Marine Pollution Bulletin xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Chemical dispersants: Oil biodegradation friend or foe?

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ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 12 April 2016
Accepted 19 April 2016
Available online xxxx

Keywords:
Biodegradation
Dispersant
Oil spill
Enhanced dissolution

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.

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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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http://dx.doi.org/10.1016/j.marpolbul.2016.04.044
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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	☑	☑	☑		☑

Please cite this article as: Rahsepar, S., et al., Chemical dispersants: Oil biodegradation friend or foe?, Marine Pollution Bulletin (2016), http://dx.doi.org/10.1016/j.marpolbul.2016.04.044

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