columns exhibited similar patterns to those of the straight-chain alkane transformations in each respective treatment. There was a ~40−60% reduction in the concentration of monocycloalkanes, independent of molecular weight, in oil samples from the nitrate-treated column when compared to the monocycloalkane concentrations in the parent crude oil. The C_{10}−C_{29} monocycloalkanes in oil samples from the perchlorate-treated column underwent a <10% change in concentration throughout the experiment. The similarities between the biotransformation patterns of both monocycloalkanes and straight-chain alkanes in oil samples from the nitrate- and perchlorate-treated columns support the conclusion that nitrate enhances crude oil hydrocarbon biotransformation while perchlorate inhibits biotransformation. The microbiological evidence illustrating the abundances of NRB and DPRB in the respective treatments, confirming the biological differences in environments with a different dominant electron acceptor, and mechanistic interpretations of hydrocarbon degradation are discussed in further detail in Shrestha et al.[35] The microbiological results from 16S rRNA sequencing suggest that the DPRB found in the columns are not hydrocarbon degraders; therefore, their presence and activity do not result in crude oil biotransformations.[35] As these microbial communities are not known to degrade crude oil hydrocarbons in order to drive their metabolic processes, it is unlikely that the DPRB communities would have adapted to transform these classes of hydrocarbons had the duration of the experiments been extended.

Biotransformations of Monoaromatic Compounds. The N_{DBE} = 4 compound class consists of monoaromatic species and steranes with four aliphatic rings. Transformation of toluene and ethylbenzene by different pure culture isolates of sulfate-reducing microorganisms has been well documented.[49,50] Here we utilize GC-VUV-HTOF data to quantify how the isomeric sum of all C_{10}−C_{29} monoaromatic compounds and steranes is transformed as a function of time, going beyond previous studies which focused on transformations of a limited number of aromatic species.[49,50] Transformations of monoaromatic species in oil samples from the biosouring control, nitrate-treated, and perchlorate-treated columns are illustrated in Figure 3. In all oil samples from the three columns, there was a ~20−40% reduction in concentration of the C_{10}−C_{11} monoaromatic species when compared to the parent oil concentrations at day 0, similar to the depletions observed in the N_{DBE} = 0 and 1 compound classes prior to treatment. In oil samples from the biosouring control

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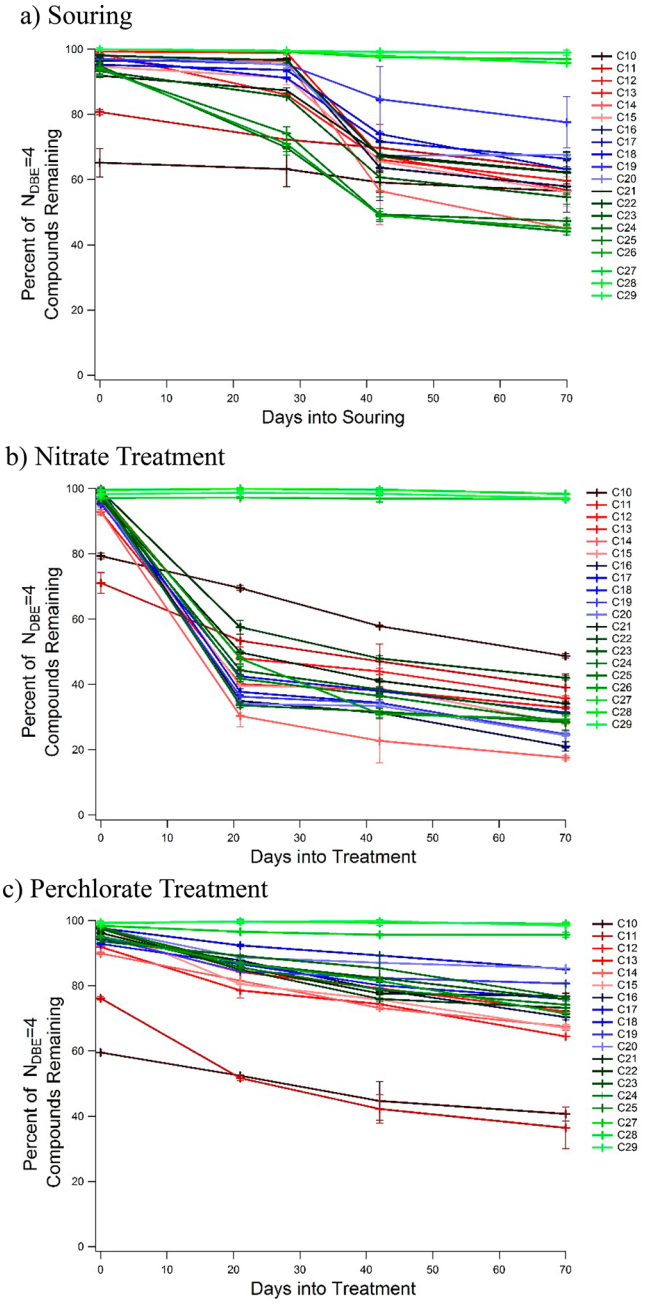

Figure 3. Transformations of isomerically summed monoaromatic compounds (N_{DBE} = 4) as a function of time in oils from the (a) souring control, (b) nitrate-treated, and (c) perchlorate-treated columns, measured by GC-VUV-HTOF. Bars indicate minimum and maximum percentages (n = 2).

