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Whole cell bioreporter application for rapid detection and evaluation of crude oil spill in seawater caused by Dalian oil tank explosion

Dayi Zhang [a], *Aizhong Ding* [b], *Shuangchao Cui* [b], *Cheng Hu* [c], *Steven F. Thornton* [a], *Junfeng Dou* [b], *Yujiao Sun* [b], *Wei E. Huang* [a,*]

[a] *Kroto Research Institute, University of Sheffield, Sheffield S3 7HQ, UK*
[b] *College of Water Sciences, Beijing Normal University, Beijing 100875, PR China*
[c] *Liaoning Academy of Environmental Sciences, Shenyang 110031, PR China*

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ABSTRACT

Accidents involving the release of crude oil to seawater pose serious threat to human and animal health, fisheries and marine ecosystems. A whole cell bioreporter detection method, which has unique advantages for the rapid evaluation on toxicity and bioavailability, is a useful tool to provide environmental risk assessments at crude oil-contaminated sites. *Acinetobacter baylyi* ADPWH_alk and ADPWH_recA are chromosomally-based alkane and genotoxicity bioreporters which can be activated to express bioluminescence in the presence of alkanes and genotoxic compounds. In this study, we applied *Acinetobacter* ADPWH_alk and ADPWH_recA bioreporters to examine six seawater and six sediment samples around the Dalian Bay four weeks after an oil tank explosion in Dalian, China in 2010, and compared the results with samples from the same sites one year after. The results of bioreporter detection suggest that seawater and sediments from five sites (DB, NT, JSB, XHP and FJZ) four weeks after the oil-spill were contaminated by the crude oil with various extents of genotoxicity. Among these six sites, DB and NT had high oil contents and genotoxicity, and JSB had high oil content but low genotoxicity in comparison with an uncontaminated site LSF, which is located at other side of the peninsula. These three sites (DB, NT and JSB) with detectable genotoxicity are within 30 km away from the oil spill point. The far-away two sites XHP (38.1 km) and FJZ (31.1 km) were lightly contaminated with oil but no genotoxicity suggesting that they are around the contamination boundary. Bioreporter detection also indicates that all six sites were clean one year after the oil-spill as the alkane and genotoxicity were below detection limit. This study demonstrates that bioreporter detection can be used as a rapid method to estimate the scale of a crude oil spill accident and to evaluate bioavailability and genotoxicity of contaminated seawater and sediments, which are crucial to risk assessment and strategic decision-making for environmental management and clean-up.

1. Introduction

With increasing demand for oil resources, the frequency of accidental oil spills also increased in recent years, which posed a serious threat to human health, fisheries and ecosystems (Peterson et al., 2003; Piatt et al., 1990). Highly publicised examples include the Exxon Valdez oil spill in Prince William Sound, Alaska, in 1989 (Bence et al., 1996;

* *Corresponding author*. Tel.: +44 114 2225 745; fax: +44 114 2225 700.
E-mail address: w.huang@shef.ac.uk (W.E. Huang).
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Bragg et al., 1994) and the Deepwater Horizon oil spill in Gulf of Mexico in 2010 (Camilli et al., 2010), which had significantly environmental impacts. Besides these large impact cases, there are many accidental crude oil spills at smaller scale, which require a rapid detection of toxicity and bioavailability of contaminated water and sediments for risk assessments. Traditional chemistry and toxicity analyses for such complex organic mixtures could be laborious, time-consuming and costly, which face a challenge to achieving a fast detection *in-situ* demanded by sudden and accidental events. Bioreporters can be useful in this respect as a complementary tool to traditional chemical analysis and ecotoxicological assessment of mixed compounds in environmental samples (Tecon and van der Meer, 2008). One case that required a rapid environmental assessment is the oil spill caused by storage-tank explosion that occurred close to Dalian China, in 2010, where the damaged pipes released about 1500 tonnes of crude oil into the nearby harbor and the Yellow Sea.

