There are two major problems that the oil industry faces while dealing with paraffinic crude oil. The first problem is of deposition of paraffin-wax in oil well tubing during the production and transport of crude oil where the oil industry loses millions of dollars due to lowered productivity of oil wells and in remedial measures. The second problem is of the disposal of oily sludge that results from the refining of the paraffinic crude oil. A peculiar case study, where acidic oily sludge was generated as a result of primitive wax refining process, has been studied here.

To address the problem of paraffin deposition in oil well tubings and pipelines, paraffin contaminated soil samples were collected from Gujarat state of western India. These soil samples were then enriched using paraffin-wax as the sole carbon source at 70°C in minimal salts medium. The high temperature enrichment would ensure that the bacteria, thus obtained, would be able to survive at the high temperatures encountered in oil well tubings and the use of minimal salts medium with wax as the carbon source was to have the enriched bacteria get acclimatized to the nutrition that would be available to it in the harsh reservoir conditions. The strains thus obtained, was purified and the most efficient paraffin-wax degrading bacterial strain (TERI NSM) was selected. This strain was characterized on the basis of its morphological characteristics, its biochemical and hydrocarbon utilization profiles and identified as *Geobacillus kaustophilus*. This strain could grow on alkanes from tetradecane to triacontane as the sole carbon source but not on lower alkanes from octane to dodecane. The nutritional and growth conditions of this strain were optimized to increase its degradation abilities. Its growth and degradation capabilities were tested with a commercial paraffin-wax and were also tested on paraffinic alkanes that represent this wax. The efficient use of a commercial wax, which represented the paraffinic alkane range of most crude oils of India, by TERI NSM ensured that this strain would be able to degrade most paraffins that cause deposition in the thermophilic conditions of oil-well tubings. The paraffin degradation pathway adopted by *G. kaustophilus* TERI NSM was determined by studying the intermediates formed during the degradation of even and odd chain paraffinic alkanes. The detection of monocarboxylic fatty acids during the growth of TERI NSM on paraffinic alkanes indicated a monoterminal oxidation pathway being
followed by this strain. This was further confirmed by studying the intermediates formed when this strain was grown on known monoterimal metabolic pathway intermediates like primary alcohols and monocarboxylic acids. Additionally, the alkane hydroxylase gene, that is responsible for the first critical step of alkane oxidation, was also detected in this strain. Of the three sets of primers (designed by Kohno et al., 2002) that detected different known groups of alkane monoxygenases, TERI NSM, did not give a positive amplification with the first set that was designed by aligning the genes of short to medium chain alkane degrading bacteria (similar to AlkB of Pseudomonas sp.). There was no amplification with the second set of primers either. These primers were designed by aligning the sequences of the mono- and di-oxygenases of Acinetobacter sp. encoded by AlkM genes. However, the chromosomal DNA of TERI NSM, gave a positive amplification using the third set of primers that included bacteria with unknown substrate specificities but encoding the alkB or alkB1 homologues. After establishing the paraffin degradation capabilities and alkane catabolic pathways adopted by TERI NSM, its degradation abilities were tested under laboratory conditions with the paraffinic crude oil collected from oil wells of Gujarat. The results were promising, with *G. kaustophilus* TERI NSM, showing efficient utilization of the paraffins of the crude oil and improved flow behaviour. After laboratory experiments, the strain was produced in mass scale and injected (along with nutrients) into selected oil wells that had a history of paraffin deposition related problems. The oil wells were then closed for seven days to allow the culture to establish in the oil well tubing. Subsequent field results provided by the oil company show a reduction in the viscosity of the paraffinic crude oil. The mechanical scraping frequency of these oil wells which was done on alternate days was not done for eight months. This indicates a saving of roughly $1500 a week by using *Geobacillus kaustophilus* TERI NSM. This technology is now being patented.