column, C_{24}−C_{26} monoaromatic species were reduced to ∼70−75% of the original parent oil concentrations after 28 days (Figure 3a), while the concentrations of C_{10}−C_{23} monoaromatic compounds underwent minimal (<10%) change compared to the parent crude oil concentrations during the same time span. Between 28 and 43 days of souring, C_{10}−C_{23} monoaromatic species in oil samples from the control column were depleted to ∼60−85% of the initial parent oil concentrations at a transformation rate ∼10-fold faster than that of the same compounds between 0 and 28 days. Larger C_{24}−C_{26} monoaromatic species continued to be transformed to ∼50−60% of the parent crude oil concentrations after 43 days of souring at a rate ∼1.5-fold faster than that of the same compounds between 0 and 28 days. The C_{10}−C_{23} monoaromatic species were depleted an additional ∼10% during the final 27 days of souring, but the C_{24}−C_{26} monoaromatic species were only depleted an additional ∼5% during the same time span. The C_{27}−C_{29} steranes in oil samples from the control column underwent minimal transformation throughout the entirety of the biosouring experiment and are considered to be recalcitrant markers.[46]

The transformation patterns of monoaromatic compounds in oil samples from the biosouring control column differed from those patterns observed in the N_{DBE} = 0 and 1 compound classes. As the amount of available aliphatic hydrocarbons to serve as electron donors and carbon sources decreased after 21 days of souring, the SRCs transformed more complex aromatic material. The total microbial community analysis, provided in further detail in Shrestha et al.,[35] revealed changes in the relative abundances of SRCs that aligned with changes in the hydrocarbon transformation patterns in the biosouring oil. Aliphatic hydrocarbon-degrading SRCs dominated the microbial communities in the first 21 days of souring in the control column. As souring continued and sulfide production increased (Figure 1), the relative abundance of monoaromatic hydrocarbon-degrading SRCs increased. This shift in community abundance coincided with a decrease in aliphatic hydrocarbon degradation and an increase in monoaromatic hydrocarbon degradation as souring progressed. The microbial community analysis confirms that the relative abundances of microbial communities is a driving factor in hydrocarbon degradation during biosouring.

Monoaromatic compound transformation patterns in oil samples from the nitrate- and perchlorate-treated columns are illustrated in Figure 3b and 3c, respectively. These transformations follow a similar pattern to that of aliphatic transformations in each respective treatment. As the nitrate treatment began, C_{12}−C_{26} monoaromatic compounds in oil samples were depleted to ∼40−60% of the original parent oil concentrations after 21 days. These transformations continued as the treatment progressed, resulting in monoaromatic hydrocarbon concentrations ∼30−50% of the original parent oil concentrations. Monoaromatic species in oil samples from the perchlorate-treated column were only depleted to ∼10−20% of the parent oil concentrations after 70 days, further supporting the conclusion that perchlorate injections hinder biotransformation of crude oil.

Biotransformations of Polycyclic Aromatic Hydrocarbons. The C_{10}−C_{26} PAHs in oil samples from the souring control column were depleted to ∼85−95% of the parent oil concentrations in the first 28 days, ∼65−90% after 43 days, and ∼60−85% after 70 days, as shown in Figure S3. Transformation of monoaromatic species in oil samples from the souring control column was ∼1.5 times greater than transformation of PAHs of the same carbon number in the souring control column over the experimental timeline, implying that SRCs favor monoaromatic compound degradation over PAH degradation. These PAHs in oil samples from the nitrate-treated column were depleted to ∼40−60% of the parent oil concentrations without any preference based on molecular weight, while PAHs in oil samples from the perchlorate-treated column were depleted to ∼10−20% of the parent oil concentrations.

This study describes those crude oil hydrocarbons susceptible to microbial degradation by illustrating the

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transformation of complete chemical classes of labile crude oil components. Rather than focusing only on specific compounds for biodegradation, such as cyclohexane or benzene, GC-VUV-HTOF allows a complete class of hydrocarbon isomers to be summed in order to understand how a complete N_{DBE} class of one molecular mass is degraded by microbial communities. Biotransformation of crude oil by microbial communities in the biosouring column was found to be highly selective. The SRCs first degraded C_{10}−C_{19} aliphatic compounds before degrading C_{10}−C_{26} monoaromatic hydrocarbons, a transformation pattern that was supported by the shift in microbial community structure. The nitrate treatment enhanced biotransformation of all crude oil hydrocarbons regardless of molecular weight, while the perchlorate treatment inhibited biotransformation of the crude oil. Previous studies have only shown microbial communities targeting a select group of hydrocarbons, such as *n*-alkanes or C_6−C_{10} monoaromatic compounds,[47−51] for degradation. The GC-VUV-HTOF technique incorporated in this study revealed that hydrocarbon-degrading microbial communities transform isomers of C_{10}−C_{30} compounds of different structural classes and that the transformation patterns vary depending on the abundances of microbial communities and the availability of the terminal electron acceptor.

