Marine biodegradation of crude oil in temperate and Arctic water samples

Mette Kristensen [a], Anders R. Johnsen [b], Jan H. Christensen [a],[*]

[a] Analytical Chemistry, Section for Environmental Chemistry and Physics, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark
[b] Geological Survey of Denmark and Greenland (GEUS), Department of Geochemistry, Øster Voldgade 10, 1350 Copenhagen K, Denmark

HIGHLIGHTS

- Biodegradation was assessed after exposure to $100\,mg\,L^{-1}$ crude oil in microcosms.
- The degradation order in Disko Bay samples were n-alkanes > alkyltoluenes > PAHs.
- The degradation order in North Sea samples were PAHs > alkyltoluenes > n-alkanes.
- The differences in degradation orders will affect risk assessment in arctic regions

GRAPHICAL ABSTRACT
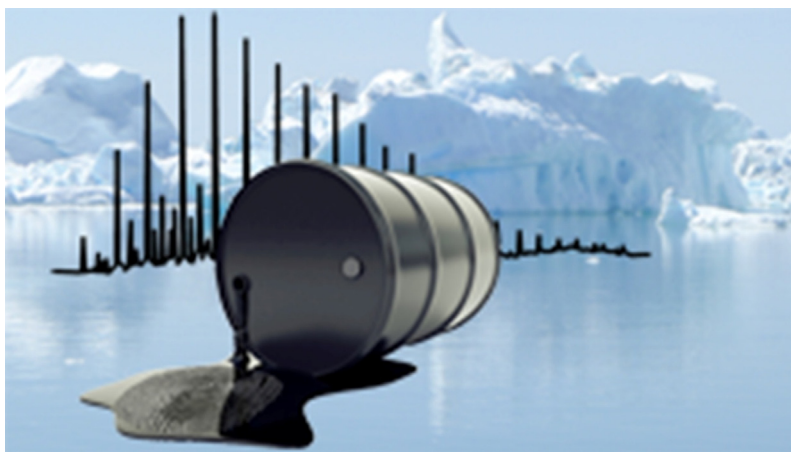
ARTICLE INFO

ABSTRACT

Despite increased interest in marine oil exploration in the Arctic, little is known about the fate of Arctic offshore oil pollution. Therefore, in the present study, we examine the oil degradation potential for an Arctic site (Disko Bay, Greenland) and discuss this in relation to a temperate site (North Sea, Denmark). Biodegradation was assessed following exposure to Oseberg Blend crude oil ($100\,mg\,L^{-1}$) in microcosms. Changes in oil hydrocarbon fingerprints of polycyclic aromatic hydrocarbons (PAHs), alkyl-substituted PAHs, dibenzothiophenes, n-alkanes and alkyltoluenes were measured by gas chromatography-mass spectrometry (GC–MS). In the Disko Bay sample, the degradation order was n-alkanes > alkyltoluenes (para- > meta- > ortho-isomers) > PAHs and dibenzothiophenes, whereas, the degradation order in the North Sea samples was PAHs and dibenzothiophenes > alkyltoluenes > n-alkanes. These differences in degradation patterns significantly affect the environmental risk of oil spills and emphasise the need to consider the specific environmental conditions when conducting risk assessments of Arctic oil pollution.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Focus on oil pollution is developing around the Arctic and other high-latitude environments due to climate change and intensified

* Corresponding author.

exploration for new oil resources. The Arctic regions differ from temperate regions in many ways, which may influence the fate of oil pollution. Low temperature, low light intensity [1,2], ice coverage [2] and lack of microbial adaptation [3,4] all indicate that oil pollution of the Arctic marine environment may be more persistent than oil pollution in temperate and tropic climates.

In 2011, marine oil exploration started west of Greenland with 20 licensed areas covering 201.131 km^2 [5]. Oil exploration and exploitation in this region is associated with an increased risk of accidental oil spills due to sea ice, drifting icebergs, and the depth at which the drillings take place (>350 m), but there is no knowledge on the fate of oil pollution in deeper parts of the water column in the Arctic. For the Macondo Deep Water Horizon blowout in the Gulf of Mexico, pre-spill adaptation of the microbial degrader community led to surprisingly fast oil degradation deep in the water column, probably caused by microbial adaptation from natural oil seeps [6]. Therefore, we wanted to test whether a similar adaptation was present in the marine water column at Disko Bay (Western Greenland).

When investigating the fate of oil spills, unfortunately, it is common to analyse only for selected compounds in the complex mixture of crude oil or to quantify only the sum of total petroleum hydrocarbon. When focusing on a few target compounds, information on the entire mixture is limited. Many compounds in crude oil show large variations in physical-chemical properties and represent different classes that are important in different time aspects of an oil spill. The composition of *n*-alkanes, alkyltoluenes and monoaromatics changes rapidly in the initial phase, whereas, polycyclic aromatic hydrocarbons (PAHs), alkyl-substituted PAHs and NSO aromatics (nitrogen-, sulphur- and oxygen containing compounds) are more recalcitrant and may be present for years or decades [7].

Since the 1980s, chemical fingerprinting of oil hydrocarbons ('oil hydrocarbon fingerprinting') has been used for the identification of oil spill sources and for assessing weathering effects [8]. Chemical fingerprinting gives the relative composition of hundreds of compounds in a complex mixture by using specific analytical methods. A general trend in oil hydrocarbon biodegradation is the degradation order of *n*-alkanes > monoaromatic compounds (including benzene, toluene, ethylbenzene and xylenes known as BTEX) > branched and cyclic alkanes > polycyclic aromatic compounds (decreased degradation with increased number of aromatic rings), and a negative correlation between degradation and degree of alkyl substitution [8–10]. *n*-alkyltoluenes are not commonly included in the chemical fingerprinting of oil spills, but have been suggested as a complementary analysis to *n*-alkanes in the preliminary screening of oil pollution [11]. The analysis of alkyltoluenes can provide additional information about biodegradation rates and patterns in the initial stage of an oil spill, as well as in source identification.

