Marine Pollution Bulletin xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

The application of a carrier-based bioremediation strategy for marine oil spills

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ARTICLE INFO

Keywords:
Carrier material
Biodegradation
DGGE
Ecotoxicity
Hydrocarbon

ABSTRACT

The application of recycled marine materials to develop sustainable remediation technologies in marine environment was assessed. The remediation strategy consisted of a shell carrier mounted bacterial consortium composed of hydrocarbonoclastic strains enriched with nutrients (Bioaug SC). Pilot scale studies (5000 l) were used to examine the ability of Bioaug-SC to degrade weathered crude oil (10 g l^{-1}; initially 315,000 ± 44,000 mg l^{-1}) and assess the impacts of the introduction and biodegradation of oil. Total petroleum hydrocarbon mass was effectively reduced by 53.3 (±5.75)% to 147,000 (±21,000) mg l^{-1} within 27 weeks. 16S rDNA bacterial community profiling using Denaturant Gradient Gel Electrophoresis revealed that cyanobacteria and Proteobacteria dominated the microbial community. Aquatic toxicity assessment was conducted by ecotoxicity assays using brine shrimp hatchability, Microtox and *Phaeodactylum tricornutum*. This study revealed the importance of combining ecotoxicity assays with oil chemistry analysis to ensure safe remediation methods are developed.

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1. Introduction

In the marine environment, petroleum is naturally found in subsurface reservoirs and other underground formations. As the global demand for energy continues to increase, the dependency on offshore drilling to extract oil or natural gas in areas such as the outer continental shelf, including regions in deepwater, continues (Skogdalen et al., 2011). In addition, transportation through vessels and pipelines is critical to ensure the consistent delivery of petroleum. Therefore, as long as it is economically viable, oil exploration in marine environments will continue in order to meet these energy demands. As a result of these activities, there can be significant impacts associated with accidental discharge of oil on the environment, human health and industries, including tourism and fisheries. Whilst new technologies in locating, extracting and exporting oil have reduced environmental impacts, such as reducing the zone of seafloor disturbance, altering drilling fluids with mineral oils and synthetic fluids and introducing double hulled vessels (Ball et al., 2012) the risk of marine oil spills are still current and therefore effective remediation treatments are vital. These remediation technologies need simplicity in application and be economic to source, to ensure their application in a global context (Simons et al., 2012).

Following an oil spill, conventional oil remediation methods such as physical removal with booms, skimmers and absorbent materials must be deployed promptly as these can transfer (remove) oil from the surface water layer, although they rarely achieve complete removal. The use of chemical methods involving mixtures of surfactants and solvents, disperse the oil into droplets relieving stress on from marine mammals and birds. However, these methods do not remove the oil from the environment and can be potentially toxic (Nikolopoulou and Kalogerakis, 2011; Zahed et al., 2011). Bioremediation is a branch of environmental biotechnology that utilizes living organisms to accelerate biological degradation and/or precipitation of pollutants (Osborne et al., 2004). Intervention via bioremediation involves two main methodologies; bioaugmentation and biostimulation. Bioaugmentation is the addition of microorganisms which have the capacity to metabolize and grow utilizing the contaminants of interest to

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http://dx.doi.org/10.1016/j.marpolbul.2014.03.044
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Please cite this article in press as: Sheppard, P.J., et al. The application of a carrier-based bioremediation strategy for marine oil spills. Mar. Pollut. Bull. (2014), http://dx.doi.org/10.1016/j.marpolbul.2014.03.044

a contaminated site (Nikolopoulou and Kalogerakis, 2011). Biostimulation is a method used to stimulate the indigenous bacteria naturally present within the contaminated site by modifying environmental conditions through actions such as addition of nutrients (Kouzuma and Watanabe, 2011). These two methods form a complementary treatment method when combined with a carrier material.

Recent studies by Simons et al. (2012) and Simons et al. (2013) selected mussel shells as a carrier material due to their highly favorable attributes including absorbance capabilities, recycling of waste products, simplicity of application and effectiveness of providing a protective niche to the bioaugmentation strains. Simons et al. (2012) showed that modified mussel shells, inoculated with hydrocarbon degrading microorganisms, were able to degrade up to 55% of hydrocarbon contaminant (123.3 mg l^{-1} from 276 mg l^{-1}) in nutrient rich medium over a 30 d period. The carrier substrate is a critical part of the bioremediation process in marine environments and must be capable of supporting high numbers of added microorganisms to achieve enhanced rates of degradation (hydrocarbon) through interaction within the oil (Yardin et al., 2000).

The surface of the carrier material encourages the natural formation of biofilms, structured microbial communities, where the microbes gain high tolerance to physical, chemical and biological stresses (Gorbushina and Broughton, 2009). These structures allow multiple species to work together providing a protective matrix for the consortia to degrade the hydrocarbon substrates (Singh et al., 2006). In addition, an advantage of the use of light weight carrier materials, such as shell grit is their buoyancy. This buoyancy is an important component as it enables carrier materials to interact with the oil through the water column and therefore, enhance degradation.