Biodegradation Products. The heteroatom class distributions, based on relative abundance in the mass spectrum, of the most abundant chemical species observable with +ESI and −ESI combined with FT-ICR MS in the parent crude oil, oil from the souring control column, oil from the nitrate-treated column, and oil from the perchlorate-treated column are shown in Figure 4. The distributions were normalized to the most abundant peak in each spectrum. Compounds containing a single pyridinic nitrogen atom (N_1) comprised the most abundant chemical class in all the oil samples (Figure 4a). The relative abundance of the N_1 class decreased from ∼60% to ∼40% in the oil after 70 days of souring. This depletion suggests selective transformation of these species into other compounds, such as oxygenated species, or degradation by SRCs. Compounds with two pyridinic nitrogen atoms (N_2) fell from ∼2.5% to ∼1.5% relative abundance in the same souring time span as the depletion of the N_1 class. This decline in the relative abundances of pyridinic nitrogen classes during souring was matched by an increase in species that contain both oxygen and nitrogen. Compounds with one oxygen and one nitrogen atom (N_1O_1) in the oil increased in relative abundance from ∼2.0% to ∼5.0% after 70 days of souring. The relative abundance of N_1O_2 compounds also grew from ∼0.3% to ∼2.0% during the same time span in the oil from the souring column. Pyridinic nitrogen species detectable in +ESI-FT-ICR MS in the oils from the nitrate or perchlorate treatments did not decrease in relative abundance to the same extent as was observed in the oil from the souring control column. There was a ∼5% decrease in the relative abundance of the N_1 class in the oils from both treatments and a minimal (<0.5%) change in the relative abundances of the N_1O_1 and N_1O_2 classes.

An increase in the relative abundances of compounds containing either 2 or 4 oxygen atoms (O_2 and O_4 classes) in the oils from both the biosouring control and the nitrate-treated columns is illustrated in Figure 4b. There was a ∼6-fold increase in the relative abundances of compounds belonging to the O_2 and O_4 classes in the oil from the biosouring control column when compared to those relative abundances in the parent crude oil. There was also a similar increase in the relative abundances of compounds belonging to the O_2 and O_4 classes

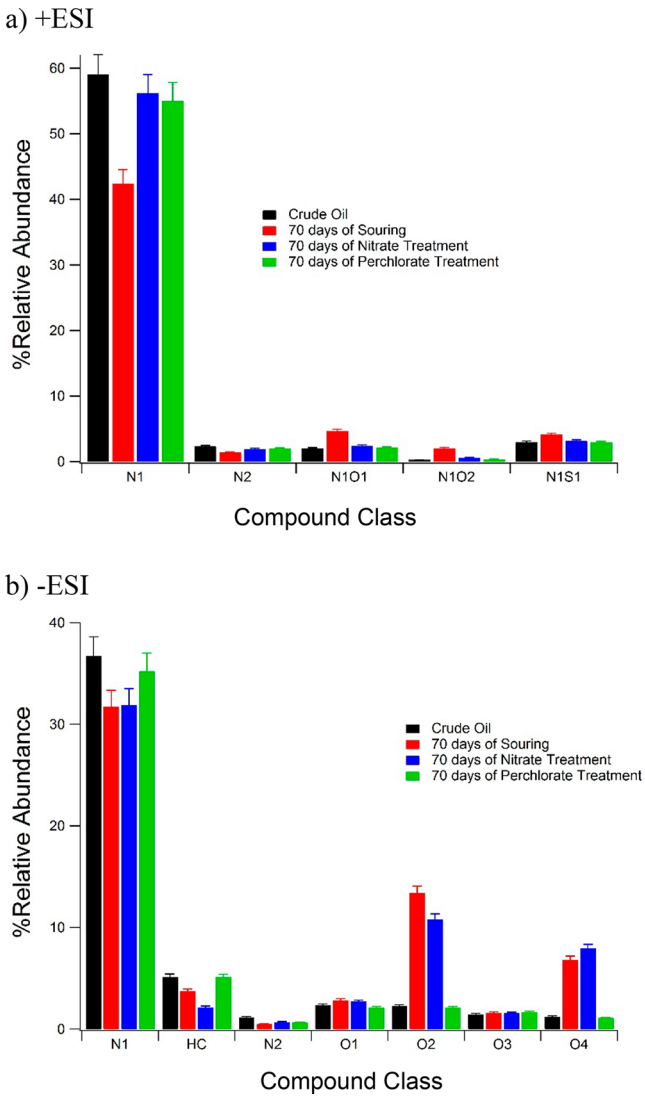

Figure 4. Relative abundance (%) of molecular formulas containing different heteroatoms observed using (a) positive electrospray ionization and (b) negative electrospray ionization combined with FT-ICR MS of parent North Sea crude oil, oil from 70 days into souring, oil from 70 days into the nitrate treatment, and oil from 70 days into the perchlorate treatment.

in the oil from the nitrate-treated column. There was not an increase in the relative abundances of compounds containing either 1 or 3 oxygen atoms (O_1 and O_3 classes) in either of these oils. There were minimal increases in oxygenated content (O_1−O_4 classes) in the oil from the perchlorate treatment, further corroborating the conclusion that the perchlorate treatment inhibited hydrocarbon degradation and product formation.

