Microbial communities related to biodegradation of dispersed Macondo oil at low seawater temperature with Norwegian coastal seawater

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Summary

The Deepwater Horizon (DWH) accident in 2010 created a deepwater plume of small oil droplets from a deepwater well in the Mississippi Canyon lease block 252 (‘Macondo oil’). A novel laboratory system was used in the current study to investigate biodegradation of Macondo oil dispersions (10 μm median droplet sizes) at low oil concentrations (2 mg l−1) in coastal Norwegian seawater at a temperature of 4–5°C. Whole metagenome analyses showed that oil biodegradation was associated with the successive increased abundances of Gammaproteobacteria, while Alphaproteobacteria (Pelagibacter) became dominant at the end of the experiment. Colwellia and Oceanospirillales were related to n-alkane biodegradation, while particularly Cycloclasticus and Marinobacter were associated with degradation of aromatic hydrocarbons (HCs). The larger oil droplet dispersions resulted in delayed sequential changes of Oceanospirillales and Cycloclasticus, related with slower degradation of alkanes and aromatic HCs. The bacterial successions associated with oil biodegradation showed both similarities and differences when compared with the results from DWH field samples and laboratory studies performed with deepwater from the Gulf of Mexico.

Introduction

After marine oil spills indigenous oil-degrading bacteria bloom and increase their abundances dramatically (Braddock et al., 1995; Yakimov et al., 2007), most of these belonging to the classes Alphaproteobacteria or Gammaproteobacteria (Yakimov et al., 2007). While both alpha- and gammaproteobacterial oil degraders are abundant during oil biodegradation in temperate seawater, Gammaproteobacteria become enriched at low seawater temperatures (Brakstad and Lødeng, 2005; Deppe et al., 2005; Coulon et al., 2007; Hazen et al., 2010; Baslum et al., 2012; Dubinsky et al., 2013). In marine oil spills aliphatic hydrocarbon (HC)-degrading bacteria like Alcanivorax are often succeeded by other bacterial populations like Cycloclasticus that attack the more slowly biodegradable oil compounds like polycyclic aromatic HCs (PAH) (Kasai et al., 2002a,b). Several HC degraders, including Colwellia, Marinobacterium, Marinomonas, Glaciecola and Pseudoalteromonas, have previously been associated with cold marine environments (Yakimov et al., 2004a; Deppe et al., 2005; Brakstad et al., 2008).

During the Deepwater Horizon (DWH) oil spill, to which the dispersant Corexit 9500 had been added at the wellhead, a deepwater plume (900–1300 m in depth) with finely dispersed oil at low concentrations and low seawater temperatures (4–6°C) was reported in several studies (Camilli et al., 2010; Reddy et al., 2012). Simulation of the plume trajectories indicated that oil droplets with diameters between 10 and 50 μm were abundant in this zone and were transported horizontally, while droplets larger than 90 μm rapidly rose to the surface (North et al., 2011). During the spill, elevated population levels of indigenous deep-sea bacteria associated with HC biodegradation (Oceanospirillales, Cycloclasticus, Colwellia, Methylphaga) were found in association with the deepwater plume (Hazen et al., 2010; Valentine et al., 2010; Redmond and Valentine, 2012; Dubinsky et al., 2013; Yang et al., 2014). Zones of oxygen anomalies, indicative of oil biodegradation, were also associated with the dispersed oil in the deepwater, resulting in a slight reduction of oxygen saturation from 67% outside to 59% inside the plume (Hazen et al., 2010).

