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Chemical dispersants: Oil biodegradation friend or foe?

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ABSTRACT

Chemical dispersants were used in response to the Deepwater Horizon oil spill in the Gulf of Mexico, both at the sea surface and the wellhead. Their effect on oil biodegradation is unclear, as studies showed both inhibition and enhancement. This study addresses the effect of Corexit on oil biodegradation by alkane and/or aromatic degrading bacterial culture in artificial seawater at different dispersant to oil ratios (DORs). Our results show that dispersant addition did not enhance oil biodegradation. At DOR 1:20, biodegradation was inhibited, especially when only the alkane degrading culture was present. With a combination of cultures, this inhibition was overcome after 10 days. This indicates that initial inhibition of oil biodegradation can be overcome when different bacteria are present in the environment. We conclude that the observed inhibition is related to the enhanced dissolution of aromatic compounds into the water, inhibiting the alkane degrading bacteria.

1. Introduction

Large oil spills in the marine environment have been occurring since the early 1900s when oil and gas industries started extracting oil offshore and using oil tankers for transportation (Burger, 1997). From 1970 to 2012, approximately 5.75 million tons of oil were released to the oceans as a result of tanker incidents (Oil Tanker Spill Statistics, 2015). Release of oil into the marine environment is the main cause of marine pollution (Holliger et al., 1997). The largest accidental marine oil spill in the history of the petroleum industry is the Deepwater Horizon oil spill, in April 2010 in the Gulf of Mexico (McNutt et al., 2012).

Once oil is discharged into the marine environment, the properties of the spilled oil change due to a variety of physical, chemical and biological processes. These processes, collectively known as weathering (Boehm et al., 2008; Wardlaw et al., 2008) change the oil's composition, its physical/chemical behaviour and its toxicity. An important weathering process is evaporation which transfers light-weight and more volatile compounds to the atmosphere (Mansuy et al., 1997). Generally, this happens at the sea surface during the first few hours after a spill (Mansuy et al., 1997).

Another important weathering process is biodegradation by which bacteria partially or completely transform oil to compounds that can be further degraded and become more soluble in water (Lepo et al., 2003; Pontes et al., 2013). The rate of biodegradation depends on many parameters, such as temperature, presence of electron acceptors and nutrients, composition of the oil, and the active microbial population. Moreover, the presence of other compounds influences the biodegradation rate by either enhancing or inhibiting the microbial conversion or by changing the bioavailability of oil and its toxicity to bacteria. Therefore, weathering processes iteratively affect the ongoing degradation of the oil.

Traditionally, oil spill management often includes the application of chemical dispersants on oil slicks to remove these from the water surface. Dispersants reduce the interfacial tension between the oil and seawater, and stabilize the smaller oil droplets that are formed. As a result, the bioavailability of the oil increases, which can enhance oil biodegradation. At oil spills like the Deepwater Horizon spill, dispersants were injected under water to the crude oil (Kujawinski et al., 2011). In this case, the application of dispersants creates oil micro-emulsions, and benzene, toluene, ethylbenzene and xylene (BTEX) and polycyclic aromatic hydrocarbons (PAHs) compounds dissolve faster. Since micro-emulsions cannot be separated easily from the water phase, and this often leads to a higher apparent water solubility of these compounds (Zheng & Obbard, 2002).

Whether the addition of dispersant enhances or decreases oil degradation is not yet clear as in literature contradicting results were published (Brakstad et al., 2015; Lindstrom and Braddock, 2002). Previous studies showed the positive effect of Corexit on the oil biodegradation by mixed bacterial communities (Hazen et al., 2010; Valentine et al., 2012). However, some other studies have reported negative effect of Corexit on the oil biodegradation (Hamdan and Fulmer, 2011). Clearly, the scientific and technical understanding of the physicochemical interactions taking place and how they affect subsequently biological activities not (yet) complete.

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We hypothesise that these contradicting results in the literature may at least partially relate to the chemical composition of different types of the oil (crude oil, weathered oil), the absolute and relative concentration of oil and dispersants, and the characteristics of the microbial population (presence or absence of active alkane and aromatic degraders) applied in the experimental work.