The O_2 and O_4 compound classes detected in -ESI-FT-ICR MS are most likely dominated by compounds containing one or two carboxylic acid functional groups, respectively. The increasing abundance of these carboxylic acids provides further support for microbiological transformations, as they are typical products of biodegradation.[52,53] These compounds form via the activation and transformation of monoaromatic hydrocarbons and PAHs by bacteria endogenously incorporating fumaric acid to a benzyl radical,[54] thereby creating benzylsuccinate derivatives by charging and detoxifying the aromatic ring.

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This microbial process results in carboxylic acid formation. Microbial communities can then utilize these products as an electron donor or a carbon source.[54−56]

Smaller molecular weight compounds of the C_{20}−C_{30} molecular size range containing two or four oxygen atoms were successfully identified in the oils from the biosouring control and nitrate-treated columns using GC-VUV-HTOF. While the position of the carboxyl group on most of the observed compounds is unknown, authentic standards (Sigma-Aldrich, St. Louis, MO, USA) confirmed the identification of a subset of the broader range of C_{20}−C_{30} carboxylic acids in these oil samples. The results of −ESI combined with FT-ICR MS illustrated that the range of carboxylic acids produced extended to C_{30}−C_{60} compounds, which are outside the analytical range of GC-VUV-HTOF. The combination of monoaromatic hydrocarbon and PAH degradation and production of $C_xH_yO_2$ and $C_xH_yO_4$ compounds supports the interpretation that fumarate addition occurred during biodegradation in this study, which is discussed in more detail in Shrestha et al.[35]

Molecular Distribution of Biodegradation Products. Compositional changes of crude oil between time points for all members of a heteroatom class simultaneously can be visualized in images of N_{DBE} versus carbon number. Isoabundance-contoured plots of N_{DBE} versus carbon number for the N_1, N_1O_1, N_1O_2, and N_1S_1 classes derived from +ESI combined with FT-ICR MS of the parent crude oil, oil from the souring control column, oil from the nitrate-treated column, and oil from the perchlorate-treated column are shown in Figure 5a. Compounds pertaining to the N_1O_1 class in the oil from the soured column increased in carbon number range from C_{20}−C_{60} to C_{10}−C_{75} and N_{DBE} range from 5−25 to 3−30 when compared to the ranges of the parent crude oil. Similarly, compounds of the N_1O_2 class in the oil from the soured column increased from C_{30}−C_{55} to C_{20}−C_{80} and from 7−17 to 7−27 N_{DBE} when compared to the ranges of the parent crude oil. The increase of species containing both nitrogen and oxygen atoms within the soured oil's chemical composition indicates that microbial catabolism of crude oil can result in the production of larger molecular weight compounds that were not present in the original parent oil. There was an increase in the carbon number and N_{DBE} ranges of compounds belonging to the N_1O_1 and N_1O_2 classes in the oils from the nitrate- and perchlorate-treated columns, but the ranges are far less than they are in the oil from the souring control column.

Isoabundance-contoured plots of N_{DBE} versus carbon number for the O_1, O_2, O_3, and O_4 classes derived from −ESI combined with FT-ICR MS of the parent crude oil, oil from the souring control column, oil from the nitrate-treated column, and oil from the perchlorate-treated column are shown in Figure 5b. While there are minimal changes in the molecular distributions of O_1 and O_3 compounds in any of the oils, the relative abundances of O_2 compounds of C_{10}−C_{60} and 5−25 N_{DBE} increased in the oils from both the souring and the nitrate-treated columns compared to the abundances in the parent crude oil. The O_4 compounds of C_{10}−C_{60} and 3−23 N_{DBE} also increased in relative abundance in the oils from both the souring and the nitrate-treated columns compared to the abundances in the parent crude oil. The increases in abundances and changes in O_2 and O_4 compound distributions were not as apparent in the oil from the perchlorate-treated column, further bolstering the conclusion that perchlorate injections inhibited biotransformation of the parent crude oil.

