

Abstract

Microbial community composition and functional potential of a petroleum refinery waste sludge from Digboi refinery, Indian Oil Corporation Limited (IOCL), Assam, India was investigated to assess the scope for its bioremediation. High total petroleum hydrocarbons (171 g/Kg), sulfur, ammonium, and heavy metal (As, Cd, Cr, etc.) content, low dissolved O₂, nitrate, nitrite, phosphate and negative oxidation reduction potential characterized the sludge. Analysis of microbial community through sequencing of 16S rRNA genes (V3 and V6 regions) from the metagenome revealed preponderance of anaerobic hydrocarbon oxidizing *Actinobacteria* (*Coriobacteriaceae*), *Chloroflexi* (*Anaerolineaceae*), along with *Proteobacteria* (*Sphingomonadaceae*), sulfur metabolizing *Caldiserica* (*Caldiseriaceae*), *Firmicutes* (*Thermodesulfobiaceae*), etc. and acetoclastic methanogenic archaea *Methanosaetaceae* within the sample. Presence of 39 bacterial and archaeal candidate phyla was also evident. Successful identification of *dsrB* and *mcrA* genes indicated the community's potential in dissimilatory sulphite reduction and methane metabolism. *Pseudomonas*, *Bacillus* and *Microvirgula* spp. populated the normal cultivable taxa while prolonged enrichment with hydrocarbons and crude oil yielded isolation of *Burkholderia*, *Brevundimonas*, *Enterobacter*, *Kocuria*, *Pandoraea*, *Paraburkholderia*, and *Rahnella* isolates. The enrichment isolates showed assemblages of versatile metabolic properties: superior aliphatic (C6-C22) and polyaromatic hydrocarbons utilization abilities, anaerobic growth with multiple terminal electron acceptors and higher biosurfactant production. Biodegradation of dodecane and total sludge by selected five bacteria (of genera *Burkholderia*, *Pandoraea* and *Paraburkholderia*) was studied by GC-FID. A systematic study towards bioremediation was adopted through both bioaugmentation and biostimulation based approaches. Amendment of the waste sludge with the consortium of the five strains yielded >85% TPH reduction. Suitability of the bioremediated waste sludge for its safe disposal was further assessed through ecotoxicity study with cultivation of *Triticum* sp. Compared to the untreated sludge, the bioremediated waste amended soil supported growth of *Triticum* sp. with increased germination index, higher chlorophyll content and plant biomass. The biomass indicators were close to that of a healthy soil. Overall study provided the first metagenome based details of microbial community composition of the Digboi refinery waste; allowed us to gain information of the bacterial hydrocarbon degradation potential through enrichment culture and evaluated the bioremediation potential of natural strains successfully.

Keywords: Digboi refinery; Oily sludge; Microbial community; 16S rRNA amplicon sequencing; Enrichment culture; *Paraburkholderia*; Candidate divisions; Bioremediation; Total petroleum hydrocarbon.