Bioresource Technology 170 (2014) 556–564

Contents lists available at ScienceDirect

Bioresource Technology

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

Biodegradation of complex hydrocarbons in spent engine oil by novel bacterial consortium isolated from deep sea sediment

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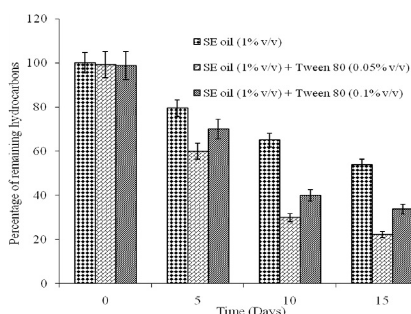
HIGHLIGHTS

- Hydrocarbon degrading bacteria was isolated from deep sea sediment (2100 m).
- Degradation of oil was confirmed by FTIR and GC–MS analyses.
- Possible application in bioremediation of oil contaminated marine environments.

GRAPHICAL ABSTRACT

Biodegradation of spent engine (SE) oil by deep sea bacterial consortium.



ARTICLE INFO

Article history:
Received 13 May 2014
Received in revised form 1 August 2014
Accepted 3 August 2014
Available online 12 August 2014

Keywords:
Aromatic hydrocarbons
Deep sea bacteria
Spent engine oil
Biodegradation
Bioremediation

ABSTRACT

Complex hydrocarbon and aromatic compounds degrading marine bacterial strains were isolated from deep sea sediment after enrichment on spent engine (SE) oil. Phenotypic characterization and phylogenetic analysis of 16S rRNA gene sequences showed the isolates were related to members of the *Pseudoalteromonas* sp., *Ruegeria* sp., *Exiguobacterium* sp. and *Acinetobacter* sp. Biodegradation using 1% (v/v) SE oil with individual and mixed strains showed the efficacy of SE oil utilization within a short retention time. The addition of non-ionic surfactant 0.05% (v/v) Tween 80 as emulsifying agent enhanced the solubility of hydrocarbons and renders them more accessible for biodegradation. The degradation of several compounds and the metabolites formed during the microbial oxidation process were confirmed by Fourier transform infrared spectroscopy and Gas chromatography–mass spectrometry analyses. The potential of this consortium to biodegrade SE oil with and without emulsifying agent provides possible application in bioremediation of oil contaminated marine environment.

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1. Introduction

Mixture of complex hydrocarbons and its residues reach the marine water and sediments in different modes which include deposition from municipal and industrial effluents, wastes burn out, accidental oil spill during transportation or deposits induced by sediment drilling (Dhananjayan et al., 2012). Hydrocarbons are persistent environment pollutants by virtue of their recalcitrant nature to biodegradation, bioaccumulation in the environment and immense health effects associated with its exposure (Allen et al., 1999). In marine environment, persistence of hydrocarbons causes long term detrimental effect on the living organisms (Sundt et al., 2011). Broad ranges of petroleum hydrocarbon mixtures, which include crude oil, engine oil, diesel fuel, creosote products and other fuel oil materials contain complex mixtures

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http://dx.doi.org/10.1016/j.biortech.2014.08.008
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of hydrocarbons, xenobiotics and other refractory organic compounds (Hamamura et al., 2006). The chemical composition of the crude oil can be classified into major fractions of aliphatics, aromatics, asphaltenes (phenols, fatty acids, ketones, esters and porphyrins) and resins (pyridines, quinolines, carbazoles, sulfoxides and amides) (Colwell and Walker, 1977). Main fractions of aliphatic hydrocarbons consist of readily biodegradable n-alkanes, less biodegradable branched alkanes and cyclic alkanes (Das and Chandran, 2011). Polycyclic aromatic hydrocarbons (PAHs) is a group of chemical compounds with two or more aromatic rings and are the most recalcitrant components of crude oil present at high percentage composition (Kanaly and Harayama, 2000; Haritash and Kaushik, 2009). Due to the recalcitrant property, bioaccumulation potential, toxic, mutagenic, carcinogenic and genotoxic activity, the PAHs have posed serious risks to the environment and human health. The complex molecular structure of asphaltenes renders them resistant to biodegradation (Tavassoli et al., 2012). In general, the susceptibility of hydrocarbons towards microbial induced degradation was rated in the order of n-alkanes > isoalkanes > low molecular weight aromatics > high molecular weight aromatics.

Microbial degradation is an eco-friendly process that results in the breakdown and complete utilization of hydrocarbons when compared to other processes like adsorption, volatilization, photolysis and chemical degradation (Haritash and Kaushik, 2009). A wide variety of microorganisms including algae, bacteria, fungi and yeasts have the ability to degrade hydrocarbons. However, bacterial community plays a pivotal role in bioremediation of hydrocarbon contamination. Several marine bacterial species, such as *Bacillus* sp., *Cycloclasticus* sp., *Mycobacterium* sp., *Pseudoxanthomonas* sp., *Rhodococcus* sp., *Acinetobacter* sp., *Pseudomonas* sp. and *Pseudoalteromonas* sp. were isolated and tested for their biodegradation potentials against different hydrocarbons.