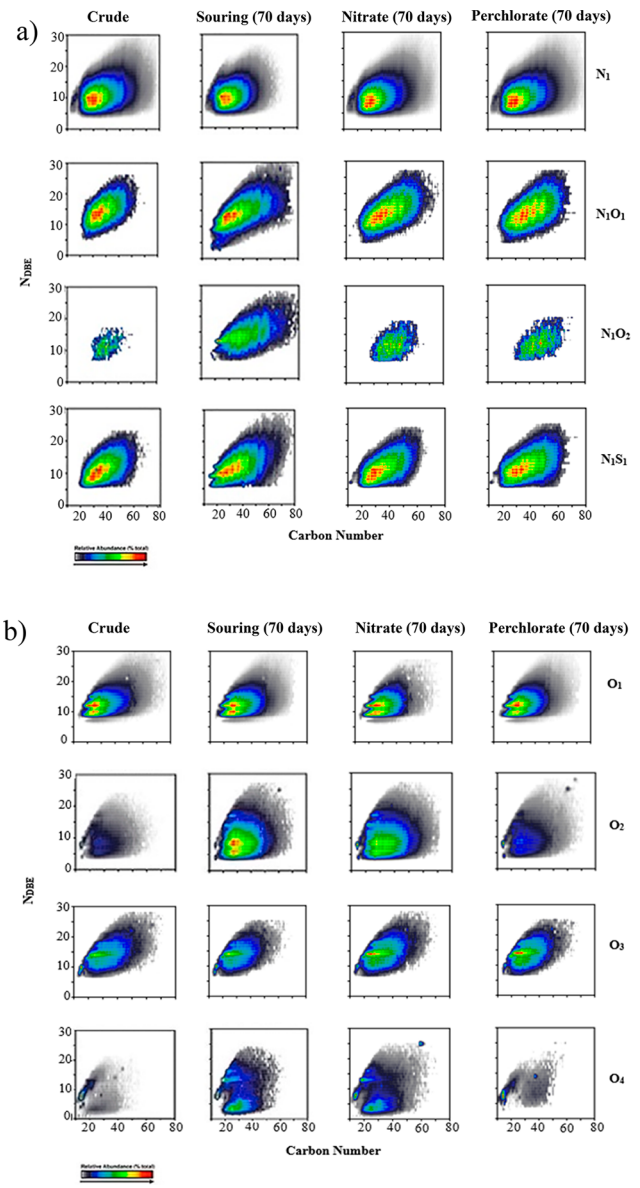

Figure 5. Isoabundance-contoured plots of double-bond equivalents (N_{DBE}) vs carbon number for parent North Sea crude oil, oil from 70 days into souring, oil from 70 days into the nitrate treatment, and oil from 70 days into the perchlorate treatment measured by (a) positive electrospray ionization and (b) negative electrospray ionization combined with FT-ICR MS. Each compositional image is normalized to the most abundant species within that heteroatom class for each mass spectrum.

Environmental and Industrial Applications. This study provides a comprehensive temporal characterization of microbial transformations of crude oil as a result of biosouring and subsequent treatments. In the oil from the souring control column, C_{10}−C_{15} aliphatic compounds were first degraded followed by transformation of C_{10}−C_{26} monoaromatic species. Both the nitrate and the perchlorate treatments mitigated sulfide production, but the nitrate treatment accelerated the rate of biotransformation of crude oil hydrocarbons, while the perchlorate treatment inhibited the majority of biotransformation. +ESI and −ESI combined with FT-ICR MS revealed increases in the relative abundances of oxygenated biodegradation products, involving the addition of two or four oxygen atoms to existing hydrocarbons, in the oils from both

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the biosouring control and the nitrate-treated columns. These products are indicative of monocarboxylic and dicarboxylic acids. No such increases were observed in the oil from the perchlorate-treated column. On the basis of the lack of hydrocarbon degradation and product formation, perchlorate injections may be the preferred treatment for biosouring.

The differences in transformation patterns observed in the oils from the souring control column, the nitrate-treated column, and the perchlorate-treated column demonstrate that the degree of microbial biotransformation of crude oil is specific to the presence of a dominant electron acceptor, the relative abundances of microbial communities,[35] and the availability of specific crude oil components as electron donors and carbon sources. While it has been shown that microbial communities can activate a range of hydrocarbons for degradation that is significantly greater than that which can be metabolized,[56] this study reveals that hydrocarbon degradation and product formation of crude oil is not limited to a narrow range of compound classes or molecular weights. Isomers of hydrocarbons from C_{10}–C_{30} were degraded, and carboxylic acid products were formed up to molecular sizes of C_{60}. The biotransformation patterns and product formations revealed in this work can constrain and strengthen existing models of crude oil degradation patterns for industrial and environmental applications.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b05346.

Additional information as described in the text including details regarding GC-VUV-HTOF analytical description and calibration, FT-ICR MS analytical description, instrumentation, and calibration; figures illustrating N_{DBE} = 1 and 7 compound class changes during souring, nitrate treatment, and perchlorate treatment (PDF)

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Notes

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■ REFERENCES

(1) Youssef, N.; Elshahed, M. S.; McInerney, M. J. Microbial processes in oil fields: culprits, problems, and opportunities. *Adv. Appl. Microbiol.* **2009**, *66*, 141–251.

(2) Gieg, L. M.; Jack, T. R.; Foght, J. M. Biological souring and mitigation in oil reservoirs. *Appl. Microbiol. Biotechnol.* **2011**, *92* (2), 263–282.

(3) Myhr, S.; Lillebo, B. L. P.; Sunde, E.; Beeder, J.; Torsvik, T. Inhibiton of microbial H_2S production in an oil reservoir model column by nitrate injection. *Appl. Microbiol. Biotechnol.* **2002**, *58* (3), 400–408.

(4) Greene, E. A.; Hubert, C.; Nemati, M.; Jenneman, G. E.; Voordouw, G. Nitrite reductase activity of sulphate-reducing bacteria prevents their inhibition by nitrate-reducing, sulphide-oxidizing bacteria. *Environ. Microbiol.* **2003**, *5* (7), 607–617.

(5) Tang, K.; Baskaran, V.; Nemati, M. Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries. *Biochem. Eng. J.* **2009**, *44*, 73–94.

(6) Williamson, N. *Applied Microbiology and Molecular Biology of Oilfield Systems*; Springer Netherlands: Dordrecht, 2008; Vol. *13*, Chapter 18, pp 151–157.

(7) Hubbard, C. G.; Cheng, Y.; Engelbrekston, A.; Druhan, J. L.; Li, L.; Ajo-Franklin, J. B.; Coates, J. D.; Conrad, M. E. Isotopic insights into microbial sulfur cycling in oil reservoirs. *Front. Microbiol.* **2014**, *5*, 1–12.