The second problem addressed in this thesis was of the bioremediation of acidic oily sludge. The acidity of the oily sludge at Digboi refinery of India was a result of a century of primitive wax refining techniques using sulphuric acid. In order to find microbes that could degrade the toxic oily sludge under acidic conditions, the oily sludge contaminated soil of Digboi refinery was used for enrichment in minimal salts medium at pH3. The consortium that was obtained as a result of this enrichment was purified and the two most efficient acidic oily sludge degrading strains were obtained. These strains were identified using biochemical and molecular techniques as a novel yeast species named *Candida digboiensis*. The selected efficient strain TERI ASN6 was characterized for its hydrocarbon utilization profile as also its peculiar morphological traits. This strain was found to be dimorphic under different environmental conditions. It developed pseudohyphae in nutrient medium containing glucose, yeast extract and peptone if the
pH dropped to 3 but showed normal yeast cell morphology at pH7. Under slight nutrient pressure as in Luria Bertani Agar, where an easily assimilable carbon source like glucose was not available, this strain formed pseudohyphae at pH3 and after a few hours incubation at pH7 as well. However, in minimal salts medium containing hydrocarbons like eicosane, fluoranthene, crude oil or oily sludge as the carbon source, this strain formed hyphae at pH3 as well as pH7. In medically important yeast strains this phenomenon is known to increase invasiveness of this strain to forage for nutrients and thereby increase its pathogenicity. The ability of TERI ASN6, to form hyphae at low pH and in the presence of hydrocarbons, gives it an advantage (mycelial access to the hydrophobic hydrocarbons) for survival on field where it would encounter both these stresses simultaneously. The hydrocarbon utilization profile of TERI ASN6, showed that it was able to grow on minimal salts medium at pH3 using alkane from octane to triacontane as the sole carbon source. However, the growth was markedly reduced when aromatic hydrocarbons were used as the sole carbon source. This pattern was also noted during the degradation studies of C. digboiensis TERI ASN6 with the even chain alkanes octadecane and eicosane; the odd chain alkane heneicosane and the aromatic hydrocarbon pyrene as the carbon source. While TERI ASN6 could efficiently utilize eicosane and heneicosane as the sole carbon source, it could not do so with pyrene. The alkane and aromatic hydrocarbon oxidation pathways for this strain were determined by growing this strain with representatives of each group of oily sludge ie alkanes, aromatics and NSO containing compounds. The intermediate metabolites formed when TERI ASN6 was grown with odd and even carbon chain alkanes indicated that a monoterminal oxidation pathway was functional in the yeast strain. This was further confirmed by growing TERI ASN6 on primary alcohols and mono carboxylic acids. There was also evidence that the dicarboxylic oxidation might be a minor pathway. However, subterminal oxidation was ruled out. During the growth of TERI ASN6 with aromatic hydrocarbons, pyrene and phenanthrene, oxidative metabolites were observed. In two separate analyses, 1- and 2- pyrenol were detected. The oxidative products of the bacterial and lignolytic oxidation pathways not being detected and the first product of oxidation being pyrenol indicated that aromatic hydrocarbon oxidation by this strain follows the non-lignolytic pathway. The growth of TERI ASN6 with dibenzothiophene (DBT), a representative of the NSO group, yielded interesting results. While the products of DBT degradation were not detected, DBT sulfone and hydroxybiphenyl of the DBT desulfurization pathway were detected. The Cytochrome P450 gene, known for alkane degradation in yeasts, was also detected in this strain using degenerate primers. After the degradation ability of this strain was established under laboratory conditions, it was tested on field. Initially, feasibility studies were conducted where various treatments were compared in a small area at the Digboi refinery site. These treatments were, the
neutralization of lime and addition of TERI ASN6, the application of only nutrients, the application of nutrients and TERI ASN6 without lime and the fourth plot was left as the untreated control. The periodic analysis of the total petroleum hydrocarbons, collected from these treatments, during the study showed the treatment of the acidic oily sludge with nutrients and *C. digboiensis* TERI ASN6 showed the maximum degradation. This treatment was then used in a full scale bioremediation study. This technology is now patented.