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Microbial community structure shifts are associated with temperature, dispersants and nutrients in crude oil-contaminated seawaters

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ABSTRACT

This study tracked structure shifts of bacterial compositions before, during and after invading by crude oil to determine the microbial response and explore how temperature, dispersants and nutrients affect the composition of microbial communities or their activities of biodegradation in artificial marine environment. During petroleum hydrocarbons exposed, the composition and functional dynamics of marine microbial communities were altered, favoring bacteria that could utilize this rich carbon source such as the *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* phyla. Low temperature as a dominant factor decreased bacterial richness and catabolic diversity due to abated enzymes activities in correlation with the process of biodegradation. Dispersants exerted no negative consequences on microbial composition, however, bacterial composition by the *Chloroflexi*, *TM6*, *OP8*, *Cyanobacteria* and *Gemmatimonadetes* phyla increased. It seemed that more frequent fertilizer application could be equally safe to bacteria and increased significantly the abundance of bacterial strains but *Actinobacteria* phyla decreased.

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1. Introduction

During oil production, transportation and other activities, a large amount of crude oil released into the marine environment, such as the *Exxon Valdez* oil spill (Bragg et al., 1994) and *Deep Horizon* oil spill (Hazen et al., 2010). Crude oil is composed mainly of petroleum hydrocarbons, roughly a third of which are alkanes, another third of which are cycloalkanes and polycyclic aromatic hydrocarbons (PAHs) make up the remaining third (Douglas Head et al., 2006). Natural occurrence of obligate hydrocarbon-degrading bacteria (Gutierrez et al., 2013) of indigenous microbial consortia is a central important method for effective bioremediation techniques when compare with physical and chemical methods gradually.

Microbial communities are of resistance and resilience (Allison and Martiny, 2008), however, petroleum hydrocarbons have a profound impact on bacterial population abundance, and all available studies recognize shifts in the composition of microbial communities in direct contact with oiled seawater and sediments in comparison with pristine environments (Atlas and Hazen, 2011; Joye et al., 2014; Kostka et al., 2014; King et al., 2015). Environmental factors such as nutrients, temperature, and dispersants have been studied to evaluate the impact of petroleum pollution on the bacterial community composition and the relative abundance of bacterial species (Chakraborty et al., 2012; Lamendella et al., 2015; Kimes et al., 2014).

Successional changes in microbial populations expose to a complex mixture of hydrocarbons could be observed. Temperature has a direct effect on microbial physiology and an effect on the physical properties of oil that influence its bioavailability (Lindstrom and Braddock, 2002; Redmond and Valentine, 2012); both factors will influence the response of hydrocarbon-degrading bacteria (Coulon et al., 2007; Teira et al., 2007; Venosa and Holder, 2007). Temperature is shown to be a determining factor controlling bacterial abundance, community structure and biodegradation, by changing the activities of degradative enzymes in correlation with degradation of petroleum hydrocarbons (Valentine et al., 2012). Enzyme activity of the hydrocarbon-degrading bacteria has been investigated and different enzymes' activity confirmed had an important influence on the degradation of oil spill (Wang et al., 2010; Lu et al., 2012; Wang and Shao, 2013).

Dispersants (Atlas and Hazen, 2011) are globally and routinely applied as an emergency response to oil spills in marine ecosystems with the goal of chemically enhancing the dissolution of oil into water, which is assumed to stimulate microbially mediated oil biodegradation (Yoshida et al., 2006). Biosurfactants (Ron and Rosenberg, 2002) act as natural dispersants that emulsify oil in water, lead to the formation of small droplets and make the oil potentially available for biodegradation.

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Pioneering studies by Atlas and Bartha (1973) demonstrated that the available concentrations of nitrogen and phosphorus in seawater are limiting factors for the growth of hydrocarbon-degrading microorganisms (Bragg et al., 1994). Thus, the addition of nitrogen and phosphorus fertilizers stimulates the rebounce of bacterial composition. Besides, the concentrations of these elements in marine environments-primarily nitrates, phosphates, and iron can affect microbial responses to crude oil and also limit rates of oil biodegradation (Ziervogel et al., 2012; N'Guessan et al., 2010; Scott et al., 2015). Moreover, High-throughput sequencing (Kappell et al., 2014) has facilitated major advances in our understanding of microbial ecology.

On one hand, the objectives of this study aimed to track microbial community structure shifts were modificated from clean seawaters before the occurrence of oil spill to seawaters after oil spill. On the other hand, bacterial composition was analyzed in response to oil-contaminated under the effect of temperature, dispersants and nutrients. More importantly, we investigated how community structure shifted in response to crude oil under different temperatures, with or without dispersants and nutrients and the changes of enzyme activities, biomass, biodegradation rate, surface tension, cell surface hydrophobicity, emulsification index and nutrients utilization corresponding to the shifts.

2. Materials and methods

2.1. Device set up

The device (Fig. S1) has two rectangular containers. One is conducted as control group while the other is inoculated and used for the experimental group. The tank size is 108 cm × 42 cm × 48 cm, which is made by PE. The device equips with a computer-controlled electrical machine, which can make the rectangular container to do reciprocating motion. The electrical machine can control the tidal range and cycles.