(8) Dunsmore, B.; Youldon, J.; Thrasher, D. R.; Vance, I. Effects of nitrate treatment on a mixed species, oil field microbial biofilm. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 454–462.

(9) Xue, Y.; Voordouw, G. Control of microbial sulfide production with biocides and nitrate in oil reservoir simulating bioreactors. *Front. Microbiol.* **2015**, *6*, 1387–1398.

(10) Voordouw, G.; Grigoryan, A. A.; Lambo, A.; Lin, S. P.; Park, H. S.; Jack, T. R.; Coombe, D.; Clay, B.; Zhang, F.; Ertmoed, R.; Miner, K.; Arensdorf, J. J. Sulfide remediation by pulsed injection of nitrate into a low temperature Canadian heavy oil reservoir. *Environ. Sci. Technol.* **2009**, *43* (24), 9512–9518.

(11) Callbeck, C. M.; Agrawal, A.; Voordouw, G. Acetate production from oil under sulfate-reducing conditions in bioreactors injected with sulfate and nitrate. *Appl. Environ. Microbiol.* **2013**, *79* (16), 5059–5068.

(12) Grigoryan, A. A.; Cornish, S. L.; Buziak, B.; Lin, S.; Cavallaro, A.; Arensdorf, J. J.; Voordouw, G. Competitive oxidation of volatile fatty acids by sulfate- and nitrate-reducing bacteria from an oil field in Argentina. *Appl. Environ. Microbiol.* **2008**, *74* (14), 4324–4335.

(13) Callbeck, C.; Dong, X.; Chatterjee, I.; Agrawal, A.; Caffrey, S.; Sensen, C. Microbial community succession in a bioreactor modeling a souring low-temperature oil reservoir subjected to nitrate injection. *Appl. Microbiol. Biotechnol.* **2011**, *91* (3), 799–810.

(14) Hubert, C.; Nemati, M.; Jenneman, G.; Voordouw, G. Containment of biogenic sulfide production in continuous up-flow packed-bed bioreactors with nitrate or nitrite. *Biotechnol. Prog.* **2003**, *19* (2), 338–345.

(15) Engelbrektson, A.; Hubbard, C. G.; Tom, L. M.; Boussina, A.; Jin, Y. T.; Wong, H.; Piceno, Y. M.; Carlson, H. K.; Conrad, M. E.; Anderson, G.; Coates, J. D. Inhibition of microbial sulfate reduction in a flow-through column system by (per)chlorarte treatment. *Front. Microbiol.* **2014**, *5*, 1–11.

(16) Baeuerle, P. A.; Huttner, W. B. Chlorate—a potent inhibitor of protein sulfation in intact cells. *Biochem. Biophys. Res. Commun.* **1986**, *141* (2), 870–877.

(17) Coates, J. D.; Achenbach, L. Microbial perchlorate reduction: rocket-fueled metabolism. A. *Nat. Rev. Microbiol.* **2004**, *2* (7), 569–580.

(18) Gregoire, P.; Engelbrektson, A.; Hubbard, C. G.; Metlagel, Z.; Csencsits, R.; Auer, M. Control of sulfideogenesis through bio-oxidation of H_2S coupled to (per)chlorate reduction. *Environ. Microbiol. Rep.* **2014**, *6* (16), 558–564.