Both field observations and laboratory experiments indicated short biodegradation half-lives for nC13-nC26...
alkanes, ranging from 1 to 8 days (Hazen et al., 2010). Water samples associated with the plume had lower microbial diversities than samples outside the plume (Hazen et al., 2010; Mason et al., 2012) and were enriched with a variety of metabolic genes involved in both aerobic and anaerobic HC degradation, including genes for motility, chemotaxis and aliphatic HC degradation, while genes encoding aromatic HC degradation were expressed only at low levels (Lu et al., 2012; Mason et al., 2012). Laboratory studies showed that mixing of the Macondo oil with the dispersant resulted in faster oil biodegradation (Hazen et al., 2010; Bælum et al., 2012). A model was suggested for the deep sea biodegradation of HCs with sequential and pulsed propagation of bacteria degrading specific HCs, including n-alkanes, aromatics and C1−C3 gas components (Valentine et al., 2012). Bacterial respiration of gas compounds (mainly propane and ethane) was suggested to accelerate the response of HC-degrading bacteria (Valentine et al., 2010), while methane was oxidized by methanotrophic bacteria, but at a slower rate than for propane (Kessler et al., 2011).

The aim of this study was to determine the relative abundances of microbes related to biodegradation of specific oil compound groups, as well as the effect of oil droplet size on biodegradation, using Macondo oil in natural seawater at a temperature relevant for the Gulf of Mexico (GoM) deepwater. We used a novel experimental system, generating dispersed oil at two different droplet size distributions in an oil droplet generator, with incubation of the dispersions in a slowly rotating carousel system (Brakstad et al., 2015). The experiment was performed with permanently cold seawater from a Norwegian fjord, and results were compared with relevant field data from the DWH spill.

Results and discussions

The biodegradation experiment with the Macondo oil pre-mixed with Corexit 9500 [dispersant-to-oil ratio (DOR) 1:100] was performed in a slowly rotating carousel system after generation of dispersions with 10 μm or 30 μm median oil droplet sizes and nominal concentrations of 2 mg l⁻¹. These dispersions were prepared with seawater pre-acclimated for 6 days (5°C), and with low oil concentration (0.2 mg l⁻¹ of Macondo oil). The experiment was performed at 5°C for 64 days (Brakstad et al., 2015). Consistency of oil droplet size distributions was measured by Coulter counter during the biodegradation period. Droplet sizes, measured by Coulter counter, were maintained in the 10 μm dispersions, while the 30 μm dispersions in unfiltered seawater showed a rapid decline in droplet size between days 0 and 8. However, the 30 μm median oil droplet diameter was maintained in the sterile dispersions, as recently reported (Brakstad et al., 2015). Although no addition of nutrients or oxygenation was performed during the experimental period, we did not experience oxygen or nutrient (NO₃⁻-N/NO₂⁻-N, NH₄⁺-N, o-P0₄²⁻-P) depletion (Brakstad et al., 2015).

Microbial concentrations

Bacterial counts of the source seawater showed cell concentrations of 1.44 ± 0.77–2.95 ± 1.19 × 10⁵ cells ml⁻¹ (Fig. 1); these concentrations are similar to results from previous samples of the same water source (Brakstad et al., 2004). During the biodegradation period average concentrations of 3.97 × 10⁵ (range 1.44 × 10⁴–5.25 × 10⁵), 10.2 × 10⁵ (range 2.95 × 10⁵–16.8 × 10⁵) and 9.61 × 10⁵ (range 2.95 × 10⁵–16.0 × 10⁵) cells ml⁻¹ were measured in seawater without oil, 10 μm dispersions and 30 μm dispersions respectively (Fig. 1). During the 6-day acclimation period increased cell concentrations were measured both in source seawater and in the 10 μm dispersion used for acclimation (Fig. 1). From the start to the end of the biodegradation experiment, cell counts increased by a factor of 1.24 in the seawater without oil and by 1.03–1.07 in the dispersions. Cell concentrations in the 10 μm and 30 μm dispersions were higher than in the seawater without oil, with average factors of 2.7 and 2.4, in favour of the 10 and 30 μm dispersions respectively. The concentrations became slightly higher in the 10 than the 30 μm dispersions, from 16 to 64 days of the experiment. The bacterial concentrations in the Norwegian water used in this experiment were higher than in samples collected from the GoM deepwater during the ongoing spill, which ranged from 1 to 5.5 × 10⁵ cells ml⁻¹ (Atlas and Hazen, 2011; Bælum et al., 2012).