Each tank was partially filled with sand. The sand was profiled to establish a beach with a slope of approximately 8 degrees and a 50 cm platform at the bottom of the tank (Fig. S1). Then each tank was filled with 10 L seawater. The sand and seawater were collected from the bathing beach of Qingdao, Shandong Province, China. The seawater has a pH of 7.95 and a salinity of 32. The sand was taken from intertidal zone. The cycle of the artificial wave was 3 s.

2.2. Sample collection and sterile operation

The MSM contains K_2HPO_4 0.5 g/L, Na_2SO_4 2.0 g/L, NH_4Cl 1.0 g/L, $MgSO_4 \cdot 7H_2O$ 0.02 g/L, $CaCl_2$ 0.07 g/L and 1.0 mL of trace salt solution per liter. The trace salt solution is defined as 30 mg/L $FeCl_3$, 0.5 mg/L $CuSO_4$, 0.5 mg/L $MnSO_4 \cdot H_2O$, and 10 mg/L $ZnSO_4 \cdot 7H_2O$·The pH is adjusted to 7.0–7.2 with 1.0 mol/L NaOH and 1.0 mol/L HCl.

Prespill samples were collected from clean seawater uncontaminated of crude oil. Then added crude oil for cultured 30 days, onset of the spill water samples were collected. The crude oil used in this study is from the Shengli oilfield. Physical parameters of the crude oil included a viscosity of 79.2 mPa s (25 °C, 50 rpm), a freezing point 23.0 °C and density 0.8552 g/cm.

With the environmental factors such as nutrients, temperature, and dispersants disturbance, aqueous phase samples are collected after 1, 3, 5, 7 days for analysis biodegradation rate, nutrient change, biomass, surface tension, cell surface hydrophobicity, emulsification index, enzyme activity and microbial community composition. Dispersants are used by GM-2.

During the process of experiments, sterile operation is an important thing for obtaining reliable results. It was inevitable to avoid contaminations from airborne microbes in laboratory, however, unnecessary contamination could be try to reduce each step of the experiment.

In order to simulate original environment, samples before and after oil spill were not necessary to keep the conditions of sterile while some operations strictly controlled the interference of airborne bacteria such as experimental materials or essential devices by steam sterilization and ultraviolet sterilization.

2.3. DNA extraction and bacterial community composition

All seawater samples were filtered through a 0.2 μm diameter filter. Microbial samples were collected from the bioreactors at the end of each test stage. Centrifuge tubes through steam sterilization were used for collecting bacterial population and then observed at −20 °C. Genomic DNA was extracted directly with the DNA extraction kit (Cwbio, China) according to manufacturer's instructions. DNA concentration and purity were monitored on 1% agarose gels. Bacterial universal primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) are used to amplify the V4 region of bacteria l6S rRNA. All PCR reactions were carried out in 30 μL reactions with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 0.2 μm of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s. Finally 72 °C for 5 min. Mix same volume of 1× loading buffer (contained SYB green) with PCR products and operate electrophoresis on 2% agarose gel for detection. Samples with bright main strip between 400 and 450 bp were chosen for further experiments. PCR products is mixed in equidensity ratios. Then, mixture PCR products were purified with Gene JET Gel Extraction Kit (Thermo Scientific). Sequencing libraries are generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes are added. The library quality is assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina MiSeq platform and 250 bp/300 bp paired-end reads were generated. Besides, extraction of DNA was conducted in clean bench.

2.4. Bioinformatic analysis

Sequences are analyzed using QIIME software package (Quantitative Insights into Microbial Ecology) (Caporaso et al., 2010), and in-house Perl scripts are used to analyze alpha- (within samples) and beta- (among samples) diversity. QIIME software package (http://qiime.org/) and UPARSE pipeline (http://drive5.com/uparse/) are used to analyze the reads and pick operational taxonomic units (OTUs). Sequences are assigned to OTUs at 97% similarity (Kostka et al., 2011). We picked a representative sequence for each OTU and used the RDP classifier (Edgar, 2010) to assign taxonomic data to each representative sequence. In order to compute Alpha Diversity, we rarified the OTU table and calculate three metrics: Chao1 estimated the species abundance; Observed Species was estimated the amount of unique OTUs found in each sample, and Shannon index. Rarefaction curves were generated based on these three metrics. We used unweighted unifrac for Principal Coordinate Analysis (PCoA) and Unweighted Pair Group Method with Arithmetic mean (UPGMA) Clustering. UPGMA Clustering was a type of hierarchical clustering method using average linkage and could be used to interpret the distance matrix.

2.5. Protein estimation and enzyme activities

2.5.1. Protein estimation

For estimation of bacteria cell protein, cells of the bacterial were suspended and washed in potassium phosphate buffer. They were further sonicated and centrifuged at 20,000 rpm at 4 °C for 30 min. The supernatant was subsequently stored at 0 °C and the determination of protein content followed the method of Lowry et al. (1951) at 660 nm by UV–visible spectrophotometer using BSA (bovine serum albumin) as a standard.

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