Chemosphere 182 (2017) 555–558

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Short Communication

Biotransformation of natural gas and oil compounds associated with marine oil discharges

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HIGHLIGHTS

- Mixtures of gas and soluble oil compounds were biodegraded in natural seawater.
- Gas compound oxidation did not stimulate biodegradation of oil compounds.
- Gas oxidation rates were comparable for methane, ethane and propane.
- Results from the seawater differed from DWH oil spill field data.

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form
28 April 2017
Accepted 8 May 2017
Available online 11 May 2017

Handling Editor: Keith Maruya

Keywords:
Natural gas
Oil
Water-soluble fractions
Biotransformation
Seawater

ABSTRACT

Field data from the Deepwater Horizon (DWH) oil spill in the Gulf of Mexico (GoM) suggested that oxidation of gas compounds stimulated biodegradation of oil compounds in the deep sea plume. We performed experiments with local seawater from a Norwegian fjord to examine if the presence of dissolved gas compounds (methane, ethane and propane) affected biodegradation of volatile oil compounds, and if oil compounds likewise affected gas compound oxidation. The results from the experiment showed comparable oil compound biotransformation rates in seawater at 5 °C between seawater with and without soluble gases. Gas oxidation was not affected by the presence of volatile oil compounds. Contrary to DWH deep sea plume data, propane oxidation was not faster than methane oxidation. These data may reflect variations between biodegradation of oil and gas in seawater environments with different history of oil and gas exposure.

1. Introduction

During the Deepwater Horizon (DWH) blowout a continuous plume of gas and soluble oil compounds were detected during the spill period at depths of 900–1300 m (Camilli et al., 2010). Field analyses suggested faster losses of propane and ethane than of methane, and it was suggested that propane and ethane were drivers of microbial respiration (Valentine et al., 2010). Propane and ethane respiration could thus stimulate bacterial populations for degradation of other hydrocarbons in the plume (Valentine et al., 2012). Also methane was depleted when measured after the spill period, probably by microbial oxidation (Kessler et al., 2011; Gutierrez and Aitken, 2014).

Empirical oxidation rate data for gases in seawater (SW) are scarce. Methane oxidation rates were faster in Arctic bottom SW than in surface SW (Griffiths et al., 1982). A study of aerobic oxidation of methane, ethane, propane and butane in marine sediments revealed that C3 and C4 were oxidized faster than C1 and C2 (Kinnaman et al., 2007), in agreement with the data from the DWH oil spill.

The objective of the current study was to determine if gas compound (methane, ethane and propane) oxidations affected degradation of soluble oil compounds in SW from a Norwegian fjord with no identified history of previous oil and gas exposure, and if oxidation rates differed between the gas compounds. These studies could further be used to investigate if gas biodegradation rates could jump-start biodegradation of oil compounds in pristine SW, in the same way as suggested for the DWH oil spill in the GoM (Valentine et al., 2010), an environment previously exposed to oil and gas.

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http://dx.doi.org/10.1016/j.chemosphere.2017.05.046

2. Materials and methods

2.1. Gases, oil and seawater

HiQ methane (CH_4), ethane (C_2H_6) and propane (C_3H_8) (99.95% purities) were purchased (Linde Gases Division, Pullach, Germany) and used to fill a self-sealing, 46 cm-diameter, Mylar balloon (nominal ratio methane:ethane:propane of 8:1:1). A crude Statfjord blend paraffinic oil was used for preparation of low-energy water-accommodated fractions (LE-WAFs) of oil. SW was collected from a depth of 80 m (below thermocline) in a non-polluted Norwegian fjord (Trondheimsfjord; 63°26′N, 10°23′E), supplied by a pipeline system from the source to our laboratories (salinity of 34‰, temperature of 6–8 °C, and dissolved oxygen (DO) of 8.5 mg/L when reaching our laboratory). The SW was acclimated at 5 °C for 7 days before start of the experiment.

2.2. Biodegradation experiment

LE-WAFs were prepared from the oil, as previously described (Aurand and Coelho, 1996). Crude oil (925 μL) was carefully applied to the surface of 9.25 L acclimated (5 °C) SW in a 10 L closed SCHOTT flask with silicone tops and stopcock in the bottom. An oil:SW ratio of 1:10,000 was used. Sterilized LE-WAFs with filtered SW (0.2 μm exclusion limit), and supplemented with $HgCl_2$ (100 mg/L $HgCl_2$), were also similarly prepared. The LE-WAFs were generated by slow magnetic stirring (5 °C, 72 h), to avoid generation of a vortex. LE-WAFs in unfiltered and sterilized SW were removed from the bottom of the flasks (stopcock) and transferred to 100 ml serum flasks (no headspace) which were sealed with butyl rubber septa. Other serum flasks were filled with acclimated (5 °C) unfiltered SW without oil. The gas mixture from the Mylar balloon was injected by a cannula into serum flasks with unfiltered or sterilized WAFs, or to flasks with SW without LE-WAFs, using a peristaltic pump at 20 ml/min flow rate for 3 min per flask. Gas injection was compensated with a cannula for removal of excess gas.

Flasks with or without injected gas, were incubated at 5 °C for up to 64 days in the dark. Flasks were removed and sacrificed (triplicate) for gas and oil compound analyses at day 0, and after 7, 14, 21, 28, 42 and 64 days of incubation.

