SUMMARY

The present investigation encompassed an approach where the bacterial diversity in various oil reservoirs situated in three geographically different locations (Assam, Gujarat, and Bombay high) were documented. The oil reservoirs were situated in the northeastern, western, and the southwestern parts of the India. The reservoirs were having different temperatures, and were having high water cut. The diversity was evaluated by cultivation based studies.

The collected formation water was analyzed for the electrical conductivity. The electrical conductivity of formation water was in the range of 6.0-7.5 Sm⁻¹ in case of Assam, whereas 6.12-7.85 Sm⁻¹ in case of the Gujarat. The total dissolved solids were 3000-5800 ppm, and 3200-6000 ppm respectively from Assam, and Gujarat. Amongst 15 anaerobic media, S-7 medium was found to be the best medium to assess the diversity. The total microflora of the formation water was determined by the isolation of anaerobic bacteria procured from different reservoir conditions. A total of 96 bacterial isolates were isolated. The bacterial strains were isolated and purified with the help of hungate roll tube method. The species Anaerobaculum, Garciella, Clostridium, Coprothermobacter sp. were predominantly procured at 50 °C, however, Thermosediminibacter, Caldanaerobacter sp., Thermodesulfovibrio sp., Thermodesulfobacterium sp., Thermodesulfotobacterium sp. were procured at 60 to 70 °C. The maximum diversity was found in northeastern part of India (Assam) than western part of the country. The Garciella nitratireducens was found to be the most dominant in the northeastern site however, Clostridium sporogenes to the western site.

The phenotypic and biochemical characterization of the particular bacterial strains were studied. The gram positive and gram negative bacteria were isolated. The isolates were found to produce volatile fatty acids, hydrogen sulfide gas. The identification of the bacterial strains was performed by sequencing the genes encoding 16S rRNA. The bacterial species were predominantly affiliated to Firmicutes. It was observed that the Clostridium and Garciella sp. were dominant.
in the western parts and northeastern parts of the country, respectively. The isolated species belonged to the genera of *Anaerobaculum, Garciella, Clostridium, Thermosediminibacter, Coprothermobacter* sp., *Thermodesulfovibrio* sp., *Thermodesulfobacterium* sp., *Thermodesulfotobacterium* sp., *Caldanaerobacter* sp. and *Thermodesulfobacterium* sp.

The analysis of the total petroleum hydrocarbon (TPH) fractions of the crude oil was estimated. The aliphatic fraction in the region of Assam was 60 – 75 %, whereas 36 % of the TPH of the Gujarat crude consist of aliphatic fraction. This could be one of the reasons for the difference between the genetic diversity among the different geographical locations.

The strain TERI SRB 1001 (*Anaerobaculum mobile*) is gram negative rods optimally growing at 50 °C was characterized. It was found that this strain was able utilize the sodium thioglycolate, sodium acetate as carbon source; nitrate as nitrogen source. The strain was able to grow between the pH 6-9, salinity 0.5-4%. The strain was able to use sodium thioglycolate, sodium sulfate, ferric sulfate, magnesium sulfate, sodium sulfate, sodium thio sulfate as the electron acceptor. The phylogenetic tree constructed with the help of Phylip 3.0 showed that this strain shared 96% homology with the *Anaerobaculum mobile*.

The strain TERI SRB 1010 (*Garciella nitratireducens*) is a spore forming rods growing at 50 °C. This strain was able to grow on sucrose, fructose, cellulobiose, sodium thioglycolate, sodium nitrate. Among the tested electron acceptors, sodium thioglycolate, sod dithionate, magnesium sulfate were optimally utilized by the strain. The optimal growth was obtained at salinity - 2.0%, pH - 7.0, though the growth was found between the pH 6.5 to 8.0. The strain was found to be 97% similar to the *Garciella nitratireducens*, the phylogenetic tree was constructed by phylip 3.0.

The intraspecies genetic diversity was studied for *Garciella nitratireducens*. The 16S rDNA restriction based analysis revealed that 18 Garciella sp. delineated into at least 9 genotypic groups. The variability amongst 16S-23S ITS (Internally transcribed spacer) regions revealed that there were at least 8 genotypic groups.
amongst the chosen *Garciella* sp. The northeastern part of India (Oil India Limited, Assam) showed the maximum intraspecies diversity amongst the strains.

The ten chemical biocides were screened for the control of these sulfate-reducing microorganisms. These biocides were found to be effective against these microbes at 50 °C, 60 °C and 70 °C. The minimum inhibitory concentration of each biocide was studied by incubating the SRB with the various concentrations of the selected biocide (25, 50, 75, 100, 150, and 200 ppm). The time kill studies of the biocide against the SRBs were studied by incubating the biocide with the SRB for different time intervals. The contact time between the SRB and biocide was found to be 2h. The effect of nutritional regime was studied by observing the effect of various concentrations of the nitrate on the sulfide production. The presence of nitrate as a competitive inhibitor of sulfate in the ratio of 1:1 could inhibit the sulfide production up to 8 days.

A full scale study on evaluation of microbiocide efficacy to control indigenous sulfide producing bacterial population in the produced water at Kathloni, Oil India Limited (OIL), Assam (northeast India) was conducted. Out of 10 microbiocides screened, three (sodium hypochlorite, benzyl trimethyl ammonium chloride, and 2-bromo-2-nitropropane-1, 3-diol) were selected to control the growth and sulfide production. The strategy was designed in which these three microbiocides were sequentially applied in an oilfield for 132 days. There was no significant difference in the chemical composition of the produced water after the treatment.

At the onset of treatment, the population of sulfidogens was $10^7$ cfu ml$^{-1}$ in the storage tank and $10^8$ cfu ml$^{-1}$ in the pump delivery sample. However, after a few days of the treatment H2S gas production had ceased completely, and no viable sulfide producing bacterial cells were detected anymore in the produced water. As a consequence, the rate of disposal of the produced water into well was maintained to $707 \pm 5.35$ kl d$^{-1}$, $722 \pm 7.69$ kl d$^{-1}$, $751 \pm 4.45$ kl d$^{-1}$, $760 \pm 3.98$ kl d$^{-1}$ respectively for each of the four sequences of the treatment. Hence no well cleaning was required after the 132 days of the treatment. However after the microbiocide treatment, there was neither plugging nor any requirement of periodic redevelopment to restore the capacity of disposal wells by physical scrubbing, acidification, pumping etc. to dispose the produced water.