DOI: 10.1021/acs.est.7b05346
Environ. Sci. Technol. 2018, 52, 1290–1300

(19) Gieg, L. M.; Davidova, I. A.; Duncan, K. E.; Suflita, J. M. Methanogenesis, sulfate reduction and crude oil biodegradation in hot Alaskan oilfields. *Environ. Microbiol.* **2010**, *12* (11), 3074−3086.
(20) Drozd, G. T.; Worton, D. R.; Aeppli, C.; Reddy, C. M.; Zhang, H.; Variano, E.; Goldstein, A. G. Modeling comprehensive chemical composition of weathered oil following a marine spill to predict ozone and potential secondary aerosol formation and constraint transport pathways. *J. Geophys. Res. Oceans* **2015**, *120* (11), 7300−7315.
(21) Aitken, C. M.; Jones, D. M.; Larter, S. R. Anaerobic hydrocarbon biodegradation in deep subsurface oil reservoirs. *Nature* **2004**, *431* (7006), 291−294.
(22) Townsend, G. T.; Prince, R. C.; Suflita, J. M. Anaerobic oxidation of crude oil hydrocarbons by the resident microorganisms of a contaminated anoxic aquifer. *Environ. Sci. Technol.* **2003**, *37* (22), 5213−5218.
(23) Townsend, G. T.; Prince, R. C.; Suflita, J. M. Anaerobic biodegradation of alicyclic constituents of gasoline and natural gas condensate by bacteria from an anoxic aquifer. *FEMS Microbiol. Ecol.* **2004**, *49* (1), 129−135.
(24) Walters, C. C.; Wang, F.C.; Qian, K.; Wu, C.; Mennito, A. S.; Wei, Z. Petroleum alteration by thermochemical sulfate reduction-A comprehensive molecular study of aromatic hydrocarbons and polar compounds. *Geochim. Cosmochim. Acta* **2015**, *153*, 37−71.
(25) Fingas, M. *Handbook of Oil Spill Science and Technology*; John Wiley & Sons, Inc., 2015.
(26) Reddy, C. M.; Quinn, J. G. GC-MS analysis of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons in seawater samples after the north cape oil spill. *Mar. Pollut. Bull.* **1999**, *38*, 126−135.
(27) White, H. K.; Xu, L.; Hartmann, P.; Quinn, J. G.; Reddy, C. M. Unresolved complex mixture (UCM) in coastal environments is derived from fossil sources. *Environ. Sci. Technol.* **2013**, *47*, 726−731.
(28) Isaacman, G.; Wilson, K.; Chan, A.; Worton, D.; Kimmel, J.; Nah, T.; Hohaus, T.; Gonin, M.; Kroll, J. H.; Worsnop, D. R.; Goldstein, A. H. Improved resolution of hydrocarbon structures and constitutional isomers in complex mixtures using gas chromatography-vacuum ultraviolet-mass spectrometry. *Anal. Chem.* **2012**, *84* (5), 2335−2342.
(29) Kaiser, N. K.; Quinn, J. P.; Blakney, G. T.; Hendrickson, C. L.; Marshall, A. G. A novel 9.4 T FTICR mass spectrometer with improved sensitivity, mass resolution, and mass range. *J. Am. Soc. Mass Spectrom.* **2011**, *22* (8), 1343−1351.
(30) Marshall, A. G.; Rodgers, R. P. Petroleomics: Chemistry of the underworld. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (47), 18090−18095.
(31) Hughey, C. A.; Hendrickson, C. L.; Rodgers, R. P.; Marshall, A. G. Elemental composition analysis of processed and unprocessed diesel fuel by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Energy Fuels* **2001**, *15* (5), 1186−1193.
(32) Marshall, A. G.; Hendrickson, C. L.; Jackson, G. S. Fourier transform ion cyclotron resonance mass spectrometry: a primer. *Mass Spectrom. Rev.* **1998**, *17* (1), 1−35.
(33) McKenna, A. M.; Williams, J. T.; Putman, J. C.; Aeppli, C.; Reddy, C. M.; Valentine, D. L.; Lemkau, K. L.; Kellermann, M. Y.; Savory, J. J.; Kaiser, N. K.; Marshall, A. G.; Rodgers, R. P. Unprecedented ultrahigh resolution FT-ICR mass spectrometry and parts-per-billion mass accuracy enable direct characterization of nickel and vanadyl porphyrins in petroleum from natural seeps. *Energy Fuels* **2014**, *28* (4), 2454−2464.
(34) Kaiser, N.; Savory, J.; McKenna, A.; Quinn, J.; Hendrickson, C. L. Electrically compensated fourier transform ion cyclotron resonance cell for complex mixture mass analysis. *Anal. Chem.* **2011**, *83* (17), 6907−6910.
(35) Shrestha, P. M.; Williamson, A. J.; Barnum, T. P.; Liu, Y.; Nowak, J.; Loutey, D.; Louie, W.; Kim, H.; Chin, W. S.; Mayilvahanan, K. S.; Shabbir, T.; Goldstein, A.; Coates, J. D. Microbial biosouring control using perchlorate treatment in crude oil reservoir simulated condition. *Manuscript in preparation*, 2018.
(36) Cline, J. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr.* **1969**, *14*, 454−458.
(37) Worton, D.; Zhang, H.; Isaacman-VanWertz, G.; Chan, A.; Wilson, K.; Goldstein, A. Comprehensive chemical characterization of hydrocarbons in NIST standard reference material 2779 Gulf of Mexico crude oil. *Environ. Sci. Technol.* **2015**, *49* (22), 13130−13138.
(38) Chan, A.; Isaacman, G.; Wilson, K.; Worton, D.; Ruehl, C.; Nah, T.; Gentner, D. R.; Dallmann, T. R.; Kirchstetter, T.; Harley, R.; Gilman, J.; Kuster, W.; de Gouw, J.; Offenberg, J.; Kleindienst, T.; Lin, Y.; Rubitschun, C.; Surratt, J.; Hayes, P.; Jimenez, J.; Goldstein, A. H. Detailed chemical characterization of unresolved complex mixtures in atmospheric organics: insights into emission sources, atmospheric processing, and secondary organic aerosol formation. *Journal of Geophysical Research: Atmospheres* **2013**, *118*, 6783−6796.
(39) McKenna, A. M.; Nelson, R. K.; Reddy, C. M.; Savory, J. J.; Kaiser, N. K.; Fitzsimmons, J. E.; Marshall, A. G.; Rodgers, R. P. Expansion of the analytical window for oil spill characterization by ultrahigh resolution mass spectrometry: beyond gas chromatography. *Environ. Sci. Technol.* **2013**, *47* (13), 7530−7539.
(40) Lobodin, V. V.; Juyal, P.; McKenna, A. M.; Rodgers, R. P.; Marshall, A. G. Tetramethylammonium hydroxide as a reagent for complex mixture analysis by negative ion electrospray ionization mass spectrometry. *Anal. Chem.* **2013**, *85* (16), 7803−7808.
(41) Ruddy, B. M.; Huettel, M.; Kostka, J. E.; Lobodin, V. V.; Bythell, B. J.; McKenna, A. M.; Aeppli, C.; Reddy, C. M.; Nelson, R. K.; Marshall, A. G.; Rodgers, R. P. Targeted petroleomics: analytical investigation of macondo well oil oxidation products from pensacola beach. *Energy Fuels* **2014**, *28* (6), 4043−4050.
(42) Rodgers, R. P.; Schaub, T. M.; Marshall, A. G. Petroleomics: MS return to its roots. *Anal. Chem.* **2005**, *77*, 20A−27A.
(43) McLafferty, F. W.; Turecek, F. *Interpretation of Mass Spectra*, 4th ed.; University Science Books: Mill Valley, CA, 1993.
(44) Worton, D. R.; Isaacman, G.; Gentner, D. R.; Dallmann, T. R.; Chan, A. W. H.; Ruehl, C.; Kirchstetter, T. W.; Wilson, K. R.; Harley, R. A.; Goldstein, A. H. Lubricating oil dominates primary organic aerosol emissions from motor vehicles. *Environ. Sci. Technol.* **2014**, *48*, 3698−3706.
(45) Mao, D.; Weghe, H. V. D.; Lookman, R.; Vanermen, G.; Brucker, N. D.; Diels, L. Resolving the unresolved complex mixture in motor oils using high-performance liquid chromatography followed by comprehensive two-dimensional gas chromatography. *Fuel* **2009**, *88*, 312−318.
(46) Aeppli, C.; Nelson, R. K.; Radović, J. R.; Carmichael, C. A.; Valentine, D. L.; Reddy, C. M. Recalcitrance and degradation of petroleum biomarkers upon abiotic and biotic natural weathering of *Deepwater Horizon* oil. *Environ. Sci. Technol.* **2014**, *48* (12), 6726−6734.
(47) Aitken, C. M.; Jones, D. M.; Maguire, M. J.; Gray, N. D.; Sherry, A.; Bowler, B. F. J.; Ditchfield, A. K.; Larter, S. R.; Head, I. M. Evidence that crude oil alkane activation proceeds by different mechanisms under sulfate-reducing and methanogenic conditions. *Geochim. Cosmochim. Acta* **2013**, *109*, 162−174.
(48) Rueter, P.; Rabus, R.; Wilkest, H.; Aeckersberg, F.; Rainey, F. A.; Jannasch, H. W.; Widdel, F. Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria. *Nature* **1994**, *372*, 455−458.
(49) Ommedal, H.; Torsvik, T. Desulfotignum toluenicum sp. nov., a novel toulene-degrading, sulphate-reducing bacterium isolated from an oil-reserovir model column. *Int. J. Syst. Evol. Microbiol.* **2007**, *57* (12), 2865−2869.
(50) Nakagawa, T.; Sato, S.; Yamamoto, Y.; Fukui, M. Successive changes in community structure of an ethylbenzene-degrading sulfate-reducing consortium. *Water Res.* **2002**, *36* (11), 2813−2823.
(51) Zedelius, J.; Rabus, R.; Grundmann, O.; Werner, I.; Brodkorb, D.; Schreiber, F.; Ehrenreich, P.; Behrends, A.; Wilkes, H.; Kube, M.; Reinhardt, R.; Widdel, F. Alkane degradation under anoxic conditions by a nitrate-reducing bacterium with possible involvement of the electron acceptor in substrate activation. *Environ. Microbiol. Rep.* **2011**, *3* (1), 125−135.