The aim of this study is a proof of principle of the effect of dispersants to oil degradation. We have systematically assessed the biodegradation of crude and weathered oil in the water phase with different dispersant to oil ratios (DORs), and different bacterial cultures for either high or low energy hydrodynamic conditions by using dynamic or static experimental systems. This allows us to get insight into the competing effects of increased bioavailability on the biodegradation process under various conditions relevant for the marine environment. This will improve our understanding of the fate of chemically dispersed oil which is essential for assessing the added value of dispersant application.

2. Materials and methods

2.1. Oil and chemical dispersant

Macondo surrogate oil (MC252), kindly provided by BP (BP Gulf Science Data, n.d.), was used in this study. MC252 is classified as a light sweet crude oil and contains a high number of light hydrocarbons, saturated n-alkanes, PAHs, with low sulphur content (Ryerson et al., 2011). To simulate the impact of the evaporative weathering process, the oil was artificially evaporated to 30% weight loss. The oil was continuously stirred with a magnet stirrer at 70 °C for 3 h, while light flow of nitrogen gas constantly flowed over the oil's surface. This resulted in a viscous oil with less lighter hydrocarbons and aromatic compounds, and without hydrocarbon compounds smaller than C14 (Zhanfei et al. 2012).

Corexit® EC9500A (Nalco Holding Company, USA) was applied as a chemical dispersant. Dispersant solutions were prepared by diluting Corexit into demineralized water to make different ratios. Before addition to the batch bottles, the dispersant solutions were filtered sterilized (0.2 μm).

2.2. Bacterial cultures

Rhodococcus qingshengii TUHH-12 (DSMZ No. 46766), an alkane degrading culture, was used as inoculum in our experiments. The culture was isolated at the Technical University of Hamburg Harburg, Germany from a seawater sample collected in Spitzbergen, Norway, with an optimal growth temperature of 28 °C. This culture was maintained in mineral medium with n-hexadecane as the sole carbon source. The medium consisted of 2.6 g Na_2HPO_4, 1.33 g KH_2PO_4, 1 g $(NH_4)_2SO_4$ and 0.20 g $MgSO_4 \cdot 7H_2O$ dissolved in 1000 mL of demineralized water. The medium was adjusted to pH 7. After sterilization, 5 mL of trace element solution and 1 mL of vitamin solution were added. The composition of both solutions is mentioned in the experimental setup section. The bacterial culture was incubated for three days, and four days prior to the experiments, the culture was transferred into artificial seawater amended with medium salts and n-hexadecane as carbon source. This resulted in an active culture in its optimal growth phase, as controlled by measuring the Optical Density (OD) with a spectrophotometer (DR3900, Hach Lange) at a wavelength of 600 nm. An OD of 0.98 was taken as a culture in its optimal growth phase.

Pseudomonas putida F1 is an aromatic degrading culture and was purchased as a freeze dried culture from the German collection of microorganisms and cell cultures (DSMZ, No. 6899). After activation according to the DSMZ suggested procedure (Opening of ampules and rehydration of dried cultures, 2014), *P. putida* F1 was transferred to the DSMZ medium No. 457 and supplemented with toluene as a sole carbon source. Four days prior to the experiments, the culture was transferred into seawater amended with medium salts and toluene. This resulted in an active culture in its optimal growth phase, as controlled by measuring the OD. An OD of 0.305 was taken as a culture in its optimal growth phase.

2.3. Experimental setup

The growth medium consisted of (per litre of water) 10.4 g Na_2HPO_4; 5.32 g KH_2PO_4; 4 g $(NH_4)_2SO_4$; 0.8 g $MgSO_4 \cdot 7H_2O$; 1 mL of trace element solution (2 g/L $FeCl_3 \cdot 4H_2O$; $CoCl_2 \cdot 6H_2O$ 2 g; 1 g/L $CaCl_2 \cdot 2H_2O$; 0.5 g/L $MnCl_2 \cdot 4H_2O$; 30 mg/L $CuCl_2 \cdot 2H_2O$; $ZnCl_2$ 50 mg/L; 50 mg/L HBO_3; 90 mg/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$; 100 mg/L $Na_2SeO_3 \cdot 5H_2O$; 50 mg/L $NiCl_2 \cdot 6H_2O$; 1 g/L EDTA; 1 mL/L 36% HCl); resazurin 0.5 g/L, and 1 mL of vitamin solution (0.106 mg/L biotin; 0.005 mg/L folic acid; 0.0025 mg/L pyridoxal-HCl; 0.015 mg/L lipoic acid; 0.0125 mg/L riboflavin; 0.266 mg/L thiamine-HCl; 0.413 mg/L Ca-D-pantothenate; 0.0125 mg/L cyanocobalamin; 0.0125 mg/L p-aminobenzoic acid; 0.0125 mg/L nicotinic acid). To avoid precipitation while mixing sea salt and growth medium, the phosphate and sulphate solutions were prepared separately and subsequently mixed while stirring.