Although marine microorganisms are known to degrade various hydrocarbons, the composition of hydrocarbons and other complex refractory organics in spent engine (SE) oils tends to change, thus there is a need to isolate novel microbes that can degrade and completely mineralize major components of oil. The uniqueness in biodegradation works depends on isolation of new microorganisms which could degrade complex mixtures of both aliphatic and aromatic hydrocarbons effectively. Therefore, marine bacterial species with broad substrate specificity for both alkanes and PAHs is of current importance. In the present study, the novel strains of hydrocarbon degrading bacteria isolated from deep sea sediment were characterized and biodegradation of the SE oil was studied in detail.

2. Methods

2.1. Sample collection, enrichment and isolation of hydrocarbon degrading bacteria

Deep sea sediment sample was collected at a depth of 2100 m from Bay of Bengal (13°14′192″N/80°49′061″E), using the Oceanographic Research Vessel Sagar Manjusha. To isolate hydrocarbons degrading bacterial consortium, the deep sea sediment was enriched on mineral salt medium (MSM) supplemented with SE oil (0.1% v/v). Enrichment was carried out with intermittent shaking at room temperature (27 ± 2 °C) for 3 months until there was growth (turbidity) by continual addition of SE oil up to 1% (v/v). After consecutive transfers, hydrocarbon degraders were isolated by plating on MSM agar plates containing the SE oil as sole carbon sources. The MSM contained 1.0 g of NaCl, 0.1 g of KCl, 1.0 g of KH_2PO_4, 1.5 g of K_2HPO_4, 0.75 g of $(NH_4)_2SO_4$, 0.3 g of $MgSO_4{\cdot}7H_2O$, 0.1 g of peptone and 1.0 mL of trace salt solution per liter. The trace salt solution composed of 20.0 mg of $CaCl_2$, 30.0 mg of $FeCl_3$, 0.5 mg of $CuSO_4$, 0.5 mg of $MnSO_4{\cdot}H_2O$, and 10.0 mg of $ZnSO_4{\cdot}7H_2O$ per liter and this solution was filter sterilized before addition. The pH was adjusted to 7.2 ± 0.2 with 1.0 M NaOH/HCl before sterilization. Isolated bacterial culture was purified to axenic culture and stocked in slants.

2.2. Molecular characterization of hydrocarbon degraders – 16S rDNA sequences analysis

The cultures were harvested during its logarithmic growth phase and the genomic DNA was extracted, using the high salt extraction method. To amplify the 16S rDNA gene, a polymerase chain reaction (PCR) was performed using two primers: the forward primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and the reverse primer 1492R (5′-GGCTACCTTGTTACGACTT-3′). Each 25 μL reaction mixture was composed of followings: 12.5 μL of ExPrime Taq Premix (2×) (GENET BIO), 1 μL of template DNA, 1 μL of 27F primer, 1 μL of 1492R primer (final concentration 10 pm/μL) and made up to a volume of 25 μL using nuclease free water. The amplification was carried out in a 96 well-thermal cycler (Veriti-Applied Biosystems, USA). The PCR conditions were set at initial denaturation for 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min and 30 s, followed by final chain-elongation at 72 °C for 10 min. The PCR amplicons were analyzed in 1.5% agarose gel electrophoresis. The PCR products were purified using PCR purification kit (Qiagen, USA) as per the manufacturer's protocol and the purified PCR products were sequenced using Applied Biosystem 3500 DNA sequencer. The DNA sequence of the PCR products was subjected to a similarity search using BLAST on the NCBI website (http://www.ncbi.nlm.nih.gov). The phylogenetic trees were constructed using the MEGA 4.0 software.

2.3. Morphological, physiological and biochemical characterization

The colony morphology was examined in cultures grown on marine agar for 7–10 days and microscopic structure was examined by phase contrast microscopy (Carl Zeiss Axioskop 2 plus, USA). The biochemical properties were tested using KB002 HiAssorted™ Biochemical Test Kit, HiCarbohydrate™ Kit KB009A, HiCarbohydrate™ Kit KB009B and HiCarbohydrate™ Kit KB009C (Hi Media, India) according to the manufacturer's instructions. Test cultures (50 μl) were added separately into individual wells in respective test kits and incubated at 37 °C for 24 h. The results were interpreted by comparing with standard result interpretation chart.

2.4. Effect of pH, temperature and NaCl concentration on bacterial growth

The effect of hydrogen ion concentration on growth of pure cultures of deep sea strains was studied in marine broth at pH 5.0–10.0. The influence of temperature (10–45 °C) was studied in marine broth at pH 7.0. The effect of NaCl concentration on bacterial growth was studied in the range of 1–10% (w/v). After appropriate incubation, the total viable count of the marine bacteria was enumerated using Zobell marine agar (ZMA; Himedia, India).

2.5. Oil degradation and effect of non-ionic surfactant

Biodegradation of oil with mono and mixed culture was performed in MSM supplemented with 1.0% (v/v) concentrations of SE oil. In monoculture and mixed culture (four-strain consortium) analysis (2.0%, v/v) was added into MSM for degradation studies. All culture flasks were incubated at 30 °C with shaking rates of

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