Previous work has shown bioaugmentation and biostimulation bioremediation technologies using mussel shells (Bioaug-SC) as a carrier material were successful for the biodegradation of a weathered hydrocarbon source in a tank mesocosm study (Simons et al., 2013). However, the research of Simons et al. (2013) was limited to testing the developed bioremediation technology (Bioaug-SC) in a small volume of contaminated seawater (50 l). Given that the technology will eventually be applied to large-scale contamination events, it is important that its effectivity be demonstrated in greater volume of contaminated seawater. In addition, previous investigations did not examine the effect of the technology on the functionality of the microbial community in the treated seawater. Improved functionality of the indigenous seawater microbial community would be desirable and beneficial to the bioremediation process. Therefore in this study, we investigated the efficacy of the application of the newly developed Bioaug-SC mounted bacterial consortium in a simulated marine remediation scenario using 5000 l of contaminated seawater. We also investigated the effects of the addition of Bioaug-SC on the functionality of the indigenous microbial community over the experimental period (27 weeks) using MT2 Biolog plates. Changes in the microbial communities associated with the degradation of weathered crude oil under natural environmental conditions and the impact of the introduction of the exogenous micro-organisms on the indigenous microbial community structure and diversity were investigated using 16S rRNA gene based PCR–DGGE-Sequencing methodologies.

2. Material and methods

2.1. Tank design

The experimental design consisted of two open system circular fibreglass tanks (dimensions 1.25 m height, 3.2 m diameter) subject to natural elements and southern hemisphere seasonal conditions (Table 1). The tanks were aerated using compressed air from a central compressor system via tubing attached with air stones that were evenly spaced around the edge of the tanks. Five thousand liters of natural Gulf of St. Vincent (South Australia) seawater (10 μm filtered) was added to each tank. Weathered crude oil sludge (1% w/v; 50 kg) was added to one of the tanks to simulate a marine oil spill scenario. The contaminated tank was left for 48 h for the water and oil to associate before the addition of the carrier material. At selected time points (Weeks 0, 6, 9,12,15,18, and 27) seawater from the uncontaminated and Bioaug-SC tank were collected in triplicate (by submerging sterile containers in the tank and allowing them to be filled up with water prior to manual mixing of tanks) for chemical and biological analyses. These include determining residual oil concentration (total petroleum hydrocarbon; TPH), soluble hydrocarbon concentration, along with DNA, nutrients, cell counts, hydrocarbon utilization potential, and ecotoxicity analysis which were performed in triplicate.

2.2. Carrier material

The biostimulation composition consisted of urea ($(NH_2)_2CO_2$) and sodium dihydrogen orthophosphate ($NaH_2PO_4^*7H_2O$) which in conjunction with the weathered crude oil (50 kg; 80% carbon) provided a molar ratio of 80:10:1 for C:N:P respectively. The bioaugmentation component consisted of six bacterial hydrocarbonoclastic strains composed of *Actinobacteria*, *Bacilli*, and *Gammaproteobacteria* (Table 2) which were isolated from hydrocarbon contaminated environments (Kadali et al., 2012a). Bacterial strains were individually cultured in nutrient broth (NB, Oxoid) to an OD reading of 0.5–1.0. The bacterial cells were concentrated and suspended in phosphate buffered saline (PBS) (total volume 6 l). The augmentation (6 l) and biostimulation (30 l) additives were combined with dry shell grit material (87 kg, mixed particle sizes; Fodder store, Adelaide, Australia) (referred throughout as Bioaug-SC; Simons et al., 2013). Preliminary studies were used to determine the water holding capacity of the shell grit to determine the saturation point. Upon saturation (approximately 48 h) the carrier material was added to the Bioaug-SC tank by manually spreading the mixture evenly over the surface of the seawater/oil.

2.3. Seawater characteristics analysis

Temperature, dissolved oxygen, electrical conductivity, salinity and pH were measured (YSI, Australia) at selected time points over a period of 27 weeks (Table 1). In addition dissolved ammonium, nitrate and phosphate levels were tested in triplicate as per manufacturers protocol (Aquaspex LF 24000). In parallel, cell counts were carried out in triplicates via serial dilution. Neat and diluted samples (10^{-2}, 10^{-4}) were plated (100 μl) onto nutrient agar (Oxoid) supplemented with 1.5% NaCl. Plates were incubated overnight at 25 °C.

2.4. Hydrocarbon preparation and extraction

Throughout the experimental period, changes in the concentration of hydrocarbons present in the dissolved phase and the residual product (remaining weathered crude oil concentration) were determined. Sample extraction and analysis was performed at external analytical laboratories (MDT-Labmark Australia and Flinders Advanced Analytical Facility- Flinders University) which are nationally accredited facilities (NATA). The extracts were analyzed using an 8200 Autosampler gas chromatograph (Column: SGE, HT5, length: 15 m, IDL 0.25 mm, film: 0.1 μm) with a 261 Varian 8200 Autosampler with flame ionization detector (FID) and mass spectrometry (MS) detector. Hydrocarbon concentrations were

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