Microbial communities

A total of 17 DNA extracts were subject to metagenome sequence analyses, including DNA extracts from four samples of seawater without oil added, seven oil dispersions of 10 μm and six dispersions of 30 μm droplet
size. Initial analyses indicated high abundances of *Pelagibacter* sp., *Colwellia psychrerythraea*, *Pseudomonas syringae*, *Pseudomonas fluorescens* and *Xanthomonas translucens* (not shown). A confirmatory reference alignment analysis was performed for *Pelagibacter* sp., *C. psychrerythraea*, *P. syringae*, *P. fluorescens*, *X. translucens* and PhiX174 (control) full-length genomes. Sequence data for *Pelagibacter* sp., *C. psychrerythraea* and the control PhiX174 revealed over 95% coverage over the full length reference genomes. Reads matching *P. syringae*, *P. fluorescens* and *X. translucens* in reference alignments revealed <1% coverage and aligned sequences matched only a few, limited genetic regions; therefore, these organisms were removed from subsequent analysis.

The bacterial communities in the seawater without oil were dominated by Alphaproteobacteria, with 51–95% abundance, while Gammaproteobacteria showed 2–42% abundances (Fig. 2A and Fig. S1). However, in the oil dispersions the Gammaproteobacteria became rapidly predominant after 2 to 32 days of biodegradation, with 54–80% and 65–96% abundances in the 10 μm and 30 μm dispersions respectively. The Gammaproteobacteria abundances peaked after day 8 in the 10 μm dispersions, and after day 16 in the 30 μm dispersions. Thereafter the abundances decreased in the dispersions, and after 64 days the Alphaproteobacteria became predominant in both dispersions (72% and 71% abundances in the 10 μm and 30 μm dispersions, respectively), similarly to the seawater without oil (Fig S1). Typically, oil biodegradation in cold marine seawater is associated with abundances of Gammaproteobacteria, shown in both laboratory and field studies (e.g. Yakimov et al., 2004a; Deppe et al., 2005; Gerdes et al., 2005; Brakstad and Bonaunet, 2006; Hazen et al., 2010; Redmond and Valentine, 2012; Dubinsky et al., 2013).
For the comparison of the microbial communities in our study with field results from the DWH oil spill, we used a grouping system recently described for DWH deepwater field samples (Redmond and Valentine, 2012). In the seawater without oil, the Alphaproteobacteria were distributed between Rhodobacterales (11–28% abundances) and ‘other Alphaproteobacteria’ (32–74% abundances), as shown in Fig. 2A. The ‘other Alphaproteobacteria’ were dominated by Pelagibacter of the SAR11 clade (21–63% abundances). Pelagibacter is accepted as the most abundant group of heterotrophic bacteria in the oceans, and represent approximately one-quarter of all 16S ribosomal RNA (rRNA) genes identified in clone libraries from marine environments (Morris et al., 2002). High abundance of this genus in the seawater without oil was therefore expected. The groups of Gammaproteobacteria in the seawater included Colwellia (0–23% abundances) and ‘other Gammaproteobacteria’ (1–18% abundances), including Marinobacter and unclassified Gammaproteobacteria, appearing at the start of the experiment (Fig. 2A).

The increasing enrichments of Gammaproteobacteria in the oil dispersions followed a succession, with Colwellia and Oceanospirillales being abundant during the first 8 days, with subsequent predominances of Cycloclasticus after 16–64 days in both dispersions (Fig. 2B and C). Abundances of Colwellia increased between days 0 and 8 from 20% to 71% in the 10 µm dispersions and were maintained between 36% and 40% in the 30 µm dispersions. From 16 to 64 days Colwellia was reduced to ≤ 4% abundance in both dispersions. Also Oceanospirillales increased during the early phase of the experiment, from 4% to 14% and from 5% to 11% between day 0 and 4 in the 10 µm and 30 µm dispersions respectively. The abundances of Oceanospirillales then declined during the rest of the period. The group described as DWH Oceanospirillales (Fig. 2) refers to uncultivated Oceanospirillales observed in DWH deepwater plume (Hazen et al., 2010; Mason et al., 2012; Redmond and Valentine, 2012) and accounted for 25–100% of the Oceanospirillales in the dispersions. Typically, the DWH Oceanospirillales emerged later than ‘other Oceanospirillales’ in the dispersions. The reductions of both groups of Oceanospirillales came later in the 30 µm than the 10 µm dispersions (Fig. 2B and C). The genera associated with the Oceanospirillales included Bermanella and Thalassolituus, which also are some of the closed cultured relatives to the DWH Oceanospirillales (Yang et al., 2014).