Biodegradation of crude and weathered oil was tested in 125 mL bottles. The bottles contained 20 mL of medium, suitable for bacterial growth, in artificial sea water (32 g of artificial coral sea salt, AquaHolland, The Netherlands) in 1 L demineralized water. After autoclaving for 25 min at 121 °C, the bottles were opened in a laminar flow cabinet, and the filter sterilized vitamin solution was added. Depending on the condition, 0.1 g crude or weathered oil, chemical dispersant (DOR 1:20 or 0:1), and 2 mL bacterial culture were added. The bottles were sealed with a Viton rubber stopper (Rubber BV, Hilversum, The Netherlands), and closed with aluminium caps. The bottles were incubated at 20 °C in the dark on a rotary shaker (120 rpm) (dynamic conditions) or under static conditions. Sterilized abiotic controls were taken along as well.

2.4. Oxygen consumption of crude oil at different DORs

Oxygen consumption by *R. qingshengii* TUHH-12 was measured in batches with different DORs. The tested DORs (w/w) were 1:1, 1:10, 1:20, 1:50, 1:100, 1:1000, and 0:1 (no dispersant). These ratios were prepared by adding 5000, 500, 250, 100, 50, 10, 5 and 0 mg dispersant per L of solution to which 0.1 g crude oil was added. Oxygen concentration was measured regularly, and pure oxygen was added when the concentration of oxygen in the gas phase dropped below 10% (v:v). Based on the results, DORs 1:20 and 0:1 were chosen for our further experiments.

2.5. Effect of chemical dispersant on the biodegradation of BTEX and n-alkanes

A total of 6 sets of experiments were conducted, with either *R. qingshengii* TUHH-12 or *P. putida* F1, dynamic or static, and abiotic control (Table 1). Each set contained 6 conditions, representing different types of oil (crude, weathered, or no oil) and two DORs (1:20 and 0:1), and were tested in duplicate.

Table 1
Overview of the experimental sets.

	Crude oil	Weathered oil	No oil	Dynamic	Static
R. qingshengii TUHH-12	☑	☑	☑	☑	☑
P. putida F1	☑	☑	☑		☑
R. qingshengii TUHH-12 and *P. putida* F1	☑	☑	☑		☑
Abiotic control	☑	☑	☑		☑

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During the incubations, the batches were monitored by regular analyses of oxygen and carbon dioxide in the headspace. In addition, the headspace of the batches was sampled for analysis of BTEX at selected time intervals, and the complete content of a batch was sacrificed for solvent extraction followed by analyses of n-alkanes (C11–C40).

2.6. Chemical analysis

Oxygen and carbon dioxide were analysed by gas chromatography (GC, Shimadzu (Shimadzu, Kyoto, Japan)). Headspace samples of 50 μL taken from batch bottles by glass syringe were injected directly into the GC. The GC was equipped with two packed columns in parallel (Porabond Q, 60–80 mesh, 2 m length, 3 mm internal diameter and Molsieve 5a, 60–80 mesh, 2 m length, 3 mm internal diameter; Varian, Middelburg, The Netherlands) and a thermal conductivity detector. Temperatures were constant with injector of 120 °C, column of 75 °C and detector of 150 °C. Helium was used as the carrier gas at a constant flow rate of 30 mL/min. Calibration was done with gas samples containing 2.98% oxygen and 24.80% carbon dioxide.