The aim of our study was to evaluate the natural potential for biodegradation of crude oil in subsurface Arctic water, presumably with limited microbial adaptation to oil hydrocarbons. Biodegradation was assessed by analysing *n*-alkanes, alkyltoluenes, PAHs and dibenzothiophenes to give a complex fingerprint of the crude oil changes. Additionally, the degradation potential of a typical shallow, temperate environment with oil production and shipping was evaluated to emphasise the significant differences in degradation due to environmental conditions.

Diagnostic ratios are a commonly used method in oil spill assessments to distinguish between degradation processes. The method is robust and insensitive to changes in extraction efficiency or analysis. Diagnostic biodegradation ratios, such as *n*C$_{17}$/pristane, *n*C$_{18}$/phytane, PAH isomers or other compounds with the same physical-chemical properties but different susceptibilities to microbial attack, are used as markers of biodegradation,

and to identify sources of hydrocarbon pollution [12–14]. A change in diagnostic ratios is evidence for biodegradation, which is especially important in subsurface spills where biodegradation is the only true degradation process.

To examine the biodegradation potential of the two environments under laboratory conditions, a microcosm study was set up with the addition of typical North Sea crude oil (Oseberg Blend) to subsurface water from the Disko Bay, Greenland (Arctic) and to surface water from the North Sea, Denmark (temperate) in 1 L microcosms. The degradation of crude oil and changes in the microbial degrader communities was followed over a 10-week incubation period.

2. Materials and methods

2.1. Chemicals: The North Sea crude oil, Oseberg Blend (Statoil, Norway), was used for oil incubations

For solid phase extraction and gas chromatography - mass spectrometry (GC–MS), methanol (HPLC grade, Rathburn), *n*-pentane (HPLC grade, VWR Chemicals), acetone (HPLC grade, Rathburn), dichloromethane (HPLC grade, Rathburn), ammonia solution (28–30%, MERCK) and ammonium acetate (≥98%, MERCK) were used. For instrument performance assessment, a GC–MS tune mixture was used. This was composed of benzidine (99.9%, Sigma), 1,1,1-Trichloro-2,2-bis(4-chlorophenyl) ethane (99.7%, Sigma), pentachlorophenol (99.9%, Sigma, Supelco) and decafluorotriphenylphosphine (99.3%, Sigma, Supelco) (50 µg mL^{-1} of each). For further quality assurance and quality control, a reference sample composed of 19 PAHs with 2–6 aromatic rings and 15 deuterated PAHs with 2–6 aromatic rings was analysed between samples. Internal standard and recovery standard solutions consisted of mixtures of deuterated PAHs and nitrogen- and oxygen-containing polycyclic aromatic compounds in methanol. The internal standard stock solution contained a mixture of d_8-naphthalene (13.42 µg mL^{-1}, 99%), d_8-dibenzothiophene (8.82 µg mL^{-1}, 98%), d_{10}-acenaphthene (7.96 µg mL^{-1}, 98%), d_{10}-phenanthrene (13.05 µg mL^{-1}, 98%), d_{10}-pyrene (8.27 µg mL^{-1}, 98%) and d_{10}-fluorene (7.68 µg mL^{-1}, 98%) from the Cambridge Isotope Lab and d_9-acridine (12.50 µg mL^{-1}, 98.7%, CHIRON AS), d_8-carbazole (12.50 µg mL^{-1}, 98.9%, CHIRON AS), d_8-antraquinonone (9.05 µg mL^{-1}) and d_5-phenol (13.78 µg mL^{-1}, 98%, Sigma–Aldrich). The recovery standard stock solution contained d_{10}-anthracene (7.07 µg mL^{-1}, 98%, Dr. Ehrenstorfer Gmb), and d_8-acenaphthylene (7.76 µg mL^{-1}, 98%), d_{10}-fluoranthene (6.91 µg mL^{-1}, 98%), d_{12}-benzo[a]anthracene (7.12 µg mL^{-1}, 98%), d_{12}-benzo[a]pyrene (8.40 µg mL^{-1}, 98%) and d_{12}-indenol(1,2,3–c and d) pyrene (6.84 µg mL^{-1}, 98%) from the Cambridge Isotope Lab, and hydrochloric acid (37%, VWR Chemicals).

Substrates used for Most-Probable-Number enumeration (MPN) were hexadecane (99%, Sigma–Aldrich), *m*-xylene (>99%, Fluka), 2-methylnaphthalene (97%, Sigma–Aldrich), 1-naphtol (≥99%, Sigma–Aldrich), 1-naphthoic acid (98%, Alfa Aesar) and silicone oil AR20 (Sigma–Aldrich). Dilutions were made in pre-mixed Bushnell–Haas minimal mediums containing magnesium sulphate (0.2 g L^{-1}), calcium chloride (0.02 g L^{-1}), monopotassium dihydrogen phosphate (1.0 g L^{-1}), diammonium hydrogen phosphate (1.0 g L^{-1}), potassium nitrate (1.0 g L^{-1}) and ferric chloride (0.05 g L^{-1}) (Difco^{TM}, Becton, Dickinson and Company, Sparks, MD21152, USA) [15].

2.2. Sampling

Two locations, the North Sea and the Disko Bay, represented typical temperate and Arctic marine environments. The North Sea