

Abstract

Remediation of large amounts of oily sludge produced by petroleum refineries poses an environmental challenge. Microbial ecology of these wastes is imperative to elucidate the nature and function of inhabitant microorganisms in attenuation of toxic hydrocarbons present in these sludge. The current study aimed to characterize the microbial community of a waste sludge from Guwahati Oil refinery, IOCL, Assam, India and evaluate the hydrocarbon remediation potential of the native populations. A hydrocarbon rich (total petroleum hydrocarbon: 440 g/kg), anaerobic sludge from the waste water lagoon of the refinery (designated as GR3) was composed strict anaerobic bacteria (*Smithella*, *Syntrophus*, *Ferredobacterium*, *Coprothermobacter*, *Caldisericum*, etc.) and *Euryarchaeaota* members; facultative anaerobic (*Thauera*, *Geobacter*, *Azovibrio*) and a few aerobic bacteria (*Pseudoxanthomonas*, *Pseudomonas*, *Tepidiphilum*, *Stenotrophomonas*). Community level physiological profiling (CLPP) and isolation based attempts were made to characterize the communities' gross abilities to metabolize selected substrates. Whole metagenome sequencing showed detailed pathways for hydrocarbon, methane, nitrogen metabolism, along with other housekeeping processes. Biostimulation of hydrocarbon degrading populations (constituted mainly by members of *Comamonadaceae*, *Rhodocyclaceae* and *Pseudoxanthomonadaceae*) was achieved successfully by addition of nitrate (as NaNO_3 ; 10 mM) allowing 80% removal of the TPH in 12 weeks. Whole metagenome sequencing confirmed a shift in the genetic repertoire of the biostimulated community with enhanced read allocations towards pathways involved in nitrogen cycling and hydrocarbon degradation. Hydrocarbon degrading biostimulated cultures were obtained through aerobic and anaerobic sub-culturing. Following a detailed characterization of their hydrocarbon metabolism, selected cultures were tested for their efficacy as bioaugmentation agent. Bioaugmentation of the refinery sludge with aerobically enriched culture composed of *Comamonadaceae*, *Pseudoxanthomonadaceae* and *Pseudomonadaceae* members allowed rapid degradation of upto 88% TPH in 1 month. The study presented an elaborate view of the composition and genomic details of the refinery waste, highlighting the potential of native populations in bioremediation.

Keywords: Refinery waste sludge, hydrocarbon biodegradation, biostimulation, enrichment, bioaugmentation, whole metagenome sequencing, amplicon sequencing