Concentrations of BTEX compounds were analysed on a GC (Fisions 8000) equipped with a CP-Sil 8CB column (25 m × 0.53 mm × 5.0 μm, Chrompack, Middelburg, The Netherlands). Samples were extracted from the headspace of the batches (10 mL) by solid phase micro extraction (SPME) with a 100 μm polydimethylsiloxane (PDMS) coated fibre. The fibre was placed in headspace of the batch bottles for 2 min, and desorbed in the injection port of the GC at 200 °C. The GC was operated in constant flow mode (2.5 mL/min). The column temperature increased from 40 °C to 130 °C at 5 °C/min, followed by a constant temperature for 5 min. BTEX compounds were detected with a Flame Ionization Detector (FID) at 300 °C. The GC performance was tested by starting each GC sequence with an external standard. The identity of each individual BTEX compound was determined by using the retention time in a standard solution. Based on the peak area of each individual BTEX compound, the relative concentration of each compound was calculated, and finally summed to calculate the total relative concentration of the BTEX compounds per sample.

Prior to n-alkanes quantification with gas chromatography, the samples were extracted with acetone and n-hexane, according to method NEN 5733. The n-hexane was dried with Na_2SO_4 and 1 μL was injected automatically by an auto sampler into a HP 6890 GC with a CP-SIMDIST column (10 m × 0.32 mm × 0.1 μm) and Flame Ionization Detector at 320 °C with helium as the carrier gas. Following 5 min at an initial temperature of 40 °C, the temperature was increased at 10 °C/min to a final temperature of 300 °C. Each individual n-alkane (C11–C40) was determined by using the retention time in a standard solution. Based on the peak area of each individual n-alkane, the relative concentration of each n-alkane was calculated per data point, and finally summed to calculate the total relative concentration of the n-alkanes. *R. qingshengii* TUHH-12 is an n-alkane degrading culture, therefore we report the degradation of n-alkanes only and not of total petroleum hydrocarbons (TPH).

3. Results

3.1. Biodegradation of crude oil at different DORs

To determine the rate and extent of biodegradation of crude oil by *R. qingshengii* TUHH-12, the oxygen consumption was measured daily in batches with different DORs (Table 2). The oxygen consumption rate changed with the amount of Corexit. At DORs of 1:10–1:100 oxygen consumption rates were approximately 0.16 mmol/day. At a high DOR of 1:1 the lowest oxygen consumption rate was seen (0.13 mmol/day) while oxygen consumption rate was highest with DORs of 1:1000 and 0:1 (0.36 mmol/day).

Based on these results, DORs of 1:20 and 0:1 were chosen for further experiments. The targeted DOR was 1:20 as it was applied in the Gulf of Mexico in the response phase after the Deepwater Horizon oil spill in 2010.

3.2. Dispersant effects on oil biodegradation by R. qingshengii TUHH-12

The degradation of both crude and weathered oil at DORs of either 1:20 or 0:1 by *R. qingshengii* TUHH-12 was monitored by measuring the oxygen consumption and the concentration of n-alkanes under both dynamic and static conditions.

3.2.1. Oxygen consumption

In absence of Corexit, high oxygen consumption rates were found already after a few days (Fig. 1), both for crude and weathered oil degradation. They behaved similar and reached their maximum oxygen consumption after 30 days. When Corexit was applied, the oxygen consumption was delayed for both crude and weathered oil. The low oxygen consumption for weathered oil lasted 10 days, after which it became comparable to the oxygen consumption in batches without Corexit. For crude oil, low oxygen consumption rates lasted at least 50 days (full incubation time), and never reached the high oxygen consumption, as the other batches. In the control batches without oil, very limited oxygen consumption was observed, most likely due to the degradation of Corexit (results not shown).

No differences were found between static and dynamic incubations (results not shown); indicating that mass transfer was not a limiting factor under static conditions.

3.2.2. N-alkanes biodegradation

The biodegradation of the n-alkanes from crude or weathered oil at DORs of either 1:20 or 0:1 was assessed by measuring the sum of individual n-alkanes (C11 to C40) during our degradation experiments (Fig. 2).