Following the reduced abundances of Colwellia and Oceanospirillales, increased levels of Cycloclasticus appeared. Cycloclasticus constituted 54% and 88% of the bacterial populations in the 10 µm and 30 µm dispersions after 16 days, with subsequent declines to 10% and 16% in the two dispersions after 64 days, and were closely affiliated to the pyrene-degrading Cycloclasticus sp. strain P1 (Lai et al., 2012), As with Oceanospirillales, the decline of Cycloclasticus was slower in the 30 µm than the 10 µm dispersions. At the end of the experimental period (32 and 64 days), additional genera of Gammaproteobacteria became abundant, shown as ‘other Gammaproteobacteria’ (Fig. 2B and C), mainly affiliated with Marinobacter (20% and 29% abundances in the 10 µm and 30 µm dispersions respectively). In contrast to the late abundance of Marinobacter in oil dispersions, this genus appeared early in seawater without oil. The predominances of ‘other Alphaproteobacteria’ in both dispersions at the end of the experiment (70–71% abundances) were associated with Candidatus Pelagibacter in both dispersions. The dispersed oil therefore resulted in stimulation of mainly Gammaproteobacteria, following a succession in three steps, with an early phase of Colwellia and Oceanospirillales, a mid-phase of Cycloclasticus and a late phase of mainly Candidatus Pelagibacter, and with contributions of Marinobacter. The differences in succession patterns between 10 µm and 30 µm dispersions were mainly related to the slower decline of some of the bacterial groups after their peak abundances, including Oceanospirillales and Cycloclasticus.

The abundant bacteria in our laboratory study with Macondo oil dispersions in Norwegian coastal seawater showed some similarity to results from the GoM field samples of the deepwater plume during the DWH spill. The field samples from the DWH deepwater plume were also dominated by a few Gammaproteobacteria, including Oceanospirillales, Colwellia and Cycloclasticus, with an early increase in the abundances of Oceanospirillales, followed by later appearances of Cycloclasticus (Hazen et al., 2010; Valentine et al., 2010; Redmond and Valentine, 2012; Dubinsky et al., 2013). The genera Bermanella and Thalassolituus, which were the closest relatives the Oceanospirillales sequences in our study, were also associated with the DWH Oceanospirillales (Yang et al., 2014), and Bermanella has also been associated with natural marine crude oil seeps (Hawley et al., 2014). Most of the Colwellia sequences in our study were related to C. psychrerythraea, in agreement with the Colwellia sequences retrieved from the DWH deepwater plume (Mason et al., 2014). Marinobacter, which appeared late in the experiment with Norwegian seawater, was detected both in DWH deepwater plume and surface water (Hazen et al., 2010; Kostka et al., 2011), mainly after the spill period in September/October 2010 (Gutierrez et al., 2013). In accordance with our results, increased abundances of SAR11 were measured in the plume after well shut in (Yang et al., 2014).

However, the bacterial successions in the GoM field samples also differed from our results. The DWH field samples showed much higher abundances of
Oceanospirillales in early plume samples than in our studies (Hazan et al., 2010; Redmond and Valentine, 2012; Dubinsky et al., 2013). Further, these field studies showed late abundances of Colwellia, similar to the Cycloclasticus (Redmond and Valentine, 2012; Dubinsky et al., 2013). In addition, the field samples were enriched with Pseudomonas and Pseudoalteromonas (Redmond and Valentine, 2012; Dubinsky et al., 2013).