2.3. Sampling, analyses and calculations

Flasks were sampled by removing 20 ml volumes for DO analyses and to generate headspace in the flasks. Flasks with headspace were shaken vigorously (1 min), incubated at room temperature for 30 min, and 2 ml headspace evacuated by a gas-tight syringe. The rest of the water was transferred to 40 ml VOC vials and acidified ($pH < 2$) for VOC analyses.

DO measurements were performed by a DO-meter (YSI, Inc., Yellow Springs, OH). Headspace gases were quantified by a gas chromatograph coupled to a flame ionization detector (GC-FID; Agilent 6890N with J&W CP-Al2O3/Na2SO4 50m, 0.32 μm × 5 μm column; Agilent Technologies). Targeted oil compounds representing C5-C9 saturates and C0-C4/C5 benzenes were analysed by a Purge & Trap unit (Teledyne Tekmar Atomx; Mason OH, U.S.A.) coupled to a GC-MS (Agilent 6890N GC and an Agilent 5975B MSD detector; Agilent Technologies) (P&T GC-MS). The response values for individual target analytes were determined, with a signal-to-noise ratio of 10 as the lower detection limit, and a lower limit of detection of 0.5 μl/L (ppmV) gas compound, or 0.01 μg/L (ppb) target compound in the WAFs.

Biotransformation kinetics were determined as first-order rate coefficients and half-lives by “one-phase exponential decay” or “plateau followed one-phase decay” (GraphPad Prism 6.01; GraphPad Software, La Jolla CA, U.S.A). Data sets were compared by paired *t*-test or one-way ANOVA analyses (GraphPad Prism).

3. Results and discussions

3.1. Depletion and biodegradation of gas compounds

The SW blank solutions (SW without LE-WAF and/or gas mixture) did not contain detectable concentrations of the gases included in this study, while low BTEX background concentrations (1 μg/L) were measured, corresponding to 0.2% of the BTEX concentrations (median values) of measured BTEX in the LE-WAFs. Higher microbial densities have been measured in the seawater used in our studies than in DWH non-plume samples, in addition to low concentrations of oil-degrading microbes (Brakstad et al., 2004; Hazen et al., 2010). Recent comparison of microbial communities in SW close to the pipeline inlet (80 m depth) and at the pipeline outlet did not show significant differences ($P > 0.05$) (Deni Ribicic, personal communication).

When the gas mixture was injected by the similar procedure in SW and LE-WAFs, the initial gas concentrations were significantly higher in SW than in the LE-WAFs (Table 1). The gas concentrations in SW were higher than determined in the DWH deep sea plume (0.6–183 μM methane, below detection limit to 16 μM ethane, and below detection limit to 7.7 μM propane), but within plume concentrations in the LE-WAFs (Valentine et al., 2010). While BTEX concentrations were lower than gas concentrations in field samples (Camilli et al., 2010; Valentine et al., 2010), these were higher in the LE-WAFs. However, this was necessary to be able to measure targeted VOC compounds.

A biodegradation experiment was performed with gas mixtures injected in SW or LE-WAFs, and with LE-WAFs without injected gas. Gas compounds were depleted in unfiltered SW and LE-WAFs, but not in sterilized LE-WAFs (Fig. S1, Supplementary Information), and we therefore considered the depletion to be the result of microbial gas oxidation. DO concentrations were 8.2 ± 0.4 mg/L at the start of the experiment, and were reduced to 6.7 ± 0.1 mg/L (lowest concentration 6.5 mg/L) at the end of the experiment (64 days), showing that oxic conditions were maintained during the study. Regression analyses resulted in oxidation half-lives of 13 and 29 days in SW and LE-WAFs, respectively (Fig. 1A). The half-lives of each gas ranged from 12 to 29 days (Table 1). Rate coefficients (Table 1) did not differ significantly between SW and LE-WAFs ($P > 0.05$; paired *t*-test), although initial gas concentrations differed significantly in the SW and LE-WAFs. The different initial gas concentrations in SW without WAF and in LE-WAFs did therefore not affect degradation rate coefficients.

The data from the current study therefore showed that gas compound oxidation rates were not significantly influenced by the presence or absence of soluble oil compounds, or different concentrations of these compounds. The rate coefficients were further not significantly different between methane, ethane and propane in SW and the LE-WAFs ($P > 0.05$; one-way ANOVA).

The preferential oxidation of propane (median 0.21 $\mu M\ d^{-1}$) over methane (median 0.01 $\mu M\ d^{-1}$), measured from the DWH deep sea plume samples (Valentine et al., 2010), was therefore not measured in our study with SW from a Norwegian fjord. In a one-dimensional time-dependent mixing, transport and reactions model, methane oxidation rate constants were estimated to peak in July with oxidation rate constants up to 0.2 d^{-1}, and returning to values of 0.0015 d^{-1} by mid-September (Kessler et al., 2011). These peak data were within the ranges of methane rate constants determined in our study (Table 1). Further, propane oxidation rates in GoM SW, following first-order rate kinetics (rate constant appr. 0.14 d^{-1}) (Valentine et al., 2010), were higher than determined by