At a DOR of 0:1, biodegradation of n-alkanes in both crude and weathered oil started immediately. After 5 days however, n-alkanes continued to be consumed from weathered oil while consumption of n-alkanes from crude oil stopped. At a DOR of 1:20, biodegradation of both crude and weathered oil was delayed and less degradation was found. However, the extent of n-alkane removal after 38 days was similar to incubations without dispersant for weathered oil.

3.3. Dispersant effects on oil biodegradation by R. qingshengii TUHH-12 and/or P. putida F1

Similar degradation experiments as described for *R. qingshengii* TUHH-12 were also performed with the aromatic compounds degrading culture *P. putida* F1 and with both pure cultures combined in the presence and absence of Corexit.

Table 2
Oxygen consumption rates at different DORs.

DORs	1:1	1:10	1:20	1:50	1:100	1:500	1:1000	0:1
Corexit (mg/L)	5000	500	250	100	50	10	5	0
O_2 consumption (mmol/day)	0.13 ± 0.08	0.17 ± 0.05	0.17 ± 0.02	0.17 ± 0.01	0.15 ± 0.02	0.25 ± 0.02	0.34 ± 0.03	0.37 ± 0.08

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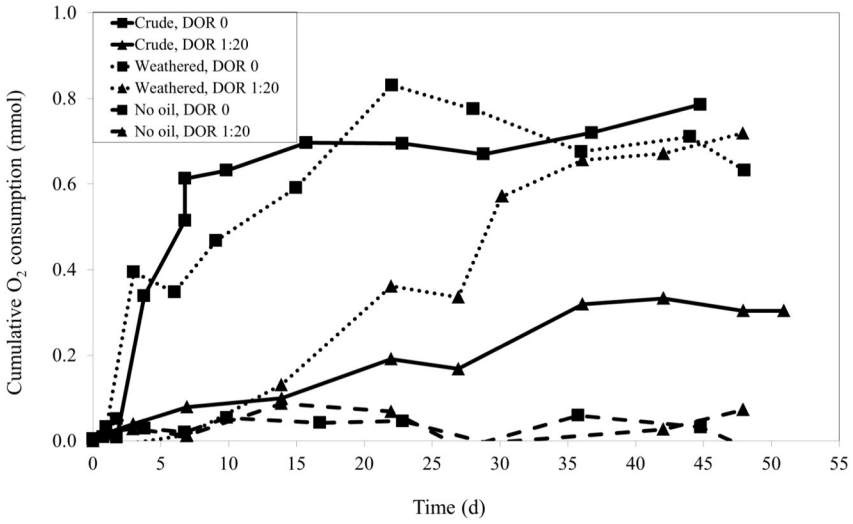

Fig. 1. Cumulative oxygen consumption by *R. qingshengii* TUHH-12 during biodegradation of crude or weathered oil with and without Corexit.

3.3.1. Oxygen consumption

Oxygen consumption profiles with *R. qingshengii* TUHH-12 or *P. putida* F1 or a combination of both pure cultures are depicted in Fig. 3.

High oxygen consumption was observed for incubations of crude oil with *R. qingshengii* TUHH-12 at DOR 0:1 and for the combination of both pure cultures at both DORs, although oxygen consumption at DOR 1:20 only increased after a lag phase of 10 days. Oxygen consumption with the aromatic compounds degrading culture *P. putida* F1 was much lower compared to the other incubations with *R. qingshengii* TUHH-12 and/or a combination of both cultures, as Macondo Crude oil is light sweet oil and contains mostly aliphatic hydrocarbons and less aromatic compounds.

3.3.2. n-Alkane biodegradation

The relative concentration of the summed individual n-alkanes for the tested conditions is given in Fig. 4. Without dispersant, a faster n-alkane removal was observed compared to the DOR 1:20. Furthermore, a slower initial n-alkane degradation was observed for *R. qingshengii* TUHH-12 and the combined culture experiments during the first 10 days of incubation for DOR 1:20 than 0:1.

3.3.3. BTEX compounds biodegradation

The relative concentration of BTEX compounds in tested conditions is given in Fig. 5.

Limited BTEX compounds degradation was observed in the presence of n-alkane degrading *R. qingshengii* TUHH-12 (see Fig. 5), since *R. qingshengii* TUHH-12 is an alkane degrading culture.