Laboratory studies of Macondo oil biodegradation with GoM deepwater at 4–5 °C showed a relatively late abundance of Colwelliaceae compared with our results (Bælum et al., 2012; Wang et al., 2014). However, early abundances of Oceanospirillales in these studies (5–10%) were more in agreement with our results than with the field data (Bælum et al., 2012; Wang et al., 2014). These differences between GoM deepwater and Norwegian shallow water are expected, due to different geographical origin, depths and environmental conditions. The continuous exposure of oil from natural seepages in the GoM may be of particular importance for the presence of oil-degrading bacterial communities in the GoM (e.g. Smith et al., 2014).

Results from the DWH deepwater plume also showed methanotrophs and methylo trophs (Methylomonas, Methylphaga), which were associated with methane oxidation and oil compound biodegradation during the DWH oil release (Valentine et al., 2010; Dubinsky et al., 2013). Methylo trophic bacteria were only measured at ≤1% abundances in the dispersions (Fig. 2). This was not surprising, since C1 substrates like methane were not expected to be present in the dispersions studied here. However, we have previously detected Methylphaga during oil biodegradation with the same seawater source as used here (Brakstad et al., 2004).

Microbial diversities estimated by Shannon–Wiener diversity indexes did not differ between communities in seawater without oil and dispersions (Table S1), and the diversities were reduced both in seawater and dispersions over time. Low diversity indices were associated with relative enrichment of abundant bacterial genera described above, in particular Pelagibacter and Cycloclasticus.

In addition to the whole genome analyses bacterial 16S rDNA denaturing gradient gel electrophoresis (DGGE) revealed banding pattern differences between oil dispersions and seawater, and similarities between the two dispersions, with a banding pattern changing in the oil dispersions between day 8 and 16, and with separate cluster of seawater with oil and dispersions (Fig. S2).

Comparison of bacterial analyses and petroleum HC biodegradation

Biotransformation data from this experiment have recently been presented for 22 volatile and semivolatile oil compounds (Brakstad et al., 2015), as defined by Reed and colleagues (2000), representing 70–80% of the oil according to true boiling point curve (Pasquini and Bueno, 2007). First-order biotransformation rates of both saturates and aromatic HCs were generally faster in the 10 μm than in the 30 μm dispersions, showing the importance of oil droplet size distribution for biodegradation (Brakstad et al., 2015). Depletion data of C25–C36 n-alkanes, decalines monoaromatic HCs and PAHs are shown in the Supporting Information as 1st order biotransformation decay curves (Figs S3 and S4), and as rate coefficients and half-lives (Table S2). Depletion of all compound groups was mainly the result of biodegradation, since sterilized controls showed only small or minor depletion during the experimental period. Both rate coefficients and half-lives of the n-alkane and aromatic HC groups were significantly different between the 10 μm and 30 μm dispersions in unfiltered seawater (P < 0.05 by non-parametric t-test analyses).

Biotransformation of the alkane and aromatic oil compound groups, based on the rate coefficients, was compared with the appearances of abundant bacterial groups in the dispersions (Table 1). The n-alkanes were mostly biodegraded between days 0 and 8, although nC25–nC36 alkanes required 16 days before >80% biotransformation was reached. This early period was related to the abundances of Oceanospirillales and Colwellia. In accordance with the generally slower biotransformation of the n-alkanes in the 30 μm than in the 10 μm dispersions, the abundances of especially Oceanospirillales were delayed in the larger oil-droplet dispersions (Table 1). The later emergence of DWH Oceanospirillales than ‘other Oceanospirillales’ may be related to different n-alkane preferences of the two groups, and single cell sequencing of DWH Oceanospirillales has revealed genes for both n-alkane and cycloalkane degradation (Mason et al., 2012). Several groups within the order Oceanospirillales are associated with n-alkane biodegradation, including members of the genus Thalassolituus reported in our study (Yakimov et al., 2004b). However, no information of alkane degradation exists for Bermanella, the other major Oceanospirillales genus in our study (Pinhassi et al., 2009). Colwellia may metabolize a variety of oil compounds, including gaseous alkanes, aromatics and alkane-derived intermediates (Valentine et al., 2010; Redmond and Valentine, 2012; Mason et al., 2012; Gutierrez et al., 2013).

