Chemical Engineering Journal 306 (2016) 60–66

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Reconstruction of bacterial community structure and variation for enhanced petroleum hydrocarbons degradation through biostimulation of oil contaminated soil

Aiyoub Shahi [a], Sevcan Aydin [a,*], Bahar Ince [b], Orhan Ince [a]

[a] Environmental Engineering Department, Istanbul Technical University, Maslak, Istanbul, Turkey
[b] Institute of Environmental Sciences, Bogazici University, Bebek, Istanbul, Turkey

HIGHLIGHTS

- Illumina analysis is an efficient monitoring approach during biostimulation process.
- Gram-negative bacteria have main role in degradation of petroleum-contaminated soil.
- *Bacteroidetes* and *Proteobacteria* are essential phyla in bioremediation process.

ARTICLE INFO

Article history:
Received 13 March 2016
Received in revised form 27 June 2016
Accepted 5 July 2016
Available online 14 July 2016

Keywords:
Petroleum contaminated soil
Illumina sequencing
Microbial community
Nutrient amendment
Biostimulation

ABSTRACT

The relative abundance of dominant bacterial phyla during a biostimulation practice of petroleum contaminated soil has been evaluated through the Illumina sequencing method. Biostimulation practice was fulfilled by amending the petroleum contaminated soil with addition of different N/P ratios. Gram-negative bacteria were found to be more dominant than gram-positive bacteria. *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria*, *Lentisphaerae*, *Planctomycetes*, *Thermotogae*, *Bacteroidetes*, *Synergistetes*, and *Verrucomicrobia* were dominant phyla with various relative abundances in all of the studied microcosms. Among the dominant phyla, *Proteobacteria* was most significant. The relative abundance of *Bacteroidetes* and *Verrucomicrobia* significantly increased with an increase in petroleum hydrocarbons and nutrient concentrations, while the relative abundance of *Firmicutes* also increased with increasing nutrient amounts. Based on the results obtained from microcosm with the highest petroleum hydrocarbons removal rate, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* phyla, which composed about 50% of dominant phyla, proved to be the most influential on the biodegradation of petroleum contaminated soil. Among all dominant phyla, the relative abundance of *Bacteroidetes* was most affected by the increase in nutrient ratio, while the relative abundance of *Acidobacteria* showed least deviation among studied microcosms.

1. Introduction

Petroleum contamination is a global environmental problem. High amounts of petroleum enter soil during extraction, transportation, and processing activities. Because of the health risk it presents to humans and wildlife and environmental problems due to petroleum hydrocarbons, petroleum polluted soil needs to be treated [1]. Common physicochemical treatment methods are not effective in complete remediation of oil contaminated sites [2] in addition to being costly and leaving site disturbances. Furthermore, they do not pose permanent solutions and cause additional environmental problems [3]. Thus, biological methods have been investigated as more economical and environmental friendly solutions. Microorganisms are the main biological agents that are used in biological treatments [4]. The acclimation and adaptation opportunity of indigenous microorganisms to toxic chemicals and their developed resistance to pollutants have made them an integral part of environmental protection plans [5]. Accelerating the biodegradation capacity of native microorganisms requires making some transactions on the target environment. The addition of necessary nutrients and oxygen as well as modification of environmental pH, temperature, and moisture are com-

* Corresponding author at: Department of Environmental Engineering, Istanbul Technical University, Maslak, 34469 Istanbul, Turkey.
E-mail address: sevcan_aydn@hotmail.com (S. Aydin).

http://dx.doi.org/10.1016/j.cej.2016.07.016
1385-8947/

mon methods for enhancing the biodegradation capability of indigenous microorganisms [6]. The environmental handling in such a way would certainly affect the microbial population and their construction in the polluted sites [7]. Providing suitable conditions for special pollution degrader strains, facilitating bacterial replication, and also horizontal transfer of involved functional genes through the indigenous bacteria will prompt changes in the construction of microbial populations in the target environment, which benefits the pollutant-resistant microorganisms [8,9]. All of these changes in the arrangement of microbial populations are needed for construction of the necessary catabolic networks for decomposition of organic pollutants [10]. To reduce unwelcome effects of environmental manipulation during modification of ecological conditions through the bioremediation process, evaluation of changes in the microbial population is an important step [9].