In the incubations with *P. putida* F1, a fast initial BTEX degradation was observed, especially when treated with Corexit. BTEX compounds degradation was highest when both cultures were present. Concentration of the BTEX compounds were increased when Corexit was applied (Table 3).

4. Discussion

We studied the effect of adding chemical dispersant to crude and weathered oil and the consequential effect on oil biodegradation in the presence of n-alkane or aromatic compounds degrading culture or a combination of both pure cultures. To mimic the effect of the applied Corexit on the oil biodegradation at the surface or under water, we used either crude or weathered oil in our experiments. Crude oil is representative for the fresh spilled oil at the sea depth/oil wellhead.

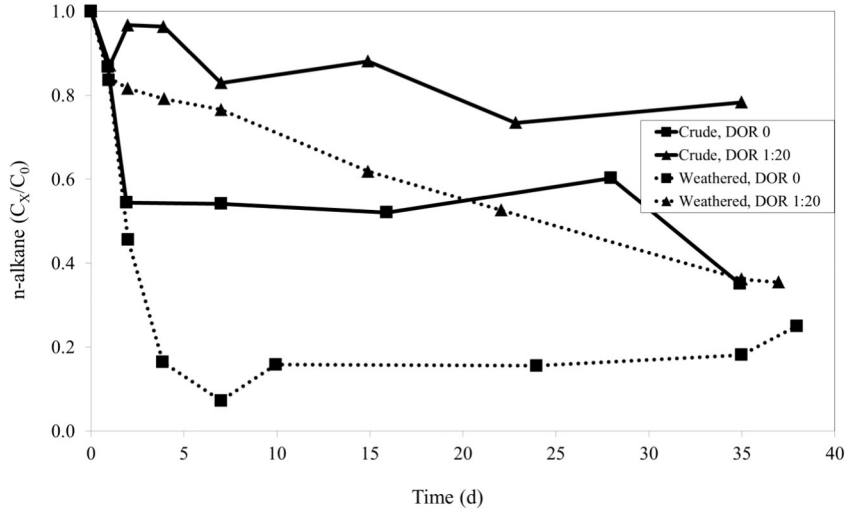

Fig. 2. Relative n-alkane concentration in the presence of *R. qingshengii* TUHH-12 during biodegradation of crude or weathered oil with and without Corexit.

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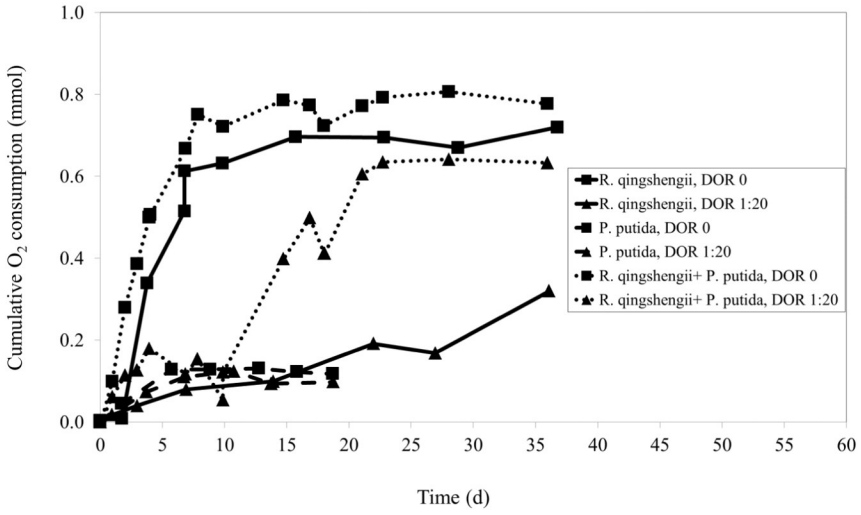

Fig. 3. Cumulative oxygen consumption by *R. qingshengii* TUHH-12 and/or *P. putida* F1 during biodegradation of crude oil with and without Corexit.

Weathered oil represents the oil slicks at the surface, where a large part of the volatile compounds was evaporated to the atmosphere. By using pure cultures, we tried to limit uncertainties caused by population dynamics that may obscure the results when working with more complex microbial systems.

