Conclusions:

It is well known that the earth’s petroleum reservoirs are finite and their declining has sparked the need for the development of many non-fossil fuel-based energy alternatives. The half of the crude oil from the world has been extracted and used. The remaining half is much more difficult to extract, the process is more costly and oil will be of poorer quality. There are many reports of using biotechnology for solving the problems in petroleum industry. The most important target of the petroleum industry is to increase and maintain a continuous oil production. It is well recognized that petroleum reservoirs contain diverse populations of microorganisms. The potentials for microbial enhanced oil recovery (MEOR) have been investigated for many decades. In the present investigation microbial consortium was developed for the enhancement of in situ methanogenesis in the matured oil reservoirs. This study has yielded information of bacterial diversity in oil reservoirs of western India. Present investigation also developed a consortium that has demonstrated crude oil degrading in the relatively anaerobic conditions to generate methane.

- Formation water samples from ten different oil reservoirs of Gujarat, India were collected. Physico-chemical analysis exhibited their slightly alkaline nature (pH 8.0). Even though the samples were collected from different geographic locations, there was not a significant difference in the physical and chemical properties of the formation water samples. All samples were of same salinity (~ 1%) and contained low level of total solids and total nitrogen. These physico-chemical parameters revealed the possible existence of microbial flora in the oil reservoir. The concentration of toxic heavy metal ions was relatively low to affect the survival of
native microorganisms. Formation water itself was an oligotrophic medium to support the growth of the native bacteria. Knowledge of composition of formation water was needed for the investigation of bacterial diversity as well as their isolation.

- The bacterial populations of these formation water samples were analyzed by denaturing gradient gel electrophoresis (DGGE) with PCR-amplified 16S rRNA fragments. The reciprocal of Simpson’s index (1 - $D$) and the Shannon Weiner Index (H) index were chosen to characterize the microbial communities in our formation water samples because they have good to moderate discriminating ability and are used widely in ecological studies. Simpson's index (1 - D) and the Shannon-Weaver index (H) revealed more diversity in Meh 71 and Limb 28. Bech 144 and GGS 1 showed relatively low values of inverse of Simpson’s index (1/D), indicating less diversity in the formation water samples. The equitability (J) indicated absence of dominant population in any of the oil reservoir tested.

- Phylogenetic analysis showed four major bacterial classes in the samples; firmicutes, gamma-proteobacteria, beta-proteobacteria and Actinobacteria. This observation revealed the presence of significantly diverse microbial population in the Indian oil reservoirs. Sequence analysis of the DGGE bands revealed that Proteobacteria were a major component of the bacterial population of formation water samples. So far, there has not been any report in the published literature for the description of microbial diversity associated with Indian oil reservoirs investigated by molecular tools.
Bacteria identified by DGGE analyses like *Brachymonas petroleovorans*, *Hydrogenophaga taeneospiralis*, *Pelomonas sp.*, *Lysobacter sp.*, *Bacillus sp.*, *Paracoccus sp.*, *Pseudomonas sp.* etc. have been reported in the literature as hydrocarbon degraders. Many of them have been reported as thermophilic hydrocarbon degrading bacteria. This diversity study gave us an indication that the formation water can be a good source for the isolation of thermophilic hydrocarbon degrading bacteria.

Many of the bacterial sequences shared less than 95% homology with the reference sequences available in Genbank database. Many of these could represent novel species/genera. Thus, Indian oil reservoirs could be effectively used as a source for isolation of novel bacterial species.

In the present investigation, by enrichment technique total 11 hydrocarbon degrading bacteria were isolated. Based on their ability to grow at or above 45°C, total five isolates (MCM B-882, MCM B-883, MCM B-884, MCM B-885 and MCM B886) were selected for further studies. MCMB-883 and MCMB-884 exhibited the closest phylogenetic affiliation to *H. hirschi* and *B. pumilus*, respectively. MCMB-882, MCM B-885 and MCMB-886 exhibited the closest phylogenetic affiliation to *A. pallidus*, on the basis of morphological, cultural and biochemical characterization; DNA base composition; FAME analysis and sequence homology of 16S rRNA genes. However, the polyphasic identification approach also revealed the significant differences in the DNA base composition, FAME profile, carbohydrate fermentation profiles of MCM B-882, MCM B-883, MCM B-885 and MCM B-886 when compared with their closest phylogenetic affiliates. These observations indicated that MCM B-882, MCM B-885 and MCM B-
886 were probably new sub species of genus *Aeribacillus*. Similarly, MCM B-883 was probably new species of genus *Hydrogenophilus*.

- MCM B-882, MCM B-883, MCM B-885 and MCM B-886 were able to grow very well at 60°C. All isolates could grow in a wide range of pH (pH 6.0- pH 10). These properties will be important for the growth of isolated cultures in the field studies.

- The evaluation of potential of petroleum hydrocarbon degradation of the isolates was assessed by various techniques like INT assay, GC and spectrophotometric analysis of residual hydrocarbons. It was observed that all isolates are appeared to be the good degraders and degraded 7-9% of the crude oil as estimated by measuring absorbance at A420 nm. These thermophilic bacteria were also displayed crude oil degradation potential when evaluated in terms of chemotaxis studies, INT assay and visual observation of oil dispersion.

- These cultures were further characterized and assessed for their ability to degrade n-alkanes. It was found that all isolates could utilize mixture of alkanes (C12-C18) as their sole source of carbon and energy at 60°C (except MCM B-884 which could grow up to 55°C).

- Production of surface active agents like biosurfactants is a desirable property of hydrocarbon degrading bacteria. In the present study all isolated bacterial cultures could produce biosurfactant in a complex as well as minimal medium. Production of biosurfactant was confirmed qualitatively and by its extraction with Chloroform: Ethanol. The hydrocarbon emulsifying ability of individual isolate was assessed in terms of emulsion index. The emulsion index revealed good emulsifying ability of all isolates when compared with published data.
In the optimization of process parameters it was observed that all isolates could effectively degrade crude oil as well as n-alkanes at elevated temperatures, at alkaline pH range when ammonium chloride provided as a nitrogen source under anoxic conditions. To the best of our knowledge this is the first report of isolation, identification and characterization of thermophilic crude oil degrading bacteria from Indian oil reservoirs.

Generation of methane was detected in the sand pack trials where crude oil was used as sole source of carbon and energy and a consortium of thermophilic cultures, under anaerobic conditions.

This observation provided the proof of concept that a microbial process can be developed for the degradation of residual oil and for the recovery of energy from matured oil reservoirs. To the best of our knowledge this is the pioneering report of its kind at least for Indian oil reservoirs.