DOI: 10.1021/acs.est.7b05346
Environ. Sci. Technol. 2018, 52, 1290−1300

(52) Atlas, R. M. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Rev.* **1981**, *45* (1), 180−209.
(53) Aeppli, C.; Carmichael, C. A.; Nelson, R. K.; Lemkau, K. L.; Graham, W. M.; Redmond, M. C.; Valentine, D. L.; Reddy, C. M. Oil weathering after the *Deepwater Horizon* disaster led to the formation of oxygenated residues. *Environ. Sci. Technol.* **2012**, *46*, 8799−8807.
(54) Bharadwaj, V.; Vyas, S.; Villano, S.; Maupin, C.; Dean, A. Unraveling the impact of hydrocarbon structure on the fumarate addition mechanism-a gas-phase ab initio study. *Phys. Chem. Chem. Phys.* **2015**, *17* (6), 4054−4066.
(55) Kühner, S.; Wöhlbrand, L.; Fritz, I.; Wruck, W.; Hultschig, C.; Hufnagel, P.; Kube, M.; Reinhardt, R.; Rabus, R. Substrate-dependent regulation of anaerobic degradation pathways for toluene and ethylbenzene in a denitrifying bacterium, strain EbN1. *J. Bacteriol.* **2005**, *187* (4), 1493−1503.
(56) Jarling, R.; Kuhner, S.; Basilio Janke, E.; Gruner, A.; Drozdowska, M.; Golding, B. T.; Rabus, R.; Wilkes, R. Versatile transformations of hydrocarbons in anaerobic bacteria: substrate ranges and regio- and stereo-chemistry of activation reactions. *Front. Microbiol.* **2015**, *6*, 5−14.

DOI: 10.1021/acs.est.7b05346
Environ. Sci. Technol. 2018, 52, 1290−1300