Chemical dispersants are often applied to manage and control oil spills in DORs that generally range from 1:1 to 1:50 (Use of dispersants to treat oil spills: Technical Information Paper 4, 2011). The DOR used depends on the chosen dispersant, the application method, the type of oil, and the environmental conditions. During the Deepwater Horizon oil spill response phase, Corexit® EC9500A was applied in a targeted overall ratio of 1:20 (Kujawinski et al., 2011).

We tested different DORs and found that the applied Corexit concentrations directly affected the oxygen consumption rate by *R. qingshengii* TUHH-12. A higher Corexit concentration (lower DOR) resulted in a lower oxygen consumption rate, indicating that the oil biodegradation was inhibited. In our detailed experiments with crude and weathered oil and the tested pure bacterial cultures we were able to show the reason of the observed inhibition.

According to our results, when *R. qingshengii* TUHH-12 was applied, the inhibition of Corexit on crude oil biodegradation lasted at least 50 days (our incubation time), whereas weathered oil biodegradation was inhibited only 10 days. Weathered oil contains less light aliphatic and aromatic compounds compared with crude oil. Our chemical analyses showed that crude oil had high concentrations of BTEX compounds (around 2000 μg/g oil), whereas our weathered oil hardly contained BTEX compounds (<0.25 μg/g oil). According to our results, the use of Corexit with crude oil resulted in higher concentrations of dissolved lighter aromatic compounds (BTEX) in the dispersed small oil droplets and in the water phase (Prince, 2015). Those higher concentrations of BTEX compounds in the water column could decrease the activity of the bacteria, which could explain the retardation effect we observed for *R. qingshengii* TUHH-12.

The experiments performed with the BTEX degrading culture, *P. putida* F1 in combination with the n-alkane degrading culture, *R. qingshengii* TUHH-12 resulted in an immediate degradation of BTEX compounds, followed by degradation of n-alkanes. With the combination of both culture the inhibitory effect of chemically dispersed crude oil on the degradation lasted only 10 days. We believe that the initial inhibition of the activity of *R. qingshengii* TUHH-12 by the dissolved light aromatic compounds is decreased through biodegradation of these compounds by *P. putida* F1. Since weathered oil is devoid of aromatic compounds, this inhibitory effect of high concentration of dissolved BTEX for *R. qingshengii* TUHH-12 did not occur in chemically dispersed

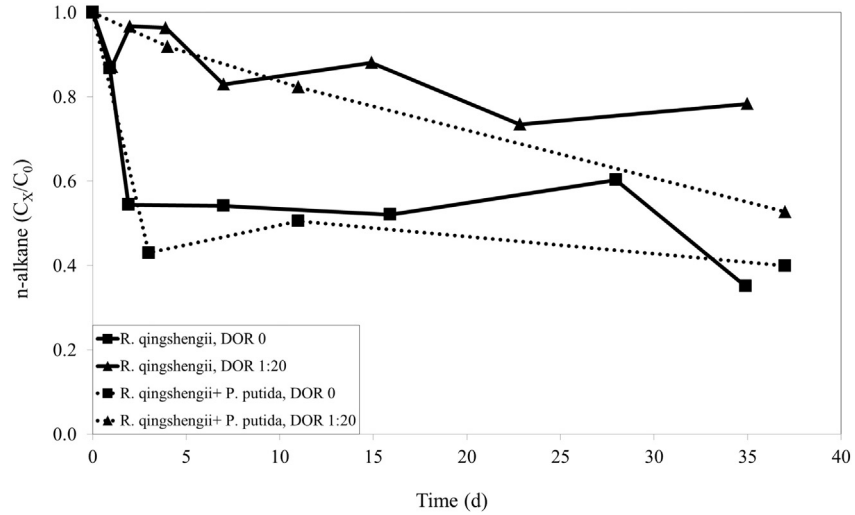

Fig. 4. Relative n-alkane concentration in the presence of *R. qingshengii* TUHH-12 and/or *P. putida* F1 during biodegradation of crude oil with and without Corexit.