Colwellia has been associated with cold oil-polluted marine environments like Antarctic and Arctic seawater and ice (Yakimov et al., 2004a; Brakstad et al., 2008), and have also been detected in water injection systems of offshore oil platforms (Korenblum et al., 2010).

Biodegradation of aromatic HCs, both monoaromatics and PAHs, was slower than for n-alkanes, with the highest
bicarbonate concentrations of 30 μm were lower than in the smaller dispersions (10 μm) due to the different settling rates of the droplets. This finding is consistent with previous studies, which have shown that droplets produced from a single nozzle experience reduced biodegradation due to their rapid settling rate and the associated sedimentation of bacteria and DOM [Peters et al., 2015]. Therefore, our study has demonstrated that the choice of oil droplet size is an important factor to consider when studying biodegradation processes in the water column.
Dispersions were transferred to 2 l borosilicate flasks capped with teflon inserts (VWR International) which were completely filled and mounted on a carousel incubation system, as recently described (Brakstad et al., 2015). The carousel system rotated in clockwise direction at a velocity of 0.75 r.p.m. (Fig. S6).

**Experimental conditions**

The seawater used (Trondheimsfjord, outside the harbor area of Trondheim, 63°26′N, 10°26′E) and the experimental conditions have recently been described (Brakstad et al., 2015). The oil used was unweathered Macondo oil collected directly from the subsea containment system at the MC252 wellhead on the Oil Barge Massachusetts (MASS oil). COREXIT 9500A (Covaris™ S220 Sonicator; Covaris, Inc., Woburn, MA) to approximately 300 base pairs (bp), and fragments were used to synthesize indexed sequencing libraries using the TruSeq DNA Sample Prep Kit V2 (Illumina, Inc., San Diego, CA), according to manufacturer’s recommended protocol. Cluster generation was performed on the cB0T using the TruSeq PE Cluster Kit v3-cBot-HS (Illumina). Libraries were sequenced with an Illumina HiSeq 2000 at Nationwide Children’s Hospital (NCH) Biomedical Genomics Core (Columbus, OH) using the TruSeq SBS Kit v3 reagents (Illumina) for paired end sequencing with read lengths of 100 bp (200 cycles). Primary analysis (image analysis and basecalling) was performed using HiSeq Control Software version 1.5.15.1 and Real Time Analysis version 1.13.48. Secondary analysis (demultiplexing) was performed using Illumina CASAVA Software v1.6 on the NCH computed cluster. Sequence data were analysed by a custom in-house basic local alignment search tool (BLAST) (National Library of Medicine, Bethesda, MD) method to probe for organism identity. Quality-filtered sequence data (bases had a phred quality of ≥ 17, i.e. the probability of a correct base call was ~ 98%) were converted to FASTA formats. Reads were searched against a database comprising > 1.4 million DNA genome sequences obtained from the RefSeq database v. 8/22/2013 (National Center for Biotechnology Information, NCBI), Bethesda, MD) from invertebrate organisms excluding plastids and plasmids. Top hit BLAST results were filtered for sequences with ≥ 97% identity and sequence length of ≥ 80 bps and post-processed by removal of top hits for any given taxa < 0.01% (1:10 000) of the total BLAST hits. Filtered BLAST results were classified to report the relative abundance of organisms identified in the sample.

On primary analysis the RefSeq database was determined to contain genome sequences of *P. syringae* and *X. translucens* that produced an abundance of false positive results in the BLAST analysis, confirmed by secondary reference alignment using CLC Genomics Workbench. A second bioinformatics analysis was therefore performed, in which *P. syringae*, *P. fluorescens* and *X. translucens* sequences had been omitted from the output data to provide reliable in-depth analysis of the data.