Indigenous microorganisms that are able to degrade petroleum hydrocarbons are ubiquitously distributed in the environment [11], and their successful role in the remediation of terrestrial and marine petroleum contaminated sites has been reported in numerous studies [12,13]. Biodegradation of complex mixtures of hundreds of hydrocarbons in petroleum wastes requires cooperation of diverse groups of microorganisms [14]. Therefore, analysing microbial community dynamics during the bioremediation process will improve the scientific knowledge concerning microbial dynamics and functional occurrence during the bioremediation process. Furthermore, by employing the obtained knowledge any probable adverse effects of environmental manipulation that come from unsuitable nutrient sources or inhibitory effects of inappropriate amounts of applied nutrients could be mitigated [15,16]. Common molecular methods, such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) that are used for analysis of the microbial community in the petroleum contaminated soil, do not provide comprehensive information about the present microorganisms. Illumina sequencing is a high-throughput sequencing technology that produces wide-range 16S RNA data of a studied site and covers a broad diversity of microbial populations [17]. Current advancements of this technique, including sensitivity enhancement, sequencing cost reduction, and its ability to scan all DNA materials, have made it a powerful tool to obtain a comprehensive picture of microbial and functional gene occurrence in contaminated sites. In the current study, the Illumina sequencing method was implemented to evaluate the reconstruction of a microbial community during the biostimulation practice of petroleum contaminated soil.

2. Methods and materials

2.1. Soil sampling and characterisation

Petroleum polluted soil samples were collected simultaneously from different areas of an old petroleum storage pit in Turkey. Samples were transferred to the laboratory and then were mixed and stored at 4 °C for future examination. Clean soil samples were collected from different areas in the Emergan Forest (Istanbul, Turkey), then were mixed and stored at 4 °C for further examination. The physicochemical characteristics of all collected samples were analysed and compared.

2.2. Experimental setup

Aerobic microcosms were set up as described in our previous work [9]. Petroleum contaminated soil and clean forest soil samples were mixed in different ratios. Four different samples with different initial total organic carbon concentrations of 5%, 10%, 15%, and 25% were prepared, and each was amended with four different nitrogen and phosphorous ratios to obtain the following C/N/P ratios: 100/5/1, 100/10/1, 100/15/1, and 100/25/1. As a result, sixteen microcosm setups were produced. The microcosms were established in triplicate and incubated for 90 days in dark condition at 24 °C. Microcosms were aerated manually every day, and the moisture content was maintained at 70% of water holding capacity by adding distilled water daily. Control tests for each setup were prepared without addition of nutrients. The established microcosms groups are summarised in Table 1.

2.3. DNA extraction and PCR amplification

DNA was extracted from 500 mg of collected samples using a PowerSoil DNA isolation kit (Mo Bio Laboratories, USA) following the manufacture's procedure. Concentrations of extracted DNA were determined via a NanoDrop UV–vis spectrophotometer (Thermo Scientifics, USA). 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′) primers specific for V4 region (length, ca. 250 bp) of the rRNA gene were selected [18], required Illumina adapters and barcode sequences were added to the primers. Extracted DNA was amplified using PCR following the amplification protocol, which is as follows: initial denaturation for 3 min at 94 °C, followed by 20 cycles of 45 s at 94 °C, 30 s at 53 °C, 90 s at 65 °C, and a final elongation step of 10 min at 65 °C ([18]). All DNA samples were further purified using the Wizard DNA Clean-Up System (Promega) in accordance with manufacture's protocol. The samples were then quantified using Qubit 2.0 Fluorometer (Invitrogen, NY, USA).

2.4. Illumina HiSeq sequencing

16S rRNA genes were sequenced following the Illlumina GAIlx method (Illumina, Inc., CA, USA) with paired-end read cycles. Sequence analysis and the identification of operational taxonomic units (OTUs) were obtained using the methods suggested by Giongo et al. [20,21] and Fagen et al. [22]. After sequencing and analysing the results, low quality bases and the first 11 bases of the primer region were removed via a script based on Trim2 (Huang et al. [23]; Trim2_Illumina.pl. available online: https://gist.github.com/1006830). The reads were filtered by taking into account 70% bases, which was used as the minimum percentage, using a minimum quality score of 20 in Phred + 33 encoding [19]. Paired reads were mapped to the reference Ribosomal Database Project (RDP) using CLC Assembly Cell v3.0.2b (CLC Bio, QIAGEN). Taxonomic descriptions were done based on the NCBI taxonomy database and entered in the RDP database using TaxCollector [20,21]. Of the fraction length matches, 80% were used for classification. At least 80% of sequence similarity was considered as the domain and phylum. OTUs abundance matrices for each taxonomic rank were created using the total number of reads, which showed 16S rRNA sequences matching with the database, and matrices of each sample were divided by the total number of pairs for normalising varying sequencing depths [19].

Table 1
Experimental groups with different initial TOC amount and different N/P ratios [9].

C/N/P	Initial TOC amount			
	T1 (5%)	T2 (10%)	T3 (15%)	T4 (25%)
N1 (100/5/1)	N1T1	N1T2	N1T3	N1T3
N2 (100/10/1)	N2T1	N2T2	N2T3	N2T4
N3 (100/15/1)	N3T1	N3T2	N3T3	N3T4
N4 (100/25/5)	N4T1	N4T2	N4T3	N4T4
C (Control)	C1	C2	C3	C4