Crude oil consists of a complex mixture of hydrocarbons and other organic compounds with various molecular weights. Alkanes are saturated hydrocarbons and the main constituents of crude oil, accounting up to 50% of the total oil composition depending on the oil source (Head et al., 2006). They can be linear (n-alkanes), circular (cyclo-alkanes), and branched (iso-alkanes), and are usually insoluble in water. Several alkane bioreporters have been constructed and applied previously. One of the first attempts was to clone *alkS* gene and corresponding *palkB* promoter from *Pseudomonas oleovorans* into plasmids of *Escherichia coli* DH5α, which was able to detect C_6–C_{11} alkanes (Sticher et al., 1997). The detection limit for short-chain octane was 0.04 μM (Tecon et al., 2010). Despite the good detection limit for individual compounds, the disadvantage of *E. coli* based bioreporters for oil sensing (alkanes or seawater aromatic compounds) is that the cells can only detect the soluble fraction of oil (van der Meer et al., 2004). In contrast, bioreporter cells that can chemotactically search and sense oil could potentially enhance the bioreporter's response (Zhang et al., 2012). Hence, an ideal alkane bioreporter should have high sensitivity, natural oil affinity, broad detection range, and be applicable to seawater or sediment samples (van der Meer and Belkin, 2010). *Acinetobacter baylyi* ADP1 is a soil bacterium that is able to degrade broad chain length of alkanes (C_6 to C_{40}) (Tanaka et al., 2010; Kennedy et al., 1975). ADP1 has an alkane hydroxylase-encoding gene *alkM* which is regulated by a regulator ALKR (Ratajczak et al., 1998a). The genetic organization of these *alk* genes is different from that in *P. oleovorans* (Ratajczak et al., 1998b). Most recently, a chromosomally-based *A. baylyi* bioreporter ADPWH_alk was reported to have high affinity and sensitivity to detect alkanes rapidly, which was able to sense and quantify a broad range of alkanes from C_7 to C_{36} within 4 hours (Zhang et al., 2012). A toxicity bioreporter ADPWH_recA was also developed previously using ADP1 as a host strain for toxicity detection (Song et al., 2009). Furthermore *A. baylyi* ADP1 naturally tolerates high salt (Sand et al., 2011) and both ADPWH_alk and ADPWH_recA are functional well in the seawater (Zhang et al., 2012).

In this study, we applied *Acinetobacter* bioreporters ADPWH_alk and ADPWH_recA to contaminated seawater and sediment samples collected from six sites around Dalian oil spill point and demonstrated that bioreporter detection can be used as a rapid method to estimate the scale of a crude oil spill event and to evaluate toxicity impact.

2. Materials and methods

2.1. Site description

On 16th July 2010 an explosion occurred at Dalian Xingang oil port, which destroyed about 200 m of oil pipeline, causing a serious oil fire, combustion of over 100,000 m^3 crude oil and significant air pollution. This explosion resulted in the release of over 1500 tonnes of crude oil into the Yellow Sea and a potential disaster for the marine environment and ecology. Dalian Xingang oil port is located at the south end of Liaotung Peninsula, on the west coast of Dayao Bay, Yellow Sea, in China. With a designed transport capability of 1.5 million tons per year, it is one of the most important bases for crude oil imports and exports, where millions of tonnes of Daqing crude oil are transferred to different places in China and countries around the world. Based on numerical modelling of the oil spill using established methods (Guo and Wang, 2009), six seawater and six sediment sampling points were selected in this study (Fig. 1 and S1). The sampling was taken twice, firstly on 28th July 2010 and secondly on 12th June 2011. The points of Dalian Bay (DB) and NanTuo (NT) are located on the north shore of Dalian Bay. These sites are respectively 27.8 km and 26.5 km from the oil release point, containing the main oil plume, and considered to be the most polluted marine area. The other three points of JinShi Beach (JSB), XingHai Park (XHP) and FuJiaZhuang (FJZ) are 16.9 km, 38.1 km and 31.1 km from the oil release point, respectively (Fig. 1). These sites are outside the marine oil plume and are evaluated as slight contamination. LaShuFang (LSF) is located in the Bohai Sea, 170 km from the oil release point, with no contamination, and represents a background control for the study (Table 1). Both seawater and sediment samples were taken from these six sites and stored at 4 °C for transportation and analysed two weeks after sampling.

2.2. Analysis of crude oil-contaminated samples

2.2.1. Chemical analysis

The chemical analysis of crude oil was carried out by gravimetric and GC/MS methods, as previously described (Zhang et al., 2012). Briefly, 200 mL of seawater and 10.0 g sediment samples were extracted by 50 mL chloroform solvent. The extracts were passed through an anhydrous sodium sulphate column to remove water and suspended solids. The total hydrocarbon content was determined gravimetrically after the solvent was evaporated in a fume cupboard for 48 h under sterile conditions. Subsequently, 30–80 mg of the crude oil/sediment extract was dissolved in 5 mL of *n*-hexane and loaded onto a glass column. The saturated fraction was eluted using 30 mL of 95% *n*-hexane, concentrated to 1 mL, and analysed using an Agilent 7890A GC coupled with an Agilent 5975C MS operated in the electron impact mode. The GC/MS was calibrated using solutions