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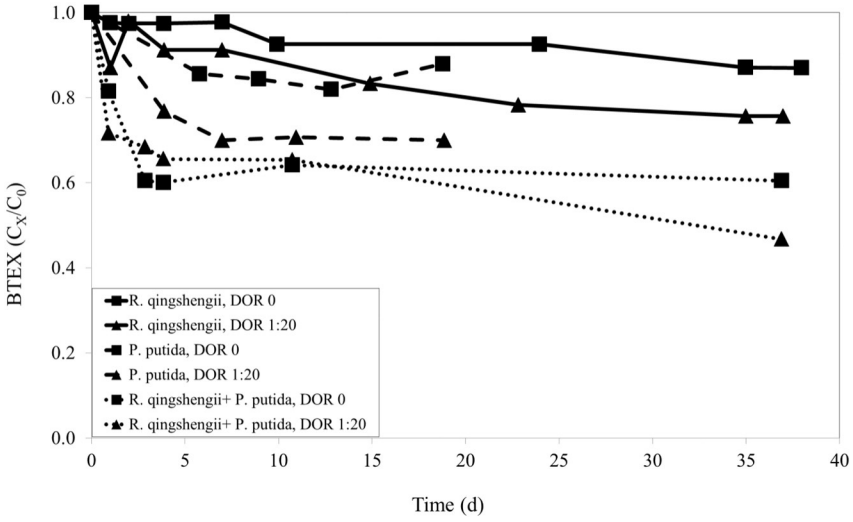

Fig. 5. Relative BTEX concentration in the presence of *R. qingshengii* TUHH-12 and/or *P. putida* F1 during biodegradation of crude oil with and without Corexit.

weathered oil. Finally, the biodegradation of crude and weathered oil was comparable under both dynamic and static conditions, indicating that mass transfer was not a limiting factor in static condition and addition of Corexit does not tackle the rate limiting step.

By the addition of Corexit, the oil water interface increases, and consequently the bioavailability of the oil increases, thus enhancing its degradation (Southam et al., 2001). Some studies showed that Corexit improves the oil biodegradation (Hazen et al., 2010), however we did not find any case of improvement on oil biodegradation by using Corexit. Our study shows that chemically dispersed crude oil inhibits the biodegradation, and not Corexit itself.

We believe that the inhibitory effect of Corexit on biodegradation of the oil reported in the literature depends on the choice of weathered or crude oil, the experimental design and the applied bacteria. There are a variety of oil degrading bacterial communities present in marine environments which are capable of degrading different oil compounds (Lloyd et al., 2010; Orcutt et al., 2010). These communities can consume oil from natural seeps and their population will increase during a spill in other to cope with the high concentration of the spilled oil (Hazen et al., 2010; Joye et al., 2014; Mason et al., 2012). When dispersants are injected under water, however, the maximum concentration of lighter aromatic compounds can be reached (Kleindienst et al., 2015). We believe that the inhibition of crude oil biodegradation due to Corexit addition that we found in our lab experiments, will not be limited in natural marine environment, as the increased dissolved aromatic compounds can be degraded by naturally existing bacterial communities.

5. Conclusion

The application of Corexit on crude oil resulted in an increased solubility of the aromatic compounds of the oil in seawater. This resulted in higher concentrations of these aromatic compounds which inhibited oil biodegradation, especially when no aromatic compounds degrading culture were present. When a combined culture was used, this retardation was overcome after 10 days of incubation, as the aromatic degrading culture decreased the BTEX compounds concentration which decreased the activity of the n-alkane degrading culture. For weathered oil this mechanism does not play a role as hardly any aromatic compounds are present.

Table 3
Individual BTEX concentration (μg/L) analysed in the headspace of the batches with and without Corexit.

	Benzene	Toluene	Ethylbenzene	*o*-, *m*-, and *p*-Xylene
DOR 0	133 ± 3.67	67 ± 1.24	9 ± 0.5	43 ± 1.06
DOR 1:20	155 ± 6.18	75 ± 1.64	10 ± 0.11	47 ± 0.22

List of abbreviations

BTEX	Benzene, toluene, ethylbenzene and xylenes
DORs	dispersant to oil ratios
OD	Optical Density
TPH	total petroleum hydrocarbon

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