The sequences from this study were uploaded on the NCBI Sequence Read Archive (accession numbers SAMN03392593 – SAMN03392608).

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**Chemical analyses and data treatment**

Water samples were solvent-solvent extracted for measurements of target analytes by gas chromatography-mass spectrometry (GC-MS), including C17-C22 n-alkanes, two- to five-ring PAHs and the recalcitrant oil biomarker compound 17x(9)β(H)-Hopane (30ab Hopane), as recently described (Brakstad et al., 2014). Volatile organic carbon (VOC) was determined by purge and trap GC-MS analyses (Brakstad et al., 2015). Aromatic VOC and SVOC target compounds included in GC-MS analyses are shown in Table S3.

Determined concentrations of target compound groups (Table S3) were normalized to 30ab Hopane (Prince et al., 1994), and biotransformation rates determined by non-linear regression analyses for calculation and 1st order rate coefficients (k1) and half-lives, including lag phases, as recently described (Brakstad et al., 2015).

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Column statistics were compared by Wilcoxon matched paired test in GraphPad Prism vs. 6.

**Other analyses**

Oil droplet concentrations and size distributions were determined by Coulter counter measurements (Beckman Coulter Multisizer 4; Beckman Coulter Inc., Brea, CA) fitted with a 280 μm aperture, for measurement of droplets within a diameter range of 5.6–100 μm (Brakstad et al., 2015).

Bacterial community diversity was evaluated by calculation of Shannon–Wiener diversity index (Shannon and Weaver, 1963).

**Conflict of Interest**

The authors have no conflict of interest to declare.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Fig. S1.** Relative abundances of bacterial classes in seawater without oil, 10 μm dispersions and 30 μm dispersions. The results are shown in seawater used for pre-acclimation 6 days prior to start of biodegradation (Pre), and in seawater and dispersions collected at the start (0) and after 2–64 days of biodegradation.
**Fig. S2.** Bacterial 16S rRNA PCR-DGGE of dispersions and seawater without oil. DGGE gels of samples at the start of acclimation (pre) and during the biodegradation period are shown in A, while comparison of samples as UPGMA dendrograms are shown in B. NC is negative control.

**Fig. S3.** Depletion of nC5-nC36 alkanes in dispersions with unfiltered or sterilized (Ster) seawater. The depletion of SVOC n-alkanes (nC10-nC36 alkanes) was determined after normalizing targeted n-alkanes against the recalcitrant biomarker 17α(H),21β(H)-Hopane (30ab Hopane). The depletion in dispersions with unfiltered seawater is shown as nonlinear decay curves, determined by 1st order rates with lag periods included. Data from non-linear regression analyses are shown in Table S3.

**Fig. S4.** Depletion of aromatic hydrocarbons in dispersions with unfiltered or sterilized (Ster) seawater. The depletion of SVOC compounds (naphthalenes and PAH) was determined after normalizing targeted n-alkanes against the recalcitrant biomarker 17α(H),21β(H)-Hopane (30ab Hopane). The depletion in dispersions with unfiltered seawater is shown as nonlinear decay curves, determined by 1st order rates with lag periods included. Data from non-linear regression analyses are shown in Table S3.

**Fig. S5.** Average oil droplet size distributions generated in the oil dispersion generator at different seawater flow rates after premixing of the oil with Corexit 9500A.

**Fig. S6.** Oil dispersion carousel with flasks mounted. Flasks are completely filled with oil dispersions, mounted on the carousel which is slowly rotated in clockwise direction at 0.75 r.p.m.

**Table S1.** Bacterial diversity determined by Shannon–Wiener index measurements.

**Table S2.** Kinetics data for groups of nC5-nC36 alkanes and aromatic compounds in 10 μm dispersions of Macondo oil determined by 1st order rate approach with lag phases included. Half-lives were determined from rate coefficients and lag phases included. The goodness of fit of non-linear regressions was determined as R².

**Table S3.** Aromatic hydrocarbon target groups from GC-MS analyses of volatile and semivolatile compounds included in this study.